South Carolina
White-nose Syndrome Response Plan

Revised November 2016

This document applies to colonial cavity roosting bat species (all *Myotis*, *Perimyotis*, *Eptesicus*, *Corynorhinus*, *Nycticeius*, and *Tadarida* with emphasis on *Myotis*, *Perimyotis*, and *Eptesicus*).

Revised by Jennifer Kindel with help from SCDNR and listed cooperators and partners.

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Tables and Figures

I. Cooperators and Partners

The mission of monitoring, survey, regulation and research cannot be met by a single entity. The response to White-nose Syndrome (WNS) will require cooperation from government, non-governmental organizations and the private sector. Cooperators and partners include:

State Agencies

- South Carolina Army National Guard, Fort Jackson - Stanley Rikard; McCrady Training Center - Layne Anderson, Bryan Hall, Chris Stone
- South Carolina Department of Health and Environmental Control - Christy Jeffcoat, Rachel Radcliffe, DVM
- South Carolina Department of Natural Resources - Mary Bunch, Jennifer Kindel, Jay Butfiloksi, Sam Chappelear, Dean Harrigal, Julie Holling, Greg Lucas, Richard Morton, Al Segars, Derrell Shipes, Willie Simmons, Sam Stokes, Tom Swayngham
- South Carolina Department of Parks, Recreation and Tourism - Terry Hurley, Joseph Lemeris, Jr.
- South Carolina Forestry Commission - Russell Hubright
- Southeastern Cooperative Wildlife Disease Study - Heather Fenton
- South Carolina Department of Transportation - Siobhan Gordon

Federal Agencies

- United States Army Corps of Engineers - Sandra Campbell
- United States Forest Service, Southern Research Station - Susan Loeb; Francis Marion National Forest - Mark Danaher; Francis Marion National Forest, Sumter National Forest, and all ranger districts therein (Andrew Pickens, Enoree, Long Cane, and Francis Marion Ranger Districts) - Jeff Magniez
- United States Fish and Wildlife Service - Jennifer Koches, Morgan K. Wolf
- United States Geological Survey - Fort Collins Science Center: Laura Ellison

Universities

- Clemson University - David Jachowski, Greg Yarrow; Campbell Museum of Natural History - Stanlee Miller
- Furman University - Travis Perry
- South Carolina Upstate - Jonathan Storm
- Anderson University - Rocky Nation

Non-governmental Organizations

- Bat Conservation International - Katie Gillies, Dan Taylor
- North Carolina Bat Working Group - Mary K Clark, Mary Frazier, Lisa Gatens
- Nuisance Wildlife Control Operators
- Palmetto Bluff Conservancy - Mary Socci
- Southeastern Bat Diversity Network - Trina Morris, Tim Carter
- The Nature Conservancy - Kristen Austin

II. Objective

To coordinate with cooperators and partners of the conservation community in creating and adhering to state and federal White-nose Syndrome Response Plan guidelines which address the prevention and spread of WNS in South Carolina.

III. Bat Species Affected that Occur in South Carolina

White-nose Syndrome is a disease characterized by the white fungus species *Pseudogymnoascus destructans* (*Pd*; previously known as *Geomyces destructans*) which forms on the noses and wing membranes of affected hibernating bats. Mortality rates attributed to WNS have reached up to 90 and 100% at hibernacula, causing the death of more than 5.7 million bats in North America since it was first documented in New York during the winter of 2006/2007. This disease has affected bat species already designated as high conservation concern, and WNS could be a major contributing factor of this classification for additional bat species.

For an extensive summary of WNS and the threat to bat species:

http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/

For the most updated nationwide WNS information (this includes a new caver decontamination video):

https://www.whitenosesyndrome.org/

WNS was first confirmed in South Carolina in Pickens County on a tri-colored bat (*Perimyotis subflavus*) during March of 2013. Since then, another case in Pickens county on an eastern small-footed myotis (*Myotis lebii*) and two other cases in Oconee and Richland counties on tri-colored bats have been reported in 2013 and 2014.

Among the bat species currently confirmed to be affected by WNS in other states, five of these occur in South Carolina. These species are all colonial cavity roosting bats, mainly from the *Myotis genus* (see Table 1). The fungus known to cause WNS has also been detected on additional bat species in other states, but they have not yet shown diagnostic signs of the disease. These species include two colonial cavity and tree roosting bat species (*Myotis* and *Corynorhinus* genus) and two bat species that generally roost in foliage (*Lasiurus* and *Lasionycteris* genus). The fungus was found on these species when they were roosting in caves.

In the Upstate of South Carolina there have been incidental records of the Indiana Bat (*Myotis sodalis*), which is also a species confirmed to be affected by WNS, and the Big Free-tailed Bat (*Nyctinomops macrotis*). However, due to their rarity, we will not address these species here unless greater numbers are found in the state.

Table 1: Conservation Status and Occurrence of WNS for South Carolina Bat Species

Common Name	Scientific Name	Global Rank	State Rank	Protection*	Affected by WNS
Big Brown Bat	***Eptesicus fuscus***	**G5**	**S5**		**Yes**
Eastern Small-footed Myotis[+]	***Myotis leibii***	**G1G3**	**S1**	**ST**	**Yes**
Little Brown Myotis	***Myotis lucifugus***	**G3**	**S3?**		**Yes**
Northern Long-eared Bat	***Myotis septentrionalis***	**G2G3**	**S4**	**FT**	**Yes**
Tricolored Bat[+]	***Perimyotis subflavus***	**G3**	**SNR**		**Yes**
Rafinesque's Big-eared Bat	*Corynorhinus rafinesquii*	G3G4	S2?	SE	**
Silver-haired Bat	*Lasionycteris noctivagans*	G5	SNR		**
Eastern Red Bat	*Lasiurus borealis*	G5	SNR		**
Southeastern Bat	*Myotis austroriparius*	G3G4	S1		**
Hoary Bat	*Lasiurus cinereus*	G5	SNR		
Northern Yellow Bat	*Lasiurus intermedius*	G4G5	SNR		
Seminole Bat	*Lasiurus seminolus*	G5	SNR		
Evening Bat	*Nycticeius humeralis*	G5	SNR		
Brazilian Free-tailed Bat	*Tadarida brasiliensis*	G5	S5		

[+] Species that have tested positive for WNS in South Carolina.

* FT = Federally Threatened, SE = State Endangered, ST = State Threatened.

** WNS has been detected on these species in other states but they have not yet shown diagnostic sign of the disease.

IV. Permit Requirements

Scientific Research Permittees and Wildlife Rehabilitators must adhere to permit requirements for state or federally listed bats, such as those listed as endangered, or threatened. State and federal authorization is required to collect and possess dead specimens, handle live bats, and/or to euthanize sick bats. Researchers/biologists conducting actions relating to capture, handling, attachment of radio transmitters, and tracking of northern long-eared bats will be required to obtain a federal scientific collection/recovery permit under Section 10(a)(1)(A) of the Endangered Species Act (ESA) and a state permit.

However, the ESA 4 (d) rule *exempts* Nuisance Wildlife Control Operators (NWCOs) from the requirement of a federal permit to handle federally threatened northern long-eared bats (*Myotis septentrionalis*).

V. WNS Prevention and Disease Surveillance

Any equipment that cannot be decontaminated according to USFWS decontamination protocols cannot be used in South Carolina if it has been used in WNS affected states for bat or cave or mine work, despite the fact that South Carolina is now considered a WNS affected state. This applies to everyone.

Sites within South Carolina: if any equipment that cannot be decontaminated according to USFWS decontamination protocols has been previously used in a South Carolina WNS affected site, it should not be used in a South Carolina WNS unaffected site.

A. Nuisance Wildlife Control Operators

South Carolina Department of Natural Resources (SCDNR) sends information and updates on WNS to all NWCOs listed for bats on its most recent NWCO list. Under current laws/regulations, SCDNR can only make the following recommendations to NWCOs:

1. All NWCOs are recommended to incorporate applicable elements of the United States Fish and Wildlife Service (USFWS) Decontamination Protocol for Bat Field Research and Monitoring (Appendix A), especially those companies which work in other states. Applicable elements would include practices such as only using exclusion devices that are amenable to decontamination in South Carolina if they were used in affected states.
2. Follow the Acceptable Management Practices for Bat Control Activities in Structures developed by the WNS Conservation and Recovery Working Group as posted on the SCDNR WNS website: https://www.whitenosesyndrome.org/sites/default/files/resource/wns_nwco_amp_1_april_2015_0.pdf
3. NWCO personnel who handle individual bats during removal are urged to reference the Reichard Wing Damage Index (WDI) and report bat species to SCDNR scoring a 2 or greater (Appendix B). If possible, submitting a picture of these bats (especially with outstretched wings) to SCDNR is encouraged. The WDI is *not* a diagnostic tool.
    a. SCDNR staff may request dead bats for submission to Southeastern Cooperative Wildlife Disease Study (SCWDS) in 2015 and 2016. In this case, see guidelines for collection of dead bats in section VII. B. 5., and submission to SCWDS in section VII. B. 6. a. Otherwise, please follow steps for safe disposal of any dead bats in section VII. B. 6. C.

B. Wildlife Rehabilitators

Wildlife rehabilitators that currently rehabilitate or transport any bats are discouraged from doing so. If persons insist on rehabilitation efforts, the following procedures are recommended:

1. Use the USFWS Decontamination Protocol (Appendix A) and isolate all colonial bats.
2. Follow the Bat Rehabilitation Guidelines developed by USFWS and adapted for South Carolina as posted on the SCDNR WNS website: http://www.dnr.sc.gov/wildlife/publications/pdf/batrehabguidelines092011.pdf. Known bat or rabies vector rehabilitators will be contacted directly with the guidelines created (but not endorsed or discouraged) by the USFWS.
3. We recommend referencing the Reichard Wing Damage Index (WDI) and reporting bat species to SCDNR scoring a 2 or greater (Appendix B). If possible, submitting a picture of these bats (especially with outstretched wings) to SCDNR is encouraged. The WDI is *not* a diagnostic tool.
    a. SCDNR staff may request dead bats for submission to Southeastern Cooperative Wildlife Disease Study (SCWDS) in 2015 and 2016. In this case, see guidelines for collection of dead bats in section VII. B. 5., and submission to SCWDS in section VII. B. 6. a. Otherwise, please follow steps for safe disposal of any dead bats in section VII. B. 6. c.

C. Scientific Research Permitees

All Scientific Research permittees who work on bats in South Carolina must follow the guidelines of the USFWS Decontamination Protocol (Appendix A) in order to retain their SCDNR Scientific Research Permit. Additionally, they must score all bats with the Reichard Wing Damage Index (WDI) (Appendix B). WDI is *not* a diagnostic tool and it is not an effective indicator of WNS, especially in warmer months.

1. Document any handled bats scoring a 2 or higher on the WDI to SCDNR and/or USFWS. Data and material to be collected should include:
    a. Photographing the wing damage and submitting to SCDNR/USFWS (include date, location, animal identification number and species).
    b. Taking tissue from live animals (see Appendix D), if requested from SCDNR or USFWS for submission to SCWDS. No requests are in place currently.
2. For dead bats, first see section VII. B. 5. for collection of bat carcasses.
    a. Submit dead bats from unusual die-offs not easily attributed to other obvious causes such as poisoning or entrapment to SCWDS via SCDNR, or to the National Wildlife Health Center (NWHC) and notify SCDNR (see VII. B. 5. a. for SCWDS submissions).
    b. For all other dead bats not suspected of WNS, send fresh bats and/or bats with intact skull to museum (section VII. B. 5. b.) or safely dispose of bat (section VII. B. 5. c.).
3. Report all bats captured along with WDI score and location to the Bat Population Database (BPD) (http://my.usgs.gov/bpd). Each record can be entered into the online form, or a standardized capture spreadsheet can be used and uploaded to the BPD. If you do not already have an account to sign into the USGS website and/or would like a standardized spreadsheet, please contact the USGS.

D. South Carolina Department of Natural Resources

SCDNR biologists shall collect bats from abnormal die-offs (5+ bats) from unknown causes in 2015 and 2016 and submit to SCWDS.

E. South Carolina Department of Health and Environmental Control

South Carolina Department of Health and Environmental Control (SCDHEC) routinely receives bats from across the state for rabies testing. SCDHEC staff are requested to assess WDI score on bats if they don't save them for submission to SCWDS. **Bats that are not positive for rabies should be refrigerated or frozen for SCDNR and submitted to SCWDS**. SCDHEC will notify SCDNR if any bats with visible fungus are received during winter months.

VI. Passive and Active Monitoring

Follow standardized protocols for bat surveys and data collection. See "A Plan for a North American Bat Monitoring Program (NABat)," published by the United State Forest Service (USFS), Southern Research Station in 2015. http://www.srs.fs.usda.gov/pubs/gtr/gtr_srs208.pdf.

A. Pre-WNS Sites

1. Conduct acoustic baseline surveys (others may be added)
    a. Acoustic statewide surveys through NABat initiated in 2015 and continued in 2016. [Done] A minimum of 30 routes will be run two times each summer.
    b. Continue survey route in Andrew Pickens Ranger District of Sumter National Forest (started in 2009) run by the Southern Research Station.
    c. Continue survey routes in Carolina Sandhills National Wildlife Refuge and Francis Marion National Forest.
    d. Conduct Lake Jocassee and Keowee shoreline point counts at selected sites by Duke Energy contractor. [DONE]

e. Continue survey route at Long Cane Ranger District.

2. Continue and/or increase netting or sampling at known maternity sites, particularly those along our northern border.
    a. Use telemetry to locate hibernacula of known little brown bat (*Myotis lucifugus*) maternity colonies. [This study was attempted in the fall of 2011/2012, but efforts to relocate bats after they left maternity site were unsuccessful].

3. Continue and/or increase infrared (IR) video photography monitoring of known roosts to detect dramatic declines in bat populations.

4. Continue and/or increase winter surveys, which will require careful decontamination of gear as per protocols.
    a. Follow guidelines detailed in section V.C.
    b. Full counts and follow-up counts at Stumphouse Tunnel (now a WNS positive site).
    c. New mine surveys and initial counts at sites without a vertical component (i.e. no rope work). Private mines: SCDNR has mapped over 200 known or potential locations (part of a State Wildlife Grant project) and most lack bat habitat. Unfortunately, most of the reported mines or prospects in the piedmont region were no longer extant or never had adits or shafts, and therefore provided no underground bat roosts. Of 48 mine sites surveyed in that project, only nine had an underground component with low numbers of tri-colored bats (*Perimyotis subflavus*) present. Most of the gold mine adits have some human entry (not always with landowner permission).
    d. Entrance counts at Santee Park when partners are available.
    e. Better temperature data could be gathered for suitability to *Pd* in the two best caves known by SCDNR which are on SCPRT land.

