Best Management Practices for Boat, Gear and Equipment Decontamination

January 2020

Contents

INTRODUCTION

This document outlines the Best Management Practices associated with the Wisconsin Department of Natural Resources Boat, Gear and Equipment Decontamination and Disinfection Manual Code. This document should be reviewed by field staff during annual trainings. The research that supports these methods should be reviewed at least every 5 years to determine whether new research has improved our understanding of disinfection efficacy and also to evaluate effectiveness of these prevention methods when new species are observed in the state.

GENERAL PRACTICES

To slow the spread of aquatic invasive species (AIS), 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

- 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 of Wisconsin.
- If a high percentage of work is done in waters with invasive species, consider dedicating certain gear to be used only in those waters.
- If possible, work with local 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.
- When working on multiple water bodies, 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, decontaminate whenever equipment crosses a barrier while going upstream.
- Consider purchasing gear with the fewest places for organisms and debris to become attached (i.e. one-piece waders with full rubber material and open cleat soles).

During

- 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 [PDF] when new occurrences are observed.
- Reduce the amount of plants, sediment, or organisms that are removed from the water into boats or sampling gear.
- Regularly inspect and clean gear while working.

After

- Fully inspect equipment and remove any organisms present.

- 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.
- 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.
- When scrubbing fabric, be careful to brush with the nap (direction of fabric), as brushing against the nap could cause small seeds to become more imbedded. Scrubbing should be followed by a rinse with clean water.

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. These BMPs have been developed and give 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. For information on the effectiveness of each method on specific species, see Appendix A.

Steam

- Steam 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.
- When 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.) to prevent clogging of steam cleaners. Scale build-up on coils within steamers can cause internal pressure to increase, thereby 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 as that will ensure the most contact time.
- All people who handle steam cleaners should 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, hot water needs to be at least 140º F 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 that 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.
- If a boat wash is being used near surface water, no permit is required for discharges incidental to the normal operations of recreational vessels under the Clean Boating Act (CBA) of 2008. The DNR wastewater program has concluded that "discharges incidental to the normal operations" includes discharges from boat washing stations for invasive species. The CBA directed EPA to evaluate recreational vessel discharges, develop management practices for appropriate discharges, and promulgate performance standards for those management practices. It then directs the U.S. Coast Guard (USCG) to promote regulations for the use of the management practices developed by EPA and requires recreational boater compliance with such practices. To date, EPA has not developed management practices for appropriate discharge and promulgation performance standards for those management practices. Additionally, the wastewater program has not developed management practices for discharges incidental to the normal operations of recreational vessels.

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.

Chlorine

- 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.
- A chlorine solution of 500ppm (1.22 fl. oz. or 2.44 tablespoons of 5.25% sodium hypochlorite solution of household bleach per gallon of water) is effective at killing many AIS and fish diseases; however, it is not effective on spiny water flea resting eggs, NZMS, or Asian clam. For this reason, it is recommended to follow chlorine solution treatments with an additional disinfection method.
- 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

- 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
- Chlorine solutions also deteriorate with exposure to light, heat, contact with air, metals, metallic ions and organic materials[1].
- 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[2].
- After opening the original bottle of bleach, it may only be used for a maximum of two months. Write the date the container was opened on the original container. Bleach is best stored out of heat and sun.
- The words "Bleach Solution" and the date and time of dilution must be written on the container holding the diluted bleach.
- If stored at a temperature between 50 and 70 ° 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[3]. 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 faster 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.
- 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.
- 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. Sodium thiosulfate can be purchased at a pool supply company.
- While bleach is effective in killing most invasive species, it will not dissolve the shells of zebra/quagga veligers. Therefore, it is imperative to use 100% vinegar to dissolve the shells from sampling nets and gear that are used for zebra/quagga mussel sampling. This will also help avoid false positive results on the next sampling event. Bleach will not kill New Zealand mudsnails (Hosea and Finlayson, 2005).
- **Do not mix chlorine bleach with other chemicals (e.g., vinegar). After using bleach, rinse well with water and then apply other chemicals. Sodium thiosulfate should not be mixed with sodium nitrite, mercury, or iodine.**

[1] 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.
[2] Johnson, B.R., and N.A. Remeik. 1993. Effective Shelf-life of Prepared Sodium Hypochlorite Solution. *Journal of Endodontic* 19(1):40-43.
[3] 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

- Dispose of unused chlorine down a sink drain and flush with water.

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 should not be used on items made of wood. This solution soaks into the wood, so the wood could 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[4].
- Negative impacts of Virkon can be reduced by rinsing equipment with clean water (municipal, bottled, well, etc.) after disinfection is complete. Rinsing might not remove residual Virkon from equipment; therefore, Virkon should not be used on water quality equipment (i.e. Van Dorn samplers, chemistry probes, etc.)
- In 2014, Stantec tested the safety of Virkon® Aquatic for the WDNR. This study found that airborne concentrations of Virkon® Aquatic are well below regulatory limits. 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: https://dnrx.wisconsin.gov/swims/downloadDocument.do?id=137688847.
- Always apply alkyl C12-16 dimethylbenzyl ammonium chloride away from surface water or wetlands.
- The 2% Virkon Aquatic® solution should be disposed of by diluting to 1% or lower and discarded as per site regulations. Please speak with the facility or lab manager to learn more about site regulations.
- Dispose of unused Virkon Aquatic down a sink drain. When disposed of down a drain, Virkon® Aquatic uses oxidative mechanisms and will use any leftover product to oxidize organic sludge in the drain.
- Use Virkon Aquatic within 7 days post mixing because the product degrades. Test strips can be purchased to test the concentration of Virkon® Aquatic solutions.
- The word "Virkon" and the date of mixing must be written on the container holding the solution.
- Always refer to the manufacturer's directions for additional guidance. The Safety Data Sheet (SDS) for Virkon® Aquatic can be found in the Additional Resources section.

Non-Manual Code Approved disinfection methods that can be used in tandem with other manual code-approved methods.

Freezing

- **Not approved in manual code so must be used in tandem with another disinfection option.** Due to the threat that fish pathogens pose on our fisheries, and the ability of these pathogens to survive freezing temperatures, freezing is not 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.

