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Survey and Evaluation of Dive and Field Gear Decontamination Protocols for Aquatic Invasive Species

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14. ABSTRACT

This report identifies existing aquatic invasive species (AIS) decontamination protocols and studies to identify information about method effectiveness and the suitability for sensitive equipment. The two goals of this study were to compile and review existing dive and aquatic field sampling gear decontamination protocols and conduct a literature review of research focused on decontamination methods. Information was compiled from these documents to help determine most studied methods, most used methods, and information gaps or methods that have potential, but are not well studied. No single best approach for AIS decontamination emerged from this analysis but some methods do appear to be more viable than others. It was made evident that having a proactive and flexible AIS program that evaluates different scenarios and tailors techniques to specific conditions, equipment, and AIS is the best approach that should be taken.

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Survey and Evaluation of Dive and Field Gear Decontamination Protocols for Aquatic Invasive Species

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Contents

Page

Executive Summary

Preventing the spread of Aquatic Invasive Species (AIS), particularly quagga and zebra mussels (dreissenids) in the western United States is a high priority for state and local governments as well as the Department of Interior and the Bureau of Reclamation (Reclamation). AIS can cause billions of dollars in damages to dam facilities and other water infrastructure. They can also cause fish kills, degrade aquatic ecosystems, and degrade recreational activities. Once established many AIS, especially dreissenids are nearly impossible to eradicate.

Watercraft are considered high risk for spreading AIS, and considerable time and resources have been devoted to developing and implementing watercraft inspection and decontamination protocols throughout much of the West. However, protocols for decontaminating underwater dive (SCUBA and surface supplied air [SSA]) and aquatic field sampling equipment are much less established despite also being high risk activities for spreading AIS. Some agencies have established dive and aquatic field sampling gear decontamination protocols but current research examining the effectiveness of protocols is often narrow in scope, either focusing on a single species or a specific type of equipment.

The two goals of this study were to – 1) compile, summarize and review existing dive and aquatic field sampling gear (including sensitive/delicate equipment) decontamination protocols, and 2) conduct a literature review of research focused on the effectiveness of decontamination methods and suitability for field use. Information was compiled to help determine most studied methods, most used methods, and information gaps or methods that have potential, but are not well studied. The findings were analyzed to identify the most appropriate existing protocols and any necessary future method development and/or studies.

Compiling protocols and studies was a helpful process for determining what decontamination protocols are currently being used and what studies have been conducted. Fortunately, the most common AIS protocols were generally aligned with the most studied methods. While studies supported most of the common decontamination methods, there were also gaps and inconsistencies in methods and studies. Saltwater for example, does not seem to be well studied for decontamination of species other than dreissenid mussels and additional studies should be considered. It is true that most decontamination methods could benefit from additional research, especially for any emerging species of concern. No single best approach for AIS decontamination emerged from this analysis. Rather, it was made evident that having a proactive and flexible AIS program that evaluates different scenarios and tailors techniques to specific conditions, equipment, and AIS is the best approach that should be taken. Identifying acceptable decontamination options, AIS risk, and developing protocols that allow situational flexibility may be the best strategy for preventing the spread of AIS.

1. Introduction

Preventing the spread of Aquatic Invasive Species (AIS), particularly quagga and zebra mussels (dreissenids) in the western United States is a high priority for state and local governments as well as the Department of Interior and the Bureau of Reclamation (Reclamation). AIS can cause billions of dollars in damages to dam facilities and other water infrastructure. They can also cause fish kills, degrade aquatic ecosystems, and degrade recreational activities. Once established many AIS, especially dreissenids are nearly impossible to eradicate. Dreissenid mussels are particularly challenging because the larvae are microscopic and can survive for weeks in a drop of water. There is a heightened urgency to protect high priority areas currently without quagga or zebra mussels, such as the Pacific Northwest. Prevention of the spread of AIS is vital to avoiding additional costs and degradation associated with these invasions.

Watercraft are considered high risk for spreading AIS, and considerable time and resources have been devoted to developing and implementing watercraft inspection and decontamination protocols throughout much of the West. However, protocols for decontaminating underwater dive (SCUBA and surface supplied air [SSA]) and aquatic field sampling equipment are much less established despite also being high risk activities for disseminating AIS.

Unlike the common habits of recreational boaters or fishermen that may typically only go to one or two waterbodies during an outing or weekend, organizational dive and aquatic sampling teams often operate in many different waterbodies and watersheds within a short amount of time leaving little dry time for equipment. Additionally, many dive or aquatic sampling teams are regional or national assets and often travel large distances over multiple watershed boundaries, further increasing the risk of spreading AIS. Dive and aquatic sampling gear can be particularly challenging to decontaminate because it often has soft, porous materials and can contain pockets and chambers that collect water and aquatic materials. This equipment can also be more sensitive than typical boating equipment – fragile sensors, cameras, fine mesh nets, intricate regulators, and specialized safety equipment may be damaged by some decontamination methods commonly used for boats.

These equipment characteristics required additional consideration and assessment when determining the most appropriate decontaminant techniques but are not as well-studied or well communicated when compared to watercraft protocols. For example, one of the most common dive equipment decontamination methods is to use a table salt and water solution. While this method is established and generally accepted as effective, it is not always a viable option due to the difficulty bringing salt into solution to an adequate concentration, disposing of the remaining saltwater, inability to thoroughly rinse salt residue off gear, and the damage it can cause some types of equipment. Lastly, there are some uncertainties as to its effectiveness in certain conditions and on different AIS of concern. Therefore, a need exists to further evaluate and establish effective protocols that can be followed by dive and aquatic sampling teams. Assessing the current methods and their validity and providing this additional information will only increase the knowledge for making the best choices for gear decontamination.

Dive and aquatic field sampling gear decontamination protocols currently exist at various agencies. Research has been conducted that examines the effectiveness of some protocols, but is often narrow in scope, either focusing on a single species or a specific type of equipment. This study identifies

existing AIS decontamination protocols and studies to assess existing information about method effectiveness on various AIS and suitability for sensitive equipment.

2. Methods

The two goals of this study were to – 1) compile and review existing dive and aquatic field sampling gear decontamination protocols, and 2) conduct a literature review of research focused on decontamination methods. Information was compiled to help determine most studied methods, most used methods, and information gaps or methods that have potential, but are not well studied. The findings were analyzed to identify the most appropriate existing protocols and any necessary future method development and/or studies. This report does not evaluate the effectiveness of protocols, or merit of studies, rather, it consolidates protocols and studies to understand the extent of methods used and any associated research.

2.1 AIS Decontamination Protocols

A literature search was conducted to find AIS decontamination protocols from organizations throughout Canada and the United States. The search focused on identifying dive and aquatic field decontamination protocols, and most of the protocols found were developed by government agencies within the western United States. Many decontamination protocols focused on watercraft were identified but were not included in this report because they did not focus on dive and aquatic sampling gear. Likewise, in most cases simplistic "clean, drain, dry" documents designed for public awareness were not included in the report because that basic level of decontamination should always be conducted, and these procedures vary little in practice. Website/internet searches were the primary method of research. While some agencies and organizations were contacted, not all the organizations and agencies were contacted directly. Therefore, some protocols or version updates may not have been identified and assessed in this document. The intent of this study was to assess the common dive and field gear protocols being used, not to compile an exhaustive list of all agency protocols.

2.2 AIS Decontamination Research Studies

A literature review of published research reports on AIS decontamination methods was also conducted, consisting of reference searches for primary materials and keyword searches. The focus of this review was to determine which decontamination methods have been evaluated and for which species, with the intent to validate existing protocols and identify research gaps. When analyzing protocols and studies, common decontamination factors such as minimum exposure/contact time and minimum concentration/threshold limits were compared. Other factors such as method availability, portability, environmental considerations, effects on equipment, and safety were also considered during this review, helping to assess the practicality of the methods. Review of the studies also focused on method efficacy (mortality rate), contact time, and concentrations. Anomalies and unique information found in each of the protocols were also noted when possible.

This literature review was focused on identifying studies and not evaluating the worthiness of the study itself.

2.3 Protocol and Study Analysis

After the literature was compiled, the protocols were assessed to determine if there was supporting research to validate use. The literature was assessed for commonalities, caveats, or specific techniques worthy of replicating. Existing studies were evaluated to identify the most studied methods and AIS, research gaps, and research needs for future AIS protocol development. This synthesis helped identify best practices based on existing and proven protocols and will serve as a tool or starting point for developing or enhancing Reclamation dive and aquatic sampling team decontamination protocols and/or to identify gaps in studies or protocol deficiencies to inform future research efforts.

3. Literature Overview

3.1 Decontamination Protocols

Not surprisingly, some of the most common protocols identified were for watercraft inspection and decontamination. These documents typically focused on on-site decontamination stations found at boat ramps and focused on hot water and high pressure washing of watercraft; many of the documents were designed for training and references for AIS inspectors. Some of these documents did have information on equipment and dive gear decontamination. Other documents contained regulatory or statutory information for state programs, providing more of a legal framework than a programmatic framework. Other documents were more intended for public outreach and awareness of AIS, which had simplistic explanations of AIS and basic "clean, drain, dry" messages, and did not provide program or protocol specific guidance. These types of documents were largely not referenced in this the study. Some of the other documents analyzed in this survey were written for internal agency or contract personnel that would be responsible for decontamination of their own gear at sites not likely to have established decontamination stations. These documents provided the best information on protocols for dive and aquatic field equipment decontamination.