B. Post-WNS Sites

1. Minimize nonessential research or educational programs without research value that involve handling of bats, but continue acoustic surveys of same route(s) for rough population trends.
2. Monitor cave/mine roosts to evaluate survivorship, using methods that minimize stress on roosting bats.
3. Cooperate with other states and researchers in gathering samples or monitoring information as requested.
4. Evaluate and consider various proposed treatment options as they develop, if necessary.
5. South Carolina Bat Blitz. During this intensive bat survey (a program of the Southeastern Bat Diversity Network), some nets, poles, ropes, and other survey items will be provided and decontamination materials will be on hand. All participants of the SC Bat Blitz should adhere to the guidelines presented in the USFWS Decontamination Protocol (Appendix A):
    a. Participants should not use any equipment that hasn't been, or cannot be, properly decontaminated if it was used for surveys in a state with suspect or confirmed WNS.
    b. Even if it has been properly decontaminated, participants returning to a state without suspect or confirmed WNS should not use any gear used at the SC Bat Blitz in that state.
    c. Unless it has been properly decontaminated, participants returning to a state with suspect or confirmed WNS should not use gear used at the SC Bat Blitz in that state. However, participants

should check with their applicable state or federal regulator agency to determine whether properly decontaminated gear may be used in their state.

d. All netting team leaders are responsible for adhering to WNS decontamination protocols, and will be required to have a state permit for research through the office of Derrell Shipes, the SCDNR Chief of Wildlife Statewide Projects, Research and Surveys (Phone: 803-734-3938, Email: ShipesD@dnr.sc.gov).

VII. Regulatory and Management Actions

A. Regulations

1. South Carolina Department of Parks, Recreation and Tourism (SCPRT) prohibits recreational caving and staff entry to caves on their parks. No permits for caving are issued.

2. Recreational caving and rock climbing is not permitted on SCDNR owned lands or Wildlife Management Areas (WMA) year round. SCDNR does not have regulatory authority over privately owned lands or non-WMA state owned lands.

3. The USFS has issued an emergency order banning public entry or use of caves and mines. Notify SCDNR Law Enforcement. [DONE]

4. The USFWS Decontamination Protocol for Bat Field Research/Monitoring (Appendix A) must be used by all bat researchers in order to retain their SCDNR Scientific Research Permit.

5. SCDNR to provide signage, “Entry Prohibited,” for a major southeastern bat (*Myotis austroriparius*) cave system. [DONE]

B. Management

1. Equip or supply field offices with appropriate decontamination and disposal protocol and supplies.

2. For WNS affected caves/mines, consider posting a sign outside the entrance identifying it as such.

3. SCDNR response to public calls:

   a. Determine if there is potential rabies exposure. Contact caller and obtain their contact information if there was potential for the caller to be exposed to rabies. If so, instruct the caller to contact DHEC state headquarters or their local DHEC office:

      i. DHEC State Headquarters Phone: 803-896-0640; DHEC contacts by county of occurrence: http://www.scdhec.gov/Health/FHPF/DiseaseResourcesforHealthcareProviders/RabiesTreatment/RabiesGuidetoManagingExposures/index.htm#contacts.

      ii. Rabies Guide to Managing Exposure: http://www.scdhec.gov/Health/FHPF/DiseaseResourcesforHealthcareProviders/RabiesTreatment/RabiesGuidetoManagingExposures/#bite

   b. Create a dead bat report for all calls regarding dead or dying bats and/or enter these reports into a spreadsheet. Fields should include date, number of bats, county, and phone number and address of person reporting dead bats.

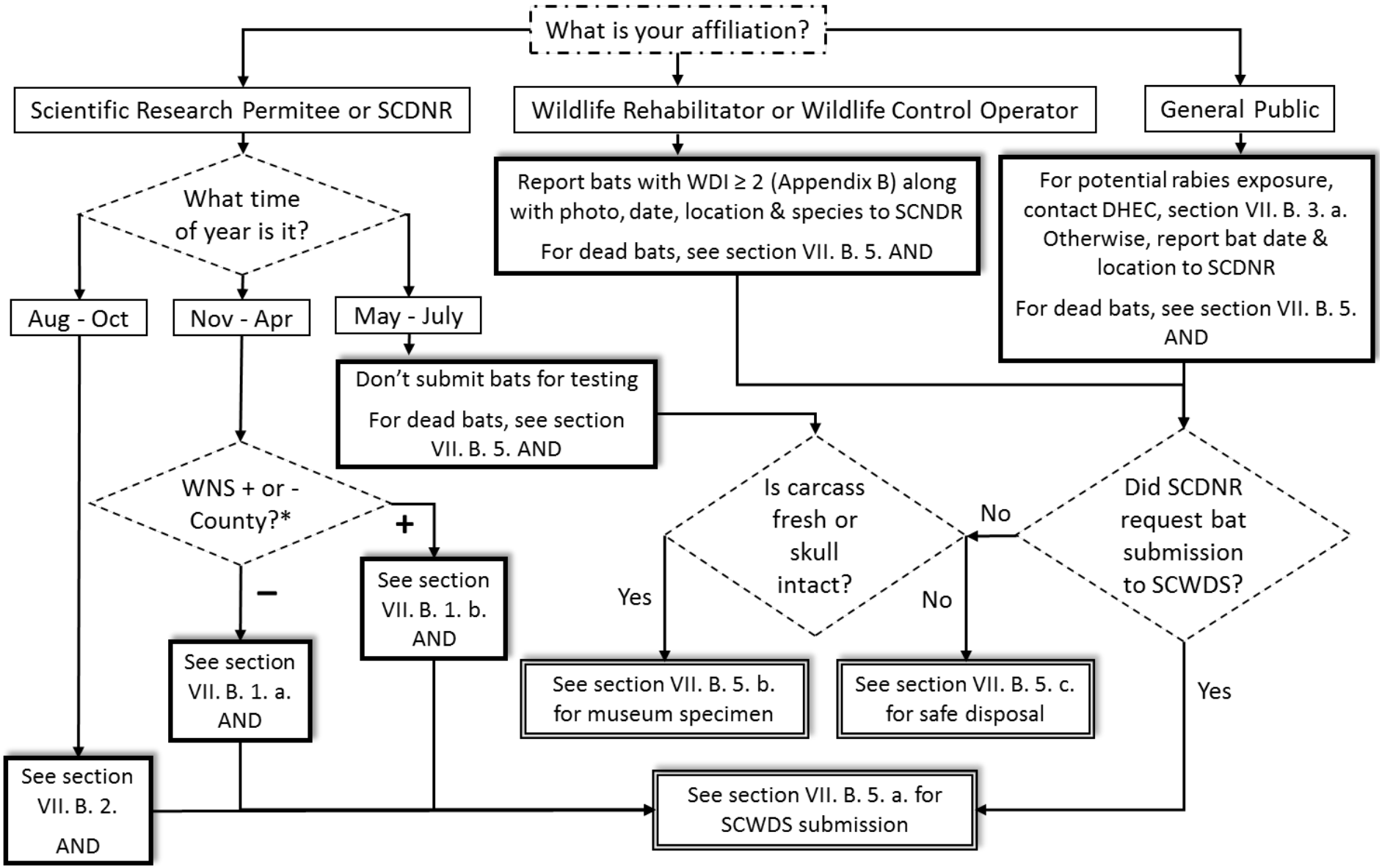
   c. For response to bats with signs of WNS, see Flowchart to Determine Response to Bats with Potential WNS.

4. Collection of dead bats:
    a. Double check the bat is dead from a safe distance by using a tool such as a shovel. If closer observation is necessary, use leather gloves or a similar protective barrier that can be washed in hot water greater than 131°F for 20 minutes.
    b. When picking a maximum of 5 to 6 total bats, choose the freshest bats and try to choose bats of different species or age classes.
    c. Open two Ziploc bags, and use latex gloves on both hands. Pick up dead bat(s) with gloved non-writing hand. Don't touch equipment or anything else with this now contaminated glove.
    d. Taking care not to contaminate the outside of the bag, use the uncontaminated glove to pick up one bag and place the bats in the bag. With the uncontaminated glove, close the bag and use a sharpie to write your name, date, location, county, and species (if known) on the bag. Continuing to use the uncontaminated glove, place this bag inside the other Ziploc bag and close it securely.
    e. Take off contaminated glove with uncontaminated glove and place both in a trash receptacle. Thoroughly wash your hands with antibacterial hand sanitizer that is at least 60% alcohol (e.g., Purell®) before picking up the Ziploc bag.
    f. Using a disinfectant such as bleach, peroxide wipes, or 70% alcohol wipes, clean the outside of the bag. For the general public - bring bat to local DNR office; if not possible, see 6. c. below for safe disposal instructions.
    g. Thoroughly wash any clothing and/or gear that come in contact with the bat in water held at 131°F for 20 minutes. Though complete decontamination may not be possible on carpeting or furniture, scrubbing and washing with hot water and antibacterial soap such as Dawn® antibacterial dish soap may help. Test a small area first to ensure there are no adverse effects.

5. What to do with dead bats:
    a. **SCWDS submission**: Keep specimen(s) on freezer pack or refrigerated, and ship within 24-36 hours. If shipping timeframe is not possible, place the bag in freezer until the next shipping opportunity. Fill out SCWDS form (Appendix E) and email to SCWDS. Be sure to CC form and email any photos to Mary Bunch (BunchM@dnr.sc.gov). Ship bats overnight to SCWDS (Monday-Thursday), only after receiving confirmation from the lab.
    b. **Museum specimen** (if WNS isn't suspected): Freeze bat specimen(s) and submit with date, location, county, and species (if known) to the Campbell Museum of Natural History at Clemson University. Fresh bats specimens are preferred, however partially decomposed bats, especially those with an intact skull, will be accepted. Museum curator: Stanlee Miller, Email: smmll@clemson.edu; Phone: 864-656-3456.
    c. **Safe disposal**: Dispose of bat(s) in bags with your garbage. An alternative to this is to bury only the bat carcass at least a foot deep so as not to be excavated by animals.

Figure 1: Flowchart to Determine Response to Bats with Potential WNS

Always adhere to permit requirements for state or federally listed bats.
**WNS + Counties in 2016: Oconee, Pickens, Richland*



VIII. Response to Bats with Potential WNS

A. See Flowchart to Determine Response to Bats with Potential WNS

B. Details for Scientific Research Permitees and SCDNR

Assess extent and distribution of WNS throughout cave or mine before collecting samples. Conduct a full count of infected and non-infected bats and record bat behavior if deemed unusual.

1. Bats encountered with field signs of WNS during Winter/Spring - **November through April**

a. If field signs of WNS are observed in areas of South Carolina where WNS **has not been documented (new county)**:

iii. A total count of all bats at colony/site and conduct WNS swab testing if possible.

iv. Collect 3-5 freshly dead bats representative of the affected species.

1) For species known to be affected by WNS (Table 1): if dead bats are not available for collection and WNS is suspected or the fungus is visible, use non-lethal sampling (Appendix D). Use of a UV light will greatly assist in wing biopsies for WNS testing (Appendix C) and is recommended.

2) For species not known to be affected by WNS (Table 1): if dead bats are not available for collection and WNS is suspected or the fungus is visible, follow accepted guidelines to humanely euthanize one of each non-federally listed species that has obvious visible fungal growth (see Guidelines of the American Society of Mammalogists for the use of wild mammals in research by Sikes et al. 2011 in the Journal of Mammalogy 92(1): 235-253). Take non-lethal samples if it is a federally listed species and you have authorization to do so (Appendix D). Using a UV light will greatly assist in wing biopsies for WNS testing (Appendix C) and is recommended.

3) For all other dead bats, use safe disposal guidelines in section VII. B. 5. c.

b. If field signs of WNS are observed in areas of South Carolina where WNS **is already confirmed**:

1) A total count of all bats at colony/site during routine winter count cycles (3-5 years).

2) Species known to be affected by WNS should be left undisturbed.

3) Collect all dead bats for species of unknown susceptibility to WNS (Table 1). If dead bats are not available for collection and WNS is suspected or the fungus is visible, follow accepted guidelines to humanely euthanize one of each non-federally listed species that has obvious visible fungal growth (see Guidelines of the American Society of Mammalogists for the use of wild mammals in research by Sikes et al. 2011 in the Journal of Mammalogy 92(1): 235-253). Take non-lethal samples if it is a federally listed species and you have authorization to do so (Appendix D). Using a UV light will greatly assist in wing biopsies for WNS testing (Appendix C) and is recommended.

4) For all other dead bats, safely dispose of them using guidelines in section VII. B. 5. c.

2. Bats encountered with field signs of WNS during Fall - **August through October**
    a. Investigate reports of unusual numbers of sick or dead bats (usually 5 or more) by surveying for increased adult and/or pup mortalities at maternity colonies. Determine which fresh, intact carcasses are representative of the affected species, and send 3-5 of those to SCWDS.
    b. If a species has evidence of severe wing damage (WDI ≥ 2) and is of unknown WNS susceptibility, take photos of wing damage.

IX. Outreach and Education

A. NWCOs, Caving Groups and Other Cooperators

1. SCDNR shall send links and hard copies of WNS information from USFWS, such as the decontamination protocol and the WNS fact sheet, to all NWCOs. [DONE]
2. SCDNR shall send updates on WNS to all NWCOs that are listed for bats on the most recent NWCO list. [ONGOING]
3. Set up workshop for SCDNR staff and Cooperators [DONE- see archived webinar at https://connect.clemson.edu/p64123383/]
4. Work with caving clubs such as the South Carolina Interstate Grotto to assist with WNS education and outreach. [ONGOING]

B. General Public

1. Create an informational SCDNR webpage [DONE 2010 - see http://www.dnr.sc.gov/wildlife/batswns.html]
2. Coordinate Press Releases with Greg Lucas, SCDNR, to educate the public and update elected officials.
3. Inform public to report unusual die-offs to their regional wildlife biologists for WNS testing.
4. Create a WNS list serve
5. Create a bat watch program where the public counts bats exiting known roosts to measure population declines [a Halloween emergence count at a site to report occupied bat boxes starts fall 2016].