Salt solution

- **Not approved in manual code so must be used in tandem with another disinfection option.**
- Table salt is an effective decontamination method for certain species and gear. Zebra and quagga mussel veligers are killed when gear is submersed in a salt solution (½ cup salt per gallon of

[4] *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.

water) for 30 minutes (Kilgour and Kepple, 1993).

- Dispose of unused salt solution down a sink drain and flush with water.

Vinegar

- **Not approved in manual code so must be used in tandem with another disinfection option.**
- Vinegar dissolves zebra/quagga veliger shells and should be used on nets or gear that are used to collect samples for zebra/quagga mussel analysis (e.g., eDNA or veliger samples) after sampling to prevent false positive detections in uninfected lakes.
- Apply by spraying or using a sponge so surface is thoroughly exposed to the vinegar. Contact time should be at least 10 minutes.
- Use white distilled vinegar without dilution.
- There have been no peer reviewed studies investigating vinegar as a disinfectant for invasive species; therefore, it must be used in tandem with another disinfection option.
  Store in a cool, dry area away from incompatible materials (e.g., bleach). Always refer to the manufacturer's directions for additional guidance.
- Shelf life is indefinite if stored properly. Small amounts of unused vinegar may be disposed of down a sink drain.
- Dispose of unused vinegar down a sink drain and flush with water.

Alkyl C12-16 Dimethylbenzyl Ammonium Chloride

- **Not approved in manual code so must be used in tandem with another disinfection option.**
- Alkyl C12-16 Dimethylbenzyl Ammonium Chloride is a quarternary compound available in many consumer products.
- 1,940 mg/L benzethonium chloride and a 50% solution of Formula 409each killed NZMS within 5 minutes. (Hosea and Findlayson 2006).
- A 10-minute submersion treatment of 100% Formula 409 causes 100% mortality in New Zealand mudsnails (Schisler *et al*., 2008).
- Formula 409 is available at most convenience stores. Contact appropriate supervisor for purchase information.
- Causes rubber toes on boots to crack but doesn't impact integrity of the boots (Hosea and Findlay 2005).
- Always apply alkyl C12-16 dimethylbenzyl ammonium chloride away from surface water or wetlands.
- Dispose of unused alkyl C12-16 dimethylbenzyl ammonium chloride down a sink drain and flush with water.
- Always refer to the manufacturer's directions for additional guidance.

GEAR SPECIFIC PRACTICES

The following methods are provided to assist with disinfecting equipment and gear commonly.

Personal Gear

- To remove debris, scrub personal gear with a stiff-bristled 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 used.
- 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[5,6,7].

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.
- Electronic sampling gear may be damaged by the disinfection methods listed above 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.
- 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.

Nets
- Organic debris must be removed prior to disinfection. The most effective way to remove organic debris from nets is via 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.

Boats
- 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 the manufacturer's recommendations.

---

[5] 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.

[6] 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*

[7] 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.

- 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.
- 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.
- <u>Every effort should be made to keep the disinfection solution and rinse water out of surface waters.</u> 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. The piece of equipment is then coated with steam/hot water after debris and mud are removed from the piece of equipment.

ADDITIONAL RESOURCES

Wisconsin 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

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

AUTHORS

Jeremy Bates, Sarah Fanning, Maureen Ferry, Brian Flynn, Amy Kretlow, Ashleigh McCord, Michelle Nault, Amanda Perdzock, Sabrina Scull, Erin Vennie-Vollrath

ACKNOWLEDGEMENTS

Contributors and Technical Advisors:

Customer and Employee Services

Safety and Risk Management

Marsha Present - *Environmental Health Specialist*

Land Division:

Wildlife

Daniel Hirchert - *Wildlife Biologist*

Parks

Craig Anderson – *Conservation Biologist*

Law Enforcement

Todd Schaller – *Chief Warden*

Sustainability and Business Support:

Environmental Analysis and Sustainability

Michael Halsted - *Energy Transportation and Environmental Analysis*

Science Services

Matt Mitro – *Fisheries Research*

Kelly Wagner – *Aquatic Plant Research*

Water Division:

Center for Limnology

Carol Warden – *AIS Specialist*

Fisheries

David Rowe - *Fisheries Supervisor*

Susan Marcquenski - *Fish Health*

Bob Hoodie – *Fisheries Supervisor*

Robert Fahey - *Operations Supervisor*

UW Extension

Tim Campbell – *AIS Communication Specialist*

Water Quality

Jeremy Bates - *AIS Monitoring and Rapid Response*

Maureen Ferry – *AIS Monitoring*

Sue Graham - *Lake Biologist*

Amy Kretlow – *AIS Monitoring and Rapid Response*

Michael Miller - *Water Quality*

Michelle Nault – *AIS Monitoring and Rapid Response*

Kevin Olson – *AIS Monitoring*

Amanda Perdzock – *AIS Rapid Response*

Tim Plude – *AIS Monitoring and Rapid Response*

Julia Riley - *Water Quality*

Michael Sorge - *Stream Biologist*

Erin Vennie-Vollrath – *AIS Rapid Response*

Bob Wakeman – *AIS Coordinator*

Sarah Fanning – *AIS Monitoring Specialist*

Ashleigh McCord – *Water Quality*

Sabrina Scull – *Water Quality*
Brian Flynn – *AIS Student Intern*
Watershed Management
Martin Griffin - *Waterway Science and Policy Leader*

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.

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.

