Wound Healing in the Flight Membranes of Wild Big Brown Bats

TYLER POLLOCK,1 Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada
CHRISTIAN R. MORENO, Department of Animal and Human Biology, Havana University, 455-25 Street, Havana 10400, Cuba
LIDA SÁNCHEZ, Department of Animal and Human Biology, Havana University, 455-25 Street, Havana 10400, Cuba
ALEJANDRA CEBALLOS-VASQUEZ, Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada
PAUL A. FAURE, Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada
EMANUEL C. MORA, Department of Animal and Human Biology, Havana University, 455-25 Street, Havana 10400, Cuba

ABSTRACT The flight membranes of bats are susceptible to damage (e.g., holes and tears) from a number of sources, including impacts with natural and man-made objects, fighting between conspecifics, and attacks by predators or pathogens. Biologists routinely biopsy bat wings as a method of tissue collection for molecular research, and sometimes for the temporary identification of animals in the field. A previous study reported that captive big brown bats (Eptesicus fuscus) rapidly and completely healed flight membrane wounds. Given that limited care is provided to animals following tissue biopsy in the field, we sought to determine whether healing times for wounds from bats in captivity were applicable to bats in the wild. We measured and compared healing times of wounds in the wing and tail membranes of 50 non-reproductive female big brown bats from a wild population in Cuba following recapture. Tail wounds healed significantly faster than wing wounds of the same size, likely because of the increased thickness and vasculature of the tail membrane. Our data are concordant with a previous laboratory study in captive big brown bats, and confirm that tail membrane biopsies are better for obtaining tissue samples for molecular work because tail wounds heal faster than wing wounds. © 2015 The Wildlife Society.

KEY WORDS big brown bat, chiropterygium, Eptesicus fuscus, tail membrane, tissue biopsy, tissue regeneration, uropatagium, wing membrane, wound repair.

The flight membranes of bats are vitally important to their survival. Bats rely on their flight membranes for several critical functions, the most important of which are aerial locomotion and execution of complex flight maneuvers during feeding, mating, and predator avoidance (Norberg 1972, Faure and Barclay 1992, Kalko 1995). Bats may also use their flight membranes for thermoregulation (Reichard and Fellows 2010), water balance (Bassett 1980, Thomas and Cloutier 1992), and cutaneous gas exchange (Makanya and Mortola 2007).

The flight membranes of bats are thin, vascularized, lateral extensions of the skin originating from the dorsal and ventral body surfaces (Quay 1970, Norberg 1972, Holbrook and Odland 1978). Bat flight membranes (i.e., patagia) can be separated into 2 distinct sections: the tail membrane and the wing membrane (Faure et al. 2009). In most species, the tail membrane (uropatagium) extends medially from the hind leg to the distal tip of the tail. The wing membrane can be further divided into 3 subsections: the protopatagium extends from the shoulder to the thumb, the chiropterygium occupies the area between each phalange and extends from the 2nd to the 5th phalange, and the plagiopterygium extends laterally from the body to the 5th phalange, ranging from the distal portion of the hind leg to the arm.

Despite possessing moderate elasticity and a puncture resistance comparable to that of a plastic sandwich bag (Studier 1972), the thinness of the patagia can lead to the frequent acquisition of holes and tears. Such wounds originate from a number of sources, including attacks by predators, fights between conspecifics, and contact with natural (e.g., thorns and spines) or man-made objects (Davis 1968, Broders et al. 2013). Flight membrane damage can also result from disease (e.g., infection with Pseudogymnoascus destructans, the fungus that causes white-nose syndrome in bats; Meteyer et al. 2009, Reichard and Kunz 2009, Fuller et al. 2011). Even with modest-sized natural holes and tears, bat flight membranes retain their ability to generate lift.
(Davis 1968), presumably because the elasticity of the tissue provides excess load-bearing capacity in these situations (Struhasker 1961). Additionally, bat researchers and wildlife biologists frequently introduce holes in bat flight membranes as a method of tissue sampling (Dixon 2011) and, more rarely, for temporary identification of animals (Bonaccorso and Smythe 1972). Excision of flight membrane tissue permits extraction of DNA, RNA, and protein for molecular analyses. Molecular techniques are becoming increasingly important in animal management and conservation because they can be used to determine relatedness between individuals, gene flow between groups, and the genetic diversity of populations (Vonhof et al. 2008, Dixon 2011). Furthermore, flight membrane biopsies produce easily identifiable holes in the short-term and enduring unpigmented tissue in the long term, both of which can be used for the identification of individual bats in the field (Bonaccorso and Smythe 1972).

