North Carolina's White-nose Syndrome Surveillance and Response Plan

April 21, 2016

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I. This plan was drafted by the NC Wildlife Resources Commission (NCWRC) in consultation with the following groups:

Eastern Band of Cherokee Indians
National Park Service (Great Smoky Mountains National Park and Blue Ridge Parkway)
NC Bat Working Group
NC Division of Parks and Recreation
NC Flittermouse Grotto
The Nature Conservancy's North Carolina Chapter
US Fish & Wildlife Service (USFWS)
US Forest Service
USDA Wildlife Services
Veterinary Public Health, NC Department of Health and Human Services

II. Contacts:

NC Wildlife Resources Commission:
Katherine Caldwell (katherine.caldwell@ncwildlife.org, 828-545-8328)
Kendrick Weeks (kendrick.weeks@ncwildlife.org, 919-609-7605)

US Fish & Wildlife Service - Asheville Field Office:
Susan Cameron (susan_cameron@fws.gov, 828-258-3939, ext 224)

III. Objective

The objective of this plan is to coordinate the conservation community's strategy for addressing White-nose Syndrome (WNS) in North Carolina as it relates to disease surveillance and response, population monitoring, and research.

IV. Surveillance and Monitoring

A. Standard Year-round Procedures:

All biologists conducting bat surveys in North Carolina must adhere to guidance presented in the document "National White-Nose Syndrome Decontamination Protocol – Version 04.12.2016," which appears in Appendix A. These protocols will be updated as new information warrants and can be found at whitenosesyndrome.org.

1) Notify NCWRC and USFWS-Asheville Field Office at the email addresses provided above (in Section II) if signs of WNS are observed.

2) Permit requirements for state or federally listed bats (i.e., endangered, threatened, or special concern; see Table 1):
   a) For state and federally listed species, authorization is needed to collect and possess dead specimens, to handle live bats, and/or to euthanize sick bats.
   b) NCWRC and USFWS–Asheville Field Office will work with all currently permitted researchers and others that are collaborating in the WNS surveillance and monitoring efforts to amend or issue permits to authorize limited collection of state and federally listed species for WNS surveillance in accordance with this plan.
   c) For federally listed species, permit conditions sent with researchers' permits will outline the specific scenarios under which it is acceptable to euthanize a federally listed bat.

3) In the situation where dead bats are not available and live bats must be taken for testing, authorized collection of bats should be done according to current American Veterinary Medical Association (AVMA) guidelines for euthanasia (www.avma.org/issues/animal_welfare/euthanasia.pdf), and submitted as directed below. For more information, please contact NCWRC.

4) Bats that are to be submitted for testing at the Southeastern Cooperative Wildlife Disease Study (SCWDS) Lab should be sent according to procedures and forms provided in Appendix B.

5) Protocol for dead bats that do not need to be sent to SCWDS for WNS diagnosis:
   a) The NC Museum of Natural Sciences should be contacted to determine if the specimens are needed (Lisa Gatens, Curator of Mammals, 11 West Jones St., Raleigh, NC 27601, lisa.gatens@naturalsciences.org, 919 707-9946)
   b) In the case that the NC Museum of Natural Sciences does not need the specimens, bat wing biopsies may be sent to the American Museum of Natural History (Dr. Nancy B. Simmons, Chair, Division of Vertebrate Zoology, Curator-in-Charge, Department of Mammalogy, American Museum of Natural History (AMNH), New York, NY 10024, simmons@amnh.org, 212-769-5483). Wing biopsies should be submitted to AMNH with the form in Appendix C.

Biopsy protocols for the AMNH are provided in Appendix D. The AMNH will supply sample tubes and cover shipping costs. A maximum of 20 specimens per year per species per locality per season can be submitted.

c) Dead bats that are not being submitted for WNS diagnosis or as specimens for a museum should be disposed of properly. If small numbers of bats need to be disposed of, the bat should be placed in a Ziploc bag with bleach, then double bagged and put in the trash. NCWRC will work closely with USDA Wildlife Services to ensure that the appropriate disposal methods are used.

6) Response to report of possible WNS infected site

a) Notify NCWRC and/or USFWS.

b) NCWRC and/or USFWS will investigate the site as outlined in Section VI (Agency Response to Suspected WNS in Caves/Mines), while strictly following the USFWS protocols for decontamination.

c) If a potential WNS infected bat is detected, follow procedures in Sections IV.B and IV.C (Winter/Summer Submission of Bat Samples).

7) All data on bats should be submitted to NCWRC. This will help in tracking long term trends and the potential effects of WNS on bat populations.

8) NCWRC will communicate with Wildlife Damage Control Agents and the Veterinary Public Health program with the NC Division of Public Health (NC DPH) to coordinate surveillance for WNS with other efforts, including rabies surveillance.

9) Researchers working in North Carolina are encouraged to band all bats captured in the normal course of surveillance, monitoring, and/or research efforts in the summer months. Lipped aluminum bands are the preferred type for use on bats in NC. We suggest using 2.9 mm bands on small bats (e.g., *Myotis leibii* and *Lasiurus borealis*) and 4.9 mm bands on larger species (e.g., *Eptesicus fuscus* and *Lasiurus cinereus*).

Table 1. Bat Species of North Carolina: Listing Status & Susceptibility to White-nose Syndrome*

Common Name	Scientific Name	Status**: Federal (State)	Susceptible to WNS
Eastern big-eared bat (coastal plain)	*Corynorhinus rafinesquii macrotis*	SC (SC)	
Rafinesque's big-eared bat (mountains)	*Corynorhinus rafinesquii rafinesquii*	SC (T)	
Virginia big-eared bat	*Corynorhinus townsendii virginianus*	E (E)	
Big brown bat	*Eptesicus fuscus*		✓
Silver-haired bat	*Lasionycteris noctivagans*	(SR)	
Eastern red bat	*Lasiurus borealis*		
Hoary bat	*Lasiurus cinereus*	(SR)	
Florida yellow bat	*Lasiurus intermedius floridanus*	(SC)	
Seminole bat	*Lasiurus seminolus*		

Common Name	Scientific Name	Status*: Federal (State)	Susceptible to WNS
Southeastern bat	*Myotis austroriparius*	SC (SC)	
Gray bat	*Myotis grisescens*	E (E)	✓
Eastern small-footed bat	*Myotis leibii leibii*	SC (SC)	✓
Little brown bat	*Myotis lucifugus*		✓
Northern long-eared bat	*Myotis septentrionalis*	T(SC)	✓
Indiana bat	*Myotis sodalis*	E (E)	✓
Evening bat	*Nycticeius humeralis*		
Tri-colored bat	*Perimyotis subflavus*		✓
Mexican free-tailed bat	*Tadarida brasiliensis*		
* There are currently 7 species of bats affected by WNS in North America ** E=Endangered; T=Threatened; SC=Special Concern; SR=State Rare			

B. Winter/Spring (November-April)

1) A 3 tier system for WNS surveillance and monitoring will be used in North Carolina. NCWRC will determine which survey tier is appropriate for each site
   a) Tier 1: Full Hibernacula Count: Enter hibernacula, check for presence of WNS, and conduct a count to document potential declines.
   b) Tier 2: Rapid Survey: Enter hibernacula and check for the presence of fungus, of bats roosting in abnormal places, etc. A count does not need to be done, but the researchers should have knowledge about the site and thus can give an estimate of the number of bats (close to previous levels, much higher or much lower).
   c) Tier 3: Entrance Survey: Visit the known hibernacula and check for bat activity or bats roosting near the cave entrances. Make sure these visits are on days that would normally be too cold for bat activity. Volunteers can be utilized at many of these sites. As time and resources allow, Anabat detectors could also be set up for more extended Tier 3 entrance surveys.

2) Hibernacula monitoring and surveillance for WNS in winter is prioritized by state biologists according to the following factors:
   a) Sites that are due to be monitored on the rotational schedule
   b) Sites that have federal and/or state listed species
   c) Sites with significant numbers of non-listed bats (particularly the little brown bat and the tri-colored bat, two species that have been hard hit by WNS)
   d) Geographic location (those closest to leading edge are higher priority)
   e) Sites with increased chance of spread by humans
   f) The potential for impacts from disturbance during surveillance and monitoring activities

3) Criteria for winter submission of bats for WNS diagnosis.

a) If field signs of WNS (Table 3) are observed in areas (i.e., sites and/or counties) of North Carolina where WNS has not been documented,
   i) Photographic evidence and a total count should be acquired in all circumstances.
   ii) For species of known susceptibility (Table 1), collect 1-5 freshly dead bats of representative species from throughout the hibernaculum (if available). If dead bats are not available, take non-lethal samples (Appendix E).
   iii) For species of unknown susceptibility (Table 1), collect 1-5 freshly dead bats (if available). If dead bats are not available, humanely euthanize 1 bat on site, based on accepted guidelines, of each non-federally listed species that has obvious visible fungal growth indicative of WNS. When dead bats are not available and it is a federally listed species, take non-lethal samples (Appendix E).

b) If field signs of WNS (Table 3) are observed in areas (i.e., sites and/or counties) of North Carolina where WNS is already confirmed,
   i) Photographic evidence and a total count should be acquired in all circumstances.
   ii) Species of known susceptibility should be released or otherwise left undisturbed.
   iii) For species of unknown susceptibility (Table 1), collect 1-5 freshly dead bats (if available). If dead bats are not available, humanely euthanize 1 bat on site, based on accepted guidelines, of each non-federally listed species that has obvious visible fungal growth indicative of WNS. When dead bats are not available and it is a federally listed species, take non-lethal samples (Appendix E).

Table 3. Field Signs of White-nose Syndrome in Winter/Spring
Excessive or unexplained mortality at/near hibernaculum
Visible fungus on flight membranes, muzzle, and/or ears of live or freshly dead bats
Abnormal behaviors including daytime activity, population shift to entrance of the hibernaculum, altered arousal with disturbance inside hibernaculum
Moderate to severe wing damage in bats*
Thin body condition*

*Nonspecific field sign

Note: not all signs must be present but confidence levels improve with increasing number of signs observed.

