South Carolina
White-nose Syndrome Response Plan

Revised February 2015

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

Contents

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 (NGOs) and the private sector. This document was revised by Jennifer Kindel for the South Carolina Department of Natural Resources (SCDNR) in cooperation with the partners below, and funded by United States Fish and Wildlife Service (USFWS) WNS State Support Grant SC-E-F14AP00731.

A. State Agencies

i. South Carolina Army National Guard, Fort Jackson - Stanley Rikard; McCrady Training Center - Layne Anderson, Bryan Hall, Chris Stone

ii. South Carolina Department of Health and Environmental Control - Stephanie Cox, Christy Jeffcoat

iii. South Carolina Department of Natural Resources - Mary Bunch, Jay Butfiloksi, Sam Chappelear, Dean Harrigal, Julie Holling, Greg Lucas, Richard Morton, Al Segars, Derrell Shipes, Willie Simmons, Sam Stokes, Tom Swayngham.

iv. South Carolina Department of Parks, Recreation and Tourism - Terry Hurley, Joseph Lemeris, Jr.

v. South Carolina Forestry Commission - Russell Hubright

vi. Southeastern Cooperative Wildlife Disease Study - Heather Fenton

B. Federal Agencies

i. United States Army Corps of Engineers - Sandra Campbell

ii. 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

iii. United States Fish and Wildlife Service - Jennifer Koches, Morgan K. Wolf

iv. United States Geological Survey - Fort Collins Science Center: Laura Ellison

F. Universities

i. Clemson University - David Jachowski, Greg Yarrow; Campbell Museum of Natural History - Stanlee Miller

ii. Furman University - Travis Perry

iii. Lander University - Austin Trousdale

iv. South Carolina Upstate - Jonathan Storm

v. Southern Wesleyan University - Rocky Nation

G. Non-governmental Organizations

i. Bat Conservation International - Katie Gillies, Dan Taylor

ii. North Carolina Bat Working Group - Mary K Clark, Mary Frazier, Lisa Gatens

iii. Nuisance Wildlife Control Operators

iv. Palmetto Bluff Conservancy - Mary Socci

v. Southeastern Bat Diversity Network - Trina Morris

vi. The Nature Conservancy - Kristen Austin

H. Independent Contractors

i. Jennifer Kindel

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* (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/

https://www.whitenosesyndrome.org/sites/default/files/resource/white-nose_fact_sheet_3-2014_1.pdf.

For the most updated nationwide WNS information:

https://www.whitenosesyndrome.org/

WNS was first confirmed in South Carolina in Pickens County on a tricolored 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 tricolored 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 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 (those affected by WNS are in bold).

Common Name	Scientific Name	Global Rank	State Rank	State Protection	Affected by WNS
Big Brown Bat	***Eptesicus fuscus***	**G5**	**S5**		**Yes**
Eastern Small-footed Myotis+	***Myotis leibii***	**G1G3**	**S1**	**ST: Threatened**	**Yes**
Little Brown Bat	***Myotis lucifugus***	**G3**	**S3?**		**Yes**
Northern Long-eared Bat	***Myotis septentrionalis***	**G2G3**	**S4**		**Yes**
Tricolored Bat+	***Perimyotis subflavus***	**G3**	**SNR**		**Yes**
Rafinesque's Big-eared Bat	*Corynorhinus rafinesquii*	G3G4	S2?	SE: Endangered	*
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.
* WNS has been detected on these species in other states but they have not yet shown diagnostic sign of the disease.

IV. Permit Requirements

A. NWCOs, Wildlife Rehabilitators and Scientific Research Permittees must all 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.

i. Regardless if the northern long-eared bat (*Myotis septentrionalis*) is listed as threatened or endangered, 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. This requirement will be in place no later than January of 2016.

V. Pre-WNS Arrival Response at Sites

A. Early Detection and Spread of WNS

i. "Soft" equipment (such as clothing and soft-sided equipment) or any equipment that cannot be decontaminated 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.

1. Sites within South Carolina: if soft equipment or any equipment that cannot be decontaminated has been previously used in a South Carolina WNS affected site, it should not be used in a South Carolina WNS unaffected site.

ii. Nuisance Wildlife Control Operators

1. All NWCOs are recommended to incorporate applicable elements of the USFWS Disinfection Protocol for Bat Field Research and Monitoring (Appendix A), especially those companies that work in other states. Applicable elements would include practices such as only using exclusion devices that are amenable to disinfection in South Carolina if they were used in affected states. SCDNR can only make these as recommendations and provide information to NWCOs under current laws/regulations.
2. Most NWCO's do not directly handle bats, but personnel who handle individual bats during removal are recommended to follow as much of the WNS protocols listed under Scientific Research permittees (section V. A. iv., page 5) as possible. Otherwise, NWCO's are encouraged to reference the Reichard Wing Damage Index (WDI), report bat species to SCDNR scoring a 2 or greater (Appendix B), and submit a picture of these bats with outstretched wings to SCDNR. The WDI is *not* a diagnostic tool.
3. SCDNR staff may request bats scoring a WDI of 2 or greater for submission to Southeastern Cooperative Wildlife Disease Study (SCWDS) in 2015 and 2016. In this case, see steps for collection of dead bats and submission to SCWDS in section V. A. iv. 3 (page 5). If bat specimens with extensive wing damage of WDI of 2 or higher are not requested for submission, please follow steps in section V. A. iv. 4 (page 6) for submission to the Campbell Museum of Natural History or disposal.
4. SCDNR shall send links and hard copies of WNS information from USFWS, such as the disinfection protocol and the WNS fact sheet. [DONE]
5. SCDNR shall send updates on WNS to all NWCOs that are listed for bats on the most recent NWCO list. [ONGOING]

iii. Wildlife Rehabilitators

1. 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:
    a. Use the USFWS Disinfection Protocol (Appendix A) and isolate all colonial bats.
    b. 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.
    c. Wildlife rehabilitators are encouraged to reference the Reichard Wing Damage Index (WDI), report bat species to SCDNR scoring a

2 or greater (Appendix B), and submit a picture of these bats with outstretched wings to SCDNR. The WDI is *not* a diagnostic tool. Wildlife rehabilitators are also urged not to release any bat with extensive wing damage (score of 3 or higher) as they may spread the fungus to unaffected healthy bats.

d. Wildlife rehabilitators are encouraged to report unusual wing damage (reference Appendix B) and any animals with a WDI of 2 or more along with ID and location information 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 Laura Ellison (ellisonl@usgs.gov). Do *not* treat WDI as an indicator or diagnosis of WNS.

iv. Scientific Research Permittees

1. All Scientific Research permittees who work on bats in South Carolina must follow the guidelines of the USFWS Disinfection 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. Submission of 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 (submission forms in Appendix E; don't ship specimens on Fridays).
2. **SCDNR biologists should collect bats from abnormal die-offs (5+ bats) from unknown causes in 2015 and 2016 and submit those bats to SCWDS via WNS submission forms in Appendix E. Ship bats overnight to SCWDS (Monday-Thursday), only after receiving confirmation from the lab.**
3. Bats encountered with field signs of WNS and/or scoring a 2 or higher on the WDI at sites in South Carolina where WNS **has not been documented** (for sites in SC where WNS **has been documented**, see section VI. A. i. 1 (page 9).
    a. During winter/early spring - November through April
        (i) A total count of all bats at colony/site and photographs of wing damage should be taken in all cases. Taking photos of all bats is recommended when possible, as review of pictures later may show more bats with fungus than what was visible in the field. Take pictures of dead bat(s) and bats with fungus from multiple angles such as the face, feet, outstretched wings, and whole body. These photos should be submitted to SCDNR/USFWS with date, location, animal identification number and species.
        (ii) For species known to be affected by WNS (Table 1), collect 1-5

freshly dead bats of representative species from throughout the hibernaculum. If dead bats are not available for collection and WNS is suspected or the fungus is visible, and if taking tissue from live animals is requested from SCDNR or USFWS for submission to SCWDS, 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. **However, no requests for tissue samples from SCDNR or USFWS are in place currently.**

(iii) For species not known to be affected by WNS (Table 1) collect 1-5 freshly dead bats of representative species from throughout the hibernaculum. 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.

