Fisheries and Oceans Canada | Pêches et Océans Canada
Ecosystems and Oceans Science | Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2022/055

National Capital Region

"Clean, Drain, Dry, and Decontaminate" treatments and protocols to prevent the introduction and spread of aquatic invasive species

A.M. Weise, N. Simard, V. Massé-Beaulne, and J.M. Hill

Fisheries and Oceans Canada
Maurice Lamontagne Institute
850 route de la mer
Mont-Joli, QC G5H 3Z4
Canada

December 2022

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-45265-4 Cat. No. Fs70-5/2022-055E-PDF

Correct citation for this publication:

Weise, A.M., Simard, N., Massé-Beaulne, V. and Hill, J.M. 2022. “Clean, Drain, Dry, and Decontaminate” treatments and protocols to prevent the introduction and spread of aquatic invasive species. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/055. x + 91 p.

Aussi disponible en français :

Weise, A.M., Simard, N., Massé-Beaulne, V. et Hill, J.M. 2022. Traitements et protocoles "Lavez, Videz, Séchez et Décontaminez" pour prévenir l'introduction et la propagation des espèces aquatiques envahissantes. Secr. can. des avis sci. du MPO. Doc. de rech. 2022/055. xi + 104 p.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Aquatic invasive species (AIS) pose a significant threat to Canadian fresh, estuarine, and marine waters and threaten Canada's biodiversity, economy, and society. To prevent the introduction and spread of AIS by water-based commercial and recreational activities, many government and non-government organizations encourage owners and operators to voluntarily Clean, Drain, and Dry (CDD) their watercraft, trailers, and equipment. In some cases, an additional Decontamination step may be applied (CDD+D) which has species-specific treatment parameters to achieve AIS mortality and/or removal. To date, a comprehensive evaluation of the effectiveness of CDD+D protocols used in Canada for marine and freshwater AIS has not been conducted. This research document provides a review, by species, of effective decontamination treatments identified in the scientific literature and suggests treatment guidelines aimed to kill the greatest number of target AIS taxa. The effectiveness of recommendations in existing freshwater and marine CDD+D protocols used in AIS management across Canada or abroad were also assessed. Lethal decontamination treatments for AIS of interest from different functional and taxonomic groups (e.g., bivalves, gastropods, zooplankton, macrophytes, macroalgae, crabs, and tunicates) were evaluated and included physical (e.g., hot water spray/immersion, pressure washing, air-drying, and freezing) and chemical (e.g., sodium hypochlorite, acetic acid, quaternary ammonium compounds, salt water, Virkon©, brine, and hydrated lime) sprays/immersions or a combination of these. The scientific literature showed that several decontamination treatments can be lethal for AIS but only if applied for specific exposure times and conditions. Recommendations in government or state protocols mostly echoed the scientific literature and underline that CDD campaigns should continue to be supported across the country. In some cases, when additional decontamination is required, (e.g., a watercraft is at high risk of transporting AIS), temperature, pressure, and/or chemical treatments may need to be adjusted to ensure 100% mortality of a greater number of target AIS. Although numerous species- or environment-specific decontamination treatments were identified as effective at killing or removing AIS, no single decontamination treatment was applicable to all freshwater and marine AIS or to all watercraft and equipment. The results from this study will help develop national CDD+D recommendations and provide advice to Fisheries and Oceans Canada's regulatory programs and to the Canadian public.

GLOSSARY[1]

Air-drying: A decontamination treatment if used under specific conditions (e.g., temperature and exposure time) that is lethal for the target aquatic invasive species. This treatment can be used alone or in combination with other treatments to enhance its effectiveness (e.g., hot water, chemical immersion, etc.). As methodologies vary between studies, air-drying may refer to drying organisms in a laboratory setting or outside (direct or indirect sunlight), exposed individually or in clusters, on tables or suspended (e.g., mussel socks), etc. Air-exposure, aerial exposure and drying are used as synonyms in the literature.

Aquatic invasive species (AIS): A non-indigenous aquatic species (e.g., fish, animal, and plant species) that has a negative ecological, human health, and/or economic impact after its introduction, establishment and/or spread into a new ecosystem. Aquatic invasive alien species, nuisance species, invaders, exotic, and introduced species are used as synonyms in the literature.

Clean, Drain, Dry (CDD): Campaigns used to raise awareness about aquatic invasive species and encourage the public to follow voluntary actions to reduce the likelihood of transporting aquatic invasive species when moving recreational watercraft and equipment between waterbodies. These three sequential steps usually refer to:

- **Clean:** Inspect, remove, and clean all plants, animals, mud, dirt, debris, surface deposits from all watercraft parts (interior and exterior), trailer and equipment.
- **Drain**: Empty all livewells, bait-wells, storage compartments, bilge areas, engine compartments, decks, ballast tanks, water storage and delivery systems, cooler or other water storage areas from the watercraft, trailer, engine, or equipment onto dry land.
- **Dry**: Dry all watercraft parts (interior and exterior), trailer, and equipment before launching into another waterbody. No standing water. Drying can be done by either air-drying over several days, or using towels, wet/dry vacuums, or pressurized air.

Decontamination: A treatment with the intent to kill, destroy, and/or remove aquatic invasive species to prevent their spread in Canadian waters. Decontamination may be achieved by a physical or chemical treatment that is specifically parameterized (e.g., exposure times, chemical concentrations, temperature, etc.) to ensure the mortality of targeted species.

Effectiveness: The level to which a decontamination treatment can kill a targeted invasive species. Effectiveness is expressed quantitatively (as percent mortality or removal) or qualitatively (effective or not effective). In the present work, the effectiveness of physical and chemical decontaminations were categorized as effective if treatments resulted in ≥ 99% mortality.

Equipment: Any material or gear that comes into contact with water. It can include items that are worn or used on a watercraft (e.g., safety equipment such as personal flotation devices (PFDs); accessories such as anchors, paddles, ropes, waders, boots, nets, buckets, coolers, scientific equipment, inflatables, beach toys, etc.) for water-related activities (e.g., boating, fishing, paddling, scuba-diving, swimming, hunting, etc.).

[1] Some definitions reproduced and/or modified from the Canadian Action Plan to Address the Threat of Aquatic Invasive Species – September 2004. and the Uniform Minimum Protocols and Standards for Watercraft Inspection and Decontamination Programs for Dreissenid Mussels in the Western United States (UMPS IV). Elwell and Phillips 2021.

Freezing: A decontamination treatment if used under specific conditions (e.g., temperature and exposure time) that is lethal for the target aquatic invasive species. Small equipment can be frozen in a freezer while watercraft (and larger equipment) can be frozen when removed from water and left outside when winter air temperatures are below freezing.

Immersion: A decontamination treatment if used under specific conditions (e.g., concentration and exposure time) that is lethal for the target aquatic invasive species. In the scientific literature, individuals or clusters of AIS were completely submerged in cold or hot water or chemical solutions. These immersions are most useful for small equipment but recently developed “dip tanks”, i.e. large heated water tanks into which boaters can back their watercraft, may allow this treatment to be applied to watercraft.

Mortality: Organism death. Mortality is achieved when organisms are dead and show no signs of movement or vital activity (e.g., cessation of growth, feeding, response to tactile stimulation, or reduction of biomass, etc.). Mortality is expressed in %.

Native species: A species that occurs naturally in a given area or habitat (i.e. historical range), as opposed to an introduced species or invasive species. Indigenous species is used as a synonym in the literature.

Natural/native range: The geographical area where a species originated from.

Non-indigenous species (NIS): Plant, animal, or microorganism occurring in an area outside of its known natural habitat or range, which may have a negative ecological, human health, and/or economic impact after its introduction, establishment and/or spread into a new ecosystem. Invasive species, alien species, nuisance species, invaders, exotic, and introduced species are used as synonyms in the literature.

Pathway: One or more routes by which an invasive species is transferred from one ecosystem to another.

Pressure washing: A decontamination treatment (freshwater and/or seawater sprays) if used under specific conditions (e.g., spray pressure, temperature, and exposure time) that is able to remove and/or kill the target aquatic invasive species. In this document, low-pressure washing refers to sprays with water pressures below 60 psi (e.g., garden hoses) while high-pressure sprays refer to greater than 400 psi. Water can be heated to increase effectiveness (i.e. mortality of AIS) of pressure washing.

Primary introduction: The first introduction of an invasive species into a non-indigenous ecosystem.

Propagule: Any material (e.g., seed, spore, larvae, etc.) that functions in propagating an organism to the next stage in its life cycle.

Propagule pressure: In invasion ecology, propagule pressure refers to the introduction effort represented by the number and frequency of release events of propagules being introduced to a new (uninvaded) area. It incorporates estimates of the absolute number of individuals involved in any one release event (propagule size) and the number of discrete release events (propagule number). As the number of releases and/or the number of individuals released increases, propagule pressure also increases.

Removal: Refers to the removal of organisms from watercraft and equipment by scrubbing, scraping, wiping and/or pressure washing. The removal of aquatic invasive species does not ensure their mortality.

Secondary spread: Where an invasive species increases its geographic range following initial invasion. Secondary spread can be facilitated by human-mediated activities such as recreational boating activities, shellfish transfers, aquarium trade.

Vector: The physical means by which an invasive species is transported from one area to another. These vectors can be natural (e.g., wind, currents, and animals) or anthropogenic (e.g., ballast water, hull fouling, aquaculture, and aquarium trade).

Watercraft: a motorized or non-motorized vessel that travels in or on water. Watercraft can include: boats, canoes, kayaks, paddle boards, etc. In the present work, these were limited to watercraft under 24 m in length.

1. INTRODUCTION

The establishment of invasive species in aquatic ecosystems is considered one of the primary drivers of biodiversity loss, with serious consequences for both ecological and economic functioning (Mack et al. 2000; Clavero and García-Berthou 2005; Costello et al. 2010; Simberloff et al. 2013; Gallardo et al. 2019). Most regions on the planet have been subjected to non-indigenous species invasions (Vitousek et al. 1996), the vast majority of which can be attributed to anthropogenic activities associated with increasing globalization and trade, creating a greater range of pathways of introduction (Ricciardi 2006; Hulme 2009). These invasions are now occurring over unprecedented temporal and spatial scales (Ruiz et al. 2000; Ricciardi 2006, 2007), often mediated by environmental and socioeconomic factors (Mills et al. 1993, 1994; Ricciardi 2006). The ecological effects of invaders may have impacts across multiple levels of organization and trophic levels (Brennan et al. 2014; Jackson 2015; Jackson et al. 2017), and include behavioral shifts in native species (Jackson et al. 2017; Langkilde et al. 2017), alteration of native habitat (Scheffer 2009; Strayer 2012; Simberloff et al. 2013), alteration of food webs and trophic dependencies (Vander Zanden et al. 1999; Strayer 2010) and, in some cases, extirpation of native biota (Lodge and Shrader-Frechette 2003; Simon and Townsend 2003). Furthermore, with the progression of climate change, the management of invasive species and their associated impacts becomes even more challenging, as geographic ranges of some invasive species are expected to shift as the climate warms (Dukes and Mooney 1999; Bradley et al. 2010), and where climate change stressors (Bellard et al. 2013) and climate extremes (Diez et al. 2012) create new opportunities for introduced species to establish and thrive (Beaury et al. 2020). Aquatic Invasive Species (AIS) that are introduced or spread to ecosystems beyond their natural range can threaten Canada's biodiversity, economy, and society.

The documented invasion history of North America spans approximately two centuries and implicates multiple vectors or pathways of invasion, the most important of which in modern times has been the release of ballast water from ocean vessels (Mills et al. 1993; Ricciardi 2006; Kelly 2007; Bailey 2015). Ballast water release has driven primary introductions of many AIS, also referred to as Non-Indigenous Species (NIS), throughout the world, and in response, there has been a global movement towards regulating ballast water release (David 2015; Chan and Briski 2017), which has resulted in an international convention for the control and management of ships' ballast water and sediments which was implemented in 2017 (International Maritime Organization 2004). Management and control of AIS introduction pathways are critical to the protection and conservation of native aquatic ecosystems (Mack et al. 2000; Ricciardi 2007; Blackburn et al. 2011; Saul et al. 2017); but introductions via deliberate and compassionate releases, range extensions via natural dispersal, hull fouling and recreational boating can complicate invasive species management (e.g., Ricciardi et al. 1995; Johnson et al. 2001; Magellan 2020). Recreational boating and hull fouling in particular have been identified as major vectors contributing to the spread and establishment of marine and freshwater AIS (Johnson et al. 2001; Rothlisberger et al. 2010; Clarke Murray et al. 2011; Lacoursière-Roussel et al. 2012; Kelly et al. 2013; Pelletier-Rousseau et al. 2019; Mohit et al. 2021). Water-based commercial and recreational activities (e.g., SCUBA, boating, fishing, paddling, swimming etc.) can unintentionally spread AIS to new locations if species hitchhike on watercraft, trailers, and equipment (e.g., smaller equipment which has contact with water, which includes, but is not limited to: boots, waders, fishing rods, inflatables, jet skis, kayaks etc.) or if they are transported in standing water (e.g., bilge water and livewells). Consequently, if not already in practice, any future AIS management strategies should consider some type of boating/water equipment cleaning guidelines and regulations, to reduce boater/water

equipment-mediated spread (Rothlisberger et al. 2010; Kelly et al. 2013; Pelletier-Rousseau et al. 2019; Mohit et al. 2021).

In most Canadian provinces, recreational users of aquatic resources (primarily in the freshwater environment) are encouraged to follow voluntary actions to reduce the likelihood of transporting AIS when moving watercraft and equipment between waterbodies and are referred to as sequential steps to “Clean, Drain, and Dry” (CDD) (e.g. Canadian Council on Invasive Species, 2021). Similar awareness campaigns exist worldwide (e.g., New Zealand Government 2020 and Great Britain Non-Native Species Secretariat 2021) to prevent the spread of AIS when moving between waterways. CDD guidelines aim to be concise and easy to follow, to encourage the participation of the general public. Although guidelines in Canada may vary between regions and organizations, they generally include the following steps:

The first step, “clean”, consists of inspecting and cleaning the watercraft, trailer, and equipment that made contact with the waterbody. All visible plant fragments, animals, mud, and other organic debris should be removed and disposed of on land. The watercraft, trailer, and equipment should be washed, scrubbed and/or rinsed. All small items that can be immersed, should be cleaned by hand washing on-site. The cleaning step should be completed on dry land, away from storm drains, ditches or waterways to limit the risks of re-introduction of organisms to aquatic ecosystems. Local car washes should be avoided if AIS are present, as they could make their way into the environment through municipal drainage systems.

The second step, “drain,” involves draining all water from the watercraft, trailer, and equipment. This includes draining all spaces or items that can hold water, such as internal compartments (ballasts, bilges, livewells, etc.) and equipment (coolers, bait buckets, ropes, etc.). Water should be drained from the engines and the watercraft tilted when stored to allow the bilge to drain. In Alberta, Manitoba, and Saskatchewan, it is illegal to transport watercrafts between waterbodies with the drain plug in place.

The third step, “dry,” consists of completely drying all parts of the watercraft and equipment and to ensure there is no standing water. Drying can be done by either air-drying over several days, or using towels, wet/dry vacuums, or pressurized air. Everything must be dry to the touch before entering a new waterbody.

In situations where there is an identified (or higher) risk that a watercraft or water-related equipment may be transporting AIS, an additional “decontamination” step may be applied (CDD+D), which involves either a temperature, pressure, or chemical treatment and/or a combination of these. CDD+D is dependent on the target species, the watercraft/equipment type to be disinfected, and is often (but not always) performed by trained personnel with specialized equipment.

CDD and CDD+D are not mutually exclusive steps and in some cases, contain similar elements. For example, drying is implicit in CDD protocols but can also be a decontamination method. While CDD provides a series of best practices for public consideration, CDD+D methods have species-specific treatment parameters that aim to ensure a particular, quantified level of AIS mortality or removal.

To date, a comprehensive evaluation of the effectiveness of CDD+D protocols used in Canada for marine and freshwater AIS has not been conducted. The need for a fulsome review is compounded by the fact that a wide variety of methods are endorsed and used by different organizations without national consistency. To address this gap, a science request was initiated by Fisheries and Oceans Canada (DFO) AIS National Core Program, the governing body responsible for the implementation of federal AIS regulations at both national and regional

levels, to develop national Clean, Drain, Dry & Decontaminate recommendations and to provide advice to DFO's regulatory programs and the Canadian public.

The objectives of this research document were to:

- Complete a review of the scientific literature on decontamination treatments for the removal and/or mortality of freshwater and marine AIS and of the existing freshwater and marine CDD+D protocols used in AIS management in Canada or abroad;
- Assess the effectiveness of decontamination treatments and existing CDD+D protocols at reducing the propagule pressure of marine and freshwater AIS along the overland transportation pathway.

The scope of this project was limited to watercraft under 24 m in length, trailers, and equipment that move from water to land before entering a new waterbody (including equipment used in work, undertakings, and activities (WUAs) which take place in water), excluding those that remain in the water. Large commercial vessels (> 24 m) were not within the scope of this work, nor were forest firefighting equipment or floatplanes.

Following this review, common elements across protocols could be identified by AIS management programs to derive best management practices for CDD+D in Canada, for use in AIS regulatory tools such as Fisheries Act S.34/35 authorizations, Conservation and Protection activities, DFO regulatory programs (i.e., Fish and Fish Habitat Protection Program, Species at Risk Program, Small Craft Harbors), and to inform the Canadian general public (recreational watercraft owners and operators). Any advice generated from this work on best management practices will be subject to the caveat that CDD+D effectiveness relies heavily on public uptake and compliance, the assessment of which is beyond the scope of this report.

2. METHODS

A literature review was completed on decontamination treatments for several freshwater and marine AIS, and existing CDD+D protocols. Literature was collected from several databases; Web of Science (Web of Knowledge), DFO's Federal Science Library, Google Scholar (Google™) and ResearchGate. All publication years were considered (earliest available – 2021). Publications included peer-reviewed journal articles, governmental and consultant reports and protocols (i.e., secondary/gray literature), relevant websites and personal communications/expert opinion where applicable. Boolean search terms (modelled after Mohit et al. 2021) are given in Appendix 1.

Representative species from various functional and taxonomic groups (e.g., bivalves, gastropods, zooplankton, parasites, macrophytes, macroalgae, crabs, and tunicates) were selected according to their presence (or their expected arrival) in Canadian freshwater and marine environments (Table 1). In some cases when data was limiting for a target AIS, data were included for nuisance/invasive species of a similar taxon.

2.1. LITERATURE REVIEW OF THE EFFECTIVENESS OF DECONTAMINATION TREATMENTS FOR FRESHWATER AND MARINE AIS

Publications were retained if they met the following criteria: 1) included a detailed description of one or several physical and/or chemical decontamination treatments used to kill or remove AIS; 2) quantitatively evaluated the effectiveness of decontamination treatments (viability, mortality, survival, removal and/or growth), and 3) were applicable to the decontamination of either watercraft or related equipment. We excluded studies on boater surveys about cleaning practices or knowledge, ballast water treatment on commercial ships, ships > 24 m, forest

firefighting equipment or floatplanes, boat wrapping, antifouling coatings, in-water decontamination treatments (e.g., copper, zinc), eradication of invasive species, invasion models, and other vectors of AIS spread.

The following most commonly identified decontamination treatments were assessed for freshwater AIS (see glossary):

Physical treatments

- Hot water (immersion or spray)
- Pressure washing
- Air-drying (or air-exposure)
- Freezing

Chemical treatments (immersions)

- Sodium hypochlorite (bleach) (NaClO)
- Acetic acid (CH_3COOH)
- Quaternary Ammonium Compounds (QAC)
- Salt water (sodium chloride, NaCl and potassium chloride, KCl)
- Virkon® (active ingredient: pentapotassium bis(peroxymonosulphate) bis(sulphate))

For marine species, numerous physical and chemical treatments retained in this review were from studies conducted for aquaculture activities (e.g., introduction and transfer operations). While developed for other purposes, we considered these studies to be relevant for this work because they assessed the effectiveness of treatments to kill AIS and could be applied in a CDD+D context. The following most commonly identified decontamination treatments were assessed for marine AIS (see glossary):

Physical treatments (sometimes combined with air-drying)

- Freshwater immersion
- Hot seawater or freshwater (immersion or spray)
- Pressure washing
- Air-drying (or air-exposure)

Chemical treatments (immersion or spray) (sometimes combined with air-drying)

- Sodium hypochlorite (bleach) (NaClO)
- Acetic acid (CH_3COOH)
- Brine solutions
- Hydrated lime (CaOH)

Decontamination was defined as a physical and/or chemical treatment (or combination of these) which defined a time component to achieve a quantified level of AIS mortality or removal (e.g., X temperature or X concentration for X minutes). Data were classified by decontamination treatment and target AIS. Treatment parameters (concentrations, exposure times, temperatures, etc.) and associated mortality (%) or removal (%) were reported for both young and adult life stages where available. Most of the existing literature on decontamination treatments focused

on mortality as an endpoint, while pressurized water sprays focused either on mortality and/or removal. The effectiveness of each physical and chemical decontamination was categorized as effective if treatments resulted in ≥ 99% mortality. Publications reporting ≥ 99% mortality are presented first with additional publications reporting lower mortality, followed by some publications stating that a treatment is qualitatively effective (no amount of mortality presented). Some ineffective treatments were retained if they resulted in contradictory results for similar combinations of parameters, if all publications were in agreement on a treatment being ineffective, or if a specific treatment was ineffective for a given species. In some cases, if very little information was available for prioritized AIS, studies which looked at similar taxa (e.g., control of Undaria sp. as a proxy for macroalgae) were included.

The most effective treatment options that were lethal to the greatest number of AIS were identified, along with measures of associated uncertainty. Levels of uncertainty were assigned to each decontamination treatment option per species and life stage, and scores were assigned based on the number of studies available (few, limited, many, or comprehensive), their quality (pers. comm., technical report, or peer reviewed), and their agreement with the identified treatment options (contradictory, different conclusions, mostly agree, or agree) (Table 2). Consequently, although a given treatment may be identified as effective for a particular species, a high uncertainty score is possible where few peer-reviewed studies were available. Similarly, low/reasonable uncertainty scores are presented where many peer-reviewed studies supported the proposed effective treatment option. Uncertainty scores were not calculated for ineffective treatments.

2.2. LITERATURE REVIEW OF CURRENTLY ADOPTED NORTH AMERICAN CDD+D PROTOCOLS

Protocols were defined as a combination of treatments and guidelines that are recommended by different governments and agencies to remove and/or kill AIS and, as such, prevent their introduction and spread. CDD+D protocols were retained if they met the following criteria: 1) the protocol was based on scientific literature and/or contained a list of references; and 2) the source was a relevant governmental (federal/state/provincial/territorial) report or legal reference. Relevant information was classified by source, type of treatment and target AIS, and by province/state. The decontamination treatments recommended within the CDD+D protocols were reviewed to determine whether existing protocols are supported by the scientific literature.

3. RESULTS

3.1. ASSESSMENT OF MORTALITY

Mortality endpoints varied between studies, taxa and organism life-stage, however general methods for assessment of viability and survival were comparable. Adult bivalves were considered dead when, after a period of recovery, organisms did not respond to tactile stimulation, did not re-attach byssal threads and/or demonstrated prolonged shell gaping (e.g., Harrington et al. 1997; Forrest and Blakemore 2006; Barbour et al. 2013; Stockton and Moffit 2013; Davis et al. 2015a; Moffit et al. 2015; Joyce et al. 2019), while death of veligers was confirmed by the absence of ciliary movement inside the shell or extended velum (e.g., Verween et al. 2009; Haque et al. 2014; Moffit et al. 2016; Haque and Kwon 2017; Davis et al. 2018). New Zealand mudsnail (*Potamopyrgus antipodarum*) mortality was confirmed when individuals with closed opercula showed no movement after 10 min, or where bodies were clearly protruding from shells (Schisler et al. 2008; Opligner and Wagner 2011; De Stasio et al. 2019).

Mortality of zooplankton was determined when individuals were stationary, demonstrated no twitching of antennae/perepods, did not respond to stimuli and/or did not hold their pereopoda under their body after a 12-48 hour recovery period (e.g., Sebire et al. 2018; De Stasio et al. 2019; Bradbeeer et al. 2020).

Green crab (*Carcinus maenas*) were considered dead when there was a lack of reflexive retractions of legs when tugged or by a lack of papillae and/antennule activity (Darbyson et al. 2009; Best et al. 2014).

Criteria to establish plant/algae mortality were more variable, with multiple approaches defined in the literature to assess survivorship and viability after decontamination treatments (see Mohit et al. 2021 for a review). Techniques included the ratio of variable to maximal fluorescence of leaves (where plants with a score < 0.3 were considered dead; Anderson et al. 2015; Shannon et al. 2018), lack of new growth in apical stems, nodes or roots over 14-35 d in recovery treatments (Basiouny et al. 1978; Evans et al. 2011; Jerde et al. 2012; Bickel 2015; Bruckerhoff et al. 2015; Baniszewski et al. 2016; Crane et al. 2019), visual estimation of degradation (MacNair 2002; Jerde et al. 2012; Barnes et al. 2013; Baniszewski et al. 2016; Crane et al. 2019), loss of biomass and/or reduction of enzyme activity (Basiouny et al. 1978; Watkins and Hammerschlag 1984; Blumer et al. 2009; Barnes et al. 2013; Bickel 2015), and estimating the probability of fragments remaining viable as a measure of the percentage of water loss following desiccation (Basiouny et al. 1978; Evans et al. 2011; Barnes et al. 2013).

Methods for determining mortality of tunicates was equally variable, but field studies generally categorized individual and/or colonies of tunicates as dead if they were absent, discoloured and putrefying, or detached from the substrate (Carman et al. 2010; 2016). In some cases, significant biomass reduction compared to control colonies was a reliable indicator of colony regression which ultimately led to mortality (see Paetzold et al. 2012; Roche et al. 2015). Laboratory studies used a lack of water siphoning, and the absence of tactical response or the inability to close valves after 48 h in recovery treatments (e.g., Hillock and Costello 2013, Hopkins et al. 2016, Sievers et al. 2019).

Mortality of both adult (free swimming triactinomyxons) and young (myxospores) stages of whirling disease were assessed using methylene blue, propridium iodide, or fluorescein diacetate stains (e.g., Hoffman and Markiw 1977, Wagner 2002, Wagner et al. 2003), where only dead cellular material takes up stain. In one study, the mortality of myxospores was assumed in the absence of production of the triactinomyxon stage from *Tubifex tubifex* cultures inoculated with treated myxospores over a 4-5-month period (Hedrick et al. 2008).

3.2. PHYSICAL DECONTAMINATION TREATMENTS FOR FRESHWATER AIS

The effectiveness of common physical treatments for freshwater AIS decontamination is summarized by treatment below and in Table 3. A total of 49 literature sources (45 primary publications and 4 technical reports) were examined which considered mortality or removal associated with hot water immersions (23), hot water sprays (11; 9 using low pressure and 2 high pressure), air-drying (23), and freezing (8). While most studies focused on dreissenid mussels (i.e. zebra and quagga mussels), the lethal effects of hot water immersion and air-drying covered a greater number of AIS.

3.2.1. Hot water immersion

Exposure to hot water is lethal for many freshwater AIS and multiple temperature and exposure combinations that could cause mortality were found in the scientific literature. In general, immersion in hot water (43°C-49°C) for short durations (1-15 min) were lethal for dreissenid mussels, several pelagic invertebrates, and some macrophytes. The temperature tolerance of

dreissenid mussels has been the most extensively studied. Lethal water temperatures have been reported to vary between 32°C (> 4 d, Elderkin and Klerks 2005) and 49°C (1 min, Beyer et al. 2011) for zebra mussel (*Dreissena polymorpha*), depending on immersion time. Similarly, the thermal limit of quagga mussel (*Dreissena bugensis*) ranged from 38°C (20 min, Garton et al. 1990) to 49°C (1 min, Beyer et al. 2011).

Immersion in hot water was found to be lethal not only for dreissenid mussels, but also for several other AIS, including New Zealand mudsnail (e.g., 45°C, 1 min, Dwyer et al. 2003), Asian clam (*Corbicula fluminea*) (e.g., 45°C, 5 min, Coughlan et al. 2019) spiny water flea (*Bythotrephes longimanus*) (e.g., 43°C, 5 min, Beyer et al. 2011), bloody red shrimp (*Hemimysis anomala)* (e.g., 45°C, 15 min, Anderson et al. 2015), and some macrophytes such as parrot's feather (*Myriophyllum aquaticum)*, floating pennywort (*Hydrocotyle ranunculoides*), and curly water-thyme (*Lagarosiphon major)* (e.g., 45°C, 15 min, Anderson et al. 2015). Variable exposure times were reported for killer shrimp (*Dikerogammarus villosus*) (e.g., 10 s at 40°C, Shannon et al. 2018; 30 s at 50°C, Sebire et al. 2018; 15 min at 45°C, Anderson et al. 2015).

Although the scientific literature showed that immersion in hot water (45°C) for 15 min was lethal for several invasive invertebrates, hotter temperatures (60°C, 5 min) were required to kill a greater number of AIS, in particular for macrophytes, as lower temperatures were ineffective. For example, Blumer et al. 2009 tested six temperatures (45-80°C) and three immersion times (2-10 min) and reported that immersion in water at 60°C for 2 min was required for complete mortality of Eurasian watermilfoil (*Myriophyllum spicatum)*. Similarly, Shannon et al. 2018 tested five temperature (40-60°C) and five time (10 s-15 min) treatments and reported that only 40% of parrot's feather were killed at 45°C (15 min) while 100% mortality was reported at temperature/time exposures of 50°C (5 min) or 60°C (10 s), which contrasts with the results (45°C, 15 min) reported by Anderson et al. (2018). Spores of *Myxobolus cerebralis*, the salmonid parasite that causes whirling disease in farmed salmon and trout, and wild fish, were not affected by temperatures of 40°C while temperatures ≥ 60°C for 10 min caused distortion and probable death of spores (Hoffman and Putz 1969). Additional studies reported that temperatures ≥ 75°C for 5 min (Wagner et al. 2003) and 90°C for 10 min (Hoffman and Markiw 1977) were lethal for spores.

Studies that examined a combination of temperatures and immersion times found that increasing water temperatures reduced time to mortality (Beyer et al. 2011; Anderson et al. 2015; Shannon et al. 2018). For example, within the 40 to 100°C temperature range, Mohit et al. (2021) showed that for every 1°C increase in water temperature, effective exposure time decreased by 9.9%.

3.2.2. Pressure washing

Pressure washing can remove and/or kill AIS. For example, high-pressure water sprays can remove encrusted organisms and/or cause them physical damage, while high temperatures sprays may cause thermal shock and thereby induce AIS mortality. The following sections describe the effects of pressure washing on AIS removal and the combination of pressure and temperature on AIS mortality.

3.2.2.1. AIS removal by pressure washing

Studies that examined pressure washing through a comparison of low and high pressures, predominantly evaluated AIS removal rather than mortality. High-pressure washing was found to be more effective than low-pressure to remove AIS from watercraft (Rothlisberger et al. 2010; Wong et al. 2014). Rothlisberger et al. (2010) reported that visual inspection with manual removal and low pressure (40 psi), 90-180 s sprays were significantly less effective at removing small-bodied organisms (e.g., spiny water flea) from watercraft, with 65% and 74% removal

rates, respectively, in contrast to high-pressure (1800 psi) 90-180 s sprays which had a 91% removal rate. These authors also reported that high-pressure spray and visual inspection and manual removal resulted in significantly greater removal rates of plant fragments (83 and 88%, respectively) than low pressure sprays (62% removal). Wong et al. (2014) examined the pressure (1500 vs 3000 psi) and time required to remove 100% of zebra and quagga mussels from watercraft and reported that the time a watercraft was out of the water, the amount of mussel fouling, and water pressure used were the primary factors determining removal rates. They reported that the time required to remove mussels from watercraft was significantly shorter when mussel densities were low and water pressure high (3000 psi in contrast to 1500 psi) and that the longer the vessel was out of the water (e.g., 1-2 weeks), the faster the mussels were removed because the byssal threads were likely to have dried out. More recently, Mohit (2021) tested the effects of 6 pressure levels ranging between 0 and 1950 psi and reported that pressures of 900-1200 psi removed 90% of the periphyton from the surfaces of suspended aluminium tiles (which had been growing algae and other organisms for a period of 3 weeks). The highest pressure (1950 psi) was reported to be less effective because it resulted in more splash back, redistributing material over the surfaces, instead of the water running off as with the lower pressure groups.

With regards to zebra mussel veligers, Davis et al. (2016) found that flushing watercraft livewells with a garden hose (60 psi) removed 90% of zebra mussel veligers.

Only one study demonstrated that pressurized spray (not temperature) induced mortality, where fragmentation and complete mortality was observed for the floating pennywort following both cold and hot water pressurized sprays (1600 psi) (Bradbeer et al. 2021).

3.2.2.2. AIS mortality with hot water sprays

It is important to note that the temperature of the water exiting a pressure washer decreases with increasing distance to the point of contact. For example, Bradbeer et al. (2021) reported that a pressurized hot water spray programmed to 90°C on a pressure washer and projected at a distance of 10 cm for 15 s resulted in a maximum on-contact temperature of 67.4°C and decreased to 52.0°C and 37.3°C at distances of 40 cm and 100 cm, respectively. The temperatures presented below, unless specified otherwise, are those at the point of contact and not the programmed temperature on the pressure washer.

Low pressure hot water sprays (<60 psi)

Three studies examined the effects of low pressure hot water sprays on dreissenid mussel mortality. Morse (2009) assessed the efficacy of low pressure (15 psi) hot water sprays (4 temperatures, 40-80°C) to kill zebra mussels by spraying them for 1, 5 or 10 s. They concluded that sprays ≤ 50°C were ineffective while sprays at 60°C for 10 s or 80°C for 5 s were 100% lethal. Comeau et al. (2011) examined the susceptibility of quagga mussel to low pressure (2 psi) hot water sprays at six temperatures (20-80°C) and durations (1-160 s). Increasing temperature reduced time to mortality, as sprays with 40°C for 40 s, 50°C for 20 s, 54°C for 10 s, and 60°C for 5 s resulted in 100 % mortality. Wong et al. (2014), in addition to assessing the effects of high pressure on removal rates, examined the time required to attain 100% zebra and quagga mussel mortality following exposure to low pressure (2 psi) hot water spray (20-80°C, 1-160 s). They suggested that exposure to water at 54°C for 10 s would be effective for zebra and quagga mussels.

Steam exposure during at least 10 s was lethal to several submergent aquatic plant species, such as the curly-leaf pondweed (*Potamogeton crispus*), according to Crane et al. (2019). Similarly, Bradbeer et al. (2021) reported 100% mortality of killer shrimp using direct steam exposures of ≥ 10 s, while shorter exposure times (5 s) resulted in only 70% mortality. Longer

exposure times of 30 s were required to cause 100% mortality in dreissenid mussels (Coughlan et al. 2020a), bloody red shrimp (Coughlan et al. 2020b) and Asian clam (Coughlan et al. 2019).