C. Summer/Fall (May-October)

1) Through collaboration with partners, continue to collect bat population data from long-term monitoring projects (i.e., mist netting and roost surveys) and when possible, expand monitoring efforts (e.g., establish and coordinate acoustic survey routes) in NC to document bat population changes and possible impacts from WNS. Table 4 is a working list of long term summer bat monitoring sites. In 2011, a total of 32 acoustic bat survey routes were set up in western North Carolina as the pilot year of the North Carolina Bat Acoustic Monitoring Program (NCBAMP). These routes will be run twice a summer by citizen scientist volunteers with the NC Wildlife Resources Commission. In 2015, an additional 32 acoustic survey routes and 24 stationary survey points were implemented statewide by the University of North Carolina at Greensboro as a pilot year of the North American Bat Monitoring Program (NABat) in NC.

2) Delay summer mist netting for regulatory purposes (e.g., presence/absence surveys for listed species) until June 1st.

3) Reichard Wing Damage Index (WDI) should be recorded for all bats captured in NC (see Appendix F).
   a) Bats with score of 0 or 1: release the bats.
   b) Bats with score of 2 or 3: get photo documentation (see Appendix F), then release the bats. If it is a species of unknown susceptibility, see 4c below.

4) Criteria for summer submission of bats for WNS diagnosis.
   a) Respond to reports of unusual numbers of sick or dead bats (typically 5 or more). This includes investigating increased adult and/or pup mortalities at maternity colonies. Collect 3-5 fresh, intact carcasses which are representative of the affected species and send to SCWDS.
   b) In the unlikely event fungal growth is observed on the muzzle, ears, or wing membranes during the summer, photograph and collect non-lethal samples (Appendix E). Send these to SCWDS for testing.
   c) If a species of unknown WNS susceptibility has evidence of severe wing damage (WDI ≥ 2),
      i) In May-June: photograph bats and collect non-lethal samples (Appendix E) from live bats or fresh, intact carcasses and submit them to SCWDS for testing. Do not euthanize live bats solely on the basis of wing damage.
      ii) In July-October: the only action necessary is to take photos of any severe wing damage.

Table 4. Long-term Summer Monitoring Sites in North Carolina

Note: AT=acoustic transect, AS=acoustic stationary, MN=mist-netting, RS= Roost Structure

Site Name	Region	County	Site Type
32 NCBAMP routes in western NC	Mountain	Multiple counties	AT
32 NABat routes across NC	All	Multiple counties	AT
24 NABat stationary sites	All	Multiple counties	AS
Linville River at Pineola	Mountain	Avery	MN
North Harper Creek	Mountain	Avery	MN
Cold Knob/FS 479H	Mountain	Buncombe	MN
FR 496/FR 210 Junction	Mountain	Burke	MN
North Shoals Creek/FS 408	Mountain	Cherokee	MN
Shuler Creek	Mountain	Cherokee	MN
John's Branch/FS 81C	Mountain	Graham	MN
A-0009A - Carver Pond	Mountain	Graham	MN
A-009N (FS 404)	Mountain	Graham	MN
Pigeon River/Twelvemile	Mountain	Haywood	MN
Hurricane Creek	Mountain	Haywood	MN
Little TN River/Hwy 28 Bridge	Mountain	Macon	MN
Nantahala Dam Road	Mountain	Macon	MN
Victor Road Cemetery	Mountain	McDowell	MN

Table 4 continued			
Site Name	**Region**	**County**	**Site Type**
Upper Curtis Creek Road	Mountain	McDowell	MN
Balsam Road	Mountain	Mitchell	MN
Twentymile 2	Mountain	Swain	MN
Nantahala River Bike Path	Mountain	Swain	MN
Alarka Laurel 1	Mountain	Swain	MN
Cherokee Tribal Hatchery	Mountain	Swain	MN
Bunches Creek Gate	Mountain	Swain	MN
Jenkins Creek	Mountain	Swain	MN
Davidson River/Pisgah Center	Mountain	Transylvania	MN
Atkins River	Mountain	Watauga	MN
Upper Neals Creek	Mountain	Yancey	MN
Stratton Meadow	Mountain	Graham	RS
Harmon Den/Hurricane Creek	Mountain	Haywood	RS
Little East Fork	Mountain	Haywood	RS
Dillsboro (Tuckaseegee River)	Mountain	Jackson	RS
Little TN River/Hwy 28	Mountain	Macon	RS
Sandlin	Mountain	Swain	RS
Fontana Lake	Mountain	Swain	RS
Linn Cove	Mountain	Watauga	RS
Howell Woods conservation area	Piedmont	Johnston	AT
Buffalo Creek (multiple sites)	Piedmont	Guilford	MN
R-2527	Piedmont	Montgomery	MN
Eno River Bridge	Piedmont	Durham	RS & MN
Goose Creek	Coastal Plain	Beaufort	MN
Croatan mitigation bank R-1015	Coastal Plain	Craven	MN
Bennett's Creek Site 1	Coastal Plain	Gates	MN
Bennett's Creek Site 2	Coastal Plain	Gates	MN
Bridge # w.o. 6.4220089	Coastal Plain	Bladen/Pender	RS
Bridge at Bladen/Sampson line	Coastal Plain	Bladen/Sampson	RS

V. Management of Caves and Mines

1) The Nature Conservancy, National Park Service, NCWRC, NC Division of Parks and Recreation, and US Forest Service have closed caves and mines in North Carolina. The US Fish & Wildlife Service has issued a cave advisory recommending suspension of activities in caves to protect bats from White-nose Syndrome (http://whitenosesyndrome.org/faq/what-us-fish-and-wildlife-service-recommending-its-cave-advisory), with the exception of agency sanctioned research or monitoring projects.

2) Meet regularly, as needed, with the NC Bat Working Group, Flittermouse Grotto, private cave owners, state and federal agencies, and other organizations to review the status of WNS and cave management in North Carolina.

3) Post signs about WNS and/or USFWS protocols at select sites.

VI. Agency Response to suspected WNS

A. In Caves and Mines:

1) Containment: Research continues on the effectiveness of potential biological control treatments and other containment measures and select North Carolina mines may be considered for future application of such measures. Otherwise, the current plan is to follow the protocols outlined below.

2) Procedure to follow:

a) Investigate extent of potential infection in the cave/mine prior to collecting any samples. Conduct a full count of infected and non-infected bats and assess distribution of WNS throughout cave/mine. Record any unusual bat behavior.

b) Collect samples (see Section IV.B.3). The bats collected should include a representative sample of species.

c) Isolate all gear used in affected cave by double bagging equipment and placing in a labeled plastic box to ensure that this gear is only used in WNS positive caves in the future.

d) Contact NCWRC and USFWS (see Section II).

e) Send bats to SCWDS lab for analysis (see Section IV.A.4).

f) Consider placing WNS affected cave/mine sign outside entrance.

B. In Other Areas:

1) Reports of Suspected WNS from the General Public: Reports of suspected WNS made to the NC Wildlife Resources Commission will be handled according to the flowchart in Appendix G.

2) Procedure to follow:

a) Contact caller, determine if there is potential rabies exposure. If there is potential rabies exposure, get their contact information and then contact the county health department. The health department will coordinate testing of bat(s) for rabies.

b) Fill out the "dead bat reports" spreadsheet for all calls regarding dead or dying bats.

c) If November-April, follow general guidelines in Section IV.B.3 for collection and submission of bats for testing. If May-October, follow guidelines in Section IV.C.4. If the guidelines require collection and submission of the bats, arrange for collection (see steps outlined below in VI.B.2.d); if not, instruct caller to dispose of bats according to guidelines in Section VI.B.2.e.

d) Steps to take when guidelines require collection of dead bats:

   a. When picking up dead bats use latex glove(s) and remember not to touch any equipment with contaminated glove(s).
   b. Take pictures of dead bat(s) from all angles (whole body, face, wing spread, and foot)
   c. Pick the freshest bats and pick different species or age classes if they are apparent (maximum of 5 to 6 total bats)

d. Place bat(s) in a Ziploc bag (do not contaminate outside of bag); Use a sharpie to label bag with your name, date, location, county, and species if known. Then place inside another bag.
e. Put bag on ice (preferably freezer pack) or keep refrigerated until shipping as soon as possible (within 24-36 hours, otherwise put in freezer until next shipping window).
f. Contact Western Wildlife Diversity staff (Katherine and Kendrick) and the Wildlife Diversity Supervisor in your region and send photos (email or phone).
g. Fill out SCWDS form, email SCWDS (Appendix B), and after receive confirmation from lab, ship bats overnight to SCWDS (Monday-Thursday)

e) Steps for disposal of dead bats:
   a. Pick up the dead bat with a plastic bag over your hand or use disposable gloves
   b. Place both the bat and the bag into another plastic bag and spray with disinfectant (such as bleach, Lysol, or 409), then close the bag securely
   c. Dispose of it with your garbage.
   d. Thoroughly wash your hands and any clothing that comes into contact with the bat.

VII. Outreach:

1) Identify key audiences who should be kept abreast of WNS developments and should have basic knowledge of WNS and who to contact if they have questions. Suggested audiences include:
   a) NCWRC Division Chief, Director's Office, and Commissioners
   b) Biologists engaged with bat work
   c) NC Bat Working Group
   d) NC WNS Listserv
   e) Private landowners with caves or important bat populations
   f) Public land managers, including appropriate US Forest Service, National Park Service, and NC State Park staff
   g) Appropriate state and federal elected officials
   h) NC grottos
   i) Amateur geologists (rockhounds)
   j) Key outdoor and environmental journalists
   k) Rehabilitation agents in NC
   l) Rabies lab/animal control/wildlife damage control agents
   m) NCSU Wildlife Extension and College of Veterinary Medicine
   n) Outdoor adventure groups and businesses

2) Develop and/or borrow outreach tools to communicate what WNS is, why we should be concerned, what people should do if bats are discovered showing signs of WNS, and recent developments.
   a) Organize a WNS listserv (already done)
   b) Develop a basic WNS brochure for NC (done, should be regularly updated)
   c) Develop WNS website (or link to USFWS WNS site)
   d) Collect and maintain contact information for all stakeholders

3) Reach out to identified audiences.
   a) Use WNS listserv and NC Bat Working Group to distribute new information about WNS

b) Contact private landowners with caves or important bat populations to make them aware of WNS and what they can do to help control its spread
c) Via e-mail, phone calls, or face to face meetings, keep private landowners, public land managers, elected official staff, grotto leadership, and reporters (e.g., press releases to western NC newspapers) abreast of new developments
d) Contact Wildlife Rehabilitators to share information about WNS and what to do if they are contacted about bats with damaged wings and/or exhibiting unusual behavior or unusual bat morbidity or mortality.
e) Communicate to animal control the signs of WNS and what to do if they suspect WNS
f) Communicate and cooperate with adjoining states (i.e., VA, TN, GA, and SC)

VIII. Plan Review:

1) Update response plan as needed and on an annual basis.
2) Keep informed of high priority research. Assist in specimen collection when feasible and appropriate justification is provided.

