

## APPENDIX F

### DECONTAMINATION PLAN

(Modified from the Declining Amphibian Task Force Fieldwork Code of Practice)

A Code of Practice was prepared by a group of scientists within the Declining Amphibian Populations Task Force to provide guidelines for use by anyone conducting fieldwork at amphibian breeding sites or in other aquatic habitats. Observations of diseased and parasite-infected amphibians are now being frequently reported from sites all over the world. This has given rise to concerns that releasing amphibians that can pick up unapparent infections may cause an increased risk of mortality in wild populations. Amphibian pathogens and parasites can also be carried in a variety of ways between habitats on the hands, footwear, or equipment of fieldworkers, which can spread them to novel localities containing species which may have had little or no prior contact with such pathogens or parasites. In some instances, such occurrences may be implicated in locations where amphibian populations have already declined. Therefore, it is extremely important for those involved in amphibian research and other types of wetland/pond studies to take steps to minimize the spread of disease agents and parasites between study sites.

The aquatic fungus *Batrachochytrium dendrobatidis* (Bd) occurs within the California Red-legged Frog Safe Harbor Agreement planning area. Bd causes amphibian chytridiomycosis and is associated with amphibian losses on multiple continents (Bosch et al. 2001, Skerratt et al. 2007, Vredenburg et al. 2010). Chytridiomycosis is an infection of the epidermis that can result in death and has been identified as a primary cause of population declines for some amphibians (Berger et al. 1998, Bosch et al. 2001, Briggs et al. 2010). Translocations under this Safe Harbor Agreement primarily involve moving egg masses of the California red-legged frog (*Rana draytonii*). Bd is a fungus that requires keratin to persist (Longcore et al. 1999). Amphibian egg masses contain no keratin but the Bd zoospores may persist in water collected from the site. To reduce or eliminate the presence of Bd zoospores in the water that will be translocated along with the egg masses, the following actions will be implemented:

1. Water from the collection site will be filtered and placed in four containers. Each partial egg mass collected will be placed in a sterile container filled with filtered water from the source pond or stream and allowed to rinse for several minutes.
2. Each egg mass will then have a repeated treatment for a total of three rinses before being placed in a fourth sterile transportation container, also filled with filtered water from the source pond or stream.

The Decontamination Protocol for equipment, shoes and clothing are as follows:

1. Before leaving each site: remove mud, snails, algae, and other debris from nets, traps, boots, vehicle tires, and all other surfaces. Rinse cleaned items with sterilized (e.g., boiled or treated) water.
2. Boots, nets, traps, etc. should then be scrubbed with 70 percent ethanol solution and rinsed clean with sterilized water between study sites. Avoid cleaning equipment in the immediate vicinity (within 50 feet) of a pond or wetland.

3. In the field, clean all equipment with 70 percent ethanol or a 5 percent bleach solution (0.5 to 1.0 cup of bleach per gallon of water), and rinse with sterile water upon return to the lab or at “base camp.” Care must be taken so that all traces of the disinfectant are removed before entering the next aquatic habitat. Elsewhere, when washing machine facilities are available, remove nets from poles and wash (in a protective mesh laundry bag) with bleach on a “delicates” cycle.
4. When working at sites with known or suspected disease problems, or when sampling populations of rare or isolated species, wear disposable vinyl or nitrile gloves and change them between handling each animal. Latex gloves are toxic to amphibians and should not be used. Dedicate sets of nets, boots, traps, and other equipment to each site being visited. Clean and store them separately at the end of each field day.
5. When amphibians are collected, ensure the separation of animals from different sites and take great care to avoid indirect contact between them (e.g., via handling or reuse of containers) or with other captive animals. Isolation from unsterilized plants or soils which have been taken from other sites is also essential. Always use disinfected and disposable husbandry equipment.
6. Examine collected amphibians for the presence of diseases and parasites soon after capture. Prior to their release or the release of any progeny, amphibians should be quarantined for a period and thoroughly screened for the presence of any potential disease agents.
7. All used cleaning materials (including liquids) should be disposed of safely and, if necessary, taken back to the lab for proper disposal. Used disposable gloves should be retained for safe disposal in sealed bags.

## References

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