

# Superior National Forest Bat Monitoring

## Summary of 2013 Survey Effort



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## EXECUTIVE SUMMARY

Until recently, there has been a limited amount of information regarding bat populations and habitat use on the Superior National Forest (SNF). In the summer of 2013, SNF biologists initiated a pilot project to document forest bat occupancy and collect demographic and habitat use data to complement an acoustic monitoring program started in 2009. Prior to this effort, few data existed to inform impact analyses and aid in the development of mitigation strategies to maintain and restore habitat for these species. Although limited by a small sample size, this effort - which included mist-netting, radio-telemetry, habitat characterization, and acoustic surveys at newly identified roost structures - has begun to provide useful data for describing bat presence and habitat use on the SNF.

We captured and processed 34 bats over nine nights of mist-netting at eight locations on the Kawishiwi District in northeastern Minnesota. Northern long-eared bats (*Myotis septentrionalis*) comprised 38.2% (n = 13) of total captures and little brown bats (*M. lucifugus*) accounted for 61.8% (n = 21). No other species were detected during the survey effort. We captured both reproductive adults and non-reproductive juveniles. The bats were found to be in good condition and tissue damage resulting from WNS exposure was not detected. Radio-transmitters were attached to five bats and multiple roost sites were detected on subsequent tracking sessions.

Three maternity roosts were identified in cracks and crevices of live aspen (*Populus* sp.). Four additional roosts were identified in dead aspen and white pine (*Pinus strobus*). Canopy closure in the surrounding stands tended to be high (62 – 98%) though all roost trees had some level of exposure to sunlight during the day. Overall stand composition was variable. Habitat use data from bat radio-telemetry efforts on the SNF are limited but suggest that Myotine bats utilize cracks and crevices in live and dead aspen (*Populus* sp.) and white pine (*Pinus strobus*). Additional survey effort will be required to draw definitive conclusions regarding northern long-eared bat occupancy on other parts of the SNF and elucidate patterns in occupancy related to habitat characteristics

If expanded and combined with ongoing acoustic survey efforts, active bat monitoring will improve our understanding of bat ecology on the SNF, help establish data-driven mitigation measures to maintain and restore bat habitat, and serve as a means for detecting the symptoms of white nose syndrome (WNS) infection and document some of the disease's impact on forest bat populations in northeastern Minnesota.

## INTRODUCTION

A significant proportion of North American bats rely on forested ecosystems for roosting, foraging, or both (Brigham 2007). Roosts are typically found in tall trees with large diameters and located in stands with open canopies and a high density of snags (Kalcounis-Rüppell et al. 2005). Whether it be foliage, exfoliating bark, or other cracks and crevices, roosts provide shelter from the elements and protection from predators, as well as locations to rear young, digest food, and interact with other individuals (Kunz and Lumsden 2003).

Despite their ecological importance in forest ecosystems, there has been little quantitative data collected for bat populations on the Superior National Forest (SNF) and major knowledge gaps exist for North American bats in general (Amelon 2006). With the emerging threat of white-nose syndrome (WNS) devastating bat populations across the eastern United States (Turner et al. 2011), and the proposed federal-listing of the northern long-eared bat (Federal Register 2013), biologists and decision makers need accurate information regarding forest bat populations and habitat use. Prior to initiating this survey effort the only information available for the SNF was that bats occur on the Forest and overwinter at the nearby Soudan Underground Mine (Nordquist et al. 2006, USDA Forest Service *unpublished data*). Little effort had been expended to identify additional hibernacula on the Forest – although hibernacula are known at a small abandoned mine on private ownership near Ely, MN and a few locations along the North Shore of Lake Superior (Knowles 1992). Two maternity colonies on the Forest are known to occur in human structures but neither had been monitored nor had the species of bats using these structures been verified.

Six acoustic monitoring routes were established on the SNF in 2009 and have been surveyed annually, including surveys in 2013 (USDA Forest Service *unpublished data*). These acoustic data provide an index of relative activity and can provide general information regarding bat species and habitat occupancy. In addition, these data can be implemented over large spatial and temporal extents and across many habitat types (Rodhouse et al. 2011). Additionally, acoustic surveys can be conducted at a low annual cost once initial equipment purchases are made (Roche et al. 2011). Acoustic survey data, while useful, do not provide information regarding demographic parameters (e.g., sex, age, reproductive condition) nor do they help to identify exposure to white-nose syndrome (WNS). Furthermore, acoustic data have proven difficult to process and analyze and the technology to draw reliable species identification from acoustic survey data has not been fully refined.

Alternatively, mist-netting, capturing and handling bats is an effective means for obtaining demographic data, may provide early evidence of the occurrence and spread of WNS on the SNF, and can be aggregated across the region to better understand impacts of WNS at broader spatial scales. Bats can be marked with unique identification bands and recaptured bats can provide information regarding survival, site fidelity, dispersal, and regional and local metapopulation dynamics though implementation and analysis challenges still exist with this technique (Ellison 2008, Perry 2011). Radio-telemetry can elucidate home range and habitat use patterns at the tree- and stand-level (Owen et al. 2003, Johnson et al. 2011) and inform forest management decisions in the context of bat conservation (e.g., Silvis et al. 2012). In addition, radio-telemetry efforts can provide information on the characteristics of summer roost habitat and the location of summer maternity colonies that would otherwise remain unidentified. When

combined with ongoing acoustic monitoring efforts, these data can inform management decisions at project- and forest-scales and provide a framework for monitoring the response of local bat populations to the potential spread of WNS – thereby contributing to regional bat conservation objectives.

### ***Survey Objectives***

The objectives of the 2013 survey effort were to:

1. Develop and expand the technical bat expertise of SNF personnel through training opportunities, equipment purchases, and pre-exposure vaccinations and titer checks. The continued development of these skill sets will better prepare the SNF for future management of bat habitat as will be required should WNS spread to the Forest;
2. Conduct mist-netting surveys with guidance and training provided by regional bat expert(s);
3. Develop bat processing and handling skills including tissue and microbial sample collection;
4. Deploy radio-tracking units on bats to identify summer roost sites; and
5. Collect tree- and stand-level data at roost sites identified during tracking efforts.

## **METHODS**

### ***Study Area***

Surveys were conducted on the Kawishiwi Ranger District of the SNF in northeastern Minnesota, USA (Figure 1). This landscape is characterized by a nearly contiguous boreal forest interspersed with forested and un-forested wetlands and numerous lakes of varying sizes. The dominant upland tree species included black spruce (*Picea mariana*), white pine (*Pinus strobus*), red pine (*P. resinosa*), jack pine (*P. banksiana*), white spruce (*P. glauca*), balsam fir (*Abies balsamea*), aspen species (*Populus* sp.) and paper birch (*Betula papyrifera*). Black spruce, northern white cedar (*Thuja occidentalis*) and black ash (*Fraxinus nigra*) comprised the dominant lowland species.

Specific survey sites (Table 1, Figure 2) were identified by Forest Service biologists familiar with bat behavior and with experience in bat capture in boreal forest ecosystems. Additional planning field visits were conducted with Katrina Schultes (USFS – Wayne National Forest) who joined SNF biologists for the mist-netting operation. Katrina has specific expertise in roost tree selection by Indiana bats (*Myotis sodalis*) and northern long-eared bats (*M. septentrionalis*) and serves on the Ohio Bat Working Group.

### ***Mist-Netting***

We used double- and triple-high net sets during mist-netting sessions to capture bats. Nets were placed across natural flyways (e.g., forest roads, edges of open lowlands) and near known roost sites to maximize captures per unit effort. Specific net placement was determined by Forest Service biologists based on bat foraging behavior and logistic constraints. Nets were opened

approximately 30 minutes before nightfall and closed at 2 a.m. Personnel checked nets every 15-30 minutes to minimize the amount of time that a bat would be restrained in the net.

### ***Bat Handling***

Exposure to WNS can cause lesions to the skin of bats (Cryan et al. 2010). Each bat was examined for damage to the wing membrane (e.g., necrotic tissue, tears, and scars) following the methodology developed by Jonathan D. Reichard at the Center for Ecology and Conservation Biology, Boston University (Appendix A). Both wings and the uropatagium were inspected for damage and scored from zero (i.e., no damage) to four (high damage) following the protocol and the scoring criteria therein.

We determined weight, sex, age, forearm length, and species for each bat captured. Prior to release, a subset of bats were swabbed for microbial community analysis following a protocol provided by research scientists at the US Forest Service Northern Research Station in Rhinelander, WI (Appendix B). We also collected two wing tissue punch biopsies from these bats for DNA analysis following a standardized protocol (Appendix B). Microbial swabs and wing biopsies were not collected from bats instrumented with radio-transmitters.

### ***Decontamination Protocol***

We utilized the National White-Nose Syndrome Decontamination Protocol – Version 06.25.2012 (Appendix C) to ensure that our survey activities did not contribute to the spread of WNS among bats.

### ***Radio-Telemetry***

Five bats were tagged with Holohil LB-2N radio transmitters weighing approximately 0.35 g and providing an estimated tracking window of 12 days. The transmitters were attached to the back of the bats between the scapulae after enough fur was trimmed to allow the surgical cement (Osto-Bond) to bond the transmitter to the skin. Attempts were made to obtain daily locations and to identify specific roost sites utilized during the tracking session. When located, field crews collected general location information about the roost site (geographic coordinates, tree or structure type, and access notation) so the site could be visited later for more detailed habitat data collection. When possible, emergence surveys were conducted at roost trees to estimate the number of bats using the structures.

### ***Habitat Data***

Basic habitat data were collected at each of the roost trees identified through radio-telemetry tracking. These data included tree species, diameter at breast height (dbh), tree height, the type of roost structure (e.g., crevice, cavity, loose bark, broken top, etc.) if identified, tree condition (e.g., live/dead, bark and limb condition, and any breakage), slope, aspect, basal area (using a 10-factor prism), and canopy cover. Given the low sample size no formal data analysis was conducted. Rather, we summarize the characteristics of roosts identified as being used by transmitted bats. Sample data forms are provided in Appendix D.

### ***Acoustic Monitoring***

We used Anabat (Titley Electronics, Ballina, Australia) broadband, frequency-division, bat detectors to passively monitor bat echolocation pulses at diurnal roost trees identified during

radio-tracking efforts. These data are currently being processed and will be included in subsequent monitoring progress reports.

## RESULTS

We captured and processed 34 bats over nine nights of mist-netting at eight locations on the Kawishiwi District (Table 2, Figure 3). Northern long-eared bats (*Myotis septentrionalis*) comprised 38.2% (n = 13) of total captures and little brown bats (*M. lucifugus*) accounted 61.8% (n = 21). No other species were detected during the survey effort. We captured both reproductive adults and non-reproductive juveniles (Figure 4, Table 3). Approximately 80 additional little brown bats were captured at the Kawishiwi Lab site but not processed due to a mass emergence from the roost that overwhelmed the personnel present. Once these bats were identified to species, they were immediately released so that the mist-nets could be closed. With the exception of the Kawishiwi Lab site, mean capture per unit effort was 0.55 bats/hour ( $\pm 0.43$  SD).

In general, forest bats were found to be in good condition (Table 2). Little brown bats had a mean weight of 9.42 g (range: 7.50 – 11.25 g). Northern long-eared bats averaged 7.33 g (range: 6.25 – 8.5 g). We did not detect signs of tissue damage due to WNS exposure on any of the bats that were handled and wing conditions were generally very good (Figure 5). We collected wing biopsies and microbial swabs from 18 bats. These samples were submitted to the US Forest Service Northern Research Station in Rhinelander, WI for analysis. The results of these analyses had not yet been reported at the time that this report was prepared.

