CANADIAN WILDLIFE HEALTH COOPERATIVE

CWHC NATIONAL OFFICE

Western College of Veterinary Medicine

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How to survey for *Pd* and WNS

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Preamble

The purpose of this document is to provide recommendations for conducting surveillance for the fungus *Pseudogymnoascus destructans* (*Pd*) and surveillance for the disease Bat white-nose syndrome (WNS). The guidance provided is by no means the only right way to conduct surveillance, but are the techniques used by WNS surveillance specialists in Canada for identifying this disease in hibernating bats. Specifically, recommendations are made on: priority surveillance sites, identifying presence and recognizing signs of WNS, and data collection during surveillance. This document also refers to several important protocols (e.g., decontamination protocol, bat submission protocol).

Pd and WNS: the difference

In popular literature, online webpages, and news articles the terms '*Pd*' and 'WNS' are often used interchangeably and confusion may arise. *Pd* and *Pd* positive describe the fungus and *presence of the fungus* on bats or in the environment. WNS and WNS positive describe the *clinical disease* in bats that is caused by the fungus: resulting in fungal lesions and subsequent physiological issues and torpor arousal that often results in the death of affected North American bats. *Pd* can be found in hibernacula and on bats while bats do not show signs of illness, and thus do not have WNS. Thus, bats can carry the fungus, be *Pd* positive, but show no signs of the disease WNS.

Pd/WNS surveillance recommendations

Surveillance period: Canada's national survey for WNS designates each annual surveillance period as the interval from November 1 of a given year to May 31 of the following year. Visible growth of *Pd* on bats becomes more noticeable and luxuriant and the lesions of WNS can be more prominent as winter progresses. **March is suggested as the optimal time to survey hibernacula** as this will increase the chances of identifying bats affected by WNS prior to spring emergence. However, *Pd* growth on bats and lesions associated with WNS can be observed as early as November, indicating that an early (January) and late (March) survey during the hibernation period can be of value to increase detection of fungal growth and potentially to collect carcasses should mortality occur. It is important to recognize that a balance must be struck between *Pd*/WNS surveillance and the importance of minimizing disturbance of hibernating bats as much as possible. The lack of visible signs of *Pd* growth on bats does not mean the fungus is not present and fungal organisms other than *Pd* can grow on bats, mimicking the appearance

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of *Pd*. Therefore, typical surveillance techniques for *Pd*, such as fungal culture and/or molecular tests (*i.e.*, quantitative real time polymerase chain reaction: qPCR), should be utilized on a statistically robust portion of the population and environment to document the presence or absence of the fungus. The fungus can arrive a year or more before behavioural changes in bats and/or visible fungal growth or WNS become apparent.

Environmental surveillance for *Pd* outside of hibernacula is ideally done at summer colonies where and when large numbers of bats congregate. **Spring, the first weeks after bats emerge from hibernation in a given area, is the best time for collection of surface swabs, substrate swabs, and guano to detect *Pd***. *Pd* has been successfully detected in guano of unknown age throughout the year, including summer and fall, but with a lower probability of detection. Note that while the presence of *Pd* can be detected in summer, at summering colonies, and on bats at swarming sites, typically WNS is not a problem at these times.

Priority sites: Hibernacula within provincial/territorial counties or districts not previously confirmed as WNS positive are given the highest priority for surveillance. High priority should also be given to hibernacula not previously confirmed as *Pd* or WNS positive, or species not previously confirmed as *Pd* or WNS positive within counties already confirmed as positive. If known, hibernacula with the largest bat populations should be prioritized for surveillance efforts. Similarly, large maternity colonies with a large amount of guano accumulation offer the best chance of detecting *Pd* if it is present at that site. Work done in Saskatchewan demonstrated that guano accumulations beneath concrete bridges crossing substantial watercourses with roosting habitat for bats in their structure were good locations for *Pd* surveillance with positive results documenting the spread of *Pd* in Canada.

