USGS National Wildlife Health Center
Winter 2012/2013 Bat Submission Reference Chart

Within the WNS Endemic Area

(Appendix A Map – Pg. 9)

Unusual bat mortality/behavior not associated with WNS (NOV-APR) Pg. 6

Priority Samples

- Any species
- Any county
- ≥ 5 dead/sick bats at one location

•For other situations- consult with NWHC

Samples to submit

(5-8 bats)

- photos AND
- fresh, intact carcasses
- MAXIMUM of 3 euthanized non-T/E bats per site

Bats with signs suggestive of WNS* (NOV-APR) Pg. 7-8

Priority Samples

- Species not previously confirmed with WNS at/near a contaminated hibernaculum in a confirmed county

•Any species at a hibernaculum of suspect or unknown status in an unconfirmed county

Samples to submit

(1-5 bats)

- photos AND
- fresh, intact carcass of any species
- wing biopsies or fungal tape from live T/E species, banded bats, or species were WNS confirmation is NOT required
- Euthanasia of sick, live bats is not advised except for species not previously confirmed with WNS (MAXIMUM of 3 euthanized bats per site)

Species confirmed with WNS- *Myotis lucifugus, M. septentrionalis, M. sodalis, M. leibii, M. grisescens, Perimyotis subflavus, Eptesicus fuscus*

* WNS signs include visible fungus, UV fluorescence, WDI ≥2, suspicious behaviors (day flight activity, entrance roosting, delayed arousal)

USGS National Wildlife Health Center
Winter 2012/2013 Bat Submission Reference Chart

Outside of the WNS Endemic Area

(Appendix A Map – Pg.9)

Unusual bat mortality/behavior not associated with WNS (NOV-APR) Pg. 6	Bats with signs suggestive of WNS* (NOV-APR) Pg. 7-8
Priority Samples • Any species • Any county • ≥ 5 dead/sick bats at one location •For other situations- consult with NWHC	Priority Samples • Species with confirmed susceptibility to WNS at a suspect positive hibernaculum • Any cave species at/near a hibernaculum of unknown status in any county of unconfirmed status
Samples to submit (5-8 bats) • photos AND • fresh, intact carcasses • MAXIMUM of 3 euthanized non-T/E bats per site	Samples to submit (1-5 bats) • photos AND • fresh, intact carcass of any species • wing biopsies or fungal tape from T/E species or banded bats • MAXIMUM of 3 euthanized non-T/E bats per site

Species confirmed with WNS- *Myotis lucifugus, M. septentrionalis, M. sodalis, M. leibii, M. grisescens, Perimyotis subflavus, Eptesicus fuscus*

* WNS signs include visible fungus, UV fluorescence, WDI ≥2, suspicious behaviors (day flight activity, entrance roosting, delayed arousal)

USGS National Wildlife Health Center

Bat "White-Nose Syndrome" (WNS) Submission Guidelines
Winter 2012/2013 (November – April)

TABLE OF CONTENTS **PAGE**

The following sample submission guidelines are for use when surveying bat hibernacula or evaluating unusual bat morbidity or mortality during the winter 2012-2013. They are meant to assist with prioritizing appropriate field samples for laboratory submission based on geographic location and prior knowledge of WNS status at survey sites. **The primary objectives of this targeted surveillance are to identify new geographic locations and bat species affected with WNS.** This document replaces the 2012 Summer Submission Guidelines for Bats and all previous winter submission guidelines from the USGS- National Wildlife Health Center (NWHC). The level of diagnostic evaluation depends on 1) the presence of unusual numbers of sick or dead bats, and 2) the distance from confirmed contaminated sites with greater emphasis on suspect WNS bats found outside the current disease boundaries. These guidelines will be periodically reviewed to ensure that they meet the needs of the field and the laboratory. Please contact your regional FIT member with any questions, suggestions, or concerns (Eastern US: Anne Ballmann, 608-270-2445, aballmann@usgs.gov; Central US: LeAnn White, 608-270-2491, clwhite@usgs.gov; Western US: Barb Bodenstein, 608-270-2447, bbodenstein@usgs.gov).

Winter field signs associated with WNS in bats:

- White or gray powdery fungus seen around the muzzle, ears, wing/limbs, and/or tail;
- Excessive/unexplained bat mortality at the winter hibernaculum;
- Delayed arousal from torpor following disturbance;
- Aberrant bat behaviors (found on ground inside or outside the hibernaculum, roosting near hibernaculum entrance, increased bat activity outside the hibernaculum during cold weather) ;
- Thin body condition and/or dehydrated (wrinkled and flaky appearance of furless areas);
- Wing damage (membrane thinning, depigmented areas, holes, tears, flaky appearance) in cave bat species found outside the hibernaculum through May

WNS has been confirmed in the following 7 species:

- Little brown bats (*Myotis lucifugus*)
- Tri-colored bats (*Perimyotis subflavus*)
- Northern long-eared bats (*Myotis septentrionalis*)
- Indiana bats (*Myotis sodalis*)
- Small-footed bats (*Myotis leibii*)
- Big brown bats (*Eptesicus fuscus*)
- NEW Gray bats (*Myotis grisescens*)

Potentially susceptible species (only *Geomyces destructans* DNA detected):

- Cave bats (*Myotis velifer*)
- Southeastern myotis (*Myotis austroriparius*)

Key components of the diagnostic effort:

1. **Hibernaculum data collection.**

   Fill out the hibernaculum data collection sheet (Appendix B.2) whenever hibernacula are surveyed, regardless of what state or county you are in and whether or not you see fungus on bats. These data will increase our understanding of the epidemiology of WNS and records of negative data (no fungus or abnormal behaviors observed) are important in this effort. Also complete the 2nd page (Individual Bat Specimen Collection Datasheet Winter 2012/2013) whenever samples are collected for laboratory analysis for WNS. If observed bat mortality does not appear to be related to WNS, please submit specimens with the NWHC Specimen History Form (Appendix B.1). E-mail the appropriate completed datasheets to the appropriate FIT contact (608-270-2415 fax) when submitting samples to NWHC.