We attached radio-transmitters (Figure 6) to one pregnant female (F001) and two male little brown bats – one which displayed signs of reproductive status (i.e., testes descended; M002) and a second that was a non-reproductive juvenile (M003). Female F001 was captured at the old Birch Lake Campground on 24 June and relocated in a timber stand improvement plot on the western shore of Birch Lake on 25 June. She was roosting in a large aspen and relocated there the following night (26 June). Three subsequent tracking efforts relocated her in a second live aspen just off of the Little Lake Road (FR 429). Male M002 was captured at a location on FR434B and was subsequently re-located twice in a large aspen snag. Juvenile M003 was captured on the road to the Big Lake Boat Access off of the Echo Trail and two roost locations were identified. Both roosts occurred in large pine snags off the Stuart River Portage.

We attached radio-transmitters to two northern long-eared bats. The first was a juvenile male (M004) and the second was a post-lactation female (F005). Juvenile M004 was tracked to a single roost site located in a large live aspen off of FR 1525. Female F005 was located in a roost tree on the southeast shore of White Iron Lake and subsequent tracking efforts identified a second roost area that was used on multiple nights on the east side of the South Kawishiwi River near its confluence with White Iron Lake. The specific roost tree was not identified and its location was estimated using triangulation due to poor access.

Roost characteristics are reported for all of the bats tracked though we acknowledge that roost tree selection varies by sex, age, and reproductive status (Broders and Forbes 2004, Broders et al. 2006, Perry and Thill 2007, Garroway and Broders 2008). We identified seven roost sites (Table 4). Little brown bats roosted in both live and dead aspen and white pine. These trees were large

(dbh > 11 inches) with heights ranging from 23.5 – 70.6 feet (Figure 7). Though it was difficult to ascertain the exact location of the roost structure within each tree, we believe that most roosts were located in cracks and crevices. Topography surrounding the roost trees was gradual (3 – 20% slope) and the condition of the trees varied. Basal area in the surrounding stand ranged from 20 – 90. Canopy closure in the surrounding stands tended to be high (62 – 98%) though the roost trees had some level of exposure to sunlight during the day.

Two northern long-eared bat roosts were located on the SNF (Table 4). One roost was located in a live aspen and the second in an aspen snag. These trees were large (dbh > 14 inches) with heights estimated at 75.6 feet. As with little brown bat roosts, topography was gradual (15 – 25 % slope) although basal area in the surrounding stands was greater for the northern long-eared bat roosts (100 – 130).

## **DISCUSSION**

Habitat use data from bat radio-telemetry efforts on the SNF are limited but suggest that Myotis bats generally utilize cracks and crevices in live and dead aspen and white pine. These mature trees located on predominantly east facing aspects although slopes were generally gradual across the survey area. Canopy closure in the surrounding stands tended to be high (62 – 98%) though the roost trees had some level of exposure to sunlight during the day. Overall stand composition was variable in nature. Other roost studies have found similar results (Ford 2006, Garroway and Broders 2008). It should be noted that these data are preliminary and based on a small sample size (n = 5). Our understanding of the specific habitat features supporting forest bats on the SNF will continue to improve as additional data are collected in subsequent years.

Similar to other studies, we found that little brown and northern long-eared bats utilized multiple roost sites in close proximity to each other (Barclay and Kurta 2007, Johnson et al. 2012). This is not surprising given the ephemeral nature of snags and roost features in live or newly dead trees and the variability of temperature and moisture conditions throughout the summer months. It should be noted, however, that some studies have shown a high degree of roost fidelity with individuals returning to utilize the same roost sites annually (Barclay and Bingham 2001, Kurta and Murray 2002 Willis et al. 2006). Furthermore, emergence counts conducted at roosts identified during radio-telemetry tracking confirmed that reproductive females occupy communal maternity roosts which are not uncommon for forest bats (Kunz and Lumsden 2003, Broders and Forbes 2004).

### ***Conclusions***

Mist netting survey efforts initiated by SNF biologists during the summer of 2013 suggest that little brown and northern long-eared bats can be detected, albeit at low numbers, across much of the forest where surveys are conducted. Acoustical monitoring data, once analyzed, may show greater use of the Forest by bats and may help identify important patterns in habitat occupancy. These preliminary surveys provide valuable information regarding forest bat habitat use in northeastern Minnesota.

While some have argued that roost availability influences bat diversity (Humphrey 1975) and that loss of roosts may be, in part, responsible for population declines (Evelyn et al. 2004) there

is no indication that roosts are a limiting factor on the SNF. That said, it is important to note that little information is available to evaluate roost quality. High-quality roosts may be limited in availability (Barclay and Kurta 2007) and future monitoring efforts will help assess this possibility. Examining roost selection at multiple spatial scales, including micro-climate features inside the roost structure (Boyles 2007), would be a major step in classifying roost quality potential.

In the meantime, forest management that favors structural and compositional diversity, a wide distribution of age classes, and the retention of large snags and live trees with suitable roost characteristics should aid in maintaining suitable roosting habitat for forest bats on the SNF. Identifying roost habitat and conserving known roost sites is important but equally important will be the management of forested landscapes to provide for the recruitment of suitable roost structures (Brigham 2007).

In terms of foraging habitat, continued acoustic surveys and radio-telemetry tracking will provide valuable bat activity data and help refine our understanding of commuting and foraging patterns. In general, bats tend to utilize vertical and horizontal edges and linear features (e.g., road openings, riparian corridors, etc.) as foraging habitat. However, additional data is needed to identify the specific features that characterize quality foraging habitat. Continuing and expanding bat monitoring efforts of the SNF will help obtain this type of information.

Given the proposed listing of the northern long-eared bat as endangered under the Endangered Species Act (Federal Register 2013), and the recent detection of the fungus *Pseudogymnoascus destructans* in the nearby Soudan Underground Mine, data regarding habitat use, habitat quality, and population status will become increasingly important for management decisions on the SNF.

### ***Recommendations***

- Continue to develop skill sets and expertise that will allow forest biologists to guide and implement future monitoring efforts on the SNF.
- Develop a formal monitoring plan with well-defined monitoring objectives to track the status of forest bats on the SNF.
- Continue to mist-net forest bats on the SNF and expand the scope of the monitoring effort to ensure adequate sampling across the forest.
- Identify key knowledge gaps regarding forest bat ecology that would inform forest management decisions.
- Seek partnerships with local universities and other research organizations to implement studies specifically designed to answer questions about forest bat habitat selection in northeastern Minnesota.
- Collaborate in the establishment of a Minnesota bat working group to acquire and share knowledge regarding bat management in the State.

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Table 1. Mist-net locations for the 2013 forest bat survey effort on the Superior National Forest, Minnesota. Coordinates are in NAD83 Zone 15.

Site Code	Date	Location	Zone	UTMe	UTMn	Habitat	Site Description
2013-1	6/22/2013	Glippi Road	15	598222	5308549	Road	
2013-2	6/23/2013	Kawishiwi Lab	15	594500	5296330	Maternity Colony / Road	Old log building that was set to be demolished; MYLU maternity roost in attic; also netted access road with excellent side and canopy cover.
2013-3	6/24/2013	Old Birch Campground	15	590759	5295975	Road	Old campground roads with excellent side cover and some overhanging canopy; mixed conifer-deciduous with dense midstory.
2013-4	6/25/2013	FR-1525	15	590346	5298894	Road	Narrow road with dense side canopy and spotty canopy cover; nets set in canopy "tunnel" plus one net parallel to the road next to an open meadow lacking canopy cover.
2013-5	6/26/2013	FR-434	15	580798	5297792	Road	Gated road with good side cover but spotty canopy cover; variable aged forest of mixed pine-hardwood.
2013-6	7/11/2013	FR-434(B)	15N	581574	5298969	Road	0.5 mile down same road as 2013-5
2013-7	7/13/2013	FR-192A	15N	586784	5293116	Road	Along narrow forest road.
2013-8	7/15/2013	Big Lake Boat Access	15N	575134	5327008	Road	Along road down to Big Lake.
2013-9	7/23/2013	FR-1525	15N	590346	5298894	Road	Narrow road with dense side canopy and spotty canopy cover; nets set in canopy "tunnel". Same location as 2013-4 moved parallel net down a bit and across road.

Table 2. Bat captures for the 2013 forest bat survey on the Superior National Forest.

Date	Site Code	Capture No.	Species	Time	Sex	Age	Reproductive Status	Weight (g)	Forearm Length (mm)	Wing Score	Comments
6/22/2013	2013-1	1	<i>M. lucifugus</i>	22:35	M	J	NR	8.5	38.2	0	2 biopsies & swab (MN001)
6/23/2013	2013-2	1	<i>M. lucifugus</i>	09:30	F	A	P	10.5	36.9	0	2 biopsies & swab (MN002)
6/23/2013	2013-2	2	<i>M. lucifugus</i>	09:30	F	A	NR	7.5	39.6	0	2 biopsies & swab (MN003); slight tear & mites L wing
6/23/2013	2013-2	3	<i>M. lucifugus</i>	09:30	F	A	NR	7.5	36.6	1	2 biopsies & swab (MN005); mites on wings & flaking forearm photos: 5147-5160
6/23/2013	2013-2	4	<i>M. lucifugus</i>	09:30	F	A	PL	8.5	34.3	0	2 biopsies & swab (MN004)
6/23/2013	2013-2	5	<i>M. lucifugus</i>	09:30	F	A	P	10.75	38.2	0	2 biopsies & swab (MN006)
6/23/2013	2013-2	6	<i>M. lucifugus</i>	09:30	F	A	P	9.75	37.2	1	
6/23/2013	2013-2	7	<i>M. lucifugus</i>	09:30	F	A	P	10.25	37	0	mite L wing
6/23/2013	2013-2	8	<i>M. lucifugus</i>	09:30	F	A	NR	8	38.1	1	splotchy (non-transluminance) but no flakiness
6/23/2013	2013-2	9	<i>M. lucifugus</i>	09:30	F	A	P	11.5	39.3	0	
6/23/2013	2013-2	10	<i>M. lucifugus</i>	09:30	F	A	PL	9.5	37.3	0	small tear L wing
6/23/2013	2013-2	11	<i>M. lucifugus</i>	01:00	F	A	P	10.75	38.4	0	little blood under right armpit
6/23/2013	2013-2	12	<i>M. lucifugus</i>	01:40	F	A	L	10.25	37.8	0	bat bug
6/24/2013	2013-3	1*	<i>M. lucifugus</i>	22:15	F	A	P	11.25	39.8	0	swab MN021; Radio-tagged freq 150.357 (ID F001); bat bug(s) & mites
6/24/2013	2013-3	2	<i>M. septentrionalis</i>	22:45	M	A	NR	7	37.2	1	2 biopsies & swab (MN007); wing plotchy (but doesn't need translumination) & slightly flakey FA
6/24/2013	2013-3	3	<i>M. lucifugus</i>	23:00	M	A	TD	9.25	38.5	0	2 biopsies & swab (MN008); splotchy wings
6/25/2013	2013-4	1	<i>M. septentrionalis</i>	21:55	M	A	TD	7	35.9	0	2 biopsies & swab (MN009); bat bugs
6/25/2013	2013-4	2	<i>M. septentrionalis</i>	22:10	F	A	PL	8	36.7	0	2 biopsies & swab (MN010); mites and scar tissue on L wing; lumps on dorsal side of ears (see photos - Kari & Dan)
6/26/2013	2013-5	1	<i>M. septentrionalis</i>	22:08	M	-	-	-	-	-	Escaped from net
6/26/2013	2013-5	2	<i>M. septentrionalis</i>	22:15	M	J	NR	6.25	35.2	0	2 biopsies & swab (MN011); small spots on wings
6/26/2013	2013-5	3	<i>M. lucifugus</i>	23:08	M	A	TD	9	38.1	0	2 biopsies & swab (MN012); small mite spots. Recaptured at 0045 h in same net.
6/26/2013	2013-5	4	<i>M. septentrionalis</i>	23:44	M	A	TD	7	36	0	2 biopsies & swab (MN013); pinprick holes and scar tissue on R wing
6/26/2013	2013-5	5	<i>M. septentrionalis</i>	00:30	M	J	NR	8.5	36.8	0	2 biopsies & swab (MN014); pinpricks and blotches on R wing