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)	Drying (5 days)	Chlorine (500 ppm, 10 min)	Virkon (2:100 solution, 20 min)	Freezing (26°F†)
Curly Leaf Pondweed	☑ [79]	Ⓡ	☑ [3, 55]	Ⓡ	Ⓡ	⊗ [52]
Curly Leaf Pondweed Turion	☑ [53]	☑ [53]	⊗ [3]	Ⓡ	Ⓡ	Ⓡ
Eurasian Watermilfoil	☑ [15]	☑ [15]	☑ [3, 12, 55]	Ⓡ	Ⓡ	⊗ [58*]
Eurasian Watermilfoil Seed	Ⓡ	Ⓡ	⊗ [56*]	Ⓡ	Ⓡ	Ⓡ
Hydrilla	Ⓡ	Ⓡ	☑ [55*, 59, 60*, 61]	Ⓡ	Ⓡ	Ⓡ
Yellow Floating Heart	Ⓡ	Ⓡ	⊗ [62*]	Ⓡ	Ⓡ	Ⓡ
Starry Stonewort	☑ [84]	Ⓡ	☑ [81*, 84]	Ⓡ	Ⓡ	☑ [81*, 82, 84]
Didymo	☑ [48]	☑ [48]	☑ [13, 48]	☑ [13, 48, 49, 50*]	Ⓡ	☑ [48]
Brazilian Egeria	☑ [79]	Ⓡ	⊗ [55]	Ⓡ	Ⓡ	Ⓡ
Parrotfeather	Ⓡ	Ⓡ	⊗ [55]	Ⓡ	Ⓡ	Ⓡ

*Additional details:
[50] Median lethal concentration at 11.5°C is 5.7 mg/L. Would need one more temperature trial.
[55] Hydrilla reported as “fasting drying plant” of 10 species tested; however, additional viability testing not done due to state transport laws
[56] EWM seeds are viable to excessive periods of desiccation.
[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
[81]This study only looks at bulbil germination and not survivability of adult SSW.

Table 2 Efficacy of treatment methods for invertebrates.

AIS	Steam Cleaning (212°F)	Hot Water (140°F)	Drying (5 days)	Chlorine (500 ppm, 10 min)	Virkon (2:100 solution, 20 min)	Freezing (26°F†)
Faucet Snail	☑ 18*	☑ 18*	⊗ 35	⊗ 18	®	®
New Zealand mud snail	☑ 4, 65*	☑ 4, 65*	☑ 6*, 66*	⊗ 76*	☑ 9, 10*, 74, 76, 83	☑ 4, 6*
Quagga Mussel (Adults)	☑ 7*,16*	☑ 7*,16*	☑ 14*	®	☑ 9	®
Quagga Mussel (Veligers)	☑ 4, 17, 80*	☑ 4, 17	☑ 69*	®	☑ 9	®
Zebra Mussel (Adult)	☑ 7*, 8*, 25	☑ 7*, 8*, 25	☑ 14*, 25*, 27	☑ 22*	®	☑ 25, 27
Zebra Mussel (Veligers)	☑ 4, 80*	☑ 4	®	☑ 22*, 25	®	®
Asian Clam	☑ 4, 37, 78	☑ 4, 37	⊗ 4	⊗ 37*, 38*	®	®
Spiny Water Flea (Adult)	☑ 7*, 47*, 80*	☑ 7*, 47*	®	☑ 76, 83	☑ 76, 83	☑ 76, 83
Spiny Water Flea (Resting Eggs)	☑ 2*, 80*	☑ 2*	☑ 2*, 4	⊗ 2	®	⊗ 2*
Bloody Red Shrimp	®	☑ 83*	☑ 83*	☑ 83*	☑ 83*	®
Rusty Crayfish	☑ †	☑ †	®	®	®	®

*Additional details:
†Based on the understanding that these organisms are regularly steamed and boiled for consumption
[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)
[37]Short exposure time (30 min) at low rates (0, 5, 7.5, & 10 mg/L), Morality at 35-43°C (95-110°F)
[38]Long exposure time (14-28 days) to low rates (0.25-0.4 mg/L)
[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
[80] Mentioned as effective in DiVittorio et al 2010, however no reference or study provided to validate claim
[83]Only tested on Nylon Mesh and Canvas

Table 3 Efficacy of treatment methods for viruses and diseases.

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

<u>*Additional details:</u>
[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

REFERENCES

1. Root, S., and C. M. O'Reilly. 2012. Didymo control: increasing the effectiveness of decontamination strategies and reducing spread. *Fisheries* 37(10):440-448.

   Tested the effectiveness of liquid dish detergent, bleach, Virkon, and salt in killing Didymosphenia geminata. Study found that longer submersion times did not significantly increase mortality and that a one-minute submersion time would be sufficient for all treatments. Exact mortality rates are not listed for each treatment, however, a graph included in the paper shows the effectiveness for 1% Virkon solution at around 80% and the effectiveness for 2% bleach around 95%.

2. Branstrator, D. K., L. J. Shannon, M. E. Brown, and M. T. Kitson. 2013. Effects of chemical and physical conditions on hatching success of *Bythotrephes longimanus* resting eggs. *Limnology and Oceanography* 58(6):2171-2184.

   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). Chlorine solutions of 3400 mg L^{-1}had no impact on hatching success when exposed for up to 5min.

3. Bruckerhoff, L., J. Havel, and S. Knight. 2013. *Survival of Invasive Aquatic Plants After Air Exposure and Implication for Dispersal by Recreation Boats.* Unpublished data.

   Studied the impacts of drying on the viability of Eurasian watermilfoil and curly-leaf pondweeds. For Eurasian watermilfoil, single stems were viable for up to 24hrs while coiled strands were viable for up to 72hrs. For curly leaf pondweed, single stems were viable for 18hrs, and turions were still viable after 28 days of drying.

4. USFS Intermountain Region Technical Guidance. 2014. Preventing Spread of Aquatic Invasive Organisms Common to the Intermountain Region.
   http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5373422.pdf

   Outlines guidance to avoid spread of AIS during fire management and suppression activities. Recommends treatments for various species based on a literature review; references are outlined in this guidance.
   For quagga and zebra mussel adults and larva ≥140°F (60°C) hot water spray for 5 to. 10 seconds, or hot water immersion of ≥120°F (50°C) for 1 minute. Freeze at 0°C for adults. Dry for 5 days. 0.5% bleach solution rinse. 2% Virkon Aquatic solution for 10 minutes.

5. Hedrick, R. P., T. S. McDowell, K. Mukkatira, E. MacConnell, and B. Petri. 2008. Effects of freezing, drying, ultraviolet irradiation, chlorine, and quaternary ammonium treatments on the infectivity of myxospores of *Myxobolus cerebralis* for *Tubifex tubifex*. *Journal of Aquatic Animal Health* 20(2)116-125.

Conducted experiments on various disinfection methods to eliminate the infectivity of Myxobolus cerebralis in its myxospore stage. The disinfection methods varied in effectivity, and closely resembled environmental conditions the myxospores may encounter.

