

**Amphibian Monitoring Protocol for Klondike Gold Rush National Historical Park**  
**Standard Operating Procedure (SOP) No. 3**  
**Hygiene Protocol for Control of Disease Transmission between Amphibian Study Sites**  
**Version 1.0 (May 2009)**

**Revision History Log**

| Previous Version # | Revision Date | Author | Changes Made | Reasons for Change | New Version # |
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### **3.1 General Principles and Background**

Conducting fieldwork at amphibian breeding sites requires a code of practice to minimize the spread of disease and parasites between sites that are not “water-connected”, or that amphibians don’t freely move between. Observations of diseased and parasite-infected amphibians are frequently reported from breeding sites throughout the world and contribute to the decline and extinction of amphibian populations and species.

Amphibian pathogens can be carried in a variety of ways between habitats, including on the hands, footwear, and equipment of fieldworkers. Of special concern in Southeast Alaska and British Columbia is the spread of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which has been documented and confirmed in western toad (*Bufo boreas*) populations in Klondike Gold Rush National Historical Park (KLGO). Subsequently, handling of amphibians during field studies should be done in a manner that does not significantly increase their risks of exposure to the fungus above those normally experienced in the absence of handling (Berger 2005).

#### **3.1.1 *Batrachochytrium dendrobatidis* fungus**

The fungus *Batrachochytrium dendrobatidis* (BD), also called chytrid fungus, is a disease agent responsible for chytridiomycosis in amphibians. Although thought to have been spread throughout the world in the last century, its extent and current distribution are unclear (Adams 2007). The disease was originally described in 1998 from observations of dead, dying, and deformed frogs and is one of the main factors attributed to the loss of amphibian biodiversity worldwide. Dispersal of the fungus is assumed to be via infected frogs, contaminated water, or an unknown host (Weldon 2007, Adams 2007). Amphibians exposed to the fungus may die soon after their skin is infected and can quickly spread through an area, causing a rapid collapse of the entire population at the site.

The amphibian chytrid fungus is extremely sensitive to temperatures above 29 deg. C and will die at 32 deg. C. *B. dendrobatidis* will not grow on human skin and complete drying will kill the chytrid fungus (Berger 2005). The greatest risk of transmission is when amphibians are placed together in contact or in the same container or in containers reused for holding amphibians without disinfection between uses (Chelgren 2005). See SOP No. 4: Collecting samples for *Batrachochytrium dendrobatidis* (BD) testing.

### 3.2 Handling Amphibians

Amphibians can be handled using bare hands as long as the handler washes their hands between amphibians in water to which the animals would normally be exposed. This will ensure that the risks to frogs of exposure are not increased above environmental levels (Berger 2005). If no water is available for washing hands between amphibians, the handler should wear unused disposable gloves and change them between water-connected sites. Amphibians do not appear to show signs of stress after handling; however, unnecessary handling should be avoided and amphibians should be handled and released as quickly as possible at the site from which they were captured (Chelgren 2005). See SOP No. 7: Amphibian Handling Guidelines.

#### 1.2.1 Handling considerations for larvae

Tadpoles normally share water and placing them in a common container does not increase their rates of physical contact. They can therefore be held in groups in containers, as long as all members of the group are from the same site. Tadpoles should not be held with batches of tadpoles collected from other sites in the same or different water bodies (Berger 2005).

### 3.3 Field Hygiene Procedures {Livo, 2005 #106}

The following equipment is required for hygiene procedures:

- Plastic bucket with handle, for sterilizing and holding cleaning gear
- Chlorine bleach (6% concentration of sodium hypochlorite)
- Stiff scrub brushes with handles, 2 (1 for sterilization, and 1 for removing soil)
- Rubber dishwashing gloves
- Spray bottle
- Isopropyl alcohol, in bottle or individual wipes

The following procedure should be conducted between any sites that are not “water-connected”, or that amphibians don’t freely move between. The procedure applies to all equipment that may have touched water or amphibians at the site. This includes, but is not limited to: waders, shoes, boots, dip nets, rulers and other instruments, specimen bags, and containers. All sterilizing is done well away from streams or ponds, preferably on gravel or asphalt surfaces where the chlorine solution will not move directly into water bodies.

1. Before leaving the pond site, use site water to wash off mud, dirt, vegetation, and other detritus attached to the equipment by shaking, rinsing, and hand picking
2. Away from the site, fill a five-gallon bucket with two gallons of clear water (can be from a natural water source or from a spigot)
3. Add 12 capfuls (6 Tablespoons or 1/3 cup) of bleach to create a 1% concentration
4. Stir the water/bleach mixture with a scrub brush to mix
5. Clean off any remaining mud, dirt, vegetation, or detritus from equipment that was missed earlier
6. Dip instruments, equipment, and shoes in the solution and scrub. Shake off and let dry in the sun.
7. Either dip and scrub waders in the solution or lay waders on the ground and pour solution over them while scrubbing.
8. Use spray bottle with the same solution concentration to apply solution where needed.
9. Sterilize brushes in solution.

10. Dispose of solution on asphalt, cement or hard roadbed, well away from other water bodies.
11. When possible, allow all gear and equipment to dry completely before reuse at the next site.  
UV light will quickly break down any residual bleach.

### 3.3.1 Field hygiene considerations

Using multiple pairs of boots and waders, such as one pair for each site, is the easiest and safest way to prevent spreading disease from footwear.

A covered, sealed bucket of bleach solution may be stored in or near the Dyea Ranger Station for convenience if the bucket is clearly labeled and if residents and employees using the station are notified.

When it is impractical to disinfect field equipment using bleach solution, isopropyl alcohol (70%) may be used for small items such as scales, calipers, plastic bags, and thermometers. Another option is to let equipment dry completely (at least 3 hours) to kill the chytrid fungus.

If conducting chytrid fungus testing by collecting swabs from individual amphibians, it is important to note that Isopropyl alcohol does not destroy DNA of the chytrid fungus. DNA testing is used to detect presence or absence of the fungus during laboratory analysis, so dipping utensils in isopropyl alcohol is not sufficient to decontaminate them for this purpose (Wood 2008). However, metal utensils may be ignited or held under a flame to destroy the DNA, as long as there are no large chunks of tissue remaining. See SOP No.4: Collecting Samples for BD testing.

## 4. References

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