Evaluate and Follow USFWS Guidelines for Containment
https://www.whitenosesyndrome.org/sites/default/files/white-nose_syndrome_national_plan_may_2011.pdf

X. Appendices

A. USFWS Decontamination Protocol (April 14, 2016)

https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_decon_protocol_04.12.2016.pdf

I. INTRODUCTION

The fungus *Pseudogymnoascus destructans* (*Pd* – formerly identified as *Geomyces destructans*) is the cause of white-nose syndrome (WNS), a disease that has resulted in unprecedented mortality of hibernating bats throughout eastern North America. Since first documented in New York in 2006, WNS continues to threaten hibernating populations of bats across the continent, having spread rapidly through the Northeast, mid-Atlantic, Midwest, and Southeast states, as well as eastern Canada.

Best available science indicates that *Pd* arrived in North America from a foreign source. Once *Pd* has been detected, either on bats or in the hibernaculum environments, the county of occurrence is considered contaminated indefinitely due to the long-term persistence of the fungus. Because of the devastating effects of WNS in North America, recommendations detailed in this document were developed to minimize the risk of human-assisted transmission. All persons who come into contact with bats, their environments, and/or associated materials for any reason (*e.g.,* research, recreation, etc.) are advised to take precautions to avoid additional, inadvertent transport of *Pd* to uncontaminated bats or habitats.

Observations of live or dead bats (multiple individuals at a single location) should be reported to local USFWS Field Office or State agency wildlife office http://www.whitenosesyndrome.org/partners. **Do not handle bats unless you are properly trained, vaccinated, and, where necessary, authorized in writing to do so by the appropriate government agency.**

II. PURPOSE:

The purpose of this document is to provide recommendations based on the best available scientific information known to effectively clean and treat (herein referred to as decontaminate, or similar derivation thereof) clothing, footwear, and/or gear (herein collectively referred to as equipment) that may have been exposed to *Pd*. When activities involve contact with bats, their environments, and/or associated materials the following decontamination procedures are designed to reduce the risk of human-assisted transmission of the fungus to other bats and/or habitats.

For the protection of bats and their habitats: 1) comply with all current cave and mine closures, advisories, and regulations on federal, state, tribal, and private lands; 2) follow relevant recommendations found in this document; and 3) **do not transport any equipment into or out of the United States of America** (USA) that has been in contact with bats or their environments.

Local, state, federal, or other management agencies may have additional requirements or clarifications for equipment used on lands under their jurisdictions[1] or work involving public trust resources. Always follow all state and/or federal permit conditions. Contact the respective agency representatives for supplemental documents or additional information.

III. PRODUCT USE:

Ensuring the safety of individuals using any of the applications and/or products identified in this document must be the first priority. Safety data sheets (SDS) for chemicals and user's manuals for equipment developed by product manufacturers provide critical information on the physical properties, reactivity, potential health hazards, storage, disposal, and appropriate first aid procedures for handling, application, and disposing of each product in a safe manner. Familiarization with the SDS for chemical products, and manufacturer's product care and use standards, will help to ensure appropriate use of these materials and safeguard human health. Read

product labels in advance of intended field use. Ensure availability of adequate emergency eye-wash supplies or facilities at intended site of use. Always store cleaning products out of the reach of children or pets.

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 SDS. Products, or their contaminated rinse water, must be managed and disposed of in accordance with local environmental requirements and, where applicable, product label, to avoid contamination of groundwater, drinking water, or non-municipal water features such as streams, rivers, lakes, or other bodies of water. Follow all local, state and federal laws. Requirements for product disposal may vary by state. Note: Quaternary ammonium wastewaters should not be drained through septic systems because of the potential for system upset and subsequent leakage into groundwater.

IV. TRIP PLANNING/ORGANIZATION:

1.) Identify the appropriate WNS Management Area (Figure 1) in which the equipment has been used and will be used in the future. Users of new or site-dedicated equipment (that has been and will be used in only one site) may skip to #3.

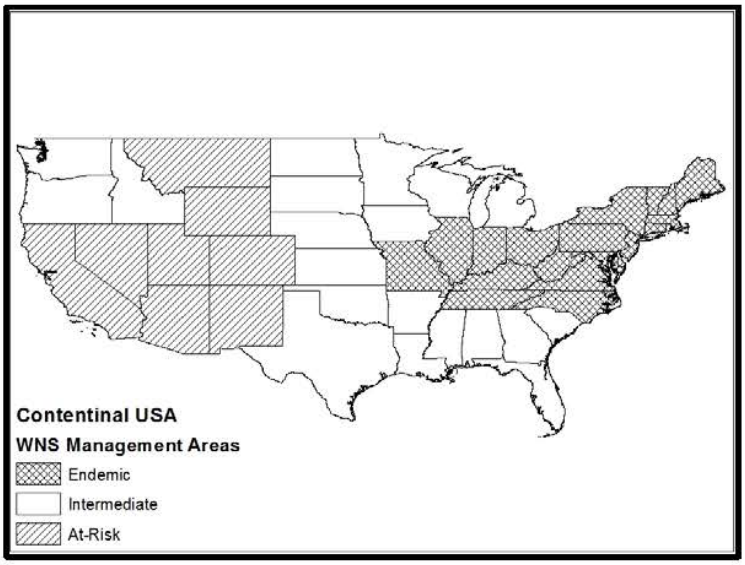


Figure 1. WNS Management Areas by state.

"Site" is loosely defined in this document as the location of a discrete bat roost (cave, barn, talus slope, etc.) or as a specific field location for mist netting or other trapping. Since conditions vary considerably, delineating sites will be at the discretion of the appropriate local regulatory or land management agency.

2.) Once the appropriate Management Areas have been determined using Figure 1, use Figure 2 to determine appropriate uses for A. Subterranean Equipment or B. Terrestrial Equipment. **"Subterranean equipment" includes any equipment that has ever been exposed to a cave/mine environment. "Terrestrial equipment" includes any equipment that has not previously been exposed to a cave/mine environment.** Regardless of the equipment designation, equipment should only be reused at similarly classified or progressively more contaminated locations[2]. In addition, given uncertainties in the distribution of *Pd* in the Pacific Northwest (i.e., ID, OR, & WA), subterranean and terrestrial equipment should not be transferred between the PNW and eastern USA (endemic/intermediate).

3.) Contact local state/federal regulatory or land management agencies for additional requirements, exemptions, or addendums on lands under its jurisdiction that supplement guidance provided in Figure 2A and 2B.

4.) Choose equipment that can be most effectively decontaminated [*e.g.*, rubber or synthetic rather than leather boots], otherwise commit use of equipment to a specific location (herein referred to as equipment dedication). Equipment should always be inspected for defects prior to use. Replace all defective or degraded equipment with new equipment. Brand new equipment can be used at any location where access is permitted, as long as it has not been stored or come in contact with contaminated equipment.

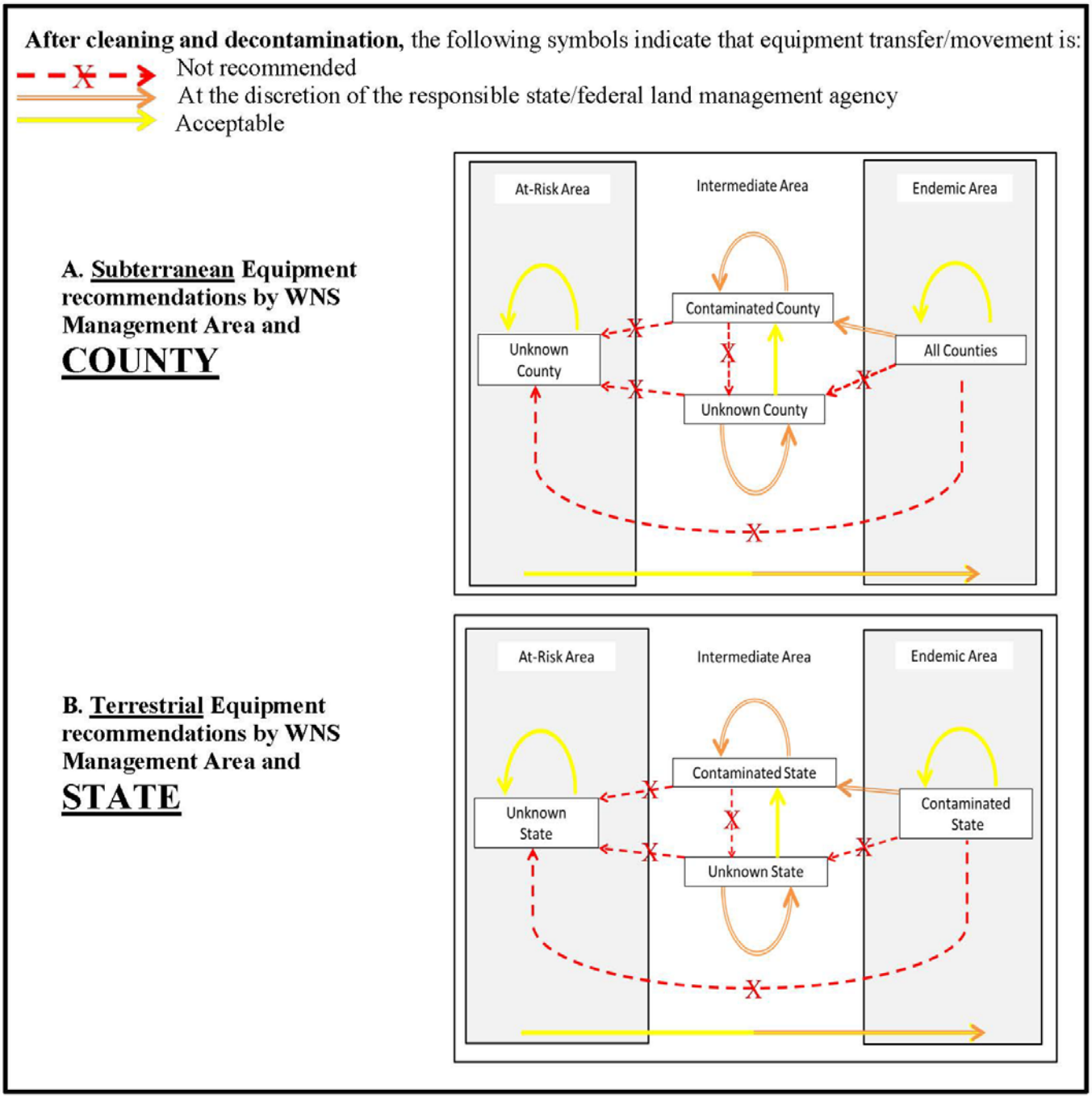


Figure 2. Movement recommendations for decontaminated (A) Subterranean and (B) Terrestrial equipment.

5.) Prepare a strategy (*i.e.*, Outline how/where all equipment and waste materials will be contained, stored, treated and/or discarded after returning to the vehicle/base area) that allows daily decontamination of equipment and, where applicable, between individual sites visited on the same day, **unless** otherwise directed by local state/federal or land management agency instructions. Confirmed *Pd* contaminated sites or those with a high index of suspicion for contamination should be visited **only after** those sites of unknown *Pd*/WNS status[2] have been visited, to further reduce the risk of inadvertent transmission.

V. PROCEDURES FOR DECONTAMINATION:

1.) On site:

a.) Thoroughly remove sediment/dirt from equipment immediately upon exiting from the site.

b.) Contain all exposed and potentially contaminated equipment in sealed bags/containers for treatment away from the location. Decontaminate the outside hard, non-porous surfaces of containers and bags prior to moving them to a secondary location (*e.g.*, vehicles, labs, or storage). Store all exposed and decontaminated equipment separately from unexposed equipment.

c.) Clean hands, forearms, and exposed skin using hand/body soaps/shampoos and, when feasible, change into clean clothing and footwear prior to entering a vehicle.

2.) Off site:

a.) REMOVE dirt and debris from the outside of vehicles (especially wheels/undercarriage) prior to additional site visits, especially when traversing WNS Management areas or scenarios categorized as "Not Recommended" (Figure 2).

b.) CLEAN submersible and non-submersible equipment according to manufacturer's specifications. Sediments and debris significantly reduce the effectiveness of treatments. Laboratory trials[3&4] demonstrate that the use of conventional cleansers like Woolite® detergent or Dawn® dish soap aided in the removal of sediments and debris prior to treatment, contributing to the effectiveness of decontamination.

c.) TREAT submersible or non-submersible equipment only in a safe manner according to the equipment and product labels using the most appropriate application or product listed in Table 1. For equipment that cannot safely be treated in accordance with both the manufacturer's recommendations and product labeled instructions, dedicate to individual sites as determined appropriate in Section IV.

i. Submersible Equipment (*i.e.*, equipment that can safely withstand submersion in water or other specified product for the recommended amount of time without compromising the integrity of the item):

Treatment of submersible equipment must be done in accordance with manufacturer's recommendations for your equipment. The preferred treatment for all submersible equipment is submersion in hot water that maintains a temperature of at least 55°C (131°F) for a minimum of 20 minutes. Ensure that all equipment surfaces remain in direct contact (*i.e.*, avoid all trapped air) with the hot water treatment for the duration of the treatment period. Consider that although many commercial and home washing machines with sanitize (or allergen) cycles may be capable of submerging gear in the recommended hot water application for the required time, it is incumbent on the user to be sure that machines to be used attain and sustain the needed temperatures throughout the process. If heat may comprise the safety and/or integrity of the otherwise submersible equipment, consider equipment dedication or other products listed in Table 1. When considering other products found in Table 1, recognize that the applicability and effect of such products on the safety and integrity of equipment remains untested. Be aware the use of preferred applications and products in Table 1 should be done with extreme caution and proper personal protective gear due to the risk of personal injury.

ii. Non-submersible Equipment (*i.e.*, equipment that may be damaged by liquid submersion):

Treat all non-submersible equipment using the most appropriate application or product in Table 1 that complies with the equipment manufacturer's recommendations and product label instructions, where applicable. The listed applications or products may not be appropriate or safe for non-submersible equipment. Dedication of equipment should always be considered the preferred application in these circumstances.

d.) RINSE equipment, as appropriate, thoroughly in clean water, particularly items that may contact humans, bats, or sensitive environments. Allow all equipment to completely dry prior to the next use.

e.) DECONTAMINATE the equipment bins, sinks, countertops and other laboratory, office, or home areas with the most appropriate applications or products in Table 1.

