

Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada

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Emerging amphibian and reptile disease in Canada

Over the past few decades, emerging infectious diseases have caused population declines and extinctions of amphibian and reptile species worldwide. Pathogens that pose a potential threat to Canadian amphibian and reptile populations include:

Chytrid Disease

Caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), this disease has driven the decline or complete extinction of over 200 species, mainly frogs, worldwide. The fungus has been detected throughout much of Canada, although disease outbreaks are not known to be common.

Salamander Chytrid Disease

Caused by the fungus *Batrachochytrium salamandrivorans* (*Bsal*), this disease recently spread from Asia to Europe where it caused over 96% mortality in wild populations of Fire Salamander. *Bsal* has not yet been reported in North America, but the potential for introduction through global trade poses a significant risk to our native species.

Ranaviruses

These viruses have caused declines in many populations of amphibians, reptiles, and fish worldwide. Ranavirus infections can cause 90-100% mortality in populations and can affect multiple species at the same time. Ranaviruses have been responsible for disease outbreaks in many areas across Canada.

Snake Fungal Disease (SFD)

Caused by the fungus *Ophidiomyces ophiodiicola*, this disease can lead to severe illness and death in snakes. It has been detected in wild snakes throughout northeastern North America, including several locations in southern Ontario.

Purpose

This protocol provides guidance that will help prevent the spread of wildlife pathogens and assist in maintaining healthy amphibian and reptile populations in Canada. This document outlines a general approach to decontamination that is effective against the amphibian and reptile pathogens discussed above, as well as most other pathogens that may affect these species. This protocol is based on the best available scientific information and will be updated as new information becomes available. It is designed to be applied flexibly depending on particular field conditions.

Why decontaminate?

Field research activities can negatively affect wild reptile and amphibian populations through the accidental spread of pathogens. Proper decontamination of boots, waders, nets, boats, and other field equipment helps keep wildlife populations healthy by ensuring that pathogens are not transported between sites. Specialized equipment like calipers or spring scales can also transmit pathogens from site to site or from one individual to another at the same site. Find out more about wildlife disease prevention and monitoring at www.cwhc-rscf.ca

When to decontaminate

Equipment should always be disinfected between sites, particularly when working with critically endangered populations or at a location with an ongoing mortality event. The definition of a **site** may vary with the scale of the project, the geography of the area, and the ecological communities.

In aquatic environments, the following are considered separate sites:

- Wetlands, ponds, lakes, or other water bodies that are separated by terrestrial areas
- Each tributary of a river or stream
- Each upstream location within a river or stream; it is not necessary to decontaminate between each location when work in rivers or streams follows a downstream direction.

In terrestrial environments, the following are considered separate sites:

- Areas that are divided by major geographical barriers (e.g. lakes or large rivers, cliffs, major highways)
- Areas that are separated by more than 1 km of unsuitable habitat (e.g. agricultural land, urban settlements) or 3 km of suitable habitat (e.g. naturally occurring habitats that are permeable to movement by amphibian or reptile species). These distances are based on NatureServe minimum separation distances.

Within a site, it is recommended that gloves and other equipment (e.g. measurement tools) be changed or disinfected between handling individual animals, unless those animals have already been in close contact with each other (e.g. in the same pitfall trap). If gloves are not being worn, hands should be washed and disinfected between individuals (note: gloves should always be worn when handling amphibians to protect their sensitive skin from chemicals, oil and sweat on your hands).

Materials

- Commercial household bleach: example, Clorox® Bleach (active ingredient 4-6% sodium hypochlorite)
- Biodegradable soap
- Large bucket or tote that will hold about 25 litres (Rubbermaid totes work well)
- Bucket or container with water-tight lid
- Container with tap water
- Spray bottles
- Scrub brushes
- Dishwashing gloves and safety glasses



Why use bleach for decontamination?

This decontamination protocol recommends bleach as a disinfectant for several reasons:

- 1) Studies have shown that immersion in a relatively dilute bleach solution is sufficient to neutralize *Bd*, Ranaviruses and SFD (see the table at the end of this document for more detail). Information regarding the effectiveness of disinfectants for neutralizing *Bsal* has

not yet been published. However, bleach is likely effective against *Bsal* at similar concentrations used for *Bd* and other amphibian and reptile pathogens.

- 2) Bleach is readily available.
- 3) Bleach breaks down relatively quickly and poses a lower risk to the environment than some other disinfectants.