   If there is no unexplained bat mortality and there is no evidence of fungal growth or unusual behaviors in live bats at the hibernaculum, no photos or diagnostic samples are requested for submission. The NWHC does not accept samples from normal populations of hibernating bats without prior knowledge of purpose and agreement to participate in healthy bat surveillance. Disturbance of hibernation sites can compromise survival of bats.

2. **Field photographs.**

   Handling bats may cause much of the visible fungus to disappear before specimens arrive at the lab. Please take good quality field photographs of representative affected bats, particularly in regions where WNS has yet to be identified, to be included with all bat submissions. Digital photos can be e-mailed to the appropriate FIT contact for further submission consultation.

   When non-lethal samples (tape-lift or biopsies) are collected, we request close-up images of individual live bats to be sampled. E-mail photos to the appropriate FIT contact (608-270-2415 fax) along with the Hibernaculum/Bat data sheets (Appendix B.2) including the date photos were taken, site name, and the photographer's name.

3. **Carcass collection.**

   Advised application- whenever laboratory confirmation of WNS is required (suspicious field signs of WNS in a species not previously confirmed with the disease or in a new geographic area).

   Lethal take of a small number of affected animals may be necessary in the absence of natural mortality to confirm WNS. Ensure you have the proper permits or authorization for specimen collection. Please see the AVMA Guidelines on Euthanasia 2007 (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) and http://www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf.

Once WNS has been confirmed in a federal or state-listed endangered species, only specimens of that species that are found dead will be accepted for diagnostic testing except in extenuating circumstances where necessary permits allow.

Collect the freshest carcasses (intact body, no evidence of scavenging, fur does not pull out easily) of all affected species. If fresh carcasses are unavailable, mummified carcasses are preferable to wet, slimy carcasses and may be accepted upon consultation with a FIT member. Follow carcass collection instructions described in Appendix F. If carcasses are being submitted for diagnostic evaluation, keep individual carcasses chilled in separate bags with ID labels containing the following information:

- date died & date collected (if different)
- location (hibernaculum or nearest town, county, state)
- collector name & phone
- species
- unique animal ID number (standard format: state, MMDDYY, collector, ###; ex: WI061610AEB001)
- 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 another site. If you plan to visit additional sites on the same day, follow the current recommendations described in the USFWS WNS Decon Supplement for Researchers (http://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf). Contact the appropriate FIT member to arrange shipping. If additional intact carcasses are being saved for future evaluation, triple-bag the labeled specimens, freeze carcasses and store locally. Keep record of frozen bat carcass inventory on datasheets (Appendix B.2).

Please contact the NWHC prior to submitting bat samples. See Appendix F for NWHC shipping instructions.

4. Non-lethal sampling techniques:

NOTE: Bats from WNS confirmed counties with visual evidence of WNS (white material on muzzle and wing membranes) are presumptively positive. Disturbance of these bats that are likely infected with WNS compromises survival and further testing is not advised unless there are special circumstances. ***Most current non-lethal sampling techniques cannot confirm WNS and may have a reduced reliability of detection as compared to whole carcass evaluation.***

☐ **Fungal tape-lift sample collection (see Appendix C for detailed instructions)**

Advised application- known susceptible bat species in an unconfirmed county within the WNS confirmed region **with visible fungus**; any threatened/endangered bat species with visible fungus on muzzle; histological confirmation of the disease **is not** necessary.

Wear clean gloves to handle each bat to reduce the risk of cross-contamination of diagnostic samples. Collect tape-lifts only from visibly affected muzzles of bats (alive or dead) with fungal growth when carcasses cannot be submitted. E-mail hibernaculum data collection sheet (Appendix B.2) and specimen history form (Appendix B.1) to the appropriate FIT contact and send fungal tape slides with a hard copy of the datasheet to the NWHC.

NEW

☐ **Ultraviolet light (UVA) screening of wing membranes (see Appendix E for detailed instructions)**

Advised application- any dead bat or live bat with physical or behavioral signs suggestive of WNS but lacking visible fungal growth examined mid-winter through spring. **This is an INVESTIGATIONAL screening technique with unknown specificity outside the WNS endemic area.**

This technique requires handling individual bats to examine extended wings and thus results in hibernation disturbance as well as unknown safety risks to bats. Detection of pale yellow-orange fluorescence spots on wings **IS NOT** definitive for diagnosing WNS and therefore should be used in conjunction with other techniques for targeted sample collection. **It is not recommended for use in apparently healthy bat populations outside of the WNS endemic area.**

☐ **Wing punch biopsy (see Appendix D for detailed instructions)**

Advised application- any threatened/endangered bat species **with visible fungus or characteristic fluorescence on wing membranes under UVA light**; known susceptible species in an unconfirmed county within the WNS confirmed area with physical evidence (visible fungus, wing damage). This non-lethal sampling is the preferred, more sensitive method to fungal tape lifts for diagnostic evaluation when fungus is present on both flight membranes and muzzle as PCR and/or histopathology may be performed.

To reduce the risk of cross-contamination among bats, all equipment (i.e.: gloves, tissue punches, biopsy boards, and forceps) should be cleaned or changed between each sampled bat. Collect wing biopsies only on live bats with visible fungal growth or characteristic UV fluorescence when whole carcasses cannot be submitted. Biopsy punches should be collected from portions of the wing membrane that exhibit fungal growth or other types of visible lesions (Appendix D & E). E-mail hibernaculum data collection sheet (Appendix B.2) and specimen history form (Appendix B.1) to the appropriate FIT contact and overnight ship samples to the NWHC.

Note: Non-lethal sampling techniques are meant to serve as adjunct or alternative diagnostic methods to evaluate for the presence of G. destructans among suspect bats at a particular location. The maximum number of individuals (in any sample combination of carcasses, tape lifts, or wing biopsies) per site that will be accepted for WNS/Gd diagnostic evaluation is 10 per season unless prior arrangements have been made with the lab. Not all of the submitted samples may be tested; this will be at the discretion of the lab.

