

DECONTAMINATION PROTOCOL TO REDUCE THE RISK OF SPREADING INFECTIOUS AMPHIBIAN DISEASES IN FRESHWATER SYSTEMS



Pathogens can be vectored within and between aquatic systems through a multitude of pathways and little human control can be exerted over most of them in the wild. Waterfowl, migratory birds, foraging ungulates, cats, dogs, domestic livestock, feral goats and pigs and even native terrestrial wildlife can act as vectors for a wide array of pathogenic microorganisms.

Asexual reproduction of *Batrachochytrium dendrobatidis*; the fungus which causes amphibian chytridiomycosis. Zoospores infect an amphibian and develop into zoosporangium in epidermal tissue. When mature, zoospores discharge through papillae into surrounding water to seek new host.



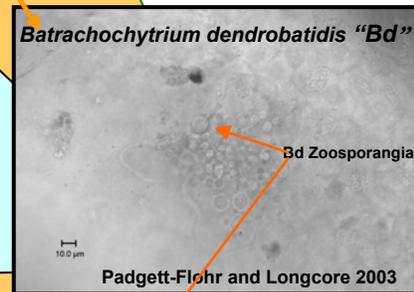
Potential Vector



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For microorganisms with a lifestage that can withstand dessication, the wind can be a vector. The single transport agent (i.e. vector) over which we have some control, and the only one which has the capacity for worldwide travel in a 24-hour period, is *Homo sapiens*. If you are a professional who regularly ventures into the field working in or around freshwater systems, it is imperative to decontaminate yourself and your equipment each and every time you go in the field (before **and** after your visit) in order to minimize your risk of transporting pathogens from one site to the next.



Located in the coastal foothills of Central California, Joseph D. Grant County Park is a 10,000-acre haven which boasts temporary and perennial ponds, lakes, and perennial and intermittent streams. Strict decontamination procedures are carried out during sampling by CCADC crew to ensure that pathogens are not transported from one waterbody to another.

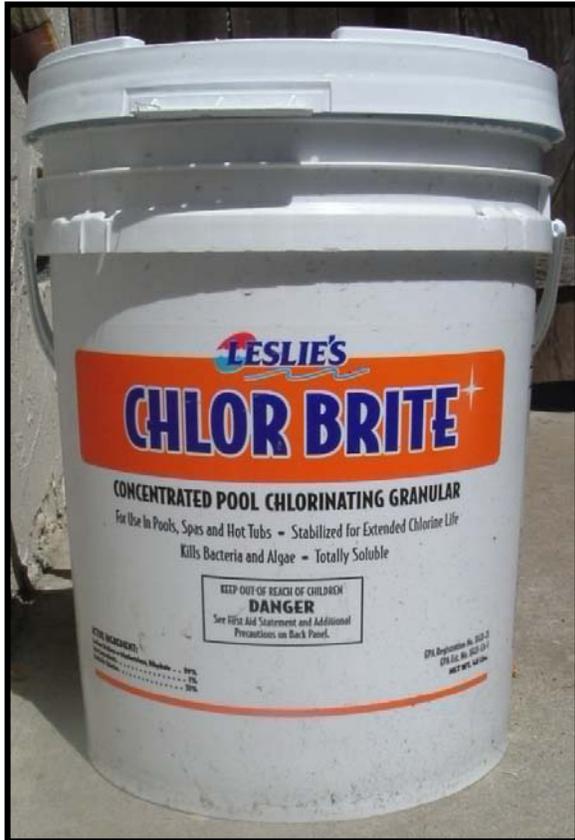
Field conditions are a far cry from the environment of the human medical institution where sepsis and sterility are tightly controlled and monitored. Although we cannot duplicate **that** level of control while in the field, we **can** set standards and practices to ensure that we, as wildlife professionals, do not spread disease or act as vectors increasing the risk of disease outbreaks in new areas by transporting pathogenic agents between sites. **Remember that the outcome of a disease outbreak is based on the number of infectious particles contained in the initial inoculating dose.** The steps outlined in this protocol may not kill all the infectious particles; however a reduction in the environment of the number of infectious particles that can invade an amphibian or its habitat will provide an opportunity for a more positive outcome

The key to any decontamination procedure is PLANNING AHEAD. If you are surveying by yourself, even more forethought will be required to effectively decontaminate yourself and your equipment properly before leaving a site.

