Decontamination Protocols: Recommendations for Research and Monitoring Activities in and around New Jersey Waters

New Jersey Water Monitoring Council

Decontamination Protocols Workgroup

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i. Introduction

This **working document** describes general best practices to reduce the spread of aquatic invasive species (AIS) and disease during fieldwork and provides a consistent set of procedures for decontamination of water monitoring equipment. The spread of AIS and disease can be reduced through the use of the physical, mechanical and chemical preventative methods discussed in this document. The New Jersey Water Monitoring Council (NJWMC) member agencies and others working in and around NJ waters are encouraged to adopt these procedures to protect the ecological integrity of New Jersey's aquatic ecosystems. It is recommended that this document be reviewed by field staff annually. The research that supports these methods should be reviewed at least every five years to determine whether

new research has improved our understanding of disinfection efficacy and also to evaluate the effectiveness of these prevention methods when new species are observed in the state.

The decontamination procedures will be widely applicable and shall be recommended for use by the NJWMC member agencies and all professional water monitoring groups (state, federal, local government agencies, academic institutions, non-profits, environmental consultants and watershed groups), unless a more stringent program or project decontamination protocol already exists.

The decontamination procedures are intended for all equipment that comes in direct contact with a waterbody including boats and boating equipment (e.g., non-motorized and motorized boats, trailers, motors, bilge, live wells, dry wells, paddles, anchors), sampling equipment (e.g., sondes/data loggers, water samplers, nets, buckets, churns), and personal equipment (e.g., waders, boots, gloves, personal floatation devices).

The goal of this document is to provide general best management practices and is organized based on level of risk. For instance, equipment used infrequently and/or on only one body of water (low risk level) does not require the same level of decontamination as equipment used on multiple bodies of water per day (medium risk level) and/or equipment used with known AIS infestations (high risk level).

ii. Acknowledgements and Contact Information

The New Jersey Water Monitoring Council (NJWMC) serves as a statewide body to promote and facilitate the coordination, collaboration and communication of scientifically sound ambient water quality and quantity information to support effective environmental management. We would like to acknowledge the NJWMC for their endorsement of this workgroup to develop decontamination protocols for water monitoring activities in and around NJ waters. The workgroup consists of representatives from the NJWMC's member agencies. Workgroup members include Alena Baldwin-Brown (*co-chair, retired*), Jenna Krug (*co-chair*), Brian Henning, and Emily Mayer from the New Jersey Department of Environmental Protection (NJDEP) Division of Water Monitoring, Standards & Pesticide Control, Joseph Bilinski from the NJDEP Division of Science and Research, Heather Desko (*co-chair*) and Angela (Gorczyca) Mostwill from the New Jersey Water Supply Authority, Mike Danko from the New Jersey Sea Grant Consortium, Heather Heckathorn (NJWMC co-chair) and Zoltan Szabo from the United States Geological Survey NJ Water Science Center, Rita Isabel Matos from the United States Environmental Protection Agency Region 2, Christa Reeves from the Musconetcong Watershed Association, Will Ruocco from Brick Township Municipal Utility Authority, Meiyin Wu from

Montclair State University and Cheryl Yao from the Meadowlands Research and Restoration Institute. Contact information for workgroup members is available in Table 1. The workgroup would like to thank additional past workgroup members for their contributions: Maria Berezin, Raritan Headwaters Association; Lisa Carper, US Geological Survey NJ Water Science Center; Nik Hansen, The Watershed Institute; Nancy Lawler, Musconetcong Watershed Association; and Marilyn Sobel, NJ Pinelands Commission. Thank you to the workgroup members' agencies for prioritizing this environmental threat and supporting this endeavor.

We would also like to thank the NJWMC Co-Chairs Heather Heckathorn (USGS-NJWSC) and Bob Schuster (NJDEP-WLM), and retired NJWMC Co-Chairs Leslie McGeorge (NJDEP-BFBM) and Bob Reiser (USGS-NJWSC), as well as the members of the NJWMC who reviewed the documents produced by the workgroup and non-members who provided invaluable feedback including Kathleen M. Foley from United States Environmental Protection Agency Region 2 and Kyle Clonan from NJWSA.

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iii. Acronyms and Definitions

Acronyms

AIS – Aquatic Invasive Species
NJWMC – New Jersey Water Monitoring Council
NJDEP – New Jersey Department of Environmental Protection
NAS – Non-indigenous aquatic species
RHM- Right-Handed Mudsnail
US EPA - United States Environmental Protection Agency
USGS - United States Geological Survey
WDNR - Wisconsin Department of Natural Resources

Definitions

"Aquatic species" means any species of fish, mollusk, crustacean, other aquatic invertebrate, amphibian, reptile or aquatic plant but is not limited to fish and fishes. (N.J. Admin. Code § 2:89-1.2)

"Aquatic organism" means an animal or plant of any species or hybrid thereof and includes gametes, seeds, eggs, sperm, larvae, juvenile and adult stages any of which is required to be in water during that stage of its life. This definition does not include birds and mammals. (N.J. Admin. Code § 2:89-1.2)

"Game animal" means any mammal for which a legal hunting or trapping season has been established in New Jersey, identified in this subchapter, or designated a game animal by the Fish and Game Council in the annual game code. (N.J. Admin. Code § 7:25-10.4)

"Invasive species" means nonindigenous plant and animal species that have been intentionally or accidentally introduced into habitats and geographical areas outside of their natural geographical range and that have the ability to reproduce and spread, thereby threatening native biological diversity and/or the integrity of natural ecosystems. (N.J. Admin. Code § 7:5A)

"Native species" means any species of any plant or animal that naturally occurs in the water of the State and is capable of surviving in the wild for 12 consecutive months. ((N.J. Admin. Code § 2:89-1.2))

"Non-native species" means any species of plant or animal that does not occur naturally in the waters of the State. (N.J. Admin. Code § 2:89-1.2)

Additional definitions are included in Appendix A.

I. Introduction to Aquatic Invasive Species

What are Aquatic Invasive Species (AIS)? Invasive species (also called exotic or nonindigenous species) are species whose populations have expanded beyond their natural range and cause damage in their invaded environment. This damage can be to native habitat, populations of other species, or ecosystem services. Aquatic Invasive Species (AIS) are invasive species that spend all or part of their life cycle in aquatic systems.

Where do AIS come from? Aquatic Invasive Species can be introduced both intentionally and unintentionally via human activities. Maritime trade vessels have been targeted as an AIS vector, with invasive species stowing in ballast water or attached to the hulls of ships. Some invasive species are intentionally introduced into the wild via home aquarium fish release or for aquaculture purposes. In some cases, native species can also be considered invasive if they have gained the ability to grow in populations larger than their ecosystem can tolerate due to external forces such as the elimination of predators, habitat modification or changes in climate patterns.

Why are AIS a problem? Fast growing, unchecked populations of invasive species can have far reaching consequences for native species, ecosystems, and ecosystem services. For example, silver carp (*Hypophthalmichthys molitrix*) are aggressive and can outcompete native species for resources, driving local populations into decline. Fast-growing invasive species such as *Hydrilla verticillata* and the Zebra mussel (*Dreissena polymorpha*) can grow to such a density that they are capable of clogging and damaging water resource infrastructure. Some invasive species, such as the clinging jellyfish (*Gonionemus vertens*) can become so abundant making waterways unsafe for human recreation. Without any natural predators to act as population control, some invasive species such as the non-aquatic spotted lanternfly (*Lycorma delicatula*) can severely damage native plant populations, resulting in the endangerment or extinction of native species as well as the loss of agricultural revenue if the prey species is a crop.

What is a native species versus a natural/wild species?

