Best Management Practices for Boat, Gear and Equipment Decontamination

State of Wisconsin
Department of Natural Resources

Bureau of Water Quality
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Contents

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BACKGROUND

This is a **working document** meant to outline recommended best management practices (BMPs) for the decontamination of boats, equipment, and gear moved between waters. It is supplemental to Manual Code #9183.1 Boat, Gear and Equipment Decontamination and Disinfection Protocol, which requires all Wisconsin Department of Natural Resources employees, agents and service providers, and some permitees to take steps to decontaminate boats, gear and equipment when transporting equipment. Members of the general public who use boats and/or participate in water recreation are required to follow the decontamination procedures in s. NR 40.02(44). Following the disinfection BMPs listed in this document is not required for members of the general public participating in recreational boating and other water activities.

These BMPs are the current best procedures to decontaminate gear to be in compliance with Manual Code #9183.1. They represent the most stringent actions water users can take to ensure AIS are not being moved by their activities, and **will be periodically updated** to reflect the latest scientific findings for decontamination. The guidelines outlined in this document cover many gear types, but do not cover all gear types. Boats, gear, and equipment not expressly mentioned in this document that come in contact with surface waters are still subject to Manual Code #9183.1.

DEFINITIONS

"Agent" a department or agency of this or another state, federal agency, county, town, corporation or individual that has been expressly delegated by statute, rule or written contract to act under full or partial authority of the department.

"Aquatic invasive species" has the meaning given in s. NR 40.02 (3m), Wis. Adm. Code, that aquatic invasive species are any invasive species that dwells in water or wetlands.

"Barrier" is a natural or human made structure which does not allow the migration of aquatic organisms up to the 100 year event. Examples include dams or waterfalls. Dams with locks are excluded from this definition as they allow for migration.

"Decontamination" is the process of removing invasive species or materials that may contain or transmit invasive species.

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

"Invasive species" has the meaning given it in s. 23.22(1)(c), Wis. Stats., and s. NR 40.02 (24), Wis. Adm. Code, where "invasive species" means nonnative species including hybrids, cultivars, sub specific taxa, and genetically modified variants whose introduction causes or is likely to cause economic or environmental harm or harm to human health, and includes individual specimens, eggs, larvae, seeds, propagules and any other viable life-stages of such species. For "invasive species" fish, s. NR 40.04 (12a), Wis. Adm. Code, includes all nonnative species, but excludes established nonnative fish species.

"Locks" a device used for raising and lowering boats, ships, and other watercraft between stretches of water or different levels on river and canal waterways.

"Manual code" will refer to Manual Code #9183.1 Boat and Gear Disinfection Protocol for the purpose of this document.

"Service provider" includes contractors, volunteers, intern, any non-DNR employee that requires access to networks, Information systems, data or facilities.

"Waterbody" means any spring, stream, pond, lake, or wetland.

GENERAL PRACTICES

To slow the spread of invasive species, it is best to take AIS into consideration during all stages of field work, including planning, while fieldwork is in progress, and cleanup. The following are suggestions to assist during each work stage. If followed properly, they will significantly reduce the possibility of transporting AIS on equipment and gear.

Before Heading to the Field

- Be aware of infestations in your management area. The Where to find aquatic invasive species (https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=126471317) document has been created to assist in finding where species that have been documented and verified across the state.
- If a high percentage of work is done in waters with invasive species, consider dedicating certain gear to be used only in those waters.
- Depending on the type of work you are doing, it may be possible to work with lake volunteers and use their boats to collect samples. If the volunteer's boat is staying on the water body, then the department's equipment will be the only items that need to be disinfected.
- If working on multiple water bodies, organize sampling so work goes in order of infestation magnitude, with least infested waters being monitored first and most infested waters last.
- Arrange sampling plans to progress from the least to the most likely to be contaminated areas when working within the same water body. When working on different reaches of the same stream, plan on decontaminating whenever equipment crosses a barrier while going upstream.
- Consider purchase of wading gear and boots with the fewest places for organisms and debris to become attached. One-piece systems with full rubber material and open cleat soles are recommended to reduce likelihood of AIS spread. Multi-piece wading systems with fabrics, detachable boots and felt soles are more likely to transport AIS. Mud/rock guards are recommended for all stocking-foot waders to minimize contamination on inside surfaces.

While on a Waterbody

- Keep an eye out for any invasive species that may not have been previously recorded but may get on your gear if present. Adjust decontamination plans and follow Wisconsin's Rapid Response Framework (https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=126471653) when new occurrences are observed.
- Sample from upstream to downstream in a watershed or from areas of less weed growth to dense weed growth.
- Minimize wading and avoid running boats into sediment or through infested areas when possible.
- Consider using bank sampling poles instead of wading.
- Reduce the amount of plants, sediment, or organisms that are removed from the water into boats or sampling gear.
- Get in the habit of regularly inspecting and cleaning gear while working.

