SCIENCE ADVICE ON "CLEAN, DRAIN, DRY AND DECONTAMINATE" TREATMENTS AND PROTOCOLS TO PREVENT THE INTRODUCTION AND SPREAD OF AQUATIC INVASIVE SPECIES

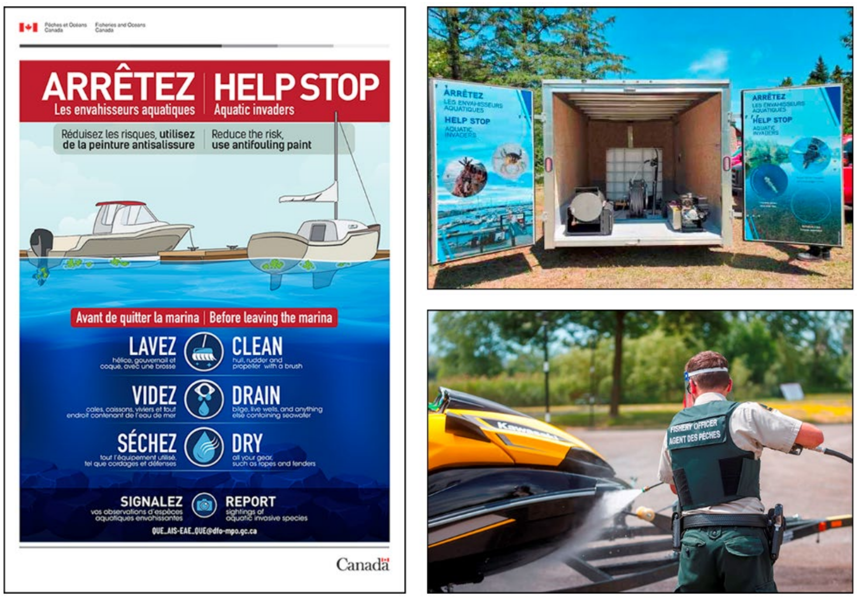

Figure 1. A DFO "Clean, Drain, and Dry" poster, a mobile decontamination unit, and a fishery officer decontaminating a watercraft. Photo credits: DFO.

Context:

To prevent the introduction and spread of Aquatic Invasive Species (AIS), many government and non-government organizations operate Clean, Drain, Dry (CDD) programs. CDD is an established best management practice targeted towards the general public and owners or operators of watercraft, trailers, and equipment used in and near water. CDD programs require watercraft operators to undertake cleaning, draining, and drying steps to reduce the likelihood of transporting AIS on their equipment. In situations where there is a higher risk that AIS could be transported, an additional decontamination step may be applied (CDD+D). Decontamination generally includes chemical or temperature treatments which may vary depending on the target species or disease.

To date, a comprehensive review of the effectiveness of CDD+D protocols used in Canada on 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.

This Science Advisory Report is from the March 30 – April 1, 2021 National Advisory Meeting for Science Advice on "Clean, Drain, Dry and Decontaminate" Treatments and Protocols to Prevent the Introduction and Spread of Aquatic Invasive Species. Additional publications from this meeting will be *posted on the [Fisheries and Oceans Canada (DFO) Science Advisory Schedule] as they become available.*

SUMMARY

- To prevent the introduction and spread of aquatic invasive species (AIS) by water-based commercial and recreational activities, many government and non-government organizations encourage owners and operators to Clean, Drain, Dry (CDD) their watercraft, trailers, and equipment. In some cases, an additional decontamination step may be applied (CDD+D) that generally includes disinfection with details varying depending on the target organism or application, and is often performed by trained personnel with specialized equipment.
- To understand the effectiveness and limitations of CDD+D for watercraft under 24 m, trailers, and equipment that are moved from water to land before entering a new waterbody, a literature review of existing treatments, protocols, and supporting scientific literature was conducted. Watercraft, trailers, and equipment that remain in water were not considered, nor was public uptake of CDD+D.
- 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.
- Numerous species- or environment-specific decontamination treatments were identified as effective at killing or removing AIS. However, no single decontamination treatment was applicable to all freshwater and marine AIS. Effective options for species of interest are presented in Tables 2 to 5.
- Key uncertainties and knowledge gaps include:
  - Making comparisons between studies that had different experimental designs, scales, and methods of measuring mortality and/or removal;
  - Applying conclusions from laboratory studies to field conditions; and,
  - Interpreting the effectiveness of decontamination treatments that were designed for different applications (e.g., aquaculture transfers, cleaning of infrastructure).
- This work also focused on select AIS of interest, but additional taxonomic groups could be considered in the future.