Native species are defined as a species of any plant or animal that naturally occurs in the State and is capable of surviving in the wild for 12 consecutive months. Non-native species are any species of plant or animal that does not occur naturally in the State. Note that not all non-native species are considered invasive. Non-native species that are not considered invasive can include the following:

- Game species that are intentionally stocked for sport. Game species generally lack the ability to permanently establish in the wild. The Fish and Game Council designates specific game species in the annual game code.
- Ornamental species, usually plants, are species that are intentionally introduced for

decorative purposes. Most ornamental species do not have the potential to become invasive, although some established invasive species such as Japanese knotweed (*Reynoutria japonica*) were first introduced as ornamentals and have escaped into the wild.

- Nuisance species, such as the deer tick (*Ixodes scapularis*), are native species that can negatively impact human activities. Nuisance species populations are often controlled despite the fact that they are native.

How can I help?

The primary focus of this document is to prevent the inadvertent spread of aquatic invasive species by groups and individuals conducting research, monitoring and/or sampling activities in and around the waters of New Jersey. As such, this document focuses on species and methodologies of particular concern to water samplers.

You can help by becoming aware of best practices, which involve carefully designing your sampling schedule to start with low-risk sites, selecting appropriate equipment, and adopting the appropriate decontamination procedure. Whether or not you are involved in aquatic sampling, you can help by observing the water bodies you habitually visit for recreational or other activities and promptly notify authorities when you observe invasive species, explained in more detail below. If you own or use a boat, please keep in mind that boats are especially effective at spreading invasive species. You can help by giving special attention to cleaning your boat and boating equipment. For detailed procedures, see section III.B, Watercraft Decontamination Procedures below.

II. General Best Practices to Reduce Risk of Spread

Recreational boating and fishing activities are a major dispersal vector for aquatic invasive species (AIS) and contribute to the spread of AIS once a species has been introduced and established (Johnstone, 1985; Kelly et al., 2012; Rothlisberger et al., 2010). Additionally, researchers, scientists, lake management consultants and environmental monitoring agencies are at risk of spreading AIS. Everyone accessing waterbodies, whether it be for recreation or scientific purposes, can take measures to help reduce the spread of AIS by practicing the following general best practices and the decontamination procedures in Section III.

i. Before Heading to the Field

- Be Aware:
  - Check for reported infestations in your study area using the USGS NAS Database (https://nas.er.usgs.gov/viewer/omap.aspx) or NJ Invasive Species Strike Team Interactive Map (https://www.fohvos.info/invasive-species-strike- team/interactive-map/)

- Know your study areas' "most wanted" invasive species of concern and learn how to identify, avoid, and decontaminate the invasive species you are most likely to encounter in your fieldwork. If species of special concern are known in your study area, review the special concern protocols in this document. Watershed (HUC8) distribution and identification information can be obtained from the USGS NAS Database: https://nas.er.usgs.gov/default.aspx or from the Mid-Atlantic Field Guide to Aquatic Invasive Species, designed by PA Sea Grant: http://seagrant.psu.edu/topics/aquatic-invasive-species/projects/ais-field- guides.

- Plan Your Sampling Strategy:
  - If working on multiple water bodies, organize sampling so work goes in order of infestation magnitude, from low to high.
  - When working on multiple sites within the same waterbody, organize sampling so that it progresses from the least to most likely to be contaminated, generally from upstream to downstream.
  - When working within different reaches of the same stream, plan to decontaminate whenever equipment crosses a barrier while going upstream.
  - Know your level of risk (see Section III) and be prepared to decontaminate your personal equipment, sampling gear and boat accordingly.
- Choose Your Equipment:
  - Avoid materials that are known to harbor and spread AIS, including absorbent materials such as felt-soled waders.
  - Choose equipment and materials with the fewest places for organisms and debris to become attached, such as attached boot waders with cleated soles.
  - If a high percentage of work is done in contaminated waters (e.g., High Risk Areas), consider dedicating certain gear to only be used in those waters.
  - Select equipment that can withstand the decontamination process required for known invasives in your sampling area. Alternative gear (i.e., rubber soled wading boots with metal studs) are recommended if conditions warrant.
- Select and prepare appropriate decontamination solutions for equipment that will be used in the field. Plan for and properly dispose of consumables and decontamination solutions.

ii. While in the Field

- Preventing the spread of aquatic invasive species begins at the parking area. Prior to sampling note any AIS that may be visible from the parking area or enroute to the sampling location. Plan your route to and from the sampling location accordingly.
- Select a location where your decontamination equipment will be placed. Prior to sampling be sure your decontamination area is ready to minimize contact when

returning to the vehicle with samples.

- Visually inspect all sampling gear and personal items prior to sampling and repeat prior to decontaminating.
- Strategize your sampling so that it progresses from areas of least to most likely to be contaminated.
- Minimize wading and boating into potentially contaminated areas and consider alternatives to wading, such as using bank sampling poles.
- Regularly inspect and clean your equipment from area to area and site to site.
- Remove plants, sediment or organisms from boats or sampling gear.

iii. After Fieldwork is Complete

- Fully inspect all equipment and manually remove any visible organisms present.
- Whenever possible, scrub equipment with a stiff-bristled brush and/or wash with soapy water. Check manufacturers' recommendations for cleaning before applying detergents, solvents, and abrasives.
- Follow the recommended decontamination procedures for the type of equipment and particular AIS of concern for your study area. (USGS NAS Database)
- Do not clean equipment in or near streams.
- Do not dispose of materials and wash water into systems that will return to a water body without treatment.

iv. Reporting

While in the field, if a new or suspected AIS population is encountered, sighting reports should be reported via the EDDMapS app or website (https://www.eddmaps.org/report/). Reports must include the observation date, name of the species, location (latitude/longitude coordinates), estimated population size, and pictures. The EDDMapS platform does require registration to submit reports. A tutorial for reporting on the EDDMapS app is available in Appendix B (FoHVOS NJ Strike Team App). Sightings can also be reported directly to Emily Mayer at Emily.mayer@dep.nj.gov.

III. Post-Sampling Decontamination Protocols

This section details post-sampling decontamination protocols and is organized by equipment type with details based on level of risk.

i. Level of Risk Definitions

Low: Sampling is conducted, or boat is used, on one waterbody or equipment (including boat) is used infrequently and can be cleaned and fully dried before next use. Field technicians, sampling equipment, and/or boats do not come into contact with any waterbodies with known AIS.

Medium: Sampling takes place on multiple waterbodies per day or week. Boat is used on multiple waterbodies per week. The presence of AIS in waterbody is either known or unknown.

High: While sampling, contact is made with AIS or takes place in waters with known AIS. Sampling takes place in multiple water bodies per day on both AIS and non-AIS waterbodies. Boat is in contact with multiple water bodies on a given day.

ii. Decontamination Level

Below is the general approach recommended to select the appropriate protocols for various situations. You should always use your best professional judgment for the situation and use the best materials available to you. Figure 1 summarizes the decontamination levels needed for each sampling risk level.

- Level 1 decontamination is conducted at all sites.
- Levels 1 and 2 decontaminations are conducted at medium and high-risk sites.
- Levels 1, 2 and 3 decontaminations are conducted at high-risk sites.

