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Mitigation Measures to Reduce the Risk of Introduction and Spread of Aquatic Invasive Species through Shellfish and Macroalgal Movements

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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.

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ABSTRACT

Aquatic Invasive Species (AIS) that are introduced or spread to ecosystems beyond their natural range can threaten Canada's biodiversity, economy, and society. Shellfish and macroalgal movements are considered to be an important vector for the introduction and spread of AIS in marine ecosystems. To date, a comprehensive evaluation of the effectiveness of treatments that could be used in Canada to kill or remove marine AIS from shellfish and macroalgae being moved and their impacts on those species has not been conducted. Thus, a wide variety of treatments are currently in use without national guidance or consistency. A literature review was undertaken to document the effectiveness of existing treatments to kill/remove AIS and their impacts on moved shellfish and macroalgal species. Effective treatments for AIS from different taxonomic groups (tunicates, bivalves, gastropods, crustaceans, sea stars, macroalgae, polychaetes, bryozoans, sponges, hydrozoans) were evaluated and included physical (pressure washing, air drying, freshwater, heat) and chemical (chlorine-based compounds, acetic acid, citric acid, brine, hydrated lime, Virkon®) sprays/immersions or combinations of these. Treatment impacts on moved species (mussels, oysters, scallops, macroalgae) were also evaluated. Numerous physical and chemical treatments were identified as being effective at killing or removing various AIS and several treatment options were also identified that have no/low impacts on moved shellfish species. Very little information was found for cultured macroalgae transfers. Although a few treatment options were applicable to a large number of AIS, no single treatment was found to be applicable to all AIS while maintaining shellfish survival. Given the context-dependency of treatment options, additional research is needed to develop national standards to mitigate AIS movements associated with shellfish and macroalgal movements in Canada.

GLOSSARY

Air drying: A treatment if used under specific conditions (e.g., temperature and exposure time) that is lethal to marine organisms (specifically targeting aquatic invasive species herein). 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. It may also be an exposure to cold or hot air, with high or low relative humidity. Air exposure, aerial exposure, desiccation, and drying are used as synonyms in the literature.

Aquatic Invasive Species (AIS): A non-indigenous aquatic species (animal, plant or microorganism) 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.

Effectiveness/Effective/Not effective: The level to which a treatment can kill a targeted AIS. Effectiveness is expressed quantitatively (as percent mortality or removal) or qualitatively (effective or not effective, without percent values). Effectiveness of physical and chemical treatments was categorized as effective if they resulted in 100% mortality (quantitative) or characterized as ‘effective’ at killing a given AIS (qualitative).

Field: Studies were classified as “field” when they were conducted on aquaculture farms where conditions were loosely monitored and/or measured and closer to "real-world" conditions of aquaculture operations.

Impacted: Term used for qualitative results for treatments deemed to have unquantified impacts (no quantified data on survival) on moved species**.** Examples of unquantified impacts found in literature: high/low mortality or mortality occurred, weight loss, byssal attachment, shell or cell damage, reduced growth or performance, or sub-lethal effects.

Immersion: Complete submersion of organisms or aquaculture gear in cold or hot water or a chemical solution, which is lethal for the target AIS (individuals or clusters) if used under specific conditions (e.g., concentration and exposure time).

Laboratory: Studies were classified as "laboratory" in cases where the experiment was conducted in a laboratory setting where all conditions were closely monitored, measured, and controlled or in cases where experiments were conducted in the field in tanks or buckets where some parameters were controlled. Controlled or laboratory conditions/settings are synonyms.

Mortality: Organism death. Mortality is achieved when organisms are dead and show no signs of movement or vital activity (e.g., cessation of growth or feeding, no response to tactile stimulation, reduction of biomass, etc.). We have expressed mortality in percent, relative to the initial number of organisms present before a treatment or controls.

Moved species: Shellfish or macroalgal organisms that are physically displaced from one location or body of water to another (for further cultivation), or to a processing facility (destined for market), or to an experimental laboratory.

Movement: The physical displacement of shellfish or macroalgal organisms from one location or body of water to another, or to processing or experimental facilities.

Non-indigenous species (NIS): Plant, animal, or microorganism occurring in an area outside of its known natural habitat or range, which may have negative ecological, human health, and/or economic impacts after its introduction, establishment, and/or spread to a new ecosystem.

Not impacted: Term used for qualitative results for treatments that were deemed to have low/no impact (no quantified data on survival) on moved species. Examples of results classified as “Not impacted” found in the literature included: unaffected, survived, low mortality, survival criterion respected, low impact, no obvious effect, no detectable impact, or growth over time (weeks or months).

OBIS: Ocean Biodiversity Information System (OBIS™)

Pressure washing: A treatment consisting of freshwater and/or seawater sprays that, if used under specific conditions (e.g., spray pressure, temperature, and exposure time), is able to remove and/or kill marine organisms (specifically targeting AIS herein). In this document, low-pressure washing refers to sprays with water pressures below 60 psi (e.g., deck wash hoses) while high-pressure washing refers to greater than 700 psi. Water can be heated to increase effectiveness (i.e., mortality of AIS) of pressure washing.

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

Practical part per thousand (ppt): Denotes one part per 1,000 parts.

Practical salinity unit (psu): 1 gram of salt per 1,000 grams of water (1 psu = 1 ppt).

Quantitative: Quantitative results on the effectiveness of AIS treatments and their impacts on moved species were indicated by percent values, where 100% refers to a treatment which killed 100% of AIS or ensured 100% survival of the moved species.

Qualitative: Qualitative results on the effectiveness of AIS treatments and their impacts on moved species were not indicated in percent values, but as effective/not effective for AIS and impacted/not impacted for moved species.

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

Spread: Where an AIS increases its geographic range following an introduction. This is often facilitated by human-mediated activities, such as shellfish and macroalgal transfers or movements, recreational/commercial boating activities, and the aquarium trade.

Survival: Organism remains alive. Survival is deemed when organisms are lively and show signs of vital activities (e.g., growth, feeding, response to tactile stimulation, increase in biomass, photosynthetic activity, natural color, etc.). Survival is expressed in percent (%).

Treatment: A method used with the intent to kill, destroy, and/or remove AIS to prevent their spread.

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

WoRMS: World Register of Marine Species (WoRMS™)

1. INTRODUCTION

Non-indigenous species (NIS), including Aquatic Invasive Species (AIS), that are introduced or spread to ecosystems beyond their natural range can threaten Canada's biodiversity, economy, and society. Shellfish and macroalgal movement is considered an important vector for the introduction of AIS in marine ecosystems whether conducted at international, interregional, regional, or local scales (Minchin 1996; Verlaque 2001; Wallentinus 2002; Wolff and Reise, 2002; McKindsey et al. 2007; Mineur et al. 2007; Verlaque et al. 2007; Haupt et al. 2010; Levings et al. 2002; Katsanevakis et al. 2013; Nunes et al. 2014; McKenzie et al. 2016; Ferguson et al. 2017; Cunningham et al. 2020; Stranga and Katsanevakis 2021). Consequently, preventing introductions, and further spread of AIS through means of common codes of practice is crucial. The International Council for the Exploration of the Sea (ICES) Code of Practice on the Introduction and Transfer of Marine Organisms provides a framework to evaluate new intentional introductions and recommends procedures for species that are part of current commercial practices to reduce the risk of unwanted introductions and translocations (ICES, 2005). In 2003, Canada's provincial, territorial, and federal governments jointly implemented the National Code on Introduction and Transfer of Marine Organisms (hereafter referred to as 'The Code') (DFO 2017). The Code adheres to guiding principles for decision making on introduction and transfers in which Introduction and Transfers Committees (consisting of provincial/territorial/ federal representatives) evaluate requests to introduce, move or transfer aquatic organisms. Fisheries and Oceans Canada's role within the Introduction and Transfers committees is to conduct risk evaluations through the committees' evaluation process, to issue licenses under the Code, and to ensure compliance monitoring and enforcement. Licenses issued under Section 6 of the Code adhere to Canada's *Fisheries Act*. Under Section 55 of the Fishery (General) Regulations of the *Fisheries Act*, DFO has the legal authority to ensure that no person may release live fish[1] into their habitat or transfer live fish to rearing facilities unless they obtain a permit from the federal Department of Fisheries and Oceans (DFO) Canada. The legal basis for requiring a license to transfer fish and shellfish is found in Section 56 of the Fishery (General) Regulations of the *Fisheries Act*. This section states that DFO may issue a permit to release live fish into any habitat or transfer live fish to rearing facilities if:

1. The release or transfer of fish is in accordance with sound fisheries management and control;
2. The fish are free from diseases and pathogens that could harm the protection and conservation of the species; and
3. The release or transfer is not likely to have an adverse effect on the size of the stock or on the genetic characteristics of the fish or fish stocks.

Mitigation measures for AIS may be recommended or required as a condition of license to reduce the risk of introduction and transfer of AIS. Although a general decrease in new introductions has been attributed to compulsory measures to control the introduction of NIS/AIS via aquaculture activities, such as Introduction and Transfers Codes of Practice (Katsanevakis et al. 2013), variability in regionally recommended mitigation measures may impact their overall success.

[1] In the Act, the term "fish" is defined to include fish proper and their parts and, by assimilation, molluscs, crustaceans, and other marine animals and their parts, as the case may be, including eggs, semen, milt, spawn, larvae, spat, and young of animals.

Although mitigation control measures (including AIS control treatments) are often used as a condition of the licensee for DFO approved Introduction and Transfer permits, there are no national DFO standards for proposed mitigation measures used to treat aquaculture shellfish or macroalgal products to reduce the risk of introduction and spread of AIS. To date, the effectiveness of treatments used in Canada on marine AIS and the impacts of particular treatments on cultured and non-cultured species has not been reviewed and a wide variety of methods are currently in use without national guidance and 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, and National Aquaculture Management Program to develop national recommendations. These two DFO Programs intend to use this advice to develop standards (or guidelines) to reduce the risk of spreading AIS through shellfish/macroalgal movements. Those standards would provide guidance related to shellfish and macroalgal movements to both programs as well as for regional DFO Introduction and Transfers committees. Recommendations herein could be used to inform decision making, including both management and policy, with regard to Canadian AIS mitigation for movement of shellfish and macroalgal species that may be vectors of marine AIS.

1.1. OBJECTIVES

This process was based on a literature review and is limited to marine invertebrate and macroalgal species, with an emphasis on epibiont AIS, which have the potential to be transported externally on shellfish/macroalgal species during movements (e.g., aquaculture transfers, scientific experimental transfers). Internally transported species (e.g., living in mantle water), as well as viruses, bacteria, phytoplankton, and protozoans, were not within the scope of this work. The ease of application and feasibility/practicality of treatments, their associated health and safety hazards, cost, and disposal were also out of scope. Representative species from various functional and taxonomic groups (e.g., bivalves, tunicates, crustaceans) were selected according to their presence (or their expected arrival) in Canadian marine environments and data availability. Impacts of treatment methods on shellfish (e.g., mussels, oysters, scallops) and macroalgae were also assessed.

The specific objectives of this advisory process are:

1. Review and characterize existing reactive control methods to mitigate the risk of spreading marine epibiont invertebrate and macroalgal AIS during shellfish/macroalgal movements.
2. Assess the effectiveness of existing methods to kill/remove marine epibiont invertebrate and macroalgal AIS and their impacts on shellfish and macroalgal species including, but not exclusively limited to, survival.
3. Provide treatment options that could kill/remove AIS while keeping alive (low impact) moved shellfish and macroalgal species.
4. Identify data gaps and sources of uncertainty.

2. METHODS

A literature review of physical and chemical treatments used as mitigation measures to control marine AIS and epibionts was completed to document the effectiveness of treatments on AIS (i.e., mortality) and their impacts on moved shellfish and macroalgal species (i.e., survival). Literature was collected from several sources including: Web of Science™ (Web of Knowledge), DFO's Federal Science Library (WAVES), Google Scholar (Google™), and ResearchGate™. All publication years were considered from the earliest available until November 2022. Publications included peer-reviewed journal articles, government and consultant reports (i.e., secondary/grey literature), relevant websites, and personal communications/expert opinions, where applicable. Boolean search terms are given in Appendix 1.

2.1. LITERATURE REVIEW: CRITERIA FOR SELECTION OF SPECIES

Representative AIS/epibiont species from various functional and taxonomic groups (e.g., tunicates, bivalves, gastropods, crustaceans, sea stars, macroalgae, polychaetes, bryozoans, sponges, and hydrozoans) were selected according to their presence (or their expected arrival) in Canadian marine environments (Table 1). Species of shellfish and macroalgae known to have been or being commonly cultivated in Canada (Atlantic and Pacific coasts) and of interest for movements among water bodies were selected for this literature review (Table 2). Species that are increasingly of interest for future Canadian aquaculture development were also considered [e.g., the bay scallop (*Argopecten irradians*); Comeau et al. 2017].

In some cases, when data were limiting for a target AIS or a moved species, data were included for nuisance/invasive species or moved shellfish/macroalgal species that could serve as suitable proxies for a particular functional or taxonomic group. Proxy species were chosen to represent target species based on their morphological similarity or taxonomic relatedness to selected species (Hilton and Richardson 2004; Loman et al. 2021), or their belonging to the same functional group (e.g., mussels, oysters, bryozoans) (Cahill et al. 2022), their similarity in habitat preferences or distribution range, and/or whether mitigation treatment effectiveness was tested on the proposed proxy species.

The taxonomic databases World Register of Marine Species (WoRMS™) and Ocean Biodiversity Information System (OBIS™) were consulted to determine taxonomic relatedness of proxy species to representative ones. Specifically, the New Zealand green-lipped mussel (*Perna canaliculus*) was selected to represent the Mediterranean mussel (*Mytilus galloprovincialis*) since these species share the same functional group (mussels) and are of similar size and distribution range (Pacific Ocean). The southern mud oyster (*Ostrea angasi*) was chosen to represent the European flat oyster (*Ostrea edulis*) since these species share the same functional group (oysters) and taxonomic genus and are therefore closely related, although their distribution ranges do not overlap. The red-rust bryozoan (*Watersipora subtorquata*) was chosen as a proxy for the bryozoan *Cryptosula pallasiana* as they are both colonial encrusting bryozoans from the suborder Flustrina (closest level of taxa). Some proxies were chosen based on the fact that some species share common taxonomic genera and are therefore closely related. This was the case for the Northern Pacific sea star (*Asterias amurensis*), the solitary tunicate *Ciona savignyi*, and the colonial tunicate *Botrylloides leachii*, which were chosen proxies for the common sea star (*Asterias rubens*), the vase tunicate (*Ciona intestinalis*), and the violet tunicate (*Botrylloides violaceus*), respectively.

2.2. LITERATURE REVIEW: CRITERIA FOR SELECTION OF TREATMENTS

Publications were retained if they met the following criteria:

1. Included a detailed description of one or several physical or chemical treatments used to kill or remove surface-dwelling AIS and/or epibionts;
2. Quantitatively or qualitatively evaluated treatment effectiveness (e.g., mortality, removal) of the AIS and/or impacts (e.g., survival, viability, and/or growth) on the species being moved; and
3. Could be applicable for the treatment of Canadian marine shellfish or macroalgae prior to their movement (e.g., aquaculture transfers, scientific experimental permits).

Based on information extracted from the available literature (a total of 203 literature sources), the following most commonly identified reactive treatments were assessed for their effectiveness on a subset of marine AIS/epibionts as well as on their impacts on a selection of moved shellfish and macroalgal species:

Physical treatments

- Pressure washing (low- and high-pressure spray)
- Air drying or air exposure
- Freshwater (immersion or spray) (sometimes combined with air drying)
- Hot seawater or hot freshwater (immersion or steam)

Chemical treatments (sometimes combined with air drying)

- Chlorine-based compounds (immersion)
- Acetic acid (CH_3COOH) (immersion or spray)
- Citric acid ($C_6H_8O_7$) (immersion)
- Brine solution (immersion)
- Hydrated lime (CaOH) (immersion or spray)
- Brine + lime solution (immersion)
- Virkon® [active ingredient: pentapotassium bis(peroxymonosulphate) bis(sulphate)] (immersion)

Studies were classified as "laboratory" in cases where the experiment was conducted in a laboratory setting where all conditions were closely monitored, measured, and controlled or in cases where experiments were conducted outside in tanks or buckets where some parameters were controlled. Studies were classified as “field” when they were conducted on aquaculture farms where conditions were likely more loosely monitored and/or measured.

For air drying treatments, there was a lack of information in the literature regarding variability of other parameters, such as temperature and humidity. These parameters were indicated when provided in publications.

Three types of results were available for chlorine-based treatments in the literature:

1. Those reporting initial concentrations of sodium hypochlorite (NaClO) [e.g., diluted sodium hypochlorite or “bleach” (these results being expressed in %)];

2. Those reporting final concentrations of total residual chlorine (TRC), labeled with the symbol "§" (these results being expressed in mg L^{-1}). According to Rajagopal et al. (2002, 2003) and Haque et al. (2014, 2015), TRC is a measurement of the sum of dissolved free and combined (with other chemicals in water) chlorine ions (Cl^-) that remain in the solution after dilution and/or treatment. Instead of using the initial concentration of sodium hypochlorite, they used the total residual concentrations of chlorine (Cl^-) of the final solution.
3. Those reporting chlorine dioxide (ClO_2) concentration (expressed in %) instead of sodium hypochlorite (e.g., Asgari and Jahangard 2012).

For saturated brine treatments, some publications mentioned a saturation, but did not specify the exact concentration used. Since a saturated brine solution is reached at a concentration near 360 ppt at room temperature (Chem LibreTexts 2022), we assumed that, in those exceptional cases where salt concentration was unspecified but indicated as saturated, this solution had reached a brine concentration of at least 300 ppt as undissolved solute starts to appear over this concentration.

Some treatments, such as biological (e.g., urchin grazing) and certain mechanical (e.g., bag flipping to allow desiccation) mitigation methods, were deemed to be longer-term control mechanisms that would not be applicable in the short-term context of movements of species (e.g., aquaculture transfers). Results from those sorts of treatments were presented as 'Proactive biofouling management options' (see Section 3.3). Proactive and preventive methods specifically used to routinely clean AIS from aquaculture equipment and product (e.g., maintenance cleaning conducted for local control to decrease the weight of structures and increase shell growth and production) are only briefly presented (see Section 3.3.3).

Mitigation measures containing metal-based compounds (e.g., copper, zinc, biocides, antifouling paints) meant for treating inorganic material and structures to avoid and control biofouling were excluded from the present review. Metal-based chemical antifoulants and bioactive netting may be detrimental to the survival and growth of the cultured organisms and pose a risk to environmental and human health (Muñoz et al. 2010; Guardiola et al. 2012). Treatments such as air freezing and extreme heat (e.g., burning), which were effective at causing AIS mortality, but also caused significant mortality of moved species, were also excluded as they would not be useful for aquaculture or scientific transfers where the commercial/scientific product must be kept alive. Results of studies targeting AIS with a Virkon® treatment, however, were included in the present document as this disinfectant/virucide is approved by Health Canada for its use in aquaculture facilities, is environmentally friendly, is safely handled by those conducting AIS treatments, and was tested directly on shellfish in very few publications. It is a multicomponent mixture comprised of 25–50% potassium hydrogen peroxymonosulphate (primary active ingredient), 10 to <25% butanedioic acid, <10% sulphamic acid, and a range of other minor ingredients. Sodium hypochlorite, acetic acid, and hydrated lime were retained as these chemicals are widely used in aquaculture practices, have low toxicity and environmental persistence compared to biocides, and were shown to have no or low risks for human and environmental health if applied under the conditions currently used in an aquaculture context (low frequency, low initial concentrations, use of safety measures) (Carver et al. 2003; Sharp et al. 2006; Piola et al. 2009; Ramsay et al. 2014; Cahill et al. 2021).

2.3. LITERATURE REVIEW: CRITERIA FOR ASSESSING EFFECTIVENESS OF TREATMENT ON MARINE AIS

Treatment was defined as a physical and/or chemical (or combination of these) method with a defined time component to achieve a quantified level of AIS mortality or removal (e.g., X temperature or Y concentration for Z minutes). Data in the results tables are presented by

treatment in headers and by target AIS or epibionts in rows. Treatment parameters (concentrations, exposure times, temperatures, etc.) and associated species mortality (%) or removal (%) were recorded. Most of the existing literature on AIS treatments focused on mortality as an endpoint, while pressurized water sprays focused either on mortality or removal. Effectiveness is expressed quantitatively (as percent mortality or removal) or qualitatively ("Effective" or "Not effective", without a percent). Effectiveness of physical and chemical treatments were categorized as effective if they resulted in 100% mortality/removal or characterized as "effective" at killing a given AIS. In the tables, publications reporting 100% mortality/removal are presented first with those reporting lower mortality second, followed by publications stating that a treatment was qualitatively effective, but no measure of mortality was provided. Ineffective treatments (quantitative or qualitative) were retained in order to identify potential conflicting results in the literature, assess the agreement on a treatment being effective or not, and to fill gaps of information if no treatment was found effective for any given species. Unpublished results provided by local experts were also considered for some physical and chemical treatments, and were identified as 'unpublished data' and labeled with two superscript asterisks (**). Reports or grey literature were labeled with one superscript asterisk character (*).

2.4. LITERATURE REVIEW: CRITERIA FOR ASSESSING IMPACTS OF TREATMENT ON SHELLFISH AND MACROALGAE

For the impacts of treatment on moved species, treatment was defined as a physical and/or chemical (or combination of these) method with a defined time component to ensure a quantified survival rate. Data on impacts of treatments on moved species (shellfish or macroalgae) were also extracted from the literature, reviewed, and classified by treatment. Quantitative results on impact of treatments on moved species were indicated in survival percentages (%), where 100% refers to a treatment that ensured keeping 100% of the moved species alive after treatment. Publications in which treatments were deemed to have low/no impact or high impact without specific survival data on the moved species (qualitative data) were referred to as "Not impacted" or "Impacted", respectively. We classified results as "Not impacted" when authors described their results with the following qualifiers: "unaffected", "survived", "low mortality", "survival criterion respected", "low impact", "no obvious effect", "no detectable impact", or "growth over time" (weeks or months). In addition, some authors used various unquantified impacts (classified as "Impacted") such as: "occurring/high mortality", "weight loss", "shell damage", and "reduced growth". In the text, publications with the highest quantitative survival percentage are presented first, followed by those with decreasing survival percentages in descending order, followed by articles with qualitative results ("Impacted" or "Not Impacted"). Unpublished results provided by local experts were also considered for some physical and chemical treatments, and were identified as 'unpublished data'.

2.4.1. Representative shellfish as moved species and AIS

The blue mussel (*Mytilus edulis*), Mediterranean mussel (*M. galloprovincialis*), Pacific oyster (*Crassostrea gigas*), and Eastern oyster (*C. virginica*) are the main shellfish species that are, or have been, cultivated in Canada. They can also be considered, however, as epibionts on moved species. For the purpose of this literature review, those shellfish are considered as being either moved species or AIS, depending on geographical location and context of the study. Thus, some results obtained for these species were transposed and presented as mortality or impact data, accordingly. The approach to determine size categories for these four bivalve species (see section 2.4.2) was used regardless of whether they were considered invasive or moved species.

2.4.2. Size categories of shellfish

Given that different life stages/sizes of shellfish are moved during transfers and that treatment effectiveness/impact may be strongly linked to size, two size categories ("large" and "small") were established for most shellfish species. Cutoff values for size categories of shellfish species are presented in Table 3 along with supporting references for the establishment of these cutoff values. The cutoff value was determined using at least three or more publications and/or reports per species, providing complementary information pertaining to biological size ranges, market or commercial size, life- and aquaculture-related stages (e.g., adult, seed/spat), and age (months or years). Using only life-stages as a criterion to determine these two size categories was challenging, as sizes for bivalve species may vary considerably depending on growth conditions at various locations (e.g., difference in climate in west versus east coast of Canada).

Cutoffs are expressed in shell lengths (mm), with the exceptions of *A. irradians*, where sizes found in the literature were generally not specified, and the deep-sea scallop (*Placopecten magellanicus*) for which sizes used to establish the cutoff were mostly specified in shell height. Literature found on *M. edulis* and *M. galloprovincialis* was used to establish the same cutoff values for both species. The following shellfish size caveats apply:

- Large size: references include mentions of "adult" and "large-sized juvenile" stages.
- Small size: references include mentions of "juvenile stages" (spat/seed) and "small-sized adults".
- When two groups of shellfish belonging to the same size category were tested in the same study and showed different results, both results were presented separately. For example, if two small-size groups of mussels of 5–25 mm and 35–45 mm were tested, results from both size groups were included in the "small" size category (note: a cutoff of 50 mm shell length was determined for mussels; Table 3).
- Range of sizes applicable for both size categories (small and large): when a range of sizes overlapped the cutoff value, results were repeated in both size categories (e.g., 20–60 mm, with a cutoff value = 50 mm).
- When no size or range of sizes was provided, nor life stage or age, but information (quantitative or qualitative) on survival or mortality was provided, results were classified in a size category following a precautionary approach. When results on AIS/epibionts indicated 100% mortality or an "effective" treatment, they were classified into the "small" size category (i.e., assumed to be more sensitive than large bivalves for a given species), whereas results indicating <100% mortality or a "not effective" treatment were classified into the "large" size category. Similarly, for moved species, when results indicated ≥90% survival or that they were "not impacted", they were classified into the "large" size category, while results indicating <90% survival or that they were "impacted" were classified into the "small" size category (i.e., assumed to be less resistant than large bivalves for a given species).

2.5. ASSESSMENT OF MORTALITY

Mortality endpoints varied among studies, taxa, and organism life stage, however, general methods for assessment of viability and survival were comparable. Methods for determining mortality of tunicates was indeed variable, but field studies generally categorized individuals and/or colonies of tunicates as dead if they were absent, discoloured, putrefied, or detached from the substrate or if cells were uncoloured after using Neutral Red vital stain (Williams and Schroeder 2004; Forrest et al. 2007; Carman et al. 2010, 2016; Ferguson et al. 2016). In some cases, significant biomass reduction compared to control colonies was a reliable indicator of colony regression, which ultimately led to mortality (see MacNair et al. 2006; Leblanc et al.

2007; Paetzold et al. 2012; Roche et al. 2015). Some laboratory studies have used a lack of water siphoning and the absence of tactical response (for tunicates) or the inability to close valves after 48 h in recovery (for bivalves) (e.g., Hillock and Costello 2013; Hopkins et al. 2016; Sievers et al. 2019).

Bivalves were considered dead when, after a period of recovery, organisms did not respond to tactile stimulation, did not re-attach byssal threads, had open shells, and/or demonstrated prolonged shell gaping (e.g., Forrest and Blakemore 2006; Leblanc et al. 2007; Joyce et al. 2019; Sievers et al. 2019; Cahill et al. 2021), while death of veligers was confirmed by the absence of ciliary movement inside the shell or on the extended velum (e.g., Haque et al. 2014; Haque and Kwon 2017). An example of a sign of mortality used for gastropods was the absence of hatching, while a closed operculum and feeding activity were identified as viability signs for this group (Gill et al. 2008).

European green crabs (*Carcinus maenas*) were considered dead when there was a lack of reflexive retractions of legs when tugged or by a lack of papilla and/or antennule activity (Darbyson et al. 2009; Best et al. 2014), while mortality of the Japanese skeleton shrimp (*Caprella mutica*) was defined as a lack of response to a mechanical stimulus (i.e., gentle prodding with a blunt probe) (Ashton et al. 2007). For amphipods, the animals were classified qualitatively as active (crawling/swimming, gills moving), inactive (twitching/no activity), or dead (floating/no gill activity) (Paetzold et al. 2008).

Criteria to establish macroalgal mortality were variable, with multiple approaches defined in the literature to assess survivorship and viability (or mortality) after treatments. Techniques included the visual estimation of degradation, discolouration, pigmentation lost, fragments disintegrating to the point where no tissue remained, necrotic appearance, absence of fluorescence, and number of shrunken and emptied cells (MacNair 2002; Smit et al. 2003; Forrest and Blakemore 2006; Sharp et al. 2006).

Mortality or impacts of treatments on polychaetes were expressed by different terminology in the reviewed literature such as a touch stimulus of radioles, gently lifting worm tubes (dead worms fall out of their tubes), decaying worms, lethargy, and stress symptoms (Velayudhan 1983; Leighton 1998; Jute and Dunphy 2017).

Bryozoans (e.g., *W. subtorquata*, *Bugula neritina*) were considered dead when they were absent (Piola et al. 2009). Sea stars were considered dead when they became soft and flabby or were partially or wholly disintegrated (Loosanoff 1960) or if no tube feet were moving upon inspection (Rolheiser et al. 2012). Death of sponges can manifest as a change in colour or colony disintegration (Loosanoff 1960; Carver et al. 2010).

2.6. LEVELS OF UNCERTAINTY

The most effective treatment options that were lethal (100% or qualitatively effective) to the greatest number of AIS with no or minimal impacts on the moved species (>90% survival or qualitatively not impacted) were identified, along with measures of associated uncertainty. Levels of uncertainty (very high uncertainty, high uncertainty, moderate uncertainty, and low uncertainty) were assigned to each treatment option for each AIS species (or taxonomic group) and moved shellfish/macroalgal species. Those uncertainty levels were assigned based on the number of studies available [few (1 study), limited (2), many (3−6), or comprehensive (≥7)], their quality (pers. comm., technical report, or peer reviewed),and agreement among studies with the identified treatment options (conflicting, different conclusions, mostly agree, or fully agree) (see Table 4). When a taxonomic group gathered multiple species (e.g., polychaetes), the level of uncertainty increased (as results are not species-specific). Consequently, although a given treatment may be identified as effective for a particular species, a higher uncertainty score is

possible when few peer-reviewed studies were available. Similarly, low/moderate uncertainty scores are generated when many peer-reviewed studies agreed on the effectiveness of a treatment option. Uncertainty scores were not calculated for ineffective treatments.

Many considerations, gaps, and sources of uncertainty were identified in this work and limited the interpretation of results. These considerations originate mainly, but not only from: limited data with many gaps, variable experimental study designs, limitation to extrapolate laboratory studies to field context, effects of environmental factors and variability of parameters on treatment effectiveness and organism survival, influence of organism size on survival or mortality, and qualitative vs quantitative data. Among other limitations, assessment of the relative contribution of specific components within combined treatments (e.g., immersion and air drying, heating, combination of two chemicals) to global effectiveness and chronic-stress effects of treatments compared to acute stress on moved species were less studied. These limitations and sources of uncertainty are further described in section 4.6.

3. RESULTS

More than 200 references were considered during this literature review, of which 115 scientific publications and grey literature reports were further reviewed to assess the effectiveness of various physical and chemical treatments for the removal and/or mortality of marine AIS/epibionts, and for their impacts on a selection of moved shellfish and macroalgal species.

3.1. ASSESSMENT OF EFFECTS OF TREATMENTS ON MORTALITY OF MARINE AIS AND EPIBIONTS

3.1.1. Effectiveness of physical treatments

A total of 64 literature sources (47 primary publications and 17 technical reports) were considered. Those included publications on using pressurized seawater (7), air drying (30), freshwater (31), and heat treatments (hot freshwater or seawater, and steam; 26), or a combination of these for the control of marine AIS and epibionts (Table 5). Unpublished results (6) provided by local experts were also considered for some physical treatments and were identified as ‘unpublished data’ and labeled with two superscript asterisks (**).

3.1.1.1. Pressure washing (low- and high-pressure sprays)

Limited information was found on the effectiveness of pressurized seawater for eliminating AIS compared to other physical treatments and most studies in this category focused on the removal of tunicates. The pressure required to remove 100% of biofouling organisms varied across AIS and ranged from 400 to 2,000 psi (2 to 10 s) (Coutts 2006; Forrest and Blakemore 2006; Paetzold et al. 2012; Ramsay 2014a). High-pressure (700 psi) water treatment was effective at reducing golden-star tunicate (*Botryllus schlosseri*) and violet tunicate (*Botrylloides violaceus*) at almost 100% (Paetzold et al. 2012) and 84% (Arens et al. 2011a), respectively. Arens et al. (2011a) showed that low pressure (40 psi) had no effect. In addition, Ramsay (2014a) found that the use of high-pressure seawater (400–600 psi) using rotary nozzles was effective in reducing levels of sea vase (*Ciona intestinalis*) on mussel socks. Coutts (2006) reported that a combination of high-pressure seawater spray and air drying was a cost-effective method for treating moorings and a variety of other artificial structures. Specifically, on-land treatment of moorings using 2,000 psi spray and 48 h of air drying removed 100% of the carpet sea squirt (*Didemnum vexillum*). Small and large *M. edulis* (here considered as AIS) were not affected by pressure washing treatments (40 or 700 psi; Arens et al. 2011a) and no mortality (0%) was observed up to 700 psi after 10 s (Arens et al. 2011b). At higher pressure (≥2,000 psi for 2 s), high pressure water spray was completely effective (100%) at removing gametophytes of

Wakame (*Undaria pinnatifida*) from mussel shells (Forrest and Blakemore, 2006). In contrast, Curtis et al. (2021) showed that high pressure washing (2,000−3,000 psi; 10, 20, 30 s) was ineffective at removing biofouling [including *B. schlosseri*, *B. violaceus*, *D. vexillum*, hooked slipper snail (*Crepidula adunca*), *C. maenas*, and sea stars] and mobile invertebrates (e.g., several species of crabs) on cultured oysters (*C. gigas*). Although many other organisms could have been part of the biofouling community examined in those studies, no specific information was found for the compound sea squirt (*Diplosoma listerianum*), clubbed tunicate (*Styela clava*), European sea squirt (*Ascidiella aspersa*), sea grapes (*Molgula* spp.), small and large bivalves species (*M. galloprovincialis*, *C. virginica*, *C. gigas*), *C. mutica*, barnacles, oyster thief (*Codium fragile*), polychaetes, bryozoans, sponges, and hydrozoans.

3.1.1.2. Air drying

Live tunicates (including *B. schlosseri, B. violaceus, D. vexillum, D. listerianum, C. intestinalis, A. aspersa, and S. clava*) were eliminated from shellfish following air exposure treatments for 24 h (Carman et al. 2010). Medcof (1961) observed that *Molgula* spp. were effectively killed when spat collectors were exposed to air for a 24-h period. MacNair et al. (2006) showed that buoys air dried for 72 h were nearly devoid (almost 100% effective) of live *B. violaceus*. An air-exposure trial of only 5 h [18–19°C; 92% relative humidity (RH)] induced 100% mortality of colonies of *B. schlosseri* on PVC monitoring plates (Bernier et al., DFO, unpubl. data). In contrast, pontoons infested with *D. vexillum* needed to be removed from the water for approximately 2 weeks to desiccate and kill the colonies (Pannell and Coutts 2007). For solitary tunicates, Hopkins et al. (2016) demonstrated that air drying can be an effective mitigation method for a broad range of fouling taxa. They showed that adults/recruits and juveniles of *Ciona savignyi* (proxy for *C. intestinalis*) were 100% killed within 24 h (18°C) and 8 h (14.5°C, 95% RH) of air exposure, respectively, under laboratory conditions (Hopkins et al. 2016). The tunicate *C. savigni* was 100% killed in field trials after only 6 h of direct sunlight exposure at ambient outdoor temperatures (in the range of 9.5–32.2°C; Hopkins et al. 2016). There is ambiguity in the literature as to the effectiveness of air drying for *S. clava*. Hillock and Costello (2013) showed that air drying under direct sunlight was effective at eliminating *S. clava*, irrespective of relative humidity, and required 24 h (25–27°C) to achieve 100% mortality. Similarly, Carman et al. (2010) found that 24 h of air drying was effective for *S. clava*. In contrast, Minchin and Duggan (1988) observed 0% mortality of this tunicate using the same treatment. Hillock and Costello (2013) observed that an air exposure for 2 weeks was required to eradicate 100% of *S. clava* from marine equipment when the air temperature was 10°C. In another study, *S. clava* was 100% killed on structures and vessels when they were removed from the water for air drying for at least 1 week (Coutts and Forrest 2005). One week air exposure was required because *S. clava* can survive exposures to air from 17 h to approximately 6 d, depending on ambient temperatures and humidity, particularly in high humidity conditions such as when growing on fouled rope (Coutts and Forrest 2005). In controlled laboratory conditions at 20°C, only 90 min was required to kill 100% of juvenile *S. clava* (Davidson et al. 2005).

Seuront et al. (2019) showed that an outdoor air exposure of 6 h at 41°C was effective in killing 100% of small and large *M. edulis*, while Leblanc et al. (2005) observed no mortality when the large bivalves were exposed to temperatures ranging from 20 to 41°C for 3 h under laboratory conditions. Leblanc et al. (2005) also found that 11 h of air exposure (27°C, 55.6% RH) resulted in 47.8% mortality of small *M. edulis*. In another study, Leblanc et al. (2007) observed no more than 38% mortality of small *M. edulis* after 40 h (21°C, 34% RH) of air exposure in field conditions comparable to industrial methods. Field work led by Comeau (DFO, unpubl. data) showed that 24 h of air exposure (17–31°C, RH not specified) was not effective (5.6% mortality) for small *M. edulis* and that a longer exposure time (5 d; 8–31°C, RH not specified) was required

to reach 99% mortality. Shorter times of exposure of 2–3 h in low RH conditions were effective in killing small *M. edulis* according to Arakawa (1980), but in high RH conditions, 5–6 h were not enough. Vickerson (2009) demonstrated, under simulated cold-transport conditions, that 24 h of air exposure (4°C, 100% RH) was not enough to kill small *M. edulis*. For large *M. galloprovincialis*, mortality started to occur after at least 4 d (18°C) of air drying, but 11 d were required for 100% mortality under laboratory conditions (Hopkins et al. 2016). In another laboratory experiment, 7 d (20.3°C) of air exposure was required to attain 100% mortality of large *M. galloprovincialis* (Hopkins et al. 2016). Asgari and Jahangard (2012) observed no mortality (0%) of large *M. galloprovincialis* after 24 h of air exposure under laboratory and field conditions (both conducted at temperatures ranging from 14 to 18°C). For small *M. galloprovincialis*, Hopkins et al. (2016) found that 24 h in the laboratory (18°C, high RH conditions) was 100% effective, whereas 6 h (18.5°C, 95% RH) in the field induced only 80% mortality. According to Mallet et al. (Mallet Research Services Ltd., unpubl. data), 8% mortality of small *M. edulis* (3–18 mm) was observed after 24 h of air drying under controlled conditions.

Information on the effect of air drying for *C. virginica* was only available for small individuals. Field trials led by Comeau (DFO, unpubl. data) demonstrated an increase in mortality percentages with increasing time of air exposure, where 1 d (with daily temperatures ranging from 17–31°C, RH not specified) was 0% effective, 5 d (8−31°C) was 32% effective, and 11 d (4−36°C) was 99% effective on small (spat) *C. virginica*. In the laboratory experiments, Mallet et al. (Mallet Research Services Ltd., unpubl. data) observed 98% mortality of small (3–18 mm) *C. virginica* after 24 h of air drying. A 72 h air exposure period, in conditions avoiding variations in sun exposure and weather, did not kill *C. virginica* (35−65 mm; 5% mortality) (Mayrand et al. 2015). In a laboratory experiment, large *C. gigas* were 100% killed after 34 d at 18°C, but mortality started to occur after 7 d (Hopkins et al. 2016). In field conditions, under a wider range of temperatures and high RH (9.5–32.2°C, 95% RH), the same authors observed that mortality occurred after 72 h, but 16 d were required for killing 100% of the individuals.

Hancock (1969) observed that individuals of the marine gastropod *Urosalpinx* spp. were not killed even after up to 8 d of air drying. In protected or enclosed spaces, *C. maenas* can survive for extended periods of time out of water where RH remains high enough to avoid gill drying (Darbyson et al. 2009). At a mean air temperature of 29°C, 50% of crabs fully exposed to air survived 59–105 h (2.5–4.4 d). No crabs survived the 7 d air-exposure experiment when left alone in fish crates at higher densities (10 or 15 individuals per crate), whereas 7% survived in the crates with a lower crab density (5 individuals per crate). However, about 60% of crabs survived for 7 d when seawater was present in fish crates with the crabs. Air drying induced 92% mortality after 10.8 d in adults of the common barnacle (*Balanus balanoides*) when exposed to desiccation in controlled laboratory conditions (10°C, 0% RH) (Foster 1971).

There is limited information available on the effects of air drying on the alga *C. fragile*, but it seems to be fairly tolerant to desiccation (MacNair 2002; Kim and Garbary 2007). Air drying for 17 h at 20°C (72–75% RH) was somewhat effective at killing the alga, where thalli lost 20% of their mass, but it still showed high levels of photosynthetic activity (Kim and Garbary 2007). The same authors showed that 5 h of air drying under the same conditions was not enough to kill *C. fragile*. A 24-h air drying period showed almost 100% mortality of *C. fragile* by fragmenting the plants, but some fragments regrew into healthy plants after months (MacNair 2002). Therefore, this latter treatment was considered ineffective at mitigating *C. fragile*.

The seaweed *U. pinnatifida* showed complete gametophyte mortality (100%) after 3 d and 12 h of air drying at 10°C and 20°C, respectively, under ambient humidity (55–85% RH; Forrest and Blakemore 2006). At high humidity (>95% RH), 6 weeks at 20°C were required to be 100% effective (Forrest and Blakemore 2006). At 10°C with high humidity (>95%), air drying was not effective, live gametophytes still being present after 8 weeks (Forrest and Blakemore 2006). As

for polychaetes, the serpulid tube worm *Hydroides elegans* mortality was 92–96.2% after 3–6 h of air drying outdoors under cloudy conditions and high RH (not specified) (Arakawa 1980). Longer durations of 1–2 d were shown to be effective at killing the worm with sun exposure and low RH (not specified) conditions (Arakawa 1980). In contrast, Forrest et al. (2007) demonstrated that 24 h of air drying in laboratory conditions (room temperature, high RH) was not effective at killing *H. elegans*. The serpulid polychaete worm (*Spirobranchus paumotanus*) had 90–100% mortality after 12 and 24 h under laboratory conditions but 24 h under field conditions were not effective (7% mortality) (Asgari and Jahangard 2012). Air drying was effective on the spionid polychaete *Polydora ciliata* in the field after 7–10 d in a shaded area (Nell 2007) and 100% effective after 14 d in cold storage (3°C) on the mud blister worm (*P. websteri*) (Brown 2012). Air drying for 24 h (room temperature, high RH) was not effective at eliminating Terebellidea worms, *B. neritina*, and *W. subtorquata (*Forrest et al. 2007). Carver et al. (2010) revealed that air exposure for 18 h (~25°C) was not an effective treatment at killing the yellow boring sponge (*Cliona celata*). No information was available for the effects of air drying on large *C. virginica*, gastropods, *C. mutica*, sea stars, or hydrozoans.

3.1.1.3. Freshwater immersion or spray (with and without air drying)

Freshwater immersion times required to induce 100% mortality varied across AIS and ranged from 3 h to more than 24 h. Based on qualitative results from Carman et al. (2010) and Davidson et al. (2005), a 5-min freshwater spray (low pressure) applied directly to oysters or aquaculture gear was the minimum time deemed effective to kill colonial (*B. schlosseri*, *B. violaceus*, *D. vexillum*, and *D. listerianum*) and solitary (*C. intestinalis*, *S. clava,* and *A. aspersa*) tunicates. However, when treating mussel seed in mesh bags in a laboratory setting, Ramsay (2015a) found that a 12 h of freshwater immersion (in a continuous flow-through system in tanks) was required to be effective at killing the colonial tunicates *B. violaceus* and *B. schlosseri*. Nearly 100% mortality resulted when these invasive species (removed from mussels) were exposed to a minimum of 6 h of freshwater immersion in laboratory-type experiments (Ramsay 2015a). Total mortality (100%) for *B. schlosseri* and *B. violaceus* was observed after a 24-h freshwater immersion of mussel socks in a continuous flow-through system in tanks at an industrial scale (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. 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, with all colonies of both species held at 5 ppt suffering 100% mortality after 24 h. Carman et al. (2016) showed that freshwater immersion (8 h) and spray (10 min) followed by 1 h of air drying were both 100% effective at killing colonial tunicates [*B. schlosseri* (larval recruits for spray + air drying), *B. violaceus*, *D. vexillum*, and *D. listerianum*] present on mussel socks. In field conditions, a 10-min freshwater exposure was found to be ineffective at killing *D. vexillum* and, in fact, the species showed an increase in fouling coverage over time, 5 weeks post-treatment (Rolheiser et al. 2012). In contrast, experiments at an aquarium scale revealed that a 4-h freshwater immersion was 100% effective at killing this species (McCann et al. 2013). 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: trials revealed 74% mortality with a 2-min dip, 84% after 5 min, and 87% after 10 min.

Ramsay (2015b) suggested that a minimum 3-h immersion in freshwater (small-scale experiment) was sufficient to cause almost 100% mortality of adult *C. intestinalis*. Preliminary results from additional trials conducted by the same laboratory in 2020 suggested that a 6-h immersion under controlled conditions was effective, but both 3- and 6-h immersions under field conditions were not effective at killing the solitary tunicate attached to mussel socks [Ramsay, Prince Edward Island (PEI) Department of Fisheries and Communities (DFC), unpubl. data).

However, 12-h freshwater immersions conducted in the field in 2020 resulted in almost 100% mortality of *C. intestinalis* on mussel socks (Ramsay, PEI DFC, unpubl. data). Ramsay (2015b) presumed that earlier life stages of *C. intestinalis* would be more vulnerable to freshwater immersion and may require shorter durations to induce 100% mortality. Bourque et al. (DFO, unpubl. data) examined the impacts of reduced salinities on *C. intestinalis* eggs and larvae over different exposure times and observed almost no metamorphosed or moving larvae following an immersion time of 1 h in freshwater (98% mortality). Carver et al. (2003) demonstrated that a 1-min immersion was ineffective (10% mortality) at eliminating all stages of *C. intestinalis* from culture equipment. While freshwater immersion for 3 h (field trial) was sufficient to cause 100% mortality of both juvenile and adult *S. clava* (Ramsay 2015c), only 15 s in laboratory conditions were required for juveniles (Davidson et al. 2005). During preliminary field trials, Coutts and Forrest (2005) showed that mortality of *S. clava* occurred within 24 h of freshwater immersion and, hence, at least 1 d of immersion was suggested for effective mitigation. They also specified that increasing salinities could, however, enhance *S. clava* survival (Coutts and Forrest 2005). Minchin and Duggan (1988) showed that a 1 h freshwater immersion was not effective for *S. clava* under laboratory conditions (0% mortality).