High pressure hot water sprays (>400 psi)

To our knowledge, only one study evaluated the combined effectiveness of high-pressure and hot water spray on AIS mortality. Although previous studies report that (low-pressure) hot water sprays of 54°C for 10 s (Wong et al. 2014) or 60°C for 10 s (Morse 2009) were lethal for zebra mussels, a recent study by Bradbeer et al. 2021 found that only 50% and 83% of zebra mussels were killed at a similar temperature (59ºC) following 10 s and 15 s exposure to pressurized (1600 psi) hot water spray. A higher temperature of 67.4°C was required during 15 s to result in 100% zebra mussel mortality since a 10 s exposure resulted in only 92% mortality. These authors reported that a temperature of ≥ 59°C for 5 s was lethal for killer shrimp (but only 83% mortality at 55.9°C for 15 s exposure) while the macrophyte Australian swamp-stonecrop (*Crassula helmsii*) survived even following 90 s exposure at 67.4°C. No data was found for other freshwater AIS.

3.2.3. Air-drying

Air-drying is one of the most researched methods in the literature for the control of freshwater AIS with a total of 23 publications (2 technical reports and 21 primary publications) spanning all the targeted freshwater AIS in the present work. However, no data were available for many young stages, including Asian clam, New Zealand mudsnail, or killer shrimp. Air-drying temperatures ranged from 5°C to 40°C and their lethality to AIS was dependent on a number of factors, including temperature, relative humidity (RH), life stage, and air-exposure time.

For dreissenid mussels, lethal air-drying times generally ranged between 1-7 d in warm temperature conditions (≥ 20°C) compared to 5-47 d in colder conditions (< 20°C) (Ricciardi et al. 1995; Ussery and McMahon 1995; Kappel 2012, Collas et al. 2014; Mohit 2021). Required air-drying times for 100% dreissenid mussel mortality decreased with increasing temperatures but was dependent on mussel size and RH (e.g., McMahon et al. 1993; Ricciardi et al. 1995). Ricciardi et al. 1995 reported that at 20°C and 50% RH (early temperate summer conditions), large zebra mussels (21-28 mm) were killed after 7 d air-exposure in contrast to cooler humid conditions (10°C, 95% RH; spring/autumn conditions) which required 15 d. These authors also noted that smaller mussels (10-18 mm) were killed faster (5 d) at 20°C and 50% RH than were larger mussels. Several studies reported that larger or older invertebrates are more resistant to drying than are smaller individuals or juveniles (Ricciardi et al. 1995; Richards et al. 2004; Collas et al. 2014; Snider et al. 2014). Higher relative humidity was also found to increase AIS tolerance to air-drying (e.g., McMahon et al. 1993; Ricciardi et al. 1995). For example, Ricciardi et al. (1995) reported that for a same given temperature (20°C) and air-drying time (5 d), zebra mussel mortality decreased with increasing relative humidity: e.g., 100% mortality at low RH (10%) with mortality decreasing to 84% and 53% with higher relative humidity of 50% and 95%, respectively.

Air-drying at 20-21°C (RH 68%) was 99% effective for killing New Zealand mudsnail in under 2 d (44-45 h; Richards et al. 2004; Collas et al. 2014), with higher temperatures killing the snail substantially quicker (29°C for 21 h and 40°C for 2 h; Richards et al. 2004). Results for Asian clam were inconsistent, with one study reporting 99% mortality at 20°C after 23 d (RH 68%; Collas et al. 2014), while another showed similar mortality (90%) after 3.5 d at the same temperature but higher RH (80% RH; Guareschi and Wood 2020). This last study reported 100% mortality at higher temperatures (25-30°C) after 48 h (80% RH).

Comparatively less information was available on the effectiveness of drying for the control of pelagic invertebrates and the parasite *M. cerebralis*. Bloody red and killer shrimps require 1 d

and 9 d of drying respectively at 14°C (Anderson et al. 2015) to achieve 90% mortality, while warmer drying temperatures can kill quicker, with 100% mortality of bloody red shrimp after 2-3 h at 20°C (De Stasio et al. 2019). Water flea (Cercopagidae spp.) are also sensitive with eggs showing 100% mortality after 6 h at 17°C (Branstrator et al. 2013) and adults 99% mortality after 3h at 20°C (Mohit 2021). Lower drying times (1h, 20°C) were required to reach 100% mortality of adult *M. cerebralis* (Wagner 2002; Wagner et al. 2003). Younger stages required 18.5 h of drying at 22°C according to Hedrick et al. (2008).

Although many studies on the effects of air-drying on macrophyte species were found in the scientific literature, substantial variation in effectiveness (from 0% to 100%) was seen across species which were related to temperature, RH, plant tissue type (e.g., stem, turion), fragment form (e.g., single stem, coiled), and air-exposure time. Drying was shown to be lethal at temperatures ≥ 20°C (RH ˜40%) for >3 h for Eurasian water milfoil (uncoiled fragments, Jerde et al. 2012; Barnes et al. 2013; Bruckerhoff et al. 2015), Carolina fanwort (*Cabomba caroliniana)* (Barnes et al. 2013; Bickel 2015), water thyme (*Hydrilla verticillata*) (Baniszewski et al. 2016) and curly-leafed pondweed (Barnes et al. 2013; Bruckerhoff et al. 2015) but not for parrot's feather (Barnes et al. 2013). Anderson et al. (2015) however reported 90% mortality of parrot's feather at 14°C if dried for 9 d. In studies where temperatures were lower (and RH higher), longer drying times were required for macrophyte mortality (Evans et al. 2011; Bickel 2015; Bruckerhoff et al. 2015). Tissue type was important, with turions demonstrating higher resistance to drying than single stems in curly-leaf pondweed (Bruckerhoff et al. 2015) and so was morphology, where single stems were killed faster when exposed to drying than coiled masses of stems of Eurasian watermilfoil (Jerde et al. 2012; Bruckerhoff et al. 2015) and curly-leafed pondweed (Bruckerhoff et al. 2015).

3.2.4. Freezing

Very little information was available on the effectiveness of freezing on AIS mortality other than for zebra mussel, New Zealand mudsnail, Cercopagidae spp. and *M. cerebralis*. Overall, the primary literature showed that effective temperatures for 100% mortality range between -1.5°C to -20°C. Individual zebra mussels experience 100% mortality after 0.5 h at -10°C but require 2 h when clustered together (McMahon et al. 1993; Payne et al. 1992). New Zealand mudsnail experience 98% mortality after 4 d of freezing at temperatures between -8°C to -14°C (Cheng and LeClair 2011). No water fleas survived after being sprayed with water and frozen for 2 h (De Stasio et al. 2019). Branstrator et al. 2013 noted that freezing waterflea eggs for 24 h was effective in water but not in air. Results for mortality of *M. cerebralis* were more complex, with some studies showing 100% mortality of adult stages after freezing in water for 100 min at -20°C (Wagner 2002; Wagner et al. 2003), but others showing variable times (from 7 d to 9 m) for younger stages (Hoffman and Putz 1969; Hedrick et al. 2008).

3.3. CHEMICAL DECONTAMINATION TREATMENTS FOR FRESHWATER AIS

The effectiveness of common chemical treatments for freshwater AIS decontamination is summarized by treatment below and in Table 4. A total of 49 literature sources (42 primary publications and 3 technical reports) were examined which considered mortality associated with sodium hypochlorite (28; including 11 which used other chlorine oxidants), acetic acid (2), QACs (10), salt water (11) and Virkon (12). Studies focused largely on dreissenid mussels, New Zealand mudsnail and Asian clam, with few studies testing associated mortality for pelagic invertebrates and only one of which considered macrophytes.

3.3.1. Sodium hypochlorite (bleach, 5% sodium hypochlorite)

The majority of studies evaluating the use of sodium hypochlorite (or other chlorine oxidants) immersion to kill 100% of adult zebra mussels focused on low dose (0.000025 – 0.0015%), long-term chronic exposure (4-45 d) in the context of decontamination of industrial water intake structures and continuous chlorination systems (Greenshields and Ridley 1957; Klerks and Fraleigh 1991; Martin et al. 1993a, 1993b; McMahon et al.1994; Matisoff et al. 1996; Harrington et al. 1997; Rajagopal et al. 2002, 2003). Zebra mussel veliger larvae died faster, with 100% mortality after 18-24 h at similar exposure concentrations (Van Benschoten et al. 1993; McMahon et al. 1994). Low dose (0.000005 – 0.0001%), long-term chronic exposure (6-36 d) was also effective at killing adult Asian clam in some studies (Bernhard 1986; Cherry et al.1986; Ramsay et al. 1988), but in others it was not (0.000025-0.001%; 28 d; Doherty et al. 1986). Higher concentrations (0.001-1.0%) for shorter exposure times (30 min – 2 d) were also not lethal to Asian clam (Tilly 1976; Mattice et al. 1982; Barbour et al. 2013; Coughlan et al. 2019). Immersion in concentrations of 0.00005% for 96-108 h was 100% lethal to veliger larvae of Asian clam (Goss et al. 1979), but similar concentrations did not reliably kill juvenile clams (Doherty et al. 1986). For both species however, treatment effectiveness was related to exposure time, concentration of chlorine and water temperature.

Immersion in or spray with 0.04-0.05 % sodium hypochlorite for 20 min killed 100% of all stages of bloody red shrimp and waterfleas (De Stasio et al. 2019), while killer shrimp were tougher, requiring immersion in 5% sodium hypochlorite for a minimum of 30 s to kill adults and 0.02% for 15 min to kill younger stages (Sebire et al. 2018). With regards to the *M. cerebralis* parasite that cause whirling disease in fish, concentrations >0.25% are effective against myxospore stages after 10-15 min of exposure (Hoffman and Putz 1969; Wagner 2002; Hedrick et al. 2008), while 0.0013-0.013% immersions for 1-10 min are effective against triactinomyxon stages (Wagner et al. 2002, 2003).

No sodium hypochlorite treatment was effective for killing any stage of New Zealand mudsnail (Dwyer et al. 2003; Hosea and Finlayson 2005; De Stasio et al. 2019) or Eurasian watermilfoil (Watkins and Hammerschlag 1984) and no data were available for any other macrophyte species.

3.3.2. Acetic acid (vinegar, 5% acetic acid)

Only two primary publications considered the effectiveness of acetic acid on killing freshwater AIS. Immersions in concentrations of 2.5%, 3.75% and 5% (i.e. vinegar) took 4, 2 and 1 hour (s) respectively to kill 100% of adult zebra mussels (Davis et al. 2015a), while immersions in 5% acetic acid (i.e. vinegar) took > 10 min to kill 100% of zebra mussel veliger larvae (Davis 2016). No information is available in the literature on the effectiveness of acetic acid as a decontamination treatment for quagga mussels, New Zealand mudsnail, Asian clam, waterfleas, bloody red shrimp, killer shrimp, whirling or any macrophyte species.

3.3.3. Quaternary ammonium compounds (QAC)

Quarternary ammonium compounds (QAC) considered in the literature for the control of freshwater AIS included BULAB 6002, BULAB 6009, Polyquat WSCP, DDAC, HDQ, Formula 409, Roccal-D, Sparquat, Hyamine 1622, Benzalkonium chloride and Stepanquat. As different QAC products contain different concentrations of quaternary ammonium compounds, results are presented here (and in Table 4) in % QAC, (and not in percentage product) for easy comparison. Most of the research on using quaternary ammonium compounds for decontamination of AIS investigates its effectiveness on New Zealand mudsnail, however, full consensus on effective treatments for this species is lacking. While two studies showed that

concentrations of 0.07-0.15% for an exposure time of 5 min was reported to be 100% lethal to adult New Zealand mudsnail (Hosea and Finlayson 2005; Stout et al. 2016), Schisler et al. 2008 reported that this same treatment was entirely ineffective. Three additional studies show that higher concentrations of 0.24-0.4 % or greater for >5 min were equally lethal (Schisler et al. 2008; Opligner and Wagner 2011; De Stasio et al. 2019).

Similar to sodium hypochlorite decontamination treatments, most studies on QAC applications for the control of zebra mussels were low dose, long-term chronic immersion exposures; with 100% lethality at 0.00003–0.0001% for 2-34 d and 0.00005-0.0004 for 22-29 d for adults and veliger larvae respectively (Martin et al. 1993a, 1993b; McMahon et al. 1994). A single study of quagga veliger larvae showed 100% effectiveness after a 10 min immersion in 0.4% QAC solution (Britton and Dingman 2011).

Concentrations of 0.15 % QAC are also 100% lethal to myxospore stages of whirling disease (*M. cerebralis*) after 10 min of immersion (Hedrick et al. 2008), although lower concentrations (0.02-0.08%) over 24 h have also been reported as effective (Hoffman and Putz 1969; Wagner 2002). As QAC interfere with gill membrane function (Schisler et al. 2008), they are not effective for the control of invasive macrophytes and no information was available for any other target AIS.

3.3.4. Salt water (sodium chloride and potassium chloride)

Immersion exposure times to achieve 100% mortality for adult zebra mussels are 5-31 d in saltwater at concentrations of 0.1-0.2 ppt KCl (potassium chloride) (Lewis et al.1997; Fernald and Watson 2014; Moffit et al. 2016), whereas concentrations of 10-30 ppt KCl only require a minimum of 12 h (Davis et al. 2018). Similar concentrations of NaCl (sodium chloride) were slower to achieve 100% lethality (30-50 ppt for 24 h – 18 d; Spidle et al. 1994; Davis et al. 2015b, 2018). Shorter acute immersions (5h) at high salinities (30 ppt) of mixed KCl-NaCl are not effective for adult mussels (Ellis and MacIsaac 2009). Zebra mussel veliger larvae showed 100% mortality after 5-24 h at KCl concentrations of 0.96-10 ppt (Waller et al. 1996; Moffit et al. 2016; Davis et al. 2018), but the same mortality over similar immersion exposure times required slightly higher concentrations of NaCl (10-20 ppt; Waller et al. 1996; Davis et al. 2018). Zebra mussel larvae were also effectively killed by a mix of salt solutions (KCl + NaCl) between 14-30 ppt in as little as 2 h (Ellis and MacIsaac 2009).

Adult quagga mussels were harder to kill, requiring upwards of 40 h of immersion at higher saltwater (natural seawater) concentrations (33 ppt) and 70 h at lower concentrations (15-21.3 ppt dilutions of natural seawater; Hofius et al. 2015) to achieve 100% mortality. Shorter time frames (5 h) at 30 ppt (Instant Ocean ®, a mix of KCl + NaCl) are also completely ineffective (Ellis and MacIsaac 2009). According to Spidle et al. (1995) immersions in 50 ppt NaCl solutions take upwards of 18 d to effectively kill adult quagga mussels and no data were available for quagga mussel veliger larvae.

Salt water immersion is ineffective for Asian clam (Barbour et al. 2013; Coughlan et al. 2019), but is lethal to fish hook (*Cercopagis pengoi*) and spiny waterfleas (24-30 ppt, 1-4 h immersion), and bloody red shrimp (30 ppt, 3-5 h) (Ellis and MacIsaac 2009). No data were available for any other target AIS.

3.3.5. Virkon®

Virkon ® is a broad spectrum germicide for cleaning and disinfection in veterinary practice and elsewhere (active ingredient: pentapotassium bis(peroxymonosulphate) bis(sulphate), see Safety Data Sheet Library for the MSDS and a list of ingredients). Ninety min of immersion in 2-4% Virkon® achieved 100% mortality for adult zebra mussel (Coughlan et al. 2020b), while

immersions in 0.5-2% were lethal for veliger larvae after 2 min (Davis 2016). Adult and veliger larvae of quagga mussel were easier to kill, with equal effectiveness after 5-10 min at the same concentration (Stockton 2011; Moffit et al. 2015). Both adult and veliger larvae of quagga mussels can be also be killed effectively at lower Virkon® concentrations (0.25-0.5 %) if immersion times are increased to 10-15 min (Stockton 2011).

Twenty min of 2% Virkon® immersion and spray applications are 100% lethal to adult and young stages of New Zealand mudsnail (Stockton 2011; Stockton and Moffitt 2013; De Stasio et al. 2019), and adult and young stages of waterfleas and bloody red shrimp (De Stasio et al. 2019). Killer shrimp require less exposure time, showing 100% mortality after either a 60 second immersion or a 2 min spray at the same concentration (Bradbeer et al. 2020). Immersions in 1% Virkon® are also effective for bloody red shrimp (1 min; Coughlan et al. 2020a) and killer shrimp (12 min: Sebire et al. 2018; 2 min: Bradbeer et al. 2020).

Conflicting results were seen for Asian clam, where 2% immersion in Virkon® was reported to induce > 93% mortality after 5 min of exposure (Barbour et al. 2013) but a second study reported high survivability even after 80 min immersions in 2-4% Virkon® (Coughlan et al. 2019). Thirty minute immersions in 2-4% Virkon® did not kill Brazilian water weed (Crane et al. 2020) and no data were available for other macrophytes or the parasite *M. cerebralis*.

3.4. PHYSICAL DECONTAMINATION TREATMENTS FOR MARINE AIS

Within the literature for management of marine invasive species, decontamination can be categorized into the application of freshwater immersion, hot water immersion, pressure washing, and air-drying. A total of 40 literature sources (25 primary publications and 15 technical reports) were included, which considered a variety of physical treatments, including pressurized seawater (9), air-drying (13), freshwater (17), hot seawater or freshwater (11), or a combination of these for the control of marine AIS (Table 5). An overview of these physical treatments and relevant literature regarding them is presented below. A few unpublished results provided by local experts were also considered for some treatments and are identified as ‘unpublished data’ in the tables below.

3.4.1. Freshwater immersion or spray

Freshwater immersion times required for 100% mortality varied across species and ranged from 3 h to more than 24 h. Based on qualitative results from Carman et al. (2010), only a 5 min freshwater spray applied directly to oysters or aquaculture gear is effective to eliminate colonial and solitary tunicates; whereas another study found that nearly 100% mortality resulted when colonial tunicates (violet tunicate (*Botrylloides violaceus*) and golden star tunicate (*Botryllus schlosseri*)) were exposed to a minimum of 6 h (laboratory scale) to 24 h (field scale) freshwater immersions respectively (Ramsay 2015a). MacNair et al. (2006) also demonstrated that colonies of *B. violaceus* held for long periods (18 to 24 h) in freshwater resulted in 100% tunicate mortality and that aquarium-scale experiments revealed that 4 h in freshwater was 100% effective at killing the carpet sea squirt *Didemnum vexillum* (McCann et al. 2013). In a study on the physiological response of *B. schlosseri* and *B. violaceus* to low salinities, Dijkstra et al. (2008) demonstrated the sensitivity of these species to freshwater, where all colonies of both species held at 5 ppt suffered 100% mortality after 24 h. Carman et al. (2016) showed that freshwater immersion (8 h) and spray (10 min) followed by 1 h air-drying were effective against colonial tunicates (*B. schlosseri*, *B. violaceus*, *D. vexillum*, and the compound sea squirt *Diplosoma listerianum*) present on aquaculture socks. Denny (2008) reported that *D. vexillum* mortality increased with longer immersion times in freshwater (followed by a 24 h air-exposure period), but did not reach 100% effectiveness: 74% mortality for 2 min, 84% for 5 min, and 87% with a 10 min dip. Ramsay (2015b) suggests that a minimum of 3 h immersion in freshwater

(small scale experiment) is sufficient to cause 100% mortality in adult vase tunicate (*Ciona intestinalis*). The larval and juvenile stages were not considered in this study, but the authors presumed that earlier life stages of the vase tunicate would be more vulnerable to freshwater immersion. However, additional trials were conducted by the same laboratory in 2020 and preliminary results suggest that both 3 and 6 h immersions were not 100% effective to kill *C. intestinalis* present on mussels socks, whereas 12 and 24 h showed promising results for 100% mortality (Ramsay, unpubl. data). Based on the results of trials on impacts of reduced salinities on eggs and larvae of *C. intestinalis* over different exposure times, almost no metamorphosis or moving larvae were observed with an immersion time of 1 h in freshwater (Bourque et al., DFO, unpubl. data). However, a 1 min immersion was ineffective (10% mortality) to eliminate *C. intestinalis* from culture equipment (Carver et al. 2003). While freshwater immersion for ≥3 h was sufficient to cause 100% mortality in both juvenile and adult clubbed tunicates (*Styela clava*) (Ramsay 2015c), another study suggested that *S. clava* mortality occurred within 24 h (Coutts and Forrest 2005). Rolheiser et al. (2012) reported that freshwater was not effective at eliminating *D. vexillum* at lower exposure times (0.5, 1, 5, and 10 min) and in fact showed an increase in *D. vexillum* fouling over time. Results for mussels were similar, with freshwater immersions for 24 h (adults) and 24-48 h (juveniles) found to be ineffective to kill blue mussels (*Mytilus edulis*) (Forrest and Blakemore 2006; Carman et al. 2016; Landry et al., DFO, unpubl. data).

No information was available for green crab. Prolonged periods of freshwater influx was not effective against the Asian shore crab *Hemigrapsus sanguineus*, with a survival rate of 65% after a two weeks immersion at 1 ppt (Hudson et al. 2018).

Thalli of the green alga *Codium fragile* (oyster thief), survived for at least 6 h in freshwater, and showed almost complete recovery of photosynthetic capacity within a few hours of return to full seawater (Kim and Garbary 2007). Other data show that a freshwater immersion of more than 24 h is needed to kill *C. fragile* (Landry et al., DFO, unpubl. data). The wakame kelp *Undaria pinnatifida* (all stages: gametophyte and plantlet) is killed within a 1 d (at 20°C) or 2 d (at 10°C) immersion in freshwater (Forrest and Blakemore 2006).

3.4.2. Hot seawater/freshwater immersion

Carver et al. (2003) showed that a 1 min immersion in freshwater at 40°C was partially effective (66%) at eliminating *C. intestinalis* in laboratory conditions and 100% effective when tested on culture equipment and oyster inventory, whereas Gill et al. (2007) found that an immersion in seawater for a few seconds at 60°C was not effective for the same species. Another study reported that immersion in seawater at 40°C for 10 or 30 s induced 66% mortality, but exposures for 60 s at 40°C, and for 10, 30 or 60 s in 50°C and 60°C seawater caused 100% mortality of the vase tunicate (Sievers et al. 2019). In the same study, low mortality (~12%) was recorded for *S. clava* for all 40°C seawater treatments. As temperature increased, so did mortality, with 50°C causing 40, 70 and 86% mortality after 10, 30 and 60 s, respectively. Similarly, 60°C resulted in 86, 100 and 100% mortality after 10, 30 and 60 s, respectively (Sievers et al. 2019). However, Davidson et al. (2005) found that an immersion for 4 s at a temperature of 80-90°C was needed to cause 100% mortality for *S. clava*.

Best et al. (2014) found that an immersion at 55°C for 1 min was not effective at killing adult mussels (*M. edulis*). Gonzalez and Yevich (1976) showed that following an acclimation period (temperature raised at a rate of approximately 1°C/day from 2.5°C until 25°C was attained), adult mussels (*M. edulis*) exposed to heated seawater showed no mortality at 26°C for 24 h, while an immersion at 28°C resulted in 100, 80 and 50% mortality after 6 d, 4 d and 3 d, respectively, and only 6% mortality at 27°C for 48 h. In addition, the same authors found that the entire mussels population present in the effluent canal died when temperatures were between

28 to 30°C for 3 d (field work). Forrest and Blakemore (2006) showed that a seawater immersion treatment for only 5 s at high temperature (55°C) was ineffective on juvenile mussel (*M. edulis*) stages, while another laboratory study found that a temperature of 30°C for 10 min was ineffective for killing young mussels (Landry et al., DFO, unpubl. data). However, Rajagopal et al. (2005) demonstrated that a temperature of 36°C for 84 min and 41°C for 1 min induced 100% mortality on juvenile mussels. However, Landry et al. (DFO, unpubl. data) measured 87% mortality on juvenile mussels at similar temperature (40°C) for 5 min, while 32.6°C (6h) resulted in 76% mortality of mussel spat (Leblanc et al. 2005).

Juvenile green crab immersed in seawater temperatures of 45 to 55°C for 1 min or 55°C for 5 s to 1 min suffered mortality (100%); while seawater immersions of 40°C for 1 min or 45 to 50°C for 5 s were ineffective or only partially effective (Best et al. 2014).

A heated seawater (50°C) treatment for 30 s is effective to kill *C. fragile* (Landry et al., DFO unpubl. data). Comparatively, a hot seawater immersion treatment for 3 s in 80 to 85°C was found to be effective to kill macroalgal propagules introduced via oyster culturing and transportation, resulting in significantly reduced algae biodiversity (in some cases zero) or only the presence of small individuals of tubular green algae *Ulva* spp. (Mineur et al. 2007). Hot water exposure times that resulted in complete mortality of *U. pinnatifida* were 10 min (35°C), 45 s (45°C) and 5 s (55°C) (Forrest and Blakemore 2006).

3.4.3. Pressure washing (low and high pressure sprays)

3.4.3.1. Low-pressure hot water sprays (<60 psi)

Davidson et al. (2005) found that a steam treatment (50 psi; 100°C) for 30 s produced 100% mortality for *S. clava*. Joyce et al. (2019) examined the efficacy of direct steam exposure (100°C; 50 psi) to induce mortality of selected biofouling species (the blue mussel *Mytilus edulis,* Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*), acorn barnacle *Semibalanus balanoides,* rockweed *Fucus vesiculosus* and *Ulva* sp). They observed total mortality for *M. edulis* (adult) and *Magellana gigas* (juveniles) (60 s), *Semibalanus balanoides* (30 s) and, *Magellana gigas* at 300 s. The application of steam also reduced the biomass of *F. vesiculosus* and significantly reduced *Ulva* sp. biomass, with complete degradation observed for *Ulva* sp. following 120 s of exposure.

3.4.3.2. High-pressure hot water sprays (>400 psi)

Comparatively, studies on *U. pinnatifida* show that high-pressure water spray is completely effective in removing *Undaria* gametophytes from shells at ≥ 2000 psi for 2 s (Forrest and Blakemore, 2006). Coutts (2006) and Coutts and Forrest (2007) report that a combination of high-pressure water-spray and air-drying is a cost-effective method for treating moorings and a variety of other artificial structures. The removal and on-land treatment of moorings using 2000 psi spray and 48 h of air-drying is capable of eliminating both *D. vexillum* and other non-target species. However, another study found that the use of high-pressure (2000-3000 psi; 10-30 s) was not 100% effective to remove fouling, including non-indigenous tunicates, and mobile organisms on cultured Pacific oysters (Curtis et al. 2021). Paetzold et al. (2012) and Arens et al. (2011) showed that high-pressure (700 psi) water treatment was effective at *reducing B. schlosseri* and *B. violaceus* and other epifauna while low pressure (40 psi) had no effect (Arens et al. 2011). In addition, Ramsay (2014) found that the use of high-pressure water using rotary nozzles (400-600 psi) was also effective to reduce *C. intestinalis* on mussels socks.

3.4.4. Air-Drying

Live tunicates (including *B. schlosseri, B. violaceus, D. vexillum, D. listerianum, C. intestinalis* and *S. clava*) were absent on shellfish and aquaculture gear following air-exposure treatments for 24 h and 3 d, respectively, simulating overland transport (Carman et al. 2010). MacNair et al. (2006) showed that buoys air-dried for 72 h were almost (<100%) free of live violet tunicate (*B. violaceus*) except for a few very small pieces mixed among the algae attached to the upper end of the buoy. Pontoons infested with *D. vexillum* needed to be removed from the water for approximately 2 weeks to desiccate colonies (Pannell and Coutts 2007). An air-exposure trial of only 6 h (18-19°C; 92% RH) induced 100% mortality of *B. schlosseri* colonies on PVC monitoring plates (Bernier et al., DFO, unpubl. data). Hopkins et al. (2016) demonstrated that air-drying can be an effective mitigation method for a broad range of fouling taxa, including the Pacific transparent sea squirt *Ciona savignyi*, where adults died within 24 h of air-exposure, while 100% mortality of *Ciona* recruits was achieved after only 8 h. There is no consensus in the literature on the effectiveness of air-drying for *S. clava* (see Hillock and Costello 2013). These authors showed that air-drying under direct sunlight is most effective, irrespective of relative humidity, and requires less time to achieve 100% mortality. Direct sunlight at 25-27°C caused mortality in *S. clava* within 24 h. As the temperature decreased, mortality also decreased and they predicted that exposure to air for two weeks could be an effective management method to eradicate *S. clava* from marine equipment when the air temperature is 10°C. In the case of structures and vessels infested with the invasive clubbed tunicate, these should be removed from the water for air-drying for at least one week (Coutts and Forrest 2005). One week air-exposure is required because *S. clava* can survive air-exposures from 17 h to approximately 6 d depending on ambient temperatures and humidity, particularly in high humidity conditions such as when growing on infected rope.

Seuront et al. (2019) showed that an air-exposure of 6 h at 41°C was effective to kill 100% of adult mussels (*M. edulis*), while no mortality was observed when the bivalves were exposed to temperatures ranging from 20 to 41°C for 3 h. Leblanc et al. (2005) found that an 11 h air-exposure (27°C) resulted in 47.8% of mortality for juvenile mussels (*M. edulis*). The effect of air-drying on young and adult stages of the Mediterranean mussel *Mytilus galloprovincialis* was tested by Hopkins et al. (2016) who reported that a 7 d outdoor air-exposure was required at an average temperature of 20.3°C to be 100% lethal for adult mussels. On the other hand, 24 and 6 h air-exposures (18.5°C; RH 95%) caused 100 and 80% mortality respectively on young mussels (*M. galloprovincialis*).

Green crab can survive for extended periods of time out of water, particularly in protected or enclosed spaces, where relative humidity remains high enough to avoid gill drying (Darbyson et al. 2009). At mean air temperatures of 29°C, 50% of crabs fully exposed to air survived 59-105 h. No crabs survived to the end of the experiment (7 d) in the crab only treatment with higher densities (10 or 15 individuals) of crabs, whereas 7% survived in the crates with lower crab density (5 individuals). However, about 60% of crabs survived to 7 d when seawater or seawater and rope were present in fish crates with crabs.

There is limited information about the effects of air-drying on the survival of *C. fragile*, but it seems to be tolerant to desiccation (MacNair 2002, Kim and Garbary 2007). After 5 h of air-drying, *C. fragile* thalli lost 20% of its mass, but still showed high levels of photosynthetic activity (Kim and Garbary 2007), while plants left to air-dry for 24 h showed <100% mortality (MacNair 2002). Comparatively, the seaweed *U. pinnatifida* showed complete gametophyte and plantlet mortality can be achieved after 3 d (10°C) and 1 d (20°C) of air-drying respectively, at ambient humidity (55-85% RH) and 6 weeks (20°C) at high humidity (>95% RH) (Forrest and Blakemore 2006). However, in the 10°C treatment at high humidity, live gametophytes were still present after 8 weeks of air-drying (Forrest and Blakemore 2006).

3.5. CHEMICAL DECONTAMINATION TREATMENTS FOR MARINE AIS

A variety of chemical treatments were considered from 28 literature sources for the control of marine AIS and included 19 primary publications and 9 technical reports. Treatments included immersion and/or spray (followed or not by an air-exposure) of sodium hypochlorite (11), acetic acid (17), brine solutions (12), and hydrated lime (12). An overview of these treatments is presented in Tables 6 and 7 and are summarized below. A few unpublished results provided by local experts were considered for some treatments and are identified as 'unpublished data' in the tables.

3.5.1. Sodium hypochlorite (bleach, 5% sodium hypochlorite)

While a 20 min immersion of 0.006% sodium hypochlorite was not effective in removing *C. intestinalis* from oyster culturing equipment (Carver et al. 2003), a pilot study (qualitative observations) suggested that more concentrated sprays (1% sodium hypochlorite) for 5 s could be effective for *C. intestinalis* and the common Mediterranean sea squirt *Botrylloides leachii* when the treated tunicates are then left for a 30 min exposure period before being rinsed with seawater (Piola et al. 2009). In the same study, although lower concentration sprays (0.5% sodium hypochlorite) followed by 6 h air-exposure were effective for *B. schlosseri*, the same concentration was ineffective for *B. leachii* and *C. intestinalis*, even when followed by a 12 h air-exposure period (Piola et al. 2009). Immersion in 0.3 or 0.6 % sodium hypochlorite solution resulted in 100% mortality of *B. violaceus* in just 15 s (MacNair et al. 2006), whereas immersion in more diluted sodium hypochlorite concentrations (0.01, 0.02 or 0.05%) took much longer (minimum 12 h) to induce 100% mortality of *S. clava* (Coutts and Forrest 2005). Aquarium-scale experiments run by McCann et al. (2013) showed that *D. vexillum* experienced 100% mortality after immersion in a 0.05% sodium hypochlorite concentration for 10 min, whereas laboratory tests by Roche et al. (2015) reported 5, 15 and 30 min at higher concentration (1%) induced only 50, 65 and 55% mortality respectively. In seed mussels fouled with *D. vexillum*, immersion in a 0.25% (for 2 min) or a 0.5% (for a minimum of 20 s) sodium hypochlorite solution eradicated 100% of the invasive tunicate (Denny 2008).

Rajagopal et al. (2002, 2003) found that immersions in very low concentrations of sodium hypochlorite were 100% effective on adult stages of *M. edulis* in continuous chlorination systems but required very long exposure times (40 d at 0.0001% and 62 d at 0.000025%). However, at higher concentration (0.0004%), shorter immersion time (150 h) was sufficient to kill adult mussels (Haque et al. 2015). Similarly, Haque and Kwon (2017) showed that the required time for 100% adult mussel mortality of two size groups (14 and 25 mm) in 0.0004% sodium hypochlorite were 124 h (14 mm) and 150 h (25 mm) respectively. Mussel veliger larvae were easier to kill, such that immersions in 0.0001% sodium hypochlorite (20 min), 0.00001% (4h) and 0.000005% (5h) caused 100% mortality (Haque et al. 2014). However, juvenile mussels (1.4 mm) required a longer exposure (7h), even at higher sodium hypochlorite concentrations (0.0004%) (Haque et al. 2015).

3.5.2. Acetic acid (vinegar, 5% acetic acid)

Immersion in acetic acid concentrations of 4% for 1 min resulted in complete mortality of the colonial tunicates *B. schlosseri* and *B. leachii*, while immersion at lower concentrations (2%) were not effective (Forrest et al. 2007). Similarly, immersion in 5% acetic acid resulted in 100% mortality of *B. violaceus* in just 15 s (MacNair et al. 2006). Another colonial tunicate, *D. vexillum*, experienced 100% mortality after a 2 min immersion in 10% acetic acid (McCann et al. 2013), whereas laboratory studies by Roche et al. (2015) suggested that a 5 min immersion at half that concentration can induce 65% mortality. In seed mussels fouled with *D. vexillum*, immersion in 4% acetic acid for 10 min resulted in ca 95% tunicate mortality, but lower concentrations (1-2%)

and associated exposure times (1-10 min) were less effective (~45-82%) in several field trials (Denny 2008). Moreover, Rolheiser et al. (2012) showed that immersion in 5% acetic acid for only 30 s was effective in inducing *D. vexillum* mortality.

Spray applications of acetic acid were less effective and/or less practical for controlling fouling on mussel socks. MacNair et al. (2006) indicated that 2 passes (30 s) of a 5% acetic acid spray applied using a commercial sprayer with multiple nozzles, is 90% effective for *B. violaceus*, but removed most other fouling organisms, while only 81% mortality was seen in *D. vexillum* with a 3 s spray of 4% acetic acid followed by a 1 h air-exposure (Denny 2008). In other cases, using a 5% acetic acid spray (5 s) on fouled plates with tunicates, followed by a 30 min air-exposure period, was effective against *B. schlosseri*, *B. leachii* and *C. intestinalis* (Piola et al. 2009). Based on full-scale acetic acid trials, the same paper also showed that a single-spray treatment of 5% acetic acid caused 100% mortality for *D. vexillum* when colonies were exposed to air for 30 min after spray application.