(iv) Keep bat specimens and tissues on freezer pack or refrigerated, and ship within 24-36 hours. If it is not possible to ship within that timeframe, keep frozen until the next shipping opportunity.

(v) Fill out SCWDS form (Appendix E) and email to SCWDS. Be sure to CC form and email any photos with date, location, animal identification number and species to Mary Bunch (BunchM@dnr.sc.gov). Ship bat specimens overnight to SCWDS (Monday-Thursday), only after receiving confirmation from the lab.

b. During fall - August through October

(i) 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. If a species has evidence of severe wing damage (WDI ≥ 2) and is of unknown WNS susceptibility, take photos of wing damage. For specimen and photo submission instructions, see section (iv) and (v) above.

c. During late spring/summer - May-July: it is not necessary to submit bats for WNS testing.

4. Freeze all other dead bat specimens and submit with date, location, county, and species (if known) to the Campbell Museum of Natural History at Clemson University. Fresh bat 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.

a. If submission of the carcass is not possible, dead bats should be properly disposed of by placing the bat(s) in a Ziploc bag, then sprayed with disinfectant (such as bleach, Lysol, or 409), double bagged, and placed in the trash. An alternative to this is to bury the bat carcass at least a foot deep so as not to be excavated by animals.

5. 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 shall notify SCDNR if any bats with visible fungus are received during winter months.

6. 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 Laura Ellison (ellisonl@usgs.gov).

B. Increase Baseline Passive and Active Monitoring

i. Follow standardized protocol below for bat surveys and data collection.

1. A continental program to monitor and track bat populations is currently in review. "A Plan for a North American Bat Monitoring Program (NABat)" will be published by the U.S. Forest Service, Southern Research Station in 2015. See https://www.fort.usgs.gov/science-tasks/2457.

ii. Conduct acoustic baseline surveys (others may be added)

1. Acoustic statewide surveys using high priority, randomized grids determined by NABat map and USGS are to be initiated in 2015. A minimum of 30 routes will be run two times each summer.

2. Continue survey routes in Carolina Sandhills National Wildlife Refuge and Francis Marion National Forest.

3. Conduct Lake Jocassee and Keowee shoreline point counts at selected sites by Duke Energy contractor. [DONE]

4. Continue survey route at Long Cane Ranger District

iii. Continue and/or increase netting or sampling at known hibernaculum, particularly those along our northern border.

1. Continue and/or increase netting or sampling at Walhalla Fish Hatchery.

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

v. Continue and/or increase winter surveys, which will require careful

decontamination of gear as per protocols.

1. Full counts and follow-up counts at Stumphouse Tunnel
2. New mine surveys and initial counts at sites without a vertical component (i.e. no rope work).
3. Entrance or emergence counts at Santee State Park when partners are available.
4. 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 the hibernacula were unsuccessful].

vi. South Carolina Bat Blitz

1. Because South Carolina is now considered a WNS affected state and the site of the SC Bat Blitz varies and may or may not be an affected SC site, please see section VI. B. iv. (page 11) in the post WNS arrival for affected sites section.

vii. Submit all bat data 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 Laura Ellison (ellisonl@usgs.gov).

C. Management or Regulatory Actions

i. South Carolina Department of Parks, Recreation and Tourism (SCPRT) prohibits recreational caving and staff entry to caves on their parks. 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. SCDNR shall provide signage, "Entry Prohibited," for a major southeastern bat (*Myotis austroriparius*) cave system. [DONE]

ii. The United States Forest Service (USFS) has issued an emergency order banning public entry or use of caves and mines. Notify SCDNR Law Enforcement. [DONE]

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

iv. Private mines: SCDNR has mapped over 200 known or potential mine locations (an objective of a State Wildlife Grant project). Unfortunately most of the reported mines or prospects in the piedmont region were no longer extant, or they never had adits or shafts and were placer mines, and therefore provided no underground bat roosts. Of 48 mine sites surveyed in that project, only 9 had an underground component with bats low numbers of tricolored bats present.

v. The 2 best caves known by SCDNR are on SCPRT land and neither is well suited to gating. However, better temperature data needs to be gathered for suitability to *Pseudogymnoascus destructans*.

vi. Response to suspected WNS in caves and mines where WNS **has not been documented** (for caves and mines where WNS **has been documented**, see section VI. C. ii., page 11).

1. 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.

2. See section V. A. iv. (page 5) to follow Scientific Research permittees guidelines for collection and submission of bat specimens.

3. To ensure that gear used in the affected cave is only used in WNS positive caves in the future, double bag all equipment and place in a labeled plastic box.

4. Posting a sign outside of the entrance to the cave or mine that identifies it as a WNS affected cave/mine is recommended.

D. Increase Awareness through Outreach and Education

i. Set up workshop for SCDNR staff and Cooperators. [DONE- see archived webinar at https://connect.clemson.edu/p64123383/]

ii. Coordinate Press Releases with Greg Lucas, SCDNR, to educate the public and update elected officials.

iii. Create an informational SCDNR webpage. [DONE 2010 - see http://www.dnr.sc.gov/wildlife/batswns.html]

iv. Inform the public to report unusual die-offs to their regional wildlife biologists for submission for testing.

v. Work with caving clubs such as the South Carolina Interstate Grotto to assist with WNS education and outreach.

VI. Post-WNS Arrival Response at Sites

A. Continue and Reinforce Spread Prevention Measures

i. Continue to adhere to permit requirements for state or federally listed bats (see section IV., page 3). Follow WNS guidelines laid out in section V. A. for scientific research permittees (page 5), and instead of section V. A. iv. 3 (sites in SC where WNS **has not been documented**), use the following:

1. Bats encountered with field signs of WNS and/or scoring a 2 or higher on the WDI at sites in South Carolina where WNS **has been documented**:

a. During winter/early spring - November through April

(i) A total count of all bats at colony/site and photographs of wing damage should be taken in all cases. Taking photos of all bats is recommended when possible, as review of pictures later may show more bats with fungus than what was visible in the field.

Take pictures of dead bat(s) and bats with fungus from multiple angles such as the face, feet, outstretched wings, and whole body. These photos should be submitted to SCDNR/USFWS with date, location, animal identification number and species.

b. Live bat species known to be affected by (Table 1), should be left undisturbed.

c. For species not known to be affected by WNS (Table 1) collect 1-5 freshly dead bats of representative species from throughout the hibernaculum. 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.

d. Keep bat specimens and tissues on freezer pack or refrigerated, and ship within 24-36 hours. If it is not possible to ship within that timeframe, keep frozen until the next shipping opportunity.

e. Fill out SCWDS form (Appendix E) and email to SCWDS. Be sure to CC form and email any photos with date, location, animal identification number and species to Mary Bunch (BunchM@dnr.sc.gov). Ship bat specimens overnight to SCWDS (Monday-Thursday), only after receiving confirmation from the lab.

