Acta Chiropterologica, 11(1): 220–223, 2009
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS
doi: 10.3161/150811009X465866

Healing rates of wing punch wounds in free-ranging little brown myotis (*Myotis lucifugus*)

KIMBERLY N. WEAVER[1], SARA E. ALFANO[1], AMANDA R. KRONQUIST[1], and DEEANN M. REEDER[1, 2]

[1]*Department of Biology, Bucknell University, Lewisburg, PA 17837, USA*
[2]*Corresponding author: E-mail: dreeder@bucknell.edu*

Key words: Chiroptera, energy balance, immune function, *Myotis lucifugus*, wing biopsy, wing damage, wound healing

INTRODUCTION

Defects in the wing membranes of bats are commonly observed as a natural consequence of a membrane-wing method of flight (Davis and Doster, 1972). This type of flight remains successful not only because bats can tolerate asymmetries resulting from these injuries, but also because they can rapidly heal rips and fill in missing membranes and even sometimes fractures of the wing bones (Davis, 1968; Bogdanowicz and Urbańczyk, 1986). The wound healing process can generally be classified into three overlapping stages: an inflammatory stage, a proliferative stage, and a protracted remodeling stage. The initial inflammatory stage typically lasts up to four days, while the proliferative stage predominates from days 4–21. Only after the wound has closed upon itself can the wound healing process advance to the final, protracted remodeling stage, which can last up to two years (Kinsey *et al*., 2003; Christian *et al*., 2006). Because this process is energy-dependent, healing rates may vary by life history stage, sex, and season, and may be especially slow in immune compromised animals (Nelson and Demas, 1996; Worthington-Wilmer and Barratt, 1996; Kinsey *et al*., 2003; Martin *et al*., 2008).

While much is known about the basic ecology and physiology of *Myotis lucifugus* (the little brown myotis), little baseline information is available regarding immune function in this or any other bat species. The primary objective of our study was to assess wound healing rates in this species. Wound healing rates can be viewed as an indicator of both immune competence and of variations in energy allocation. Additionally, because the use of sterile biopsy punches to collect tissue for DNA analysis (as originally described by Worthington-Wilmer and Barratt, 1996) has become quite common, it is important to understand how quickly the resulting wounds heal. While the rate of wound healing has not been closely monitored in *M. lucifugus*, previous studies in free ranging *M. bechsteinii* have indicated that 3 mm wounds heal in 3–4 weeks (Kerth *et al*., 2000, 2002). Church and Warren (1968) reported that 2 cm × 2 cm holes took approximately 24 days to heal in captive *Eidolon helvum* while Davis and Doster (1972), working with captive *Antrozous pallidus*, reported that 14 mm holes in the wings required up to 33 days to heal. Davis and Doster (1972) noted that the regenerated region often remains pale, which they and others have suggested explains the pale splotches often found on the wings of free-ranging bats.

We created 3.0 mm circular wounds in the wing membranes of free-ranging *M. lucifugus* and monitored the healing process. Based on previous research on bats and on the general timeline of the wound healing process described from other mammals, we hypothesized that little healing would occur during the first week, but that wounds would close completely within two to three weeks. Wound healing rates in the field were assessed using calipers and digital photographs for later analysis with ImageJ software. A secondary objective of our study was to evaluate the reliability of these two methods. We hypothesized that the use of digital photographs would yield more accurate and precise measurements of wound area.

MATERIALS AND METHODS

Harp traps were used to capture 150 female (140 lactating and 10 non-reproductive) *M. lucifugus* from a summer maternity colony at dusk from a farm outbuilding in Lycoming County in Central Pennsylvania, USA. Bats were captured in July 2008, banded, weighed, and had their forearms measured. Both wing membranes were punctured with a sterile 3.0 mm biopsy punch (Miltex, Inc., USA) (Worthington-Wilmer and Barratt, 1996; Kerth *et al*., 2000). Although multiple sizes of biopsy punches exist, the size most commonly used for smaller bat species is 3.0 mm. Harp traps were deployed at this field site twice a week for three weeks following initial wound creation and 32 of the 150 bats were recaptured, all of which were lactating.

Wound size was quantified using two methods: calipers and digital photographs. A 150 mm dial plastic caliper was used to measure the diameter of the wound at greatest width. A digital photograph (Canon S5IS), including a 3.0 mm paper reference circle, band number, and date was taken of each wounded wing. In an effort to reduce potential stretching of the membrane, and thus of the wound, bats were laid on their backs with their wings arranged such that the upper margin was perpendicular to their body prior to measurement. The margins of the wounds on the digital photographs were traced on a tablet PC. ImageJ software (http://rsbweb.nih.gov/ij) was then used to calculate the areas of the wounds in relation to the paper reference circle.