The vase tunicate appeared to be more sensitive to acetic acid than colonial tunicates, where 5% concentrations of acetic acid were highly effective at killing *C. intestinalis*, with immersion times of as little as 10 s (Sievers et al. 2019), 15 s (Gill et al. 2007), 1 min (Carver et al. 2003) and 4 min (Forrest et al. 2007) demonstrating 100% mortality. Moreover, immersions of 5% acetic acid for 30 s (Carver et al. 2003) and 5-10 s (Locke et al. 2009) were 95% and 70-95% effective against *C. intestinalis*, respectively. Lower concentrations of acetic acid (2%) took 60 s for *C. intestinalis* to effect 100% mortality, although including heat treatments (40˚C) in combination with immersion at the same concentration reduces required immersion time to 10 s (Sievers et al. 2019). In other cases immersions in 2 % acetic acid and spray (5%) were substantially less or not effective (Gill et al. 2007; Forrest et al. 2007; Sievers et al. 2019).

A combination of immersion with periods of air-exposure was extremely effective at removing tunicates, with a 24 h drying period (in temperature control cabinets to simulate inter-regional transport) after 1 min of immersion in 2 or 4% acetic acid solutions resulting in 100% mortality for *C. intestinalis*, *B. schlosseri* and *B. leachii* (Forrest et al. 2007). However, with air-exposure limited to 1 hour, a longer immersion (5 min) in 5% acetic acid is required to reach 100% mortality for multiple tunicate species namely *B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. diplosoma*, *C. intestinalis* and European sea squirt *Ascidiella aspersa* (Carman et al. 2016).

Styela clava was more variable in its response to acetic acid, the mildest treatment of which achieved 100% mortality, with a 40°C, 2% acetic acid solution immersion of 60 s (Sievers et al. 2019) or with 5% acetic acid for 15 s (Davidson et al. 2005), whereas Coutts and Forrest (2005) indicate that a 1 min immersion at low concentration (2%) was not effective at killing *S. clava*. Furthermore, they show that a concentration of 5% can kill 100% of *S. clava* after 1 min, while 1% and 2% solutions take a minimum of 10 and 5 min respectively (Coutts and Forrest 2005). Moreover, acetic acid immersions (2 and 5%) for 60 s were only 50% effective (Sievers et al. 2019), whereas a 5% acetic acid spray was only 5-60% effective against *S. clava* (Davidson et al. 2005) when mussel socks were immediately returned to the water after treatment.

Short immersion or spray treatments with 5% acetic acid (5-30 s) were shown to be ineffective on blue mussels (*M. edulis*) in several studies (Carver et al. 2003; MacNair et al. 2006; Gill et al. 2007; Locke et al. 2009). Only one study measured 100% mortality on young mussels when organisms were immersed in 5% acetic acid for 5 min (Carman et al. 2016).

Very little information is available on the effects of acetic acid immersion or spray on the mortality of invasive macroalgae. One comprehensive experiment investigated the effects of 5% acetic acid spray on eleven species of algal species which included *U. pinnatifida*, ulvoids and red algae and demonstrated that a spray followed by a 10 min air-exposure resulted in almost (< 100%) complete mortality of all algal species, with the exception of *Ulva linze* (Piola et al.

2009). Forrest et al. (2007) showed highly variable responses in mortality of the plant pest *U. pinnatifida* (all stages) to acetic acid immersion, but overall a 4% solution was 100% effective for the majority of tissues (gametophytes, plantlets and sporophyll) after 1 min. In addition, field trials results showed that 5% acetic acid immersions for 15 s were very effective for killing a species of *Cladophora*, a type of filamentous green macroalga. (MacNair 2009), while Sharp et al. (2006) found that the same treatment was not effective (12-79%) against the same macroalgae.

3.5.3. Brine solutions

Mortality associated with brine immersion (70 ppt) on tunicate species attached to oysters was investigated by Carman et al. (2010) and showed that 10 min immersions followed by air-drying for 2 h were effective against multiple tunicate species (*B. schlosseri*, *B. violaceus*, *Didemnum albidum* (white crust tunicate), *D. vexillum*, *Diplosoma listerianum*, *Molgula manhattensis (sea grape)*, *S. clava*, *A. aspersa*). However, a more recent study indicated that some of the same species (*B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. listerianum*, *C. intestinalis*, *A. aspersa*) were killed after only a 10 s brine (70 ppt) immersion, followed by 1 h air-drying (Carman et al. 2016). At comparable brine concentrations (62 ppt), McCann et al. (2013) found that immersion times for *D. vexillum* of more than 4 h were required to be 100% effective. Furthermore, Rolheiser et al. (2012) showed that *D. vexillum* was not affected by 40, 50, and 70 ppt brine concentrations (from 0.5 to 10 min of exposure) because fouling increased five weeks post-treatment after being returned to water. Similarly, experiments conducted with brine solutions (300 ppt) showed that 15 s immersion alone, without air-exposure (MacNair et al. 2006), was not effective against *B. violaceus*, while 30 s (Gill et al. 2007) and 8 min (Carver et al. 2003) was also ineffective to kill *C. intestinalis*. MacNair et al. (2006) also carried out several trials on aquaculture gear and tunicate-fouled mussel socks testing immersions in brine solution (300 ppt), followed by a period of air-exposure. At these saturated concentrations, brine was effective in reducing violet tunicate fouling, where 5 min of immersion followed by 1 h of air-drying appeared to be 100% effective, but 1 min of immersion followed by the same period of air-drying was not long enough to ensure total mortality. Similarly, a 15 s of immersion (300 ppt) followed by 1 h of drying was also not effective to kill *C. intestinalis* (Gill et al. 2007).

Brine immersion (300 and 70 ppt) treatments (with and without air-drying period) on adult and juvenile mussels were completely ineffective (0%) or resulted in very low mortality (MacNair et al. 2006; Sharp et al. 2006; Bourque et Myrand 2007; Carman et al. 2016; Landry et al., DFO, unpubl. data). The most effective treatment (39% mortality for young stage mussels) was an immersion at 300 ppt (10 min) followed by 24 h of air-drying (MacNair et al. 2006).

Brine immersions (300 ppt) of 15 min followed by 1 h of air-drying is a promising treatment for killing *C. fragile* according to Landry et al. (DFO, unpubl. data) and is supported by similar findings (100% mortality after 15 min immersion in 300 ppt) in a study by MacNair (2002). Additional data showed immersions for 15 or 10 min, combined with air-drying for 2 or 24 h, respectively, were also 100% effective combinations (MacNair 2002). For other macroalgal species, 400 ppt brine immersion for 30 min significantly reduced survival, with the exception of a few resistant taxa (e.g., *Cladophora* spp., tubular *Ulva* sp) (Mineur et al. 2007). In comparison, a 15 s immersion in a 300 ppt brine solution was found to be effective to kill *Cladophora* sp. (MacNair 2009), while Sharp et al. (2006) determined that this treatment was not totally effective (69-96%) against that macroalgae.

3.5.4. Hydrated lime

Immersion in solutions (ranging between 4 to 20%) of hydrated lime (calcium hydroxide) for the control of tunicates and algae on aquaculture gear gave mixed results. Ramsay et al. (2014)

found that 2 min of immersion at 4% was moderately effective (80%) for the tunicates *C. intestinalis* and *S. clava*. Similar results were seen for *C. intestinalis* on culture equipment and oysters (Carver et al. 2003) and on collectors (Gill et al. 2007), where immersions at 4% for 8 min and 15 s caused 70 and 50-80% mortality, respectively.

Fouling by *D. vexillum* was reduced by 80 to 96% after a 2 to 4 min immersion in 4-5% lime solution (Denny 2008; Switzer et al. 2011). Denny (2008) also demonstrated that a 10% lime solution was ca 99% effective on *D. vexillum* with similar exposure times. Field and laboratory experiments conducted by Rolheiser et al. (2012) supported these findings and demonstrated that exposure to 4% hydrated lime for 5 min was the most effective (92%) for removing *D. vexillum*. MacNair et al. (2006) tested 4% lime immersions on mussel socks for shorter durations of 15 s to control *B. violaceus*, but all tunicates made a full recovery after being returned to the water for 7 d post-treatment.

Air-exposure following lime immersion can sometimes ensure higher mortality and is commonly used to kill tunicates on fouled gear to give consistently effective results (Ramsay et al. 2014; MacNair et al. 2006). Buoys exposed to air for 10 and 15 min after a 15 s lime immersion showed 80% and 90% of *B. violaceus* mortality, 7 d post-treatment, respectively (MacNair et al. 2006). Moreover, a 4% hydrated lime immersion for 15 s followed by 20 min air-exposure resulted in 100% mortality on buoys fouled with vase tunicate (Gill et al. 2007). Based on qualitative visual assessments, hydrated lime sprays (20% for 5s) were also effective on *B. schlosseri* and *B. leachii* when treated fouled plates were left during 6 h (air-exposure), but the same treatment required longer exposures (12 h) for *C. intestinalis* (Piola et al. 2009). Sprays combined with air-exposure (45 s) were applied to control tunicates on mussel socks and were shown to be effective on *S. clava* (Ramsay et al. 2014).

Hydrated lime (4%) immersion (up to 1 min) and spray (15 s) treatments were shown to be ineffective on adult blue mussels (MacNair et al. 2006; Gill et al. 2007; Locke et al. 2009; Comeau et al. 2017). Similarly, a 2 min immersion (Ramsay et al. 2014) and 30 s followed by 1 h air-drying (Landry et al., DFO, unpubl. data) in 4% lime solution did not affect the survival of juvenile mussels; although longer exposure times (15 and 30 min) tended to increase mortality (53-78%) (Landry et al., DFO, unpubl. data).

Hydrated lime immersion (4% for 2 min) was not lethal to green crab (Ramsay et al. 2014), whereas a 4% immersion (5 min) was found to be >90 % effective to kill *C. fragile* (MacNair 2002). A short immersion (30 s) in 4% hydrated lime combined with 1 hour of air-drying was also an effective means of killing *C. fragile* according to Landry et al. (DFO, unpubl. data). In addition, MacNair (2002) also observed almost 100% mortality on *C. fragile* after immersions of 15 min and 1 min, followed by 2 h and 24 h of air-drying, respectively.

3.6. CDD+D PROTOCOLS USED IN CANADA AND ELSEWHERE

A total of 15 publications for freshwater were retained for this review as well as multiple personal communications with experts from various Canadian provinces. Most freshwater protocols used by the provinces and states were developed for watercraft inspection and decontamination (WID) and target dreissenid mussels through the use of pressurized hot water sprays. The use of chemicals was mainly associated with species-specific decontamination (e.g., against the whirling disease parasite *M. cerebralis*) and for the decontamination of small equipment. Very few protocols were found for the marine environment and usually involved a combination of physical and chemical treatments, but were mainly developed for different applications (e.g., aquaculture-related activities) and treatment details (concentrations and exposure times) were often not specified. A summary of the most commonly used freshwater decontamination protocols is presented in Table 8.

3.6.1. Decontamination protocols for freshwater AIS

3.6.1.1. "Clean, Drain, Dry" (CDD) guidelines aimed at the general public

Most Canadian provinces endorse CDD programs, which are aimed at the general public and watercraft owners to minimize the transfer of invasive species by cleaning, draining, and drying watercraft and equipment when moving between waterbodies (OMNRF 2017; MFFP 2018; NBISC 2019; Government of Saskatchewan 2020; NSISC 2020; Government of Alberta 2021; CCIS 2021; Government of British Columbia 2021a; Government of Manitoba 2021a; PEIISC 2021; and Government of Yukon 2021). However, specific conditions (e.g., spray temperatures and pressures, air-drying, and treatment exposure times) were not specified in most guidelines and were mainly developed for recreational watercraft in freshwater environments. Very little information was found on CDD for the Northwest Territories (Government of Northwest Territories 2021), Nunavut (Government of Nunavut 2021), and Newfoundland and Labrador (Government of Newfoundland and Labrador 2021).

3.6.1.2. Decontamination protocols applied at watercraft inspection stations

All watercraft must stop for an inspection when stations are open in British Columbia, Alberta, Saskatchewan, and Manitoba. When required, watercraft and equipment is decontaminated by trained personnel and involves the use of pressurized hot water and/or chemicals to kill AIS without damaging watercraft and water-related equipment.

The western provinces (Alberta, British Columbia, Manitoba, and Saskatchewan) have centered their protocols on the recommendations in the "Uniform Minimum Protocols and Standards for watercraft inspection and decontamination programs for dreissenid mussels in the western United States" ("UMPS IV", Elwell and Phillips, 2021), which was developed by the Pacific States Marine Fisheries Commission. It has been adopted by the Western Regional Panel on Aquatic Nuisance Species and is applied by most watercraft inspection and decontamination (WID) programs in the western United States. UMPS IV recommends the use of lethal water temperatures (see below) as the preferred decontamination method for dreissenid mussels. High-pressure washing or flushing allows the removal of mussels while lethal water temperatures kill veligers and adults. Although developed for dreissenid mussels, it is also used for other types of decontaminations (e.g., standing water, plant, and bait decontamination).

UMPS IV recommends the use of pressurized hot washes for decontaminating watercrafts and the pressure (low or 3000 psi), temperature (49° or 60°C), and duration (≥10, 130, or 132 s) are adjusted for equipment/surface type compatibilities (exterior surfaces, propulsion system, interior areas, equipment, and trailer). For example, high-pressure (3000 psi) hot water (60˚C) for ≥ 10 s is recommended for the hull while a lower pressure hot (60˚C) spray and/or flush for at least 2 min is recommended for the propulsion system (gimbal and engine) to prevent damage to the watercraft. A lower temperature (49˚C) low pressure spray and a longer contact time is recommended for more sensitive interior areas such as the ballast tanks, live/bait wells, and bilge areas. The protocols emphasize that water temperature must be monitored at the point of contact to ensure that the correct temperature is being applied during the decontamination process to account for temperature losses in water with distance. Several detailed manuals describe step-by-step decontamination protocols and procedures, see for example Brown and Walters (2021), as well as those from the governments of Alberta (2020) and British Columbia (2020a). The Aquatic Nuisance Species Task Force (ANSTF 2013), as well as several US states (e.g., Utah Department of Natural Resources 2012; Michigan Department of Environmental Quality 2014; Washington Department of Fish and Wildlife 2016; Minnesota Department of Natural Resources 2017; Wisconsin Department of Natural Resources, 2020; Brown and Walters 2021) and Quebec (MFFP 2018) have developed similar protocols, albeit with some variation (e.g., different pressures and/or exposure times).

The decontamination protocols used at British Columbia's watercraft inspection stations directly follow the UMPS IV guidelines. British Columbia's 2020 Watercraft inspection and decontamination training manual (Government of British Columbia 2020b) provides detailed step-by-step methods for watercraft inspections and decontaminations, risk assessment flow charts, and information on legislation and enforcement. AIS inspectors are equipped with mobile decontamination unit (hot water high pressure washers) to enable decontamination at roadside inspection stations or through scheduled inspections by roving inspection crews.

Similarly, the Alberta government has adopted a modified UMPS IV protocol, implementing a decontamination protocol for work in or near water (Government of Alberta 2020). Two principal decontamination protocols are followed: partial (also known as standing water/plant decontaminations or hot washes) and full (used only on mussel-fouled watercraft) (Mcleod, R., pers. comm.) decontaminations. Partial decontaminations are performed when standing water or unverifiable water (such as ballast tanks) are present on a high risk watercraft using the recommendations in the UMPS IV protocol. When invasive mussels are found on a watercraft, a full decontamination is performed by a trained professional with WID level certification. Furthermore, chemical disinfectants may be required for AIS that are difficult to control. For example, high-pressure hot water is used for whirling disease, but in combination with a QAC soak/spray and at much higher temperatures (90°C steam treatment versus 60°C usually applied for dreissenid mussels) (Mcleod, R., pers. comm.). Decontamination treatments for whirling disease depend on whirling disease risk zones (white, yellow, and red) and associated decontamination levels.

Watercraft inspectors in Saskatchewan also follow UMPS IV guidelines to decontaminate watercraft at their inspection and decontamination stations, or any time a watercraft is directed to them by conservation officers or the Canadian Border Services Agency (CBSA) for follow-up (Geiger, J., pers. comm.).

Manitoba's Aquatic Invasive Species Regulation under The Water Protection Act (Government of Manitoba 2021b) requires decontamination when water-related equipment cannot be completely dried before placing it in another water body and/or a watercraft or water-related equipment is removed from a control zone (area where AIS already occurs or where it is expected to spread due to downstream connectivity). Under these regulations, decontamination of watercraft and equipment can be conducted at a watercraft inspection station run by the Manitoba government or users can do a self-decontamination (LeGal, M., pers. comm.). The decontamination treatment for watercraft completed by the Manitoba government involves low pressure (40-60 psi) hot water (50° or 60°C) sprays which are applied for varying durations (≥10, 70, or 130 s) at close range (i.e., ≤ 10 cm) from the surface, where temperature, pressure and duration combinations are adjusted for the type of watercraft and/or components being decontaminated (e.g., livewells, ballast tanks, and motor), to prevent equipment damage. If visible AIS are present, a high-pressure wash (3000-3500 psi) is done. Equipment is typically decontaminated by submerging in hot water (50 or 60°C) for a minimum of 10 min. Users can conduct a decontamination on their own following the similar procedures outlined in Schedules B and C of the Government of Manitoba AIS regulation for watercraft and equipment, respectively (Government of Manitoba 2021a).

In contrast to the west coast, no watercraft inspection stations are currently in place in eastern Canada. In a recently published best practices guide to prevent the introduction and spread of AIS (plants, animals, and micro-organisms), the Quebec Government (MFFP 2018) similarly recommends the use of pressurized hot water sprays (60 ˚C, 10 s), albeit at a lower pressure (2600 psi) than that applied in the western provinces, but similar to ANSTF (2013) recommendations.

3.6.1.3. Decontamination protocols for equipment

Several provinces and states recommend a series of options to decontaminate equipment, including immersing equipment in hot water or chemical solutions, air-drying, and/or freezing. Rinsing equipment in water at a temperature of 60°C for 5 or 10 min has been recommended by the Michigan Department of Environmental Quality (2014) and the Government of Manitoba (2021a), respectively. Recommended air-drying times were generally 5 d (ANSTF 2013; Minnesota Department of Natural Resources, 2017; MFFP, 2018; Wisconsin Department of Natural Resources, 2020), but ranged up to 14 d for colder winter periods (Utah Department of Natural Resources, 2012). Recommended freezing times ranged from as little as 4 h (DiVittorio et al. 2012) to 3 d (Utah Department of Natural Resources, 2012; Government of Manitoba, 2021a).

With regards to chemical options, the Government of Manitoba (2021a) recommends the following options to decontaminate equipment: bleach (100 ml of bleach to 1L of water = 0.525% sodium hypochlorite, 30 min), vinegar (no dilution, equivalent to 5% acetic acid, 60 min), hydrogen peroxide (64 ml of 7% H_2O_2 in 1L of water = 0.448%, 60 min), salt (10ml of NaCl dissolved in 1L of water = 10 ppt, 24h), and freezing (-10 °C, 3 d). Similarly, the MFFP (2018) also lists several options including: bleach (100 ml of bleach to 1L of water = 0.525% sodium hypochlorite, 10 min), vinegar (750 ml of vinegar (5% acetic acid) in 1L of water = 3.75% acetic acid, 20 min), air-drying (5 d), and freezing (≤ -9°C, 8h).

In some cases, the choice is dependent on the level of risk and watercraft components and equipment. For example, in Wisconsin, when working in waterbodies known to contain specific invasive species, it is mandatory to use a disinfection method that is effective for the AIS of concern (Wisconsin Department of Natural Resources 2016), as outlined in their best management practices available at the Wisconsin Department of Natural Resources.

3.6.2. Decontamination protocols for marine AIS

Very few protocols and/or biofouling management guidelines (out of water cleaning) were found for the marine environment to reduce the risks of spreading marine AIS. Although mainly developed for different applications (e.g., aquaculture-related activities, risk assessments upon watercraft arrival), they present relevant elements about biofouling management practices that are consistent with the “Clean, Drain, and Dry” (CDD) approach.

On the east coast of Canada, a draft protocol was developed by DFO personnel for field operations in AIS infested coastal and inland waters of the Maritime provinces, Quebec, and Newfoundland and Labrador, to mitigate the risks of spreading AIS by watercraft and other equipment (DFO, C. Mills, pers. comm.). This protocol recommends that DFO personnel inspect their watercraft, engines, and trailers; remove and dispose of fouling plants and animals in a garbage container; drain water from the engine, bilge, wells, and other areas that hold water; clean watercraft and equipment using low pressure (hand-pump garden sprayer) vinegar (4-5% acetic acid) or freshwater sprays followed by a minimum 1 h air-drying; and that anti-fouling paint be applied to watercraft.

In Newfoundland and Labrador, DFO’s AIS Science group (DFO, C. McKenzie, pers. comm.) developed a protocol for researchers to prevent the spread of AIS by research vessels. This document outlines recommendations for proper planning, inspection, cleaning, draining, and drying to mitigate the risk of AIS invasions by reducing the likelihood of new introductions to unaffected areas. As an example of proper planning, when sampling work is conducted in areas with AIS, the protocol recommends dedicating certain equipment to be used exclusively in that area. They also recommend to fully inspect watercraft (motor, propeller, anchor, hull, deck, bilge, etc.), trailer and equipment (ropes, chains, floats, bumpers, sampling equipment), and

hand-remove any visible aquatic organisms present. Finally, the last steps of this protocol are consistent with the CDD approach and recommend to: clean and drain watercraft, trailers and equipment on land; remove all organic material and drain water from livewells, bilges, and pumps with freshwater (e.g., flush motor with freshwater for two min), soak equipment in vinegar (5% vinegar), and let watercraft, trailer and equipment dry completely before entering another water body.

Although not developed for decontaminating watercraft, DFO's Introductions and Transfers (I&T) Committee (PEI), in collaboration with PEI's Department of Fisheries and Communities (Aquaculture Division) and the PEI Aquaculture Alliance, recommend several treatment options to reduce the risk of transferring AIS on shellfish and aquaculture equipment between waterbodies (A. Ramsay, pers. comm.). For example, to reduce the transfer risk of colonial tunicates (*B. schlosseri* and *B. violaceus*) on shellfish and aquaculture equipment being introduced to other non-AIS infested bodies of water, they suggest either a combined brine (300 ppt) and 4% hydrated lime immersion (30 s) followed by 1 h air-drying minimum (for oyster spat and adults), or a freshwater soak for 24 h with continuous freshwater flow (for mussel spat), or a 100% brine immersion (30 s) followed by a minimum of one hour air-drying (mussel adults). Similarly, for oyster transfers only, they recommend immersion in 4% hydrated lime (30 s) followed by 1 h air-drying minimum to kill solitary tunicates (*S. clava*) on spat and adults.

The Western Regional Panel on Aquatic Nuisance Species, which regroups 19 US states and 4 Canadian provinces, developed best practices for biofouling management which recommend to: 1) apply an approved antifouling coating to minimize biofouling growth, 2) clean watercraft and equipment before moving between regions to reduce the likelihood of introducing AIS, 3) clean watercraft at certified boat yards, and 4) develop a plan for managing biofouling for watercraft (e.g., biofouling logbook).

The Australian government has developed national biofouling guidelines to assist recreational vessel owners and operators to reduce the risk of spreading marine AIS by managing biofouling on their boats and trailers (Marine Pest Sectoral Committee 2018a, b). These guidelines, similar to CDD in freshwater, recommend the following precautions before moving boating equipment to another location: remove entangled or attached biofouling (e.g., seaweeds) or mud/sediment from the vessel and trailer; rinse the vessel (internal and external) and trailer with freshwater; wash vessel using a soft cloth to remove slime layer; rinse internal seawater systems by cleaning intake and outlet points and by flooding with freshwater; drain completely and dry for 48 h.

In Sweden, a guide on best practices of biofouling management was developed for the Baltic Sea (Watermann et al. 2021). When moving boats to another water region via land, they recommend to remove any attached material (not allowed to enter the water) and to clean boats (hull and niche areas) and trailers using sponges and pressure washers. The best method identified to remove biofouling, particularly for niche areas, is a high-pressure hot water spray with a duration of several seconds (>60°C for 5 s). It is also recommended to let the trailer dry before transporting it to a new waterbody.

At the international level, the International Maritime Organization (IMO) has developed voluntary guidelines, resembling CDD in freshwater, for the control and management of recreational boat biofouling to minimize the transfer of AIS from one marine area to another via watercraft, trailers, or equipment (IMO 2012). After removing the vessel from the water and before transporting it to another water body or storing it on land, these guidelines recommend removing attached biofouling (e.g., seaweeds, barnacles, mussels) from the watercraft, equipment and trailer; drain hull compartments, pipework, and outboard engines; rinse the craft inside and out with freshwater and, if possible, dry all areas before moving; disposing biofouling and

wastewater ashore where it cannot drain back into the water or drains; and inspect, clean, and dry the equipment after each journey or trip.

4. DISCUSSION

In the present document, detailed information is provided on decontamination treatments that are lethal to individual AIS species and while multiple treatments were identified as effective, they were fundamentally species- and environment-specific, with large ranges in associated mortality. Effectiveness was a function of watercraft or equipment type, treatment type, duration/intensity/method of application, and species, among other factors. In order for a given treatment to be effective at killing the greatest number of target of AIS, harsher treatments were typically required than those needed for any particular AIS, e.g., hotter temperatures, increased chemical concentrations, and longer exposure times. Based on results of species-specific decontamination treatments presented in Tables 3-7, effective decontamination treatments to remove/kill the greatest number of freshwater and marine AIS were identified to help future management decisions. Options for watercraft and equipment decontamination are presented in Tables 9 and 10 for freshwater AIS, and in Tables 11 and 12 for marine AIS. Associated levels of uncertainty are presented for each AIS and decontamination treatment. A summary of treatment compatibilities and feasibility are presented in Tables 13 and 14, as not all treatments will be easily applicable to all situations.

4.1. LETHAL DECONTAMINATION TREATMENTS FOR FRESHWATER AIS

For freshwater invasive species, the majority of protocols and decontamination treatments in the scientific literature focused on the control of zebra and quagga mussels, presumably linked to their long invasion history and substantial ecological and socio-economic impacts in North America. With few exceptions, the primary literature supported the recommendations in provincial protocols and hot water sprays/immersions, air-drying, and sodium hypochlorite applications were the most commonly studied.

Pressurized hot water sprays are widely used to decontaminate watercraft but scored high uncertainty due to the limited number of studies and contradictory results. In the freshwater environment, only three studies were found on effective pressures to remove and/or kill AIS. These showed that high-pressure sprays were more effective and/or faster than low pressure sprays in removing freshwater AIS such as zebra mussel, plant fragments, and small organisms from watercraft (1800 vs 40 psi, Rothlisberger et al. 2010; 3000 vs 1500 psi, Wong et al. 2014). However, a recent study by Mohit (2021) suggested that high pressures (1950 psi) may be less effective than mid-range pressures (900-1200 psi), as the higher pressures caused splash back and the redistribution of material over the surfaces. Contrasting results may be due to different methodologies (field studies using high-pressure sprays on fouled watercraft (Wong et al. 2014) vs. manipulative field experiments where periphyton was grown on surfaces or plant material artificially attached to surfaces (Mohit 2021)). Uncertainty not only surrounded pressures but also temperatures that are lethal to AIS. For example, three studies (Morse et al. 2009; Comeau et al. 2011; Wong et al. 2014) reported that 60°C sprays for 10 s were lethal for dreissenid mussels while a recent study (Bradbeer et al. 2021) reported that these were insufficient (50% mortality at 59°C for 10 s) and that hotter (68°C) and longer (15 s) exposure times were required to kill zebra mussels with 100% effectiveness. This highlights the need for further research (ideally field-based) on effective pressures and temperatures to kill zebra mussels and other target AIS and consequently ensure that the recommendations in currently applied protocols (e.g., 60°C, 10 s, 3000 psi in the “UMPS IV” protocol, Elwell and Phillips, 2021) are effective. Overall, the scientific literature suggests that pressurized hot water spray (68°C, 15 s, 1600 psi)

will be effective at killing zebra mussel and killer shrimp (high uncertainty) as well as quagga mussel (very high uncertainty). No data were available for other target species.

Hot water immersions were also found to be lethal for several freshwater AIS. Although not currently easily applicable for watercraft decontamination, hot water immersion can be effective in decontaminating equipment and hard-to-reach areas of watercraft (e.g., ballast tanks, live/bait wells, and bilges). Different temperature/exposure combinations were found in the scientific literature to be effective for several AIS. Although short exposure times in warm water (50°C) were lethal for zebra and quagga mussels (1 min, Beyer et al. 2011) and New Zealand mudsnail (15 s, Dwyer et al. 2003), longer immersion times and/or higher temperatures were required for other AIS. A recent review of decontamination practices in North America (Mohit et al. 2021) concluded that immersion in water ≥ 50°C for 15 min resulted in 100% mortality for mussels, small invertebrates and some plants. Our review revealed that higher temperatures (e.g., 60°C) reduced the time (e.g., 5 min) required for mortality and was lethal for a greater number of species, in particular some macrophytes for which temperatures below 60°C where ineffective (e.g., Eurasian watermilfoil). Even higher temperatures, 75°C and 90°C, respectively, were required to kill myxospores (young stages) and triactinomyoxins (adults) of *M. cerebralis*, the salmonid parasite that causes whirling disease in farmed salmon, trout, and wild fish. Several state and provincial protocols currently recommend decontaminating equipment by immersion in hot water (60°C) for 10 min, which aligns with the scientific literature on lethal treatments for several AIS. Standing water of recreational boats can also harbor AIS, especially planktonic stages (Johnson et al. 2001; Kelly et al. 2013), and flooding these parts with hot water can be an appropriate means of decontamination. Several state and provincial protocols (e.g., “UMPS IV”, Elwell and Phillips, 2021) recommend flushing watercraft ballast tanks, live/bait wells, and bilges for 120-130 s with warm water (49°C). However, these immersion times would need to be increased to 15 min (at 50°C) or to 5 min (at 60°C) to kill the greatest number of AIS. It is important to note that the appropriate temperature must be maintained for the complete duration of the immersions for decontamination to be effective. Consequently, in agreement with several CDD+D protocols, hot water immersion (60°C, 5 min) could be effective to kill zebra mussel, quagga mussel, killer shrimp, waterfleas, and some macrophytes (with reasonable uncertainty), and Asian clam, New Zealand mudsnail, and bloody red shrimp (with high uncertainty). No data were available for several young invertebrate stages and some macrophytes.

Drying watercraft and equipment is one of the three important steps of the public oriented CDD outreach programs but many do not clearly define what exactly is meant by “drying” (e.g., towels, wet/dry vacuums, pressurized air, or air-drying time). The scientific literature showed that air-drying can be an effective means of decontamination but only if applied for the appropriate time for the given temperature and relative humidity conditions and for the AIS life stage. Generally, lethal air-drying times for freshwater AIS were shorter in warm conditions (e.g., 1-7 d) than in colder conditions (6-15 d) but were influenced by RH. High RH allows AIS to tolerate drying for longer time periods, and among invertebrates, larger or older individuals were more resistant to drying than smaller individuals or juveniles (Ricciardi et al. 1995; Richards et al. 2004; Collas et al. 2014; Snider et al. 2014). Macrophyte fragment morphology also influenced effectiveness, with coiled fragments remaining viable longer than single stems or uncoiled fragments (Jerde et al. 2012; Bruckerhoff et al. 2015). Although dependent on a number of factors (e.g., RH, life stage, and temperature), air-drying for 7 d (warm temperatures, 20–35°C) could be lethal, with reasonable uncertainty, for dreissenid mussels, New Zealand mudsnail, *M. cerebralis*, and several macrophytes, and with higher uncertainty for Asian Clam, bloody red shrimp, and waterfleas. Longer drying times were required to be effective in cooler conditions, and greater uncertainty, ranging from reasonable to very high, was associated with air-drying at 10°C-19°C for 15 d, especially for Asian clam and killer shrimp (very high uncertainty), as this treatment was not 100% lethal or results were contradictory between

studies. No data were available for many young invertebrate stages. Recommendations in government or state protocols generally echoed the scientific literature suggesting to air-dry watercraft and equipment for 5 d in the summer months or 14 d in the winter months. These recommendations could be increased to 7 d (summer) and 15 d (winter) to be effective for a greater number of freshwater invasive species and life stages.

Freezing could be a decontamination option if watercraft can be left in cold environments or equipment placed in freezers for the appropriate temperature and time. Studies on the effectiveness of freezing were found for only a handful of species and these showed that freezing was lethal from as little as 30 min exposure (e.g., zebra mussel at -10°C; Payne et al. 1992) while some species required 4 d (e.g., New Zealand mudsnail at -8°C to -14°C; Cheng and LeClair 2011). Although lethal freezing times were shorter for zebra mussel, these depended on temperature and whether mussels were separate or clustered (McMahon et al. 1993). Recommendations for freezing generally ranged from 4 h to 3 d in government and state applied protocols and, as such, may be insufficient to kill all AIS (e.g., New Zealand mudsnail). Overall, the literature suggests that freezing (-20°C) for 4 d will be effective at killing zebra mussel, New Zealand mudsnail, spiny waterflea (sprayed adults and frozen eggs in water – not air), and *M. cerebralis* (high uncertainty). However, no data were available for quagga mussels, Asian clam, bloody red shrimp, killer shrimp, or any macrophyte species.

In North America, sodium hypochlorite is used for killing dreissenid mussels, as chlorination is an effective, economical, and traditionally practiced biofouling control method for industrial water intake structures (see McMahon et al. 1994). Consequently, sodium hypochlorite is often cited as an effective method, usually at concentrations ranging from 0.25-5% (bleach = 5-6% sodium hypochlorite) for 5-30 min, in reports and governmental sources, or as a good alternative to more effective decontamination methods (e.g., steam, freezing) if they are not accessible (Miller et al. 2006; Cockman et al. 2012; Michigan Department of Environmental Quality 2014; New York State Department of Environmental Conservation 2015; Alberta Environmental and Parks 2017; MFFP 2018; Lake Stewards of Maine 2019; Wisconsin Department of Natural Resources 2020). While there is a long history of research on low level, continuous chlorination for control of industrial biofouling including mussels (e.g., Greenshields and Ridley 1957; Klerks and Fraleigh 1991; Harrington et al. 1997) and Asian clam (e.g., Doherty et al. 1968; Bernhard et al. 1986; Ramsay et al. 1988), comparatively fewer studies have investigated acute, higher concentration applications for the decontamination of watercraft and equipment in the context of biosecurity, despite its inclusion in many CDD+D protocols. There was no scientific literature on short-term sodium hypochlorite immersion for the control of zebra or quagga mussels, or the majority of macrophyte species and only two studies investigated its lethality on zooplankton species after 20 min immersions in concentrations of between 0.02-5% (Sebire et al. 2018; De Stasio et al. 2019). Both young and adult stages of whirling disease were effectively killed after immersions at lower concentrations (0.00026- 0.5%) in a similar timeframe (Wagner et al. 2002, 2003; Hedrick et al. 2008), but the same treatments were ineffective for New Zealand mudsnail (De Stasio et al. 2019) and Asian clam (Mattice et al 1982; Barbour et al. 2015; Coughlan et al. 2019). Consideration of the data suggest that that short-term, higher concentration immersions are likely also effective for zebra mussels (being several orders of magnitude higher than the concentrations tested in the literature, albeit at shorter exposure times). Consequently, in agreement with some CDD+D protocols, the literature suggests that 20 min of immersion in 0.25% sodium hypochlorite will be effective at killing all stages of whirling disease (reasonable uncertainty), zebra mussels, bloody red shrimp, and both waterflea species (high uncertainty), as well as killer shrimp (very high uncertainty), but is unlikely to kill Asian clam, New Zealand mudsnail or Eurasian watermilfoil. No data were available quagga mussels or for any other macrophyte species.