Table 1. Applications and products with demonstrated efficacy against Pd [3, 4, 5, 6, & 7]. Remember to consult equipment labels, registered product labels, and the appropriate SDS for regulations on safe and acceptable use.

	Tested Applications & Products [3, 4, 5, 6, & 7]	Federal Reg No.:	Laboratory Results
Preferred Applications	Equipment Dedication	N/A	Clean according to manufacturer standards and dedicated to a site
	Submersion in Hot Water[4, 6, & 7]	N/A	Laboratory effectiveness demonstrated upon submersion in water with sustained temperature $\geq 55^{o}C$ *(131°F)* for 20 minutes.
Other Products	Ethanol (60% or greater)[4, 6, & 7]	CAS - 64-17-5	Laboratory effectiveness demonstrated upon exposure in solution for at least 1 minute.
	Isopropanol (60% or greater)[4, 6, & 7]	CAS - 67-63-0	
	Isopropyl Alcohol Wipes (70%)[4, 6, & 7]	CAS - 67-63-0	Laboratory effectiveness demonstrated immediately following contact and associated drying time.
	Hydrogen Peroxide Wipes (3%)[4, 6, & 7]	CAS - 7722-84-1	
	Accel®[4, 5, 6, & 7]	EPA - 74559-4	Laboratory effectiveness demonstrated when used in accordance with product label.
	Clorox® Bleach[3, 4, 5, 6, & 7]	EPA - 5813-100	
	Clorox® Wipes[4, 5, 6, & 7]	EPA - 5813-79	
	Clorox® Clean-Up Cleaner + Bleach[4, 5, 6, & 7]	EPA - 5813-21	
	Hibiclens®[4, 5, 6, & 7]	NDA - 017768	
	Lysol® IC Quaternary Disinfectant Cleaner [3, 4, 5, 6, & 7]	EPA - 47371-129	

Other effective treatments with similar water based applications or chemical formulas (e.g., a minimum of 0.3% quaternary ammonium compound) may exist but remain untested at this time. Find more information on the EPA or FDA registered product labels by accessing the individual hyperlink or searching EPA or FDA Registration Numbers at: http://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1 or http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm.

Products with USEPA registration numbers mitigate persistence of living organisms on surfaces and are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 USC 136, et seq.). FIFRA provides for federal regulation of pesticide distribution, sale, and use. Within FIFRA, pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. FIFRA further defines pests as any insect, rodent, nematode, fungus, weed, or 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). Find more information on FIFRA at: http://www.epa.gov/oecaagct/lfra.html.

VI. EQUIPMENT AND ACTIVITY SPECIFIC RECOMMENDATIONS:

It is the responsibility of the users of this protocol to read and follow the product label and SDS. The product label is the law!

A. Clothing & Footwear:

IMPORTANT: All clothing (*i.e.*, inner and outer layers) and footwear should be decontaminated after every site visit using the most appropriate Application/Product in Table 1 or otherwise cleaned and dedicated for use at individual sites or areas as determined appropriate in Section IV.

Use of a disposable suit (*e.g.*, Tyvek® or ProShield®) or site-dedicated, reusable suit (*i.e.*, coveralls) is an appropriate strategy to minimize sediment/soil accumulation on clothing during a cave/mine or bat research activity. As stated earlier, all clothing layers should still be decontaminated or otherwise cleaned and dedicated after every use.

Disposable items, regardless of condition, should not be reused. Contain all used equipment in plastic bags upon final exit from a site, separating disposable materials from reusable equipment. Seal and store plastic bags in plastic containers until trash can be properly discarded, and/or exposed reusable equipment can be properly decontaminated off site.

B. Cave/Mine and other Subterranean Equipment:

Dedicate, as necessary, or decontaminate all cave/mine equipment (*e.g.*, backpacks, helmets, harness, lights, ropes, etc.) using the most appropriate guidance in Section V. Most types of equipment, including but not limited to, technical and safety equipment, have not undergone testing for safety and integrity after decontamination. Therefore carefully review and adhere to the manufacturer's care and use standards to maintain equipment functionality and safety protective features. If the application/product options in Table 1 are not approved by the manufacturer's care and use standards for the respective type of equipment, clean and inspect equipment according to manufacturer's specification and dedicate to similarly classified caves/mines/bat roosts and only reuse in progressively more contaminated caves/mines/bat roosts.

C. Scientific Equipment:

Always consider the use of disposable scientific equipment and materials between individual bats. All disposable scientific equipment (*e.g.*, work surfaces, bags/containers/enevelopes, exam gloves, etc.) should only be used on one bat, then discarded after use. Re-useable equipment (e.g., cotton bags, plastic containers, etc.) must be decontaminated between individual bats using the most appropriate application or product in Table 1. In all cases, use breathable bags (*e.g.*, paper, cotton, mesh, etc.).

At the completion of daily activities and when allowable by equipment and product labels, equipment may be autoclaved before reuse; otherwise use the guidance in Section V to determine the relevant procedure for decontamination of all work surface area(s) and equipment (*e.g.*, light boxes, banding pliers, holding bags, rulers, calipers, scale, scissors, wing biopsy punches, weighing containers, etc.).

D. Mist-Nets:

Contamination of trapping equipment is possible year-round when used at *Pd* contaminated hibernacula (NWHC, unpublished data). Dedicate, as necessary, or decontaminate all netting equipment (*e.g.*, netting, tie ropes, poles, stakes, etc.) using the most appropriate guidance in Section V for the particular equipment. All nets that are contacted by one or more bats must be decontaminated after each night of use according to the submersion in hot water application (Table 1). All nets should be completely dry prior to the next use.

E. Harp Traps:

Contamination of trapping equipment is possible year-round when used at *Pd* contaminated hibernacula (NWHC, unpublished data). Dedicate, as necessary, or decontaminate all trapping equipment (*e.g.*, lines,

frame, feet, bags, etc.) using the most appropriate guidance in Section V for the particular equipment. All trapping equipment that comes in contact with one or more bats OR enters a cave/mine/bat roost must be decontaminated after each night of use according to the most appropriate application or product (Table 1). Explore the use of disposable trap bags or liners to reduce transmission risks throughout each trapping effort. Disposable trap bags should be discarded at the end of each night.

F. <u>Acoustic Monitor, Camera, and Related Electronic Equipment:</u>

Dedicate, as necessary, or decontaminate all acoustic monitoring, camera, and related electronic equipment (*e.g.*, detector, camera, tablets, cell phones, laptops, carrying case, lenses, microphone(s), mounting devices, cables, etc.) using the most appropriate guidance in Section V for the particular equipment. The material composition of this equipment requires careful review and adherence to the manufacturer's care and use standards to maintain their functionality and protective features. If application/product options in Table 1 are not approved by the manufacturer's care and use standards for the respective type of equipment, clean equipment accordingly and dedicate to similarly classified caves/mines/bat roosts or only reuse in progressively more contaminated caves/mines/bat roost. Electronic devices used as terrestrial equipment, independent of bat handling work, pose a limited risk of transmission (*i.e.*, driving transects or fixed point detector surveys not associated with a cave/mine/bat roost entrance).

Equipment used in a cave/mine/bat roost may be placed in a sealed plastic casing, plastic bag, or plastic wrap to reduce the potential for contact/exposure with contaminated environments. Prior to opening or removing any plastic protective wrap, first clean, then remove, and discard all protective wrap. This technique has not been tested and could result in damage to, or the improper operation of, equipment.

These recommendations are 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 approved and 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. The protocol will be updated as necessary to include the most current information and guidance available.

1 To find published addenda and/or supplemental information, visit http://www.whitenosesyndrome.org/topics/decontamination.

2 Visit http://www.whitenosesyndrome.org/resources/map for the most updated information on the status of county and state. County and state level determination is made after a laboratory examination and subsequent classification of bats according to the current WNS case definitions. Definitions for the classification can be found at http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/Case%20Defintions%20for%20WNS.pdf . Contaminated determination includes both confirmed and suspect WNS classifications.

3 Information from : V. Shelley, S. Kaiser, E. Shelley, T. Williams, M. Kramer, K. Haman, K. Keel, and H.A. Barton – Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of White-Nose Syndrome (WNS) Journal of Cave and Karst Studies, v. 75, no. 1, p. 1–10. DOI: 10.4311/2011LSC0249

4 Efficacy of these agents and treatments are subject to ongoing investigation by the Northern Research Station, USDA Forest Service Cooperative Agreement 13-IA-11242310-036 (U.S. National Park Service and U.S. Forest Service) & 16IA11242316017 (U.S. Fish and Wildlife Service and U.S. Forest Service). Information contained in this protocol from work associated with either agreement will continue to be revised, as necessary, pending results of these investigations.

5 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.

6 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.

7 Final determination of suitability for any decontaminant is the sole responsibility of the user. All users should read and follow all labeled instructions for the products/applications and/or understand associated risks prior to their use. Treatments and the corresponding procedures may cause irreversible harm, injury, or death to humans, bats, equipment or the environment when used improperly. Always use personal protective equipment in well-ventilated spaces to reduce exposure to these products or applications.

B. Reichard Wing Damage Index (WDI)

Protocol:

http://www.fws.gov/northeast/PDF/Reichard_Scarring%20index%20bat%20wings.pdf

Published paper:

https://www.whitenosesyndrome.org/sites/default/files/resource/reichard_and_kunz_2009.pdf

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

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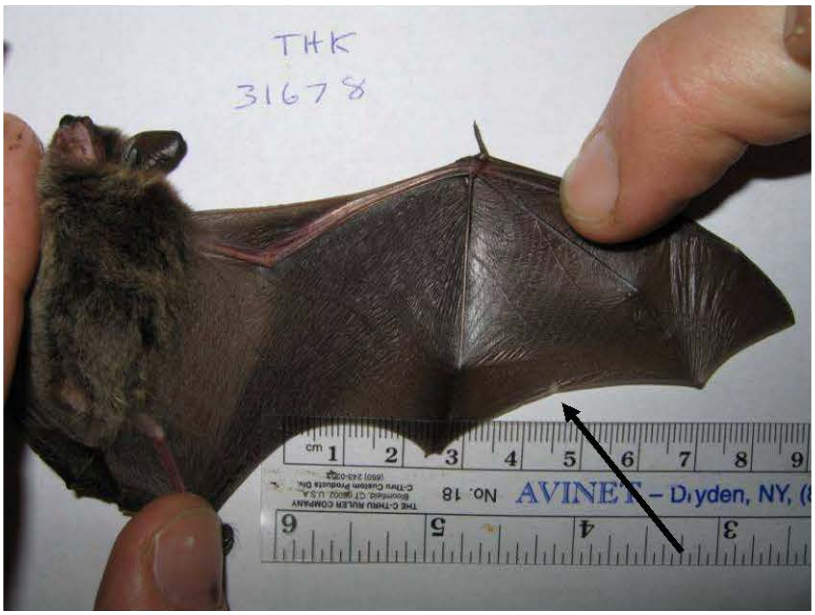
White-nose Syndrome (WNS) is characterized by the growth of one or more species of fungus on the rostrum, ears, and flight membranes of hibernating bats. During the warm months of the year, damage to these membranes may be manifested by the appearance of necrotic tissue, tears, and scars in these membranes. 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.

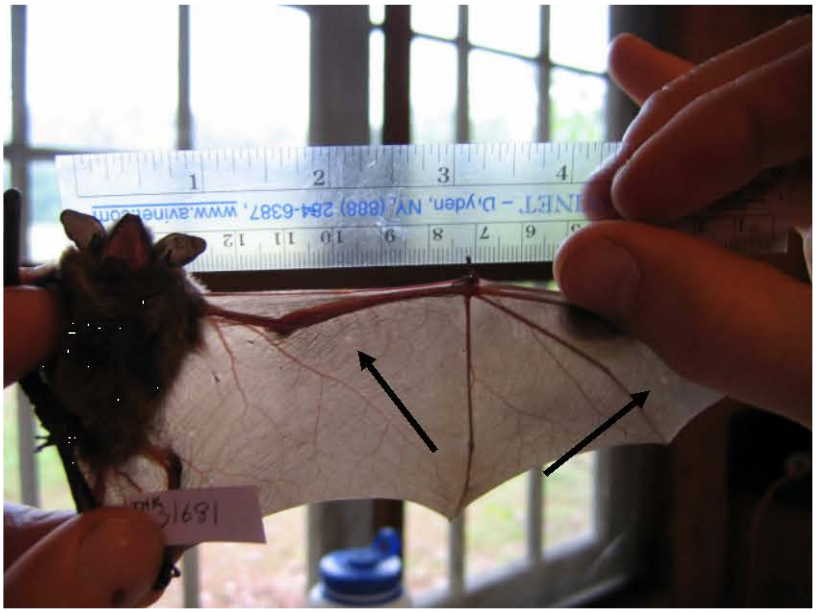
The damage to membranes and the forearms are scored 0 (none) to 3 (high) according to the criteria listed below and digital photographs are taken to document any damage. Each photograph should include a reference scale and the bat ID number (specimen number if collected dead or band or ID number if alive 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 pslotching 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:

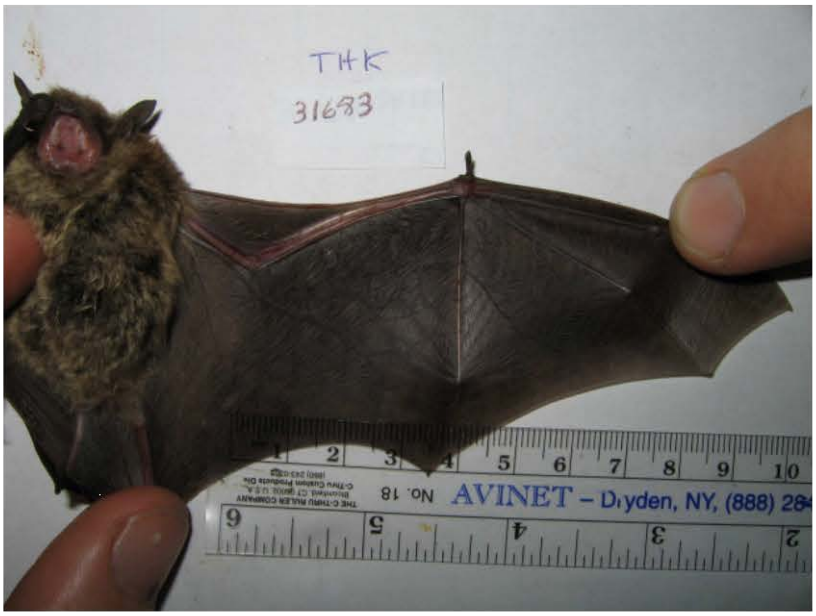
Each bat is assigned the score for which it exhibits one or a combination of the characteristics designated to that score. Some minor physical damage may be normal. See notes on physical damage not associated with necrosis at the end of this document.