Previous observations suggest that the healing cascade in bat flight membranes is similar to that of cutaneous lacerations in other mammals (Faure et al. 2009, Weaver et al. 2009, Meteyer et al. 2011). Injury to the skin leads to the disruption of the tissue matrix and blood vessels, causing bleeding and the subsequent formation of a clot (Singer and Clark 1999). The blood clot re-establishes homeostasis and provides a temporary matrix for the migration of cells and blood-mediated healing factors. Healing begins by restoring the connection between healing factors and the dermis, which permits the animal to induce inflammatory or immune responses (Campos et al. 2008). The presence of blood vessels near the injury site facilitates the first step in the healing cascade (Martin 1997, Singer and Clark 1999). Blood vessels supply many factors to the wound site (e.g., platelets, neutrophils, growth factors, macrophages, fibroblasts, cytokines, and chemokines; Singer and Clark 1999, Barrientos et al. 2008) that are required to clean the wound, prevent infection, and initiate the process of tissue regeneration and repair. Over the next several days, wound contraction and closure occurs through mitosis, the migration of epithelial cells (re-epithelization), and a complex reorganization of the extracellular matrix (Martin 1997, Singer and Clark 1999).

Given that the flight membranes are essential for survival and reproduction in bats but are also susceptible to damage, wound healing is clearly evolutionarily advantageous. Several studies reported that flight membrane wounds heal rapidly and completely in a variety of bat species (Church and Warren 1968, Davis and Doster 1972, Kerth et al. 2002, Faure et al. 2009, Weaver et al. 2009). Standard practice in the field is to biopsy the wing membrane in areas with less vascularization (Bonaccorso and Smythe 1972, Kleiman and Davis 1974), resulting in little or no bleeding from the wound site. As such, most studies on healing in bats examine only wing membrane wounds (Church and Warren 1968, Davis and Doster 1972, Kerth et al. 2002, Weaver et al. 2009, Meteyer et al. 2011). Working with a captive colony of big brown bats (Eptesicus fuscus), Faure et al. (2009) reported that the tail membrane healed significantly faster than the wing membrane for wounds of the same size. This observation was likely due to the increased vascularization of the uropatagium, as evidenced by greater bleeding from tail wounds after membrane biopsy. Tissue excised from the uropatagium was also thicker and, as a result, weighed more and contained more DNA than same-sized biopsies from the chiropatagium. Based on these findings, Faure et al. (2009) concluded that researchers should consider biopsying the tail membrane for studies needing to obtain DNA, RNA, or protein for molecular analyses, and to biopsy the wing membrane for the short-term marking and identification of bats in the field. Given that researchers, conservation biologists, and wildlife managers routinely perform flight membrane biopsies in the field, with little or no direct observation of the effects of this procedure, our goal was to determine if the wound healing observations of Faure et al. (2009) measured for bats in captivity were concordant for free-living bats in the wild.

**STUDY AREA**

We conducted this study between 25 March and 9 May 2014 at the Botanical Gardens located south of Havana, Cuba (22°59′29.5″ N, 82°20′13.7″ W). We captured big brown bats from a colony that was roosting in the outdoor portion of a 2-story residence building.

**METHODS**

We used mist nets to capture 50 non-reproductive female big brown bats at dusk. Following capture, we weighed and marked each bat with a uniquely colored plastic split-ring band. Prior to membrane biopsy, we placed each bat in a prone position on a plastic sheet (Acrylite FF 3.0 mm; CRYO Industries, Parsippany, NY) and extended the wing or tail membrane to a standard, outstretched position (Ceballos-Vasquez et al. 2014). Using a ScopeTek DCM900 digital camera (9 MP resolution; ScopeTek Opto-Electric, Zhejiang, China) mounted on an Olympus stereomicroscope (Olympus, Tokyo, Japan), we photographed the intact flight membrane prior to biopsy. We then punched the membrane following the procedures of Faure et al. (2009). Briefly, we used a 4.0-mm diameter Sklar Tru-Punch circular disposable biopsy tool (Sklar Instruments, West Chester, PA) to excise tissue from either the chiropatagium or uropatagium (n = 25/group). Immediately following biopsy, which marked day 0 of the experiment, we cleaned wounds with a cotton swab soaked in 70% ethanol. Consistent with the increased vascularization of the uropatagium, we observed some bleeding in tail wounds but not wing wounds. We permitted bleeding to cease naturally before photographing wounds and releasing bats near their site of capture.