After Fieldwork on Water Body is Complete

- Fully inspect equipment and hand-remove any visible aquatic or terrestrial organisms present. This step can reduce the amount of macrophytes on a boat by 88% [1].

[1] Rothlisberger, J.D., W.L. Chadderton, J. McNulty, and D.M. Lodge. 2010. "Aquatic Invasive Species Transport via Trailered Boats: What is Being Moved, Who is Moving It, and What Can Be Done". *Fisheries* 35(3):121-132.

- Only use pressure washing if it's used in conjunction with hot water or on the site where work took place. Otherwise it can aid in the spread of AIS since it removes organisms, but does not kill them.
- Whenever possible, scrub equipment with a stiff-bristled brush and/or wash with soapy water. This simple step will aid in the removal of small organisms and seeds, as well as remove organic materials that make disinfection less effective. Scrubbing could damage the anti-fouling paint/coating of some boat hulls so check manufacturers' recommendations.
- When brushing fabric, be careful to brush with the nap, as brushing against the nap could cause small seeds to become more imbedded[2]. Brushing should be followed by a rinse with clean water.
- Do not clean equipment or gear in or near streams – it may promote the spread of invasive species downstream. An exception can be made for cleaning mud and debris off of gear before exiting the same waterbody that the mud and debris came from.
- The manual code only requires one disinfection method; however, multiple disinfection methods can be used to treat for a wider range of species.
- When cleaning equipment, make sure to keep wind to your back to prevent being sprayed with disinfectant. Any gear and clothing that needs cleaning should be taken off before applying a disinfectant spray to decontaminate.

DISINFECTANT SPECIFIC PRACTICES

While simple decontamination methods, such as hand removal, can reduce the majority of AIS found on gear and equipment, additional disinfection methods are still required to get rid of any elements that may not be seen. The manual code has been developed with this in mind and gives employees a range of effective methods for disinfecting equipment, as well as the ability to choose which options are practical for specific situations. The following section will give more detail on each disinfection option outlined in the manual code. For information on the effectiveness of each method on specific species, see Appendix A.

Steam Cleaning

- Heated water is effective in killing a wide range of organisms and fish pathogens.
- Steam cleaners can work well in small spaces, and on items such as small boat hulls, clothing and heavy equipment. To be the most effective, all sides of equipment being treated should be sprayed, as well as the inside of equipment.
- When setting something on the ground to steam clean, make sure to steam the ground before setting the equipment down.
- Be careful when steaming over items held together with adhesives, since high temperatures can melt bonds. Inflatable PFDs can also be melted by the use of steam.
- Using quick strokes instead of lingering in one place with steam cleaner will decrease the likelihood of causing damage to equipment.
- If using a low pressure steam cleaner, steam clean in an enclosed area to ensure proper contact with equipment.
- Orange cones should be used to mark off areas where steaming is taking place.
- Use clean water (i.e. municipal, bottled, well, etc.) when steam cleaning to prevent clogging of steam cleaners.

[2] DiVittorio, J., M. Grodowitz, J. Snow. 2010. Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species [2010 Edition]. U.S. Department of the Interior Bureau of Reclamation. Technical Memorandom No. 86-68220-07-05

- Scale build up on coils within steamers can cause internal pressure to increase, decreasing the efficiency of the unit. It is possible to add a pressure gage to larger steamer units. When unit pressures begin to increase, run a descaler through the unit to get rid of buildup. Softened water can also be used to decrease the likelihood of scale buildup.
- When you have an option of nozzle types, make sure you pick one that is suited to the surface being steamed and that will ensure the most contact time.
- All employees who handle steam cleaners shall wear heat resistant gloves. Depending on the type of steamer used, additional heat resistant personal protective equipment (PPE) may be required as well. Refer to the equipment's operation manual for suggested PPE. Be aware that scalding can occur if PPE is not used.

Hot Water

- Hot water works by physically removing AIS and killing some AIS. While some species are killed at lower temperatures, the manual code requires hot water to be at least 140 º F in order to kill the most species.
- Suggested contact time to kill the most species is 10 minutes.
- This method becomes more effective when applied with high pressure.
- It is important to note, most self-serve car washes do not get hot enough to meet the manual code's temperature requirement.
- To verify that the hot water spray is effectively heating the contact area, a non-contact infrared thermometer can be purchased at home supply stores for around $30. The distance of reading depends on the product purchased. Be sure to read the product label.
- Wear heat resistant gloves when cleaning equipment with hot water.

Drying

- Make sure equipment and gear is completely dried during drying period. Surfaces may appear dry while the interior is still wet. Waders, boots, wetsuits, fabric and wood may be difficult to dry thoroughly.
- If using shared equipment, it is recommended to keep a log of when things are used to ensure the minimum drying period has been met. If there is any possibility of another individual using the shared equipment before the five day drying period is reached, it is safer to disinfect via other means.