IX. Appendices

Appendix A: National White-Nose Syndrome Decontamination Protocol (04/12/2016)
Appendix B: Protocol and Submission Form for SCWDS
Appendix C: American Museum of Natural History (AMNH) Submission Form
Appendix D: Tissue Sampling Protocols for AMNH
Appendix E: Alternate Sampling Methods for P.d. Testing
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Appendix I: NC WNS Decontamination Justification and Guidance

National White-Nose Syndrome Decontamination Protocol - Version 04.12.2016

I. INTRODUCTION

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

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

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

II. PURPOSE:

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

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

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

III. PRODUCT USE:

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

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

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

IV. TRIP PLANNING/ORGANIZATION:

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

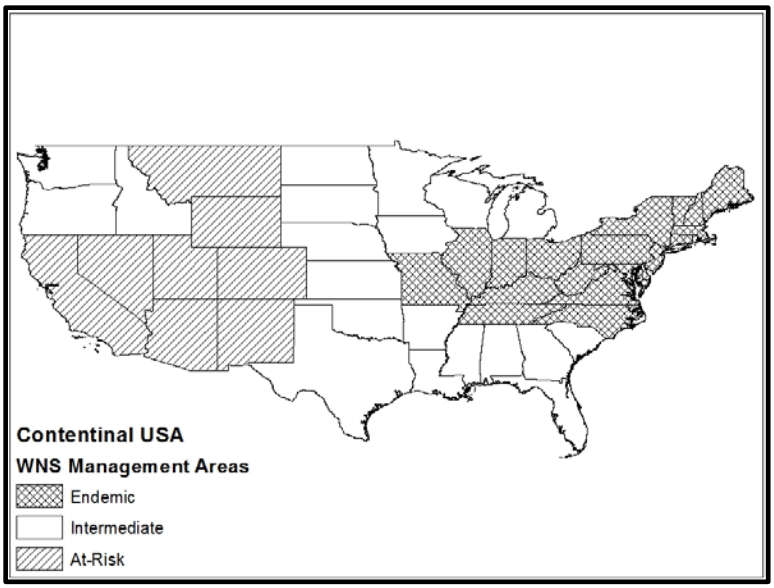


Figure 1. WNS Management Areas by state.

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

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

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

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

After cleaning and decontamination, the following symbols indicate that equipment transfer/movement is:

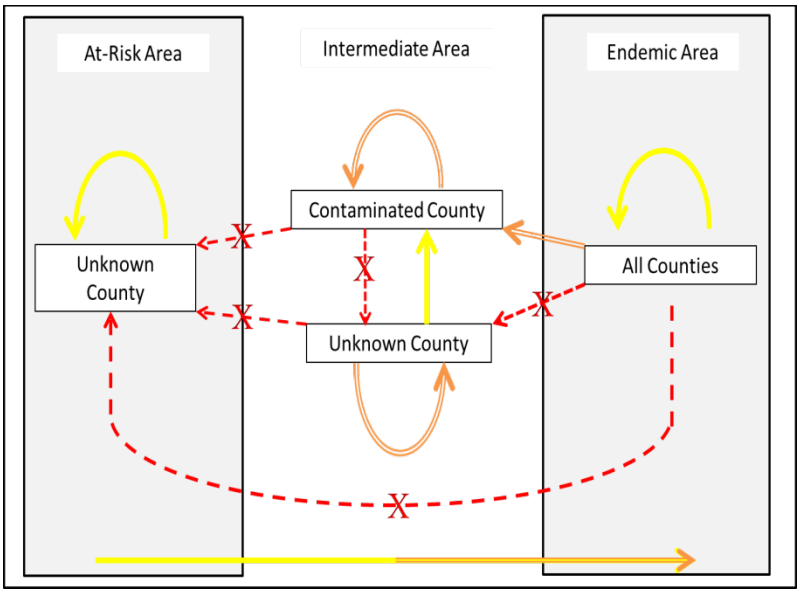
Not recommended

At the discretion of the responsible state/federal land management agency

Acceptable

A. <u>Subterranean</u> Equipment recommendations by WNS Management Area and

<u>COUNTY</u>



B. <u>Terrestrial</u> Equipment recommendations by WNS Management Area and

<u>STATE</u>

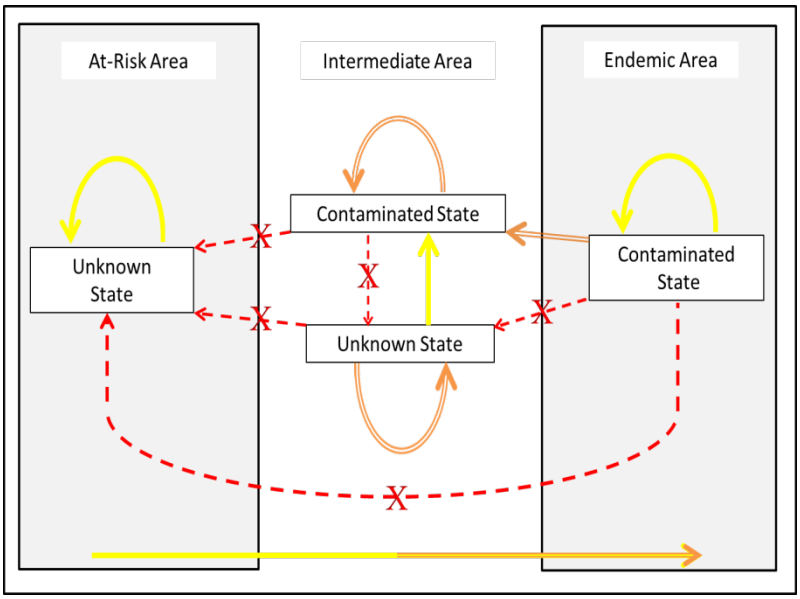


Figure 2. Movement recommendations for decontaminated (A) Subterranean and (B) Terrestrial equipment.

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

V. PROCEDURES FOR DECONTAMINATION:

1.) On site:

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

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

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

2.) Off site:

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

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

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

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

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

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

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

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

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

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

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

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

Products with USEPA registration numbers mitigate persistence of living organisms on surfaces and are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 7 USC 136, et seq.). FIFRA provides for federal regulation of pesticide distribution, sale, and use. Within FIFRA, pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. FIFRA further defines pests as any insect, rodent, nematode, fungus, weed, or any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 25(c)(1). Find more information on FIFRA at: http://www.epa.gov/oecaagct/lfra.html.

VI. EQUIPMENT AND ACTIVITY SPECIFIC RECOMMENDATIONS:

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

A. Clothing & Footwear:

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

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

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

B. Cave/Mine and other Subterranean Equipment:

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

C. Scientific Equipment:

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

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

D. Mist-Nets:

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

E. Harp Traps:

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

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

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

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

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

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

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

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

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

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

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

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

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

Appendix B. Protocol and Submission Form for SCWDS

Protocol:

Collection in field:
For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats. When euthanization is authorized and necessary, please see the AVMA Guidelines on Euthanasia at http://www.avma.org/issues/animal_welfare/euthanasia.pdf.

Collect whole bats, making sure to collect the freshest specimens that are available (intact body, no evidence of scavenging, fur does not pull out easily), for submission to the Southeast Cooperative Wildlife Disease Study (SCWDS). Photographs should be taken as the bats are collected because the appearance of the fungus can change during shipment; these photos should be sent to SCWDS. Bats should be sorted by species and stored individually in zip-lock type bags, and then double bagged and immediately placed on ice until they can be shipped (bring a cooler containing ice into the field to immediately chill carcasses). Note that a blue ice pack container is preferred but frozen water in soda bottles is also acceptable; do not use wet ice. Sample bags should be labeled with 1) date collected; 2) location (hibernaculum, nearest town, county, state); 3) collector name & phone; 4) species; 5) your reference number for that animal; and 6) found dead or method of euthanasia. Group all individually bagged carcasses destined for laboratory shipment in a 2nd clean bag upon exiting the hibernaculum but prior to traveling to the next site.

Storage, Package, and Shipment:
Freezing/thawing impedes isolation of some pathogens and damages tissues. Unfrozen specimens are preferred if they can be sent within 24-36 hours of collection or death. As a general guideline: if you cannot call or ship within 24-36 hours, freeze the animal(s).

To package for shipping, line a hard-sided shipping cooler with a thick plastic bag and place absorbent material inside the bag to absorb any liquids that might leak during shipping. Then place the double-bagged bat samples inside the cooler with blue ice packs or frozen plastic soda and/or water bottles (do NOT use wet ice or dry ice). Place the completed SCWDS Submission Form (below, in Appendix C) and return shipping label in a ziplock bag and tape to the inside lid of the cooler (if you want the cooler returned). Using packing or duct tape, tape the cooler shut around the lid and at each end using a continuous wrap around the cooler.

Ship the package for overnight, next morning delivery on Monday – Thursday only because the lab is not open on weekends. Dr. Lisa Last with SCWDS (or other staff if she's not available) should be notified that samples are being sent (see contact information below).

Notification:
Notifying all staff listed in the table below ensures that someone on duty is expecting a shipment. In the event that the sample is collected and sent by someone other than NCWRC or FWS-Asheville field office, someone in NCWRC (i.e., Gabrielle Graeter, Kendrick Weeks) should be notified when the sample is shipped. SCWDS will be notified that the NCWRC is aware of the submission.

SCWDS is currently evaluating the process for diagnosing WNS, and all parties will be notified of any changes in the current process.

Table: SCWDS personnel and contact information.