Well over 60 AIS decontamination protocols were found and evaluated in this study. Of those found, 54 were reviewed and analyzed for this project. Protocols were not selected for review for the reasons stated above - either they were very simplistic or general (e.g., "clean, drain, dry"), were law and statute focused with little focus on protocols or were strongly focused on watercraft inspection and decontamination stations using hot, pressurized water. All dive gear decontamination protocols that were found were reviewed and assessed in this document. The 54 reviewed and analyzed documents were developed by 17 different States (US), 7 federal agencies (US), 2 Canadian provinces, and 5 other organizations. The protocols developed and used by the states within Reclamation boundaries were a focus of the search, and most of their protocols were analyzed for this document. Some states and agencies had multiple protocols that were used by different internal

agencies or for different decontamination situations. Many organizational protocols contained multiple methods that could be used to decontaminate equipment. 186 methods were listed in the 54 protocols that were reviewed; many of these methods were the same or like methods found in other protocols.

Protocols varied widely in complexity. Some were basic and simple to follow, while others contained somewhat complex tiered levels that would require some training. Most agency guidance/protocols contained multiple decontamination options. For example, guidance may list the procedures for using either a saltwater solution or hot water to decontaminate equipment.

The four most utilized methods were hot water, recommended in 44 of the agency protocols; Quaternary Ammonium Compounds (QACs) were listed 30 different times; and saltwater solution was the third most common method with 20 occurrences (Table 1). Air dry/desiccate was almost always recommended at some level, at a minimum as part of the "clean, drain, dry" message. However, some protocols offered specific dry time and/or temperature requirements. It was difficult to quantify total number of uses since almost all agencies had some form of "clean, drain, dry", messaging. Drying/desiccation will be discussed as a viable decontamination method, but the number of instances found was not counted.

Table 1 lists the methods found more than once during literature research. Several other decontamination methods were only cited by a single protocol (see Table 3). Most decontamination protocols were not species specific; those that were primarily focused on quagga and/or zebra mussels, whirling disease, and chytrid fungus. Appendix A lists the most common decontamination types with reference to the agency/organization documents where they were found. The most used protocols are discussed in the following sections.

Table 1: Most frequently referenced decontamination methods in AIS decontamination protocols.

Decontamination Method	Times referenced
Hot water (with or without high pressure)	44
QACs	30
Saltwater solution	20
Bleach	13
Freeze	9
Virkon™	9
Vinegar	9
Steam	5
Ethanol	2

Protocols were also evaluated on their relevance to dive and aquatic field equipment. Several organizations had dive gear decontamination protocols established. The follow is a reference list of documents found with dive specific recommendations.

- California Department of Fish and Game. (2009)
- California Department of Fish and Wildlife. (a)
- California Department of Fish and Wildlife. (b)
- Colorado Division of Parks and Wildlife. (2012)

- U.S. Environmental Protection Agency. (2016).
- U.S. Environmental Protection Agency. (2006).
- U.S. Fish and Wildlife Service. (2018)
- National Oceanic and Atmospheric Administration. (b)
- Minnesota Department of Natural Resources. (2015)
- Wisconsin Department of Natural Resources.
- Pennsylvania Sea Grant
- Bureau of Reclamation. (2021)
- US Geological Survey. (2016)
- US Geological Survey. (2017)
- Diving Unlimited International. (2009)
- Nevada Department of Wildlife.

3.2 Decontamination Research Studies

A total of 77 AIS focused research studies were reviewed to evaluate the amount of information and data that supports AIS protocols. The most research studies were found for: desiccation/drying (57), Hot water (spray and immersion) (29), Virkon™ Aquatic (17), chlorine bleach (sodium hypochlorite) (14), steam (10) and QACs (10). This is by no means an exhaustive list of the research that has been conducted on AIS decontamination methods, however, it does represent some of the most available and cited AIS studies. Appendix B provides a list of decontamination methods and the AIS that has been examined along with the literature source. The following sections provide an overview of the most studied decontamination methods.

4. Results

4.1 Thermal Treatments

4.1.1 Hot water

Hot water was the most utilized gear decontamination method identified in the reviewed protocols. This method is consistent with most watercraft decontamination protocols and recommended in the Uniform Minimum Protocols II (UMPS II) (Pacific States Marine Fisheries Commission, 2012). The protocol for watercraft decontamination is to use 60°C water at high pressure (3,000 pound-force per square inch (psi)) to decontaminate the hull and low pressure to decontaminate motors and engines. Interior compartments are decontaminated with 49°C at low pressure. These standards were developed from research completed by Morse (2009).

Comeau et al. (2011) found that zebra and quagga mussels can be effectively treated with hot water at or above 60°C for 10 seconds. A review of decontamination methods by Mohit et al. (2021) found that immersion in water ≥ 50°C for 15 minutes resulted in 100% mortality among mussels, small invertebrates and some plant species. A higher temperature of 60°C was required for hot water

spray applications lasting ≥ 5 seconds to achieve the same mortality rate among dreissenid mussels. Cultures of the fungus that causes chytrid disease in amphibians were also found to be sensitive to heating and within 4 h at 37°C, 30 min at 47°C and 5 minutes at 60°C, 100% mortality occurred (Johnson, et al. 2003).

High pressure-washing eliminated significantly more entangled plants, and small organisms and seeds than low pressure (Mohit, et al. 2021). Watters (2014) found that using 3,000 psi of water to remove dreissenids from watercraft is accomplished at a faster rate when the vessel has been out of the water for at least one week in the summer and two weeks in the winter compared to being fresh out of the water. Watters also exposed zebra mussels to hot-water sprays at 20, 40, 50, 54, 60, 70, and 80°C for 1, 2, 5, 10, 20, 40, 80, and 160 s. Sprays at 54°C for 10 s were shown to be 100% lethal.

There was variation observed in the recommendations for treatment temperature and exposure times. Recommended exposure time varied from 15 seconds to 15 minutes at 60°C. Other temperature recommendations included 40°C, 43°C, 45°C, 47°C, 49°C, 90.5°C, and 93°C with exposure time varying from 5 to 40 minutes for these different temperatures. Some of these variations were for targeting specific species or limiting damage to sensitive equipment. For example, viruses such as whirling disease may have higher thresholds for hot water. A 90°C hot water treatment for ten-minutes was the most common recommendation for whirling disease (Government of Alberta, 2017; Cockman et al., 2012; National Ecological Observatory Network, 2015; Oregon Department of Fish and Wildlife). This treatment is much hotter than UMPSII or common watercraft decontamination protocols. This temperature would most certainly cause damage to gear and creates additional safety concerns for those conducting the decontamination since it is near boiling temperatures.

There are many benefits to the use of hot water to decontaminate equipment – no chemical or toxicity dangers, no hazardous waste, relatively low costs, readily available. Despite all these benefits and hot water being the most common and likely the most generally agreed upon effective decontamination method (besides drying/desiccation), hot water may not be acceptable or available for decontamination of aquatic field or dive equipment. Heating water reliably to 60°C is not always an option at remote sites, either requiring long drives to facilities or bringing water and heating equipment onto a field/dive site. Hot water treatment can also be water intensive and handling hot water and heating elements have safety risks.

Hot water is also not always appropriate for field and dive gear because it may cause damage to sensitive equipment resulting in equipment failure, expensive repair costs, loss of data and time, or inaccurate data. Damage to dive gear could result in a catastrophic safety issue. The National Oceanic and Atmospheric Administration (NOAA) cautions that hot water will damage equipment made of Gore-Tex (National Oceanic and Atmospheric Administration, a). The Bureau of Reclamation dive gear decontamination guidance warns of potential damage to dive gear using water over 40°C (Bureau of Reclamation, 2021), while the California Department of Fish and Wildlife (CDFW), the U.S. Geological Survey (USGS), and Apeks (Apeks, 2016) warn against use of water over 49°C for dive gear decontamination (California Department of Fish and Wildlife, and U.S. Geological Survey, 2016). Despite the potential for damage to equipment, several dive specific decontamination options did include the use of hot water. However, the temperature recommendations were either 40°C or 43 to 49°C (California Department of Fish and Game, 2009; California Department of Fish and Wildlife, b; U.S. Geological Survey, 2016; U.S. Geological Survey, 2017).

4.1.2 Freezing

Freezing was listed in several protocols. Freezing is simple, non-toxic, and often readily available. Freezing durations are also much lower than typical drying or desiccation recommendations. Freezer temperature requirements ranged from -9 to 0°C for 24 hours (Government of Quebec, 2018) to -3°C for 6 hours (Oregon Department of Fish and Wildlife), to -9 to 0°C for 24 hours or -10°C for 8 hours (U.S. Fish and Wildlife Service, 2018). The FWS warned to make sure salt crystals (if applicable) are removed before freezing (U.S. Fish and Wildlife Service, 2018). Wisconsin DNR warns that freezing is not effective against viruses or bacteria (Wisconsin Department of Natural Resources, 2020).

Four dive teams used freezing as a decontamination option. One protocol listed 72 hours freeze time (Minnesota Department of Natural Resources, 2015), while another listed overnight as the freeze time for dive suits, fins, and gloves (Bureau of Reclamation, 2021). Freezing would only be a viable option for durable equipment such dive suits and fins, and sensitive equipment should not be frozen.