Virkon® Aquatic

- Virkon® Aquatic is a powder disinfectant in the peroxygen (hydrogen peroxide) family that is 99.9% biodegradable and breaks down to water and oxygen.
- Virkon® Aquatic is a good option for small equipment that has come into contact with sediments, and is the best method to use on equipment that has been used in areas that are known to have New Zealand mud snail (*Potamopytrgus antipodarum*, NZMS) populations or might be vulnerable to NZMS.
- Virkon® Aquatic should not be used on items made of wood. Since solution soaks into the wood, wood may carry residues that could be harmful to fish.
- Labeling for Virkon® Aquatic says it is not corrosive at the recommended dilution, however, solutions have been shown to cause degradation to gear and equipment when used repeatedly[3].

[3] *Stockton, K.A., and C.M. Moffitt. 2013. Disinfection of Three Wading Boot Surfaces Infested with New Zealand Mudsnails. North American Journal of Fisheries Management.* 33:529-538.

- Negative impacts of Virkon can be reduced by rinsing equipment with clean water (municipal, bottled, well, etc.) after disinfection is complete.
- The DNR has had Virkon® Aquatic tested for safety by Stantec. This study found that airborne concentrations of Virkon® Aquatic are well below regulatory limits, but that employees should always wear nitrile gloves, chemical splash goggles and/or face shields when mixing solutions. The final report on the safety of Virkon® Aquatic can be found here: http://intranet.dnr.state.wi.us/int/at/af/safety/PeshtigoVirkon_IH_Report.pdf
- The 2% Virkon Aquatic® solution should be disposed of by diluting to 1% or lower and dispose as per site regulations. Please speak with the facility or lab manager to learn more about site regulations.
- When disposed of down a drain, Virkon® Aquatic uses oxidative mechanisms and will use any leftover product to oxidize organic sludge in the drain.
- As is outlined in the manual code, test strips can be purchased to test the concentration of Virkon® Aquatic solutions.
- Virkon® Aquatic and Virkon® Aquatic test strips are available from Western Chemical.
- The Safety Data Sheet (SDS) for Virkon® Aquatic can be found in the Additional Resources section.

Chlorine solution

- A chlorine solution of 500ppm (1.22 fl. oz. or 2.44 Tablespoon of 5.25% sodium hypochlorite solution of household bleach per gallon water) is recommended by the manual code because it is effective at killing many AIS and fish diseases at this concentration. As is seen in Appendix A, however, it is not effective on spiny water flea resting eggs or NZMS. For this reason, it is recommended to follow chlorine solution treatments with an additional disinfection method. In the event of an emergency, treatment with chlorine solution on its own will be sufficient to meet manual code requirements.
- When using chlorine solutions, it is best to note that chlorine concentrations in solutions deteriorate with time, exposure to light and heat and on contact with air, metals, metallic ions and organic materials[4].
- There are no differences in disinfection abilities between solutions using tap water versus sterile water to mix the diluted chlorine solution, and the cleaning and disinfection abilities of diluted chlorine solutions are not impacted by the temperature of the water used[5].
- Chlorine solutions will begin to lose disinfecting properties after 24 hours, and the more diluted the chlorine solution, the quicker it will deteriorate. Based on this information, it is important to use 0.5% bleach solutions that are less than 24 hours old.
- When using household bleach as a chlorine source, it is also important to be aware of bleach shelf life. If stored at a temperature between 50 and 70 º F, household bleach retains its disinfection properties for about six months, after which, it degrades into salt and water at a rate of 20% each year[6]. If bleach is stored in locations with higher temperatures, such as a garage or the back of a truck, it will lose its disinfection properties at a quicker pace. Therefore, new bleach should be purchased for purposes of decontamination at the beginning of each field season. If using bleach year round for decontamination, new bleach should be purchased every 6 months.

[4] Clarkson, R.M., A.J. Moule, and H.M. Podlich. 2001. The Shelf-life of Sodium Hypochlorite Irrigating Solutions. *Australian Dental Journal* 46(4):269-276.
[5] Johnson, B.R., and N.A. Remeik. 1993. Effective Shelf-life of Prepared Sodium Hypochlorite Solution. *Journal of Endodontic* 19(1):40-43.
[6] Brylinski, M. 2003. Clorox@casupport.com Email to the Director of WCMC EHS Dated February 6, 2003. http://weill.cornell.edu/ehs/forms_and_resources/faq/biological_safety.html

- Make sure old bleach is disposed of properly and away from surface waters.
- Chlorine solutions may have corrosive effects on certain articles of equipment; however, these effects can be reduced by rinsing equipment with clean water after disinfection is complete.
- Chlorine solution in the form of household bleach (5.25% sodium hypochlorite) can be purchased from a grocery or convenience store. Granular chlorine (70% calcium hypochlorite) can be purchased from a pool supply company.
- When using a large quantity of chlorine solution to disinfect equipment, any excess solution must be inactivated with sodium thiosulfate prior to disposal. Enough sodium thiosulfate should be added to create an 800 ppm solution (3 grams per gallon of water) to neutralize the chlorine solution. Equipment that was treated with chlorine solution does not need to be sprayed with a sodium thiosulfate solution. Sodium thiosulfate is available through pool and chemical supply companies.
- Because different brands of bleach vary on the amount of sodium hypochlorite used, different amounts of bleach are needed to create a disinfection solution of 500ppm (Table 1).