INTRODUCTION

The establishment of invasive species in aquatic ecosystems is considered one of the global primary drivers of biodiversity loss, with serious negative consequences to ecological and ecosystem function. Aquatic invasive species (AIS) pose a significant threat to Canadian fresh, estuarine, and marine waters and threaten Canada's biodiversity, economy, and society. Water-based commercial and recreational activities can unintentionally spread AIS to new locations if AIS hitchhike on watercraft, trailers, and equipment, or if they are transported in standing water (e.g., bilge water and live wells).

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) that generally includes disinfection with the details of treatment applications dependent on the target organism and/or the watercraft/equipment type to be disinfected, and is often performed by trained personnel with specialized equipment. CDD and decontamination 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 treatment. While CDD provides a series of best practices for public consideration, decontamination methods have species-specific treatment parameters which aim to ensure a particular level of AIS mortality or removal. Both CDD and decontamination 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.

To date, a comprehensive review of the effectiveness of CDD+D protocols used in Canada 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. This science request was initiated by Fisheries and Oceans Canada (DFO) AIS National Core Program, the governing body responsible for the implementation of 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 to the Canadian public.

The objectives of this Science Advisory Report were to:

1. Complete a review of the scientific literature on decontamination treatments for removal and/or mortality of freshwater and marine AIS, and of the existing freshwater and marine CDD+D protocols used in AIS management across Canada or abroad.
2. 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 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 (e.g., Fish and Fish Habitat Protection Program, Species at Risk Program, Small Craft Harbours), and for the Canadian general public (recreational watercraft). Any advice generated from this work on best management practices will be subject to the caveat that the success of CDD+D relies heavily on public uptake and compliance, the assessment of which is beyond the scope of this Science Advice.

METHODS

A review of the scientific literature and an assessment of the effectiveness of decontamination treatments were completed for several freshwater and marine AIS. Representative species from different functional and taxonomical groups (e.g., bivalves, gastropods, zooplankton, parasites, macrophytes, macroalgae, crabs, and tunicates) were selected according to their presence (or their expected arrival)[a] in Canadian freshwater and marine environments (Table 1).

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 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)* and in some cases similar nuisance species
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*)

Studies included in the literature review for freshwater AIS used the treatments listed below.

- Physical treatments
  - Hot water (immersion or spray)
  - Pressure-washing
  - Air-drying
  - Freezing

[a] Erratum July 2022: "presence or probability of presence" was replaced by "presence (or their expected arrival)".

- Chemical treatments (immersions)
  - Sodium hypochlorite
  - Acetic acid
  - Quaternary ammonium compounds (QAC)
  - Salt water (sodium chloride and potassium chloride)
  - Virkon®

For marine AIS, the treatments are listed below.

- Physical treatments
  - Freshwater immersion
  - Hot seawater or freshwater (immersion or spray)
  - Pressure-washing
  - Air-drying
  - A combination of the above
- Chemical treatments (immersion or spray)
  - Sodium hypochlorite
  - Acetic acid
  - Brine
  - Hydrated lime
  - Sometimes combined with air-drying

Data were classified by treatment and target AIS. Treatment parameters (concentrations, exposure times, temperatures, etc.) and associated removal or mortality (%) were reported for both young (veligers and/or juveniles) and adult life stages when available. The effectiveness of each physical and chemical decontamination treatment for freshwater and marine AIS was classified, where effective treatments were considered to result in ≥ 99% mortality or removal of AIS.

Existing CDD+D protocols were also reviewed and the effectiveness of treatments recommended therein were reviewed to determine whether existing protocols are supported by the scientific literature.

ASSESSMENT

Reducing propagule pressure using CDD+D can be achieved by physically removing (e.g., pressure-washing, scrubbing, hand-picking) and/or killing invasive species (e.g., temperature or chemical treatments). More than 130 scientific publications and grey literature reports were reviewed to assess the effectiveness of various physical and chemical treatments for the removal and/or mortality of freshwater and marine AIS.

Decontamination treatments

The scientific literature on decontamination treatments in both marine and freshwater environments was highly species-specific. While most existing literature focused on mortality as a measure of effectiveness, pressurized water sprays in the marine literature focused on removal. Freshwater literature mainly considered controlling dreissenid mussels, and hot water treatments and air-drying were the most comprehensively studied. Marine literature focused primarily on controlling tunicates through freshwater, acetic acid or brine immersions.