Freshwater treatments were less effective at killing shellfish (here considered as AIS) compared to tunicates (Ramsay 2015a; Carman et al. 2016; Rolheiser et al. 2012). Freshwater immersions for 12 and 24–48 h, and immersions or sprays (8 h or less) followed by 1 h of air drying, were ineffective (0−10% mortality) at killing small *M. edulis* under field or laboratory conditions (Ramsay 2015a; Carman et al. 2016; Landry et al., DFO, unpubl. data). Small *Mytilus* sp. were not killed (c.a. 0% mortality in a laboratory setting) even after 5 d of immersion in freshwater (Forrest and Blakemore 2006). Similarly, 30-min and 48-h freshwater immersions were ineffective at killing large *M. galloprovincialis* (0% mortality; Asgari and Jahangard 2012) and small *C. virginica* (0−4% mortality; Landry et al., DFO, unpubl. data) in laboratory conditions, respectively. Denny (2008) showed that a freshwater immersion of 10 min followed by 24 h of air drying was ineffective (1–2% mortality) at killing large *M. galloprovincialis* in field conditions. A laboratory study by Brown (2012) showed that a freshwater immersion of 72 h was not effective on *C. virginica* (size not specified, but assumed to be large), even if followed by a period of air drying for 8 d in cold storage (3°C). A 10-min freshwater immersion was not effective (20% mortality) at killing large *C. gigas* under field conditions (Rolheiser et al. 2012). Nel et al. (1996) observed that 12-h freshwater immersions only achieved 11.5 and 4.2% mortality of small *C. gigas* in field and laboratory conditions, respectively. Furthermore, Nell (2007) showed that a 12-h freshwater immersion in the field was not effective on *C. gigas* (size not specified but assumed to be large).

Freshwater immersion for 1 h was not effective on adult of *C. maenas* and, moreover, they have been found in freshwater streams in Newfoundland (Canada), demonstrating their tolerance to freshwater (McKenzie et al., DFO, unpubl. data) for at least short periods of time in laboratory conditions. Prolonged periods of freshwater influx were deemed to be ineffective to kill the Asian shore crab (*Hemigrapsus sanguineus*), with 65% survival after a 2-week immersion at 1 ppt (Hudson et al. 2018; results not presented in Table 5). According to Ashton et al. (2007), 100% mortality of *C. mutica* occurred following a 48-h immersion in water at salinities ≤15 ppt, while Takeuchi et al. (2003) achieved 100% mortality after 24 h in freshwater (0–8 ppt). With the same exposure time (24 h), Takeuchi et al. (2003) demonstrated that increasing salinity decreased treatment effectiveness for *C. mutica*, with immersions at 13–16 ppt resulting in 10–64% mortality and no mortality for salinities >21 ppt.

Even though the duration of freshwater treatment varied among algal species, the treatment time needed to effectively cause algal mortality was typically longer (several hours to days) than for other taxonomic groups. Thalli of *C. fragile* 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). However, the same authors showed that an immersion for 14 h was effective at killing *C. fragile*. Other data showed that a freshwater immersion of more than 24 h was required to kill *C. fragile* (Landry et al., DFO, unpubl. data). Gametophytes of *U. pinnatifida* were killed within 22 h (at 20°C) or 43 h (at 10°C) of immersion in freshwater, while a 10 min immersion was sufficient to kill all plantlets at both temperatures (Forrest and Blakemore 2006).

Freshwater treatment effectiveness for polychaete worms varied across species and within species of the same genus. Arakawa (1980) showed that 64.9% mortality resulted when the tube worm *H. elegans* was exposed to a 2-h freshwater immersion. Asgari and Jahangard (2012) showed that only 40% mortality was achieved with *S. paumotanus* with a 30-min immersion. For mud worms, under controlled laboratory conditions, 100% mortality was achieved with 6-h immersion for *P. ciliata* (Velayudhan 1983) but 6–12 h only killed up to 25.9% of *Polydora hoplura* (Nel et al. 1996). For oyster farming management in the Canadian Maritimes, Medcof (1961) observed that a 12–16-h freshwater immersion was effective to kill *P. websteri*. Under various salinity tolerance experiments led by Brown (2012), 25−60% mortality of *P. websteri* was observed in one experiment when burrowed worms inside oyster shells were immersed in freshwater for 72 h. To reach 100% mortality, *P. websteri* needed to be removed from burrows and immersed directly in freshwater. Experiments in a laboratory by Ruellet (2004) showed that the mud worm *Boccardia polybranchia* had 100% mortality after 15 min when directly immersed in freshwater, but a 8.5-h immersion was not effective when the worms were burrowed in oyster shells. In a different experiment, Brown (2012) observed 100% mortality when a 72-h immersion was followed by a period of air drying (in cold storage) for at least 8 d, but with a shorter drying period of 4 d they observed ~95% mortality (Brown 2012). Field experiments showed that a 2-d freshwater immersion was effective at killing *P. websteri* (Nell 2007), whereas only 12 h was required to effectively kill *P. hoplura* (Nel et al. 1996; Nell 2007). For worms of the family Sabellidae, 100% mortality was achieved with a 20-min (Jute and Dunphy 2017) or a 4-h (Moore et al. 2007) freshwater immersion for adults and 64 s for juveniles (Moore et al. 2007). Only one research paper was found in the literature for *Cliona* boring sponges (Medcof 1961), and it showed that a 12–16-h freshwater immersion was effective at killing these species. Freshwater immersions (30 s) were not effective (0% mortality) for tubular hydroid *Ectopleura crocea* (Fitridge et al. 2012). No freshwater immersion or spray treatment information was found for *Molgula* spp., large *M. edulis*, small *M. galloprovincialis*, gastropods, barnacles, sea stars, or bryozoans.

3.1.1.4. Hot freshwater immersion

Few studies have focused on heated freshwater immersions in comparison to heated seawater immersions. Carver et al. (2003) showed that a 1-min immersion in freshwater at 40°C was partially effective (66% mortality) at eliminating *C. intestinalis* under laboratory conditions. Forrest and Blakemore (2006) showed that a freshwater immersion treatment for only 5 s at high temperature (55°C) was ineffective on small *M. edulis*.

Landry et al. (DFO, unpubl. data) showed that hot freshwater immersions were not effective at killing small *C. virginica*. When *C. virginica* were acclimated at 10°C prior to being exposed to a heated (30°C) freshwater treatment for 10 min, 11% mortality was achieved, compared to 0% mortality when the organisms were pre-acclimated to 4°C (differences not significant). A shorter freshwater exposure time (5 min) at higher temperature (40°C) was also not effective (0% mortality) (Landry et al., DFO, unpubl. data). As for Sabellidae polychaetes, the treatment was 100% effective at 29.5°C, 33°C, and 36°C for 24-h, 3-h, and 30-min exposure times, respectively (Leighton 1998). No hot freshwater immersion treatment information was found for other targeted AIS.

3.1.1.5 Hot seawater immersion

Literature pertaining to heated seawater immersion treatments were more numerous than those for heated freshwater treatments and covered a wider range of species. Piola and Hopkins (2012) found that heated seawater immersion treatments (37.5°C, 60 min; 40°C, 30 min; 42.5°C, 20 min) were 100% effective at killing the tunicates *Botrylloides leachii*, *D. vexillum*, and *C. intestinalis*. Gill et al. (2007) found that an immersion in seawater for a few seconds at 60°C was not effective at killing *C. intestinalis*. Another study reported that immersion in seawater at 40°C for 10 s induced 66% mortality, but exposures at 40°C for 60 s and at 50 or 60°C for 10 s caused 100% mortality of *C. intestinalis* (Sievers et al. 2019). In the same study, low mortality (~12–25%) was recorded for *S. clava* for all 40°C seawater treatments. As time of heated seawater immersion increased, so did mortality of *S. clava*, with 50°C causing 40–50, 60–70, and 86% mortality after 10, 30, and 60 s, respectively (Sievers et al. 2019). Similarly, a heated seawater exposure at 60°C resulted in 86% mortality after 10 s (Sievers et al. 2019), but 100% mortality after 15 s (Minchin and Duggan 1988) and 30 s (Sievers et al. 2019). Immersion in 70°C seawater for 10 s was also 100% effective on *S. clava* (Minchin and Duggan 1988). Davidson et al. (2005) found that a shorter, 4-s seawater immersion at higher temperatures (80–90°C) caused 100% mortality of *S. clava*.

Heated seawater immersion experiments were conducted on various sizes of mussels (*M. edulis* and *M. galloprovincialis*) and oysters (*C. virginica* and *C. gigas*). Best et al. (2014) found that an immersion in seawater at 55°C for 1 min was not effective at killing large nor small *M. edulis*. McDonald (2010) showed that 20 s was needed to reach 100% mortality of large *M. edulis* immersed in seawater at 60°C, while an immersion for 5 s resulted in only ~35% mortality. Koganezawa (1972), cited in Arakawa (1980), showed that the effectiveness of heated seawater treatment was affected by mussel size in the field. They demonstrated that small *M. edulis* (size 40−50 mm) were not killed (0% mortality) following seawater immersions at 50°C for 5 s, 55°C for 20 s, and 60°C for 1 s. In contrast, heated seawater at 50°C for 30 s, 50°C for 15 s, and 60°C for 5 s were 100% effective treatments for small *M. edulis* (size 10–20 mm), while 60 s at 60°C was required for another group of small *M. edulis* (size 40–50 mm). A 60°C seawater immersion for 15–30 s was only partially effective (20–60%) at killing small *M. edulis* (40–50 mm), and 50°C for 15−20 s was also partially effective (10–30%) at killing small *M. edulis* (10–20 mm) (Koganezawa 1972). Under controlled experiments, McDonald (2010) showed 100% mortality of small *M. edulis* at 60°C after 15 s, but only ~40% mortality when the immersion time was reduced to 5 s. In another study, Leach (2011) showed that heated immersion at 60°C for 15 min was 100% effective on small *M. edulis*, whereas an immersion at 40°C for 30 min resulted in approximately 40% mortality.

Gonzalez and Yevich (1976) showed that, following an acclimation period (temperature raised at a rate of ~1°C/d from 2.5 to 25°C), small *M. edulis* mussels exposed to heated seawater showed no mortality (0%) at 26°C after 24 h. This study also demonstrated that a 28°C post-acclimation exposure resulted in 100, 80, and 50% mortality of *M. edulis* after 6, 4, and 3 d, respectively, and only 6% mortality at 27°C after 48 h. Under field conditions, the entire mussel population present in an effluent canal died after 3 d when temperatures were between 28 and 30°C (Gonzalez and Yevich 1976). Rajagopal et al. (2005a), through acclimation laboratory experiments, demonstrated that brackish water (19–20 ppt) at a temperature of 36°C for 84 min and 41°C for 1 min induced 100% mortality of small *M. edulis*. In addition, a laboratory study found that a temperature of 30°C for 10 min was ineffective (0% mortality) at killing small *M. edulis* (Landry et al., DFO, unpubl. data). However, Landry et al. (DFO, unpubl. data) measured 87% mortality (pre-acclimated to 10°C) and 33% mortality (pre-acclimated to 4°C) of small *M. edulis* at 40°C for 5 min, whereas 32.6°C (6 h) resulted in 76% mortality of mussel spat (Leblanc

et al. 2005). Higher seawater temperatures, 60–80°C, were found to be effective at killing small *M. edulis* after only 4 s (Davidson et al. 2005).

Results for large *M. galloprovincialis* differed from those of *M. edulis* for heated seawater immersion. Asgari and Jahangard (2012) showed that trials in field conditions with heated seawater were not entirely successful at killing large *M. galloprovincialis*, where 54–58% mortality was achieved with temperatures of 60–65°C after 30 s and 0–3% mortality with temperatures of 45–51°C for 40–45 s. Laboratory experiments conducted by the same authors at 45–48°C (80 s) or 51–53°C (55–70 s) achieved no more than 13% or 0% mortality of *M. galloprovincialis*, respectively (Asgari and Jahangard, 2012). According to Sievers et al. (2019), large *M. galloprovincialis* incurred 100% mortality after being immersed at 50°C for 60 s, while immersions at 50°C for 30 s or 60°C for 10 s were sufficient to kill all small mussels (100% mortality). Heated immersions were not effective (0% mortality) with both decreasing exposure times and temperatures at killing large (40°C after 60 s or 50–60°C after 10 s) and small (40°C after 60 s) *M. galloprovincialis* (Sievers et al. 2019). In addition, the same authors showed that heated immersions at 50 and 60°C were 40% effective at killing large mussels when the duration was increased to 30 s, while a treatment at 50°C for 10 s was also not effective on small mussels (25% mortality). For a longer exposure duration (5 min), small and large *M. galloprovincialis* were killed (100% mortality) at 50°C, while only 1 and 5% mortality were measured for large and small mussels, respectively, at 35°C (Piola and Hopkins 2012).

High mortality (~95%) was seen with small *C. virginica* exposed to 60°C seawater for 30 s under controlled conditions (McDonald 2010). However, results from other trials found that heated seawater immersions of 60°C for 5–15 s (small oysters) and 30 s (large oysters) were not effective (a maximum of 10% mortality) (McDonald 2010). In another study, heated seawater immersions (60°C for 15 s) tested on two size groups (35–45 mm and 55−65 mm) of oysters (*C. gigas*), which we classified into the ‘small’ category, differed in effectiveness: 50% mortality (35–45 mm) and less than 5% mortality (55–65 mm) (Mayrand et al. 2015). Depending on season and size, the effectiveness of heated seawater on small *C. virginica* varied from 30 to 50% (35–45 mm) and from 40 to 90% (55−65 mm) at 60°C after 15 s (Rousselle 2012). A comprehensive study by Piola and Hopkins (2012) showed that *C. gigas* spat (classified as ‘small’) were more sensitive to heated seawater than were juveniles (also classified as ‘small’) and adults (large), with 23.3% mortality (37.5°C, 60 min), 50% mortality (40°C, 30 min), and 86.7% mortality (42.5°C, 20 min) for oyster spat, no mortality (0%) for large oysters for the three temperature/duration treatments, and only 2% mortality for juvenile oysters at 42.5°C for 20 min.

A 100% mortality was achieved on three small *C. gigas* size groups (11, 35, and 54 mm) for an immersion at 43°C for 60 min (Rajagopal et al. 2005b) under laboratory conditions. The authors also observed that two size groups of small *C. gigas* required significantly different times of immersion at 40°C to achieve 100% mortality, where the 11-mm and 54-mm groups required 96 and 167 min, respectively (Rajagopal et al. 2005b). In field conditions, immersions in seawater heated to 60°C only induced 60% and 8–20% mortality of small *C. gigas* after 60 s and 15–30 s, respectively, while a temperature of 50°C for 60 s achieved 0% mortality (Koganezawa 1972). Furthermore, Nel et al. (1996) showed that the effectiveness of short immersions (30–45 s) in 70°C seawater on small *C. gigas* ranged from 0 to 11.2% mortality in field or laboratory conditions.

Juvenile *C. maenas* immersed in 45 to 55°C seawater for 1 min or 55°C for 5 s suffered 100% mortality, while 40°C for 1 min or 50°C for 5 s were ineffective or only partially effective at causing mortality of this species (Best et al. 2014). Furthermore, McKenzie et al. (DFO, unpubl. data) observed that an immersion of 1 h in 32−45°C heated seawater was effective on adult *C. maenas*. Another crustacean, *C. mutica*, faced 100% mortality when immersed in seawater at 30°C for 48 h (Ashton et al. 2007). In another study, hot seawater immersion (60°C for 25 s)

was ~99% effective at killing *Balanus* sp. (McDonald 2010), but completely effective (100% mortality) for Balanidae species held at 40°C for 30 min (Leach 2011).

Heated seawater immersion at 50°C for 30 s was 100% effective at killing *C. fragile* (Landry et al., DFO unpubl. data), while a lower temperature of 40°C for a shorter time exposure (10 s) was sufficient (100% mortality) at killing the hydrozoan *E. crocea* (Sievers et al. 2019). An immersion for 3 s in 80 to 85°C seawater was effective at killing most macroalgae (45 out of 46 taxa) introduced via oyster transfers, leaving only tubular green algae *Ulva* spp. present on some oyster shells (Mineur et al. 2007). Hot seawater exposure times resulting in complete mortality (100%) of *U. pinnatifida* were 10 min (35°C), 45 s (45°C), and 5 s (55°C) (Forrest and Blakemore 2006). Williams and Schroeder (2004) found that seawater immersion at 72°C for 1 h was effective to kill the green alga *Caulerpa taxifolia* (not yet detected in Canada).

Heated seawater immersion (dip) at >25°C was effective at killing adult *A. rubens* sea star but not juveniles (Medcof 1961). However, field experiments showed that immersion in a warmer temperature of 40°C for 60 s was not effective at killing *A. amurensis* (proxy for *A. rubens*) (Fitridge et al. 2012). One study provided results on heated seawater treatments for *S. paumotanus* on *M. galloprovincialis* shells (Asgari and Jahangard 2012). Based on results of a range of temperatures and exposure times tested in field and laboratory conditions, the authors ascertained that decreasing temperatures and exposure times reduced treatment effectiveness on the worm. Under laboratory conditions, the highest mortality (99%) was achieved after a seawater immersion at 53°C for 60 s and the lowest mortality (~60%) at 45°C for 40 s. Under field conditions, the highest mortalities achieved ranged from 92% (51°C for 45 s, offshore in a closed, circulated seawater-heating system) to 98.4% (56°C for 30 s, onshore; Asgari and Jahangard 2012). A laboratory study on the mud worm *P. hoplura* demonstrated that seawater immersion at 70°C for 30–45 s was ineffective (30.3 to 39.2% mortality) at killing this species (Nel et al. 1996). Bryozoans immersed in heated seawater (37.5°C for 60 min, 40°C for 30 min, or 42.5°C for 20 min) were eliminated (100% mortality) (Piola and Hopkins 2012). Leach (2011) showed that a hot seawater immersion (40°C, 30 min) was 100% effective for unspecified polychaete species, bryozoans, and sponges. No information was available on hot seawater immersion treatments for *B. schlosseri*, *D. listerianum*, *A. aspersa*, *Molgula* spp., or gastropods.

3.1.1.6. Steam

Few studies (2) on the effects of steam on AIS were found (Davidson et al. 2005; Joyce et al. 2019). Davidson et al. (2005) found that a steam treatment (100°C, 50 psi) for 30 s resulted in 100% mortality of *S. clava*, but it was not effective on small mussels *(M. edulis).* Joyce et al. (2019) examined the effectiveness of steam exposure (100°C, 50 psi) to induce mortality of selected biofouling species [*M. edulis, C. gigas*, *Semibalanus balanoides*, rockweed (*Fucus vesiculosus*), and *Ulva* sp.]. They observed total mortality (100%) of small *M. edulis* and *C. gigas* (60 s), large *C. gigas* (300 s), and *S. balanoides* (30 s). The application of steam for 60 s also reduced the biomass of *F. vesiculosus* and *Ulva* sp., with complete degradation (100% mortality) observed for the latter following 120 s of exposure. No information was available on the effects of steam treatment for colonial tunicates (*B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. listerianum*), *C. intestinalis*, *A. aspersa*, *Molgula* spp., large *M. edulis*, small and large *M. galloprovincialis*, small and large *C. virginica*, gastropods, *C. maenas*, *C. mutica*, *C. fragile*, sea stars, polychaetes, bryozoans, sponges, or hydrozoans.

3.1.2. Effectiveness of chemical treatments

A variety of chemical treatments were considered from 51 literature sources for the control of marine AIS and included 32 primary publications and 19 technical reports. Treatments included immersion and/or spray of chlorine-based compounds (16), acetic acid (24), citric acid (3), brine

solutions (32), brine and lime solutions (2), hydrated lime (20), and Virkon® (2), which were occasionally followed by an air-exposure period. An overview of these treatments is presented in Tables 6 and 7 and is summarized below. A few unpublished (6) results provided by subject matter experts were considered for some treatments and are identified as 'unpublished data' and identified by two superscript asterisks (**) in Tables 6 and 7.

3.1.2.1. Chlorine-based compound immersion

Results on the effectiveness of chlorination treatments were found in the literature (Rajagopal et al. 2002, 2003; Carver et al. 2003; Ruellet 2004; Williams and Schroeder 2004; Coutts and Forrest 2005; MacNair et al. 2006; Denny 2008; Piola et al. 2009; Asgari and Jahangard 2012; McCann et al. 2013; Haque et al. 2014, 2015; Roche et al. 2015; Haque and Kwon 2017), but studies tested different chlorine compounds (i.e., sodium hypochlorite, TRC, chlorine dioxide) measured in non-comparable units (% and mg/L). During laboratory experiments, an immersion in 0.006% sodium hypochlorite for 20 min was not effective (0% mortality) to kill *C. intestinalis* (Carver et al. 2003). A pilot study (qualitative observations in the field) by Piola et al. (2009) suggested that a 1% sodium hypochlorite spray for 5 s could effectively kill *C. intestinalis* and *B. leachii* when the treated tunicates were left for an additional 30-min air-drying exposure period before being rinsed with seawater. In the same study, lower concentration sprays (0.5% sodium hypochlorite) followed by 6 h of air drying were effective for *B. schlosseri*, but not when followed by 3 h of air drying, suggesting that air exposure time was critical. However, 0.5% sodium hypochlorite sprays were ineffective for *B. leachii* and *C. intestinalis*, even when followed by a 12-h air-drying exposure period (Piola et al. 2009). Under laboratory conditions, immersion in 0.3 or 0.6% sodium hypochlorite solutions resulted in 100% mortality of *B. violaceus* in just 15 s (MacNair et al. 2006) without air drying. In field trials, 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 the solitary tunicate *S. clava* (Coutts and Forrest 2005). Laboratory experiments showed that *D. vexillum* experienced 100% mortality after being immersed in a 1% sodium hypochlorite solution for 10 min (McCann et al. 2013) and in a 1% sodium hypochlorite solution for only 30 s (Denny 2008). In contrast, McCann et al. (2013) showed that 2 min of immersion in 1% was only 50% effective on *D. vexillum*. Laboratory tests by Roche et al. (2015) reported that 5-, 15-, and 30-min immersions in 1% sodium hypochlorite significantly reduced the biomass of *D. vexillum* by 50, 65, and 55%, respectively. The same authors observed that mortality had occurred for the remaining biomass (discoloration, unhealthy look). On seed mussels fouled with *D. vexillum*, laboratory immersion in lower concentrations (0.1% sodium hypochlorite) for 2 min achieved at least 90% mortality (Denny 2008). Furthermore, 2-min immersions in 0.25% or 0.5% (for a minimum of 20 s) sodium hypochlorite solutions eradicated 100% of *D. vexillum* (Denny 2008). The same study also demonstrated that an immersion in 0.25% sodium hypochlorite solution for 2 min, followed by 5 h of air drying, killed 100% of *D. vexillum*.

Rajagopal et al. (2002, 2003) found that immersions in very low concentrations of TRC were 100% effective on small *M. edulis* in continuous chlorination systems but required very long exposure times (17 d at 3 mg/L and 40 d at 1 mg/L, respectively). However, at a higher concentration (4 mg/L TRC), all small *M. edulis* were killed (100% mortality) after immersions of 6.3 d (25 mm) or 7 h (1.4 mm) (Haque et al. 2015). Similarly, Haque and Kwon (2017) showed that the required times for 100% mortality of two small-size groups, 14 and 25 mm, of *M. edulis* in 4 mg/L TRC were 124 h (5.2 d) and 150 h (6.3 d), respectively. Veliger larvae of *M. edulis* were easier to kill, such that immersions in 1 mg/L TRC (20 min), 0.1 mg/L (4 h), and 0.05 mg/L (5 h) caused 100% mortality (Haque et al. 2014). However, concentrations of 0.7 mg/L TRC were not effective (only 16% mortality) on larvae after 10 min of immersion (Haque et al. 2014; Haque and Kwon 2017). Less information was available on the effectiveness of chlorination for

M. galloprovincialis compared to *M. edulis*. A higher initial concentration of 0.5% sodium hypochlorite for 30 s to 2 min (followed or not by a 24-h air-drying period) on small *P. canaliculus* (proxy for *M. galloprovincialis*) was not effective, inducing just over 6% mortality (Denny 2008). Asgari and Jahangard (2012) tested chlorination with chlorine dioxide (not TRC concentrations) and found that an immersion for 9 min, at concentrations ranging from 0.14 to 0.28%, was not effective (only 3% mortality) on large *M. galloprovincialis*. In the context of a field study where sodium hypochlorite was pumped and contained within wraps enclosing wharf pilings, a 0.05% sodium hypochlorite immersion was not effective for *C. gigas* (size not specified, but assumed to be large) even following 12 h of exposure (Coutts and Forrest 2005).

A 1-h sodium hypochlorite (5%) immersion was not effective to induce death of *C. maenas*, according to trials executed by McKenzie et al. (DFO, unpubl. data). Sodium hypochlorite was shown to be 100% effective (7-d post treatment) at a concentration of 0.25% on the invasive green seaweed *Caulerpa taxifolia* after 60 min of immersion (Williams and Schroeder 2004). Field trials lead by Ruellet (2004) showed that a 0.5% sodium hypochlorite immersion of 5 min was not effective at killing *Polydora* sp. on oyster shells, even if the shells were cleaned of mud prior to the immersion. Asgari and Jahangard (2012) tested the effectiveness of chlorine dioxide on *S. paumotanus* at a concentration of 0.28% for an immersion of 9 min and had no success (0% mortality) killing the worms.

Finally, no information was found on the use of chlorination treatments for *D. listerianum*, *A. aspersa*, *Molgula* spp., large *M. edulis*, small and large *C. virginica*, small *C. gigas*, gastropods, *C. mutica*, barnacles, sea stars, *C. fragile*, bryozoans, sponges, or hydrozoans.

3.1.2.2. Acetic acid immersion or spray (with and without air drying)

Immersion in 4% acetic acid for 1 min in laboratory tests was effective at killing the colonial tunicates *B. schlosseri* and *B. leachii*, while immersion at a lower concentration (2%) for 4 min was not effective (Forrest et al. 2007). 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 being immersed for 2 min in 10% acetic acid (McCann et al. 2013), whereas a study by Roche et al. (2015) suggested that a 5 min immersion at half that concentration (5%) could induce 65% mortality. However, Roche et al. (2015) demonstrated conflicting results in the same study, where *D. vexillum* exhibited only 45 to 50% mortality after longer exposure times (15 and 30 min at the same 5% concentration). In the field, tunicate fouled mussel seed immersed in 4% acetic acid for 10 min resulted in approximately 95% mortality of *D. vexillum*, but laboratory experiments showed that 1–3 min at the same concentration resulted in 80–85% mortality of the tunicate (Denny 2008). In the field, Rolheiser et al. (2012) showed that immersion in 5% and 0.25% acetic acid for only 30 s was effective at inducing mortality of *D. vexillum*. Results from several field trials by Denny (2008) showed that lower concentrations (2%) and their associated exposure times (1–10 min) were less effective (~45–82%) at causing *D. vexillum* mortality.

Under controlled conditions, a 1-min immersion in 4% acetic acid followed by a period of 24-h air-drying exposure (in temperature-controlled cabinets to simulate inter-regional transport) was effective at killing *B. schlosseri*, *B. leachii,* and the solitary tunicate *C. intestinalis* (Forrest et al. 2007). A longer immersion (5 min) in 5% acetic acid, along with an air-drying exposure of 1 h, was required in laboratory experiments to reach 100% mortality for adult stages of *B. schlosseri*, *B. violaceus*, *D. vexillum*, and juvenile stages of *D. listerianum*, *C. intestinalis*, and *A. aspersa* (Carman et al. 2016). Field trials by Denny (2008) showed that adding a period of air drying (ranging from 1 to 41 h) did not improve the effectiveness (mean of 77% mortality) of a 2% acetic acid treatment on *D. vexillum*, compared to results for 2% acetic acid immersions only (45–82% mortality).

Forrest et al. (2007) tested the effect of rinsing tunicates with seawater between an acetic acid immersion and a drying period. They found that rinsing between the two periods influenced the effectiveness of the treatment, depending on the species, concentration, and the duration of immersions. Indeed, 1-min immersions in 2% acetic acid followed by 24 h of air drying were not effective on *B. schlosseri* and *B. leachii* when the tunicates were rinsed between the two steps. Without the rinsing step, the same treatment (2%, 1 min) was shown to be effective on those tunicate species, as well as *C. intestinalis*. When the duration of the immersion was 3 min instead of 1 min, the treatment was effective on both species whether or not a rinsing step was added before the air-drying period of 24 h. Forrest et al. (2007) also observed that rinsing did not affect the effectiveness of a 1-min immersion in 2% acetic acid, followed by a drying period of 24 h, on *C. intestinalis*. The same authors also tested the effect of reversing the steps of air drying and immersion, finding that 24 h of air drying followed by a 1-min immersion in 4% acetic acid was effective on many AIS, including *B. schlosseri, B. leachii, C. intestinalis, Cladophora* sp., Terebellidae worms*, B. neretina, and W. subtorquata* (proxy for *C. pallasiana*). With a concentration of 2%, the same reversed treatment was effective on Terebellidae immersed for 1 min, but *B. neretina* required an immersion of 3 min to be effective (Forrest et al. 2007).

Spray applications of acetic acid were less effective than immersions for controlling fouling on mussel socks. MacNair et al. (2006) indicated that 1 min (2 passes of 30 s each pass) of a 5% acetic acid spray applied with a commercial sprayer with multiple nozzles resulted in 90% *B. violaceus* mortality and removed most other fouling organisms. Only 81% mortality was seen in *D. vexillum* with a 3-s spray of 4% acetic acid followed by a 1-h air-drying exposure (Denny 2008). Based on controlled field acetic acid trials, a 5% spray (5 s) on tunicate fouled plates followed by a 30-min air-exposure period (in shade) was 65% effective at killing *B. schlosseri*, *B. leachii*, and *C. intestinalis* and a 10-min period was ~95% effective at killing *D. vexillum* (Piola et al. 2009). Piola et al. (2009) also showed that a 30-min air-exposure period (in shade) was totally effective (100% mortality) at killing *D. vexillum*. Additionally, those authors tested the effect of higher concentrations of acetic acid and found that a treatment of 10% acetic acid for 5 s, followed by 30 min of air-drying exposure (in shade), was 95% effective at removing *B. schlosseri* and *B. leachii*.

The vase tunicate *C. intestinalis* appears to be more sensitive to acetic acid than colonial tunicates. In laboratory experiments, 5% acetic acid immersions for 5–10 s were deemed to be not effective for the species (Carver et al. 2003), whereas immersions for 10 s, 1 min, and 4 min (at the same concentration), were indeed effective (Carver et al. 2003; Forrest et al. 2007; Sievers et al. 2019). In the field, Gill et al. (2007) observed 99–100% mortality of *C. intestinalis* when immersing them in 5% acetic acid for 15 s. In contrast, immersions in 5% acetic acid for 30 s in the laboratory (Carver et al. 2003) and 5–10 s in the field (Locke et al. 2009) were only 95% and 70–95% effective at killing *C. intestinalis*, respectively. According to Sievers et al. (2019), a 30-s immersion with lower concentrations of acetic acid (2%) induced 66% mortality of *C. intestinalis*, although a 4-min immersion at the same concentration was not effective (Forrest et al. 2007). In other treatment applications, a 5% acetic acid spray was substantially less (10–20% mortality) or not effective on *C. intestinalis* compared to immersion (Forrest et al. 2007; Gill et al. 2007, 2008; Sievers et al. 2019). However, in field conditions, when spraying oyster bags with 5% acetic acid for 30 s and leaving them exposed for 30 s before returning to the water, Carver et al. (2003) measured a large range of effectiveness (60 to 100% mortality) for *C. intestinalis*, depending largely on the density of the tunicate settlement (Carver et al. 2003).

As for *C. intestinalis*, the clubbed tunicate, *S. clava*, showed variable responses to acetic acid immersions. In the field, using wrapped wharf piles, Coutts and Forrest (2005) indicated that a 1% acetic acid immersion for 1 min was not effective at killing *S. clava*, but that 10 min was 100% effective at that concentration. The authors showed that 2% and 5% acetic acid

immersions of 5 min and 1 min, respectively, were 100% effective at killing *S. clava*. Davidson et al. (2005) showed that only 15 s of immersion in 5% acetic acid was enough to kill 99–100% of *S. clava* on mussel socks in the field. In contrast, 2 and 5% acetic acid immersions for 60 s in laboratory tests were respectively 0% and only ~50% effective on *S. clava* (Sievers et al. 2019), whereas a 5% acetic acid spray on mussel socks in the field was weakly (5–60%) or not effective at killing *S. clava* (Davidson et al. 2005; Gill et al. 2008). Only Sievers et al. (2019) tested the effectiveness of heated acetic acid in the laboratory on several AIS attached to commercial shellfish, including the solitary tunicates *C. intestinalis* and *S. clava*. They showed that a heated 2% acetic acid immersion at 40°C or 50°C achieved 100% mortality of *C. intestinalis* after 10 s, but *S. clava* required a duration of 60 s at both temperatures to reach 100% mortality. If they increased the acetic acid concentration to 5%, temperatures of 40–50°C and duration of 10 s were equally effective (100%) at killing *C. intestinalis*. A 2% heated acetic acid immersion at 40°C for 30 s was not effective at killing *S. clava* (54% mortality), but 5% heated acetic acid at 40°C for 60 s killed nearly 100% of the tunicate.

Short (15–30 s) immersion or spray treatments with 5% acetic acid were not effective (only 7.7–60% mortality) on small *M. edulis* in a few studies (MacNair et al. 2006; Sharp et al. 2006; Gill et al. 2007). Only one study achieved 100% mortality of small *M. edulis* and this was accomplished by immersion in 5% acetic acid for 5 min, followed by air drying for 1 h (Carman et al. 2016). Under field conditions, immersions in 5% acetic acid were found effective at killing small *M. edulis* after 15 s (Davidson et al. 2005; Gill et al. 2007). In contrast, MacNair et al. (2006) observed the opposite after 30 s at the same concentration. Nevertheless, treatment effectiveness may be size dependent within the small size category of *M. edulis*. According to Carver et al. (2003), immersion times in 5% acetic acid in laboratory tests ranging from 5 s to 1 min were effective (mortality occurred, unquantified) for 10-mm mussels, but not for the 20-mm mussels (unaffected). Furthermore, a 5-s immersion in 5% acetic acid in field conditions was not more than 10–15% effective on large *M. edulis*, according to Locke et al. (2009). To test the effect of a simulated transport on small *M. edulis*, Vickerson (2009) showed that a 30-s immersion in 4% acetic acid, followed by a rinsing step and a 24-h period of cold (4°C, 100% RH) air exposure did not kill the mussels.

The most effective treatment on large and small *P. canaliculus* was at the highest concentration tested (10% acetic acid) for 1 min, followed by 24 h of air drying, achieving 69–87% mortality (Denny 2008). Studies in which only acetic acid immersions (up to 4 min) were tried (without drying periods) did not yield effective mortality results for *P. canaliculus* or *M. galloprovincialis*. According to Sievers et al. (2019), no mortality (0%) occurred in small or large *M. galloprovincialis* after a straight immersion of 30 s in 2 or 5% acetic acid in laboratory experiments (no air drying), while a 2-min immersion in 4 or 8% acetic acid was less than 5% effective on small and large *P. canaliculus* (Forrest et al. 2007). Under field conditions, a 4% acetic acid immersion was 9% effective on large and small *P. canaliculus* (Forrest et al. 2007). Cahill et al. (2021) observed that the mean mortality of small *P. canaliculus* was 62–65% after immersions in 8% acetic acid for times of 10, 30, and 60 s (durations pooled; Cahill et al. 2021). Forrest et al. (2007) evaluated the effect of a rinsing step between an immersion in acetic acid and an air-drying period on *P. canaliculus* under field conditions. Under controlled laboratory conditions, Forrest et al. (2007) showed that immersions of 2 min in 4 and 8% acetic acid, followed by air drying (24 h) directly (no rinsing step between), resulted in at least 43% and 74% mortality, respectively, of small and large *P. canaliculus*. Adding a rinsing step between a 4-min immersion in 4% acetic acid and 24 h air drying decreased the effectiveness of the treatment on small and large *P. canaliculus* to under 9% mortality under field conditions (Forrest et al. 2007). Drying for 24 h prior to a 4% acetic acid immersion (reversed steps) of 4 min (field conditions) and 2 min (laboratory conditions) were also not effective on small and large *P. canaliculus*, with ~10 and 5% mortality observed, respectively (Forrest et al. 2007). Times of immersions tested

(2, 4, or 10 min) with concentrations of 0.5 or 1% acetic acid followed by 24 h of air drying were not effective on large and small *P. canaliculus* (10% mortality or less; Denny 2008). A low mortality percentage of 5% was also observed by Denny (2008) in laboratory tests on large *P. canaliculus* after a quick (3 s) 10% acetic acid spray followed by 26 h of air drying.

Heated acetic acid was not more effective at killing large *M. galloprovincialis*, but did affect small-sized mussels. The highest mortality percentage observed for large *M. galloprovincialis* was ~60% at a concentration of 5% acetic acid heated to 50°C and immersed for 10 s (Sievers et al. 2019). That study obtained one conflicting result where a 5% acetic acid immersion at 50°C for 30 s induced only ~25% mortality of large *M. galloprovincialis*. The same authors showed that lower concentration (2%) or lower temperature (40°C) induced 0% mortality of large individuals. Sievers et al. (2019) demonstrated that small *M. galloprovincialis* mussels had 100% mortality in 2% acetic acid heated to 50°C after 30 s and 90% mortality in 5% heated acetic acid at 50°C after 10 s. No mortality (0%) was recorded with small *M. galloprovincialis* at 40°C in 2% (30 s) or 5% (10 s) acetic acid immersions (Sievers et al. 2019).

For large *C. virginica*, an immersion in 5% acetic acid for 30 s induced 56% mortality, while a concentration of 10% for 10 min or 20% for 5 min were considered effective (Carver et al. 2010). In several field trials by Gill et al. (2008), 5% acetic acid immersions of up to 10 min were not effective at killing small *C. virginica*. Total mortality (100%) of large *C. gigas* was observed after a 5 min immersion in 5% acetic acid in field conditions (Rolheiser et al. 2012). The same study reported that immersion in 5% acetic acid for 30 s or in lower concentrations of 0.25% or 1.25% acetic acid for 10 min were not more than 40% effective on large *C. gigas*. Immersions of 1 min and 30 s at concentrations of 0.25% and 1.25%, respectively, induced only 20% mortality of *C. gigas* (Rolheiser et al. 2012). No acetic acid (1, 2, 4, or 8%) immersions (15–60 s) tested in laboratory experiments were effective (0% mortality) on small *C. gigas* (Cahill et al. 2021). Cahill et al. (2021) also observed 0% mortality when the small oysters were immersed for 30 s in 4% acetic acid in the field. Field experiments led by Coutts and Forrest (2005) on wrapped wharf piles showed no effectiveness on *C. gigas* (no size specified but assumed to be large) for the immersion times tested (up to 10 min), no matter the concentration (1–5%) of acetic acid.

Under field conditions, an immersion in 5% acetic acid was not effective at killing *Balanus* sp. (1 min; some barnacles remained alive after treatment; McDonald 2010) nor effective on adults or eggs of the Atlantic oyster drill (*Urosalpinx cinerea*) (10 min; Gill et al. 2008). Sprays of 5% acetic acid (5−10 s) followed by 45 s of air drying were tested on *C. mutica* settled on ropes (Paetzold et al. 2008). Although only 89% mortality was observed directly after treatment (2–3 h), 100% mortality was eventually observed after 5–9 d post-treatment. In field conditions on aquaculture lines, a 5% acetic acid immersion of 5 min or 30 s was 100% effective on the mottled star (*Evasterias troschelli*) (Rolheiser et al. 2012) and on *A. amurensis*, a proxy of *A. rubens* (Fitridge et al. 2014), respectively. The use of a lower concentration (2%) in laboratory experiments for 30 s required a greater temperature (40°C) to kill 100% of *A. amurensis* (Fitridge et al. 2012). Laboratory trials (Sharp et al. 2006) showed that 5% acetic acid immersions for 15 s were not effective at killing *Cladophora* sp. In addition, a comprehensive experiment investigated the effects of 5% acetic acid spray on 11 macroalgal species, including *U. pinnatifida* and various Ulvoid and Rhodophyta species, and demonstrated that a spray followed by a 10-min air-drying exposure was almost completely effective (almost 100% mortality) at killing all species, with the exception of the green alga *Ulva linza* (Piola et al. 2009). Forrest et al. (2007) tested the effects of various acetic acid immersions (durations and concentrations) on young stages of *U. pinnatifida*. A 4% acetic acid immersion for 1 min or 2% for 4 min were 100% effective at killing the majority of young-stage (gametophytes, plantlets, and sporophyll) tissues in both laboratory and field conditions (on ropes). Forrest et al. (2007) also showed that immersions at lower concentrations (2% acetic acid) were effective on

Cladophora sp. after at least 3 min, but quickly became ineffective as soon as the time of exposure was decreased under 3 min (1–2 min). The same study demonstrated that a 1-min immersion in 4% acetic acid followed by a period of 24 h of air drying was effective for *Cladophora* sp. Forrest et al. (2007) also tested the influence of a rinsing step between a lower concentration (2%) of acetic acid immersion and air drying on *Cladophora* sp. mortality. They showed that immersion for 1 min without rinsing the algae before drying was effective, whereas it required at least 3 min of immersion in 2% acetic acid to be effective if the alga was rinsed before the drying period.

For treating biofouling on oyster cages, Chinnadurai et al. (2019) showed that immersion in 5% acetic acid for 10 min was effective on many AIS, including *H. elegans*, *Polydora* sp., the bryozoans *Membranipora* sp., *B. neritina*, and the sponge *Leucosolenia* sp. For *H. elegans*, simple immersions for 4 min in 4 or 2% acetic acid were not effective, but adding a drying period of 24 h after the immersion in 4% acetic acid was effective at killing the worm (without rinsing the worm between the immersion and drying periods; Forrest et al. 2007). In contrast, immersion in 2% acetic acid (4 min) followed by a period of 24 h of air drying was not effective for *H. elegans* (Forrest et al. 2007). For Terebellidae worms, Forrest et al. (2007) showed that, at concentrations of 4 and 2% acetic acid, at least 2 and 3 min, respectively, were required to be effective (1 min was not effective on Terebellidae). They also showed that immersions in 4 or 2% acetic acid for 1 min were effective for Terebellidae only when followed by a 24-h air-drying period (Forrest et al. 2007). Forrest et al. (2007) also found that a 1-min immersion in 4% acetic acid was effective at killing the bryozoans *B. neritina* and *W. subtorquata* (proxy for *C. pallasiana*), but 4 min in 2% acetic acid was ineffective. Immersions in 2% were effective at killing the two bryozoans after 1 min followed by 24 h of air drying (without rinsing between the immersion and air-drying periods; Forrest et al. 2007). If both bryozoans were rinsed with seawater between the immersion and air-drying exposure, the treatment required a longer immersion period of 4 min in acetic acid (2%) to be effective (Forrest et al. 2007). Oyster bags fouled with *B. neritina*, *W. subtorquata*, and hydroids treated with a 4% acetic acid solution for 30 s resulted in the total elimination (100%) of biofouling cover (Cahill et al. 2021). Piola et al. (2009) showed that a 5-s immersion in a 5% acetic acid solution followed by 12 h of air drying was not effective at killing *B. neritina*, and this result didn't change at a higher concentration (20%) of acetic acid. A treatment was >90% effective in field experiments on *C. celata*, but required high concentrations of acetic acid for relatively long periods (10 and 20% for 10 and 5 min, respectively) (Carver et al. 2010). Acetic acid induced 100% mortality of *E. crocea* in only 10 s, with immersions in 2 or 5% acetic acid alone and 2% heated acid acetic at 40–50°C (Sievers et al. 2019). No information was found on the use of acetic acid treatments for *C. fragile*.

3.1.2.3. Citric acid immersion (with and without heat)

In general, citric acid was found to be less effective at eliminating AIS than acetic acid. Three studies tested the effectiveness of citric acid on only a relatively small number of AIS, namely *C. intestinalis*, *S. clava*, *M. galloprovincialis*, *Cladophora* sp., and *E. crocea*, but most of the results came from Sievers et al. (2019). An immersion in 5% citric acid for 10 s was only 33% effective at killing *C. intestinalis* (Sievers et al. 2019). For *C. intestinalis*, a concentration of 2% citric acid was not effective after 5 s (Locke et al. 2009) and 10 s (0% mortality; Sievers et al. 2019). Heating the citric acid gave better results, where 100% mortality of *C. intestinalis* was achieved when immersed for 10 s in a 2% solution heated to 50°C, or for 10 s in a 5% solution heated to 40°C (Sievers et al. 2019). Furthermore, an immersion of 10 s in a 2% solution heated to 40°C induced 66% mortality of *C. intestinalis* (Sievers et al. 2019). For *S. clava*, results were less consistent regarding citric acid immersions, just as it was for acetic acid. Sievers et al. (2019) showed that ~75% mortality of *S. clava* was achieved in a 10% citric acid solution after 30 s.