Acetic acid is a low cost chemical which is readily available to the general public (vinegar = 5% acetic acid) and is identified in some provincial (MFFP 2018; Government of British Columbia 2020b, 2020c; Government of Manitoba 2021a) and state (DiVittorio et al. 2012; Michigan Department of Environmental Quality 2014; Department of Natural Resources 2020) CDD+D protocols as effective, primarily for the control of zebra mussel larvae on equipment (see Table 8; 3.75-5% for 10-60 min). Only one primary research paper and one thesis were found in the literature that quantified the lethality of acetic acid immersion on zebra mussels (adults: Davis et al. 2015a; veliger larvae: Davis 2016), which suggests that a 1 h immersion at a concentration of 5% (i.e. vinegar) is effective to kill adult and young stages of zebra mussels, however with high associated uncertainty. No data were available for any other target species.

Quaternary ammonium compounds (QACs) are substances regularly used as biocides, pesticides and disinfectants, which interfere with gill membrane function of aquatic organisms (Schisler et al. 2008). They are a key ingredient in industrial molluscicides used to prevent biofouling in cooling systems (Dobbs et al. 1995). Only two CDD+D protocols recommend their use: the Government of Alberta (2020) targeting whirling disease on watercraft and equipment (0.15% soaking or 0.3% spraying for 10 min) and the Michigan Department of Environmental Quality (2014) for targeting New Zealand mudsnail on small equipment (10 min immersion in 0.3%). The use of QACs for the control of New Zealand mudsnail is well represented in the literature, and despite full consensus on effective treatment regimes, the data support the Michigan Department of Environmental Quality's protocol, with concentrations of 0.24-0.4% or greater for >5 min effecting 100% mortality on adult New Zealand mudsnail, with reasonable uncertainty. Only one (Hedrick et al. 2008) of three peer-reviewed studies on QAC applications for killing whirling disease supports the protocol proposed by the Government of Alberta, and indicates immersions in 0.15% QAC for 10 min would be lethal to both adult and younger stages, with high uncertainty. Similar to sodium hypochlorite treatments, of the four studies on QAC applications for the control of dreissenid mussels in the literature, three (2 primary publications, 1 technical report) were low dose, long-term chronic immersion exposures to control zebra mussels (Martin et al. 1993a, 1993b; McMahon et al. 1994). A single study was available on acute immersion, where quagga mussel veliger larvae showed 100% mortality after a 10 min immersion in a 0.4% QAC solution (Britton and Dingman 2011).Consideration of the data suggests a 10 minute immersion in a 0.4% QAC solution are likely also effective for both stages of zebra and quagga mussel veliger larvae and young stages of whirling disease with high uncertainty, driven by the quantity and quality of data available in the literature. This treatment represents a short-term, higher concentration immersion, several orders of magnitude higher than those tested on zebra mussels in the literature, but at shorter exposure times. Similarly, New Zealand mudsnail will show 100% mortality with reasonable uncertainty. Immersion in QAC solutions is however not effective for any invasive macrophyte and data on the other target species are lacking.

Immersion in saltwater is an approach identified in several CDD+D protocols, primarily aimed at killing dreissenid mussel larvae. However, the immersion times and salt concentrations identified therein are not fully supported by the literature. While 24 h immersions at salt concentrations of 10 ppt (as promoted by the Government of Manitoba 2021a and DiVittorio et al. 2012) would be 100% lethal to zebra mussel veliger larvae, 30 minute immersions in 35 ppt (ANSTF 2013) or 4 ppt (Michigan Department of Environmental Quality 2014) salt solutions would not. Comparatively, adult dreissenid mussels are much harder to kill, requiring extensive immersion times (even at high salt concentrations), which may be explained by behavioural responses such as valve closure in response to osmotic stressors (Nicastro et al. 2010; McFarland et al. 2015). Although short immersions were shown to be effective at killing some zooplankton (Ellis and MacIsaac 2009), in most cases salt water treatments were either entirely ineffective (Asian clam; Barbour et al. 2013; Coughlan et al. 2019) or required particularly long

exposure times (adult mussels) to be lethal. This is perhaps not surprising, as many freshwater AIS have inherently wide salinity tolerances (Ricciardi 2006; Ricciardi and Rasmussen 1998; MacIsaac et al. 2002; Ellis and MacIsaac 2009; Pagnucco et al. 2015), often owing to long-term changes in climate or life-history (Strayer and Smith 1993; Reid and Orlova 2002). This, in combination with a lack of data for the other target AIS, suggests that immersions in saltwater are not useful decontamination treatments for the control of freshwater AIS.

While Virkon® is typically used as a broad spectrum germicide for cleaning and disinfection, it has recently been considered in the context of biosecurity (see Barbour et al. 2013). Four CDD+D protocols advocate its use at concentrations of 2% for 20 min, primarily for the control of New Zealand mudsnail (Michigan Department of Environmental Quality, 2014; Washington Department of Fish and Wildlife, 2016; Government of Alberta, 2020; Wisconsin Department of Natural Resources, 2020). The literature supports this treatment application, showing 100% mortality of adult and young stages of New Zealand mudsnail (Stockton 2011; Stockton and Moffitt 2013; De Stasio et al. 2019), both waterflea species, bloody red shrimp (De Stasio et al. 2019) and killer shrimp (Bradbeer et al. 2020). Comparatively, adult quagga mussels and veliger larvae of both dreissenid mussel species were killed much quicker (2-10 min) after immersion in similar concentrations (Stockton 2011; Moffit et al. 2015; Davis 2016), but upwards of 90 min was required to kill adult zebra mussels (Coughlan et al. 2020b). Conflicting results were seen for Asian clam, where immersion at the same concentration for 5 min was either highly effectively (Barbour et al. 2013) or completely ineffective (even after 80 min; Coughlan et al. 2019). Considering the longer timeframe required to induce 100% mortality in adult zebra mussels, a 90 min immersion in a higher concentration of 4% Virkon® will be lethal to the largest number of target species, including both stages of zebra mussel, New Zealand mudsnail, bloody red and killer shrimp (reasonable uncertainty), Brazilian waterweed and both stages of quagga mussel and waterflea species (high uncertainty) and potentially adult Asian clam (high uncertainty). No data were available for the majority of macrophytes or the parasite *M. cerebralis*.

4.2. LETHAL DECONTAMINATION TREATMENTS FOR MARINE AIS

Although marine protocols were mainly developed for different applications (e.g., aquaculture-related activities, risk assessments upon watercraft arrival), they include management practices that are consistent with a CDD+D approach. Pressurized hot water spray, freshwater immersion/spray, acetic acid, brine, and hydrated lime immersions are recommended in several decontamination protocols. These methods are effective at killing several AIS if appropriate exposure times are used. However, it was not always possible to determine if a given protocol was supported by the scientific literature as detailed information was sometimes lacking (e.g., exposure time or concentration).

Freshwater treatments are safe and easy to apply and could be a useful tool for controlling numerous marine AIS. Several primary publications and technical reports identified freshwater immersion as an effective treatment against colonial and solitary tunicates, with low to reasonable uncertainty scores. However, there is no consensus in the literature on effective immersion/duration times to achieve 100% mortality, with variation (> 3 h to up to 24 h) across and within tunicate species (Coutts et Forrest 2005; MacNair et al. 2006; Dijkstra et al. 2008; Carman et al. 2010; McCann et al. 2013; Ramsay 2015a, b, c; Carman et al. 2016). Several studies reported that effectiveness was increased when tunicates were exposed to air following freshwater immersion (Denny 2008; Carman et al. 2016; Rolheiser et al. 2012). Freshwater immersion treatments were generally also effective, with high uncertainty, to kill macroalgal taxa (Forrest and Blakemore 2006; Kim and Garbary 2007; Landry at al. unpubl. data), but ineffective to kill blue mussels (Forrest and Blakemore 2006; Carman et al. 2016; Landry et al., DFO,

unpubl. data). Although effective for tunicates (albeit based on only two studies), no studies on the effects of freshwater sprays on other taxa (with the exception of one study on blue mussels) were found in the literature (Denny 2008; Carman et al. 2016). Overall, freshwater immersion for 24 h followed by 1 h air-drying would likely be effective at killing marine AIS including colonial tunicates (low uncertainty), solitary tunicates (reasonable uncertainty), as well as oyster thief and other macroalgae (high uncertainty) present on water-related equipment, as immersions are not currently easily applicable for watercraft. However, this treatment is likely ineffective against blue mussel (adults and juveniles) and data on green crab were lacking. A few marine protocols recommend decontaminating watercraft and related-equipment with freshwater immersion/spray to mitigate the risks of spreading AIS, but only one (A. Ramsay, pers. comm.) provides exposure times and recommends a freshwater immersion for 24 h with continuous freshwater flow to control colonial tunicates, which aligns with the scientific literature on lethal treatments for several marine AIS.

Heated seawater treatments were effective for several taxonomic groups of marine AIS, with reasonable to high uncertainty (Gonzalez and Yevich 1976; Rajagopal et al. 2005; Forrest and Blakemore 2006; Best et al. 2014; Sievers et al. 2019). However, treatment temperatures and duration times were highly variable, and conclusions differed between solitary tunicates (Gill et al. 2007; Sievers et al. 2019) and adult blue mussels (Gonzalez and Yevich 1976; Rajagopal et al. 2005; Forrest and Blakemore 2006; Best et al. 2014). Mortality of solitary tunicates (*C. intestinalis* and *S. clava*) increased with temperature and exposure times and an immersion at 60°C for 30 s is sufficient to cause 100% mortality (with high uncertainty) to both species (Sievers et al. 2019). Overall, this same protocol may also be effective (with high uncertainty) for killing blue mussels (Rajagopal et al. 2005), juvenile green crab (Best et al. 2014), oyster thief (Landry et al., DFO, unpubl. data) and some other macroalgae (Forrest and Blakemore 2006). No data on mortality associated with heated seawater immersion exists for adult green crab or colonial tunicates and this treatment was not recommended for controlling marine AIS in reviewed protocols.

Very little information on pressurized seawater (for both low and high pressures) treatments was available in the published literature for marine AIS, but available data focused on removing organisms from infrastructures rather than effecting mortality. Identifying a decontamination treatment using pressure which is applicable across species is thus very challenging and associated uncertainty scores for this treatment are high in most cases. Low pressure spray (40-50 psi) could be an effective treatment however, if combined with high temperature (Davidson et al. 2005; Joyce et al. 2019). The application of low pressure seawater spray at 100°C (or steam) for 120 s could kill solitary tunicates, adult blue mussels, and macroalgae present on water-related equipment and watercraft, with high uncertainty, but no information was available for similar treatments on colonial tunicates, juvenile blue mussels, green crab and oyster thief.

A few primary publications reported that high pressurized seawater (400-3000 psi) for various durations (up to 30 s) could be a highly effective method (but not always 100%) to eliminate macroalgae and tunicates on shells in aquaculture systems (Forrest and Blakemore 2006; Paetzold et al. 2012; Curtis et al. 2021). A technical report and a primary publication showed that the combination of pressure washing (2000 psi) and air-drying (48 h) is a cost-effective method to treat moorings and a variety of other artificial structures against tunicates (*D. vexillum*) and other non-target species (Coutts 2006; Coutts and Forrest 2007) infestations and infers that this method may also be effective to decontaminate watercraft and related equipment. Inglis et al. (2012) reviewed options for managing fouled vessels and identified pressure washing (2000 psi or greater) as a common technique for removing biofouling, although niche areas (e.g., inlet pipes, gratings) may require additional chemical treatments.

Based on this limited number of studies, high-pressure (2000 psi) spray for 15 s followed by 48 h air-dry may be effective to eliminate tunicates and macroalgae (reasonable to high uncertainty) from fouled equipment/watercraft, but no data were found for blue mussels, green crabs and oyster thief. No studies combining high pressure and hot water were found for marine AIS. However, as multiple studies showed that both pressurized seawater (Forrest and Blakemore 2006; Paetzold et al. 2012; Ramsay 2014) and hot water (Rajagopal et al. 2005; Forrest and Blakemore 2006; Best et al. 2014; Sievers et al. 2019) were effective for several marine AIS, their combination is likely to be equally or possibly more effective. During a rapid response intervention, McKenzie et al. (2016) found that the application of steam, detergent, and then high-pressure spray (time and pressure not specified) on a boat heavily fouled with *C. intestinalis* was effective to kill this tunicate. Given the lack of data, additional research on the efficacy of low- and high-pressure sprays and temperatures on marine AIS is required. Despite the recommendations from multiple freshwater protocols, only the guide of best practices developed by Sweden (Watermann et al. 2021) suggests the use of a high-pressure hot water spray (60ºC for 5s) to remove marine AIS.

Air-drying is commonly identified in the primary and secondary literature as a control method for marine AIS, and was found to be effective (albeit with long exposure times) for fouling taxa, in particular for colonial and solitary tunicates (Coutts and Forrest 2005; MacNair et al. 2006; Pannell and Coutts 2007; Carman et al. 2010; Hillock and Costello 2013; Hopkins et al. 2016, Bernier et al., DFO, unpubl. data). As described by Hillock and Costello (2013) and Inglis et al. (2012), this method can be easily applied in many situations, such as dry-docking of boats, moorings, and aquaculture and fishing equipment. However, depending on the quantity of organisms present, the species, stages, and local environmental conditions (temperature, relative humidity), it could take up to two or eight weeks to be 100% effective for tunicates and macroalgae, respectively (Forrest and Blakemore 2006; see review of Hilliard and Polglaze 2006 for examples of air-drying times for specific groups). As such, the 48 h drying period recommended by the Australian Marine Pest Sectoral Committee (2018a, b), is likely insufficient. For an effective decontamination treatment across species, an air-drying treatment for 7 d should be sufficient, with reasonable to high uncertainly, to kill most tunicates, green crabs, blue mussels and multiple macroalgae species, including oyster thief, present on water-related equipment and watercraft. However, note that *M. galloprovincialis* was used as a proxy for blue mussels (Hopkins et al. 2016) and the 7 d treatment will only be effective against green crabs if the animals are fully exposed to air at 29°C (Darbyson et al. 2009). Moreover, some macroalgae gametophytes could require more than 8 weeks of air-drying under certain conditions (10 °C; 95% relative humidity; Forrest and Blakemore 2006).

The wide range of sodium hypochlorite concentrations (0.000025-1%) and exposure times (15 s to 62 d) investigated in the literature complicate the comparison of mortality on various marine AIS (Rajagopal et al. 2002; Coutts and Forrest 2005; MacNair et al. 2006; Denny 2008; McCann et al. 2013; Haque et al. 2014, 2015; Haque and Kwon 2017). In addition, when sodium hypochlorite is added to seawater, hypobromite ions and hypobromous acid (the primary biocides) are quickly formed, and any organic matter in the seawater will bind with these oxidants, inactivating them (Taylor 2006) and diminishing the effectiveness of sodium hypochlorite as a biocide (Piola et al. 2009). Some studies on colonial tunicates and blue mussels suggest an inverse relationship between sodium hypochlorite concentration and immersion time, with higher sodium hypochlorite concentrations requiring shorter exposure times for 100% mortality (Rajagopal et al. 2002, 2003; MacNair et al. 2006; McCann et al. 2013; Haque et al. 2015). However, low concentrations required very long times of exposure (days) to kill blue mussel adults (Rajagopal et al. 2002, 2003; Haque and Kwon 2017), and mussel size (or stages) is an important factor when determining the exposure duration required for 100% mortality (Haque et al. 2005). There was no consensus in the literature on effective

concentrations or immersion times for higher concentration studies, with some conflicting results on the most lethal concentrations and exposure times for *D. vexillum* (Denny 2008; McCann et al. 2013; Roche et al. 2015). The effectiveness of sodium hypochlorite spray was also poorly studied, with only one study reporting that a period of exposure to air is required after the spray treatment to be effective over similar time frames against some tunicates, while the same treatment was not effective against other tunicate species (Piola et al. 2009). Although no decontamination protocol recommending sodium hypochlorite was found in the literature for marine AIS, a 0.05% sodium hypochlorite immersion for 6 h may be effective at killing colonial and solitary tunicates (reasonable uncertainty) as well as blue mussels (high uncertainty) present on water-related equipment. However, more research on other AIS taxa (e.g., green crab, oyster thief, macroalgae) is required for a better understanding of its overall effectiveness.

Contrary to freshwater AIS, acetic acid (immersion/spray with or without air-exposure) is one of the most studied treatments in the literature for marine AIS. It has been shown to be highly effective for controlling a large number of cosmopolitan fouling species, including solitary and colonial tunicates, blue mussels and some macroalgae (Carver et al. 2003; Coutts and Forrest 2005; MacNair et al. 2006; Forrest et al. 2007; Gill et al. 2007; Denny 2008; MacNair 2009; Piola et al. 2009; Rolheiser et al. 2012; Sievers et al. 2019). Based on studies which showed 100% mortality, an immersion in 5% acetic acid for 10 min is a good option for treating water-related equipment fouled with tunicates, macroalgae and juvenile blue mussels (reasonable to high uncertainty). However, no data were available for green crab and oyster thief. Spray applications of acetic acid were less effective and the addition of a subsequent air-drying (air-exposure) step is required to reach 100% mortality (Piola et al. 2009). Immersions in acetic acid (2 and 4%) followed by air-exposure (1 and 24 h) were also lethal to colonial and some solitary tunicates (Forrest et al. 2007; Carman et al. 2016), and this combined treatment reduced immersion times. Sievers et al. (2019) reported that combining heat and acid treatments was more effective against solitary tunicates than either treatment alone. Studies on blue mussels that considered acetic acid were mainly developed to control tunicates (or other AIS) in cultured mussel stocks to reduce their spread during aquaculture activities (e.g., transfers). As such, concentrations and exposure times tested are generally low as they were explicitly chosen to be tolerated by mussels. However, higher concentrations and longer exposure times may also be effective to kill molluscs. Although there is no data to show if *M. edulis* will react similarly, a 4% acetic acid treatment (2 min + 24 h air-exposure) caused substantial mortality of green lipped mussel (*Perna canalicus*) (Denny 2008). This suggests that acetic acid could be a promising treatment for blue mussel adults but additional research is needed to establish the most effective immersion times (with or without air-drying combination). In terms of the efficiency of acetic acid on biofouling in general, Cahill et al. (2021) showed that 4% acetic acid immersions for 30 s were highly effective for the total elimination of biofouling cover (mainly bryozoans and polychaete worms). There is also evidence that acetic acid can reduce the cover of the Australian droplet tunicate *Eudistoma elongatum*, an invasive ascidian in New Zealand, to near zero (Page et al. 2011), and cause high mortality to *Caprella* spp. (Paetzold et al. 2008). Two protocols (DFO personnel for field operations and Newfoundland and Labrador DFO's AIS Science group) recommend the use of 5% acid acetic immersion to prevent the spread of marine AIS, but information on exposure time were not provided.

Although somewhat variable between tunicate species, 100% effectiveness was reported in almost all studies using a combined brine (70 ppt or 300 ppt) immersion and air-drying (air-exposure) treatment (MacNair et al. 2006; Carman et al. 2010, 2016). However, brine immersion alone was not consistently effective at controlling tunicate infestations (McCann et al. 2013; Rolheiser et al. 2012). Even at high concentrations (300 ppt), MacNair et al. (2006) noted that brine immersion treatments were only effective in reducing tunicate cover on aquaculture gear and mussel socks when followed by a period of air-exposure. Brine (300 and 70 ppt)

immersion treatments (with and without air-exposure period) on adult and juveniles of mussels were completely ineffective (0%) or resulted in very low mortality (MacNair et al. 2006; Sharp et al. 2006; Bourque et Myrand 2007; Carman et al. 2016; Landry et al., DFO, unpubl. data). However, as for acetic acid, additional research is required to determine the effectiveness of this chemical on the survival of mussels when used at higher concentration and/or longer immersion times. Killing macroalgae using a combination of brine immersion and air-drying requires higher concentrations (300-400 ppt as seen in MacNair 2002 and Mineur et al. 2007) and more research is needed to evaluate macroalgal survival across a range of concentrations and exposure times. The overall literature indicated that a brine (300 ppt) immersion for 15 min followed by a 2 h air-exposure period may be effective at killing colonial and solitary tunicates and macroalgae (reasonable uncertainty) and oyster thief (high uncertainty) present on water-related equipment (e.g., fishing gear). However, this treatment was not effective against blue mussels and no data were found on green crabs.

Hydrated lime is commonly used in mussel and oyster aquaculture industries for controlling predators (e.g., starfish) and fouling tunicates on mussel seed collectors, mussel socks, and aquaculture gear, such as buoys (Ramsay et al. 2014). Hydrated lime (4%) immersion alone was not a 100% effective control method for all targeted species (MacNair 2002; Carver et al. 2003; Denny 2008; MacNair et al. 2006; Locke et al. 2009; Switzer et al. 2011; Rolheiser et al. 2012; Ramsay et al. 2014), but air-exposure after treatment enhances this technique's effectiveness for tunicates (MacNair et al. 2006; Gill et al. 2007) and oyster thief (MacNair 2002). A solution of brine (300 ppt) and 4% lime immersions are recommended by PEI DFO's Introductions and Transfers (I&T) Committee, who suggest a 30 s soak in this solution followed by 1 h air-drying to kill tunicates on aquaculture stock and gear. No scientific study combining both chemicals was found in the literature, however as separate studies showed that both brine and hydrated lime immersions at similar concentrations followed by air-exposure were effective against tunicates and macroalgae (MacNair 2002; Mineur 2007; MacNair et al. 2006; Gill et al. 2007; Carman et al. 2010, 2016), we can assume that their combination would be equally effective. In summary, for a decontamination treatment applied across species, a hydrated lime (4%) immersion for 15 min followed by a 2 h air-dry period could be effective at killing colonial tunicates (reasonable uncertainty) and solitary tunicates and oyster thief (high uncertainty) present on fouled equipment. However, this treatment was not effective against blue mussels and green crab adults and no data were found on green crab juveniles and macroalgae.

In general, there is not enough information provided in the reviewed protocols to determine if these are supported by the scientific literature. Consequently, there is a clear need to develop more detailed CDD+D protocols for marine waters.

4.3. LIMITATIONS AND SOURCES OF UNCERTAINTY

There were multiple limitations and sources of uncertainty identified by this research document, which complicated the development of common guidelines for both marine and freshwater ecosystems. The single species (or similar taxa) approach to many studies challenged comparisons across studies (conflicting or counter-intuitive results likely owing to unreported differences in experimental design). Consequently, no single decontamination treatment was found to be applicable to all freshwater and marine AIS, as while multiple treatments were found to be effective at killing some AIS, they were fundamentally species - and environment-specific, with large ranges in associated mortality. Effectiveness was a function of watercraft or equipment type, treatment type, duration/intensity/particular method of application, and species, among other factors. Consequently, any treatment applied across these factors will impose variable levels of mortality and subsequent control of associated AIS introductions.

The majority of published studies had different experimental designs, scales, and methods of measuring mortality and/or removal, which contributes significant uncertainty to the assessment and comparison of effectiveness (defined here as removal or mortality). Air-drying, for example, is a well-documented decontamination treatment for the control of AIS, but its effectiveness is strongly dependent on animal size (Ricciardi et al. 1995), air temperature, and relative humidity (Ricciardi et al. 1995; Mohit et al. 2021). This link is often overlooked in the primary literature, where studies report effectiveness of drying on either one AIS size class or one temperature/humidity combination, skewing interpretations for future management use. This problem is confounded by the fact that much of the scientific work on decontamination available in the primary literature has been completed under laboratory conditions (see Tables 3-7), and results may not necessarily translate into equally effective 'real world' practical applications. Drying macrophyte fragments in a laboratory for example does not represent the same environment as drying macrophytes fragments caught in humid boat spaces. Further research is required to understand how effective decontamination treatments tested in the laboratory (e.g., air-drying, steam applications, freezing etc.) can be used effectively in a field setting.

Additionally, a substantial subset of scientific studies considered in this document were designed to answer questions for different applications (e.g., aquaculture transfers, cleaning of infrastructure). Freshwater decontamination focused on cleaning transient recreational boats and equipment, while marine decontamination treatments focused primarily on cleaning mussels socks and/or equipment and infrastructure deployed over the longer-term (e.g., floating docks), which may be more heavily fouled. Interpreting removal and mortality from these data contributes some uncertainty to the effectiveness of these techniques in the context of CDD+D. Moreover, to our knowledge the majority of CDD+D protocols are geared towards freshwater recreational activities, with no protocols directly linked to similar marine boating activities.

There is also some uncertainty associated with pressure washing techniques – a widely recommended decontamination treatment in provincial and state CDD+D protocols (e.g., "UMPS IV", Elwell and Phillips 2021). Because of the limited number of scientific studies, additional research on field applications is needed to identify which pressures are more effective at removing marine and freshwater AIS, which temperatures ensure mortality of zebra mussels and other target AIS, and what contact times are needed for pressure and temperature combinations to be 100% effective. Long contact times and high temperatures for example, are difficult to apply in the field. Furthermore, details on recommended nozzle head configuration such as type of spray (e.g., fan-like instead of pinpoint) and angle (e.g., 40°), as well as flow rate (e.g., 5 GPM) and application distance, were seldom identified in the primary literature and can influence the effectiveness of pressurized water treatments. While some freshwater protocols provide this type of guidance (see details in WID training manuals from Brown and Walters 2021; Government of British Columbia 2020a), similar information was not available in marine protocols. More primary research on pressure washing application approaches (e.g., nozzle, angle, GPM etc.) are required to ensure maximum application effectiveness

Treatment combinations may also be more effective than single treatment approaches for many species, although research on this is largely lacking. The inclusion of an air-drying step after hydrated lime (Ramsay et al. 2014; MacNair et al. 2006) and pressure washing techniques (Coutts 2006; Coutts and Forrest 2007) were shown to be more lethal to tunicates than either application alone and findings from Mohit (2021) suggest that hot water immersion followed by air-drying was also more effective than immersion alone for killing invertebrates and macrophytes. There is additional evidence to suggest that combining heat and chemical applications may also increase treatment effectiveness, where warmer acetic acid immersions required less time to kill solitary tunicates (Sievers et al. 2019). Current knowledge gaps on the combination of treatments preclude its consideration in this research document, but future

research should consider the cumulative effects of decontamination treatments on AIS, as this may shape future CDD+D recommendations.

Environmental acclimation can also substantially affect an organism's chance of survival during decontamination treatments. Species acclimated to higher (or lower) temperatures or salinities, for example, often have a higher tolerance to hot water immersions (or freezing), desiccation or changes in osmotic stress (e.g., mussels: Gonzalez and Yevich 1976; McMahon and Ussery 1995; Elderkin and Klerks 2005; Rajagopal et al. 2005; Ellis and MacIsaac 2009; New Zealand mudsnail and Asian clam: Matthews and McMahon 1999; molluscs: Wada and Matsukura 2011; Peck et al. 2014; green crab: Muňoz et al. 2017; macroalgae: Atkinson et al. 2020; waterfleas and bloody red shrimp: Ellis and MacIsaac 2009). As such, longer-term seasonal patterns in water temperature and salinity of the waterbody where a particular population originates from may have additional implications on how effective a decontamination treatment is for many AIS.

Some species (e.g., oysters and barnacles) were not considered in this review and not all considered treatments or species were well represented in the primary literature. Very little data were available for most juvenile stages. Species with an older invasion history were better studied and very little information was available overall for macrophytes and macroalgae within the context of decontamination. Some treatments were very well studied (e.g., hot water immersion, air-drying, sodium hypochlorite for freshwater AIS; freshwater and acetic acid for marine AIS), but others were very poorly studied (e.g., high-pressure hot water sprays and acetic acid for freshwater AIS; high-pressure hot water sprays, Virkon®, and QACs for marine AIS) and control strategies for some groups of AIS such as macrophytes, macroalgae and green crab which were extremely data poor. Additional taxonomic groups may be considered in the future that may require different treatment techniques, and the Science Advice based on this research document will need to be updated accordingly.

4.4. COMPATIBILITIES AND FEASIBILITY

Although a large number of effective treatment options were identified from the primary literature for the decontamination and control of a variety of marine and freshwater AIS, there are a number of considerations that may limit their usefulness in real world settings. Any future protocols recommended by management will need to assess what is feasible to do in the field, with available equipment and tools. These include the ease of application and practicality (e.g., for watercraft or equipment), compatibility with numerous materials, potential damage to watercraft/equipment, associated health and safety hazards, cost, and disposal. Consequently, the application of any treatment should include compatibility (Table 13) and feasibility (Table 14) considerations.

Hot water sprays and immersions are effective, low cost, ecologically friendly decontamination methods (e.g., Schisler et al. 2008; Morse 2009; Shannon et al. 2018) that can remove or be lethal to several marine and freshwater AIS taxa. Removal however can be complicated by the type and complexity of the watercraft (e.g. where some internal compartments cannot be visually inspected) and the effectiveness of removal can be challenging to assess when fouling organisms are small. Hot water sprays are typically recommended for watercraft (Elwell and Phillips 2021), while hot water immersions are effective at killing AIS on equipment (e.g., Shannon et al. 2018). With the advent of dip tank technology, it is possible that hot water immersions may eventually be possible for watercraft, but much work is still needed to assess its feasibility in field applications. The review of the scientific literature suggests that hot water immersion/spray temperatures and exposure times may need to be increased to be effective at killing the greatest number of target of AIS. However, these changes need further testing to assess the viability of implementation in the field as well as the potential for damage to sensitive materials and/or internal compartments of watercraft and equipment. Various materials may be

damaged by hot water sprays and immersions (aluminium, plastics, Gore-Tex, paints, HDPE, acrylic: Miller et al. 2006; Brown and Walters 2021) and temperatures higher than 50°C may damage pumps, engines and cooling systems (Elwell and Phillips 2021). Some nozzle types may also damage watercraft and equipment (Brown and Walters 2021). Outside of professional watercraft inspection, and decontamination stations, the high water temperatures needed to kill most AIS (49 or 60°C) may not be easily obtainable to the general public for a number of reasons. Firstly, very few residential homes or marinas have exterior hot water taps. Secondly, required temperatures may be difficult to achieve and maintain for the required contact times, as residential hot water heaters are required by the National Plumbing Code of Canada (NRCC, 2015) to be set at 60°C, but temperatures at point of contact will be lower (approx. 49°C) because of the water's heat loss to pipes, hoses, ambient temperature, etc. (Lévesque et al. 2004). Thirdly, sufficient freshwater may not be easily accessible in some home or marina locations. Moreover, improper application of hot water decontamination treatments could result in burns (DiVittorio et al. 2012; Anderson et al. 2015).

Pressurized sprays are a low cost, ecologically friendly approach to decontamination. Low pressure flushing (e.g., garden hose, <60 psi) is suitable for PFDs, anchors, ballast tanks, and interior compartments (Elwell and Phillips 2021; Morse 2009; Adirondack Park Invasive Plant Program 2014), while high pressure (e.g., >1000 psi) can be applied to boat hulls, trailers, and exterior (non-porous) surfaces of larger equipment (ANSTF 2013; Elwell and Phillips 2021), but it can damage delicate equipment, neoprene, pontoons, Gore-Tex, painted surfaces, glued seals, and inflatables (Wong et al. 2014; Elwell and Phillips 2021), thus the use of pressurized sprays should consider compatibility with the target equipment/part of watercraft before application (see Table 13). Pressure washing with hot water or the application of steam is most effective for killing AIS (Minnesota Department of Natural Resources 2013; Crane et al. 2019; Wisconsin Department of Natural Resources 2020), but, in many cases, may require specialized equipment (hoses, water connections, pressure washers, electrical connections) which may not be readily accessible. Furthermore, these approaches are not only labour intensive, but can also use substantial amounts of water (Miller et al. 2006; Brown and Walters 2021). In addition, as long contact times and required temperatures are often hard to achieve in the field, and as success of decontamination may be contingent on the ability to direct the intended pressure into niche spaces, on the ground field applications may be less effective than other treatments. Despite the suggestion by some CDD programs, using car wash facilities for decontamination is not recommended as water temperatures and pressures, typically ≤ 37°C and ≤ 1500 psi, may be inadequate to effectively kill AIS but more importantly because surviving organisms may be spread to a water body through municipal infrastructure and storm sewers.

Air-drying is a simple, low cost decontamination method that is environmentally friendly (Coutts 2006; Alonso et al. 2016; Hillock et Costello 2013) and kills several marine and freshwater AIS taxa, although effective exposure times are long. This approach is effective for both small and large equipment types (watercraft, moorings, aquaculture equipment, etc.). Air-drying does not remove organisms, but is a more effective decontamination tool when used in combination with other approaches (e.g., hot water and/or pressurized spray). The scientific literature indicates that 7 d is the best option to kill the greatest number of target AIS. However management will need to consider that many recreational users are weekend boaters, and a shorter drying time, while less effective, may fit better in the work week and result in better public participation.

Freezing is a low cost, environmentally friendly decontamination method for smaller equipment which has been shown to be effective at killing dreissenid mussels (McMahon et al. 1993; Payne et al. 1992), New Zealand mudsnail (Cheng and LeClair 2011), some pelagic zooplankton (Branstrator et al. 2013; De Stasio et al. 2019) and *M. cerebralis* (Wagner 2002; Wagner et al. 2003; Hedrick et al. 2008). However exposure times can be long (4 d in some

cases) and it is impractical for the decontamination of watercraft and larger equipment, unless completed in winter if temperatures fall below 0°C.

The majority of effective chemical decontamination methods identified for the control of both marine and freshwater AIS require immersion, which, while feasible for small equipment, is not feasible for watercraft, trailers and larger equipment (Davis et al. 2018). Costs are generally higher than other treatments and require the ability to manage specific concentrations (e.g., Piola et al. 2009; Elwell and Phillips 2021). There are multiple dangers to human health ranging from inhalation, burns, accidental ingestion and dermal irritation. The effects of chemical treatments on equipment integrity is also of concern, where corrosion of rubber, fabrics, metals, and plastics is common with bleach, salt and/or QAC applications (e.g., Hosea and Finlayson 2005; Schisler et al. 2008; Elwell 2010; Stockton and Moffitt 2013; Joyce et al. 2019). Many chemical disinfectants can also have serious impacts to local waters (MacNair et al. 2006; Miller et al. 2006; Locke et al. 2009). Quaternary ammonium products, for example, can persist in municipal water systems (Boethling 1984; Zhang et al. 2015) and widespread use can affect aquatic and soil systems (Garcia et al. 2001; Li and Brownawell 2010; Sarkar et al. 2010), potentially with genotoxic (Ferk et al. 2007) or other effects on non-target organisms (Waller et al. 1993) and bleach can also be hazardous to human as well as ecosystem health (Miller et al. 2006; Utah Department of Natural Resources 2012; Brown and Walters 2021). Moreover, chemical disposal for many of the identified treatments (Virkon®, bleach, QACs, hydrated lime) is not straightforward and improper disposal may cause ecosystem harm over the longer term (Sebire et al. 2018; Bradbeer et al. 2020). Legal issues concerning the use of broad-spectrum disinfectants as biosecurity agents for AIS will also need to be addressed in the future (e.g., herbicide or insecticide; Cuthbert et al. 2018, 2019; Sebire et al. 2018). The use of chemical treatments should be limited to situations in which guidelines can only be partially followed or are impractical (such as when drying times are limited and known AIS are present) and be done preferably by qualified personnel. If chemical treatments are unavoidable, the most effective environmentally friendly option should be chosen for the species of concern (e.g., salt water immersion to kill dreissenid mussels or acetic acid immersions to kill tunicates) and care should be taken to wear appropriate protective clothing.