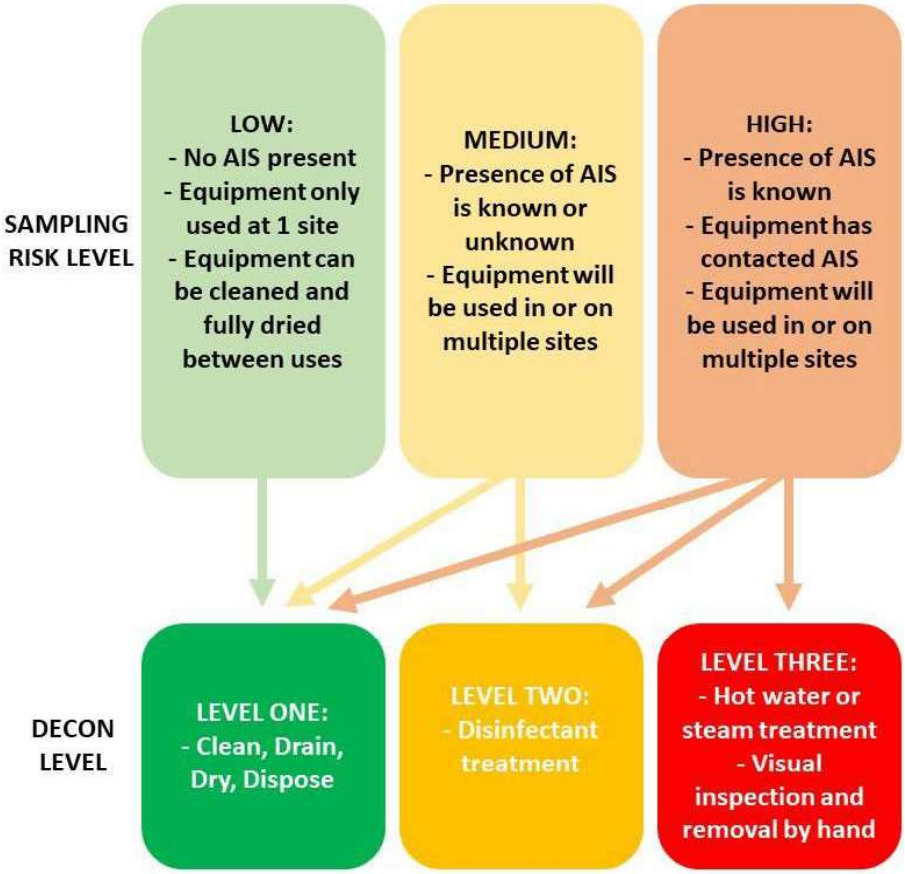

Figure 1. Flow chart for sampling risk level and decontamination level (based on Alberta Environment and Parks, 2020).

A. Personal Gear and Sampling Equipment

Personal gear can include, but is not limited to waders, boots, raingear, diving equipment, PFDs, and other similar items. Sampling equipment can include, but is not limited to nets, buckets, sieves, poles, sensors, water samplers and other similar equipment used to collect samples.

Level 1 (All Risk Levels)

1. **Clean**: Visually inspect all surfaces of waders, boots, raingear, dive equipment, PFDs, other personal gear and all sampling equipment for any organisms before leaving the sampling site. Fragments of aquatic plants should be removed and discarded at the sampling location as these fragments can remain viable for days and can reproduce and become established when entering a new waterbody (Bruckerhoff et al., 2015; Johnstone, 1985; Madsen, 1988). Scrub all surfaces of personal equipment and accessories with stiff bristled brush with a non-wooden handle and rinse with clean water. Waders, boots, raingear, dive equipment and other gear are best decontaminated while being worn. If working with another field crew member, crew members should take turns spraying each other's personal gear paying particular

attention to the soles of boots. Decontamination is considered complete when all visible debris is removed from the gear.

2. **Drain**: Drain water from personal gear and sampling equipment back into the waterbody. Be sure to stow gear in a manner that allows water to run off and promote drying (O'Hara, 2018).

3. **Dry**: Towel, sponge or dry in direct sunlight. Make sure all areas of personal gear are completely dry. It is recommended they remain dry for a minimum of 24 hours; however longer dry times are encouraged whenever possible (Alberta Environment and Parks, 2020). Ensure that all gear is completely dry as some AIS can persist in moist conditions. If drying may not be a feasible solution for personal gear and sampling equipment requires daily use, you should consider following the protocols for the higher risk category.

4. **Dispose**: Properly dispose of any water or other disinfectant solutions and single use items such as gloves, Tyvek suits, etc. in the trash.

Level 2 (Medium & High Risk)

Follow the basic **Low Risk** decontamination procedures outlined in **Level 1** of **Clean**, **Drain**, and **Dry,** AND **Disinfect** all surfaces using an appropriate solution for the types of AIS you are likely to encounter (see **Decontamination Solutions** at the end of this section). Disinfect with attention to the bottom of boots, seams, and other areas where plants and organisms could attach.

1. Select and prepare one or more solutions from the options listed in the **Decontamination Solutions** section. Keep in mind the holding time of the prepared solution and amount needed to minimize waste and reduce costs. Rinse water must be disposed of properly.
2. After leaving the waterbody, rinse off waders and any equipment (i.e., meter sticks, nets, poles) that come into contact with the water to remove any organic residues. The treads of boot soles usually require more attention.
3. After rinsing with water, stand in a bin and rinse off waders and equipment with the decontamination solution you selected and let the solution remain on waders and equipment for the solution recommended contact time (See Table 2).
4. When using decontamination solutions such as bleach it is recommended to rinse personal gear and sampling equipment off with clean water. Always check labeling on solutions and manufacturers' suggestions to prevent damage to gear and equipment.

Level 2 Considerations for Sensitive Equipment

1. Clean with a soft bristle toothbrush and rinse with distilled or deionized water. This will not decontaminate, but it will remove seeds, vegetative material, and turions. Rinsing will also remove some bacteria. It has the advantage of being completely safe for the equipment.
2. Clean with bleach. This can be done for robust equipment such as YSI sondes (1:1 solution, rinse with tap water).
3. Wipe item with paper towel soaked with 2% Virkon Aquatic solution (2.7 ounces per gallon). Check with the manufacturer and operating manual before using Virkon.
4. Before storing decontamination solution containers, rinse containers and nozzles with water and air dry.

Level 3 (High Risk)

Initially, follow the personal gear and sampling equipment decontamination procedures for **Level 1** and **Level 2**. Hot water is the most environmentally friendly and cost-efficient means of decontaminating field gear, especially boats (Anderson et al., 2015). Use of a high-pressure washer will also aid in removing vegetation, mud, debris, and any organisms attached to personal gear. Visual inspection and hand removal is responsible for reducing the transport of aquatic invasive vegetation by 88% (Rothlisberger et al., 2010). Hot water should not be used on materials such as Gore-Tex. It is recommended that you always check manufacturers' labels and operating manuals prior to exposing gear and sampling equipment to hot water. Sensitive equipment that cannot be subjected to high temperature water should be cleaned with bleach or Virkon.

1. **Heat/hot water:** Hot water is effective at killing a wide range of organisms and pathogens. Use of an infrared thermometer is recommended to verify temperature. Always check labels and suitability of gear equipment before exposing it to hot water. Always clean the ground prior to placing items on the ground for cleaning. Staff should wear protective equipment (gloves and face shield) to prevent exposure to hot water or steam.
    a. Hot water (140°F or 60 °C) or steam clean (212°F or 100 °C) for 10 seconds contact time (Anderson et al., 2015; WDNR, 2020a).
    b. Hot water (113°F, 45°C for 15 minutes) has been found to be an effective decontaminant (Anderson et al., 2015).
    c. Do not use on materials such as Gore Tex.
2. **Dry:** Dry thoroughly for as long as possible; 5 days is recommended, preferably in direct sunlight, before using gear in a different waterbody (Michigan DEQ, 2014).

Decontamination Solutions

It is necessary to have access to and use multiple decontamination solutions because certain types of gear and equipment could be damaged or have their performance impacted if manufacturers' recommendations are not followed. Therefore, we have provided a comprehensive list of currently used decontamination solutions to prevent the spread of invasive species. Always check labels and suitability of gear equipment before exposing it to any of the decontamination solutions. Always check Material Safety Data sheets and labels to avoid exposure and wear the recommended personal protective equipment when mixing and using the solutions. When mixing solutions, employees should wear the appropriate PPE such as nitrile gloves, chemical splash goggles, and/or face shields. Products and/or brands mentioned in this document are for reference purposes only and do not constitute an endorsement.

