Aquatic Invasive Species and Aquatic Pathogen Decontamination Guidelines

Protocol for Aquatic Invasive Species (AIS) and aquatic pathogen decontamination when in contact with ponds, creeks, rivers, and wetlands.

New Zealand mudsnails (*Potamopyrgus antipodarum*), Chytrid fungus (*Batrachochytrium dendrobatidis*), Ranavirus, and turtle-shell (fungal) disease (*Emydomyces testavorans* (*Emte*)) have been documented in Santa Clara County and it is critical that these guidelines be followed to reduce further spread of these detrimental AIS and aquatic pathogens. New Zealand mudsnails, Chytrid fungus, Ranavirus, and *Emte* have the potential to decimate populations of native amphibian, aquatic reptiles, and fish species if preventative actions are not implemented. The salamander chytrid fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*) has not yet been detected in the United States but has the potential to devastate California tiger salamanders and other salamanders if introduced; the proposed decontamination procedures would likely also reduce the potential for the spread of *Bsal*. Snake fungal disease (SFD) has not been detected in Santa Clara County, however it has been detected in numerous other counties across the Bay Area. Additionally, several (though not all) of the decontamination methods described in the guidelines below are also effective methods for eliminating *Phytophthora* plant pathogens.

In an effort to minimize the spread of AIS and aquatic pathogens all Habitat Agency staff and Contractors in contact with ponds, creeks, rivers and wetlands (e.g., biologists, engineers, construction and biological site monitors, and surveyors) must adhere to the most current guidance for equipment decontamination and sanitization provided by the appropriate Wildlife Agencies at the time of activity implementation. Guidance may require that all materials that come in contact with water, including boot and tire treads, be cleaned of all organic matter and scrubbed with an appropriate sanitizing solution. Care should be taken so that all traces of the disinfectant are removed and appropriately disposed of to avoid environmental contamination before entering the next work site. Employees should follow the guidance outlined below for disinfecting equipment and clothing before/after entering creeks, rivers, ponds, wetlands, floodplains, or any other wet or muddy habitats that could harbor AIS or aquatic pathogens.

1. General protocols

- Cleaning and sanitation are required before entering work areas including creeks, rivers, ponds, wetlands, floodplains, or any other wet or muddy habitats that could harbor AIS or aquatic pathogens to prevent introduction of contamination from other locations.

- Cleaning and sanitation are required between leaving a waterbody and entering a new waterbody to prevent pathogen spread to other locations. A good rule of thumb to follow is any time the name of the waterbody is different, gear should be decontaminated (e.g. between Pacheco Creek and Coyote Creek or any tributary).
- For locations known or highly suspected to be contaminated with AIS or aquatic pathogens, use dedicated equipment that is only used at contaminated sites. Store this equipment separately from equipment used at other sites.
- If possible, choose boots with rubber soles. Felt soled boots are more difficult to decontaminate than rubber soled boots because they can retain moisture for more than a month. Additionally, New Zealand mudsnails are more attracted to the felt sole texture.
- Biologists will be gloved when, and replace used pairs of gloves between, handling any sensitive amphibians and reptiles to inhibit transmission of chytrid fungus, *Bsal*, ranavirus, *Emte*, or SFD.
- If sensitive amphibians and reptiles need to be moved out of harm's way, then they should be moved within the same waterbody *when possible,* to limit inadvertent spread of disease.
- If sensitive amphibians and reptiles must be relocated outside of the waterbody in which they were found, then biologists should work with the Wildlife Agencies to ensure the necessary precautions (e.g. pathogen testing through Environmental DNA sampling) are taken prior to the relocation across waterbodies.
- To prevent the spread of *Emte*, avoid relocating northwestern pond turtles more than 500 meters (1,640 feet) or to a different watershed unless approved by the Wildlife Agencies and/or all native and nonnative turtles at the project site have tested negative for *Emte*. Any turtle-transport containers will be decontaminated between uses and not used to transport individual turtles from differing water bodies together. A basic health assessment will be completed on all captured animals, and any suspected observations of disease including turtle-shell disease in northwestern pond turtle will immediately be reported to the Wildlife Agencies before relocating any northwestern pond turtle suspected of turtle-shell disease.

2. Sanitizing footwear, waders, equipment, and tools

- Before arrival at a new waterbody, equipment and tools must be appropriately sanitized (see #5 Procedures below).

3. Worker training

- Appropriate trainings on AIS and aquatic pathogens and how to prevent their spread by following approved sanitation procedures will be provided as a component of other Habitat Agency trainings. Additionally, trainings may be requested from a qualified biologist.

4. Minimize unnecessary movement of AIS

- When practical, begin work upstream/upslope and work downstream/downslope to encourage the practice of working from low risk to high-risk areas. This avoids transporting pathogens and AIS to non-infested upstream/upslope areas.