Field or Clinical Signs of *Pd* or Bat WNS: The following are field or clinical signs that could indicate the presence of *Pd* on bats or the disease WNS itself. Note: none of these signs are conclusive in the diagnosis of WNS or in determining the presence or absence of *Pd*. Collected samples (environmental and/or bat swabs and/or bat carcasses) need to be tested in a lab to formulate an accurate categorization of the situation.

- Excessive or unexplained mortality at or near a hibernaculum.
- Visible fungus on flight membranes, muzzle, or ears of live or fresh dead bats.
- Yellow-orange fluorescent pattern of non-haired skin under UVA light [Turner et al. 2014].
- Abnormal behaviours including daytime activity, premature egression from the hibernaculum or population shift to entrance of the hibernaculum.
    - The general public can report winter day flying bats. These sightings can sometimes reveal unknown hibernacula when multiple sightings are reported from one area. The media should be utilized to encourage such reporting and also to educate the public on WNS and bats in general. Appropriate provincial and territorial contacts are listed on the

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CWHC's bat health regional outlook page (http://www.cwhc-rcsf.ca/bat_health_regional_outlook.php).

- Bats suffering from WNS may roost closer to cave entrances than normal, but this does not always happen. This phenomenon can greatly increase population counts if bats are moving from inaccessible areas of the hibernaculum to accessible entrance areas. Because of these factors, baseline data on over-wintering bat populations is important to assess the impact of WNS.

- Moderate to severe wing damage in non-torpid bats [Reichard and Kunz 2009].
- Thin body condition
- NOTE: Moderate to severe wing damage in non-torpid bats or thin body condition are considered nonspecific field signs when they are observed alone.
- Dead bats found on the landscape in the winter. These will not have obvious fungal growth. When surveying hibernacula be sure to search around the immediate area (~100m radius) for bat carcasses. However, the absence of carcasses does not mean the cave is *Pd* or WNS-free. There may or may not be bat carcasses within the hibernaculum itself. In some cases bats simply leave the hibernaculum and die undetected on the landscape. Carcasses may also be removed by scavengers (*e.g.*, raccoons).

For more details, see the case definitions under diagnostic categories for reporting cases of bat WNS in the Bat Necropsy Protocol: http://www.cwhc-rcsf.ca/bat_health_resources.php#white-nose-syndrome

Data collection in hibernaculum:

1. Record the percentage of bats with visible fungal growth
2. Record the number of live bats
3. Record the number of dead bats
4. Record the species of bats
5. Collect swab samples of bats and/or the environment for detection of *Pd* (see below for instructions)
6. Collect dead bats inside or near the hibernacula for detection of WNS (see "Bat White-Nose Syndrome Submission Protocol" for details on priority species, number of carcasses to submit and contact details: http://www.cwhc-rcsf.ca/bat_health_resources.php

Dead bats should be collected for diagnostic labs, researchers, and local natural history museums. Be aware of the risks of contamination when handling, storing, or sending potential *Pd* infected bat carcasses, especially to places that are still free of *Pd* and WNS. Always contact the recipient and discuss risk mitigation.

It can be difficult to tell live bats from dead bats hanging on cave walls. Dead bats can remain hanging on walls for years as they slowly decompose. It can also be difficult to differentiate between *Pd* on a bat and other fungal growth, dirt, dust or pieces of gypsum. Look for growth of *Pd* on any exposed skin

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surface of bats; do not focus exclusively on the muzzle. It has been observed that several years after *Pd* becomes established in caves, visible growth on bats is more often on the wings and uropatagium (tail membrane) as opposed to the muzzle. The fungus *Trichophyton* sp. can look very similar to *Pd* growth on hibernating bats. Other fungi, such as *Mucor* sp., can grow on guano stuck to a hibernating bat. Surveyors should be aware of these facts that confound *Pd* and WNS surveillance and take pictures for documentation and swabs of any fungi seen growing on hibernating bats for later *Pd* testing (qPCR or culture) or fungal identification (culture). Once a bat dies, a suite of other fungi start growing on the carcass, but this does not necessarily interfere with the detection of *Pd* on the carcass or the potential confirmation of the disease WNS.