Gear contaminated with dreissenid mussels is recommended to be frozen at a temperature ≤ -6°C for at least 5 hours (McMahon, et al. 1993). Freezing has also been examined as a potential decontamination method for New Zealand mud snail (NZMS). LaFond et al. (2021) froze snails in a household chest freezer in time increments of 0, 5, 10, 30, 60, 120, 180, and 240 minutes in dry conditions (water removed) and in water. Snails in dry conditions reached 100% mortality after 30 minutes while snails in water reached 80% mortality after 240 minutes. Larger snails required longer times to achieve mortality. Snails in water could tolerate freezing temperatures longer than snails in dry conditions. Freezing is a viable decontamination method for contaminated equipment, but longer freezing times are necessary for larger snails, especially when equipment is wet.

The effectiveness of freezing for 24 hours on the macro-algae Starry stonewort (*Nitellopsis obtuse*), which propagates via vegetative propagules (bulbils) was investigated by Gottschalk and Karol (2020). A significant negative impact on the survivability of bulbils was observed after freezing as there were significant reductions in bulbil viability after 24-hour treatment durations. Freezing is also effective for whirling disease if gear is frozen at ≤ -20°C for 7 days (Hedrick, et al. 2008).

4.1.3 Steam

Steam was listed in five different AIS decontamination protocols. One method prescribed a steam temperature of greater than 60°C while the other protocol suggested the use of 100°C. Steam is well studied at 100°C and has been proven to effectively kill a variety of AIS (Wisconsin Department of Natural Resources, 2020). However, steam is created from water at or near boiling and manufacturers list temperatures of over 93°C are produced by household steam cleaners. Contact times were listed as either 1 minute or 5-10 seconds. Steam has the benefit of being portable, creates no harmful byproducts, and is quick. However, the use of steam for one minute on anything electronic, sensitive, or with adhesive would likely have detrimental effects and use of high temperature steam would be a safety consideration. No dive teams listed steam as a decontamination option.

Coughlan et al. (2020) assessed the effectiveness of steam spray (>100°C; < 120 s) and found complete mortality of both dreissenid species following 30 seconds of steam exposure. Steam can

also be an effective treatment for plants; Gottschalk and Karol (2020) found significant reduction in Starry stonewort survivability after exposure to steam for 24 hours.

4.2 Desiccation

Desiccation of AIS through drying is a common recommendation. It is effective for most AIS, given sufficient time, heat, and dryness (Wisconsin Department of Natural Resources, 2020). Drying is by far the most simple, safe, and environmentally friendly decontamination method when time allows. However, time is usually the issue for dive and aquatic field sampling teams. Multiple protocols have dry times of at least 5 days. Some species-specific recommendations are shorter; however, it is challenging to determine appropriate dry times because equipment porosity, humidity, and sunlight exposure and other ambient conditions can change dry times significantly.

The legacy *100th Meridian Initiative* website, *www.100thmeridian.org*, previously provided a *dry time calculator* tool, which is frequently cited in dry/desiccation protocols, but the calculator has been deleted along with the website. The removal of the calculator was likely due to the inability to ensure that it would provide accurate and effective numbers for all equipment and conditions, potentially creating false assurance of satisfactory decontamination. An email inquiring about the calculator was sent to the US Fish and Wildlife Service contact listed on the webpage; no response was received as of the submission of this report.

Several dive gear specific decontamination protocols listed 5-day dry times after the initial decontamination treatment. A common dive specific recommendation was to thoroughly evaluate the dryness by feel of dive suit stitching and seams.

A recent review of decontamination methods by Mohit et al. (2021) found that the recommended air-drying duration of up to one week produced high mortality (≥ 90%) among several invertebrate and macrophyte species, although survival was high for certain aquatic snails. Larger and/or older invertebrates were more resistant to desiccation. Aquatic plant survival and growth were inversely related to water loss (a function of drying time and relative humidity), and short or single fragments were less resistant to air-drying than larger or clustered fragments. Starry stonewort survivability was significantly reduced after desiccation for a 24-hour period (Gottschalk and Karol, 2020). The chytrid disease fungus also did not survive complete drying after < 3 hours at room temperature (Johnson, et al. 2003).

4.3 Quaternary Ammonium Compounds (QACs) and Alkyl Dimethyl Ammonium Chloride (ADBACs)

Decontamination protocols using QACs were the second most common protocols. They were included in 30 different organizational protocols. QACs are a family of organic compounds produced by a variety of manufacturers and are comprised of numerous different solutions, which adds to the challenge of assessing their effectiveness. Alkyl Dimethyl Ammonium Chloride compounds (ADBACs) are a subset of QACs and are sometimes used interchangeably. According to Melin et al. (2016), ADBACs (along with didecyl dimethyl ammonium chloride) are commonly used household cleaners and are also common medical and food industry sanitizers. One of the

most recognizable products containing QACs may be Formula 409®, a household disinfectant which was also recommended as a decontamination agent in some protocols. QACs are also used as an algaecide and in veterinary and other animal operations (Melin et al., 2016). They are often used as a fungicide, bactericide, and protozoacide (Melin et al, 2016). Common brand names of chemical solutions that use QACs that were recommended in decontamination protocols included Sparquat 256 (no longer manufactured), Formula 409®, Quat 128, Quat Plus, T-San, Roccal-D (plus), Odaban, Parvosol, Green Solution High Dilution 256, and Super HDQ.

Despite their widespread use, "QAC toxicity from consumer products are well documented" (Melin, et al. 2016). According to the Environmental Protection Agency (EPA) dive protocols, "If quats are mixed with chlorine bleach, the exothermic reaction is potentially explosive, and the resultant chlorine gas may be hazardous. Quats are also corrosive to the skin and eyes, and proper PPE and disposal of wash fluid is required" (U.S. Environmental Protection Agency, 2016). There are also environmental concerns with QACs. According to EPA, "quats are highly toxic to fish and aquatic plants, and care should be taken not to allow decontamination fluids to enter any body of surface water" (U.S. Environmental Protection Agency, 2016). This toxicity risk should be taken into consideration when determining if QACs should be used, especially with dive equipment for the face or mouth such as dive masks and SCUBA regulators.

Both Ocean Technology Systems and Apeks dive equipment manufacturers warn against uses harsh chemicals or solvents when cleaning their dive equipment (OTS, 2014; Apeks, 2016). Additionally, as many of these are registered pesticides they should not be used outside of their designated purposes as stated on the Safety Data Sheets (SDS) and by the EPA.

Some QAC protocols warn that QACs may not be effective against adult quagga mussels (Utah Department of Natural Resources, 2012). Despite the potential hazards of using QACs, they are included in many available protocols available. Frequently recommended QAC brands, concentrations, and contact times found in protocols are listed in Table 2.

Table 2. Commonly utilized QACs in decontamination protocols and their suggested concentrations and contact times.

QAC	Concentration	Contact Time	References
Formula 409®	100%	10 minutes	Michigan Department of Environmental Quality, 2014; Washington Department of Fish and Wildlife, 2016; Wisconsin Department of Natural Resources, 2020
QACs	4.6%	10 minutes	National Ecological Observatory Network, 2015
QAT 128	7.7%	10 minutes + 1 hour in sun	Utah Department of Natural Resources, 2012
Quat Plus	1500 PPM	10 minutes (submerged)	Alberta Environment and Parks, 2017

Sparquat 256	12.5%	10 minutes + 1 hour in sun	Utah Department of Natural Resources, 2012
Green Solutions HD 256	2.5oz/1 gallon	10 minutes	Santa Clara Valley Habitat Agency, 2021
Roccal-D	250mg/L	15 minutes	National Oceanic and Atmospheric Administration, b
Sparquat 256	4.3oz/1 gallon	10-15 minutes	Cockman et al., 2012
Quat 128	1/8tspn/1 gallon	30 seconds	Cockman et al., 2012
Quat 128	0.1%	2-5 minutes	National Park Service

Multiple protocols also recommended the use of commercially available QAC-neutralizing compounds to eliminate the chemical hazards after their use. Neutraquat was the primary commercially available product mentioned. One protocol recommended using 12 tablespoons of bentonite to one gallon of water to neutralize Green Solutions High Dilution disinfectant in 3 to 5 hours (Santa Clara Valley Habitat Agency, 2021).

Although no dive organization protocols recommended the use of QACs as an option for dive gear decontamination, a variety of QACs have been studied for use in killing AIS on contaminated gear. Several studies have examined the effectiveness of Formula 409® as a decontamination method for NZMS. Acy (2015) examined the differences of applying full strength Formula 409® as a spray vs immersion and how mud interferes with effective decontamination. Immersion killed all snails after 10 minutes; effectiveness was more variable with spraying. Mortality was decreased by the presence of mud, and the type of material that is being decontaminated was found to impact the effectiveness of the treatment. The author suggests that Formula 409® treatment is the most economical and accessible option for NZMS.

Schisler et al. (2008) also found complete mortality of NZMS when a Formula 409® immersion technique was utilized at full strength for 10 minutes. In the study, NZMS were allowed to recover from the exposure in baths for up to 56 days and none of the treated mussels recovered. Toxicity of NZMS to Formula 409® is likely due to QACs, as they interfere with gill membrane function. Since Formula 409® is a degreaser, it is also thought to aid in the effectiveness by loosening the snail's operculum seal (Schisler, et al. 2008).

