

ECOSYSTEMS BRANCH, MINISTRY OF ENVIRONMENT, BRITISH COLUMBIA

Interim Hygiene Protocols for Amphibian field staff and researchers

PURPOSE

To describe hygiene protocols to be used by amphibian field staff and researchers to reduce risk of disease transmission among sites and among animals within a site.

DESCRIPTION

Over the past few decades emerging infectious diseases such as the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) and ranavirus have been implicated in the decline of amphibian populations from many sites around the world. It is thought that field staff could act as potential vectors of transmission introducing these diseases into new sites and to naïve species. The risk of this mode of transmission is not fully understood compared to other modes of transmission, for example by vectors such as waterfowl and other wildlife. However, to reduce the risk, it is essential that all field workers incorporate the hygiene protocol into their research plans as Standard Operating Procedure (SOP).

To encourage compliance, the SOP below aims to strike a balance between reducing risk of disease transmission and increasing ease of protocol implementation under field conditions.

The protocols below address two levels of disease transmission: 1) the risk of disease transmission between sites; and 2) the risk of disease transmission among individuals within a site.

RESPONSIBILITY

This protocol applies to all field staff working in aquatic habitats sampling amphibians in freshwater lentic and lotic wetlands.

MATERIALS

- Plastic tote or box to store field equipment.
- Buckets and totes for mixing bleach solution and soaking equipment
- Spray bottle for bleach solution
- Scraper, brushes, hand-brushes
- Rubber dishwashing gloves, rubber apron
- Clean plastic containers and bags for temporarily holding animals
- Household bleach (contains active ingredient 5-6.5% sodium hypochlorite solution)
- Vinyl or nitrile gloves for handling amphibians
- Sterilizing solutions (ethanol) and alcohol burner/lighter for disinfecting instruments if toe-clipping or marking animals.
- Container for waste disposal

STANDARD OPERATING PROCEDURES (SOP)

I. SOP when moving between sites

Definition of site

At geographic scales of tens of kilometres, watersheds and major geographical barriers should be used to designate separate sites.

Each tributary of a river should be considered a separate site. Wetlands, ponds and lakes separated by dry land should be considered separate sites.

Site designation is particularly difficult at smaller geographic scales and with small isolated water bodies. At scales less than 500 m, if the water bodies remain separate under high water/flood conditions then they should be considered separate sites. Within a stream (at distances less than 500 m) sampling should occur in a downstream direction where possible. Each stream and each upstream location should be considered separate sites.

Equipment treatment

Equipment such as wetsuits, waders, footwear, nets, buckets, and traps may act as vectors of disease spread. In this SOP we recommend household bleach for disinfection because it is widely available and it is easy to dispose under field conditions if the

precautions below are followed. However, there are a number of other disinfectants that have been tested (Table 1) that offer a variety of other advantages. The SOP can be used with these chemical disinfectants instead of bleach, but manufacturer's recommendations and Material Safety Data Sheet requirements for use and disposal must be followed. It is important to ensure that disinfectants do not leave a residue on equipment, as the residue can be harmful to amphibians

1. Before leaving a site, field workers must scrub using a hand brush and rinse using the pond/stream water to remove mud, algae, plants, snails and other invertebrates from all equipment. Disinfection procedures work best on cleaned equipment, free of debris.
2. A bleach solution with 0.2 % sodium hypochlorite and exposure time of 10 minutes has been shown to be effective against *Bd* (Johnson et al 2003). Commercial household bleach sold in North America often contains 6.15% sodium hypochlorite, but concentrations can vary. To prepare the disinfectant solution, add 32 ml of household bleach to 1 litre of water. This translates to approximately 3.5 cups (0.85 litre) of bleach to one tall bucket or tote (~ 25 litres) of water. In the absence of municipal/well water supply, water from the pond or stream can be used.
3. All equipment must be soaked in the bleach solution for a minimum of 15 minutes. Small items such as dipnets, and sample containers can be immersed in the bleach solution in a bucket or plastic tote. Larger items such as chest waders, paddles, boats, canoes, meter sticks and other survey equipment should be thoroughly soaked with the bleach solution using a spray bottle.
4. The bleach solution can be rinsed off after 15 minutes with clean water from a well or municipal supply. However, if clean treated water is not available, the items can be hung out to dry, preferably in sunlight, so that the bleach evaporates completely from the equipment.
5. The bleach solution can damage exposed skin and clothing. Dishwashing gloves and rubber aprons should be worn to protect clothing and skin from exposure to the bleach solution.