5. **Biosecurity concerns:**

   A site contaminated with *Geomyces destructans* retains this designation indefinitely regardless of the presence of affected bats. Follow the most current **protocols for containment and decontamination of field gear and personnel** described in "National White-Nose Syndrome Decontamination Protocol Version 06.25. 2012)" [http://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf] prior to leaving each survey site. If you plan to visit a potentially uncontaminated hibernaculum after conducting survey work at a contaminated hibernaculum, use clothing, footwear, gear, and vehicles dedicated for use at clean sites.

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UNUSUAL BAT MORTALITY/BEHAVIOR NOT ASSOCIATED WITH WNS

Before entering hibernacula of endangered Indiana bats or any other listed bat species, appropriate Federal and State permits (or authorizations) must be obtained. For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats.

Priority samples to submit for laboratory diagnostics:

1. Any species in any county nationwide where 5 or more dead or sick bats are observed at one location within 1-2 weeks.

- **If no fungal growth on live bats is observed at the site where unexplained bat mortalities are detected**, collect 5 - 8 freshly dead bats, chill and ship to NWHC as soon as possible for evaluation according to packaging and shipping instructions in Appendix F. A maximum of 3 affected non-T/E species may be euthanized per site for submission if the quality of available carcasses is questionable. Complete a specimen history form (Appendix B.1).

BATS WITH CLINICAL SIGNS SUGGESTIVE OF WNS

Before entering hibernacula of endangered Indiana bats or any other listed bat species, appropriate Federal and State permits (or authorizations) must be obtained. For listed species, authorization is needed to collect and possess dead specimens, to handle live bats, or to euthanize sick bats.

☐ **Sites within the WNS confirmed/endemic area (see Appendix A)-**

Priority samples to submit for laboratory diagnostics:

1. Bat species not previously confirmed with WNS with suspicious fungal lesions or aberrant behavior in a confirmed county
2. Any bat species with suspicious signs at/near a hibernaculum of suspect or unknown status in an unconfirmed county

Site prioritization recommendations:

Only hibernacula of critical biological or management significance that require conclusive laboratory confirmation of WNS should be surveyed for clinically affected bats within the WNS confirmed area. **Notification of need for diagnostic confirmation at sites within this region should be communicated to the laboratory prior to collection of bats.** Take field photos and submit 3-5 bats (fresh dead or euthanized) with physical or concurrent behavioral evidence suggestive of WNS along with a completed Hibernaculum/bat datasheets (Appendix B.2). Once WNS is confirmed in the county, only bat species of unknown susceptibility will typically be accepted for WNS diagnostic evaluation from that county.

☐ **Sites outside the WNS confirmed/endemic area (see Appendix A)-**

Note: It is recommended that all previously identified *G. destructans* contaminated hibernacula outside the WNS endemic area be surveyed between late February-March for the development of WNS. Do not submit samples if no signs of WNS are observed in the bat population without prior consultation with NWHC.

Priority samples to submit for laboratory diagnostics:

1. Species with confirmed susceptibility to WNS at a suspect positive hibernaculum
2. Any cave bat species with suspicious fungal lesions or aberrant behavior at/near a hibernaculum of unconfirmed status

Site prioritization recommendations:

To be determined by the land resource management agency. Please consult the National Surveillance Implementation Plan (Dec 2011) for prioritization recommendations.

The following sample collection descriptions apply to bats with clinical signs suggestive of WNS regardless of the area they are detected. Consult the NWHC Bat Submission Quick Reference Charts (pg. i-ii) for a summary of sample prioritization recommendations.

- **If fungus, wing damage or characteristic UV fluorescence on wing membranes is observed on dead bats,** fill out hibernaculum/bat datasheets (Appendix B.2) and e-mail to the appropriate FIT contact (608-270-2415 fax). Submit 3-5 fresh carcasses of new bat species of unknown susceptibility only that appear affected from a confirmed county. If county is of suspect or unknown WNS status, submit 3-5 carcasses of any affected species. (See pg. 2 for list of confirmed susceptible species).

- **If live bats have behavioral or physical evidence of suggestive of WNS but no mortality is observed AND**

  ☐ **Histological confirmation IS required**, euthanize up to 3 bats (representative of affected non-T/E species) with evidence of fungus for submission to NWHC. Please see AVMA Guidelines on Euthanasia 2007 at http://www.avma.org/issues/animal_welfare/euthanasia.pdf and www.michigan.gov/documents/emergingdiseases/Humane_Euthanasia_of_Bats-Final_244979_7.pdf. Submit photos (clusters and live individuals) and bat carcasses to NWHC (Appendix F). Include completed hibernaculum/bat datasheets (Appendix B.2). *NOTE: UV-guided wing punch biopsies stored in 10% formalin may be used in lieu of euthanasia (Appendices D & E) although diagnostic reliability for WNS detection may be reduced as compared to whole carcass evaluation.*