2. 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. If a species has evidence of severe wing damage (WDI ≥ 2) and is of unknown WNS susceptibility, take photos of wing damage. For specimen and photo submission instructions, see section d. and e. above.

3. During late spring/summer - May-July: it is not necessary to submit bats for WNS testing.

B. Monitoring Response

i. Follow standardized protocol for bat surveys and data collection (see section V. B. i., page 7).

1. Minimize nonessential research or educational programs without research value that involves handling or disturbance of bats, but continue acoustic surveys of same route(s) for rough population trends.

ii. Cooperate with other states and researchers in gathering samples or monitoring information as requested.

iii. Monitor cave/mine roosts to evaluate survivorship, using methods that minimize stress on roosting bats.

iv. South Carolina Bat Blitz. During this intensive bat survey (a program of the Southeastern Bat Diversity Network), some nets, 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 Disinfection Protocol (Appendix A).

1. Even if "soft" gear such as clothing and soft-sided equipment has been properly decontaminated, participants should not use soft gear or any equipment that cannot be decontaminated if it was used for surveys in a state with suspect or confirmed WNS.
2. Unless it has been properly decontaminated, participants should not use any "hard gear" with hard, non-porous surfaces if it was used for surveys in a state with suspect or confirmed WNS.
3. 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.
4. 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 soft and hard gear may be used in their state.
5. All netting team leaders are responsible for adhering to WNS disinfection 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).

v. Submit all bat data 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 Laura Ellison (ellisonl@usgs.gov).

C. Management or Regulatory Actions

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

ii. Response to suspected WNS in caves and mines where WNS **has been documented** (for caves and mines where WNS **has not been documented**, see section V. C. vi., page 9).

1. 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.

2. See section VI. A. (page 9) to follow guidelines for collection and submission of bat specimens at sites where WNS has been documented.
3. To ensure that gear used in the affected cave is only used in WNS positive caves in the future, double bag all equipment and place in a labeled plastic box.
4. Posting a sign outside of the entrance to the cave or mine that identifies it as a WNS affected cave/mine is recommended.

iii. Response to public calls

1. Determine if there is potential rabies exposure. If so, instruct the caller to contact DHEC state headquarters or their local DHEC office (Phone: 803-896-0640; http://www.scdhec.gov/Health/DiseasesandConditions/InfectiousDiseases/InsectAnimalBorne/Rabies/ContactUs/) to arrange testing of bat(s) for rabies.
2. Ask caller to fill out web-based report form to report any bat sightings in South Carolina (http://www.dnr.sc.gov/wildlife/bats/index.html). This form is used for informational purposes only; it is not monitored on a daily basis and should not be used for emergency reports.
3. 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 bat(s).
4. According to the time of year, determine if bat specimens should be submitted for WNS testing:
    a. August-April: collect and submit bats for testing. Arrange for collection following step 5 outlined below.
    b. May-July: it is not necessary to submit bats for WNS testing. Ask that the caller bring the bat(s) to their local DNR office, using steps a. through g. of section 5 outlined below for proper collection of the dead bat. If the specimen is fresh or is decomposed but the skull is still intact, they should be submitted to the Campbell Museum of Natural History (see section V. A. iv. 4., page 6).
        (i) If the caller is unable to bring in the bat(s), encourage them to take photos of dead bats and email them to Mary Bunch (BunchM@dnr.sc.gov), and dispose of bat(s) according to guidelines following all steps in section 6 outlined below.
5. Steps to take when bat specimens should be submitted for WNS testing:
    a. Before proceeding, double check that 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.
    b. Take pictures of dead bat(s) from multiple angles such as the face, feet, outstretched wings, and whole body.
    c. Use latex gloves to pick up dead bat(s) and do not touch any

equipment with contaminated gloves. Pick up dead bats with the glove on your non-writing hand, and attempt to keep the glove on your writing hand uncontaminated.

d. When picking a maximum of 5 to 6 total bats, choose the most recently deceased bats. Also try to choose bats of different species or age classes if possible.

e. Open two Ziploc bags. Taking care not to contaminate the outside of the bag, use the uncontaminated glove to pick up one bag and use the contaminated glove to place the bat(s) 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.

f. 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.

g. Keep bag on freezer pack or refrigerated.

h. Ship within 24-36 hours. If it is not possible to ship within that timeframe, place the bag in freezer until the next shipping opportunity.

i. Thoroughly wash any clothing and/or gear that come in contact with the bat in hot water greater than 122 °F for 20 minutes.

j. 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).

k. Ship bats overnight to SCWDS (Monday-Thursday), only after receiving confirmation from the lab.

6. Steps for disposal of dead bats if unable to bring to local DNR office:

a. Before proceeding, double check that 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.

b. Using disposable gloves or a plastic bag over your hand, pick up the dead bat.

c. Place both the bat and the bag or disposable glove into another plastic bag. Using a disinfectant such as bleach, Lysol, or 409, spray inside the bag. Securely close the bag.

d. Dispose of the bag with your garbage, making sure that the garbage lid is secured to prevent entry by animals and small children.

e. Thoroughly wash any clothing and/or gear that come in contact with the bat in hot water greater than 122 °F for 20 minutes.

f. 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. Be sure to test a small area of furniture or carpet first to make sure there are no adverse affects.

iv. Develop a web-based report form for the public to report dead/dying bats or bat colonies. [DONE]

v. Evaluate and consider various proposed treatment options as they develop, if necessary.

D. Increase Awareness through Outreach and Education

i. Coordinate Press Releases with Greg Lucas, SCDNR, to educate the public and update elected officials.

ii. Update informational SCDNR webpage [created 2010] at http://www.dnr.sc.gov/wildlife/batswns.html. [ONGOING]

iii. Create a WNS list serve.

iv. Ideally use a bat watch program similar to that of Pennsylvania where the public counts bats exiting known roosts to measure population declines [this would require a set-up of a data file and an online reporting page for the public].

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

VII. Appendices

APPENDIX A: USFWS Disinfection Protocol (June 25, 2012). NOTE: In this document, the fungus currently known as *Pseudogymnoascus destructans* is referred to using the old nomenclature of *Geomyces destructans*.

https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf

National White-Nose Syndrome Decontamination Protocol - Version 06.25.2012

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

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

I. TREATMENTS TO REDUCE RISK OF TRANSFERRING *GEOMYCES DESTRUCTANS*[2]:

Applications/Products:

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

Submersion in Hot Water: Effective at sustained temperatures ≥50ºC (122ºF) for 20 minutes

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

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

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

[2] 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.

[3] 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.

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

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

REMEMBER, the product label is the law!

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

Products must be used in accordance with the label:

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

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

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

II. PLAN AHEAD AND CAVE CLEAN:

Dedicate your Gear: Many types of rope and webbing have not been thoroughly tested for integrity after decontamination. Dedicate your gear to a single cave/mine or don't enter caves/mines that require this gear.
Bag it Up: Bring bags on all of your trips. All gear not decontaminated on site should be isolated (quarantined) in a sealed plastic bag/s or container/s to be cleaned and disinfected off-site.

Before Each Cave/Mine or Site Visit:

1.) Determine *G.d.*/WNS status[5] of the state/county(s) where your gear was previously used.