The same immersion technique using full strength Formula 409® for 10 minutes has also been shown to be effective against whirling disease (Hendrick, et al. 2008). Formula 409® was tested using soaking and spraying and at two application durations (10 and 20 minutes) for the use of decontaminating fishing waders for NZMS (Ethaiya, 2018). The treatment achieved 100% mortality and neither application method nor duration had a significant effect on mortality. In this study Formula 409® was tested alongside Virkon and bleach and performed significantly better than both at the same application durations.

Sparquat 256® is another QACs that was tested and proven to be effective for NZMS, but it has since been discontinued by the manufacturer. Immersion of gear in a 3.1% solution of Sparquat 256® for 10- 15 minutes was effective for NZMS (Schisler, et al. 2008). Britton and Dingman (2010)

examined survival of quagga mussel veligers in 3% solution for 5 and 10 minutes. They found a 5-minute exposure duration was insufficient to kill 100% of tested veligers. However, a 10-minute exposure was effective in killing all tested veligers. Veligers were not dead immediately after treatment, an additional 60 minutes were required after the Sparquat 256® solution was removed before 100% mortality was achieved.

Several other solutions containing QACs have also been examined for NZMS and dreissenid mussels including Quat 4, Green Solutions Hi Dilutions, Super HDQ Neutral, Quat™ 128 and Quat™ 256. Britton and Dingman's (2010) research found that a 1.8% solution of Green Solutions High Dilution 256® for 10 minutes was effective at killing quagga mussels. NZMS were exposed to Quat 4, Green Solutions Hi Dilutions Disinfectant (1.8% solution for 10 minutes) and Super HDQ Neutral (1% solution for 5 minutes) to examine the efficacy (Stout, et al. 2016). Regardless of the QAC used, Stout et al. (2016) recommends an immersion disinfection rate of 0.4% and a spray disinfection rate of 0.8% in solution with an exposure duration of 10 minutes. For immersion disinfection purposes, GS 256 and Super HDQ were the most effective. Super HDQ caused higher mortality rates at 48 h post-exposure and was therefore tested and found to be highly effective for spray disinfection to prevent transporting NZMS on field equipment. These concentrations meet or exceed minimum effective disinfection requirements for quagga mussels, zebra mussels, whirling disease, and chytrid fungus.

The effectiveness of Quat™ 128 and Quat™ 256 on killing adult dreissenids was examined over time at four concentrations: 0, 1%, 3%, and 5% (Watters 2014). The results of the study show that all treatment concentrations of Quat™ 256 are 100% lethal to adult dreissenids within 36 hours. Dreissenid veligers were also examined over time at different concentrations of Quat™ 128 and Quat™ 256 (0.25%, 0.5%, and 0.75%, 0.1%, 0.25%, and 0.5%, respectively) at different solution temperatures (2, 16, and 30°C) and at different ambient temperatures (2, 15, 30, and 43°C). Complete mortality of dreissenid veligers was achieved in treatments with 40 minutes of exposure time to 0.25% Quat™ 128 or 0.1% Quat™ 256.

4.4 Saltwater Solution

Saltwater solution treatments are widely used and accepted for decontamination of quagga and zebra mussels and was the third most common recommended decontamination option discovered in the literature review. The concentration and duration of almost every protocol was a 3.5% (½ cup table salt (NaCl) to 1 gallon of freshwater) solution soaked for 30 minutes (when contact time was listed). However, several protocols did not list saltwater treatment duration. Reclamation additionally listed a 1% (2/3 cup salt to 5 gallons of water) solution soaked for 24 hours option for general equipment decontamination (Bureau of Reclamation, 2021).

Salt water is safe to handle, easily attainable, and can be disposed of in most wastewater treatment systems. However, large volumes of salt water can be difficult to bring to a remote field site and to dispose of properly; salt crystal residue can also be problematic for cleanup. It can also be water intensive, which can be problematic at remote sites and salt can be difficult to fully bring into solution. Multiple protocols suggest rinsing gear in fresh water after saltwater decontamination, especially dive suits to avoid damage from salt crystals. Salt crystals from evaporated solution can also generally be an inconvenience at the decontamination site. Salt water was one of the most

common dive gear decontamination recommendations. All dive gear decontamination protocols' concentration and duration were 3.5% saltwater solution soaked for 30 minutes (if a time was specified). NaCl was the only salt listed in dive equipment decontamination protocols using saltwater solutions. Studies indicated that saltwater is effective against dreissenid mussels, but not for all AIS.

Acy (2015) examined the effectiveness of salt solutions for decontamination of NZMS. NZMS remained viable after immersion in 35 ppt salt solution for up to 30 minutes. This decontamination method was not found to be effective since 0% survivorship was never achieved (Acy, 2015). The National Voluntary Guidelines to Prevent the Introduction and Spread of Aquatic Invasive Species (U.S. Fish and Wildlife Service, 2014) recommends that scuba diving gear utilized in freshwater dives be soaked in a 3.5% salt solution for 30 minutes to clean the equipment before leaving it to dry for 5 days. However, research conducted by Acy (2015), indicated mud snails could survive this treatment.

Hofius et al. (2015) investigated if submerging veliger contaminated boats in salt and brackish water could be an effective decontamination method. They exposed quagga mussels to water collected from different locations within the California Delta, with salinities ranging from 4 parts per thousand (ppt) to 33.4 ppt for up to fifteen days. The water with the highest salinity content killed 100% of the quagga mussels within 40 hours. One hundred percent mortality was not observed until 70 hours of exposure for quagga mussels exposed to lower salinity concentrations of 21.3 ppt and 15.3 ppt. However, 99% of mussels exposed to 4.0 ppt salinity brackish water remained alive for the 16-day duration of the study.

The toxicity of potassium salts to bivalves was first documented in native unionid mussels (Imlay, 1973) and the invasive Asian clam *Corbicula* (Anderson 1976). Potassium salts were shown to have similar toxicity in zebra mussels shortly after their introduction to the United States (Fisher, et al. 1991). Both potassium chloride (KCl), also known as potash, and potassium phosphate (KH2PO4) show comparable toxicity, destroying the gill epithelium and leading to asphyxiation (Fisher, et al. 1991, Fisher, 1994, O'Donnell, et al. 1996, Wildridge, et al. 1998). Water quality, especially sodium content can influence the effectiveness of potassium chloride as a decontamination method for dreissenid mussels (Moffitt, et al. 2016).

A variety of studies have investigated which concentrations of potassium chloride (KCl), and sodium chloride (NaCl) are effective against dreissenid mussels. Pucherelli et al. (2014) investigated the use of KCl and formalin for decontamination of fish haul trucks and found that 12 hours of KCL at 1,500 milligrams per liter (mg/L) plus 2-hour 50 mg/L dose of formalin was 100% effective for control of dreissenid mussels. Davis et al. (2016) found that exposure of adult zebra mussels to various concentrations of NaCl for 24 hours resulted in 97%-100% mortality. Davis et al. (2018) examined the toxicity of each salt to both adult zebra mussels and veliger larvae. Sodium chloride was less effective at causing mortality than KCl within the exposure periods tested. Adult mussels required a 4× longer exposure period to exhibit complete mortality when exposed to NaCl at 30,000 mg/L (24 hours) compared to KCl (6 hours). At 10,000 mg/L, NaCl took 8 times longer (96 hours) than KCl (12 hours) to cause 100% mortality of adult mussels. Veligers that were exposed to KCl at 1,250 mg/L required a 12-hour exposure to attain complete mortality, while those exposed to NaCl at 10,000 mg/L required an 18-hour exposure to exhibit the same result. It is not recommended to combine KCl, formalin, and NaCl as a decontamination method (Edwards, 2000).

4.5 Chlorine Bleach (Sodium Hypochlorite)

The fourth most common decontamination chemical was chlorine bleach. Bleach concentrations and contact times varied widely among the different protocols. Despite bleach being a commonly used household item, it is known to be harsh on equipment and poses safety and disposal concerns. The volatility of bleach can also make it difficult to achieve proper concentrations. The Wisconsin Department of Natural Resources (WI DNR) states that bleach is not effective against NZMS, spiny waterflea eggs, faucet snails (*Bithynia tentaculate*), or Asian clams (Wisconsin Department of Natural Resources, 2020).

Bleach is not often used for dive gear decontamination because of its damaging effects to equipment and potential health risks. Reclamation has determined that bleach is not an acceptable dive gear decontamination method because of the breathing equipment and potential health issues with residual chlorine gas inhalation (Bureau of Reclamation, 2021). Other dive team protocols list bleach as an option. California Department of Fish and Wildlife dive protocols list it as a cleaning option, but without concentration or duration. NOAA distinguishes sensitive and non-sensitive equipment with different bleach recommendations (non-sensitive: 10% solution for 10 minutes, sensitive: 0.1% solution for 10 minutes) (National Oceanic and Atmospheric Administration, b).