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

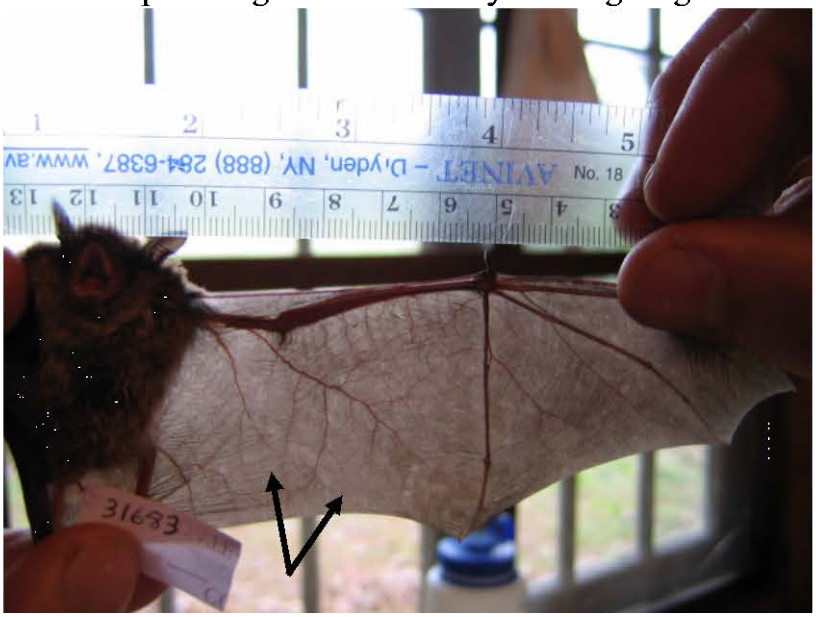




Score = 1 *Light damage*. Less than 50% of flight membrane is depigmented (splotching), which is often visible only with translumination. The membranes are entirely intact. Some discoloration or flaking is visible on forearms. Such flaking on the forearm may exist even if the patagium appears unaffected.



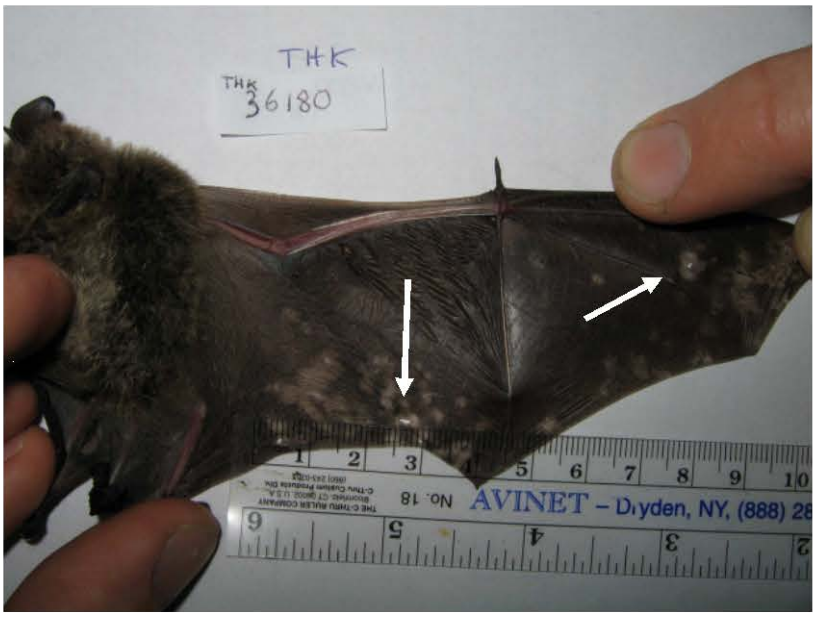
Note: no splotching visible with only front lighting.



Translumination reveals the splotchy flight membrane.

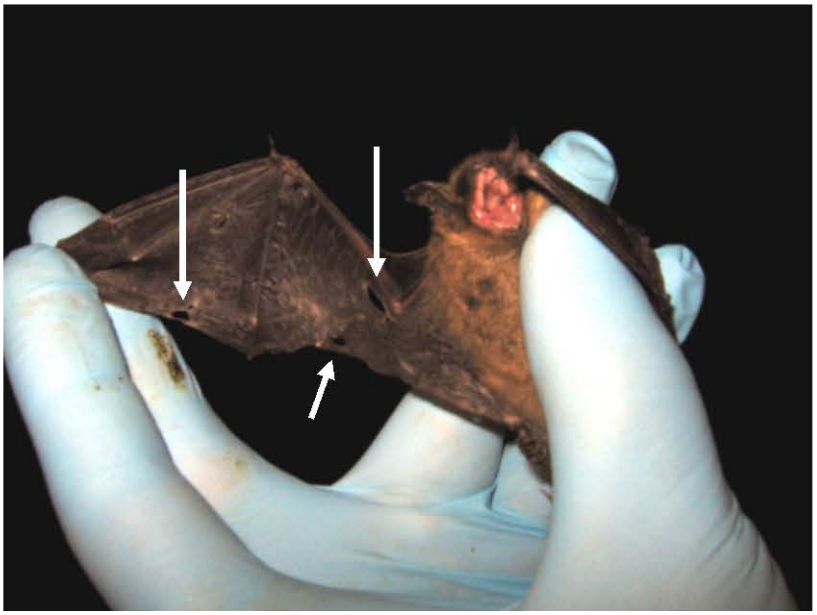
Forearms may have flaking skin or discolored areas.

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 ≥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.

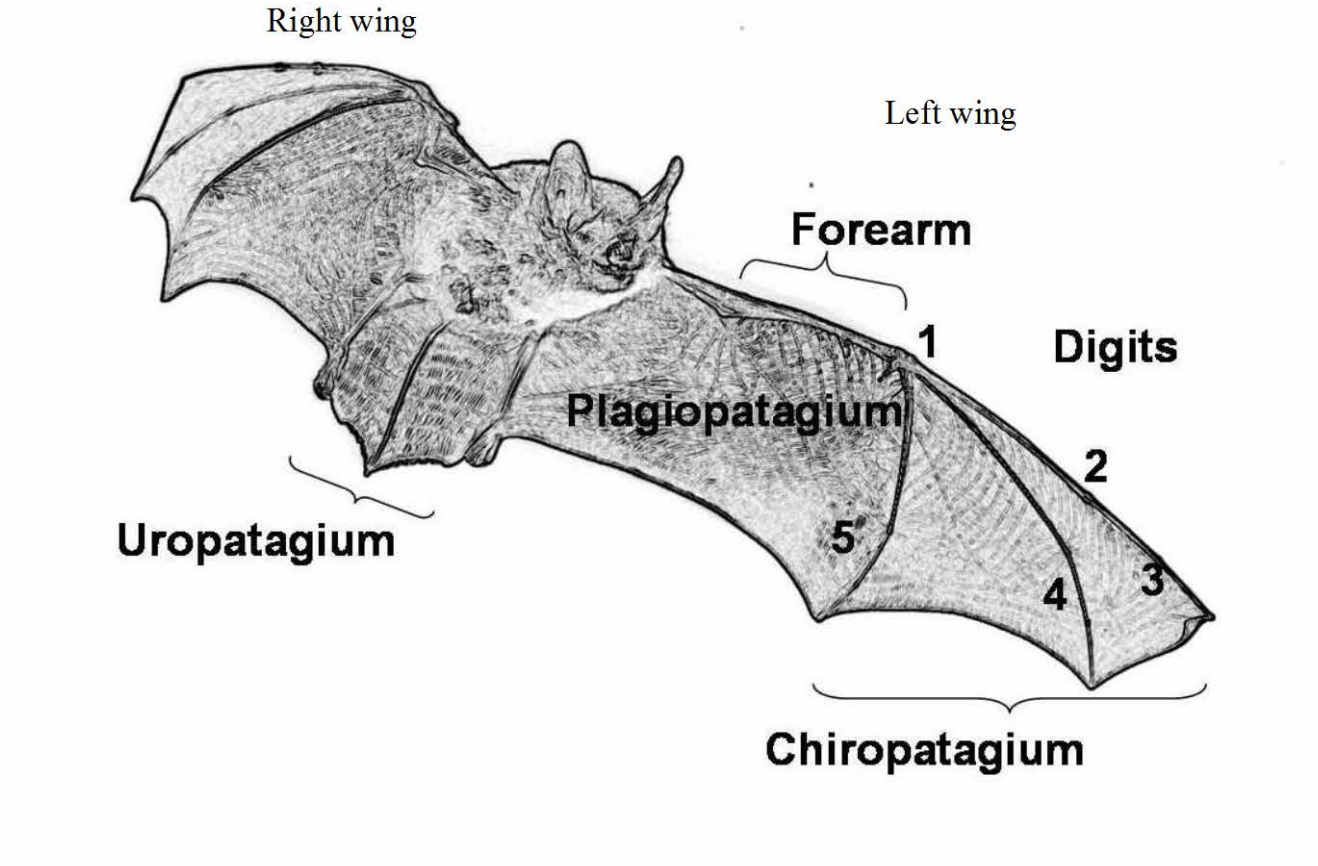
Plagiopatagium loss may be severe.

Physical Damage

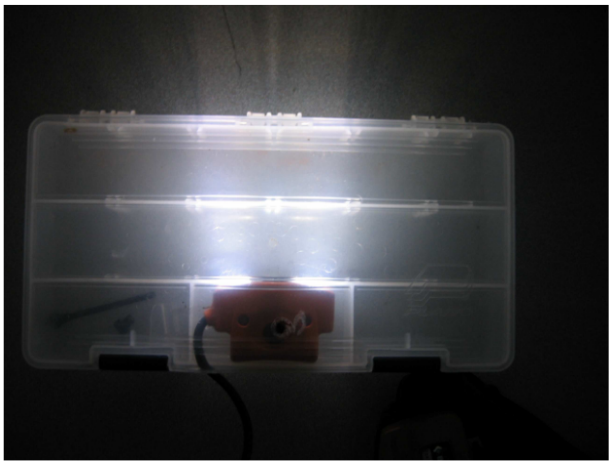
We have encountered bats that have obvious physical damage to wings, but no associated splotching or necrotic tissue. These conditions are important to document as well. We suggest these be recorded in concordance with the above scores followed by a postscript "P" for "physical damage." For example, 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.

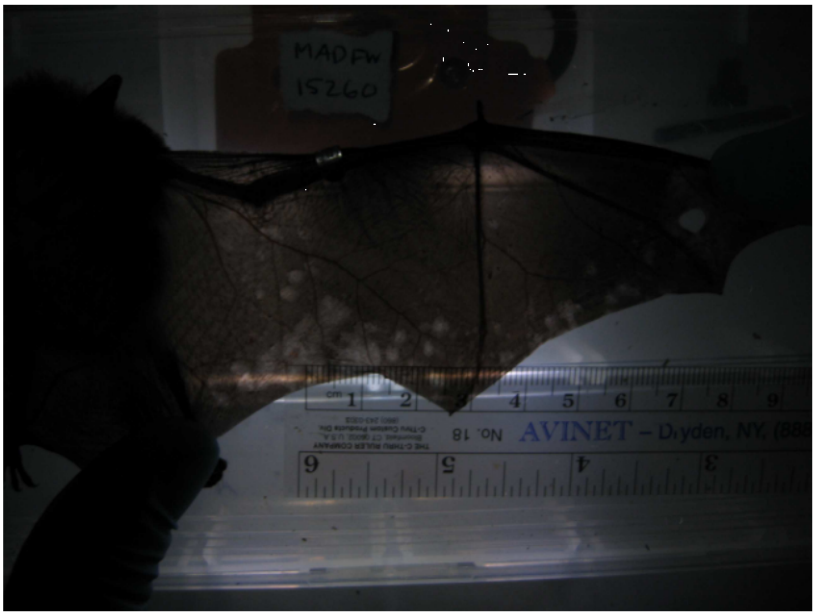
Appendix A: Reference for flight membranes and digits of bats. Image adapted from J. S. Altenbach's photograph of *Myotis thysanodes*.



Appendix B: We are working with an inexpensive light box in the field. The following model is an early effort to create an in expensive, transportable light box for transluminating 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.

C. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome.

Citation: Gregory G. Turner, Carol Uphoff Meteyer, Hazel Barton, John F. Gumbs, DeeAnn M. Reeder, Barrie Overton, Hana Bandouchova, Tomáš Bartonička, Natália Martínková, Jiri Pikula, Jan Zukal, and David S. Blehert. 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases 50: 566-573.

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NONLETHAL SCREENING OF BAT-WING SKIN WITH THE USE OF ULTRAVIOLET FLUORESCENCE TO DETECT LESIONS INDICATIVE OF WHITE-NOSE SYNDROME

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ABSTRACT: Definitive diagnosis of the bat disease white-nose syndrome (WNS) requires histologic analysis to identify the cutaneous erosions caused by the fungal pathogen *Pseudogymnoascus* [formerly *Geomyces*] *destructans* (*Pd*). Gross visual inspection does not distinguish bats with or without WNS, and no nonlethal, on-site, preliminary screening methods are available for WNS in bats. We demonstrate that long-wave ultraviolet (UV) light (wavelength 366–385 nm) elicits a distinct orange–yellow fluorescence in bat-wing membranes (skin) that corresponds directly with the fungal cupping erosions in histologic sections of skin that are the current gold standard for diagnosis of WNS. Between March 2009 and April 2012, wing membranes from 168 North American bat carcasses submitted to the US Geological Survey National Wildlife Health Center were examined with the use of both UV light and histology. Comparison of these techniques showed that 98.8% of the bats with foci of orange–yellow wing fluorescence ($n=80$) were WNS-positive based on histologic diagnosis; bat wings that did not fluoresce under UV light ($n=88$) were all histologically negative for WNS lesions. Punch biopsy samples as small as 3 mm taken from areas of wing with UV fluorescence were effective for identifying lesions diagnostic for WNS by histopathology. In a nonlethal biopsy-based study of 62 bats sampled (4-mm diameter) in hibernacula of the Czech Republic during 2012, 95.5% of fluorescent ($n=22$) and 100% of nonfluorescent ($n=40$) wing samples were confirmed by histopathology to be WNS positive and negative, respectively. This evidence supports use of long-wave UV light as a nonlethal and field-applicable method to screen bats for lesions indicative of WNS. Further, UV fluorescence can be used to guide targeted, nonlethal biopsy sampling for follow-up molecular testing, fungal culture analysis, and histologic confirmation of WNS.