The effectiveness dropped to ~60% mortality in a 5% citric acid immersion after 10 s and in a 2% citric acid after 30 s (Sievers et al. 2019). They found conflicting results in the 5% citric acid immersions, where an immersion of 30 s achieved only ~45% mortality of *S. clava* (Sievers et al. 2019). Heating the citric acid was more effective and gave more consistent results, where 100% mortality was achieved for *S. clava* in 5 or 10% citric acid immersions heated to 40°C or 50°C after 10 s and in a 2% solution heated to 50°C after 30 s (Sievers et al. 2019). Decreasing temperature to 40°C induced only 60% mortality of *S. clava* in a 2% citric acid immersion after 30 s (Sievers et al. 2019).

On large and small *M. galloprovincialis*, the greatest concentration of citric acid tested (10%) was not effective (0% mortality) after a 10 s immersion, but 40–50% mortality was observed after 30 s (Sievers et al. 2019). At a concentration of 2% citric acid, a 30-s immersion was also not effective (0% mortality) on large *M. galloprovincialis* (Sievers et al. 2019). Heating the solution did not produce better results, where an immersion of 30 s in 10% citric acid heated to 50°C achieved no more than 50% mortality of large *M. galloprovincialis* (Sievers et al. 2019). No mortality (0%) was observed for large *M. galloprovincialis* after a 30-s immersion in a 10% citric acid solution heated to a lower temperature (40°C) or in a 5% citric acid solution at 50°C for 10 s (Sievers et al. 2019). Total mortality (100%) was observed for small *M. galloprovincialis*, however, when they were subjected to a 2% citric acid immersion heated to 50°C for 30 s (Sievers et al. 2019). However, results were not consistent for the effectiveness of heated 5 and 10% citric acid solutions on small *M. galloprovincialis*, where immersions in 5% citric acid gave a moderate range of 45 to 50% mortality when heated to 40°C for 30 s (Sievers et al. 2019). Furthermore, exposure to 10% citric acid concentrations resulted in approximatively 85% Mediterranean mussel mortality in immersions heated to 50°C (10 s), 60–70% mortality in 40°C (30 s), and 0% mortality in 40°C (10 s) (Sievers et al. 2019).

Citric acid was not effective on *Cladophora* sp. when it was immersed for 15 to 30 s in a 5% solution, either when heated to 30°C (30 s) or not (Sharp et al. 2006). Sievers et al. (2019) demonstrated that immersion in 2% citric acid was 60% effective at killing *E. crocea* after 10 s, but 5% (10 s) was 100% effective (Sievers et al. 2019). The same study showed that heating 2% citric acid to 40–50°C was 100% effective (10 s) at killing *E. crocea*.

No studies were found for citric acid treatment of many species groups, including colonial tunicates, *A. aspersa*, *Molgula* spp., *M. edulis*, oysters (*C. virginica* and *C. gigas*), gastropods, *C. maenas*, *C. mutica*, sea stars, *C. fragile*, polychaetes, bryozoans, or sponges.

3.1.2.4. Brine immersion (with and without air drying)

The effectiveness of brine immersion (with and without air drying) is well documented in the literature, with 28 different sources. Mortality of tunicate species attached to oysters exposed to a brine immersion (70 ppt) was investigated by Carman et al. (2010) who showed that 10-min immersions followed by air drying for 2 h were effective for multiple species, including *B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. listerianum*, *C. intestinalis*, *S. clava*, *A. aspersa*, and the sea grape *Molgula manhattensis*. A more recent study indicated that some of the same species (*B. schlosseri*, *B. violaceus*, *D. vexillum*, juveniles of *D. listerianum*, juveniles of *C. intestinalis*, and *A. aspersa*) had 100% mortality after only a 10-s brine (70 ppt) immersion, followed by 1 h of air drying (Carman et al. 2016). To reduce the risk of transferring AIS on shellfish between waterbodies, a protocol that recommends treatment options was developed by DFO's PEI Introductions and Transfers Committee (ITC), hereafter DFO PEI ITC, in collaboration with PEI's DFC (Aquaculture Division) and the PEI Aquaculture Alliance (C. Mills, DFO, unpubl. data). Based on this protocol, a 30-s immersion in 300 ppt brine followed by 1 h of air drying was effective at killing *B. schlosseri* and *B. violaceus*. At lower brine concentrations (62 ppt), McCann et al. (2013) found that immersion times of more than 4 h were required to be 100%

effective for *D. vexillum* in the laboratory when immersions were not followed by an air-drying exposure. Furthermore, Rolheiser et al. (2012) showed that *D. vexillum* on *C. gigas* in the field was not affected by immersion in 70-ppt brine concentration for 10 min (with no air exposure) as fouling of the species increased 5 weeks post-treatment after being returned to the water. Similarly, laboratory experiments with saturated brine solutions (300 ppt) showed that 15-s and 8-min immersions without air-drying exposure were not effective at killing *B. violaceus* (MacNair et al. 2006) and *C. intestinalis* (25% mortality; Carver et al. 2003), respectively. Additionally, Gill et al. (2007) observed that a 30-s immersion in a 300-ppt saturated brine solution was not effective at killing *C. intestinalis* in the field.

MacNair et al. (2006) carried out several trials, using saturated brine (300 ppt) immersion followed by an air-drying period, on invasive-tunicate-fouled mussel socks and aquaculture gear. Saturated brine was effective in reducing *B. violaceus* fouling, where a 5-min immersion followed by 1 h of air drying was 100% effective, but 1 min of immersion followed by the same period of air drying, or longer (24 h), was not long enough to ensure total mortality (almost 100%) of the tunicate species. Gill et al. (2007), however, found that a 15-s immersion in a 300-ppt solution followed by 1 h of air drying was not effective at killing *C. intestinalis*. Davidson et al. (2005) observed that a saturated brine (concentration not specified, but considered to be 300 ppt) immersion for 10 s was 75% effective at killing *S. clava* juveniles under laboratory conditions. Under field conditions, a longer immersion time of 5 min in saturated brine (300 ppt) followed by 30 min of air drying was 100% effective at killing *S. clava* (Minchin and Duggan 1988). Laboratory experiments demonstrated that saturated brine immersions alone (without subsequent air drying) were almost 100% effective at causing *Molgula* spp. mortality after 10 min (Loosanoff 1960), and yet were considered effective after only 3 min according to MacNair and Smith (1999). Medcof (1961) found that a 3 min saturated brine immersion followed by 1 h of exposure to air was sufficient to kill *Molgula* spp., and that air exposure was not required if the immersion time was increased to 10 min. A 1-min immersion in saturated brine (300 ppt) followed by 1 h of air drying was 100% effective at killing *Molgula* spp. (Loosanoff 1960).

A saturated brine (300 ppt) immersion of 30 s followed by 1 h of air drying was not effective at killing large *M. edulis,* since this recommended treatment option taken from the DFO PEI ITC protocol was meant to treat market-sized mussels for *B. schlosseri* and *B. violaceus* (Mills, DFO, unpubl. data). The most effective treatment for newly settled *M. edulis* (18 mm) fouling on oysters and oyster bags was an immersion in 300-ppt brine for 6 min followed by 24 h of air drying, which induced a mean mortality of 97% (Mallet et al., Mallet Research Services Ltd., unpubl. data). However, MacNair et al. (2006) showed that a 300-ppt brine immersion for 10 min followed by 24 h of air drying was only 39% effective at killing small *M. edulis* under field conditions. The same authors showed that an immersion in 300-ppt brine for 2 min followed by 1 h of air drying was effective on *M. edulis*, though the size of mussels was not specified for that treatment. Most brine immersion treatments (70 to 360 ppt) of 30 min or less (with and without an air-drying period) on small *M. edulis* and on large and small *C. virginica*, as well as immersions of 1 h or less on *C. gigas* were completely ineffective (0% mortality or not effective) or resulted in very low mortality (less than 30%) (Minchin and Duggan 1988; MacNair and Smith 1999; Ruellet 2004; MacNair et al. 2006; MacNair 2009; Sharp et al. 2006; Bourque and Myrand 2007; Gill et al. 2008; Carver et al. 2010; Rolheiser et al. 2012; Carman et al. 2016; Landry et al., DFO, unpubl. data; Mallet et al., Mallet Research Services Ltd., unpubl. data). Vickerson (2009) tested the effects of a simulated mussel transport in cold humid conditions after brine immersions. The study showed that an immersion in 300 ppt for 30 s followed by 24 h of cold humid air exposure (4°C, 100% RH) did not kill small (30−40 mm) *M. edulis*. Adding a rinsing step between the immersion and the air exposure did not make a difference and reversing the steps of air exposure (24 h, 4°C, 100% RH) and immersion in saturated brine (30 s) was not more effective (Vickerson 2009). For large *M. galloprovincialis*, immersions in 350-ppt brine (in

the field or laboratory) for 30 min or less were not effective, inducing only 0–21% mortality (Asgari and Jahangard 2012). The only effective treatment for large *M. galloprovincialis* in laboratory experiments was a quick 10-s immersion in a 350-ppt chilled (-20°C) brine solution, which induced 90% mortality of mussels (Asgari and Jahangard 2012). However, the same treatment was less effective (17% mortality) with an immersion of only 5 s (Asgari and Jahangard 2012).

A saturated brine immersion (300 ppt) for 3 min followed by 30 min of air drying was 100% effective at killing the common slipper shell (*Crepidula fornicata*) (Loosanoff 1960). For young stages of *U. cinerea* and the thick-lip drill (*Eupleura caudata*), a saturated brine immersion (300 ppt) for 5 min alone or 3 min followed by several hours of air drying were considered effective (Loosanoff 1960). Gill et al. (2008) showed that immersion in 300-ppt brine for up to 10 min (without air drying) was not effective on both adults and eggs of *U. cinerea*. For *C. maenas*, trials by McKenzie et al. (DFO, unpubl. data) showed that a saturated brine immersion (300 ppt) for 1 h followed by several hours of air drying was not effective, as the crabs recovered after treatment when they were returned to seawater. Similarly, an immersion in saturated brine (300 ppt) for 1 min was not effective at killing *Balanus* sp. (McDonald 2010). Saturated brine (300 ppt) was effective at killing *A. rubens* after 2 min (Medcof 1961) or 30 s (Loosanoff 1960) and 100% effective after 1 min followed by a drying period (duration not specified) (Loosanoff 1960).

Saturated brine immersion (300 ppt) for 15 min followed by 1 h of air drying was a promising treatment for killing *C. fragile* (100% mortality) in laboratory tests (Landry et al., DFO, unpubl. data) and is supported by similar findings (100% mortality after 15 min of immersion in 300 ppt) in a field study by MacNair (2002). Immersions for 15 or 10 min, combined with air drying for 2 or 24 h, respectively, were also 100% effective combinations (MacNair 2002). However, MacNair and Smith (1999) showed that 3 min in saturated brine alone (without air drying) was not effective at killing *C. fragile* on *C. virginica* collectors in the field. For numerous macroalgal species (46 taxa), immersions in 400-ppt brine for 30 min was effective at reducing algal diversity on shells of *C. gigas*, except for a few resistant taxa (e.g., *Cladophora* spp., *Ulva* spp.; Mineur et al. 2007). In contrast, a 15-s immersion in a 300-ppt brine solution was found to be effective at killing *Cladophora* sp. in both laboratory and field conditions (Sharp et al. 2006; MacNair 2009).

Saturated brine immersion, followed by air drying or not, can be effective to kill polychaetes. An exception is *H. elegans*, where immersion for 20 min in saturated brine was only 59.4% effective (Arakawa 1980). Velayudhan (1983) showed through laboratory experiments that increasing the brine concentration required less time to be 100% effective at killing *P. ciliata*. They found that brine solutions of 42.3, 60, and 78 ppt required 19–21, 8.5, and 7.75 h, respectively, to be 100% effective at killing the worm. Similarly, a saturated brine (300 ppt) immersion for 5 min was effective for *P. ciliata* (Medcof 1961). Another laboratory study showed that a 50-ppt brine immersion was 100% effective at killing the feather duster worm (*Sabella spallanzanii*) in 24 h (Jute and Dunphy 2017). Asgari and Jahangard (2012) observed in laboratory experiments that a quick 10-s immersion in a 350-ppt chilled (-20°C) brine solution was 100% effective at killing *S. paumotanus*, but at the same concentration at ambient temperature for 30 min, the effectiveness decreased to 94.1%. In the field, they found that a 20-min immersion in 350-ppt brine was 79.1% effective at killing the tube worm (Asgari and Jahangard 2012). A saturated 300- ppt brine immersion for 1 min was effective at killing *P. websteri* living on *C. virginica* shells in the field, when followed by at least 2 h of air drying (Nell 2007). However, longer immersions of 6 and 15 min in saturated brine followed by 24 h of air drying were 85% and 90% effective at killing *P. websteri*, respectively, in trials by Carver and Mallet (Mallet Research Services Ltd., unpubl. data). In laboratory trials, a 30-min immersion in saturated brine (360 ppt) followed by an air-drying period of several hours (overnight) was near 100% effective at killing *Polydora* spp.

on oyster shells (Ruellet 2004). Gryder (2002) showed that a brine solution over 70 ppt for 15 min followed by 15 min of air drying was effective at killing *Polydora* spp. worms. Medcof (1961) found that drying for 1 h first and then dipping in saturated brine for 1 min was also an effective method for killing *Polydora* spp. worms and *Cliona* spp. sponges. For *C. celata* fixed to shells of *C. virginica*, a 5-min immersion in a 270-ppt brine solution was 100% effective in field experiments (Carver et al. 2010). With a 6-min immersion, they observed that an additional drying period of 1 h ensured 100% mortality of this sponge (Carver et al. 2010). Although the species was not specified, *Cliona* spp. had 100% mortality after being immersed for 10 min in saturated brine (300 ppt) followed by 1 h of air drying when treating cultured oysters (Loosanoff 1960). A 5-min immersion in saturated brine with no drying period was also considered effective on boring sponges (Medcof 1961). No information was found on the use of brine solution treatments on small *M. galloprovincialis*, *C. mutica*, bryozoans, or hydrozoans.

3.1.2.5. Saturated brine and hydrated lime immersion (with air drying)

Immersion in a mixture of saturated brine (300 ppt) and hydrated lime (calcium hydroxide) (4%), followed by a period of air drying was a treatment recommended by the DFO PEI ITC for treating *C. virginica* infested with colonial tunicates prior to transfers (C. Mills, DFO, unpubl. data). A 30-s immersion in this solution followed by 1 h of air drying was effective at killing *B. schlosseri* and *B. violaceus* according to this protocol. As expected, small and large *C. virginica* were not killed after an immersion of 30 s in this solution followed by 1 h of air drying (C. Mills, DFO, unpubl. data). According to Ramsay (2022), at least 1 min of immersion in this solution followed by at least 30 min of air drying is required to be effective at killing *C. intestinalis*. The author demonstrated that an immersion of 30 s induced high mortality, although it was not completely effective at killing *C. intestinalis*, with some individuals surviving despite their unhealthy condition. No information on this brine and lime mixture treatment was available for any other AIS.

3.1.2.6. Hydrated lime immersion or spray (with and without air drying)

Most of the information found in the literature for hydrated lime focused on tunicates, with less of a focus on other shellfish epibionts. Immersion in solutions ranging between 4 and 20% of hydrated lime for controlling tunicates on aquaculture gear has provided mixed results. Ramsay et al. (2014) found that a 2-min immersion at 4% was moderately effective (80%) at killing the tunicates *C. intestinalis* and *S. clava*. Similar results were observed for *C. intestinalis* in laboratory trials (Carver et al. 2003) and on mussel socks (Gill et al. 2007), where immersions at 4% caused 70% mortality after 8 min and 50–80% mortality after 15 s, respectively. To eradicate several species from *C. virginica* spat collectors, McDonald et al. (2010) reported that an immersion in 4% hydrated lime for 1 min was effective to kill *B. schlosseri, B. violaceus*, *C. intestinalis*, *S. clava*, *Molgula* spp., sea stars, bryozoans, and hydroids (species not specified for the three latter groups). A 4% immersion in lime for 1 or 3 min was also effective at killing *Molgula* spp. (MacNair and Smith 1999; Locke et al. 2009).

Fouling by *D. vexillum* was reduced by 80 to 96% after a 2 to 4-min immersion in 4–5% hydrated lime solution in the field (Denny 2008; Switzer et al. 2011). Denny (2008) also demonstrated that a 10% hydrated lime solution was 99% effective at killing *D. vexillum* with similar exposure times, but a 20% lime solution for only 20 s was not effective. Field and laboratory experiments by Rolheiser et al. (2012) supported these findings and demonstrated that exposure to 4% hydrated lime for 5 min was most effective (92.3%) to reduce *D. vexillum* cover. MacNair et al. (2006) tested 4% lime immersions on mussel socks for shorter durations (15 s) to control *B. violaceus*, but all tunicates made a full recovery (0% mortality) after being returned to the water 7 d post-treatment.

Air exposure following lime immersion or spray can sometimes ensure higher mortality and is commonly used to kill tunicates on fouled gear to give consistently effective results (MacNair et al. 2006; Ramsay et al. 2014; Ramsay 2014b). Buoys exposed to air for 10 or 15 min after a 15-s lime (4%) immersion showed 80 and 90% *B. violaceus* mortality 7 d post-treatment, respectively (MacNair et al. 2006). Moreover, a 4% hydrated lime immersion for 15 s followed by a 20-min air exposure resulted in 100% mortality of *C. intestinalis* on fouled buoys (Gill et al. 2007). Based on qualitative visual assessments, 20% hydrated lime sprays for 5 s were also effective at killing *B. schlosseri* and *B. leachii* growing on fouled plates that were left for 6 h of air exposure post-treatment, but this same treatment required longer exposures (12 h) to be effective for *C. intestinalis* (Piola et al. 2009). The same authors also found that 5% hydrated lime spray (5 s) was sufficient to kill *B. leachii* when subsequently exposed to air for 12 h. Gill et al. (2008) showed that a 1-min immersion in 4% lime followed by 5 min of air exposure was effective at causing mortality of *C. intestinalis* and *S. clava*, but it was not effective for *B. violaceus*. Sprays of 4% hydrated lime (5 s) followed by air exposure (at least 45 s) were applied to control tunicates on mussel socks and were shown to be effective for *S. clava* (Ramsay et al. 2014; Ramsay 2014b).

Hydrated lime (4%) immersions up to 3 h were shown to be ineffective (0–78% mortality) for *M. edulis*, irrespective of their size (MacNair et al. 2006; Gill et al. 2007; Ramsay et al. 2014; Comeau et al. 2017; Landry et al., DFO, unpubl. data; MacNair, PEI DFC, unpubl. data) and for small and large *C. virginica* (0–15% mortality or ineffective; MacNair and Smith 1999; Gill et al. 2008; Locke et al. 2009; Carver et al. 2010; Comeau et al. 2017; Landry et al., DFO, unpubl. data). An immersion in 4% hydrated lime for 30 s followed by air exposure for 24 h in simulated cold transport conditions (4°C, 100% RH) was also not effective to kill small *M. edulis* (Vickerson 2009). Reversing the steps by exposing the mussels to air first (24 h) then the immersion (30 s) under the same conditions was likewise not effective (Vickerson 2009). Similarly, a 5-s spray exposure to 4% lime followed by 90 s of air drying was ineffective (0% mortality) for large *M. edulis* (Comeau et al. 2017). A 30-s immersion followed by 1 h of air drying (Landry et al., DFO, unpubl. data) induced very low mortality of small *M. edulis* (2%) and small *C. virginica* (0%). Alternatively, longer exposure times (15 and 30 min) tended to increase mortality (53–78%) of small *M. edulis* in the same study (Landry et al., DFO, unpubl. data). Hydrated lime immersions (1–4%) in both laboratory and field conditions were not very effective (0–60% mortality) on large *C. gigas* when immersed for 10 min or less (Switzer et al. 2011; Rolheiser et al. 2012).

Under field conditions, an immersion in 4% hydrated lime was not effective at killing barnacles (15 min; McDonald 2010) and *U. cinerea* adults or eggs (10 min; Gill et al. 2008). In field trials, a 4% hydrated lime immersion for 2 min was not lethal to *C. maenas* (Ramsay et al. 2014). A 5-min immersion in a 4% solution was found to be 100% effective at killing *C. fragile* (MacNair 2002), whereas shorter exposures (1 min) were not (MacNair and Smith 1999). A short immersion (30 s) in 4% hydrated lime combined with 1 h of air drying was 100% effective at killing *C. fragile* (Landry et al., DFO, unpubl. data). In addition, MacNair (2002) observed almost 100% mortality of *C. fragile* after immersions in 4% hydrated lime for 15 and 1 min, followed by 2 and 24 h of air drying, respectively. In the laboratory, a 10-min immersion in 4% hydrated lime was only 32% effective at killing *C. celata* (Carver et al. 2010). Sprayed hydrated lime (5 s) followed by 12 h of air drying was not effective at removing *B. neritina* (in 5%, 10%, 20% lime solutions) or *W. subtorquata* (proxy for *C. pallasiana*) (5% lime solution) from settlement plates (Piola et al. 2009). However, a 10% hydrated lime spray for 5 s followed by 30 min of air drying and a 20% hydrated lime spray for 5 s followed by 3 h of air drying was effective at killing *W. subtorquata* (Piola et al. 2009). No information was available for hydrated lime treatments on *D. listerianum*, *A. aspersa*, *M. galloprovincialis*, small *C. gigas*, *C. mutica*, macroalgae, or polychaetes.

3.1.2.7. Virkon® immersion

Very little information was available for Virkon® treatments; only two publications provided results for treating *C. intestinalis* on mussel (*M. edulis*) socks. Paetzold and Davidson (2011) achieved 100% mortality of *C. intestinalis* juveniles in laboratory experiments with a 3% Virkon® immersion for 30 s. They also observed that the effectiveness for killing *C. intestinalis* juveniles decreased to 95% when immersing these tunicates in a 1% solution for 60 s. Gill et al. (2007) tested immersions in a 3% Virkon® solution for 15 s and achieved between 5 to 13% mortality for *C. intestinalis* on socking material and cages in field conditions. They also observed that the Virkon® treatment was not effective at killing the tunicate when applied on buoys. Immersions in a 1% solution for 60 s and a 3% solution for 30 or 60 s were not effective for *M. edulis* (size not specified but assumed to be large) inducing only 0–16.7% mortality (Paetzold and Davidson 2011).

3.2. ASSESSMENT OF IMPACTS OF TREATMENTS ON SURVIVAL OF MOVED SHELLFISH AND MACROALGAE

3.2.1. Impacts of physical treatments on moved shellfish species

Physical treatments described in the literature for the mitigation/control of marine AIS on product destined to be moved were grouped into the following categories: (1) pressure washing, (2) air drying, (3) freshwater immersion or spray, and (4) hot water immersions and steam. A total of 32 literature sources (25 primary publications and 7 technical reports) were included, which considered a variety of physical treatments, including pressurized seawater (3), air drying (11), freshwater (11), and heat treatments (hot freshwater or seawater, and steam; 19), or a combination of these, for the treatment of transferred species (Table 8). An overview of each physical treatment, supported by relevant literature, is presented herein. A few unpublished results (3) provided by subject matter experts were also included for some physical treatments. Results are provided by moved species present on both Canadian coasts (Atlantic, Pacific, or both) and for small and large size categories of moved species (see Section 2.1).

3.2.1.1. Pressure washing (low- and high-pressure sprays)

Only three papers were found with information on the impacts of pressurized water on cultured species. In a field study by Arens et al. (2011a), high (700 psi) and low (40 psi) pressure treatments (using a single rotary nozzle commercial pressure washer) applied to mussel socks showed no detectable impact on small or large *M. edulis*. In another study, Arens et al. (2011b) showed 100% survival of small *M. edulis* exposed to 700 psi for 10 s. Curtis et al. (2021) observed that treating large *C. gigas* with a higher pressure nozzle at 2000 psi for 30 s caused a significant loss of oysters from clusters on drop lines, but it was unclear if pressure washing itself would have led to actual oyster mortality (i.e., oysters could still be viable). No information was found on the impacts of pressure washing on *O. edulis*, *C. virginica*, *A. irradians*, *P. magellanicus*, or *M. galloprovincialis*.

3.2.1.2. Air drying

In a thermal tolerance experiment conducted under field conditions, Seuront et al. (2019) observed that none (0%) of the small or large *M. edulis* survived an outdoor air-drying exposure of 6 h at 41°C. In heat tolerance laboratory experiments, Leblanc et al. (2005) observed 100% survival when large *M. edulis* were exposed to temperatures ranging from 20 to 41°C for 3 h, but found that an 11-h air-drying exposure at 27°C resulted in only 52.2% survival of small mussels. Mallet et al. (Mallet Research Services Ltd., unpubl. data) observed 92% survival of small *M. edulis* after 24 h of air exposure under laboratory controlled conditions. To test the impacts of a simulated transport period on mussels, Vickerson (2009) showed that small *M.*

edulis were not impacted after a cold and humid air exposure of 24 h (4°C, 100% RH). While trying to determine a compatible industrial standard for treating *M. edulis* socks, Leblanc et al. (2007) observed only 62% survival of small *M. edulis* after 40 h of air-drying exposure (21°C, 34% RH) under field conditions. According to Arakawa (1980), survival of small *M. edulis* would be more dependent on the weather than the duration of exposure to air. They reported that short periods of air exposure (2–3 h) in low RH conditions impacted small *M. edulis* survival, but they were not impacted when exposed to high RH air conditions for longer exposure periods (5–6 h). When testing air-drying tolerance of small *M. edulis* and *C. virginica* on Chinese-hat spat collectors in the field, Comeau (DFO, unpubl. data) determined that an exposure duration as short as 1 d (17–31°C) had very low impact on small *M. edulis* (94.6% survival), but that increasing the time of exposure increased the number of mussels lost from these spat collectors and resulted in only 1% survival after 5 d (8–31°C). Air drying for 1 d (17–31°C) had no impact (100% survival) on small (spat) *C. virginica*, but exposures of 5 d (8–31°C) decreased survival of oysters to 68% and exposures of 11 d (4–36°C) to 1% survival (Comeau, DFO, unpubl. data). Additional studies, which demonstrated that *C. virginica* had a higher tolerance to air exposure than *M. edulis* (Mayrand et al. 2015), further support Comeau's (DFO, unpubl. data) results. However, results from Mallet et al. (Mallet Research Services Ltd., unpubl. data) showed that small *C. virginica* (1–2 mm) were highly impacted (only 2% survival) after 24 h of air drying, contrasts with Comeau's results. Other field experiments showed that 95% of small (35–65 mm) *C. virginica* survived after being exposed to air for 72 h, stored in a wharf building to avoid weather variations caused by sun, wind, and rain (Mayrand et al. 2015).

Resistance of *M. galloprovincialis* to air drying was tested under laboratory conditions by Hopkins et al. (2016) to determine the length of time needed to reach 100% mortality. For large *M. galloprovincialis*, mortality (impacted) started to occur after 4 d of air drying and no survival (0%) was recorded after 11 d of exposure at 18°C (Hopkins et al. 2016). In another laboratory experiment from the same study, large *M. galloprovincialis* did not survive (0%) an air exposure of 7 d at a mean temperature of 20.3°C (Hopkins et al. 2016). Small *M. galloprovincialis* were less resistant than large ones and showed a low survival of 20% after 6 h of air exposure under field conditions (18.5°C, 95% RH) and did not survive (0%) a 24-h exposure in similar conditions in the laboratory (Hopkins et al. 2016). However, Asgari and Jahangard (2012) observed 100% survival of large *M. galloprovincialis* after 24 h under both field and laboratory conditions at temperatures of 14–18°C (RH not specified).

Crassostrea gigas appeared to be the most resistant species to air drying under laboratory conditions at 18°C, where large individuals survived up to 7 d before mortality started to occur and lasted up to 34 d before recording 0% survival (Hopkins et al. 2016). In outdoor trials to simulate field conditions, under a wider range of temperatures (9.5–32.2°C) and high RH (95%), the same authors observed that mortality started to occur sooner, that is after 72 h (3 d), with some *C. gigas* surviving up to 16 d before dying (Hopkins et al. 2016). No information was found on the impacts of air drying on *O. edulis*, *A. irradians*, or *P. magellanicus*.

3.2.1.3. Freshwater immersion or spray (with and without air drying)

According to most results found in the literature, freshwater treatments appear to be less harmful to shellfish than other physical treatments (Nel et al. 1996; Asgari and Jahangard 2012; Ramsay 2015a; Landry et al. DFO, unpubl. data). Freshwater immersions for up to 12 h in continuous-flow tanks (controlled conditions; Ramsay 2015a) or 48 h in laboratory tests (Landry et al., DFO, unpubl. data) resulted in 100% survival of small *M. edulis*. During several trials, Ramsay (2015a) also observed 100% survival of *M. edulis* during freshwater immersions (up to 24 h; 11–14°C), whether the mussels were kept in mesh bags, PVC pipes, or socks, or whether they were clumped together. In addition, 100% of socked small *M. edulis* survived when immersed for 24 h in freshwater in continuous flow tanks at an industrial scale (Ramsay 2015a).

A freshwater immersion for 24 h in laboratory tests or sprays with a garden hose for 10 min, both followed by 1 h of air drying, showed high survival (over 90%) of small, socked *M. edulis* one week post-treatment (Carman et al. 2016). Small *C. virginica* also showed high survival (97–100% and 96%) under laboratory conditions after freshwater immersions of 24 h and 48 h, respectively (Landry et al., DFO, unpubl. data). In another laboratory experiment, it was observed that *C. virginica* (size not specified but assumed to be large) could survive exposures to reduced salinities with negligeable or no impacts, including a freshwater immersion for 72 h alone or when followed by a period of cold air storage (3°C) for up to 14 d (Brown 2012). Freshwater was one of the few treatments for which survival results were available for small *P. magellanicus*. A 10-min immersion in low-salinity water (4–6 ppt), after being pre-acclimated to 10°C, resulted in 100% survival after 24 h, although this dropped to 80% after 1 week (Landry et al. DFO, unpubl. data). However, the authors also observed that survival decreased to 80% after 24 h for the same treatment when the scallops were pre-acclimated to only 4°C (Landry et al., DFO, unpubl. data).

A short (30 min) freshwater immersion in laboratory tests resulted in 100% survival of large *M. galloprovincialis* (Asgari and Jahangard 2012). Almost 100% of *Mytilus* sp. individuals (unidentified, from Pacific) subjected to over 5 d in freshwater at 10°C survived for months post-treatment (Forrest and Blakemore 2006). Based on their findings, the authors suggested that a 2-d freshwater immersion was a safe treatment for meeting their 90% survival criterion for mussels (Forrest and Blakemore 2006). Under field conditions, small and large *P. canaliculus* (proxy for *M. galloprovincialis*) survived 98–99% after an immersion in freshwater for 10 min followed by a period of 24 h of air drying (Denny 2008).

A 10-min freshwater immersion under field conditions resulted in 80% survival of large *C. gigas*, although these were more resistant at a higher salinity, where 100% survival was recorded for the same immersion duration at a salinity of 5 ppt (Rolheiser et al. 2012). Nell (2007) showed that *C. gigas* (size not specified but assumed to be large) was not impacted after a freshwater immersion for 12 h under field conditions. A high survival (95.8%) was observed for small *C. gigas* after a freshwater immersion for 12 h in laboratory tests, but the survival decreased to 88.5% when conducted under field conditions (Nel et al. 1996). In addition, small *O. angasi* (proxy for *O. edulis*) showed 100% survival following a 30-s freshwater immersion (Fitridge et al. 2014). No information was found on the impacts of freshwater treatments on *O. edulis* or *A. irradians*.

3.2.1.4. Hot freshwater/seawater immersions and steam

Results from the literature for heated treatments showed that temperature, duration, and size of organisms all affected survival, where higher temperatures and longer durations tended to be more harmful to shellfish, and where smaller animals were in most cases more vulnerable to heat treatments than were larger ones (Rajagopal et al. 2005b; Asgari and Jahangard 2012; Sievers et al. 2019; Landry et al., DFO, unpubl. data). While most results are related to heated seawater immersion, two studies (Forrest and Blakemore 2006; Landry et al., DFO, unpubl. data) tested the impacts of heated freshwater immersions on shellfish.

3.2.1.4.1. Hot freshwater immersion

Forrest and Blakemore (2006) observed in laboratory tests that small *M. edulis* were minimally impacted by a 55°C freshwater immersion for 5 s, where the mussels maintained their survival criterion of a 90% attachment. The authors also observed, at lower temperatures (10–20°C), that longer immersions (3–5 d) in freshwater decreased mussel attachment under their target criterion of 90% (results not shown, Forrest and Blakemore 2006). In other laboratory experiments, Landry et al. (DFO, unpubl. data) showed that small *C. virginica* completely survived (100% survival) 40°C freshwater immersions for 5 min (for both pre-treatment

conditions at 4 and 10°C) and 30°C for 10 min (for pre-treatment condition at 4°C). When the oysters were pre-acclimated to 10°C, the latter treatment (30°C, 10 min) decreased their survival to 89% (Landry et al., DFO, unpubl. data). The authors also tested the impacts of heated freshwater immersions on *P. magellanicus*, observing that pre-acclimation temperature had no effect on the results, with 100% of small *P. magellanicus* surviving an immersion in 30°C heated water for 10 min (Landry et al., DFO, unpubl. data). Nevertheless, a small temperature increase to 40°C for 1 min resulted in very low survival (only 3%) (Landry et al., DFO, unpubl. data).

3.2.1.4.2. Hot seawater immersion

Heated seawater immersion was the most documented of physical treatments, in terms of number of sources and the range of treatment variation tested in the literature. Results for *M. edulis* were well documented compared to other species. Large *M. edulis* had 0% survival in experiments at a farm site after an immersion of only 20 s at 60°C, while mussels immersed for only 5 s experienced ~65% survival (McDonald 2010). Best et al. (2014) observed either no effect or low impact on the survival of large and small *M. edulis* after a 1 min immersion at 55°C in a laboratory setting. Arakawa (1980) provided a guide for the removal of fouling on cultured oysters, including unwanted mussels attached to collector ropes. Results from Koganezawa (1972), presented in Arakawa (1980), provided data for two small size groups of mussels (10−20 and 40−50 mm). Koganezawa (1972) showed that 40–50 mm *M. edulis* fully survived (100%) a heated immersion at 50°C for 60 s, 55°C for 20 s, and 60°C for 10 s under field conditions. Mussel survival decreased to 90% at 55°C for 30 s. Field results from the same study demonstrated that mussels (*M. edulis*, 40–50 mm) were vulnerable to heated seawater immersions at 50°C for 15–20 s (70 to 90% survival) and at 60°C for 15–30 s (40 to 80% survival), decreasing to 0% survival at 60°C for 1 min (Koganezawa 1972). For the smaller *M. edulis* group (10–20 mm), 100% survival was observed after immersions at 55°C for 5 s and 60°C for an immersion for 1 s (Koganezawa 1972). An immersion at 50°C for 15 s resulted in 90% survival for that size group (10–20 mm) and after 30 s there was no survival (0%). Likewise, an immersion at 55–60°C for 15 s was also fatal (0% survival) for small (10–20 mm) mussels (Koganezawa 1972). In another study, Leach (2011) showed that a heated immersion at 60°C for 15 min had negative impacts (0% survival) on small *M. edulis*, whereas an immersion at 40°C for 30 min resulted in approximately 60% survival. According to Davidson et al. (2005), small *M. edulis* attached to styrofoam buoys were also impacted, noting a high mussel mortality after a 4-s immersion in heated seawater at 60–80°C. In controlled experiments, McDonald (2010) observed no survival (0%) of small *M. edulis* after an immersion in seawater at 60°C for 15 s, while they obtained ~60% survival when the immersion time was reduced to 5 s.

Immersion experiments conducted at 40°C did not provide better survival for small *M. edulis*. As shown by Landry et al. (DFO, unpubl. data), mussels only experienced 13% (pre-acclimated to 10°C) and 67% (pre-acclimated to 4°C) survival after being immersed in seawater heated to 40°C for 5 min under laboratory conditions. However, Landry et al. (DFO, unpubl. data) obtained 100% survival of small *M. edulis* after an immersion for 10 min in 30°C seawater (at both pre-acclimated conditions: 4 and 10°C). Increasing the duration of a heated seawater immersion to 6 h and raising the temperature slightly to 32.6°C negatively affected survival of small *M. edulis*, reducing it to 24% (Leblanc et al. 2005). In addition, Gonzalez and Yevich (1976) observed that an entire population of small *M. edulis* in an effluent canal (natural field conditions) did not survive (0%) after being exposed for 3 d to seawater temperatures ranging from 28 to 30°C.

Gonzalez and Yevich (1976) showed that following an acclimation period (temperature raised at a rate of approximately 1°C/d from 2.5 to 25°C), small *M. edulis* mussels exposed to heated seawater showed 100% survival at 26°C after 24 h and 94% survival at 27°C after 48 h. The

same study also demonstrated that immersion at 28°C resulted in 50, 20, and 0% survival of small *M. edulis* after 3, 4, and 6 d, respectively (Gonzalez and Yevich 1976). Rajagopal et al. (2005a), through acclimation laboratory experiments (prior acclimation at 20°C for 2 weeks), demonstrated that a temperature of 36°C for 70 min or more and 41°C for 1 min resulted in no survival (0%) of small *M. edulis*.

In laboratory experiments, heated seawater immersions at 45–48°C for 80 s (Asgari and Jahangard 2012), 40°C for 60 s, and 50 and 60°C for a shorter duration of 10 s all resulted in 100% survival of large *M. galloprovincialis* (Sievers et al. 2019). Asgari and Jahangard (2012) observed survival ranging from 93 to 95% for large *M. galloprovincialis* after immersions of 55–65 s in seawater heated to 51°C. A small increase of 2°C to 53°C was enough to slightly decrease survival of the large mussels to 87–93%, with durations of 55–70 s (Asgari and Jahangard 2012). Furthermore, for immersions at 50°C, increasing the duration to 30 s lowered the survival of large *M. galloprovincialis* to ~60% (Sievers et al. 2019). With durations of 60 s, heated immersions in seawater at 50°C resulted in 0% survival of large *M. galloprovincialis* (Sievers et al. 2019). Small *M. galloprovincialis* were more impacted by both temperature and immersion durations than large ones, yet they still survived exposures of 60 s at 40°C (Sievers et al. 2019). Immersion at 50°C for 10 s decreased survival of small *M. galloprovincialis* to ~75%, while 0% survived seawater heated immersions at 50°C for 30 s and 60°C for 10 s (Sievers et al. 2019). Under field conditions, Asgari and Jahangard (2012) showed that survival of large *M. galloprovincialis* ranged from 97 to 100% after immersions in closed circulated seawater heating systems at 46–51°C for 40–45 s. At higher temperatures of 60–65°C, survival decreased to 42–46% for large *M. galloprovincialis* after an immersion of 30 s (Asgari and Jahangard 2012). For a longer exposure (5 min), small and large *M. galloprovincialis* did not survive (0%) at 50°C, while 99 and 95% survival were respectively measured for large and small mussels at 35°C (Piola and Hopkins 2012).

Mayrand et al. (2015) tested the impact of heated seawater immersions on killing two size groups of small *C. virginica* in culture bags under field conditions and found that 35–45 mm oysters were more sensitive (~50% survival) than 55–65 mm oysters (95% survival) to a 15-s immersion in 60°C seawater. The same authors also observed that all oysters had greater survival (assessed 1 month post-treatment) when the treatment was applied in August compared to June (results not presented in the tables). Heat trials conducted at 60°C on small *C. virginica* immersed for 5–15 and 30 s showed ~95–99% and 5% survival, respectively (McDonald 2010). However, the same report showed that ~90% of large *C. virginica* survived an immersion at 60°C for 30 s (McDonald 2010). Also, depending on the month of application of the treatment, Rousselle (2012) obtained variable survival results for two small size groups (35–45 mm, 55–65 mm) of *C. virginica* placed in bags and immersed in 60°C seawater for 15 s. Survival of the larger group of oysters ranged from 40% (August) to 60% (June) while that of the smaller group remained around 50% for both months (Rousselle 2012). Heated seawater treatments (40°C, 60 s; 50°C, 10 s) had no impact (100% survival) on small *O. angasi*, whereas increases in duration (50°C, 30 s; 0% survival), temperature (60°C, 10 s; 60% survival), or both parameters (60°C, 30 s; 0% survival) reduced the survival of oysters (Fitridge et al. 2012).

The oyster *C. gigas* appeared to be more resistant to heat in terms of durations of exposure to higher temperatures compared to other shellfish species. All small *C. gigas* survived (100%) immersions in seawater heated to 50°C for 60 s (Koganezawa 1972) or 70°C for 30–40 s (Nel et al. 1996) in laboratory tests. However, this latter study showed that survival of oysters decreased to 91.3% after an exposure time of 45 s at 70°C (Nel et al. 1996). Under field conditions, the same authors showed that small *C. gigas* were more vulnerable to heat treatment, where only 88.8% survival was achieved after an immersion in seawater at 70°C for 40 s (Nel et al. 1996). Koganezawa (1972) showed that seawater heated to 55°C for 60 s, 60°C

for 15–30 s, and 60°C for 60 s decreased survival of small *C. gigas* to 90%, 80–92%, and 40%, respectively. In the context of their study, Rajagopal et al. (2005b) tested the thermal tolerance of *C. gigas* and found that they had a higher upper temperature tolerance than other marine fouling animals, including *M. edulis*. They demonstrated that three size groups of *C. gigas* (11, 35, and 54 mm – small category), which were acclimated to 20°C for 2 weeks prior to treatment, had different thermal resistance capacities (Rajagopal et al. 2005b). They showed that at 40°C, the 11-mm group survived 96 min of exposure before death, compared to the 54-mm group that survived up to 167 min (Rajagopal et al. 2005b). No oysters from the three size groups survived an immersion in seawater at 43°C for 60 min (Rajagopal et al. 2005b). At similar temperatures, Piola and Hopkins (2012) showed that spat (classified as small size) of *C. gigas* were more sensitive to heated seawater treatments than juveniles (also classified as small size) and adults (large size). They observed 76.7% survival (37.5°C, 60 min), 50% survival (40°C, 30 min), and 13.3% survival (42.5°C, 20 min) for oyster spat, while the large oysters completely survived (100%) all three temperature/duration treatments and the juvenile oysters were only slightly impacted (still 98% survival) by the most severe treatment (42.5°C, 20 min).

3.2.1.4.3. Steam

Socked small *M. edulis* exposed for 30 s of steam at 100°C (applied at 50 psi) and immediately returned to water after treatment were not impacted (Davidson et al. 2005). Joyce et al. (2019) showed that, where *M. edulis* and *C. gigas* were considered as AIS, a continuous jet of steam for 60 s induced 0% survival of small individuals. A 0% survival of large *C. gigas* was also noted, but only after 300 s of exposure with the same steam jet intensity. No information was found on the impacts of steam treatments on *O. edulis*, *A. irradians*, or *P. magellanicus*.

3.2.2. Impacts of chemical treatments on moved shellfish species

A variety of chemical treatments tested on moved shellfish species were considered from the literature, including a total of 31 sources (22 primary publications and 9 technical reports were consulted). A few unpublished results (4) provided by subject matter experts or unpublished work were considered for some treatments. Treatments included immersion in chlorine-based compounds (8), acetic acid (16), citric acid (1), brine solutions (16), brine and lime solutions (1), hydrated lime (12), and Virkon® (1), which were occasionally followed by an air-drying exposure period. An overview of the impacts of these treatments on shellfish as species moved is presented in Tables 9 and 10 and are summarized below. Results are provided for moved species present on both Canadian coasts (Atlantic, Pacific, or both) and for small and large size categories of moved species (see Section 2.1).

3.2.2.1. Chlorine-based compounds immersion or spray (with or without air drying)

For chlorination treatments, different types of compounds (e.g., sodium hypochlorite, chlorine dioxide) and units (%, mg/L) have been used. Studies on the control of fouling mussels in industrial cooling water systems by continuous chlorination showed that immersions in very low concentrations of TRC resulted in 0% survival of small *M. edulis* after exposure times of 40 d at 1 mg/L and 17 d at 3 mg/L (Rajagopal et al. 2002, 2003). Haque et al. (2015) showed that increasing chlorine concentration had physiological impacts on two small-sized groups of *M. edulis* (1.4 and 25 mm) and decreased survival over shorter time intervals. At a higher concentration of 4 mg/L TRC, they observed 0% survival in 1.4 and 25 mm mussels after immersion times of 7 and 6.3 d, respectively. Similarly, at the same TRC concentration (4 mg/L), Haque and Kwon (2017) observed 0% survival of two small-sized groups (14 and 25 mm) after 5.2 and 6.3 d, respectively. Under laboratory conditions, Haque et al. (2014) focused on impacts of chlorination as a control method on *M. edulis* veliger larvae. They found that the larvae did not survive (0%) after immersions in solutions of 1 mg/L (20 min), 0.1 mg/L (4 h), and 0.05 mg/L

(5 h) TRC. However, a very short exposure time of 10 min to a concentration of 0.7 mg/L TRC resulted in 84% survival (Haque et al. 2014; Haque and Kwon 2017).

Less information was available on impacts of chlorination on *M. galloprovincialis* compared to *M. edulis*. Asgari and Jahangard (2012) tested the impacts of chlorine dioxide as a control method for polychaetes on *M. galloprovincialis*. They found that an immersion of 9 min in initial concentrations of chlorine dioxide ranging from 0.14 to 0.28% was not harmful to large *M. galloprovincialis*, resulting in a high survival (97%). Immersion in 0.5% sodium hypochlorite (initial concentration) for 30 s (followed or not by a 24-h air-drying period) of small *P. canaliculus* (proxy for *M. galloprovincialis*) resulted in a survival of over 94%, as did immersion for 2 min in 0.5% sodium hypochlorite alone (Denny 2008). In a field study by Coutts and Forrest (2005) to reduce fouling on submerged wharf structures, solutions of sodium hypochlorite at initial concentrations ranging between 0.01 and 0.05% were contained within wraps enclosing wharf piles. The authors observed that *C. gigas* (size not specified but assumed to be large) survived the treatments after 12 h of exposure (Coutts and Forrest 2005).

No information was found on the impacts of chlorine-based compound treatments on *O. edulis*, *C. virginica*, *A. irradians*, or *P. magellanicus*.