Name	Position	E-mail
Dr. Heather Fenton	Wildlife Veterinarian	hfenton@uga.edu
Dr. Justin Brown	Assistant Research Scientist	jubrown1@uga.edu
Jennifer Ballard	Wildlife Disease Diagnostician & Graduate Student	jballard@uga.edu
Dr. Sonia M. Hernandez-Divers	Assistant Professor	shernandez@warnell.uga.edu
Jeanenne Brewton	Administrative Assistant	brewton@uga.edu
Cindy McElwee	Administrative Specialist	cmcelwee@uga.edu

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 is not available 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 Lisa Last by e-mail at lalast@uga.edu.

Bats should be sent to:

Dr. Heather Fenton
589 D.W. Brooks Drive
Southeastern Cooperative Wildlife Disease Study
College of Veterinary Medicine, University of Georgia
Athens, Georgia 30602-4393

Form Updated 9-16-15

Appendix C. AMNH Form

American Museum of Natural History
Central Park West at 79th Street
New York, NY 10024-5192

SPECIMEN TRANSFER FORM

The objects described below have been sold/given to AMNH by:

Name _____ Tel: _____

Institution of Affiliation, if relevant: _____

Address:

Fax: _____

email: _____

To the American Museum of Natural History, Department of Mammalogy. These specimens are hereby transferred with no limiting conditions or restrictions. I hereby represent that I have full right and title to the objects hereby transmitted and authority to dispose thereof.

Specimen # or Number of Specimens with Description:

I collected/obtained the material through legal means from:

If the material was obtained from outside the United States of America, I verify that it was imported into the US by legal mean and I have provided copies of all relevant documentation (permits, field notes, etc.)

If these specimens were collected on State or Federal lands within the US, please attach a letter specifying where and when. Include copies of all permits or relevant correspondence.

Date of Delivery of object(s) to the AMNH: ____/____/____

Seller's/ Donor's Signature: _____ Date: ____/____/____

Curator's Signature: _____ Date: ____/____/____

Gift ☐ Exchange ☐ Purchase ☐ Other ☐

1/1999 rev'd 12/2006

Appendix D. Tissue Sampling Protocols for AMNH

WING PUNCH AND HAIR SAMPLING PROTOCOLS

Tissue and hair samples can be taken from live bats. Follow normal protocols for safe and humane handling of the animals. If you are going to take wing punches or hair samples, plan ahead and make sure you have the necessary equipment.

See http://research.amnh.org/vz/mammalogy/donating-bat-tissue-and-hair-samples-genomic-and-stable-isotope-studies/protocol-donating-specimens for more information on donating samples.

List of Equipment:

Lighter (to flame instruments)
Vials containing storage solution for membrane punches
Empty vials for hair samples
Storage box for vials
Fine-point or tissue forceps
Iris scissors
Biopsy punches (3 mm)
Bottle of alcohol or alcohol swabs for wiping instruments and surface
Latex gloves (optional)

To request vials for storing samples, contact Nancy Simmons (simmons@amnh.org)

Biopsy punches can be obtained from many sources. One source is VWR
http://www.vwrsp.com/catalog/product/index.cgi?catalog_number=82030-344&inE=1&highlight=82030-344

Wing Punches:

Wing punches are small (3mm) circles of skin removed from the wing membrane using a biopsy punch. Based on recaptures of sampled bats, the holes in the membrane usually grows back within 2-3 weeks, so there are no long-term effects. Bats are commonly captured while mistnetting with holes in their wings that are much larger than those inflicted by wing punching, and these holes don't appear to result in a loss of flight ability. When taking tissue from the wing membranes, take the samples from close to the body (between the leg and the fifth digit in the wing); this is thought to minimize the effect on flight performance. Do not punch areas with large blood vessels.

1. Flame the biopsy punch and forcep thoroughly to sterilize them and ensure that no tissue or hair from the last bat remains. The instruments should get hot.

2. Let the instruments cool by placing them on the vial box in such a way that the business ends do not touch anything and therefore remain sterile. If you don't let them cool, you will cauterize the bat's skin when you take the punch, which may prevent proper healing of the hole.

3. Wipe the instruments with an alcohol swab to remove any residue from the flaming and let the instruments dry for a few seconds.

4. Remove the bat from its holding bag and stretch the wing over a flat, hard or semi-hard surface (cutting board, clipboard, binder, cardboard, etc.). While the membrane is stretched, press the punch down onto the membrane of one wing close to the legs (between the legs and the fifth digit), and twist and/or rock the punch slightly until you can tell the punch has gone through the membrane on all sides. There is no need to hammer the punch down through the membrane, and doing so will decrease the life of the punch. Each punch can be reused multiple times (5-40 depending on how hard you are on it), but please use your judgement as to how well the punch is cutting, and dispose of punches as soon as they start to dull.

5. The cut tissue will now be sitting on the surface you punched on, or may be in the hollow portion of the punch. If the wing tissue is still in the punch, use the forceps to extract it. Transfer the membrane to an O-ring vial containing liquid preservative. The tissue tends to stick to the forceps, so you might have to shake the forceps semi-vigorously in the solution in the vial to dislodge the sample, or wipe it off onto the side of the vial.

6. Repeat for the other wing. Place both pieces of membrane from an individual into the same. When finished, please make sure that both pieces of tissue are sitting in the solution. You may have to shake the vial (with the cap on!) to dislodge them from the sides of the vial.

7. Make sure to label all vials with your unique identifier for that bat, the date (day/month/year, with the month written out, e.g., 12/Aug/2001, or Aug/12/2001), bat species, sex, reproductive condition, and age. Please also fill out the data sheet provided with the necessary information. Please do not write on the cap.

8. Between bats, please make sure that you clean the punching surface well, either by flushing with a spray bottle containing alcohol (70-95% ethanol or isopropyl) or wiping down the surface well with an alcohol swab. The goal is to minimize the chances of contaminating future samples.

9. If you ever have the opportunity to collect from dead bats, please collect a decent amount of membrane from each wing (1cm × 1 cm area) and place it in a vial with preservative. Please also take some muscle tissue (it is easiest to take it from the pectoral muscles) and store it in a separate vial with preservative. Take a minimum of a 2 mm^3 piece of tissue (a small cube), but if you can, collect as much as will fit into the vial and still allow sufficient solution to preserve the specimen. Do not overstuff vials; use multiple vials for the same individual if necessary.

Hair Samples:

1. Clean the scissors by dipping in alchol or wiping them with an alchol swab. If you are in doubt as to their cleanliness, flame the scissors as described above under the wing punch protocol. Allow them to cool and dry.

2. Clip a small amount of fur (1 cm × 1 cm area) from the area between the scapulae using scissors. Get as much of the length of the hair as possible, but you do not necessarily have to cut down to the base.

3. Store the hair in an EMPTY vial. Do not put hair into liquid preservative. .

4. Label the vial with your unique identifier for that bat, the date (day/month/year, with the month written out, e.g., 12/Aug/2001, or Aug/12/2001), bat species, sex, reproductive condition, and age. Please also fill out the data sheet provided with the necessary information. Please do not write on the cap.

5. Once finished, please wipe any remaining hair off of the scissors with an alcohol swab. Be very careful to avoid cross-contamination.

Appendix E: Alternate Sampling Methods for P.d. Testing

Method 1: Swabbing Protocol for Bats

Protocol: Swabbing of Bats for Identification of *Pseudogymnoascus destructan*s 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 B)

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

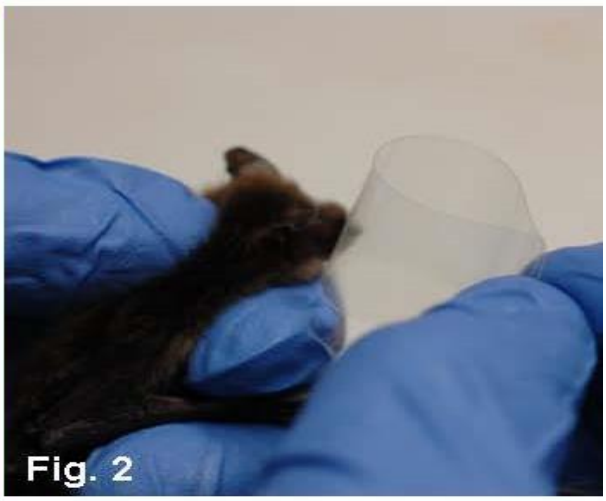


Fig. 2

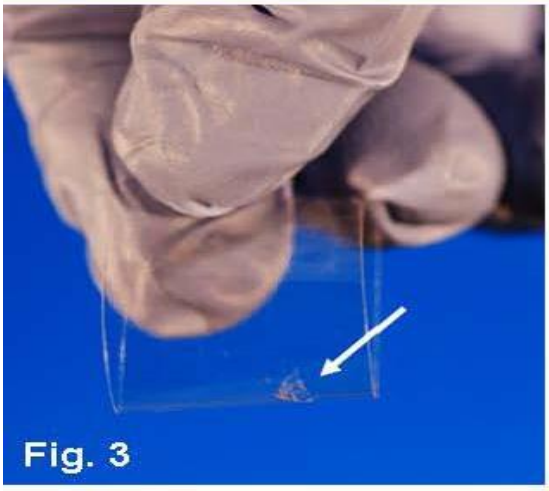


Fig. 3

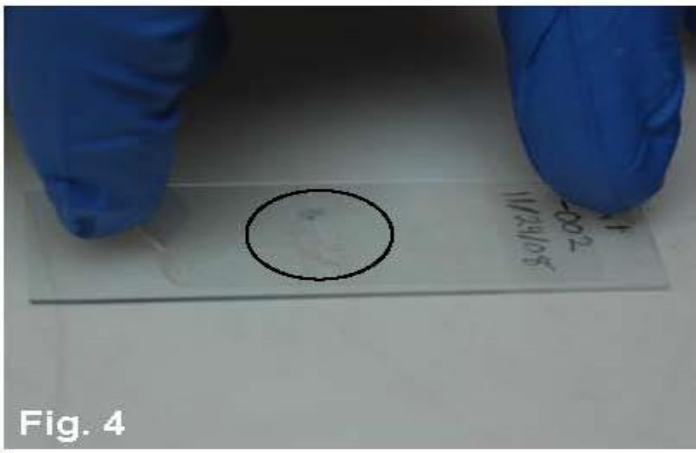


Fig. 4

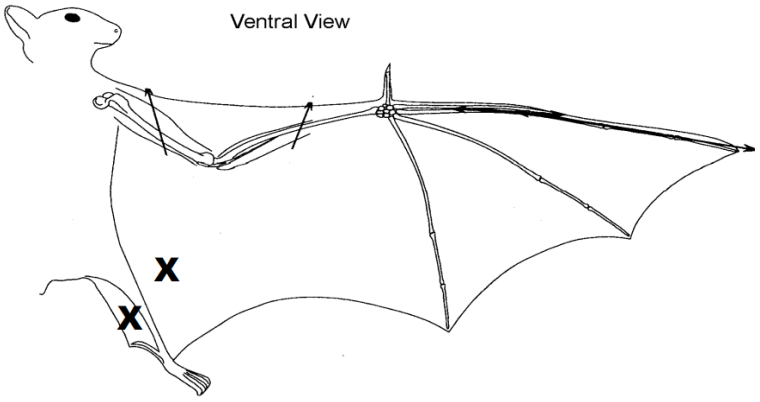
Method 3: Instructions for Taking a Wing Membrane Biopsy