Measurements from the left and the right wing of each bat were averaged prior to analysis and no animal was represented more than once in the data set. For the subset of bats that was recaptured more than once, only one datapoint was randomly selected. Differences in wound size between days 5–7 and 12–16 were analyzed with a *t*-test for both measurement types. The correlation between the two methods used to measure wound size was assessed with Pearson's correlation coefficient. To assess accuracy and precision for both measurement techniques, 3.0 mm wounds were made in both wings of 22 captive *Eptesicus fuscus*. As was the case for *M. lucifugus*, wounds were measured with both calipers and with ImageJ analysis of digital photographs, but measurements for left and right wings were not averaged prior to analysis. Research followed the guidelines for the use of live animals published by the American Society of Mammalogists (Gannon *et al*., 2007) and was approved by the Institutional Animal Care and Use Committee at Bucknell University.

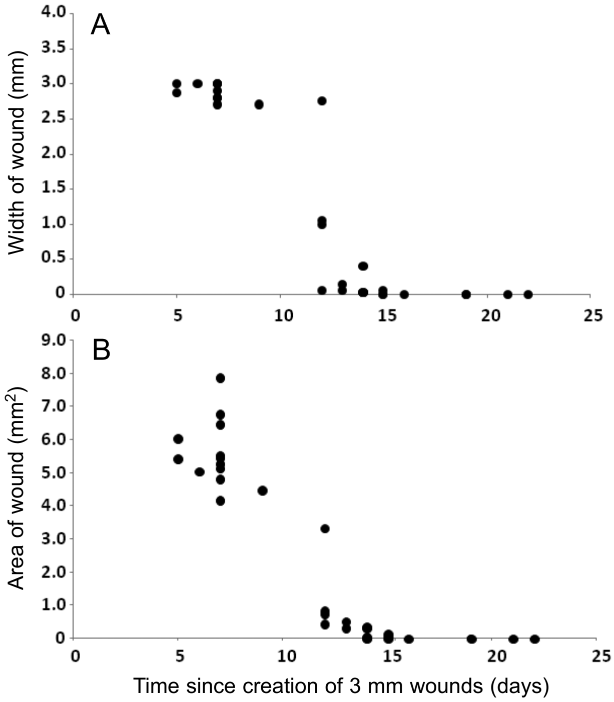

FIG. 1. Individual wound size measurements: widest width of wound as measured by calipers (A) and area of the wound as calculated using image analysis of digital photographs (B). Little healing occurred during the first week but all wounds were closed by 16 days following creation with a 3.0 mm sterile biopsy punch

RESULTS

Wounds created with 3.0 mm sterile biopsy punches displayed little healing in the first 6–7 days after wound creation, as determined both by caliper measurement and by image analysis of digital photographs (Fig. 1A–B). Wounds remained relatively round and resembled the 3.0 mm paper reference in both size and shape (Fig. 2A–B). Although the individual rates of healing varied among recaptured bats, wound size rapidly and significantly decreased in all bats after the first week (width of wound as measured by calipers days 5–7 versus 12–16: $t = 11.2$, $d.f. = 24$, $P < 0.001$; surface area of wound as measured by image analysis: $t = 14.2$, $d.f. = 24$, $P < 0.001$). We observed non-uniform healing during this time period, as wounds were no longer circular in shape (Fig. 2C). All wounds were fully closed by day 16, leaving behind a light colored, partially unpigmented area of scar tissue (Fig. 2D).

Measurements of healing wounds taken by calipers and calculated from digital photographs were strongly and significantly correlated (Pearson's $r = 0.94$, $n = 48$, $P < 0.001$). However, the measurement of wounds via image analysis was more accurate than measurements by calipers. For a 3.0 mm circle, the exact area is 7.068 mm^2. On average, measurement of fresh wounds via image analysis yielded a wound area of 7.014 ± 0.134 mm^2 ($\bar{x} \pm$ SE, $n = 44$), an underestimate of 0.8%. In contrast, measurement of the same 44 wounds with calipers indicated an average wound width of 3.180 ± 0.041 mm, as overestimate of 6.0%. The lower standard error for caliper measurements suggests that although these are less accurate, they are more precise.

