

**INTERAGENCY CONSERVATION
STRATEGY
FOR MOUNTAIN YELLOW-LEGGED
FROGS IN THE SIERRA NEVADA**

**Attachment 4: Equipment Decontamination
Protocol**

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Version 1.0

This protocol was adapted by Sequoia and Kings Canyon National Parks from the decontamination protocol established at Yosemite National Park.

PROTOCOL

All field personnel need to minimize the human spread of parasites and non-native organisms between water bodies. The fungal pathogen, *Batrachochytrium dendrobatidis*, often known as “Bd” or “chytrid fungus,” can be lethal to amphibians. This fungus is now in most lakes and water bodies throughout the Sierra Nevada. It is transmitted by aquatic zoospores, and a factor responsible for recent mass mortalities and declines of both *Rana muscosa* and *R. sierrae*.

*****IMPORTANT***. ALL FIELD STAFF MUST ADHERE TO THE FOLLOWING PROCEDURES TO DISINFECT ALL EQUIPMENT (E.G., FOOTWEAR, CLOTHING, AND EQUIPMENT) BETWEEN EACH POND, LAKE, MEADOW, STREAM, TRIBUTARY, ETC.**

(The only exception to this rule is when the next survey site is in the same immediate drainage: directly connected by stream to the site you just surveyed. Between sites in the same connected area, all equipment should be rinsed of organic debris):

- 1) **Before leaving any aquatic site, thoroughly remove all organic matter (e.g., mud, plants, algae) from nets, sampling devices, boots (especially the tread), and any other equipment or clothing that has come into contact with water or aquatic sediments.** A stiff bristle brush (e.g., old toothbrush) is very helpful. Rinse with clean water.
- 2) **While at the same aquatic site of exposure, disinfect equipment on land, at least 100 feet away from the edge of any water sources.** Submerge all gear (e.g., boots, waders, measuring and sampling devices, traps, nets, etc.) in a bucket or sturdy garbage bag for **5 minutes**, using one of the two disinfecting agents listed below. Use a spray bottle to saturate any equipment too large to submerge in available containers. After soaking or spraying, allow the equipment to dry as much as possible before departing.
 - a) **PREFERRED:** Quaternary ammonium compound 128 (Quat-128®). USE **0.01% OF THE ACTIVE INGREDIENT, DIDECYL DIMETHYL AMMONIUM CHLORIDE (DDAC).**
 - i) Quat-128 is about 5% DDAC. Therefore, use about ~0.25 oz. (7.5 mL) Quat-128 per gallon of water to make a 0.01% active ingredient solution.
 - ii) Note: The recommended dilution for Quat-128 on the bottle is 1 oz. per gallon, which is a concentration of about 0.04% active ingredient. Using this concentration to eliminate Bd is likely overkill, because under laboratory conditions, Quat-128 kills Bd at concentrations of 0.001% active ingredient (Johnson et al. 2003). However, we recommend at least 0.01% active ingredient to be conservative, especially when dealing with field equipment.
 - b) **ALTERNATIVE: FRESH,** Household bleach (USE **5% OF THE BLEACH SOLUTION IN THE BOTTLE**).

- i) Make sure to use a standard bleach solution (e.g., Clorox® Regular Bleach), which should be about 6–8% sodium hypochlorite (Do not use a less concentrated formula). Therefore, for regular bleach, use a 1:20 bleach solution-to-water ratio. For example, use about 6 oz. (~180 mL or ¾ cup) of bleach per gallon of water to make a 5% bleach solution.
 - ii) *****WARNING: BLEACH BREAKS DOWN RAPIDLY*****. IF YOU USE BLEACH, DO NOT USE AN EXPIRED BOTTLE, OR A BLEACH BOTTLE THAT HAS BEEN OPEN FOR MORE THAN ONE MONTH. Although it's environmentally beneficial that bleach breaks down quickly, using expired bleach solution would be pointless for disinfecting, and put amphibian populations at risk! You must use fresh bleach.
 - iii) Note: A 3% bleach solution (i.e., 3% of what's in the bottle) has been found to deactivate both Ranavirus (Bryan et al. 2009) and Bd (Gold et al. 2012). However, we suggest at least 5% bleach solution to be conservative, especially when dealing with field equipment. Some biologists will use up to 10% bleach solution, which is likely unnecessary, especially if you are properly scrubbing and washing equipment beforehand.
- 3) **Used disinfecting chemicals should be disposed of safely:** When using Quat-128 or bleach, wear disposable, protective gloves. Dispose of the remaining cleaning solution at least 100 feet from water, where the compound will break down (e.g., trail soil, decomposing log, duff).
 - 4) **Rinse off equipment at least 100 feet from water AT THE NEXT WATER BODY/SITE TO WHICH YOU MOVE.** Use a clean garbage bag (i.e., not previously contaminated at another site OR containing disinfectant residue) to move water from the water body to the upland rinsing area.
 - 5) **Ensure all equipment used to capture and handle amphibians is thoroughly rinsed before use.** Equipment will have very little diluted disinfectant residue remaining after rinsing in the bag. However, as an added precaution, vigorously rinse the net and any other equipment in site water prior to capturing any amphibians.

Please contact the SEKI Aquatic Ecologist (danny_boiano@nps.gov, 559-565-4273) or CDFW High Mountain Lakes Environmental Scientist (Isaac.Chellman@wildlife.ca.gov) with any questions or concerns.

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For additional information about limiting risk for amphibian disease transfer:

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