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# AMPHIBIAN AND REPTILE DISEASES

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## Disinfection Protocols for Herpetofaunal Pathogens

The spread of disease-causing pathogens is a major threat to amphibians and reptiles worldwide (Converse and Greene 2005; Picco et al. 2007; Picco and Collins 2008; St-Amour et al. 2008; O'Hanlon et al. 2018; Scheele et al. 2019). The World Organisation for Animal Health's global list of notifiable animal diseases includes herpetofaunal diseases caused by infection with *Ranavirus* spp. (*RV*) and two chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*; WOAAH 2023). Scientists began testing the efficacy of disinfection protocols

even before human-assisted transmission of these pathogens was documented (Johnson et al. 2003; Johnson and Speare 2003; Brem et al. 2007; Bryan et al. 2009), and now guidance exists to determine the appropriate biosecurity measures as personal gear moves between habitats, construction equipment moves between project areas, and investigators handle multiple animals within a population (Phillot et al. 2010; Gray et al. 2017, 2018; Julian et al. 2020; Olson et al. 2021). People who are engaged in educational, recreational, commercial, or professional activities in wetlands and aquatic habitats play an important role in helping prevent the spread of pathogens and should know how and when to employ appropriate disinfecting procedures. In particular, working groups and regional chapters of Partners in Amphibian and Reptile Conservation (PARC) have developed a variety of educational materials on biosecurity and disinfection for a wide audience (e.g., PARC 2023). Herein, we provide the instructions for the disinfection of field equipment that were recently revised by the Emerging Disease Working Group of Northeast PARC (NEPARC 2022). The impetus for revision was to inform field personnel of the use of a stronger bleach concentration (ca. 10× stronger) that is needed for effectiveness against *Bsal* (Van Rooij et al. 2017). This is important for regions where *Bsal* is currently known to occur (e.g., central Europe) as well as regions where it is presumed absent but could arrive at any time (e.g., North America).

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### BEFORE LEAVING FOR THE FIELD

In addition to chemical disinfectants, we provide a list of supplies to facilitate effective decontamination of field equipment (Tables 1 and 2, Fig. 1A and B). Multiple chemicals are effective for inactivating *Bd*, *Bsal*, and most *RV* spp., including Virkon Aquatic®, bleach, and ethanol (Table 2; Appendix 1). To ensure maximum efficacy, prepare fresh chemical solutions daily, with only as much solution as will be needed for that day's activity (e.g., sampling event, wetland survey, or fishing trip). If disinfection between sites visited within a day is not feasible, different sets of equipment (e.g., nets, buckets, boots) could be designated per site. Brightly colored, removable painter's tape is also helpful for labeling reusable containers, nets, and other equipment that have been disinfected and are ready to hold or capture new animals.

### AFTER SAMPLING AND BEFORE MOVING TO ANOTHER SITE

Both mud and vegetation reduce the effectiveness of the disinfection process, so therefore, brush off both from field equipment (Fig. 1C). Generously spray or immerse all items,



FIG. 1. Key steps in the disinfection process: A) secure each animal in new disposable bags and use new gloves for handling; B) equipment used for disinfection; C) scrub debris off gear, then rinse with water prior to applying disinfectant; D) apply disinfectant and allow 5 min of contact time; E) rinse disinfectant off with clean water.

TABLE 1. Field equipment for biosecurity and equipment disinfection

Supply item	Purpose and notes
Brushes	Use to scrub and remove mud and vegetation from equipment
Hand sanitizers and antiseptic alcohol wipes	Be careful that residues do not contact animals
Handheld bottles and/or pump sprayers	Use to apply disinfectant and water to equipment; clearly label each dispenser
Powderless nitrile gloves	Use for handling animals; these should be discarded between animals
Small plastic bags to house animals	Avoid direct contact of hands or nets with animals to minimize pathogen transfer and stress; discard bags after use with one animal
Clean rinse water	Bring extra quantities in case your dispenser runs empty
Trash bags	To dispose of single-use items
Separate bags, containers, or totes	To store re-usable equipment to be disinfected later; clearly label as “dirty”
Painter’s tape	Use to label items as disinfected and ready-for-use; use the same color of tape for all “clean” items.