Key words: Bat, Chiroptera, dermatomycosis, fungal infection, *Pseudogymnoascus* (*Geomyces*) *destructans*, ultraviolet (UV) fluorescence, white-nose syndrome.

INTRODUCTION

White-nose syndrome (WNS) is caused by the psychrophilic fungus *Pseudogymnoascus* [formerly *Geomyces*] *destructans* (*Pd*) (Lorch et al. 2011; Minnis and Lindner 2013). Mortality from *Pd* infection has been confirmed for six species of North American bats, including little brown myotis (*Myotis lucifugus*), northern myotis (*Myotis septentrionalis*), Indiana myotis (*Myotis sodalis*), Eastern small-footed myotis (*Myotis leibii*), tricolored bat (*Perimyotis subflavus*), and big brown bat (*Eptesicus fuscus*) (Turner et al. 2011). *Pd* has also been isolated from bats in Europe (Puechmaille et al. 2011a), with documentation of characteristic invasive

lesions diagnostic for WNS (Pikula et al. 2012); unusual mortality has not been reported among European bats infected by *Pd* (Martínková et al. 2010; Puechmaille et al. 2011b; Sachanowicz et al. 2014).

White-nose syndrome is the first invasive cutaneous ascomycosis reported in mammals. Currently, histopathology is required to diagnose WNS (Meteyer et al. 2009). To collect an adequate sample of wing membrane (skin) to conduct a thorough histopathologic analysis, euthanasia is typically required. A rapid, field-applicable, and nonlethal technique to identify presumptive WNS would reduce the need to euthanize bats to obtain a diagnosis. Such a technique would additionally serve to enhance ability to expand diagnostic activities to assess the presence of disease in new species and additional regions of the world, and to screen bats rapidly to determine efficacy of potential mitigation strategies.

Since the historic observation in 1925 that typical fungal dermatophyte infections fluoresce under long-wave ultraviolet (UV) light, this technique has been used as aid for diagnosing keratinaceous fungal infections, including ringworm in domestic animals (Koeing and Schneckenburger 1994) and tinea capitis in humans (Margarot and Deveze 1925). Applying this technique to wing membranes of bats with suspect WNS, long-wave (366–385 nm) UV light was shown to be a rapid, reliable, and field-applicable diagnostic tool for preliminary identification of WNS in bat-wing membranes and an accurate guide for targeted, nonlethal biopsy sampling for subsequent histologic confirmation.

MATERIALS AND METHODS

Paired assessments with the use of UV illumination and histology in the laboratory

The fluorescence of bat wings in response to long-wave UV light was compared to the histologic gold standard for diagnosing WNS. Three different UV light sources were used in these studies described below; a hand-held flashlight for quick detection of fluorescence in the laboratory, a stationary Wood's lamp for photography in the laboratory, and a stationary 9-watt UV light for transillumination in the field. These light sources are described in detail below and all had wavelengths of 366–385 nm.

The wings of 168 bats of 11 species submitted to the US Geological Survey National Wildlife Health Center Madison, Wisconsin, USA (USGS NWHC) from 21 states between March 2009 and April 2012 were evaluated for fluorescence with the use of a hand-held 51-LED 385-nm UV flashlight (model 7202 UV-385 nm, LED Wholesalers, Hayward, California, USA) in a darkened room. Laboratory personnel wore UV-protective eyewear when illuminating bat wings and the same individual performed all visual assessments for fluorescence to ensure consistency. Photography was performed in a darkened room with the use of a Nikon (Tokyo, Japan) D80 digital SLR camera (F-stop 3.3, ISO 200, shutter speed 8 sec) with an AF 60 mm lens with no filter and a Wood's lamp (366 nm; BLAK-RAY Model UVL-56, San Gabriel, California, USA) mounted approximately 13 cm above the bat at a 35–40-degree angle as the sole light source to illuminate the outstretched wing from above.

After external examination, the entire membrane was removed from a wing for histologic evaluation with the use of periodic acid–Schiff stain as described by Meteyer et al. (2009). All samples were coded for impartial histologic assessment for WNS and later compared with the UV-fluorescence status. Fisher's exact test (SigmaPlot 11.0, Systat Software, Inc., San Jose, California, USA) was used to determine whether there was a relationship between fluorescence and WNS lesions.

UV fluorescence for targeted sample collection for WNS confirmation

A field study was conducted to determine if UV fluorescence could provide a preliminary diagnosis of WNS and guide nonlethal collection of wing tissue to determine WNS status by histopathology. Torpid bats were removed from roosts during surveys, captured in flight while exiting hibernacula, or found dead at hibernacula entrances. Methods and equipment used in the field for UV illumination of bat wings were the same in the US and the Czech Republic. White or UV light was used to illuminate wing membrane of bats either from above (light on the same side as the person viewing) or below (transilluminating the wing with the light source on the opposite side of viewing). A GloBox (Artograph, Delano,

Minnesota, USA) was used for white light transillumination, and a field-portable 9-watt 368-nm fluorescent light (WTC 9L-110, Way Too Cool, from Fluorescents.com [www.fluorescents.com]) was used for UV transillumination. The use of white light illumination was discontinued after the effectiveness of UV fluorescence was established. During transillumination of live bats in the field, bats were kept in the dark, placed on the working surface of the light unit with wings extended. Photographs were then taken of wings with the use of a Canon (Melville, New York, USA) EOS 350D digital SLR camera (F-stop 5–10, ISO 200, and shutter speeds 0.5–30 sec) equipped with an EFS 18–55 mm or EF 100-mm lens with 58-mm ultraviolet filter (in Pennsylvania); or a Nikon D300 digital SLR camera (F-stop 5.3–5.8, ISO 1000, and shutter speeds 0.15–0.4 sec) with AF NIKKOR 28–80-mm lens (in the Czech Republic). Cameras were mounted on a tripod (Fig. 1A). Bats were rapidly processed to reduce handling time and minimize stress. To prevent cross-contamination, field equipment was either sanitized between bats or covered with a disposable plastic sheet (Shelley et al. 2013). Dedicated "clean" equipment was used in uninfected sites to decrease risk for inadvertent introduction of a pathogen.

To characterize ability of field biologists to assess WNS-related fluorescence accurately, wings of *M. lucifugus* ($n=6$) from two Pennsylvania sites known to harbor bats with WNS were collected in 2010 and 2011, transilluminated with UV light, and multiple 1-cm^2 regions of wing membrane were outlined on each bat with permanent marker and labeled as either fluorescent ($n=14$) or nonfluorescent ($n=13$). Marked wings were then photographed during UV transillumination, and bats were euthanized by isoflurane overdose. Carcasses were shipped overnight (chilled) to the NWHC for histologic evaluation as described above.

To evaluate the effectiveness of UV transillumination-guided biopsy sampling for WNS testing, four sizes of sterile biopsy punches (McKesson, Richmond, Virginia, USA) were used. One biopsy punch of each size (3, 4, 5, and 6 mm) was used to collect areas of wing fluorescence from each of five bats providing 20 skin biopsy samples of different sizes for histopathology evaluation.

Single biopsy samples (4-mm diameter) guided by UV transillumination were collected from each of 62 live bats of six different species in the Czech Republic as they exited their hibernacula in spring 2012. Following collection, all biopsy samples were placed into individually labeled vials containing 10% neutral buffered formalin for histopathology processing.

RESULTS

The effectiveness of long-wave UV light for detection of lesions consistent with WNS was tested with the use of a combination of field and laboratory studies. Roosting bats with distinct foci of orange–yellow fluorescence could be identified when bats were illuminated from above with UV light (Fig. 1B), but this was infrequent. Wings of bats extended and illuminated from above with white light occasionally showed indistinct white fungal growth (Fig. 1C), but evidence of fungal growth or wing damage was not apparent when the wings of the bats were transilluminated with white light (Fig. 1D). However, when long-wave UV light was used to illuminate outstretched bat wings from above (Fig. 1E) or transilluminate wings from below (Fig. 1F), distinct areas of orange–yellow fluorescence were seen. Photography in the laboratory was most successful with a Wood's lamp illuminating the wing from above (Fig. 1E). When photographing live bats under field conditions, UV transillumination (as opposed to UV illumination from above) provided the most expedient and reliable approach for detecting the orange–yellow fluorescence (Fig. 1F). When white fungal growth was seen on the wings of bats illuminated from above with white light, it corresponded to the pattern of orange–yellow fluorescence seen during UV transillumination (Fig. 1C, F). Computer magnification of digital images enhanced the ability to detect isolated pinpoint areas of fluorescence.

Paired assessments with the use of UV illumination and histology in the laboratory

Of the 168 bats submitted to the NWHC for diagnostic investigation, 80 had areas of characteristic orange–yellow fluorescence when the wings were illuminated from above with a hand-held 51-LED 385-nm UV flashlight; 79 of these were histologically positive and one histologically negative for

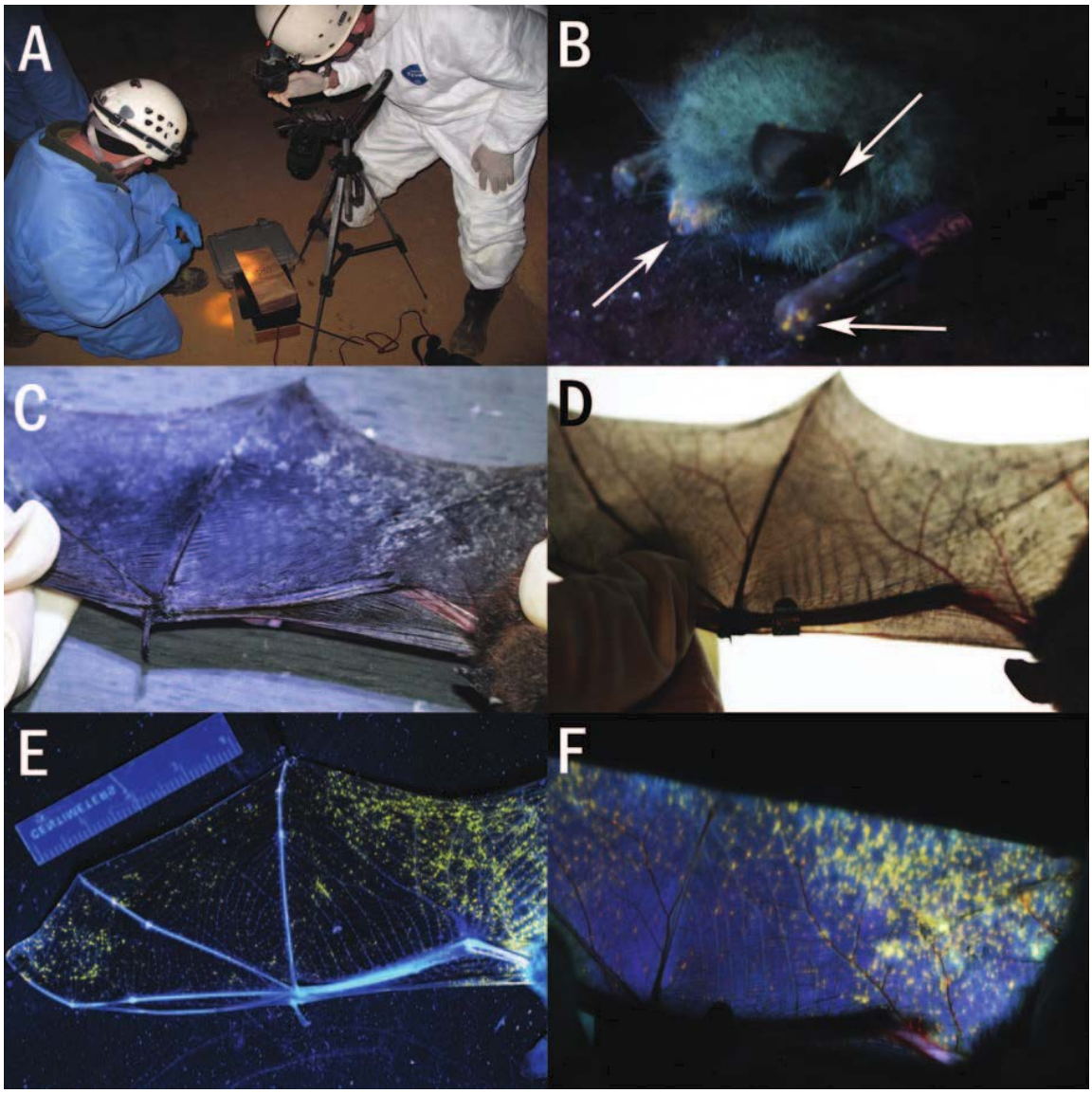


FIGURE 1. Long-wave ultraviolet (UV) and white-light illumination of lesions associated with white-nose syndrome. All photographs are from bats of the US; blurring in photos of live bats in C, D, and F is due to animal movement during long exposure. (A) Camera in cave, mounted on tripod directed at platform constructed to transilluminate bat wings with UV light (photo by Craig Stihler with permission). (B) Points of orange–yellow fluorescence (arrows) detected on a roosting Indiana myotis (*Myotis sodalis*) following surface illumination with a field-portable 9-watt 368-nm fluorescent UV light (photo by Tina Cheng with permission). (C) Wing from live little brown myotis (*Myotis lucifugus*) lit from above in cave with white light shows dispersed pattern of fungal growth. (D) White-light transillumination of wing from the live bat in C shows no obvious pattern of fungal infection or wing damage. (E) Wing from dead tricolored bat (*Perimyotis subflavus*) lit from above with hand-held 51 LED 385-nm UV flashlight shows points of orange–yellow fluorescence. (F) Transillumination of wing from live bat in C with the use of a field-portable 9-watt 368-nm fluorescent UV light. The pattern of orange–yellow fluorescence follows the distribution of surface fungal growth seen in C.