We recaptured bats at dusk, twice weekly, over 6.5 weeks to monitor wound healing. We recaptured bats from their original roost and 2 nearby roosting sites in the same building. Following recapture, we placed bats in a wire mesh holding cage (26 × 14 × 12 cm) until we could weigh and measure them as described above, and photograph the entire wound area and its surrounding tissue.

We measured wound areas as described by Faure et al. (2009) using ImageJ software (Abramoff et al. 2004). Image
contrast was enhanced by 20–80% so that the wound perimeter could be outlined with the auto-trace tool. We measured wound areas in pixels and converted them to mm² using a calibrated scale (pixels/mm) standardized to the microscope objective (1.0×) and zoom magnification (6.3–10×). To confirm that the auto-trace tool was both accurate and reliable for measuring wound areas, we manually traced wound perimeters in approximately 30% of the images. Because wound areas measured manually and with the auto-trace tool were very similar (<0.8% difference), for consistency, we used only the auto-trace values in our figures and numerical analyses.

We calculated the number of days post-biopsy to reach 10%, 25%, 50%, 75%, 90%, and 100% wound closure as an index of the healing rate; however, the low recapture rate for some bats led to an overestimation of healing times to reach these arbitrary values. To increase the sensitivity of our data, we performed linear interpolations for each bat. We first calculated the theoretical wound areas corresponding to 10%, 25%, 50%, 75%, and 90% wound closure based on the initial wound area following biopsy (day 0) for each bat. We then found the 2 closest healing times bracketing each theoretical wound size, 1 data point corresponding to a smaller wound size and the other corresponding to a larger wound size, and calculated the number of days to reach each arbitrary percent wound closure value assuming a linear rate of healing for each bat. Although healing curves are best described as sigmoidal functions, interpolations were conservative for estimating the number of days to reach between 50% and 90% wound closure when healing was relatively uniform and linear. We did not use data interpolation to extrapolate beyond our actual recorded measures to estimate the time required to reach 100% wound closure. All procedures adhered to the guidelines for the care and use of wild mammals in research approved by the American Society of Mammalogists (Sikes and Gannon 2011) and the Canadian Council on Animal Care, and were approved by the Animal Research Ethics Board of McMaster University.

**Statistical Analyses**

We performed statistical analyses using the R software environment (R Core Team 2015). Data are presented as the mean ± standard deviation. The data were normally distributed with equal variances based on Shapiro–Wilk and Bartlett’s tests (Bartlett 1937, Shapiro and Wilk 1965). We compared initial wound areas in the wing and tail membrane made on day 0 with a 2-sample t-test (equal variance model). For both non-interpolated and interpolated data, we

---

**Figure 1.** Wound healing in the wing membrane of a wild big brown bat in Cuba in 2014 before and after membrane biopsy with a 4-mm diameter circular punch. (A) Before biopsy (day 0). (B) Day 7. (C) Day 21. (D) Day 31. (E) Day 38. (F) Day 42. Day number is printed below the letter in each panel. Scale bar = 1 cm.
compared healing rates using a linear mixed model with the number of days to reach 10%, 25%, 50%, 75%, and 90% wound closure as a function of biopsy site (wing vs. tail), treating each time point as a repeated measure. We used the Huynh–Feldt estimate of sphericity (e) to adjust P-values of within-subject variables (Huynh and Feldt 1976). When this model produced a significant main effect of biopsy site, we individually compared the number of days to reach each point of percent wound closure between wing and tail wounds using 2-sample t-tests (equal variance model). For all statistical tests, we employed a comparison-wise error rate of \( \alpha < 0.05 \).