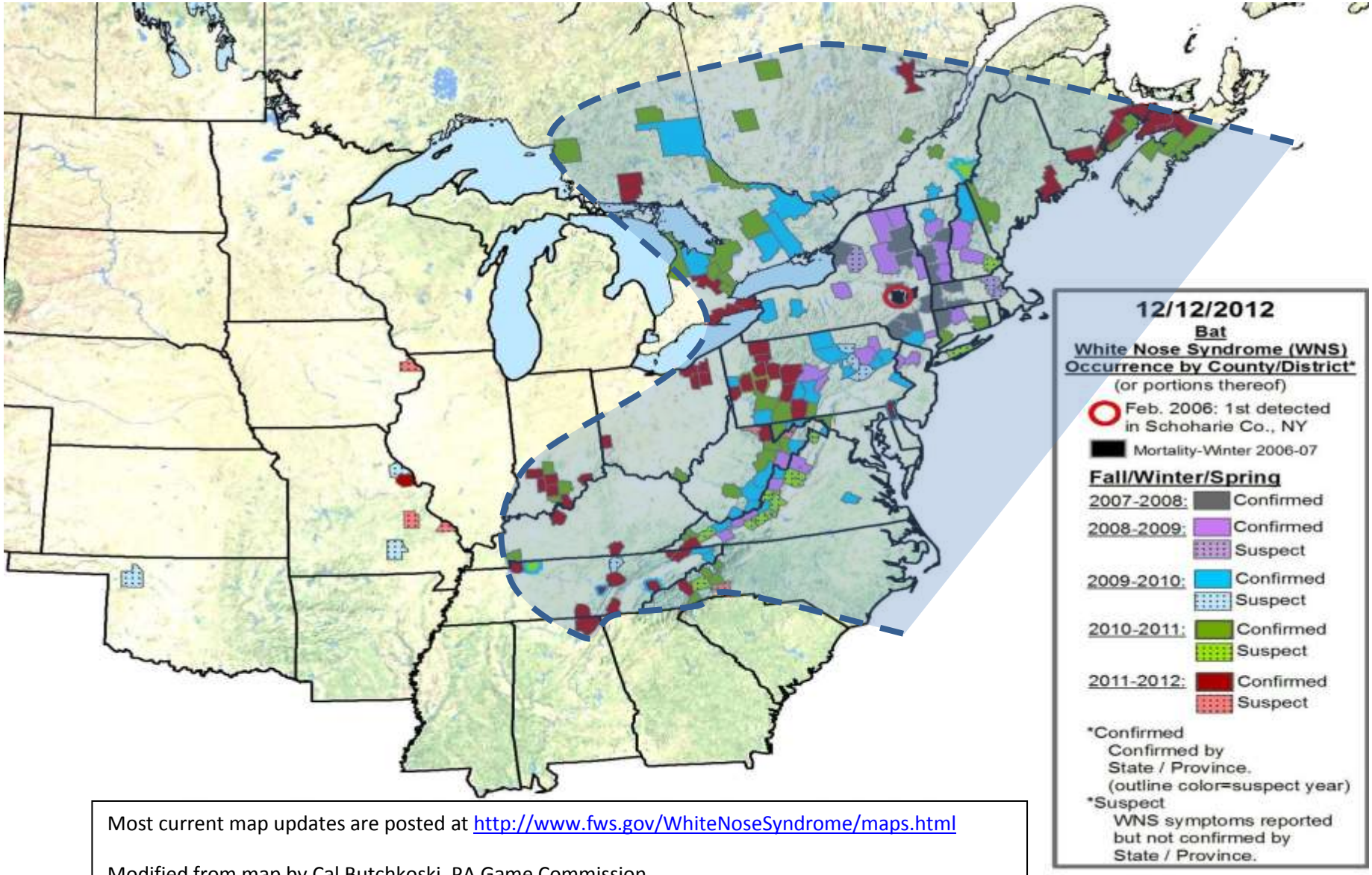
  ☐ **Histological confirmation is NOT required**, follow one of the methods below:

  1. Perform punch biopsies on 3-5 individuals (2 biopsies per each individual – See Appendix D) per field site from an affected portion of the flight membranes only and store samples chilled or frozen. Photograph the bat prior to biopsy and record associated geographic, demographic, and physical data (Appendix B.2). *NOTE: Wing punch biopsies continue to be evaluated as a definitive diagnostic & surveillance method for detecting Geomyces destructans, the fungus causative of WNS. Thus, negative results do not rule out the possibility of an animal being infected.*

  2. Collect fungal tape-lifts of grossly visible white fungal growth on the muzzles of 3 - 5 affected live bats (See Appendix C for detailed instructions). A new tape strip and gloves should be used for each individual bat. Tape-lift slides can be stored and shipped at room/ambient temperature. Follow packaging and shipping instructions for slides only in Appendix C. Include the completed datasheets from Appendix B.2. *NOTE: The sensitivity of tape-lift samples to detect Geomyces destructans, the fungus causative of WNS, is highly dependent on the slide quality; thus, negative results do not rule out the possibility of an animal being infected.*

APPENDIX A

MAP A: Counties and districts with confirmed or suspect (likely) WNS since disease emergence. WNS-confirmed (endemic) region is denoted by shaded area.



Most current map updates are posted at http://www.fws.gov/WhiteNoseSyndrome/maps.html

Modified from map by Cal Butchkoski, PA Game Commission

APPENDIX B.1

National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
Phone: 608.270.2400
FAX: 608.270.2415

SPECIMEN HISTORY FORM

For mortality events please e-mail a USGS Field Investigation Team member before shipping
Western States: Barb Bodenstein, bbodenstein@usgs.gov, 608-270-2447
Central States: LeAnn White, clwhite@usgs.gov, 608-270-2491
Eastern States: Anne Ballmann, aballmann@usgs.gov , 608-270-2445
For single animal cases, Nationwide: Jennifer Buckner, jbuckner@usgs.gov, 608-270-2443

Submitter's name: **Telephone:**
Address:

E-mail:

Collector's Name: **Affiliation:**
Telephone:
E-mail:

Date collected:

Method of animal collection: ☐ **Found Dead,** ☐ **Died in Hand,** ☐ **Euthanized**
Method of euthanization:

Species:
Number Submitted: **Condition:** ☐ **Chilled,** ☐ **Frozen,** ☐ **Preserved Tissues**

Specific die-off location (refuge unit, pond, address, intersection, park, etc):

State: **County:** **Nearest City:**

Latitude/longitude (Decimal degree in WGS 84): **Zone:**

Disease onset date: (Best estimate) **Disease end date:** (best estimate)

Species affected: (The diversity of species affected may provide clues to the disease involved.)

Age/sex: (Any pattern noticed that is related to age and sex?)

Known dead: (Actual number counted) **Known sick:**

Estimated dead: **Estimated sick:**
(Consider removal by scavengers or other means, density of vegetation, etc.)

Clinical signs: (Any unusual behavior and physical appearance.)

Population at risk: (Number of animals in the area that could be exposed to the disease.)

Population movement: (Recent changes in number of animals on area and their source or destination, if known.)

Problem area description: (Land use, habitat types, and other distinctive features.)

