Original Contribution

Free-Ranging Little Brown Myotis (*Myotis lucifugus*) Heal from Wing Damage Associated with White-Nose Syndrome

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Abstract: White-nose syndrome (WNS) is having an unprecedented impact on hibernating bat populations in the eastern United States. While most studies have focused on widespread mortality observed at winter hibernacula, few have examined the consequences of wing damage that has been observed among those bats that survive hibernation. Given that WNS-related wing damage may lead to life-threatening changes in wing function, we tested the hypothesis that reduced abundance of free-ranging little brown myotis (*Myotis lucifugus*) with severe wing damage as the summer progresses is due to healing of wing tissue. Photographs of captured and recaptured adult females were examined for wing damage and healing rates were calculated for each category of wing damage index (WDI = 0–3). We found that free-ranging bats with severe wing damage were able to heal to a lower WDI score within 2 weeks. Bats with the most severe wing damage had faster healing rates than did individuals with less damage. We also found a significant relationship between body condition and WDI for adult females captured in the early weeks of the active season. Our results support the hypothesis that some bats can heal from severe wing damage during the active season, and thus may not experience increased mortality associated with reduced functions of wings. We urge researchers and wildlife managers to use caution when interpreting data on WDI to assess the impact of WNS on bat populations, especially during the later months of the active season.

Keywords: white-nose syndrome, WNS, *Myotis lucifugus*, little brown bat, wing membrane healing, wing damage

INTRODUCTION

The normal structure and function of wings is vital to the survival of bats. Not only do bats rely on their wings for flight and feeding, but these thin, highly vascularized structures also facilitate gas exchange (Herreid et al. 1968; Makanya and Mortola 2007), water balance (Kallen 1964;

Bassett 1980; Thomas and Cloutier 1992), and thermoregulation (Reichard et al. 2010). It is suspected that *Geomyces destructans* (*Gd*), the psychrophilic fungus associated with white-nose syndrome (WNS) in hibernating bats (Blehert et al. 2009), is responsible for some of the wing damage observed in bats with this devastating disease (Reichard and Kunz 2009; Meteyer et al. 2009). Because bats depend on their wings for aerial locomotion (dispersal, foraging, and migration), predator avoidance, and homeostatic regulation of temperature and water balance

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(Cryan et al. 2010), it is important to understand whether damaged wings are able to heal and regain their normal functions following severe damage.

Histopathological examination of wing tissue from bats affected by WNS indicates that skin lesions are associated with *Gd*, the putative causative agent of WNS (Gargas et al. 2009). The hyphae of *Gd* invade hair follicles and skin glands, which may become filled with the proliferating hyphae and conidia (Chaturvedi et al. 2011; Meteyer et al. 2009). The fungus can then further invade the underlying connective tissue and capillary beds, severely eroding this tissue and causing infarctions (Meteyer et al. 2009; Cryan et al. 2010). The resulting necrotic tissue is prone to tearing or may slough off, resulting in loss of wing surface area (Reichard and Kunz 2009).

When WNS emerged in the northeastern US in 2006, researchers began to record evidence of abnormal wing damage, including perforations, scarring, and necrosis, in bats with and without clinical signs of WNS (Fig. 1; Reichard and Kunz 2009). However, as the active season progressed, the prevalence of wing damage declined, raising questions about the ultimate fate of bats with extensive wing damage (Reichard and Kunz 2009). Some of these bats could be dying because of complications resulting from poor wing condition. Alternatively, others could experience healing of their wing membranes, reducing the likelihood that wing damage will be observed later in the season.

Current understanding of healing in wing membranes is based on only two studies of free-ranging bats (Davis

Figure 1. Wing damage was defined following Reichard and Kunz (2009) and included the following criteria: discoloration, tears, holes, flaking, necrosis, receded wing margins, and missing tissue.

1972; Weaver et al. 2009), although other studies have focused on wing healing in captive or rehabilitated bats (Church and Warren 1968; Iverson et al. 1974; Faure et al. 2009; Meteyer et al. 2011). Free-ranging bats naturally incur damage from encounters with objects in the environment, and as a consequence of failed predation attempts (Davis 1968). Both captive and free-ranging bats may also be subjected to human-induced wing damage resulting from wing biopsies collected by researchers for genetic studies (e.g., Worthington-Wilmer and Barratt 1996; Turmelle et al. 2011), dietary analysis (e.g., Sullivan et al. 2006; Cryan and Diehl 2009), or from injuries associated with marking bats (Kunz and Weise 2009). Despite the sometimes severe nature of initial wounding, many injuries to wing tissue appear to heal given sufficient time. For example, free-ranging pallid bats (*Antrozous pallidus*) healed from relatively large 15 mm^2 wounds to 1 mm^2 wounds in less than 33 days (Davis 1972). There is convincing evidence that bats exhibit an ability to recover from such injuries with minor negative impact on foraging success and ultimate survivorship (Worthington-Wilmer and Barratt 1996; Faure et al. 2009; Weaver et al. 2009).