6. The SOP is most easily carried out back at the laboratory or field station. All equipment should be stored in a waterproof box or tote during transportation to prevent contaminating the vehicle and preventing the vehicles from acting as secondary sources of cross contamination.
7. If the equipment needs 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 away from the waterbody, so that no bleach contaminated water enters surface water bodies. If time permits, all equipment should be dried completely between sites. This is facilitated by having two sets of gear, one of which is drying while the other is in use. If the equipment needs to be used immediately at another site, residual bleach from nets and other equipment should be rinsed off using water from the second site, again working away from the waterbody. Even trace amounts of residual bleach can adversely affect amphibians on contact.
8. In the field, the bleach solution is best disposed far from the waterbody by pouring over an asphalt, hard roadbed or concrete surface where it breaks down in sunlight and evaporates. Ensure no bleach water enters surface waters directly.
9. Washing road vehicles at a carwash between watersheds is desirable. All off-road vehicles, boats, canoes, and other floatation devices should be subject to the same SOP as sampling equipment. Large equipment, such as boats and seine nets, are most easily handled by spraying with or soaking in the bleach solution and then rinsing off at a carwash using high pressure hot water rinses.

Special Sites

The above SOP should be sufficient to reduce the risk of disease transmission under most circumstances. However, at sites where there have been known disease outbreaks or sites with highly endangered amphibian populations extra precautions are necessary. The simplest solution would be to have dedicated field gear that is used only at that site.

II. SOP under Special Circumstances

The above SOP should be implemented under all normal operating conditions. However, if for unforeseen and unplanned reasons it is not possible to adhere strictly to

the above SOP the following precautions can be taken to reduce the risk of disease transmission.

1. At a minimum, all equipment should be scrubbed and rinsed thoroughly to remove debris, algae, invertebrates and mud.
2. Complete drying between sites can reduce the risk of transmission of chytrid fungus (*Bd*) but is not effective against ranavirus.
3. If it is possible to heat water, equipment should be soaked in water > 60° C for 30 minutes. This method may be practical for small equipment such as dipnets that come in direct contact with the animals.

III. SOP for handling amphibians within a site

It is important to assume that not all animals from a site are infected and that handling multiple animals without adequate caution will significantly increase the risk of between animal disease transmissions. The following SOP should be followed to ensure that handling by field staff does not increase the risk of an animal being exposed to a pathogen.

1. All field staff must ensure that their hands are well cleaned and free of residual chemicals such as sunscreen and insecticides at the start of the survey.
2. All field staff must wear disposable gloves when capturing amphibians. Non-powdered vinyl and nitrile gloves are preferred, as latex has been shown to have toxicity towards frog embryos and tadpoles.
3. Gloves should be changed between animal captures. If under unforeseeable circumstances access to gloves is limited, it is better to capture animals with bare hands and wash hands thoroughly in water to which the animals are habitually exposed, rather than to use the same pair of gloves to handle multiple animals.
4. Captured animals must be housed in separate disinfected containers or disposable bags such as plastic Ziploc bags.
5. The duration of restraint should be as short as possible. The greatest stress and risk of disease transmission between animals is during restraint. Every effort should be made

to process animals at the site of capture and avoid captivity. Containers used to house individuals should not be reused unless the containers have been treated using 4% bleach solution as described in SOP #1 above.

6. Fresh disposable gloves must be worn when measuring, swabbing or marking the animal.
7. Surgical instruments such as scissors used for toe clipping should be dipped in 70% ethanol and flamed briefly between uses. A *bic* lighter is useful and effective for flaming surgical instruments in the field. Wiping down with ethanol alone kills the pathogens but does not completely destroy the pathogen DNA. This may increase the number of false positives if the animals are being sampled for disease prevalence.
8. Instruments used for inserting PIT tags and other tags should also be sterilized between animals. Fresh disposable syringes should be used for each animal.
9. The tuberculin syringes used for fluorescent elastomer paint injections present a special problem. The needles can neither be changed between animals nor is it practical to use a fresh syringe for each marked animal. At this time the recommendation is that the needles should be sterilized with appropriate viricide/bactericide (or dipped in 70% ethanol) and rinsed with distilled water between making animals. The efficacy of this method in preventing between animal disease transmission needs to be assessed.

FURTHER INFORMATION

When in doubt, field staff should use the precautionary principle and use the highest standards of cleanliness possible. An additional half hour spent following the most stringent SOP is well worth the effort. A number of other protocols, using a variety of disinfection agents are listed below.