Date	Site Code	Capture No.	Species	Time	Sex	Age	Reproductive Status	Weight (g)	Forearm Length (mm)	Wing Score	Comments
7/11/2013	2013-6	1*	<i>M. lucifugus</i>	00:15	M	A	TD	10.5	37.2	0	1 small scar spot, radio-tagged frequency 150.378 (ID M002)
7/13/2013	2013-7	1	<i>M. septentrionalis</i>	23:10	M	A	TD	7.5	35.8	0	2 biopsies & swab (MN015)
7/15/2013	2013-8	1*	<i>M. lucifugus</i>	21:45	M	J	NR	8.5	37.6	0	Radio-tagged frequency 150.397 (ID M003)
7/15/2013	2013-8	2	<i>M. lucifugus</i>	23:40	M	A	TD	9	38.4	0	2 biopsies & swab (MN016), left punch too close to knee
7/23/2013	2013-9	1*	<i>M. septentrionalis</i>	22:00	M	J	NR	7.5	36	0	1 small spot on wing, radio-tagged frequency 150.716 (ID M004). Hair sample to Steve Windels VNP
7/23/2013	2013-9	2*	<i>M. septentrionalis</i>	22:40	F	A	PL	8	37.4	0	Mites, fleas, radio-tagged frequency 150.737 (F005). Hair sample to Steve Windels VNP.
7/23/2013	2013-9	3	<i>M. septentrionalis</i>	22:40	F	J	NR	7	37.1	0	
7/23/2013	2013-9	4	<i>M. septentrionalis</i>	23:15	M	J	NR	7	34.7	0	2 biopsies & swab (MN017)
7/23/2013	2013-9	5	<i>M. lucifugus</i>	23:15	F	J	NR	8.5	38.5	0	
7/23/2013	2013-9	6	<i>M. septentrionalis</i>	00:30	M	A	TD	7.25	35.2	0	
7/23/2013	2013-9	7	<i>M. lucifugus</i>	00:30	M	J	NR	8.5	38.4	0	

Table 3. Age and sex distribution of forest bats captured during nine nights of mist-net surveys on the Superior National Forest in 2013.

Sex	Adult	Percent of Total	Juvenile	Percent of Total
Male	9	37.5	7	77.8
Female	15	62.5	2	22.2
Total:	24	100.0	9	100.0

Table 4. Roost site characteristics for forest bats tracked on the Superior National Forest in 2013.

RoostID	Bat Species	Date	Tree Species	dbh	Height	Roost Structure	Live / Dead	Percent Slope	Aspect	Basal Area	Canopy Cover	Bark Condition	Limbs	Breakage
F001A	<i>M. lucifugus</i>	8/25/2013	<i>P. tremulooides</i>	14.4	70.62	possible crevice with small cavity	live	12	44	50	62.14	tight, intact	mostly present	< 1/4 broken
F001B	<i>M. lucifugus</i>	8/20/2013	<i>P. tremulooides</i>	11.2	63	crevice	live	3	164	80	84.76	tight, intact	mostly present	no breakage
M002A	<i>M. lucifugus</i>	8/22/2013	<i>P. tremulooides</i>	11	51.5	unknown	dead	5	168	60	66.56	tight, intact	mostly present	no breakage
M003A	<i>M. lucifugus</i>	8/23/2013	<i>P. strobus</i>	49.2	23.5	unknown	dead	20	80	20	86.58	tight, intact	limbs absent	>3/4 broken
M003B	<i>M. lucifugus</i>	8/23/2013	<i>P. strobus</i>	22.5	24	unknown	dead	16	158	90	92.3	over 75% missing	limbs absent	>3/4 broken
F005A	<i>M. septentrionalis</i>	8/27/2013	<i>P. tremulooides</i>	14.8	75.6	unknown	live	25	0	130	98.54	.	.	.
M004A	<i>M. septentrionalis</i>	8/27/2013	<i>P. spp (aspen)</i>	14.9	.	unknown	dead	15	20	100	93.34	tight, intact	mostly present	no breakage

Figure 1. The Superior National Forest in Minnesota, USA.

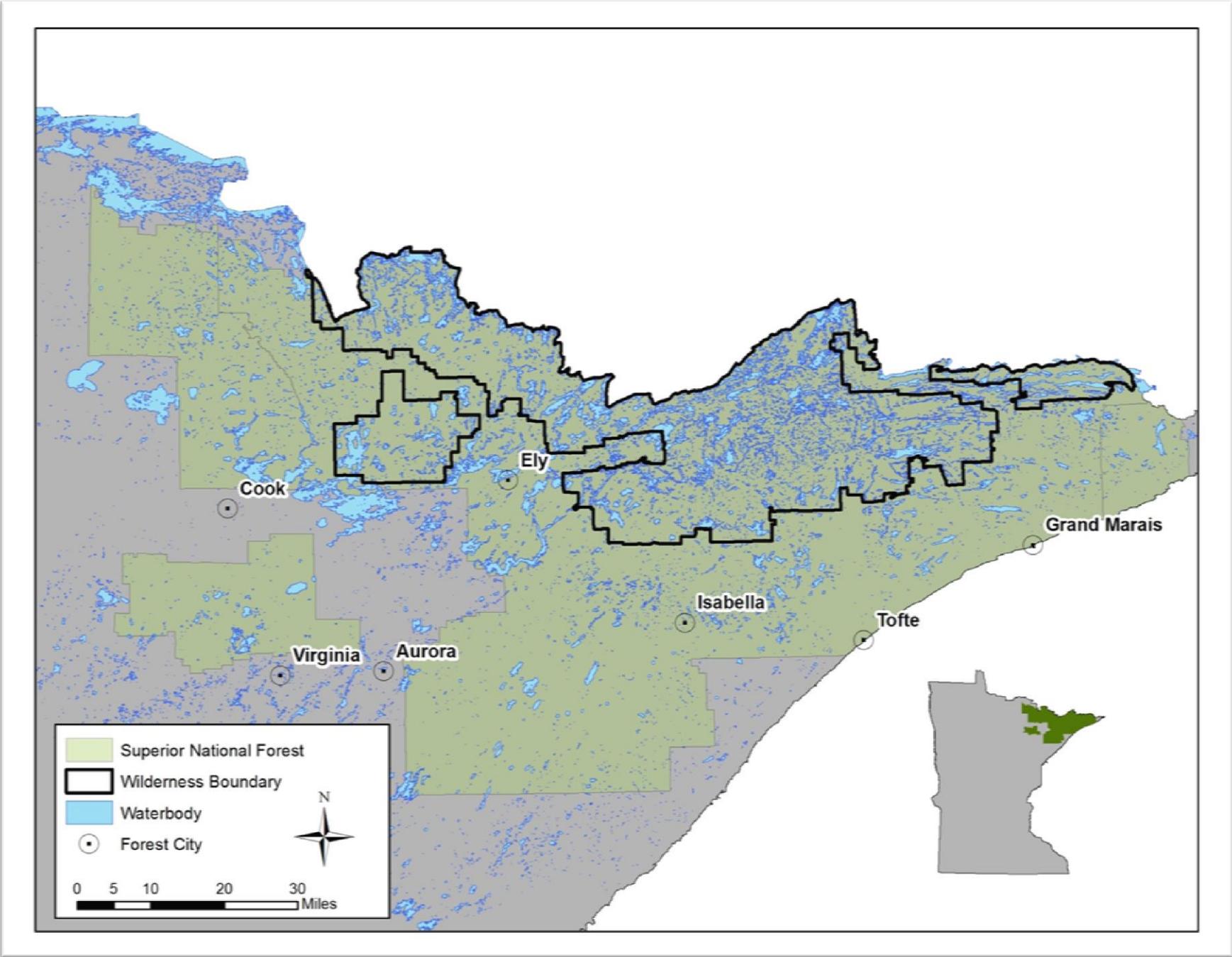


Figure 2. Mist-netting sites on the Superior National Forest, Minnesota, USA.

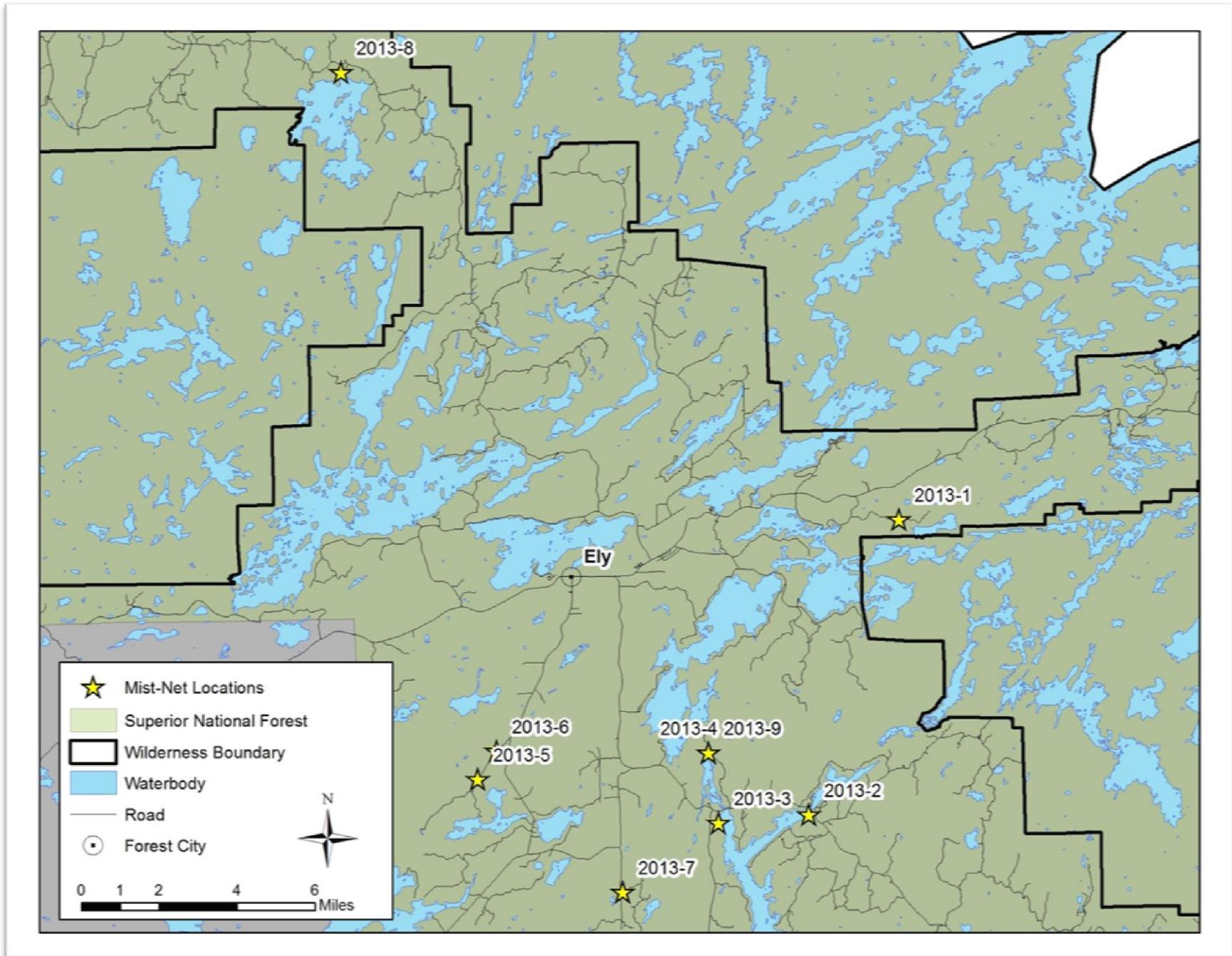


Figure 3. Little brown bat (*Myotis lucifugus*) captured in a mist-net during the 2013 forest bat survey on the Superior National Forest.