It is best not to handle live bats during surveillance. Instead, minimize disturbance by carefully swabbing bats *in situ* on the hibernaculum wall or ceiling. Also, be sure to follow the recommended decontamination procedures (see WNS Decontamination Protocol: http://www.cwhc-rcsf.ca/bat_health_resources.php), especially if surveillance involves entering multiple sites. *Pd* can persist in hibernacula during all seasons for years without the presence of bats. The concern is not just the spread of *Pd* to new areas, but also the intermixing of different strains of the fungus which may lead to new, possibly more virulent, genetic variants.

Surveillance techniques: Many surveillance techniques have been described in the literature for *Pd*/WNS surveillance, including wing damage scoring [Reichard and Kunz 2009] and Ultraviolet fluorescence of WNS lesions [Turner et al. 2014]. However, in Canada, it is recommended that only approved qPCR or fungal culture be utilized as confirmatory diagnostic tests for *Pd* detection. Surveillance for the disease WNS requires additional diagnostic evaluation of dead bats, including necropsy and histology. *Pd* surveillance requires taking swabs of the environment within hibernacula, swabbing live bats, or collecting guano or substrate at hibernacula or summer roosting sites. In regions with unknown *Pd* or WNS status it is important to swab live bats and the hibernaculum environment as well as collect soil samples beneath hibernating bats, if possible. However, in *Pd* or WNS positive sites, to minimize disturbance of live bats, we recommend only swabbing the hibernaculum environment and those species of bats for which the *Pd* or WNS status are currently unknown.

Collecting environment samples

This section provides instructions on swab, guano, and substrate collection and is adapted from 'WNS/Pd Continental Transmission Study' prepared by Winifred Frick. A minimum of 10 samples per site is recommended but sample size will vary based on hibernaculum size, number of bats, and logistic capacity of field personnel and laboratories. Substrate sampling can include soil and guano samples or swabs of hibernaculum walls. We recommend that substrate sampling be done in areas where bats routinely roost as opposed to hibernacula entrances.

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Pd Sampling

(1) Live Bat sampling
   a. Record if fungal growth is visible
   b. Swab bat
      i. Use dry and sterile polyester swabs (do not use cotton swabs as they can contain PCR inhibitors)
      ii. Swab bat 5x on wing and 5x on muzzle
      iii. Store swabs dry (e.g., in a sterile whirl pak bag or vial)
      iv. Apply a unique ID tag to bag or vial
   c. Record unique ID and any other data (location, species, gender, visible fungus, etc.)

(2) Substrate sampling
   a. Swab substrate / collect soil
      i. Use dry and sterile polyester swabs to test substrate or scoop a soil sample into a sterile vial or whirl pak bag
      ii. Swab 5x on cave substrate and/or collect roughly 1 gram of soil
      iii. Store swabs / soil samples dry (e.g. in a zip-lock bag or vial)
      iv. Apply a unique ID tag to bag or vial and a label "SUBSTRATE"
   b. Record unique ID and any other data (e.g. location, how far from cave entrance, presence of bats with or without *Pd* visible, etc.)

(3) Guano sampling (Cory Olson, WCS Canada, pers. comm.)
   a. Collect guano
      i. Wear proper PPE including a NIOSH-approved respirator (TC-84A particulate filters) to prevent inhalation of guano dust, in addition to disposable exam gloves, and rubber boots
      ii. Scoop guano directly into a 50 ml plastic centrifuge container (*e.g.*, VWR cat# 10160-140, Ultra High Performance 50mL Centrifuge Tubes, rated to 20,000 x g) or use a bucket (attached to telescopic pole if out of reach) to scoop it up and transfer it to centrifuge container. Decontaminate reusable containers with soap water and bleach to kill *Pd* AND destroy DNA between sampling sites.
         - Note that methods for storing guano for single-species DNA barcoding or other research may differ from guano collected for *Pd* surveillance.
      iii. Collect at least 5 ml, but ideally 25 ml of guano (filling the container no more than halfway facilitates homogenization later in the process)
      iv. If not shipped immediately or frozen for long term storage immediately, ensure samples are kept chilled and dried, preventing buildup of moisture. Moisture is better absorbed when a sample is kept in a paper envelope inside a zip-lock bag with a desiccant pack. Transfer the sample to the 50 ml plastic centrifuge container before shipping or freezing.
      v. Apply a unique ID tag to the vial and a label "GUANO"
   b. Homogenization
      i. The Animal Health Laboratory at the University of Guelph homogenizes guano samples for a $10 fee (as of January 2022).