3.2.2.2. Acetic acid immersion or spray (with and without air drying)

A number of studies and reports tested acetic acid as a mitigation and control method for tunicates and algae on socks, collectors, and buoys at mussel farms (Carver et al. 2003; Davidson et al. 2005; MacNair et al. 2006; Sharp et al. 2006; Gill et al. 2007; Locke et al. 2009). Generally, those studies observed that small *M. edulis* were weakly impacted (85–92.3% survival) after spray treatment or not impacted after 5% acetic acid immersions for durations ranging from 5 to 30 s (Carver et al. 2003; MacNair et al. 2006; Gill et al. 2007; Locke et al. 2009). Survival of 85–90% was observed in large *M. edulis* after a 5 s immersion in 5% acetic acid (Locke et al. 2009), but Davidson et al. (2005) and Gill et al. (2007) observed impacts on small *M. edulis* under field conditions following a similar treatment. These authors described the impacts (qualitative results) in terms of mortality, weight loss, and reduced growth. Sharp et al. (2006) tested a 20-s immersion in 5% acetic acid on spat collectors for *M. edulis* (collectors were rinsed with seawater after treatment) and observed that only 40% of mussels remained attached and did not show signs of gaping (considered as survival) 24 h post-treatment. Carver et al. (2003) tested the impacts of 5% acetic acid immersions in the laboratory on two size groups (10 and 20 mm, small category) of *M. edulis* for 5–10 s, 30 s, and 1 min. They observed that the 10-mm group was impacted, where mortality occurred in one trial for each duration, but the 20-mm group remained unaffected in all trials. Carman et al. (2016), whose purpose was to control tunicates on mussel farms, observed 0% survival of small *M. edulis* when socks were immersed in the laboratory in 5% acetic acid baths for 5 min, followed by a period of air drying for 1 h at room temperature. Vickerson (2009) tested the impacts on byssal attachment activity of small *M. edulis* of 4% acetic acid immersions followed by an air-exposure period simulating interregional transport under laboratory conditions. The author observed that 40 mm *M. edulis* (small) were impacted after an immersion without air drying for 30 s in 4% acetic acid. However, the mussels were not impacted after an immersion for 30 s if they were rinsed prior to 24 h exposure to cold and humidity (4°C; 100% RH; Vickerson 2009). In contrast, byssal attachment activity was impacted (i.e., detachment) if the small mussels were not rinsed between the immersion (30 s) and air-exposure (24 h; 4°C; 100% RH) periods. Vickerson (2009) also observed that using ice during the 24-h air exposure (transport simulation at 1−2°C instead of 4°C, 100% RH) after the immersion (30 s) decreased the byssal activity significantly (impacted), and that this situation only happened when treating the mussels with acetic acid and not with lime or brine. Reversing the 24-h air exposure step (4°C, 100% RH) and the immersion (of 30 s) also impacted the mussels (Vickerson 2009).

Sievers et al. (2019) tested the impacts of 2 and 5% acetic acid immersions in laboratory experiments on large and small *M. galloprovincialis* and assessed their survival 48 h post-treatment. They observed that 100% of the individuals survived 30-s immersions in both concentrations.

Under field conditions, Forrest et al. (2007) tested 4% acetic acid immersions, with and without an air-drying period of 24 h, to assess the survival (1 month post-treatment) of large and small *P. canaliculus* (proxy for *M. galloprovincialis*). They observed that survival was consistently ≥91% whether or not a 4-min immersion in 4% acetic acid was followed by 24 h of air drying. In those field experiments, whether mussels were de-clumped or attached to crop lines did not affect survival, but this only held true when the mussels were rinsed with water prior to air drying (Forrest et al. 2007). They observed that not rinsing the mussels prior to transport reduced their survival to <67% for attached mussels and <37% for de-clumped mussels (results not presented in the tables; Forrest et al. 2007). By reversing the steps of this treatment, which is air drying for 24 h first followed by an immersion for 4 min in 4% or 8% acetic acid, they observed >90% survival for both large and small *P. canaliculus*. The same authors also tested the impacts of 4 and 8% acetic acid immersions for 2 min on large and small *P. canaliculus* in laboratory experiments (Forrest et al. 2007). They assessed the attachment of mussels on ropes 24 h post-treatment and observed no major impact in either treatment as mussel attachment was consistently >95%. However, they showed that adding a 24 h air-drying period after a 2 min acetic acid immersion (4 and 8%) impacted *P. canaliculus*, particularly when individuals were not rinsed with seawater before the exposure to air. They observed that the no-rinse treatments impacted the mussels and reduced byssal attachment to <57% and <26% at 4 and 8% acetic acid concentrations, respectively (Forrest et al. 2007). To counter the decrease in mussel attachment and maximize survival, they switched the steps and undertook the immersion treatments (4 and 8%, 2 min) after the 24-h air-exposure phase. The results were consistent with the field observations where mean survival was ~95% for both large and small *P. canaliculus* under laboratory conditions (Forrest et al. 2007).

During several experiments with different types of treatments, including acetic acid, Denny (2008) treated *P. canaliculus* to mitigate for *D. vexillum* to reduce its spread through aquaculture transfers. The author observed 98.5% survival of small and large *P. canaliculus* after immersion in 0.5% acetic acid for 10 min, followed by 24 h of air drying (Denny 2008). With a slightly higher concentration of 1% acetic acid, the same treatment (10 min + 24 h of air drying) reduced survival to 90–95% (Denny 2008). At a high (10%) acetic acid concentration, survival of *P. canaliculus* dropped to 13–31% after an immersion for 1 min followed by 24 h of air drying (Denny 2008). Nevertheless, at the same concentration (10%), the application of acetic acid by spray for 3 s, instead of immersion, followed by 26 h of air drying resulted in more than 95% survival of *P. canaliculus*. Denny's (2008) spray experiment was conducted using mesh bags spread flat in a bin and sprayed with acetic acid using a garden hose with a spray nozzle attachment, and where the air-drying period was intended to simulate transport time. Cahill et al. (2021) observed that the mean survival of small *P. canaliculus* was 35–38% after immersions in 8% acetic acid for 10, 30, and 60 s (durations pooled). The authors suggested a treatment of 2% acetic acid for 60 s for small *P. canaliculus* to ensure its survival.

Sievers et al. (2019) tested the impacts of heated acetic acid (2 or 5%) on two small groups (15 and 50 mm) of *O. angasi* and small and large *M. galloprovincialis*. For *O. angasi*, laboratory experiments showed that both groups of oysters completely survived (100%) an immersion for 30 s in 5% acetic acid heated to 40°C. The 50-mm group also completely survived (100%) after 10 s in 2% acetic acid heated to 50°C, but the same treatment decreased the survival of the 15-mm group to ~40%. Both groups of oysters showed 0% survival after an immersion for 30 s in 2% acetic acid heated to 50°C (Sievers et al. 2019). Heating acetic acid to 50°C in laboratory

experiments also reduced survival of small and large *M. galloprovincialis* (Sievers et al. 2019). Sievers et al. (2019) observed that 100% of small and large *M. galloprovincialis* survived immersions for 30 or 10 s in 2 or 5% acetic acid heated to 40°C, but an immersion in 5% acetic acid heated to 50°C reduced survival to ~75% after 30 s and ~10% after 10 s, for large and small mussels, respectively. However, for large *M. galloprovincialis*, Sievers et al. (2019) obtained ~40% survival when immersed for 10 s in the same treatment (5%, 50°C) while a 10 s-immersion at 40°C in 5% acetic acid ensured 100% survival of small mussels. An increase in immersion time to 30 s was enough to decrease survival to 80%. At concentrations of 2% acetic acid, survival of small *M. galloprovincialis* was 0% after a 30 s immersion at 50°C (Sievers et al. 2019).

Acetic acid was the treatment for which the most information was available for treating *O. edulis* and *O. angasi* (proxy for *O. edulis*), albeit it was only two papers (Carver et al. 2003; Sievers et al. 2019). In laboratory trials, immersions for 30 s in 2 and 5% acetic acid assured 100% survival of *O. angasi* (Sievers et al. 2019). Also in laboratory trials, Carver et al. (2003) found that a 1-min immersion in 5% acetic acid had no impact on a 20-mm group of *O. edulis* but impacted a 10-mm group (both small groups). Under field conditions, Carver et al. (2003) observed 80% survival of small *O. edulis* after a 30-s spray of acetic acid (5%) followed by a 30-s air-drying period, while large oysters were not impacted.

As for large *C. virginica*, Carver et al. (2010) showed that only 44% survival was obtained after a short (30 s) immersion in 5% acetic acid at a culture site while treating shells for boring sponges. They also found that the species was impacted after an immersion for 10 min in 10% acetic acid or 5 min in 20% acetic acid, noting a decline in large *C. virginica* survival (unquantified) (Carver et al. 2010).

In floating oyster culture trays, Rolheiser et al. (2012) observed 60% survival of large *C. gigas* following an immersion for 30 s in 4% acetic acid, while a 5 min immersion resulted in 0% survival. At lower concentrations, an immersion for 30 s at 1.25% acetic acid had no impacts (100% survival) on large *C. gigas*, but immersions of 1 (1.25% acetic acid) and 10 min (0.25% acetic acid) resulted in 80 and 60% survival of oysters, respectively (Rolheiser et al. 2012). Following exposure to a range of concentrations of acetic acid (1, 2, 4, and 8%) and immersion times (15, 30, 45, and 60 s) under laboratory conditions, Cahill et al. (2021) observed 100% survival of small *C. gigas* and no impacts over months after all the treatments. They also observed under field conditions that 100% of small *C. gigas* survived an immersion in 4% acetic acid for 30 s (Cahill et al. 2021) and suggested that this (4%, 30 s) was a promising treatment to ensure species survival (Cahill et al. 2021). The Coutts and Forrest (2005) study on wrapped wharf piles, *C. gigas* (size not specified but assumed to be large) was a non-target species, but was not impacted and survived 10-min exposures to acetic acid concentrations ranging from 1 to 5%.

No information was found on the impacts of acetic acid treatments on *A. irradians*, or *P. magellanicus*.

3.2.2.3. Citric acid immersion (with and without heat)

All results on citric acid were extracted from Sievers et al. (2019), whose purpose was to treat cultured bivalve biofouling. Among multiple treatments, they tested impacts of non-heated and heated citric acid on *O. angasi* (proxy for *O. edulis*) and *M. galloprovincialis*.

Sievers et al. (2019) also found that large *M. galloprovincialis* had 100% survival following 2% citric acid immersions for 30 s and 10% for a shorter duration of 10 s. A 30-s immersion in 10% citric acid decreased the survival to ~50–60%. Heating the solution did not impact the mussels as 100% survival was observed for large *M. galloprovincialis* immersed for 30-s in 10% citric

acid heated to 40°C. They also survived (100%) 10 s at a lower concentration (5%) citric acid, while simultaneously increasing the temperature to 50°C. However, large *M. galloprovincialis* had only 50–60% survival following a 10- or 30-s immersion in 10% citric acid heated to 50°C. Complete survival (100%) was observed for small *M. galloprovincialis* after a 10-s immersion in 10% citric acid, but an increase in exposure time to 30 s decreased survival (~60%). Small *M. galloprovincialis* were more vulnerable to heated citric acid compared to large ones. In a 10% citric acid immersion heated to 40°C, small *M. galloprovincialis* completely survived (100%) a 10-s immersion, but a longer one (30 s) reduced survival to only 30–40%. A 30-s immersion in 5% citric acid heated to 40°C gave a range of survival values (50–55%). Furthermore, increasing concentration of citric acid to 10% and temperature to 50°C with a 10-s exposure decreased the survival of small *M. galloprovincialis* to approximatively 15%. Zero percent survival of small *M. galloprovincialis* was recorded after being immersed for 30 s in 2% citric acid solution heated to 50°C.

Sievers et al. (2019) also tested the treatment on two size groups (15 and 50 mm, small category) of *O. angasi*. Both size groups fully survived (100%) an immersion for 30 s in 10% citric acid, but the 50-mm group showed inconsistent results as survival dropped to 75% when the immersion time was reduced to 10 s. As for heated citric acid, both size groups survived at 100% in a 2% citric acid immersion heated to 50°C for 10 s. At a concentration of 10%, the oysters survived a 30-s immersion only when the temperature was lowered to 40°C. However, *O. angasi* did not survive immersions in 2% citric acid at 50°C for 30 s.

3.2.2.4. Brine immersion (with and without air drying)

Most shellfish easily survived brine treatments, resulting in survival close to 100% (MacNair et al. 2006; Carver et al. 2010; Rolheiser et al. 2012; Landry et al., DFO, unpubl. data). In a laboratory environment, immersions in 300 ppt saturated brine for 15 min resulted in high survival (98–100%) of small *M. edulis* (Landry et al., DFO, unpubl. data). Adding 1 h of air drying after that treatment (300 ppt, 15 min) had no impact on survival (100%) (Landry et al., DFO, unpubl. data). Immersions in 300 ppt brine for 1 min and 30 s followed by 1 h and 24 h of air drying, respectively, had no impacts (100% survival) on *M. edulis*, although size of the mussels was not specified (MacNair et al. 2006). However, Landry et al. (DFO, unpubl. data) observed that survival of small *M. edulis* decreased to 77–82% after an immersion for 30 min in 300-ppt brine. Mallet et al. (Mallet Research Services Ltd., unpubl. data) showed that, under laboratory conditions, survival of small (3–18 mm) mussels decreased to 83% after a 6-min immersion in 300 ppt saturated brine and to 3% when the same immersion was followed by air drying for 24 h. Their results showed that brine immersion (83% survival) or air drying (92% survival) alone resulted in greater survival of mussel spat than when the two treatments were combined. According to Bourque and Mayrand (2007), survival of small (9–15 mm) *M. edulis* on collectors that were immersed for up to 60 s in a 300 ppt brine solution under field conditions varied depending on temperature and ranged from 84% (25°C) to 95% (18°C). Sharp et al. (2006) assessed the impacts of brine immersion on small *M. edulis* 24 and 48 h post-treatment in a laboratory setting and observed that 30 s in 300 ppt brine had no impact on them. Under field conditions, MacNair (2009) observed that treating mussel spat collectors with 300 ppt brine immersions for 15 s also had no impacts on small *M. edulis* and noted that treated mussel seed were larger at the end of trials compared to untreated ones. Adding an air-drying period after immersion in brine seems to decrease survival of small *M. edulis* in two studies. Carman et al. (2016) obtained 70–92% survival for small *M. edulis* after an immersion for 20 s in 70 ppt brine followed by 1 h of air drying in laboratory conditions. To treat spat collectors attached to longlines to kill tunicates under field conditions, a 300 ppt saturated brine immersion for 10 min followed by 24 h of air drying reduced survival of *M. edulis* (size was not specified) to 61% (MacNair et al. 2006). The same study showed that some *M. edulis* mortality occurred

(unquantified) in one trial involving an immersion for 2 min (300 ppt), followed by 1 h of air drying (MacNair et al. 2006). Vickerson (2009) showed that small *M. edulis* were not impacted following an immersion for 30 s in saturated (300 ppt) brine followed by an air exposure of 24 h, simulating transport conditions (4°C, 100% RH). The same author showed that reversing the air-exposure step (24 h) and the immersion (30 s) also had no impact on small *M. edulis* survival. The DFO PEI ITC protocol recommends treating small mussels for colonial tunicates with a 30-s immersion in 300 ppt brine followed by 1 h of air drying prior to transfers.

All results for *C. virginica* showed very high survival (>90%) following brine immersions. Under field conditions, 100% of large *C. virginica* in meshed sleeves survived a single brine immersion (270 ppt) for 10 min and an immersion for 6 min followed by an18-h period of air drying (Carver et al. 2010). Laboratory trials by Landry et al. (DFO, unpubl. data) showed 100 and 90% survival for small *C. virginica* after immersions in 300 ppt saturated brine for 15 and 30 min, respectively, and 100% after 30 s followed by 1 h of air drying. Mallet et al. (Mallet Research Services Ltd., unpubl. data) showed that small (spat, 1–2 mm) *C. virginica* completely survived (100%) after an immersion in saturated brine (300 ppt) for 6 min, but adding an air-drying period of 24 h resulted in no survival (0%). MacNair and Smith (1999) investigated the effectiveness of brine and lime treatments to kill *C. fragile* and *Molgula* sp. on oyster spat collectors and observed that 100% of small *C. virginica* survived when immersed in saturated brine (300 ppt). Furthermore, small *C. virginica* were not impacted after an immersion in a 300 ppt brine solution for up to 10 min, according to Gill et al. (2008), whose purpose was to kill oyster drills by exposing them to several treatment types. Only one result was available for small *O. edulis*, where an immersion for 1 h in saturated brine (300 ppt) assured 100% survival of the oysters (Minchin and Duggan 1988). As for *P. magellanicus*, results from Landry et al. (DFO, unpubl. data) indicate lower survival compared to other shellfish. In laboratory experiments, they obtained 87–89% and 24% survival after immersions for 1 and 5 min in 300 ppt brine, respectively (Landry et al. DFO, unpubl. data).

Only one report provided results on impacts of saturated brine solutions on large *M. galloprovincialis*. Asgari and Jahangard (2012) examined survival under laboratory conditions with treatments to eliminate polychaetes and showed that 100% of large *M. galloprovincialis* survived a 20-min immersion in 350 ppt brine, but extending the treatment duration to 30 min reduced survival to 96.3%. In addition, the same treatment (350 ppt, 20 min) had impacts (79% survival) on large *M. galloprovincialis* under field conditions (Asgari and Jahangard 2012). They also tested the impacts of a 350 ppt brine solution chilled to -20°C on large mussels, but found that even at very short exposure times (5 and 10 s of exposure) survival dropped to 83% and 10%, respectively.

Rolheiser et al. (2012) reported 100% survival of large *C. gigas* when they were immersed for up to 10 min in 70 ppt brine solution at a shellfish aquaculture site. Ruellet (2004) showed that survival of large *C. gigas* decreased to 75% after an immersion for 30 min in 300 ppt saturated brine followed by several hours of air drying (overnight). Another study showed that under field conditions, 100% of small *C. gigas* survived an immersion for 1 h in saturated brine (300 ppt) (Minchin and Duggan 1998). No information was found on the impacts of brine on *A. irradians*.

3.2.2.5. Saturated brine and hydrated lime immersion (with air drying)

Immersion in a solution of saturated brine (300 ppt) and hydrated lime (4%), followed by a period of air drying, is recommended by DFO's PEI ITC for treating *C. virginica* to mitigate for colonial tunicates prior to transfers. As suggested by the protocol, large and small *C. virginica* are not impacted after an immersion for 30 s in the brine and lime solution followed by 1 h of air drying.

3.2.2.6. Hydrated lime immersion (with and without air drying)

Ramsay et al. (2014) executed field trials to test hydrated lime treatments as a management strategy for mussel growers to kill *S. clava*. They showed that 100% of small *M. edulis* on spat collectors survived immersions in 4% lime for 1–2 min. Large *M. edulis* survived (85−90%) an immersion in 4% lime for 1 min (MacNair, PEI DFC, unpubl. data). In similar field conditions, MacNair et al. (2006) obtained 98% survival of socked *M. edulis* (size not specified but assumed to be large) after a 15-s immersion in 4% hydrated lime. Using the same treatment (4%, 15 s) under field conditions, Gill et al. (2007) obtained similar results to those of MacNair et al. (2006) on small socked *M. edulis* with survival of 98–100%. In laboratory experiments, most results from different trials showed lower survival for small *M. edulis*, ranging from 31 to 47% and from 22 to 23% after immersions in 4% hydrated lime for 30 and 15 min, respectively. Locke et al. (2009) suggest that, according to MacNair (pers. comm.), mortality occurred for socked *M. edulis* (size not specified but assumed to be large) after immersions in 4% hydrated lime for 1 min and that mortality increased if the valves of the mussels remained open during treatments (which also applies for other immersion treatments). Through several laboratory trials, Vickerson (2009) showed that an immersion for 30 s in 4% lime caused stress and started to impact small *M. edulis*, where they observed the byssal attachment activity decreased compared to their control (24 h post-treatment). The mussels were directly returned to water after the immersion and impacts were observed 24 h post-treatment (Vickerson 2009). The same author also tested the addition of an air-exposure period of 24 h in cold and humid conditions (4°C, 100% RH) before and after a 30-s immersion in lime (4%). In contrast to the immersion alone treatment (30 s), they observed no impact on the byssal attachment activity when the air- exposure step (24 h) was done before or after the immersion.

In preliminary laboratory experiments, Comeau et al. (2017) showed that 100% of *M. edulis* (size not specified but assumed to be large) survived repeated 30-min 4% hydrated lime exposures over a 3-h period for 3 consecutive days (albeit with few individuals and varying lime concentrations). By conducting experiments within a portable laboratory stationed dock side, Comeau et al. (2017) showed that 100% of large *M. edulis* survived after having been subjected to manual sprays of 4% hydrated lime for 5 s followed by 90 s of air exposure. Landry et al. (DFO, unpubl. data) also observed a high survival of 98% for small *M. edulis* after an immersion in 4% hydrated lime for 30 s followed by a 1-h air-drying exposure.

Hydrated lime did not result in observable impacts on *C. virginica*, where immersions in 4% solutions under laboratory or field conditions for 1 to 30 min had no impacts (i.e., 100% survival) of large and small oysters (MacNair and Smith 1999; Gill et al. 2008; Locke et al. 2009; Carver et al. 2010; Landry et al., DFO, unpubl. data). Comeau et al. (2017) did not detect any mortality (100% survival) of C. *virginica* and *A. irradians* (sizes not specified but assumed to be large) in their preliminary laboratory experiment 14 days following repeated 30-min 4% hydrated lime exposures over a 3-h period for 3 consecutive days. However, these latter results were based on very few individuals (maximum of 6 per species) and the concentrations of lime varied during this trial (Comeau et al. 2017). Small *C. virginica* had 100% survival in laboratory experiments where they were immersed in 4% hydrated lime for 30 s followed by 1 h of air drying (Landry et al., DFO, unpubl. data). Hydrated lime appeared to be the most harmful to *P. magellanicus* compared to other shellfish, as observed in laboratory experiments conducted by Landry et al. (DFO, unpubl. data). The authors showed that immersion in a 4% solution for 30 s reduced the survival of small scallops to 37% (scallops pre-acclimated to 10°C) or 14% (pre-acclimated to 4°C).

Rolheiser et al. (2012) tested different hydrated lime concentrations (1, 2, and 4%) and immersion times on large *C. gigas* in laboratory (1 and 5 min exposure times) and field conditions (0.5, 1, 5, and 10 min) at an aquaculture site in submerged trays attached to long

lines. They found that 100% of large *C. gigas* survived an immersion in 4% hydrated lime for 5 min in the laboratory. In contrast, immersions in 4% hydrated lime for 30 s and 1 min in the field decreased the survival of the oysters to 80% and 40%, respectively. Oysters subjected to only 2% hydrated lime for 10 min in the field had 100% survival. Switzer et al. (2011), also using submerged oyster trays at an aquaculture site, achieved only 64% survival of large *C. gigas* after 4 min of immersion in 4% hydrated lime.

No information was found on the impacts of hydrated lime on *O. edulis* or *M. galloprovincialis*.

3.2.2.7. Virkon®

Only one publication provided results on Virkon® and its impacts on shellfish. Paetzold and Davidson (2011) observed, in laboratory experiments, that 100% of *M. edulis* (size not specified, but assumed to be large) survived an immersion in a 1% Virkon® solution for 60 s. However, increasing the concentration to 3% reduced survival to 94.4 and 83.3% after 30 and 60 s, respectively. No information on impacts of Virkon® was found in the literature for other cultured species.

3.2.3. Impacts of physical and chemical treatments on moved macroalgae

Only one report was found on the impacts of treatments on survival of cultured macroalgae in Canada, which described a disinfection method using sodium hypochlorite for *Saccharina* spp. in a hatchery setting (Tamigneaux et al. 2013). Control strategies on cultured macroalgae or seaweed covered in the literature are mostly alternative or preventive methods (see section 3.3.; e.g., biocontrol, manual cleaning, choice of location) to minimize the impact of biofouling species on cultured macroalgae (e.g., Bannister et al. 2019). A few publications provided information on physical and chemical treatments tested on macroalgae cultured elsewhere (not in Canada) (Yan et al. 2011; Li et al. 2018a; Meichssner et al. 2020; Du et al. 2021; Kang and Kim 2022). The effectiveness of some treatments on AIS or other macroalgae, described in Section 3.1, were also considered in the present section as examples of potential impacts of these treatments on macroalgal survival (even if they are not cultured macroalgae) (Table 11).

3.2.3.1. Air drying

For the main purpose of keeping macroalgae alive while removing most of the fouling organisms from the thalli, Meichssner et al. (2020) tested air-exposure treatments on cultured *F. vesiculosus* and *F. serratus* over a whole season at an aquaculture site. They showed that growth of macroalgae kept in baskets was slightly impacted (not more than 10–20% of algal wet weight loss) if they were air dried three times a week through a whole season, either in a shaded area or during nighttime to avoid direct sun exposure and extreme temperatures. According to aquaculture protocols for the red alga *Pyropia yezoensis*, air drying by emersion may be used as a control method for epibionts (Li et al. 2018a). That species is highly resistant to desiccation (2 to 40 min) and can recover after only 1 h of rehydration (return to seawater) as indicated by measured optimum photochemical efficiency (Li et al. 2018a). Indeed, air-dried thalli fully recovered even after incurring relative water losses between 40 and 70%. In another laboratory study, Du et al. (2021) observed a rapid decrease in photosynthetic activity with increased water loss when thalli of *P. yezoensis* were treated by air drying. However, recovery of thalli after this treatment was not indicated. Kim and Garbary (2007) showed that a 1-h air-drying treatment reduced the survival (lost 10% of their mass) of the invasive alga *C. fragile*, and survival of *U. pinnatifida* was also impacted (~40% survival) after a 6-h exposure to air at 20°C (Forrest and Blakemore 2006).

3.2.3.2. Freshwater immersion

Smit et al. (2003) showed that freshwater immersions for 3 h under laboratory conditions caused no visual damage to the red macroalga *Gracilaria gracilis*, but a significant decrease in growth rate was measured 1 week post-treatment. Thalli of the invasive green alga *C. fragile* exposed to a 3-h freshwater immersion (0 psu; followed by a 60-h recovery period) or to a 3-h immersion in a hyposaline solution (8 psu; 50-h recovery period), completely recovered after being returned to full seawater (Kim and Garbary 2007). In another study, Forrest and Blakemore (2006) observed 0% survival of plantlets of the invasive seaweed *U. pinnatifida* after a freshwater immersion for 10 min.

3.2.3.3. Hot seawater immersion

Heated seawater immersions can have strong impacts on macroalgal survival (Mineur et al. 2007; Forrest and Blakemore 2006; Landry et al., DFO unpubl. data). Williams and Schroeder (2004) found almost all fragments of the invasive seaweed *C. taxifolia* died following a 1 hr immersion in seawater at 72°C, whereas *C. fragile* was impacted following an immersion for 30 s in seawater at 50°C (Landry et al., DFO, unpubl. data). Hot (35°C) seawater exposure started to have impacts on survival (80% survival) of *U. pinnatifida* after approximately 1 min (Forrest and Blakemore 2006), while an immersion for 3 s in 80 to 85°C seawater also negatively affected *Ulva* spp. survival (Mineur et al. 2007).

3.2.3.4. Sodium hypochlorite immersion (with or without hand cleaning and air drying)

Sodium hypochlorite is used on the kelps *Saccharina latissima* and *S. longicruris* in Canadian hatcheries in a two-step process. The first step is to physically clean fouling organisms (e.g., epiphytic macroalgae, bryozoans, tube worms, hydrozoans, gastropods, barnacles) from the kelp sori by hand picking and wiping with cotton or absorbent paper (Tamigneaux et al. 2013; Clark, Cascadia Seaweed Corp., unpubl. data). After this hand-cleaning step, one of the options consists of immersing the sori for 1 min in 0.1% sodium hypochlorite (diluted with sterilized seawater), then rinsing multiple times in sterilized seawater before leaving the sori to air dry for 12−16 h (Clark, Cascadia Seaweed Corp., unpubl. data). The alternate option consists of a 2-min immersion in 0.003% sodium hypochlorite, followed by a rinsing step (duplicate rinse in separate baths) using sterilized seawater, before manually wiping the sori dry with blotting paper (Tamigneaux et al. 2013).

The only other macroalgal treatment study found in the literature using sodium hypochlorite showed that, 7 d post-treatment, this chemical had no detectable acute effects (100% survival) on fragments of the seaweed *C. taxifolia* at a concentration of 0.001% after 30 min of immersion (Williams and Schroeder 2004). However, the authors observed mortality occurring after 14 d, reaching 40% mortality at 77 d post-treatment suggesting a chronic effect.

3.2.3.5. Acetic acid immersion or spray

Forrest et al. (2007) showed that immersions in 2% acetic acid for 1−2 min did not impact *Cladophora* sp. survival. The same authors also showed that an immersion (2% acetic acid) for 1−2 min followed by a 24-h air exposure did not impact the survival of the species, as long as a rinse was applied between the immersion and the air exposure. However, when the 24-h air exposure occurred prior to the same treatment, *Cladophora* sp. did not survive (qualitative data). Forrest et al. (2007) assessed the survival of gametophytes of *U. pinnatifida* 2 weeks post-treatment after immersion in 2% acid acetic for 1 min (followed by a post-treatment seawater rinse) and found no impacts on this macroalgal species (100% survival).

One comprehensive experiment investigated the effects of 5% acetic acid spray on 11 macroalgal species, which included *U. pinnatifida*, *U. linza*, and various Rhodophyta species

(Piola et al. 2009). Those authors demonstrated that a 5% acetic acid spray for 1 min, followed by a 1-min air-drying exposure, eliminated (absence) all macroalgal species, except for *U. linza* (Piola et al. 2009).

3.2.3.6. Citric acid immersion

The only study in the literature that described the treatment of a cultured macroalga (*Porphyra haitanensis*) with citric acid (pH=2.0) showed that a 3-min immersion resulted in 90.9% survival (Yan et al. 2011).

3.2.3.7. Brine immersion

A study on the photosynthetic response (sub-lethal impacts) of *P. yezoensis* to high salinity immersions showed that this macroalga had 30 and 40% water loss after a 10-min immersion in brine solutions of 80 and 100 ppt, respectively (Du et al. 2021). Although optimum photochemical efficiency was not inhibited at 40% water loss, other photosynthetic parameters were interrupted, which indicates some level of impact (albeit not described in Du et al. 2021). The same authors also observed that a brine solution ≥100 ppt concentration caused obvious thallus deformation. Sharp et al. (2006) and MacNair (2009) showed that a 15-s immersion in a 300-ppt brine solution had impacts on the survival of *Cladophora* sp. under laboratory conditions. In addition, MacNair (2009) mentioned that shorter immersion times greatly reduced the negative impacts on the alga. Mineur et al. (2007) tested a brine treatment (400 ppt, 30 min) on macroalgal survival, but only mentioned that this chemical significantly reduced the survival of macroalgal assemblages and that only a few resistant taxa were able to survive, including *Cladophora* spp., *Ulva* spp., and *Porphyra* sp.

3.3. PROACTIVE BIOFOULING MANAGEMENT OPTIONS: SPATIAL AND TEMPORAL PREVENTION STRATEGIES, AND OTHER HUSBANDRY PRACTICES

Although a combination of several AIS-mitigation strategies contributes to sustainable, integrated pest management in marine aquaculture systems (see Cahill et al. 2022), the focus of the present literature review was to provide information on existing reactive methods used to mitigate the risk of spreading epibiont invertebrate and macroalgal AIS during shellfish and macroalgal movements and to assess the effectiveness of these methods to kill or remove AIS biofouling quickly. Consequently, the effectiveness of other proactive biofouling management methods—which generally require a longer time to have an impact on undesired AIS, such as antifouling coatings and bioactive netting (copper alloy), biological controls, preventative measures such as seasonal maintenance of aquaculture gear and equipment, manual/mechanical removal, and repetitive treatments—were not assessed here. Nevertheless, some of these proactive measures could be applied prior to transport of moved organisms or used in conjunction with reactive physical and/or chemical treatments to remove AIS from shellfish and macroalgae mentioned in Sections 3.1.1 and 3.1.2. Several of these proactive biofouling management strategies may potentially reduce the presence and density/abundance of AIS on cultured species, thereby augmenting the effectiveness of physical and chemical treatments at removing AIS when cultured species are moved. Consequently, these methods are briefly mentioned below.

3.3.1. Spatial and temporal prevention strategies

One of the foremost preventative biofouling measures for bivalve and macroalgal culture is to avoid placing culture leases in locations where biofouling risks are high (Sievers et al. 2014; Watts et al. 2015; Bannister et al. 2019), but this is often not feasible as aquaculturists are often constrained by permitted space allocations. However, choosing culture areas with increased

water movement seems to reduce the biofouling infestation on cultured product (Bannister et al. 2019; IOC-UNESCO and GEF-UNDP-IMO GloFouling Partnerships 2022). Since water temperatures and phytoplankton blooms influence the larval settlement period of fouling species, a common practice in shellfish and macroalgal culture in temperate climates is to conduct harvesting of cultured product in the spring before sea temperatures rise and phytoplankton blooms occur, when feasible, to avoid large fouling events (Getchis 2014; Stévant et al. 2017; Bannister et al. 2019; IOC-UNESCO and GEF-UNDP-IMO GloFouling Partnerships 2022). Choosing healthy macroalgal seedlings, free of biofoulers, as well as timing the out-planting of the macroalgae, are common industrial seaweed farming practices used to control biofouling impacts for the duration of cultivation (Bannister et al. 2019). Year-round seaweed cultivation in some areas is limited, as some seaweed producers are forced to harvest in May-June to avoid heavy biofouling by epiphytes in the summer (see Stévant et al. 2017 for details).

Direct avoidance of natural peak recruitment periods of biofouling organisms may not be feasible in areas where fouling pressure is persistent, but this mitigation strategy may be useful in locations where the settlement timing of certain fouling species is predictable. Direct preventative measures in off-bottom oyster culture, such as raising the height at which oysters are grown (to above the mid-tide level or at least 0.5 m above the mud substratum), seems to reduce continued mud blister worm infestations (Bower 2004; Nell 2007), especially in areas with good tidal range and flushing (Nell 2007). Indirect, preventative mitigation measures, such as retrieval and transfer of shellfish, should be conducted in spring before new biofouling settlement occurs (Medcof 1961). Modifying the timing of culture routines to avoid peak fouling recruitment and/or temporarily removing cultured product from the depth at which fouling organisms prefer to settle has been used as a preventative mitigation measure for both bivalve and macroalgal culture (Arakawa 1980; PEI DAFA 2003; NB DAA 2008; Getchis 2014; Sievers et al. 2014; Atalah et al. 2017; Bannister et al. 2019). In addition, the postponement of bivalve grading and re-socking—until after peaks of intense biofouling settlement—can further reduce the amount of fouling on culture gear and product (Sievers et al. 2014).

Increasing stocking densities of cultured species has also been shown to decrease AIS recruitment, including the reduction of barnacle fouling rates on cockles (Dunham and Marshall 2012) and clubbed tunicate abundances on blue mussels (N. MacNair, PEI DFC, unpubl. data), as well as being used in seaweed farming to allow fronds to outcompete or exclude potential nuisance biofoulers (Getchis 2014). Medium *M. edulis* density stocking (~250 individuals per 30 cm of mussel sock), compared to low (90 individuals per 30 cm of sock) and high (500 individuals per 30 cm of sock) density stocking, was associated with the lowest biomass of newly settled *C. intestinalis* during the summer months (Ramsay et al. 2008). Varying the stocking density (226, 453, and 679 individuals/m^2) for grow-out of *C. gigas*, however, did not significantly reduce biofouling intensity (Marshall and Dunham 2013). Even though the impact of increased shellfish stocking density on biofouling rates and abundance may vary with a number of factors (e.g., cultured species, temperature, season, life stage), this proactive husbandry practice may help reduce the intensity of bivalve/macroalgal biofouling at aquaculture sites, thereby improving the likelihood that effective chemical treatments will kill whatever biofouling remains on cultured species prior to their movement for introduction and transfer purposes.

Other standard husbandry practices to control AIS in marine bivalve culture in Canada include sinking cultured bivalves in deeper waters and periodic rotating or flipping shellfish culture enclosures at the surface to promote regular desiccation of biofouling. Sinking long-line mussel (*M. edulis*) socks for natural de-fouling by rock crabs (*Cancer irroratus*) was tried and believed to be an efficient biocontrol method by some mussel growers as they visually observed reduced fouling on mussels in the field (Leblanc et al. 2003). Consequently, this preventative biofouling

control method was commonly conducted two to three times during the growing season by the PEI mussel aquaculture industry. Although mussel wet weight was increased and shell length was longer for de-fouled mussel socks compared to control ones, this method resulted in mussel condition indices from de-fouled socks being lower than controls and the method was proven inefficient at significantly reducing fouling on mussels over a 7-month period (LeBlanc et al. 2003). Although, the periodic rotating or flipping of shellfish enclosures may be more efficient at reducing fouling on shellfish and/or culture gear than altering depth of shellfish and gear for natural biocontrol, it is mostly restricted to robust cultured species such as oysters. In New Brunswick and PEI, native fouling organisms are thus controlled by some oyster culturists by flipping over oyster enclosures 1 d (full 24 hr) per week from May/June to October, thus exposing various sides of the enclosures and oysters to air exposure (Gill et al. 2008; Mallet et al. 2009). The overturning of the OysterGro® system, as well as oyster bags, proved efficient at controlling not only invasive *B. violaceus*, *S. clava*, and *C. intestinalis*, but also native fouling on oysters and culture gear in PEI in most environments, with minimal oyster mortality (<5%) (Gill et al. 2008). In areas with heavy barnacle settlement, flipping floating bags to allow for surface desiccation was effective to eliminate settled barnacles (*Balanus improvisus*) and mussels (*M. edulis*) on oyster (*C. virginica*) bags, but not harmful for the oysters within the bags (Mallet et al. 2009). Turning floating oyster bags post-barnacle settlement (once in mid-August) and post-mussel settlement (once in mid-October) was recommended as likely sufficient to control most of the biofouling on oyster bags (Mallet et al. 2009). In subsequent trials (2012−2014), Mallet (Mallet Research Services Ltd., unpubl. data) determined that the optimal flipping frequency to eliminate fouling barnacles, mussels, and oysters on cultured oysters was every 2 weeks, but noted that oyster growth was impacted. Based on their results, the most recommended desiccation protocol for biofouling control is to flip the OysterGro® cages for a desiccation period of 48 h for large (>50 mm) oysters, while floating bags containing small (<50 mm) oysters can be flipped for only 24 h every 3 weeks during June to September. This preventive procedure must be done while taking into account the biofouling intensity and avoiding excessive temperature stress by reducing the exposure time when air temperatures exceed 25°C (Mallet, Mallet Research Services Ltd., unpubl. data). In some areas with heavy AIS tunicate fouling, Gill et al. (2008) showed that flipping oyster floating bags over bi-weekly, once tunicates begin to settle, should keep floating bags clean and free of fouling. While this desiccation method may reduce fouling of problematic biofouling species on small to large oysters at an aquaculture farm scale over a longer period of time, it may not be applicable to all life stages (e.g., shellfish spat or smaller juveniles) before these are moved to another waterbody to grow to maturity. When transferring shellfish spat or very small juveniles to another site or farm for grow-out purposes, a different reactive mitigation method (i.e., physical or chemical treatment) for these smaller life stages is most likely required to reduce the risk of AIS introduction or transfer.

3.3.2. Depth gradients and biological control

In suspended bivalve culture, biofouling density tends to decrease with increasing depth (Claereboudt et al. 1994; MacNair et al. 2006; Nell 2007; Fitridge et al. 2012; Watts et al. 2015), presumably because of species-specific settlement requirements, which are related to various environmental drivers such as light intensity, temperature, pressure, food, and nutrients (Cowie 2009). For example, adjusting the depth of macroalgal longlines can minimise settlement or survival of some biofouling organisms (Getchis 2014). In addition to depth gradients, surface orientation may be an important factor to consider as some species used as biocontrol agents were found to be most effective on vertical surfaces compared to diagonal ones or on the underside of surfaces (Atalah et al. 2016).

Management of marine biofouling in aquaculture by means of biological control has shown variable results, depending on the cultured species, culture methods and locations, and the

choice and density of biological control species. Benthic invertebrate fauna may be useful to naturally control biofouling AIS growth. For example, small snails of the Cypraeidae and Lamellariidae families, along with certain crab species, are known to feed on *B. violaceus*, *B. schlosseri*, and other colonial ascidians in Japan (Arakawa 1980). In PEI, mussel socks on longlines are lowered temporarily (7−10 d) in the water column until the socks are just touching the bottom of the seafloor to encourage the removal of fouling, such as the second set of mussel spat, from socks by rock crabs (*C. irroratus*) and sea stars (PEI DAFA 2003). Given that indigenous rock crabs consumed significantly more *C. intestinalis* than invasive green crabs (*C. maenas*) (Carver et al. 2003), lowering fouled mussel socks closer to the seabed (i.e., giving crabs greater access to fouled socks) was presumed to be a potentially beneficial natural biocontrol method for removal of fouling AIS, as well as indigenous ones (MacNair, PEI DFC, pers. comm.).

The grazing effect of the Chilean native rock shrimp (*Rhynchocinetes typus*) was tested on Peruvian scallops (*Argopecten purpuratus*) held in pearl nets, showing that *R. typus* lowered both the percent cover of the invasive bryozoan *B. neritina* by ~25% and densities of the tunicates *C. intestinalis* and *Pyura chilensis* by ~15%, which represented a 50% overall decrease of fouling on the nets (Dumont et al. 2009). Moreover, scallops showed lower mortality and a slightly increased growth when grazing shrimp were present compared to when they were absent (Dumont et al. 2009). The native collector sea urchin (*Tripneustes gratilla*) was found to substantially reduce the abundance of the invasive alga *Kappaphycus* spp. in a semi-controlled environment (fenced enclosures built around three 0.25-m^2 natural reef plots) within a 5-month span (Conklin and Smith 2005). In the Irish Sea, sea urchins (*Echinus esculentus* and *Psammechinus miliaris*) and hermit crabs (*Pagarus* spp.) effectively removed fouling from king scallop (*Pecten maximus*) pearl nets, reducing the fouling weight on the nets by ~50% and significantly reduced hydroid fouling (to between 0 and 10%) on scallop shells (Ross et al. 2004). In fact, sea urchins were deemed the most effective biological control organism tested, removing hydroids (*Tubularia* sp. and *Bougainvillia* sp.) and solitary tunicates (*Ascidiella scabra*) efficiently from scallop netting, thereby eliminating the need for labour-intensive manual cleaning of nets (Ross et al. 2004). Consequently, the polyculture of scallops (*P. maximus*) and sea urchins was suggested as an efficient and environmentally friendly biofouling mitigation method for scallop cultivation (Ross et al. 2004). In Venezuela, the variegated sea urchin (*Lytechinus variegatus*) also reduced fouling on both suspended culture nets (by 74%) and on shells of the pearl oyster (*Pinctada imbricata*) (by 71%) (Lodeiros and García 2004). In British Columbia (Canada), green sea urchins (*Strongylocentrotus droebrachiensis*) have been cultured with mussels (*Mytilus* spp.) to mitigate biofouling on anti-predator nets surrounding the mussels (Sterling et al. 2016). While urchin density did not significantly affect mussel growth, higher urchin densities (90 and 120 individuals/net) reduced fouling intensity (i.e., percent net occlusion) on nets by approximately 40% compared to those with lower urchin densities (30 individuals/net) (Sterling et al. 2016). A Canadian laboratory study found that while the sea urchin *S. droebrachiensis* was efficient at grazing invasive ascidians (*S. clava*, *B. schlosseri*, *B. violaceus*, and *D. vexillum*), it chose to graze upon other preferred food (i.e., kelp) over ascidians when given the choice (Epelbaum et al. 2009). Furthermore, urchin grazing may be less effective at reducing biofouling when fouling is well established; one Canadian study found that *S. droebrachiensis* failed at controlling fouling of the colonial tunicate *D. vexillum* on heavily fouled *C. gigas* (Switzer et al. 2011).

Although grazers may contribute to successful biofouling management for particular shellfish cultivation, they may in some cases also contribute to the accidental spread of some biofouling organisms. Given the optimal environmental conditions in which to flourish, periwinkles (*Littorina littorea*) showed great potential for algal control in oyster *(C. gigas*) culture in northwest Spain by reducing grow-out time, labour, and production costs due to grazing on various macroalgal

species (*Enteromorpha compressa*, *E. prolifera*, *Ulva* sp., *Ceramium nodulosum*, and *Polysiphonia* sp.) (Cigarria et al. 1998). However, periwinkle (*Littorina* spp.) grazing algal fronds of the non-indigenous *Sargassum muticum* in southern England was suspected to have promoted the spread of this alga by severing fertile fragments that went adrift (Critchley et al. 1986).

Trials in China involving the pinspotted spinefoot fish (*Siganus fuscescens*) have been successful at reducing biofouling on bivalves while improving their growth performance over 6 months (Li et al. 2018b). Some fish species, as well as herbivorous invertebrates (e.g., amphipods and isopods), have been used as biological control methods for macroalgal culture (see Bannister et al. 2019 for specific studies and authors) with mixed results.

3.3.3. Manual and mechanical removal methods

Removal of biofouling organisms can be done by manually/mechanically cleaning the cultured organisms and the structures on and in which they are cultivated or simply moving cultured species from fouled to clean structures. Although costly and time-consuming, cleaning or changing culture enclosures (e.g., netting, mesh bags, trays, cages) may benefit some cultured species. For instance, systematic net changing to remove fouling organisms caused a 68% increase in adductor muscle mass of the deep-sea scallop (*P. magellanicus*) in the Baie des Chaleurs (Canada) (Claereboudt et al. 1994). Net changing is the standard practice for dealing with the heavy settlement of *C. intestinalis* and other ascidians in east coast scallop culture, although scallops tend to be more sensitive to stress than various fouling organisms and require being handled with care (Carver, Mallet Research Services Ltd., pers. comm.). Hand-scraping and rinsing with seawater might be the only option for treating scallops prior to movements (Carver, Mallet Research Services Ltd., pers. comm.). Enclosure changing is an established practice in the commercial suspended tray culture of *C. gigas* in British Columbia (Canada).