RELEVANT LITERATURE

- Daszak P, Cunningham AA, Hyatt AD. 2001. Draft guidelines for international translocation of amphibians with respect to infectious diseases. Attachment 6. *In*: Speare R and Steering Committee of Getting the Jump on Amphibian Disease. Developing management strategies to control amphibian diseases: Decreasing the risks due to communicable diseases. School of Public Health and Tropical Medicine, James Cook University: Townsville. 2001: 150-156.
- Department of Environment and Heritage. 2006. Threat abatement plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis. Department of Environment and Heritage, Commonwealth of Australia.
<http://www.deh.gov.au/biodiversity/threatened/publications/tap/chytrid/>
- Gutleb, A. C., M. Bronkhorst, J. H. J. van den berg, and A. J. Murk. 2001. Latex laboratory gloves: an unexpected pitfall in amphibian toxicity assays with tadpoles. *Environmental Toxicology and Chemistry* **10**:119-121.
- Johnson M, Berger L, Philips L, Speare R. 2003. Fungicidal effects of chemical disinfectants, UV light, dessication and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* **57**:255-260.
- Speare, R., L. Berger, L. F. Skerratt, R. A. Alford, D. Mendez, S. Cashins, N. Kenyon, K. Hauselberger, and J. J. L. Rowley. 2004. Hygiene protocol for handling amphibians in field studies. Amphibian Disease Group, James Cook University, Townsville 4811, Australia. <http://www.jcu.edu.au/school/phtm/PHTM/frogs/field-hygiene.pdf>
- US Forest Service Intermountain Region. Technical Chemical Information for Disinfecting Aquatic Invasive Species (excellent resource for calculating disinfectant dilutions)
http://www.fs.fed.us/r4/resources/aquatic/guidelines/chemical_tech_final.xls
- Webb, R., D. Mendez, L. Berger, and R. Speare. 2007. Additional disinfectants effective against the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* **74**:13-16.
- Wellington, R. and R. Haering. 2001. Hygiene protocol for the control of diseases in frogs. New South Wales National Parks and Wildlife Service Threatened Species Management Information Circular No 6, Hurstville, Australia.
www.npws.gov.au/wildlife/licence/frog.html

Table 1: Summary of disinfectant concentrations and exposure times found to be effective against *Bd*. Table reproduced with modification from Speare et al (2004) with additional information from Johnson et al (2003) and Webb et al (2007).

DISINFECTANT	CONCENTRATION	TIME	REFERENCE
Disinfecting surgical equipment and scales			
Ethanol	70%	1 min	Speare et al 2004 Webb et al 2007
Virkon	1mg/ml	1 min	Speare et al 2004
Benzalkonium chloride	1 mg/ml	1 min	Speare et al 2004
Disinfecting collection equipment, containers, footwear, waders, boats, nets and other field gear			
Household bleach (sodium hypochlorite 4% to 6%)	4% to 1% (0.2 to 0.01% sodium hypochlorite)	10 min to 30 sec	Speare et al 2004 Johnson et al 2003 Webb et al 2007
Didecyl dimethyl ammonium chloride	1 to 1000 dilution	30 sec	Speare et al 2004
Quaternary ammonium compound 128	Full strength to 1×10^{-3}	5 mins to 30 sec	Johnson et al 2003
Virkon	1 mg/ml 2 g/litre	5 min, 20 sec 1 min	Johnson et al 2003 Webb et al 2007
F10 Super Concentrate Disinfectant	0.7 ml/litre	1 min	Webb et al 2007
TriGene Viricidal Surface Disinfectant Cleaner	0.2 ml/litre	1 min	Webb et al 2007
DDAC	2 ml/litre	1 min	Webb et al 2007
Sodium chloride	10%	5 min, 2 min	Johnson et al 2003
Potassium permanganate	2%	10 min, 5 min	Johnson et al 2003
Hot wash for cloth bags and clothing	60°C or greater	15 mins	Speare et al 2004
Heat	60°C	30 minutes	Speare et al 2004 Webb et al 2007
Complete drying (footwear only)	Effective against <i>Bd</i> but not ranavirus	3 hrs or greater	Speare et al 2004
Sterilising UV light (1000 mW m ⁻² , wavelength 254 nm)	Effective against ranavirus but not <i>Bd</i>	1 min	Speare et al 2004 Johnson et al 2003