The primary objective of this study was to test the hypothesis that free-ranging bats are capable of healing following severe wing damage that is associated with WNS. We predicted that recaptured bats would show signs of recovery, with the fastest rates of healing being observed in individuals with the most damage and thus more area to heal. A secondary objective was to evaluate the relationship between body condition and wing damage index scores (WDI). We tested this relationship in very early pregnancy, before a noticeable fetus is present, to remove a potential bias that could be introduced by body mass gained by pregnant females. We also predicted that bats with high WDI would have a poorer body condition, based on derived body mass indices (BMI = length of forearm/mass).

METHODS

Study Site

This study was conducted between 13 May and 10 August of 2009 at two maternity roosts of *M. lucifugus* in New England (Framingham, Massachusetts and Milford, New Hampshire). These maternity colonies are located in barns that are used to shelter livestock and to store hay and varied household items. *Myotis lucifugus* is the most common
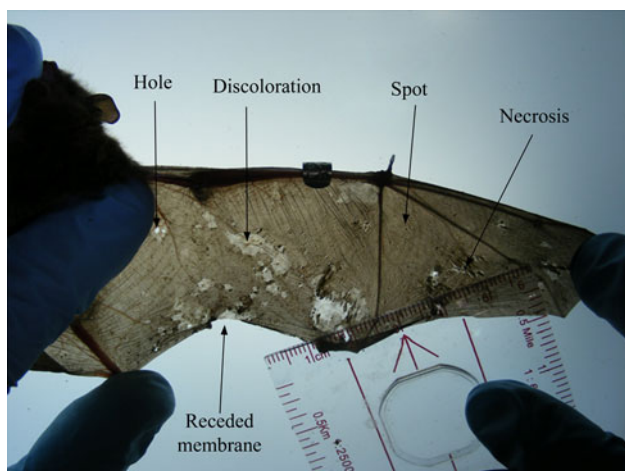
species at these locations along with small numbers of *Eptesicus fuscus*, *M. septentrionalis*, and *Perimyotis subflavus*. The surrounding habitat is mixed hardwood forest, light residential, and some agriculture. Both sites are located near wetlands and waterways (Sudbury River in Framingham, Massachusetts and Nashua River in Milford, New Hampshire). In both colonies, *M. lucifugus* adult females and pups form small clusters during the day in small crevices or along the ridgepole of the barns and depart and return through the main doors or from openings between side-boards and eaves. These are the same colonies sampled by Reichard and Kunz (2009) in the development of the WDI, and thus are ideal for investigating the dynamics of wing healing within the affected range of WNS.

Trapping and Field Measurements

Each colony was trapped at bi-weekly intervals using two portable, double-frame harp traps (0.9 m wide by 1.0 m high or 1.5 m wide by 1.9 m high). The traps were placed side by side in front of one of the main open doors of the barns, while the second main entrances were closed and other large passageways were obstructed by plywood or cotton sheets to encourage bats to depart from a single portal (Kunz et al. 2009). Traps were left standing for approximately 1 h or until no bats were captured over a 10-min period. Each captured bat was placed into a clean, individually marked cotton bag and placed inside a heated holding container for later processing. Only adult bats were used in this study; juveniles were sexed and noted in the total number captured but were immediately released outside the building.

Sex, age, reproductive condition, length of forearm, and body mass were recorded (Brunet-Rossinni and Wilkinson 2009; Racey 2009). Bats were banded with 2.9 mm, individually numbered, lipped alloy bands (Porzana Ltd., UK; Kunz and Weise 2009). Wings and uropatagia were transilluminated using a portable light box (GloBox Lightbox, Artograph, Inc., Delano, Minnesota) and assigned a WDI score following methods described by Reichard and Kunz (2009; Fig. 1).

Quantifying Recovery of Wings

Both wings and the uropatagium of each bat were transilluminated and photographed with a digital camera (Fujifilm FinePix S700) by extending the wing and leg away from the body so that the leg was fully extended in its natural position and the forearm and propatagium were

perpendicular to the axis of the body. Photographs were taken with the camera's automatic macro setting and no flash. A metric ruler or another object of known dimensions was included in each image for scale (Fig. 1).

All photographs were characterized for the proportion of total damaged wing area using ImageJ (v. 1.43u, National Institutes of Health). First, the scale of each photograph was established using a scaling item (i.e., metric ruler or the radius of an US penny). Next, the entire pixel area of each wing was established by outlining the visible wing area. When part of the total wing area was occluded by the handler, we estimated this area and included it in the total. Wing area for each wing was summed for each bat. Finally, the damaged area was outlined and the total damaged area for each wing was summed for each bat. We considered damage as one or more of the following conditions: discoloration (i.e., white areas, abnormal brown areas, black spots, red areas), tears, holes, flaking, necrosis, receded wing margins, and missing tissue (Figs. 1, 2). Healing was defined as a reduction in total damaged area between initial capture and recapture date, and was usually identified as a change from the above criteria to uniformly colored and structured tissue. Healing rate was calculated by dividing the total area of wing tissue that healed by the number of days between initial capture and recapture.