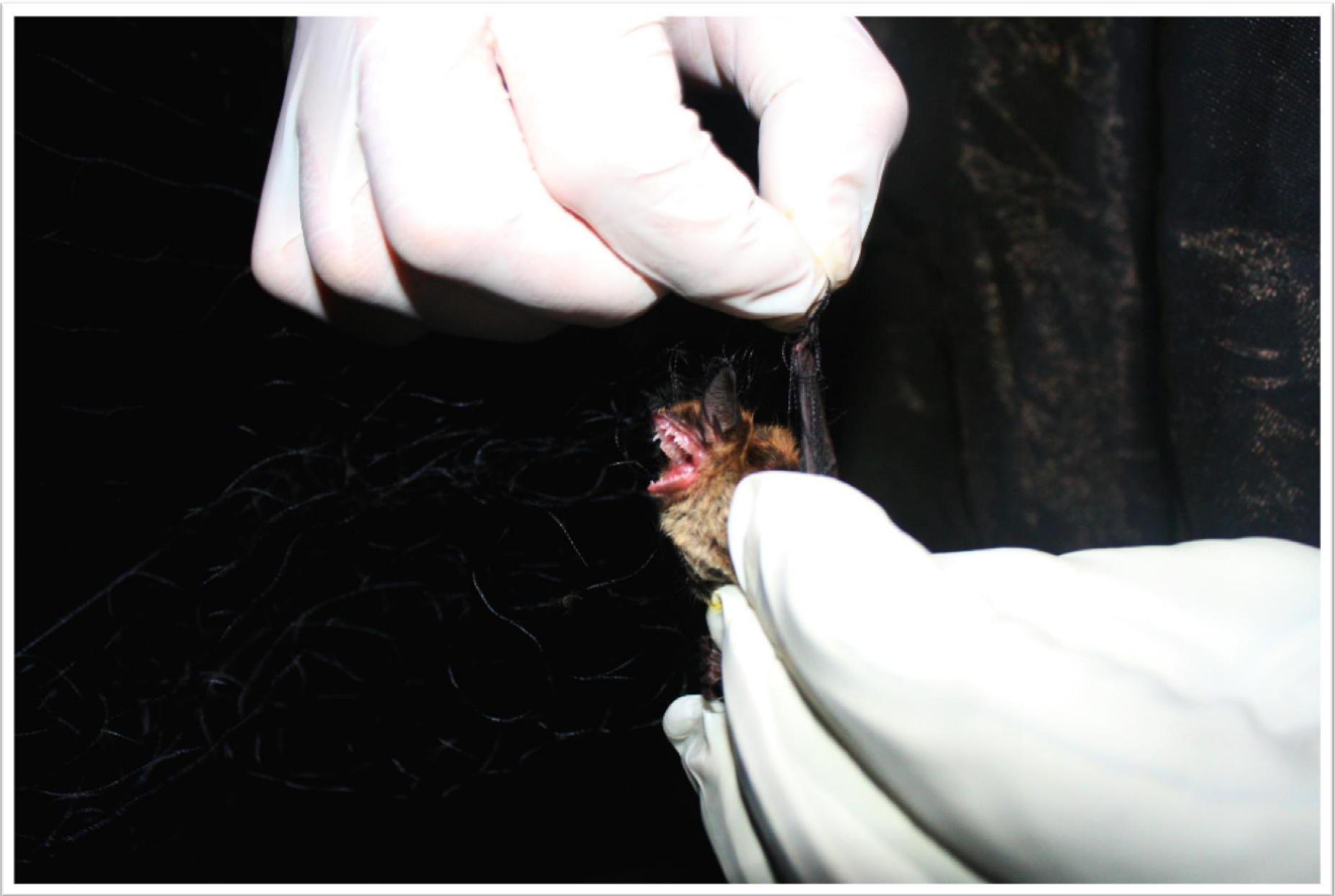


Figure 4. Reproductive condition of forest bats captured during nine nights of mist-net surveys on the Superior National Forest in 2013.

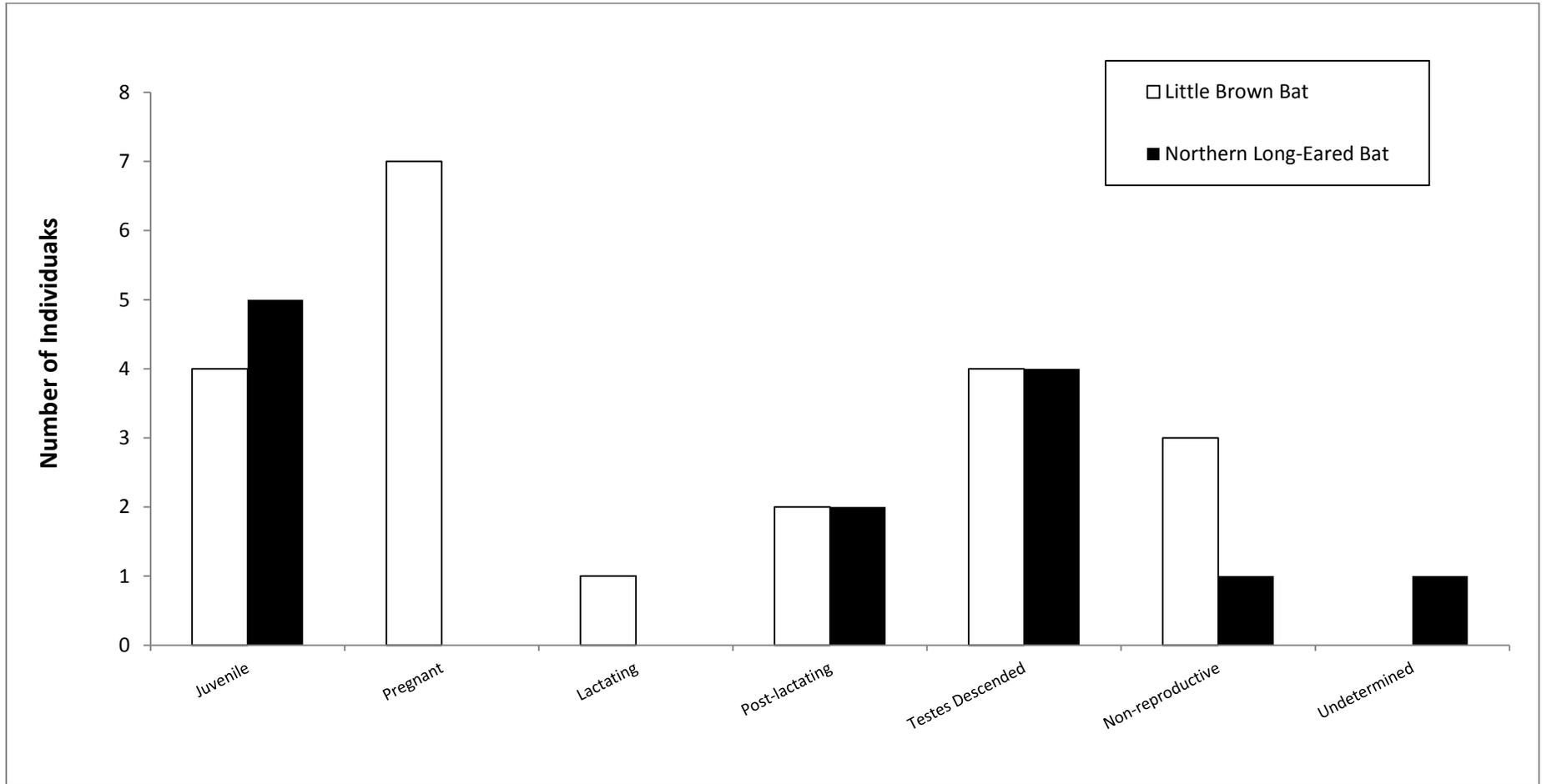


Figure 5. Wing score data for forest bats captured during nine nights of mist-net surveys on the Superior National Forest in 2013.

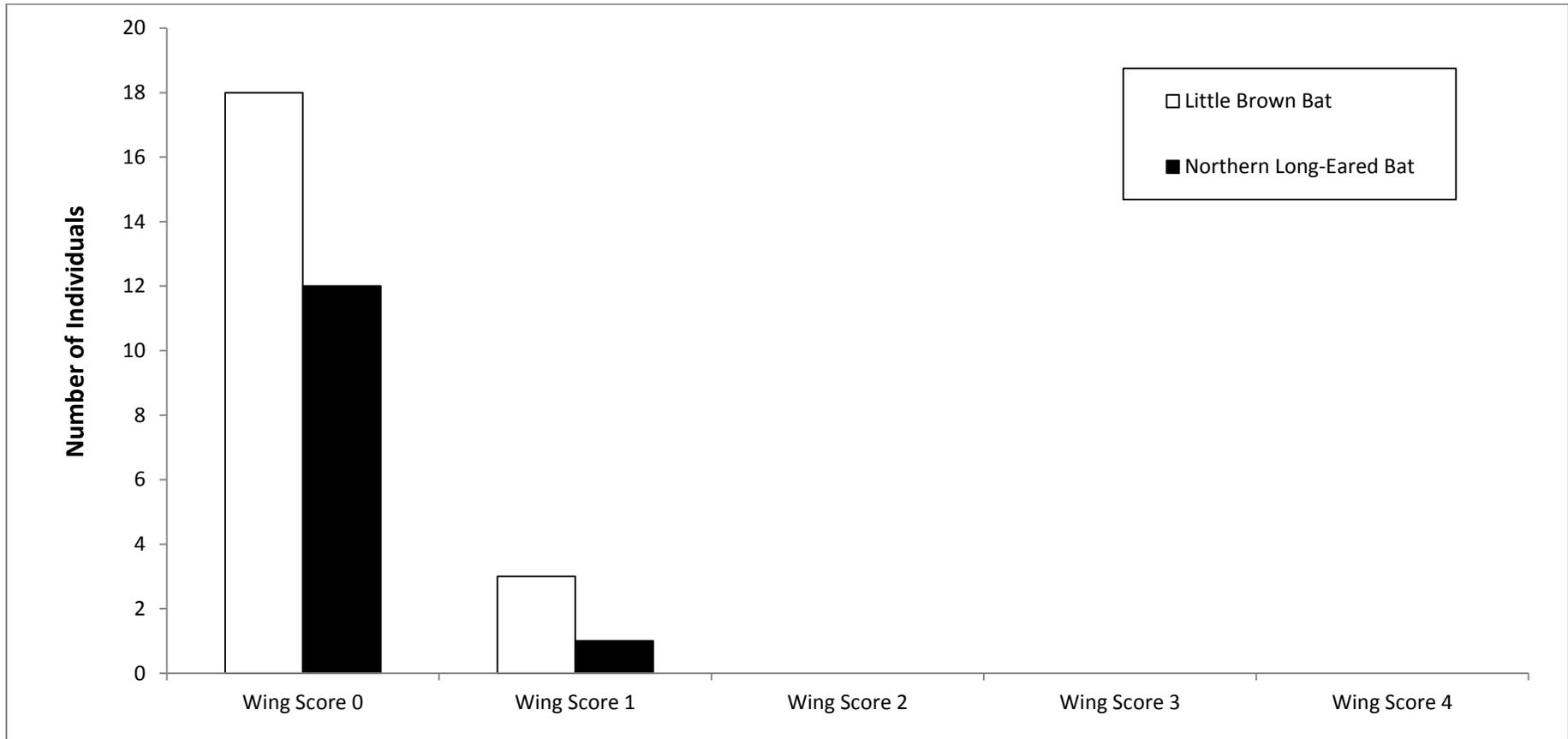


Figure 6. Radio-transmitter attachment on a little brown bat (*Myotis lucifugus*) bat captured on the Superior National Forest in 2013.