WNS (98.8% agreement between UV and histopathology assessments; Table 1). The 88 bats that were UV-fluorescence negative were all histologically negative for WNS (Table 1). There was a strong Fisher's exact test association between UV fluorescence and WNS lesions ($P<0.001$) in these 168 bats.

TABLE 1. Summary of paired ultraviolet (UV) fluorescence and histologic analyses for bats from North America and UV-targeted biopsy-based study for bats from Europe.

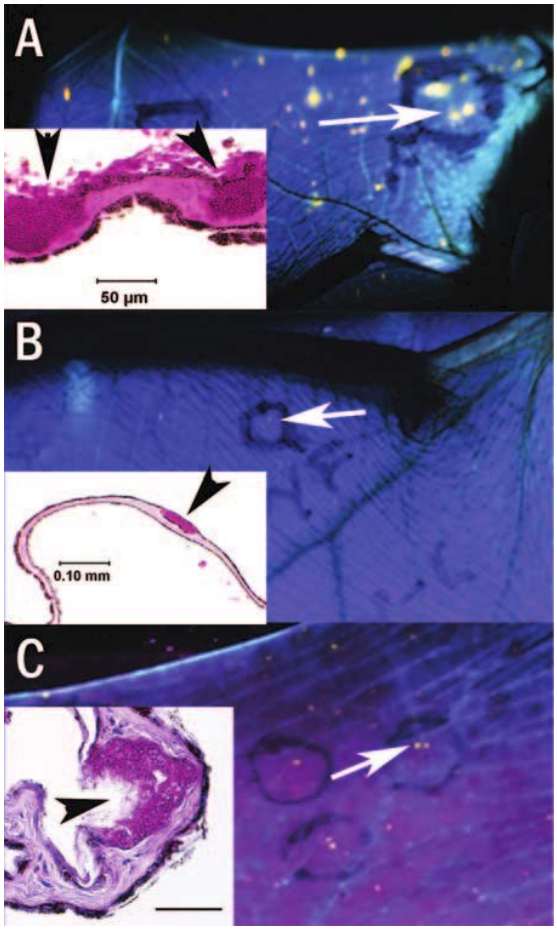
Bat species	Positive		Negative		Total
	Fluorescence	Histology	Fluorescence	Histology	
US (whole carcasses)					
Myotis lucifugus	59	58	40	41	99
Eptesicus fuscus	1	1	1	1	2
Myotis leibii	1	1	0	0	1
Myotis septentrionalis	5	5	7	7	12
Perimyotis subflavus	11	11	16	16	27
Myotis grisescens	0	0	7	7	7
Myotis velifer	0	0	11	11	11
Myotis sodalis	0	0	1	1	1
Myotis yumanensis	0	0	1	1	1
Myotis austroriparius	0	0	3	3	3
Tadarida brasiliensis	0	0	1	1	1
Unidentified *Myotis* sp.	3	3	0	0	3
Total	80	79	88	89	168
Czech Republic (biopsy samples)					
Myotis myotis	17	16	13	14	30
Myotis daubentonii	2	3	10	9	12
Myotis nattereri	2	2	5	5	7
Myotis bechsteinii	0	0	6	6	6
Myotis alcathoe	0	0	5	5	5
Myotis emarginatus	1	1	1	1	2
Total	22	22	40	40	62

Of the 88 bats that were UV-fluorescence negative and histologically negative, 22 had microscopic evidence of fungal colonization in the superficial keratin layer of wing skin that was morphologically distinct from WNS, and these fungi were considered to be different from *Pd*.

Use of UV fluorescence to target sample collection for WNS confirmation

Histologic examination of all 1-cm^2 targeted samples of fluorescent wing membrane collected from bats in Pennsylvania (n=14) were positive for the dense aggregates of fungal hyphae that form cupping erosions, which define WNS (Fig. 2A, B). When these 1-cm^2 skin samples encompassed single, pinpoint dots of fluorescence, microscopic examination identified individual fungal erosions diagnostic for WNS as small as 20–40 μm in diameter (Fig. 2B). Nine of 13 1-cm^2 regions of wing membrane marked as nonfluorescent had no cupping erosions when examined microscopically. The remaining 4 of 13 nonfluorescent samples examined microscopically had a single fungal cupping erosion (20–40-μm diameter) diagnostic for WNS. Retrospective computer magnification of the digital images taken in the field of these four fluorescence-negative bats subsequently detected scattered small pinpoint fluorescent areas that were not initially detected, suggesting that the reliable margin of accuracy in assessing unmagnified digital images may be lesions approximately 20–40 μm in diameter.

The utility of nonlethal UV-targeted biopsy sampling and biopsy size requirements was evaluated with the use of wing skin samples from bats in Pennsylvania. Biopsy samples of four diameters (3, 4, 5, and 6 mm) from each of the five bat carcasses provided adequate tissue for diagnosing cupping erosions characteristic of WNS, confirming the usefulness of this nonlethal sampling technique for biopsies as small as 3 mm in diameter.



pping erosion (arrowhead).

Consistent with samples analyzed from North America, 21 of 22, 4-mm targeted biopsy samples from UV-fluorescent wing skin of bats from the Czech Republic also contained dense aggregates of fungal hyphae filling cupping erosions that are diagnostic for WNS (95.5% agreement between UV and histopathology assessments; Fig. 2C; Table 1). Retrospective review of digital images indicated that, for the histology-negative animal, the circled region of wing skin targeted for biopsy sampling had missed the point of fluorescence. For reporting purposes, however, this animal was classified as fluorescence-positive and histology negative. Additionally, a biopsy sample from 1 of 40 fluorescence-negative bats from the Czech Republic was positive for WNS by histology.

DISCUSSION

The gold standard for diagnosing bat WNS is the histologic identification of aggregates of fungal hyphae that form characteristic cupping erosions and ulceration of wing membrane (Meteyer et al. 2009). The large amount of wing membrane needed to detect these lesions histologically necessitates euthanasia of the bat. Given the detrimental effect that WNS has had on bat populations (Blehert et al. 2009; Frick et al. 2010; Turner et al. 2011), detection protocols that do not require euthanasia would be advantageous.

Illumination/transillumination of wing membranes of bats with WNS with the use of long-wavelength UV light (366–385 nm) elicited a distinct orange–yellow fluorescence that correlated with the presence of fungal cupping erosions used to diagnose WNS by histopathology (Figs. 1, 2). This correlation of fluorescence to WNS histologic lesions was observed in wings from five North American and four European species of bats (Table 1), with 98.8 and 95.5% agreement between UV and histopathology assessments for bats of North America and Europe, respectively. In addition, the 22

of 88 fluorescence negative bats that had fungi along the superficial keratin of wing skin were also histologically negative for the cupping erosions that confirm WNS. This supports our hypothesis that it is the lesion of cupping erosion, characteristic of WNS, that is fluorescing with UV light, and not superficial fungal hyphae. We thus conclude that observation of orange–yellow fluorescence following illumination/transillumination of wing membranes with UV light facilitates identification of bats with WNS. *Pd* is an ascomycete fungus, as are numerous plant pathogens. Ascomycete plant pathogens change morphologically as they penetrate the plant cuticle and the distinct subsurface hyphae release novel products related to virulence at the fungal–tissue interface (Valent and Khang 2010). A similar scenario might explain fluorescence associated with the invasive lesion of WNS and not surface hyphae. Once penetration of the epidermis occurs, *Pd* hyphae may secrete novel proteins, metabolic products, and enzymes that contribute to the erosion of living tissue and fluorescence.

Bats severely affected by WNS had numerous conspicuous large, coalescing regions of fluorescence distributed over much of the wing membrane and were readily identifiable (Fig. 1E, F). In North American bats with mild WNS (Fig. 2B), as in the WNS-positive bats in Europe (Fig. 2C), the random, sparse, and pinpoint pattern of fluorescence was more difficult to see, particularly when environmental white light was not eliminated. In addition, ability to discern sparse, subtle fluorescence often varied by observer, potentially because of factors such as inexperience with the technique, red–green color blindness, or other differences in visual acuity. Because of these difficulties, UV technique may miss individual bats with mild cases of WNS. Laboratory tests including PCR for detection of *Pd* (Muller et al. 2013), culture for *Pd* (Lorch et al. 2010), and histology to diagnose WNS (Meteyer et al. 2009) continue to play a definitive role in confirming WNS. The ability to observe sparse points of fluorescence can be enhanced by using digital photography with extended exposure time and augmentation by computer magnification of the digital images. The smallest points of fluorescence that could be visually detected with the unaided eye correlated to cupping erosions >20 μm in diameter.

In addition to the demonstrated utility of long-wave UV light as a rapid field assessment technique to obtain a preliminary diagnosis for WNS, this technique can also be used to optimize nonlethal collection of small (4-mm) biopsy samples for testing by histology, PCR, or culture. Another benefit of the enhanced accuracy afforded by UV-guided sampling is the ability to identify bats with fluorescent lesions (Fig. 1B) while limiting disturbance to nonfluorescent bats within a hibernaculum. This nonlethal assessment technique can also assist natural resource managers and researchers investigating WNS by facilitating the ability to track progression of disease in individual bats and by providing the potential, in the hands of trained field personnel, to generate accurate preliminary on-site results to inform mitigation strategies more quickly. The ability to perform targeted and nonlethal sampling of bats for WNS offers a needed tool to facilitate enhanced surveillance and research for this disease.

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Permits

In Pennsylvania, work with live bats was conducted by personnel of the Pennsylvania Game Commission in compliance with Pennsylvania Statute Title 34, Section 322, and procedures for sampling and euthanasia of bats in the US were conducted in accordance with US Geological Survey National Wildlife Health Center (NWHC) Institutional Animal Care and Use Committee Experimental Protocol 081124-A2. In the Czech Republic, live bats were sampled as they left hibernacula, and work was conducted in accordance with the Czech Academy of Sciences Ethics Committee Animal Use Protocol 169/2011 in compliance with Law 312/2008 on Protection of Animals against Cruelty adopted by the Parliament of the Czech Republic. Nonlethal sampling was in compliance with Law 114/1992 on nature and landscape protection, and was based on permits 01662/MK/2012S/00775/MK/2012, 866/JS/2012, and 00356/KK/2008/AOPK issued by the Nature Conservation Agency of the Czech Republic.

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D. Alternative Sampling Methods for *P.d.* Testing.

These guidelines are from Appendix E (page 12) of North Carolina's White-nose Syndrome Surveillance and Response Plan, and can be found at:

http://www.ncwildlife.org/Portals/0/Conserving/documents/WildlifeDiversity/NCWNS_Surveillance%20ResponsePlan.pdf

Method 1: Swabbing Protocol for Bats

Protocol: Swabbing of Bats for Identification of *Pseudogymnoascus destructans* Fungus

Authors: Gabrielle J. Graeter, North Carolina Wildlife Resources Commission; based on protocols written by Winifred Frick at University of California – Davis.

Date: 10 December 2013

Purpose: The following procedure is designed to collect fungi from the skin of bats for later microscopic analyses while minimizing harm to the sampled bat.

List of supplies needed

General Supplies

- Latex gloves - Use new glove for each bat
- Lysol wipes – for decontamination of supplies, gear, datasheets, etc.
- Plastic clipboard – easy to decontaminate with Lysol
- Ziplock bags - Double bag all sample vials after decontaminated prior to shipping.
- Garbage bags – use to dispose gloves, swab handles, used dipping vials, etc.

Sampling Supplies

- Swabs – 1 used per bat
- Storage tubes - are 2ml tubes with RNALater (a preservative)
- Dipping vials – are tubes filled with sterile water. Use these to moisten swab head prior to rubbing on bat. Plan on using 1 dipping vial for every 10-20 bats. Discard used dipping vials after each site survey. Any unopened dipping vials can be used at another site.
- Labels – prepare labels in advance that have a unique ID on them (NC14-01, NC14-02, NC14-03, etc.). Make sure they will fit on the vials and will stick when wet and muddy.

Step-by-Step Instructions

1. Prior to site entry, place unlabeled storage tubes, swab supplies, and labels into ziplock bags (recommend 2-5 items per bag) to prevent needing to decon unused supplies after site exit.
2. Locate focal bat (needs to be within reach)
    a. On page 2 of the NCWRC Winter Hibernacula Survey Datasheet, fill out the "Submitted Bats/Samples" section for each bat swabbed. Do this prior to swabbing the focal bat. In the Comments section, note where on the bat you see visible fungus.
    b. Take several photos of the bat (record photo #'s on datasheet)
3. Handling instructions:
    a. Use a new pair of gloves for each bat.
    b. Leave bat in place on wall and perform swab instructions as indicated in Step 4.
4. Swabbing instructions:
    a. Remove unlabeled 2ml storage tube from ziplock bag and place label sticker on tube.
    b. Remove swab from sterile packaging (open packaging from end without the swab to avoid contaminating swab head).
    c. Dip swab head in sterile water in dipping vial.
    d. Hold one hand under the bat in case it loses its grip on the wall during swabbing.

e. Firmly rub the swab across the forearm of the right wing with the wing folded starting at the caudal end of the forearm and moving toward the head and then back toward the caudal end (back/forth = 1 X).
f. Repeat this procedure four more times (total of 5 X) twirling the swab as you move it across the forearm.
g. Repeat the procedure on the top of the bat's muzzle 5X (back/forth = 1 X) – do not return the swab to dipping vial or storage tube between forearm and muzzle.
h. If necessary, repeat the procedure on any other portions of the bat's body with visible fungus that was not already swabbed.
i. Place the swab head into the 2ml storage tube and break off the section you have touched so that only the polyester swab tip remains in the vial.
j. Close and lock tube tightly and place into a Ziploc.

5. Make sure to finish recording information on the Datasheet
6. Disposal and Decontamination Procedures:
    a. All swab handles and packaging, used dipping vials, used gloves, used Lysol wipes, etc. can be disposed of in a garbage bag
    b. Decontaminate with Lysol: all ziplock bags used to carry unused supplies
    c. Decontaminate with Lysol: any unused supplies inside any ziplock bags that were opened underground.
    d. Remove and discard used dipping vials
7. Storage and Shipment Procedures:
    a. Double bag and label each Ziploc with:
        i. State
        ii. Collector's Name
        iii. Site Name(s)
        iv. Date
        v. Number of samples collected
    b. Store sample in a refrigerator or freezer until shipment.
8. Ship to SCWDS for testing (see Appendix E)

Method 2: Fungal Tape-lift Protocol for Bats

Protocol: Tape-Strip Sampling of Bats for Identification of *Geomyces destructans* Fungal Infection

Authors: David S. Blehert and Anne Ballmann, USGS – National Wildlife Health Center
Date: 7 October 2009 (modified)

Purpose: The following procedure is designed to collect fungi from the skin of bats for later microscopic analyses while minimizing harm to the sampled bat.