Statistical Analyses

Statistical analyses of healing rate required that some of the recaptured bats be removed due to lack of photographs, or poor quality images that could not be used in our analysis. Thus, we assessed healing rates of 29 recaptured bats. Groupings by wing damage were based on initial WDI. Average healing rates among WDI cohorts were compared using nonparametric statistical analyses.

The relationship between BMI and WDI was calculated using a subset of the total sample. To account for the effect of reproductive condition (i.e., body mass gained during pregnancy) on BMI, only female bats that were captured between 13 May 2009 and 27 May 2009 were included in this analysis. This time period was selected to avoid confounding effects of growing fetuses in pregnant bats; colonies first consisted of palpably pregnant bats on 27 May. We assumed that most females captured were pregnant between 13 May and 27 May, and that the small fetus during this period did not have a measurable effect on body mass or BMI. All statistical analyses were performed using JMP v. 9.0.0 (SAS Institute Inc., S. Cary, North Carolina).
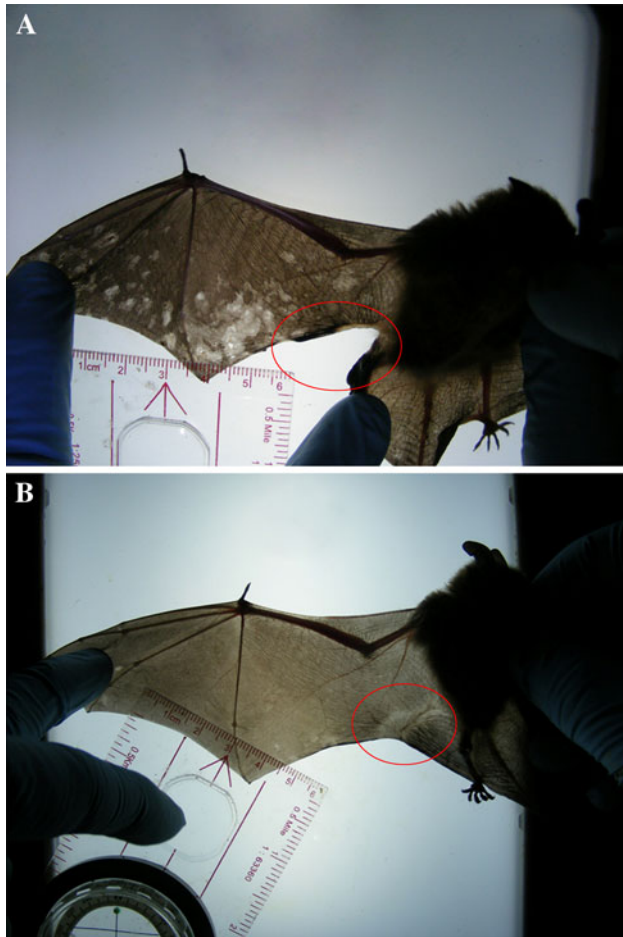
Figure 2. Photographs of a transilluminated wing of an adult female *Myotis lucifugus* showing the most extensive healing recorded in this study. The *highlighted region* shows an area of lost wing tissue that healed over a period of time extending from 10 June 2009 (**a**) to 6 August (**b**) 2009 (57 days). This particular bat was captured in lactation on 23 June 2009 and was observed in post-lactation on 6 August 2009.

RESULTS

We captured a total of 324 bats (including 71 that had been banded in previous years) and recaptured 37. Many of the recaptured adult females were observed in lactation after 9 June. The greatest occurrence of pregnant females was on 27 May at the Massachusetts site ($n = 46$) and on 3 June at the New Hampshire site ($n = 19$). Throughout our study, only ten adult females showed no apparent signs of reproduction. Among these individuals, seven had BMI values that were lower than the average for all individuals captured between 13 and 27 May (average BMI $= 0.21$). The relative abundance of adult females with different WDI scores varied with time (Pearson's $\chi^2 = 96.4$, $P < 0.001$, $N = 291$; Fig. 3). The relative abundance of the most severe wing damage (WDI $= 2$ or 3) was higher in early summer (13 May–10 June) than late-summer (18 June–6 August). In May, the relative abundance of bats with WDI $= 2$ and 3 was almost 50% of the total number captured. After 4 June, the relative abundance of bats with WDI $= 2$ was less than 0.25 and after 10 July no bats were observed with WDI $= 2$ or 3, except for one individual on 6 August. The abundance of moderate to severe wing damage was greatest during the time period coinciding with early to late pregnancy (13–27 May; Fig. 3).