6. Richards, D.C., P. O'Connell, and D. Cazier Shinn. 2004. Simple control method to limit the spread of the New Zealand Mudsnail *Potamopyrgus antipodarum*. *North American Journal of Fisheries Management* 24(1)114-117.

   Experimented with different temperature and humidity parameters to observe effects on the mortality of seven size classes of Potamopyrgus antipodarum. Mortality increased with exposure time for all of the size classes. P. antipodarum did not survive experimental freezing or desiccation.

7. Beyer, J., P. Moy, and B. De Stasio. 2011. Acute upper thermal limits of three aquatic invasive invertebrates: hot water treatment to prevent upstream transport of invasive species. *Environmental Management* 47(1):67-76.

   Tested upper thermal tolerance limits of three aquatic invasive invertebrate species through hot water immersion of boat parts and gear. Mortality was assessed after a 20 minute recovery period following immersion.

8. Morse, J. T. 2009. Assessing the effects of application time and temperature on the efficacy of hot-water sprays to mitigate fouling by *Dreissena polymorpha* (zebra mussels Pallas). *Biofouling* 25(7):605-610.

   Assessed the lethality of various hot water spray treatments on D. polymorpha adults. Experimented with different temperatures of water applied over varying lengths of time. Sought the heat and time treatments which would ensure 100% mortality.

9. Stockton, K.A. 2011. Methods to assess, control, and manage risks for two invasive mollusks in fish hatcheries. M.S. Thesis, University of Idaho.

   Tested the efficacy of Virkon Aquatic solution in achieving 100% mortality for New Zealand mudsnails and quagga mussel adults and veligers. Also tested the risk of Virkon to steelhead trout and found that it was minimal, implying that Virkon residues on boats would not be problematic to fish.

10. 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(3):529-538.

    Tested efficacy of Virkon Aquatic spray or bath treatments in disinfecting different materials of wading boots from New Zealand mudsnails. They also tested to see if contamination of organic materials had any impact on disinfection success.

11. Cope, W. G. T. J. Newton, and C. M. Gatenby. 2003. Review of techniques to prevent introduction of zebra mussels (*Dreissena polymorpha*) during native mussel (Unionoidea) conservation activities. *Journal of Shellfish Research* 22(1):177-184.

    Literature review recommends use of chlorine solutions with concentrations ranging from 25-250 mg/L for disinfecting equipment and supplies.

12. Jerde, C. L., M. A. Barnes, E. K. DeBuysser, A. Noveroske, W. L. Chadderton, and D. M. Lodge. 2012. Eurasian Watermilfoil fitness loss and invasion potential following desiccation during simulated overland ransport. *Aquatic Invasions* 7(1):135-142.

Tested the viability of Eurasian watermilfoil fragments after desiccation treatment periods of 1, 3, 5, and 24 hours. Viability was measured by survival and root formation. All fragments died after desiccation periods of 3, 5, and 24 hours.

13. Kilroy, C. 2005. Tests to determine the effectiveness of methods for decontaminating materials that have been in contact with *Didymosphenia geminata*. Christchurch: National Institute of Water & Atmospheric Research Ltd. Client Report CHC 2005-005.

1% bleach solution resulted in 100% mortality after 30 seconds. Tested efficacy of several disinfection methods including drying, hot water, chlorine, and salt, nappy cleaner, household antiseptics, or detergent. Effectiveness was determined by examining intact chloroplasts, measuring chlorophyll a, and observing stained cells.

14. Ricciardi, A., R. Serrouya, and F. G. Whoriskey. 1995. Aerial exposure tolerance of zebra and quagga mussels (Bivalvia, Dressenidae) – implications for overland dispersal. *Canadian Journal of Fisheries and Aquatic Sciences* 52(3):470-477.

Examines the effects of ambient temperature (10, 28, and 30°C) and relative humidity (20, 50, and 95% RH) on the aerial exposure tolerance of adult zebra mussel (Dreissena: polymorpha) and quagga mussel (D. bugensis) collected from the St. Lawrence River.

15. Blumer, D. L., R. M. Newman, and F. K. Gleason. Can hot water be used to kill Eurasian Watermilfoil? *Journal of Aquatic Plant Management* 47:122-127.

Submerged at ≥60°C (140°F) for at 2-10 min. Submerged 20 cm fragments of Eurasian watermilfoil in hot water at six different temperatures between 45 and 80°C for 2, 5, and 10 minutes each. Discerned viability of fragments after 30 days based upon living tissue, biomass, and enyzme activity. All fragments killed at ≥60°C (140°F) regardless of exposure time.

16. Comeau, S., S. Rainville, W. Baldwin, E. Austin, S. Gerstenberger, C. Cross, and W.H. Wong. 2011. Susceptibility of quagga mussels (*Dreissena rostriformis bugensis*) to hot-water sprays as a means of watercraft decontamination. *Biofouling* 27(3): 267-274.

Determined quagga mussel viability after variable hot water spray temperature and time exposure treatments. Treatments at or above 60°C for five minutes ensured 100% mortality, as did 40°C for 40 seconds.

17. Craft, C. D., and C. A. Myrick. 2011. Evaluation of quagga mussel veliger thermal tolerance. Colorado Division of Wildlife Task Order # CSU1003.

Evaluated the heat tolerance of veliger quagga mussels under conditions realistic to boat travel between lakes. Used static water baths at increasing temperatures to assess the veligers' survivability.

18. Mitchell, A. J., and R. A. Cole. 2008. Survival of the faucet snail after chemical disinfection, pH extremes, and heated water bath treatments. *North American Journal of Fisheries Management* 28(5):1597-1600.

Exposed faucet snails to various chemicals, temperatures and pH levels. Virkon was only tested at a 0.16 and 0.21% solution. 100% of Snails exposed to a 1% solution of household bleach for 24hrs survived.

19. Harrington, D. K., J. E. VanBenschoten, J. N. Jensen, D. P. Lewis, and E. F. Neuhauser. 1997. Combined use of heat and oxidants for controlling adult zebra mussels. *Water Research* 31(11): 2783-2791.

Tested different parameters of heat and oxidants, chlorine and ozone, upon zebra mussels to determine their lethal thresholds. Their research informed recommendations for heat and oxide usage in controlling zebra mussels.