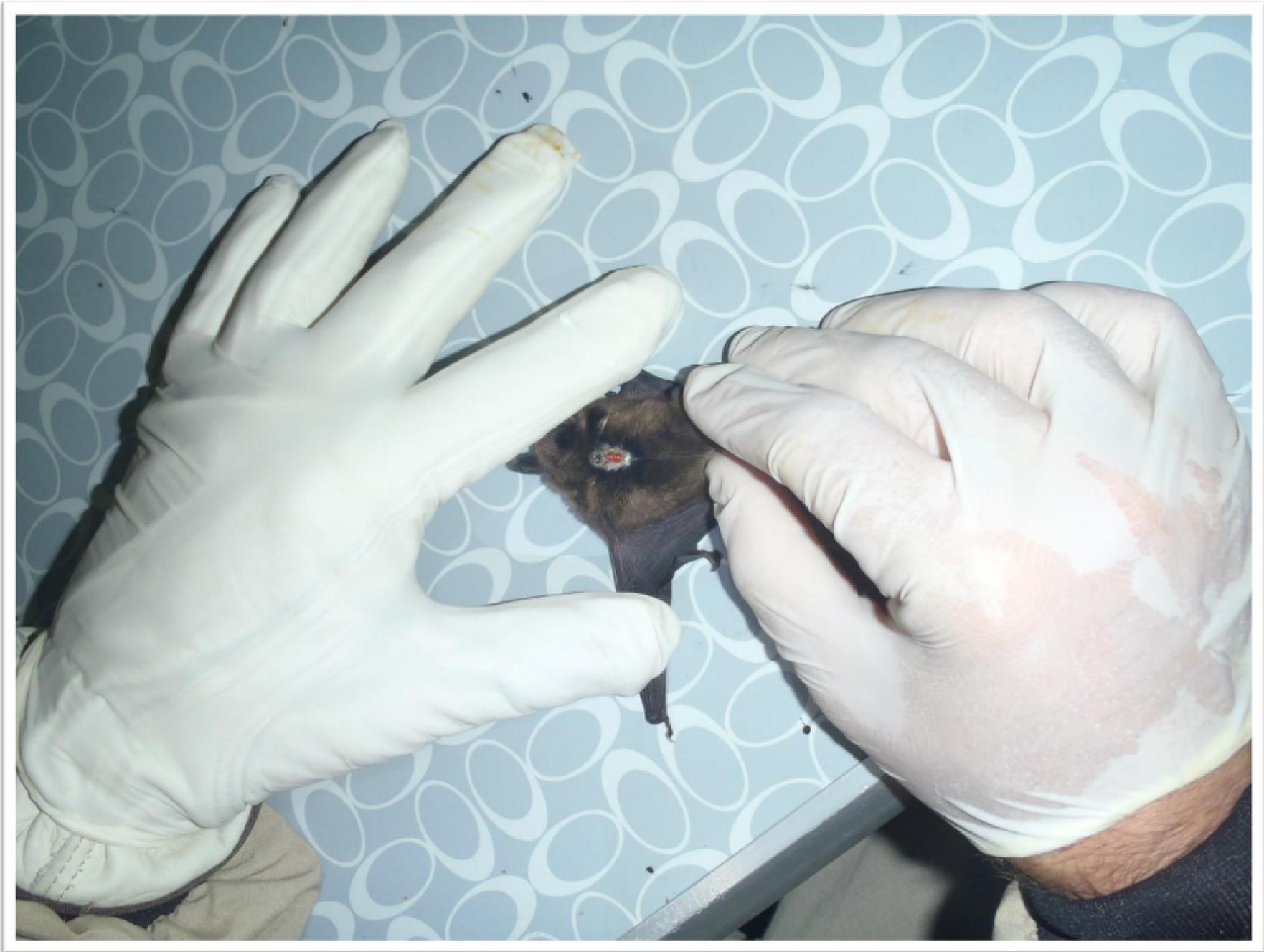


Figure 7. Quaking aspen (*Populus tremuloides*) roost on the Superior National Forest.



## **Wing-Damage Index Used for Characterizing Wing Condition of Bats Affected by White-nose Syndrome**

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Boston, MA 02215**

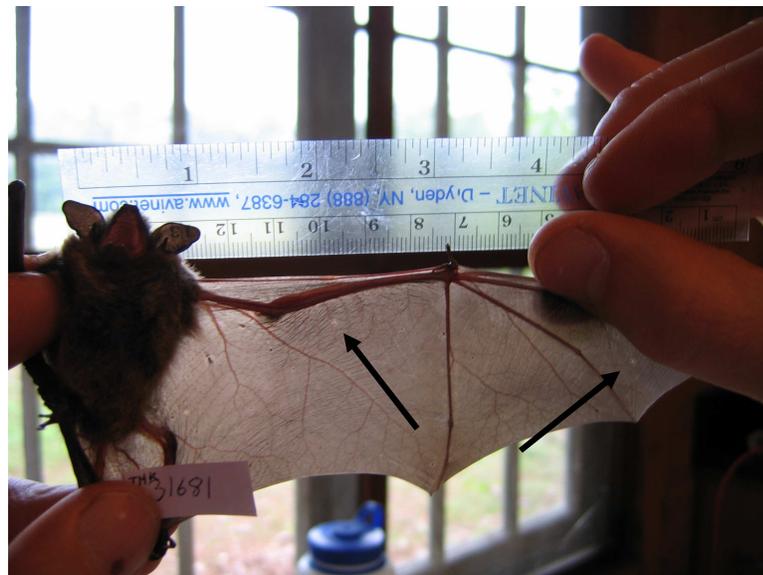
White-nose Syndrome (WNS) is characterized by the growth of one or more species of fungus on the rostrum, ears, and flight membranes of hibernating bats. During the warm months of the year, damage to these membranes may be manifested by the appearance of necrotic tissue, tears, and scars in these membranes. To assess the occurrence and severity of damage to flight membranes, researchers authorized to handle bats should inspect the membranes of both wings and the uropatagium for each bat handled. Each bat is assigned a single score based on the collective condition of these membranes as described below. Affected membrane areas are estimated as the percent of the total membrane area (including both wings and the uropatagium). Translumination of membranes helps to reveal damage that is not otherwise visible. Damage also has been observed on the forearms of some bats and has been included in these scoring criteria. A general diagram of bat anatomy is included in Appendix A for reference.

The damage to membranes and the forearms are scored 0 (none) to 4 (high) according to the criteria listed below and digital photographs are taken to document any damage. Each photograph should include a **reference scale** and the **bat ID number** (specimen number if collected dead or band or ID number if alive and released). Place the **animal on its back on a flat surface with wings and leg extended**. Record images of both wings and the uropatagium either simultaneously or individually. This is best accomplished if one person grasps the tips of the wings and spreads them fully, while a second person extends the bat's legs and uropatagium with one hand and takes the photo with the other. Alternatively, each wing and the uropatagium can be photographed separately, making sure that each photo includes the reference scale and ID number. You may need to experiment with camera settings to achieve quality images; we have had success recording images of flight membranes using a Canon PowerShot A95 (5 MP) digital camera against a white background using the Macro setting, a low intensity, built-in flash, F7.0, shutter speed = 1/800. These settings highlight some of the pspotching and all of the necrosis and holes described below. If possible, translumination may highlight more scarring, but this may be difficult in the field. For translumination, we have used a modified Plano Stowaway tackle box insert (translucent white plastic box) with an LED headlamp inside (see Appendix B). If digital images cannot be recorded, sketches of damaged wings will be helpful.

**Scoring Criteria:**

Each bat is assigned the score for which it exhibits one or a combination of the characteristics designated to that score. Some minor physical damage may be normal. See notes on physical damage not associated with necrosis at the end of this document.

**Score = 0** *No damage.* Fewer than 5 small scar spots are present on the membranes. The membranes are fully intact and pigmentation is normal.

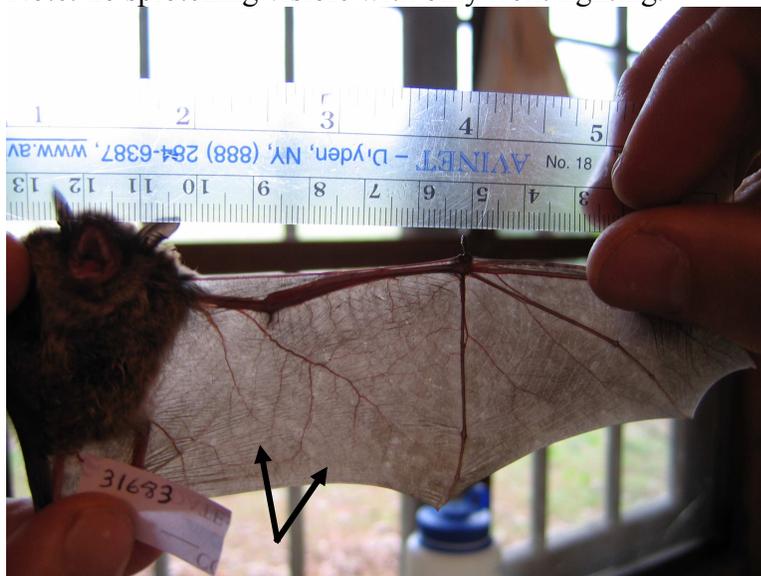


**Score = 1**

*Light damage.* Less than 50% of flight membrane is depigmented (spotching), which is often visible only with translumination. The membranes are entirely intact. Some discoloration or flaking is visible on forearms. Such flaking on the forearm may exist even if the patagium appears unaffected.



Note: no spotching visible with only front lighting.



Translumination reveals the splotchy flight membrane.



Forearms may have flaking skin or discolored areas.



**Score = 2** *Moderate damage.* Greater than 50% of wing membrane covered with scar tissue (splotching). Scarring is visible without translumination. Membrane exhibits some necrotic tissue and possibly few small holes (<0.5 cm diameter). Forearm skin may be flaking and discolored along the majority of the forearm, but this condition alone *does not* earn this score level.