Manual cleaning by brushing, scraping, tumbling, or pressure washing has been used to remove biofouling invertebrates and macroalgae (Nell 2007; NB DAA 2008; Paetzold and Davidson 2010; Arens et al. 2011a, b; Paetzold et al. 2012; Getchis 2014; Bannister et al. 2019; Hood et al. 2020; IOC-UNESCO and GEF-UNDP-IMO GloFouling Partnerships 2022). Indirect manual cleaning methods (e.g., Wave-Brush), moving freely and independently under the influence of waves and currents, have been used to successfully self-clean stacked oyster containers (Sala and Lucchetti 2008). Direct, regular manual cleaning of biofouling on pearl oyster (*Pinctata* spp.) shells was consistently linked to improved oyster performance with no deleterious effects on their survival (Taylor et al. 1997; Southgate and Beer 1997, 2000). Taylor et al. (1997) recommended monthly cleaning of oysters to maximize growth and reduce shell deformities.

Altering siltation levels near some cultured species may affect their survival or prevent their infestation. For example, smothering was demonstrated as a very effective manual method for killing oyster drills by burying these molluscs in a thin layer of bottom deposit (Loosanoff 1960). The depth at which oyster cages are placed seems to be directly proportional to the level of mud worm infestation (i.e., oysters in cages closest to the seafloor tend to be more infested) (Ruellet 2004). In bottom-grown oyster cultivation, siltation of oysters has shown conflicting results with inconsistent success at curbing mud blister worm infestations (Morse et al. 2015). Furthermore, a few studies have shown that infestations of mud blister worms still occur in surface-cultured oysters, even when water column siltation levels are high (Loosanoff and Engle 1943; Clements et al. 2018).

In Canada, mechanical cleaning of organisms at shellfish farm sites can occur multiple times within the growing season as well as during the final processing of adult shellfish prior to packaging for transport or sale. Typically, mussels may be mechanically or manually cleaned

when spat are collected and harvested for declumping, grading, and re-socking during crop maintenance (double socking as required) and when adults are harvested for sale. Oysters may be similarly cleaned when spat are collected and harvested for grading and re-bagging or moving to larger and/or cleaner enclosures during regular crop maintenance (tumbling and thinning as required) and at final harvest (Ramsay, PEI DFC, pers. comm.). However, manual or mechanical removal of some biofouling organisms in the marine environment can cause fragmentation (Paetzold and Davidson 2012), thus, spreading AIS even further. Due to rapid re-growth of residual tissue and the lack of native herbivorous predators, the invasive alga *Kappaphycus* spp. rapidly recovered after a labour-intensive manual removal in Hawaii (Conklin and Smith 2005). Hand-picking of the non-indigenous alga *S. muticum* in southern England was deemed very labour-intensive and time-consuming (Gray and Jones 1977), thus abandoned as a removal method for large-scale clearance (Critchley et al. 1986). Depending on the ecological sensitivity of affected areas and in the absence of chemical or biological control strategies, Critchley et al. (1986) recommended using mechanical clearance by suction as a removal strategy only as a last resort, since regeneration due to fragmentation could still occur. To prevent re-establishment of undesirable macroalgae, manual removal of *Sargassum horneri* from rocky reefs in southern California (USA) was deemed most effective when conducted over larger areas (>60 m^2) in targeted, novel introduction sites and during cooler-water years favouring growth and production of native algae (Marks et al. 2017).

Even though periodic manual removal of biofouling epiphytes and animals is commonly practiced on infrastructure and/or for some species of cultured macroalgae/bivalves, it remains labour-intensive and may damage cultured species (Switzer et al. 2011; Bannister et al. 2019; see IOC-UNESCO and GEF-UNDP-IMO GloFouling Partnerships 2022 for further references). Consequently, species-specific biological controls are increasingly being considered as the least damaging biofouling cleaning method for cultured algae/bivalves (Bannister et al. 2019; IOC-UNESCO and GEF-UNDP-IMO GloFouling Partnerships 2022).

4. DISCUSSION

Although some proactive methods were briefly mentioned in the present work, this literature review mainly summarizes the effectiveness of existing physical and chemical treatments to remove/kill various AIS (reactive methods) as well as the impacts of such treatments on moved shellfish and macroalgal species. Multiple treatments (e.g., air drying, freshwater, heated seawater, acetic acid, brine) were identified as effective at killing some AIS, while potentially not impacting moved species. However, treatments were fundamentally species- and environment-specific, with large ranges in percent mortality of AIS and survival of moved species relative to duration/intensity/method of application, location, time of year, and species. This context-dependency limits the ability to draw broad conclusions related to the treatment of AIS in Canada's three oceans and their impacts on species of interest that are being moved. In many cases, harsher or more severe treatments are typically required (e.g., hotter temperatures, increased chemical concentrations, and longer exposure times) for a given treatment to be effective at killing the greatest number of target AIS. Based on results of species-specific treatments presented in Tables 5–10, those that met the criteria of being "effective" at killing AIS (100% mortality) while not impacting the moved species (90–100% survival), were identified to help inform future management decisions (Tables 12 and 13 for physical and chemical treatments, respectively). Associated levels of uncertainty are presented for each AIS or functional group for mortality of AIS and survival of moved species.

4.1. LETHAL PHYSICAL TREATMENTS FOR AIS AND EPIBIONTS WITH NO/LOW IMPACTS ON SHELLFISH

Among several options for physical treatments in the literature, only those that were fully effective (100% mortality) at killing a large range of AIS while ensuring survival (90−100%) of moved species were considered for further evaluation and are presented in a summary table (Table 12). Following this selection criteria, at least one treatment option for each physical treatment type (pressure washing, air drying, freshwater, heat) was retained and the most useful treatments for each treatment type are presented (Table 12). For each retained treatment option, results obtained ('effective', 'not effective', or 'no data') for each AIS/taxonomic group and moved species are presented in the summary table, with their associated level of uncertainty (when applicable). Since limited data were found for some physical treatments (i.e., freshwater spray, freshwater spray + air drying, heated freshwater), they could not be considered as viable options to treat a large range of AIS.

4.1.1. Pressurized seawater

Relatively little information on pressurized seawater (either low or high pressures) treatments for marine AIS (or epibionts) was available in the published literature and the limited available data focused primarily on removing organisms from infrastructure (e.g., mussel socks, oyster bags/trays) rather than the effects of the treatment on the mortality of AIS or the impacts on the shellfish. Identifying a specific treatment using pressurized seawater that could be applicable across AIS with no/low impacts (>90% survival) on moved shellfish is thus very challenging (Table 12). Only four publications reported that high pressurized seawater (700–2,000 psi) for various durations (up to 30 s) could be an effective method (but not always 100%) to eliminate some macroalgae and tunicates on cultured shellfish (Coutts 2006; Forrest and Blakemore 2006; Paetzold et al. 2012; Ramsay 2014a). High pressure (2,000 psi) would completely remove young stages of AIS macroalgae (Forrest and Blakemore 2006) and *D. vexillum* (when followed by a 48-h air drying period) (Coutts 2006). However, studies that evaluated the impacts of high pressure washing on survival of moved species mainly used a pressure of 700 psi or lower, which led to the suggestion that this pressure could be a viable option (Table 12). Based on the limited number of studies, high-pressure sprays at 700 psi for 10 s were not effective at eliminating *B. schlosseri* and *B. violaceus*, small and large *M. edulis*, *C. maenas*, gastropods, or sea stars; while a slightly lower pressure of 400−600 psi was found effective on *C. intestinalis*, although no duration for the treatment was provided.

No data on mortality were found for other AIS taxa and on survival of almost all selected cultured shellfish. Curtis et al. (2021), however, found that pressure washing had negative consequences on large oysters grown in clumps on drop lines, where important losses (due to oysters being "blown" off the clumps by the spray) of *C. gigas* were observed with high pressure washing (≥2,000 psi) after only 10 s, and were significantly greater after 30 s. Despite the loss, the authors observed that the oyster shells remained in good condition under a pressure of 2,000 psi, but shells were left in fair/poor condition when increasing the pressure to 3,000 psi. To prevent loss of oysters, they suggested placing nets behind the oyster clumps to catch blown-off individuals during the treatment. They also observed that the oysters were significantly more impacted by the pressure-washing treatment in June compared to July, signifying some seasonality variation in impacts (Curtis et al. 2021) so other factors could also be important if this method were adopted broadly in Canada.

Another negative consequence of pressure washing for the removal of biofouling from cultured bivalves in the marine environment is the fragmentation of removed organisms (Paetzold and Davidson 2010; Paetzold et al. 2012; Curtis et al. 2021), which may increase the risk of the spread of AIS, especially colonial/solitary tunicates. This method was abandoned in some

regions of Canada, since unintended effects observed in trials testing high pressure washing indicated that undamaged *C. intestinalis* had the potential to re-attach to a new substrate, as could damaged individuals, likely enhancing the release of gametes (Carver, Mallet Research Services Ltd., pers. comm.). Therefore, when pressure washing is used as a treatment, control measures are required to avoid the release of AIS propagules into the marine environment.

Pressurized seawater at 700 psi for 10 s was the only option that ensured the survival of at least one moved species (*M. edulis*). This treatment option did not impact small (moderate uncertainty) or large (high uncertainty) *M. edulis*, but also was not effective on AIS for which information was available. Pressurized seawater with greater force (e.g., 2,000 psi) is likely effective on a few AIS (*D. vexillum* and macroalgal gametophytes), but no data were available on the impacts of this treatment on most moved species. Given the lack of data, additional research on the efficacy of low- and high-pressure sprays on marine AIS and on the survival of moved shellfish is required.

4.1.2. Air drying

Air drying is commonly identified in the primary and secondary literature (e.g., technical reports) as a control method for marine AIS. It was found to be effective (albeit with long exposure times) at killing numerous fouling taxa in many different application contexts (Medcof 1961; Arakawa 1980; MacNair 2002; Coutts and Forrest 2005; Davidson et al. 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, including aquaculture transfers. However, the effectiveness of this treatment depends on the abundance of organisms present, the species, life-history stage, and local environmental conditions (temperature, relative humidity). For example, it could take 2 to 8 weeks at 10°C and in high relative humidity conditions to be 100% effective at killing *S. clava* and macroalgae, respectively (Forrest and Blakemore 2006; see review of Hilliard and Polglaze 2006 for examples of air-drying times for specific groups). Such long exposure times cannot be considered efficient or effective in the context of movements of cultured shellfish and macroalgae and shorter durations for air drying exposure treatments need to be investigated further prior to their suggested use.

As a potential lethal treatment for a wide range of AIS taxa with no/low impacts (≥90% survival of moved species), an air drying treatment for 24 h would likely be sufficient to kill *C. intestinalis* (low uncertainty); *B. schlosseri*, *B. violaceus*, and *S. clava* (moderate uncertainty); and *D. listerianum*, *D. vexillum*, *A. aspersa*, small *M. galloprovincialis*, some polychaetes, *Codium fragile*, and some macroalgae (high uncertainty) (Table 12). However, this treatment is likely not effective for small *M. edulis*, large *M. galloprovincialis*, small *C. virginica*, gastropods, barnacles, bryozoans, and sponges. Note that conflicting levels of effectiveness (i.e., ‘Effective’ and ‘Not effective’) from air-drying treatments were found in different studies for the same species or within the same taxonomic group, including for *C. fragile* (MacNair 2002; Kim and Garbary 2007) and various polychaete species (Forrest et al. 2007; Asgari and Jahangard 2012). For example, Kim and Garbary (2007) found that a 17-h air exposure was effective at killing *C. fragile*, whereas a 24-h period was considered ineffective by MacNair (2002). These conflicting effectiveness results for the same species could be explained by the fact that air drying effectiveness is strongly correlated to temperature and humidity conditions, which are likely to between studies. Likewise, conflicting results for polychaetes could be an artifact of grouping all biofouling polychaetes together, including those protected by tubes and/or bivalve shells (boring species) and those that are unprotected by a hardened casing. An air-drying treatment for 24 h would allow the survival of small *M. edulis* and *C. virginica* (moderate uncertainty) and large *M. galloprovincialis* (high uncertainty), although small *M. galloprovincialis* would likely be impacted.

No data were available for many taxa (large *C. Virginica*, small *C. gigas*, *O. edulis*, *A. irradians*, *P. magellanicus*, *C. mutica*, *H. sanguineus*, sea stars, hydrozoans), but large *M. edulis* (with heat addition; Seuront et al. 2019), large *C. gigas* (72 h to 16 d; Hopkins et al. 2016), and *C. maenas* (7 d at 29°C; Darbyson et al. 2009) were killed effectively using extended drying times or by adding heat.

In summary, air drying, followed by heated seawater immersion, were the most studied physical treatments in the literature. The 24-h air-drying option was effective at killing several AIS while impacting few moved species. Mostly based on qualitative results, this treatment option was mainly effective on tunicates, but not on shelled, hard-cased, or calcareous organisms. More research on the impacts of air drying as a treatment for bivalves is required as no survival data were found for the majority of the moved species examined (Table 12).

4.1.3. Freshwater immersion or spray (with and without air drying)

Freshwater treatments have been shown to be generally safe for the cultured species (e.g., *M. edulis* and *C. virginica*) and may be easily applied in some situations and could be a useful tool for controlling numerous marine AIS. Several primary publications and technical reports identified freshwater immersion as an effective treatment to kill tunicates. However, there was no consensus in the literature on effective immersion times to achieve 100% mortality, with times spanning from 3 h up to 24 h among and within tunicate species (Coutts and Forrest 2005; MacNair et al. 2006; Dijkstra et al. 2008; McCann et al. 2013; Ramsay 2015a, b, c; Carman et al. 2016). Overall, a 24-h freshwater immersion treatment would likely be effective at killing numerous marine AIS, including *S. clava*, *C. fragile*, and *C. mutica* (low uncertainty); *B. schlosseri*, *B. violaceus*, various polychaetes (moderate uncertainty), *D. vexillum*, and macroalgae (high uncertainty), and sponges (very high uncertainty) (Medcof 1961; Takeuchi et al. 2003; Forrest and Blakemore 2006; Ashton et al. 2007; Kim and Garbary 2007; Moore et al. 2007; Nell 2007; Jute and Dunphy 2017; Landry et al., DFO, unpubl. data) (Table 12). However, it is important to note that this treatment is likely ineffective at killing small *M. edulis*, small or large *C. virginica*, and *H. sanguineus*. Although a 24-h immersion was not tested on the following species, freshwater immersions were not effective for *C. intestinalis* after 12 h (Ramsay, PEI DFC, unpubl. data), large *M. galloprovincialis* after 30 min (Asgari and Jahangard 2012), small/large *C. gigas* after 12 h (Nel et al. 1996; Nell 2007), *C. maenas* after 1 h (McKenzie et al., DFO, unpubl. data), and hydrozoans after 30 s (Fitridge et al. 2014). Data on mortality for other AIS taxonomic groups were lacking.

In terms of survival of moved organisms, a 24-h freshwater immersion treatment would have no impact (>90% survival or not impacted) on small *M. edulis* (moderate uncertainty) and large (high uncertainty) and small (very high uncertainty) *C. virginica* (Gill et al. 2008; Brown 2012; Mayrand et al. 2015; Ramsay 2015a; Comeau, DFO, unpubl. data; Landry et al. DFO, unpubl. data). Although *C. gigas* (Nel et al. 1996; Rolheiser et al. 2012), *M. galloprovincialis* (Asgari and Jahangard 2012), *O. angasi* (proxy for *O. edulis*; Fitridge et al. 2014), and *P. magellanicus* (Landry et al., DFO, unpubl. data) survived shorter freshwater immersion times (30 s to 12 h), it is unknown whether these species would survive a 24-h immersion treatment, and no data were available in the literature for the other moved species.

A few studies reported that the probability of eliminating colonial tunicates was increased when they were exposed to air drying following freshwater immersion (Denny 2008; Brown 2012; Carman et al. 2016). Based on these studies, a freshwater immersion for 8 h followed by 1 h of air drying could be 100% effective at killing colonial tunicates (*B. schlosseri*, *B. violaceus*, *D. vexillum*, and *D. listerianum*) (high uncertainty), but this treatment was not effective for small *M. edulis* and large *C. virginica* (Brown 2012; Carman et al. 2016). A 10-min freshwater spray followed by 1 h of air drying would likely be effective for the four colonial tunicates (high

uncertainty) but remained ineffective for small *M. edulis*. No information was found for all other selected AIS and epibionts for treatment options adding air drying with freshwater immersion or spray.

In terms of survival of moved shellfish, no data were found on most species, with the exception of small *M. edulis* and large *C. virginica*, which were not impacted (high uncertainty) after an immersion for 8 h followed by 1 h of air drying (Table 12). With different parameters, *P. canaliculus* (proxy for *M. galloprovincialis*) survived a 10-min freshwater immersion followed by 24 h of air drying (Denny 2008). *Mytilus edulis* (small) was the only species for which information was available for a 10-min spray followed by 1 h of air drying and it was not impacted (high uncertainty).

In summary, a 24-h freshwater immersion remains an effective option to control many AIS and the literature suggests very low impacts on mussels and oysters. If colonial tunicates are the target AIS, a freshwater immersion or spray treatment combined with air drying would likely be effective. However, less information on the effectiveness of this combined treatment (i.e., freshwater immersion or spray with air drying) was available for other AIS. In addition, the levels of uncertainty for an 8-h immersion followed by 1 h of air drying as well as the 10-min spray followed by 1 h of air drying increased for both AIS and moved species compared to the longer 24-h immersion without air drying.

4.1.4. Hot seawater immersion

Several studies showed that heated seawater treatments were effective for numerous taxonomic groups of marine AIS and epibionts, but effective temperatures (35 to 90°C) and durations (3 s to 2.7 h) were highly variable across species and studies (Koganezawa 1972; Gonzalez and Yevich 1976; Carver et al. 2003; Williams and Schroeder 2004; Davidson et al. 2005; Rajagopal et al. 2005a, b; Forrest and Blakemore 2006; Mineur et al. 2007; McDonald 2010; Asgari and Jahangard 2012; Best et al. 2014; Sievers et al. 2019; Landry et al., DFO, unpubl. data). Results suggested that temperature, duration, species, and organism size had variable effects on percent mortality, where higher temperatures and longer durations tended to be more harmful, and smaller sizes were usually more vulnerable to heat treatments (Rajagopal et al. 2005b; Asgari and Jahangard 2012; Sievers et al. 2019; Landry et al., DFO, unpubl. data).

Among several options for heated seawater treatments in the literature, only those that were most effective at both killing a large range of AIS and assuring survival of moved species were considered for further evaluation and are presented in a summary table (Table 12). From the three options presented in Table 12 (immersions at 50°C for 60 s, 60°C for 10 s, and 60°C for 30 s), an immersion at 60°C for 10 s seems to be the most effective at eliminating the highest number of AIS while keeping the highest number of moved species alive. Macroalgae (Forrest and Blakemore 2006), *C. intestinalis* (Sievers et al. 2019; Piola and Hopkins 2012), small and large *M. edulis*, and small *M. galloprovincialis* (Koganezawa 1972; McDonald 2010; Sievers et al. 2019) were all effectively killed (moderate uncertainty) using this treatment, as well as juvenile *C. maenas* and hydrozoans, albeit with high uncertainty (Asgari and Jahangard 2012; Best et al. 2014; Sievers et al. 2019). That same treatment could be used to kill sea stars (Medcof 1961) and *C. fragile* (Landry et al., DFO, unpubl. data), although with very high uncertainty. However, this treatment option (60°C for 10 s) would likely be ineffective for *S. clava*, large *M. galloprovincialis*, small and large *C. virginica*, small *C. gigas*, and for various species of polychaetes. No data on mortality associated with heated seawater immersion (60°C for 10 s) were found for *H. sanguineus* and certain selected taxonomic groups (gastropods and sponges) (Table 12).

Although the following were not tested with the same parameters, heated seawater immersion seems to be a promising method (100% mortality) to kill *B. leachii* (proxy for *B. violaceus*), *D. vexillum*, and bryozoans (42.5°C for 20 min; Piola and Hopkins 2012); adult *C. maenas* (32–45°C for 1 h; McKenzie et al. DFO, unpubl. qualitative data); *C. mutica* (30°C for 48 h; Ashton et al. 2007); and barnacles (40°C for 30 min; Leach 2011).

In terms of impacts on the moved species, a few shellfish species are likely to survive (≥90%) if they are exposed to the treatment (60°C for 10 s) before being moved (Nel et al. 1996; McDonald 2010; Piola and Hopkins 2012). Those moved species included small *C. gigas* (low uncertainty), small *C. virginica* (55–65 mm; moderate uncertainty), and large *C. virginica* (very high uncertainty, based on qualitative results). However, the same treatment (60°C for 10 s) would likely have an impact on small and large *M. galloprovincialis* and *M. edulis*, small (35–45 mm) *C. virginica*, and small *O. edulis* (Koganezawa 1972; Fitridge et al. 2014; McDonald 2010; Rousselle 2012; Mayrand et al. 2015; Sievers et al. 2019) (Table 12).

Heated immersions were often short (60 s or less) and decreasing the duration to 10 s required the temperature to be 60°C to effectively kill AIS. Increasing the immersion time to 30 s at 60°C increased the number of taxa that could be killed, however, small *C. virginica* (55–65 mm) falls from a moderate uncertainty of survival to being impacted. According to the uncertainty scores obtained, oysters (treatment not effective to cause mortality) seem to be more resistant than mussels (moderate uncertainty for mortality) to most heated immersions (Table 12). The hotter the water temperature in which bivalves are immersed, the more likely they will be impacted, which, in turn, results in a higher level of uncertainty for their survival. This was especially relevant for small bivalves. Caution is advised when determining immediate versus long-term impacts of heated seawater treatment on shellfish. Indeed, preliminary results from treatment trials in the literature from aquaculture facilities showed that oysters treated with heated seawater (60°C immersion) were highly impacted and often entirely killed because of the long-term effects of treatment (Mallet et al., Mallet Research Services Ltd., unpubl. data; Ramsay, PEI DFC, pers. comm.) – a finding not evident from short duration trials.

4.1.5. Steam

The application of steam (hot seawater spray at 50 psi) at 100°C for 5 min would likely be effective at killing various marine AIS (or epibionts) present on moved species, with moderate to very high uncertainty, including *S. clava*, small *M. edulis*, both sizes of *C. gigas*, barnacles, and several macroalgal species (Davidson et al. 2005; Joyce et al. 2019). However, no information was available for similar treatments on other species or taxonomic groups or on the survival of most moved shellfish (with the exception of small *M. edulis* and both sizes of *C. gigas*, which were impacted; Table 12). As very little information on survival of shellfish to steam treatments was available, additional research is required before this treatment should be considered as an option for treating moved shellfish.

4.2. LETHAL CHEMICAL TREATMENTS FOR AIS AND EPIBIONTS WITH NO/LOW IMPACTS ON SHELLFISH

Although a wide variety of chemical treatments has been tested and found to be effective at reducing certain biofouling species, not all chemical treatment types are feasibly applied in a field environment at shellfish farming scales. Chemical treatment options that were effective at killing multiple AIS (Tables 6−7) and those having no or low impact on the survival of moved shellfish (Tables 9−10) were compared to identify which treatment options could cause 100% mortality on a wide range of AIS while ensuring 90–100% survival of moved species. At least one treatment option for each chemical treatment type (sodium hypochlorite, acetic acid, citric acid, brine, hydrated lime, brine + hydrated lime, Virkon®) was identified and options meeting

those criteria are summarized in Table 13. Results for each AIS/taxonomic group (i.e., effective, not effective, or no data) and moved species (i.e., not impacted, impacted, and no data) are presented in this table with their associated level of uncertainty (when applicable).

4.2.1. Sodium hypochlorite immersion

A wide assortment of sodium hypochlorite treatments with varying concentrations (0.006–5%) and exposure times (5 s to 12 h) were found in the literature (Rajagopal et al. 2002, 2003; Carver et al. 2003; William and Schroeder 2004; Coutts and Forrest 2005; Davidson et al. 2005; MacNair et al. 2006; Denny 2008; Asgari and Jahangard 2012; McCann et al. 2013; Haque et al. 2014, 2015; Haque and Kwon, 2017; McKenzie, DFO, unpubl. data), which rendered the comparability of mortality of AIS and survival of moved shellfish challenging. Furthermore, when sodium hypochlorite is added to seawater, hypobromite ions and hypobromous acid (the primary biocides) are quickly formed, such that any organic matter in the seawater will bind with these oxidants, inactivating them (Taylor 2006), hence diminishing the effectiveness of sodium hypochlorite as a biocide (Piola et al. 2009).

An inverse relationship between sodium hypochlorite concentration and immersion time was found by a few studies on colonial tunicates and blue mussels, suggesting that higher sodium hypochlorite concentrations required shorter exposure times to induce 100% mortality (Rajagopal et al. 2002, 2003; MacNair et al. 2006; Denny 2008; Haque et al. 2014, 2015). However, considering mussel size (or stages) is an important consideration when determining the exposure duration required for 100% mortality, as larger mussels may require greater exposure time compared to smaller mussels to attain this same level of mortality (Haque et al. 2005). Consensus was not obtained from the literature on effective concentrations or immersion times for studies examining higher concentrations, with some conflicting results on the most lethal concentration (~1%) and exposure times for *D. vexillum* (Denny 2008, 100% mortality at 1% for 30 s; McCann et al. 2013, 50−65% mortality at 1% for 5−30 min). Nevertheless, an immersion in 0.5% sodium hypochlorite for 20 s was deemed effective at killing *D. vexillum* (high uncertainty) and *B. violaceus* (very high uncertainty) while an immersion in 0.01% for 12 h may kill *S. clava* (very high uncertainty) (Table 13). Sodium hypochlorite was also effective at killing 100% of the macroalgae under other parameters (0.0125% for 60 min; Williams and Schroeder, 2004) (Table 6). However, the first aforementioned treatment option (0.5% sodium hypochlorite for 20 s) would not be effective at killing small *M. galloprovincialis*, various polychaete species, and *C. maenas*. The second sodium hypochlorite treatment option presented in Table 12 (0.01% for 12 h) was also ineffective for large *C. gigas*. In terms of survival of moved species, small *M. galloprovincialis* and large *C. gigas* are likely to survive this treatment (0.01% for 12 h) with high and very high uncertainty, respectively, and large *M. galloprovincialis* would survive (>90% survival), but under different parameters (0.5%, 30 s–2 min), whereas small *M. edulis* would likely be impacted (0% survival) when exposed to 3 mg/L of TRC (Table 9).

The effectiveness of sodium hypochlorite spray was poorly examined, with only one study (Piola et al. 2009) reporting that a period of exposure to air following a 5-s spray treatment (1% sodium hypochlorite) was effective at killing some tunicate species. However, a lower concentration (0.5% sodium hypochlorite) was generally not effective at killing these tunicates and small mussels, even when followed by a longer period of exposure to air (Piola et al. 2009).

In summary, treatment options for sodium hypochlorite immersions were effective on some species of colonial and solitary tunicates without impacting two moved species (*M. galloprovincialis* and *C. gigas)*, but all results were accompanied with a high or very high uncertainty level. More research on the effectiveness of sodium hypochlorite as a mitigation method for other AIS taxa is required for a better understanding of its overall effectiveness (see taxa with no data in Table 13).

4.2.2. Acetic acid immersion or spray (with and without air drying)

One of the best studied treatments in the literature for killing marine AIS was acetic acid (immersion/spray with or without air exposure). It has been shown to be highly effective for controlling a large number of cosmopolitan fouling species, including solitary and colonial tunicates, mussels (*M. edulis*), oysters (*C. virginica*, *C. gigas*), and *C. mutica*, as well as some macroalgae, bryozoans, hydrozoans, sponges, and polychaete worms (Carver et al. 2003, 2010; Coutts and Forrest 2005; Davidson et al. 2005; MacNair et al. 2006; Sharp et al. 2006; Forrest et al. 2007; Gill et al. 2007; Paetzold et al. 2008; Piola et al. 2009; Rolheiser et al. 2012; McCann et al. 2013; Carman et al. 2016; Chinnadurai et al. 2019; Sievers et al. 2019; Cahill et al. 2021). Although several acetic acid immersion treatment options (4–5% for durations varying from 5 to 30 min; 1–2% for 30 s to 10 min) were tested in the literature (Table 6), only those (4–5% for 30 s, 1 min, and 5 min) and found to be efficient at killing AIS and keeping moved species alive were considered for further evaluation.

Based on studies that showed 100% mortality, an immersion in 4–5% acetic acid for 5 min is likely a good treatment option for killing *C. intestinalis*, *B. violaceus*, small *M. edulis*, and bryozoans (low uncertainty); *D. vexillum, S. clava*, some young stages of macroalgae, hydrozoans (moderate uncertainty); and *B. schlosseri*, large *C. gigas*, sea stars, and polychaetes (high uncertainty), but is not effective at killing small and large *M. galloprovincialis*, small *C. virginica*, and gastropods (Table 12). However, large oysters (*C. gigas* and *C. virginica*) and small and large mussels (*M. edulis*) may be impacted when treating for biofouling species at 4–5% acetic acid for 30 s, or 1 or 5 min (Table 13). No acetic acid immersion data were available for many taxa (several tunicates, *C. maenas*, *H. sanguineus*, *C. mutica*, and *C. fragile*), although that chemical treatment type caused 100% mortality for sponges when using other treatment parameters (10% for 10 min; Carver et al. 2010) (Table 13). Acetic acid immersions at 4–5% for 15 s were considered not effective (10–15% mortality) at killing large *M. edulis* when considered as an epibiont, yet their survival was still considered impacted (85–90%) as it was under the 90% survival threshold requirement for moved species (Table 13). Using other parameters, acetic acid immersions were not effective for large *M. edulis* (4−5% for 5 s; Locke et al. 2009), large *C. virginica* (4−5% for 30 s; Carver et al. 2010), small *C. gigas* (4−5% for 15−60 s; Cahill et al. 2021), and barnacles (4−5% for 1 min; McDonald et al. 2010). The high uncertainty, which reflects conflicting results, suggests that polychaete mortality effectiveness for two treatment options (30 s and 1 min, 4–5%) may be an artifact of grouping all biofouling polychaete species together, including those protected by tubes and/or bivalve shells (boring species) and those that are unprotected by a hardened casing. In terms of survival, small and large *M. edulis*, large *C. virginica*, and large *C. gigas* were impacted by the three treatment options (30 s, 1 and 5 min) in 4–5% acetic acid. Information on some moved species was available for the two shorter durations (30 s and 1 min), where small and large *M. galloprovincialis* and small *O. edulis* survived up to a 1-min immersion with low and moderate uncertainty, respectively. Small *C. gigas* survived a 30-s immersion (low uncertainty), but uncertainty increased to moderate for a 1-min immersion. Effectiveness of acetic acid immersion trials by Sievers et al. (2019) on *M. galloprovincialis* were mainly developed to control solitary tunicates (*C. intestinalis* and *S. clava*) and the hydroid *E. crocea* on cultured mussel socks to reduce their spread during aquaculture activities (e.g., transfers). Consequently, concentrations and exposure times tested were generally low to keep cultured mussels alive. Their results showed that *M. galloprovincialis* completely survived (100%), but after only a very short immersion for 30 s in acetic acid. Noteworthy are the conflicting survival results in the literature for small *C. gigas* exposed to 4–5% acetic acid concentrations for 30 s and 1 min (Cahill et al. 2021), which led to them being placed in the low and moderate uncertainty categories compared to large *C. gigas* being impacted after only 30 s (Rolheiser et al. 2012). Experimental designs and environmental conditions could explain the conflicting

results, but another potential explanation may be the slight difference in concentrations used in each study, where Cahill et al. (2021) used 4% acetic acid on small oysters, Rolheiser et al. (2012) tested 5% acetic acid on larger oysters. Caution is thus advised when choosing duration times and concentrations for a 4–5% acetic acid immersion when treating different sizes of oysters.

Immersions in a higher concentration of acetic acid (4–5% rather than 1–2%) for 4 and 5 min followed by air exposure (24 and 1 h, respectively) were lethal to a wide range of taxa (Table 6). An immersion for 5 min in 4–5% acetic acid followed by 1 h of air drying could be a good treatment option as it caused 100% mortality of tunicates (*B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. listerianum*, *C. intestinalis*, and *A. aspersa*) (Forrest et al. 2007; Carman et al. 2016) as well as 100% mortality of small *M. edulis* (Carman et al. 2016), but with high uncertainty (Table 13). However, there was a lack of data for many taxa for that treatment option (Table 13). Using another treatment option (4 min immersion with 24 h of air drying), *C. intestinalis*, *B. schlosseri*, *B. violaceus*, and various species of macroalgae, polychaetes (high uncertainty), and bryozoans (very high uncertainty) were killed effectively (Forrest et al. 2007). An acetic acid (4–5%) immersion for 4 min followed by 24 h of air drying was not effective at causing mortality of small or large *M. galloprovincialis*, but survival of this species was impacted under field settings (Forrest et al. 2007). Small *M. edulis* would be impacted following both combined treatments (4 min followed by 24 h of air drying and 5 min followed by 1 h of air drying) (Carmen et al. 2016; Vickerson 2009).

When comparing the most effective options selected for acetic acid (Table 13), adding an air-drying period is no more advantageous than the short immersion without air drying with respect to the range of AIS that can be killed and the survival of moved species. After a 5-min immersion in 4–5% acetic acid without air drying, moved species started to be impacted and they remained impacted after the addition of air drying. Acetic acid can be harmful to mussels and Vickerson (2009) demonstrated that acetic acid was the harshest treatment for small *M. edulis*, compared to lime and brine treatments. However, Vickerson (2009) observed lower impacts on the attachment of mussels when the air exposure came before the immersion, or when the mussels were rinsed prior to the air-exposure period. Similar results were observed with *P. canaliculus* (proxy for *M. galloprovincialis*) by Forrest et al. (2007), where the survival in a field context was maximized by undertaking the immersion after the air exposure and/or by the addition of a rinsing step in between.

In summary, acetic acid treatments are effective on the largest range of AIS taxa, with or without air drying. Time of immersion in 4–5% acetic acid is an important factor to consider allowing survival of the moved species, particularly with small organisms, which tend to be more vulnerable to longer exposures. A 5-min acetic acid immersion (without air drying) showed that moved shellfish were already being impacted and remained impacted with the addition of an air-drying period (Table 13). Acetic acid was the most studied chemical treatment in the literature and could be a promising treatment for cultured shellfish, according to the few studies that tested short (under 1 min) immersions. However, further research is needed to more precisely assess the optimal acetic acid concentrations, immersion times, and air-exposure times for effectiveness at killing AIS and the resulting impact on moved species (survival), as well as the effects of the addition of a rinsing step or the reversal of the air-exposure and immersion steps.

4.2.3. Citric acid immersion

Based on the only three studies found in the literature for citric acid (Sharp et al. 2006; Locke et al. 2009; Sievers et al. 2019) its effectiveness (5% citric acid) on biofouling AIS via an immersion for 10 s without air drying was 100% effective at killing hydrozoans (high uncertainty), but was not effective at killing *C. intestinalis*, *S. clava*, macroalgae, or *M.*

galloprovincialis, although it may ensure the survival (high uncertainty) of small *O. edulis* (as indicated by survival of its proxy *O. angasi*) and *M. galloprovincialis* (Table 13). Heated citric acid immersion seems a better option when treating shellfish, specifically to kill *C. intestinalis*, *S. clava*, and *E. crocea* (100% mortality using 2 or 5% acetic acid at 50°C for 10 s), however, small *M. galloprovincialis* were also killed (100% mortality) when using this treatment for longer than 20 s (Sievers et al. 2019). The effectiveness of citric acid as a mitigation treatment remains unknown for many biofouling species such as colonial tunicates, *M. edulis*, *C. virginica*, *C. gigas*, gastropods, *C. maenas*, *H. sanguineus, C. mutica*, barnacles, sea stars, *C. fragile*, polychaetes, bryozoans, and sponges (Table 13).

Citric acid is 100% effective on hydrozoans but is not a widely covered treatment in the literature. Further research is required to assess its effectiveness on a wider range of AIS taxa, and to understand the impact on survival of moved species.

4.2.4. Brine immersion (with and without air drying)

Brine immersion without air drying was not consistently effective at controlling tunicate infestations (McCann et al. 2013; Rolheiser et al. 2012). Even at a saturated concentration (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. Of the many combinations of brine treatments tested in the literature and considered herein (see brine columns in Table 7), three (immersion in 300 ppt for 15 min, immersion in 300 ppt for 15 min plus 2 h of air drying, immersion in 300 ppt for 30 s plus 1 h of air drying) were considered as possible treatment options for further evaluation (Table 13). Of these three options, the combined brine immersion (300 ppt for 15 min) and air drying (2 h) treatment seemed to ensure 100% mortality of a wider range of unwanted biofouling species but this treatment was not tested on moved species (Table 13). However, small *M. edulis* survived a combined brine immersion and air-drying treatment under different parameters: 30 s followed by 24 h of air exposure (at 4°C and 100% RH; Vickerson 2009), 15 min followed by 1 h of air drying (Landry et al. DFO, unpubl. data), and 30 s plus 1 h of air drying (Mills, DFO, unpubl. data). Colonial (*B. schlosseri*, *D. vexillum*, and *D. listerianum*) and solitary (*C. intestinalis*, *S. clava*, *A. aspersa*, and *Molgula* spp.) tunicates (low uncertainty); *B. violaceus*, *C. fragile*, and sponges (*Cliona* spp.) (moderate uncertainty); polychaetes (*Polydora* spp., qualitative result, high uncertainty); and gastropods (very high uncertainty) were effectively killed using this combined treatment. However, this treatment would not be effective at killing small *M. edulis* (when considered as an epibiont), large *C. gigas*, and *C. maenas*. Many AIS did not have any data for this combined brine/air-drying treatment (immersion in 300 ppt for 15 min plus 2 h of air drying; Table 13). Note that the third treatment option (immersion in 300-ppt brine for 30 s followed by 1 h of air drying) is also likely effective on colonial tunicates (*B. schlosseri*, *B. violaceus*, *D. vexillum*, *D. listerianum*), and *C. intestinalis* (low or moderate uncertainty). Although a combined brine and air-drying treatment was not found in the literature for treatment of macroalgal AIS, a brine immersion (300 ppt for 15 min) was deemed effective at causing mortality (based on qualitative results only) for some species of macroalgae (low uncertainty) (Table 13).

The 15-min, 300-ppt brine immersion treatment option without air drying was ineffective (0−30% mortality) at killing small *M. edulis*, *C. virginica*, small *C. gigas*, and large *M. galloprovincialis* (Minchin and Dungan 1988; MacNair et al. 2006; Sharp et al. 2006; Bourque and Myrand 2007; Gill et al. 2008; MacNair 2009; Carver et al. 2010; Carman et al. 2016; Landry et al., DFO, unpubl. data). Nevertheless, the survival of some cultured bivalves, such as small *P. magellanicus*, was still impacted (24 to 89% survival when immersed in 300 ppt for 5 and 2 min, respectively) using saturated brine treatments, presumably because of their inability to close their valves completely during treatments. Based on laboratory trials, small *M. edulis* and *C.*

virginica would be less impacted by a short-term exposure to a brine immersion without air drying (6 min) if they could expel the brine by being re-immersed immediately in seawater after the treatment (Mallet et al., Mallet Research Services Ltd., unpub. Data). Similarly, the same treatment had no impact on *O. edulis*. Otherwise, when the immersion was combined with an air-drying period (24 h), high mortality would likely occur for both species (Mallet et al., Mallet Research Services Ltd., unpubl. data). The two shellfish species showed 100% survival with moderate to very high uncertainty for the third option (30 s in brine followed by 1 h of air drying), while immersion for 15 min without air drying assured the survival of a higher number of moved species (when compared to the other two options) and with lower uncertainty for *M. edulis* and *C. virginica*.

The treatment option of a 30-s immersion followed by 1 h of air drying was only effective on tunicates, including *B. schlosseri* (low uncertainty), *B. violaceus*, *D. vexillum*, *D. listerianum*, and *C. intestinalis* (moderate uncertainty). That treatment could ensure the survival of some moved species including small *M. edulis* and small/large *C. virginica* (moderate to very high uncertainty, respectively) (Table 13). No data on mortality associated with brine immersion (with or without air drying) were found for *H. sanguineus*, *C. mutica*, bryozoans, and hydrozoans.

4.2.5. Hydrated lime immersion (with and without air drying)

The use of hydrated lime is very common in mussel and oyster aquaculture industries for controlling predators (e.g., sea stars) and fouling tunicates on mussel seed collectors, mussel socks, and aquaculture gear (Ramsay 2014b; Ramsay et al. 2014). Hydrated lime immersion followed by air drying was found to enhance treatment effectiveness for only two solitary tunicates (*C. intestinalis* and *S. clava*) (MacNair et al. 2006; Gill et al. 2007) and *C. fragile* (MacNair 2002). Likewise, various hydrated lime sprays followed by air-exposure treatments were found to be effective at killing very few AIS. Consequently, neither of these treatment options were further assessed or presented in the summary table as they were not effective at killing a large range of AIS while ensuring the survival of most moved species.

Of the many hydrated lime immersion treatment options (20% for 20 s; 10% for 2 min; 5% for 2 min; 4% for 15 s and 1, 2, 4, 5, 8, 10 min and 3 h; 2% for 5–10 min; 1% for 30 s) found in the literature (Table 6), only the most efficient at killing a wide range of AIS (i.e., immersion in a 4% solution for 5 min) was considered further (Table 13). This treatment was deemed to be effective to kill *Molgula* spp. (low uncertainty), *C. fragile* (moderate uncertainty), *B. violaceus* and *S. clava* (high uncertainty), *B. schlosseri*, sea stars, bryozoans, and hydrozoans (very high uncertainty) (Table 13). The very high uncertainty score given to the effectiveness of this treatment at killing sea stars, bryozoans, and hydrozoans was mainly due to there being only one study available for species within these diverse invertebrate groups (Table 13). Hydrated lime immersions were not effective at killing *C. intestinalis*, *D. vexillum*, gastropods, *C. maenas*, barnacles, or sponges (Table 12). Nevertheless, that treatment method ensured the survival (>90%) of most cultured bivalves, including small and large *C. virginica* (low uncertainty), large *M. edulis* (moderate uncertainty), and large *C. gigas* and *A. irradians* (high uncertainty), with the exception of small *P. magellanicus*, whose survival was lower than 37% when immersed in 4% hydrated lime concentrations for 1 min or less (Table 13). No data were found for the effectiveness of hydrated lime immersions on small/large *M. galloprovincialis*, small/large *O. edulis*, small *A. irradians*, large *P. magellanicus*, and small *C. gigas*, or on *D. listerianum, A. aspersa*, polychaetes, *C. mutica*, *H. sanguineus*, and macroalgae (Table 13).

A 5-min immersion was effective at killing numerous tunicates, bryozoans, hydrozoans, and *C. fragile* while keeping several moved shellfish species alive, including *A. irradians*, for which lime immersion was the only treatment option available in the literature (100% survival of very few

individuals exposed to variable lime concentrations for 3 h; Comeau et al. 2017). However, a 5-min immersion treatment was not effective at killing several AIS (i.e., gastropods, *C. maenas*).

4.2.6. Brine and hydrated lime mixture immersion (with air drying)

Only one report was available in the literature on the effects of a brine and lime solution on AIS (Ramsay 2022). Nevertheless, DFO's PEI ITC has been recommending immersion in a 300-ppt brine and 4% lime solution as an effective treatment to control some invasive tunicates (*B. violaceus* and *B. schlosseri*) on the grounds that a 30-s soak in this solution followed by a minimum of 1 h of air drying kills these tunicates without impacting *C. virginica* (Mills, DFO, unpubl. data). Based on qualitative results, the selected option of 1 min immersion in this solution followed by 1 h of air drying is required to be effective for *C. intestinalis* (very high uncertainty), since it was demonstrated that 30 s was insufficient to kill this species (Ramsay 2022). As for the tunicates *B. schlosseri* and *B. violaceus*, a 1-min immersion is effective, although with high uncertainty due to the lack of studies combining brine and lime solutions.

Although only one such study combining both chemical treatments was found in the literature, (Ramsay 2022), several studies showed that both brine and hydrated lime immersions, used separately but at similar concentrations, followed by air exposure were effective at killing tunicates and macroalgae (MacNair 2002; MacNair et al. 2006; Gill et al. 2007; Mineur et al. 2007; Carman et al. 2010, 2016). Therefore, it is assumed that their combination would be at least equally effective as reduced effectiveness is unlikely. However, this brine and hydrated lime treatment combination needs further testing to assess comparability to other treatment types described herein and its impact on other moved species.

4.2.7. Virkon®

The limited data on the effectiveness of Virkon® (3%, 30 s) suggests that it is likely effective at causing 100% mortality of juvenile *C. intestinalis* (high uncertainty; Paetzold and Davidson 2011) while ensuring survival (94.4%) of large *M. edulis* (high uncertainty) (Table 13). However, adult *C. intestinalis* is not effectively killed in an immersion for 15 s at 3%, showing the importance of immersion time (Gill et al. 2007). Since that disinfectant and virucide is more commonly used in the finfish aquaculture industry, its application and effectiveness at causing mortality of many biofouling AIS and its impacts on many moved species remains unknown (see taxa with no data in Table 13). Therefore, the use of Virkon® needs to be investigated further for many species to evaluate its potential suitability as a treatment method for the movement of cultured shellfish and macroalgae.

4.3. CONCEPTUALIZATION OF A DECISION TOOL

The selection of the most appropriate treatment option to maximize both mortality of the AIS and survival of the moved shellfish species, in the context of movements of marine organisms, is conditional on which AIS, or range of AIS, need to be treated and what species are to be moved. Table 14 is a conceptualization of the process to derive treatment advice depending on AIS and species being moved and could be used as a management decision tool to respond to two different scenarios: mitigating a range of AIS or targeting just one AIS.