BMI varied among bats with different WDI scores (Kruskal–Wallis test, $\chi^2 = 11.66$, $df = 3$, $P = 0.0086$) captured between 13 May 2009 and 27 May 2009 (Fig. 4). Bats with WDI $= 2$ or 3 had significantly lower BMI than bats with WDI $= 0$ or 1 (pairwise comparisons using Wilcoxon Method).
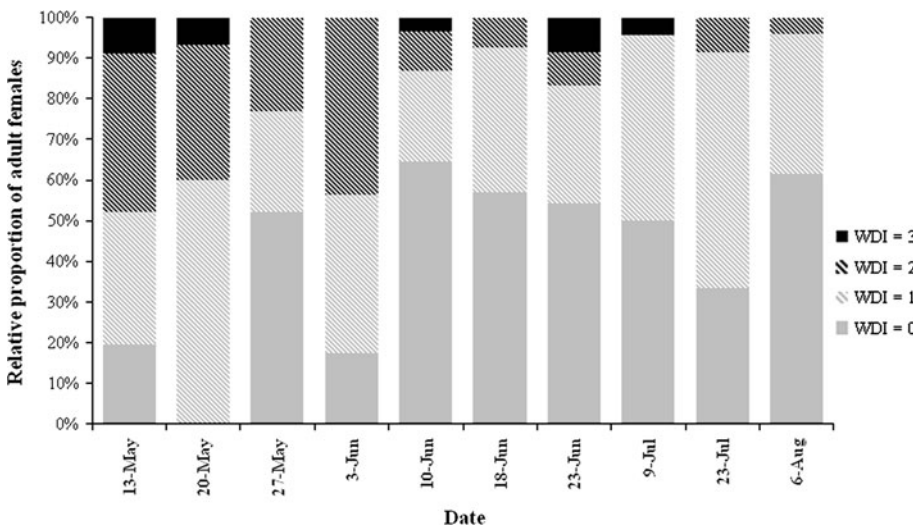
Figure 3. Relative proportion of adult female *Myotis lucifugus* with various degrees of wing damage recorded at two summer maternity roosts in New England. Trapping events that yielded fewer than five individuals were excluded from this analysis.
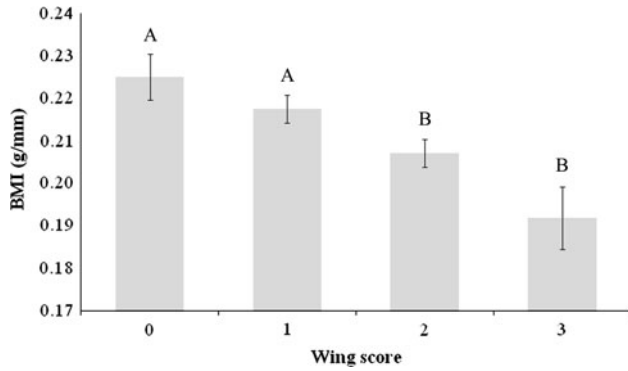
Figure 4. Mean BMI [BMI = M_b (g)/length of forearm (mm)] of adult female *Myotis lucifugus* with different WDI scores at summer maternity roosts in New England from 13 May to 27 May 2009. BMI was calculated before visibly or palpably pregnant females were observed at study colonies to control for gain in body mass during pregnancy. *Bars* labeled with similar letters are not significantly different. *Error bars* are standard errors.
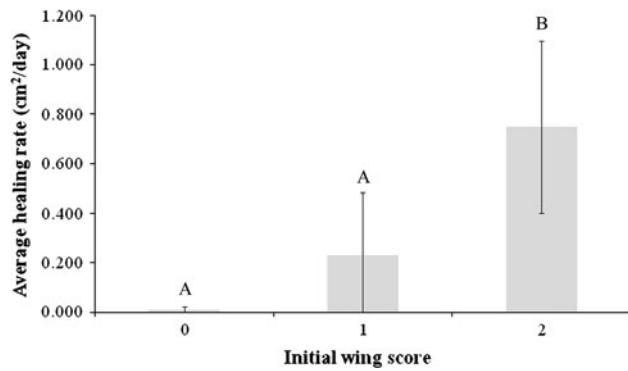
Figure 5. Average healing rates (cm^2/day) of wings of adult female *Myotis lucifugus* at two summer maternity roosts in New England. *Bars* labeled with similar letters are not significantly different. *Error bars* are standard errors.