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. destructan*s 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 F. Reichard Wing Damage Index

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White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*)

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White-nose syndrome (WNS) is an emerging disease causing massive mortality of hibernating bats in the northeastern United States. At hibernacula, bats affected with WNS typically exhibit growth of a white psychrophylic fungus (*Geomyces destructans*) on the nose, wings and ears; many individuals seem to prematurely die of starvation owing to depleted fat reserves. Conspicuous scarring and necrosis of the wings on WNS-affected bats that survive hibernation may have lasting consequences for survival and reproductive success during the active season. We monitored two maternity colonies of little brown myotis, *Myotis lucifugus*, in Massachusetts and New Hampshire from 14 May to 8 August 2008 to assess body conditions after expected exposure to WNS over the previous winter. We developed a 4-point wing damage index (WDI = 0 to 3) to assess the incidence and severity of wing damage in the months following emergence from hibernation. Severe wing damage was observed up to 4 June and moderate damage was observed through 9 July. Light wing damage was observed on both adult and juvenile bats throughout the study period, but was not exclusively attributed to WNS. The most severe wing damage was associated with a lower body mass index which may reflect reduced foraging success. Overall, reproductive rate was 85.1% in 2008; slightly lower than reported in previous studies. The incidence, timing, and geographic range of wing damage observed on little brown myotis in 2008 correspond to the occurrence of WNS at hibernacula. Monitoring wing conditions of affected and healthy bats will be important tool for assessing the spread of this disease and for establishing baseline data for unaffected bats. The simple scale we propose should be useful for monitoring wing conditions in any bat species.

Key words: disease monitoring, flight performance, white-nose syndrome, wing damage index, WNS

INTRODUCTION

White-nose syndrome (WNS) is an unprecedented, recently described condition that affects hibernating bats in the northeastern United States (Blehert *et al*., 2009). First reported from Howe Cavern near Albany, New York in February 2006 and in a handful of nearby hibernacula in the winter of 2006–2007, WNS had spread to 37 counties in New Hampshire, Vermont, New York, Massachusetts, Connecticut, New Jersey, Pennsylvania, West Virginia, and Virginia by the end of the winter of 2008–2009. WNS is linked to massive mortality of four hibernating species in the region — *Myotis lucifugus*, *M. septentrionalis*, *M. leibii*, and *M. sodalis*, and expected mortality in two other species — *Perimyotis* (formerly *Pipistrellus*) *subflavus* and *Eptesicus fuscus* (Blehert *et al*., 2009). Local declines at several hibernacula reach 90% in New England (J. Reichard, personal observation; S. Darling, personal communication; T. French, personal communication) and 100% in New York State (A. Hicks, personal communication). WNS is associated with a psychrophilic, or cold-adapted fungus (*Geomyces destructans*) growing on the nose, ears and membranes of hibernating bats (Gargas *et al*., 2009); individuals that succumb to WNS presumably die of starvation owing to prematurely depleted fat reserves during winter. At present, the cause and consequences of this syndrome are not fully understood.

Premature depletion of fat reserves during hibernation has implications that threaten the survival and sustainability of affected bat populations. Upon approaching depletion of critical fat reserves, some bats may emerge and attempt to forage (Turbill and Geiser, 2008) or relocate to warmer microclimates within the hibernaculum, presumably to conserve energy (Boyles and Willis, 2009). Bats may also vacate affected hibernacula prematurely to seek alternate roosts for the remainder of the winter and early spring. In cold climates, these behaviors exact high

energetic costs and risk injuries such as frostbite (Thomas *et al*., 1991). At the end of hibernation, bats rely on their remaining fat reserves to complete migration to summer roosts (Kunz *et al*., 1998). Moreover, females rely on fat reserves for the production of leptin to induce the cascade of other hormones that lead to ovulation and subsequent gestation (Zhao *et al*., 2003). Thus, the adverse impacts of WNS likely extend beyond the hibernation period by limiting spring migration and potentially reducing reproductive success during the summer.

A large proportion of bats leaving WNS-affected hibernacula exhibit varying degrees of scarring, necrosis, and atrophy of flight membranes. Insectivorous bats rely on the unique mechanical properties of their wings to capture prey, evade predators, and to access roosts (Swartz *et al*., 2003). Wings are also important for circulatory regulation (Wiegman *et al*., 1975; Davis, 1988*a*, 1988*b*), thermoregulation (Thomas and Suthers, 1972), gas exchange (Herreid *et al*., 1968; Makanya and Mortola, 2007), and water balance (Kluger and Heath, 1970; Thomson and Speakman, 1999; Bassett *et al*., 2009). Wounds or infections on the wing membranes of bats can adversely affect these properties or functions, and ultimately may affect foraging success. In this way, WNS poses another threat to affected bat populations during the active season.

Our study was designed to characterize the physical damage to wing membranes and to document phenological changes in wing conditions in little brown myotis (*Myotis lucifugus*) at maternity roosts in the spring and summer months following emergence from hibernation. We postulated that bats affected by WNS during winter, but that survived and arrived at maternity roosts with damaged wing membranes, would have poorer body condition than bats with healthier flight membranes. We predicted that bats with the most severely damaged wings may succumb to starvation or predation during the summer. We also predicted that bats affected by WNS would be at increased risk of failed reproduction.

Materials and methods

Study Sites

The study was conducted from 14 May and 8 August 2008 at two maternity colonies of *M. lucifugus* within 60 km of each other in the northeastern US (Framingham, Massachusetts and Milford, New Hampshire). Both sites are within 160 km of Aeolus Cave, East Dorset, Vermont and Chester Emery Mine, Chester, Massachusetts, where hibernating bats experienced high prevalence of WNS in the winter of 2007–2008 and 2008–2009. Thus, the distances between the summer colonies and two highly affected hibernacula are within the putative seasonal migratory range of this species in eastern North America (Davis and Hitchcock, 1965; Griffin, 1970; Fenton, 1970; Humphrey and Cope, 1976). The maternity colonies are located in barns used for hay and household storage and for housing assorted livestock (e.g., chickens, geese, and sheep). The landscape surrounding these sites is composed of mixed hardwood forest, agricultural grassland, and residential communities. These roosts are also inhabited by smaller numbers of the northern long-eared myotis (*M. septentrionalis*), tri-colored bat (*P. subflavus*), and big brown bat (*E. fuscus*). Because *M. lucifugus* is the most common of the species affected by WNS and has a rich history of scientific study in this region, it is an ideal species for the current study. The study period we report spans the early active season of *M. lucifugus* in the northeastern US, extending from arrival at maternity roosts following spring migration to departure for swarming sites and hibernacula in late summer.

Field Methods

Except for two weeks in late June, colonies were visited at biweekly intervals and bats were trapped with double-frame harp traps (0.9 m wide by 1.0 m high or 1.5 m wide by 1.9 m high) placed in a doorway of the barn at dusk (Kunz *et al*., 2009). Other large openings were partially obstructed with coarse nylon nets to increase trapping success. Captured *M. lucifugus* were transferred to and temporary held in individual cotton bags until trapping was complete at the end of the evening emergence period. Other species, when captured, were transported several meters away from the barn and released without further processing. Traps and nets used for blocking alternate exit routes were removed once 60 *M. lucifugus* were trapped or after one hour, to allow bats to return and emerge freely from the barn.

Sex, age, reproductive condition, body mass (M_b), and length of forearm were recorded. Bats were banded with 2.9 mm numbered and lipped alloy bat bands (Porzana Ltd. Icklesham, UK). The wings and uropatagium were inspected by transillumination, using a 3-LED light source (Dot-It, OSRAM Sylvania, Billerica, MA, US). Alternatively, portable light boxes from arts and crafts suppliers provide excellent transillumination of wings (D. Reeder, personal communication). Each bat was assigned a single wing damage index (WDI) to describe scarring and necrosis on the flight membranes (see below). For each bat that was scored with a WDI ≥ 1, we recorded digital photographs of the transilluminated wings (Fig. 1). Wings were photographed on the camera's automatic setting with the flash turned off, by extending the wing on the translucent surface that was positioned above the diffuse LED light source (or portable light box). The identification number (band number) of each individual, the date of capture, and a metric ruler were included in each digital photograph. All methods were conducted in accordance with American Society of Mammalogists Guidelines for the Capture, Handling, and Care of Mammals, Boston University's Institutional Animal Care and Use Committee, and the US Fish and Wildlife Service's Disinfection Protocol for Bat Field Studies.

Wing Damage Index

Five types of wing damage were identified: splotching, flaking, necrosis, holes, and membrane loss (Table 1 and Figs. 1–5).

The wing damage index, described below, is a four-point scale ranging from 0 (no / minimal damage) to 3 (severe damage) for recording the occurrence of these symptoms. After examining both wings and the uropatagium, each bat was assigned a single WDI corresponding to the highest score for which it exhibited one or more types of damage for that level (Table 2). Thus, the WDI is a composite assessment for the wing membranes and uropatagium. Because the severity of forearm flaking, when present, was fairly consistent, other categories of damage characteristic of WDI = 2 and WDI = 3 were considered for assigning these scores.