If the intent is to mitigate for a range of AIS, the first step would be to identify possible effective physical and chemical treatment options in Tables 12 and 13 that would include all AIS groups of concern (e.g., colonial tunicates, bryozoans, *Codium fragile*), which also ensures a high survival of the moved species (e.g., small *Mytilus edulis*) (see scenario A, step 2A in Table 14). However, if the goal is to ensure the mortality of a particular AIS (e.g., *Ciona intestinalis*) on a single moved species (e.g., small *Mytilus edulis*), possible effective physical and chemical

treatment options will first need to be determined from Tables 12 and 13 for *C. intestinalis* (see scenario B, step 2B in Table 14). If a more AIS encompassing treatment to ensure the desired level of *C. intestinalis* mortality or small *M. edulis* survival is not found, then an optimal treatment option for these species could be chosen from Tables 5–7 for AIS mortality and Tables 8–10 for moved species survival (see scenario B, step 3B in Table 14).

From these treatment options identified in the scenarios A and B, the feasibility/applicability of treatments will need to be evaluated (outside the scope of this work), since they are context dependent, and optimal treatment(s) for a given situation must be determined.

4.4. LETHAL TREATMENTS FOR AIS AND EPIBIONTS WITH NO/LOW IMPACTS ON MACROALGAE

Most mitigation measures found in the literature for macroalgal culture dealt with preventative methods related to avoidance of biofouling (e.g., timing, location, biological control) (Smit et al. 2003; Førde et al. 2015; Bannister et al. 2019). However, other mitigation treatment methods are required to further mitigate the risk of transferring AIS when moving macroalgae from farming sites to new locations.

Only one report was found on the impacts of treatments on the survival of cultured macroalgae in Canada (Tamigneaux et al. 2013). Based on information received from experts on both Atlantic and Pacific coasts (Clark, Cascadia Seaweed Corp., pers. comm; Tamigneaux, Cégep de la Gaspésie et des Îles, pers. comm.), one protocol, including several steps for decontamination, is applied to clean sori (reproductive fragments) of *Saccharina* spp. in hatcheries prior to cultivation at a farm site. To avoid settlement of *Ulva* spp. and *Ectocarpus siliculosus* on culture ropes, the protocol is applied at the source in the laboratory/hatchery to ensure a monospecific culture of *Saccharina* spp., instead of treating fronds and ropes after growth (Tamigneaux, Cégep de la Gaspésie et des Îles, pers. comm.). The transfer of cultivated plantlets from hatcheries to farm sites generally involves small individuals (2−4 mm) previously cultured in sterilized seawater. A harsh physical or chemical treatment could easily have devastating impacts on the survival of such small plantlets and is hence avoided (Tamigneaux, Cégep de la Gaspésie et des Îles, pers. comm.). Even though this protocol is not used for the same application (i.e., cleaning sori in hatcheries prior to cultivation), we assume that it would likely be effective for movements of macroalgae.

Based on results of the impacts of physical and chemical treatments on macroalgae cultured elsewhere and on other non-cultured macroalgal species, only a few effective treatments could ensure the survival of moved macroalgae. Overall, survival of several macroalgal species seems to be greatly affected by physical treatments including air drying (Forrest and Blakemore 2006; Kim and Garbary 2007; Meichssner et al. 2020), freshwater (Smit et al. 2003; Forrest and Blakemore 2006), and hot seawater (Williams and Schroeder 2004; Forrest and Blakemore 2006; Mineur et al. 2007; Landry et al., DFO, unpubl. data). For example, as an intertidal cultured species, *P. yezoensis* experiences periodical emersion and submersion during culture practices and is known to be tolerant to desiccation and high-water loss (up to 70%) (Li et al. 2018a; Du et al. 2021). Similar results were also observed for other intertidal macroalgal species in the same genus that may experience 90% weight loss and still recover rapidly after being resubmerged (Contreras-Porcia et al. 2011; Guajardo et al. 2016; Li et al. 2018a). Li et al. (2018a) showed that the thalli of *P. yezoensis* had an increased tolerance to water loss when periodically exposed to air drying compared to ones never exposed to the air (submerged). However, although this red alga showed resistance to air drying and can recover quickly from dehydration, high water loss impacts remained observable at a cellular level (Li et al. 2018a; Du et al. 2021). These existing sub-lethal impacts should be considered when thinking of air drying as a possible treatment for macroalgae.

Limited studies were found on the effectiveness of chemical treatments to eliminate epiphytes associated with cultured macroalgae and the impacts of those treatments on them. Sodium hypochlorite immersions (Williams and Schroeder 2004; Tamigneaux et al. 2013; Clark, Cascadia Seaweed Corp., unpubl. data), acetic acid (Forrest et al. 2007; Piola et al. 2009), brine (Sharp et al. 2006; Mineur et al. 2007; MacNair 2009; Du et al. 2021), and citric acid immersions (Yan et al. 2011) were chemical options that might be promising treatments for some cultured species. In fact, some Asian-cultivated macroalgal species such as *Porphyra haitanensis* and *Neopyropia yezoensis*, have survived immersions in high concentrations of ~10% hydrochloric acid (Yan et al. 2011; Kang and Kim 2022). Brine immersions were shown to be less harmful to macroalgae than air drying, even at equivalent water loss rates (e.g., 40%), since the macroalga remains in full contact with water (Du et al. 2021). Hence, air drying could be more damaging to thalli than chemical immersions in general, even for the most resistant taxa, because of impacts that might occur at the cellular level. An important factor to consider is that some intertidal marine macroalgal species (e.g., *Porphyra* spp., *P. yezoensis*, *Ulva* spp., and *Cladophora* spp.) showed more resistance to stress than some subtidal macroalgal species, some of which are cultured in Canada (e.g., *Saccharina* spp.) (Hansen et al. 2006; Li et al. 2018a; Du et al. 2021).

In summary, since impacts of these treatments on macroalgae cultivated in Canada are unknown and could potentially affect their survival, it is not possible to recommend any of the above treatments and new research to determine appropriate treatments to eliminate AIS epiphytes on moved macroalgae is required.

4.5. SUITABILITY OF PROACTIVE BIOFOULING MANAGEMENT PRACTICES RELATIVE TO MITIGATION OF MOVED CULTURED SPECIES

While many biofouling prevention and removal techniques exist (see general techniques listed in IOC-UNESCO and GEF-UNDP-IMO 2022), they may not all be applicable to marine shellfish and macroalgal aquaculture activities and/or transfers of these cultured organisms to other waterbodies. Because methods of biological control, as opposed to chemical ones, rarely produce unwanted side effects such as pollution or the development of pesticide resistance, they may appear to be the most environmentally friendly choice to eliminate fouling organisms attached to cultured shellfish and macroalgae. However, in some cases, the use of targeted biological agents for the control of unwanted AIS biofouling has been shown to have no immediate effect on unwanted biofouling, including AIS (Critchley et al. 1986; Sumi and Scheibling 2005; Switzer et al. 2011) and/or adverse effects on non-targeted indigenous or cultured species (Sumi and Scheibling 2005; Epelbaum et al. 2009). In addition, the movements of mobile organisms as biological control agents may be particularly challenging when used in mussel culture or drop-line oyster culture as they may not remain on the culture infrastructure long enough to reduce biofouling (e.g., Comeau et al. 2012). Biological control, even when effective at controlling biofouling species on shellfish and/or macroalgae, often requires a lengthy time (i.e., up to several months) before an effect is observable (Cigarria et al. 1998; Carver et al. 2003; Conklin and Smith 2005; Dumont et al. 2009; Atalah et al. 2016; Li et al. 2018a) and may be best used in conjunction with other fouling mitigation strategies.

Manual (hand picking, brushing, filing) and mechanical (harvesting and tumbling with motorized equipment) biofouling treatment methods may be done repeatedly at aquaculture sites or only when product is harvested before transferring to another waterbody. These manual methods vary with cultured organisms, are often time-consuming and costly, must be conducted repeatedly throughout the growing season, and may stress some cultured organisms intended for transfer to other waterbodies or destined for market.

While repetitive treatments are often husbandry practices that can reduce biofouling on cultured species (Davidson et al. 2016), they are not a logistical option when considering a mitigation treatment prior to moving a cultured species in the context of Introduction and Transfers of shellfish and macroalgae due to the rather immediate need for a mitigation treatment that is often species-specific.

4.6. LIMITATIONS AND SOURCES OF UNCERTAINTY

This literature review was limited to marine invertebrate and macroalgal species, with an emphasis on epibiont AIS. Internally transported species (e.g., living in mantle water) and viruses, bacteria, phytoplankton, and protozoans were not within the scope of this work. A separate literature review on the topic would be required to answer the need to identify mitigation measures for those types of organisms.

There were multiple limitations and sources of uncertainty identified in this research document. The single-species (or similar taxa) approach to many studies made comparisons across studies challenging and often led to conflicting or counter-intuitive results, likely owing to unreported differences in experimental design or other factors. 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. Consequently, no single treatment with low impact on moved species was found to be applicable to all marine AIS.

Many information gaps were encountered regarding target AIS, moved species, and treatments. Very little data were available for most juvenile stages of AIS (possibly the most susceptible stage). For all species that were not classified in size categories, it was assumed that most results were for adult stages, based on evidence provided in the publications, yet not properly stated. It was also surmised that if a treatment worked for an adult AIS it would also be effective on juveniles (Loosanoff 1960; Medcof 1961).

Species with longer invasion histories were generally studied more extensively than those with shorter ones. Likewise, some treatments were relatively well studied by multiple studies (e.g., hot water immersion, air drying, freshwater, and acetic acid), while others were poorly studied (e.g., pressurized seawater, chlorination, citric acid, and Virkon®). Control strategies for some groups of AIS—such as crustaceans (including *C. maenas* and *H. sanguineus*), sea stars, bryozoans, sponges, and bryozoans—were relatively data poor. This led to using literature for proxy species as substitutes to fill some gaps. No information in the literature was available for many targeted moved species, such as Pacific clams [Manila clam (*Ruditapes philippinarum* formerly *Venerupis philippinarum*), varnish clam (*Nuttallia obscurata*), Pacific geoduck clam (*Panopea generosa*)], *C. islandica*, and *O. edulis* (mostly information from the proxy *O. angasi* was used in the present work, with the exception of Carver et al. 2003 and Fitridge et al. 2014) (Table 2).

Very little information was available for macroalgae, either as AIS/epibionts or moved species (Tables 1, 2). Additional invertebrate/macroalgal 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.

4.6.1. Qualitative results

Information on mortality and survival extracted from the literature was divided between two types of results: quantitative or qualitative. Qualitative results on mortality (effective/not effective) or survival (impacted/not impacted) increased the level of uncertainty, as it was unclear as to whether these results met the criteria for effectiveness to kill AIS (100% mortality) or keep moved species alive (>90% survival). Some authors may have considered a treatment

to be qualitatively effective, based on their own criteria; however, these results were not always quantitatively specified in the study and were left as qualitatively effective herein for categorical purposes. For instance, one laboratory study recommended a hot-seawater immersion at 70°C for 40 s as an effective treatment for field applications to kill the mud worm *P. hoplura*, while avoiding mortality of the cultured host species. However, their statement about effectiveness was misleading as their laboratory trial results demonstrated that the treatment only induced 30–39% mortality in the worm (Nel et al. 1996). Although this particular study may be an exception, the stated mortality was considered insufficient according to the acceptable mortality threshold (100%) for AIS in the present work. Furthermore, some qualitative studies or reports did not always mention whether controls were included in their experimental design, compared to quantitative studies, which generally included such details on controls in their methodology. Thus, caution should be used when considering qualitative results alone.

4.6.2. Experimental designs

Most published studies differed in terms of experimental design, location (laboratory or field), and method of measuring survival of moved species or mortality of AIS, all of which contributed significant uncertainty to the assessment and comparison of effectiveness (defined here as removal or mortality) and survival among studies. Other parameters are known to influence the effectiveness of treatments. Air drying, for example, is a well-documented treatment for the control of AIS, but its effectiveness is strongly dependent on animal size (Rajagopal et al. 2005b; Asgari and Jahangard 2012; Sievers et al. 2019; Landry et al., DFO, unpubl. data), air temperature, and relative humidity (Arakawa 1980; Hillock and Costello 2013; Hopkins et al. 2016). 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, potentially skewing interpretations for future management use. This problem is confounded by the fact that much of the scientific work on treatments available in the primary literature has been completed under laboratory conditions (see Tables 5–11), with the results not necessarily translating into equally effective and practical field applications. Drying macroalgal fragments in a laboratory or under controlled conditions, for example, does not represent the same environment as drying them in humid or shaded outdoor conditions. A few studies obtained matching results between laboratory and field settings (e.g., Forrest et al. 2007; Cahill et al. 2021), although the stability and effectiveness of chemical solutions may be widely variable under different environmental conditions (Piola et al. 2009; Cahill et al. 2021). Further research is required to understand how various treatments (e.g., air drying, steam applications, chemical immersion, etc.) that have been deemed as being effective under laboratory conditions will function under field settings.

Based on the whole suite of literature presented in the detailed results tables above, there are obvious gaps between treatment parameters (e.g., duration, temperature, concentration, etc.). Results of mortality and survival are not consistently based on continuous fully-scaled parameters within papers (e.g., full-scale range of increasing concentrations or durations tested within one study) and different publications may not necessarily complement each other in terms of full-scale ranges. This limitation made it more difficult in some cases to assess the level of uncertainty associated with the effectiveness on AIS and the impacts on moved species. Data gaps were also observed for the size ranges of shellfish tested in the literature (see Table 3), where most publications provided a measured size (in millimetres or centimetres), whereas others provided only size categories based on qualitative information (e.g., adult or juveniles, small or large, seed/spat, market size), which also contributed to uncertainty. The type of shell measurements (height, length, width; for example, see Rajagopal et al. 2005a, b; Rolheiser et al. 2012; Cahill et al. 2021) in millimetres/centimetres were not consistently specified for shellfish. Often only one size was provided, and the terms ‘height’ and ‘length’ were, at times, used interchangeably. It was assumed that all measurements provided in the literature referred

to the longest dimension (maximum distance between the umbo and the ventral valve margin) and were all described as shell length. Some research also evaluated different size groups within the same category (e.g., two small groups), but since not all papers specified the size of the organisms used, it occasionally limited the interpretation of results for a given category (mostly the small category) or may have induced conflicting results.

Another experimental design consideration concerns the four species of shellfish that were considered both as moved species and as invasive for the purpose of the present work: *M. edulis*, *M. galloprovincialis*, *C. virginica*, and *C. gigas*. Most papers focused on keeping shellfish alive and adapted their experimental design to ensure survival and lesser impacts post-treatment, rather than focusing purposely on their mortality. Even though a few papers focused on inducing mortality of these shellfish, there was a lack of information on treatments that could be effective for these four species.

4.6.3. Timing of assessment of mortality, long-term or sub-lethal effects

Publications included in this literature review assessed post-treatment mortality or survival of organisms on a large range of time scales (e.g., immediate or minutes, hours, days, weeks, or months). For example, some studies tested short treatment durations in seconds (e.g., 3, 5–10, or 60 s), but assessed mortality (effectiveness of treatment) after 48 h (Guenther et al. 2011), 8 and 13 d (Locke et al. 2009), or 24 h (Joyce et al. 2019), respectively. A few studies focused on seasonal assessment of mortality, based on weekly preventive treatments, which were not applicable in the context of this work. In some cases, studies observed that mortality evolved with time during post-treatment assessment. It was noted that after using a treatment, biofouling organisms might not die at first, but the effects of the treatment would gradually appear after a few days (Arakawa 1980). For example, an acetic acid spray for a few seconds followed by a quick period of air exposure induced 89% mortality *of C. mutica* 2 to 3 h post-treatment, but 100% mortality was observed after more than 5 d (Paetzold et al. 2008). In another example, following a daily assessment schedule, an air-drying treatment for *M. galloprovincialis* took 4 d for mortality to first occur, but took 11 d to reach 100% mortality (Hopkins et al. 2016). Such delayed mortality also occurred in trials with heated seawater when stocks of oysters (cultured species) were completely lost because of long-term effects of heat (Mallet et al., Mallet Research Services Ltd., unpubl. data). Furthermore, MacNair (2002) showed that acute effectiveness of air drying was almost 100% effective at killing *C. fragile* by fragmenting the plants after 1 month, but some algae pieces regrew into healthy plants after 3 months. Though these cases were pointed out in the results, timing of treatment assessment of mortality/survival of AIS/moved product is a variable aspect of experimental design in studies that introduces uncertainty regarding the effectiveness/impacts of a treatment applied in the field on species intended for transfer/movement.

Most studies assessed acute impacts on shellfish and rarely longer-term (chronic) and sub-lethal effects (e.g., Haque et al. 2015) of a treatment over farming production timeframes. A few papers assessed long-term impacts of repeated treatments for *in situ* maintenance through and after a season (Gallo-García et al. 2004; Gill et al. 2008; Hood et al. 2020), but not in the context of a single treatment during a transfer or movement of organisms. Vickerson (2009), however, studied the long-term impacts of a one-time air-drying treatment for 24 h on small (30−40 mm) *M. edulis* and observed no acute impacts based on byssal attachment activity, indicating a low level of stress. In the longer term, the mussels showed great performance roughly 8 months after being returned to the water following the air-drying treatment. Though qualitative ‘impacted’ results for moved species encompass a few cases of sub-lethal effects in the literature (e.g., shell damage, decrease in growth, reduced byssal attachment), caution

should be used in the context of survival of moved species, where long-term effects of treatment stress on moved species remain generally unknown.

4.6.4. Variability of parameters

Parameter variability in natural environments can affect the stability of a soluble chemical, as well as its effectiveness to kill an AIS, depending on their physiological tolerances. Many laboratory experiments were conducted under controlled conditions, including ambient temperature, which can influence the effectiveness of a given chemical treatment or air drying, the effects of the latter being known to be highly dependent on both temperature and relative humidity (Arakawa 1980; MacNair 2002; Forrest and Blakemore 2006). Caution must be taken to avoid extrapolating results from laboratory experiments to field-scale applications. It is known that the stability of sodium hypochlorite, for example, is influenced by many environmental factors (e.g., light, evaporation, salinity, time in solution, other chemical compounds; Delaruelle and Claes 1996; Nicoletti and Magalhães 1996; Piola et al. 2009; Haque et al. 2014) that have the potential to decrease its effectiveness, mostly in field contexts. Coutts and Forrest (2005) demonstrated that initial concentrations of sodium hypochlorite can decrease by 50% or more after mixing with seawater and will continue to decline throughout the duration of the treatment. Their observations showed the importance of keeping the residual (free) available chlorine (part of total residual chlorine; TRC) in solution stable or above the effective residual concentration, by choosing the proper initial concentration (Coutts and Forrest 2005). According to Bourque and Mayrand (2007), survival of small *M. edulis* that were immersed in brine solution under field conditions varied depending on temperature and ranged from 84% at 25°C to 95% at 18°C. Keeping parameters constant during the application of a treatment is important and parameter discrepancy can lead to sources of uncertainty. For example, an increase in salinity during a freshwater treatment can increase the time required to kill fouling organisms that are more tolerant to low or brackish salinities (Coutts and Forrest 2005; Forrest and Blakemore 2006; Brown 2012; Vercaemer et al. 2011; Rolheiser et al. 2012). In addition, the potency of some chemicals can diminish with time, making them less effective at killing AIS. For instance, sodium hypochlorite should be freshly opened and mixed prior to treatment due to its instability and chemical reactivity (Piola et al. 2009). This is also the case for hydrated lime powder that will react with carbon dioxide when exposed to air and slowly revert back to limestone (Ramsay et al. 2014). Hydrated lime bags should only be used when freshly opened before mixing with seawater (Ramsay et al. 2014).

As mentioned previously, caution is advised when extrapolating treatment results obtained from laboratory-scale studies to an industrial scale, without performing the relevant field research. Industrial settings can result in conditions that enable fouling organisms to survive by not being sufficiently exposed to a treatment, such as in areas deep within clumps of mussels or oysters (Carver et al. 2003; Ramsay 2015a). Saltwater leaching from interstitial spaces among large quantities of cultured species and their associated biofouling community can alter the initial concentration of treatment baths or sprays (e.g., freshwater or chemical) or change the temperature of a treatment and reduce its effectiveness (MacNair et al. 2006). This possible dilution effect could explain variation in results obtained from different studies that tested a treatment with the same parameters. It should also be noted that ensuring flow circulation is important to keep parameters constant (Rajagopal et al. 2002; Forrest and Blakemore 2006; Ramsay 2015a). Flow-through systems (even better when flushing out quickly to avoid increasing salinity; Ramsay, PEI DFC, pers. comm.) are more efficient to maintain constant parameters (e.g., concentration, temperature, salinity) compared to standing-water systems (Arakawa 1980) or spray applications, and help maximize the effectiveness of a treatment and/or survival of a given moved species. In a real-world context, it is also important to consider the extent of AIS colonization (thickness and cover) on cultured species, especially for

tunicates. Furthermore, AIS that are aggregated (compared to a single specimen) or those protected by thicker outer layers are more likely to survive, thus reducing the effectiveness of a given treatment.

Environmental acclimation and seasonality can also substantially affect an organism's mortality or survival during treatments. Species (AIS or moved) acclimated to higher temperatures or lower salinities, for example, often have a higher tolerance to hot-water immersions, freshwater immersions, desiccation, or changes in physiological stress (e.g., *M. edulis*: Gonzalez and Yevich 1976; Joyce et al. 2019; Rajagopal et al. 2005a; *C. gigas*: Rajagopal et al. 2005b; Joyce et al. 2019; *C. maenas*: Best et al. 2014; macroalgae: William and Schroeder 2004). Special consideration should be given when choosing the timing of year to treat different sizes of cultured/moved organisms to remove AIS prior to moving them to other locations as the survival of small oysters was assessed at 50% after a hot seawater immersion in June compared to 89% for the same treatment type conducted in August (Mayrand et al. 2015). These factors should be considered when choosing the type of treatment and when it is best to apply it.

4.6.5. Susceptibility of valve-gaping shellfish

Most chemical treatments (e.g., sodium hypochlorite, acetic acid, hydrated lime) are suspected to be more harmful to shellfish with gaping valves (William and Schroeder 2004; MacNair et al. 2006; MacNair 2009; World Organisation for Animal Health 2009; Roche et al. 2015; Landry et al., DFO, unpubl. data) and gaps in knowledge on their survival after a direct exposure of soft tissues to chemicals limits applications of harsh treatments. Since scallops tend to be more sensitive to stress than fouling organisms and require being handled with care (Carver, Mallet Research Services Ltd., pers. comm.), hand-scraping and rinsing with seawater might be the only option for treating scallops prior to movements (Carver, Mallet Research Services Ltd., pers. comm.). High mortality has also occurred with the mussel *P. canaliculus* during chemical immersions when their valves were open and shaking prior to treatment could reduce the chemical exposure of internal tissues by inducing valve closure (Forrest et al. 2007).

4.6.6. Effect of organism size

It has been demonstrated that size is statistically correlated to survival of shellfish in various treatments (Rajagopal et al. 2002; Haque and Kwon 2017; Cahill et al. 2021). Results from the literature for heated treatments, for example, showed that temperature and durations affected survival, but results were size-specific, smaller individuals being more vulnerable to heat treatments than larger ones (Rajagopal et al. 2005b; Asgari and Jahangard 2012; Rousselle 2012; Mayrand et al. 2015; Sievers et al. 2019; Landry et al., DFO, unpubl. data). As smaller-sized organisms are generally more vulnerable to heat or chemicals, the bigger they get, the more tolerant they become to a treatment, and this can sometimes be observed with a relatively small size difference, such as 10 mm or less (Rajagopal et al. 2002; Haque and Kwon 2017; Hopkins et al. 2016; Cahill et al. 2021). The size of moved species may not always be the same (e.g., the variation in size in a mussel sock) and represents a source of uncertainty that needs to be considered regarding the harshness of any given treatment, on smaller-sized organisms in particular.

Since specific shellfish life cycle stage was rarely provided in the literature, shellfish were separated into two size categories (i.e., small and large) herein to facilitate the interpretation of results. Indeed, conflicting interpretations of sizes were also seen between publications [e.g., adults of 25 mm (Haque and Kwon 2017) and seeds of 15−30 mm (Vickerson 2009), and sometimes size in millimetres was unknown]. Thus, results were occasionally conflicting and different small-sized groups (e.g., 8−10 mm, 35−45 mm) of the same species tested in the literature fall into the same size category. Our small-size category (i.e., seed, juveniles, and

small adults) for shellfish species remains unprecise to provide accurate treatment recommendations to managers for particular life stages. This uncertainty is mostly due to the lack of precision in the literature itself, limiting the possibility to break it down further into smaller categories for more precision.

In some cases, industry may focus on removing newly settled organisms on cultured species, including small mussels and oysters and other epibionts (Carver, Mallet Research Services Ltd., pers. comm.). However, there is a significant lack of data for young stages of AIS and epibionts and that treatments in industry practices would mostly be applied on small (young) moved species, which tend to be more vulnerable. In that context, it is assumed that effective treatments on adult AIS will be effective on their younger stages, but the uncertainties remain for the survival of small-sized moved species.

4.6.7. Combined treatments

Treatment combinations may be more effective than single treatment approaches for many AIS/epibiont species, although research on this is largely lacking. The inclusion of an air-drying step after hydrated lime dipping (MacNair et al. 2006; Ramsay et al. 2014) and pressure washing (Coutts 2006; Coutts and Forrest 2007) was shown to be more lethal to tunicates than either application alone. Adding an air-drying period prior to and after immersion in 4% lime was also tested by Fitridge et al. (2014), who showed that treatment effectiveness at killing *S. clava* was increased compared to immersion alone. There is additional evidence to suggest that combining heat/cold and chemical applications may also increase treatment effectiveness, where warmer acid immersions (acetic and citric) required less time to kill solitary tunicates (Sievers et al. 2019) and where chilled immersions (-20°C) increased the effectiveness of brine to kill polychaetes (Asgari and Jahangard 2012). Information on chilled brine treatments was scarce in the literature (Asgari and Jahangard 2012; Cox et al. 2012), but is convincing, showing the ability to kill a range of fouling organisms, including oysters (Cox et al. 2012). However, the impacts of chilled combined treatments on shellfish requires further investigation, since conflicting results have been obtained regarding survival (Fitridge et al. 2014; Asgari and Jahangard 2012; Cox et al. 2012). Several examples of combinations or mixing two chemicals at once were tested. Since brine immersion mortality results varied by species, researchers introduced lime to brine solutions to test its effectiveness to cause mortality of unwanted fouling tunicate species. Recent trials by PEI DFC revealed that 1-min immersion in a saturated 300-ppt brine solution mixed with 4% hydrated lime, followed by 1 h of air drying, was effective at causing *C. intestinalis* mortality (Ramsay 2022). In another study, MacNair et al. (2006) suggested a combination of two chemicals to kill *B. violaceus* on infested mussel socks (e.g., a 5% acetic acid dip followed by a 300-ppt brine dip and a period of air drying).

The specific relative contribution of each chemical within a combined chemical treatment cannot be easily verified and thus limits the assessment of each of these chemicals to its global effectiveness. Furthermore, the global effectiveness and impacts of combined treatments may differ depending on the order of the steps (e.g., air drying before an immersion as opposed to after an immersion; Forrest et al. 2007; Vickerson 2009). Current knowledge gaps on the impact /effectiveness of potential combined treatments preclude their consideration in this research document, but future research should consider the cumulative effects of treatments, and their order, on AIS and their impacts on survival of moved species.

4.7. FEASIBILITY AND OTHER CONSIDERATIONS

Although many effective treatment options were identified from the literature for the control of a variety of marine AIS, while assuring the survival of moved species, there are a number of considerations that may limit their usefulness in real-world settings in the context of moving

species for introduction and transfers. These considerations include, but are not limited to, the ease of application and practicality under field conditions, associated health and safety hazards, cost, and disposal. For example, sufficient freshwater may not be easily accessible in some locations, and/or it may not be possible to heat or chill immersion treatments at some sites, and/or it could be too costly or timely for some small operations (Ramsay, PEI DFC, pers. comm.). Although beyond the scope of this research document, specific treatments will have logistical considerations.

Some treatments identified as being ineffective (or may be effective but have high levels of uncertainty) may be effective under different concentrations, pressures, temperatures, and/or exposure times. Future research could refine these treatment methods and discern the point at which they become effective (e.g., sodium hypochlorite treatment effectiveness on all life stages of mussels).

5. CONCLUSIONS

This literature review identified (1) several treatment options applicable to many AIS while keeping moved species alive, (2) key uncertainties and knowledge gaps that remain, and (3) future research needs.

- Based on published studies reviewed in this work, no single treatment was found applicable to all AIS while maintaining shellfish or macroalgal survival in the context of species movements.
- Numerous physical and chemical treatment options were identified as effective (100% mortality or effective) at killing various AIS or epibiont species.
- Numerous physical and chemical treatment options were identified as having no/low impacts (>90% survival) on moved shellfish species.
- Although a large number of effective treatment options were identified from the literature for the control of a variety of marine AIS, while assuring the survival of moved species, there are a number of considerations that may limit their usefulness in real-world settings. These considerations include, but are not limited to, the ease of application and practicality, associated health and safety hazards, cost, time, and disposal.
- Few physical and chemical treatment options applicable to a large number of AIS, while keeping moved shellfish species alive, were identified (see Tables 12 and 13).
- The most appropriate treatment type (from the most applicable treatment options summarized in Tables 12 and 13) is conditional on which AIS, or range of AIS, need to be treated and the species to be moved (see Table 14 for a conceptualization of the process to derive treatment advice depending on AIS and species being moved).
- There are likely other AIS that may become a challenge in the future that are not covered in this document.
- Key uncertainties and knowledge gaps include:
    - Uncertainty in interpretation when comparing studies with different experimental designs, scales, and methods of measuring AIS/epibiont mortality and/or removal and with the use of both quantitative and qualitative data.
    - Uncertainty when extrapolating results from laboratory studies to field conditions.
    - Very little information on macroalgal survival was found and no effective treatment for killing AIS associated with cultured macroalgae in Canada was identified. More research is required on treatment methods for moved macroalgae.

- Based on these uncertainties and knowledge gaps, some specific types of movements/AIS concern may require a specific laboratory/field experiment to determine treatment effectiveness.
- Standards should be developed for identified invasive and moved key species.

- Future research needs to include:
  - Combined treatments and their effectiveness at killing AIS/epibionts and their impacts on moved species is needed (e.g., combining chemical immersions/spray and heat or different types of chemicals). This includes partitioning the level of mortality associated with both steps.
  - Sub-lethal and chronic effects of treatments on moved species.
  - Treatments that could be applied to shellfish and macroalgae in the field or at industry scales (as opposed to laboratory-scale studies).
  - The effect of seasonal variation of various physical/chemical treatments on both the effectiveness on AIS and the impacts on moved species.
  - The effect of AIS and moved species sizes on various physical/chemical treatment effectiveness and impacts, respectively.
  - The effectiveness of treatments for eliminating AIS while ensuring the survival of cultured shellfish/macroalgae under future ocean conditions (i.e., increasing temperature and [CO_2] and decreasing dissolved oxygen and salinity).

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8. TABLES

Table 1. Marine Aquatic Invasive Species (AIS) and epibionts that were assessed in the present work.

Representative group	**AIS and epibiont species**
Colonial tunicates	Golden star tunicate (*Botryllus schlosseri*), violet tunicate (*Botrylloides violaceus*), carpet sea squirt (*Didemnum vexillum*), and compound sea squirt (*Diplosoma listerianum*)
Solitary tunicates	Clubbed tunicate (*Styela clava*), vase tunicate (*Ciona intestinalis*), European sea squirt (*Ascidiella aspersa*), and sea grape (*Molgula manhattensis*)
Bivalves	Blue mussel (*Mytilus edulis*), Mediterranean mussel (*Mytilus galloprovincialis*), Eastern oyster (*Crassostrea virginica*), and Pacific oyster (*Crassostrea gigas*)
Gastropods	Slipper snails (*Crepidula fornicata*, *Crepidula adunca*) and oyster drills (*Urosalpinx cinerea*, *Eupleura caudata*)
Crustaceans	European green crab (*Carcinus maenas*), Asian shore crab (*Hemigrapsus sanguineus*), Japanese skeleton shrimp (*Caprella mutica*), and common rock barnacle (*Semibalanus balanoides*)
Sea stars	Common sea star (*Asterias rubens*) and mottled star (*Evasterias troschelii*)
Macroalgae	Oyster thief (*Codium fragile), Undaria* sp., *Cladophora* sp., *Ulva* sp., Rhodophyta, *Fucus* spp., *Gracilaria* sp., and *Caulerpa* sp.
Polychaetes	Tube worms [*Hydroides elegans, Spirobranchus paumotanus (=Pomatoceros taeniata), Sabella spallanzanii*], mud worms (*Polydora ciliata*, *Polydora hoplura*, *Polydora websteri*, *Boccardia polybranchia*), and Terebellidae worms.
Bryozoans	Kelp encrusting bryozoan (*Membranipora* sp.), brown bryozoan (common Bugula) (*Bugula neritina*), red crust bryozoan (*Cryptosula pallasiana*), and *Schizoporella* spp.
Sponges	Boring sponge (*Cliona celata*) and calcareous sponge (*Leucosolenia* sp.)
Hydrozoans	Pink-mouth hydroid (*Ectopleura crocea*)

Table 2. Shellfish and macroalgal species cultivated in Canada (Atlantic and Pacific coasts) that were assessed in the present work.

Region	Group	Moved and cultured species
Atlantic and Pacific	Mussels	Blue mussel (*Mytilus edulis*)
	Macroalgae	Sugar kelp (*Saccharina latissima*)
Atlantic	Oysters	Eastern oyster (*Crassostrea virginica*) and European flat oyster (*Ostrea edulis*)
	Scallops	Bay scallop *(Argopecten irradians)*, giant scallop (*Placopecten magellanicus*), and Iceland scallop (*Chlamys islandica*)
	Macroalgae	Atlantic kelp (*Saccharina longicruris*)
Pacific	Mussels	Mediterranean mussel (*Mytilus galloprovincialis*)
	Oysters	Pacific oyster (*Crassostrea gigas*)
	Scallops	Japanese weathervane scallop *(Mizuhopecten yessoensis)*
	Clams	Manila clam (*Ruditapes philippinarum* formerly *Venerupis philippinarum*), varnish clam (*Nuttallia obscurata*), and Pacific geoduck *(Panopea generosa)*
	Macroalgae	Bull kelp (*Nereocystis luetkeana*), giant kelp (*Macrocystis pyrifera*), Pacific dulse (*Develarea mollis*), sieve kelp (*Neoagarum fimbriatum*), and winged kelp (*Alaria marginata*)

Table 3. Established cutoffs for shellfish species and size ranges tested across literature for all treatments. NS: not specified.

Coast (Atlantic/ Pacific)	**Moved species**	**Large size category cutoff (≥ mm)**	**Measurement**	**Range of size tested for treatments (mm)**		**References**
				Small	**Large**	
Atlantic and Pacific	**Blue mussel** *Mytilus edulis*	50	Length	[1.4–44.95]	[50–59.3]	Mallet and Myrand 1995; Myrand et al. 2000; DFO 2003; Lamb and Handby 2005; Drapeau et al. 2006; Sukhotin et al. 2007; Lachance et al. 2008; Gosling 2015; Guillou et al. 2020
	Mussels *Mytilus* spp.	50	Length	[16–36]	-	Mallet and Myrand 1995; Myrand et al. 2000; DFO 2003; Lamb and Handby 2005; Drapeau et al. 2006; Sukhotin et al. 2007; Lachance et al. 2008; Gosling 2015; Guillou et al. 2020
Atlantic	**European flat oyster** *Ostrea edulis*	65	Length	[<10–40]	[70–80]	Raimbault 1964; Boghen 1995; Gosling 2015; Lemasson 2019
	Eastern oyster *Crassostrea virginica*	70	Length	[6.23–65]	NS	Boghen 1995; Kozlofff 1995; Lavoie 1995; NB DAA 2008; Gosling 2015
	Bay scallop *Argopecten irradians*	40	NS	-	-	Boghen 1995; MacDonald et al. 2006; Gosling 2015; Robinson et al. 2016; Wei et al. 2021
	Sea scallop *Placopecten magellanicus*	70	Height	[6.63–22.97]	-	Black et al. 1993; Boghen 1995; Stokesbury and Himmelman 1995; Stokesbury et al. 2006; Robinson et al. 2016
Pacific	**Mediterranean mussel** *Mytilus galloprovincialis*	50	Length	30	[60–67]	Mallet and Myrand 1995; Myrand et al. 2000; DFO 2003; Lamb and Handby 2005; Drapeau et al. 2006; Sukhotin et al. 2006; Lachance et al. 2008; Gosling 2015; Porri et al. 2016; Guillou et al. 2020
	Pacific oyster *Crassostrea gigas*	70	Length	[14.9–60]	[90.94–146.61]	Goulletquer 1995; Kozloff 1995; Diederich et al. 2005; Fey et al. 2010; Cilenti et al. 2018; Hick et al. 2018; Lagarde 2018; Lemasson 2019

Table 4. Uncertainty score calculations for effective treatments to kill the greatest number of target marine AIS. Levels of uncertainty were assigned to each 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 (conflicting results, 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	Multiple species	Score	Final score	
Few (1 study)	0	Personal communication	0	Conflicting results	0	Yes	-1	-	no data
Limited (2 studies)	1	Technical report	1	Different conclusions	1	No	0	Very high uncertainty	0–1
Many (3 to 6 studies)	2	Peer-reviewed	2	Mostly agree	2	-	-	High uncertainty	2–3
Comprehensive) (≥ 7 studies)	3	-	-	Fully agree	3	-	-	Moderate uncertainty	4–5
-	-	-	-	-	-	-	-	Low uncertainty	6–8

*Table 5. Effectiveness of physical treatments for marine AIS, where "100%" refers to 100% mortality for a particular treatment combination on adult organisms (unless otherwise specified). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. "Removal" means that the result is not expressed in terms of mortality, but cover reduction or removal of the species. NS: not specified; *: technical reports; **: unpublished data or not peer-reviewed; Δ: acclimation laboratory experiments; juv.: treatment was on juveniles or young stage; a: Botrylloides leachii, proxy for B. violaceus; c: Ciona savignyi, proxy for Ciona intestinalis; e: Watersipora subtorquata, proxy for Cryptosula pallasiana; g: tested on different size groups from the same size category; h: Asterias amurensis, proxy for Asterias rubens; i: several crab spp., proxies for Carcinus maenas; F: field experiment. Laboratory experiments are presented by default. References are enumerated in superscript.*

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature ; time)	(temperature; time)	(time)
COLONIAL TUNICATES										
Golden star tunicate *Botryllus schlosseri*	**Not effective** F 40 psi; NS[29]	**Almost 100%** F 700 psi; 10 s[30] **84%** F 700 psi; NS[29] **Not effective removal** F 2000–3000 psi; 10,20,30 s[45]	**100%** 5 h (18–19°C; RH 92%)[19**] **Effective** F 24 h[41]	**100%** F 24 h[22*] 24 h[71] **Almost 100%** 6 h[22*] **Effective** 12 h[22*]	**Effective** F 5 min[41]	**100%** 8 h; 1 h[10]	**100%** 10 min; 1 h (juv.)[10]	-	-	-
Violet tunicate *Botrylloides violaceus*	**Not effective** F 40 psi; NS[29]	**Almost 100%** F 700 psi; 10 s[30] **84%** F 700 psi; NS[29] **Not effective removal** F 2000–3000 psi; 10,20,30 s[45]	**Almost 100%** F 72 h[2*] **Effective** F 24 h[41]	**100%** F 18–24 h[2*] F 24 h[22*] 24 h[71] **Almost 100%** 6 h[22*] **Effective** 12 h[22*]	**Effective** F 5 min[41]	**100%** 8 h; 1 h[10]	**100%** 10 min; 1 h[10]	-	[a]**100%** 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96]	-

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Carpet sea squirt *Didemnum vexillum*	-	**100% removal** F 2000 psi; NS (+48 h air drying) [44*] **Not effective removal** F 2000–3000 psi; 10,20,30 s[45]	**Effective** F 2 weeks[51*] **Effective** F 24 h[41]	**100%** 4 h[49] **Not effective** F 10 min[9]	**Effective** 5 min[41]	**100%** 8 h; 1 h[10] **87%** F 10 min; 24 h[5] **84%** F 5 min; 24 h[5] **74%** F 2 min; 24 h[5]	**100%** 10 min; 1 h[10]	-	**100%** 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96]	-
Compound sea squirt *Diplosoma listerianum*	-	-	**Effective** F 24 h[41]	-	**Effective** F 5 min[41]	**100%** 8 h; 1 h[10]	**100%** 10 min; 1 h[10]	-	-	-
SOLITARY TUNICATES										
Vase tunicate *Ciona intestinalis*	-	**Effective** F 400–600 psi; NS[48*]	**100%** [c]24 h (18°C)[21] [c]8 h (juv.14.5°C, 95% RH)[21] [c]F 6 h (9.5–32.2°C)[21] **Effective** F 24 h[41]	**Almost 100%** 3 h[23*] F 12 h[25**] **98%** 1 h (larvae)[70**] **10%** 1 min[1] **Effective** 6 h[25**] **Not effective** F 3–6 h[25**]	**Effective** F 5 min[41]	-	-	**66%** 40°C; 60 s[1]	**100%** 40°C; 60 s[11] 50,60°C; 10 s[11] 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96] **66%** 40°C; 30 s[11] **Not effective** 60°C; few s[50*]	-

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Clubbed tunicate *Styela clava*	-	-	**100%** F 24 h (25–27°C)[47] F 2 weeks (10°C)[47] F At least 1 week[42*] 90 min (juv. 20°C)[46*] **0%** F 24 h[112] **Effective** F 24 h[41]	**100%** F 3 h[24*] 15 s (juv.)[46*] **0%** 1 h[112] **Effective** 24 h[42*]	**Effective** 5 min[46*] F 5 min[41]	-	-	-	**100%** 60°C; 30 s[11] 60°C; 15 s[112] 70°C; 10 s[112] 80–90°C; 4 s[46*] **86%** 50°C; 60 s[11] 60°C; 10 s[11] **60–70%** 50°C; 30 s[11] **40–50%** 50°C; 10 s[11] **~12–25%** 40°C; 10, 30, 60 s[11]	**100%** 30 s[46*]
European sea squirt *Ascidiella aspersa*	-	-	**Effective** F 24 h[41]	-	**Effective** F 5 min[41]	-	-	-	-	-
Sea grape *Molgula* spp.	-	-	**Effective** 24 h[79*]	-	-	-	-	-	-	-

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
MOLLUSKS											
Blue mussel *Mytilus edulis*	LARGE ≥50 mm	**Not effective** F 40 psi; NS[29]	**Not effective** F 700 psi; NS[29]	**100%** F 6 h (41°C)[62] **0%** 3 h (20–41°C)[60]	-	-	-	-	-	**100%** 60°C; 20 s[26*] **~35%** 60°C; 5 s[26*] **Not effective** 55°C; 1 min[40]	-
	SMALL < 50 mm	**Not effective** F 40 psi; NS[29]	**0%** F 700 psi; 10 s[67] **Not effective** F 700 psi; NS[29]	**100%** F 6 h (41°C)[62] **99%** F 5 d (8–31°C)[18**] **47.8%** 11 h (27°C; RH 55.6%)[60] **38%** F 40 h (21°C; 34% RH)[4] **5.6%** F 24 h (17–31°C)[18**] **Effective** F 2–3 h (low RH)[89*] **Not effective** F 5–6 h (high RH)[89*]	**0%** 12 h[22*] 24–48 h[39**] F 24 h (11–14°C)[22*]	-	**10%** 8 h; 1 h[10]	**10%** 5 min; 1 h[10]	**Not effective** 55°C; 5 s[35]	**100%** [g]F 50°C; 30 s (10–20 mm)[95*] [g]F 55°C; 15 s (10–20 mm)[95*] [g]F 60°C; 5 s (10–20 mm)[95*] [g]F 60°C; 60 s (40–50 mm)[95*] 60°C; 15 s[26*] 60°C; 15 min[99] 28°C; 6 d[56Δ] 41°C; 1 min[54Δ] 36°C; 84 min[54Δ] F 28–30°C; 3 d[56] **87%** 40°C; 5 min (10°C)[39**] **80%** 28°C; 4 d[56Δ] **76%** 32.6°C; 6 h[60]	**100%** 60 s[36] **Not effective** 30 s[46*]

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
				24 h (4°C, 100% RH)[111]						**50%** 28°C; 3 d[56Δ] **~40 %** 60°C; 5 s[26*] 40°C; 30 min[99] **20–60%** [g]F 60°C; 15–30 s (40–50 mm)[95*] **33%** 40°C; 5 min (4°C)[39**] **10–30%** [g]F 50°C; 15–20 s (10–20 mm)[95*] **6%** 27°C; 48 h[56Δ] **0%** 30°C; 10 min[39**] 26°C; 24 h[56Δ] [g]F 50°C; 5 s (40–50 mm)[95*] [g]F 55°C; 20 s (40–50 mm)[95*] [g]F 60°C; 1 s (40–50 mm)[95*] **Effective** 60–80°C; 4 s[46*] **Not effective** 55°C; 1 min[40]	

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Mediterranean mussel *Mytilus galloprovincialis*	LARGE ≥ 50 mm	-	-	**100%** 11 d (18°C)[21] 7 d (20.3°C)[21] **0%** 24 h (18°C)[17*] F 24 h (14–16°C)[17*] **Not effective** 4 d (18°C)[21]	**0%** 30 min[17*]	-	**1–2%** F 10 min; 24 h[5]	-	-	**100%** 50°C; 60 s[11] 50°C; 5 min[96] **54–58%** F 60–65°C; 30 s[17*] **~40%** 50,60°C; 30 s[11] **7–13%** 53°C; 55–70 s[17*] **5–7%** 51°C; 55–65 s[17*] **0–3%** F 45–51°C; 40–45 s[17*] **1%** 35°C; 5 min[96] **0%** 45–48°C; 80 s[17*] 40°C; 60 s[11] 50,60°C; 10 s[11]	-