1. **Bleach**: Household Bleach (5.25% sodium hypochlorite) is inexpensive but can be harmful to sensitive organisms. A chlorine solution of 500 ppm or 1.22 fl. oz. of household bleach per gallon of water is effective at killing many aquatic invasive species and fish diseases (WDNR, 2020). Write the date on the bottle of bleach when it is opened, store out of sun and heat, and only use it for 2 months (Michigan 2014). Diluted bleach will begin to degrade after 24 hours. Therefore, it is recommended to only mix small quantities that can be used within 24 hours of mixing.
    a. If purchasing household bleach in bulk quantities it should be purchased in amounts that will be used within 6 months and stored at temperatures between 50- and 70-degrees Fahrenheit (WDNR, 2020a). Bleach stored over 6 months will begin to deteriorate and lose its disinfection properties.
    b. It should be noted that household bleach will not kill right-handed mudsnails (Holea, 2005), spiny water flea resting eggs or Asian clams. When working in areas where there is the potential to encounter or that have documented populations of these species it is recommended that an additional disinfection method be used.
    c. Recommended bleach solution is 1.22 ounces bleach to 1 gallon of water (WDNR, 2020a). Recommend using solutions less than 24 hours old. Recommended contact time is 10 minutes. Personal gear and equipment may require a freshwater rinse. When applying additional chemicals, it is imperative to thoroughly rinse the items with water.
2. **Virkon Aquatic**: More expensive but generally considered safer for aquatic organisms. Avoid using wood handled equipment or unscrew wood handles prior to soaking. Virkon Aquatic can be absorbed into wood and then leach back into the water during the next sampling and may be harmful to fish (WDNR, 2020a). Virkon should not be used on

sensitive equipment like Van Dorn samplers or chemistry probes as repeated use of the product may lead to degradation over time (WDNR, 2020a). Store at room temperature. Keep the solution away from extreme cold or heat. Virkon Aquatic solution remains stable for 1 week if diluted with tap water. Mix Virkon Aquatic into a solution at the ratio of 39 cc (cup size) per gallon of water. A 15–20-minute bath immersion of 20 grams per liter (g/L) Virkon Aquatic is effective in killing 99% of right-handed mudsnails (Stockton and Moffit, 2013).

a. From Boat Procedures - Virkon Aquatic (2% solution, 20-minute contact time; WDNR, 2020). https://www.fishersci.com/shop/products/virkon-aquatic-10-tub/nc0398532 .

b. Mix 20 grams per liter of water and use within 7 days. Apply directly to the surface by spraying or using a sponge so that the surface is thoroughly exposed to the Virkon Aquatic solution. Contact should be at least 20 minutes. Dispose unused Virkon solution in the sanitary sewer.

3. **Alconox/Liquinox in conjunction with Bleach/Virkon or other Decontamination Method.** Alconox/Liquinox are degreasers/cleaning agents that can be used to remove solids and organic material on aquatic sensors (Parker, 2015). Prepare at 0.2% solution using either 378.5 mL of Liquinox or 37.85g Alconox phosphate-free detergent into a 5-gallon bucket. Fill bucket with tap water, mixing halfway through filling. Wash with kimwipes and/or lint-free swabs. Triple rinse with tap water, followed by triple rinse with deionized water. Use of these products would require secondary treatment by another decontamination solution (Bleach/Virkon) or method (freezing/hot water) to kill targeted AIS and/or pathogens if present, and therefore are not included in Table 2. As some of these solutions/methods are not appropriate for some sensitive equipment, the removal of solids and organic material with Alconox/Liquinox is encouraged beyond simply rinsing with water for this equipment. Always follow manufacturers' instructions.

Table 2. Personal Gear and Sampling Equipment Decontamination Reference

Equipment Type ↓	Level of Risk			Notes
	Low	**Medium**	**High**	
Waders/wading boots, snorkeling equipment	Clean, Drain, Dry	**Clean using one of the below methods then drain and dry** 1. Soak in chlorine bleach or Virkon aquatic for 20 minutes. Rinse with fresh water. 2. Soak in chlorine bleach solution. Rinse with fresh water. Freeze for 8 hours. 3. Freeze for 8-24 hours.	**Clean using one of the below methods then drain and dry** 1. Hot water pressure washer (>140°F) or handheld steamer (10 seconds per spot) 2. Soak in hot water (120°F) for 20 minutes 3. Use alternate gear that has been disinfected according to protocol and is completely dry for 5 days	1. Car washes typically do not reach lethal temperatures for AIS. 2. High temperatures may melt adhesives and seams on Gore-Tex and similar fabrics. 3. Freezing may not kill pathogens, thus freezing should be used in addition with another decontamination method (i.e., chlorine bleach)
Nets (e.g., D-nets, siene nets, fish nets, block nets, etc.)	Clean, Drain, Dry	**Clean using one of the below methods then drain and dry** 1. Soak in chlorine bleach or Virkon aquatic for 20 minutes. Rinse with fresh water. 2. Soak in chlorine bleach solution. Rinse with fresh water. Freeze for 8 hours. 3. Freeze for 8-24 hours.	**Clean using one of the below methods then drain and dry** 1. Hot water pressure washer (>140°F) or handheld steamer (10 seconds per spot) 2. Soak in hot water (120°F) for 20 minutes 3. Use alternate gear that has been disinfected according to protocol and is completely dry for 5 days	1. Car washes typically do not reach lethal temperatures for AIS. 2. High temperatures may melt adhesives and seams on Gore-Tex and similar fabrics. 3. Freezing may not kill pathogens, thus freezing should be used in addition with another decontamination method (i.e., chlorine bleach)
Electronics (probes, sondes, data loggers, flow meters)	Clean, Drain, Dry (Follow manufacturer's instructions)	**Clean, Drain, Dry (Follow manufacturer instructions)** 1. Some water quality meters can be stored in pH 4 buffer solution which may kill some invasives 2. Wipe item with paper towel soaked with bleach solution	**Clean, Drain, Dry (Follow manufacturer instructions)** 1. Wipe item with paper towel soaked with bleach solution	1. Virkon may not be completely removed with rinsing, and should not be used on water quality equipment (WDNR 2020)
Portable pumps	Clean, Drain, Dry	Flush with 2% solution of Virkon aquatic or bleach for 20 minutes	flush with hot water >100°F-120°F exit temperature for 2 minutes	
Boats, trailers, and outboard motors	See section B. Watercraft Decontamination Procedures			

B. Watercraft Decontamination Procedures

Recreational boating and fishing activities are a major dispersal vector for aquatic invasive species (AIS) and contribute to the spread of AIS once a species has been introduced and established (Johnstone, 1985; Kelly et al., 2012; Rothlisberger et al., 2010). Additionally, researchers, scientists, lake management consultants and environmental monitoring agencies are at risk of spreading AIS. All boaters can take measures to help reduce the spread of AIS by practicing the following decontamination procedures.

Preventing the spread of aquatic invasive species begins at the boat launch. Prior to launching a watercraft, visually inspect the watercraft and its trailer. This will ensure that there are no aquatic organisms or vegetation clinging to the boat or trailer that may be introduced into the waterbody. Conduct the same visual inspection upon removing your watercraft from the water and remove all organisms and vegetation at the boat launch. Know your level of risk and be prepared to decontaminate your boat accordingly (summarized in Table 3). Table 4 summarizes the options by treatment method and boat equipment.