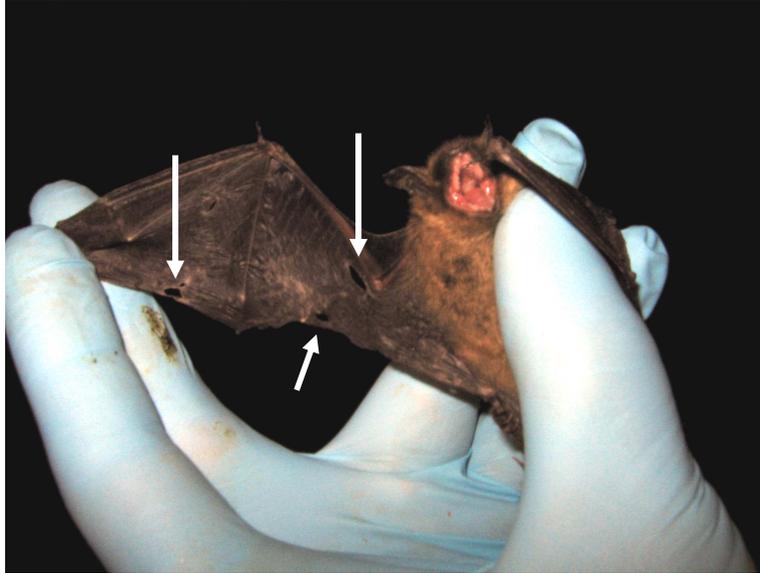


Small holes are surrounded by discolored tissue. Necrotic tissue is sometimes associated with less severe splotching.



**Score = 3**

*Heavy damage.* Deteriorated wing membrane and necrotic tissue. Isolated holes  $\geq 0.5$  cm are present in membranes. Necrotic or **receding plagiopatagium** and/or chiropatagium are evident. This score is characterized by notable loss of membrane area and abundant necrosis.



Flight membranes show damage similar to level 2 damage with additional loss of flight membrane area due to holes and/or receding edges of the wings.





Plagiopatagium loss may be severe.

## Physical Damage

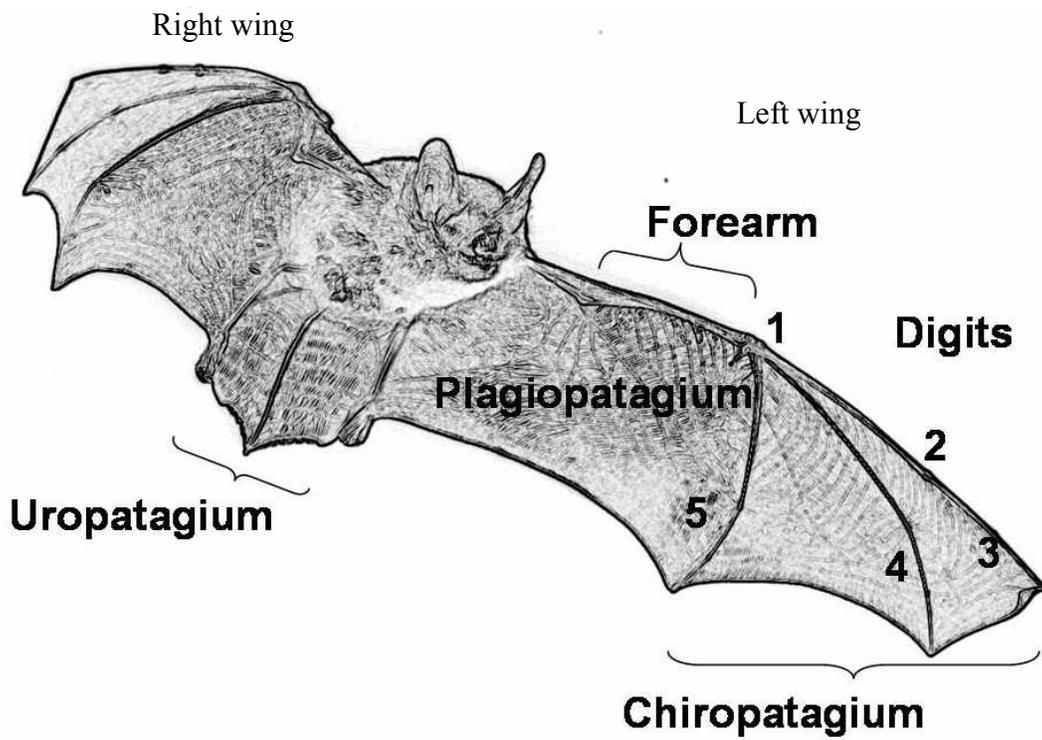
We have encountered bats that have obvious physical damage to wings, but no associated splotching or necrotic tissue. These conditions are important to document as well. We suggest these be recorded in concordance with the above scores followed by a postscript “P” for “physical damage.” For example, an animal which has no noticeable splotching or flaking, but does have a tear in the wing membrane would be scored “0-P.” An animal that has moderate splotching and a tear or puncture would be scored “2-P.” Along with these scores, a description of the physical damage should be included on the data sheet.



Example: **Score = 1-P** due to light splotching (not shown in photo) and a physical tear in the membrane. **Description:** Right plagiopatagium appears to have torn from trailing edge of the membrane to about 1

cm proximal to the elbow.

Appendix A: Reference for flight membranes and digits of bats. Image adapted from J. S. Altenbach's photograph of *Myotis thysanodes*.



**Appendix B:** We are working with an inexpensive light box in the field. The following model is an early effort to create an inexpensive, transportable light box for transilluminating wings. The Plano Stowaway tacklebox insert (~\$3.00) is a good size and the headlamp in this model may be replaced with small LED keychain lights (~\$3.00 each).



The 23 cm x 12 cm tackle box insert is cut to fit the light of a headlamp, creating a diffuse light source.



In this model, images are a bit underexposed, but splotching is highlighted nicely. Brighter lights or more LEDs may solve this problem and a tripod would allow for slower shutter speed. This image was taken using F2.8, shutter speed = 1/30.

## Protocol for Swabbing Bats for microbial community analysis

Modified by Paula Marquardt (USFS-NRS13) June 20, 2013 (original: Kevin Keel, UC Davis; January 8, 2011).

NOTE: Swabs are to be submitted to the lab, collect 1 swab per bat. This non-lethal sampling method will be used for diagnostic PCR evaluation of microbial communities.

- Put on a clean pair of gloves before swabbing a new bat or handling the storage tubes.
- Plastic tubes will be pre-labeled with sample ID numbers that correspond to data sheets.
- Complete data sheet with state, date, collector initials, bat number; species, sex, age class, sample type, GPS coordinates, and if there is evidence of WNS (visible fungus on flight membranes, ear, tail and muzzle, wing damage), band number.
- Open the package containing the sterile swab so that the handle is presented first (Fig 1)
  - Be certain not to contact the swab (or shaft nearest the swab) with gloved hands, clothing, work surfaces, etc.
  - The procedure may be easier if one person opens the swab wrapper and presents the sterile swab to the person collecting the sample.



- With a gentle sweeping motion, swab both sides of the muzzle three to five times. Gently swab the dorsal surface of each distended wing five times, moving the swab from the body towards the outer margin of the patagium.
  - Rotate the swab  $\frac{1}{4}$  turn with each stroke of the swab.
- Place the tip of the swab in the appropriately labeled tube, being certain not to touch any external surfaces of the tube (Fig 2).
  - Sterilize scissors with alcohol wipe.
  - Cut swab to fit in the tube and close tightly.
  - Place excess garbage (swab wrapping, remaining swab stick, alcohol wipe, and gloves) in “garbage” Ziploc bag.
- Place capped tubes in a freezer box with ice packs to keep the samples cool.
- When the freezer box is full, or all samples have been collected, carefully tape the lid shut.
  - Place the box in a clean plastic bag, evacuate excess air and secure the bag.

- For more samples, repeat this process with a second freezer box and clean bag.
- Refrigerate the swabs and ship as soon as possible in a cooler, on ice packs, for delivery by next business afternoon.
  - Maintenance of the cold chain is critical for accurate diagnostics.
- If samples are not shipped within 24 to 36 hours, samples should be frozen until submitted to the lab for PCR analysis.
- Specimens should be chilled or frozen and shipped overnight in a cooler with ice packs to Rhinelander USFS.
  - Saturday delivery is not available unless we make prior arrangements.
  - Attach the shipping document (airbill) with the DOT information to the outside of cooler in a re-sealable pouch
  - Tear off top copy (Sender's Copy) of FedEx shipping label and place inside of shipping box.
  - Using packing or duct tape, tape the cooler shut around the lid at each end using a continuous wrap around the cooler.
  - Please forward the tracking number by e-mail to [ddonnerwright@fd.fed.us](mailto:ddonnerwright@fd.fed.us)

Using enclosed Fed-ex shipping label, the swabs and data sheets should be shipped overnight to:

Deahn Donner  
USDA Forest Service  
5985 HWY K  
Rhinelander, WI 54568  
Telephone: 715-362-1146

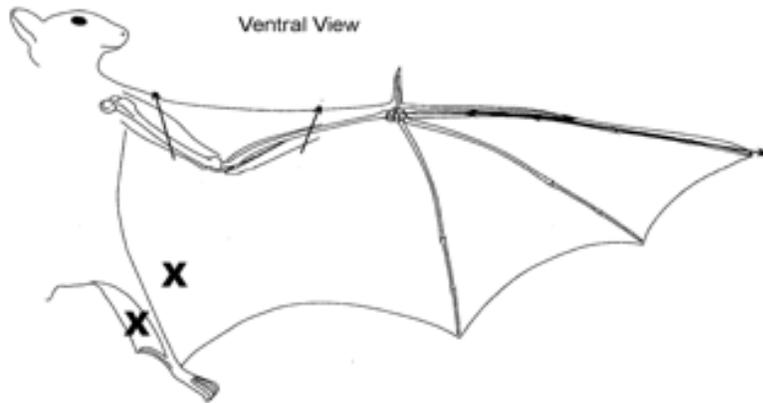
## **Instructions for Taking a Wing Tissue Punch Biopsy**

Updated by Paula Marquardt (USFS-NRS13) 6/20/2013: (original: Shonene Scott, Portland State University 5/2003; updated by Pat Ormsbee (NFS) and Jan Zinck 5/14/09; modified by Anne Ballmann (USGS-NWHC) 12/21/12)

NOTE: Punch biopsies are to be submitted to the lab for testing of bat DNA; collect 2 biopsies per bat (from different wings). Biopsy punches should be collected from portions of the wing membrane that do not exhibit fungal growth or other types of visible lesions, if possible. Please note on data sheets if abnormalities are present in sample. Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings. To reduce the risk of cross-contamination among bats, all equipment (i.e.: gloves, tissue punches, biopsy boards, and needles) should be cleaned or changed between each sampled bat.

1. When taking biopsies it is important to reduce the potential for cross-contamination between bats. In order to do this, use a small clean piece of sturdy cardboard that can be discarded after each animal, a new tissue punch for each bat, a new sterile needle to transfer sample, and disposable gloves.
2. Sterile tubes will be pre-labeled with sample numbers that correspond to the data sheet
3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a syringe needle ready for each bat. Do not touch (contaminate) the end of the punch, the needle, or the inside of the tube lid with fingers or environmental debris. (if it is easier to simply use the plastic clipboard and not the cardboard square...make sure to sterilize clipboard with alcohol wipes in-between samples).
4. Identify 2 representative areas to biopsy on the wings/tail of the bat. Place the bat on the cardboard on its back and extend one wing membrane (avoid sampling from bats with large wing tears). For people inexperienced in this technique, it works best when one person holds the bat and another person collects the biopsy.
5. When collecting wing tissue biopsies, avoid bones and major blood vessels. (Figure 1). If possible, locate an area near the body wall within the lower half of the wing membrane or uropatagium. These locations have been demonstrated to have faster healing rates and are less disruptive to flight aerodynamics (Faure PA et al. 2009. J Mammalogy 90(5): 1148-56.) Press the punch firmly through the membrane and twist the punch slightly to ensure a complete punch. Apply direct pressure to biopsy site for several minutes if bleeding occurs.