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
	SMALL < 50 mm	-	-	**100%** 24 h (18°C; RH 95%)[21] **80%** F 6 h (18.5°C; RH 95%)[21] **8%** 24 h (3–18 mm)[107**]	-	-	-	-	-	**100%** 50°C; 30 s[11] 60°C; 10 s[11] 50°C; 5 min[96] **~25%** 50°C; 10 s[11] **5%** 35°C; 5 min[96] **0%** 40°C; 60 s[11]	-
Mussels *Mytilus* spp.	SMALL < 50 mm	-	-	-	**Almost 0%** 5 d (10°C)[35]	-	-	-	-	-	-
Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	-	-	-	**Not effective** 72 h[91]	-	**Not effective** 72 h + 8 d (3°C)[91]	-	-	**~10%** 60°C; 30 s[26*]	-

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
	SMALL < 70 mm	-	-	**99%** F 11 d (spat; 4–36°C)[18**] **98%** 24 h (3–18 mm)[107**] **32%** F 5 d (spat; 8–31°C)[18**] **5%** F 72 h (35–65 mm)[20] **0%** F 1 d (spat; 17–31°C)[18**]	**0–4%** 24–48 h[39**]	-	-	-	**11%** 30°C; 10 min (10°C)[39**] **0%** 40°C; 5 min[39**] 30°C; 10 min (4°C)[39**]	**~95%** 60°C; 30 s[26*] **40–90%** [g]F 60°C; 15 s (45–55 mm)[27] **30–50%** [g]F 60°C; 15 s (35–45 mm)[27] **~50%** [g]F 60°C; 15 s (35–45 mm)[20] **~1–5%** 60°C; 5–15 s[26*] **5%** [g]F 60°C; 15 s (55–65 mm)[20]	-
Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	-	-	**100%** 34 d (18°C)[21] F 16 d (9.5–32.2°C; RH 95%)[21] **Not effective** 7 d (18°C)[21] F 72 h (9.5–32.2°C; RH 95%)[21]	**20%** F 10 min[9] **Not effective** F 12 h[82*]	-	-	-	-	**0%** 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96]	**100%** 300 s[36]

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
	SMALL < 70 mm	-	-	-	**11.5%** F 12 h[85] **4.2%** 12 h[85]	-	-	-	-	**100%** [g]43°C; 60 min (11, 35, 54 mm)[94] [g]40°C; 96 min (11 mm)[94] [g]40°C; 167 min (54 mm)[94] **60%** F 60°C; 60 s[95*] **86.7%** [g]42.5°C; 20 min (spat)[96] **50%** [g]40°C; 30 min (spat)[96] **23.3%** [g]37.5°C; 60 min (spat)[96] **8–20%** F 60°C; 15–30 s[95*] **11.2%** F 70°C; 40 s[85] **8.7%** 70°C; 45 s[85] **2%** [g]42.5°C; 20 min (juv)[96]	**100%** 60 s[36]

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
									0% F 50°C; 60 s[95*] 70°C; 30–40 s[85] [g]37.5°C; 60 min (juv)[96] [g]40°C; 30 min (juv)[96]	
Slipper snails *Crepidula fornicata* *Crepidula adunca*	-	**Not effective removal** 2000–3000 psi; 10,20,30 s[45]	-	-	-	-	-	-	-	-
Oyster drills *Urosalpinx* spp.	-	-	**Not effective** 8 d[109*]	-	-	-	-	-	-	-
CRUSTACEANS, SEA STAR AND MACROALGAE										
Green crab *Carcinus maenas*	-	[i]**Not effective removal** 2000–3000 psi; 10,20,30 s[45]	**100%** F 7 d (29°C)[53] **50%** F 2.5–4.4 d (29°C)[53]	**Not effective (adults)** 1 h[104**]	-	-	-	-	**100%** (juv) 45–55°C; 1 min[40] 55°C; 5 s[40] **Effective (adult)** 32–45°C; 1 h[104**] **Not effective** (juv) 40°C; 1 min[40] 20–50°C; 5 s[40]	-

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Japanese skeleton shrimp *Caprella mutica* *Caprella* spp.	-	-	-	**100%** [≤ 15 ppt]; 48 h[88] 24 h[92] **10–64%** [13–16 ppt]; 24 h[92] **0%** [> 21 ppt]; 24 h[92]	-	-	-	-	**100%** 30°C; 48 h[88]	-
Barnacles *Balanus* spp. Balanidae *Semibalanus balanoides*	-	-	**92%** 10.8 d (0% RH; 10°C)[97]	-	-	-	-	-	**100%** 40°C; 30 min[99] **~99%** 60°C; 25 s[26*]	**100%** 30 s[36]
Sea stars *Asterias rubens* Ophiuroidea	-	**Not effective removal** 2000–3000 psi; 10,20,30 s[45]	-	-	-	-	-	-	**Effective** 25°C; NS (dip)[79*] **Not effective** 27°C; NS (juv.; dip)[79*] [h]F 40°C; 60 s[98]	-
Oyster thief *Codium fragile*	-	-	**Effective** 17 h (20°C; 72–75% RH)[37] **Not effective** 5 h (20°C; 72–75% RH)[37] F 24 h[33*]	**100%** 24 h[39**] **Effective** 14 h[37] **Not effective** 6 h[37]	-	-	-	-	**100%** 50°C; 30 s[39**]	-

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Macroalgae *Undaria* sp. *Cladophora* sp. *Ulva* sp. *Rhodophyta* sp. *Fucus* spp. *Caulerpa* sp.		-	**100% removal** 2000 psi; 2 s (gametophytes)[35]	**100%** (gametophyte) 3 d (10°C; RH 55–85%)[35] 12 h (20°C; RH 55–85%)[35] 6 weeks (20°C; > RH 95%)[35] **Not effective** (gametophyte) 8 weeks (10°C; > RH 95%)[35]	**100%** (gametophyte) 22 h (20°C)[35] 43 h (10°C)[35] (plantlet) 10 min (10 or 20°C)[35]	-	-	-	-	**100%** 35°C; 10 min[35] 45°C; 45 s[35] 55°C; 5 s[35] **Effective** 80–85°C; 3 s[38] 72°C; 1 h[77]	**100%** 120 s[36] **Not effective** 60 s[36]
POLYCHAETES											
Tube worms	**Serpulidae** *Hydroides elegans* *Spirobranchus paumotanus (=Pomatoceros taeniata)*	-	-	**100%** 24 h[17*] **90–100%** 12 h[17*] **7%** F 24 h[17*] **92–96.2%** F 3–6 h (high RH)[89*] **Effective** F 1–2 d (low RH)[89*] **Not effective** 24 h (ambient, high RH)[3]	**64.9%** 2 h[89*] **40%** 30 min[17*]	-	-	-	-	**99%** 53°C; 60 s[17*] **98%** F 56°C; 30 s[17*] **97%** 51°C; 80 s[17*] **94%** F 51°C; 30 s[17*] **89–92%** F 51°C; 40–45 s[17*] **~60%** 45°C; 40 s[17*]	-

AIS and epibionts		Seawater		Air drying	Freshwater				Heat		
		Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
		(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
	Sabellidae *Sabella spallanzanii*	-	-	-	**100%** 20 min[68] 64 s (juv.)[83] 4 h[83]	-	-	-	**100%** 29.5°C; 24 h[87] 33°C; 3 h[87] 36°C; 30 min[87]	-	-
Mud worms (Spionidae)	*Polydora ciliata*	-	-	**Effective** F 7–10 d (in shade)[82*]	**100%** 6 h[78]	-	-	-	-	-	-
	Polydora hoplura	-	-	-	**9.3–25.9%** 6–12 h[85] **Effective** F 12 h[82*, 85]	-	-	-	-	**30.3–39.2%** 70°C; 30–45 s[85]	-
	Polydora websteri	-	-	**100%** 14 d (3°C)[91]	**25–60%** 72 h[91] **Effective** 12–16 h[79*] F 2 d[82*]	-	**100%** 72 h; 8 d (3°C)[91] **~95%** 72 h; 4 d (3°C)[91]	-	-	-	-
	Boccardia polybranchia	-	-	-	**100%** 15 min[110] **Not effective** 8.5 h[110]	-	-	-	-	-	-
Terebellidae		-	-	**Not effective** 24 h (high RH)[3]	-	-	-	-	-	-	-
Polychaeta (unidentified)		-	-	-	-	-	-	-	-	**100%** 40°C; 30 min[99]	-

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
BRYOZOANS										
Kelp encrusting bryozoan *Membranipora* sp.	-	-	-	-	-	-	-	-	-	-
Brown bryozoan (common bugula) *Bugula neritina*	-	-	**Not effective** 24 h (high RH)[3]	-	-	-	-	-	**100%** 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96]	-
Red crust bryozoan *Cryptosula pallasiana*	-	-	**Not effective** [e]24 h(high RH)[3]	-	-	-	-	-	-	-
Bryozoa	-	-	-	-	-	-	-	-	**100%** 40°C; 30 min[99]	-
SPONGES										
Boring sponge *Cliona celata*	-	-	**Not effective** 18 h (~25°C)[81]	-	-	-	-	-	-	-
Boring sponge *Cliona* spp.	-	-	-	**Effective** 12–16 h[79*]	-	-	-	-	-	-
Calcareous sponge *Leucosolenia* sp.	-	-	-	-	-	-	-	-	-	-
Porifera (species not specified)	-	-	-	-	-	-	-	-	**100%** 40°C; 30 min[99]	-

AIS and epibionts	Seawater		Air drying	Freshwater				Heat		
	Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Spray	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
	(pressure; time)	(pressure; time)	(time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
HYDROZOANS										
Pink-mouth hydroid *Ectopleura crocea*	-	-	-	**0%** 30 s[98]	-	-	-	-	**100%** 40°C; 10 s[11]	-
Hydroid (species not specified)	-	-	-	-	-	-	-	-	-	-

[1]Carver et al. (2003), [2*]MacNair et al. (2006), [3]Forrest et al. 2007, [4]Leblanc et al. 2007, [5]Denny (2008), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [11]Sievers et al. (2019), [17*]Asgari and Jahangard (2012), [18**]Comeau (DFO, unpubl. data), [19**]Bernier et al. (DFO, unpubl. data), [20]Mayrand et al. (2015), [21]Hopkins et al. (2016), [22*]Ramsay (2015a), [23*]Ramsay (2015b), [24*]Ramsay (2015c), [25**]Ramsay (PEI DFC, unpubl. data – 2020 trials), [26*]McDonald (2010), [27]Rousselle (2012), [29]Arens et al. (2011a), [30]Paetzold et al. (2012), [33*]MacNair (2002), [35]Forrest and Blakemore (2006), [36]Joyce et al. (2019), [37]Kim and Garbary (2007), [38]Mineur et al. (2007), [39**]Landry et al. (DFO, unpubl. data), [40]Best et al. (2014), [41]Carman et al. (2010), [42*]Coutts and Forrest (2005), [44*]Coutts (2006), [45]Curtis et al. (2021), [46*]Davidson et al. (2005), [47]Hillock and Costello (2013), [48*]Ramsay (2014a), [49]McCann et al. (2013), [50*]Gill et al. (2007), [51*]Pannell and Coutts (2007), [53]Darbyson et al. (2009), [54]Rajagopal et al. (2005a), [56]Gonzalez and Yevich (1976), [60]Leblanc et al. (2005), [62]Seuront et al. (2019), [67]Arens et al. (2011b), [68]Jute and Dunphy (2017), [70**]Bourque et al. (DFO, unpubl. data), [71]Dijkstra et al. (2008), [77]Williams and Schroeder (2004), [78]Velayudhan 1983, [79*]Medcof (1961), [81]Carver et al. (2010), [82*]Nell (2007), [83]Moore et al. (2007), [85]Nel et al. (1996), [87]Leighton (1998), [88]Ashton et al. (2007), [89*]Arakawa (1980), [91]Brown (2012), [92]Takeuchi et al. (2003), [94]Rajagopal et al. (2005b), [95]Koganezawa (1972), [96]Piola and Hopkins (2012), [97]Foster (1971), [98]Fitridge et al. (2014), [99]Leach (2011), [104**]McKenzie et al. (unpubl. data), [107**]Mallet et al. (Mallet Research Services Ltd., unpubl. data –2008 trials), [109*]Hancock, (1969), [110]Ruellet (2004), [111]Vickerson (2009), [112]Minchin and Duggan (1988).

*Table 6. Effectiveness of chemical treatments (chlorination, acetic acid and citric acid) for marine AIS, where "100" refers to 100% mortality for a particular treatment combination on adult organisms (unless otherwise specified). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. Results with chemical concentrations above those indicated in titles of columns are provided within square brackets. For chlorination treatments, all results are for sodium hypochlorite by default, other chlorine-based compounds are identified with symbols (§, ♯) in subscript. Results for chlorination immersion or spray with air drying are presented in the same column with spray presented as the default option, and immersion being in brackets when used. NS: not specified; conc.: concentration of chemical; *: technical reports; **: unpublished data or non peer-reviewed; §: Total residual chlorine of sodium hypochlorite solution (TRC); ♯: Chlorine dioxide; a: Botrylloides leachii, proxy for Botrylloides violaceus; b: Perna canaliculus, proxy for Mytilus galloprovincialis; e: Watersipora subtorquata, proxy for Cryptosula pallasiana; g: tested on different size groups from the same size category; h: Asterias amurensis, proxy for Asterias rubens; F: field experiment. Laboratory experiments are presented by default. References are enumerated in superscript.*

AIS and epibionts	Chlorination		Acetic acid							Citric acid	
	Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
	(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
COLONIAL TUNICATES											
Golden star tunicate *Botryllus schlosseri*	-	**Effective** F [0.5%]; 5 s; 6 h[8] **Not effective** F [0.5%]; 5 s; 3 h[8]	**Effective** 1 min[3]	**Not effective** 4 min[3]	-	**100%** 5 min; 1 h[10] **Effective** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	**Effective** 1 min; 24 h (no rinse)[3] 3 min; 24 h[3] **Not effective** 1 min; 24 h (+ rinse)[3]	**95%** [10%]; 5 s; 30 min[8] **65%** 5 s; 30 min[8]	-	-	-
Violet tunicate *Botrylloides violaceus*	**100%** [0.3 or 0.6%]; 15 s[2*]	[a]**Effective** F [1%]; 5 s; 30 min[8] [a]**Not effective** [0.5%]; 5 s; 12 h[8]	**100%** 15 s[2*] **Effective** [a] 1 min[3]	[a]**Not effective** 4 min[3]	**90%** F 1 min[2*]	**100%** 5 min; 1 h[10] **Effective** [a]1 min; 24 h[3] [a]24 h (dry first); 1 min[3]	[a]**Effective** 3 min; 24 h[3] 1 min; 24 h (no rinse)[3] [a]**Not effective** 1 min; 24 h (+ rinse)[3]	[a]**95%** [10%]; 5 s; 30 min[8] [a]**65%** 5 s; 30 min[8]	-	-	-

AIS and epibionts	Chlorination		Acetic acid							Citric acid	
	Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
	(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
Carpet sea squirt *Didemnum vexillum*	**100%** [0.5%]; 20 s[5] [0.25%]; 2 min[5] [1%]; 10 min[49] [1%]; 30 s[5] **>90%** [0.1%]; 2 min[5] **50%** [1%]; 2 min[49] [1%]; 5 min[52] **65%** [1%]; 15 min[52] **55%** [1%]; 30 min[52]	**100%** [0.25%]; 2 min (immersion); 5 h[5]	**100%** [10%]; 2 min[49] **95%** F 10 min[5] **80–85%** 1–3 min[5] **65%** 5 min[52] **45–50%** 15–30 min[52] **Effective** F 30 s[9]	**45–82%** F 1–10 min[5] **Effective** F [0.25%]; 30 s[9]	-	**100%** 5 min; 1 h[10]	**77% (mean)** F 20 s–10 min; 1–41 h[5]	**100%** 5 s; 30 min[8] **~95%** 5 s; 10 min[8] **81%** F 3 s; 1 h[5]	-	-	-
Compound sea squirt *Diplosoma listerianum*	-	-	-	-	-	**100%** 5 min; 1 h (juv.)[10]	-	-	-	-	-
SOLITARY TUNICATES											
Vase tunicate *Ciona intestinalis*	**0%** [0.006%]; 20 min[1]	**Effective** F 1%; 5 s; 30 min[8] **Not effective** F 0.5%; 5 s; 12 h[8]	**100%** 1 min[1] 10 s[11] **99–100%** F 15 s[50*] **95%** 30 s[1]	**66%** 30 s[11] **Not effective** 4 min[3]	**10–20%** F (NS)[50*] **Not effective** F (NS)[6*]	**100%** 5 min; 1 h (juv.)[10] **Effective** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	**Effective** 1 min; 24 h (rinse/no rinse)[3]	**60–100%** F 30 s; 30 s[1] **65%** 5 s; 30 min[8]	**100%** [2 %]; 40ºC, 50ºC; 10 s[11] [5%]; 40ºC, 50°C; 10 s[11]	**33%** [5%]; 10 s[11] **0%** [2%]; 10 s[11] **Not effective** [~2%]; 5 s[7]	**100%** [2 %]; 50°C; 10 s[11] [5%]; 40°C; 10 s[11] **66%** [2%]; 40°C; 10 s[11]

AIS and epibionts	Chlorination		Acetic acid							Citric acid	
	Immersion	**Spray or immersion + air drying**	**Immersion**		**Spray**	**Immersion + air drying**		**Spray + air drying**	**Heated immersion**	**Immersion**	**Heated immersion**
	(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
			70–95% F 5–10 s[7] **Effective** 4 min[3] **Not effective** 5–10 s[1]								
Clubbed tunicate *Styela clava*	**100%** F [0.01%]; 12 h[42*] F [0.02%]; 12 h[42*] F [0.05%]; 12 h[42*]	-	**100%** F 1 min[42*] **99–100%** F 15 s[46*] **~50%** 60 s[11]	**100%** F 5–10 min[42*] **0%** 60 s[11] **Not effective** F 1 min[42*]	**5–60%** F NS[46*] **Not effective** F NS[6*]	-	-	-	**100%** [2%]; 40°C, 50°C; 60 s[11] **Almost 100%%** [5%]; 40°C; 60 s[11] **54%** [2%]; 40°C; 30 s[11]	**~75%** [10%]; 30 s[11] **~60%** [5%]; 10 s[11] [2%]; 30 s[11] **~45%** [5%]; 30 s[11]	**100%** [5%]; 40, 50°C; 10 s[11] [2%]; 50°C; 30 s[11] [10%]; 40°C; 10 s[11] **60%** [2%]; 40°C; 30 s[11]
European sea squirt *Ascidiella aspersa*	-	-	-	-	-	**100%** 5 min; 1 h (juv.)[10]	-	-	-	-	-
Sea grape *Molgula* spp.	-	-	-	-	-	-	-	-	-	-	-

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
MOLLUSKS												
Blue mussel *Mytilus edulis*	LARGE ≥ 50 mm	-	-	**10–15%** F 5 s[7]	-	-	-	-	-	-	-	-
	SMALL < 50 mm	**100%** [1 mg/L]; 40 d[61§] [g][4 mg/L]; 7 h (1.4 mm)[59§] [4 mg/L];5.2 d (14 mm)[57§] [4 mg/L];6.3 d (25 mm)[57§,59§] [0.1 mg/L]; 4 h (larvae)[58§] [1 mg/L]; 20 min (larvae)[58§] [0.05 mg/L]; 5 h (larvae)[58§] [3 mg/L]; 17 d[75§] **16%** [0.7 mg/L]; 10 min (larvae)[57§, 58§]	-	**60%** 20 s[34] **12.2%** 15 s[2*] **Effective** [g]5–10 s, 30 s, 1 min (10 mm)[1] F 15 s[46*, 50*] **Not effective** [g]5–10 s, 30 s, 1 min (20 mm)[1] F 30 s[2*]	-	**15%** F NS[50*] **7.7%** 30 s[2*]	**100%** 5 min; 1 h[10] **Not effective** 30 s (+rinse); 24 h (4°C; 100% RH)[111]	-	-	-	-	-

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
Mediterranean mussel *Mytilus galloprovincialis*	LARGE ≥ 50 mm	**3%** [0.14–0.28%]; 9 min[17*#]	-	[b]**9%** F 4 min[3] [b]**5%** [4, 8%]; 2 min[3] **0%** 30 s[11]	**0%** 30 s[11]	-	[b]**69–87%** [10%]; 1 min; 24 h[5] [b]**74%** [8%]; 2 min (no rinse); 24 h[3] [b]**43%** [4%]; 2 min (no rinse); 24 h[3] [b]**10%** F 24 h (dry first); 4 min[3] [b]**9%** F [4%]; 4 min (rinse); 24 h[3] [b]**5%** 24 h (dry first); 2 min[3]	[b]**5–10%** 10 min; 24 h[5] [b]**1.5%** [0.5%]; 10 min; 24 h[5]	[b]**5%** [10%]; 3 s; 26 h[5]	**~60%** [5%]; 50°C; 10 s[11] **~25%** [5%]; 50°C; 30 s[11] **0%** [2%]; 50°C; 30 s[11] [5%]; 40°C; 30 s[11]	**~40–50%** [10%]; 30 s[11] **0%** [2%]; 30 s[11] [10%]; 10 s[11]	**~40–50%** [10%]; 50°C; 30 s[11] **0%** [10%]; 40°C; 30 s[11] [5%]; 50°C; 10 s[11]

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	**Spray or immersion + air drying**	**Immersion**		**Spray**	**Immersion + air drying**		**Spray + air drying**	**Heated immersion**	**Immersion**	**Heated immersion**
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
	SMALL < 50 mm	[b]**6%** [0.5%]; 30 s–2 min[5]	[b]**6%** [0.5%]; 30 s (immersion); 24 h[5]	**62–65% (mean)** F [8%]; 10, 30, 60 s (pooled)[12] [b]**9%** F 4 min[3] **5%** [4, 8%]; 2 min[3] **0%** 30 s[11]	**0%** 30 s[11]	-	[b]**69–87%** [10%]; 1 min; 24 h[5] [b]**74%** [8%]; 2 min (no rinse); 24 h[3] [b]**43%** [4%]; 2 min (no rinse); 24 h[3] [b]**10%** F 24 h (dry first); 4 min[3] [b]**9%** F [4%]; 4 min (+ rinse); 24 h[3] [b]**5%** 24 h (dry first); 2 min[3]	[b]**5–10%** 10 min; 24 h[5] [b]**1.5%** [0.5%]; 10 min; 24 h[5]	-	**100%** [2%]; 50°C; 30 s[11] **90%** [5%]; 50°C; 10 s[11] **0%** [2%]; 40°C; 30 s[11] [5%]; 40°C; 10 s[11]	**~40%** [10%]; 30 s[11] **0%** [10%]; 10 s[11]	**100%** [2%]; 50°C; 30 s[11] **~85%** [10%]; 50°C; 10 s[11] **~60–70%** [10%]; 40°C; 30 s[11] **~45–50%** [5%]; 40°C; 30 s[11] **0%** [10%]; 40°C; 10 s[11]
Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	-	-	**56%** F 30 s[81] **Effective** F [10%]; 10 min[81] F [20%]; 5 min[81]	-	-	-	-	-	-	-	-
	SMALL < 70 mm	-	-	**Not effective** F 10 min[6*]	-	-	-	-	-	-	-	-

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	**Spray or immersion + air drying**	**Immersion**		**Spray**	**Immersion + air drying**		**Spray + air drying**	**Heated immersion**	**Immersion**	**Heated immersion**
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	**Not effective** F [0.05%]; 12 h[42*]	-	**100%** F 5 min[9] **40%** F 30 s[9] **Not effective** F 10 min[42*]	**40%** F [0.25, 1.25%];10 min[9] **20%** F [0.25%]; 1 min[9] F [1.25%]; 30 s[9] **Not effective** F 10 min[42*]	-	-	-	-	-	-	-
	SMALL < 70 mm	-	-	**0%** F 30 s[12] [4,8%]; 15–60 s[12]	**0%** 15–60 s[12]	-	-	-	-	-	-	-
Slipper snails *Crepidula fornicata* *Crepidula aduncа*		-	-	-	-	-	-	-	-	-	-	-
Oyster drills *Urosalpinx cinerea* *Eupleura caudata*		-	-	**Not effective** F 10 min[6*]	-	-	-	-	-	-	-	-
CRUSTACEANS, ECHINODERMS AND MACROALGAE												
Green crab *Carcinus maenas*		**Not effective** [5%]; 1 h[104**]	-	-	-	-	-	-	-	-	-	-
Japanese skeleton shrimp *Caprella mutica*		-	-	-	-	-	-	-	**89–100%** 5–10 s; 45 s[66]	-	-	-

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
Barnacles *Semibalanus balanoides* *Balanus* sp.		-	-	**Not effective** F 1 min[26*]	-	-	-	-	-	-	-	-
Sea stars *Evasterias troschellii* *Asterias rubens*		-	-	**100%** F 5 min[9] [h]F 30 s[98]	-	-	-	-	-	[h]**100%** [2%]; 40°C; 30 s[98]	-	-
Oyster thief *Codium fragile*		-	-	-	-	-	-	-	-	-	-	-
Macroalgae *Caulerpa* sp. *Cladophora* sp. *Rhodophyceae* sp. *Undaria* sp.		**100%** [0.25%]; 60 min[77]	-	**100%** (young stages) F 1 min[3] 1 min[3] **Not effective** 15 s[34]	**Effective** 4 min (young stages)[3] **Not effective** 1–2 min[3]	-	**100%** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	**Effective** 1 min (no rinse); 24 h[3] 3 min (+ rinse); 24 h[3]	**Almost 100%** F 5 s; 10 min[8]	-	**Not effective** [5%]; 15–30 s[34*]	**Not effective** [5%]; 30°C; 30 s[34*]
POLYCHAETES												
Tube worms	**Serpulidae** *Hydroides elegans* *Spirobranchus paumotanus (=Pomatoceros taeniata)*	**0%** [0.28%]; 9 min[17*#]	-	**Effective** F 10 min[63] **Not effective** 4 min[3]	**Not effective** 4 min[3]	-	**Effective** 4 min (no rinse); 24 h[3] **Not effective** 4 min (+ rinse); 24 h[3] 24 h (dry first); 4 min[3]	**Not effective** 4 min; 24 h[3]	-	-	-	-

AIS and epibionts		Chlorination		Acetic acid							Citric acid	
		Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
		(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
	Sabellidae *Sabella spallanzanii*	-	-	-	-	-	-	-	-	-	-	-
Mud worms (Spionidae)	*Polydora ciliata*	-	-	-	-	-	-	-	-	-	-	-
	Polydora hoplura	-	-	-	-	-	-	-	-	-	-	-
	Polydora websteri	-	-	-	-	-	-	-	-	-	-	-
	Polydora spp.	**Not effective** F [0.5%]; 5 min[110]	-	**Effective** F 10 min[63]	-	-	-	-	-	-	-	-
Terebellidae		-	-	**Effective** 2 min[3] **Not effective** 1 min[3]	**Effective** 3 min[3] **Not effective** 1–2 min[3]	-	**Effective** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	**Effective** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	-	-	-	-
BRYOZOANS												
Kelp encrusting bryozoan *Membranipora* sp.		-	-	**Effective** F 10 min[63]	-	-	-	-	-	-	-	-
Brown bryozoan (common bugula) *Bugula neritina*		-	-	**100%** F 30 s[12] **Effective** F 10 min[63] 1 min[3]	**Not effective** 4 min[3]	-	**Effective** 1 min; 24 h[3] 24 h (dry first); 1 min[3]	**Effective** 1 min (no rinse); 24 h[3] 4 min (+ rinse); 24 h[3] 24 h (dry first); 3 min[3]	**Not effective** 5 s; 12 h[8] [20%]; 5 s; 12 h[8]	-	-	-

AIS and epibionts	Chlorination		Acetic acid							Citric acid	
	Immersion	Spray or immersion + air drying	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
	(conc.; time)	(conc.; time; dry time)	[4-5%] (conc.; time)	[1-2%] (conc.; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	(conc.; temp.; immersion time)	(conc.; time)	(conc.; temp.; immersion time)
Red crust bryozoan *Cryptosula pallasiana*	-	-	**100%** [e]F 30 s[12] **Effective** [e]1 min[3]	**Not effective** [e]4 min[3]	-	**Effective** [e]1 min; 24 h[3] [e]24 h (dry first); 1 min[3]	**Effective** [e]1 min (no rinse); 24 h [3] [e]4 min (+ rinse); 24 h [3]	-	-	-	-
SPONGES											
Boring sponge *Cliona celata*	-	-	**90%** F [10%]; 10 min[81] F [20%]; 5 min[81]	-	-	-	-	-	-	-	-
Boring sponge *Cliona* spp.	-	-	-	-	-	-	-	-	-	-	-
Calcareous sponge *Leucosolenia* sp.	-	-	**Effective** F 10 min[63]	-	-	-	-	-	-	-	-
HYDROZOANS											
Pink-hearted hydroid *Ectopleura crocea*	-	-	**100%** 10 s[11]	**100%** 10 s[11]	-	-	-	-	**100%** [2%]; 40°C, 50°C; 10 s[11]	**100%** [5%]; 10 s[11] **60%** [2%]; 10 s[11]	**100%** [2%]; 40°C, 50°C; 10 s[11]
Hydroid (species not specified)	-	-	**100%** F 30 s[12]	-	-	-	-	-	-	-	-

[1]Carver et al. (2003), [2*]MacNair et al. (2006), [3]Forrest et al. (2007), [5]Denny (2008), [6*]Gill et al. (2008), [7]Locke et al. (2009), [8]Piola et al. (2009), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [11]Sievers et al. (2019), [12]Cahill et al. (2021), [17*]Asgari and Jahangard (2012), [26*]MacDonald et al. 2010,[34]Sharp et al. (2006), [42*]Coutts and Forrest (2005), [46*]Davidson et al. (2005), [49]McCann et al. (2013), [50*]Gill et al. (2007), [52]Roche et al. (2015), [57]Haque and Kwon (2017), [58]Haque et al. (2014), [59]Haque et al. (2015), [61]Rajagopal et al. (2003), [63]Chinnadurai et al. (2019), [66]Paetzold et al. (2008), [75]Rajagopal et al. (2002), [77]William and Schroeder (2004), [81]Carver et al. (2010), [98]Fitridge et al. (2014), [104**]McKenzie et al. (unpubl. data), [110]Ruellet (2004), [111]Vickerson (2009).

*Table 7. Effectiveness of chemical treatments (saturated brine, hydrated lime, and Virkon®) for marine AIS, where "100%" refers to 100% mortality for a particular treatment combination on adult organisms (unless otherwise specified). "Effective" treatments refer to studies where % mortality was deemed sufficient but not quantified. Results with chemical concentrations above those indicated in titles of columns are provided within square brackets. NS: not specified; conc.: concentration of chemical; *: technical reports; **: unpublished data or non peer-reviewed; a: Botrylloides leachii, proxy for Botrylloides violaceus; e: Watersipora subtorquata, proxy for Cryptosula pallasiana; F: field experiment. Laboratory results are presented by default. References are enumerated in superscript.*

AIS and epibionts	Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
	Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
	([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
COLONIAL TUNICATES							
Golden star tunicate *Botryllus schlosseri*	-	**100%** [70 ppt]; 10 s; 1 h[10] **Effective** [70 ppt]; 10 min; 2 h[41] [300 ppt]; 30 s; 1 h[102**]	**Effective** 30 s; 1 h[102**]	**Effective** F 1 min[26*]	-	**Effective** F [20%]; 5 s; 6 h[8]	-
Violet tunicate *Botrylloides violaceus*	**Not effective** 300 ppt; 15 s[2*]	**100%** F [300 ppt]; 5 min; 1 h[2*] [70 ppt]; 10 s; 1 h[10] **Almost 100%** F [300 ppt]; 1 min; 1 h[2*] F [300 ppt]; 1 min; 24 h[2*] **Effective** [70 ppt]; 10 min; 2 h[41] [300 ppt]; 30 s; 1 h[102**] **Not effective** F [300 ppt]; 15 s; 24 h[2*]	**Effective** 30 s; 1 h[102**]	**0%** F 15 s[2*] **Effective** F 1 min[26*]	**80–90%** F 15 s; 10–15 min[2*] **Not effective** F 1 min; 5 min[6*]	[a]**Effective** F [20%]; 5 s; 6 h[8] F [5%]; 5 s; 12 h[8]	-
Carpet sea squirt *Didemnum vexillum*	**100%** [62 ppt]; 4 h[49] **Not effective** F [70 ppt]; 10 min[9]	**100%** [70 ppt]; 10 s; 1 h[10] **Effective** [70 ppt]; 10 min; 2 h[41]	-	**99%** F [10%]; 2 min[5] **92.3%** 5 min[9] **85–96%** F 4 min[13]	-	-	-

AIS and epibionts	Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
	Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
	([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
				80% F [5%]; 2 min[5] **Not effective** F [20%]; 20 s[5]			
Compound sea squirt *Diplosoma listerianum*	-	**100%** [70 ppt]; 10 s; 1 h (juv.)[10] **Effective** [70 ppt]; 10 min; 2 h[41]	-	-	-	-	-
SOLITARY TUNICATES							
Vase tunicate *Ciona intestinalis*	**25%** [300 ppt]; 8 min[1] **Not effective** F [300 ppt]; 30 s[50*]	**100%** [70 ppt]; 10 s; 1 h (juv.)[10] **Effective** [70 ppt]; 10 min; 2 h[41] **Not effective** F [300 ppt]; 15 s; 1 h[50*]	**Effective** 1 min; 30 min, 1h[64*] **Not effective** 30 s; 30 min[64*]	**80%** F 2 min[14*] **70%** 8 min[1] **50–80%** F 15 s[50*] **Effective** F 1 min[26*]	**100%** F 15 s; 20 min[50*] **Effective** F 1 min; 5 min[6*]	**Effective** F [20%]; 5 s; 12 h[8]	**100%** [3%]; 30 s (juv.)[69] **95%** [1%]; 60 s (juv.)[69] **5–13%** F [3%]; 15 s[50*]
Clubbed tunicate *Styela clava*	**75%** [300 ppt]; 10 s (juv.)[46*]	**100%** F [300 ppt]; 5 min; 30 min[112] **Effective** F [70 ppt]; 10 min; 2 h[41]	-	**80%** F 2 min[14*] **Effective** F 1 min[26*]	**Effective** F 1 min; 5 min[6*] **86%** (mean) F 1 min; 5 min[6*]	**Effective** F NS; 45 s[14*] 5 s; 45 s[103*]	-
European sea squirt *Ascidiella aspersa*	-	**100%** [70 ppt]; 10 s; 1 h[10] **Effective** F [70 ppt]; 10 min; 2 h[41]	-	-	-	-	-

AIS and epibionts		Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
		Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
		([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Sea grape *Molgula manhattensis* *Molgula* spp.		**Almost 100%** [300 ppt]; 10 min[80*] **Effective** [300 ppt]; 3 min[65] [300 ppt]; 10 min[79*]	**100%** [300 ppt]; 1 min; 1 h[80*] **Effective** [300 ppt]; 3 min; 1 h[79*] **Effective** F [70 ppt]; 10 min; 2 h[41]	-	**Effective** 1 min[7, 65] 3 min[65] **Effective** F 1 min[26*]	-	-	-
MOLLUSKS								
Blue mussel *Mytilus edulis*	LARGE ≥ 50 mm	-	**Not effective** [300 ppt]; 30 s; 1 h[102**]	-	**10–15%** 1 min[100**] **< 2%** F 15 s[2*] **0%** 3 h (variable pH)[15]	-	**0%** 5 s; 90 s[15]	**16.7%** [3%]; 60 s[69] **5.6%** [3%]; 30 s[69] **0%** [1%]; 60 s[69]
	SMALL < 50 mm	**18–23%** [300 ppt]; 30 min[39**] **17%** [300 ppt]; 6 min (3–18 mm)[107**] **5–16%** F [300 ppt]; 60 s (9–15 mm)[55*] **3–5%** F [300 ppt]; 30–60 s[55*]	**97%** [300 ppt]; 6 min; 24 h (18 mm)[107**] **39%** F [300 ppt]; 10 min; 24 h[2*] **8–30%** [70 ppt]; 20 s; 1 h[10] **0%** [300 ppt]; 15 min; 1 h[39**] **Not effective** [300 ppt]; 30 s; 24 h (4°C; 100% RH; 30–40 mm)[111]	-	**77–78%** 15 min[39**] **53–71%** 30 min[39**] **0–2%** F 15 s[50*] **0%** F 1–2 min[14*]	**Not effective** 30 s; 24 h (4°C; 100% RH; 30–40 mm)[111] 24 h (dry first; 4°C, 100% RH); 30 s (30–40 mm)[111]	**2%** 30 s; 1 h[39**]	-

AIS and epibionts		Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
		Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
		([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
		Not effective [300 ppt]; 30 s[34] F [300 ppt]; 15 s[32*]	24 h (dry first; 4°C; 100% RH); [300 ppt]; 30 s (30–40 mm)[111]					
	NS	-	**0%** F [300 ppt]; 30 s; 24 h[2*] F [300 ppt]; 1 min; 1 h[2*] **Effective** F [300 ppt]; 2 min; 1 h[2*]	-	-	-	-	-
Mediterranean mussel *Mytilus galloprovincialis*	LARGE ≥ 50 mm	**90%** [350 ppt] (-20°C); 10 s[17*] **21%** F [350 ppt]; 20 min[17*] **17%** [350 ppt] (-20°C); 5 s[17*] **3.7%** [350 ppt]; 30 min[17*] **0%** [350 ppt]; 20 min[17*]	-	-	-	-	-	-
	SMALL < 50 mm	-	-	-	-	-	-	-

AIS and epibionts		Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
		Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
		([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	**0%** F [270 ppt]; 10 min[81]	**0%** F [270 ppt]; 6 min; 18 h[81]	**Not effective** 30 s; 1 h[102**]	**0%** F 10 min[81] **0%** 3 h (variable pH)[15]	-	-	-
	SMALL < 70 mm	**0–10%** [300 ppt]; 30 min[39**] **0%** [300 ppt]; 15 min[39**] [300 ppt]; 3 min[65] [300 ppt]; 6 min[107**] **Not effective** F [300 ppt]; 10 min[6*]	**0%** [300 ppt]; 30 s; 1 h[39**] [300 ppt]; 6 min; 24 h[107**]	**Not effective** 30 s; 1 h[102**]	**10–15%** 30 min[39**] **0%** 1 min[65] **Not effective** F 10 min[6*] 1 min[7]	**0%** 30 s; 1 h[39**]	-	-
Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	**0%** F [70 ppt]; 10 min[9]	**25%** [360 ppt]; 30 min; several hours[110]	-	**60%** F 30 s [9] **36%** F 4 min[13] **20%** F [1%]; 30 s[9] **0%** F [2%]; 5, 10 min[9]	-	-	-
	SMALL < 70 mm	**0%** F [300 ppt]; 1 h[112]	-	-	-	-	-	-
Slipper snails *Crepidula fornicata* *Crepidula adunca*		-	**100%** [300 ppt]; 3 min; 30 min[80*]	-	-	-	-	-

AIS and epibionts	Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
	Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
	([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Oyster drills *Urosalpinx cinerea* *Eupleura caudata*	**Effective** [300 ppt]; 5 min (juv)[80*] **Not effective** F [300 ppt]; 10 min[6*]	**Effective** [300 ppt]; 3 min; several h (juv.)[80*]	-	**Not effective** F 10 min[6*]	-	-	-
CRUSTACEANS, SEA STARS AND MACROALGAE							
Green crab *Carcinus maenas*	-	**Not effective** [300 ppt]; 1 h; several hours[104**]	-	**Not effective** F 2 min[14*]	-	-	-
Japanese skeleton shrimp *Caprella mutica*	-	-	-	-	-	-	-
Barnacles *Semibalanus balanoides* *Balanus* spp.	**Not effective** F [300 ppt]; 1 min[26*]	-	-	**Not effective** F 15 min[26*]	-	-	-
Sea stars *Asterias rubens* Sea star (species not specified)	**Effective** [300 ppt]; 30 s[80*] [300 ppt]; 2 min[79*]	**100%** [300 ppt]; 1 min; NS[80*]	-	**Effective** F 1 min[26*]	-	-	-
Oyster thief *Codium fragile*	**100%** F [300 ppt]; 15 min[33*] **Not effective** [300 ppt]; 3 min[65]	**100%** F [300 ppt]; 15 min; 2 h[33*] F [300 ppt]; 10 min; 24 h[33*] [300 ppt]; 15 min; 1 h[39**]	-	**100%** F 5 min[33*] **Not effective** 1 min[65]	**100%** 30 s; 1 h[39**] F 1 min; 24 h[33*] F 15 min; 2 h[33*]	-	-

AIS and epibionts		Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
		Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
		([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Macroalgae *Cladophora* sp. Other Macroalgae spp.		**Effective** F [300 ppt]; 15 s[32*] **Effective** [300 ppt]; 15 s[34] **Effective** [400 ppt]; 30 min[38]	-	-	-	-	-	-
POLYCHAETES								
Tube worms	**Serpulidae** *Hydroides elegans* *Spirobranchus paumotanus (=Pomatoceros taeniata)*	**100%** [350 ppt] (-20°C); 10 s[17*] **94.1%** [350 ppt]; 30 min[17*] **79.1%** F [350 ppt]; 20 min[17*] **59.4%** [300 ppt]; 20 min[89*]	-	-	-	-	-	-
	Sabellidae *Sabella spallanzanii*	**100%** [50 ppt]; 24 h[68]	-	-	-	-	-	-

AIS and epibionts		Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
		Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
		([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Mud worms (Spionidae)	*Polydora ciliata*	**100%** [42.3 ppt]; 19–21 h[78*] [60 ppt]; 8.5 h[78*] [78 ppt]; 7.75 h[78*] **Effective** [300 ppt]; 5 min[79*]	-	-	-	-	-	-
	Polydora hoplura	-	-	-	-	-	-	-
	Polydora websteri	-	**90%** [300 ppt]; 15 min; 24 h[106**] **85%** [300 ppt]; 6 min; 24 h[106**] **Effective** F [300 ppt]; 1 min; 2 h[82*]	-	-	-	-	-
	Polydora spp.	-	**Almost 100%** [360 ppt]; 30 min; several hours[110] **Effective** F [70 ppt]; 15 min; 15 min[74*] 1 h (dry first); [300 ppt]; 1 min (dip)[79*]	-	-	-	-	-
Terebellidae		-	-	-	-	-	-	-

AIS and epibionts	Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
	Immersion	Immersion + air drying	Immersion ± air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
	([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
BRYOZOANS							
Kelp encrusting bryozoan *Membranipora* sp.	-	-	-	-	-	-	-
Brown bryozoan (common bugula) *Bugula neritina*	-	-	-	-	-	**Not effective** F [20%]; 5 s; 12 h[8]	-
Red crust bryozoan *Cryptosula pallasiana*	-	-	-	-	-	**Effective** [e]F [10%]; 5 s; 30 min[8] [e]F [20%]; 5 s; 3 h[8] **Not effective** [e]F [5%]; 5 s; 12 h[8]	-
Bryozoans (species not specified)	-	-	-	**Effective** F 1 min[26*]	-	-	-
SPONGES							
Boring sponge *Cliona celata*	**100%** F [270 ppt]; 5 min[81]	**100%** F [270 ppt]; 6 min; 1 h[81]	-	**32%** 10 min[81]	-	-	-
Boring sponge *Cliona* spp.	**Effective** [300 ppt]; 5 min[79*]	**100%** [300 ppt]; 10 min; 1 h[80*] **Effective** 1 h (dry first); [300 ppt]; 1 min (dip)[79*]	-	-	-	-	-
Calcareous sponge *Leucosolenia* sp.	-	-	-	-	-	-	-

AIS and epibionts	Brine		Brine [300 ppt] and hydrated lime [4%]	Hydrated lime [4%]			Virkon®
	Immersion	**Immersion + air drying**	**Immersion ± air drying**	**Immersion**	**Immersion + air drying**	**Spray + air drying**	**Immersion**
	([conc.]; time)	([conc.]; immersion time; dry time)	(immersion time; ± dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
HYDROZOANS							
Pink-hearted hydroid *Ectopleura crocea*	-	-	-	-	-	-	-
Hydroid (species not specified)	-	-	-	**Effective** F 1 min[26*]	-	-	-

[1]Carver et al. (2003), [2*]MacNair et al. (2006), [5]Denny (2008), [6*]Gill et al. (2008), [7]Locke et al. (2009), [8]Piola et al. (2009), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [13]Switzer et al. (2011), [14*]Ramsay et al. (2014), [15]Comeau et al. (2017), [17*]Asgari and Jahangard (2012), [26*]McDonald (2010), [32*]MacNair (2009), [33*]MacNair (2002), [34]Sharp et al. (2006), [38]Mineur et al. (2007),[39**]Landry et al. (DFO, unpubl. data), [41]Carman et al. (2010), [46*]Davidson et al. (2005), [49]McCann et al. (2013), [50*]Gill et al. (2007), [55*]Bourque and Myrand (2007), [64*]Ramsay (2022), [65]MacNair and Smith (1999), [68]Jute and Dunphy (2017), [69]Paetzold and Davidson (2011), [74*]Gryder (2002), [78*]Velayudhan (1983), [79*]Medcof (1961), [80*]Loosanoff (1960), [81]Carver et al. (2010), [82*]Nell (2007), [89*]Arakawa (1980), [100**]MacNair (IPE DFC, unpubl. data), [102**]Mills (DFO, unpubl. data), [103*]Ramsay (2014b), [104**]McKenzie et al. (unpubl. data), [106**]Carver and Mallet (Mallet Research Services Ltd., unpubl. data), [107**]Mallet et al. (Mallet Research Services Ltd., unpubl. data – trials 2008), [110]Ruellet (2004), [111]Vickerson (2009), [112]Minchin and Duggan (1988).