RESULTS

We documented wound healing in the wing (Fig. 1) and tail membranes (Fig. 2) of wild big brown bats. Photographs show similar milestones of wound healing for the chiropatagium and uropatagium, albeit on different time-scales. The intact wing membrane (Fig. 1A) was thinner and less resistant to stretching than the intact tail membrane (Fig. 2A). Both flight membranes contained thin muscle striations within the tissue that became nearly undetectable when the intact membrane was outstretched (Figs. 1A and 2A). Both membranes also contained blood vessels, which seemed less numerous and noticeable in the chiropatagium (Fig. 1A) compared to the uropatagium (Fig. 2A). A thick, dark, red border was observed along the wound perimeter after biopsy in both membranes (Figs. 1B and 2B). This border was noticeably more evident in tail wounds than in wing wounds, likely reflecting an increased blood supply and clotting in the tail membrane. After 3–10 days, this clot slowly disappeared and the wound began to close (Figs. 1C and 2C). The addition of new cells around the wound perimeter resulted in the new membrane taking on a pale, translucent appearance that was especially prominent in wing wounds (Fig. 1C and D), whereas in tail wounds, the coloration was more similar to that of the surrounding non-biopsied tissue (Fig. 2C and D). Bands in the skin and connective tissue near the wound site became more distinct and new blood vessels permeated the new tissue as healing progressed (Figs. 1D, 1E, 2D, and 2E). In later stages of healing, wing and tail wounds became noticeably inflamed and a distinct thickened ridge formed around the wound perimeter (Figs. 1E and 2E). The inflammation and ridge persisted throughout wound closure, at which point a scab formed at the center of the newly healed tissue (Figs. 1F and 2F). Following closure, wing wounds displayed a distinct patch of pale tissue surrounding the scab and that tended not

Figure 2. Wound healing in the tail membrane of a wild big brown bat in Cuba in 2014 before and after membrane biopsy with a 4-mm diameter circular punch. (A) Before biopsy (day 0). (B) Day 7. (C) Day 14. (D) Day 17. (E) Day 21. (F) Day 28. Day number is printed below the letter in each panel. Scale bar = 1 cm.
Table 1. Mean (±SD) number of days to reach 10%, 25%, 50%, 75%, and 90% wound closure following tissue excision with a 4.0-mm diameter circular biopsy punch in the wing and tail membrane of wild big brown bats (n = no. recaptured bats) in Cuba in 2014. To account for low recapture rates and increase sensitivity, we estimated average healing times with linear interpolations.

<table>
<thead>
<tr>
<th>Punch</th>
<th>% wound closure</th>
<th>n</th>
<th>Healing time ± SD (days)</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td></td>
<td>18.1 ± 8.4</td>
<td>3.6</td>
<td>32.7</td>
</tr>
<tr>
<td>25</td>
<td>21</td>
<td></td>
<td>21.8 ± 7.5</td>
<td>9.0</td>
<td>36.4</td>
</tr>
<tr>
<td>50</td>
<td>19</td>
<td></td>
<td>25.2 ± 5.7</td>
<td>17.9</td>
<td>36.9</td>
</tr>
<tr>
<td>75</td>
<td>19</td>
<td></td>
<td>29.7 ± 5.5</td>
<td>20.4</td>
<td>40.0</td>
</tr>
<tr>
<td>90</td>
<td>17</td>
<td></td>
<td>34.2 ± 4.9</td>
<td>27.0</td>
<td>41.9</td>
</tr>
<tr>
<td>Tail</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td></td>
<td>12.1 ± 4.7</td>
<td>4.6</td>
<td>21.2</td>
</tr>
<tr>
<td>25</td>
<td>21</td>
<td></td>
<td>16.2 ± 4.3</td>
<td>6.8</td>
<td>25.2</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td></td>
<td>20.8 ± 5.8</td>
<td>10.5</td>
<td>34.2</td>
</tr>
<tr>
<td>75</td>
<td>20</td>
<td></td>
<td>25.6 ± 6.5</td>
<td>14.2</td>
<td>38.4</td>
</tr>
<tr>
<td>90</td>
<td>19</td>
<td></td>
<td>29.5 ± 6.9</td>
<td>16.4</td>
<td>42.4</td>
</tr>
</tbody>
</table>

to be present in tail wounds (Figs. 1F and 2F). Tissue repigmentation occurred slowly (Faure et al. 2009, Ceballos-Vasquez et al. 2014) and was not quantified in this study.