Table 1 Converting household bleach to 500 parts per million of chlorine solution.

Sodium hypochlorite concentration (%)	Ounces chlorine solution per gallon water	Tbsp. chlorine solution per gallon water
5.0	1.28	2.56
5.25	1.22	2.44
8.25	0.78	1.55

Freezing

- Due to the threat that fish pathogens pose on our fisheries, and the ability of these pathogens to survive freezing temperatures, freezing is not being allowed on its own as a method for disinfection. It can, however be used as an extra step in tandem with other disinfection methods.
- Using chlorine solution in tandem with freezing will be sufficient to address most invasive species.

GEAR SPECIFIC PRACTICES

The following methods are provided to assist staff when disinfecting equipment and gear commonly used by department staff.

Nets

- Organic debris must be removed prior to disinfection. The most effective way to remove organic debris from nets is via of method of rinsing. Power washing is not required, but nets could be sprayed with a garden hose to remove debris.
- Nets may be steam cleaned, washed and dried thoroughly for five days, or washed and treated with a disinfection solution. Nets should be placed in the disinfection solution for the appropriate contact time for the solution being used. After rinsing, the nets can be used immediately, or hung to dry.

Personal Gear and Clothing

- To remove debris, scrub personal gear with a stiff bristle brush and rinse with clean water (municipal, bottled, well, etc.), and then refer to one of the disinfection options outlined in the manual code.

- An adhesive roller can be used on clothing to remove seeds and plant materials that could spread.
- Note that hot water and steam can damage gortex (rain gear) and melt seams of waders/boots.
- Heat resistant gloves, nitrile gloves, splash goggles, face shield, emergency eyewash stations and other personal protective equipment should be purchased at a department approved vendor outlined in state contracts.
- When using chlorine or Virkon® Aquatic solution on personal equipment, some individuals spray and place equipment in plastic bags to maintain a wet surface for the desired contact time, however, soaking has been found to be more effective with certain species/disinfectant combinations, and bagging sprayed equipment does not increase the efficacy of spray applications[7][8,][9].

Dip nets, measuring boards and other sampling gear

- There are several options for disinfecting smaller gear while in the field, but the first step is to always remove any organic material from sampling gear. Scrubbing gear with a stiff bristled brush is helpful.
- Dissolved oxygen probes and other sensitive electronic sampling gear may be damaged by the disinfection methods listed in the manual code and should only be rinsed with clean water (municipal, bottled, well, etc.). See manufacturer's instructions for further directions on the cleaning of sensitive gear such as sondes, hydrolabs and dataloggers.
- For other gear used in water choose one of the following options after scrubbing and rinsing:
    - Use steam, hot water, chlorine solution or Virkon® Aquatic solution to disinfect equipment.
    - If using Chlorine or Virkon® Aquatic solution, fill a tub with disinfection solution and place all equipment in the tub for the appropriate contact time. While soaking is preferred, it is also possible to spray gear with a disinfection solution so a wet surface is maintained for the appropriate contact time, however, this method is not as effective as soaking. The gear should be rinsed with clean water (i.e. municipal, bottled, well, etc.) after applying disinfection to maintain the integrity of the equipment.
    - Use a completely new set of gear for each waterbody during the workday and disinfect all gear at the end of the day using the options outlined in the manual code.

Boats, trailers, and live wells

- Remove organic material from boats, trailers, and live wells.
- Drain water from live wells, bilges and pumps.
- Scrub all exterior surfaces with a long-handled stiff bristled brush to remove sediments. Scrubbing could damage the anti-fouling paint/coating of some boat hulls so check manufacturers recommendations.
- The outside and inside of the boat, trailer, live wells, bilges and pumps should be steam cleaned or sprayed with the disinfection solution and left wet for the appropriate contact time.
- The inside of the live wells, bilges and pumps should be in contact with disinfection solution for the appropriate time as well.

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[7] Stockton, K.A., and C.M. Moffitt. 2013. Disinfection of Three Wading Boot Surfaces Infested with New Zealand Mudsnails. *North American Journal of Fisheries Management.* 33:529-538.

[8] DeStasio, B. 2016. Effectiveness of decontamination procedures for reducing the spread of small-bodied aquatic invertebrates [Draft]. *Project summary and update for DNR surface water grant # AIRD-106-15*

[9] Schreiner, L., K. Stepenuck, and L. Albright. 2016. *2% Virkon Aquatic Spray Applications to Wading Boots Infested with New Zealand Mudsnails [Poster Presentation]*. National Water Quality Monitoring Council 10th National Monitoring Conference. Tampa, FL.