Species-specific decontamination treatments reviewed from the scientific literature are presented in Weise et al. (In press[1]; see Tables 3-7 for detailed information). Effective decontamination treatments to remove and/or kill the greatest number of freshwater and marine AIS were identified; options for watercraft and equipment decontamination are presented in Tables 2 and 3 for freshwater AIS, and in Tables 4 and 5 for marine AIS. Associated levels of uncertainty are presented for each AIS and decontamination treatment. Uncertainty scores were based on a combination of the quantity and quality of the data available, and the suitability of the identified effective treatment option. 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 effective treatment options (contradictory, different conclusions, mostly agree or agree). 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. Given the strong species and treatment specific variability, reference to Weise et al. (In press)[1] for species-specific decontamination methods is recommended, as requirements (exposure times, temperatures etc.) may be different than those presented in the summary of the present work.

It is important to note that numerous studies in the literature were developed for different applications. The majority of marine decontamination treatments focused on removing and/or killing AIS on aquaculture infrastructure, but were considered applicable for the decontamination of fouled watercrafts and equipment. Similarly, because very little data exists for sodium hypochlorite and QAC in the context of CDD + D for freshwater and marine AIS, treatments developed for industrial water intake structures were also considered. However, these focused on extremely low concentrations and long exposure times (days to months), and as such complicated the interpretation of these results in the CDD + D context and the calculation of associated uncertainty scores.

Although a large number of effective treatment options were identified, not all will be easily applicable to all situations. To help future management decisions, a summary of treatment feasibility is presented in Table 6.

CDD+D protocols

More than 50 CDD+D protocols currently in use were evaluated to assess whether their recommendations were supported by the scientific literature.

Only some provinces currently 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 III", Elwell and Phillips, 2016) which are used at their watercraft inspection and decontamination stations.

Pressurized hot water spraying is commonly recommended in protocols to decontaminate recreational watercraft/equipment in freshwater environments. Temperature, pressure, and

[1] Erratum July 2022: Reference was previously cited as "In preparation" and has since been approved for publication.

exposure time vary between protocols and are adjusted for equipment/surface type compatibilities to prevent damage to watercraft and equipment. Despite its recommendation in many protocols, only a handful of peer-reviewed studies were found to support this technique. Three studies supported the most widely used recommendations (60 °C, 10 s), while a new study suggested that higher temperatures and longer exposure times (68 °C, 15 s) were required for 100 % mortality of zebra mussels. Overall, appropriate pressure levels required to remove and/or kill AIS were poorly studied.

Hot water immersion, air-drying, and freezing have been recommended in several protocols for the decontamination of equipment. While these methods are effective at killing several AIS, greater temperatures and/or longer exposure times are often required than those recommended in the protocols.

Guidelines for controlling AIS with chemicals were not consistent across protocols and were not always supported by the scientific literature. Sodium hypochlorite, Virkon®, quaternary ammonium compounds, and acetic acid were found to be effective for certain AIS if used at the appropriate concentrations and exposure times, which sometimes differed from protocol recommendations.

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 the CDD+D approach. Pressurized hot water spray, freshwater immersion/spray, acetic acid spray, 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).

For detailed information on protocols and comparisons with the scientific literature, please refer to Weise et al. (In press).

Table 2. Summary of watercraft decontamination treatments for 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 provided and are based on the 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 ineffectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments. See Weise et al. (In press)[1] for detailed results.*

Treatments for watercraft		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages)	No data (adults)
Air-drying [1] [b]	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 [c]	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).

[b] Erratum July 2022: Treatment "5 d (20-35°C)" replaced by "7 d (20-35°C)", "MP" modified from "Reasonable uncertainty" to "Low uncertainty", "some MP" added to "No data (adults)"; Treatment "7 d (10-17°C)" replaced by "15 d (10-19°C)".
[c] Erratum July 2022: "WF[3]" in "Ineffective" replaced by "WF[2] (eggs in air)".

Table 3. Summary of equipment decontamination treatments for 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 provided and are based on the 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 ineffectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments. See Weise et al. (In press)[1] for detailed results.*

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages)	No data (adults)
Air-drying [1] [d]	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 [e]	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 [f]	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

[d] Erratum July 2022: Treatment "5 d (20-35°C)" replaced by "7 d (20-35°C)", "MP" modified from "Reasonable uncertainty" to "Low uncertainty", "some MP" added to "No data (adults)"; Treatment "7 d (10-17°C)" replaced by "15 d (10-19°C)".
[e] Erratum July 2022: "WD[2]" removed from "High uncertainty" and "WD (adults)" in "Ineffective" replaced by "WD[2]".
[f] Erratum July 2022: "AC" modified from "High uncertainty" to "Ineffective" and "WD" removed from "No data (adults)".