Average wound areas measured immediately after biopsy on day 0 did not differ between wing (14.75 ± 1.88 mm²) and tail wounds (14.26 ± 1.92 mm²; t₁₈ = 0.92, P = 0.363). Average areas for wing and tail wounds were slightly larger than the expected area for a 4.0-mm diameter circular biopsy (12.57 mm²). The healing rates of non-interpolated data differed between wing and tail wounds (F₁₄,₁₃₆ = 20.86, e = 0.53, P < 0.001); tail wounds healed faster than wing wounds for 10% (t₄₀ = 2.30, P = 0.027), 25% (t₄₀ = 2.63, P = 0.012), and 50% wound closure (t₃₇ = 2.13, P = 0.040). Because of low numbers of recaptures of certain individual bats, the observed healing data were less sensitive and overestimated flight membrane healing times compared to the interpolated data, especially during the later stages of healing. When we re-calculated the number of days to reach 10%, 25%, 50%, 75%, and 90% closure for wing and tail wounds using linear interpolated data (Table 1), healing times differed between wing and tail wounds (F₁,₃₄ = 9.70, P = 0.004). The number of days to reach arbitrary points of percent wound closure also differed (F₄,₁₃₆ = 168.73, e = 0.37, P < 0.001); tail wounds healed faster than wing wounds for 10% (t₄₀ = 2.84, P = 0.007), 25% (t₄₀ = 2.94, P = 0.005), 50% (t₃₇ = 2.46, P = 0.019), 75% (t₃₇ = 2.11, P = 0.041), and 90% wound closure (t₃₄ = 2.33, P = 0.026).

We used non-interpolated data to illustrate wound areas (Fig. 3) and describe percent wound closure (Fig. 4) as a function of post-biopsy time. Both wing and tail wounds followed a similar healing pattern (Fig. 3). Wound closure was not evident in the first several days following membrane biopsy, and in some cases, wound areas increased during this initial period. This was especially noticeable for tail membrane healing, as reflected by negative values of percent wound closure (Fig. 4B). Despite tail wounds expanding to be larger in area than expected for a 4-mm diameter circular punch, they began healing earlier than wing wounds. Once new cells were formed at the wound perimeter, healing continued at a steady pace until the wound was nearly closed, at which point the rate of healing progressed more slowly until the wound fully healed. In 6 cases, wing (n = 3) and tail (n = 3) wounds expanded during the healing process as reflected by increased values of wound area (Fig. 3) and decreased values of percent wound closure (Fig. 4). Because wound expansion was a natural consequence of tissue biopsy in free-ranging bats, the data from these 6 animals were retained in our analyses. We never observed wounds reopening after they initially closed.

Of 50 bats biopsied at the start of the experiment, 42 were recaptured at least once. Of the 8 bats that were never recaptured, 4 were biopsied in the wing and 4 were biopsied in the tail membrane. The number of recaptured bats per attempt

![Figure 3. Wound healing times of 4-mm diameter circular biopsies in the (A) wing and (B) tail membranes of wild big brown bats in Cuba in 2014 as a function of time post-biopsy (day 0). We present the mean (thick black line) ± standard deviation (thin black lines) non-interpolated wound area (mm²) of individual bats (thin gray lines).](image-url)
ranged from 9 to 24, with an average of 16/capture attempt. In our first attempt, we recaptured only 12 bats, suggesting that some moved from the original roost site after they were biopsied. On subsequent attempts we recaptured bats from the original roost and 2 nearby roosts in the same building. Over the next 7 attempts, we recaptured between 15 and 24 bats/attempt. In the final 5 attempts, we recaptured between 9 and 13 bats/attempt. Sample sizes reflect the measured wound areas of individual bats (i.e., non-interpolated data points) as a function of time after biopsy (Fig. 5).