5. Procedures for sanitizing tools, surfaces, and footwear

- Surfaces, tools, and footwear should be clean and sanitized before use. Wood handles on tools should be sealed with a waterproof coating to make them easier to sanitize. Options for sanitization and decontamination include drying, a chemical treatment, and freezing in combination with a dilute chemical treatment, dependent on pathogen. See below for details on specific protocols.
- Remove all soil and organic material (roots, sap, *etc.*) from the surface of nets, buckets and equipment that have come in contact with water or mud. If necessary, use a detergent solution and brush to scrub off surface contaminants. The sanitizing agent may also be used as a detergent solution. A screwdriver, wire brush, hoof pick, or similar implement may be needed to clean soil out of crevices or shoe treads. Brushes and other implements used to help remove soil need to be cleaned and decontaminated after use.

Option 1: Dry*

- Scrub gear with a stiff-bristled brush to remove all organisms and soil. Thoroughly brush small crevices such as boot laces, seams, net corners, *etc.* and rinse with fresh water.
- Place equipment into a drying cabinet or other method of controlling temperature to maintain equipment at 14°C (57°F) for 8 days OR 35°C (95°F) for 30 hours OR 70°C (158°F)* for 30 minutes. Please note that the drying clock does not begin until gear is completely dry.

NOTE: New Zealand mudsnails and other mollusks can take several days to lethally desiccate due to their protective shells. The post-dry clock begins following internal tissue desiccation of mollusks. Removing mollusks before drying reduces the time needed to achieve effective results for all other species.
*Drying for 30 minutes at 70°C (158°F) is also effective against *Phytophthora* plant pathogens. Note that ONLY a constant temperature of 150°F or more for 30 minutes will effectively kill *Phytophthora* plant pathogens.
**As of finalization of CDFW's 10/3/2022 Aquatic Invasive Species Decontamination Protocol, there were no data or research to demonstrate the efficacy of the drying method against Ranavirus, *Emte*, *Bsal*, or SFD.

Option 2: Chemical treatment*

- Scrub gear with a stiff-bristled brush to remove all organisms and soil. Thoroughly brush small crevices such as boot laces, seams, net corners, *etc.* and rinse with fresh water.
- Soak gear in Virkon S or Virkon Aquatic (2% solution) for 20 minutes. Appropriate guidelines should be followed regarding appropriate handling and disposal of this

chemical. The following personal protective equipment should be used while handling this chemical: splash goggles, face shield, Tychem apron, chemical-resistant gloves, and rubber boots.

- Ensure that all items being decontaminated are fully submerged in the chemical treatment for the entire 20 minutes of contact time.
- All decontaminated equipment should then be rinsed clean after exposure to chemical treatment. By all practicable means, decontamination should be completed in an area where the sanitizing solution can be disposed of safely. If decontamination must be completed in the field, sanitize gear in an area that is at least 150 feet from aquatic features, storm drains, wetlands, or other sensitive habitats. Care should be taken so that all traces of the disinfectant are removed before entering the next aquatic habitat.
- Used sanitizing solution (liquids, *etc.*) should be disposed of safely at an appropriate disposal site.
- Transport contaminated equipment in sealed plastic bags and keep separate from clean gear.

*The chemical treatment method is also effective against *Phytophthora* plant pathogens.
**As of finalization of CDFW's 10/3/2022 Aquatic Invasive Species Decontamination Protocol, there were no data or research to demonstrate the efficacy of the chemical treatment method against *Emte*, *Bsal*, or SFD.

Option 3: Dilute chemical treatment and freeze (not recommended to treat *Phytophthora* plant pathogens)

- Scrub gear with a stiff-bristled brush to remove all organisms and soil. Thoroughly brush small crevices such as boot laces, seams, net corners, *etc.* and rinse with fresh water.
- Following the safety procedures included in "Option 1: Chemical treatment" above, soak gear in 1% Virkon S solution for 5 min.
- All gear should then be rinsed clean after exposure to chemical treatment.
- Place in a freezer -3°C (26.6°F) or colder for a minimum of 2 hours.

* As of finalization of CDFW's 10/3/2022 Aquatic Invasive Species Decontamination Protocol, there were no data or research to demonstrate the efficacy of the dilute chemical treatment and freeze method against *Phytophthora* plant pathogens, *Emte, Bsal*, or SFD.

Reporting Aquatic Invasive Species and Pathogens

If you suspect you have found New Zealand mudsnails, quagga mussels, zebra mussels, Chytrid fungus, Ranavirus, *Emte*, *Bsal*, SFD, or other AIS or pathogens, please immediately notify a qualified biologist connected with the Habitat Agency. Please provide your contact information, specific location of discovery, and photographs of the organisms (if possible).

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