DISCUSSION

Wound healing requires activation of the immune system and allocation of energy, both of which are affected by factors such as photoperiod, energy stores, and stress (Nelson and Demas, 1996; Christian *et al*., 2006; Martin *et al*., 2008). Despite the fact that the bats in this study were lactating, and thus in a period of peak energy demand (Kurta *et al*., 1989), wounds in the wing membrane healed rapidly. This suggests both that sufficient energy was available and that the immune system was able to be

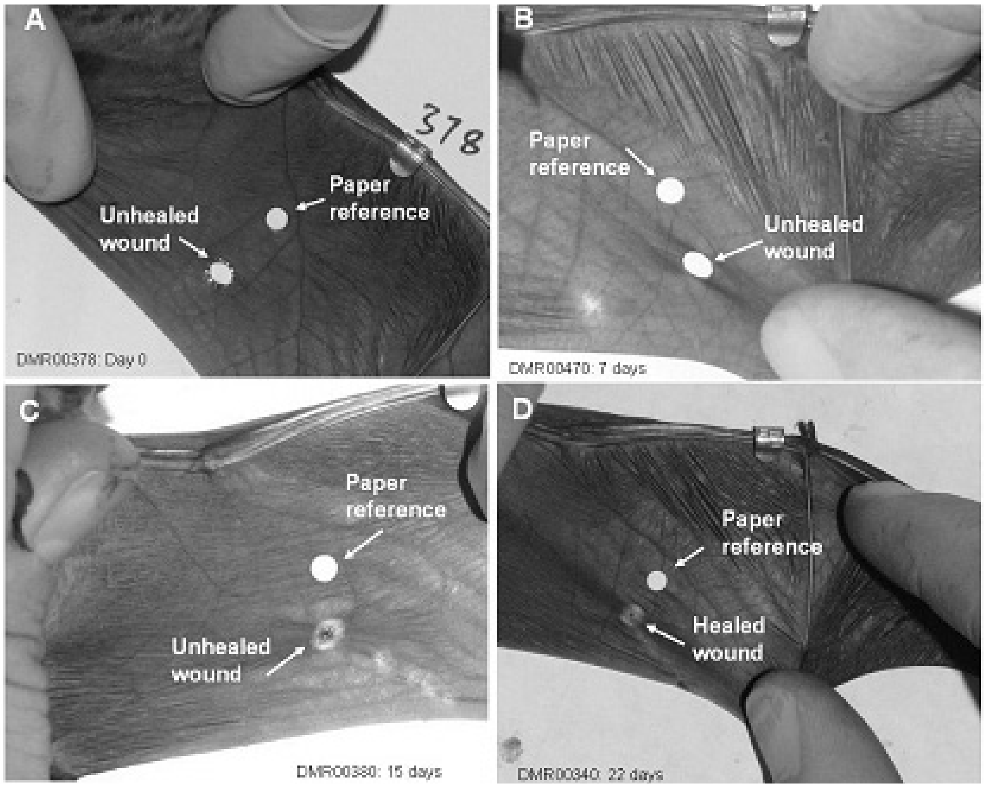

FIG. 2. Digital photographs of wounds used for measuring area using ImageJ software (http://rsbweb.nih.gov/ij), showing paper reference and healing over time (A–D)

mobilized at this time. Rapid wound healing has also been noted in long-day, but not short-day Siberian hamsters (*Phodopus sungorus*), which suggests that the results of our study may have differed if it were conducted in another season (Kinsey *et al.*, 2003). Worthington-Wilmer and Barratt (1996) stated, without providing data, that the wing's healing capacity is reduced just prior to hibernation. Indeed, data from our laboratory also support the idea that the prehibernatory period, as well as hibernation, represent a time of immune compromise for bats (D. M. Reeder and R. Jacob, unpublished data).

The time course of wound healing in our study suggests that healing progressed readily through the three known phases: the inflammatory stage, proliferative stage, and remodeling stage (Church and Warren, 1968; Christian *et al.*, 2006). During the initial inflammatory stage, a favorable wound-healing environment is created but the wound size does not decrease, as was shown during the first week of healing in our bats. Wounds rapidly healed between days 7–14, during which the second, proliferative stage presumably predominated. New non-pigmented tissue formed around the edge of the wound and grew inward until the wing membrane was once again continuous. All wounds were fully closed by day 16; however, tissue remodeling was most likely still occurring because scar tissue began to regain pigment. The time course and pattern of healing is similar to that found with captive *E. helvum* (Church and Warren, 1968). However, healing in our study occurred sooner than the 3–4 weeks reported for healing in *M. bechsteinii* sampled at roughly the same time of year by Kerth *et al.* (2000, 2002; but note that assessing the precise time of healing was not the goal of these studies).

Because bat wings are made of living skin-membrane, the wound is subject to infection, which could potentially increase wound area (Davis, 1968) and delay healing (Bucknall, 1980). Several wounds were larger than the initial biopsy punch width, 3.0 mm, and initial wound area, 7.068 mm^2 (Fig. 1). These may have become infected or torn, causing holes to grow larger in size, rather than shrinking. Alternatively, wounds may have been recorded as being larger than the initial wound due to stretching of the wing prior to caliper measurement or photograph, or even stretching of the wing when the initial wounds were made.