DISCUSSION

Most biologists extract tissue from the wing when performing flight membrane biopsies on bats. Likely explanations for this bias include the ease with which a single researcher can manipulate a bat’s wing with one hand while using the other hand for tissue excision. Others may prefer to biopsy the wing instead of the tail membrane to reduce potential bleeding from the wound site in an attempt to minimize the impact of tissue excision on the animal. Although not quantified, we observed a higher density of blood vessels in the uropatagium of big brown bats compared to the chiropatagium and, in turn, noticed more bleeding from tail wounds compared to wing wounds following flight membrane biopsy. We also found that tail wounds healed significantly faster than wing wounds of the same size, thus replicating the results of Faure et al. (2009) for wound healing in captive big brown bats. These observations are consistent with the idea that proximity to blood vessels is tightly linked to the speed of the healing process (Martin 1997, Singer and Clark 1999, Campos et al. 2008). Despite resulting in less bleeding immediately following tissue excision, wing membrane biopsy may not necessarily reduce long-term trauma to the animal.

Given that it is standard practice to biopsy bat flight membranes in areas with little or no prominent vascularization, most studies investigating wound healing have focused solely on wing wounds. In studies of captive bats, Church and Warren (1968) reported that oval holes measuring approximately 2 × 2 cm in the wings of straw-colored fruit bats (Eidolon helvum) healed in approximately 24 days, and Davis and Doster (1972) reported that 14-mm diameter circular holes in the wings of pallid bats (Antrozous pallidus) healed between 22 and 33 days. In studies of free-ranging (wild) bats, Kerth et al. (2002) reported that 3-mm diameter circular holes in the wings of Bechstein’s bats (Myotis bechsteinii) endured for up to 3–4 weeks, whereas Weaver et al. (2009) reported that 3-mm diameter circular holes in the wings of little brown bats (M. lucifugus) healed by 16 days. Pierce and Keith (2011) reported that 3-mm diameter circular holes in the wings of free-ranging African Vespertilionids (Hyptoxy anchietae, Neoromicia zuluensis, and Pipistrellus rusticus) healed to between 65% and 95% closure within 11 days. Furthermore, Fuller et al. (2011) reported healing over a 2-week period in little brown bat wings damaged by white-nose syndrome. Direct comparisons between wound healing studies are limited, given differences in biopsy size, shape, study conditions, and the anatomical structure of flight membranes across different species.
species. Nevertheless, these studies demonstrate that wing wounds heal rapidly, and in most cases completely, in a variety of bats.

To the best of our knowledge, Faure et al. (2009) is the only other study to directly compare wound healing in the chiropatagium and uropatagium. Faure et al. (2009) made 4-mm and 8-mm diameter circular holes in the flight membranes of captive big brown bats, and reported that tail wounds healed faster than wing wounds of the same size. Our study has replicated these results for 4-mm diameter circular biopsies in the chiropatagium and uropatagium of free-ranging big brown bats in the wild. Although similar trends were observed in both studies, flight membrane wound healing was notably faster in captivity. For example, captive big brown bats reached 10% wound closure in an average of 5.0 days and 4.6 days and 90% closure in an average of 17.3 days and 12.0 days for wing and tail wounds, respectively, compared to 18.1 days and 12.1 days for 10% closure and 34.2 days and 29.5 days for 90% closure in the present study. Wound closure also progressed more quickly in captive bats throughout the entire healing process. Given the metabolic demands associated with wound healing, we suggest that the faster healing times observed for bats in captivity may be related to these animals having unrestricted access to food and water compared to bats in the wild. Free-ranging bats were also subject to different ambient conditions that may have slowed the healing process compared to bats in captivity.

Because tissue excised from the tail contained a higher concentration of DNA compared to same-sized excisions from the wing, Faure et al. (2009) suggested that biologists and researchers should consider biopsying the tail membrane for the purposes of obtaining DNA, RNA, or protein for molecular analyses but biopsying the wing membrane for the short-term identification of bats in the field. The laboratory study of Faure et al. (2009) demonstrated the importance of considering which flight tissue to biopsy to maximize research impact while minimizing long-term trauma and membrane healing times. Whenever possible, it is important to verify such recommendations in the field where there is less direct observation of animals following tissue biopsy and no human intervention or animal care. For example, Broders et al. (2013) reported that a small number of northern long-eared bats (M. septentrionalis) experienced adverse effects (i.e., became stuck on car antennas or tore a hole in the trailing edge of the uropatagium) following tail membrane biopsy. Conversely, we observed no overt adverse consequences of tail or wing membrane biopsy in wild big brown bats despite having no direct observations of animals between recaptures. Differences in the foraging behavior of northern long-eared bats and big brown bats may be related to the observations of Broders et al. (2013) because northern long-eared bats glean insect prey off surfaces using their uropatagium (Faure et al. 1993), whereas big brown bats have rarely been observed to employ a substrate-gleaning foraging strategy (Kurta and Baker 1990).