2.) Determine *G.d.*/WNS status[5] of state/county(s) to be visited.

3.) Determine whether your gear is permitted for your cave/mine visit or bat related activity, as defined by the current WNS case definitions[6] and the flowchart below.

4.) Choose gear that can be most effectively decontaminated [i.e., rubber wellington type (which can be treated with hot water and/or secondary treatment options in section I.) vs. leather boots] or dedicated to a specific location**. Remember, under no circumstances should any gear that was used in a WNS-affected state or region be used in a WNS-unaffected state or region.** Brand new gear can be used at any location where access is otherwise permitted.

5.) Determine if any state/federal regulatory or land management agency addendum or supplemental document[1] provides additional requirements or exemptions on lands under its jurisdiction that supplement the final instruction identified in the flowchart below.

6.) Prepare a "Clean Caving" strategy (i.e., how and where all gear and waste materials will be stored, treated and/or disposed after returning to your vehicle and base area) for your particular circumstances that provides for cleaning and treatment of gear on a daily basis **unless** instructed above to do so more frequently throughout the day.

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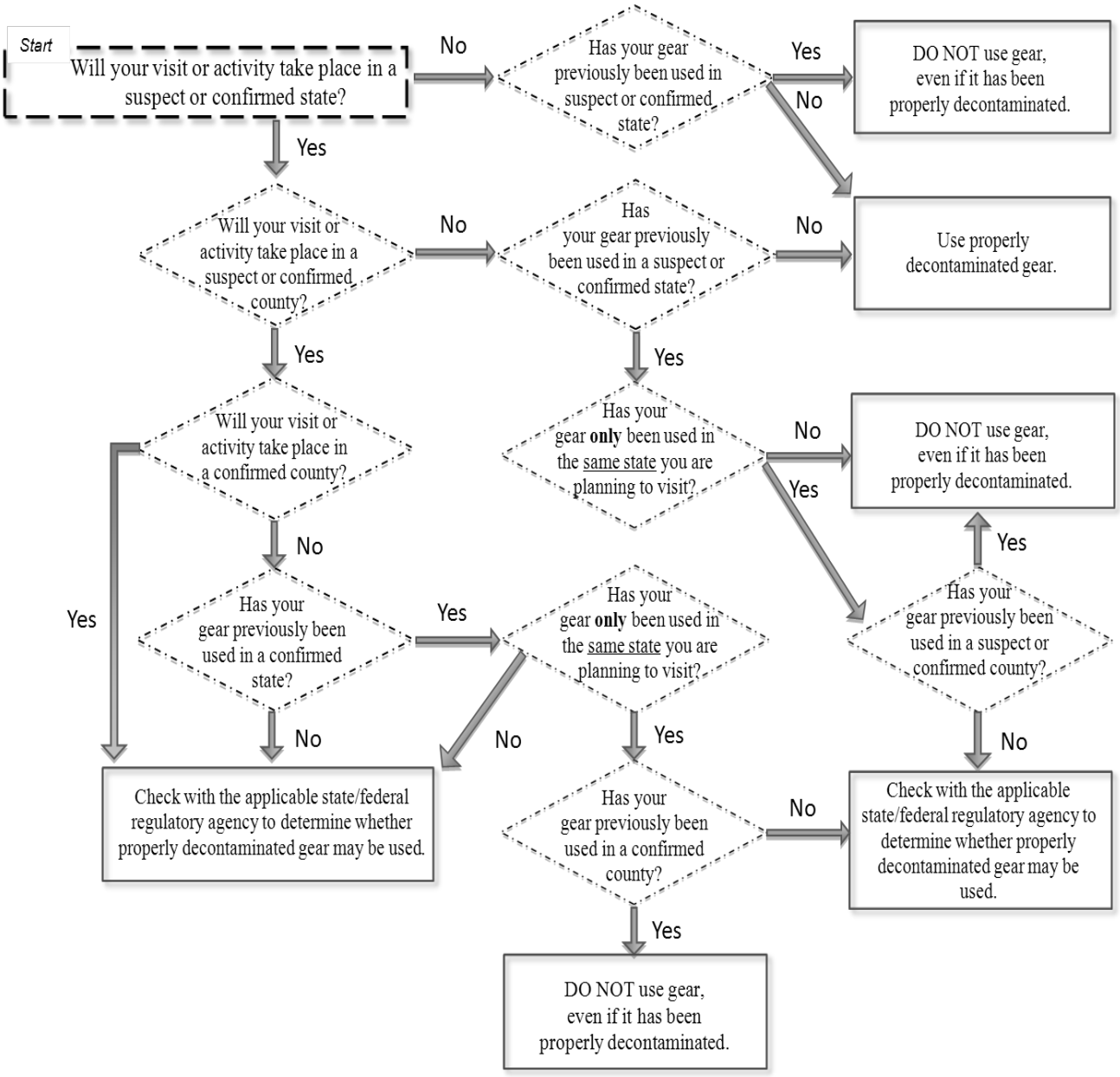
prior to entering cave environments and understand that products and corresponding procedures may cause irreversible harm. Always use personal protective equipment to reduce contact with these products, particularly when recommended by the manufacturer.

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

[6] Visit http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/wns_definitions.jsp for current WNS case definitions.

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

Flowchart to Determine Gear Use or Decontamination



After Each Cave/Mine or Site Visit*:*

1.) Thoroughly scrub and remove sediment/dirt from clothing, footwear, and other gear immediately upon emerging from the cave/mine or bat research site. Avoid contamination of vehicles; store exposed gear separately from unexposed gear.

2.) Once fully scrubbed and rinsed of all soil and organic material, clothing, footwear, and any appropriate gear should be sealed, bagged in a plastic container and once at home, machine or hand-washed/cleaned using a conventional cleanser like Woolite® detergent or Dawn® antibacterial dish soap in water (the use of Dawn® antibacterial dish soap is **not intended** for use in conventional washing machines.) Once cleaned, rinse gear thoroughly in water. Clean/treat gear used in a suspect or confirmed state prior to transport when traveling back to or through a state **without** known cases of *G.d.*/WNS. Use the treatments listed under Applications/Products on page 1 for a minimum of 10 (products) or 20 (hot water) minutes.

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

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

Clothing, footwear, and other submersible gear:

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

B.) Non-submersible Gear:

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

Cameras and Electronic Equipment:

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

3.) Reduce the risk of vehicle contamination and transport of *G.d.* to new areas by making sure to

A) transport gear in clean containers,

B) remove outer clothing/footwear and isolate in a sealed plastic bag or container prior to entering a vehicle. Storage container options vary considerably depending on the type of vehicle; but **always clean and disinfect the outside surfaces of storage containers prior to putting them in the vehicle**.

C) remain outside of the vehicle after exiting a cave/mine or completing field work,

D) change into clean clothing and footwear prior to entering the vehicle, and

E) clean dirt and debris from the outside of vehicles (especially wheels/undercarriage).

OBSERVATION OF LIVE OR DEAD BATS

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

Note on the use of Pesticides/Products listed above:

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. §136 et seq. (1996))
http://www.epa.gov/oecaagct/lfra.html

defines a pesticide as follows:

(u) Pesticide
The term "pesticide" means (in part)
(1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.

FIFRA defines a pest at §136:
(t) Pest
The term "pest" means (in part)
(1) any insect, rodent, nematode, fungus, weed, or (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1).