20. Wagner, E. J. 2002. Whirling disease prevention, control, management: a review. *American Fisheries Society* 29:217-225.

This is a literature review of different chemical and physical control methods of the parasite that causes whirling disease. Studies identified in this review indicate that 5,000 ppm chlorine for 10 min killed the intermediate spores that infect tubifex worms that lead to whirling disease in fish. 130-260 ppm chlorine was recommended in treatment of the direct spores that infect fish. Temperature is effective treatment at 75^{o}C for 10 minutes, but 70^{o}C for 100 minutes was not effective.

21. Hosea, R. C. and B. Finlayson. 2005. Controlling the spread of New Zealand Mud Snails on wading gear. State of California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005-02.

NZMS exposed to various dilutions of household bleach for 5 minutes. The only concentration to show an impact was undiluted bleach.

22. Sprecher, S. L., and K. D. Getsinger. 2000. Zebra mussel chemical control guide. U.S. Army Corps of Engineers – Engineer Research and Development Center. ERDC/EL TR-00-1.

U.S. Army Corps of Engineers report on various chemical control methods for zebra mussels.

23. Barbour, J. H., S. McMenamin, J. T. A. Dick, M. E. Alexander, and J. Caffrey. 2013. Biosecurity measures to reduce secondary spread of the invasive freshwater Asian clam, *Corbicula fluminea* (Müller, 1774).

Management of Biological Invasions 4(3):219-230. *C. fluminea of varying size were treated either with Virkon® Aquatic, common household bleach or salt at a variety of concentrations for a range of immersion times. Virkon® emerged as the most effective of the three treatment types and achieved 93.3% mortality when used at 2% for 5 minutes. There was no significant difference in mortality among clam sizes.*

24. Kipp, R. M., A. K. Bogdanoff, and A. Fusaro. 2014. Ranavirus. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. Revision Date: 8/17/2012. http://nas.er.usgs.gov/queries/GreatLakes/SpeciesInfo.asp?NoCache=5%2F6%2F2011+6%3A17%3A25+PM&SpeciesID=2657&State=&HUCNumber=DGreatLakes>

Description of Ranavirus, also known as Largemouth Bass virus. Includes its identification, life history and ecology, impacts, and management strategies.

25. Boelman, S. F., F. M. Neilson, E. A. Dardeau Jr., and T. Cross. 1997. Zebra mussel (*Dreissena polymorpha*) control handbook for facility operators, First Edition. US Army Corps of Engineers, Zebra Mussel Research Program. Miscellaneous Paper EL-97-1.

U.S. Army Corps of Engineers report on various control methods for zebra mussels, including chemical and physical treatments. Provided specific recommendations and explanations of methods' efficacy.

26. Batts, W. N., and J. R. Winton. 2012. Viral hemorrhagic septicemia. USGS Western Fisheries Research Center. http://afs-fhs.org/perch/resources/14069231582.2.7vhsv2014.pdf

27. McMahon, R. F., T. A. Ussery, and M. Clarke. 1993. Use of emersion as a zebra mussel control method. US Army Corps of Engineers Contract Report EL-93-1 http://el.erdc.usace.army.mil/elpubs/pdf/crel93-1.pdf

Zebra mussels were emersed in varying conditions of temperature and relative humidity (RH) to determine desiccation and freezing control methods. At near zero RH at 25 °C (77 °F), an exposure duration of at least 3 days would be required to kill 100 percent of adult mussels attached to a boat hull.

28. Yanong, R. P. E. and C. Erlacher-Reid. 2012. Biosecurity in Aquaculture, Part 1: An Overview. Southern Regional Aquaculture Center, SRAC Pub. No. 4707.

This publication provides an overview of major concepts in biosecurity for aquaculture and is not a scientific study. Based on research (Bowker, et al. 2011), recommends Chlorine 500 mg/L for 15 minutes or Virkon® Aquatic 0.5 to 1% for 10 minutes to disinfect Whirling disease virus, VHS, LMBv, and SVCv.

29. World Organization for Animal Health. 2012. Manual of Diagnostic Tests for Aquatic Animals. http://www.oie.int/international-standard-setting/aquatic-manual/access-online/

Direct quotes:
"The virus is inactivated at 56°C for 30 minutes, at pH 12 for 10 minutes and pH 3 for 2 hours (Ahne,1986)."
"The following disinfectants are also effective for inactivation… 540 mg litre–1 chlorine for 20 minutes, 200–250 ppm (parts per million… (Ahne, 1982; Ahne & Held, 1980; Kiryu et al., 2007)."
"The virus is most stable at lower temperatures, with little loss of titre for when stored for 1 month at –20°C, or for 6 months at –30 or –74°C (Ahne, 1976; Kinkelin & Le Berre, 1974)."
VHSv reference in the above source was quote from another study Arkush, et. Al 2006, this reference has been added.

30. Iowa State University: College of Veterinary Medicine. 2007. Spring Viremia of Carp. http://www.cfsph.iastate.edu/Factsheets/pdfs/spring_viremia_of_carp.pdf

Direct Quote:
"It can be inactivated with…chlorine (500 ppm)… SVCV can also be inactivated by heating to 60°C (140°F) for 30 minutes…" No contact time was given for the bleach solution.

31. Kiryu, I., T. Sakai, J. Kurita, and T. Iida. 2007. Virucidal effect of disinfectants on spring viremia of carp virus. *Fish Pathology* 42(2):111-113.

This study reviewed past literature and displayed the following results: using a Bleach concentration of 7.6ppm for a contact time of 20 min. resulted in 99-99.9% inactivation of SVCv and a concentration of 540 ppm for a 20 min. contact time resulted in >99.9% inactivation of SVCv. This paper also reveals that 45°C heat treatments for 10 min. have been found SVCv to be 99-99.9% inactivated, while 60°C heat treatments for 30 min. was recommended for *sterilization.*

32. Plumb, J. A. and D. Zilberg. 1999. Survival of largemouth bass iridovirus in frozen fish. *Journal of Aquatic Animal Health* 11:1, 94-96.

    This study found LMBv to be very stable when frozen at -10°C in fresh fish tissue. Infectious doses were still found after freezing for 155 days in fish tissue.