BASIC SUPPLIES

1. Granulated swimming pool chlorine in a puncture-proof container.

Granulated chlorine bleach can be purchased at any swimming pool supply store in a variety of quantities, up to and including 40 pounds. Recent pricing (April 2004) for this chemical is approximately \$90.00 for 40 pounds and \$68.00 for 25 pounds.



Granulated chlorine bleach has the distinct advantage of being in dry form and is therefore much more portable than the gallons of liquid bleach currently being carted around by some folks. Besides being heavy and cumbersome, liquid bleach loses most of its effectiveness within 2 weeks of the container being opened. The abbreviated active life of opened liquid bleach solutions can mean that although folks are following the decontamination procedure—they are not accomplishing the task of killing pathogens if they are using bleach which has lost its effectiveness. Old liquid bleach is not decontaminating anything.

Storing disinfecting agents at home safely is hard enough, but trying to transport chemicals, even for the best of reasons, out into the field can be a real challenge. At the CCADC we have found the best container to store and transport the granulated bleach to be the Backpackers Cache®. This is a bear-proof container that is therefore puncture-proof and is light enough (2.7 pounds) to place in a backpack for easy transport into the field.



Chlorine granules are poured into gallon-size Ziploc® plastic bags, which are sealed and then placed in the Backpackers Cache® with a measuring cup for easy mixing of the disinfecting solution when on site. The Backpackers Cache® runs about \$70.00 and retailers carrying the item can be located by checking at <http://www.backpackerscache.com/dealers.asp>.



2. **Pop-up waterproof garden trimming container.** Although large, the best model we have found is manufactured by Fiskars, runs \$19.95 and is called the “Kangaroo Utility Container”. This container is waterproof, 22” X 28” and will fold down to a 3” stack. (A smaller version would be preferable, but at this time only the large version is available in waterproof form). The Kangaroo container can easily be attached to a backpack by the handles as it weighs less than 1 pound. It is available at: http://www.fiskars.com/en_US/garden/category.do?cat=5.



3. **Plastic tarp at least 8’ x 8’ in size.**
4. **Hard bristled scrub brushes (preferably with an extra long handle).**
5. **Plastic laboratory aprons.**
6. **Collapsible 1-gallon jugs (2-3).** These can be purchased relatively inexpensively at local camping or outdoor gear supply stores.
7. **Extra change of clothes in case your clothes get wet.** Bring the change of clothes in a plastic bag so that you can isolate your wet “contaminated” clothing in the bag after you have donned your dry clean clothes. If you are doing extensive field work, more than one change of clothes and extra plastic bags may be needed, so plan ahead!

FIELD DECONTAMINATION PROTOCOL

This protocol assumes that there is more than one individual going out in the field. We are also assuming that some type of sampling in or around a waterbody will occur during the field visit.

General Policies

1. **Always start your fieldwork with sterilized equipment.** Prior to going in the field decontaminate (“decon”) all equipment (dip nets, two-pole seines, hip boots, chest waders etc.) using a 5% bleach solution or Quat 128¹ (didecyl dimethyl ammonium chloride) diluted 1 oz/ 1 gal of water. **This means a two-minute soak in the sterilizing solution!** A 2-second dunk merely gets the item wet- it does not render it instantly sterile.



¹ Very effective, less corrosive on equipment. Can be obtained at any local medical supply store. Not effective on whirling disease of fish.

2. **Don't forget your field vehicle.** In published literature on veterinary medicine, vehicles have been identified as vectors spreading livestock diseases between ranches and farms. Careful cleaning of vehicles is routinely practiced by veterinary professionals traveling between populations of livestock. Wildlife populations are vulnerable to disease as well; therefore, for field biologists, scrubbing down the tires on the field vehicle is a necessary practice, especially if you have had to drive through a stream or creek. Scrubbing down your tires not only helps prevent spreading wildlife disease pathogens, it also assists in preventing the spread of plant diseases such as SODS (Sudden Oak Death Syndrome).



