

**MINISTRY OF ENVIRONMENT AND MINISTRY OF FOREST LANDS AND NATURAL
RESOURCE OPERATIONS, BRITISH COLUMBIA**

Decontamination SOPs for working around bats and bat habitats

PURPOSE

To describe Standard Operating Procedures (SOPs) for field staff to reduce the risk of introduction of the agent that causes White Nose Syndrome in bats, *Pseudogymnoascus destructans* (formerly known as *Geomyces destructans*), into B.C., and to reduce the risk of disease transmission among sites and among animals within a site

DESCRIPTION

White Nose Syndrome (WNS), a serious disease that has killed over 6 million bats in eastern and central North America (<http://www.whitenosesyndrome.org/>), is caused by the fungus, *Pseudogymnoascus destructans* (*Pd*). The method of transmission of the *Pd* fungal spores is not fully understood but both human associated transmission and bat-to-bat transmission are possible. WNS was detected in Washington State near the B.C. border in March 2016. It has not yet been found in B.C., but bat species found here are known to be susceptible. Therefore, it is prudent to implement measures to lower the risk of human-associated transportation of fungal spores.

If signs of WNS are detected (large numbers of dead or dying bats, day flights of bats observed in the middle of winter, bats with damaged wing membranes etc.), please report them immediately to,

B.C. Community Bat Program

1-855-922-2287 or info@bcbats.ca

Please follow protocols set out on Page 2 of the Factsheet on WNS in bats, posted on the BC Wildlife Health website at <http://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/wildlife/wildlife-health/wildlife-diseases/white-nose-syndrome>.

In addition, if bat work is conducted in or near fresh water bodies there is a potential to inadvertently move amphibian diseases including *Batrachochytrium dendrobatidis* (*Bd*) the causal agent of Chytridiomycosis, and ranavirus strains among wetlands and streams (<http://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/wildlife/wildlife-health/wildlife-health-matters/amphibian-health>).

RESPONSIBILITY

It is essential that all staff working with bats incorporate these decontamination SOPs into their field and research plans. Even if bats are not directly captured or handled, please implement Section 1 and Section 2 of this protocol when working in or around habitats where bats congregate (caves, mines, roost sites etc). It is recommended that these decontamination protocols be implemented in B.C. when people, equipment and materials are moving between mines and caves or other potential bat roosts or hibernacula.

Research to date suggests the highest spore-loads on bats are found in winter and spring, although spores have been found on some bats in summer, especially bats in close vicinity to hibernation areas. Because the viability of spores on bats in summer is not fully understood, it is suggested that decontamination SOPs be implemented year-round.

When working around wetland habitats, the Interim Hygiene Protocols for Amphibian Field Staff and Researchers applies (<http://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/wildlife/wildlife-health/wildlife-health-matters/amphibian-health>). even if animals are not directly sighted or handled.

STANDARD OPERATING PROCEDURES

The SOP has been divided for ease of use; Section 1 of the SOP addresses the general decontamination procedures, followed by more specific recommendations to address three levels of transmission risk:

Section 1: General procedures to decontaminate field gear, equipment and clothing.

Section 2: Procedures to reduce the risk of introduction of *Pd* into B.C.

Section 3: Procedures to reduce the risk of disease transmission among sites within B.C.

Section 4: Procedures to reduce the risk of disease transmission among individual bats within a site.

Section 1 – General procedures for field gear, equipment and clothing

The first step in the decontamination procedure is to thoroughly wash all mud and other debris from equipment, as these can reduce the efficiency of the disinfection procedure. A limited number of disinfectants have been laboratory tested in the US and

shown to be effective in making *Pd* spores nonviable

(<http://www.whitenosesyndrome.org/topics/decontamination>). However, some of these products are not available in Canada or the Canadian formulations of certain products do not appear to contain adequate quantities of active agent. We recommend the following decontamination procedures but these may change with time. Please read and follow all safety and health recommendations on disinfectant products.

1. Soaking in disinfectant containing at least 0.26% ammonium quaternary compounds for 10 minutes. There is currently much debate about what products available in Canada can be used at this concentration, as most manufacturers do not provide safety information regarding mixing their products at this strength. Until further guidance can be provided by the US Fish and Wildlife Service and/or the Canadian White Nose Syndrome Interagency Committee, we recommend you employ caution with whatever chemical you end up using. Some possible concentrates that you may consider dilution include: Zep Aqua San, Virkon, Aseptol2000, etc.