- Due to the difficulty of ensuring appropriate contact times, steam cleaning is the preferred method for decontamination when possible.
- Run pumps so they take in the disinfection solution and make sure that the solution comes in contact with all parts of the pump and hose.
- The boat, trailer, live well, bilges and pumps should be rinsed with clean water after the appropriate contact time.
- Every effort should be made to keep the disinfection solution and rinse water out of surface waters. Pull the boat and trailer off the ramp and onto a level area where infiltration can occur and away from street drains to minimize potential runoff into surface waters.

Motors

- After removing from the water, scrub sediments off the exterior of the motor and then tip the motor down and allow water to drain from engine.
- Alternatively and especially for motors moored in water for several days or more, submerge the lower unit in a container of disinfectant and run the motor to ensure contact with all internal parts and allow for the appropriate contact time.
- Or, rig up a bucket with a thru hull fitting on the bottom and attach that fitting to a short (6-foot) piece of garden hose to lower unit muffs.
- Install a small valve between the hose and the muffs to control the flow of disinfectant. The pail of the disinfectant can then be set in the back of the boat and gravity fed into the lower unit.
- Next start the engine and run it long just enough to see the solution to run out the exhaust and the tell-tale.
- Never run the engine without disinfectant or fresh water flowing into the lower unit.
- Allow solution to remain in motor for the appropriate contact time
- A non-corrosive (Virkon® Aquatic) is recommended for use to protect the impeller.
- Rinse external surfaces with clean water after disinfection.
- Flush motor with fresh water for 2 minutes following instructions outlined in owner's manual.

Heavy Equipment

- Scrub equipment with a stiff bristled brush or spray with pressurized water to remove any sediment.
- Steam-cleaning or hot water (≥140) is an effective method for disinfecting heavy equipment.
- Steam-cleaning will not be effective if soil and other organic matter is present so be sure to scrub equipment with a stiff bristled brush.
- Decontamination should take place in areas where equipment is unloaded and loaded.
- Before transporting a piece of heavy equipment from one project site to the next, debris and soil must be cleaned off the tracks, tires and other portions of the piece(s) of equipment by hand with hand tools or with high pressurized water.
- If the heavy equipment comes in contact with stream or lake water while either working in the waterbody or using stream or lake water to clean equipment, a portable steamer should be brought to the site with clean water (i.e. municipal, bottled, well, etc.). The piece of equipment is then coated with steam/hot water after debris and mud are removed from the piece of equipment.

ADDITIONAL RESOURCES

Species of Concern

Invasive species of concern are outlined in Wis Adm, Code ch NR 40. Further information about NR 40 and the species outlined by the administrative code can be found through the DNR's website:

- http://dnr.wi.gov/topic/Invasives/classification.html

Additional information on AIS can be found at the following sites:

- Statewide Aquatic Invasive Species Efforts- http://dnr.wi.gov/lakes/invasives/
- WI DNR Invasive Species Resources-http://dnr.wi.gov/topic/invasives/
- UW Seagrant Invasive Species Fact Sheets- http://seagrant.wisc.edu/home/Default.aspx?tabid=639

Safety Data Sheets for Disinfection Chemicals used for Control of AIS:

- Sodium hypochlorite (4-6% solution): http://avogadro.chem.iastate.edu/MSDS/NaOCl-6pct.htm
- HTH Dry Chlorine Granular (70%): http://www.pollardwater.com/pdf/MSDS_Sheets/HTH%20Granular%20Chlorine%20MSDS.pdf
- Sodium thiosulfate (800 ppm): http://avogadro.chem.iastate.edu/MSDS/Na_thiosulfate-5H2O.htm
- Virkon-Aquatic Powder: http://www.syndel.com/Assets/file/Virkon_Aquatic_MSDS-2014-CAN.pdf
- Virkon-Aquatic Solution: http://www.cygnetenterprises.com/files/msds/VirkonsolutionMsds.pdf http://www.wchemical.com/downloads/dl/file/id/72/virkon_aquatic_msds.pdf

Nationally Accepted Disinfection Guidelines

Boat and trailer cleaning guidelines to prevent the spread of aquatic invasive species have been widely distributed to the public through a variety of publications, pamphlets, signs, etc. The distributed guidelines consist of a nationally-accepted set of prevention steps.