Healing rate among recaptured bats varied within the grouped WDI measures (Fig. 5). Healing rates among bats with moderate to severe wing damage (WDI = 2 or 3) were significantly faster than in bats with lesser damage (WDI = 0 or 1; Kruskal–Wallis test, χ^2 = 16.729, *df* = 2, *P* = 0.0002). Bats with an initial capture WDI of 0 (*n* = 5) had an average healing rate of 0.010 cm^2/day. Bats with an initial WDI score of 1 (*n* = 10) healed at an average rate of 0.232 cm^2/day. Half of these individuals healed to level 0. The group containing bats with an initial score of 2 (*n* = 12) had an average healing rate of 0.750 cm^2/day. Of these, eight individuals healed to level 1 and four healed to level 0. Due to the small sample size of bats with WDI = 3 and unusual healing patterns within this group, an average healing rate could not be calculated and compared.
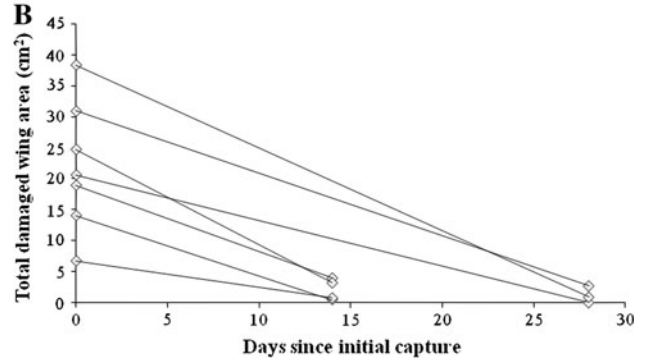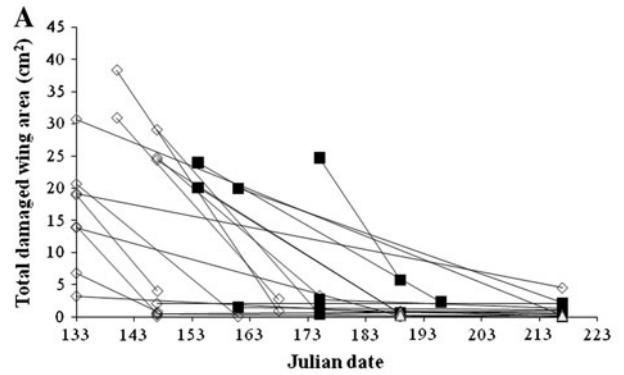
Figure 6. Healing rates of adult female *Myotis lucifugus* at two summer maternity roosts in New England. Each connected pair of points represents an individual bat. **a** Healing rates of each recaptured bat that was included in photographic wing analysis. *Markers* represent month of initial capture. *Open diamonds* May, *closed squares* June, *shaded triangles* July. **b** Healing rates of bats that were initially captured between 13 May 2009 and 27 May 2009 (day 0) and recaptured within 28 days of initial capture.

However, both of the individuals in this group showed extensive healing, reaching levels 2 and 1. The maximum healing rate observed in this study was 1.293 cm^2/day in a bat that repaired 37.498 cm^2 of damaged tissue in 29 days, transitioning from level 2 to 0 (Fig. 6).

DISCUSSION

Implications of Rapid Wound Healing in Free-Ranging Bats

We present evidence that some bats affected by WNS during hibernation are capable of rapid healing of their wing membranes and survive the effects of WNS-associated wing damage upon arrival at maternity roosts. We describe healing for 29 of 37 (78%) recaptured bats. Of the remaining eight bats that were recaptured, four showed a decrease in WDI, but we were unable to document these

changes due to lack of photographs. The remaining four bats that showed no evidence of healing had low initial WDI scores (0 or 1). Our study demonstrates the value of mark-recapture data and thus reveals resilience to wing injuries among free-ranging *M. lucifugus* subjected to wing damage associated with WNS.

An hypothesis proposed by Reichard and Kunz (2009) states that the reduction in relative abundance of moderate to severe wing damage late in the active season was due to increased mortality from predation or starvation resulting from reduced flight maneuverability and foraging efficiency of these individuals. In support of this hypothesis, they reported only two recaptured bats during the summer of 2008 that had improved wing conditions and observed numerous dead bats in and nearby their study colonies. While we offer evidence to the contrary, we cannot fully reject this hypothesis. Recapture rates for this study were relatively low (∼0.10) but were similar to those in past studies on *M. lucifugus* (0.10–0.35; Frick et al. 2010b), Thus, we cannot account for bats that were not recaptured, and it is possible that some or all of these bats died, although evidence from the Massachusetts site suggests that at least one bat that was banded in 2009 had survived until the spring of 2011 (K. Langwig, personal communication).

We observed a faster healing rate among bats with moderate to severe wing damage (WDI = 2 or 3) than in bats with lesser damage (WDI = 0 or 1). This result matches previous results of wing healing studies in free-ranging and captive bats (Church and Warren 1968; Iverson et al. 1974; Davis 1972; Faure et al. 2009; Weaver et al. 2009; Meteyer et al. 2011) and patterns of mammalian wound healing in general (Singer and Clark 1999). Cutaneous wound healing, as understood from clinical studies, generally progresses as follows: clotting, inflammation, re-epithelialization, wound contraction, and angiogenesis (Singer and Clark 1999). Within each step there is a complex interaction of gene regulation, cell migration, and cytokine secretions (i.e., epidermal growth factors) that promotes cell proliferation, microbial clearing, and tissue restructuring (Martin 1997). The accelerated healing we observed in free-ranging bats with severely damaged wing tissues likely represents rapid epithelialization paired with wound contraction. However, individuals with lesser damage, including spotting and lack of pigmentation, most likely exhibit signs of melanocyte repopulation, a process that occurs in the final stages of wound healing (Cox et al. 1989).

The cellular mechanisms of wing membrane healing in bats are not known, nor is there information on whether wings of bats regenerate, such as have been observed in skin tissues of other mammals (Breedis 1954; Williams-Boyce and Daniel 1986). Whether bats regenerate wing tissue completely, including replacement of hair follicles and skin glands, is an important component to our understanding of how wing damage affects the physiology of bats during hibernation (Cryan et al. 2010). If bat wings do not recover completely, leaving them with poorly functioning or abnormally distributed skin appendages (e.g., hair follicles and glands), then survivors of one hibernation season may suffer cumulative injury as their wings become increasingly compromised with each successive winter of WNS-induced damage followed by tissue replacement in the active season.