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

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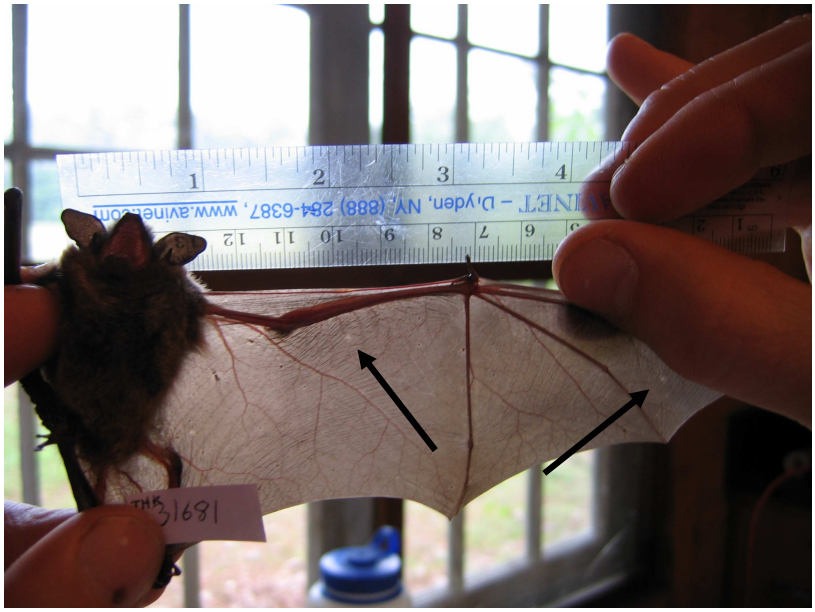
White-nose Syndrome (WNS) is characterized by the growth of one or more species of fungus on the rostrum, ears, and flight membranes of hibernating bats. During the warm months of the year, damage to these membranes may be manifested by the appearance of necrotic tissue, tears, and scars in these membranes.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

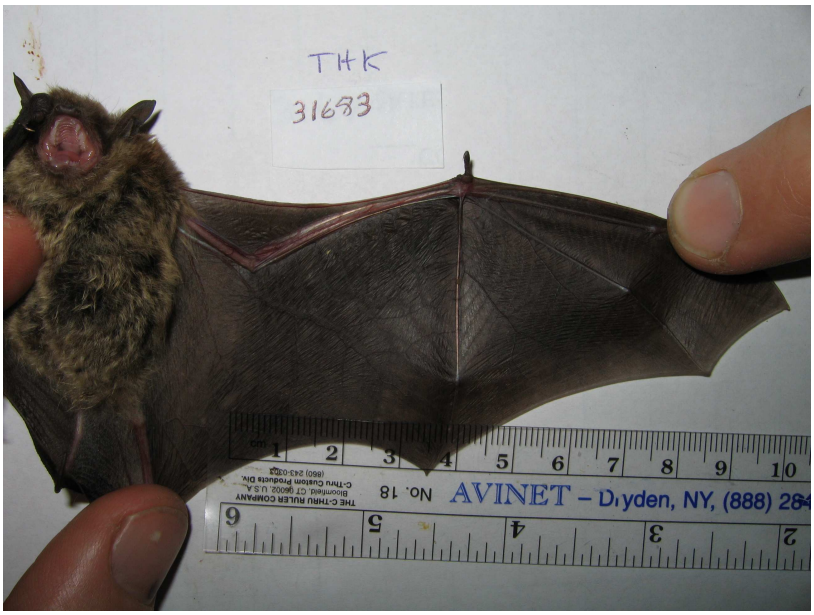
Scoring Criteria:

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.

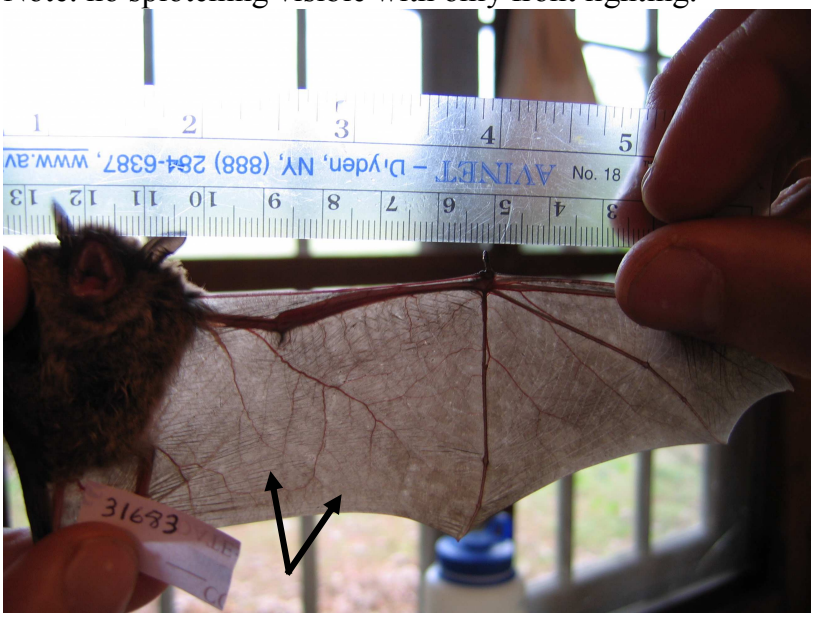
Score = 0 *No damage*. Fewer than 5 small scar spots are present on the membranes. The membranes are fully intact and pigmentation is normal.



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.



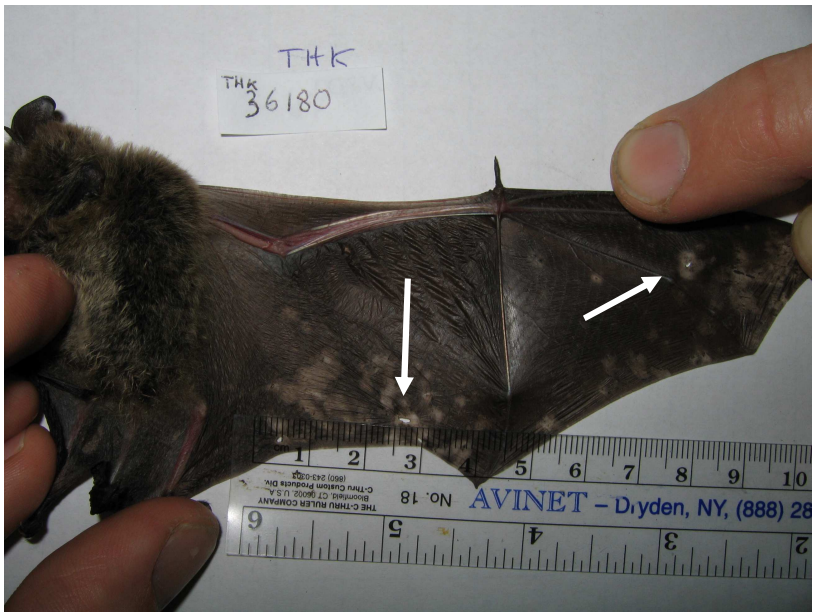
Note: no splotching visible with only front lighting.