Level 1 (All Risk Levels)

1. **Clean**: Visually inspect all surfaces of boat, trailer, anchor, lines, paddles, and sampling equipment for any organisms. Fragments of aquatic plants should be removed and discarded at the boat ramp as these fragments can remain viable for days and can reproduce and become established when entering a new waterbody (Bruckerhoff et al., 2015; Johnstone, 1985; Madsen, 1988). Scrub all surfaces of boat, trailer and accessories with stiff bristled brush and rinse with clean water.

2. **Drain**: Upon exiting the ramp, pull all livewell, bilge and drain plugs. Remove any ballast or standing water.

3. **Dry**: Towel, sponge or dry in sun. Make sure all areas of the boat are completely dry for 5 days before next use. Ensure that the boat is completely dry because some AIS can live in standing water. Drying may not be a feasible solution for frequent users that require using the same equipment daily.

4. **Dispose**: If you are angling, be sure to dispose of unused bait in the trash. Never dispose of bait (i.e., minnows, shiners, worms, crayfish) into a waterbody.

Level 2 (Medium & High Risk)

Follow the basic boat decontamination procedures of clean, drain, and dry **AND** disinfect all surfaces using one of the methods below. Disinfect with attention to livewells, bilges, and other areas of standing water.

1. **Bleach** (500 ppm or 1.22 fl. Oz. or 2.44 tablespoons of 5.25% sodium hypochlorite per one gallon of water, 10-minute contact time) (WDNR, 2020a)

2. **Virkon Aquatic** (2% solution, 20-minute contact time) (WDNR, 2020a)

3. **Hot Water** (140°F) or **steam clean** (212°F) for 10 seconds contact time (Anderson et al., 2015; WDNR, 2020a)

Level 3 (High Risk)

Follow the boat decontamination procedures for the Low and Medium Risks **AND** perform a motor flush of 140°F (exiting temperature) for 2 minutes (WDNR, 2020a). If no hot water is available, flush the motor with clean water to expel any organisms. Boat accessories such as anchors, lines, paddles, etc. should also be decontaminated by either soaking in hot 140°F water, bleach, or Virkon Aquatic.

Hot water is the most environmentally friendly and cost-efficient means of decontaminating field gear, especially boats (Anderson et al., 2015). Most commercial car washes do not reach the temperatures necessary to fully decontaminate. However, using a high-pressure washer will aid in removing vegetation, mud, debris, and any organisms attached to the boat. Visual inspection and hand removal is responsible for reducing the transport of aquatic invasive vegetation by 88% (Rothlisberger et al., 2010).

Table 3. Boat decontamination options based on level of risk.

Level of Risk	Trailer	Boat hull	Livewell	Bilge pump	Holding tanks/ballast/ standing water	Motor
Low	Clean, Drain, Dry					
Medium	Clean, Drain, Dry **AND** disinfect with Bleach, Virkon Aquatic, or handheld steamer					
High	High pressure, 140°F temperature for 10 seconds per spot	High pressure, 140°F temperature for 10 seconds per spot	low pressure, 120°F temperature for 2 minutes	120°F exit temperature for 2 minutes	low pressure, 120°F temperature for 2 minutes	flush with water >100°F- 120°F exit temperature for 2 minutes. If no hot water available, flush motor with clean water to expel any organisms

Table 4. Boat decontamination options by treatment method and boat equipment.

Decontamination Method	Trailer	Boat hull	Livewell	Bilge pump	Holding tanks/ballast/ standing water	Boat Accessories (anchors, lines, ect.)	Motor Flush
Hot water	High pressure, 140°F temperature for 10 seconds per spot	High pressure, 140°F temperature for 10 seconds per spot	low pressure, 120°F temperature for 2 minutes	>100°F-120°F exit temperature for 2 minutes	low pressure, 120°F exit temperature for 2 minutes	Soak in water >120°F temperature for 30 minutes	flush with water >100°F- 120°F exit temperature for 2 minutes. If no hot water available, flush with available clean water to expel any organisms
Steam Cleaner	Steam 212°F, 30 seconds contact time						NA
Bleach	500 ppm solution, spray on or apply with sponge, 10 minute contact time. Rinse with potable water						NA
Virkon Aquatic	2:100 solution, spray on or apply with sponge, 20 minute contact time. Rinse with potable water						NA

C. Special Consideration Protocols

For these species and special situations, utilize the high-risk decontamination protocols for all equipment.

i. **Right-Handed Mudsnail:** Right-handed Mudsnail (RHM) (*Potamopyrgus antipodarum*) is a very small highly invasive snail. RHM reproduction in the United States is parthenogenic; each snail can make 250 clones in a year. The snail has an operculum. This and other adaptations make it resistant to digestive acids and can pass through the gut of most fish and avian predators unharmed. After visiting a site, first clean your waders and then treat them using either physical or chemical methods. Physical methods: **Freeze** gear for 6 hours is the best-known treatment to kill all snails. Freezer temperatures should be 26°F (-3 °C) OR **Soak** in hot water for 10 minutes. Hot water should be 120-140°F (46°C). This method is not recommended for Gore-Tex OR **Dry** gear before reuse. A drying time of at least 48 hours is recommended to remove all dampness. Gear should be completely dry for an additional 24 hours to limit snails. Check boots to ensure that they are completely

dry before reuse. Chemical methods: Soak wading gear in 2% **Virkon Aquatic Cleaner** for 20 minutes is the best chemical treatment to kill all snails. Soak gear in **Formula 409® Cleaner Degreaser Disinfectant** at full strength for 10 minutes is known to kill snails; however, these compounds may result in surface cracking of the rubber and loss of water repellency on wading gear. Some treatments are not effective against RHM. Bleach solutions and salt solutions have not been found to be effective.

ii. **Zebra mussels/quagga mussels:** 100% white vinegar should be used to dissolve the shells of zebra/quagga mussels on sampling nets and equipment (WDNR 2020). Boat decontamination after use in water bodies known to contain zebra and/or quagga mussels should follow the Uniform Minimum Protocols and Standards for Decontamination Programs for Dreissenid Mussels in the Western United States ("Uniform Minimum Protocols and Standards for Watercraft Inspection and Decontamination Programs for Dreissenid Mussels in the Western United States (UMPS III).", 2016).

iii. **Cyanobacteria:** Cyanobacteria can generally be decontaminated by submerging equipment and supplies in a 10% bleach solution for 30 minutes. Afterward, the bleach solution can be poured down the drain. However, akinetes or spore-like dormant/resting cells are able to survive harsh environmental conditions (i.e., cold temperature, nutrient deficiency, and low light availability). Akinetes are protected by a thickened cell wall/multilayered extracellular envelope; these resting cells can be resistant to cold, heat, desiccation, oxidative stress, and osmotic stress. Akinetes are often found hiding at the bottom of a waterbody or in the sediment. Unfortunately, there are no sound treatment methods available to decontaminate akinetes at this time. Proper disposal of any sediment samples or sediment trapped in gears to avoid germination of cyanobacteria akinetes is the best approach.

iv. **Pinelands Commission Decontamination Procedures for Pond Research**

Materials: Household bleach, 2 or 3 gallon pump sprayer for bleach solution, 2 or 3 gallon pump sprayer for tap water (DB SMITH Shoulder Strap Sprayer, Backpack Sprayer Type - 14U075|190216), plastic bin, deionized (DI) or distilled water.

Disinfection procedure:

1. Prepare 1:10 ratio solution of household bleach (5.25% sodium hypochlorite) and tap water (1 part bleach: 9 parts water = 10% solution) before leaving to sample sites. Solution will last about a week if stored in a dark place at the end of the day.
2. After leaving the pond, rinse off waders and any equipment (i.e., meter sticks) that comes into contact with pond water to remove any organic residues. The treads of boot soles usually require more attention.