In addition to the direct effects of wing damage on flight aerodynamics and wing physiology, rapid wing tissue healing may reflect a greater allocation of nutrients to wound healing in those bats that have severe wing damage. Rapid healing of small wounds in bats is likely an adaptive process and is usually beneficial, considering that bats regularly incur minor injuries (Davis 1968). While healing to this degree increases the probability of short-term survival by facilitating flight, long-term survival may be compromised when individuals allocate more nutrients to healing than they normally would. Wound healing is energetically expensive to mammals and depends considerably upon the status of the immune system (Lee 2006). Thus, bats may experience energetic trade-offs by allocating more energy to regenerating lost tissue rather than to other important processes such as reproducing, mounting an immune response to challenges other than wound healing, or migration (Bernardo and Agosta 2005). A trade-off between reproduction and immune function has been shown in a number of vertebrate taxa, including small mammals (French et al. 2007, 2009). If limited energy resources are allocated to repair wing tissue, then bats will face increased risks to survival and reproduction during the early weeks of the active season, a trade-off that would be exacerbated by low ambient temperatures (increasing thermoregulatory costs) and reduced insect availability during the spring in New England (Hoying and Kunz 1998).

Considerations for WNS Research

WNS is having an unprecedented impact on hibernating bat populations in the eastern United States (Frick et al. 2010a) but the actual mechanism of mortality remains

elusive. However, an hypothesis recently proposed by Cryan et al. (2010) suggests that damaged wing membranes may play a significant role in the death of WNS-affected bats during hibernation. As the hyphae of *Gd* invade and erode wing tissue, specifically the sebaceous glands that produce secretions that aid in waterproofing, wings may lose their ability to regulate water balance. Consequently, affected bats may be forced to arouse from torpor more frequently because of their need to replenish body water (Cryan et al. 2010; Németh et al. 2010). If this postulated mechanism is indeed the cause of mortality among bats with WNS, then healing of wing tissue during the active season represents a life-saving process to the few bats that survive hibernation after being infected with *Gd*. Additional research is needed to understand the physiology of healthy and damaged wing tissue during the hibernation and active season.

Records of WDI are invaluable in establishing a baseline level of wing damage in a region and may also be used to assess the lasting impact of WNS into the active season. For example, large-scale studies offer the potential to determine the relationship between the occurrence of wing damage and regions of *Gd* infection (Francl et al. 2011). It is tempting, however, to diagnose "confirmed presence" of WNS in a region where a higher frequency of wing damage from *Gd* is observed, but wing condition alone is not a diagnostic tool to confirm WNS (Meteyer et al. 2009; Reichard and Kunz 2009). Until a temporal model of wing membrane healing and a reliable field test of *Gd* presence are developed, factors such as date of observation must be considered before attempting to correlate wing damage with WNS. We note that little damage was discernable on most individuals by mid- to late-summer (i.e., during post-lactation; Fig. 2); any damage observed during or after late July should be carefully scrutinized for cause.

Researchers may attempt to correlate intensity of *Gd* infection (i.e., fungal load) with degree of wing damage. Again, some evidence supports this hypothesis, but these observations must be validated with histopathological analysis to demonstrate fungal penetration of wing tissue (Meteyer et al. 2009). Given the fragility of wing tissue and the gregarious nature of most hibernating bats, a number of other sources of wing damage could be misinterpreted as damage from *Gd*. Assessing baseline occurrence of WDI in geographic regions that are unaffected by WNS will permit researchers to identify changing patterns of wing damage in bat populations that incur wing damage more regularly.

Maternity Colonies Corroborate WNS-Related Population Declines

One trend revealed from comparing the 2008 study (Reichard and Kunz 2009) and our 2009 findings is the notable decrease in total captures in 2009. This pattern may be explained by large-scale mortality due to WNS at nearby hibernacula (Aeolus Cave, East Dorset, Vermont and the Chester Mines, Chester, Massachusetts). The hibernating population at the upper Chester Mine before the winter of 2008/2009 was estimated to be 8,000–10,000 individuals, but has since declined to just 116 bats by mid winter in 2009/2010 (T. French, personal communication). The population at Aeolus Cave before it was infected with *Gd* may have been as high as 300,000 individuals (Trombulak et al. 2001) but the majority of the bats hibernating in the accessible portions of this site had died by late January 2009 (S. Darling, personal communication). We can further illustrate the decline at these colonies using the Schnabel Method (1938) for estimating population size based on mark-recapture data of adult female bats. Using Reichard and Kunz's (2009) data for summer of 2008, we calculated total occupancy of bats in the Massachusetts site to be 4,570 individuals, while the total at the New Hampshire site was 657 individuals. Unfortunately, these numbers appear to be greatly inflated compared to direct emergence counts at these colonies. Notwithstanding, these values illustrate the point that bats were sufficiently numerous in 2008 that the probability of recapturing an individual bat was extremely low, which will bias Schnabel estimations. In the summer of 2009, we calculated the population of bats in the Massachusetts site to be 281 individuals and 41 individuals in the New Hampshire site using the same trapping and population estimation procedures. Although this value may still overestimate colony size because of inherit limitations and assumptions of mark-recapture methods (O'Shea et al. 2004), the difference between years is striking, showing a precipitous population decline at each colony, which corroborates observations of reduced activity at acoustic monitoring stations (Dzal et al. 2010; Brooks 2011).

