

Amphibian Chytrid Disinfection Protocol

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Background

Chytrid fungi are widespread in the environment where they act as decomposers of keratin, chitin, cellulose, and other plant material. Chytrids are also known parasites of fungi, algae, higher plants, protozoa, and invertebrates, but none were known to infect vertebrates until recently. Since amphibian chytrid (*Batrachochytrium dendrobatidis* = Bd) was described in 1999, it has been found in many amphibian populations around the world, including many that are declining. While Bd is probably moved naturally from one water body to another on dispersing amphibians, or on waterbirds and flying insects, it is important to assure that researchers are not spreading Bd between sites as part of research or monitoring programs.

The basic assumption when doing field work on pond- or stream-dwelling amphibians is that salamanders, frogs, and tadpoles living in the same pond/lake/stream have all been exposed to Bd if it is present at that site. Hence, uninfected individuals have almost certainly been exposed, or will be exposed regardless of their handling by biologists.

Field protocol

Make sure that any lotions, mosquito repellent, or sunscreen are washed off your hands. This can be done with tap water or local pond water, but it should be done without soap since a soap residue might remain after washing.

After capturing and handling each amphibian, rinse your hands in the local pond water where the frog was captured. Dipnets, plastic bags, rulers, calipers, or other equipment that touches any amphibian, should also be cleaned between individual captures by dipping the item in the pond and vigorously swishing it around to dislodge any skin that might have been sloughed off the animal and may be adhering to the equipment. There is no need or benefit to doing this away from the pond/lake/stream the frog came from, as the skin from an infected individual would have been sloughed off in the pond anyway.

Disinfection between sites

All equipment that has been in contact with an amphibian or the local water need to be disinfected using either bleach or Quat-128 when moving between sites that are further away than an amphibian might travel on its own (approximately 1 km), or sites that are in different drainages. Equipment includes boots, nets, rulers, thermometers, plastic bags, etc. It is not necessary to disinfect one's hands, even if frogs have been handled bare handed since amphibian chytrid will not survive on bare skin for more than 6 min. While this amount of time will be longer if one's hands have been regularly rinsed, any pond that can be reached in such a short

period of time is likely within the distance that a frog might move during its normal activities throughout the year.

Disinfection recommendations - bleach

1. Prior to bleaching, clean everything that has been in touch with amphibians and/or the local water. This primarily involves removing wet or dried mud from boots and other equipment. Bleach kills fungus, but bleach is not a cleaning solution. A stiff scrub brush and an old screwdriver are useful tools for removing mud from shoe treads.
2. Make a solution of 1 part bleach to 16 parts water. Completely immerse anything that needs to be disinfected in the diluted bleach solution. It is best if the equipment soaks for 15 min. Alternatively, dip nets, boots, etc. in the bleach for a few minutes, shake off the excess, and let everything air dry while you travel to the next site. If you are working in the field near a vehicle, it is useful to have a bucket (large enough to accommodate the hoop of a dipnet) for bleaching. Bleach can be discarded on site, well away from any water source. At a field station, it is best to dedicate a 45 gallon plastic garbage can (half full and tightly sealed) for this procedure. If it has been more than a few days between uses, you should add enough bleach to compensate for evaporation and dilution from wet equipment.

While it would be good to bleach everything that has been in the water (especially anything that has touched a frog) when you leave each site, that is not always necessary at sites that are in close proximity. All equipment must be cleaned and then disinfected:

1. Immediately after visiting a site where frogs appear to be infected (based on mouth part deformities) or if the site has a history of known or suspected Bd,
2. Prior to visiting a site with a known population of a particularly rare amphibian, and
3. When moving equipment between drainage basins, or sites separated by more than 1 km.

Clearly these guidelines leave room for interpretation and implementation, but it provides a basis for making decisions. If one is working from a vehicle, it is not much trouble to clean and bleach at each site. When working miles from the nearest road, it is much less practical, but it is still essential under the three conditions noted above.

Amphibians captured as part of a Bd study

If the goal of a study is to evaluate the prevalence of Bd in a local population, then it is essential that a new set of gloves be used for each individual frog. Use vinyl gloves rinsed with local pond or stream water (Cashins et al. 2008. Lethal effect of Latex, Nitrile and vinyl gloves on tadpoles. *Herpetological Review* 39:298-301). Additionally, testing the response of frogs to the specific brand and lot, is highly recommended as a precautionary step.