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For information on guano collection methods used in Saskatchewan and Alberta in 2021, see the online presentation "Bats and Bridges: Using guano sampling for species inventories and Pd surveillance" by Cory Olson of the Wildlife Conservation Society Canada and Alberta Community Bat Program.

Mailing *Pd* samples

(1) Decontaminate everything using appropriate disinfecting wipes (see WNS Decontamination Protocol: http://www.cwhc-rcsf.ca/bat_health_resources.php), including datasheets which should be printed on waterproof paper such as "write-in-rain" to facilitate this process.

(2) *Pd* samples: Please refrigerate the samples if they are not to be submitted immediately, or freeze the samples for long term storage before submission.

(3) Mailing: Ship samples with an ice pack because cold storage/shipping minimizes background growth and makes testing more reliable. Complete shipping and handling instructions for entire carcasses are available at http://www.cwhc-rcsf.ca/bat_health_resources.php.
If you are not involved with a project or collaborative research that provides qPCR testing for *Pd*, in Canada field samples for *Pd* qPCR testing can be shipped as diagnostic specimens to Animal Health Laboratory-Molecular Biology, University of Guelph (419 Gordon Street, Guelph, Ontario, Canada N1G2W1 (ahlmolec@uoguelph.ca, A519-824-4120 ext 54086, https://www.uoguelph.ca/ahl) or to the Animal Health Centre, Abbotsford, BC (http://www.agf.gov.bc.ca/ahc/; Toll Free 1-800-661-9903 [BC Only]; 604-556-3003). Please contact these laboratories prior to sending specimens so their personnel can be aware of and make appropriate arrangements for the submission. For up to date cost of sample analysis, please contact the respective laboratories at the phone numbers or email addresses as listed below.

(4) If samples are to be submitted from a bat confirmed with rabies or from a bat displaying behaviour suggestive of rabies, please contact the diagnostic lab before shipping the samples in viral inactivated reagent, *e.g.* inactivated VTM, Tripure or TriReagent.

Direct Links

WNS Decontamination Protocol

Recommendations for WNS decontamination during summer activities

Recommended Culture Method for *Pseudogymnoascus destructans*

Shipping and Handling Instructions Bat submission protocol

Evolving approach to white-nose syndrome diagnostic standards in Canada

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CWHC Contacts

Jordi Segers, Canadian White Nose Syndrome Program Coordinator (jsegers@cwhc-rcsf.ca; 902-566-0744)

Dr. Hugh Cai, Animal Health Laboratory, University of Guelph (hcai@uoguelph.ca; 519-824-4120 ext 54316)

Animal Health Centre, Abbotsford, BC (http://www.agf.gov.bc.ca/ahc/; Toll Free 1-800-661-9903 [BC Only]; 604-556-3003)

Regional bat surveillance experts

See the CWHC website for regional office contact details and see the CWHC Bat Health Regional Outlook page for provincial and territorial WNS surveillance expert contact details.

References

Turner GG, Uphoff Meteyer C, Barton H, Gumbs JF, Reeder DM, Overton B, Bandouchova H, Bartonička T, Martínkov N, Pikula J, Zukal J, Blehert DS. 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases, 50(3): 566-573.

Reichard JD, Kunz TH. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterologica, 11(2): 457-464.