CONCLUSION

This study represents important evidence that some bats that incur wing damage during hibernation (presumably from exposure to WNS, in this instance) are able to heal rapidly and may also successfully reproduce in spite of such

damage. However, while this evidence is a positive note among mostly negative trends in WNS research, it is important that conservation efforts for bats focus on year-round strategies both during hibernation and throughout the active season. With the severe declines in summer populations observed in this and other studies (Dzal et al. 2010; Brooks 2011), and the prediction of regional extinction of *M. lucifugus* within 16–20 years (Frick et al. 2010a), the few maternity colonies that remain represent vital islands of reproduction and genetic variation. If human interventions, such as increased utility-scale wind-energy development (Kunz et al. 2007; Arnett et al. 2008) and "pest control" practices threaten the viability of these colonies, then local extinction of populations of *M. lucifugus* may occur sooner than predicted. Further research is needed to assess the long-term impacts of WNS on summer colonies of bats in the northeastern US and the effect of wing damage on reproductive success, foraging ability, flight maneuverability, physiological functions of wings, and energetic costs of healing.

ACKNOWLEDGMENTS

REFERENCES

Arnett EB, Brown WK, Erickson WP, Fiedler JK, Hamilton BL, Henry TH, et al. (2008) Patterns of bat fatalities at wind energy facilities in North America. *Journal of Wildlife Management* 72:61–78

Bassett JE (1980) Control of postprandial water loss in *Myotis lucifugus lucifugus*. *Comparative Biochemistry and Physiology* 65:497–500

Bernardo J, Agosta SJ (2005) Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. *Biological Journal of the Linnean Society* 86:309–331

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier B, Buckles EL, et al. (2009) Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227

Breedis C (1954) Regeneration of hair follicles and sebaceous glands from the epithelium of scars in the rabbit. *Cancer Research* 14:575–579

Brooks R (2011) Declines in summer bat activity in central New England 4 years following the initial detection of white-nose syndrome. *Biodiversity and Conservation* (doi:10.1007/s10531-011-9996-0) [Online 28 January 2011]

Brunet-Rossinni AK, Wilkinson GS (2009) Methods for age estimation and study of senescence in bats. In: *Ecological and Behavioral Methods for the Study of Bats*, Kunz TH, Parsons S (editors), Baltimore, MD: Johns Hopkins University Press, pp 315–325

Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li X, Peck MK, et al. (2011) Morphological and molecular characterizations of psychrophilic fungus *Geomyces destructans* from New York bats with white nose syndrome (WNS). *PLoS One* 5:e10783 . doi:10.1371/journal.pone.0010783

Church JCT, Warren DJ (1968) Wound healing in the web membrane of the fruit bat. *British Journal of Surgery* 55:26–31

Cox PM, Dhillon AP, Howe S, Pittilo RM, Rode J (1989) Repopulation of guinea-pig skin by melanocytes during wound healing: a morphometric study. *British Journal of Experimental Pathology* 70:679–689

Cryan PM, Diehl RH (2009) Analyzing bat migration. In: *Ecological and Behavioral Methods for the Study of Bats*, Kunz TH, Parsons S (editors), Baltimore, MD: Johns Hopkins University Press, pp 467–488

Cryan PM, Meteyer CU, Boyles JG, Blehert DS (2010) Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology* 8:135 (doi:10.1186/1741-7007-8-135) [Online November 11, 2010]

Davis R (1968) Wing defects in a population of pallid bats. *American Midland Naturalist* 79:388–395

Davis R (1972) Wing repair in pallid bats. *Journal of Mammalogy* 53:377–378

Dzal Y, McGuire LP, Veselka N, Fenton MB (2010) Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown bat (*Myotis lucifugus*). *Biology Letters* (doi:10.1098/rsbl.2010.0859) [Online November 24, 2002]

Faure PA, Reeder DE, Clare EL (2009) Wound healing in the flight membranes of big brown bats. *Journal of Mammalogy* 90:1148–1156

Francl KE, Sparks DW, Brack V Jr, Timpon J (2011) White-nose syndrome and wing damage index scores among summer bats in northeastern United States. *Journal of Wildlife Diseases* 47:41–48

French SS, DeNardo DF, Moore MC (2007) Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *The American Naturalist* 170:79–89

French SS, Moore MC, Demas GE (2009) Ecological immunology: the organism in context. *Integrative and Comparative Biology* 49:246–253

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GR, et al. (2010) An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679–682

Frick WF, Reynolds DS, Kunz TH (2010) Influence of climate and reproductive timing on demography of little brown myotis *Myotis lucifugus*. *Journal of Animal Ecology* 79:128–136