33. Wagner, E. J., M. Smith, R. Arndt, and D. W. Roberts. 2003. Physical and chemical effects on viability of the *Myxobolus cerebralis triactinomyxon. Diseases of Aquatic Organisms* 53(2):133-142.

    Various chemical and physical methods for destroying the triactinomyxon (TAM) stage of the myxozoan parasite Myxobolus cerebralis were tested at different exposure/doses. Freezing or drying for 1 h, Chlorine concentrations of 130 ppm for 10 min, immersion in 75oC water bath for 5 min all produced 0% viability of parasite which causes whirling disease. However at 58oC water bath for 5minutes, as much as 10% remain possibly viable.

34. DNR/ GLFC guidance. 2005. http://dnr.wi.gov/topic/fishing/documents/fishhealth/heterosporis_factsheet.pdf

    Direct Quote:
    "Immerse gear in a chlorine bleach solution for five minutes (3 cups of household bleach in 5 gallons of water). Freezing at -4 °F for 24 hours (home freezer) will also kill the spores….completely dry for a minimum of 24 hours for dessication to effectively kill the spores."

35. Wood, A. M., C. R. Haro, R. J. Haro, and G. J. Sandland. 2011. Effects of desiccation on two life stages of an invasive snail and its native cohabitant. *Hydrobiologia* 675:167-174.

    Compared the effects of desiccation on adults and egg viability on Faucet snails and a native snail. Results found desiccation for 7 days produced 73% mortality in faucet snail eggs, and only 62% mortality in adult faucet snails.

36. Ramsay, G. G., J. H. Tackett, and D. W. Morris. 1988. Effect of low-level continuous chlorination on *Corbicula fluminea. Environmental Toxicology and Chemistry* 7:855-856.

    The time required for continuous chlorination to produce 100% mortality in adult Corbicula fluminea was determined. The total residual chlorine concentrations maintained were lower than any previously tested: 0.05, 0.10 and 0.20 mg/L.

37. Mattice, J. S., R. B. McLean, and M. B. Burch. 1982. Evaluation of short-term exposure to heated water and chlorine for control of the Asiatic clam (*Corbicula fluminea*). Technical Report ORNL/TM-7808. Oak Ridge National Lab., TN (USA).

    Tested combined short-term treatments of hot water and chlorine on the mortality of Asian clams. Found that chlorine does not affect mortality, while hot water is effective.

38. Belanger, S. E., D. S. Cherry, J. L. Farris, K. G. Sappington, J. Cairns Jr. 1991. Sensitivity of the Asiatic clam to various biocidal control agents. *Journal – American Water Works Association* 83(10):79-87.

39. Doherty, F. G., J. L. Farris, D. S. Cherry, and J. Cairns Jr. 1986. Control of the freshwater fouling bivalve

Corbicula fluminea by halogenation. *Archives of Environmental Contamination and Toxicology* 15(5):535-542.

40. Chandler, J. H. and L. L. Marking. 1979. Toxicity of fishery chemicals to the Asiatic clam, *Corbicula manilensis*. *Progressive Fish-Culturist* 41:148-51.

    Tested concentrations of various chemicals on Asiatic clam. Clorine solutions derived from Calcium hypochlorite had a 96-hr LC_{50} of 1450mg/L.

41. Habel, M. L. 1970. Oxygen consumption, temperature tolerance, filtration rate of introduced Asiatic clam *Corbicula manilensis* from the Tennessee River. MS Thesis, Auburn University, Auburn, Alabama, 66 pp.

42. Coldiron, D. R. 1975. Some aspects of the biology of the exotic mollusk *Corbicula* (Bivalvia: Corhiculidae). MS Thesis, Texas Christian University, Fort Worth, Texas, 92 pp.

43. Cherry, D. S., J. H. Rodgers Jr., R. L. Graney, and J. Cairns Jr. 1980. Dynamics and control of the Asiatic clam in the New River, Virginia. Bulletin 123, Virginia Water Resources Research Center, Virginia Polytechnic Institute & State University, 72 pp.

44. McMahon, R. F. 1979. Tolerance of aerial exposure in the Asiatic freshwater clam *Corbicula fluminea* (Muller). In Proceedings, First International Corbicula Symposium, ed. by J. C. Britton, 22741, Texas Christian University Research Foundation.

45. Dudgcon, D. 1982. Aspects of the dessication tolerance of four species of benthic Mollusca from Plover Cove Reservoir, Hong Kong. *Veliger* 24:267-271.

46. Müller, O., and B. Baur. 2011. Survival of the invasive clam *Corbicula fluminea* (Müller) in response to winter water temperature. *Malacologia* 53(2):367-371.

    Studied the cold tolerance of C. fluminea in a laboratory setting, simulating winter water temperatures. Found that clam cold tolerance is higher than previously understood, which may allow the species to increase its range as streams continue to warm.

47. Garton, D. W., D. L. Berg, and R. J. Fletcher. 1990 Thermal tolerances of the predatory cladocerans *Bythotrephes cederstroemi* and *Leptodora kindti*: relationship to seasonal abundance in Western Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 47:731–738.

    Tested whether thermal tolerance is related to seasonal abundance (summer versus autumn) of introduced Bythotrephes cederstroemi (Spiny water flea) and the native Leptodora kindti. Observed that B. cederstroemi has a lower ability to acclimate at warmer [summer] temperatures.

48. Kilroy, C., A. Lagerstedt, A. Davey, and K. Robinson. 2007. Studies on the survivability of the invasive diatom *Didymosphenia geminata* under a range of environmental and chemical conditions. Biosecurity New Zealand NIWA Client Report: CHC2006-116. National Institute of Water and Atmospheric Research LTD. Christchurch, New Zealand.

    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°C water, but 60°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.

49. Jellyman, P. G, S. J. Clearwater, B. J. F. Biggs, N. Blair, D. C. Bremner, J. S. Clayton, A. Davey, M. R. Gretz, C. Hickey, and C. Kilroy. 2006. *Didymosphenia geminata* experimental control trials: Stage One (screening of biocides and stalk disruption agents) and Stage Two Phase One (biocide testing). Christchurch: National Institute of Water & Atmospheric Research Ltd.