Two brands of disinfecting wipes sold in Canada have the minimum concentration of ammonium quaternary compound required to kill *Pd* ($\geq 0.26\%$ quats): Clorox and Lysol disinfecting wipes.

MSDS:

<http://www.thecloroxcompany.com/downloads/msds/wipes/cloroxdisinfectingwipes-freshscentjwcanadafca.pdf> and [http://www.rbnainfo.com/MSDS//CA/CA%20-%20LYSOL%20Disinfecting%20Wipes%20\(all%20sizes,%20all%20scents\)%20-%20English%20\(Feb%202012\).pdf](http://www.rbnainfo.com/MSDS//CA/CA%20-%20LYSOL%20Disinfecting%20Wipes%20(all%20sizes,%20all%20scents)%20-%20English%20(Feb%202012).pdf)

Due to the uncertainty that still surrounds the safety and efficacy of Canadian fungicidal products, it is recommended that you use the hot water method (below) when possible.

2. Soaking in 10% bleach (1:10 or one part bleach to 9 parts water) for 10 minutes (approximately half cup bleach in a litre of water). The MSDS is at:

<http://www.iaprisoinind.com/downloads/msds/IPI-ChlorineBleach10.pdf>

3. Submersing in water at a temperature of at least 55⁰C for >20 minutes.

4. Steam-cleaning of large pieces of equipment may be used where other methods are impractical but its efficiency has not been tested.

5. New as of April 2016 – four new products are now recommended for inclusion in decontamination procedures: ethanol (60% or greater), isopropanol (60% or greater),

isopropyl wipes (70%), or hydrogen peroxide wipes (3%). The latter two wipes have been found effective against *Pd* on contact and do not require soaking. Use of ethanol and isopropanol requires at least one minute of soaking.

Smaller equipment such as boots, ropes, climbing harnesses, mistnets, etc., may be submerged in the above decontamination liquids for the advised time, then rinsed and dried. Larger equipment or non-submersibles such as headlamps and acoustic equipment, should be sprayed where possible or wiped down with decontamination liquids or Clorox/Lysol wipes, ensuring that the surfaces are wet for the minimum time required before being wiped dry. Clothing should be washed in hot water with bleach in a washing machine, hand washed with a pre-soak of 10 minutes in the decontamination liquids above or immersed in a >15 minute pre-soak in water $\geq 60^{\circ}$ C.

It is important to note that the information on the efficacy of the recommended chemical disinfection agents is rapidly evolving. So, it is important to consult the Canadian National White Nose Syndrome Plan (<http://www.cwhc-rcsf.ca/docs/BatWhiteNoseSyndrome-NationalPlan.pdf>) for the latest information and ensure that the most up to date information on chemical disinfection procedures are followed.

The products above may damage gear, pose health risks, and cause environmental damage. It is the responsibility of the users to read the MSDS sheets, follow safety protocols, use protective gear and follow appropriate procedures for disposal.

Section 2 - Procedures to reduce the risk of introduction of *Pd* into B.C.

Although bat to bat contact is the primary mode of *Pd* transmission, human mediated transport of disease spores can potentially occur. **Any equipment or gear that has been previously used in states or provinces where WNS has been found must not be used in B.C. For a map of current locations where WNS has been detected please visit, <http://www.whitenosesyndrome.org/about/where-is-it-now>.** Currently we do not know if the western coastal strain is the same as the one found in eastern Canada/US and thus we need to treat these areas as separate infections and not spread spores between these 2 separately infected areas. Other equipment used previously in a bat roost or potential bat roost such as a mine or cave (e.g. boots, clothing, headlamp, mining equipment, ropes, climbing harnesses) and acoustic bat detection

equipment should be thoroughly decontaminated using the procedures in Section 1 after use and before moving to another site.

Section 3 – Procedures to reduce the risk of disease transmission among sites

As *Pd* has recently been detected in western North America, we are instituting these SOPs as a precaution against spreading as yet undetected *Pd* in B.C.

These procedures should be adhered to when moving between sampling sites. For example, when bat habitats that potentially contain *Pd* spores (caves and mines) are entered, equipment and footwear/clothing should be cleaned and decontaminated, especially if bats are observed/handled. Summer roost sites such as attics have been shown to support only low levels of *Pd* spores and decontamination procedures are thus not required at this time. Same-day decontamination between-entry is not required if mines/caves entered are <10 km apart, within the same watershed (along the same river/tributary), and no bats have been handled. Mines/caves that are >10 km apart, are in different watersheds, or with major geographical barriers between them are considered separate sites and between-entry decontamination is required.