Figure 1: "X" marks ideal sample locations for collecting tissue biopsies from bat flight membranes.



6. Carefully lift the bat off the biopsy board and look for the tissue sample. It should either be on the board or inside the tip of the punch. Be careful on windy days since the wind can blow the tissue off of the board. A new 25 gauge needle should be used to pick up the tissue and transfer each biopsy to separate storage tubes without media.
7. Deposit used needles in the provided "garbage" Ziploc bag along with the tube it came in, biopsy punches and gloves. DO NOT try to place the used needle back into its vial. Send "garbage" Ziploc bags back with samples to Rhinelander and they will be disposed of according to established safety procedures. Dispose of the used biopsy punch after each animal. DO NOT reuse the same biopsy punch or needle on multiple bats. Change into new gloves before handling each bat.
8. Release the bat only after tissue samples have been placed into the tubes, the tubes have been closed, and any bleeding has stopped. The number of biopsies has been limited to 2 per bat to prevent compromising flight.
9. While in the field, sample tubes should be stored on ice (or ice packs). If samples are not shipped within 24 to 36 hours, samples should be frozen until submitted to the lab for PCR analysis.
10. Specimens should be chilled or frozen and shipped overnight in a cooler with ice packs to Rhinelander USFS.
  - a. Saturday delivery is not available unless we make prior arrangements.
  - b. Attach the shipping document (airbill) with the DOT information to the outside of cooler in a resealable pouch
  - c. Tear off top copy (Sender's Copy) of FedEx shipping label and place inside of shipping box.
  - d. Using packing or duct tape, tape the cooler shut around the lid at each end using a continuous wrap around the cooler.
  - e. Please forward the tracking number by e-mail to [ddonnerwright@fd.fed.us](mailto:ddonnerwright@fd.fed.us)

Using enclosed Fed-ex shipping label, the tissue samples and data sheets should be shipped overnight to:

Deahn Donner  
 USDA Forest Service  
 5985 HWY K  
 Rhinelander, WI 54568  
 Telephone: 715-362-1146

## National White-Nose Syndrome Decontamination Protocol - Version 06.25.2012

The fungus *Geomyces destructans* (*G.d.*) is the cause of white-nose syndrome (WNS), a disease that has devastated populations of hibernating bats in eastern North America. Since its discovery in New York in 2007, WNS has spread rapidly through northeastern, mid-Atlantic, and Midwest states and eastern Canada. It continues to threaten bat populations across the continent. For the protection of bats and their habitats, comply with all current cave and mine closures, advisories, and regulations on the federal, state, tribal, and private lands you plan to visit. In the absence of cave and mine closure policy, or when planned activities involve close/direct contact with bats, their environments, and/or associated materials, the following decontamination procedures should be implemented to **reduce the risk of transmission** of the fungus to other bats and/or habitats. For the purposes of clarification, the use of the word “decontamination,” or any similar root, in this document entails both the 1) cleaning and 2) treatment to disinfect exposed materials.

**Under no circumstances should clothing, footwear, or equipment that was used in a confirmed or suspect WNS-affected state or region be used in a WNS-unaffected state or region.** Some state/federal regulatory or land management agencies have supplemental documents<sup>1</sup> that provide additional requirements or exemptions on lands under their jurisdiction.

### I. TREATMENTS TO REDUCE RISK OF TRANSFERRING *GEOMYCES DESTRUCTANS*<sup>2</sup>:

#### Applications/Products:

The most universally available option for treatment of submersible gear is:

**Submersion in Hot Water: Effective at sustained temperatures  $\geq 50^{\circ}\text{C}$  (122°F) for 20 minutes**

Secondary or non-submersible treatment options (for a minimum of 10 min.) include:

	<b>Clorox® (6% HOCl) Bleach</b>	<b>Lysol® IC Quaternary Disinfectant Cleaner</b>	<b>Professional Lysol® Antibacterial All- purpose Cleaner</b>	<b>Formula 409® Antibacterial All- Purpose Cleaner</b>	<b>Lysol® Disinfecting Wipes</b>
<b>APPROVED USES</b>	<b>Hard, non-porous surfaces</b>	Yes	Yes	Yes	Yes
	<b>Non-porous personal protective safety equipment</b>	No	Yes (headgear, goggles, rubber boots, etc.)	No	No
	<b>All surfaces, including: porous clothing, fabric, cloth footwear, rubber boots</b>	Yes (Do not use on ropes, harnesses or fabric safety gear.)	No	No	No
<b>DILUTION / TREATMENT (as per label)</b>	Effective at 1:10 dilution (bleach : water) <sup>3,4</sup>	Effective at 1:128 dilution (1 ounce: 1 gallon of water) <sup>3,4</sup>	Effective at 1:128 dilution (1 ounce: 1 gallon of water) <sup>3,4</sup>	Effective at concentrations specified by label <sup>3,4</sup>	Effective at 0.28 % dimethyl benzyl ammonium chloride <sup>3,4</sup>

<sup>1</sup> To find applicable addenda and/or supplemental information, visit <http://www.whitenosesyndrome.org/topics/decontamination>

<sup>2</sup> The use of trade, firm, or corporation names in this protocol is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by state and/or federal agencies of any product or service to the exclusion of others identified in the protocol that may also be suitable for the specified use.

<sup>3</sup> Product guidelines should be consulted for compatibility of use with one another before using any decontamination product. Also, detergents and quaternary ammonium compounds (i.e. Lysol® IC Quaternary Disinfectant Cleaner) should not be mixed directly with bleach as this will inactivate the bleach and in some cases produce a toxic chlorine gas. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

<sup>4</sup> Final determination of suitability for any decontaminant is the sole responsibility of the user. Use of some treatments which utilize such method need to be applied carefully, especially in confined spaces, due to inhalation or contact risks of the product. All users should be aware of these risks

*Other effective disinfectant(s) with similar chemical formulas (e.g., a minimum of 0.3% quaternary ammonium compound) or water based applications may exist but are unknown and not recommended at this time.*

**REMEMBER, the product label is the law!**

***It is the responsibility of the users of this protocol to read and follow the product label and MSDS.***

**Products must be used in accordance with the label:**

Ensuring the safety of those who use any of the above products for treatment is of utmost importance. Material safety data sheets (MSDS) developed by product manufacturers provide critical information on the physical properties, reactivity, potential health hazards, storage, disposal, and appropriate first aid procedures for handling or working with substances in a safe manner. Familiarization with MSDS for chemical products prior to use will help to ensure appropriate use of these materials and assist in emergency response.

It is a violation of federal law to use, store, or dispose of a regulated product in any manner not prescribed on the approved product label and associated MSDS.

- Disinfectant products, or their contaminated rinse water, should be managed and disposed of as per product label directions to avoid contamination of groundwater, drinking water, or non-municipal water feature such as streams, rivers, lakes, or other bodies of water. Follow all local, state and federal laws. State-by-state requirements for product disposal may vary. Note: Quaternary ammonium wastewaters should not be drained through septic systems because of the potential for system upset and subsequent leakage into groundwater.

**II. PLAN AHEAD AND CAVE CLEAN:**

**Dedicate your Gear:** Many types of rope and webbing have not been thoroughly tested for integrity after decontamination. Dedicate your gear to a single cave/mine or don't enter caves/mines that require this gear.

**Bag it Up:** Bring bags on all of your trips. All gear not decontaminated on site should be isolated (quarantined) in a sealed plastic bag/s or container/s to be cleaned and disinfected off-site.

**Before Each Cave/Mine or Site Visit:**

- 1.) Determine *G.d./WNS* status<sup>5</sup> of the state/county(s) where your gear was previously used.
- 2.) Determine *G.d./WNS* status<sup>5</sup> of state/county(s) to be visited.
- 3.) Determine whether your gear is permitted for your cave/mine visit or bat related activity, as defined by the current WNS case definitions<sup>6</sup> and the flowchart below.
- 4.) Choose gear that can be most effectively decontaminated [i.e., rubber wellington type (which can be treated with hot water and/or secondary treatment options in section I.) vs. leather boots] or dedicated to a specific location. **Remember, under no circumstances should any gear that was used in a WNS-affected state or region be used in a WNS-unaffected state or region.** Brand new gear can be used at any location where access is otherwise permitted.
- 5.) Determine if any state/federal regulatory or land management agency addendum or supplemental document<sup>1</sup> provides additional requirements or exemptions on lands under its jurisdiction that supplement the final instruction identified in the flowchart below.
- 6.) Prepare a "Clean Caving" strategy (i.e., how and where all gear and waste materials will be stored, treated and/or disposed after returning to your vehicle and base area) for your particular circumstances that provides for cleaning and treatment of gear on a daily basis **unless** instructed above to do so more frequently throughout the day.

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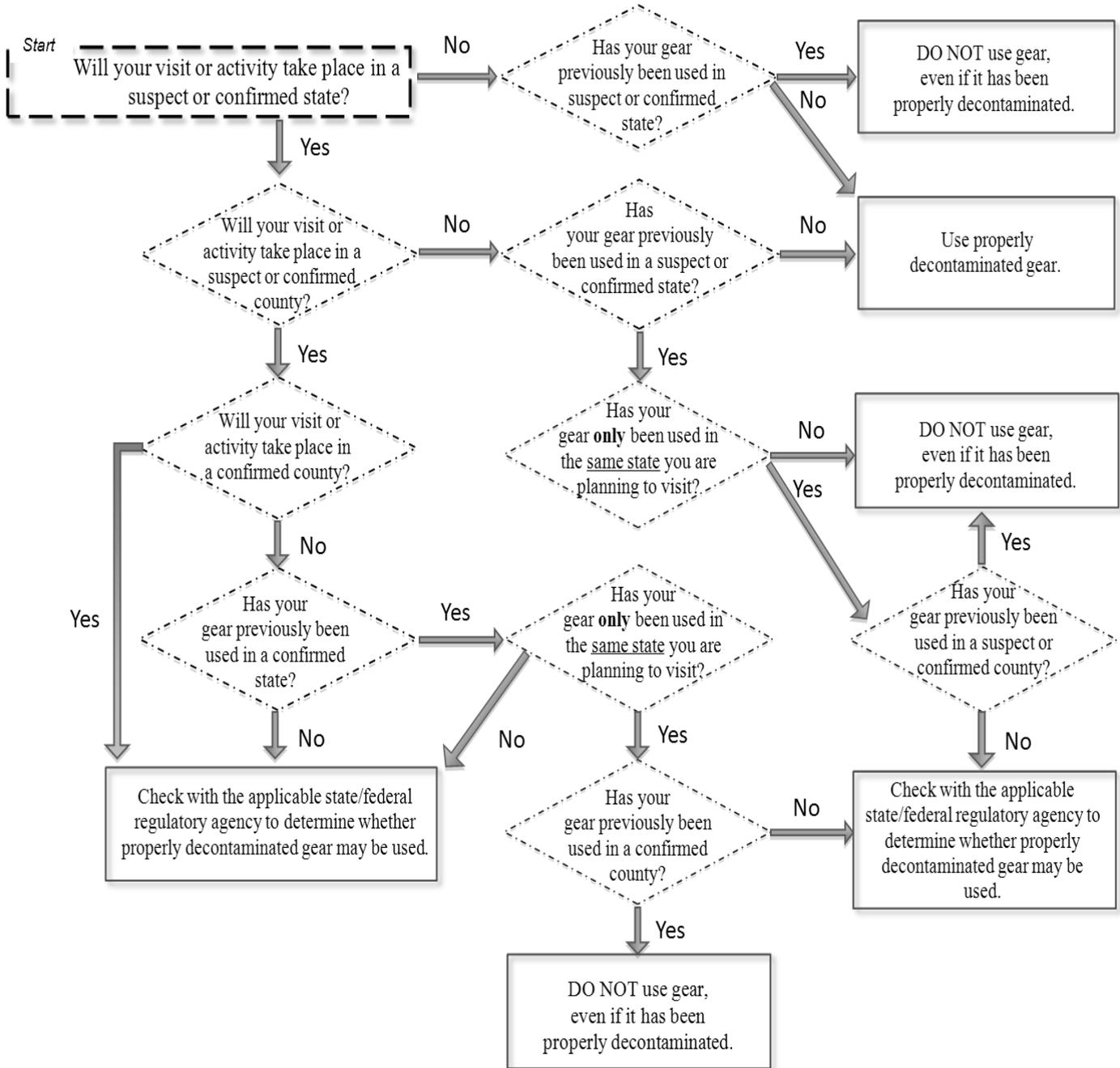
prior to entering cave environments and understand that products and corresponding procedures may cause irreversible harm. Always use personal protective equipment to reduce contact with these products, particularly when recommended by the manufacturer.