    Tested various chemical treatments on viability of Didymosphenia geminata. Observed effects of adding the biocide to bins of river water containing mats of D. geminata, pebbles and cobble substrate, with aeration to simulate turbulence. Samples were later examined for viability in the laboratory using staining techniques.

50. Beeby, J. 2012. Water quality and survivability of *Didymosphenia geminata*. Colorado State University, Master's Thesis Dissertation.

    Colonized Didymo in the lab in an artifiicial stream, then exposed it to different water quality parameters to test survivability. Tested the impact of chlorine solutions at various doses. Chlorine had a significant effect on Didymo viability, but copper showed the greatest effect on Didymo. Tested the impact of chlorine solutions at the doses of 1.3, 2.5, 5.0, and 10 mg/L.

51. Jellyman, P. G., S. J. Clearwater, J. S. Clayton, C. Kilroy, C. W. Hickey, N. Blair, and B. J. F. Biggs. 2010. Rapid screening of multiple compounds for control of the invasive diatom *Didymosphenia geminata*. *Journal of Aquatic Plant Management* 48:63-71.

    Applied 10 different biocides to Didymo in toxicity trials in artificial stream channels. Determined biocides' efficacy through three replicate exposures. Chlorine significantly reduced Didymo viability but was the least effective of the top five biocides, with Gemex being most effective.

52. USDA-NRCS, 2009. Curly-leaf pondweed. The PLANTS Database Version 3.5. Baton Rouge, USA: National Plant Data Center. http://plants.usda.gov

    Minimum temp of -33°F; freezing unlikely to cause mortality.

53. Barr, T.C. III. 2013. Integrative control of curly leaf pondweed propagules employing benthic bottom barriers: physical, chemical and thermal approaches. University of California – Davis. Ph.D Dissertation.

    Study tested the pumping of heated water under bottom barriers to inhibit turion sprouting. Turions were exposed to treatments and then given recovery period. Those that did not sprout were believed to be unviable. Water of temperatures between 60-80°C (140-176°F) for 30 seconds was sufficient to inhibit growth.

54. Rajagopal, S., G. Van Der Velde, M. Van Der Gaag, and H. A. Jenner. 2005. Factors influencing the upper temperature tolerances of three mussel species in a brackish water canal: size, season and laboratory protocols.

    Biofouling 21:87-97. *Led thermal tolerance experiments to determine if the following factors have any effect on mussel mortality: mussel size, season, nutritional status, acclimation temperature, acclimation salinity.*

55. Barnes, M. A., C. L. Jerde, D. Keller, W. L. Chadderton, J. G. Howeth, D. M. Lodge. 2013. Viability of

aquatic plant fragments following desiccation. *Invasive Plant Science and Management* 6(2):320-325.

Hydrilla reported as "fastest drying plant" of 10 species tested; however, additional viability testing not done due to state transport laws

56. Standifer, N. E. and J. D. Madsen. 1997. The effect of drying period on the germination of Eurasian watermilfoil seeds. *Journal of Aquatic Plant Management* 35:35-36.

EWM seeds are viable to excessive periods of desiccation

57. Watkins, C. H., and R. S. Hammerschlag. 1984. The toxicity of chlorine to a common vascular aquatic plant. *Water Research* 18(8):1037-1043.

Studied impact of low chlorine concentrations (0.02, 0.05,0.1, 0.3,0.5, and 1.0mgL^{-1}) on Eurasian watermilfoil growth over 96-hr period. Rate reductions ranged from 16.2% for plants grown with chlorine concentrations of 0.05 mgL^{-1} to 88.2% reduction in growth in a chlorine concentration of 1.0 mgL^{-1}.

58. Patten, B.C. Jr. 1955. Germination of the seed of *Myriophyllum spicatum* L. *Bulletin of the Torrey Botanical Club* 82(1):50-56.

EWM seeds likely experience increased viability after freezing

59. Silveira, M. J., S. M. Thomaz, P. R. Mormul, and F. P. Camacho. 2009. Effects of desiccation and sediment type on early regeneration of plant fragments of three species of aquatic macrophytes. *International Review of Hydrobiology* 94(2):169-178

Fragments of Hydrilla was left on trays of sand and clay for 1-4 days inside a greenhouse. Samples left in clay were still viable after 1-4 days of desiccation, however, not sprouts were produced in the sand treatment after one day of drying.

60. Kar, R. K., and M. A. Choudhuri. 1982. Effect of desiccation on internal changes with respect to survival of *Hydrilla verticillata. Hydrobiological Bulletin* 16(2-3):213-221.

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.

61. Basiouny, F. M., W. T. Haller, and L. A. Garrard. 1978. Survival of Hydrilla (*Hydrilla verticillata*) plants and propagules after removal from the aquatic habitat. *Weed Science* 26:502–504.

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.

62. Smits, A. J. M, R. Van Ruremonde, and G. Van der Velde. 1989. Seed dispersal of three nymphaeid macrophytes. *Aquatic Botany* 35:167-180

N. peltata seeds show high tolerance to desiccation.

63. Arkush, K. D., H. L. Mendonca, A. M. McBride, S. Yun, T. S. McDowell, and R. P. Hedrick. 2006. Effects

of temperature on infectivity and of commercial freezing on survival of the North American strain of viral hemorrhagic septicemia virus (VHSV). *Diseases of Aquatic Organisms* 69(2-3):145-151.

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

64. Ahne, W., H. V. Bjorklund, S. Essbauer, N. Fijan, G. Kurath, J. R. Winton. 2002. Spring viremia of carp (SVC). *Diseases of Aquatic Organisms* 52:261-272.

    Review of the properties, histopathology, and epizootiology of Spring Viremia of Carp virus (SVCV). The authors list various methods by which virus infectivity may be destroyed, including heat treatments and chlorine.

65. Dwyer, W., B. Kerans, and M. Gangloff. 2003. Effects of acute exposure to chlorine, copper sulfate, and heat on survival of New Zealand mudsnails. *Intermountain Journal of Sciences* 9:53-58.

    No description available. Details found in USFS outline of disinfection guidelines to avoid spread of AIS during fire management and suppression activities. Recommends treatments for various species based on a literature review; references are outlined in this guidance.