We used a circular biopsy punch to excise flight membrane tissues in big brown bats, a tool that is commonly employed by researchers in the field. The expected initial area (A) of a circular wound can easily be calculated with the formula $A = \pi r^2$, where $r$ is the radius of the biopsy tool and $\pi$ is a mathematical constant representing the ratio of a circle's circumference to its diameter. For a 4-mm diameter circular biopsy, the theoretical wound area is 12.57 mm$^2$, yet the average initial wound areas measured in the wing (14.75 mm$^2$) and tail membranes (14.26 mm$^2$) of our wild bats were both larger than expected. Several studies have reported larger than expected initial wound areas following tissue excision with circular punch tools (Davis and Doster 1972, Faure et al. 2009, Weaver et al. 2009). Using the same 4-mm diameter circular biopsy tool, Faure et al. (2009) measured average starting areas of 16.72 mm$^2$ for wing wounds and 21.65 mm$^2$ for tail wounds. Their results indicate that overstretching of the flight membrane and wound expansion can occur even under laboratory conditions when working with anesthetized animals. Experimental wounds in our study increased in area over the first several days following biopsy (Figs. 3 and 4), and this was especially prominent for wounds made in the uropatagium. Although we cannot rule out the possibility that wounds expanded because of interactions (e.g., fighting) with conspecifics or contact with objects in the environment, another possible explanation is that human investigators over extended the flight membranes during biopsy, causing the collagen and elastin bundles in the tissue to be overstretched (Holbrook and Odland 1978). Because of their tensile nature, the collagen and elastin fibers would contract following biopsy and this would result in wound expansion (Gosline et al. 2002). Over-stretching of the flight membrane during biopsy, in addition to interactions with conspecifics or contact with objects in the environment, may also explain why wing biopsies in 3 bats took more than 30 days to reach 10% and 25% wound closure. Of these 3 bats, 2 reached 90% wound closure by the end of the study, whereas the other failed to reach 50% wound closure.

In summary, our results support the experimental work of Faure et al. (2009) who reported faster healing times for tail membrane wounds compared to wing membrane wounds in a population of captive big brown bats. Although overall healing times were faster for bats in captivity, the differential rates of healing for the chiropatagium and uropatagium were maintained and thus valid for this species in the wild.

**MANAGEMENT IMPLICATIONS**

Insectivorous bats are able to rapidly and completely heal holes in their wing and tail membranes, without extensive animal care or direct individual observations that realistically can only be provided in a laboratory (i.e., captive) setting. Our results support the use of different biopsy locations, depending on the research purpose. We recommend considering the flight membrane biopsy suggestions of Faure et al. (2009). In brief, tail membrane biopsies are recommended to obtain samples for molecular analyses given the higher tissue mass and concentration of DNA, whereas wing membrane biopsies are better for the marking and identification of bats in field because the wound and scar...
persist longer. Tail membrane biopsies offer a more efficient and humane method of tissue collection owing to higher excised tissue masses and faster wound healing times in bats; however, the foraging strategy of the bat should also be considered when selecting a suitable biopsy location.

ACKNOWLEDGMENTS

We thank the staff of the Botanical Gardens south of Havana, Cuba for nocturnal access to the bats and facility, and A. H. Abad, A. Cádiz, and D. F. Turuceta for assistance with data collection. Research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC), Canada, and infrastructure grants from the Canada Foundation for Innovation and the Ontario Innovation Trust awarded to P. A. Faure. T. Pollock was supported by an NSERC Canada Graduate Scholarships Michael Smith Foreign Study Supplement.

LITERATURE CITED


Associate Editor: Amy Kuenzi.