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages)	No data (adults)
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.
[2g] *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).

[g] Erratum July 2022: "Whirling Disease requires 90 ˚C, 10 min" replaced by "*M. cerebralis* adults and young stages require 75 ˚C (5 min) and 90 ˚C (10 min), respectively".

Table 4. 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 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 ineffectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments. See Weise et al. (In press)[1] for detailed results.

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 5. 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 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 ineffectiveness of the treatment. Note that uncertainty scores were not calculated for ineffective treatments. See Weise et al. (In press)[1] for detailed results.

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages and adults)
Freshwater immersion [h]	24 h + 1h (air-drying)	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-dry	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
Hydrated lime immersion + air-drying	4 %, 15 min + 2h (air-dry)	-	CT	ST, OT	-	BM, GC (adults)	MA, GC (young stages)

[h] Erratum July 2022: “CT” modified from “Reasonable uncertainty” to “Low uncertainty”; “ST” modified from “High uncertainty” to “Reasonable uncertainty”.

Treatments for equipment		Low uncertainty	Reasonable uncertainty	High uncertainty	Very high uncertainty	Ineffective	No data (young stages and adults)
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 6. Decontamination treatment feasibility with regards to practicality, equipment requirements, human health and ecosystem risks, and disposal.

Treatments	Practicality (boats, 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 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

Treatments	Practicality (boats, large equipment)	Practicality (small equipment)	Special equipment required	Human health risks	Ecosystem risks	Special disposal	Notes
Hydrated lime (immersion)	NO	YES	YES	Chemical burns	NO	NO	Insoluble; difficult to get accurate concentrations
Brine (immersion)	NO	YES	NO	NO	NO	NO	-

Sources of Uncertainty

No single decontamination treatment is applicable to all freshwater and marine AIS or to all watercraft and equipment. While multiple treatments were found to be effective at killing some AIS, they were fundamentally species - and environment-specific. Consequently, any treatment applied across species, ecosystems and/or equipment type will impose variable levels of control.

The majority of studies reviewed 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).

Numerous knowledge gaps were identified in the review of scientific literature. Some species (e.g., oysters and barnacles) and treatments (zinc and copper) were not considered and not all treatments or species were well represented. 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.

Much of the scientific work on decontamination was completed under laboratory conditions, and results may not necessarily translate into equally effective (or practical) field applications. Further research is required to understand how effective decontamination treatments tested in the laboratory can be effectively used in a field setting.

A substantial subset of scientific studies considered in this Science Advice were designed to answer questions for different applications (e.g., aquaculture transfers, cleaning of infrastructure). Interpreting removal and mortality from these data contributes some uncertainty to the effectiveness of these techniques in the context of CDD+D.

This work also focused on select AIS of interest, as defined above, but additional taxonomic groups could be considered in the future which may require different treatments techniques, and this Science Advice will need to be updated accordingly.

CONCLUSIONS AND ADVICE

- Outreach and education campaigns such as "Clean, Drain, and Dry" (CDD) and "Pull the plug" are easily accessible to the general public, are essential in preventing the introduction and spread of AIS, and should continue to be supported and implemented.
- Current 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.
- CDD and decontamination are not mutually exclusive; decontamination is an additional step which 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 (see Tables 2 to 5).

- No single decontamination treatment is applicable to all freshwater and marine AIS or to all watercrafts 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.
- 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.

OTHER CONSIDERATIONS

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.

Some of the treatments which were identified as ineffective (or may be effective but have high levels of uncertainty) here and in the supporting research document (Weise et al. In press)[1] may be effective under different concentrations/pressures/temperatures and/or exposure times. Future research could aim to refine these treatment methods, to discern the point at which they become effective (e.g., sodium hypochlorite treatment effectiveness on all life stages of mussels).

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SOURCES OF INFORMATION

This Science Advisory Report is from the March 30 – April 1, 2021 National Advisory Meeting for Science Advice on "Clean, Drain, Dry and Decontaminate" Treatments and Protocols to Prevent the Introduction and Spread of Aquatic Invasive Species. Additional publications from this meeting will be posted on the Fisheries and Oceans Canada (DFO) Science Advisory Schedule as they become available.

Elwell, L.C., and Phillips, S. (Eds) 2016. Uniform minimum protocols and standards for watercraft inspection and decontamination programs for dreissenid mussels in the Western United States (UMPS III). Pacific States Marine Fisheries Commission, Portland, Oregon (USA). 53 p.

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

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