66. Alonso, A., and P. Castro-Diez. 2012. Tolerance to air exposure of the New Zealand mudsnail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca) as a prerequisite to survival in overland translocations. *NeoBiota* 14:67-74.

    Tested different time lengths of air exposure to P. antipodarum in vessels within a controlled climatic chamber. The snails were then rehydrated, and mortality was measured at 24, 96, 168 and 264 hours, to assess lethal air exposure treatments.

67. McMahon, R. F. 1996. The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. *American Zoologist* 36(3):339-363.

    Review of physiological responses of zebra mussels due to temperature, respiration and metabolism, hypoxia/anoxia, salinity, desiccation and freezing, starvation, and bioenergetics.

68. Clarke, M. 1993. Freeze sensitivity of the zebra mussel (*Dreissena polymorpha*) with reference to dewatering during freezing conditions as a mitigation strategy. M.S. Thesis, The University of Texas at Arlington, Arlington, Texas.

69. Choi, W. J., S. Gerstenberger, R. F. McMahon, and W. H. Wong. 2013. Estimating survival rates of quagga mussel (*Dreissena rostriformis bugensis*) veliger larvae under summer and autumn temperature regimes in residual water of trailered watercraft at Lake Mead, USA. *Management of Biological Invasions* 4(1):61-69.

    Measured quagga mussel veliger survival rates on boats outside of water bodies in summer and autumn weather conditions. Recommended to reach 100% veliger mortality before transporting a boat out of quarantine and into another water body.

70. Hoffman, G.L., and M. E. Marliw. 1977. Control of whirling disease (*Myxosoma cerebralis*): use of methylene blue staining as a possible indicator of effect of heat on spores. *Journal of Fish Biology* 10:181-

183.

71. Bovo, G., B. Hill, A. Husby, T. Hästein, C. Michel, N. Olesen, A. Storset, and P. Midtlyng. 2005. Pathogen survival outside the host, and susceptibility to disinfection- Work Package 3, Report QLK2-Ct-2002-01546 Fish Egg Trade, VESO, Oslo, Norway.

Outline of disinfection guidelines to avoid spread of AIS during fire management and suppression activities. Recommends treatments for various species based on a literature review; references are outlined in this guidance.

72. Jørgensen, P.1974. A study of viral diseases in Danish rainbow trout: their diagnosis and control. Thesis, Royal Veterinary and Agricultural University, Copenhagen. 101pp.

73. Pietsch, J., D. Amend, and C. Miller.1977. Survival of infectious hematopoietic necrosis virus held under various conditions. *Journal of Fisheries Research Board of Canada* 34:1360-1364.

Outline of disinfection guidelines to avoid spread of AIS during fire management and suppression activities. Recommends treatments for various species based on a literature review; references are outlined in this guidance.

74. Acy, C.N. 2015. *Tolerance of the Invasive New Zealand Mud Snail to Various Decontamination Procedures.* Thesis submitted in candidacy for Honors at Lawrence University.

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.

75. 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.

76. De Stasio BT, Acy CN, Frankel KE, Fritz GM, Lawhun SD. Tests of disinfection methods for invasive snails and zooplankton: effects of treatment methods and contaminated material. Lake Reserv Manage. 35:156–166.

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.

77. 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^{o}C, nor did any survive 20 hours of immersion at 35^{o}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^{o}C and seven days at 25^{o}C.

78. Coughlan, N., Cuthbert, R., Dickey, J., Crane, K., Caffrey, J., Lucy, F. E., Dick, J. (2019). Better biosecurity: spread-prevention of the invasive Asian clam, Corbicula fluminea (Müller, 1774). Management of Biological Invasions. https://doi.org/10. 3391/mbi.2019.10.1.07.

This article examines the efficacy of a range of biosecurity techniques, including recommended (aquatic disinfectants, bleach and salt solutions) and more novel (hot water and direct steam) approaches, to induce adult C. fluminea mortality.

79. Crane, K., Cuthbert, R.N., Dick, J.T.A. et al. Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. Biol Invasions 21, 1311–1321 (2019). https://doi.org/10.1007/s10530-018-1901-2.

Examines the use of direct steam exposure to induce substantial fragment (i.e. propagule stage) degradation of seven invasive macrophytes: Ceratophyllum demersum, Crassula helmsii, Egeria densa, Elodea canadensis, Elodea nuttallii, Lagarosiphon major and Potamogeton crispus.

80. DiVittorio, J., Grodowitz, M., Snow, J. (2010) Inspection and Cleaning Manual for Equipment and Vehicles to Prevent the Spread of Invasive Species. Technical Memorandum No. 86-68220-07-05, U.S. Department of the Interior, Bureau of Reclamation, Denver, Colorado.

Procedures have been developed in this manual to address the transport of invasive species and pests through equipment movement. This manual provides guidance for inspecting and cleaning vehicles and equipment to help prevent the spread of noxious invasive species during Bureau of Reclamation activities.

81. Gottschalk, Stephen D., and KENNETH G. Karol. "Survivability of starry stonewort bulbils using commonly available decontamination strategies." J. Aquat. Plant. Manage 58 (2020).

This study tested the effects of freezing, desiccation, steam, and bleach on starry stonewort bulbils. Bulbils subjected to desiccation or freezing were tested by two measures of viability: the germination assay and the tetrazolium assay."

82. Glisson, Wesley & Wagner, Carli & Verhoeven, Michael & Muthukrishnan, Ranjan & Contreras-Rangel, Rafael & Larkin, Daniel. (2020). Desiccation tolerance of the invasive alga starry stonewort (Nitellopsis obtusa) as an indicator of overland spread risk. Journal of Aquatic Plant Management. 58. 7-18.

This study conducted laboratory and outdoor experiments to evaluate desiccation tolerance of starry stonewort propagules, including single stem fragments, small and large clumps of fragments, and bulbils (asexual reproductive structures).

83. Wisconsin Department of Natural Resources. "Effectiveness of disinfection methods for reducing the spread of invasive snails and zooplankton." Factsheet. Wisconsin Department of Natural Resources. Madison, WI. Oct. 2020. Web.

84. Wisconsin Department of Natural Resources. "Efficacy of decontamination strategies on the survivability of starry stonewort bulbils." Factsheet. Wisconsin Department of Natural Resources. Madison, WI. Oct. 2020. Web.