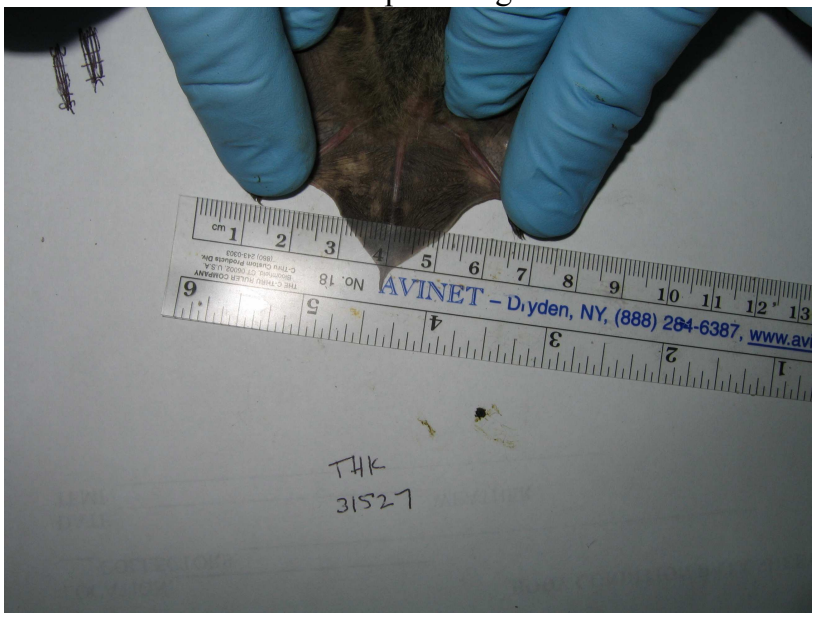
Translumination reveals the splotchy flight membrane.

Forearms may have flaking skin or discolored areas.

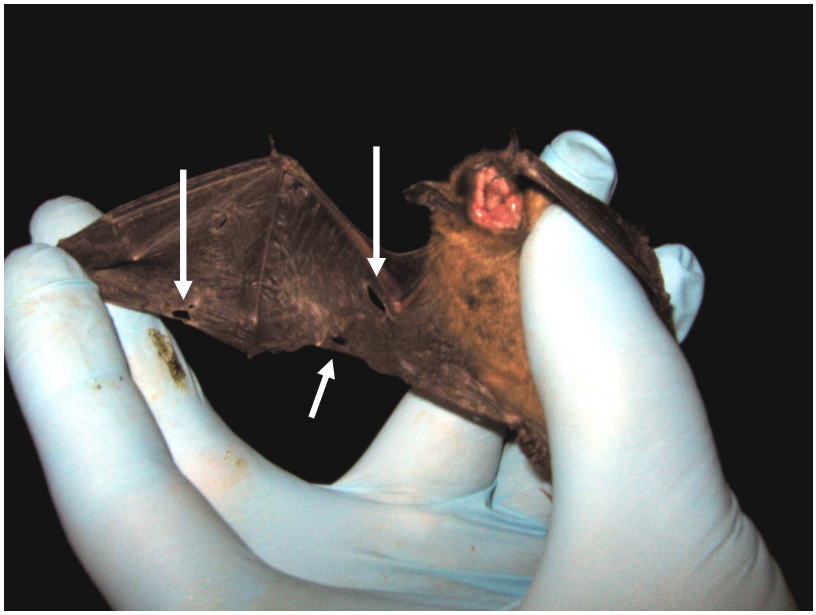
Score = 2 *Moderate damage*. Greater than 50% of wing membrane covered with scar tissue (splotching). Scarring is visible without translumination. Membrane exhibits some necrotic tissue and possibly few small holes (<0.5 cm diameter). Forearm skin may be flaking and discolored along the majority of the 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.

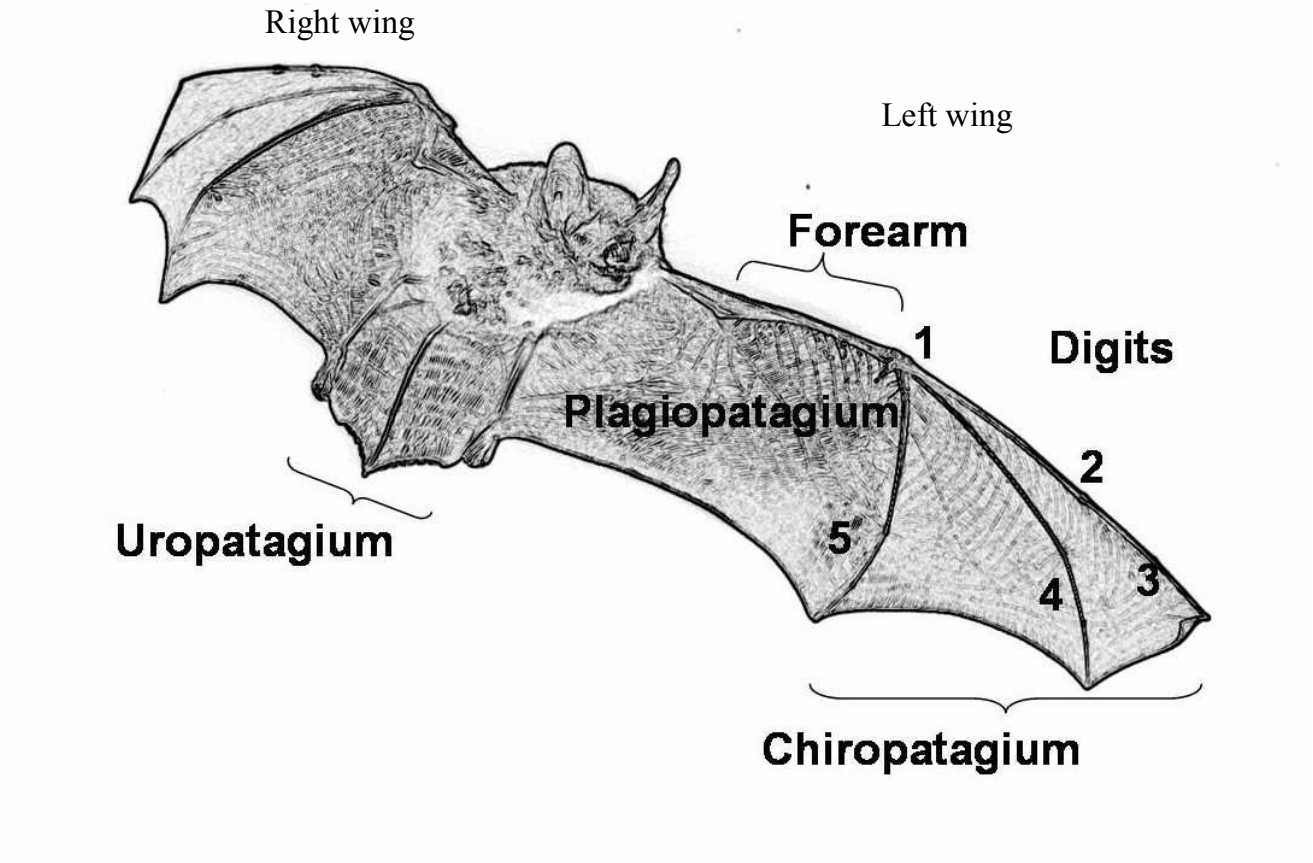
Plagiopatagium loss may be severe.