Chlorination, using a variety of chlorine containing compounds (e.g., chlorine gas, hypochlorite, chloramine, and chlorine dioxide) are effective at killing dreissenid mussels through oxidation (Klerks and Fraleigh, 1991, Van Benschoten, et al. 1995, Brady, et al. 1996, Matisoff, et al. 1996, Rajagopal, et al. 2002, Takeguchi, et al. 2012). Chlorination trials with adult zebra and quagga mussels indicate significantly higher mortality for quagga compared to zebra mussels (Brady, et al. 1996). Chlorination is widely used for water treatment, and it is largely in this context that it has been employed for control of dreissenid mussels. Chlorine treatments for dreissenid mussels are most used at hydropower plants or in flow-through systems to prevent and remove settlement. Matisoff et al. (1996) suggest using a 0.5%-2% (250 – 1000 parts per million (ppm)) bleach solution for 10 minutes for decontamination of dreissenid mussels from gear. It is important to consider that water temperature can impact the effectiveness of chlorine dioxide.

Chlorine has been examined as a decontamination method for other AIS and specific doses and immersion durations lethal to small organisms established (Mohit, et al. 2021). It is unclear how well chlorine works for species like starry stonewort, which propagates via vegetative propagules (Gottschalk and Karol, 2020). Acy (2015) found that chlorine is not an effective treatment for adult NZMS using realistic exposure times. NZMS remained viable after immersion in to 200 and 400 ppm bleach solution for up to 30 minutes. Chlorine has been proven to be effective for didymo where ≥90% mortality was observed using a 2% solution for 1 minute (Root and O'Reilly, 2012). Research by Hendrick et al. (2008) found immersion in 1% (500 ppm) bleach solution for 15 minutes is effective against whirling disease. Similarly, Johnson et al. (2003) found concentrations of 1% sodium hypochlorite and above are effective at treating the fungus *Batrachochytrium dendrobatidis,* which causes chytrid disease in amphibians.

4.6 Virkon™ (Potassium Peroxymonosulfate Compounds)

Potassium Peroxymonosulfate (PPMS), like QACs are also common in household cleaners and have been more recently used for AIS decontamination. The Lanxess company developed a product line using PPMS called Virkon™, that is listed as a disinfectant and virucide. Virkon™ Rely+On is another product in this line that was referenced in some decontamination protocols that is sold in tablet form. However, Virkon™ Rely+On is not currently available in the United States (Lanxess, 2022). Virkon™ S is designed for use in terrestrial animal operations, such as the cattle and poultry industries (Lanxess, 2022) was also mentioned in some protocols. Virkon™ S is not recommended for aquatic applications because the manufacturer has developed an aquatic specific version of Virkon™ S (Lanxess, 2022). Virkon™ Aquatic, which has the same active ingredients as Virkon™ S, but without dyes, was developed for the aquaculture industry, and listed uses include cleaning and disinfecting aquaculture equipment.

Decontamination protocols listed concentrations of PPMS compounds at 0.5%, 1%, or 2% with contact times of 10 or 20 minutes. FWS protocols state that the 1-2% solution will maintain efficacy for up to 7 days (U.S. Fish and Wildlife Service, 2018). USGS states that Virkon™ negatively reacts with metals and any equipment with metal components should be rinsed with freshwater immediately after decontamination is completed (U.S. Geological Survey, 2017).

Virkon™ was referenced for use in dive gear decontamination protocols by USGS and NOAA. USGS protocols are 0.5% Virkon™ Rely+On or Virkon™ S for 10 minutes contact time (U.S. Geological Survey, 2017). NOAA protocols are for a 1% Rely+On solution or a 0.5% Virkon S solution for 10 minutes (National Oceanic and Atmospheric Administration, b).

Virkon Aquatic (Virkon) effectiveness for a range of AIS has been studied (Mohit, et al. 2021). Recent research has focused on determining the effectiveness for NZMS and dreissenid mussels. Immersion of equipment in a Virkon™ bath has been found to be more reliable than a spray application to provide complete mortality (Stockton and Moffitt, 2013; De Stasio, et al. 2019). Stockton and Moffitt (2013) found a 15–20-minute bath application of 20 g/L Virkon™ resulted in 100% mortality of both adult and neonate NZMS on boot surfaces and wading gear surfaces. Wading gear exposed to repeated bath disinfections showed little deterioration. The presence of mud reduced the chemical's effectiveness after 4-24 hours of exposure. Acy (2015) found that all NZMS treated with a 2% solution were killed after immersion for 15 min.

Virkon has also been shown to be an effective decontamination method for quagga and zebra mussels. Stockton (2011) found a bath immersion of 20 g/L Virkon™ for 20 minutes is effective as a disinfectant method for quagga mussel adults and veligers. Whereas Coughlan et al. (2020) examined the effectiveness of immersion (<90 minutes) with 2% or 4% solutions of Virkon™ and found complete mortality of zebra mussels following exposure for 90 min at both concentrations. However, high but incomplete mortality (40–90%) was recorded for quagga mussel across other disinfectant treatments.

Moffitt et al. (2015) used calcium hydroxide and sodium hydroxide to elevate the pH of Virkon™ and found it may provide a more economical way to disinfect large surfaces. Aqueous solutions of pH 12 were created with sodium hydroxide or calcium hydroxide and tested at 16°C and 20°C, and three aqueous concentrations of Virkon™ were tested at 20°C. Complete mortality was observed

within a 10-minute exposure in solutions of pH 12 prepared with calcium hydroxide and within a 30-minute exposure in solutions prepared with sodium hydroxide. Solutions of 5 g/L of Virkon™ killed all veligers within a 10-minute exposure.

Virkon™ has also been studied as a disinfectant for NZMS and quagga mussels at aquaculture facilities with a focus on the risks to fish if exposed to low residues of the chemical remaining on equipment, or if containers with disinfecting concentrations of 20 g/L were spilled into raceways or around fish holding systems (Stockton-Fiti and Moffitt, 2017). The study found that the Virkon™ treatment was effective for both species and there is very little risk to fish when exposed.

The effectiveness of Virkon™ against a variety of other AIS species including Asian clams, faucet snail, bloody-red shrimp, killer shrimp, and spiny water flea has been examined and is presented in a literature review by Mohit et al. (2021). Additional research on Asian clam by Barbour et al. (2013) found that Virkon™ achieved 93% mortality when used at 2% concentration for 5 minutes. Virkon™ at 2% for 105 minutes has also been found to be 80% effective for didymo (rock snot) (Root and O'Reilly, 2012) and for the fungus *Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis in amphibians. Exposure of cultured fungus to 1 mg of Virkon™ resulted in 100% death of zoospores and zoosporangia (Johnson, et al. 2003).

4.7 Vinegar (Acetic Acid)

Five percent acetic acid (white vinegar) was recommended in 8 of the protocols examined. Six of the protocols recommended no dilution (full strength); however, two recommended dilutions at a 1:1 ratio and a 75 mL/L ratio with tap water. Ten- or twenty-minute contact times were recommended for all protocols that specified a contact time. Montana Fish, Wildlife and Parks requires a 5% acetic acid soak for 10 minutes for all plankton net samplers and environmental DNA field equipment (Montana Fish, Wildlife and Parks, 2019). California Department of Fish and Wildlife protocols use a 1:1 ratio vinegar to tap water (no time specified) and 24-hour dry time for survey equipment (California Department of Fish and Wildlife, 2013). Vinegar is widely available, but the potential to damage equipment makes this an unpopular choice for decontamination.

Only an older California Department of Fish and Wildlife protocol mentioned vinegar as an option for cleaning dive gear but did not provide concentration or duration information (California Department of Fish and Wildlife, 2009). However, another California Department of Fish and Wildlife protocol states vinegar or other acids on should not be used on dive gear (California Department of Fish and Wildlife, a).

Acetic acid is a common decontamination method for AIS that have calcium carbonate shells, especially zebra and quagga mussels. Acetic acid may not be as effective in hard water because it has a higher buffering capacity (Stockton, 2011). Complete adult zebra mussel mortality was observed when exposed for four hours or more to 100, 75, 50 or 25% vinegar (5% acetic acid). Stockton (2011) found that soaking equipment exposed to veligers for 20 minutes in undiluted white vinegar was sufficient to kill all veligers. Another benefit of treating equipment with acetic acid is that the vinegar also degrades the shell, so that veligers can no longer be detected by microscopy.

4.8 Alcohol/ Ethanol (ETOH)/ Isopropyl Alcohol (IPA)

Alcohols are easily attainable, evaporate quickly without residue, and contact time is near instantaneous. However, they are a flammable solvent and can be dangerous in large quantities. Alcohols would also have a negative effect on adhesives and other products that react or dissolve in the presence of alcohol solvents.

The EPA dive team protocols states that using 70% isopropyl alcohol (the typically available concentration of commercially available) is "… ideal for wiping down areas under the seals of a diver's AGA mask [full face mask] (the latex seal around the diver's face mask where the mask meets the dry suit), or around the area where the diver's helmet mates to the dry suit" (U.S. Environmental Protection Agency, 2006). While it is not practical to consider using alcohol to decontaminate entire sets of gear, it may be an effective option for smaller more sensitive parts of equipment that would not be affected by use of a solvent.

4.9 Other Methods

Other decontamination methods were only listed once (Table 3). These protocols were not commonly used and therefore did not contain much information on their use. However, more time could be spent exploring some of these decontamination methods.

Table 3. Other decontamination methods mentioned in only a single agency protocol.