3. After rinsing with water, stand in bin and rinse off waders and equipment with bleach solution and let solution remain on waders and equipment for at least 1 minute.
4. Rinse waders and equipment off with water again to remove residual bleach.
5. Before storing bleach containers, rinse containers and nozzles with water and air dry.

v. **Aquatic Macrophytes**: Aquatic macrophytes serve as critical habitat for fish and various aquatic biota, a source of oxygen for all organisms, a refuge for prey as well as a foraging area for predators, a buffer against erosion and sediment resuspension from both waves and shoreline inputs, and significantly contribute to overall lake primary productivity. Invasive aquatic macrophytes can be introduced through various pathway vectors of spread including seeds, fragmentation, transport/hitchhike through wildlife. aquarium dumps, and contaminated equipment.

Remove all aquatic macrophyte fragments on watercraft and gear. The shape of the fragments (coiled vs. uncoiled) when attached to gear or watercraft impacts the survivability during decontamination. Studies have shown that achieving 100% mortality among uncoiled fragments of Eurasian watermilfoil (*Myriophyllum spicatum*) compared to only 76% mortality for coiled fragments subjected to the same decontamination treatment. Coiled fragments can remain viable for longer periods of time after air drying versus single or uncoiled fragments (Bickel, 2015; Bruckerhoff et al., 2015). Most successful mortality rates for aquatic macrophytes occur during hot water immersion (pressure washing, steam, etc.) at 55 degrees Celsius (or 131°F) for 15 minutes (Figure 2) (Mohit et al., 2021).

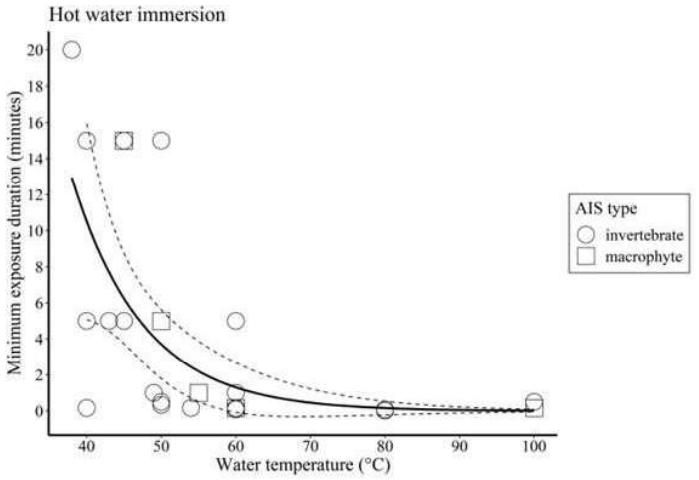

Figure 2. Water temperature and exposure duration resulting in 100% mortality. The regression line shows the relationship between water temperature and exposure duration among all AIS types collectively. Dashed lines represent the 95% confidence bands.

Figure 2. AIS decontamination curve for hot water immersion. (Mohit et al., 2021)

Note that each treatment identified below effectively decontaminates equipment and watercraft vessels; however, implementing more than one can improve efficacy and save time and effort (e.g. inspection, removal, steam cleaning/washing and drying). When conducting inspections be vigilant about the presence of species-specific seeds, turions or bulbils which may require a specific decontamination method as referenced in Table 4. Seeds, turions and bulbils may have differing survivability based on the decontamination method (Gottschalk, 2020; Patten, 1955; Smits et al., 1989). Weather and humidity can impact duration of drying rates and ultimately impact mortality rates based on each species survivability. Higher humidity levels can allow various AIS to tolerate air-drying for longer (Coughlan et al., 2018; Havel, 2011; McMahon et al., 1993; Ricciardi et al., 1995).

Table 4. Specific Aquatic Macrophyte Species Decontamination Recommendations.

Common Name	**Scientific Name**	**References**	**Recommended Decontamination Methods**
Brazilian Elodea	*Egeria densa*	(Crane et al., 2019)	Steam cleaning at 212°F
Curly-leaf pondweed	*Potamogeton crispus*	(Barr, 2013; Bruckerhoff et al., 2015; Crane et al., 2019)	Hot water at 140°F **OR** drying for more than >5 days. **Note:** If turions are present, steam cleaning or hot water methods as listed above are recommended. 5 days of drying was not effective and requires longer drying periods.
Curly Water Thyme	*Lagarosiphon major*	(Anderson et al., 2015; Crane et al., 2019)	Hot water at 140°F (Crane et al., 2019) **OR** Remove fragments, power wash, 4 – 9 days air-drying (Anderson et al., 2015).
Eurasian Watermilf oil (EWM)	*Myriophyllum spicatum*	(Barnes et al., 2013; Blumer et al., 2009; Bruckerhoff et al., 2015; Jerde et al., 2012; Patten, 1955)	Steam cleaning at 212°F **OR** Hot water at 140°F **OR** drying for more than >5 days. **Note:** If EWM seeds are present, drying method is not recommended as these seeds are viable for excessive periods of desiccation. EWM seeds likely experience increased viability after freezing (26°F).

Fanwort	*Cabomba caroliniana*	(Anderson et al., 2015)	Remove fragments, power wash, 4 – 9 days air-drying **OR** Steam cleaning at 212°F (more research is needed to confirm).
Floating Pennywort	*Hydrocotyle ranunculoides*	(Anderson et al., 2015)	Remove fragments, power wash, 4 – 9 days air-drying **OR** Steam cleaning at 212°F (more research is needed to confirm).
Hydrilla	*Hydrilla verticillata*	(Barnes et al., 2013; Basiouny et al., 1978; Kar & Choudhuri, 1982; Silveira et al., 2009)	Drying for >5 days **and** Steam cleaning at 212°F (more research is needed).
Parrot Feather	*Myriophyllum aquaticum*	(Anderson et al., 2015; Havel, 2011; Montalto & Drago, 2003)	Remove fragments, power wash at 131°F and >7 days for air-drying.
Starry Stonewort (Macroalgae)	*Nitellopsis obtusa*	(Glisson et al., 2020; Gottschalk, 2020; WDNR, 2020a, 2020b)	Steam cleaning at 212°F **OR** drying for >5 days **OR** Freezing (26°F). **Note**: According to study, bulbil desiccation was analyzed via bulbil germination. Drying for >5 days was effective.
Yellow Floating Heart (YFH)	*Nymphoides peltata*	(Smits et al., 1989)	Steam cleaning at 212°F is recommended, however more research is needed on efficacy of treatment method. **Note:** Drying for 5 days was not effective and may need a longer drying period (>5) as YFH seeds show high tolerance to desiccation.

Although limited research has been conducted on aquatic macrophyte decontamination protocol effectiveness, recent studies have shown a combination of power washing or steam cleaning (temperatures >131°F) along with "clean, drain, dry" is the most successful (Table 4). Future research should include the determination of survival and viability by more than one means to ascertain the sensitivity and specificity of the chosen criteria (Mohit et al., 2021).

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Appendices

Appendix A: Definitions

Aquatic Invasive Species - invasive species that spend all or part of their life cycle in aquatic ecosystems.

Decontamination – the process of removing invasive species and propagules (e.g., seeds, eggs, tubers, turions), or materials that may contain or transmit invasive species and/or their propagules.

Detergents – surfactant or a mixture of surfactants with cleansing properties in dilute solutions.

Disinfection- a method of decontamination that destroys or kills all forms of an invasive species that may be present, whether the presence is known or not.

EDDMapS- a web-based mapping system for documenting invasive species and pest distribution. Eddmaps.org

Exotic- not native to the place where it was found.

Indigenous – occurring naturally in a particular region or environment.

Infestations – the state of being invaded or overrun by pests or parasites.

Invasive species - a non-native species that has been introduced into a habitat and geographical area outside of its natural geographical range and that can reproduce prolifically and spread with little or no natural control, thereby displacing native biological diversity and threatening the integrity of natural ecosystems.