<sup>5</sup> Visit <http://www.whitenosesyndrome.org/resources/map> to determine the WNS status of a county or state.

<sup>6</sup> Visit [http://www.nwhc.usgs.gov/disease\\_information/white-nose\\_syndrome/wns\\_definitions.jsp](http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/wns_definitions.jsp) for current WNS case definitions.

7.) When visiting multiple caves/mines or bat research sites on the same day, clean and treat all gear between **each** cave/mine/site, **unless** otherwise directed in an agency/landowner addendum. It is recommended that known confirmed or suspect caves/mines be visited only after those sites of unknown *G.d.* status have been visited, to further reduce the risk of inadvertent transmission.

**Flowchart to Determine Gear Use or Decontamination**



### After Each Cave/Mine or Site Visit:

- 1.) Thoroughly scrub and remove sediment/dirt from clothing, footwear, and other gear immediately upon emerging from the cave/mine or bat research site. Avoid contamination of vehicles; store exposed gear separately from unexposed gear.
- 2.) Once fully scrubbed and rinsed of all soil and organic material, clothing, footwear, and any appropriate gear should be sealed, bagged in a plastic container and once at home, machine or hand-washed/cleaned using a conventional cleanser like Woolite<sup>®</sup> detergent or Dawn<sup>®</sup> antibacterial dish soap in water (the use of Dawn<sup>®</sup> antibacterial dish soap is **not intended** for use in conventional washing machines.) Once cleaned, rinse gear thoroughly in water. Clean/treat gear used in a suspect or confirmed state prior to transport when traveling back to or through a state **without** known cases of *G.d./WNS*. Use the treatments listed under Applications/Products on page 1 for a minimum of 10 (products) or 20 (hot water) minutes.

**Remember:** Many types of rope and webbing have not been thoroughly tested for integrity after decontamination. Dedicate your gear to a single cave/mine or don't enter caves/mines that require this gear.

#### A.) Submersible Gear (i.e. clothing, footwear, and/or equipment that can be submerged in liquid):

Clothing, footwear, and other submersible gear:

Following steps 1 and 2 above, the primary treatment for all submersible gear should always be submersion in **water of at least 50°C (122°F) for a minimum of 20 minutes, where possible**. Some submersible gear (depending on material) could be soaked for a minimum of 10 minutes in the appropriate products listed in the Applications/Products chart on page 1, rinsed thoroughly in water again, and air dried. Note: Although commercially available washing machines with sanitation cycles often sustain desirable water temperatures, their efficacy for killing the conidia of *G.d.* is unknown.

#### B.) Non-submersible Gear:

Gear that may be damaged by liquid submersion should be cleaned according to the manufacturer's recommendation between cave/mine visits and when appropriate, follow steps 1 and 2 above in addition to following:

Cameras and Electronic Equipment:

Until effective techniques are developed to comprehensively disinfect cameras and electronics, it is recommended that these items only be used in caves when absolutely necessary. Regardless of the cave/mine visited, clean/treat cameras and electronics after each visit using an appropriate product listed in the Applications/Products chart on page 1. Equipment that must be used in the cave/mine may be placed in a sealed plastic casing (i.e., underwater camera housing), plastic freezer bag, or plastic wrap that permits operation of the equipment (i.e., glass lens is exposed) and reduces the risk of exposure to the cave environment. Prior to opening or removing any plastic protections, wipe the outside surfaces with an appropriate product described in the Applications/Products chart on page 1. Plastic freezer bag or wrap should be removed and discarded after each visit. A sealed plastic casing may be reusable if properly submersed in appropriate product as described in the Applications/Products chart and the functionality and protective features of the casing are not sacrificed (check with manufacturer). After removal of any outside plastic protection, all non-submersible equipment surfaces (i.e., camera body, lens, etc.) should be wiped using an appropriate product described in the Applications/Products chart.

- 3.) Reduce the risk of vehicle contamination and transport of *G.d.* to new areas by making sure to
  - A) transport gear in clean containers,
  - B) remove outer clothing/footwear and isolate in a sealed plastic bag or container prior to entering a vehicle. Storage container options vary considerably depending on the type of vehicle; but **always clean and disinfect the outside surfaces of storage containers prior to putting them in the vehicle**.
  - C) remain outside of the vehicle after exiting a cave/mine or completing field work,
  - D) change into clean clothing and footwear prior to entering the vehicle, and
  - E) clean dirt and debris from the outside of vehicles (especially wheels/undercarriage).

## OBSERVATION OF LIVE OR DEAD BATS

If you observe live or dead bats (multiple individuals in a single location) that appear to exhibit signs of WNS, contact a wildlife professional in your nearest state (<http://www.fws.gov/offices/statelinks.html>) or federal wildlife agency (<http://www.fws.gov/offices/>, <http://www.fs.fed.us/>, <http://www.blm.gov/wo/st/en.html>, or <http://www.nps.gov/index.htm>). **Do not handle bats unless authorized in writing to do so by the appropriate government agency.**

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### Note on the use of Pesticides/Products listed above:

**The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)** (7 U.S.C. §136 et seq. (1996))

<http://www.epa.gov/oecaagct/lfra.html>

**defines a pesticide as follows:**

#### **(u) Pesticide**

The term “pesticide” means (in part)

**(1)** any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.

**FIFRA defines a pest at §136:**

#### **(t) Pest**

The term “pest” means (in part)

**(1)** any insect, rodent, nematode, fungus, weed, or (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1).

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*This document is the product of the multi-agency WNS Decontamination Team, a sub-group of the Disease Management Working Group established by the National WNS Plan (A National Plan for Assisting States, Federal Agencies, and Tribes in Managing White-Nose Syndrome in Bats, finalized May 2011). On 15 March 2012 a national decontamination protocol was adopted by the WNS Executive Committee, a body consisting of representatives from Federal, State, and Tribal agencies which oversees the implementation of the National WNS Plan. This version of the protocol contains some modifications to the 15 March version, intended to clarify the recommendations for the appropriate use of treatment options. This decontamination protocol will continue to be updated as necessary to include the most current information and guidance available.*

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## Superior National Forest Bat Roost Structure Data Form

Roost Tree ID: \_\_\_\_\_ Date: \_\_\_\_\_ Observer(s): \_\_\_\_\_

UTM E: \_\_\_\_\_ UTM N: \_\_\_\_\_ (NAD83)  Photo of Data Sheet

Tree Characteristics				Stand Characteristics				
1. Species				8. Slope (%)		15. Basal Area (sqft/ac)		
2. DBH (in)				9. Aspect		16. Basal Area direction		21. Canopy Cover
3. Height Measurements	% T	% B	Dist.	10. Roost Photos		17. Density N		x 1.04 =
4. Height (ft)				11. Photo N		18. Density E		x 1.04 =
5. Relative Height				12. Photo E		19. Density S		x 1.04 =
6. Roost Structure				13. Photo S		20. Density W		x 1.04 =
7. Live/Dead				14. Photo W				22. AVG =

Decay				
23. Bark	Tight, intact	50% loose or missing	50% -75% missing	Over 75% missing
24. Limbs	Mostly present	Small limbs missing	Few limbs remain	Limbs absent
25. Canopy Breakage	No breakage	< ¼ broken off	¼ to ¾ broken	> ¾ broken off

26. Please use this space to provide a brief description of the surrounding stand and any notable features nearby (roads, trails, streams, lakes, etc.). If roost structure is identified, please provide a description, including size, direction facing.

# Superior National Forest Bat Roost Structure Data Form

## Instructions

- Species: Identify tree or snag to species. If unidentifiable, record as "Unk." If not a tree, record type of structure and provide detailed description in box 15.
- Diameter at breast height in inches. Measure using d-tape. If visual estimate, denote as "est." Example: 16" (est.)
- Height Measurements: Record % slope to top of roost tree (%T), % slope to bottom of roost tree(%B), and distance from roost tree (feet). Make sure to include appropriate "+" and "-" signs for slope. If the base of the tree is downhill from you, the slope to the base should be negative. Take measurements from a distance of at least 50 feet from the roost for greatest accuracy.
- Height (ft): Total height of tree or snag measured in feet. This can be calculated from the measurements in #3 using the following formula:
  - Height = Distance x (%T - %B). If height is a visual estimate (not preferred), denote as "est." Example: 45' (est.)
- Relative Height: Code height of the roost tree relative to the predominant surrounding canopy within approximately 40 feet of roost.

Code	Description
1	Top of roost tree greater than 10 feet above top of surrounding canopy
2	Top of roost tree within 10 feet of surrounding canopy
3	Top of roost tree greater than 10 feet below top of surrounding canopy

- Roost structure: If possible, identify structural characteristic used by bats. These could include, crevices, cavity, loose bark, broken top, other (identify), or unknown. Provide a description of the structure in box #26.
- Live = L, Dead = D
- Slope: Measure average slope of ground within 40 foot plot centered at roost tree using a clinometer. Record in %.
- Aspect: Record average aspect within 40 foot plot centered at roost tree. Record in degrees.
- Roost Photos: Take photos of roost trying to capture as much of roost as can. If specific roost structure can be identified, take close-up. Record photos numbers.
- 14. Take a photo from the base of the roost facing each cardinal direction. Record the photo number or ID.
- Basal Area: Record live basal area using a center point 5 feet from the roost in a random direction.
- Basal Area direction: Record random direction from roost at which basal area was measured.
- 20. Using convex densitometer, record the density measurement approximately 20 feet from the roost in each cardinal direction. To use the densitometer: Hold densitometer level, with head just outside the grid. Mentally divide each of the 24 boxes into four sub-boxes with a dot at the center of each sub-box (96 total dots). Count the number of points occupied by canopy.
- Multiply each densitometer reading by 1.04 to estimate canopy cover.
- Average canopy cover. Add the four canopy cover readings and divide the total by four.
- Bark condition. Circle most applicable description
- Limbs. Circle most applicable description
- Top breakage: Circle best estimate of portion of original canopy remaining.
- Provide any additional information that may be useful in describing the roost.

### Supply List

- Camera
- Prism (10-factor)
- Convex densitometer
- Compass
- Diameter tape
- Linear tape
- GPS