Gargas A, Trest MT, Christensen M, Volk TJ, Blehert DS (2009) *Geomyces destructans* sp. nov associated with bat white-nose syndrome. *Mycotaxon* 108:147–154

Herreid CF, Bretz WL, Schmidt K (1968) Cutaneous gas exchange in bats. *American Journal of Physiology* 215:506–508

Hoying KM, Kunz TH (1998) Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Journal of Zoology (London)* 245:15–27

Iverson OH, Bhangoo KS, Hansen K (1974) Control of epidermal cell renewal in the bat web. *Virchows Archiv-B: Cell Pathology* 16:157–179

Kallen FC (1964) Some aspects of water balance in the hibernating bat. *Annales Academiæ Scientiarum Fennicæ* 71:259–267

Kunz TH, Weise CD (2009) Methods and devices for marking bats. In: *Ecological and Behavioral Methods for the Study of Bats*, Kunz TH, Parsons S (editors), Baltimore, MD: Johns Hopkins University Press, pp 36–56

Kunz TH, Arnett EB, Erickson WP, Johnson GD, Larkin RP, Strickland, et al. (2007) Ecological impacts of wind energy development on bats: questions, hypotheses, and research needs. *Frontiers in Ecology and the Environment* 5:315–324

Kunz TH, Hodgkison R, Weise CD (2009) Methods of capturing and handling bats. In: *Ecological and Behavioral Methods for the Study of Bats*, Kunz TH, Parsons S (editors), Baltimore, MD: Johns Hopkins University Press, pp 3–35

Lee KA (2006) Linking immune defenses and life history at the levels of the individual and the species. *Integrative and Comparative Biology* 46:1000–1015

Makanya AN, Mortola JP (2007) The structural design of the bat wing web and its possible role in gas exchange. *Journal of Anatomy* 211:687–697

Martin P (1997) Wound healing—aiming for perfect skin. *Science* 276:75–81. doi:10.1126/science.276.5309.75

Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn-Bochsler V, et al. (2009) Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 21:411–414

Meteyer CU, Valent M, Kashmer J, Buckles EL, Lorch JM, Blehert DS, Lollar A, Berndt D, Wheeler E, White CL, Ballmann AE (2011) Recovery of little brown bats (*Myotis lucifugus*) from natural infection with *Geomyces destructans*, white-nose syndrome. *Journal of Wildlife Diseases* 47:618–626

Németh I, Nyitrai V, Németh A, Altbäcker V (2010) Diuretic treatment affects the length of torpor bouts in hibernating European ground squirrels (*Spermophilus citellus*). *Journal of Comparative Physiology B* 180:457–464

O'Shea TJ, Ellison LE, Stanley TR (2004) Survival estimation in bats: historical overview, critical appraisal, and suggestions for new approaches. In: *Sampling Rare or Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters*, Thompson WL (editor), Washington, DC: Island Press, pp 297–336

Racey PA (2009) Reproductive assessment of bats. In: *Ecological and Behavioral Methods for the Study of Bats*, Kunz TH, Parsons S (editors), Baltimore, MD: Johns Hopkins University Press, pp 249–264

Reichard JD, Kunz TH (2009) White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). *Acta Chiropterologica* 11:457–464

Reichard JD, Fellows SR, Frank AJ, Kunz TH (2010) Thermoregulation during flight: body temperature and sensible heat transfer in free-ranging Brazilian free-tailed bats (*Tadarida brasiliensis*). *Physiological and Biochemical Zoology* 83:885–897

Schnabel ZE (1938) Estimation of total fish population of a lake. *The American Mathematical Monthly* 45:348–352

Sikes RS, Gannon WLAnimal Care, Use Committee of the American Society of Mammalogists (2011) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 92:235–253

Singer AJ, Clark RAF (1999) Cutaneous wound healing. *The New England Journal of Medicine* 341:738–746

Sullivan JC, Buscetta KJ, Michener RH, Whitaker JO Jr, Finnerty JR, Kunz TH (2006) Models developed from δ^{13}C and δ^{15}N of skin tissue indicate non-specific habitat use by the big brown bat (*Eptesicus fuscus*). *Ecoscience* 13:11–22

Thomas DW, Cloutier D (1992) Evaporative water loss by hibernating little brown bats, *Myotis lucifugus*. *Physiological Zoology* 65:443–456

Trombulak SC, Higuera PE, DesMeules M (2001) Population trends of wintering bats in vermont. *Northeastern Naturalist* 8:51–62

Turmelle AS, Kunz TH, Sorenson MD (2011) A tale of two genomes: contrasting patterns of phylogeographic structure in a widely distributed bat. *Molecular Ecology* 20:357–375

Weaver KN, Alfano SE, Kronquist AR, Reeder DM (2009) Healing rates of wing punch wounds in free-ranging little brown myotis (*Myotis lucifugus*). *Acta Chiropterologica* 11:220–223

Williams-Boyce PK, Daniel JC Jr (1986) Comparison of ear tissue regeneration in mammals. *Journal of Anatomy* 149:55–63

Worthington-Wilmer J, Barratt E (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News* 37:1–3