WDI scores were determined based on the physical conditions of the wings, without consideration of the causes of observed damage. When a cause could be hypothesized (e.g., bites from ectoparasites or tears from assorted environmental hazards) these notes were recorded in addition to WDI.

Analytical Methods

Separate contingency tables were created for adult females and juveniles to test for changes in the relative abundance of

TABLE 1. Wing conditions observed in *M. lucifugus* used for developing the wing damage index (WDI) for assessing the physical condition of flight membranes

Symptom	Description	Example
Spotting, splotching and depigmented membrane	Light spots appear on the darker wing and tail membranes. These spots are often more visible when the membrane is backlit	Fig. 1
Flaking and depigmented forearm	Dry skin appears along the forearm. Some spots appear lighter brown or pink where skin appears to have flaked off	Fig. 2
Necrotic tissue	Membranes may have visible scabs, open wounds, or infections. In more severe cases, large sections of membrane are sloughing from the wing	Fig. 3
Holes	Some very small pin-holes appear to be associated with ectoparasite wounds. Other holes are larger and often surrounded by depigmented or necrotic tissue. The appearance of the edges of holes may be likened to singed nylon	Fig. 4
Membrane loss	Wing areas are notably reduced along edges. Most commonly, the trailing edge of the plagiopatagium is receded in an arc from the leg to the fifth digit. Such damage may be severe, greatly reducing the overall surface area of the wings	Fig. 5

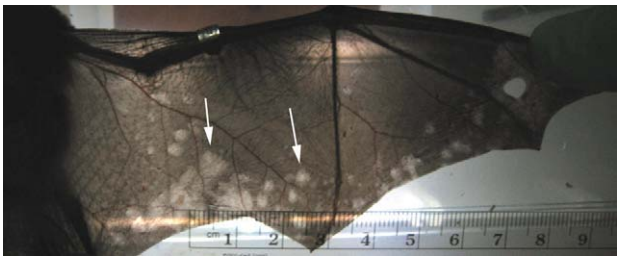

FIG. 1. Spotting, splotching, and depigmented tissue associated with scarring on wings of *M. lucifugus*

FIG. 2. Depigmentation and flaking skin along the forearm of *M. lucifugus*

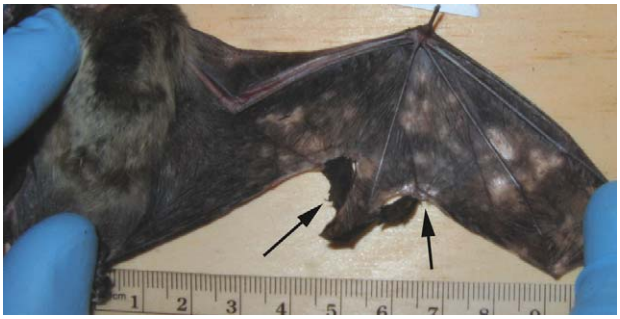

FIG. 3. Necrotic tissue and sloughed membrane on *M. lucifugus*

FIG. 4. Small holes surrounded by necrotic tissue and spots on *M. lucifugus*

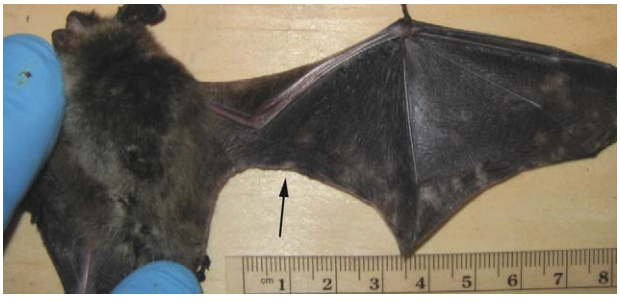

FIG. 5. Loss of flight membrane on *M. lucifugus*

TABLE 2. Criteria used for the wing damage index (WDI) to assess bat flight membrane conditions. Each bat received the highest WDI for which it exhibits one or more of the indicated conditions for that level. The WDI score is recorded as a single composite score for both wings and the uropatagium, as a whole

	Wing condition	Condition			
		Spots / splotches	Discolored / flaking forearm	Necrotic tissue	Holes	Membrane loss
WDI = 0	No damage / Minimal damage	≤ 5 small spots visible with trans-illumination	Not present	Not present	No holes, or possibly very small pin-sized holes	Fully intact
WDI = 1	Light damage	Present on < 50% of flight membranes	Present	Not present	No holes, or possibly very small pin-sized holes	Fully intact
WDI = 2	Moderate damage	Present on > 50% of flight membranes	Present (this condition alone scores WDI = 1)	Few areas of necrosis	Small holes < 0.5 cm diameter – often associated with necrotic tissue	Necrosis on edges of patagium, but no loss of membrane area Tears < 1cm
WDI = 3	Severe damage	Present on > 90 % of flight membranes	Present (this condition alone scores WDI = 1)	Abundant necrosis	Large holes > 0.5 cm diameter – often associated with necrotic tissue	Noticeable loss of membrane, often along trailing edge of plagiopatagium Tears > 1 cm

bats with different WDI over time. Body mass index (BMI = M_b (g) / length of forearm (mm)) was calculated for adult females and for juveniles captured up to 9 July (when WDI ≥ 2 was last observed) to compare relative body conditions among WDI scores with a Kruskal-Wallis test. Reproductive rate of each colony was estimated by maximum percentage of adult females that were pregnant on a given sample night.

RESULTS

A total of 603 *M. lucifugus* were captured between 14 May and 8 August 2008. Pregnant females were captured in the greatest proportions on 28 May in Framingham (89.2%) and 4 June in Milford (81.1%). Mean M_b was 8.6 ± 1.0 g for pregnant females (n = 91), 7.6 ± 0.9 g for nonpregnant adult females (including undetectable pregnant females in early summer; n = 338), 6.8 ± 1.0 g for adult males (n = 8), and 6.6 ± 0.6 g for juveniles (n = 166). Volant juveniles were first captured on 2 July in Milford.

Bats with WDI ≥ 1 were captured on each sampling night. For adult females, the incidence of different WDI scores was not independent of date (G = 107.96, $d.f.$ = 27, P < 0.001 — Fig. 6). Relative abundance of bats with obvious wing damage peaked in June when more than 60% of bats in the colonies had WDI ≥ 1. Bats with WDI = 3 were most prevalent in May and were not observed after 4 June. Bats with WDI = 2 were not observed after 9 July. The incidence of different WDI scores for juveniles was not independent of date (G = 12.05, $d.f.$ = 5, P < 0.05 — Fig. 7). Juveniles exhibited WDI ≤ 1 throughout the study period; wing damage on juveniles was most abundant from late July to early August when about 20% of juveniles had WDI = 1.

Body mass index (BMI) differed among WDI scores for adult females (χ^2 = 15.04, $d.f.$ = 3, P < 0.01, Kruskal-Wallis test) (Fig. 8). Median BMI (range) was greatest for bats with WDI = 0 (n = 173) and WDI = 1 (n = 108), being 0.22 g/mm (0.17–0.29 g/mm) and 0.22 g/mm (0.16–0.31 g/mm), respectively. Median BMI was 0.20 g/mm (0.16–0.28 g/mm) for adult female bats with WDI = 2 (n = 29) and 0.19 g/mm (0.15–0.20 g/mm) for WDI = 3 (n = 6). BMI did not differ among juveniles with different WDI (χ^2 = 0.01, $d.f.$ = 1, P = 0.92, Kruskal-Wallis test); median BMI was 0.17 g/mm (0.14–0.23 g/mm) and 0.17 g/mm (0.17–0.20 g/mm) for juveniles with WDI = 0 (n = 152) and WDI = 1 (n = 16), respectively.

Of the 603 bats captured, 549 bats (380 adults, 166 juveniles) were banded. However, all adult bats that were recaptured were initially banded on or

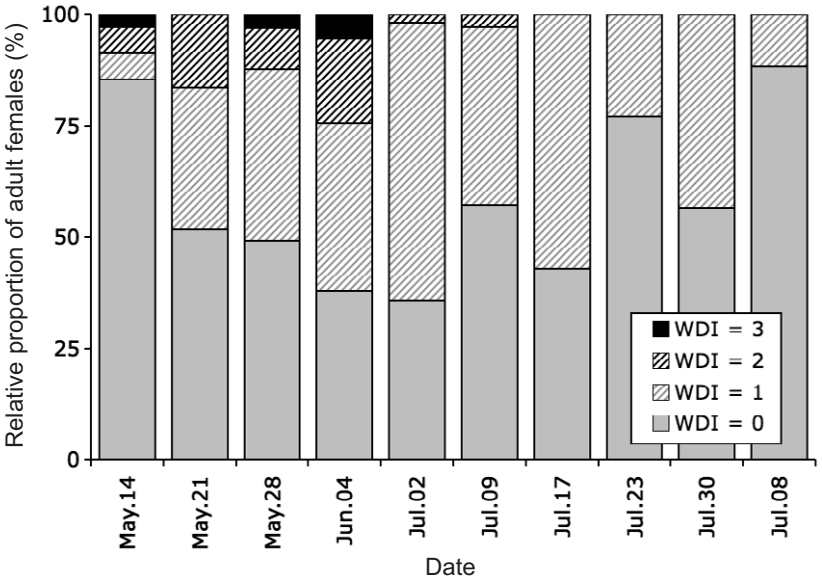

FIG. 6. Relative proportion of adult female *M. lucifugus* exhibiting various degrees of wing damage (WDI) at summer maternity colonies in the northeastern US

before 9 July. Thus, of 362 adult bats banded up to that date, 34 (9.4%) were recaptured. Recapture rates differed among wing damage scores with borderline significance (G = 6.89, *d.f.* = 3, *P* = 0.08 — Table 3). Wing conditions of only three recaptured bats improved over the study period; one from WDI = 2 to WDI = 1 and two from WDI = 1 to WDI = 0. All other recaptured bats had the same WDI as recorded at the time of initial capture.