4.5. IMPORTANCE OF CDD, WATERCRAFT INSPECTION, AND DECONTAMINATION PROGRAMS

Recent scientific reviews have confirmed the significant risks posed by recreational boating as a vector for secondary AIS spread in Canadian freshwater and marine ecosystems (Drake 2017; Drake et al. 2017; Simard et al. 2017). Recreational fishing surveys from 2020 in Ontario suggest that while >85% of anglers always drain their bilges/livewells/motors and visually inspect their boats to remove plants, organisms and mud before moving their watercraft, only 70% dry their boats and gear thoroughly, and less than 55% wash their boats with hot water and/or high pressure (Len Hunt, pers. com.). Furthermore, several studies indicate that the effectiveness of boat cleaning is greatly influenced by boater behavior (Jensen 2010; Rothlisberger et al. 2010; Cimino and Strecker 2014; Drake 2017). As such, the implementation of CDD and programs are essential in preventing the introduction and spread of AIS. Outreach and education campaigns such as CDD and “Pull the plug” are easily accessible to the general public and should continue to be supported and implemented. Completing the CDD steps when working in or near water will likely promote the removal of AIS organisms visible to the naked eye (e.g., adult crabs, zebra mussels, aquatic vegetation) from watercraft and equipment, but smaller organisms (including larval stages) may be harder to see and more easily trapped in hard-to reach areas. Consequently, where there is an identified higher risk of AIS hitchhikers (e.g., watercraft leaving a waterbody where zebra mussel is established), a thorough watercraft inspection is a critical first step for identifying risk and determining if decontamination is

required. If a decontamination step is necessary in addition to CDD, it could be completed either by boat owners/operators or by professional watercraft inspection and decontamination stations. Currently, only few provinces apply and/or recommend decontamination treatments in addition to CDD. The western provinces (Alberta, British Columbia, Manitoba, and Saskatchewan) have centered their decontamination protocols on the Uniform Minimum Protocols and Standards for Inspection and Decontamination Programs for Dreissenid Mussels in the Western United States ("UMPS IV", Elwell and Phillips, 2021) which are used at their watercraft inspection and decontamination stations.

These watercraft inspection and decontamination (WID) stations, which employ trained personnel, appear to be effective in reducing the spread of AIS. For example, the Saskatchewan government reported that no invasive mussels were detected in the province in 2020 (Government of Saskatchewan 2021). In British Columbia, auxiliary conservation officers conducted 29,900 inspections in 2020 and stopped 16 mussels-fouled boats. Similarly in 2019, 22 mussels-fouled boats were intercepted and decontaminated. These came from Ontario, Michigan, Utah, and North Carolina (Government of British Columbia 2021c). The BC program received advanced notification of 17 of the 22 mussel-fouled boats either from another jurisdiction or by Canada Border Services agents (Government of British Columbia 2021c).

The continued implementation of CDD programs as well as mobile decontamination units at key entry points and contaminated waterbodies will help prevent the spread of AIS to new waterbodies. As such, the western provinces and states are targeting boats coming into their jurisdictions at key entry points along highways (Martina Beck, Government of British Columbia, pers. comm.). Optimizing the location of future watercraft inspection and/or cleaning stations may also be important. For example, Drury and Rothlisberger (2008) and Rothlisberger and Lodge (2011) reported that mandatory watercraft cleaning during outbound trips from infested lakes ("offensive" AIS management), was most effective at preventing spread early in the invasion process but that implementing cleaning stations at uninvaded lakes to target inbound trips was most effective as the invasion progressed ("defensive" AIS management). Haight et al. 2021 similarly highlighted the importance of optimizing the location of watercraft inspection and decontamination stations.

4.6. OTHER CONSIDERATIONS

Although beyond the scope of this research document, there are multiple management considerations which need to be evaluated. CDD and decontamination are not mutually exclusive steps; decontamination is an additional step which may be required by management. How CDD and decontamination steps are implemented will depend on management priorities which will need to consider which areas (e.g., waterbodies) or events (e.g., fishing tournaments) are high risk, where and by whom CDD+D should be completed (at entries or exits of waterbodies, provincial boundaries, etc.), which species are targeted, and the feasibility of effective treatment options at WID stations and/or for the general public. Moreover, public and proponent uptake/participation will play an integral part in the successful management of AIS in marine and freshwater ecosystems.

A further consideration is that decontamination could lead to 1) removal without mortality of AIS, 2) mortality without removal of AIS, and 3) both removal and mortality of AIS (the preferable endpoint). If AIS are removed but not killed, these can be washed and spread to waterways. Conversely, if AIS are killed but not removed (e.g., encrusted organisms), questions may remain regarding their viability. As such, these different endpoints could have important consequences for the enforcement of AIS regulations in certain provinces and territories.

The implemented decontamination treatments will need to be reviewed and adjusted as new scientific information becomes available or new AIS establish.

5. CONCLUSIONS

- Outreach and education campaigns such as “Clean, Drain, and Dry” (CDD) and “Pull the plug” are easily accessible to the general public, are important in helping prevent the introduction and spread of AIS, and should continue to be supported and implemented.
- Reducing propagule pressure using CDD+D can be achieved by physically removing (e.g., cleaning, scrubbing, hand-picking) and/or killing AIS (e.g., pressure washing, temperature or chemical treatment). Most existing literature focused on mortality as a measure of effectiveness.
- Current government and state applied CDD+D protocols are generally supported by the scientific literature, although these are often centered on controlling one species in particular. Protocols should be reviewed regularly to assess results from recent scientific literature and their potential effectiveness/feasibility in field applications.
- Most CDD+D protocols were aimed at freshwater AIS while complete and detailed protocols for marine AIS were not available in the literature or were mainly developed for different applications and these require further development to be implemented.
- CDD and decontamination are not mutually exclusive; decontamination is an additional step that may be required by management. These decisions will need to consider which areas are of high risk, where CDD+D should be completed (at entries or exits of waterbodies, provincial boundaries, etc.), which species are targeted, and the feasibility of effective treatment application.
- Numerous species - or environment-specific (marine or freshwater) decontamination treatments were identified as effective (≥ 99%) at killing and/or removing AIS.
- No single decontamination treatment is applicable to all freshwater and marine AIS or to all watercraft and equipment.
- Chemical decontamination treatments should be limited to situations in which other treatment options are not achievable. If chemical treatments are unavoidable, the most effective environmentally friendly option should be chosen for the species of concern and should preferably be done by qualified personnel.
- Key uncertainties and knowledge gaps include:
    - Comparing studies with different experimental designs, scales, and methods of measuring mortality and/or removal;
    - Extrapolating results from laboratory studies to field conditions;
    - Interpreting the effectiveness of decontamination treatments that were designed for different applications (e.g., aquaculture transfers, cleaning of infrastructure).
- This work describes decontamination treatments that are lethal for representative groups of AIS based on currently available scientific data. As additional information on treatments or new species become available, this science advice will need to be updated.
- Public uptake and compliance is beyond the scope of this work, but will play an integral part in the successful management of AIS in marine and freshwater ecosystems.

6. ACKNOWLEDGEMENTS

We wish to thank the steering committee and meeting participants who provided useful comments and advice: Andrew Drake, Martina Beck, Renée Bernier, Claudio DiBacco, Andrew Drake, Timothy Johnson, Sophie Foster, James Kristmanson, Andrea Locke, Lynn Lush, Cynthia McKenzie, Chris McKindsey, Aaron Ramsay, Stephanie Sardelis, Brendan Spearin, and Thomas Therriault. We also thank provincial contacts for providing insightful information: Jeff Brinsmead, Laureen Janusz, Nicole Kimmel, and Rob Mcleod. The chair and rapporteur, Gilles Olivier and Alex Tuen, respectively, ensured a smooth and efficient meeting. Finally, we thank Martina Beck and Chris McKindsey for the final review of the document.

7. REFERENCES CITED

Adirondak Park Invasive Plant Program 2014. Boat inspection and decontamination for Aquatic Invasive Species prevention. Boat inspection and decontamination for Aquatic Invasive Species prevention. Accessed October 2020. Recommendations for the Adirondack Region (USA). 64 p.

Alberta Environment and Parks 2017. Decontamination protocol for watercraft & equipment. ISBN 978-1-4601-3498-6. Accessed October 2020. Alberta Government (Canada). iii + 28 p. + appendices.

Alonso, A., Valle-Torres, G., and Castro-Diez, P. 2016. Survival of an invasive aquatic snail to overland translocation in non-aquatic media: Implications for spreading. Limnologica 57: 60-65.

Anderson, L.G., Dunn, A.M., Rosewarne, P.J., and Stebbing, P.D. 2015. Invaders in hot water: a simple decontamination method to prevent the accidental spread of aquatic invasive non-native species. Biol. Invasions 17: 2287-2297.

ANSTF (Aquatic Nuisance Species Task Force) 2013. Voluntary guidelines to prevent the introduction and spread of aquatic invasive species: recreational activities. Accessed November 2020. Stop aquatic hitchhikers! (USA). 16 p.

Aquatic Invasive Species Network 2021. Level II inspector and decontamination training (WIT II). Accessed online February 2021. WIT/WID Western States Training Program, Pacific States Marine Fisheries Commission, Nevada/Arizona (USA).

Arens, C.J., Paetzold, S.C., Ramsay, A., and Davidson, J. 2011. Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture. Aquat. Invasions 6(4): 465-476.

Atkinson, J., King, N., Wilmes, S.-B., Moore, P. 2020. Summer and winter marine heatwaves favor an invasive over native seaweeds. J. Phycol. 56: 1591-1600.

Bailey, S.A. 2015. An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. Aquat. Ecosyst. Heath 18(3): 261-268.

Banha, F., Gimeno, I., Lanao, M., Touya, V., Durán, C., Peribáñez, M., and Anastácio, P. 2016. The role of waterfowl and fishing gear on zebra mussel larvae dispersal. Biol. Invasions 18: 115-125.

Baniszewski, J., Cuda, J. P., Gezan, S. A., Sharma, S., and Weeks, E. N. I. 2016. Stem fragment regrowth of *Hydrilla verticillata* following desiccation. J. Aquat. Plant Manag. 54: 53-60.

Barbour, J. H., Dick, J. T. A., Alexander, M. E., and Caffrey, J. 2013. Biosecurity measures to reduce secondary spread of the invasive freshwater Asian clam, Corbicula fluminea (Müller, 1774), Manag. Biol. Invasions 4(3): 219-230.

Barnes, M.A., Jerde, C.L., Keller, D., Chadderton, W.L., Howeth, J.G., and Lodge, D.M. 2013. Viability of aquatic plant fragments following desiccation. Invasive Plant Sci. Manag. 6: 320–325.

Basiouny, F. M., Haller, W. T., and Garrard L. A. 1978. Survival of hydrilla (*Hydrilla verticillata*) plants and propagules after removal from the aquatic habitat. Weed Sci. 26(5): 502–504.

Beaury, E. M., Finn, J. T., Corbin, J. D., Barr, V., and Bradley, B. A. 2020. Biotic resistance to invasion is ubiquitous across ecosystems of the United States. Ecol. Lett. 23: 476-482.

Bellard, C., Thuiller, W., Leroy, B., Genovesi, P., Bakkenes, M., and Courchamp, F. 2013. Will climate change promote future invasions? Glob. Chang. Biol. 19: 3740– 3748.

Bernhard, H.F.M. 1986. Asiatic clams tested at Edison stations. Edison Elec. Biocurr. 2: 12.

Best K., McKenzie C. H., and Couturier, C. 2014. Investigating mitigation of juveniles European green crab *Carcinus maenas* for mussels to prevent transfer during Newfoundland mussel aquaculture operations. Manag. Biol. Invas. 5(3): 255-262.

Beyer, J., Moy, P., and Stasio, B.D. 2011. Acute upper thermal limits of three aquatic invasive invertebrates: hot water treatment to prevent upstream transport of invasive species. Environ. Manag. 47: 67–76.

Bickel, T.O. 2015. A boat hitchhiker's guide to survival: *Cabomba caroliniana* desiccation resistance and survival ability. Hydrobiologia 746:123–134.

Blackburn, T.M., Pysek, P., Bacher, S., Carlton, J. T., Duncan, R.P., Jarosik, V., Wilson, J.R.U., Richardson, D.M. 2011. A proposed unified framework for biological invasions. Trends Ecol. Evol. 26: 333–339.

Blumer, D.L., Newman, R.M., Gleason, F.K. 2009. Can hot water be used to kill Eurasian watermilfoil? J. Aquat. Plant Manag. 47: 122–127.

Boethling, R.S. 1984. Environmental fate and toxicity in wastewater treatment of quaternary ammonium surfactants. Water Res. 18(9): 1061-1076.

Bourque, F. et Myrand, B. 2007. Traitement des collecteurs de moule à la saumure pour contrer la prédation par les étoiles de mer. MAPAQ, DIT. Rapport de R-D #160. 20 p.

Bradbeer, S.J., Coughlan, N.E., Cuthbert, R.N., Crane, K., Dick, J.T.A., Caffrey, J.M., Lucy, F.E., Renals, T., Davis, E., Warren, D.A., Pile, B., Quinn, C., and Dunn, A.M. 2020. The effectiveness of disinfectant and steam exposure treatments to prevent the spread of the highly invasive killer shrimp, *Dikerogammarus villosus*. Sci. Rep. 10: 1919.

Bradbeer, S.J., Renals, T., Quinn, C., Warren, D.A., Pile, B., Hills, K., and Dunn, A.M. 2021. The effectiveness of hot water pressurized spray machines in field conditions to slow the spread of invasive alien species, Manag. Biol. Invasions 12(1): 125-147. ISSN 1989-8649.

Bradley, B.A., Blumenthal, D.M., Wilcove, D.S., Ziska, L.H. 2010. Predicting plant invasions in an era of global change. Trends Ecol. Evol. 25(5): 310-8. PMID: 20097441.

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

Brennan, A.C., Woodward, G., Seehausen, O., Muñoz-Fuentes, V. Moritz, C. Guelmami, A., Abbott, R.J., Edelaar P. 2014. Hybridization due to changing species distributions: adding problems or solutions to conservation of biodiversity during global change? Evol. Ecol. Res.16:475-491.

Britton, D.A., Dingman, S. 2011. Use of quaternary ammonium to control the spread of aquatic invasive species by wildland fire equipment. Aquat. Invasions 6(2): 169-173.

Brown, E., Walters, R. (Eds.) 2021. Official Colorado Watercraft Inspection and Decontamination (WID) Procedures. Accessed March 2021. Colorado Parks and Wildlife Invasive Species Program 2021, Colorado (USA).

Bruckerhoff, L., Havel, J., Knight, S. 2015. Survival of invasive aquatic plants after air exposure and implications for dispersal by recreational boats. Hydrobiologia 746: 113–121.

Cahill, P.L., Atalah, J., Cunningham, S., Day, A., Fletcher, L., South, P., Forrest, B., Hopkins, G. 2021. Acetic acid immersion – A reactive pest treatment for bivalve aquaculture. Aquaculture 533: 1-11.

California Department of Fish and Wildlife 2013. Aquatic Invasive Species Disinfection/Decontamination Protocol. Accessed November 2020. California (USA). 10 p.

Canadian Council on Invasive Species 2021. Clean, Drain, Dry: Prevent the Spread of Aquatic Invasive Species. Accessed February 2021.

Carman, M.R., Morris, J.A., Karney, R.C., Grunden, D.W. 2010. An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. J. Appl. Ichthyol. 26: 8-11.

Carman, M.R., Lindell, S., Green-Beach, E., Starczak, V.R. 2016. Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture socks. Manag. Biol. Invasions 7(1): 101-110.

Carver, C.E., Chrisholm, A., Mallet, A.L. 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.) biofouling on shellfish production. J. Shellfish Res. 22(3) : 621-631.

Chan, F.T., Briski, E. 2017. An overview of recent research in marine biological invasions. Mar. Biol. 164 (121).

Cheng, Y.W., LeClair, L.L. 2011. A quantitative evaluation of the effect of freezing temperatures on the survival of New Zealand mudsnails (*Potamopyrgus antipodarum*, Gray, 1843), in Olympia Washington's Capitol Lake. Aquat. Invasions 6(1): 47-54.

Cherry, D.S., Roy, R.L., Lechleitner, R.A., Dunhardt, P.A., Peters, G.T., Cairns, J. Jr. 1986. *Corbicula* fouling and control measures at the Celco plant, Virginia. Proceedings of the Second International Corbicula Symposium (Special Edition). Am. Malocol. Bull. pp. 69-82.

Choi, W.J., Gerstenberger, S., McMahon, R.F., Wong, W.H. 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. Manag. Biol. Invasions 4: 61–69.

Cimino, S., Strecker, A.L. 2014. OSMB Final Report: Task 6. Tenmile Lake Boat Wash Effectiveness Monitoring. Portland State University. 18 p + appendices.

Clarke Murray, C., Pakhomov, E.A., Therriault, T.W. 2011. Recreational boating: a large unregulated vector transporting marine invasive species. Divers. Distrib. 17: 1161-1172.

Clavero, M., García-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. Trends Ecol. Evol. 20(3):110. Epub. PMID: 16701353.

Cockman, J., Gibbons, A., Kelly, S. 2012. Decontamination for whirling disease on the seeley fire in Utah 2012: Sharing an Important Process with our Fellow Teams. Accessed December 2020. United States Department of Agriculture, U.S. Forest Service (USA). 27 p. + appendices.

Collas, F.P.L., Koopman, K.R., Hendriks, A.J., van der Velde, G., Verbrugge, L.N.H., Leuven, R.S.E.W. 2014. Effects of desiccation on native and non-native molluscs in rivers. Freshwater Biol. 59: 41-55.

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

Comeau, L.A., Sonier, R., Guyondet, T., Landry, T., Ramsay, A., Davidson, J. 2017. Behavioural response of bivalve molluscs to calcium dioxide. Aquaculture 466: 78-85.

Costello, M.J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H., Miloslavich, P. 2010. A census of marine biodiversity knowledge, resources, and future challenges. PLoS ONE 5(8): e12110.

Coughlan, N.E., Cuthbert, R.N., Dickey, J.W.E,. Crane, K., Caffrey, J.M., Lucy, F.E., Davis, E., Dick, J.T.A. 2019. Better biosecurity: spread-prevention of the invasive Asian clam, *Corbicula fluminea* (Muller, 1774). Manag. Biol. Invasions 10: 111-126.

Coughlan, N.E., O'Hara, S., Crane, K., Dick, J.T.A., MacIsaac, H.J., Cuthbert, R.N. 2020a. Touch too much: aquatic disinfectant and steam exposure treatments can inhibit further spread of invasive bloody-red mysid shrimp *Hemimysis anomala*. Wetl. Ecol. Manag. 28: 397-402.

Coughlan, N.E., Bradbeer, S.J., Cuthbert, R.N., Cunningham, E.M., Crane, K., Potts, S., Caffrey, J.M., Lucy, F.E., Dunn, A.M., Davis, E., Renals, T., Quinn, C., Dick, J.T.A. 2020b. Better off dead: assessment of aquatic disinfectants and thermal shock treatments to prevent the spread of invasive freshwater bivalves. Wetl. Ecol. Manag. 28: 285-295.

Coutts, A.D.M., Forrest, B.M. 2005. Evaluation of eradication tools for the clubbed tunicate *Styela clava*. Report No. 1110. Cawthron Institute, Nelson (New-Zealand). 48 p.

Coutts, A.D.M. 2006. An evaluation of incursion response tools for invasive species; a case study of *Didemnum vexillum* in the Marlborough Sounds. Cawthron Institute, Nelson (New-Zealand). Report No. 1093. 84 p.

Coutts, A.D.M., Forrest, B.M. 2007. Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*. J. Exp. Mar. Biol. Ecol. 342: 154-162.

Craft, C.D., Myrick. C.A. 2011. Evaluation of quagga mussel veliger thermal tolerance. Colorado Division of Wildlife Task Order. Report # CSU1003, Colorado State University, Colorado (USA). 21 p.

Crane, K., Cuthbert, R.N., Dick, J.T.A., Kregting, L., MacIsaac, H.J., Coughlan, N.E. 2019. Full steam ahead: direct steam exposure to inhibit spread of invasive aquatic macrophytes. Biol. Invasions 21: 1311-1321.

Crane, K., Cuthbert, R., Cunningham, E., Bradbeer, S.J., Eagling, L., Kregting, L., Dick, J.T.A., Dunning, A.M., Smith, E.R.C., Shannon, C., Caffrey, J., Lucy, F.E., Davis, E., Coughlan, N. 2020. Tomorrow Never Dies: biodegradation and subsequent viability of invasive macrophytes following exposure to aquatic disinfectants. Manag. Biol. Invasions 11:26-43.

Curtis, L.J.F., Pearce, C.M., Hodes, V., Nelson, J.C., Wasser, C., Savery, J., Therriault, T.W. 2021. Mitigating non-indigenous species movements: effects of pressure-washing intensity and duration on the removal of biofouling and mobile invertebrates from cultured Pacific oysters (*Crassostrea gigas* (Thunberg, 1793)). Manag. Biol. Invasions 3: 618-639.

Cuthbert, R.N., Coughlan, N.E., Crane, K., Caffrey, J.M., MacIsaac, H.J., Dick, J.T.A. 2018. A dip or a dab: assessing the efficacy of Virasure® Aquatic disinfectant to reduce secondary spread of the invasive curly waterweed *Lagarosiphon major*. Manag. Biol. Invasion. 9:259–265.

Cuthbert, R.N., Crane, K., Dick, J.T.A., Caffrey, J.M., MacIsaac, H.J., Coughlan, N.E. 2019. Die Hard: survival and viability of invasive *Elodea nuttallii* following submergence in aquatic disinfectants. Aquat. Bot.154:11–17.

Darbyson, E.A., Hanson, J.M., Locke, A., Willison, J.H.M. 2009. Survival of European Green Crab (*Carcinus maenus L.*) exposed to simulated overland and boating-vector transport conditions. J Shellfish Res. 28: 377-382.

David, M. 2015. Vessels and ballast water. *In:* Global maritime transport and ballast water management: issues and solutions. Invading nature – Springer series in invasion ecology, David, M., Gollasch, S. (Eds). pp. 13-34, Springer, Dordrecht.

Davidson, J., Arsenault, G., MacNair, N., Landry, T., Bourque, D. 2005. Reproduction, epidemiology and control of the clubbed tunicate, *Styela clava*. AFRI Project Final Report #043AR15. PEI Aquaculture Alliance and PEI Aquaculture and Fisheries Research Initiative, Prince Edward Island (Canada). 33 p.

Davis, E.A., Wong, D., Harman, W.N. 2015a. Distilled white vinegar (5% acetic acid) as a potential decontamination method for adult zebra mussels. Manag. Biol. Invasions 6(4): 423-428.

Davis, E.A., Wong, W.H., Harman, W.N. 2015b. Comparison of three sodium chloride chemical treatments for adult zebra mussel decontamination. J. Shellfish Res. 34: 1029-1036.

Davis, E.A. 2016. Determining effective decontamination methods for watercraft exposed to zebra mussels, *Dreissena polymorpha* (Pallas 1776), that do not use hot water with high pressure spray. Occasional Paper, State University of New York Oneonta, New York (USA). 85 p.

Davis, E.A., Wong, W.H., Harman, W.N. 2016. Livewell flushing to remove zebra mussel (*Driessena polymorpha*) veligers. Manag. Biol. Invasions 7: 399-403.

Davis, E.A., Wong, W.H., Harman, W.N. 2018. Toxicity of potassium chloride compared to sodium chloride for zebra mussel decontamination. J. Aquat. Anim. Health 30: 3-12.

De Stasio, B.T., Acy, C.N., Frankel, K.E., Fritz, G.M., Lawhun, S.D. 2019. Tests of disinfection methods for invasive snails and zooplankton: effects of treatment methods and contaminated materials. Lake Reserv. Manag. 35: 156-166.

Denny, C.M. 2008. Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. ICES J. Mar. Sci. 65 : 805-810. DOI :

DFO. 2016. Review of potential impacts of hydrated lime treatments associated with proposed expansion of mussel production in Malpeque Bay, PEI. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2016/014.

Dijkstra, J., Dutton A., Westerman, E., Harris, L. 2008. Heart rate reflects osmostic stress levels in two introduced colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus*. Mar. Biol. 154: 805-811.

Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J., Blumenthal, D.M., Bradley, B.A., Early, R., Ibáñez, I., Jones, S.J., Lawler, J.J., Miller, L.P. 2012. Will extreme climatic events facilitate biological invasions? Front. Ecol. Environ. 10: 249-257.

DiVittorio, J., Grodowitz, M., Snow, J. 2012. Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species. U.S. Department of the Interior, Bureau of Reclamation, Managing water in the West, Technical Memorandum #86-68220-07-05. Accessed December 2020. Xii + 221 p.

Dobbs, M.G., Cherry, D.S., Scott, J.C., Petrille, J.C. 1995. Environmental assessment of an alkyl dimethyl benyzl ammonium chloride (ADBAC) based molluscicide using laboratory Tests. Proceedings, Fifth International Zebra Mussel and Other Aquatic Nuisance Organisms Conference, February 21-24, 1995, Toronto, Canada, pp 87– 101.

Doherty, F.G., Farris, J.L., Cherry, D.S., Cairns. J.Jr. 1986. Control of the freshwater fouling bivalve *Corbicula fluminea* by halogenation. Arch. Environ. Contam. Toxicol. 15:535–542.

Drake, D.A.R. 2017. Overland spread of aquatic invasive species among freshwater ecosystems due to recreational boating in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/031. Vi + 38 p.

Drake, D.A.R., Bailey, S.A., Mandrak, N.E. 2017. Ecological Risk Assessment of Recreational Boating as a Pathway for the Secondary Spread of Aquatic Invasive Species in the Great Lakes Basin. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/030. V + 85 p.

Drury, K.L.S., Rothlisberger, J.D. 2008. Offense and defense in land- scape-level invasion control. Oikos. 117:182-190.

Dukes, J.S., Mooney, H.A. 1999. Does global change increase the success of biological invaders? Trends Ecol. Evol. 14(4): 135-139. PMID: 10322518.

Dwyer, W., Kerans, B., Gangloff, M. 2003. Effects of acute exposure to chlorine, copper sulfate, and heat on survival of New Zealand mudsnails. Intermt. J. Sci. 9:53-58.

Elderkin, C.L., Klerks, P.L. 2005. Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, *Dreissena polymorpha*. J. Shellfish Res. 24(1): 221-226.

Ellis, S., MacIsaac, H.J. 2009. Salinity tolerance of Great Lakes invaders. Freshw. Biol. 54: 77-89.

Elwell, L.C. 2010. Gear cleaning, does it really make a difference? Accessed October 2020. Invasive species action network, Livingston, Montana (USA). 4 p.

Elwell, L.C., Phillips, S. (Eds) 2021. Uniform minimum protocols and standards for watercraft inspection and decontamination programs for dreissenid mussels in the Western United States (UMPS IV). Pacific States Marine Fisheries Commission, Portland, Oregon (USA). 55 p.

Evans, C.A., Kelting, D.L., Forrest, K.M., Steblen, L.E. 2011. Fragment viability and rootlet formation in Eurasian watermilfoil after desiccation. J. Aquat. Plant Manag. 49: 57-62.

Ferk, F., Misik, M., Hoelzl, C., Uhl, M., Fuerhacker, M., Grillitsh, B., Parazefall, W., Nersesyan, A., Micieta, K., Grummt, T., Ehrlich, V., Knasmüller, S. 2007. Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations. Mutagenesis 22(6): 363-370.

Fernald, R., Watson, B.T. 2014. Eradication of Zebra Mussels (*Dreissena polymorpha*) from Millibrook Quarry, Virginia – Rapid response in the real world. Quagga and zebra mussels: biology, impacts, and control, chapter 13. Pp. 195-213.

Forrest, B. M., Blakemore, K. A. 2006. Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. Aquaculture 257(1-4): 333-345.

Forrest, B. M., Hopkins, G. A., Dodgshun, T. J., Gardner, J. P. A. 2007. Efficacy of acetic acid treatments in the management of marine biofouling. Aquaculture 262 : 319-332. DOI :

Gallardo, B., Bacher, S., Bradley, B., Comín, F.A., Gallien, L., Jeschke, J.M., Sorte, C.J.B., Vilà, M. 2019. InvasiBES: Understanding and managing the impacts of Invasive alien species on biodiversity and ecosystem services. NeoBiota 50:109–122.

García, M.T., Ribosa, I., Guindulain, T., Sánchez-Leal, J., Vives-Rego, J. 2001. Fate and effect of monoalkyl quaternary ammonium surfactants in the aquatic environment. Environ. Pollut. 111:169-175.

Garton, D.W., Berg, D.L., Fletcher, R.J. 1990. Thermal tolerances of the predatory cladocerans *Bythotrephes cederstroemi* and *Leptodora kindti*: relationship to seasonal abundance in Western Lake Erie. Can. J. Fish. Aquat. Sci. 47 :731-738. DOI :

Gill, K., MacNair, N., Morrison, A. 2007. Investigation into the life cycle, impact on mussel culture and mitigation strategies for the vase tunicate (*Ciona intestinalis*), a new invasive species in the Montague/Brudenell River systems. Final Report #062AR20. PEI Aquaculture Alliance and PEI Department of Fisheries and Aquaculture, Prince Edward Island (Canada). 73 p.

Gonzalez, J.G., Yevich, P. 1976. Response of an estuarine population of the blue mussel *Mytilus edulis* to heated water from a steam generating plant. Mar. Biol. 24: 177-189.

Goss, L.B., Jackson, J.M., Flora, H.B., Isom, B.G., Gooch, C., Murray, S.A., Burton, C.G., Bain, W.S. 1979. Control studies on Corbicula for steam-electric generating plants. *In:* Britton, J.C. (ed). Proceedings, First International *Corbicula* Symposium. Texas Christian University Research Foundation, Fort Worth, Texas, p 139.

Government of Alberta 2020. Decontamination protocol for work in or near water. Accessed February 2021. Alberta (Canada). 24 p. + appendices.

Government of Alberta 2021. Clean, drain, dry your gear: Help prevent the spread of aquatic invasive species and diseases. Accessed Feb 2021.

Government of British Columbia 2020a. Watercraft inspection and decontamination training manual. Ecosystems Branch and Conservation Officer Services, BC Ministry of Environment and Climate Change Strategy. British Columbia Invasive Mussel Defence Program. British Columbia (Canada). 162 p.

Government of British Columbia 2020b. British Columbia Dreissenid Mussel Lake Monitoring Field Protocol. British Columbia (Canada). 48 p.

Government of British Columbia 2021a. What can you do? – Practice clean, drain, and dry. Accessed online February 2021. B.C. Conservation Officer Service, British Columbia (Canada).

Government of British Columbia 2021b. B.C. watercraft Inspection stations. Accessed online February 2021. B.C. Conservation Officer Service, British Columbia (Canada).

Government of British Columbia 2021c. Invasive mussel defence program. Accessed online February 2021. B.C. Conservation Officer Service, British Columbia (Canada).

Government of Manitoba 2021a. Decontamination. Accessed online February 2021. Manitoba (Canada).

Government of Manitoba 2021b. The Water Protection Act – Aquatic Invasive Species Regulation. #173/2015. Accessed February 2021. Manitoba (Canada). 33 p.

Government of Newfoundland and Labrador 2021. Exotic and alien invasive species. Accessed online February 2021. Ministry of Fisheries, Forestry and Agriculture, Newfoundland and Labrador (Canada).

Government of Northwest Territories 2021. Invasive alien species. Accessed online February 2021. Ministry of Environment and Natural Resources, Northwest Territories (Canada).

Government of Nunavut 2021. Non-native invasive species in Nunavut (brochure). Accessed online February 2021. Department of Environment, Nunavut (Canada).

Government of Saskatchewan 2020. Stop aquatic invasive species – Clean drain dry your boat. Accessed online October 2020. Ministry of Environment, Saskatchewan (Canada).

Government of Saskatchewan 2021. No invasive mussels found in Saskatchewan waters in 2020. Accessed online February 2021. Aquatic invasive species monitoring program, Ministry of Environment, Saskatchewan (Canada).

Government of Yukon 2021. Aquatic invasive species. Accessed online February 2021. Science and Natural Resources, Fish and Wildlife species, Yukon (Canada).

Greenshields, F., Ridley, J.F. 1957. Some researches on the control of mussels in water pipes. J. Inst. Water. Eng. 11: 300-6.

Great Britain Non-Native Species Secretariat 2021. Check, Clean, Dry – Help stop the spread of invasive plants and animals in our waters! Accessed online March 2021. Department for Environment, Food, and Rural Affairs, Scottish Government and the Welsh Assembly Government (United Kingdom).

Guareschi, S., Wood, P.J. 2020. Exploring the desiccation tolerance of the invasive bivalve *Corbicula fluminea* (Müller 1774) at different temperatures. Biol. Invasions 22: 2813-2824.

Haight, R.G., Kinsley, A.C., Kao, S.Y., Yemshanov, D., Phelps, N.B.D. 2021. Optimizing the location of watercraft inspection stations to slow the spread of aquatic invasive species. Biol. Invasions 23:3907–3919.

Haque, N., Cho, D., Mee Lee, J., Su Lee, D., Kwon, S. 2014. Proactive approach for biofouling control: Consequence of chlorine on the veliger larvae of *Mytilus edulis* under laboratory condition. Environ. Eng. Res. 19(4): 375-380.

Haque, N., Mahabub, A., Cho, D., Kwon, S. 2015. Sensitivity of veliger larvae of Mytilus edulis and mussel of various sizes to chlorination. Toxicol. Environ. Chem. 97(7):931-945.

Haque, N., Kwon, S. 2017. Assessing the hydrogen peroxide effect along with sodium hypochlorite against marine blue mussels aimed at antifouling usage. Environ. Eng. Res. 22(1): 108-115.

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

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

Hilliard, R., Polglaze, J. 2006. Review and evaluation of the biofouling protocol for vessels less than 25 m in length. Australian Quarantine and Inspection Service, URS Australia (Australia). Xiv + 136 p.

Hillock, K.A., Costello, M.J. 2013. Tolerance of the invasive tunicate *Styela clava* to air exposure. Biofouling 29(10): 1181-1187. DOI :

Hoffman, G.L., Putz, R.E. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. Prog. Fish Cult. 31: 35-37.