including brushes, in the disinfection solution (Fig. 1D). Disinfectants are highly toxic to aquatic organisms; apply disinfectants at least 50 m (150 ft) from any natural water source, including wetlands. We suggest 5 min of contact time in field situations, which is sufficient time for all three recommended disinfectants (Table 1, Appendix 1). Rinse treated items well with water to minimize damage to equipment and to prevent exposing the next location to residual disinfectant (Fig. 1E). If disinfectant is disposed of in the field, disposal should occur >50-m away from natural water sources on gravel roads, train tracks, or roadside areas. Use alcohol wipes or ethanol to disinfect calipers, measuring boards, and other sensitive equipment that was in contact with water or animals.

INSTRUCTIONS FOR LARGE MACHINERY

Large equipment that encounters soils or organic matter at field sites can potentially transmit disease-causing pathogens between field sites and may warrant disinfection. Julian et al. (2020) addressed this need previously in detail. In summary, brush and scrub off mud (bio-degradable soap optional), disinfect with Virkon Aquatic® or bleach, and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires.

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

TABLE 2. Quick reference for disinfectants effective against *RV* spp., *Bd*, and *Bsal*. Concentrations are for the percent of active ingredient in the solution prepared. Note: bleach can be sold at various concentrations, be sure to check this and make the correct dilution.

Virkon Aquatic®	Bleach	Ethanol
1% solution for personal gear and large equipment	1.6% solution for personal gear and large equipment	70% solution for delicate equipment

or scrub off any soil or vegetation and rinse with water. Immerse in disinfectant and leave for 5 min of contact time. Rinse thoroughly with water. Hang equipment and gear and allow them to air dry completely. You may find it useful to label some re-usable equipment with removable painter's tape after it has been disinfected to signify it is ready-to-use. Dispose of used disinfection solutions as per directions on their labels. In particular, large quantities of bleach may be harmful to some city sewage treatment facilities. A 2-page, user-friendly protocol is available at: [http://northeastparc.org/wp-content/uploads/2023/04/NEPARC\\_Pub\\_2022-02\\_Disinfection\\_Protocol.pdf](http://northeastparc.org/wp-content/uploads/2023/04/NEPARC_Pub_2022-02_Disinfection_Protocol.pdf).

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APPENDIX 1. Disinfection options for three amphibian disease-causing pathogens: *Ranavirus* spp., *Batrachochytrium dendrobatidis*, and *B. salamandrivorans*. Minimum concentrations and contact times from Johnson and Speare (2003), Van Rooij et al. (2017), and Green et al. (2009).

	Virkon Aquatic®			Bleach			Ethanol
Active ingredient (AI)	Potassium peroxymonosulfate			Sodium hypochlorite (NaOCl)			Ethyl alcohol
Min. AI concentration	1.0%			1.6%			70%
Min. contact time	2 min			5 min			1 min
Preparation of working solution	type	1 gal	1 L	purchased	1 gal	1 L	Effective when applied at 70% (or stronger). If purchased at 100%, mix 700 mL with 300 mL of water to make 1L of solution or 3 cups of ethanol with 1.25 cups of water
	purchased	water +	water +	stock conc.	water +	water +	
	powder	1.3 oz	9.6 g	5.25%	7 cups	440 mL	
	tablets	8	2	6.0%	6 cups	360 mL	
				8.25%	4 cups	240 mL	
Toxicity to humans	Harmful if swallowed Irritating to respiratory system and skin May cause serious eye damage			Harmful if swallowed Vapor may cause irritation or damage to eyes and skin			May be fatal if swallowed or inhaled Can damage organ systems by repeated exposure May be absorbed through skin Repeated/prolonged contact can cause eye irritation or dermatitis
Toxicity to Amphibians	Non-toxic and non-irritant at working solution concentrations			Fatal at high concentrations			Fatal at high concentrations May destroy mucus and cause dehydration
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
Special notes	Store at room temperature and avoid extreme cold and heat 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 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
References	Antec International 2019 Pharmaceutical Research Laboratories 2014 Schmidt et al. 2009 Syndel 2019			Rutala and Weber 1997			Schmidt et al. 2009 Simmons et al. 1990