When bats are captured, at the end of each night, or between uses, all nets and equipment that has been in contact with bats must be decontaminated. Any bags or covers for mistnets need to also be decontaminated if they have been in contact with a potentially contaminated net. Harp traps should be restricted for use at roosts only. Such traps should not be used in foraging situations where bats from different roosts may be forced into close contact during capture in the harp trap thereby potentially increasing bat to bat disease transmission. Plastic collection bags under harp traps are preferable to cloth collection bags as plastic is easier to disinfect or discard between sites/nights due to the smooth, quick-dry surface.

All equipment that has come in contact with a bat or that has been inside a potential bat roost (mines/caves) and has not been decontaminated should be stored in a waterproof box/tote during transportation to prevent contaminating the vehicle and to prevent the vehicles from acting as secondary sources of cross contamination. All potentially contaminated clothing should also be stored in this manner. After a night of bat capture for example, all clothing worn during the capture, handling and release of bats needs to be removed on site, placed into containers (e.g. construction grade garbage bags) and sealed until they can be washed and decontaminated (as outlined in Section 1). Use of Tyvec or disposable overalls over coats and clothing is recommended to reduce the

exposure of clothing and hair to spores. Rain jacket, rain pants, and rubber boots are versatile field clothing, and may make excellent outer layers as they are easily washed/decontaminated. Always clean and disinfect the outside surfaces of storage containers prior to putting in a vehicle.

If the equipment is to be used immediately at another site prior to returning to the laboratory or field station, the SOP should be carried out on a road or other impermeable surface and away from water bodies to prevent environmental contamination of the disinfectant solutions. If there is no time to ensure complete drying, residual solution should be rinsed off with clean water, again working away from water bodies. Even very low residual amounts of some decontamination solutions may adversely affect bats on contact or contaminate water.

Section 4 - Procedures to reduce the risk of disease transmission among individuals within a site

It is recognized that bats roosting together have a high likelihood of bat-to-bat contact and transmission of spores. However, when capturing bats at foraging sites or during travel through daily, seasonal or migration corridors, there is a potential for researchers to increase the level of disease transmission by bringing bats into closer contact with each other. Compared to the winter season, the density or viability of *Pd* spores on bats during the summer is expected to be low given higher daily average temperatures and the ability of bats to groom frequently to prevent *Pd* proliferation. However, bat researchers are obligated to implement as high a level of decontamination procedures as is logistically feasible.

i. Extracting bats from nets

Latex/vinyl/nitrile disposable gloves should be used for all extractions of bats from nets. A new pair of gloves should be used for each bat. The teeth of small bats may be felt through latex gloves, but they are less likely to puncture the glove material. Leather gloves may be necessary when handling larger bats such as Big Brown bats and Pallid bats. If non-disposable gloves are used, they must be decontaminated (as described in Section 1) and rinsed between individual bats, thus requiring many pairs of gloves per trap session. Alternatively, large disposable gloves may be worn over non-disposable gloves, and changed between individual animals. Dexterity can be compromised with the use of gloves, especially when two pairs of gloves are worn, one over the other. If bare hands are in contact with a bat or contaminated equipment, wash with soap and water

(preferred) or thoroughly wipe with several hand wipes (e.g. diaper wipes) if water is unavailable (not the preferred method). Decontamination liquids in Section 1 may pose health risks when exposed to bare skin.

Professional judgement should be used to strike a balance between reducing bat to bat disease transmission and risking the safety and health of the bat and human. This is particularly the case for captures of bats emerging from roosts. For example, bats using the same maternity roost are likely to be in contact with each other and sharing a roosting surface. If a large number of bats emerging from this roost into a mistnet or harp trap makes changing gloves between bats an obvious added stressor to captured bats that remain in nets longer due to this procedure, the researcher may determine that gloves not be changed in between each bat. In this situation the researcher may also determine that equipment to measure bats will come into contact with multiple bats to ensure that the processing time of a large capture number from a roost is completed in a timely manner. The most important thing to remember when netting a roost is that all precautions are taken to not transport spores into the roost site or out of the roost site. In other words, all equipment arriving to the site and leaving the site need to be decontaminated or contained.

ii. Holding and processing of bats

Captured bats should be placed in holding bags, one bat per bag. Bags should be new or cleaned using the decontamination procedures described in Section 1. Care must be taken to not touch the outside of the bag with potentially contaminated gloves which would cause contamination to any other surface that it subsequently touches. All holding bags are porous to some extent to enable bats to breath. As such, if spores are present, they may escape the bag. In general, bats in bags, while they await processing, should be placed somewhere that can be easily decontaminated.