Hoffman, G.L., Markiw, M.E. 1977. Control of whirling disease (*Myxosoma cerebralis*): use of methylene blue staining as a possible indicator of effect of heat on spores. J. Fish Biol. 10:181-183.

Hofius, J., Mandella, C., Rackl, S.M. 2015. Evaluation of watercraft quagga mussel decontamination in saltwater. Manag. Biol. Invasions 6: 277-286.

Hopkins, G.A., Prince, M., Cahill, P.L., Fletcher, M., Atalah, J. 2016. Desiccation as a mitigation tool to manage biofouling riks: trials on temperate taxa to elucidate factors influencing mortality rates. Biofouling 32(1): 1-11.

Hosea, R.C., Finlayson, B. 2005. Controlling the spread of New Zealand Mud Snails on wading gear. Report #2005-02. Accessed November 2020. State of California Department of Fish and Game, Office of Spill Prevention and Response, California (USA). Vii + 17 p. + appendices.

Hudson D.M., Sexton, D.J., Wint, D., Capizzano, C., Crivello, J.F 2018. Physiological and behavioral response of the 49rgan shore crab, *Hemigrapsus sanguineus*, to salinity: Implications for estuarine distribution and invasion. Peer J. 6: e5446.

Hulme, P.E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46: 10-18.

Inglis, G., Floerl, O., Woods, C. 2012. Scenarios of vessel biofouling risk and their management. Technical paper #2012/07. Accessed March 2021. Ministry of Agriculture and Forestry, New Zealand Government (New-Zealand). Pp. 41-93.

International Maritime Organization (IMO). 2004. International convention for the control and management of ships' ballast water and sediments, 2004. International Conference on Ballast Water Management for Ships, BWM/CONF/36, London, UK. 36 p.

International Maritime Organization (IMO) 2012. Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft. Document #MEPC.1/Circ.792. Accessed March 2021. 7 p.

Jackson, M.C. 2015. Interactions among multiple invasive animals. Ecology 96: 2035-2041.

Jackson, M.C., Evangelista, C., Zhao, T., Lecerf, A., Britton, R., Cucherousset, J. 2017. Between-lake variation in the trophic ecology of an invasive crayfish. Freshwater Biol. 62: 1501-1510.

Jensen, D.A. 2010. Assessing the effectiveness of aquatic invasive species outreach influencing boater behavior in five states. Retrieved from the University of Minnesota Digital Conservancy. 313 pp..

Jerde, C.L., Barnes, M.A., Debuyssen, E.K., Noveroske, A., Chadderton, W.L., Lodge, D.M. 2012. Eurasian watermilfoil fitness loss and invasion potential following desiccation during simulated overland transport. Aquat. Invasions 7: 135-142.

Johnson, L.E., Ricciardi, A., Carlton, J.T. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecol. Appl. 11: 1789-1799.

Joyce, P.W.S., Cuthbert, R.N., Kregting, L., Crane, K., Vong, G.Y.W., Cunningham, E.M., Dick, J.T.A., Coughlan, N.E. 2019. Stay clean: direct steam exposure to manage biofouling risks. Mar. Pollut. Bull. 142: 465-469.

Kappel, M. 2012. *Dreissena rostiformis bugensis*: desiccation of adult quagga mussels found in Lake Mead as a preventive measure against overland dispersal in the western United States. Thesis, University of Nevada, Las Vegas, Nevada (USA). Paper 1744. X + 81 p.

Kelly, D.W. 2007. Vectors and pathways for nonindigenous aquatic species in the Great Lakes. Transportation Research Board Special Report #291 – Great Lakes shipping, trade, and aquatic invasive species. Accessed March 2021. Report commissioned by The National Academies Committee on the St. Lawrence Seaway: Options to eliminate nonindigenous species into the Great Lakes, Phase 2. 27 p.

Kelly, N.E., Wantola, K., Weisz, E., Yan, N.D. 2013. Recreational boats as a vector of secondary spread for aquatic invasive species and native crustacean zooplankton. Biol. Invasions 15: 509-519.

Kilgour, B.W., Mackie, G.L., Baker, M.A., Keppel, R. 1994. Effects of salinity on the condition and survival of zebra mussels (*Dreissena polymorpha*). Estuaries 17(2): 385-393.

Kim, K.U., Garbary, D.J. 2007. Photosynthesis in *Codium fragile* (Chlorophyta) from a Nova Scotia estuary: responses to desiccation and hyposalinity. Mar. Biol. 151: 99-107.

Klerks, P.L., Fraleigh, P.C. 1991. Controlling adult zebra mussels with oxidants. J. Am. Water Works Assoc. 83: 92-100.

Lacoursière-Roussel, A., Bock, D., Cristescu, M., Guichard, F., Girard, P., Legendre, P., McKindsey, C.W. 2012. Disentangling invasion processes in a dynamic shipping - boating network. Mol. Ecol. 21:4227–4241.

Lake Stewards of Maine 2019. Beyond clean, drain and dry: advanced decontamination protocols for boats, trailers, and gear. Accessed November 2020. Maine (USA). 1 p.

Langkilde, T., Thawley, C., Robbins, T. 2017. Behavioral adaptations to invasive species: benefits, costs, and mechanisms of change. Adv. Study Behav. 49:199-235.

Leblanc, N., Landry, T., Stryhn, H., Tremblay, R., McNiven, M., Davidson, J. 2005. The effect of high air and water temperature on juvenile *Mytilus edulis* in Prince Edward Island, Canada. Aquaculture 243: 185-194.

Lewis, D.P., Piontkowski, J.M., Straney, R.W., Knowlton, J.J., Neuhauser, E.F. 1997. Use of potassium for treatment and control of zebra mussel infestation in industrial fire protection water system. Fire Technol. 33(4): 356-371.

Lévesque, B., Lavoie, M., Joly, J. 2004. Residential water heater temperature: 49 or 60 degrees Celsius? Can. J. Infect. Dis. 15:11-12.

Li, X., Brownawell, B.J. 2010. Quaternary ammonium compounds in urban estuarine sediment environments—a class of contaminants in need of increased attention. Environ. Sci. Technol. 44:7561-7568.

Locke, A., Doe, K.G., Fairchild, W.L., Jackman, P.M., Reese, E.J. 2009. Preliminary evaluation of effects of invasive tunicate management with acetic acid and calcium hydroxide on non-target marine organisms in Prince Edward Island, Canada. Aquat. Invasions 4(1): 221-236.

Lodge, D.M., Shrader-Frechette, K. 2003. Nonindigenous species: ecological explanation, environmental ethics, and public policy. Conserv. Biol. 17(1): 31-37.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecol. Appl. 10: 689-710.

MacNair, N. 2002. Codium fragile treatment trials. Report #AIN10.2002. Accessed November 2020. Aqua Info – Aquaculture Notes. Government of Prince Edward Island (Canada). 4 p.

MacNair, N., Morison, A., Mills, C., Campbell, E. 2006. Investigation into the life cycles, impact on mussel culture and mitigation strategies for two new invasive colonial tunicates, the golden star and the violet tunicate. Savage Harbour PEI: AFRI Report 060AR18. PEI Department of Agriculture, Fisheries, and Aquaculture, Fisheries and Aquaculture Division, Charlottetown, PEI (Canada). Iv + 20 + appendices.

MacNair, N. 2009. Control of Green Algae on Mussel Seed Collectors. Report #AIN22.2009. Accessed March 2021. Aqua Info-Aquaculture Notes. Government of Prince Edward Island (Canada). 4 p.

MacIsaac, H.J., Robbins, T.C., Lewis, M.A. 2002. Modelling ships' ballast water as invasion threats to the Great Lakes. Can. J. Fish. Aquat. Sci. 59:1245–1256.

Magellan, K. 2020. Prayer animal release: An understudied pathway for introduction of invasive aquatic species. Aquat. Ecosyst. Health. 22(4): 452-461.

Marine Pest Sectoral Committee 2018a. National biofouling management guidelines for recreational vessels. Accessed March 2021. Australian Government, Department of Agriculture and Water Resources, Canberra (Australia). 9 p.

Marine Pest Sectoral Committee 2018b. National biofouling management guidelines for non-trading vessels. Accessed March 2021. Australian Government, Department of Agriculture and Water Resources, Canberra (Australia). 43 p.

Martin, M.D., Mackie, G.L., Baker, M.A., 1993a. Control of the biofouling mollusk, *Dreissena polymorpha* (Bivalvia: Dreissenidae), with sodium hypochlorite and with polyquaternary ammonia and Benzothiazole compounds. Arch. Environ. Contam. Toxicol. 24: 381-388.

Martin, M.D., Mackie, G.L., Baker, M.A. 1993b. Acute toxicity tests and pulsed-dose delayed mortality at 12 and 22°C in the zebra mussel (*Dreissena polymorpha*). Arch. Environ. Contam. Toxicol. 24: 389-398.

Matthews, M.A., McMahon, R.F. 1999. Effects of temperature and temperature acclimation on survival of zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia: J. Molluscan Stud. 65:317-325.

Matisoff, G., Brooks, G., Bourland, B.I. 1996. Toxicity of chlorine dioxide to adult zebra mussels. J. Am. Water Works Assoc. 88: 93-106.

Mattice, J.S., McLean, R.B., Burch, M.B. 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 Laboratory, Tennassee (USA).

McCann, L.D., Holzer, K.K., Davidson, I.C., Ashton, G.V., Chapman, M.D., Ruiz, G.M. 2013. Promoting invasive species control and eradication in the sea: Options for managing the tunicate invader *Didemnum vexillum* in Sitka, Alaska. Mar. Pollut. Bull. 77: 165-171.

McFarland, K., Baker, S., Baker, P., Rybovich, M., Volety, A.K. 2015. Temperature, salinity, and aerial exposure tolerance of the invasive mussel, *Perna viridis*, in estuarine habitats: Implications for spread and competition with native oysters, *Crassostrea virginica*. Estuar. Coasts 38, 1619–1628.

McKenzie, C.H., Matherson, K., Reid, V., Wells, T., Mouland, D., Green, D., Pilgrim, B., and Perry, G. 2016. The development of a rapid response plan to control spread of the solitary invasive tunicate, *Ciona intestinalis* (Linnaeus, 1767), in Newfoundland and Labrador, Canada. Manag. Biol. 7(1): 87-100.

McMahon, R.F., Ussery, T.A., Clarke, M. 1993. Use of emersion as a zebra mussel control method. Technical report EL-93-1. U.S. Army Corps of Engineers, Experiment Station, Vicksburg, Mississippi (USA). V + 27 p. + appendix.

McMahon, R.F., Ussery, T.A., Clarke, M. 1994. Review of zebra mussel control methods. Technical Note ZMR-2-14, U.S. Army corps of Engineers, Experiment Station, Vicksburg, Mississippi (USA). 12 p.

McMahon, R.F., Ussery, T.A. 1995. Thermal tolerance of zebra mussels (*Dreissena polymorpha*) relative to rate of temperature increase and acclimatation temperature. Technical report EL-95-10, U.S. Army corps of Engineers, Experiment Station, Vicksburg, Mississippi (USA). V + 19 p. + appendices.

MFFP (Ministère des Forêts, de la Faune et des Parcs) 2018. Guide to best practices in aquatic environments to prevent the introduction and propagation of aquatic invasive species. Accessed October 2020. Gouvernement du Québec, Québec (Canada). Xi + 35 p.

Michigan Department of Environmental Quality 2014. Policy and procedure: Invasive Species Decontamination for Field Operations in Michigan. Report #QOL-2-2014. Accessed November 2020.. Accessed November 2020. Michigan Department of Environmental Quality, Department of Natural Resources, and Department of Agriculture and Rural Development. Michigan (USA). 25 p.

Miller, N., Richardson, P., Collins, E., Young, K. 2006. Design and feasibility of wash-down stations for boats entering the Rotorua Lakes. Doc Research and Development Series #247. Accessed October 2020. Science and Technical Publishing Department of Conservation (Pub.), Wellington (New-Zealand). 25 p.

Mills, E.L., Leach, J.H., Carlton, J.T., Secor, C.L. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions J. Great Lakes Res. 19(1): 1-54.

Mills, E.L., Edward, L., Leach, J.H., Carlton, J.T., Secor, C.L. 1994. Exotic species and the integrity of the Great Lakes: Lessons from the past. Bioscience 44(10): 666-676.

Mineur, F., Belsher, T., Johnson, M.P., Maggs, C.A., Verlaque, M. 2007. Experimental assessment of oyster transfers as a vector for macroalgal introductions. Biol. Conserv. 137 : 237-247. DOI :

Minnesota Department of Natural Resources 2013. Aquatic Invasive Species (AIS) Watercraft Decontamination Handbook for Lake Service Providers. Accessed October 2020. Minnesota Government, Minnesota (USA). 11 p.

Minnesota Department of Natural Resources 2017. Aquatic invasive species and the watercraft inspection program, watercraft decontamination manual. Accessed October 2020. Minnesota Government, Minnesota (USA). Ix + 68 p.

Moffitt, C.M., Barenberg, A., Stockton, K.A., Watten, B.J. 2015. Efficacy of two approaches for disinfecting surfaces and water infested with quagga mussel veligers. Biology and management of invasive quagga and zebra mussels in the Western United States, CRC Press, USA, 467-477.

Moffitt, C.M., Stockton-Fiti, K.A., Claudi, R. 2016. Toxicity of potassium chloride to veliger and byssal stage of dreissenid mussels related to water quality. Manag. Biol. Invasions 7(3): 257-268.

Mohit, S., Johnson, T.B., Arnott, S.E. 2021. Recreational watercraft decontamination: can current recommendations reduce aquatic invasive species spread? Manag. Biol. Invasions 12 (1):148-164.

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: 605-610.

Muñoz L., Kelley A.L.D., Rivera, C.E. 2017. The effect of salinity acclimation on the upper thermal tolerance threshold of the European Green Crab. Ocean & Fish Open Access J. 4: 555627.

National Research Council of Canada (NRCC), 2015. « National Plumbing Code of Canada: 2015 ». Canadian Commission On Building And Fire Codes.

NBISC (New Brunswick Invasive Species Council 2019). Clean drain dry – Prevent the spread of aquatic invasive species. Accessed online February 2021. New Brunswick (Canada).

New York State Department of Environment Conservation 2015. A New York boaters guide to cleaning, drying and disinfecting boating equipment. Accessed October 2020. New York (USA). 6 p.

New Zealand Government 2020. Check, Clean, Dry: Preventing didymo and other pests. Accessed online February 2021. Ministry for Primary Industries (New Zealand).

NSISC (Nova Scotia Invasive Species Council) 2020. Clean drain dry. Accessed online February 2021. Nova Scotia (Canada).

Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Stephens, L., Radloff, S., Blatch, G.L. 2010. The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. BMC Ecol. 10:17.

OMNRF (Ontario Ministry of Natural Resources and Forestry) 2017. Boater Action Plan. Accessed February 2021. Government of Ontario, Ontario (Canada). 1 p.

Oplinger, R.W., Wagner, E. 2011. Tests of the ability of five disinfectants to kill New Zealand mudsnails. J. Appl. Aquacult. 23(2): 187-197.

Paetzold, S.C., Hill, J., Davidson, J. 2012. Efficacy of high-pressure seawater spray against colonial tunicate fouling in mussel aquaculture: inter-annual variation. Aquat. Invasions 7(4): 555-566.

Page, M.J., Morrisey, D.J., Handley, S.J., Middleton, C. 2011. Biology, ecology and trials of potential methods for control of the introduced ascidian *Eudistoma elongatum* (Herdman, 1886) in Northland, New Zealand. Aquat. Invasions 6(4) : 515-517. DOI :

Pagnucco, K.S., Maynard, G.A, Fera, S., Yan, N.D, Nalepa, T.F, Ricciardi, A. 2015. The future of species invasions in the Great Lakes-St. Lawrence River basin. J. Great Lakes Res. 41:96–107. DOI :

Pannell, A., Coutts, A.D.M., 2007. Treatment methods used to manage *Didemnum vexillum* in New Zealand. New Zealand Marine Farming Association Incorporated Report. Submitted to M.A.F. Biosecurity New Zealand. 29 p.

Payne, B.S. 1992. Freeze survival of aerially exposed zebra mussels. Technical Note ZMR-2-09, U.S. Army Engineer Waterways Experiment Station (WES), Arlington,Texas (USA). 2 p.

Peck, L.S., Morley, S.A., Richard, J., Clark, M.S., Davies, S.A., Dow, J.A.T., Lukowiak, K. 2014. Acclimation and thermal tolerance in Antarctic marine ectotherms. J. Exp. Biol. 217 (1): 16–22.

PEIISC (Prince Edward Island Invasive Species Council) 2021. PEI invasive species council. Accessed February 2021. Prince Edward Island (Canada).

Pelletier-Rousseau, M., Bernier, R., Clarke Murray, C., Drolet, D., Lacoursière-Roussel, A., Locke, A., Martin, J.L., McKenzie, C.H., Mckindsey, C.W., Therriault, T.W., Simard, N. 2019. Assessment of recreational boating as a vector for marine non-indigenous species on the Atlantic coast of Canada. Biol. Invasions 21: 2447-2470.

Piola, R.F., Dunmore, R.A., Forrest, B.M. 2009. Assessing the efficacy of spray delivered 'ecofriendly' chemicals for the control and eradication of marine fouling pests. Biofouling 26: 187-203.

Rajagopal, S., Van der Velde, G., Jenner, H.A. 2002. Effects of low-level chlorination on zebra mussel, *Dreissena polymorpha*. Water Res. 36: 3029-3034.

Rajagopal, S., Van der Velde, G., Van der Gaap, M., Jenner, H.A. 2003. How effective is intermittent chlorination to control adult mussel fouling in cooling water systems? Water Res. 37: 329-338.

Rajagopal, S., Van Der Velde, G., Van Der Gaag, M., Jenner, H.A. 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.

Ramsay, G.G., Tackett, J.H., Morris, D.W. 1988. Effect of low-level continuous chlorination on *Corbicula fluminea*. Environ. Toxicol. Chem. 7 : 855-856. DOI :

Ramsay, A. 2014. High-pressure water application to control the vase tunicate and increase mussel productivity. Report #AIN23.2014. Accessed July 2021. Aqua Info – Aquaculture Notes. Government of Prince Edward Island (Canada). 4 p.

Ramsay, A., Gill, K., Morrison, A., MacNair, N. 2014. Hydrated lime application by the PEI aquaculture industry. Technical Report #253. Government of Prince Edward Island, Department of Fisheries, Aquaculture and Rural Development, Aquaculture Division, Prince Edward Island (Canada). 35 p.

Ramsay, A. 2015a. Fresh water treatment as a management tool to control the spread of colonial tunicates. Report #AIN 25. Accessed November 2020. Aqua Info – Aquaculture Notes. Government of Prince Edward Island (Canada). 4 p.

Ramsay, A. 2015b. Fresh water immersion to control de Vase Tunicate, Ciona intestinalis. Report #AIN 26. Accessed November 2020. Aqua Info, Aquaculture Notes. Government of Prince Edward Island (Canada). 4 p.

Ramsay, A. 2015c. Fresh water immersion to control the Clubbed Tunicate, Styela clava. Report #AIN 27. Accessed November 2020. Aqua Info – Aquaculture Notes. Government of Prince Edward Island (Canada). 2 p.

Reid, D.F., Orlova M. 2002. Geological and evolutionary underpinnings for the success of Ponto-Caspian species invasions in the Baltic Sea and North American Great Lakes. Can. J. Fish. Aquat. Sci. 59:1144–1158.

Ricciardi, A., Serrouya, R., Whoriskey, F.G. 1995. Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. Can. J. Fish. Aquat. Sci. 52: 470–477.

Ricciardi, A., Rasmussen, J.B. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can. J. Fish. Aquat. Sci. 55:1759-1765.

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. Divers. Distrib. 12 : 425-433.

Ricciardi, A. 2007. Are modern biological invasions an unprecedented form of global change? Conserv. Biol. 21: 329-336.

Richards, D.C., O'Connell, P., Shinn, D.C. 2004. Simple control method to limit the spread of the New Zealand mudsnail *Potamopyrgus antipodarum*. N. Am. J. Fish. Manag. 24: 114-117.

Roche, R.C., Monnington, J.M., Newstead, R.G., Sambrook, K., Griffith, K., Holt, R.H.F., Jenkins, S.R. 2015. Recreational vessels as a vector for marine non-natives: developing biosecurity measures and managing risk through an in-water encapsulation system. Hydrobiologia 750(1): 187-199.

Rolheiser, K.C., Dunham, A., Switzer, S.E., Pearce, C.M., Therriault, T.W. 2012. Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. Aquaculture 364-365: 53-60.

Rothlisberger, J.D., Chadderton, W.L., McNulty, J. 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries 35: 121-132.

Rothlisberger, J.D., Lodge, D.M. 2011. Limitations of gravity models in predicting the spread of Eurasian watermilfoil. Conserv. Biol. 25:64–72.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J., Hines, A.H. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. 31(1): 481-531.

Sarkar, B., Megharaj, M., Xi, Y., Krishnamurti, G.S., Naidu, R. 2010. Sorption of quaternary ammonium compounds in soils: implications to the soil microbial activities. J. Hazard. Mater. 184:448-456.

Saul, W.-C., Roy, H.E., Booy, O., Carnevali, L., Chen, H.-J., Genovesi, P., Harrower, C.A., Hulme, P.E., Pagad, S., Pergl, J., Jeschke, J.M. 2017. Assessing patterns in introduction pathways of alien species by linking major invasion data bases. J. Appl. Ecol. 54: 657-669.

Scheffer, M., 2009. Critical Transitions in Nature and Society. Princeton University Press, Princeton, New Jersey (USA). 400 p.

Schisler, G.J., Vieira, N., Walker, P.G. 2008. Application of household disinfectants to control New Zealand mudsnails. N. Am. J. Fish. Manage. 28(4): 1172-1176.

Sebire, M., Rimmer, G., Hicks, R.J., Parker, S., Stebbing, P.D. 2018. A preliminary investigation into biosecurity treatments to manage the invasive killer shrimp (*Dikerogammarus villosus*). Manag. Biol. Invasions 9: 101-113.

Seuront, L., Nicastro, K.R., Zardi, G.I., and Goberville, E. 2019. Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel *Mytilus edulis*. Sci. Rep. 9: 17498.

Shannon, C., Quinn, C.H., Stebbing, P.D., Hassall, C., Dunn, A.M. 2018. The practical application of hot water to reduce the introduction and spread of aquatic invasive alien species. Manag. Biol. Invasions 9: 417–423.

Sharp, G.J., MacNair, N., Campbell, E., Butters, A., Ramsay, A., Semple, R. 2006. Fouling of mussel (*Mytilus edulis*) collectors by algal mats, dynamics, impacts and symptomatic treatment in P.E.I. Canada. Sci. Asia 32(1): 87-97.

Schlisler GJ, Vieira NK, Walker PG (2008) Application of household disinfectants to control New Zealand mudsnails. N. Am. J. Fish. Manage. 28: 1172– 1176, 10.1577/M07-028.1

Sievers, M., Dempster, T., Keough, M.J., Fitridge, I. 2019. Methods to prevent and treat biofouling in shellfish aquaculture. Aquaculture. 505: 263-270.

Simard, N., Pelletier-Rousseau, M., Clarke Murray, C., McKindsey, C.W., Therriault, T.W., Lacoursière-Roussel, A., Bernier, R., Sephton, D., Drolet, D., Locke, A., Martin, J.L., Drake D.A.R., McKenzie, C.H. 2017. National risk assessment of recreational boating as a vector for Marine Non-indigenous Species. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/006. Vi + 95 p.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M. 2013. Impacts of biological invasions: what's what and the way forward. Trends Ecol. Evol. 28(1): 58-66.

Simon, K.S., Townsend, C.R. 2003. Impacts of freshwater invaders at different levels of ecological 56rganization, with emphasis on salmonids and ecosystem consequences. Freshw. Biol. 48: 982-994.

Snider, J.P., Moore, J.D., Volkoff, M.C., Byron, S.N. 2014. Assessment of quagga mussel (*Dreissena bugensis*) veliger survival under thermal, temporal and emersion conditions simulating overland transport. Calif. Fish Game 100(4): 640-651.

Spidle, A.P., May, B., Mills, E.L. 1995. Limits to tolerance of temperature and salinity in the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*D. polymorpha*). Can. J. of Fish. And Aquat. Sci. 52(10): 2108-2119.

Stebbing, P., Sebire, M., Lyons, B. 2011. Evaluation of a number of treatments to be used as biosecurity measures in controlling the spread of the invasive killer shrimp (*Dikerogammarus villosus*). Report C5256. Centre for Environment, Fisheries and Aquaculture Science. Weymouth (England). 41 p.

Stockton, K.A. 2011. Methods to assess, control, and manage risks for two invasive mollusks in fish hatcheries. M.S. Thesis, University of Idaho, Idaho (USA). Xix + 170 p.

Stockton, K.A., Moffitt., C.M. 2013. Disinfection of three wading boot surfaces infested with New Zealand mudsnails. N. Am. J. Fish. Manage. 33: 529-538.

Stout, J.B., Avila, B., Fetherman, E. 2016. Efficacy of commercially available quaternary ammonium compounds for controlling New Zealand mudsnails *Potamopyrgus antipodarum*. N. Am. J. Fish. Manage. 36: 277-284.

Strayer, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshw. Biol. 55: 152-174.

Strayer, D.L., Smith, L.C. 1993. Distribution of the zebra mussel (*Dreissena polymorpha*) in estuaries and brackish waters. *In*: Zebra Mussels: Biology, Impacts and Control. Napela, T.F., Schloesser, D.W. (Eds), pp. 715–727. Lewis Publishers, Ann Arbor.

Strayer, D.L. 2012. Eight questions about invasions and ecosystem functioning. Ecol. Lett. 15(10): 1199-1210.

Switzer, S.E., Therriault, T., Dunham, W.A., Pearce, C.M. 2011. Assessing potential control options for the invasive tunicate *Didemnum vexillum* in shellfish aquaculture. Aquaculture 318(1-2): 145-153.

Taylor, C.J.L. 2006. The effects of biological fouling control at coastal and estuarine power stations. Mar. Pollut. Bull. 53:30–48.

Tilly, L.J. 1976. Clam survival in chlorinated water. Report No. DP-1398. E. I. DuPont de Nemours & Company, Savannah River Laboratory, Aiken, SC.

Ussery, T.A., McMahon, R.F. 1995. Comparative study of the desiccation resistance of zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*). Technical Report EL-95-6. U.S. Army Corps of Engineers, Experiment Station, Vicksburg, Mississippi (USA). iv + 19 p. + appendices.

Utah Department of Natural Resources 2012. Utah decontamination protocols to minimize risk for introduction or spread of aquatic invasive species. Accessed February 2021. State of Utah (USA). 5 p.

Van Benschoten, J.E., Jensen, J.N., Brady, T.J., Lewis, D.P., Sferrazza, J., Neuhauser, E.F. 1993. Response of zebra mussel veligers to chemical oxidants. Water Res. 27(4): 575-582.

Vander Zanden, M.J., Casselman, J.M., Rasmussen, J.B. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401(6752): 464-467.

Verween, A., Vincx, M., Degraer, S. 2009. Comparative toxicity of chlorine and peracetic acid in the biofouling control of *Mytilopsis leucophaeata* and *Dreissena polymorpha* embryos (Mollusca, Bivalvia). Int. Biodeterior. Biodegradation. 63(4): 523-528.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R. 1996. Biological invasions as global environmental change. Am. Sci. 84: 468-478.

Wada, T., Matsukura, K. 2011. Linkage of cold hardiness with desiccation tolerance in the invasive freshwater apple snail, *Pomacea canaliculata* (Caenogastropoda: Ampullariidae). J. Molluscan Stud. 77:149-153.

Waller, D.L., Rach, J.J., Cope, W.G., Marking, L.L. 1993. Toxicity of candidate chemicals for zebra mussel control to selected nontarget organisms. J. Great Lakes Res. 19(4): 695-70.

Waller, D.L., Fisher, S.W., Dabrowska, H. 1996. Prevention of zebra mussel infestation and dispersal during aquaculture operations. Prog. Fish-Cult. 58: 77-84.

Wagner, E.J. 2002. Whirling disease prevention, control, management: a review. Am. Fish. Soc. 29: 217-225.

Wagner, E.J., Smith, M., Arndt, R., Roberts, D.W. 2003. Physical and chemical effects on viability of the *Myxobolus cerebralis* triactinomyxon. Dis. Aquat. Org. 53: 133-142.

Washington Department of Fish and Wildlife 2016. Invasive Species Management Protocols. Accessed February 2021. WDFW Invasive Species Management Committee, Washington (USA). 15 p.

Watermann, B.T., Broeg, K., Krutwa, A., Heibeck, N. 2021. Guide on best practices of biofouling management in the Baltic sea. Complete Report. 31p. Available at

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

Wisconsin Department of Natural Resources 2020. Best management practices for boat, gear and equipment decontamination. Accessed October 2020. Wisconsin (USA).

Wong, W.H., Gerstenberger, S., Watters, A. 2014. Using pressurized hot water spray to kill and remove dreissenid mussels on watercraft: Field testing on the efficacy of water temperature, high pressure, and duration of exposure. Accessed October 2020. Report to U.S. Fish and Wildlife Service (USA). 20 p.

Wyoming Game and Fish Department 2016. State of Wyoming aquatic invasive species watercraft inspection manual. Wyoming Game and Fish Department, Cheyenne, Wyoming (USA). 26 p. + Appencices.

Zhang, C., Cui., F., Zeng, G.-M., Jiang, M., Yang, Z.-Z., Yu, Z-G., Zhu, M.-Y., Shen, L.-Q. 2015. Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. Sci. Total Environ. 518-519: 352-362.

8. TABLES

Table 1. Summary of the freshwater and marine aquatic invasive species (AIS) that were assessed in the present work.

Representative group	AIS species
Bivalves	Zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena rostriformis bugensis*), Asian clam (*Corbicula fluminea*), and blue mussel (*Mytilus edulis*)
Gastropods	New Zealand mud snail (*Potamopyrgus antipodarum*)
Zooplankton	Bloody-red shrimp *(Hemimysis anomala),* spiny waterflea *(Bythotrephes longimanus),* fishhook waterflea *(Cercopagis pengoi),* and killer shrimp (*Dikerogammarus villosus*)
Parasites	*Myxobolus cerebralis* which causes whirling disease
Macrophytes	Eurasian watermilfoil (*Myriophyllum spicatum*), parrot's feather (*Myriophyllum aquaticum*) water thyme (*Hydrilla verticillata*), fanwort (*Cabomba caroliniana*), and curly-leaf pondweed (*Potamogeton crispus*)
Macroalgae	Oyster thief (*Codium fragile)*
Crabs	European green crab (*Carcinus maenas*)
Solitary tunicates	Clubbed tunicate (*Styela clava*), vase tunicate (*Ciona intestinalis*), and European sea squirt (*Ascidiella aspersa*)
Colonial tunicates	Violet tunicate (*Botrylloides violaceus*), golden star tunicate (*Botryllus schlosseri*), carpet sea squirt (*Didemnum vexillum*), and compound sea squirt (*Diplosoma listerianum*)

Table 2. Uncertainty score calculations for effective decontamination treatments to kill the greatest number of target freshwater or marine AIS. Levels of uncertainty were assigned to each decontamination treatment option per species, and scores were assigned based on the number of studies available (few, limited, many or comprehensive), their quality (personal communication, technical report or peer reviewed), and their agreement with the identified treatment option (contradictory, different conclusions, mostly agree or agree). Uncertainty scores were not calculated for ineffective treatments. The final score is based on the sum of scores obtained for the data sources, their quality, and their agreement with the identified decontamination treatment option.