DISCUSSION

Damaged wings may lose surface area, elasticity and dexterity, thus compromising maneuverability and foraging success (Arita and Fenton, 1997). If their flight abilities were compromised during the active season, bats would be less likely to achieve sufficient energy and nutrient intake to sustain gestation and lactation. Increasing severity of wing

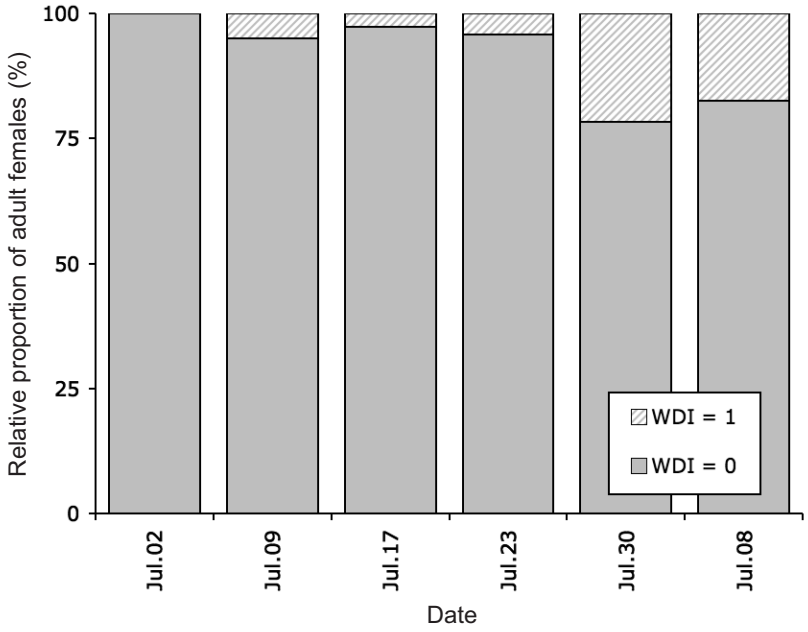

FIG. 7. Relative proportion of juvenile *M. lucifugus* exhibiting various degrees of wing damage (WDI) at summer maternity colonies in the northeastern US

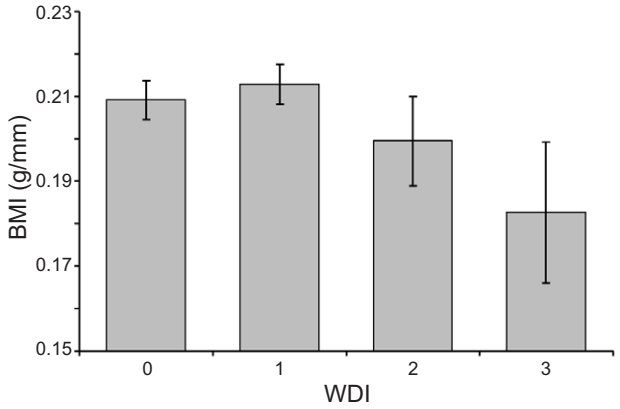

FIG. 8. Mean body mass index [BMI = M_b (g) / forearm length (mm)] of adult female *M. lucifugus* with different wing damage indices (WDI) at summer maternity colonies in the northeastern US from 14 May to 9 July 2008. Error bars are 95% confidence intervals

damage was associated with poorer body condition, suggesting foraging success may have been compromised. Moreover, reproductive rate in the current study (~85%) was slightly lower than previously reported (> 93%) for *M. lucifugus* (Humphrey and Cope, 1976; Reynolds, 1998). Although wing damage, low body mass, and a decline in reproductive success may result from many possible factors, including, but not limited to WNS, this study reveals an unexpectedly high prevalence of wing damage on little brown myotis in the affected range of the recent syndrome. Further research is needed to clarify the connection between WNS and wing damage and to fully quantify the impact that wing damage during spring and early summer has on subsequent reproductive success and survival.

Numerous dead bats were found on floors of barns and surrounding landscapes during this study period (J. Reichard, personal observation). Unfortunately, these were in various stages of decay that prevented accurate assessment of WDI or BMI. However, we expect that wing damage led to poorer survival of affected bats during the active season. Reduced flight performance of bats would compromise foraging success and make them more vulnerable to predators and other environmental hazards (Norberg and Rayner, 1987; Norberg, 1998). We suggest that the decrease in proportion of captured bats with WDI ≥ 2 into early July likely reflects either fatalities or emigration rather than recovery from damage. Mean M_b of pregnant females in 2008 was lower than for pregnant females in 1995 (9.69 g), before WNS had been reported (Reynolds and Kunz, 2000). While it is possible that poorer body condition in the summer of 2008 is associated with reduced insect abundance or other factors not measured in this study, we predict that it is more likely associated with WNS exposure in winter and wing conditions or foraging success in spring and summer. Bats that survive hibernation at affected sites may be unable to fully recover from emaciated conditions. Moreover, poor body condition may continue through the swarming and prehibernation fattening period. If the wing damage experienced by little brown myotis compromises their ability to recover lost energy and nutrient reserves incurred during pregnancy and lactation, then we can expect that these compounding factors directly and indirectly associated with WNS will lower their survival.

Wing Damage and WNS

In most cases, light wing damage (WDI = 1) on adult bats occurred in similar locations on the wings to more severe damage (WDI > 1). However, since BMI for these bats was not significantly different from bats with WDI = 0, we do not expect that light wing damage affects foraging success. It is important to note that some wing damage is likely to occur independently of WNS-related infections, and light damage may reflect 'normal' wing conditions. Documenting wing conditions at control sites not affected with WNS will elucidate the incidence and impact of wing damage in affected populations.

Bats occasionally sustain injuries from agonistic encounters with conspecifics, would be predators, and environmental obstacles in roosts and in foraging areas. Although such injuries may be acknowledged (Sachanowicz *et al*., 2006), they are probably underrepresented in the published literature (but, see Davis, 1968). Exceptions include investigations of injuries caused by wing bands (e.g., Kunz and Weise, 2009). Rapid regeneration time of damaged wings may be triggered by naturally occurring injuries to membranes or from taking wing biopsies

TABLE 3. Banding and recapture rates for adult *M. lucifugus* banded up to 9 July grouped by wing damage index (WDI) during the first capture. The bats banded up to 9 July included all adultbats recaptured through the entirety of the study

WDI	Bats banded before 9 July	Recaptured bats (%)
0	213	15 (7.0)
1	111	17 (15.3)
2	33	2 (6.1)
3	5	0 (0)
Total	362	34 (9.4)

that may heal in less than four weeks (Worthington Wilmer and Barratt, 1998), but may be delayed by bacterial or fungal infections of wounded tissue. Although damaged membranes are capable of healing, greater than 80% of recaptured bats that initially scored WDI $\geq$ 1 showed no obvious change in wing conditions. Thus, we expect that reduced abundance of bats with severe and moderate damage (WDI $\geq$ 2) as the summer progressed may be due to death from starvation or predation. Alternatively, bats with severe wing damage could have emigrated from maternity roosts if their conditions prevented successful pregnancies. The rate and extent to which wings of free-ranging bats recover following injury are not well understood and deserve further study.

Most of the scarring observed in the present study was markedly different from wounds inflicted by environmental obstacles and far more abundant than has been previously reported. The location of scars and necrotic tissue on active bats captured in spring and early summer is consistent with areas of fungal growth observed in hibernating *M. lucifugus* in the winter of 2007–2008. Histopathologic investigation of wing injuries on bats captured outside of WNS-affected hibernacula has linked fungal infection to severe inflammatory responses and sloughing of serocellular crusts containing hyphae of *Geomyces* sp. (Meteyer *et al*., 2009). Moreover, the timing and geographic distribution of wing damage is consistent with the known geographic range of WNS. Thus, it is likely that the scars and necrotic tissue observed in *M. lucifugus* in the summer of 2008 are consequences associated with WNS. We suggest that most of the wounds and scars observed on bats at summer colonies are a direct consequences of exposure to *G. destructans* causing fungal infection, associated bacterial infections, or necrosis resulting from frostbite incurred at times when bats flew outside hibernacula during subfreezing conditions. Bats observed flying during extreme cold periods near WNS-affected hibernacula may also be prone to collisions with trees, rocks, and buildings, and freezing, thus risking further injury to flight membranes.

Wing damage is not limited to bats exposed to WNS. For example, Davis (1968) reported 28 of 63 pallid bats (*Antrozous pallidus*) exhibited varying degrees of wing damage. The gleaning behavior of this species makes it more likely to encounter thorns and cactus spines, or suffer bone fractures than aerial insectivores. Juveniles of *M. lucifugus* in the current study also showed varying degrees of light scarring on the wings, but they had not previously hibernated at sites affected by WNS. We expect that many of these spots were caused by bites from ectoparasites (e.g., mites), a condition that, in another study, did not seem to effect flight performance (Fenton, 1970).

The recent emergence and spread of WNS has drawn special attention to wing conditions, both within and outside of the affected geographic range. Bat researchers and wildlife managers studying and monitoring WNS should record wing conditions to determine the impact wing damage has on bats during the active season. Researchers and managers not directly involved in WNS research will also benefit from recording WDI to establish a baseline for wing damage in healthy populations. Early detection of changes in wing conditions in these populations will be critical for assessing the future spread of WNS. Although the vector or mode of transmission of *G. destructans* has not been determined, hypotheses suggest that movements of bats among roosts and differential degrees of sociality may lead to transmission at summer roosts. Thus, dispersal of bats from the WNS-affected hibernacula may explain the continued spread of the syndrome beyond its current range. This protocol for monitoring wing damage provides a standard for quantifying wing damage quickly and consistently among different researchers.