Processing equipment must be decontaminated between bats, or better yet, it should not touch the bat. Having several sets of processing equipment will allow time for decontamination between uses. Equipment should only touch the bat if absolutely necessary. Placing the bat in a thin plastic sandwich bag (foldable baggies work best) and measuring through the bag is one method to avoid direct contact between callipers, weigh scales, rulers etc., and avoids treating equipment between individual bats. Care must be

taken to ensure the plastic never covers the bat's nose or otherwise interferes with breathing.

The researcher must be aware of what his/her hand touches after it has been in contact with the bat, i.e. a gloved or bare hand that has been in contact with a bat should not touch processing equipment, headlamps, record books, data sheets etc. as these may cross contaminate. A dedicated record keeper that does not touch handling equipment will avoid cross contamination of pens, field books, etc. Since headlamps need constant adjustment while extracting and processing bats, they can be covered with a disposable shower cap which can be changed between handling individual bats. Practicing the steps involved in processing a bat and minimizing cross-contamination is recommended prior to field deployment.

iii. Genetic sampling

If genetic sampling is performed, the tip of the biopsy punch should be dipped in ethanol and flamed between individual bats, unless new punches are used for each bat. If more than the tip of the biopsy punch comes in contact with the bat or contaminated surfaces, the punch should be discarded or the entire punch decontaminated (as in Section 1). The boards used as backing when biopsy punching must be decontaminated between bats or the board can be wrapped in several layers of cling-wrap plastic such that the biopsy punch does not penetrate to the board, and the cling-wrap plastic must be changed between processing individual bats.

MATERIALS

- Plastic tote or box to store contaminated field equipment or clothing
- Buckets and totes for mixing disinfectant solution and soaking equipment
- Spray bottle and instructions (MSDS) for disinfectant solution mixing
- Scraper, brushes, hand-brushes
- Rubber dishwashing gloves, rubber apron
- Clean cloth bags for temporarily holding animals
- Household bleach (6% sodium hypochlorite solution) or disinfectant with $\geq 0.26\%$ quaternary ammonium compounds as active ingredients
- Latex, vinyl or nitrile gloves for handling animals
- Plastic sheets, wax paper or cling-wrap plastic to cover working surfaces
- Biopsy punches (adequate for all animals) and sterilizing solutions (ethanol) and alcohol burner/lighter for disinfecting instruments when animals are biopsied for genetic sampling
- Container for waste disposal and contaminated gloves, plastic covers, etc.
- Tyvec/disposable overalls and/or change of clothes and footwear

RELEVANT LITERATURE

Decontamination procedures for bat-related activities – Canada’s national plan with focus on eastern Canada and infected areas:

http://www.cwhc-rcsf.ca/docs/WNS_Decontamination_Protocol-Nov2016.pdf

<http://www.cwhc-rcsf.ca/docs/BatWhiteNoseSyndrome-NationalPlan.pdf>

National white-nose syndrome decontamination protocol – Version 04.12.2016. US Fish and Wildlife Service. Available:

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

Human spread of White-Nose Syndrome: Why decontamination is important.

<http://www.whitenosesyndrome.org/resource/human-spread-white-nose-syndrome-why-decontamination-important>

Decontamination procedures for cavers, miners and other users of habitats associated with bats in western Canada:

http://www.cwhc-rcsf.ca/docs/WNS_Western_Transmission_Prevention.pdf

ACKNOWLEDGEMENTS

This protocol was initially drafted by Dr. Cori Lausen, Wildlife Conservation Society Canada, in consultation with Dr. Helen Schwantje and Cait Nelson, Ministry of Forests, Lands, and Natural Resource Operation, Dr. Purnima Govindarajulu, Ministry of Environment. It has been reviewed by the BC Bat Action Team Bat Health Committee. Unpublished sources of information were obtained from Jordi Segers, Canadian White Nose Syndrome Coordinator, and Canadian Wildlife Health Cooperative.