Data sources	Score	Quality	Score	Agreement	Score	Final score	
Few (≤ 1 study)	0	Pers. comm.	0	Contradictory	0	No data	0
Limited (2 studies)	1	Technical report	1	Different conclusions	1	Very high uncertainty	1-2
Many (3 to 6 studies)	2	Peer-reviewed	2	Mostly agree	2	High uncertainty	3-5
Comprehensive (≥ 7 studies)	3	-	-	Agree	3	Reasonable uncertainty	6-7
	-	-	-	-	-	Low uncertainty	8

*Table 3. Effectiveness of physical decontamination treatments for freshwater AIS, where "100%" refers to 100% mortality (unless otherwise specified) for a particular treatment combination, specified below by life stage, where possible. "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. NS: not specified, RH: relative humidity, *: technical reports, Δ: acclimation laboratory experiments. **Lethal air-drying times are dependent on RH and size (e.g., dreissenid mussels). References are enumerated in superscript and all field experiments are italicized.*

Freshwater AIS	Hot water immersion	Hot water spray		Air-drying**		Freezing
		Low pressure (<60 psi)	High pressure (>400 psi)	(≥ 20°C)	(< 20°C)	
	(temperature; time)	(temp.; pressure; time)	(temp.; pressure; time)	(temperature; RH; time)	(temperature; RH; time)	(temperature; time)
MOLLUSCS						
Zebra mussel (adults) *Dreissena polymorpha*	**100%** 32°C; > 4 d[35Δ] 36°C; 38 min[65Δ] 40°C; 10 s[71]; 30 min[65Δ] 43°C; 5 min[9] 45°C; 15 min[2] 49°C; 1 min[9] **99%** 58°C; 10 s, 61°C, 2 s[61] ***95%*** *30°C; 275 h[41]* *32°C; 20 h[41]* *36°C; 30 min[41]* **Ca. 90%** 38°C; 20 min[9] **Not effective** 32°C; 20 min[9]	***100%*** *40-50°C; 2 psi; 40 s[86*]* *54-60°C; 2 psi; 10 s[86*]* 60°C; 15 psi; 10 s[62] *70-80°C; 2 psi; 5 s[86*]* 80°C; 15 psi; 5 s[62] 100°C; steam; 30 s[26] **Not effective** 20°C; 15 psi; 10 s[62] *20°C; 2 psi; 160 s[86*]* *40°C; 2 psi; 10 s[86*]* **38-80%** *50°C; 2 psi; 5-10 s[86*]*	**100%** *67.4°C; 1600 psi; 15 s[14]* Effective removal *NS; 3000 psi; 32-52 s[86*]* *NS; 1500 psi; 41-472 s[86*]* **58-92%** *67.4°C; 1600 psi; 5-10 s[14]* **25-83%** *59°C; 1600 psi; 5-15 s[14]* **17-33%** *55.9°C; 1600 psi; 5-15 s[14]*	**100%** 20°C; 10-95%; 5-7 d[67] 25°C; 5-95%; 3-4 d[58*] 30°C; 10-95%; 1-5 d[67] **99%** 20°C; NS; 16.2-58 h[61] 20°C; 68%; 42 h[21] **53-84%** 20°C; 50, 95%; 5 d[67] **Not effective** Several temp/RH combinations[58*, 67]	**100%** 5°C; 5-95%; 15-47 d[58*] 10°C; 10-95%; 5-15 d[67] 14°C; RH NS; 6 d[2] 15°C; 5-95%; 5-12 d[79*] **97%** 10°C; 50%; 5 d[67] **90%** 14°C; NS; 7 d[2] **75%** 10°C; 10%; 5 d[67] **Not effective** Several temp/RH combinations[58*, 67]	**100%** -1.5°C (air); 15 h (separate)[12, 58*] -3°C (air); 5 h (separate) or 7 h (clustered)[12, 58*] -10°C (air); 0.5 h (separate) or 2 h (clustered)[12, 58*] **Not effective** -1.5°C (air); 48 h (clustered)[12, 58*]
Zebra mussel (veliger larvae) *Dreissena polymorpha*	-	**90% removal** *NS, 60 psi, 150 s[91]*	-	**90%** *27.5°C; 30-80%; 65-189 min[3]*	**90%** *17.5°C; 30-80%; 100-192 min[3]*	-
Quagga mussel (adults) *Dreissena bugensis*	**100%** 38°C; 20 min[9] 43°C; 5 min[9] 49°C; 1 min[9] **Not effective** 32°C; 20 min[9]	**100%** *40°C; 2 psi; 40 s[23, 86*]* *50°C; 2 psi; 20 s[23, 86*]* *54°C; 2 psi; 10 s[23, 86*]* *60°C; 2 psi; 5 s[23, 86*]* *80°C; 2 psi; 5 s[86*]* 100°C; steam; 30 s[26] **Not effective** 20°C; 2 psi; 160 s[23]	**Effective removal** *NS; 3000 psi; 43-48 s[86*]* *NS; 1500 psi; 37-430 s[86*]*	**100%** 20°C; 10- 95%; 3-5 d[67] 20°C; 20-80%; 3 d[48] 30-40°C; 20-80%; 1 d[48] **99%** 20°C; 68%; 45 h[21]	**100%** 10°C; 95%; 10-15 d[67] 15°C; 5-95%; 5-13 d[79*] **Not effective** 10°C; 20-80 %; 5 d[48]	-

Freshwater AIS	Hot water immersion	Hot water spray		Air-drying**		Freezing
		Low pressure (<60 psi)	High pressure (>400 psi)	(≥ 20°C)	(< 20°C)	
	(temperature; time)	(temp.; pressure; time)	(temp.; pressure; time)	(temperature; RH; time)	(temperature; RH; time)	(temperature; time)
Quagga mussel (veliger larvae) *Dreissena bugensis*	**100%** 30°C; > 5 d[20] 35°C; 20 h[72*], 24 h[27*] 37°C; 1 h[72] **Not effective** 5-30°C; 20 h[72] 25°C; 7 d[72] 30°C; 20 h[72] 35-36°C; 1 h[72]	-	-	**100%** 30°C; 95%; 20 h[72] 35-40°C; > 95%; 4 h[72] **Not effective** 5-30°C; 95%; 4 h[72]	-	-
New Zealand mudsnail (adults) *Potamopyrgus antipodarum*	**100%** 45°C; 1 min[34] 50°C; ≥ 15 s[34]	-	-	**99%** 20°C; 68%; 44 h[21] 21°C; 90-100%; 45 h[68] 29°C; 90-100%; 21 h[68] 40°C; 90-100%; 1 h [68]	**99%** 9°C; 20-25%; 60 h[68] 14°C; 20-25%; 68 h[68] 15°C; 69%; 53 h[1]	**98%** *-8 to -14°C (air); 4 d[18]*
New Zealand mudsnail (young stages) *Potamopyrgus antipodarum*	-	-	-	-	-	-
Asian clam (adults) *Corbicula fluminea*	**100%** 41°C; 40 min[56*] 45°C; 5 min[24]	**100%** 100°C; steam; 30 s[24]	-	**100%** 25-30°C; 80%; 48 h[40] **99%** 20°C; 68%; 23 d[21] **90%** 20°C; 80%; 3.5 d[40]	**90%** 11°C; 80%; 8.5 d[40] 15°C; 80%; 6 d[40] **Not effective** 4°C; 80%; 10 d[40]	-
Asian clam (juveniles) *Corbicula fluminea*	-	-	-	-	-	-
ZOOPLANKTON						
Fishhook and spiny waterfleas (adults) *Cercopagis pengoi* and *Bythotrephes longimanus*	**100%** 35°C; > 12 h[38] 38°C; 20 min[9] 43°C; 5 min[9] 49°C; 1 min[9] 50°C; 2 s[61] **10%** 32°C; 20 min[9]	-	-	**99%** ˜20°C; NS; 3 h[61]	-	**100%** NS; 2 h (sprayed with water and frozen)[33]

Freshwater AIS	Hot water immersion	Hot water spray		Air-drying**		Freezing
		Low pressure (<60 psi)	High pressure (>400 psi)	(≥ 20°C)	(< 20°C)	
	(temperature; time)	(temp.; pressure; time)	(temp.; pressure; time)	(temperature; RH; time)	(temperature; RH; time)	(temperature; time)
Fishhook and spiny waterfleas (eggs) *Cercopagis pengoi* and *Bythotrephes longimanus*	**100%** 43°C; 10 min[9] 49°C; 1 min[9] 50°C; 5 min[15] **50%** 38°C; 20 min[9] **Not effective** 32°C; 20 min[9] 40°C; 10 min[15]	-	-	-	**100%** 17°C; 45%; 6 h[15]	**100%** -10 to -20 °C (water); 24 h[15] **Not effective** -10 to -20°C (air); 24 h[15]
Bloody red shrimp (adults) *Hemimysis anomala*	**100%** 45°C; 15 min[2] 60°C; 5 min[33]	**100%** 100°C; 10-30 s[25]	-	**100%** 20.8°C; 60%; 2-3 h[33]	**90%** 14°C; NS; 1 d[2]	-
Bloody red shrimp (eggs) *Hemimysis anomala*	**100%** 60°C; 5 min[33]	-	-	**100%** 20.8°C; 60%; 2-3 h[33]	-	-
Killer shrimp (adults) *Dikerogammarus villosus*	**100%** 36°C; 15 min[74*] 40°C; 10 s[71] 45°C; 15 min[2] 50°C; 30 s[70]	**100%** 100°C; steam; >10 s[13] **70%** 100°C; steam; 5 s[13]	**100%** *59°C; 1600 psi; 5 s[14]* *67.4°C; 1600 psi; 5 s[14]* **75-83%** *55.9°C; 1600 psi; 5-15 s[14]*	-	**90%** 14°C; NS; 9 d[2]	-
Killer shrimp (juvniles) *Dikerogammarus villosus*	-	-	-	-	-	-
MACROPHYTES						
Eurasian watermilfoil *Myriophyllum spicatum*	**100%** 60°C; > 2 min[11] **Effective** 58°C; 2 s[61] **Not effective** 45-50°C; 10 min[11]	-	-	**100%** *21°C; low RH; 13 h[36]* 25°C; 40%; 3 h[6, 47] (uncoiled fragments) *More resistant when coiled[17, 47]* **96%** *21°C; low RH; 6 h[36]* **87%** *21°C; low RH; 3 h[36]*	**100%** 19°C; 75%; 36 h (single stems)[17] **Not effective** 19°C; 75%; 18 h (single stems)[17]	-

Freshwater AIS	Hot water immersion	Hot water spray		Air-drying**		Freezing
		Low pressure (<60 psi)	High pressure (>400 psi)	(≥ 20°C)	(< 20°C)	
	(temperature; time)	(temp.; pressure; time)	(temp.; pressure; time)	(temperature; RH; time)	(temperature; RH; time)	(temperature; time)
				Effective ˜20°C; NS; 3.5 d[61] **Not effective** *21°C; 75%; 140 h (coiled wads)*[17]		
Parrot's feather *Myriophyllum aquaticum*	**100%** 45°C; 15 min[2] 50°C; 5 min[71] 55°C; 1 min[71] 60°C; 10 s[71] **15-40%** 40-45°C; 15 min[71]	-	-	**Not effective** 25°C; 40%; 3 h[6]	**90%** 14°C; NS; 9 d[2]	-
Brazilian waterweed *Egeria densa*	-	**100%** 100°C; 10 s[28]	-	**~90%** 25°C; 40%; 3 h[6]	-	-
Fanwort *Cabomba caroliniana*	**Effective** 60°C; 2 – 10 s[61]	-	-	**100%** 25°C; 40%; 3 h[6, 10] 25°C; 60-90%; 3.6-9.1 h[10] 20-30°C; 60%; 3.5-3.8 h[10] **Effective** ˜20°C; NS; 6.5 d[61]	-	-
Water thyme *Hydrilla verticillata*	-		-	**97%** 26°C; NS; 4 h[4] **Effective** 30°C; 40%; >16 h[7] **Not effective** 30°C; 40%; 16 h[7]	-	-
Curly-leaf pondweed *Potamogeton crispus*	-	**100%** 100°C; 10 s[28]	-	**100%** 25°C; 40%; 3 h[6]	**100%** 13°C; 73%; 24 h (single stems)[17] **Not effective** 13°C; 73%; 12 h (single stems)[17] 17°C; 81%, 28 d (turions)[17]	-

Freshwater AIS	Hot water immersion	Hot water spray		Air-drying**		Freezing
		Low pressure (<60 psi)	High pressure (>400 psi)	(≥ 20°C)	(< 20°C)	
	(temperature; time)	(temp.; pressure; time)	(temp.; pressure; time)	(temperature; RH; time)	(temperature; RH; time)	(temperature; time)
PARASITES						
***Myxobolus cerebralis* (adults: triacti-nomyxons)**	**100%:** 75°C; 5 min[82, 83]	-	-	**100%** 20°C; NS; 1 h[82, 83]	-	**100%** -20°C; >100 min (water)[82, 83]
***Myxobolus cerebralis* (young stages: myxospores)**	**100%** 90°C; 10 min[43] **Effective** 60-100°C; 10 min[44] Not effective 40°C; 10 min[44]	-	-	**100%** 22°C; NS; > 18.5 h[42] 18-42°C (sun); NS; 105 min[42]	-	**100%** -20°C, 7 d (water)[42], 60 d (water)[42] **Not effective** -20°C, 18 d (water)[44]

[1]Alonso and Castro-Diez (2012), [2]Anderson et al. (2015), [3]Banha et al. (2016), [4]Baniszewski et al. (2016), [6]Barnes et al. (2013), [7]Basiouny et al. (1978), [9]Beyer et al. (2011), [10]Bickel (2015), [11]Blumer et al. (2009), [12]Payne 1992, [13]Bradbeer et al. (2020), [14]Bradbeer et al. (2021), [15]Branstrator et al. (2013), [17]Bruckerhoff et al. (2015), [18]Cheng and LeClair (2011), [20]Choi et al. (2013), [21]Collas et al. (2014), [23]Comeau et al. (2011), [24]Coughlan et al. (2019), [25]Coughlan et al. (2020a), [26]Coughlan et al. (2020b), [27*]Craft and Myrick (2011), [28]Crane et al. (2019), [33]De Stasio et al. (2019), [34]Dwyer et al. (2003), [35]Elderkin and Klerks (2005), [36]Evans et al. (2011), [38]Garton et al. (1990), [40]Guareschi and Wood (2020), [41]Harrington et al. (1997), [42]Hedrick et al. (2008), [43]Hoffman and Markiw (1977), [44]Hoffman and Putz (1969), [47]Jerde et al. (2012), [48]Kappel (2012), [56*]Mattice et al. (1982), [58*]McMahon et al. (1993), [61]Mohit (2021), [62]Morse (2009), [65]Rajagopal et al. (2005), [67]Ricciardi et al. (1995), [68]Richards et al. (2004), [70]Sebire et al. (2018), [71]Shannon et al. (2018), [72]Snider et al. (2014), [74*]Stebbing et al. (2011), [79*]Ussery and McMahon (1995), [82]Wagner et al. (2003), [83]Wagner (2002), [86*]Wong et al. (2014), [91]Davis et al. (2016).

*Table 4. Effectiveness of chemical decontamination treatments for freshwater AIS, where "100%" refers to 100% mortality (unless otherwise specified) for a particular treatment combination. "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. NS: not specified, RH: relative humidity, *: technical reports, Δ: acclimation laboratory experiments, § refers to studies which used chlorine oxidants rather than sodium hypochlorite. All treatments were immersions unless otherwise specified. References are enumerated in superscript and all field experiments are italicized.*

Freshwater AIS	Sodium hypochlorite	Acetic acid	QAC (Quaternary ammonium compounds)	Salt (NaCl or KCl)	Virkon®
	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)
MOLLUSCS					
Zebra mussel (adults) *Dreissena polymorpha*	**100%** 0.000025-0.0003%; 11-45 d[64] *0.00005-0.00025%; 5-9 d[50§]* > 0.00005%; 4 d[55§] 0.0001%; 25 d (20°C)[139] 0.0002%; 7 d (21°C)[39§] 0.00025-0.0005%; 19 d (22°C)[54], > 6 d (22°C)[53] 0.00025-0.0008%; 29 d (12°C)[54] **95%** 0.0005%; 40 h (30°C)[41§], 1 h (34°C)[41§], 30 min (36°C)[41§] 0.00005-0.00025%; 19 d (25°C)[64], 43 d (10°C)[64] 0.00003%; 14-21 d[52§*] **75%** 0.00005%; 7 d[52§*] **Not effective** 0.00005%; 7 d (21°C)[39§] 0.002%; 30 min[55§]	**100%** 5%; 1 h[29] 3.75%; 2 h[29] 1.25-2.5%; 4 h[29]	**100%** 0.0001-0.0008% (BULAB 6002); 6-10 d (22°C)[53] 0.00005-0.004% (BULAB 6009); 4-8 d (22°C)[53] 0.00003% (Polyquat WSCP); 34 d[52*] 0.00012% (Polyquat WSCP); 13 d[52*] 0.00048% (Polyquat WSCP); 8 d[52*] 0.0001% (DDAC); 24h[52*]	**100%** KCl 10-30 ppt; > 12 h (15°C)[31] *KCl 0.1 ppt; 31 d[37], >6 d (15°C)[51],* 5-23 d (19°C)[60] KCl 0.2 ppt, 10 d (19°C)[60] NaCl 30 ppt; 24 h[30, 31] NaCl 50 ppt; 18 d[73] **Effective** NaCl 4 ppt; 5d[49Δ] **Not Effective** Mix KCl + NaCl, 30 ppt; 5 h[87] Mix KCl + NaCl, 30 ppt; 5 h[87Δ]	**100%** 2-4%; 90 min[26] **>90%** 2%; 15-60 min[26] **>70%** 4%; 15-60 min[26]
Zebra mussel (veliger larvae) *Dreissena polymorpha*	***100%*** *0.00005-0.0001%; 18 h (18-22°C)[80]* 0.00005%; 24 h[52*§] **Not effective** 0.00002-0.00006%; 0.5-4 h[81]	**100%** 5%>10 min[32]	**100%** 0.0004% (BULAB 6002); 22 d (12°C)[54] 0.0001-0.0002% (BULAB 6002); 29 d (12°C)[54] **Not effective** 0.00005%; (BULAB 6002); 29 d (12°C)[54]	**100%** KCl 0.96 ppt, 5-12 h (19°C)[60] KCl 10 ppt; 3 h (12°C)[84] KCl 2.5 ppt; 24 h (12-17°C)[84] KCl 1.25 ppt; 12 h[31] NaCl 10 ppt; 24 h (15°C)[31] NaCl 10 ppt; 6 h (17°C), 24 h (12°C)[84] NaCl 20 ppt; 6 h (17°C)[84] Mix KCl + NaCl, 14 ppt; 3 h[87] Mix KCl +NaCl, 30 ppt; 2 h[87Δ]	**100%** 0.5-2%; > 2 min[32]
Quagga mussel (adults)	-	-	-	**100%** 15-21.3 ppt; 70 h[45]	**100%** 2%; 10 min[76], 5 min[59]

Freshwater AIS	Sodium hypochlorite	Acetic acid	QAC (Quaternary ammonium compounds)	Salt (NaCl or KCl)	Virkon®
	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)
Dreissena rostriformis bugensis				33 ppt; 40 h[45] NaCl 50 ppt; 18 d[73] **Not Effective** Mix KCl + NaCl, 30 ppt; 5 h[87] Mix KCl + NaCl, 30 ppt; 5 h[87Δ]	0.25%; 15-20 min[59] 0.5%; 10 min[59] **>73%** 2%; 90 min[26] **>56%** 4%; 90 min[26]
Quagga mussel (veliger larvae) *Dreissena bugensis*	-	-	**100%** 0.4% (Sparquat 256); 10 min[16]	-	**100%**: 0.25%; >15 min[76] 0.5%; 10 min[76] 2%; 5 min[76]
New Zealand mudsnail (adults) *Potamopyrgus antipodarum*	**Not effective** 0.04% (or spray); 20 min[33] *0.3-1%; 5 min[46*]* 0.05-0.3%; 15-90 sec[34]	-	**100%** 0.07% (HDQ); 5 min[77] *0.15% (Formula 409); 5 min[46*]* 0.3% (Formula 409); 20 min[33] 0.3% (Formula 409); 10 min[69] 0.045-0.24% (Roccal-D, Hyamine 1622, Benzalkonium Chloride, Stepanquat); 15 min[63] >0.4% (Sparquat 256); >5 min[69] **Not effective** 0.15% (Formula 409); 5 min[69]	-	**100%** 2%; 20 min (or spray, but immersion is more effective)[33, 75, 76] 1%; >15 min[76]
New Zealand mudsnail (young stages) *Potamopyrgus antipodarum*	**Not effective** 0.04% (or spray); 20 min[33]	-	-	-	**100%** 2%; 20 min (or spray, but immersion is more effective)[33,]
Asian clam (adults) *Corbicula fluminea*	**100%** *0.0001%; 6-12 d (temperatures between 18-29°C)[8§]* 0.00001%; 26 d[66§] 0.00002%; 13 d[66§] 0.000005%; 36 d[66§] *0.00005%; 28 d (temperatures between 9-39°C)[19§]* **60-95%** 0.00005-0.001%; 28 d (>18°C)[88§] *0.000025%; 28 d (temperatures between 20-25°C)[88§]* ***35-90%*** *0.001-0.004%; 2 d[78*§]*	-	-	**Not effective** 70 ppt; 72 h[24], 60 min[5]	**> 93%** 2%; 5 min[5] **Not effective** 2-4%; 80 min[24]

Freshwater AIS	Sodium hypochlorite	Acetic acid	QAC (Quaternary ammonium compounds)	Salt (NaCl or KCl)	Virkon®
	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)
	76% 0.5%; 1 h[5] **<53%** 0.00005-0.001%, 28 d (<16°C)[88§] **Not effective** 0.25-1%; 80 min[24,] 30 min[56*] *0.00005%; 28 d (temperatures between 12-19°C)[88§]*				
Asian clam (juveniles) *Corbicula fluminea*	**100%** *0.00005%; 96-108 h (25-28°C) (veligers only)[89§]* **60-95%** 0.00005-0.001%; 28 d (>18°C)[88§] **<53%** 0.00005-0.001%; 28 d (<16°C)[88§]	-	-	-	-
ZOOPLANKTON					
Fishhook and spiny waterfleas (adults) *Cercopagis pengoi* and *Bythotrephes longimanus*	**100%** 0.04%; 20 min (or spray)[33]	-	-	**100%** 30 ppt; 1 h[87] 24 ppt; 4 h[87Δ]	**100%** 2% (or spray, but immersion is more effective); 20 min[33]
Fishhook and spiny waterfleas (eggs) *Cercopagis pengoi* and *Bythotrephes longimanus*	**100%** 0.04%; 20 min (or spray)[33]	-	-	-	**100%** 2% (or spray, but immersion is more effective); 20 min[33]
Bloody red shrimp (adults) *Hemimysis anomala*	**100%** 0.05%; 20 min (or spray)[33]	-	-	**100%** 30 ppt; 3 h[87] 30 ppt; 5 h[87Δ]	**100%** 1% (or spray); 1 min[25] 2% (or spray); 20 min[33]
Bloody red shrimp (eggs) *Hemimysis anomala*	**100%** 0.05%; 20 min (or spray)[33]	-	-	-	**100%** 2% (or spray); 20 min[33]
Killer shrimp (adults) *Dikerogammarus villosus*	**100%** 5%; > 30 s[70]	-	-	-	**100%** 1%; 2 min[13]; 12 min[70] 2%; 60 s[13], 2 min; (spray)[13] 4%; 15 s[13]

Freshwater AIS	Sodium hypochlorite	Acetic acid	QAC (Quaternary ammonium compounds)	Salt (NaCl or KCl)	Virkon®
	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)	(concentration; time)
Killer shrimp (juveniles) *Dikerogammarus villosus*	**Effective** 0.02%, 15 min[70]	-	-	-	-
MACROPHYTES					
Eurasian watermilfoil *Myriophyllum spicatum*	***Not effective*** *0.00005%; 4 d[85§]*	-	-	-	-
Parrot's feather *Myriophyllum aquaticum*	-	-	-	-	-
Brazilian waterweed *Egeria densa*	-	-	-	-	**Not effective** 2-4%; 5-30 min[90]
Fanwort *Cabomba caroliniana*	-	-	-	-	-
Water thyme *Hydrilla verticillata*	-	-	-	-	-
Curly-leaf pondweed *Potamogeton crispus*	-	-	-	-	-
PARASITES					
Myxobolus cerebralis (adults: triactinomyxons)	**100%** 0.013%, 1 min[82] 0.0013-0.0262%, 10 min[82, 83] **> 97%** 0.0026%, 1 min[82] **Effective** > 0.5%; > 10 min[83] 0.00026%, 10 min[82]	-	-	-	-
Myxobolus cerebralis (young stages: myxospores)	**100%** > 0.25%; 15 min[42] **Effective** 0.16%; 24 h[44] > 0.5%; > 10 min[83]	-	**100%** 0.15% (alkyl dimethyl benzyl ammonium chloride); 10 min[42] **Effective** 0.02-0.08% (Roccal-D); 24 h[83, 44]	-	-

[5]Barbour et al. (2013), [8]Bernhard (1986),.[13]Bradbeer et al. (2020), [16]Britton and Dingman (2011), [19]Cherry et al. (1986), [24]Coughlan et al. (2019), [25]Coughlan et al. (2020a), [26]Coughlan et al. (2020b), [29]Davis et al. (2015a), [30]Davis et al. (2015b), [31]Davis et al. (2018), [32]Davis (2016), [33]De Stasio et al. (2019), [34]Dwyer et al. (2003), [37]Fernald and Watson (2014), [39]Greenshields and Ridley (1957), [41]Harrington et al. (1997), [42]Hedrick et al. (2008), [44]Hoffman and Putz (1969), [45]Hofius et al. (2015), [46*]Hosea and Finlayson (2005), [49]Kilgour et al. (1994), [50]Klerks and Fraleigh (1991), [51]Lewis et al. (1997), [52*]McMahon et al. (1994), [53]Martin et al. (1993a), [54]Martin et al. (1993b), [55]Matisoff et al. (1996), [56*]Mattice et al. (1982), [59]Moffit et al. (2015), [60]Moffitt et al. (2016), [63]Opligner and Wagner (2011), [64]Rajagopal et al. (2002), [66]Ramsay et al. (1988), [69]Schisler et al. (2008), [70]Sebire et al. (2018), [73]Spidle et al. (1995), [75]Stockton and Moffitt (2013), [76]Stockton (2011), [77]Stout et al. (2016), [78*]Tilly (1976), [80]Van Benschoten et al. (1993), [81]Verween et al. (2009), [82]Wagner et al. (2003), [83]Wagner (2002), [84]Waller et al. (1996), [85]Watkins and Hammerschlag (1984), [87]Ellis and MacIsaac 2009, [88]Doherty et al. 1986, [89]Goss et al. 1979, [90]Crane et al. 2020, [139]Rajagopal et al. (2003).

*Table 5. Effectiveness of physical decontamination treatments for marine AIS, where "100%" refers to 100% mortality (unless otherwise specified) for a particular treatment combination on adult organisms (except for Mytilus edulis, where adult and young stages are presented). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. NS: not specified, *: technical reports, Δ: acclimation laboratory experiments, a: Mytilus galloprovincialis, and b: Ciona savignyi. References are enumerated in superscript and all field experiments are italicized.*

Marine AIS	Seawater		Air-drying	Freshwater				Hot seawater immersion
	Low pressure spray (<60 psi)	High pressure spray ± air-drying (>400 psi)		Immersion	Spray	Immersion + air-drying	Spray + air-drying	
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)
COLONIAL TUNICATES								
Golden star tunicate *Botryllus schlosseri*	**Not effective** *40 psi; NS*[101]	**Almost 100%** *700 psi; 10 s*[136] **80%** *700 psi; NS*[101]	**100%** 6 h (18-19°C; RH 92%)[102*] **Effective removal** *24 h-3 d*[106]	**100%** 24h[142*,153] **Almost 100%** 6 h[142*]	**Effective removal** *5 min*[106]	**100%** 8 h; 1 h[107]	**100%** 10 min; 1 h[107]	-
Violet tunicate *Botrylloides violaceus*	**Not effective** *40 psi; NS*[101]	**Almost 100%** *700 psi; 10 s*[136] **80%** *700 psi; NS*[101]	**<100%** 72 h[131*] **Effective removal** *24 h-3 d*[106]	**100%** 18-24 h[131*] 24h[142*, 153] **Almost 100%** 6 h[142*]	**Effective removal** *5 min*[106]	**100%** 8 h; 1 h[107]	**100%** 10 min; 1 h[107]	-
Carpet sea squirt *Didemnum vexillum*	-	**100% removal** *2000 psi; NS (+48 h air-drying*[112*]	**Effective** *2 weeks*[137*] **Effective removal** *24 h-3 d*[106]	**100%** 4 h[134] **Not effective** *10 min*[147]	**Effective removal** *5 min*[106]	**100%** 8 h; 1 h[107] **87%** *10 min; 24 h*[116]	**100%** 10 min; 1 h[107]	-
Compound sea squirt *Diplosoma listerianum*	-	-	**Effective removal** *24 h-3 d*[106]	-	**Effective removal** *5 min*[106]	**100%** 8 h; 1 h[107]	**100%** 10 min; 1 h[107]	-
SOLITARY TUNICATES								
Vase tunicate *Ciona intestinalis*	-	**Effective** *400-600 psi; NS*[141*]	**100%** [b]24 h[125] [b]8 h (juv.)[125] **Effective removal** *3 d*[106]	**100%** 3 h[143*] 12-24 h[140*] **98%** *1 h (larvae)*[152] **10%** 1 min[108]	**Effective removal** *5 min*[106]	-	-	**100%** 40°C; 60 s[150] 50°C; 10,30,60 s[150] 60°C; 10,30,60 s[150] **66%** 40°C; 10,30 s[150]

Marine AIS	Seawater		Air-drying	Freshwater				Hot seawater immersion
	Low pressure spray (<60 psi)	High pressure spray ± air-drying (>400 psi)		Immersion	Spray	Immersion + air-drying	Spray + air-drying	
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)
				Not effective 3-6 h[140*]				**66-100%** 40ºC (freshwater); 1 min[108] **Not effective** *60°C; few s*[119*]
Clubbed tunicate *Styela clava*	**100%** 50 psi; 30 s (steam; 100°C)[115*]	-	**100%** *24 h (25-27°C)*[124] *2 weeks (10°C)*[124] *At least 1 week*[110*] **Effective removal** *24 h-3 d*[106]	**100%** *3 h*[144*] **Effective** *1 d*[110*]	**Effective removal** *5 min*[106]	-	-	**100%** 60°C; 30,60 s[150] 80-90°C; 4 s[115*] **86%** 50°C; 60 s[150] 60°C; 10 s[150] **70%** 50°C; 30 s[150] **40%** 50°C; 10 s[150] **~12%** 40°C; 10,30,60 s[150]
European sea squirt *Ascidiella aspersa*	-	-	**Effective removal** *24 h-3 d*[106]	-	**Effective removal** *5 min*[106]	-	-	-

Marine AIS	Seawater		Air-drying	Freshwater				Hot seawater immersion
	Low pressure spray (<60 psi)	High pressure spray ± air-drying (>400 psi)		Immersion	Spray	Immersion + air-drying	Spray + air-drying	
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)
MOLLUSCS								
Blue mussel (adults) *Mytilus edulis*	**100%** 50 psi; ≥ 60 s (steam; 100°C)[126]	-	**100%** *6 h (41°C)*[148*] [a]7 d (20.3°C)[125] **0%** *3 h (20-41°C)*[148*]	**Not effective** 24 h[107]	**Not effective** 10 min[107]	-	-	**100%** *28-30°C; 3 d*[120] 28°C; 6 d[120Δ] **80%** 28°C; 4 d[120Δ] **50%** 28°C; 3 d[120Δ] **6%** 27°C; 48 h[120Δ] **Not effective** 26°C; 24 h[120Δ] 55°C; 1 min[103]
Blue mussel (young stages) *Mytilus edulis*	-	-	[a]**100%** 24 h (18.5°C; RH 95%)[125] [a]**80%** 6 h (18.5°C; RH 95%)[125] **47.8%** 11 h (27°C; RH 55.6%)[129]	**0%** 24-48 h[128*] **Not effective** 48 h[117] 24 h[107]	**Not effective** 10 min[107]	-	-	**100%**: 36°C; 84 min[65Δ] 41°C; 1 min[65Δ] **87%** 40°C; 5 min[128*] **76%** 32.6°C; 6 h[129] **Not effective** 30°C; 10 min[128*] 55°C; 5 s[117]
CRABS AND MACROALGAE								
Green crab *Carcinus maenas*	-	-	**Almost 100% (adults and juv.)** *7 d (29°C)*[114] ***50%*** *60 h (29°C)*[114]	-	-	-	-	**100% (juv.)** 45-55°C; 1 min[103] 55°C; 5 s[103] **Not effective (juv.)** 40°C; 1 min[103] 45-50°C; 5 s[103]

Marine AIS	Seawater		Air-drying	Freshwater				Hot seawater immersion
	Low pressure spray (<60 psi)	High pressure spray ± air-drying (>400 psi)		Immersion	Spray	Immersion + air-drying	Spray + air-drying	
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)
Oyster thief *Codium fragile*	-	-	**< 100%** *24 h*[133*] **Not effective** 5 h[127]	**100%** 24 h[128*] **Not effective** 6 h[127]	-	-	-	**100%** 50°C; 30 s[128*]
Macroalgal taxa	**Effective** 50 psi; 120 s (steam; 100°C)[126]	**100% (all stages)** > 2000 psi; 2 s[117]	**100% (all stages)** 3 d (10°C; RH 55-85%)[117] 1 d (20°C; RH 55-85%)[117] 6 weeks (20°C; > RH 95%)[117] **100% (plantlet)** 8 weeks (10°C; > RH 95%)[117] **Not effective (gametophytes)** > 8 weeks (10°C; > RH 95%)[117]	**100%** 2 d (10°C)[117] 1 d (20°C)[117]	-	-	-	**100%** 35°C; 10 min[117] 45°C; 45 s[117] 55°C; 5 s[117] **Effective** 80-85°C; 3 s[135]
Biofouling	**100%** 50 psi; 30-300 s (steam; 100°C)[126]	**< 100%** *2000-3000 psi; 30 s*[113] **100% removal** *2000 psi; NS (+ 48 h air-dry)*[112*]	-	-	-	-	-	-

[65]Rajagopal et al. (2005), [101]Arens et al. (2011), [102*]Bernier et al. (DFO, unpubl. data), [103]Best et al. (2014), [106]Carman et al. (2010), [107]Carman et al. (2016), [108]Carver et al. (2003), [110*]Coutts and Forrest (2005), [111]Coutts and Forrest (2007), [112*]Coutts (2006), [113]Curtis et al. (2021), [114]Darbyson et al. (2009), [115*]Davidson et al. (2005), [116]Denny (2008), [117]Forrest and Blakemore (2006), [119*]Gill et al. (2007), [120]Gonzalez and Yevich (1976), [124]Hillock and Costello (2013), [125]Hopkins et al. (2016), [126]Joyce et al. (2019), [127]Kim and Garbary (2007), [128*]Landry et al. (DFO, unpubl. data), [129]Leblanc et al. (2005), [131*]MacNair et al. (2006), [133*]MacNair (2002), [134]McCann et al. (2013), [135]Mineur et al. (2007), [136]Paetzold et al. (2012), [137*]Pannell and Coutts (2007), [140*]Ramsay (unpubl. data), [141*]Ramsay (2014), [142*]Ramsay (2015a), [143*]Ramsay (2015b), [144*]Ramsay (2015c), [147]Rolheiser et al. (2012), [148*]Seuront et al. (2019), [150]Sievers et al. (2019), [152]Bourque et al. (DFO, unpubl. data), [153]Djikstra et al. (2008).