- Stop Aquatic Hitchhikers, ANS Task Force- http://protectyourwaters.net/

Protocols Recommended to the Public

While working in the field, DNR employees may come across members of the general public who are unaware of state disinfection protocols and BMPs. Members of the general public can be directed to the following resources to learn about their responsibilities while enjoying the state's water resources:

- Best Management Practices- http://dnr.wi.gov/topic/Invasives/bmp.html
- Boat Disinfection- http://dnr.wi.gov/lakes/invasives/BoatDisinfection.aspx
- Boat Transportation and Bait Laws- http://dnr.wi.gov/topic/Invasives/boat.html
- UW Sea Grant Institute- http://seagrant.wisc.edu/home/Topics/InvasiveSpecies.aspx
- ANS Task Force- http://www.anstaskforce.gov/Documents/AIS_Recreation_Guidelines_Final_8_29-_3.pdf

Hazards Communication SOP #12

http://intranet.dnr.state.wi.us/int/water/safety/SOP12_HazardsCommunications_1_15.pdf

REFERENCES

[1] Rothlisberger, J.D., W.L. Chadderton, J. McNulty, and D.M. Lodge. 2010. "Aquatic Invasive Species Transport via Trailered Boats: What is Being Moved, Who is Moving It, and What Can Be Done". *Fisheries* 35(3):121-132.

[2] DiVittorio, J., M. Grodowitz, J. Snow. 2010. Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species [2010 Edition]. U.S. Department of the Interior Bureau of Reclamation. Technical Memorandom No. 86-68220-07-05

[3] Stockton, K.A., and C.M. Moffitt. 2013. Disinfection of Three Wading Boot Surfaces Infested with New Zealand Mudsnails. *North American Journal of Fisheries Management.* 33:529-538.

[4] Clarkson, R.M., A.J. Moule, and H.M. Podlich. 2001. The Shelf-life of Sodium Hypochlorite Irrigating Solutions. *Australian Dental Journal* 46(4):269-276.

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[6] Brylinski, M. 2003. Clorox@casupport.com Email to the Director of WCMC EHS Dated February 6, 2003. http://weill.cornell.edu/ehs/forms_and_resources/faq/biological_safety.html

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[9]Schreiner, L., K. Stepenuck, and L. Albright. 2016. *2% Virkon Aquatic Spray Applications to Wading Boots Infested with New Zealand Mudsnails [Poster Presentation]*. National Water Quality Monitoring Council 10th National Monitoring Conference. Tampa, FL.

APPENDIX: LITERATURE REVIEW ON EFFICACY OF DISINFECTION METHODS BY SPECIES

The following appendix outlines the effectiveness of various disinfection methods on specific species, and includes citations for determinations. It is a working document that will be updated as new findings are made. If you have any new citations to add, please send suggestions to Maureen Ferry at maureen.ferry@wisconsin.gov.

Key:

☑= Effective- Eliminates spp when applied at rates outlined in the manual code.

⊗=Not Effective- Requiring higher rates and/or longer time periods than outlined in code to eliminate spp.

Ⓡ= Research Needed- No/insufficient sources or references found.

?= Lit Review Needed- Existence of sources or references not yet known.

Supporting references are enumerated in superscript. Symbols shown without references depict commonly shared knowledge wherein references or studies to validate may exist but have not yet been found.

Table 1 Efficacy of treatment methods for macrophytes and algae.

AIS	Steam Cleaning (212°F)	Hot Water (140°F, ≤10 min)	Drying (5 days)	Chlorine (500 ppm, ≤10 min)	Virkon (2:100 solution, ≤20 min)	Freezing (26°F, ≤24hrs†)
Curly Leaf Pondweed	Ⓡ	Ⓡ	☑[3,55]	Ⓡ	Ⓡ	⊗[52]
Curly Leaf Pondweed Turion	☑	☑[53]	⊗[3]	Ⓡ	Ⓡ	?
Eurasian Watermilfoil	☑	☑[15]	☑[12,55]	Ⓡ[57*]	Ⓡ	⊗[58*]
Eurasian Watermilfoil Seed	?	?	⊗[56]	?	?	?
Hydrilla	?	?	☑[55*,59,60*, 61]	?	?	?
Yellow Floating Heart	?	?	⊗[62*]	?	?	?
Starry Stonewort	?	?	?	?	?	?
Didymo	☑	☑[13,70]	☑[13,70]	☑[13,48,49,50 ,51]	☑[1]	☑[70]

*Additional details:

†Freezing times vary therefore specific citation should be consulted for appropriate time

[55] Hydrilla reported as "fasting drying plant" of 10 species tested; however, additional viability testing not done due to state transport laws
[57] Study looked at substantially lower concentrations.
[58] EWM seeds likely experience increased viability after freezing
[60]Study only tested twigs for up to 24hrs
[62]*N. peltata* seeds show high tolerance to desiccation

Table 2 Efficacy of treatment methods for invertebrates.