Other Decontamination Methods
Bright water
Chlorohexidine gluconate (FGNMS)
EasyDECON DF200
Simple Green
Betadine
Cold water high pressure
Copper compounds
Dive in the ocean with contaminated gear (dry 24 hours)
Formalin
Grapefruit seed extract solution
Hydrogen Peroxide
Lysol
Mild soap, water, and soft brush
Potassium
Potassium permanganate

4.9.1 EasyDecon DF200

One option that has potential is Intelagard EasyDECON DF200 (DF200). It is a compound created for biohazards and chemical weapons for the US military (Intelagard, 2022). It is effective against fungus, bacteria, and viruses and. The manufacturer's website states that it is peroxide based and biodegradable leaving no hazardous waste (Intelagard, 2022). DF200 is currently available for purchase by public safety divers through Diving Unlimited International (DUI), which states that "EasyDECON DF200 is ideal for dive gear and dry suit decontamination" (DUI website). DUI also created decontamination protocols using DF200 (Diving Unlimited International, 2009). DF200 has potential to be an option for decontamination for fungi, bacteria, and viruses and should be investigated further. Additional studies could determine its effectiveness on plant and invertebrate AIS.

4.9.2 Copper

Copper compounds including cupric chloride (Rao and Khan, 2000) and copper sulfate (Kennedy, et al. 2006) have been shown to be toxic to zebra mussels. More recently, commercial copper-based formulations (EarthTec) have been developed which are intended to more specifically target dreissenid mussels. Watters et al. (2013) tested the effectiveness of EarthTec for decontamination and achieved 100% mortality of adult quagga mussels after 96 hours with 17 and 5 ppm, and 100% mortality of veligers within 30 minutes at 3 ppm.

4.9.3 Dedicated Equipment

The use of dedicated equipment for specific waterbodies is an excellent strategy to avoid the spread of AIS. This option was listed in some of the protocols. The use of dedicated equipment avoids the need to decontaminate, and if done properly would eliminate the risk of spreading AIS altogether. Unfortunately, this may be cost and space prohibitive, especially with expensive equipment such as SSA equipment, submersible remotely operative vehicles, and multiprobes. However, this may be a feasible option for known infested areas that are frequently accessed, such as the Lower Colorado River system. It may also be feasible for smaller or more localized operations and teams with limited amounts of expensive field equipment. Care would need to be taken to ensure separate sets of gear are isolated to avoid cross contamination. Even if dedicated equipment is only used for the higher risk vectors such as dive suits or waders, it would still reduce the risk of spreading AIS. Reclamation mentions this as a dive gear contamination prevention option (Bureau of Reclamation, 2021).

5. Summary

The most studied and used methods for decontamination of equipment included: hot water, desiccation/air drying, chlorine bleach, QACs, freezing, saltwater, steam, and Virkon™. No single method stood out as the best solution when analyzing the practicality for dive and field gear decontamination. Additionally, some commonly used methods had data gaps regarding their efficacy for a variety of AIS. Vinegar (acetic acid) was one of the more common methods suggested for use by the protocols, but few research studies investigating its effectiveness were found. Likewise, saltwater was commonly recommended and well-studied for quagga and zebra mussels, but little research could be found on its effectiveness for other species.

Many of the effective methods used for decontamination appear to lack practicality for use with dive and aquatic sampling gear. For example, it may not be possible to achieve recommended drying (desiccation) times during an active field season or in humid climates. Additionally, definitive appropriate drying times become difficult to determine when species, humidity, sun exposure, and material types are highly variable. Other methods would be damaging to sensitive, porous, and safety equipment. The most studied and most used methods are discussed in the following paragraphs to assess their practicality for dive and field team situations.

Table 4. Comparison of the number of times a decontamination type was recommended by a protocol and the number of studies conducted on that decontamination type.

Decontamination Method Included in Protocols	# Of Protocols	# Of Studies
Desiccation/ Drying	Numerous	57
Hot Water	44	29
QACs	30	10
Saltwater Solution	20	9
Bleach	13	14
Virkon™	9	17
Freezing	9	4
Acetic Acid	9	1
Steam	5	10

5.1 Desiccation

Desiccation is well-studied, effective, cost-free, and waste-free method for decontamination of many species. Most agencies and organizations utilize desiccation to some degree. The basic message of "clean, drain, dry" that is communicated to the public includes air drying. This is a good solution for most AIS' and the least resource intensive. This was also the most studied decontamination process, and many studies show its effectiveness. Although larger macroinvertebrates were found to be more resistant to desiccation, most dive and aquatic sampling gear would not harbor larger individuals after cleaning and visual inspections are performed. Drying does require time however, which can be the most limiting factor to field going teams. Additionally, the many variables of drying/desiccation make determining minimum dry times difficult.

5.2 Hot Water

Use of hot water is an effective, fast, well studied, semi-portable, and waste free method. Hot water is one of the most studied decontamination methods because of its ubiquitous availability and use in watercraft decontamination. It is possible to take portable water heaters into the field, making field decontamination possible in certain situations. Hot water also has no hazardous waste, although the fate of rinsate must be considered before operations. Hot water has proven to be a good option for non-sensitive equipment, such as anchoring devices, rope, equipment housing, etc. when conditions

permit its use. It does pose a safety risk due to heater and hot water temperatures that may cause burns, as well as gas powered engine exhaust inhalation concerns. Hot water can also damage sensitive equipment and delaminate adhesives. Hot water is not compatible with much of the field and dive gear and can degrade adhesives and damage sensors.

5.3 QACs

QACs can be effective and are portable, QACs provide a convenient option that can be used with spray or dunk stations in the field. The effects of QACs on AIS are well studied. However, they are EPA regulated chemicals and most are not designated as a pesticide for AIS. Therefore, some legal issues may exist with use of QACs. QACs also have a waste disposal and exposure components that needs to be addressed if utilized. They are known to be hazardous and toxic to fish. Additionally, QACs are sold by a variety of manufacturers with different formulations and strengths, making it difficult to brand to brand interchangeability, a therefore, not allowing inference of study results among the different QAC solutions.

5.4 Saltwater

Saltwater solution is one of the most used decontamination methods for dive gear. Saltwater does not pose a health risk and is readily available. However, it can be messy to clean up and its effectiveness against species other than dreissenid mussels is not well studied. Studies indicated that it was not effective against the decontamination of NZMS.

5.5 Freezing

Freezing is affordable and waste free. Freezing may be second only to desiccation in simplicity. It is cheap after the initial purchase of a freezer. It has proven to be effective against multiple AIS. It also has a low time requirement compared to drying/desiccation, with an overnight freeze generally used. However, it is not field expedient and does not decontaminate some plants or viruses effectively. Sensitive equipment also may not tolerate freezing.

5.6 Steam

Steam is affordable, effective, semi-portable, and waste free. Steam has the potential to be a viable option. It is like water in its effectiveness, and it would be possible to transport portable units into the field. There would be some personnel safety concerns due to the high temperatures of the steam, but decontamination is near instantaneous. The high temperatures would not serve sensitive equipment well, as delamination of adhesives is a concern.

5.7 Virkon™

Virkon™ is also affordable, portable, and effective. Virkon has the potential for use in dive and aquatic field sampling gear since it can be taken to the field. It is also designed for aquaculture and decontaminating aquaculture equipment, so organizations may be able to appropriately use the product for decontamination of field and sampling equipment. Virkon is one of the most studied chemical decontaminants and may pose lesser environmental and/or human health risks than QACs.

5.8 Dedicated Equipment

Dedicated equipment provides the best assurance of preventing the spread of AIS. The only way dedicated equipment could become contaminated is if it were improperly managed and contaminated by unintentionally taking out of the designated waterbody or by storing with other field equipment. The costs and storage space will be higher with redundant sets of equipment which may not be feasible with high cost or bulky equipment.

6. Discussion

Compiling protocols and studies was a helpful process for determining what decontamination protocols are currently being used and what studies have been conducted. Fortunately, the most common AIS protocols were generally aligned with the most studied methods. While studies supported use of most of the common methods, there were also gaps and inconsistencies in methods and studies. Saltwater for example, does not seem to be well studied for decontamination of species other than dreissenid mussels and additional studies should be considered. It is true that most decontamination methods could benefit from additional research, especially for any emerging species of concern. No single best approach for AIS decontamination emerged from this analysis, although some are more viable for specific situations than others. Rather, it was made evident that having a proactive and flexible AIS program that evaluates different scenarios and tailors' techniques to specific conditions, equipment, and AIS is the best approach that should be taken. Identifying acceptable decontamination options, AIS risk, and developing protocols that allow situational flexibility may be the best strategy for preventing the spread of AIS.

Some agencies have implemented Hazard Assessment and Critical Control Points (HACCP) programs to their AIS decontamination process. This type of system offers a framework to incorporate decontamination methods or strategies that help minimize the risk of spread based on the specific situation. Since there does not seem to be a one-size-fits-all decontamination method, a systematic approach to analyzing the specific conditions for events should be developed. Whether this is through the HACCP process or some similar risk analysis process, the specific factors of events should be identified and evaluated to help choose the best options.

Factors such as known and potential AIS, number of waterbodies to be visited, time between events, environmental conditions (ambient temperatures, humidity), necessary equipment, local AIS

guidance and requirements should all be considered during the analysis process. A "toolkit" of options could be developed that lays out AIS decontamination effectiveness, hazardous waste, public health, environmental toxicity, and resource availability. The goal of the toolkit and process would be to efficiently identify key specifics of an event and the best available options to match the specific conditions. This would allow teams to incorporate this process into field planning in much the same way as a job hazard analysis is conducted.