Environmental factors: (Record conditions such as storms, precipitation, temperature changes, or other changes that may contribute to stress.)
Comments: (Additional information/observations of value such as past occurrences of disease in area, photographs or videos)

APPENDIX B.2 **Winter 2012/2013 Hibernaculum Survey Datasheet**

Investigator Name(s): _____ **Date: _____**

Phone /e-mail: _____

State:	**County:**	**Site Name:**
Latitude:	**Longitude:**	Datum:

Observations at the hibernaculum entrance (within area impacted by daylight)

# of bats observed flying at entrance in 5 minutes _____

Bat species	**Bands observed**	**# live[1]**	**# dead[1]**	**# moribund[1]**	**# with fungus visible[1]**	**Distribution of affected bats** (Solitary, Clustered[2])	**Photo #(s) of affected bats**
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	

[1]**Indicate if number is an estimate count;** [2]**Cluster defined as ≥2 bats in direct contact**

Bat observations inside the hibernaculum

Bat species	**Bands observed**	**# live[1]**	**# dead[1]**	**# moribund[1]**	**# with fungus visible[1]**	**Distribution of affected bats** (Solitary, Clustered[2])	**Photo #(s) of affected bats**
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	
						S C	

Comments:

PLEASE ATTACH A MAP OF THE HIBERNACULUM WITH LOCATIONS OF BATS WITHIN THE SITE MARKED &
COMPLETE THE INDIVIDUAL BAT DATASHEET (ON BACK) FOR ALL SPECIMEN COLLECTIONS

APPENDIX B.2- USGS NWHC Individual Bat Specimen Collection Datasheet Winter 2012/2013

ID or Band# (state, MMDDYY, collector, ###) *Ex:WI103111AB001*	**Species** (4 letter code)	**Onsite location** (Outside, Entrance, Inside)	**Sex** (Male, Female)	**Status** (Live, Dead, Euth)	**Age Class** (Juv, Adult, Unk)	**Weight (g)**	**Forearm length (mm)**	**Visible fungus** (Muzzle, Ear, Wing, Tail)	**Wing Damage Score** *(circle one)*	**Photo file ID**	**Sample Type** (Fungal Tape, Wing Biopsy, Whole Carcass, Archived)	**Comments/ Notes Key**
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	
		O E I	M F	L D E	J A U			M E W T	0 1 2 3		T B C A	

Additional Notes/Diagrams: [use key code in last column to link this information to specific animal(s)]

APPENDIX C - 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: 6 December 2010 (modified)

Purpose: The following procedure is designed to collect visible fungi from the muzzles of bats for later microscopic analyses while minimizing harm to the sampled bat. **This technique will NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed in the bat population.**

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 and permanent marker

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. Muzzle samples yield the clearest results and are the preferred 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 grossly visible areas of fungal growth on the muzzles of bats. 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(s) on the tape with a permanent marker containing the material sampled from the bat.

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 clean Ziploc bag to store or mail to the lab.

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 the appropriate FIT contact. Contact your regional FIT if you have any additional questions (Eastern US: Anne Ballmann, 608-270-2445; Central US: LeAnn White, 608-270-2491; Western US: Barb Bodenstein, 608-270-2447).

APPENDIX C. Illustrations – Fungal tape-lift protocol for bats
-Photographs by D. Berndt and D. Johnson, USGS – NWHC