AIS	Steam Cleaning (212°F)	Hot Water (140°F, ≤10 min)	Drying (5 days)	Chlorine (500 ppm, ≤10 min)	Virkon (2:100 solution, ≤20 min)	Freezing (26°F, ≤24hrs†)
Faucet Snail	☑	☑[18*]	⊗[18,35]	⊗[18]	Ⓡ[18]	☑
New Zealand mud snail	☑	☑[4,65*]	☑[6*,66*]	⊗[21, 78*]	☑[10*, 76, 77]	☑[4,6*]
Quagga Mussel (Adults)	☑†	☑[7*,16*]	☑[14*,67]	☑	☑[9]	☑
Quagga Mussel (Veligers)	☑†	☑[4,17]	☑[69*, 79*]	☑	☑[9]	☑
Zebra Mussel (Adult)	☑†	☑[7*,8*,54,67]	☑[14*,25*,67]	☑[11,19,22]	Ⓡ	☑[25,27,67,68]
Zebra Mussel (Veligers)	☑†	☑[4]	Ⓡ	☑	Ⓡ	☑
Asian Clam	☑	☑[4,37,41,42,43]	⊗[4,44*,45]	⊗[36*,37*,38*,39*,40]	☑[23]	☑[46*]
Spiny Water Flea (Adult)	☑	☑[7*,47*]	☑[4]	☑[78]	☑[78]	☑[78]
Spiny Water Flea (Resting Eggs)	☑	☑[2*]	☑[2*]	⊗[2, 78*]	☑[78]	☑[2*]
Bloody Red Shrimp	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ	Ⓡ
Rusty Crayfish	?	?	?	?	?	?

*Additional details:
†Freezing times vary therefore specific citation should be consulted for appropriate time
[2] Frozen in water, not just in air; Hot water: 50°C (122°F) for >5 min (or 1 min at >50°C); Drying: ≥ 6 hr @ 17°C (63°F)
[6]Drying: Must ensure hot and dry environment (>84°F for 24hrs; ≥ 104°F (40°C) for >2 hours); Freezing: ≤ 27°F (-3°C) for 1 to 2 hours
[7] >43°C (110°F) for 5-10 min
[8] ≥ 140°F (60°C) for 13 to 10 seconds
[10] 2% solution (77 grams/1 gal water) for 15-20 min
[14]Adult *Dreissena* may survive overland transport for 3-5 days
[16] ≥ 140°F (60°C) for 5 to 10 seconds

[18] 50°C (122°F) for ≥ 1 min
[25]Must ensure hot and dry environment (>25 C for at least 2 days, or 5 days when humidity is high)
[36]Long exposure times (2-28 days) at low rates (0.2-40 mg/L)
[37]Short exposure time (30 min) at low rates (0, 5, 7.5, & 10 mg/L)
[37,41-43] Morality at 35-43°C (95-110°F)
[38]Long exposure time (14-28 days) to low rates (0.25-0.4 mg/L)
[39]Long exposure time (28-32 days) to low rates (0.2-1 mg/L)
[44]2 weeks need for mortality
[46] Lethal temperature reported at 0°C; freezing is a possible control method which warrants research
[47] >38°C (100°F) for 12 hrs
[65] >50°C (122°F) for 15 seconds
[66] Dry in full sunlight for ≥ 50 hrs
[69]Veligers experienced 100% mortality after 5 days under summer temperature conditions, and after approximately 27 days under autumn temperature conditions
[78]Bleach solution applied at a concentration of 400ppm
[79]Veligers survived for at least 7 days at approxamately 77°F
[†] Mentioned as effective in DiVittorio et al 2010, however no reference or study provided to validate claim

Table 3 Efficacy of treatment methods for viruses and diseases.

AIS	Steam Cleaning (212°F)	Hot Water (140°F, ≤10 min)	Drying (5 days)	Chlorine (500 ppm, ≤10 min)	Virkon (2:100 solution, ≤20 min)	Freezing (26°F, ≤24hrs[†])
Spring Viremia of Carp virus (SVCv)	☑	☑[29*,30,31*,64]	⊗[4*]	☑[28*,29*,30,64]	☑[28*]	⊗[29]
Largemouth Bass virus (LMBv)	®	®	®	☑[24*,28*]	☑[24,28*]	⊗[32]
Viral Hemorrhagic Septicemia virus (VHSv)	☑	☑[4,72,73*]	☑[4,72,74*]	☑[28*]	☑[28*,72]	☑[26,29,63*] ⊗[75]
Lymphosarcoma	®	®	®	☑	®	®
Whirling Disease	☑[33*]	⊗[20*,33*,71]	☑[5,33*]	☑[5*,20*,28*,33*]	®	☑[5*,33*]
Heterosporis	®	®	☑[34*]	☑[34*]	®	☑[34*]

*Additional details:
[†]Freezing times vary therefore specific citation should be consulted for appropriate time
[4] Drying of >28 days at 70°F needed
[5] Bleach 500 mg/L for >15min; Freezing at either -20°C or -80°C for 7 days or 2 months
[20]Heat @ 90°C for 10 min; Bleach at 1600 ppm for 24hrs, or 5000 ppm for 10 min
[24]10% bleach/water solution