Davis and Doster (1972) suggested that punctured wing membranes never regain their original coloration. However, Bonaccorso *et al.* (1976)

reported that wounds created with 'punch marks' completely healed with no discoloration within six months in captive *Carollia perspicillata* and under field conditions in a variety of species. Accordingly, for at least some months following injury (the time course of which likely depends upon the season in which the wound was created, whether the species hibernates, etc.), holes and discolored blotches found on bat wings may be attributed to prior injury and/or infection. While small holes and tears may readily heal, larger tears and other healed injuries may be permanent, as documented for *A. pallidus* (Davis, 1968). Understanding wound healing, and the range of 'normal' wing injuries is especially relevant when trying to assess the health of a bat that has wings in poor condition, as is occurring with the often fatal white-nose syndrome in cave-dwelling bats in the northeastern USA (Reeder and Turner, 2008; Blehert *et al*., 2009).

We expected that measurement of wounds via image analysis would provide more accurate and precise measurements. While image analysis of digital photos did prove more accurate, caliper measurement of wound width was more precise. However, measurements using both of these methods were strongly correlated, suggesting that either can be used reliably. Despite this, we recommend the use of digital photographs and image analysis software when possible as measurement with calipers is subject to individual user variation. Additionally, digital photographs allowed for easier measurement of non-uniformly healing wounds. Using ImageJ software, we were able to trace the wound boundaries and determine their surface areas. When using this or comparable software, we recommend tracing wound margins using the 'pen mouse' of a tablet PC for accurate outlining of wounds.

With this knowledge of baseline wound healing rates in *M. lucifugus*, as well as data supporting the use of either of the two measurement methods, bat biologists can better assess and evaluate wing damage, the effects of tissue sampling for DNA analysis, and overall bat health. Future studies of wound healing rates in different life history stages, particularly in hibernating species, may be fruitful. For example, wounds made just prior to hibernation or during hibernation may take significantly longer to heal.

Acknowledgements

This work was supported by Bucknell University Undergraduate Summer Research Fellowships in the Biological Sciences to KNW, SEA, and ARK. Additional field assistance was provided by J. B. Kobilis, K. J. Piatt, and R. Jacob.

Literature cited

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome: an emerging fungal pathogen? Science, 323: 227.

Bogdanowicz, W., and Z. Urbańczyk. 1986. A case of self-healing of a broken forearm in *Myotis brandtii* (Eversmann, 1845). Acta Theriologica, 31: 180–181.

Bonaccorso, F. J., N. Smythe, and S. R. Humphrey. 1976. Improved techniques for marking bats. Journal of Mammalogy, 57: 181–182.

Bucknall, T. E. 1980. The effect of local infection upon wound healing: an experimental study. British Journal of Surgery, 67: 851–855.

Christian, L M., J. E. Graham, D. A. Padgett, R. Glaser, and J. K. Kiecolt-Glaser. 2006. Stress and wound healing. Neuroimmunomodulation, 13: 337–346.

Church, J. C. T., and D. J. Warren. 1968. Wound healing in the web membrane of the fruit bat. British Journal of Surgery, 55: 26–31.

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

Davis, R., and S. E. Doster. 1972. Wing repair in pallid bats. Journal of Mammalogy, 53: 377–378.

Gannon, W. L., R. S. Sikes, and The Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy, 88: 809–823.

Kerth, G., F. Mayer, and B. Konig. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. Molecular Ecology, 9: 793–800.

Kerth, G., F. Mayer, and E. Petit. 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). Molecular Ecology, 11: 1491–1498.

Kinsey, S. G., B. J. Prendergast, and R. J. Nelson. 2003. Photoperiod and stress affect wound healing in Siberian hamsters. Physiology & Behavior, 78: 205–211.

Kurta, A., G. P. Bell, K. A. Nagy, and T. H. Kunz. 1989. Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). Physiological Zoology, 62: 804–818.

Martin, L. B., Z. M. Weil, and R. J. Nelson. 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philosophical Transactions of the Royal Society of London, 363B: 321–339.

Nelson, R. J., and G. E. Demas. 1996. Seasonal changes in immune function. Quarterly Review of Biology, 71: 511–548.

Reeder, D. M., and G. R. Turner. 2008. Working together to combat white-nose syndrome: a report of a meeting on 9–11 June 2008, in Albany, New York. Bat Research News, 49: 75–78.

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

Received 06 April 2009, accepted 07 May 2009