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Appendix G. NCWRC Contacts for Bat Calls

Bat Calls to Headquarters

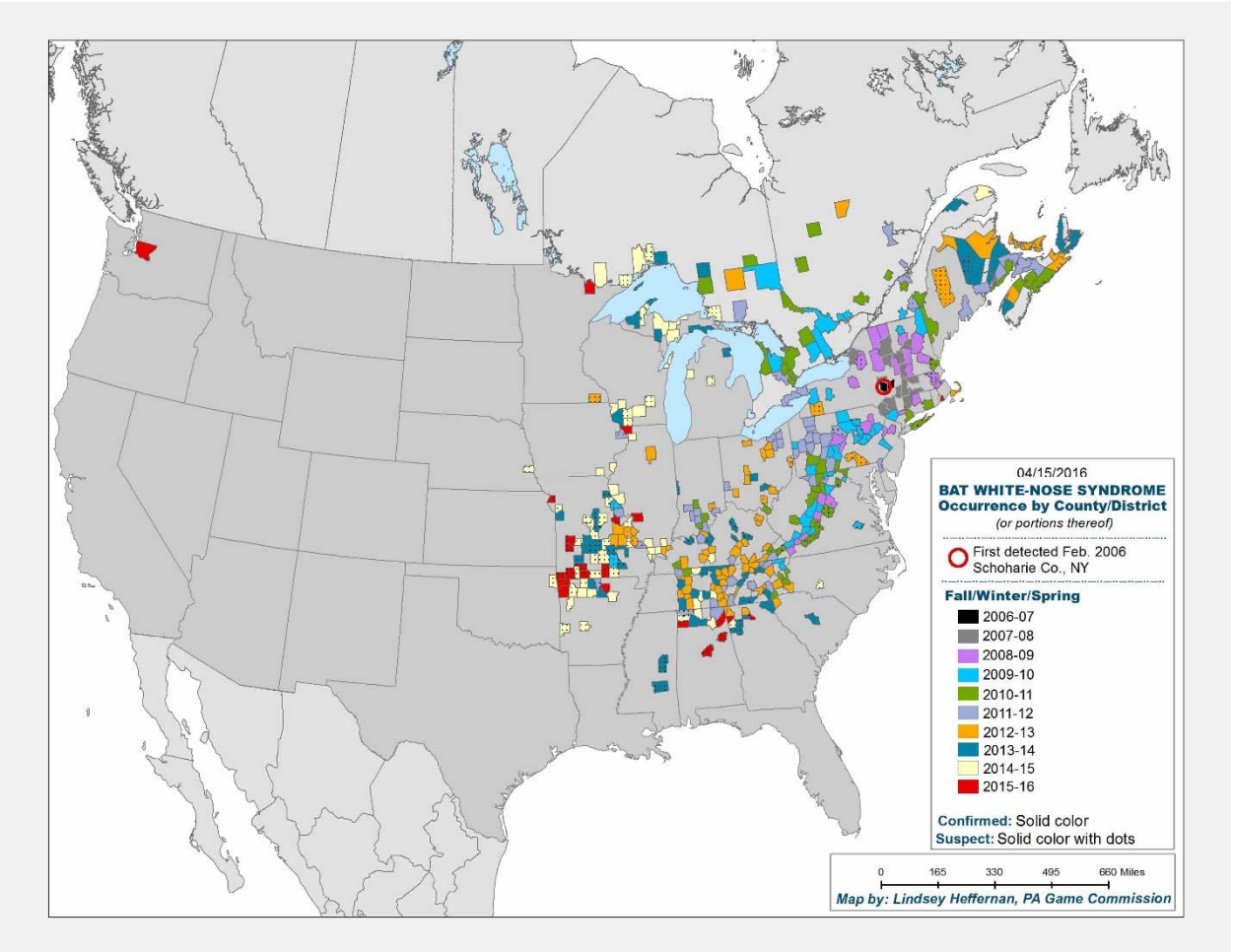
- Forward caller to Katherine Caldwell (828-545-8328, katherine.caldwell@ncwildlife.org)

Alternatively, forward caller to:

- Mountains (Districts 7-9): Kendrick Weeks (919-609-7605, kendrick.weeks@ncwildlife.org)
- Piedmont (Districts 3, 5, 6): Brandon Sherrill (919-208-9200, brandon.sherrill@ncwildlife.org)
- Coast (Districts 1, 2, 4): David H. Allen (252-448-1546, david.h.allen@ncwildlife.org)

Or appropriate district biologist

Appendix H. White-nose Syndrome Occurrence Map (04/15/2016)

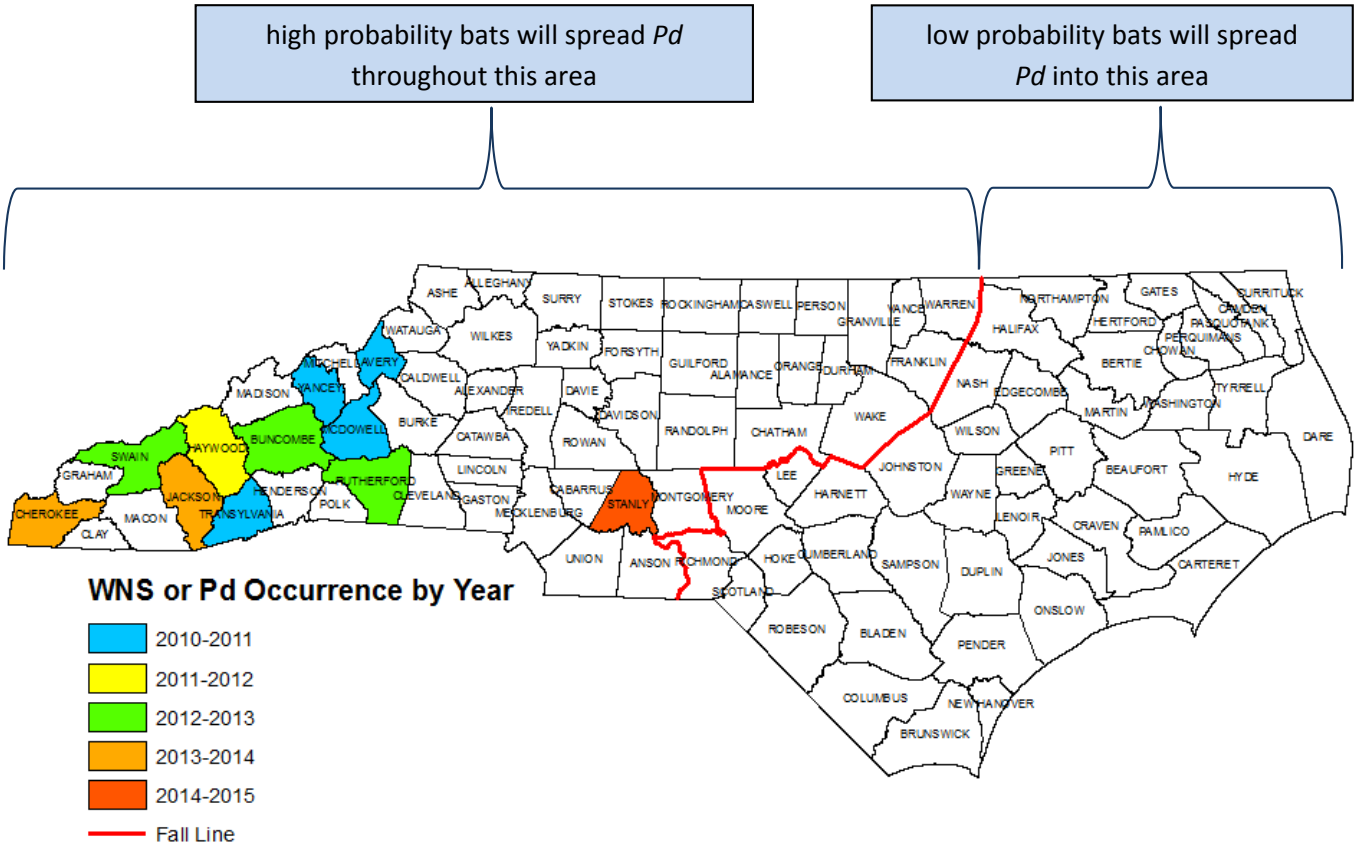


Appendix I. Guidance and Justification for Decontamination in North Carolina

Decontamination guidance:

- Terrestrial equipment that can be completely decontaminated using the hot water method (see reverse; e.g., mist nets, ropes) can be used throughout the state. Extra care must be taken to make sure items are thoroughly decontaminated.
- For terrestrial equipment that cannot be completely submerged in hot water and is more difficult to decontaminate (e.g., headlamps, chairs) and for all subterranean equipment, two sets of gear are recommended: 1) one set for use only in areas with a high probability of becoming WNS+ via bat movement and 2) a second set for use only in areas with a low probability of becoming WNS+ via bat movement (see map below).

Note: Surveyors must decontaminate their gear following the National WNS Decontamination Protocol (www.whitenosesyndrome.org/topics/decontamination) after each activity involving contact with bats, their environments, and/or associated materials in all areas of the state. Surveyors will need to evaluate the type of gear they use and their decontamination methods when deciding what new items they need to purchase.



Justification

We believe following the national protocol and additional guidance specific to North Carolina helps minimize human-assisted transmission of *Pseudogymnoascus destructans* (*Pd)*, the fungus that causes white-nose syndrome (WNS), to areas believed to be free of this deadly disease. Given the very different geology between eastern and western portions of North Carolina and limits on the distances cave bats can travel, we are hopeful the eastern part of the state will remain WNS free. To help ensure this, we have divided the state into two regions based on known bat movements, bat distribution, and geology in North Carolina. The objective of dividing the state into two regions is to highlight areas that are unlikely to become WNS+ via bat movement. These are the areas most in need of protection from accidental transport of the fungus by humans. The map above depicts these two regions. The dividing line sits roughly along the fall zone, which forms the boundary between the Coastal Plain and Piedmont. This line was selected for the following reasons: 1) there is evidence that at least some species of bats in the Coastal Plain are staying in the Coastal Plain year round and 2) there may be a break in the distribution of some bat species between the eastern Piedmont and western Coastal Plain in North Carolina with spatially separated populations (Matina Kalcounis-Rueppell, University of North Carolina at Greensboro, personal communication).

There is still much to learn about bats in North Carolina, particularly in areas outside of the mountains, and future research is being planned to determine where coastal cave bats are hibernating. Decontamination guidance for North Carolina is based on the best available current information and will be updated as we learn more about North Carolina's bats and the spread of *Pd*.

Decontamination Using the Hot Water Method

This decontamination method includes the following steps:

1. Thoroughly scrub and remove sediment/dirt from clothing, footwear, and other gear.
2. Machine or hand-wash/clean gear 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.
3. Decontaminate gear by submersing in hot water $\geq$ 55ºC (131ºF) for a minimum of 20 minutes.
4. Decontaminate equipment bins, sinks, countertops, etc. with appropriate method (e.g., Clorox® wipes).

The complete national decontamination protocol can be found at http://www.whitenosesyndrome.org/topics/decontamination.