*Table 6. Effectiveness of chemical decontamination treatments (sodium hypochlorite and acetic acid) for marine AIS, where "100%" refers to 100% mortality (unless otherwise specified) for a particular treatment combination on adult organisms (except for Mytilus edulis, where adult and young stages are presented). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. NS: not specified, *: technical reports, a: Botrylloides leachii, b: veligers, and c: acetic acid 10%. References are enumerated in superscript and all field experiments are italicized.*

Marine AIS	Sodium hypochlorite		Acetic acid					
	Immersion	Spray + air-drying	Immersion		Spray	Immersion + air-drying		Spray + air-drying
	(conc.; time)	(conc.; spray time; dry time)	[4-5%] (time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[2%] (immersion time; dry time)	[4-5%] (spray time; dry time)
COLONIAL TUNICATES								
Golden star tunicate *Botryllus schlosseri*	-	**Effective removal** *0.5%; 5 s; 6 h*[138] **Not effective** *0.1%; 5 s; 12 h*[138]	**100%** *1 min*[118]	**Not effective** *4 min*[118]	-	**100%** 5 min; 1 h[107] *1 min; 24 h*[118]	**100%** *1 min; 24 h*[118]	**Effective removal** 5 s; 30 min[138]
Violet tunicate *Botrylloides violaceus*	**100%** 0.3%; 15 s[131*]	[a]**Effective removal** *1%; 5 s; 30 min*[138] **Not effective (removal)** *0.5%; 5 s; 12 h*[138]	**100%** *15 s*[131*] [a]*1 min*[118]	[a]**Not effective** *4 min*[118]	**90%** *30 s*[131*]	**100%** 5 min; 1 h[107] [a]*1 min; 24 h*[118]	[a]**100%** *1 min; 24 h*[118]	[a]**Effective removal** 5 s; 30 min[138]
Carpet sea squirt *Didemnum vexillum*	**100%** *0.5%; 20 s*[116] *0.25%; 2 min*[116] 0.05%; 10 min[134] **50%** 1%; 5 min[146] **65%** 1%; 15 min[146] **55%** 1%; 30 min[146]	-	[c]**100%** 2 min[134] **Effective** *30 s*[147] **95%** *10 min*[116] **65%** 5 min[146]	**45-82%** *1-10 min*[116]	-	**100%** 5 min; 1 h[107]	-	**100%** 5 s; 30 min[138] **81%** *3 s; 1 h*[116]

Marine AIS	Sodium hypochlorite		Acetic acid					
	Immersion	Spray + air-drying	Immersion		Spray	Immersion + air-drying		Spray + air-drying
	(conc.; time)	(conc.; spray time; dry time)	[4-5%] (time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[2%] (immersion time; dry time)	[4-5%] (spray time; dry time)
Compound sea squirt *Diplosoma listerianum*	-	-	-	-	-	**100%** 5 min; 1 h[107]	-	-
SOLITARY TUNICATES								
Vase tunicate *Ciona intestinalis*	**Not effective** 0.006%; 20 min[108]	**Effective** *1%; 5 s; 30 min[138]* **Not effective (removal)** *0.5%; 5 s; 12 h[138]*	**100%** *4 min[118]* 1 min[108] 10 s[150] **99-100%** *15 s[119*]* **95%** 30 s[108] **70-95%** *5-10 s[130]*	**100%** 60 s[150] 10 s (40ºC)[150] **66%** 10, 30 s[150] **Not effective** *4 min[118]*	**10-20%** *(NS)[119*]*	**100%** *1 min; 24 h[118]* 5 min; 1 h[107]	**100%** *1 min; 24 h[118]*	**Effective removal** *5 s; 30 min[138]*
Clubbed tunicate *Styela clava*	**100%** *0.01%; 12 h[110*]* *0.02%; 12 h[110*]* *0.05%; 12 h[110*]*	-	**100%** *1 min[110*]* **99-100%** *15 s[115*]* **50%** 60 s[150]	**100%** 60 s (40°C)[150] *5-10 min[110*]* **50%** 60s[150] **Not effective** *1 min[110*]*	**5-60%** *NS[115*]*	-	-	-
European sea squirt *Ascidiella aspersa*	-	-	-	-	-	**100%** 5 min; 1 h[107]	-	-

Marine AIS	Sodium hypochlorite		Acetic acid					
	Immersion	Spray + air-drying	Immersion		Spray	Immersion + air-drying		Spray + air-drying
	(conc.; time)	(conc.; spray time; dry time)	[4-5%] (time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[2%] (immersion time; dry time)	[4-5%] (spray time; dry time)
MOLLUSCS, CRABS, AND MACROALGAE								
Blue mussel (adults) *Mytilus edulis*	**100%** 0.0004%; 150 h [123] 0.0004%; 124-150 h[121] 0.0001%; 40 d[139] 0.000025%; 62 d[64]	-	**10-15%** *5-10 s (stage NS)[130]* **Not effective** 5-10 s[108]	-	**15%** *15 s[119*]* **Not effective** *15-30 s[131*]*	-	-	-
Blue mussel (young stages) *Mytilus edulis*	**100%** 0.0004%; 7 h[123] [b]0.0001%; 20 min[122] [b]0.00001%; 4 h[122] [b]0.000005%; 5 h[122] **16%** 0.00007%; 10 min (veliger)[121, 122]	-	**100%** 5 min[107]	-	-	-	-	-
Green crab *Carcinus maenas*	-	-	-	-	-	-	-	-
Oyster thief *Codium fragile*	-	-	-	-	-	-	-	-
Macroalgal taxa	-	-	**100%** *1 min[118]* **Effective** *15 s[132*]* **12-79%** *15 s[149]*	-	-	-	-	**Almost 100%** *5 s; 10 min[138]*
Biofouling	-	-	**100%** *30 s[105]*	-	-	-	-	-

[64]Rajagopal et al. (2002), [105]Cahill et al. (2021), [107]Carman et al. (2016), [108]Carver et al. (2003), [110*]Coutts and Forrest (2005), [115*]Davidson et al. (2005), [116]Denny (2008), [118]Forrest et al. (2007), [119*]Gill et al. (2007), [121]Haque et Kwon (2017), [122]Haque et al. (2014), [123]Haque et al. (2015), [130]Locke et al. (2009), [131*]MacNair et al. (2006), [132*]MacNair (2009), [134]McCann et al. (2013), [138]Piola et al. (2009), [139]Rajagopal et al. (2003), [146]Roche et al. (2015), [147]Rolheiser et al. (2012), [149]Sharp et al. (2006), [150]Sievers et al. (2019).

*Table 7. Effectiveness of chemical decontamination treatments (brine and hydrated lime) for marine AIS, where "100%" refers to 100% mortality (unless otherwise specified) for a particular treatment combination on adult organisms (except for Mytilus edulis, where adult and young stages are presented). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. NS: not specified, *: technical reports, a: Botrylloides leachii. References are enumerated in superscript and all field experiments are italicized.*

Marine AIS	Brine		Hydrated lime			
	Immersion	Immersion + air-drying	Immersion	Spray	Immersion + air-drying	Spray + air-drying
	(conc.; time)	(ppt; immersion time; dry time)	(conc.; time)	(conc.; time)	(conc.; immersion time; dry time)	(conc.; spray time; dry time)
COLONIAL TUNICATES						
Golden star tunicate *Botryllus schlosseri*	-	**100%** 70 ppt; 10 s; 1 h[107] **Effective removal** 70 ppt; 10 min; 2 h[106]	-		-	**Effective** *20%; 5 s; 6 h[138]*
Violet tunicate *Botrylloides violaceus*	**Not effective** 300 ppt; 15 s[131*]	**100%** 300 ppt; 5 min; 1 h[131*] 70 ppt; 10 s; 1 h[107] **< 100%** 300 ppt; 1 min; 1 h[131*] **Effective removal** 70 ppt; 10 min; 2 h[106]	**Not effective** 4%; 15 s[131*]	-	**80-90%** 4%; 15 s; 10-15 min[131*]	[a]**Effective** *20%; 5 s; 6 h[138]*
Carpet sea squirt *Didemnum vexillum*	**100%** 62 ppt; > 4 h[134] **Not effective** *40,50,70 ppt; 0.5,1,5,10 min[147]*	**100%** 70 ppt; 10 s; 1 h[107] **Effective removal** 70 ppt; 10 min; 2 h[106]	**99%** *10%; 2 min[116]* **92%** *4%; 5 min[147]* **85-96% removal** *4%; 4 min[151]* **80%** *5%; 2 min[116]*	-	-	-
Compound sea squirt *Diplosoma listerianum*	-	**100%** 70 ppt; 10 s; 1 h[107] **Effective removal** 70 ppt; 10 min; 2 h[106]	-	-	-	-

Marine AIS	Brine		Hydrated lime			
	Immersion	Immersion + air-drying	Immersion	Spray	Immersion + air-drying	Spray + air-drying
	(conc.; time)	(ppt; immersion time; dry time)	(conc.; time)	(conc.; time)	(conc.; immersion time; dry time)	(conc.; spray time; dry time)
SOLITARY TUNICATES						
Vase tunicate *Ciona intestinalis*	**25%** 300 ppt; 8 min[108] **Not effective** *300 ppt; 30 s[119*]*	**100%** 70 ppt; 10 s; 1 h[107] **Not effective** *300 ppt; 15s; 1h[119*]*	**80%** *4%; 2 min[145*]* **70%** 4%; 8 min[108] **50-80%** *4%; 15 s[119*]*		**100%** *4%; 15 s; 20 min[119*]*	**Effective** *20%; 5 s; 12 h[138]*
Clubbed tunicate *Styela clava*	-	**Effective removal** 70 ppt; 10 min; 2 h[106]	**80%** *4%; 2 min[145*]*	-	-	**Effective** *NS; 45 s[145*]*
European sea squirt *Ascidiella aspersa*	-	**100%** 70 ppt; 10 s; 1 h[107] **Effective removal** 70 ppt; 10 min; 2 h[106]	-	-	-	-
MOLLUSCS						
Blue mussel (adults) *Mytilus edulis*	**6-8%** 70 ppt; 10-20 s[128*]	**0%** 300 ppt; 30 s; 1 h (stage NS)[131*]	**10-15%** *4%; 1 min[130]* **0-2%** 4%; 15 s[119*, 131*]	**0%** 4%; 5 s[109]	-	-
Blue mussel (young stages) *Mytilus edulis*	**3-16%** *300 ppt; 10-120 s[104*]* **6-8%** 70 ppt; 10-20 s[107] **0-23%** 300 ppt; 15-30 min[128*] **Not effective** *300 ppt; 30 s[149]*	**>39%** 300 ppt; 10 min; 24 h [131*] **0%** 300 ppt; 30 s; 1 h[128*]	**77-78%** 4%; 15 min[128*] **53-71%** 4%; 30 min[128*] **0%** *4%; 2 min[145*]*	-	**2%** 4%; 30 s; 1 h[128*]	
GREEN CRAB AND MACROALGAE						
Green crab *Carcinus maenas*	-	-	**Not effective** *4%; 2 min[145*]*	-	-	-

Marine AIS	Brine		Hydrated lime			
	Immersion	Immersion + air-drying	Immersion	Spray	Immersion + air-drying	Spray + air-drying
	(conc.; time)	(ppt; immersion time; dry time)	(conc.; time)	(conc.; time)	(conc.; immersion time; dry time)	(conc.; spray time; dry time)
Oyster thief *Codium fragile*	**100%** *300 ppt; 15 min*[133*]	**100%** *300 ppt; 15 min; 2 h*[133*] *300 ppt; 10 min; 24 h*[133*] 300 ppt; 15 min; 1 h[128*]	**>90%** *4%; 5 min*[133*]		**100%** 4%; 30 s; 1 h[128*] *4%; 1 min; 24 h*[133*] *4%; 15 min; 2 h*[133*]	-
Macroalgal taxa	**Effective** *300 ppt; 15 s*[132*] **68-96%** *300 ppt; 15 s*[149] **Effective removal** 400 ppt; 30 min[135]	-	-	-	-	-

[104*]Bourque and Myrand (2007), [106]Carman et al. (2010), [107]Carman et al. (2016), [108]Carver et al. (2003), [109]Comeau et al. (2017), [116]Denny (2008), [119*]Gill et al. (2007), [128*]Landry et al. (DFO, unpubl. data), [130]Locke et al. (2009), [131*]MacNair et al. (2006), [132*]MacNair (2009), [133*]MacNair (2002), [134]McCann et al. (2013), [135]Mineur et al. (2007), [138]Piola et al. (2009), [145*]Ramsay et al. (2014), [147]Rolheiser et al. (2012), [149]Sharp et al. (2006), [151]Switzer et al. (2011).

Table 8. Recommended decontamination treatments used for recreational watercrafts and water-related equipment in Canada and selected US states for freshwater AIS. Low pressure: garden hose flow; WID: water inspection and decontamination programs operated by the provinces/states; NS: not specified. References are enumerated in superscript. Product concentrations in protocols were converted to chemical concentrations when needed to allow comparisons with the scientific literature in Table 4 (see notes below).

Province or state	Watercraft decontamination				Equipment decontamination							
	Pressurized hot water spray				Hot water	Air-drying	Freezing	Sodium hypochlorite[1]	Acetic acid[2]	QAC[3]	Salt	Virkon®
	(location)	(pressure)	(temp.)	(time)	(temp.; time)	(time)	(temp.)	(conc.; time)	(conc.; time)	(conc.; time)	(conc.; time)	(conc.; time)
UMPS III[212]	Hull Trailer Gear Gimbal Engine[4] Ballast tanks Live and bait wells Bilge	3000 psi Low Low Low Flush Flush Low/flush Low/flush	60ºC 60ºC 60ºC 60ºC 60ºC 49ºC 49ºC 49ºC	10 s 10 s 10 s 132 s 130 s 130 s 130 s	-	-	-	-	-	-	-	-
BC[213, 214]	Centered on UMPS IV, by trained WID personnel: Pressure (low or 3000 psi), temperature (49° or 60°C), and duration (≥ 10, 130, or 132 s) dependent on watercraft components.				Immersion: 60ºC, 10 min Spray: 60ºC, ≥ 10 s	-	48 h	5% acetic acid; 2 h (rinse); followed by 0.5% sodium hypochlorite; 10 min		-	-	-
AB[215]	Centered on UMPS IV, by trained WID personnel: Pressure (low or 3000 psi), temperature (49° or 60°C), and duration (≥ 10, 130, or 132 s) dependent on watercraft components. Whirling disease: high pressure (3000 psi) hot spray (90 °C, 60°C for engines), 10 min followed by QAC Dustbane QUAT Plus (0.30 %, 10 min).				-	-	-	0.5%; 15 min	-	Whirling disease: hot water treatment followed by Dustbane QUAT Plus, 0.15%; 10 min (soaking); 0.3%; 10 min (wiping/ spraying)	-	2%; 20 min
SK[216]	Centered on UMPS IV, by trained WID personnel: Pressure (low or 3000 psi), temperature (49° or 60°C), and duration (≥ 10, 130, or 132 s) dependent on watercraft components.				-	-	-	-	-	-	-	-
MB[217]	By trained WID personnel: Low pressure (40-60 psi) hot rinse with temperature (50 or 60 °C), and duration (≥ 10, or 130 s) dependent on watercraft components. If visible AIS present, followed by high pressure (3000-3500 psi) wash (temp. NS).				Immersion: 60ºC, 10 min Spray: 60ºC, ≥ 10 s	-	-10 °C; 3 d	0.525%; 30 min	5%; 60 min	-	10 ppt; 24h	-

Province or state	Watercraft decontamination				Equipment decontamination							
	Pressurized hot water spray				Hot water	Air-drying	Freezing	Sodium hypochlorite[1]	Acetic acid[2]	QAC[3]	Salt	Virkon®
	(location)	(pressure)	(temp.)	(time)	(temp.; time)	(time)	(temp.)	(conc.; time)	(conc.; time)	(conc.; time)	(conc.; time)	(conc.; time)
QC[218]	Boat, trailer, + gear	2600 psi 2600 psi	60ºC Cold	10 s 30 s	-	5 d	0 to -9°C; 24 h or ≤ 9°C; 8 h	0.525%; 10 min	3.75%, 20 min	-	-	-
ANSTF[219]	Watercraft + gear Motor Interior parts	2600 psi	60ºC 60ºC 60ºC	10 s 2 min 2 min	-	5 d	-	-	-	-	35 ppt; 30 min	-
CO[220]	Boat/trailer Gimbal Interior + standing water Engine[4]	2500 psi Low Low	60ºC 60ºC 49ºC 60ºC	NS 45 s 1 min	-	-	-	-	-	-	-	-
CO[221]	Watercraft + gear	3000 psi	60°C	30 s	-	-	-10°C; 4 h	5%; 1 h	5%, 20 min	-	10 ppt; 24 h	-
MI[222]	Watercraft + gear	High (NS)	60ºC	10 s	60ºC; 5 min	-	-	0.6%; 10 min	5%; 10 min	Formula 409 (0.3%); 10 min	4 ppt; 30 min	2%; 15-20 min
MN[223]	Watercraft + gear Interior compartments Engine	2500 psi Low Low/flush	60ºC 49ºC 60ºC	10 s 10 s 3-10 min	-	5 d	-	-	-	-	-	-
UT[224]	Watercraft + gear	3000 psi	60ºC	10 s	-	7-14 d (summer-winter)	Air freeze, 3 d (winter)	-	-	-	-	-
WA[225]	Watercraft + gear Hard non-porous. Porous materials Whirling disease	High (NS)	60ºC 60ºC 60ºC 75ºC	10 s 15 s 5 min 5 min	-	-	0 to -9 °C, 24h or ≤ 10°C, 8h	-	-	-	-	1%; 10 min or 2%; 20 min
WI[226]	Watercraft + gear	High better, NS	60ºC	NS	60ºC, NS steam, NS	5 d	-	0.5%;10 min	5%; 10 min	-	-	2%; 20 min

Note 1: bleach contains 5% sodium hypochlorite; a dilution of 100 ml bleach to 1 L of water is equivalent to 0.525% sodium hypochlorite; 5000 ppm is equivalent to 0.5%; Note 2: vinegar contains 5% acetic acid; Note 3: product concentrations (%) have been converted to QAC concentrations (%); Note 4: time required until engine exit temperature reaches 60ºC

[212]Elwell and Phillips (2021), [213]Government of British Columbia (2020c), [214]Government of British Columbia (2020b), [215]Government of Alberta 2020, [216]Government of Saskatchewan (2020), [217]Manitoba Government (2021a), [218]Ministère des Forêts, de la Faune et des Parcs (2018), [219]ANSTF (2013), [220]Brown and Walters (2021), [221]DiVittorio et al. (2012), [222]Michigan Department of Environmental Quality (2014), [223]Minnesota Department of Natural Resources (2017), [224]Utah Department of Natural Resources (2012), [225]Washington Department of Fish and Wildlife (2016), [226]Wisconsin Department of Natural Resources (2020).

Table 9. Summary of watercraft decontamination treatments effective at killing the greatest number of target freshwater aquatic invasive species. Effective treatments (≥ 99% mortality) are based on a review of the scientific literature of lethal treatments for zebra mussel (ZM), quagga mussel (QM), Asian clam (AC), New Zealand mudsnail (NZMS), killer shrimp (KS), bloody red shrimp (BRS), waterfleas (WF), macrophytes (MP), and Myxobolus cerebralis which causes Whirling disease (WD). Associated levels of uncertainty are based on the quantity of data available, their quality, and agreement. "-" refers to occurrences where no species were classified in a particular uncertainty category or where no data was found on the effectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments.

Treatments for watercraft		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages)	No data (adults)
Air-drying[1]	7 d (20–35 °C)	MP	ZM, QM, WD, NZMS	AC, BRS, WF	-	-	AC, NZMS, KS, WF, some MP	KS, some MP
	15 d (10–19 °C)	-	ZM, NZMS	QM, BRS, WF, MP	AC, KS	-	QM, AC, NZMS, KS, BRS, some MP, WD	WF, some MP, WD
Freezing	4 d (air, -20 °C)	-	-	ZM, NZMS, WF[2], WD[3]	-	WF[2] (eggs in air)	ZM, QM, NZMS, AC, KS, BRS, MP	QM, AC, KS, BRS, MP
High pressure hot water spray	68 °C, 15 s, 1600 psi	-	-	ZM, KS	QM	-	ZM, QM, AC, NZMS, KS, BRS, WF, MP, WD	AC, NZMS, BRS, WF, MP, WD
Low pressure hot water spray	100 °C (steam), 30 s	-	ZM, QM	AC, KS, BRS, MP	-	-	ZM, QM, NZMS, AC, WF, KS, BRS, some MP, WD	NZMS, WF, some MP, WD

[1] Drying times are affected by temperature and relative humidity.
[2] Freezing eggs in air is ineffective but freezing in water is effective (eggs and adults).
[3] No data for freezing in air but effective in water (both stages).

Table 10. Summary of equipment decontamination treatments effective at killing the greatest number of target freshwater aquatic invasive species. Effective treatments (≥ 99% mortality) are based on a review of the scientific literature of lethal treatments for zebra mussel (ZM), quagga mussel (QM), Asian Clam (AC), New Zealand mudsnail (NZMS), killer shrimp (KS), bloody red shrimp (BRS), waterfleas (WF), macrophytes (MP), and Myxobolus cerebralis which causes Whirling disease (WD). Associated levels of uncertainty are based on the quantity of data available, their quality, and agreement. "-" refers to occurrences where no species were classified in a particular uncertainty category or where no data was found on the effectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments.

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages)	No data (adults)
Air-drying[1]	7 d (20–35 °C)	MP	ZM, QM, WD, NZMS	AC, BRS, WF	-	-	AC, NZMS, KS, WF, some MP	KS, some MP
	15 d (10–19 °C)	-	ZM, NZMS	QM, BRS, WF, MP	AC, KS	-	QM, AC, NZMS, KS, BRS, some MP, WD	WF, some MP, WD
Hot water immersion	60 °C, 5 min	-	ZM, QM, KS, WF, MP	AC, NZMS, BRS	-	WD[2]	ZM, AC, NZMS, KS, some MP	Some MP
Freezing	4 d (air, -20 °C)	-	-	ZM, NZMS, WF[3], WD[4]	-	WF[3] (eggs in air)	ZM, QM, NZMS, AC, KS, BRS, MP	QM, AC, KS, BRS, MP
High pressure hot water spray	68 °C, 15 s, 1600 psi	-	-	ZM, KS	QM	-	ZM, QM, AC, NZMS, KS, BRS, WF, MP, WD	AC, NZMS, BRS, WF, MP, WD
Low pressure hot water spray	100 °C (steam), 30 s	-	ZM, QM	AC, KS, BRS, MP	-	-	ZM, QM, NZMS, AC, WF, KS, BRS, some MP, WD	NZMS, WF, some MP, WD
Sodium hypochlorite	0.25%, 20 min	-	WD	ZM, BRS, WF	KS	NZMS, AC, some MP	QM, some MP	QM, some MP
Virkon®	4 %, 90 min	-	ZM, NZMS, KS, BRS	QM, AC, WF	-	-	AC, KS, MP, WD	MP, WD
Quaternary ammonium compounds	0.4 %, 10 min	-	NZMS	ZM, QM, WD	-	-	AC, NZMS, KS, BRS, WF, MP	QM, AC, KS, BRS, WF, MP, WD
Acetic acid	5 %, 1 h	-	-	ZM	-	-	QM, AC, NZMS, KS, BRS, WF, MP, WD	QM, AC, NZMS, KS, BRS, WF, MP, WD

[1] Drying times are affected by temperature and relative humidity.
[2] *M. cerebralis* adults and young stages require 75˚C (5 min) and 90˚C (10 min), respectively.
[3] Freezing eggs in air is ineffective but freezing in water is effective (eggs and adults).
[4] No data for freezing in air but effective in water (both stages).

Table 11. Summary of watercraft decontamination treatments for marine aquatic invasive species. Effective treatments (≥ 99% mortality or removal) are based on a review of the scientific literature of lethal treatments for colonial tunicates (CT), solitary tunicates (ST), blue mussel (BM), green crab (GC), oyster thief (OT), and macroalgae (MA). Associated levels of uncertainty are provided and are based on the quantity of data available, their quality, and agreement. "-" refers to occurrences where no species were classified in a particular uncertainty category or where no data was found on the effectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments.

Treatments for watercraft		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages and adults)
Low pressure hot seawater spray	100 °C (steam), 120 s	-	-	ST, MA, BM (adults)	-	-	CT, GC, OT, BM (young stages)
High pressure cold seawater spray followed by air-drying	15 s, 2000 psi + 48 h air-dry	-	CT	ST, MA	-	-	BM, GC, OT
Air-drying[1]	7 d	-	ST, OT	CT, GC[2], BM, MA[3]	-	-	-

[1] Drying times are affected by temperature and relative humidity.
[2] Only if fully exposed to air (29 °C).
[3] Effective only after 8 weeks of air-drying for some macroalgae gametophytes (10 °C; 95% relative humidity).

Table 12. Summary of equipment decontamination treatments for marine aquatic invasive species. Effective treatments (≥99% mortality or removal) are based on a review of the scientific literature of lethal treatments for colonial tunicates (CT), solitary tunicates (ST), blue mussel (BM), green crab (GC), oyster thief (OT), and macroalgae (MA). Associated levels of uncertainty are provided and are based on the quantity of data available, their quality, and agreement. "-" refers to occurrences where no species were classified in a particular uncertainty category or where no data was found on the effectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments.

Treatments for equipment		**Low uncertainty**	**Reasonable uncertainty**	**High uncertainty**	**Very high uncertainty**	**Ineffective**	**No data (young stages and adults)**
Freshwater immersion	24 h + 1h (air-dry)	CT	ST	OT, MA	-	BM	GC
Air-drying[1]	7 d	-	ST, OT	CT, GC[2], BM, MA[3]	-	-	-
Low pressure hot seawater spray	100 °C (steam), 120 s	-	-	ST, MA, BM (adults)	-	-	CT, GC, OT, BM (young stages)
High pressure cold seawater spray + air-drying	15 s, 2000 psi + 48 h (air-dry)	-	CT	ST, MA	-	-	BM, GC, OT
Hot seawater immersion	60 °C, 30 s	-	BM	ST, OT, MA, GC (young stages)	-	-	CT, GC (adults)
Brine immersion + air-drying	300 ppt, 15 min + 2h (air-dry)	-	CT, ST, MA	OT	-	BM	GC
Acetic acid immersion	5 %, 10 min	-	CT, ST, MA	BM (young stages)	-	BM (adults)	GC, OT

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages and adults)
Hydrated lime immersion + air-drying	4 %, 15 min + 2h (air-dry)	-	CT	ST, OT	-	BM, GC (adults)	MA, GC (young stages)
Sodium hypochlorite immersion	0.05 %, 6 h	-	CT, ST	BM	-	-	GC, OT, MA

[1] Drying times are affected by temperature and relative humidity.
[2] Only if fully exposed to air (29 °C).
[3] Effective only after 8 weeks of air-drying for some macroalgae gametophytes (10 °C; 95% relative humidity).

*Table 13. Summary of decontamination treatment compatibilities/incompatibilities with various materials (fiberglass, plastic, metal, fabric, rubber, neoprene, and carpets), as well as overall advantages and disadvantages. References are identified in superscript. *: technical reports, NS: not specified.*

Treatment	Compatibility	Incompatibility	Advantages	Disadvantages
Temperature	Immersion of small water sports equipment, footwear, and porous material[2, 71, 205, 225] Exterior parts of watercraft, trailers and equipment[211, 212] < 50-60°C: engines, interior compartments, hull fittings[138, 201, 208, 211, 220]	> 50-60°C: pumps, engines, internal and cooling systems [211, 212] Various watercraft materials (e.g. aluminium, plastics, Gore-Tex, paints, HDPE, acrylic)[207, 220] > 80°C: watercraft[220]	One of the most effective, environmentally sound, fast, low-cost and widely recommended cleaning methods for watercraft and fishing equipment[26, 71, 62, 207, 209, 111] > 60°C is the most widely accepted method of cleaning invasive mussels[62, 221*] No chemicals needed if hot water is available[225] Good for standing water in compartments [211, 220]	Could degrade water sports equipment and watercraft parts[2] Could cause burns and requires operator safety measures[2, 207, 221*] Cost of equipment, may not be locally available, and most car washes cannot reach the recommended 60°C[209, 221*, 222] Maintaining higher temperatures (>60C) for longer periods of time can be difficult to achieve with available pressure washing equipment[71]
Pressure washing	Low pressure: PFDs, anchors, paddles, gimbals, engines, ballast tanks, interior compartments, trailers[201, 208, 211, 212, 220] High pressure: Boat hull and exterior surfaces, trailers, equipment and non-porous materials[211, 212, 219, 220, 225] Floating docks, wharf, mooring line, equipment, and vessel cleaning[112*]	High pressure: Inflatables, Gore-Tex, neoprene, life jackets, PFDs, dry/wet/survival suits, throwbags[202], wooden boats and delicate equipment such as electronics, gimbal and glued seals[9, 87*, 202, 208, 212]	Reasonably effective and economical solution[207] Effective at removing encrusted organisms and residuals[9, 201, 221*] Good for standing water[211, 220]	May be expensive (gear purchase, water and electricity costs) and could waste water if used without a fan nozzle[207, 209, 221*] Labor-intensive method, requires operator safety measures[207,221*] Some nozzle types can cause damage to boats and equipment[220] Sensitive items should be decontaminated with other methods[201, 221*]
Air-drying	Watercraft and water sports equipment[1, 2, 205] Equipment requiring gentle care[219] Dry docking, moorings, and aquaculture equipment[124]	NS	Simple and low-cost method[1, 112*, 124, 207, 218, 112*, 124] Mortality during on-land transportation[1]	As mussels are tolerant to emersion, air-drying may not be effective over shorter time frames[106] Effective exposure times are strongly dependent on air temperature and relative humidity[58*, 61, 67, 114, 117]

Treatment	Compatibility	Incompatibility	Advantages	Disadvantages
Freezing	Small equipment[205]	NS	Applicable in winter for equipment and clothes, and when hot temperatures are unsuitable[202]	
Sodium hypochlorite (bleach)	NS	Can cause damage to equipment made of rubber, metal, fabrics, and plastics[46, 202, 207, 211, 221*, 222, 226]	Low cost widely used biocide that attacks living cells vigorously[207, 222] Good alternative if material is not heat resistant[202]	Can cause health and/or environmental hazards. Requires the use of protective equipment and proper storage[207, 224, 226] Requires neutralization afterwards with sodium thiosulfate[202, 226] Deteriorates with time, exposure to light and heat and on contact with air, metals, metallic ions and organic materials[228] Limited shelf-life, best used within 6 months and diluted solutions should be used within 24 h[226] Should not be mixed with vinegar[222]
Acetic acid (vinegar)	Aquaculture equipment and infrastructures[150]	NS	Effective and fast[29] Low-cost, accessible, and easy to apply[150] Low environmental impacts[150] Remains stable in the presence of organic matter[118, 138]	Do not mix with bleach[222] Rinse thoroughly and use self-protection[218] Health and safety of individuals[132*] Dilute with a large solution of water before disposal[218]
Quaternary ammonium compounds (QAC)	Gore-Tex, neoprene, life jackets, PFDs, dry/wet/survival suits, throw bags, ropes[202] Does not cause damage to gear[46, 222]	Can cause corrosion on metals[202, 211, 221*] Not recommended with: porous materials, field electronics and probes, all terrain/off road vehicles[202] Unsuitable for: pumps, unsealed wooden components, non-removable soles (must dry completely), inflatables, enclosed floors[202]	Common cleaning agent[202] Acute toxicity on most aquatic organisms and kills fast[46, 204]	Can cause health/genotoxic and/or environmental hazards[206, 207] Potential consequences on non-target organisms[210] Must be used in tandem with another disinfection option[226] Presence of mud reduces effectiveness[33] Must be disposed down a sewage drain[225]

Treatment	Compatibility	Incompatibility	Advantages	Disadvantages
Salt	Non corrosive on cooling engine systems[209] Equipment requiring gentle care[219]	Can cause corrosion on metals[221*]	Low-cost and low toxicity[207]	Not a practical option for watercraft[31] Not always accessible and prolonged exposure time may be required[207] Must be used in tandem with another disinfection option[226]
Virkon®	NS	NS	Non corrosive and biodegradable[222] Effective for large recreational boats and cooling systems[25]	Must be used in a well-ventilated area, preferably outdoors. Safety apparel must be worn[225]
Hydrated lime	Aquaculture equipment and buoys[131*]	Toxic for some shellfish larvae at undiluted concentrations[131*]	Eco-friendly due to its low toxicity and reduced environmental persistence compared to synthetic biocides[108] Quickly diluted in water, resulting only in short term, small scale effects on water pH in the vicinity of spraying activity[130, 227]	Difficulties associated with the insoluble nature of the hydrated lime powder[108] Substantial quantities of undissolved lime and associated impurities remain in treatment solutions leading to inaccuracies in estimates of the effective concentrations[108] Undissolved matter remaining in the lime solutions can block the nozzle of the pressure spray units, inhibiting the effective delivery of the solutions[108]
Brine	Aquaculture equipment and buoys[106, 131*]	NS	Easy and safe to use, environmentally friendly, and relatively inexpensive[115*]	NS

[1]Alonso and Castro-Diez(2012), [2]Anderson et al. (2011), [9]Beyer et al. (2011), [23]Comeau et al. (2011), [25]Coughlan et al. (2020a), [26]Coughlan et al. (2020b), [29]Davis et al. (2015a), [31]Davis et al. (2018), [33]De Stasio et al. (2019), [46]Hosea and Finlayson (2005), [58*]McMahon et al. (1993), [61]Mohit (2021), [62]Morse (2009), [67]Ricciardi et al. (1995), [71]Shannon et al (2018), [86*]Wong et al. (2014), [106]Carman et al. (2010), [112*]Coutts (2006), [114]Darbyson et al. (2009), [115*]Davidson et al. (2005), [117]Forrest and Blakemore (2006),[118]Forrest et al. (2007), [124]Hillock and Costello (2013),[130]Locke et al., 2009, [131*]MacNair et al (2006), [132*]MacNair (2009), [138]Piola et al. (2010), [147]Rolheiser et al. (2012), [150]Sievers et al. (2019), [201]Adirondak Park Invasive Plant Program (2014), [202]Alberta Environment and Parks (2017), [203]California Department of Fish and Wildlife (2013), [204]Cockman et al. (2012), [205]Elwell (2010), [206]Ferk et al (2007), [207]Miller et al. (2006), [208]Minnesota Department of Natural Resources (2013), [209]New York State Department of Environmental Conservation (2015), [210]Waller et al. (1993), [211]Wyoming Game and Fish Department (2016), [212]Elwell and Philipps (UMPS IV; 2021), [218]Ministère de la Forêt, de la Faune et des Parcs (2018), [219]ANSTF (2013), [220]Brown and Walters (2021), [221*]DiVittorio et al. (2012), [222]Michigan Department of Environmental Quality (2014), [224]Utah Department of Natural Resources (2012), [225]Washington Department of Fish and Wildlife (2016), [226]Wisconsin Department of Natural Resources (2020), [227]DFO 2016, [228]Clarkson et al. 2001.

Table 14. Decontamination treatment feasibility with regards to practicality, equipment requirements, human health and ecosystem risks, and disposal.

Treatments	Practicality (watercraft, large equipment)	Practicality (small equipment)	Special equipment required	Human health risks	Ecosystem risks	Special disposal	Notes
Air-drying	YES	YES	NO	N/A	N/A	N/A	Long exposure required; mussels may be emersion tolerant
Freezing	YES	YES	NO	N/A	N/A	N/A	Long exposure required; impractical
Hot water (immersion)	NO	YES May damage some materials	YES	Burns	NO	NO	-
Pressurized hot water sprays Low = e.g., PFDs, anchors, ballast tanks, interior compartments High = e.g., hulls, trailers, etc.	YES May damage pumps, engines, cooling systems, pontoons, glued seals, electronics etc.	YES May damage some materials	YES	Burns	Uses a lot of water	NO	Labour intensive
Steam	NO	YES May damage some materials	YES	Burns	N/A	N/A	Labour intensive: difficult to attain these temperatures
Sodium hypochlorite (immersion)	NO	YES May damage some materials	NO	Chemical burns	Persistence, non-target organisms, toxic to some shellfish larvae	YES	Use in well-ventilated areas
Acetic acid (immersion)	NO	YES May damage some materials	NO	Chemical burns	NO	YES	Use in well ventilated areas
QAC (immersion)	NO	YES May damage some materials	YES	YES	Persistence, non-target organisms	YES	Legal issues with broad-scale spectrum disinfectants Use in well ventilated areas
Virkon® (immersion)	NO	YES	YES	YES	NO	YES	Use in well ventilated areas
Hydrated lime (immersion)	NO	YES	YES	Chemical burns	Toxic to some shellfish larvae when undiluted	NO	Insoluble; difficult to get accurate concentrations
Brine (immersion)	NO	YES	NO	NO	NO	NO	-

APPENDIX 1. SEARCH TERMS

1	(decontaminat* OR “hot water” OR steam* OR clean* OR disinfect* OR spray* OR heat* OR dry* OR prevent* OR immers* OR manage* OR antifoul* OR biofoul* OR foul OR sun* OR hot OR inspect* OR airdry* OR rins* OR salinity OR pressure* OR desiccat* OR expos* OR control OR eradicate* OR biosecurity
2	(invasive OR non-native OR non-indigenous OR exotic OR foreign OR alien OR spread* OR invad*)
3	(aquatic OR freshwater OR lake* OR pond* OR river* OR stream* OR aquaculture OR ocean OR sea OR coastal OR “introductions and transfers“)
4	(species OR organism* OR animal* OR plant* OR invertebrate* OR zooplankton OR mollusc* OR bivalve* OR mussel* OR crab OR pest OR macrophyte or alga* or macroalga* OR disease OR parasite)
5	(viability OR viable OR mortality OR death OR removal OR surviv* OR reproduc* OR dispersal OR “overland transport” OR tolerance OR resistance OR lethal* OR “acute upper lethal temperature” OR temperature OR heat OR hot OR “critical maximum temperature”)
6	(protocols OR standards OR guidelines OR “clean drain dry” OR “boating hygiene” OR “pull the plug”
7	1 AND 2 AND 3 AND 4 AND 5 AND 6 AND 7