3. **In lentic (pond) habitats always work from the “cleanest” site to the “dirtiest” site.** Find out beforehand if there have been any disease occurrences detected in the area you will be working in and organize your sampling sites from clean to dirty.
4. **In lotic (stream) situations begin your surveys from the highest upstream point and work your way downstream.** If you do have to go from a downstream to an upstream area, decon after you have completed the downstream work and then proceed to the upstream site. Decon again after completing work at the upstream area.
5. **If no data are known- assume all sites are “contaminated”.** It is better to err on the side of caution. Few places have actually been specifically sampled for pathogens and/or disease occurrences. Of those places which have been sampled, the data are slow coming in. CCADC will continue to record and make available all reported occurrences (contact: gpadgettflohr@aol.com).
6. **Decontaminate all equipment and clothing between lentic water bodies.** It cannot be assumed that if one pond has been found to be positive for a specific pathogen or disease occurrence that all ponds in the vicinity are similarly afflicted. Therefore follow the “decon procedure” between ponds if you are visiting multiple sites.
7. **As the disease status of most amphibian populations is currently unknown, do not translocate amphibians of any lifestage at this time.** Given the current, very serious situation with newly emerging infectious diseases affecting amphibians worldwide, any translocations must necessarily involve a simultaneous testing of amphibian populations at both recipient and donor sites. Human initiated movements of frogs, toads and/or salamanders without testing the health of amphibians at both donor and recipient sites, have the potential to introduce pathogens and disease into otherwise healthy habitats and populations.

DECONTAMINATION SETUP AND PROCEDURE

When on-site- designate a “clean” crew and a “dirty” crew. The “dirty” crew enters the water for sampling and passes specimens to the “clean” crew who do not enter the water or muddy areas. It is helpful to have a couple of extra buckets, filled with pond water and placed next to the tarp to deposit animals into.

1. At the site the “clean” crew sets up the 8’ X 8’ tarp on the soil which will outline the clean work area. Any equipment (Pesola® spring scales, scalpels, scissors, etc.) used for sampling amphibians should be placed on the tarp. “Clean” crew dons plastic aprons to protect clothing from getting wet and they stay on the tarp for the duration of the sampling.
2. The pop-up Kangaroo container is opened up and placed at the edge of the tarp. The “dirty” crew members enter the water, fill the collapsible jugs with water and hand them to the “clean” crew who use it to fill the pop up container. The “dirty” crew members also fill the specimen buckets and place them between the pond and within reach of the “clean” crew on the tarp.
3. Once filled about 1/3 of the way with water, the “clean” crew adds approximately 1 cup of bleach granules to the water.



4. Sampling proceeds with the “dirty” crew doing the seining, electroshocking, and/or dipnetting etc. Animals to be examined can be taken directly from, and then replaced back into, the specimen buckets by “clean” crew members. The “dirty” crew members replace the contents of the buckets back into the ponds at the conclusion of sampling.

5. “Clean” crew members should disinfect hands after handling each animal and then rinse thoroughly before touching the next animal. Pond water is acceptable to rinse the hands if no other water source is available. *Gloves are not recommended due to the sensitive nature of amphibian skin.*

6. Use new individual Baggy® for each animal being retained. Bag up and toss out all used Baggies® at the end of your sampling.

7. When PIT-tagging or toe-clipping, use sterile instruments and disinfect them between animals. A small bottle of 70% ethanol can be kept handy to sterilize instruments between specimens. Seal wounds so there is no avenue for infection. Swab wound with 0.1% iodine then seal with medical grade cyanoacrylate.

8. Do not allow disinfectants to contact amphibians.

9. **Decontaminate all equipment before leaving any site.** The “clean” crew assists the “dirty” crew. To properly decontaminate means **WASH AND SCRUB FIRST** to remove **all** clumps of dirt. Lots of elbow grease!! **THEN DISINFECT.**





10. Don't forget to soak the plastic aprons, the specimen buckets, and the plastic tarp (2 minutes!). Everything should be clean when you leave the site.

11. If "dirty" crew clothing has gotten wet, clothing should be changed and the contaminated clothing bagged and isolated.

12. **The last step is to dump out the bleach solution a safe distance from the waterbody.**

13. **WASH YOUR FIELD CLOTHES AFTER EACH VISIT.**

***Note:** The photos used in this protocol show a county park vehicle with a hydraulic lift and a pumper truck to provide water for the decon solution mix. This is a fantastic setup, if available. The protocol described above assumes that specialized equipment like this is not typically available or may not have access to the site.*