Disinfection of Field Equipment for Amphibian Pathogens

Minimizing Spread of Chytrid Fungi (Bd & Bsal) and Ranavirus

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Importance of Disinfection

The spread of pathogens is a major threat to amphibians and reptiles worldwide.[1-5] This is particularly true for ranavirus (RV) and the two chytrid fungi, *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), responsible for the disease, chytridiomycosis. Humans can move pathogens from one place to another and from one organism to another in little time and over great distances. With the increasing threat of infectious diseases among amphibians and reptiles worldwide, anyone engaged in educational, recreational, commercial, or professional activities in wetlands and aquatic habitats can play an important role in helping prevent the spread of pathogens by employinng basic disinfecting procedures to prevent pathogen spread.

Before Leaving for the Field

Multiple chemicals are effective for inactivating Bd, Bsal and most RVs including Virkon Aquatic ®, bleach and ethanol [6-13] (see page 2 for detailed comparisons). To ensure maximum efficacy, prepare fresh only as much solution as you will need for that day's activity (e.g., sampling event, wetland survey, or fishing trip).

Suggested equipment:

- Brushes for scrubbing and removing mud and vegetation from equipment.
- Hand sanitizers and antiseptic alcohol wipes.
- Handheld bottles and/or pump sprayers for applying disinfectant and water.
- Clean rinse water.

- Powderless, nitrile gloves for handling animals. These should be discarded between animals.
- Small plastic bags. Avoiding direct contact with animals minimizes pathogen transfer and stress.
- Different sets of equipment for each site if disinfecting between sites is not possible.
- Trash bags.

Quick Reference

Virkon Aquatic	Bleach	Ethanol
1% solution	1.6% solution	70% solution
recommended for large equipment and gear	*recommended for large equipment and gear*	*recommended for delicate equipment*

Read table for addition details and instructions

Instructions for Large Machinery

Brush and scrub off mud (bio-degradable soap optional), disinfect with Virkon or bleach and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires. See Julian *et al.* 2020[14] for more details on heavy equipment disinfection.

After Sampling & Before Moving to Another Site

1. Brush off mud and vegetation from field equipment (e.g., nets, buckets, boots). Soil or mud reduces effectiveness of the disinfection process.
2. Generously spray or immerse all items in disinfection solution.
   - Disinfectants are highly toxic to aquatic organisms; apply disinfectants at least 50 m (150 ft) from any natural water source.
   - NEPARC suggests 5 minutes of contact time in field situations, which is sufficient time for all 3 recommended disinfectants (table).
3. Rinse treated items well with water to minimize damage to equipment and to prevent exposing the next location to residual disinfectant (see pg. 2).
4. Use alcohol wipes or ethanol to disinfect calipers, measuring boards, and other sensitive equipment that was in contact with water or animals.

Scrub and Rinse

Disinfect

Rinse

End of the Day

After returning from the field, all equipment should be washed and thoroughly disinfected. Set up two buckets or large tubs: one with water and one with disinfection solution.

- Brush or scrub off any soil or vegetation and rinse with water.
- Immerse in disinfectant and leave for 5 minutes of contact time.
- Rinse thoroughly with water.
- Hang equipment and gear, and allow them to air dry completely.

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Disinfection Options for Amphibian Pathogens

RV ● *Bd* ● *Bsal*

	Virkon Aquatic ®	Bleach	Ethanol
Active Ingredient (AI)	Potassium peroxymonosulfate	Sodium hypochlorite (NaOCl)	Ethyl alcohol
Minimum AI Concentration in Working Solution[7,9,10]	1.0%	1.6%	70%
Minimun Contact Time[7,9,10]	2 minutes	5 minutes	1 minute
Preparation of Working Solution	type purchased: powder — 1 gallon water + 1.3 oz — 1 liter water + 10 gr; tablets — 8 — 2	purchased stock conc. / 1 gallon water + / 1 liter water +: 5.25% — 7 cups — 440 ml; 6.0% — 6 cups — 360 ml; 8.25% — 4 cups — 240 ml	Effective when applied at 70% (or stronger). If purchased at 100%, mix 700 ml with 300 ml of water to make 1 L of solution or 3 cups of ethanol with 1 1/4 cup of water.
Toxicity to Humans	● Harmful if swallowed ● Irritating to respiratory system and skin ● May cause serious eye damage	● Vapor may cause severe irritation or damage to eyes and skin ● Harmful if swallowed	● May be fatal if swallowed or inhaled ● Can damage organ systems by repeated exposure ● May be absorbed through skin ● Repeated or prolonged contact can cause eye irritation or dermatitis[18]
Toxicity to Amphibians	● Non-toxic at working solution concentations[15]	● Fatal at high concentrations	● Fatal at high concentrations ● May destroy mucus and cause dehydration[13]
Effects on Equipment	● Preferred over bleach because it is safe on fabric and metals ● May cause pitting on galvanized or soft metal if not rinsed	● Corrodes metals ● Will fade colors and break down fibers	● May damage rubber and plastics ● May cause deterioration of glues[18]
Special Notes	● Store at room temperature and avoid extreme cold and heat[16] ● Shelf life for tablets is 2 years and for powder is 3 years ● Remains stable for 1 week if diluted with tap water	● Inactivated by organic material and sunlight ● If in an opaque container, diluted bleach will last 1 month[17] If exposed to sunlight or air, it will last 5 days	● Highly flammable ● Use and store in a well ventilated area ● Evaporation may diminish effective concentration[15]

Special Instructions for Disinfectants

- Remove debris from equipment prior to treatment.[19]
- Keep at least 150 feet away from lakes, streams, and ponds when disinfecting gear.
- Wear safety glasses and gloves when handling chemicals.
- Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water.

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Citations for Disinfection of Field Equipment for Amphibian Pathogens

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