There is not one decontamination method that has been thoroughly studied for its effectiveness against all AIS. Additionally, studies need to isolate variables and therefore, even if a study has been conducted on a species, it likely eliminated some variables that may factor into the method's effectiveness in the multitude of potential real world scenarios. Studies on the use of saltwater to decontaminate for AIS other than dreissenids and NZMS is very limited. This is concerning since saltwater is one of the most used decontamination methods for dive teams. It may not provide true AIS decontamination, rather only decontamination of dreissenid mussels, where dive and aquatic field sampling teams should be conducting AIS decontamination for all AIS' of concern in a waterbody. Several studies of Virkon™ have been conducted. However, the effectiveness of Virkon on several AIS has not been conducted. EasyDECON DF200 appears to have potential, at least for viral and fungal AIS, and studies on additional AIS should be conducted. It would be impractical to test all methods on all AIS species but replicating decontamination studies on different species or functional groupings could be relatively simple and important step toward expanding the understanding of a decontamination method's overall effectiveness. Better AIS decontamination decisions can be made as additional research is conducted to fill in data gaps.

- Data Availability:
    - Share Drive folder name and path where data are stored: \\bor\do\TSC\Jobs\DO_NonFeature\Science and Technology\2021-PRG-AIS Decontamination Methods
    - Data Contact: Sherri Pucherelli, spucherelli@usbr.gov, 303-445-2015
    - Description of the data: Agency protocols, manuscripts, final reports, and data summary tables

7. Literature Cited

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Appendix A

Most referenced methods in agency decontamination protocols

Decontamination Method	Agency/Organization
Acetic acid (vinegar)	Bureau of Reclamation, 2021 Bureau of Reclamation, 2022 California Department of Fish and Wildlife, a Government of Quebec, 2018 Michigan Department of Environmental Quality, 2014 Montana Fish, Wildlife and Parks, 2019 National Oceanic and Atmospheric Administration, a State of Oklahoma Water Resources Board, 2017 U.S. Forest Service Wisconsin Department of Natural Resources, 2020
Bright water	U.S. Forest Service
Chlorine bleach (sodium hypochlorite)	Bureau of Reclamation, 2021 Cockman et al., 2012 Declining Amphibian Task Force Government of Quebec, 2018 Michigan Department of Environmental Quality, 2014 Montana Fish, Wildlife and Parks, 2019 National Ecological Observatory Network, 2015 National Oceanic and Atmospheric Administration, b National Park Service State of Oklahoma Water Resources Board, 2005 U.S. Fish and Wildlife Service, 2018 U.S. Forest Service Utah Department of Natural Resources, 2012 Wisconsin Department of Natural Resources, 2016 Wisconsin Department of Natural Resources, 2020
Chlorohexidine gluconate (FGNMS)	National Oceanic and Atmospheric Administration, b
Cold water high pressure	Government of Quebec, 2018
Copper ions	Utah Department of Natural Resources, 2012
Dedicated equipment	Bureau of Reclamation, 2021
Dry/ Desiccate	California Department of Fish and Wildlife, 2013 Cockman et al., 2012

	Government of Quebec, 2018 Oregon Department of Fish and Wildlife Pennsylvania Sea Grant National Ecological Observatory Network, 2015 State of Oklahoma Water Resources Board, 2005 Utah Department of Natural Resources, 2012 U.S. Forest Service Washington Department of Ecology Wisconsin Department of Natural Resources, 2016 Wisconsin Department of Natural Resources, 2020
Easydecon DF200	Diving Unlimited International, 2009
Ethanol	Declining Amphibian Task Force State of Oklahoma Water Resources Board, 2005
Formalin	National Oceanic and Atmospheric Administration, a
Freeze	Bureau of Reclamation, 2021 California Department of Fish and Wildlife, 2013 Government of Quebec, 2018 Oregon Department of Fish and Wildlife Santa Clara Valley Habitat Agency, 2021 South Dakota Department of the Environment and Natural Resources Watershed Protection Program, 2020 U.S. Fish and Wildlife Service, 2018 U.S. Forest Service Washington Department of Ecology Washington Department of Fish and Wildlife, 2016 Wisconsin Department of Natural Resources, 2020
Grape seed oil extract	State of Oklahoma Water Resources Board, 2005
Hot water	Alberta Environment and Parks, 2017 Bureau of Reclamation, 2021 California Department of Fish and Wildlife, a California Department of Fish and Game, 2009 California Department of Fish and Wildlife, 2013 Cockman et al., 2012 Colorado Parks and Wildlife, 2012 Government of Quebec, 2018 Michigan Department of Environmental Quality, 2014 Montana Fish, Wildlife and Parks, 2019 National Ecological Observatory Network, 2015 National Oceanic and Atmospheric Administration, a

	Oregon Department of Fish and Wildlife Santa Clara Valley Habitat Agency, 2021 South Dakota Department of the Environment and Natural Resources Watershed Protection Program, 2020 Texas Parks and Wildlife Department, 2013 Texas Parks and Wildlife Department, 2015 Utah Department of Natural Resources, 2012 U.S. Forest Service U.S. Geological Survey, 2016 U.S. Fish and Wildlife Service, 2018 Washington Department of Ecology Washington Department of Fish and Wildlife, 2016 Wisconsin Department of Natural Resources, 2016 Wisconsin Department of Natural Resources, 2020 Wyoming Game and Fish Department, 2019 California Department of Fish and Wildlife, a
Hydrogen peroxide	National Oceanic and Atmospheric Administration, a
Isopropyl alcohol wipes	National Oceanic and Atmospheric Administration, b
Lysol	National Oceanic and Atmospheric Administration, b State of Oklahoma Water Resources Board, 2005
Desiccation/ Air drying	Gottschalk and Karol, 2020
Mild soap, water, and brush	State of Oklahoma Water Resources Board, 2005
Potassium	California Department of Fish and Game, 2009
Potassium permanganate	Bureau of Reclamation, 2021 National Oceanic and Atmospheric Administration, a
QACs	Alberta Environment and Parks, 2017 Bureau of Reclamation, 2021 Cockman et al. 2012 Colorado Parks and Wildlife Department of Natural Resources, 2015 Idaho Department of Environmental Quality Michigan Department of Environmental Quality, 2014 National Ecological Observatory Network, 2015 National Oceanic and Atmospheric Administration, a National Oceanic and Atmospheric Administration, b National Park Service Santa Clara Valley Habitat Agency, 2021

	South Dakota Department of Environment and Natural Resources Watershed Protection Program, 2016 State of Oklahoma Water Resources Board, 2005 Utah Department of Natural Resources, 2012 Washington Department of Fish and Wildlife, 2016 Wisconsin Department of Natural Resources, 2020 Wyoming Game and Fish Department, 2019
Saltwater solution (sodium chloride)	Aquatic Nuisance Species Task Force, 2013 Bureau of Reclamation, 2021 California Department of Fish and Wildlife, a California Department of Fish and Game, 2009 Colorado Parks and Wildlife, 2012 Michigan Department of Environmental Quality, 2014 Minnesota Department of Natural Resources, 2015 Montana Fish, Wildlife and Parks, 2019 Nevada Department of Wildlife Pennsylvania Sea Grant State of Oklahoma Water Resources Board, 2005 U.S. Fish and Wildlife Service, 2018 U.S. Forest Service Wisconsin Department of Natural Resources Wisconsin Department of Natural Resources, 2020
Steam	Government of Quebec, 2018 National Oceanic and Atmospheric Administration, a Michigan Department of Environmental Quality, 2014 Wisconsin Department of Natural Resources, 2016 Wisconsin Department of Natural Resources, 2020
Virkon	Michigan Department of Environmental Quality, 2014 National Oceanic and Atmospheric Administration, b U.S. Fish and Wildlife Service, 2018 U.S. Geological Survey, 2016 U.S. Geological Survey, 2017 Washington Department of Fish and Wildlife, 2016 Wisconsin Department of Natural Resources, 2016 Wisconsin Department of Natural Resources, 2020

Appendix B

List of decontamination methods and the aquatic invasive species that have been investigated, including literature source.