Required materials:

NOTE-Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline.

1) Glass microscope slides with white label (25 mm (W) X 75 mm (L); 1 mm thick). Fisher Scientific Catalog #12-552. Fisher list price $58.34 pack (144/pack).
2) Fungi-Tape (25 yards X 1 inch; approximately 1 mm thick). Fisher Scientific Catalog #23-769-321 (Scientific Device Laboratory No. 745). Fisher list price $35.59 per box.
3) Plastic 5-slide transport mailers. (Maximum capacity is 10 slides per mailer – see instruction #9 below). Fisher Scientific Catalog #12-569-35 ($31.00 for pack of 25) or #12-587-17B ($185.35 for pack of 200).
4) Pencil

Procedure:

1) Wear new disposable gloves when handling each individual bat to reduce the risk of cross-contamination.
2) Label the end of a microscope slide in pencil with an animal ID number, date, and anatomical sample location.
3) Remove a precut piece of Fungi-Tape from the box being careful not to contaminate the adhesive surface.
4) Bend the tape-strip (without creasing), adhesive-side out, between your thumb and index finger so that the tape forms the shape of a "U" (Fig. 1).
5) Sample muzzles of bats with grossly visible blooms of fungal growth. When possible, avoid collecting samples from wing membranes as analyses of unfurred skin have not been reliable in detection of *Geomyces destructans*.
6) Lightly touch the adhesive surface of the tape-strip, at the bottom of the "U", to an area of suspect fungal growth on bat surface (Fig. 2). DO NOT use your finger to press the tape down onto the bat's muzzle. Attempt to maximize adherence of fungus to the tape adhesive while minimizing adherence of hair (Fig. 3).
7) If only a small area is transferred to the tape, use a different portion of the same tape "U" to touch another area of visible fungal growth on the bat. DO NOT attempt to obtain more than 3 lifts per tape strip. **Collect only 1 tape-strip per live bat.**
8) Align the tape-strip containing the fungal sample, adhesive-side down, over the microscope slide. Ensure that the edges of the tape-strip do not protrude beyond the edges of the microscope slide when laid flat, and do not remove any portion of the tape-strip from the glass slide once it has adhered (Fig. 4).

9) Lightly wipe over the top surface of the tape-strip using a clean paper or cloth towel to consistently adhere the strip to the slide. Circle the area of tape used to transfer the fungus with a permanent marker.
10) Place each slide into a slide mailer for safe transport. If 2 slides are placed per slot, ensure that the tape surfaces of each slide are facing outwards (only the non-tape sides should be in contact so as not to crush the tape). Seal the slide mailer shut with standard tape or rubber bands prior to shipment.
11) Place slide mailer(s) into a clean Ziploc bag and seal closed to transport from the hibernaculum. Place in a second Ziploc bag
12) The slide mailers can now be held at ambient temperature and shipped to the NWHC for microscopic examination. Ship mailers in a padded envelop with a completed specimen history form. If including slide mailers in a cooler shipment with bat carcasses, ensure that the slide mailers are not in contact with the blue ice. Send an electronic copy of the completed specimen history form to LeAnn White (clwhite@usgs.gov) or Anne Ballmann (aballmann@usgs.gov). Contact Anne (608-270-2445) or LeAnn (608-270-2491) if you have any additional questions.

Illustrations – Fungal tape-lift protocol for bats

-Photographs by D. Berndt and D. Johnson, USGS - NWHC

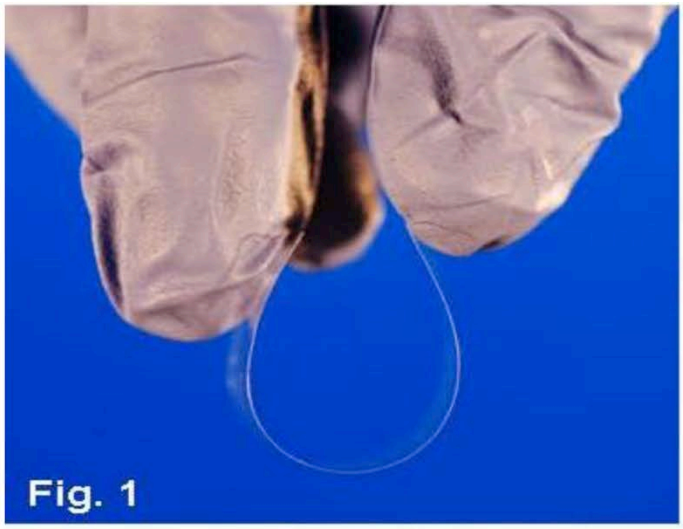


Fig. 1

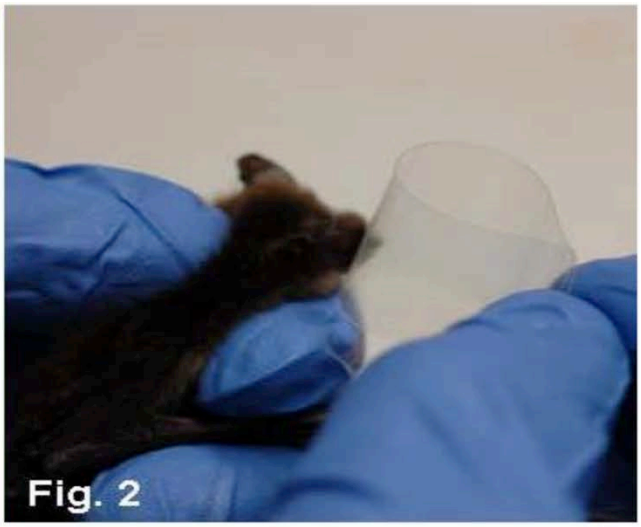


Fig. 2

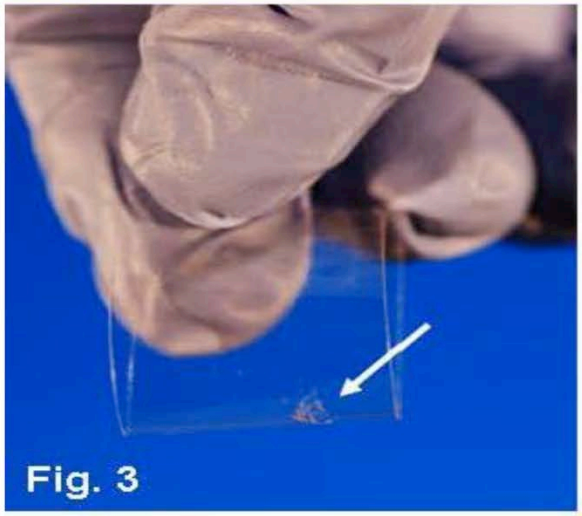


Fig. 3

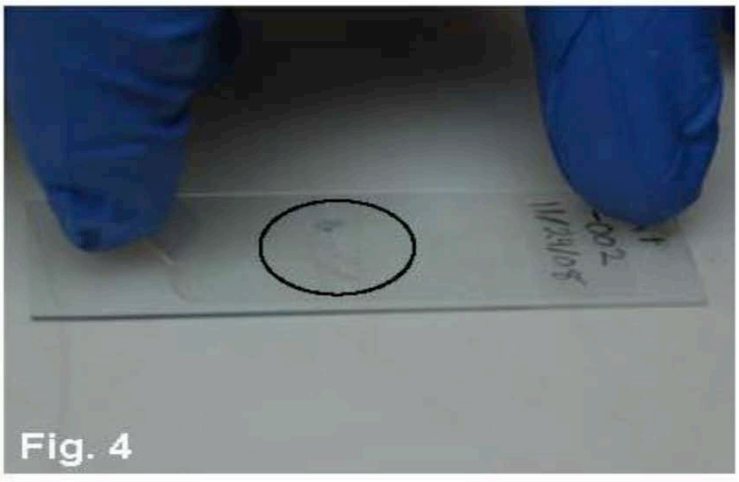


Fig. 4

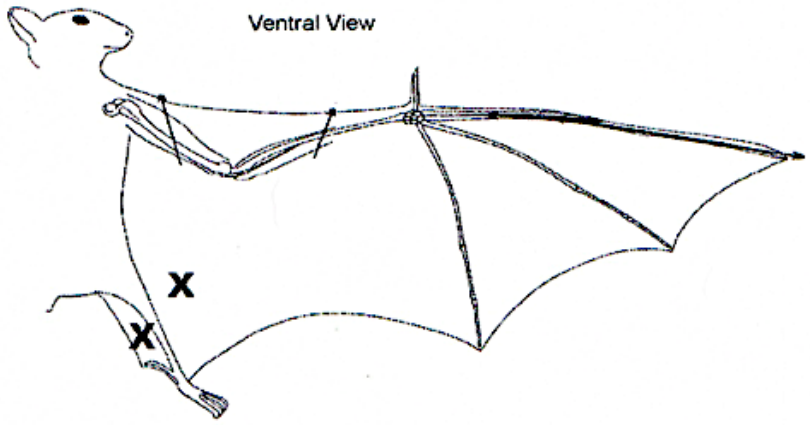
Method 3: Instructions for Taking a Wing Membrane Biopsy

Updated by Pat Ormsbee and Jan Zinck 5/14/09 (original: Shonene Scott, Portland State University 5/2003)
Modified by Anne Ballmann 6/10/10

NOTE: If punch biopsies are the only sample type to be submitted to the lab for PCR testing of *G. destructans* in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings.

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 sample, sterilized forceps, and disposable gloves.
2. Label a sterile vial: Use a black ultra-fine Sharpie permanent marker and a sticky paper label. Be careful that once the label is adhered to the tube the entire identifier is visible. Use the following naming convention to uniquely identify the bat: State, Date (MMDDYY), Collector initials, bat number (ex: WI061609AEB001)
3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.
4. Identify 2 representative lesions to biopsy on the affected 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 affected area near the body wall within the lower half of the wing membrane or uropatagium. 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 ga needle or sterile forceps can be used to pick up the tissue and transfer each biopsy to separate storage vials which contain no storage media.
7. 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.
8. While in the field, sample tubes should be stored on ice. Subsequently, samples should be frozen until submitted for fungal PCR analysis.
9. Dispose of the used biopsy punch after each animal. DO NOT reuse the same biopsy punch on multiple bats. The punches are very sharp. Be careful to not cut yourself. Change into new gloves before handling each bat.
10. Before reusing forceps while in the field, follow the flame sterilization protocols described in "Disinfection Protocol for Bat Field Research/Monitoring, June 2009" (http://www.fws.gov/northeast/wnsresearchmonitoring.html). Upon returning to the office, perform a more thorough cleaning and disinfection of nondisposable biopsy equipment with detergent washing followed by soaking in a 10% bleach solution for 10 min with a thorough clean water rinse. Once dry, forceps can be placed into a clean hard surface container (not plastic bags), free of contaminates, marked for cleaned forceps, and with handles all pointing in the same direction.
11. Ship wing tissues to NWHC: ensure that all cryovials are labeled and lids are secured in place to prevent cross-contamination of samples. Wrap lid of cryovials in parafilm and place in a Ziploc bag. If parafilm is not available double-bag specimens before placing in cooler. Specimens should be chilled and shipped overnight in a cooler with blue ice. If samples cannot be shipped overnight freeze them and ship as soon as possible. Send an electronic copy of the completed specimen history form or datasheet to the appropriate NWHC contact . Specimen history form, shipping address, and examples of appropriate shipping materials are in Appendix E. Contact Anne Ballmann (aballmann@usgs.gov , 608-270-2445) if you have any additional questions.

SUPPLIES: *NOTE-Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline*

- 2 mm biopsy punches Fisher Scientific Catalog # NC9515874 ($106.73/pack of 50)
- Forceps **OR** 25 gauge needles and sharps collection container
- 10% bleach solution (can be made fresh each time, or can be stored in opaque containers for 24 hours, it begins to break down after this)
- Sterile rinse water
- 5 ml sterile plastic vials with caps
- 95% ethanol and flame source such as cigarette lighter (for sterilizing metal sampling equipment)
- Fine point permanent marker
- Vial labels
- Disposable gloves
- Paper towels/gauze
- Nonporous cutting board
- Ziploc bags and cooler with blue ice.

E. Southeastern Cooperative Wildlife Disease Study WNS Surveillance Form

This form can be found at:

http://vet.uga.edu/population_health_files/WNS-surveillance-submission-form2014.pdf

Or use National Wildlife Health Center Bat WNS Surveillance Submission Guidelines and notify SCDNR.

http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/USGS_NWHC_Bat_WNS_submission_protocol.pdf

White-Nose Syndrome Submission Form

State ID Number_____ **SCWDS ID Number**_____
(Enter reference numbers assigned by the submitting agency here. Optional) (Leave blank. For use by SCWDS personnel)

Date Collected: _____/_____/_____ Date Shipped for testing: _____/_____/_____
(Ship for next day delivery – receipt of packages is not available at SCWDS on weekends)

Person completing this form:

Name:_____ Date: _____/_____/_____

Agency:_____ Phone:_____ Fax:_____ Email:_____

Date of initial report: _____/_____/_____ Date bat(s) were discovered: _____/_____/_____

Name of initial observer: _____ Phone:_____

Number of sick or dead bats seen:_____ Total number of bats present in cave:_____

Species of bats submitted (number):_____
(If multiple species are present please provide a label on the bats with their appropriate species)

Brief History: _____

_____

Location of bat(s):

Name of the cave:_____ UTM Coordinates:_ _____

Address (if available): _____

City:_____ County:_____ Zip code:_____

Bats should not be submitted if decomposed **(only ship freshly dead bats)**. Approximately 10 animals from each site should be sufficient for evaluation. They should be in a water-tight bag with the species written on the bag. They should be placed in a second water-tight bag and shipped overnight on sufficient ice packs to keep them cold for the duration of shipping. Use plastic coolers or Styrofoam coolers designed for shipping. Ship samples overnight so that they arrive on a week day. Prior to shipping, please notify **Heather Fenton by e-mail at hfenton@uga.edu.**

Bats should be sent to:

Dr. Heather Fenton
589 D.W. Brooks Drive
SCWDS - College of Vet Med - UGA
Athens, Georgia 30602-4393
706-542-1741