Monitor or monitoring - collection of information over time, generally on a sampling basis by measuring change in the value of one or more parameters, to determine the extent to which the implementation of a project plan is resulting in progress toward one or more of the plan's objectives.

Nonindigenous - not growing, living or occurring naturally in a particular region or environment.

Nonindigenous Aquatic Species Alert System - this site has been established as a central repository for accurate and spatially referenced biogeographic accounts of nonindigenous aquatic species. The program provides scientific reports, online/real-time queries, spatial data sets, regional contact lists, and general information. The data is made available for use by biologists, interagency groups, and the public. The geographical coverage is the United States. NAS.ER.USGS.Gov

Native - with reference to a species of plant, animal, or other living organism, indigenous and occurring naturally within a region, either evolving there or arriving and becoming established without human assistance.

Naturalized - with reference to a species of plant, animal, or other living organism that has established a presence in an area where it is not an indigenous species.

New Jersey Invasive Species Strike Team (NJISST) - created in 2011, a non-profit organization working to protect our state's natural heritage and economy from the threats posed by invasive species. The NJISST utilizes public and private land stewards to search for emerging invasive species, map locations where populations are detected and eradicate the invasive species that are found.

Nonnative - with reference to a species of plant, animal or other living organism that is not indigenous to a region.

Nuisance - when a native or non-native species proliferates without natural controls and negatively impacts the ecosystem and human-dependent activities such as boating, fishing, farming, swimming and other similar activities.

Personal Equipment – waders, boots, raingear, diving equipment, PFDs, and other similar items.

Sampling Equipment - Sampling equipment can include, but is not limited to nets, buckets, sieves, poles, sensors, water samplers and other similar equipment used to collect samples.

Turion - A bud that is produced by an aquatic plant as a means of overwintering or surviving extreme conditions. The bud is capable of growing into a complete plant when favorable conditions return.

Waterbody – any spring, river, stream, pond, lake, impoundment, wetland, coastal embayment or ocean.

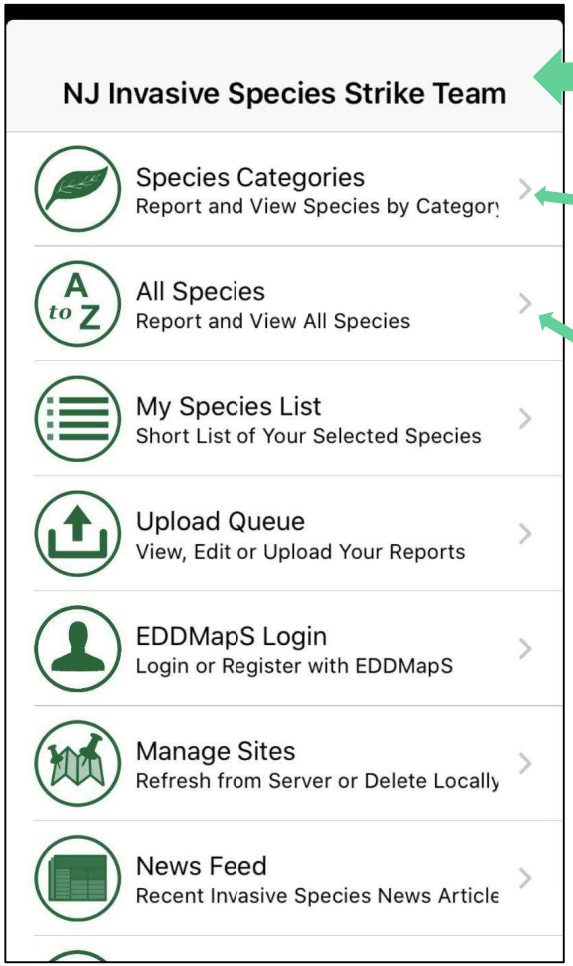
Wild - with reference to a species of plant, animal, or other living organism, that is indigenous or nonindigenous and is occurring naturally within a region, either evolving there or arriving and becoming established without human assistance.

Appendix B: FoHVOS NJ Strike Team App / EDDMapS Reporting Tutorial

FoHVOS
NJ Strike Team
Smartphone App

FoHVOS

FRIENDS OF HOPEWELL VALLEY OPEN SPACE



FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE

Upon opening the app, you will find the "General Menu"

There are two ways to view and report an invasive species:

By "Species Categories"

There are various types of invasive species. While reporting and do not know the species name, please narrow the species down by using the broad categories. Categories are generic enough for the non-botanist to choose from and then narrow down to species *(e.g. aquatic, shrub, grass, wildlife).*

OR

By "All Species"

If you know the name of the species you would like to report, please use "All Species" Option. Here, you can select using either the Scientific or Common Name and report directly. You can toggle between name types by clicking on the word in the top right.

Species Categories

Select a category to view or to help identify and report a possible invasive sighting.

All Species

FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE

Colors to the left of the images indicate the species' stage of invasion.

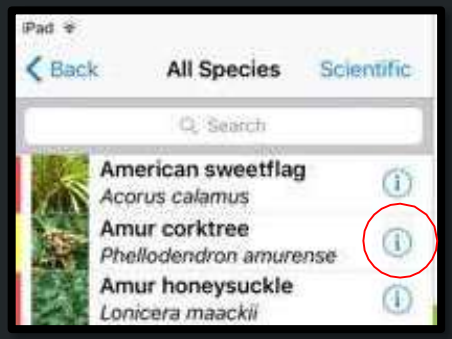
- Stage 0-1
- Stage 2
- Stage 3 or Widespread

Search or view directly for a species using either the common name or scientific name by typing in the 'Search' bar or toggling between listing types in the top right.

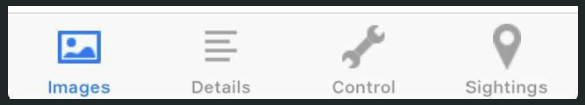
To view more information about a species….

FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE

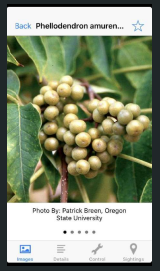
1. Click on the blue “i” icon.

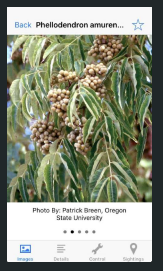


2. The screen will show an image of the species with the options below.

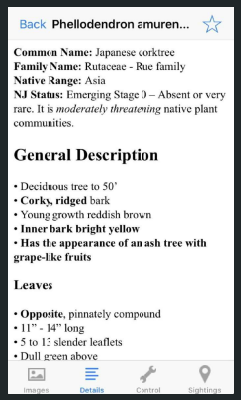


3. If available, the “Images” option will display pictures of the species such as fruits, flowers, berries, leaves, and the overall species.

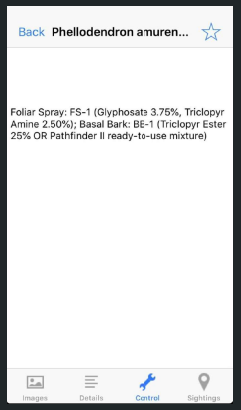




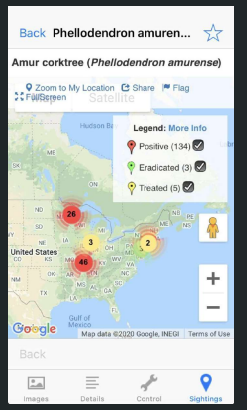
4. The “Details” option will provide more in-depth information about the species such as the status, habitat preference and identification tips.