[28] For SVC: Bleach = 500mg/L for 10 min; Virkon = 0.5-1% for 10 min, or 0.1% for 30 min
For VHS: Bleach = 200-500mg/L for 5 min; Virkon=0.5-1% for 10 min
For Whirling Disease: Bleach = 500 mg/L for 10-15 min; Virkon = 0.5-1% for 5 min
For Ranavirus (LMBv): Bleach = 500 mg/L for 15 min; Virkon = 0.5-1% for 1 min
[29]Hot water = 56°C for 30 min; Bleach =520 mg/L for 20 min
[31]Hot water 60°C (140°F) for 30 min = 99.9% mortality
[33]Freeze = 105 min @ -20°C; Desiccation = 60 min @ 19-21°C; Hot water (submerged in test tubes) = 5 min @ 75°C;
Bleach = 13ppm for >10 min, 131ppm for >1 min
[34]Freeze 24 hrs @ -4°F; Bleach=3cups/5 gal of water; Dry = > 24hrs
[63]Will not completely kill virus but will reduce infectivity or virus titres by >90%
[73]122°F (50°C) for 10 minutes, or 122°F (50°C) for 10 minutes
[74]study done on IHNH virus (similar to VHSv); dry gear for 4 days at 21°C (70°F)

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Twigs of Hydrilla verticillata were dried for periods of up to 24hrs and then analyzed for signs of life. Respiration continued for at least 20hrs.

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Hydrilla plants and propagules were dried for up to 7 days, and then replanted. 16hrs of drying resulted in no regeneration of plant fragments, while drying tubers 120 hrs and turions for 32 hrs resulted in new knew sprouting.

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N. peltata seeds show high tolerance to desiccation

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Studied the survivability of D.geminata to determine optimum growing conditions. Then tested the use of disinfection methods on D geminata being grown in optimum conditions. 100% Cell mortality occurred after20min with 40^{o}C water, but 60^{o}C for at least one minute is recommended for rapid treatment. Freezing is stated to be effective at killing D. geminata, however, this study does not list treatment times.A 1% chlorine solution was effective after 1 minute, and a 0.5% solution took 100 minutes to kill ~90% of specimens.

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In 2006, Arkush et al. found that commercial freezing (held at -20^{o}C for 2 weeks after blastfreezing at-40^{o}C) of in vitro VHSv shown a significant 99.9% reduction of the active virus post thaw.

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Virkon was found to be effective after trials of 1, 5, and 10 minute exposures to a 2% solution. Bleach and 409 were also tested. Bleach was found to be effective at 5, 10, and 20 minute exposures to a 400ppm solution.

77. Schreiner, L., K. Stepenuck, and L. Albright. 2016. *2% Virkon Aquatic Spray Applications to Wading Boots Infested with New Zealand Mudsnails [Poster Presentation]*. National Water Quality Monitoring Council 10th National Monitoring Conference. Tampa, FL.
Spray applications of 2% Virkon Aquatic solutions were applied to New Zealand mudsnails placed on waders. Waders where placed in plastic bags post spray application for exposure durations of 10 and 20 minutes. Mortality rates ranged from 87-93% for both exposure times. Study did not test the effectiveness of the spray and bag method when paired with pre-treatment cleaning methods required by the DNR's manual code.

78. DeStasio, B. 2016. Effectiveness of decontamination procedures for reducing the spread of small-bodied aquatic invertebrates [Draft]. *Project summary and update for DNR surface water grant #AIRD-106-15 Study analyzed the effectiveness of decontamination methods on spiny water flea (SWF) and New Zealand Mudsnail (NZMS). Methods tested included Virkon Aquatic, bleach, and freezing, with solutions tested via both spray and immersion application methods. Preliminary results show that immersion applications were more effective than spray applications for both disinfectants. Bleach decontamination was not effective on NZMS when applied at a concentration on 400ppm and exposure time of 25 min. 100% Mortality was seen in SWF immersed in bleach solution for 10 minutes and Virkon Aquatic for 15 min, though live embryos were still observed in brood sacs after both spray and immersion bleach treatments. Freezing was effective at killing all SWF after 2hrs of application.*

79. Snider, J.P., J. D. Moore, M.C. Volkoff, and S.N. Byron. 2014. Assessment of quagga mussel (*Dreissena bugensis*) veliger survival under thermal, temporal and emersion conditions simulating overland transport. *California Fish and Game* 100(4):640-651
Quagga mussel veligers were exposed to a gradient of water and air temperatures over a variation of time periods to determine tolerances. No veligers survived immersion for an hour at a temperature of 37°C, nor did any survive 20 hours of immersion at 35°C or greater. Overall, no veligers survived emersion or immersion and an air temperature of 35 or greater, however, veligers immersed in a small volume of water survived for at least 20 hours at 30°C and seven days at 25°C.