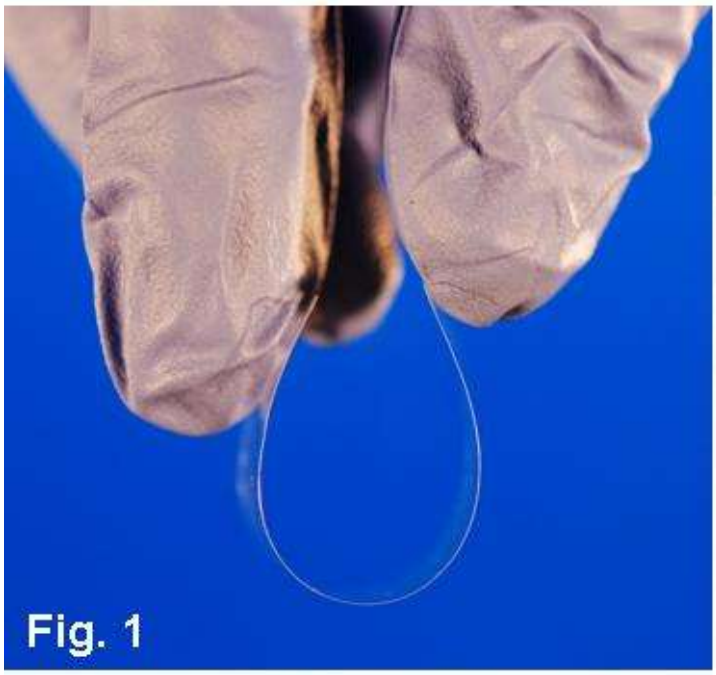


Fig. 1

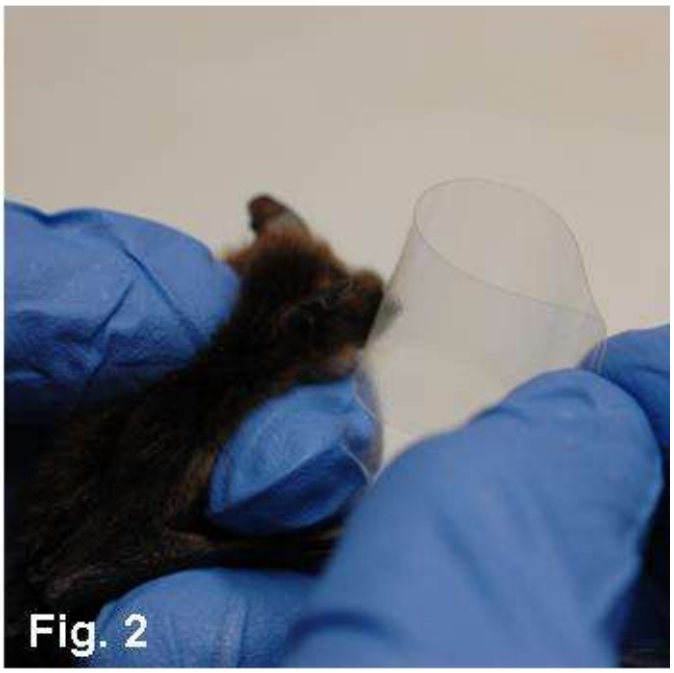


Fig. 2

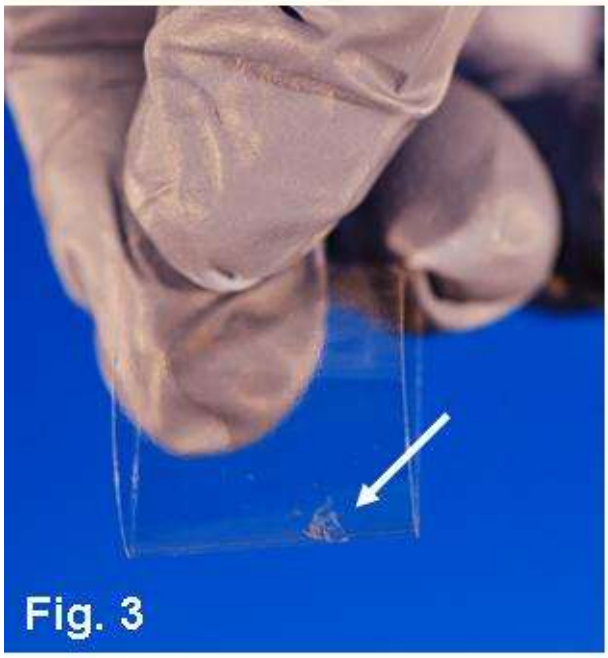


Fig. 3

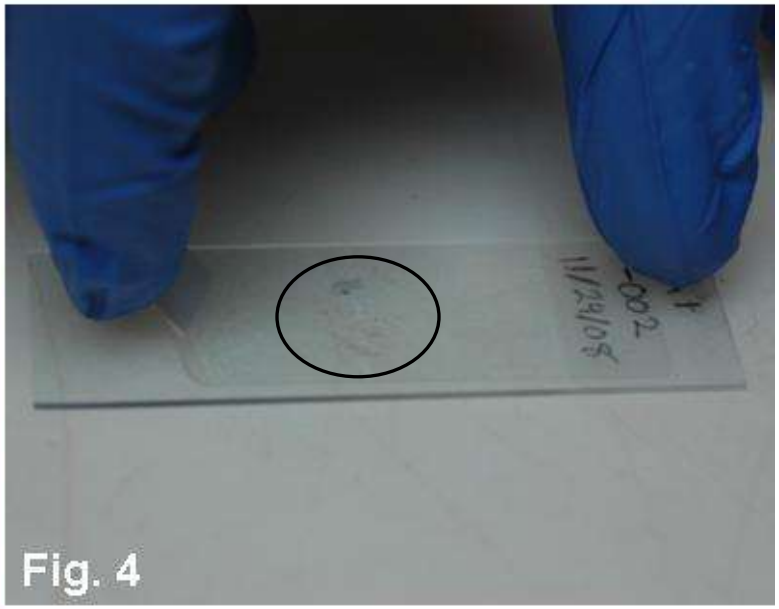


Fig. 4

APPENDIX D - Instructions for Taking a Wing Tissue Biopsy

Updated by Pat Ormsbee (NFS) and Jan Zinck 5/14/09 (original: Shonene Scott, Portland State University 5/2003)
Modified by Anne Ballmann (USGS-NWHC) 12/21/12

NOTE: If punch biopsies are the only sample type to be submitted to the lab for PCR testing of *G. destructans* in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings. **This technique may NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed in the bat population.**

1. When taking biopsies it is important to reduce the potential for cross-contamination between bats. In order to do this, use a small clean piece of sturdy cardboard that can be discarded after each animal, a new tissue punch for each bat, 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:

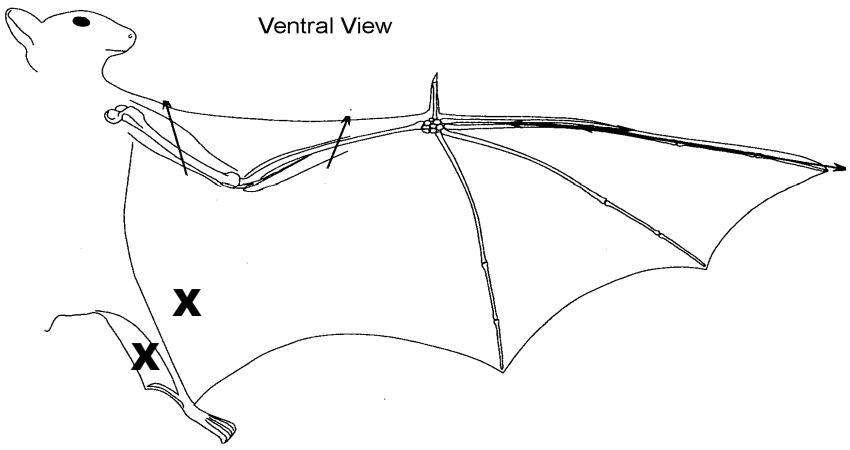
   State, Date (MMDDYY), Collector initials, bat number (ex: WI061609AEB001)

3. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready for each bat. 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). ***Long-wave UV light can optimize biopsy placement and allows for additional histopathological evaluation (target areas exhibit faint yellow-orange fluorescent spotting-See APPENDIX E).*** <u>If possible, locate an affected area near the body wall</u> within the lower half of the wing membrane or uropatagium. These locations have been demonstrated to have faster healing rates and are less disruptive to flight aerodynamics (Faure PA et al. 2009. J Mammalogy 90(5): 1148-56.) Press the punch firmly through the membrane and twist the punch slightly to ensure a complete punch. Apply direct pressure to biopsy site for several minutes if bleeding occurs.

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



APPENDIX D - Instructions for Taking a Wing Tissue Biopsy - con't

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. If fungal PCR is desired for diagnostic analysis, place tissue into an empty vial (no storage media) for storage. If histopathological evaluation is desired, place tissue into a storage vial containing 10% buffered neutral formalin (1 part tissue to 10 parts formalin).

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, unfixed samples should be frozen until submitted for fungal PCR analysis. Formalin-fixed samples should be held at room temperature (not frozen).