Physical Damage

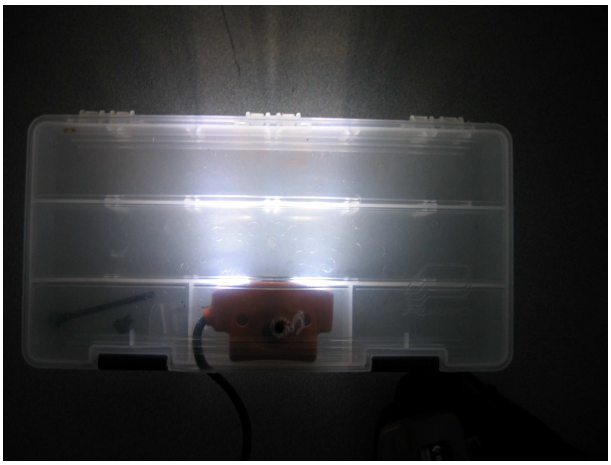
We have encountered bats that have obvious physical damage to wings, but no associated splotching or necrotic tissue. These conditions are important to document as well. We suggest these be recorded in concordance with the above scores followed by a postscript "P" for "physical damage." For example,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

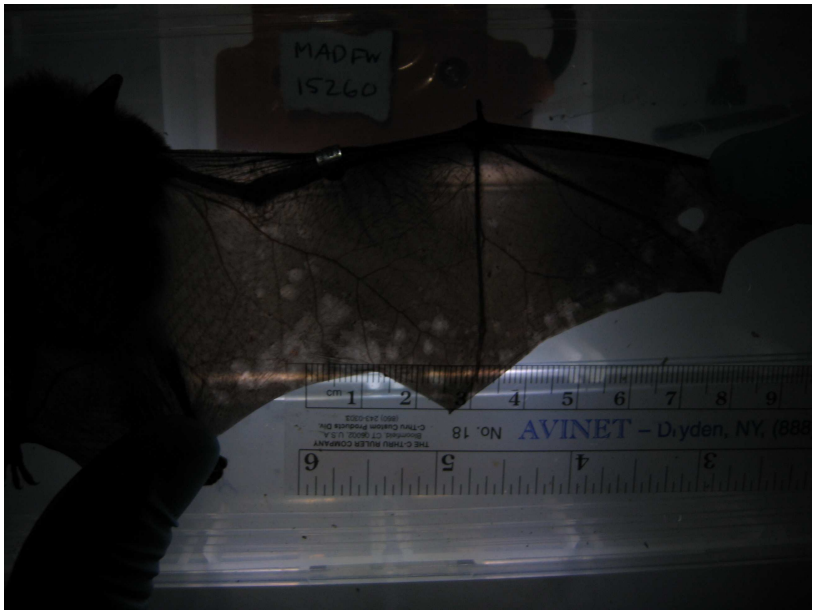
Appendix A: Reference for flight membranes and digits of bats. Image adapted from J. S. Altenbach's photograph of *Myotis thysanodes*.



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.

APPENDIX C: Nonlethal WNS Screening Using UV Florescence

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.

This document can be found at:

http://www.jwildlifedis.org/doi/pdf/10.7589/2014-03-058

DOI: 10.7589/2014-03-058 *Journal of Wildlife Diseases*, 50(3), 2014, pp. 566–573

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

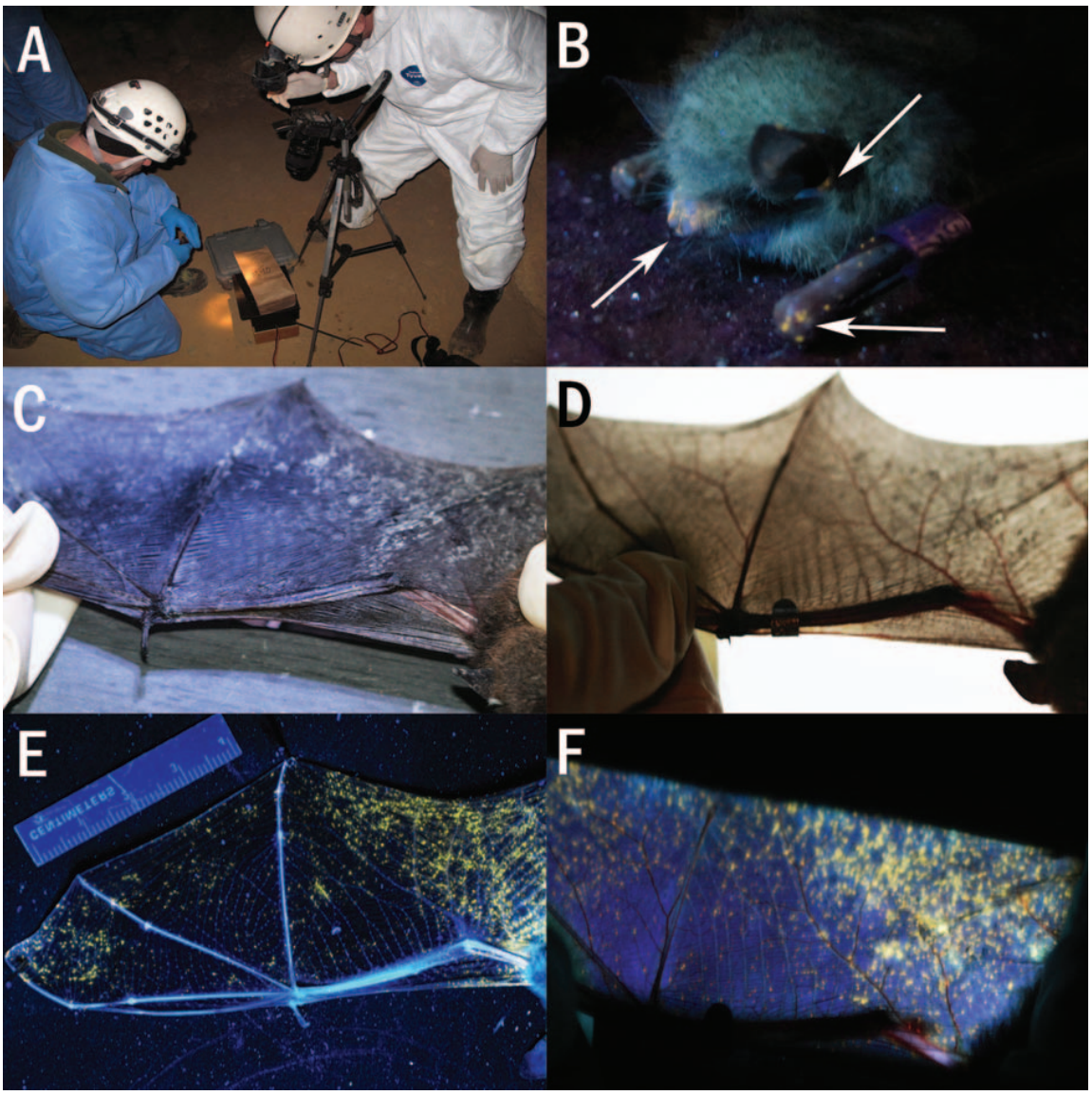

FIGURE 1. Long-wave ultraviolet (UV) and white-light illumination of lesions associated with white-nose syndrome. All photographs are from bats of the US; blurring in photos of live bats in C, D, and F is due to animal movement during long exposure. (A) Camera in cave, mounted on tripod directed at platform 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.