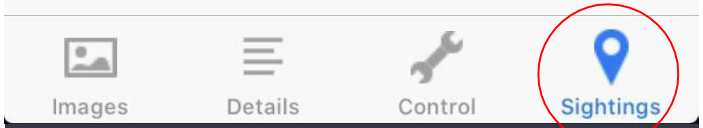
5. The “Control” option tells how you can help to eliminate or contain the species.



6. The “Sightings” option will show areas where it has been spotted and reported throughout the U.S. along with sighting statuses.

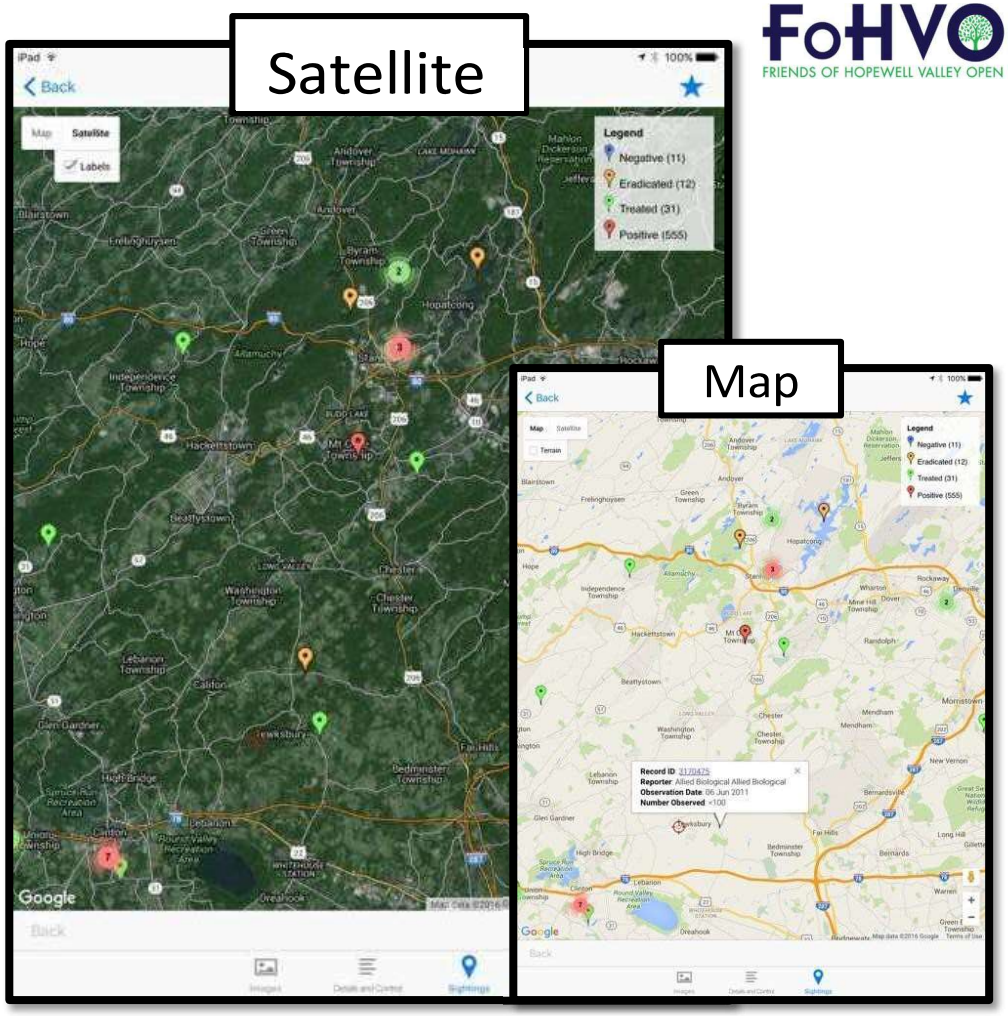


Sightings



The Sightings options will display a Google map of where the species has been reported. The map can be viewed in either satellite or standard map view. The color of the pinpoints indicate the status of the report.

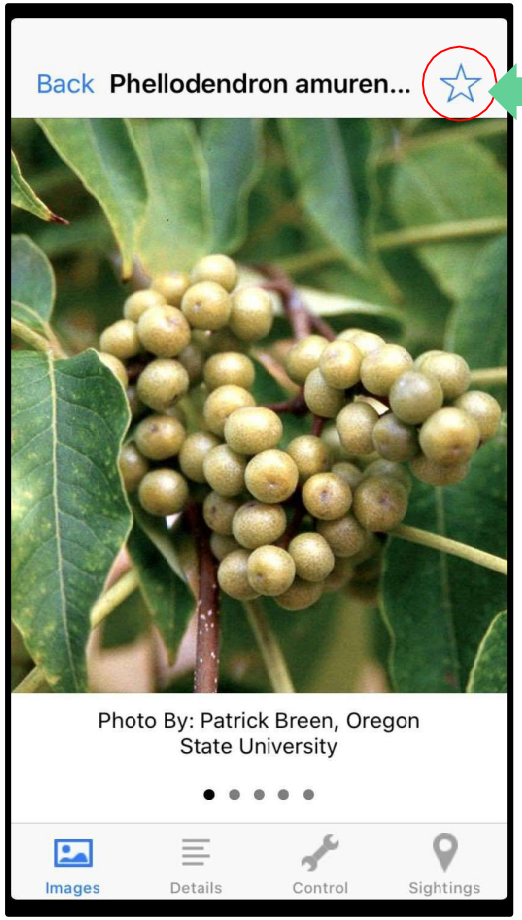
- *Negative*
  Subject was not present during a past survey.
- *Eradicated*
  Subject was present was present during a past survey, but has since been eliminated.
- *Treated*
  Subject was not present during a past survey, but control efforts have been initiated.
- *Positive*



FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE

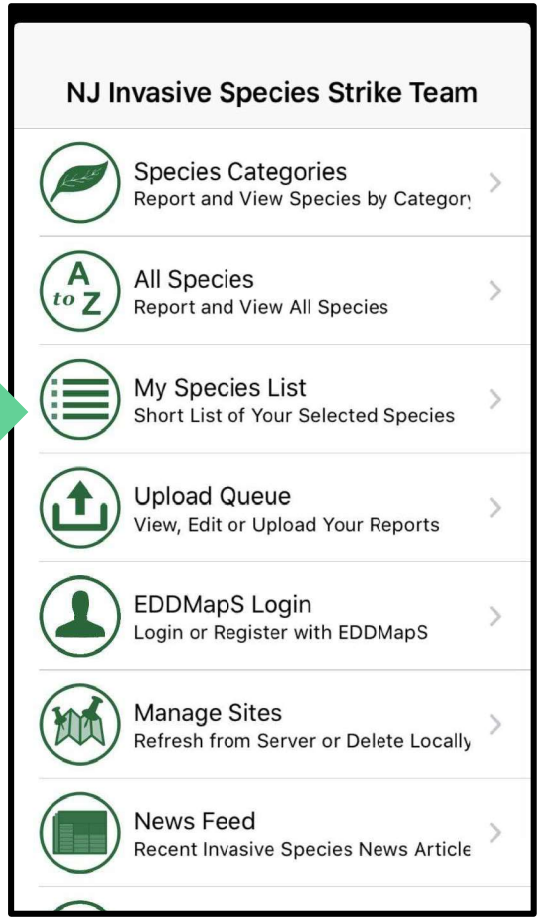
My Species List

FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE



When viewing a species, you can add species to your list for easier reporting later on. Simply click the star in the top right corner. When the star is filled in blue, it is on your list!

You can access this list later from the “General Menu” under “My Species List”



Reporting a Sighting

FoHVOS
FRIENDS OF HOPEWELL VALLEY OPEN SPACE

Fast and Simple!

1. Take picture
2. Provide Population Size
   1, 2-10, 11-100, 101-1000, >1000
3. NJ Strike Team Staff will verify