Steps for decontamination

- 1) Before you travel to your research site, ensure all your equipment has been decontaminated.
- 2) When you first arrive at the research site, identify a sunny location away from surface water (ponds, streams, ditches, etc.) that has an asphalt or hard-packed surface (e.g. a parking lot). This location will be your decontamination area after you finish your work. The direct sunlight will help break down the bleach before it can enter surface waters.
- 3) After you complete your work, return to your decontamination area and clean all equipment with tap water (do not rise with water from a local pond, lake, etc.), using a scrub brush to remove organic material (mud or vegetation) from your boots and other equipment. If done for the day, thoroughly wash equipment with soapy water and rinse with clean tap water.
- 4) Put on safety glasses and make your decontamination solution in a large bucket / tote by adding 1 part bleach to 19 parts water (e.g. add approximately 1 litre of bleach to 19 litres of tap water or, for smaller batches, 50 ml of bleach to 950 ml of water).
- 5) Immerse equipment (including scrub brushes) in the bleach solution. Use a spray bottle to soak larger items thoroughly. Soak equipment for five minutes.
- 6) For sensitive equipment like calipers, cameras, electronic scales, etc., rinse or wipe with 70% ethanol.
- 7) Rinse the bleached items with clean tap water to prevent exposing the next site to residual bleach and to minimize damage to the equipment. If clean tap water is not available, rinse the bleached items with water from the next site (away from aquatic habitats). Do not rinse with water from the current site. If you are done for the day, allow the equipment to dry completely.



- 8) Whenever possible, remove the bleach solution and soapy water from the site using a bucket with a water-tight lid and dispose of them appropriately. Otherwise, dispose of them at the decontamination site (note: these solutions should not be disposed of in the field if it is raining or if a suitable decontamination area is not available). Ensure full compliance with local laws and safe disposal regulations
- 9) Once home, wash clothing in hot water.

Additional considerations

- 1) As an alternative to decontaminating all equipment between sites, pack separate sets of equipment (e.g. bags or containers for animals, marking and measuring tools) for each site. Clean equipment after use and seal it in a plastic bag until it can be decontaminated.
- 2) It may not be possible or safe to decontaminate with bleach at some sites. In these situations, rinse and /or wash your equipment, seal it in a garbage bag and decontaminate off-site.
- 3) Some hiking boots or shoes are difficult to decontaminate thoroughly, but full immersion in bleach solution does work. Selecting footwear that is easy to clean (i.e. rubber boots) will help to keep the decontamination process effective.
- 4) When working at sites where there have been disease outbreaks or sites with critically endangered populations, have dedicated field equipment that will only be used at that site.
- 5) See the California Centre for Amphibian Disease Control's protocol for additional tips on how to make decontamination more portable:
<http://www.ccadc.us/docs/DeconForProfessionals.pdf>
- 6) Powdered bleach and other concentrated disinfectants can be highly toxic to the environment and to humans; it is recommended that these disinfectants be avoided.

Considerations when using Bleach

- 1) See manufacturer's recommendations and Material Safety Data Sheet requirements for safe handling and disposal.
- 2) Bleach contains the active ingredient sodium hypochlorite. The concentration of sodium hypochlorite in commercially available bleach varies among brands but is usually close to 6%. Check the concentration and adjust the ratio of your solution if necessary. For quick calculations, use an online calculator like this one:
<https://www.publichealthontario.ca/en/ServicesAndTools/Tools/Pages/Dilution-Calculator.aspx>
- 3) Bleach can be fatal to amphibians and reptiles, and amphibians are particularly sensitive. Ensure that bleach is kept away from these animals and their habitats.
- 4) Bleach is deactivated by prolonged exposure to organic material, air, water, and direct sunlight. Once a store-bought container of bleach is opened, it may only remain effective for 1 month. Once mixed, a bleach solution should be used within 5 days to ensure efficacy. Ideally, store bleach and solutions in a sealed, opaque, container. Solutions should be mixed and used fresh to ensure that they will be effective.

- 5) If it is raining, disposing of the decontamination solution on site may result in active bleach entering surface waters. In situations like this, it is preferable to soap and rinse on site, and seal up equipment into bags for decontamination with bleach off-site.

