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***Hygiene Protocols for field Staff working in aquatic environments***

**PURPOSE**

To describe hygiene protocols to be used by field staff to reduce risk of disease transmission among aquatic ecosystems.

**DESCRIPTION**

Over the past few decades emerging infectious diseases and parasites have caused declines in native freshwater organisms including fish and amphibians. It is thought that field staff could act as potential vectors of spread 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 aquatic field staff incorporate the hygiene protocol as Standard Operating Procedure (SOP).

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

**RESPONSIBILITY**

Field staff working in all freshwater aquatic habitats.

**MATERIALS**

- Plastic tote or box to store field equipment.
- Buckets or totes for mixing bleach solution and soaking equipment
- Spray bottle for bleach solution
- Scraper, brushes, hand-brushes
- Household bleach (contains active ingredient 5-6.5% sodium hypochlorite solution)
- Rubber dishwashing gloves, rubber apron

## **STANDARD OPERATING PROTOCOL 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 if possible. Each stream and each upstream location should be considered separate sites.

### **Equipment treatment**

Equipment such as wetsuits, waders, footwear, nets, buckets, and traps can act as major 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 any of these 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 that can be harmful to animals

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 bucket (~ 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 used 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. 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 aquatic organisms on contact.

8. In the field, the bleach solution is best disposed off far from the waterbody by poring over an asphalt, hard roadbed or concrete surface where it quickly breaks down in sunlight and evaporates. Ensure no bleach solution enters surface waters.
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 species extra precautions are necessary. The simplest solution would be to have dedicated field gear that is used only at that site.

### **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 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 of all equipment between sites can reduce the risk of transmission of some pathogens.
3. If it is possible to heat water, equipment should be soaked in water  $> 60^{\circ} \text{C}$  for 15 minutes. This method may be practical for small equipment such as dipnets that come in direct contact with the animals.

Table 1: The table summarizes information for two amphibian pathogens, *Batrachochytrium dendrobatidis* and ranavirus. However, the concentrations and times given may be effective against most other aquatic pathogens. 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
<b>Disinfecting surgical equipment and scales</b>			
Ethanol	70%	1 min	Speare et al 2004 Webb et al 2007
Virkon	1 mg/ml	1 min	Speare et al 2004
Benzalkonium chloride	1 mg/ml	1 min	Speare et al 2004
<b>Disinfecting collection equipment, containers, footwear, waders, boats, nets and other field gear</b>			
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 $1 \times 10^{-3}$	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 <sup>-2</sup> , wavelength 254 nm)	Effective against ranavirus but not <i>Bd</i>	1 min	Speare et al 2004 Johnson et al 2003

## RELEVANT LITERATURE

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- 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](http://www.fs.fed.us/r4/resources/aquatic/guidelines/chemical_tech_final.xls)
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