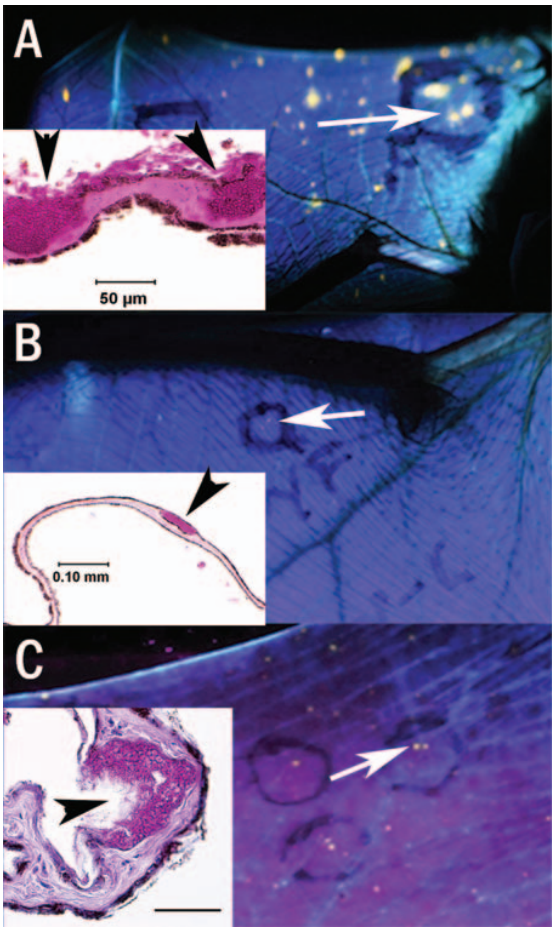

FIGURE 2. Ultraviolet fluorescence in wings of live bats (main images) and periodic acid–Schiff stained histologic sections (insets) of bat-wing skin with lesions diagnostic of white-nose syndrome; blurring in photos is due to animal movement during long exposure. (A) Black circle outlines an approximately 1-cm^2 area of wing from a little brown myotis (*Myotis lucifugus*), Pennsylvania, USA with foci of fluorescence (white arrow). Inset shows the histologic section of this 1-cm^2 area of tissue with densely packed fungal hyphae in cupping erosions (arrowheads). (B) Black circle outlines a 1-cm^2 area of wing from a little brown myotis, Pennsylvania, with a single fluorescent dot (white arrow). Inset shows the only fungal cupping erosion (arrowhead) found in the histologic section from this labeled area of wing membrane. (C) Black circles outline foci of fluorescence on the wing skin of a greater mouse-eared myotis (*M. myotis*) from the Czech Republic (white arrow). Inset (scale bar = 50 µm) shows the histologic section from a 4-mm biopsy sample taken from an area of fluorescence with densely packed fungal hyphae in cupping 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.

ACKNOWLEDGMENTS

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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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APPENDIX 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

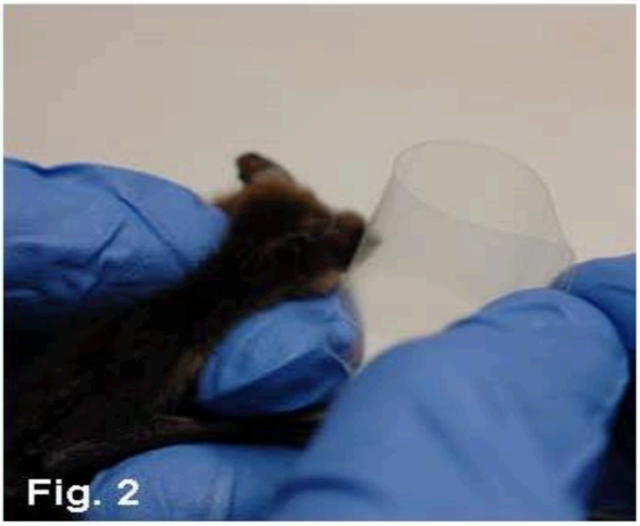


Fig. 2

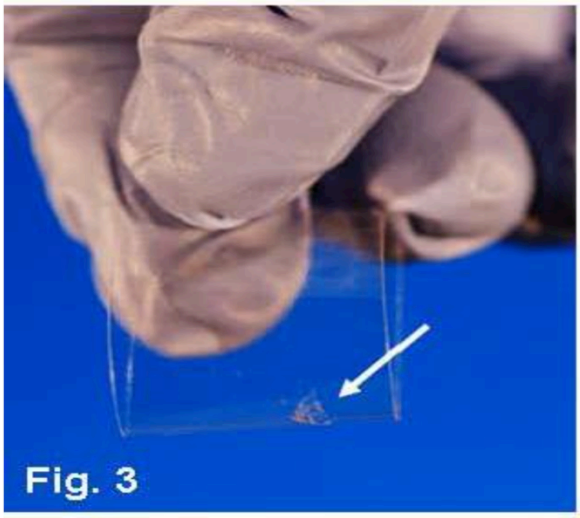


Fig. 3

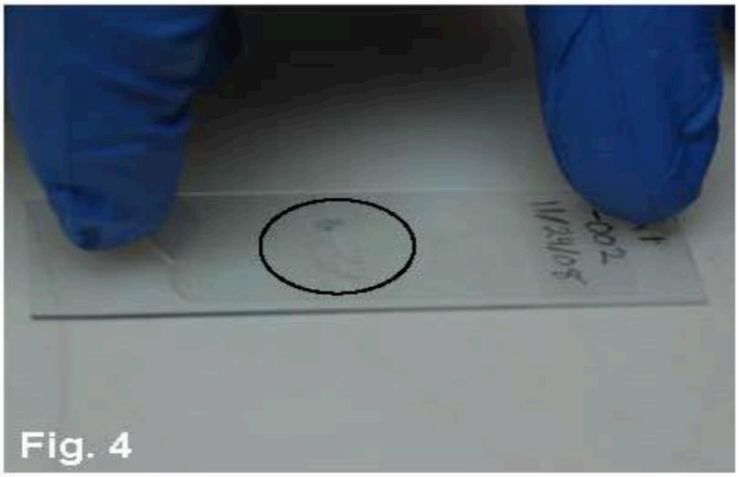


Fig. 4

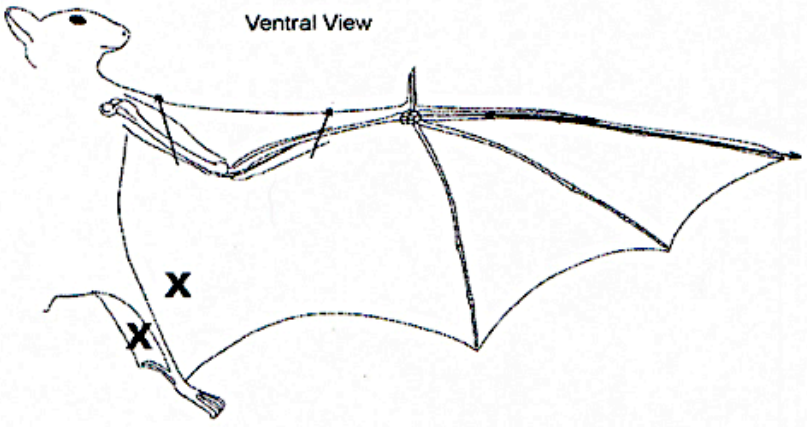
Method 3: Instructions for Taking a Wing Membrane Biopsy

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). <u>If possible, locate an affected area near the body wall</u> 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.

APPENDIX 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