Considerations when using Ethanol

- 1) See manufacturer's recommendations and Material Safety Data Sheet requirements for safe handling and disposal.
- 2) Undiluted ethanol (70% concentration of ethyl alcohol) has been proven to be effective at removing some pathogens with exposures of 20 seconds to 2 minutes.
- 3) Ethanol may be fatal to humans if swallowed or inhaled. Repeated or prolonged exposure can damage liver, kidneys and nervous system or cause eye irritation or dermatitis. Ethanol may be absorbed through the skin.
- 4) Ethanol can harm amphibians by destroying mucus and wax, resulting in dehydration and microbial infection.
- 5) Ethanol may damage rubber and plastics, and may deteriorate glues.

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Appendix 1: Tested efficiency of decontamination products

Product (% active ingredient)	Tested concentration	Exposure time (min)	Effective against	Notes	Source* (see references)
Bleach (6% Sodium Hypochlorite)	1:32 (0.2% Sodium Hypochlorite)	1-10	<i>Bd</i> RV SFD	Vapor may cause severe irritation or damage to eyes and skin; harmful if swallowed. Fatal to amphibians at high concentrations. Corrodes metals; fades colours and breaks down cloth fibers.	1, 5, 9, 13
70% Ethanol	70% ethyl alcohol	2	<i>Bd</i> RV SFD	May be fatal if swallowed or inhaled; can damage liver, kidneys and nervous system by repeated or prolonged exposure; may be absorbed through skin; repeated or prolonged contact can cause eye irritation or dermatitis. May harm amphibians by damaging epidermal coating. May damage rubber and plastics; may cause deterioration of glues.	1, 5, 9, 13
Benzalkonium chloride	1mg/ml	10	<i>Bd</i> RV SFD	Requires extreme care in handling (see M.S.D.S.). Very toxic to aquatic organisms. Essential to ensure this chemical does not enter the environment.	5, 13, 15
Virkon S® (20.4% Potassium peroxymonosulfate)	1mg/ml	1	<i>Bd</i> RV	Not tested against SFD. Harmful if swallowed; irritating to respiratory system and skin; may cause serious eye damage. Non-toxic to amphibians. Safe for fabric; may cause pitting on galvanized or soft metal if not rinsed with water.	1, 5, 8, 9, 15, 18
Nolvasan® (2% Chlorohexidine)	1:127	1	RV	Not effective against SFD. May be fatal if inhaled; causes irreversible eye damage; harmful if swallowed. Safe for amphibians for short durations. No reported effects on equipment.	1, 9, 13
Lysol® Power Bathroom Cleaner	100%	10	SFD	Not tested against <i>Bd</i> , RV.	13
Lysol® All Purpose Cleaner	100%	10	SFD	Not tested against <i>Bd</i> , RV.	13
NPD®	100%	10	SFD	Not tested against <i>Bd</i> , RV.	13
CLR® Bath & Kitchen Cleaner	100%	10	SFD	Not tested against <i>Bd</i> , RV.	13
409®	100%	10	SFD	Not tested against <i>Bd</i> , RV.	13
Didecyl dimethyl ammonium chloride	2ml/L	1	<i>Bd</i> RV	Not tested against SFD.	5, 15, 18
Quaternary ammonium compound 128	Full strength to 1X10 ⁻³	5	<i>Bd</i> RV	Not tested against SFD.	5, 8
F10® Super Concentrate Disinfectant	0.7 ml/litre	1	<i>Bd</i> RV	Not tested against SFD.	5, 18
TriGene® Virucidal Surface Disinfectant Cleaner	0.2 ml/L	1	<i>Bd</i> RV	Not tested against SFD.	5, 18
10% sodium chloride	10%	5	<i>Bd</i> RV	Not tested against SFD.	5, 8
2% potassium permanganate	2%	10	<i>Bd</i> RV	Not tested against SFD.	5, 8
Sterilizing UV light	1000 mW m ⁻² wavelength 254nm	1	RV	Not effective against <i>Bd</i> . Not tested against SFD.	5, 8, 15

Hot wash for cloth bags and clothing	≥60°C	15	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 15
Heat	≥60°C	30	<i>Bd</i> <i>RV</i>	Not tested against SFD.	5, 15, 18
Complete drying (footwear only)		3 hours or more	<i>Bd</i>	Not effective against <i>RV</i> . Not tested against SFD.	5, 15

*Includes documents that recommend and/or experimentally test decontamination efficacy.