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, rinse in alcohol and flame sterilize. Allow forceps to cool before contacting bat tissue. 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 contaminants, 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 unfixed samples cannot be shipped overnight freeze them and ship as soon as possible.

    Send an electronic copy of the completed hibernaculum/bat datasheets (Appendix B.2) to the appropriate FIT contact. Shipping address and examples of appropriate shipping materials are in Appendix F. 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.*

- ☐ 3-5 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)
- ☐ 10% buffered neutral formalin (if histopathological analysis is desired)
- ☐ Sterile rinse water
- ☐ 2 ml sterile plastic vials with caps
- ☐ 95% ethanol and flame source such as cigarette lighter (for sterilizing metal sampling equipment)
- ☐ Fine point permanent marker
- ☐ Vial labels
- ☐ Disposable gloves
- ☐ Paper towels/gauze
- □ Nonporous cutting board
- ☐ Ziploc bags and cooler with blue ice

APPENDIX E – Longwave ultraviolet (UVA) fluorescence screening of bat wings

Authors: Anne Ballmann, Carol Meteyer (modified from G. Turner & J. Gumbs 2011)

Date: 5/7/2012, revised 12/21/12

Purpose: To examine bat wings with little to no visible fungal growth for evidence of yellow-orange fluorescence areas suggestive of an infection by *Geomyces destructans*. **This is an INVESTIGATIONAL screening technique with unknown specificity outside the WNS endemic area. It will NOT confirm White-nose Syndrome (WNS) on bats and should not be used as the sole sampling methodology in areas where WNS has not been previously confirmed.**

Equipment:

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.

- 380-385 nm wavelength UV 51 bulb LED flashlight (LED Wholesaler #7202UV385-$35) or 368 nm wavelength 9 V UV box (Contact Greg Turner [grturner@pa.gov] for more details on UV box system)
- Disposable exam gloves
- Digital camera
- Permanent marker
- PPE: UVA blocking safety glasses, SPF15+ sunblock on exposed human skin

Additional equipment for non-lethal sample collection-

- 2 ml sterile vials with screw cap lids
- 10% buffered neutral formalin
- 3-5 mm sterile punch biopsies

Procedure: ***(To reduce potential cross-contamination, use clean exam gloves when handling each bat.)***

1. In complete darkness, shine the UV flashlight facing down approximately 3-5 inches (7.5-12.5 cm) above the extended ventral surface of the flight membranes (Fig. 1A). If using a UV box, place the bat on its back and extend the wing and corresponding foot over the UV light source to transilluminate the wing surface. Disinfect surface of UV box between bats. Avoid shining the light into the unprotected eyes of the bat or people or exposing bat skin to UV light for more than 3 minutes.

2. Examine wing membrane for circular areas of yellow-orange fluorescence (Fig. 1B). Fluorescence will be faint when viewed with the naked eye using a hand-held UV flashlight. Visualization is greatly enhanced by examining a digital photograph of the UV-illuminated wing surface when using the UV box. Photography does not improve visualization with the UV flashlight.

3. If the bat is to be euthanized, use a permanent marker to circle representative areas of fluorescence on the wing membrane to target sampling in the laboratory. Place marks outside of the fluorescent border.

4. If live-sampling techniques are used, collect paired wing punch biopsies (3-5 mm diameter, See Appendix D) that incorporate areas of UV fluorescence. Place one wing biopsy into a 2ml vial containing 1.5 ml of 10% buffered neutral formalin for histology. Place the second wing biopsy into an empty vial for PCR and keep chilled in the field. Label vials with the unique bat ID number.

5. Submit samples along with any digital photos of fluoresced wings to the appropriate FIT contact at NWHC.

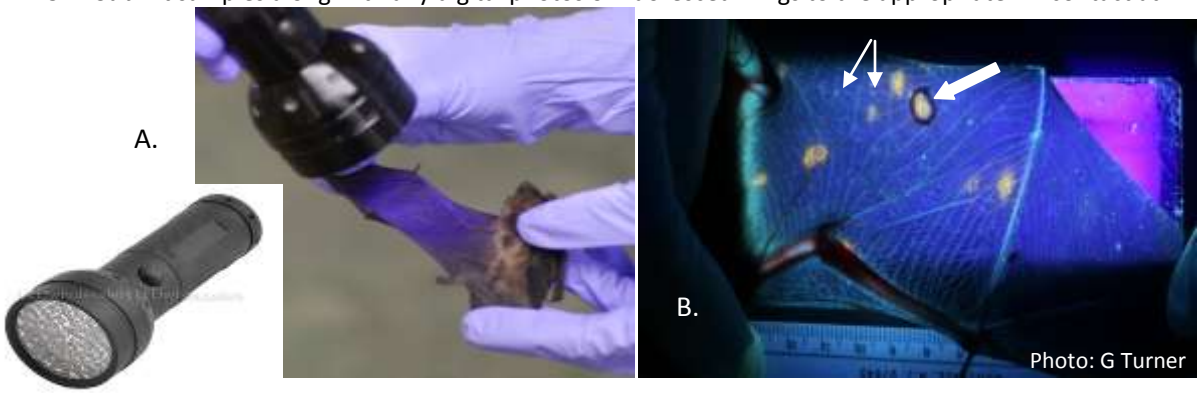


Figure 1. A) UV flashlight examination of ventral bat wing to be conducted in total darkness. B) Digital photo of backlit extended wing held over 368 nm UV light box. Arrows identify yellow-orange fluorescent areas of various diameters associated with suspect *G. destructans* infection.

USGS – National Wildlife Health Center

INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF AVIAN AND MAMMALIAN CARCASSES

Contact your USGS Field Investigation Team (FIT) member first!
Eastern states – Dr. Anne Ballmann aballmann@usgs.gov, 608-270-2445
Central states - Dr. LeAnn White, clwhite@usgs.gov, 608-270-2491
Western states– Barb Bodenstein, bbodenstein@usgs.gov, 608-270-2447
Single animal cases, Nationwide: Jennifer Buckner jbuckner@usgs.gov, 608-270-2443
Emergency Contact Number 608-270-2400

The following instructions should be used for collecting and shipping wildlife carcasses, carcass parts, and samples extracted from animals to the National Wildlife Health Center (NWHC) to insure adequate and well preserved specimens.