Decontamination Method	AIS Examined	Literature
Acetic Acid	Zebra mussel (*Dreissena polymorpha*)	Davis et al. 2015
Alum	Quagga mussel (*Dreissena rostriformis bugensis*)	Takeguchi et al. 2012
Benzalkonium Chloride	Zebra mussel	Waller et al. 1996
Calcium Chloride	Zebra mussel	Edwards et al. 2000, Waller and Fisher 1998
Calcium hydroxide	Quagga mussel	Moffitt et al. 2015
Chloramine	Quagga mussel	Takeguchi et al. 2012
Chloride Salts	Zebra mussel	Waller et al. 1996
Chlorine Bleach (Sodium Hypochlorite)	Asian clam (*Corbicula fluminea*)	Barbour et al. 2013
	Bloody-red shrimp (*Hemimysis anomala*)	De Stasio et al. 2019
	Didymo (*Didymosphenia geminata*)	Root and O'Reilly 2012
	Killer shrimp (*Dikerogammarus villosus*)	Sebire et al. 2018
	New Zealand mud snail (*Potamopyrgus antipodarum*)	Acy 2015; Ethaiya 2018
	Quagga mussel	Takeguchi et al. 2012
	Spiny waterflea (*Bythotrephes cederstroemi*)	De Stasio et al. 2019
	Starry stonewort (*Nitellopsis obtusa*)	Gottschalk and Karol 2020
	Water flea (Cladocera)	Tremblay et al. 2019
	Whirling disease (*Myxobolus cerebralis*)	Hedrick et al. 2008

	Zebra mussel	Brady et al. 1996; Van Benschoten et al. 1995;
Chlorine Dioxide	Quagga mussel/ zebra mussel	Takeguchi et al. 2012; Matisoff et al. 1996
Desiccation/ air drying	Starry stonewort	Gottschalk and Karol 2020
	Asian clam	Collas et al. 2014 a
	Dark false mussel (*Mytilopsis leucophaeata*)	Collas et al. 2014 a
	Golden mussel (*Limnoperna fortunei*)	Montalto and de Drago 2003; Darrigran et al. 2004
	Quagga mussel	Ricciardi et al. 1995; Collas et al. 2018; Snider et al. 2014
	Zebra mussel	Anderson et al. 2015; Banha et al. 2016; Collas et al. 2014 a; McMahon 1993; Ricciardi et al. 1995; Collas et al. 2018; Mohit et al. 2021
	Red swamp crayfish (*Procambarus clarkii*)	Banha and Anastacio 2014; Piersanti et al. 2018 a
	Signal crayfish (*Pacifastacus leniusculus*)	Banha and Anastacio 2014
	Bladder snail (*Physa acuta)*	Collas et al. 2014 a
	Banded mystery snail (*Vivaparus georgianus*)	Havel et al. 2014; Mohit et al. 2021
	Channeled applesnail/ golden applesnail (*Pomacea canaliculata*)	Bernatis et al. 2016; Yoshida et al. 2014
	Chinese mystery snail (*Cipangopaludina chinensis*)	Havel 2011
	Island apple snail (*Pomacea maculate*)	Bernatis et al. 2016; Yoshida et al. 2014
	New Zealand mud snail	Collas et al. 2014 a; Richards et al. 2004
	Faucet snail (*Bithynia tentaculate*)	Wood et al. 2011
	Killer shrimp	Anderson et al. 2015

	Bloody-red shrimp	Anderson et al. 2015; De Stasio et al. 2019
	Water flea	Tremblay et al. 2019; Mohit et al. 2021
	Copepod	Tremblay et al. 2019
	Parrot's feather (*Myriophyllum aquaticum*)	Anderson et al. 2015
	Floating pennywort (*Hydrocotyle ranunculoides*)	Anderson et al. 2015
	Least duckweed (*Lemna minuta*)	Coughlan et al. 2018
	Water fern (*Azolla filiculoides*)	Coughlan et al. 2018
	Canadian waterweed (*Elodea canadensis*)	Coughlan et al. 2018
	Coontail (*Ceratophyllum demersum*)	Barnes et al. 2013
	Curly water-thyme/ African elodia (*Lagarosiphon major*)	Coughlan et al. 2018; Anderson et al. 2015
	Curly-leaf pondweed (*Potamogeton crispus*)	Barnes et al. 2013; Bruckerhoff et al. 2015
	Eurasian watermilfoil (*Myriophyllum spicatum*)	Barnes et al. 2013; Jerde et al. 2012; Evans et al. 2011; Bruckerhoff et al. 2015; Mohit et al. 2021
	Nuttall's waterweed (*Elodea nuttallii*)	Coughlan et al. 2018
	Waterthyme (*Hydrilla verticillate*)	Banizewski et al. 2016
	Carolina fanwort (*Cabomba caroliniana*)	Barnes et al. 2013; Bickel 2015; Mohit et al. 2021
	New Zealand pygmyweed/ swamp stonecrop (*Crassula helmsii*)	Anderson et al. 2015

	European frogbit (*Hydrocharis morsus-ranae*)	Mohit et al. 2021
Dish liquid detergent	Didymo	Root and O'Reilly 2012
Dry Ice	Zebra mussel/ quagga mussel	Coughlan et al. 2020
EarthTec	Quagga mussel	Watters et al. 2013
Ferric Chloride	Quagga mussel	Takeguchi et al. 2012
Formula 409®	New Zealand mud snail	Acy 2015; Schisler et al. 2008; Ethaiya 2018
	Whirling disease	Hedrick et al. 2008
Freezing	Starry stonewort	Gottschalk and Karol 2020
	Quagga mussel/ zebra mussel	McMahon et al. 1993
	Whirling disease	Hedrick et al. 2008
	Faucet snails	LaFond et al. 2021
Green Solutions Hi Dilution 256®	New Zealand mud snail	Stout et al. 2015;
	Zebra mussel/ quagga mussel	Britton and Dingman 2010
Hot air	Zebra mussel/ quagga mussel	Coughlan et al. 2020
Hot water (spray and immersion)	Zebra mussel/ quagga mussel	Comeau et al. 2011, 2015; Morse 2009; Beyer et al. 2011; Snider et al. 2014 a; Anderson et al. 2015; Shannon et al. 2018; Watters 2014; Jerde et al. 2012; Mohit et al. 2021
	Asian clam	Coughlan et al. 2019 a b
	Signal crayfish	Anderson et al. 2015
	Killer shrimp	Anderson et al. 2015; Sebire et al. 2018
	Bloody-red shrimp	Anderson et al. 2015; De Stasio et al. 2019
	Spiny water flea	Beyer et al. 2011
	Water flea	Tremblay et al. 2019; Mohit et al. 2021
	Copepod	Tremblay et al. 2019

	Parrot's feather	Anderson et al. 2015; Shannon et al. 2018
	Floating pennywort	Anderson et al. 2015
	Curly water-thyme/ African elodia	Anderson et al. 2015
	Eurasian watermilfoil	Blumer et al. 2009; Mohit et al. 2021
	New Zealand pygmyweed/ swamp stonecrop	Shannon et al. 2018
	Banded mystery snails (*Viviparus georgianus*)	Mohit et al. 2021
	Carolina fanwort	Mohit et al. 2021
	European frogbit	Mohit et al. 2021
Hydrogen Peroxide	Zebra mussel	Waller and Fisher 1998
Hypochlorite	Zebra mussel	Klerks and Fraleigh 1991
Ozone	Quagga mussel	Takeguchi et al. 2012
Path X	Chytrid disease (*Batrachochytrium dendrobatidis*)	Johnson et al. 2003
Permanganate	Zebra mussel	Klerks and Fraleigh 1991
	Chytrid disease	Johnson et al. 2003
Peroxide with Iron	Zebra mussel	Klerks and Fraleigh 1991
PolyDADMAC	Quagga mussel	Takeguchi et al. 2012
Potassium Chloride	Zebra mussel	Davis et al. 2018, Moffitt et al. 2016; Edwards et al. 2000; Waller and Fisher 1998
Potassium Chloride and Formalin	Quagga mussel	Pucherelli et al. 2014
	Zebra mussel	Edwards et al. 2000
Quat™ 128	Quagga mussel	Watters 2014
	Chytrid disease	Johnson et al. 2003
Quat™ 256	Quagga mussel	Watters 2014
Quat 4	New Zealand mud snail	Stout et al. 2015
Salt water	New Zealand mud snail	Acy 2015
	Asian clam	Barbour et al. 2013
	Quagga mussel	Hofius et al. 2015
	Island applesnail (*Pomacea maculate*)	Underwood et al. 2019

	Water flea	Tremblay et al. 2019
	Didymo	Root and O'Reilly 2012
Sodium Chloride	Zebra mussel	Davis et al. 2018, 2016; Edwards et al. 2002, 2000
	Chytrid disease	Johnson et al. 2003
Sodium Hydroxide	Quagga mussel	Moffitt et al. 2015
Sparquat 256®	Zebra mussel/ quagga mussel	Britton and Dingman 2010
	New Zealand mud snail	Schisler et al. 2008
Steam	Zebra mussel/ quagga mussel	Coughlan et al. 2020
	Starry stonewort	Gottschalk and Karol 2020
	Asian clam	Coughlan et al. 2019 a b
	Brazilian waterweed (*Egeria densa*)	Crane et al. 2019
	Canadian waterweed	Crane et al. 2019
	Coontail	Crane et al. 2019
	Nuttall's waterweed	Crane et al. 2019
	Curly water-thyme/African elodea	Crane et al. 2019
	Curly-leaf pondweed	Crane et al. 2019
	New Zealand pygmyweed/swamp stonecrop (*Crassula helmsii)*	Crane et al. 2019
Super HDQ Neutral	New Zealand mud snail, Whirling disease	Stout et al. 2016
Virasure Aquatic	Zebra mussel/ quagga mussel	Coughlan et al. 2020
Virkon™ Aquatic	Zebra mussel	Coughlan et al. 2020
	Asian clam	Barbour et al. 2013
	Quagga mussel	Stockton-Fiti and Moffitt 2017; Stockton 2011; Moffitt et al. 2015; Coughlan et al. 2020;
	Didymo	Root and O'Reilly 2012
	Faucet snail	De Stasio et al. 2019
	New Zealand mud snail	De Stasio et al. 2019; Stockton and Moffitt 2013; Stockton-Fiti and Moffitt 2017; Ethaiya 2018

	Bloody-red shrimp	De Stasio et al. 2019
	Killer shrimp	Sebire et al. 2018
	Spiny waterflea	De Stasio et al. 2019
	Chytrid disease	Johnson et al. 2003