Freezing/thawing impedes isolation of some pathogens and damages tissues. NWHC prefers unfrozen specimens if they can be sent within 24-36 hours of collection or death. We will provide guidance on freezing samples on a case-by-case basis. As a general guideline: if you cannot call or ship within 24-36 hours, freeze the animal(s).

☐ Contact FIT to get shipping approval and discuss shipping arrangements. Typically, ship specimens by 1-day (overnight) service, Monday through Wednesday, to guarantee arrival at NWHC before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday, special arrangements can be made.

☐ Email/fax history and tracking number to FIT. Packages will not be opened if history does not arrive first!

☐ Use rubber, vinyl, or nitrile gloves when picking up sick or dead animals. If you do not have gloves, insert your hand into a plastic bag.

☐ More than one disease may be affecting the population simultaneously. When possible, collect both sick and dead animals. Note behavior of sick animals before euthanizing.

☐ Collect specimens that are representative of all species affected and geographic areas.

☐ Collect the freshest dead specimens. Decomposed or scavenged carcasses are usually of limited diagnostic value. If you plan to collect animals in the field, take along a cooler containing ice to immediately chill carcasses.

☐ Collect animals under the assumption that an infectious disease or toxin is involved and other animals may be at risk. Protect yourself as some diseases and toxins are hazardous to humans.

☐ Place each animal in a plastic bag, close, and seal the bag. Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape. Label the outside of this bag with the following information in waterproof ink:

- Date collected
- Location (specific site, town, county, state)
- Collector (name/address/phone)
- Species
- Found dead or euthanized
- Your reference #

☐ Place 1st bag inside a 2nd bag, close and seal. More than one individually bagged animal can be placed in the 2nd bag. This prevents cross-contamination of individual specimens and leaking shipping containers.

☐ Tag the outside of 2nd bag and number of animals and type, date collected, location, and name of collector. Reminder order: TAG, BAG, BAG, TAG.

☐ Use a hard-sided cooler in good condition for shipment. Close the drain plug of cooler and tape over inside. Line cooler with a thick bag (1 mil thickness, 3rd layer of bags).

☐ Place absorbent material in the 3rd plastic bag to absorb any liquids that might leak during shipping.
See appendix for examples of bags and absorbent materials.

☐ Pack the individually bagged animal(s) that are contained within the 2nd sealed bag into the 3rd bag with enough FROZEN BLUE ICE PACKS or similar coolant to keep carcasses cold. Use enough coolant to keep samples chilled if there is a delay in delivery.
- Blue ice (unfrozen) can be obtained at hardware, sporting goods, or grocery stores.
- Wet ice can be used if frozen in a sealed plastic container (i.e., soda or water bottle).
- DO NOT USE DRY ICE.

☐ Seal the 3rd bag with methods described for 1st bag.

☐ Place the completed specimen history and return shipping label in a ziplock bag and tape to the inside lid of the cooler (if you want the cooler returned). NWHC CANNOT PAY FOR SHIPPING.

☐ Using packing or duct tape, tape the cooler shut around the lid and at each end using a continuous wrap around the cooler.

☐ Attach the shipping document (airbill) with the DOT information below to the outside of each cooler in a resealable pouch:

Address:

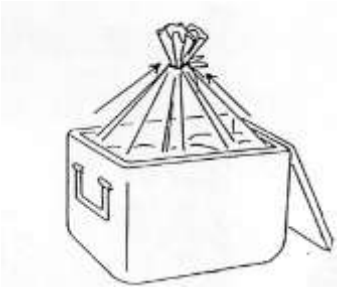
National Wildlife Health Center
Necropsy Loading Dock
6006 Schroeder Road
Madison, WI 53711

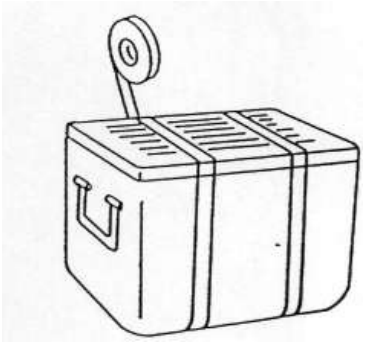
Emergency Contact:

NWHC FIT emergency
608-270-2400

Supplementary Labels**:**

Keep Cold





☐ Mark the cooler with the appropriate information:
(See Pg. 3 for printable marking labels)
- Carcasses of animals that died of unknown causes:
  BIOLOGICAL SUBSTANCE, CATEGORY B and **UN 3373.**
- Blood and tissue samples from apparently healthy animals (hunter-killed, live captured):
  EXEMPT ANIMAL SPECIMENS.
- Blood and tissue samples from dead or sick animals:
  BIOLOGICAL SUBSTANCE, CATEGORY B and UN 3373.

☐ Note the tracking number in case packages are delayed.

☐ These instructions cover federal shipping regulations for commercial carriers.

Appendix:

Example of bags available at large supermarkets (list not all inclusive):

Inner and second layer bags:
- Hefty Big Bag – 22 gal
- Hefty Freezer – 1 gal
- Hefty Jumbo – 2.5 gal
- Ziplock Freezer – 1 gallon
- Ziplock Big Bag – 20 gallon
- Glad Freezer – 1 qt, 2 qt, 1 gal

Third layer for cooler liner:
- Hefty Cinch Sak (1.1 mil) – 33 and 39 gal
- Hefty Lawn and Leaf (1.1 mil) – 33 and 39 gal
- House brand large trash (1.1 mil) – 30 gal
- Glad Force Flex (1.05 mil) – 25 gal
- Hefty Ultra Flex (1.3 mil) – 30 gal
- House Lawn - Leaf (1.2 mil) – 39 gal

Absorbent material:
- Super absorbent packet or pads for water
- Paper towels
- Do not use packing peanuts or shredded paper.
- Cellulose wadding
- Cotton batting or cotton balls

UN3373

BIOLOGICAL SUBSTANCES, CATEGORY B

EXEMPT ANIMAL SPECIMENS