*Table 8. Impacts of physical treatments on survival of moved species, where "100%" means 100% survival of organisms subjected to a particular treatment combination. "Impacted" refers to studies where survival of moved species was affected but not quantified. NS: not specified; *: technical reports; **: unpublished data or non peer-reviewed; Δ: acclimation laboratory experiments; (juv.): treatment was on juveniles or young stage; g: tested on two different sizes within the same size category; b: Perna canaliculus, proxy for Mytilus galloprovincialis; d: Ostrea angasi, proxy for Ostrea edulis; RH: relative humidity; F: field experiment. Laboratory experiments are presented by default. References are enumerated in superscript.*

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Atlantic and Pacific	**Blue mussel** *Mytilus edulis*	LARGE ≥ 50 mm	**Not impacted** F 40 psi; NS[29]	**Not impacted** F 700 psi; NS[29]	**100%** 3 h (20–41°C)[60] **0%** F 6 h (41°C)[62]	-	-	-	-	**~65%** 60°C; 5 s[26*] **0%** 60°C; 20 s[26*] **Not impacted** 55°C; 1 min[40]	-
		SMALL < 50 mm	**Not impacted** F 40 psi; NS[29]	**100%** F 700 psi, 10 s[67] **Not impacted** F 700 psi; NS[29]	**94.6%** F 1 d (17–31°C)[18**] **92%** 24 h[107**] **62%** F 40 h (21°C; 34% RH)[4] **52.2%** 11 h (27°C; RH 55.6%)[60] **1%** F 5 d (8–31°C)[18**]	**100%** 48 h[39**] 12 h[22*] F 24 h (11–14°C)[22*]	**90%** 24 h; 1 h[10]	**90%** 10 min; 1 h[10]	**Not impacted** 55°C; 5 s[35]	**100%** 30°C; 10 min[39**] 26°C; 24 h[56Δ] [g]F 50°C; 60 s (40–50 mm)[95*] [g]F 55°C; 5 s (10–20 mm)[95*] [g]F 55°C; 20 s (40–50 mm)[95*] [g]F 60°C; 1 s (10–20 mm)[95*] [g]F 60°C; 10 s (40–50 mm)[95*] **90%** [g]F 50°C; 15 s (10–20 mm)[95*] [g]F 55°C; 30 s (40–50 mm)[95*]	**0%** 60 s[36] **Not impacted** 30 s[46*]

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
					0% F 6 h (41°C)[62] **Not impacted** F 5–6 h (high RH)[89*] 24 h (4°C; 100% RH)[111] **Impacted** F 2–3 h (low RH)[89*]					**94%** 27°C; 48 h[56Δ] **70–90%** [9]F 50°C; 15–20 s (40–50 mm)[95*] **40–80%** F 60°C; 15–30 s (40–50 mm)[95*] **67%** 40°C; 5 min (4°C)[39**] **~60 %** 60°C; 5 s[26*] 40°C; 30 min[99] **50%** 28°C; 3 d[56Δ] **24%** 32.6°C; 6 h[60] **20%** 28°C; 4 d[56Δ] **13%** 40°C; 5 min (10°C)[39**] **0%** 60°C; 15 s[26*]	

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
										28°C; 6 d[56Δ] 36°C; 70 min[54Δ] 41°C; 1 min[54Δ] F 28–30°C; 3 d[56] [g]F 50°C; 30 s (10–20 mm)[95*] [g]F 55-60°C; 15 s (10–20 mm)[95*] [g]F 60°C; 1 min (40–50 mm)[95*] 60°C; 15 min[99] **Not impacted** 55°C; 1 min[40] **Impacted** 60–80°C; 4 s[46*]	
Atlantic	**European flat oyster** *Ostrea edulis*	LARGE ≥ 65 mm	-	-	-	-	-	-	-	-	-
		SMALL < 65 mm	-	-	-	[d]**100%** 30 s[98]	-	-	-	[d]**100%** 40°C; 60 s[98] 50°C; 10 s[98] [d]**60%** 60°C; 10 s[98] [d]**0%** 50°C; 30 s[98] 60°C; 30 s[98]	-
	Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	-	-	-	**Not impacted** 72 h[91]	**Not impacted** 72 h + 14 d (3°C)[91]	-	-	**~90%** 60°C; 30 s[26*]	-

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
		SMALL < 70 mm	-	-	**100%** F 1 d (spat; 17–31°C)[18**] **95%** F 72 h (35–65 mm)[20] **68%** F 5 d (spat; 8–31°C)[18**] **2%** 24 h (1–2 mm)[107**] **1%** F 11 d (spat; 4–36°C)[18**]	**96–100%** 24–48 h[39**]	-	-	**100%** 40°C; 5 min (4°C, 10°C)[39**] 30°C; 10 min (4°C)[39**] **89%** 30°C; 10 min (10°C)[39**]	**~95–99%** 60°C; 5–15 s[26*] **95%** [g]F 60°C; 15 s (55–65 mm)[20] **~50%** [g]F 60°C; 15 s (35–45 mm)[20,27] **40–60%** [g]F 60°C; 15 s (55–65 mm)[27] **~5%** 60°C; 30 s[26*]	-
	Bay scallop *Argopecten irradians*	LARGE ≥ 40 mm	-	-	-	-	-	-	-	-	-
		SMALL < 40 mm	-	-	-	-	-	-	-	-	-
	Giant scallop *Placopecten magellanicus*	LARGE ≥ 70 mm	-	-	-	-	-	-	-	-	-
		SMALL < 70 mm	-	-	-	**100%** 10 min (4–6 ppt; 10°C)[39**] **80%** 10 min (4–6 ppt; 4°C)[39**]	-	-	**100%** 30°C; 10 min[39**] **3%** 40°C; 1 min[39**]	-	-

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Pacific	**Mediterranean mussel** *Mytilus galloprovincialis*	LARGE ≥ 50 mm	-	-	**100%** 24 h (18°C)[17*] F 24 h (14–16°C)[17*] **0%** 11 d (18°C)[21] 7 d (20.3°C)[21] **Impacted** 4 d (18°C)[21]	**100%** 30 min[17*]	[b]**98–99%** F 10 min; 24 h[5]	-	-	**100%** 45–48°C; 80 s[17*] 40°C; 60 s[11] 50,60°C; 10 s[11] **99%** 35°C; 5 min[96] **97–100%** F 46–51°C; 40–45 s[17*] **93–95%** 51°C; 65 s[17*] **87–93%** 53°C; 55–70 s[17*] **42–46%** F 60–65°C; 30 s[17*] **~60%** 50°C; 30 s[11] **0%** 50°C; 60 s[11] 50°C; 5 min[96]	-

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
Pacific		SMALL < 50 mm	-	-	**20%** F 6 h (18.5°C; RH 95%)[21] **0%** 24 h (18°C; RH 95%)[21]	-	[b]**98–99%** F 10 min; 24 h[5]	-	-	**100%** 40°C; 60 s[11] **95%** 35°C; 5 min[96] **~75%** 50°C; 10 s[11] **0%** 50°C; 30 s[11] 60°C; 10 s[11] 50°C; 5 min[96]	-
	Mussel sp. *Mytilus* sp.	SMALL < 50 mm	-	-	-	**Almost 100%** 5 d (10°C)[35]	-	-	-	-	-
	Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	-	**Impacted** F 2000 psi; 30 s[45]	**0%** 34 d (18°C)[21] F 16 d (9.5–32.2°C; RH 95%)[21] **Impacted** 7 d (18°C)[21] F 72 h (9.5–32.2°C; RH 95%)[21]	**100%** F 10 min (5 ppt)[9] **80%** F 10 min (0 ppt)[9] **Not impacted** F 12 h[82*]	-	-	-	**100%** 37.5°C; 60 min[96] 40°C; 30 min[96] 42.5°C; 20 min[96]	**0%** 300 s[36]

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
		SMALL < 70 mm	-	-	-	**95.8%** 12 h[85] **88.5%** F 12 h[85]	-	-	-	**100%** 50°C; 60 s[95*] 70°C; 30–40 s[85] [g]37.5°C; 60 min (juv)[96] [g]40°C; 30 min (juv)[96] **98%** [g]42.5°C; 20 min (juv)[96] **91.3%** 70°C; 45 s[85] **90%** 55°C; 60 s[95*] **88.8%** F 70°C; 40 s[85] **80–92%** 60°C; 15–30 s[95*] **76.7%** [g]37.5°C; 60 min (spat)[96] **50%** [g]40°C; 30 min (spat)[96] **40%** 60°C; 60 s[95*]	**0%** 60 s[36]

Coast (Atlantic/Pacific)	Moved species	Size category	Seawater		Air drying	Freshwater			Heat		
			Low pressure spray (<60 psi)	High pressure spray (>700 psi) ± air drying		Immersion	Immersion + air drying	Spray + air drying	Freshwater immersion	Seawater immersion	Steam (100°C; 50 psi)
			(pressure; time)	(pressure; time)	(time)	(time)	(immersion time; drying time)	(spray time; drying time)	(temperature; time)	(temperature; time)	(time)
										13.3% [9]42.5°C; 20 min (spat)[96] **0%** [9]43°C; 60 min (11, 35, 54 mm)[94] [9]40°C; 96 min (11 mm)[94] [9]40°C; 167 min (54 mm)[94]	

[4]Leblanc et al. 2007, [5]Denny (2008), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [11]Sievers et al. (2019), [17*]Asgari and Jahangard (2012), [18**]Comeau (DFO, unpubl. data), [20]Mayrand et al. (2015), [21]Hopkins et al. (2016), [22*]Ramsay (2015a), [26*]McDonald (2010), [27]Rousselle (2012), [29]Arens et al. (2011a), [35]Forrest and Blakemore (2006), [36]Joyce et al. (2019), [39**]Landry et al. (DFO, unpubl. data), [40]Best et al. (2014), [45]Curtis et al. (2021), [46*]Davidson et al. (2005), [54]Rajagopal et al. (2005a), [56]Gonzalez and Yevich (1976), [60]Leblanc et al. (2005), [62]Seuront et al. (2019), [67]Arens et al. (2011b), [82*]Nell (2007), [85]Nel et al. (1996), [89*]Arakawa (1980), [91]Brown (2012), [94]Rajagopal et al. (2005b), [95*]Koganezawa (1972), [96]Piola and Hopkins (2012), [98]Fitridge et al. (2014), [99]Leach (2011), [107**]Mallet et al. (Mallet Research Services Ltd., unpubl.data – trials 2008), [111]Vickerson (2009).

*Table 9. Impacts of chemical treatments (sodium hypochlorite, acetic acid and citric acid) on survival of moved species, where "100%" means 100% survival of organisms subjected to a particular treatment combination. "Impacted" refers to studies where survival of moved species was affected but not quantified. For chlorination treatments, all results are for sodium hypochlorite by default, other chlorine-based compounds are identified with symbols (§, ♯) in subscript. NS: not specified; *: technical reports; **: unpublished data or non peer-reviewed; §: total residual chlorine (TRC); ♯: Chlorine dioxide; b: Perna canaliculus, proxy for Mytilus galloprovincialis; d: Ostrea angasi, proxy for Ostrea edulis; g: tested on different size groups from the same size category; F: field experiments. Laboratory experiments are presented by default. References are enumerated in superscript.*

Coast (Atlantic/Pacific)	Moved species	Size category	Chlorination	Acetic acid							Citric acid	
			Immersion	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
			([conc.]; time)	[4-5%] ([conc.]; time)	[1-2%] ([conc.]; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	([conc.]; temp.; immersion time)	([conc.]; time)	([conc.]; temp.; immersion time)
Atlantic and Pacific	**Blue mussel** *Mytilus edulis*	LARGE ≥ 50 mm	-	**85–90%** F 5 s[7]	-	-	-	-	-	-	-	-
		SMALL < 50 mm	**84%** [0.7 mg/L]; 10 min (veliger)[57§, 58§] **0%** [3 mg/L]; 17 d[75§] [1 mg/L]; 40 d[61§] [g][4 mg/L]; 7 h (1.4 mm)[59§] [g][4 mg/L]; 5.2 d (14 mm)[57§] [g][4 mg/L]; 6.3 d (25 mm)[57§,59§] [1 mg/L]; 20 min (veliger)[58§] [0.1 mg/L]; 4 h (veliger)[58§] [0.05 mg/L]; 5 h (veliger)[58§]	**87.8%** F 15 s[2*] **40%** 20 s[34] **Not impacted** [g]5–10 s, 30 s, 1min (20 mm)[1] F 30 s[2*] **Impacted** F 15 s[46*,50*] [g]5–10 s, 30 s, 1 min (10 mm)[1] 30 s[111]	-	**92.3%** F 30 s[2*] **85%** F NS[50*]	**0%** 5 min; 1 h[10] **Not impacted** 30 s (rinse); 24 h (4°C, 100% RH)[111] **Impacted** 30 s (no rinse); 24 h (4°C, 100% RH)[111] 30 s (rinse); 24 h (1–2°C; 100% RH)[111] 24 h (air first; 4°C; 100% RH); 30 s[111]	-	-	-	-	-

Coast (Atlantic/Pacific)	Moved species	Size category	Chlorination	Acetic acid							Citric acid	
			Immersion	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
			([conc.]; time)	[4-5%] ([conc.]; time)	[1-2%] ([conc.]; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	([conc.]; temp.; immersion time)	([conc.]; time)	([conc.]; temp.; immersion time)
Atlantic	**European flat oyster** *Ostrea edulis*	LARGE ≥ 65 mm	-	-	-	-	-	-	**Not impacted** F 30 s; 30 s[1]	-	-	-
		SMALL < 65 mm	-	[d]**100%** 30 s[11] **Not impacted** [g]1 min (20 mm)[1] **Impacted** [g]1 min (10 mm)[1]	[d]**100%** 30 s[11]	-	-	-	**80%** F 30 s; 30 s[1]	[d]**100%** [5%]; 40°C; 30 s (15 & 50 mm)[11] [g][2%]; 50°C; 10 s (50 mm)[11] [d]**~40%** [g][2%]; 50°C; 10 s (15 mm)[11] [d]**0%** [2%]; 50°C; 30 s (15 & 50 mm)[11]	[d]**100%** [g][10%]; 30 s (15 & 50 mm)[11] [d]**75%** [g][10%]; 10 s (50 mm)[11]	[d]**100%** [2%]; 50°C; 10 s (15 & 50 mm)[11] [10%]; 40°C; 30 s (15 & 50 mm)[11] [d]**0%** [2%]; 50°C; 30 s (15 & 50 mm)[11]
	Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	-	**44%** F 30 s[81] **Impacted** F [10%]; 10 min[81] F [20%]; 5 min[81]	-	-	-	-	-	-	-	-
		SMALL < 70 mm	-	-	-	-	-	-	-	-	-	-

Coast (Atlantic/Pacific)	Moved species	Size category	Chlorination	Acetic acid							Citric acid	
			Immersion	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
			([conc.]; time)	[4-5%] ([conc.]; time)	[1-2%] ([conc.]; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	([conc.]; temp.; immersion time)	([conc.]; time)	([conc.]; temp.; immersion time)
	Bay scallop *Argopecten irradians*	LARGE ≥ 40 mm	-	-	-	-	-	-	-	-	-	-
		SMALL < 40 mm	-	-	-	-	-	-	-	-	-	-
	Giant scallop *Placopecten magellanicus*	LARGE ≥ 70 mm	-	-	-	-	-	-	-	-	-	-
		SMALL < 70 mm	-	-	-	-	-	-	-	-	-	-
Pacific	**Mediterranean mussel** *Mytilus galloprovincialis*	LARGE ≥ 50 mm	**97%** [0.14–0.28%]; 9 min[17‡*]	**100%** 30 s[11] [b]**91%** F 4 min[3] [b]**95%** [4.8%]; 2 min[3]	**100%** 30 s[11]	-	**95%** 24 h (dry first); 2 min[3] **90%** F 24 h (dry first) [4, 8%]; 4 min[3] [b]**91%** F 4 min (rinse); 24 h[3] [b]**57%** 2 min (no rinse); 24 h[3] [b]**26%** [8%]; 2 min (no rinse); 24 h[3] [b]**13–31%** [10%]; 1 min; 24 h[5]	[b]**98.5%** [0.5%]; 10 min; 24 h[5] [b]**90–95%** 10 min; 24 h[5]	**95%** [10%]; 3 s; 26 h[5]	**100%** [2%]; 40–50°C; 30 s[11] [5%]; 40°C; 30 s[11] **~75%** [5%]; 50°C; 30 s[11] **~40%** [5%]; 50°C; 10 s[11]	**100%** [2%]; 30 s[11] [10%]; 10 s[11] **~50–60%** [10%]; 30 s[11]	**100%** [10%]; 40°C; 30 s[11] [5%]; 50°C; 10 s[11] **~50–60%** [10%]; 50°C; 10, 30 s[11]

Coast (Atlantic/Pacific)	Moved species	Size category	Chlorination	Acetic acid							Citric acid	
			Immersion	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
			([conc.]; time)	[4-5%] ([conc.]; time)	[1-2%] ([conc.]; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	([conc.]; temp.; immersion time)	([conc.]; time)	([conc.]; temp.; immersion time)
		SMALL < 50 mm	[b]**94%** [0.5%]; 30 s, 2 min[5] [0.5%]; 30 s (+ 24 h air drying)[5]	**100%** 30 s[11] **95%** [4, 8%]; 2 min[3] [b]**91%** F 4 min[3] [b]**35–38% (mean)** F [8%]; 10, 30, 60 s (pooled)[12]	**100%** 30 s[11] [b]**Not impacted** 60 s[12]	-	[b]**~95%** 24 h (dry first); 2 min[3] **90%** F 24 h (dry first) [4, 8%]; 4 min[3] [b]**91%** F 4 min (rinse); 24 h[3] **57%** [4%] 2 min (no rinse); 24 h[3] **26%** [8%]; 2 min (no rinse); 24 h[3] [b]**13–31%** [10%]; 1 min; 24 h[5]-	[b]**98.5%** [0.5%]; 10 min; 24 h[5] [b]**90–95%** 10 min; 24 h[5]	**95%** [10%]; 3 s; 26 h[5]	**100%** [2%]; 40°C; 30 s[11] [5%]; 40°C; 10 s[11] **80%** [5%]; 40°C; 30 s[11] **~10%** [5%]; 50°C; 10 s[11] **0%** [2%]; 50°C; 30 s[11]	**100%** [10%]; 10 s[11] **~60%** [10%]; 30 s[11]	**100%** [10%]; 40°C; 10 s[11] **~50–55%** [5%]; 40°C; 30 s[11] **~30–40%** [10%]; 40°C; 30 s[11] **~15%** [10%]; 50°C; 10 s[11] **0%** [2%]; 50°C; 30 s[11]

Coast (Atlantic/Pacific)	Moved species	Size category	Chlorination	Acetic acid							Citric acid	
			Immersion	Immersion		Spray	Immersion + air drying		Spray + air drying	Heated immersion	Immersion	Heated immersion
			([conc.]; time)	[4-5%] ([conc.]; time)	[1-2%] ([conc.]; time)	[4-5%] (time)	[4-5%] (immersion time; dry time)	[1-2%] (immersion time; dry time)	[4-5%] (spray time; dry time)	([conc.]; temp.; immersion time)	([conc.]; time)	([conc.]; temp.; immersion time)
	Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	**Not impacted** F [0.05%]; 12 h[42*]	**60%** F 30 s[9] **0%** F 5 min[9] **Not impacted** F 10 min[42*]	**100%** F [1.25%]; 30 s[9] **80%** F [1.25%]; 1 min[9] **60%** F [0.25%]; 10 min[9] **Not impacted** F 10 min[42*]	-	-	-	-	-	-	-
		SMALL < 70 mm	-	**100%** F 30 s[12] [4, 8%]; 15–60 s[12]	**100%** 15–60 s[12]	-	-	-	-	-	-	-

[1]Carver et al. (2003), [2*]MacNair et al. (2006), [3]Forrest et al. (2007), [5]Denny (2008), [7]Locke et al. (2009), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [11]Sievers et al. (2019), [12]Cahill et al. (2021), [17*]Asgari and Jahangard (2012), [32*]MacNair (2009), [34]Sharp et al. (2006), [42*]Coutts and Forrest (2005), [46*]Davidson et al. (2005), [50*]Gill et al. (2007), [57]Haque and Kwon (2017), [58]Haque et al. (2014), [59]Haque et al. (2015), [61]Rajagopal et al. (2003), [75]Rajagopal et al. (2002), [81]Carver et al. (2010), [111]Vickerson (2009).

*Table 10. Impacts of chemical treatments (saturated brine, hydrated lime, and Virkon®) on survival of moved species, where "100%" refers to 100% survival for a particular treatment combination on moved organisms. "Impacted" treatments refers to studies where survival of moved species was affected but not quantified. NS: not specified; *: technical reports; **: unpublished data or non peer-reviewed; F: field experiment. Laboratory experiments are presented by default. References are enumerated in superscript.*

Coast (Atlantic/ Pacific)	Moved species	Size category	Brine		Brine [300 ppt] X hydrated lime [4%]	Hydrated lime [4%]			Virkon®
			Immersion	Immersion + air drying	Immersion + air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
			([conc.]; time)	([conc.]; immersion time; ± dry time)	(immersion time; dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
Atlantic and Pacific	**Blue mussel** *Mytilus edulis*	LARGE ≥ 50 mm	-	-	-	**100%** 3 h[15] **98%** F 15 s[2*] **85–90%** 1 min[100**]	-	**100%** 5 s; 90 s[15]	**100%** [1%]; 60 s[69] **94.4%** [3%]; 30 s[69] **83.3%** [3%]; 60 s[69]
		SMALL < 50 mm	**98–100%** [300 ppt]; 15 min[39**] **84–95%** F [300 ppt]; 60 s (9–15 mm)[55*] **83%** [300 ppt]; 6 min (3–18 mm)[107**] **77–82%** [300 ppt]; 30 min[39**] **Not impacted** [300 ppt]; 30 s[34] F [300 ppt]; 15 s[32*]	**100%** [300 ppt]; 15 min; 1 h[39**] **70–92%** [70 ppt]; 20 s; 1 h[10] **3%** [300 ppt]; 6 min; 24 h[107**] **Not impacted** [300 ppt]; 30 s; 24 h (4°C; 100% RH)[111] 24 h (dry first 4°C, 100% RH); [300 ppt]; 30 s[111]	-	**100%** F 1-2 min[14*] **98–100%** F 15 s[50*] **31–47%** 30 min[39**] **22–23%** 15 min[39**] **Impacted** 1 min[7] 30 s[111]	**Not impacted** 30 s; 24 h (4°C, 100% RH)[111] 24 h (dry 4°C, 100% RH); 30 s[111]	**98%** 30 s; 1 h[39**]	-

Coast (Atlantic/ Pacific)	Moved species	Size category	Brine		Brine [300 ppt] X hydrated lime [4%]	Hydrated lime [4%]			Virkon®
			Immersion	Immersion + air drying	Immersion + air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
			([conc.]; time)	([conc.]; immersion time; ± dry time)	(immersion time; dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
				[300 ppt]; 30 s; 1 h[102**]					
		[NS]	-	**100%** F [300 ppt]; 30 s; 24 h[2*] F [300 ppt]; 1 min; 1 h[2*] **61%** F [300 ppt]; 10 min; 24 h[2*] **Impacted** F [300 ppt]; 2 min; 1 h[2*]	-	-	-	-	-
Atlantic	**European flat oyster** *Ostrea edulis*	LARGE ≥ 65 mm	-	-	-	-	-	-	-
		SMALL < 65 mm	**100%** F [300 ppt]; 1 h[112]	-	-	-	-	-	-
	Eastern oyster *Crassostrea virginica*	LARGE ≥ 70 mm	**100%** F [270 ppt]; 10 min[81]	**100%** F [270 ppt]; 6 min; 18 h[81]	**Not impacted** 30 s; 1 h[102**]	**100%** F 10 min[81] 3 h[15]	-	-	-
		SMALL < 70 mm	**100%** [300 ppt]; 15 min[39**] [300 ppt]; 6 min (1–2 mm)[107**] F [300 ppt]; 3 min[65]	**100%** [300 ppt]; 30 s; 1 h[39**] **0%** [300 ppt]; 6 min; 24 h (1-2 mm)[107**]	**Not impacted** 30 s; 1 h[102**]	**100%** F 1 min[65] 30 min[39**] **Not impacted** F 10 min[6*] 1 min[7]	**100%** 30 s; 1 h[39**]	-	-

Coast (Atlantic/ Pacific)	Moved species	Size category	Brine		Brine [300 ppt] X hydrated lime [4%]	Hydrated lime [4%]			Virkon®
			Immersion	Immersion + air drying	Immersion + air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
			([conc.]; time)	([conc.]; immersion time; ± dry time)	(immersion time; dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
			90% [300 ppt]; 30 min[39**] **Not impacted** F [300 ppt]; 10 min[6*]						
	Bay scallop *Argopecten irradians*	LARGE ≥ 40 mm	-	-	-	**100%** 3 h (variable pH)[15]	-	-	-
		SMALL < 40 mm	-	-	-	-	-	-	-
	Giant scallop *Placopecten magellanicus*	LARGE ≥ 70 mm	-	-	-	-	-	-	-
		SMALL < 70 mm	**87–89%** [300 ppt]; 1 min[39**] **24%** [300 ppt]; 5 min[39**]	-	-	**37%** 30 s (10°C)[39**] **14%** 30 s (4°C)[39**]	-	-	-
Pacific	**Mediterranean mussel** *Mytilus galloprovincialis*	LARGE ≥ 50 mm	**100%** [350 ppt]; 20 min[17*] **96.3%** [350 ppt]; 30 min[17*] **83%** [350 ppt] (-20°C); 5 s[17*] **79%** F [350 ppt]; 20 min[17*]	-	-	-	-	-	-

Coast (Atlantic/ Pacific)	Moved species	Size category	Brine		Brine [300 ppt] X hydrated lime [4%]	Hydrated lime [4%]			Virkon®
			Immersion	Immersion + air drying	Immersion + air drying	Immersion	Immersion + air drying	Spray + air drying	Immersion
			([conc.]; time)	([conc.]; immersion time; ± dry time)	(immersion time; dry time)	([conc.]; time)	([conc.]; immersion time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)
			10% [350 ppt] (-20°C); 10 s[17*]						
		SMALL < 50 mm	-	-	-	-	-	-	-
	Pacific (Giant) oyster *Crassostrea gigas*	LARGE ≥ 70 mm	**100%** F [70 ppt]; 10 min[9]	**75%** [360 ppt]; 30 min; several hours[110]	-	**100%** F [2%];10 min[9] 5 min[9] **80%** F [1%]; 30 s[9] **64%** F 4 min[13] **40%** F 30 s[9]	-	-	-
		SMALL < 70 mm	100% F [300 ppt]; 1 h[112]	-	-	-	-	-	-

[1]Carver et al. (2003), [2*]MacNair et al. (2006), [6*]Gill et al. (2008), [7]Locke et al. (2009), [9]Rolheiser et al. (2012), [10]Carman et al. (2016), [13]Switzer et al. (2011), [14*]Ramsay et al. (2014), [15]Comeau et al. (2017), [17*]Asgari and Jahangard (2012), [32*]MacNair (2009), [34]Sharp et al. (2006), [39**]Landry et al. (DFO, unpubl. data), [50*]Gill et al. (2007), [55*]Bourque and Myrand (2007), [65]MacNair and Smith (1999), [69]Paetzold and Davidson (2011), [81]Carver et al. (2010), [100**]MacNair (unpubl. data), [102**]Mills (DFO, unpubl. data), [107**]Mallet et al. (Mallet Research Services Ltd., unpubl.data – trials 2008), [110]Ruellet (2004), [111]Vickerson (2009), [112]Minchin and Duggan (1988).

*Table 11. Impacts of physical and chemical treatments on macroalgae, where "100%" refers to 100% survival for a particular treatment combination on macroalgae. "Impacted" treatments refer to studies where % survival was affected but not quantified. NS: not specified; *: technical reports; **: unpublished data or not peer-reviewed. F: field experiment. Laboratory experiments are presented by default. References are enumerated in superscript.*

Macroalgae	Air drying	Freshwater	Hot seawater	Sodium hypochlorite	Manual removal + sodium hypochlorite	Acetic acid		Citric acid	Brine
		Immersion	Immersion	Immersion	Manual wiping + immersion ± air-dry	Immersion ± air-dry	Spray ± air-dry	Immersion	Immersion
	(time)	(time)	(temp.; time)	([conc.]; imm. time)	([conc.]; imm. time; dry time)	([conc.]; imm. time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)	([conc.]; time)
Saccharina latissima, *Saccharina longicruris*	-	-	-	-	**Not impacted** Manual wiping (sori); [0.1%]; 1 min (rinse); 12–16 h (sori)[108**] Manual wiping (sori); [0.003%]; 2 min (rinse + wiped dried)[113*]	-	-	-	-
Fucus vesiculosus, Fucus serratus	**Impacted** F 3x / week[76]	-	-	-	-	-	-	-	-
Gracilaria gracilis	-	**Impacted** 3 h[86]	-	-	-	-	-	-	-
Porphyra haitanensis	-	-	-	-	-	-	-	**90.9%** [pH 2]; 3 min[101]	-
Porphyra sp.	-	-	-	-	-	-	-	-	**100%** NS[38]
Pyropia yezoensis	**Not impacted** 2–40 min [114]	-	-	-	-	-	-	-	**Impacted** 80 ppt; 10 min (30% water loss)[115] **Impacted** 100 ppt; 10 min (40% water loss)[115]

Macroalgae	Air drying	Freshwater	Hot seawater	Sodium hypochlorite	Manual removal + sodium hypochlorite	Acetic acid		Citric acid	Brine
		Immersion	Immersion	Immersion	Manual wiping + immersion ± air-dry	Immersion ± air-dry	Spray ± air-dry	Immersion	Immersion
	(time)	(time)	(temp.; time)	([conc.]; imm. time)	([conc.]; imm. time; dry time)	([conc.]; imm. time; dry time)	([conc.]; spray time; dry time)	([conc.]; time)	([conc.]; time)
Caulerpa taxifolia	-	-	**0%** 72°C; 1 h[77]	**100%** [0.001%]; 30 min[77]	-	-	-	-	-
Cladophora sp.	-	-	-	-	-	**Not impacted** [2%]; 1–2 min[3] [2%]; 1–2 min (rinse); 24 h[3] **Impacted** 24 h (air); [2%] 1–2 min[3]	-	-	**100%** 400 ppt; 30 min[38] **Impacted** 300 ppt; 15 s[32*, 34]
Codium fragile	**Impacted** 1 h[37]	**100%** 3 h[37]	**Impacted** 50°C; 30 s[39**]	-	-	-	-	-	-
Rhodophyta spp.	-	-	-	-	-	-	**0%** [5%]; 1 min; 1 min[8]	-	-
Ulva linza	-	-	-	-	-	-	**100%** [5%]; 1 min; 1 min[8]	-	-
Ulva spp.	-	-	**Impacted** 80–85°C; 3 s[38]	-	-	-	-	-	**Not impacted** 400 ppt; 30 min[38]
Undaria pinnatifida	**~40%** 6 h (20°C)[35]	**0%** 10 min[35]	**80%** 35°C; 1 min[35]	-	-	**100%** [2%]; 1 min (rinse)[3]	**0%** [5%]; 1 min; 1 min[8]	-	-

[3]Forrest et al. (2007), [8]Piola et al. (2009), [32*]MacNair (2009), [34]Sharp et al. (2006), [35]Forrest and Blakemore (2006), [37]Kim and Garbary (2007), [38]Mineur et al. (2007), [39**]Landry et al. (DFO, unpubl. data), [76]Meichssner et al. (2020), [77]Williams and Schroeder (2004), [86]Smit et al. (2003), [101]Yan et al. 2011, [108**]Clark, J. (unpubl. data), [113*]Tamigneaux et al. (2013), [114]Li et al. 2018a, [115]Du et al. 2021.

Table 12. Summary of physical treatments for marine aquatic invasive species and moved species. Effective treatments on selected AIS or epibionts (100% Mortality/removal or Effective) and survival of moved shellfish (≥90% Survival or Not impacted) are based on a review of the scientific literature of treatments for Argopecten irradians (AI), Ascidiella aspersa (AA), barnacles (BA), Botrylloides violaceus (BV), Botryllus schlosseri (BS), bryozoans (BZ), Caprella mutica (CMU), Carcinus maenas (CM), Ciona intestinalis (CI), Codium fragile (CF), Crassostrea gigas (CG), Crassostrea virginica (CV), Didemnum vexillum (DV), Diplosoma listerianum (DL), gastropods (GA), Hemigrapsus sanguineus (HM), hydrozoans (HZ), macroalgae (MA), Molgula spp. (MO), Mytilus edulis (ME), Mytilus galloprovincialis (MG), Ostrea edulis (OE), Placopecten magellanicus (PM), polychaetes (PL), sea stars (SS), sponges (SP), and Styela clava (SC). Associated levels of uncertainty are provided and are based on the data available, their quality, and agreement among studies with the identified treatment options. Note that uncertainty scores were not calculated for ineffective treatments. (s/l): small/large. (juv.): treatments was on juveniles or young stages.

Physical treatments		AIS Mortality						Moved species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
Pressurized water	700 psi; 10 s	-	-	-	-	BS, BV, CM[5], GA[5], ME(l)[5], ME(s), SS[5]	AA, BA, BZ, CF, CG(s/l), CI[1,5], CMU, CV(s/l), DL, DV[1], HM, HZ, MA[1], MG(s/l), MO, PL, SC, SP	-	ME(s)	ME(l)[5]	-	-	AI(s/l), CG(l)[3], CG(s), CV(s/l), MG(s/l), OE(s/l), PM(s/l)
Air drying	24 h	CI	BS, BV[5], SC	AA[5], CF[5], DL[5], DV[5], MA, MG(s), PL	MO[5]	BA, BZ[5], CV(s), GA[5], ME(s) MG(l), SP[5]	CG(l)[1], CG(s), CM[1], CMU, CV(l), HM, HZ, ME(l)[1], SS	-	CV(s), ME(s)	MG(l)	-	MG(s)	AI(s/l), CG(l)[3], CG(s), CV(l), ME(l)[3], OE(s/l), PM(s/l)
Freshwater immersion	24 h	CF, CMU, SC	BS, BV, PL	DV, MA	SP[5]	CV(l)[5], CV(s), HM, ME(s)	AA, BA, BZ, CG(s/l)[2], CI[2], CM[2,5], DL, GA, HZ[2], ME(l), MG(l)[2], MG(s), MO, SS	-	ME(s)	CV(l)	CV(s)	-	AI(s/l), CG(s/l)[4], ME(l), MG(l)[4], MG(s), OE(l), OE(s)[4] PM(l), PM(s)[4]

Physical treatments		AIS Mortality						Moved species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
Freshwater immersion + Air drying	8 h + 1 h	-	-	BS, BV, DL, DV	-	CV(I)[5], ME(s)	AA, BA, BZ, CF, CG(s/l), CI, CM, CMU, CV(s), GA, HM, HZ, MA, ME(l), MG(l)[2], MG(s), MO, PL[1], SC, SP, SS	-	-	CV(l), ME(s)	-	-	AI(s/l), CG(s/l), CV(s), ME(l), MG(s/l)[4], OE(s/l), PM(s/l)
Freshwater spray + Air drying	10 min + 1 h	-	-	BS, BV, DL, DV	-	ME(s)	AA, BA, BZ, CF, CG(s/l), CI, CM, CMU, CV(s/l), GA, HM, HZ, MA, ME(l), MG(s/l), MO, PL, SC, SP, SS	-	-	ME(s)	-	-	AI(s/l), CG(s/l), CV(s/l), ME(l), MG(s/l), OE(s/l), PM(s/l)
Heated seawater immersion	50°C; 60 s	CI	MA, ME(s/l), MG (s/l), SC	CM(juv.), HZ	CF, SS[5]	CG(s), CV(s/l), PL	AA, BA[1], BS, BV[1], BZ[1], CM(adult)[1,5], CMU[1], CG(l)[2], DL, DV[1], GA, HM, MO, SP	CG(s)	ME(l)[5]	-	-	ME(s), MG(s/l), OE(s)	AI(s/l), CG(l)[4], CV(l)[4,5], CV(s)[4], OE(l), PM(s/l)

Physical treatments		AIS Mortality						Moved species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
	60°C; 10 s	-	CI, MA, ME(s/l), MG(s)	CM(juv.), HZ	CF, SS[5]	CG(s), CV(s/l), MG(l), PL, SC	AA, BA[1], BS, BV[1], BZ[1], CG(l)[2], CM(adult)[1,5], CMU[1], DL, DV[1], GA, HM, MO, SP	CG(s)	CV(s; 55–65 mm)	-	CV(l)[5]	CV(s; 35–45 mm), ME(s/l), MG(s/l) OE(s)	Al(s/l), CG(l)[4], OE(l), PM(s/l)
	60°C; 30 s	SC	CI, MA, ME(s/l), MG(s)	CM(juv.), HZ	BA, CF, SS[5]	CG(s), CV(s/l), MG(l), PL	AA, BS, BV[1], BZ[1], CG(l)[2], CM(adult)[1,5], CMU[1], DL, DV[1], GA, HM, MO, SP	CG(s)	-	-	CV(l)[5]	CV(s), ME(s/l), MG(s/l), OE(s)	Al(s/l), CG(l)[4], OE(l), PM(s/l)
Steam	100°C; 50 psi	-	-	-	MA, SC	-	AA, BS, BV, CI, DL, DV, HM, MO	-	-	-	-	-	-

[1]No data with these parameters; 100% mortality with other parameters (see detailed Table 5)

[2]No data with this parameter; not effective with other parameters (see detailed Table 5)

[3]No data with these parameters; impacted with other parameters (see detailed Table 8)

[4]No data with these parameters; ≥90% survival with other parameters (see detailed Table 8)

[5]Based on qualitative results only

Table 13. Summary of chemical treatments for marine aquatic invasive species and moved species. Effective treatments on selected AIS or epibionts (100% Mortality/removal or Effective) and survival of moved shellfish (≥90% Survival or Not impacted) are based on a review of the scientific literature of treatments for Argopecten irradians (AI), Ascidiella aspersa (AA), barnacles (BA), Botrylloides violaceus (BV), Botryllus schlosseri (BS), bryozoans (BZ), Caprella mutica (CMU), Carcinus maenas (CM), Ciona intestinalis (CI), Codium fragile (CF), Crassostrea gigas (CG), Crassostrea virginica (CV), Didemnum vexillum (DV), Diplosoma listerianum (DL), gastropods (GA), Hemigrapsus sanguineus (HM), hydrozoans (HZ), macroalgae (MA), Molgula spp. (MO), Mytilus edulis (ME), Mytilus galloprovincialis (MG), Ostrea edulis (OE), Placopecten magellanicus (PM), polychaetes (PL), sea stars (SS), sponges (SP), and Styela clava (SC). Associated levels of uncertainty are provided and are based on the data available, their quality, and agreement among studies with the identified treatment options. Note that uncertainty scores were not calculated for ineffective treatments. [ ]: concentration of the chemical. (s/l): small/large. (juv.): treatment was on juveniles or young stages.

Chemical treatments		AIS Mortality						Moved Species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved Species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
Sodium hypochlorite immersion	[0.5%]; 20 s	-	-	DV	BV	CM[5], MG(s), PL[5]	AA, BA, BS, BZ, CF, CG(l)[2], CG(s), CI[2], CMU, CV(s/l), DL, GA, HM, HZ, MA[1], ME(l), ME(s)[1], MG(l)[2], MO, SC[1], SS, SP	-	-	MG(s)	-	-	AI(s/l), CG(l)[4], CG(s), CV(s/l), ME(l), ME(s)[3], MG(l)[4], OE(s/l), PM(s/l)
	[0.01%]; 12 h	-	-	-	SC	CG(l)	AA, BA, BS, BV[1], BZ, CF, CG(s), CI[2], CM[2,5], CMU, CV(s/l), DL, DV[1], GA, HM, HZ, MA[1], ME(l), ME(s)[1], MG(l)[2], MG(s)[1], MO, PL[2,5], SP, SS	-	-	MG(s)	CG(l)[5]	-	AI(s/l), CG(s), CV(s/l), ME(l), ME(s)[3], MG(l)[4], OE(s/l), PM(s/l)
Acetic acid [4−5%] immersion	30 s	-	BZ, CI, DV[5], HZ, ME(s)	BV, SS	-	BA[5], CG(s/l), CV(l), CV(s)[5], GA[5], MG(s/l), PL[5]	AA, BS[1,5], CF, DL, CM, CMU, HM, MA[1], ME(l)[2], MO, SC[1], SP[1,5]	CG(s), MG(s/l)	OE(s)	-	-	CG(l), CV(l), ME(s/l)	AI(s/l), CV(s), OE(l), PM(s/l)

Chemical treatments		AIS Mortality						Moved Species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved Species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
	1 min	BV, BZ, CI	DV[5], HZ, ME(s), SC	BS[5], MA, PL[5], SS	-	BA[5], CG(s/l), CV(s)[5], GA[5], MG(s/l)	AA, CF, CM, CMU, CV(l)[2], DL, HM, ME(l)[2], MO, SP[1,5]	MG(s/l)	CG(s), OE(s)	-	-	CG(l), CV(l), ME(s/l)	AI(s/l), CV(s), OE(l), PM(s/l)
	5 min	BV, BZ, CI, ME(s)	DV[5], HZ, MA, SC	BS[5], CG(l), PL[5], SS	-	CV(s[5]), GA[5], MG(s/l)	AA, BA[2], CF, CG(s)[2], CM, CMU, CV(l)[2], DL, HM, ME(l)[2], MO, SP[1,5]	-	-	-	-	CG(l), CV(l), ME(s/l)	AI(s/l), CG(s)[4], CV(s), MG(s/l)[4], OE(l), OE(s)[4], PM(s/l)
Acetic acid [4−5%] immersion + Air drying	5 min + 1 h	-	-	AA, BS, BV, CI, DL, DV, ME(s)	-	-	BA, BZ[1,5], CF, CG(s/l), CM, CMU, CV(s/l), GA, HM, HZ, MA[1], ME(l), MG(s/l)[2], MO, PL[1,5], SC, SP, SS	-	-	-	-	ME(s)	AI(s/l), CG(s/l), CV(s/l), ME(l), MG(s/l)[3], OE(s/l), PM(s/l)
	4 min + 24 h	-	-	BS[5], BV[5], CI[5], MA, PL[5]	BZ[5]	MG(s/l)	AA[1], BA, CF, CG(s/l), CM, CMU, CV(s/l), DL[1], DV[1], GA, HM, HZ, MO, ME(l), ME(s)[1], SC, SP, SS	-	-	-	-	ME(s)[5], MG(s/l)	AI(s/l), CG(s/l), CV(s/l), ME(l), OE(s/l), PM(s/l)
Citric acid [5%] immersion	10 s	-	-	HZ	-	CI, MA, MG(s/l), SC	AA, BA, BS, BV, BZ, CF, CG(s/l), CM, CMU, CV(s/l), DL, DV, GA, HM, ME(s/l), MO, PL, SP, SS	-	-	MG(s/l), OE(s)	-	-	AI(s/l), CG(s/l), CV(s/l), ME(s/l), OE(l), PM(s/l)

Chemical treatments		AIS Mortality						Moved Species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved Species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
Saturated brine [300ppt] immersion	15 min	-	CF, MA[5], MO[5], SP[5], SS[5]	-	-	BA[5], CG(s), CV(s/l), ME(s), MG(l), PL	AA, BS, BV[2,5], BZ, CG(l)[2], CI[2], CM, CMU, DL, DV[1], GA[2,5], HM, HZ, ME(l), MG(s), SC[2]	ME(s)	CV(s)	CG(s), CV(l), OE(s)	MG(l)	PM(s)	AI(s/l), CG(l)[4], ME(l), MG(s), OE(l), PM(l)
Saturated brine [300 ppt] immersion + Air drying	15 min + 2 h	AA, BS, CI[5], DL, DV, MO, SC	BV, CF, SP	PL[5]	GA	CG(l), CM[5], ME(s)	BA, BZ, CG(s), CMU, CV(s/l)[2], HM, HZ, MA, MG(s/l), SS[1], ME(l)[2,5]	-	-	-	-	-	AI(s/l), CG(l)[3], CG(s), CV(s/l)[4], ME(s)[4], ME(l), MG(s/l), OE(s/l), PM(s/l)
	30 s + 1 h	BS	BV, CI[5], DL, DV	-	-	CG(l), CM[5], CV(s/l), ME(l)[5], ME(s)	BA, BZ, CF[1], CG(s), CMU, GA[1], HM, HZ, MA, MG(s/l), MO[1], PL[1,5], SC[1], SP[1], SS[1]	-	ME(s)	CV(l)	CV(s)	-	AI(s/l), CG(l)[3], CG(s), ME(l), MG(s/l), OE(s/l), PM(s/l)
Hydrated lime [4%] immersion	5 min	MO[5]	CF	BV[5], SC[5]	BS[5], BZ[5], HZ[5], SS[5]	BA[5], CG(l), CI, CM, CV(s/l), DV, GA[5], ME(s/l), SP	AA, CG(s), CMU, DL, HM, MA, MG(s/l), PL	CV(s/l)	ME(l)	AI(l), CG(l)	-	PM(s)	AI(s), CG(s), ME(s)[4], MG(s/l), OE(s/l), PM(l)

Chemical treatments		AIS Mortality						Moved Species Survival					
		AIS (100% Mortality or Effective)				Not effective	No data	Moved Species (≥90% Survival or Not impacted)				Impacted	No data
		Uncertainty						Uncertainty					
		Low	Moderate	High	Very high			Low	Moderate	High	Very high		
Saturated brine [300 ppt] X hydrated lime [4%] + Air drying	1 min + 1 h	-	-	BS[5], BV[5]	CI[5]	-	AA, BA, BZ, CF, CG(s/l), CM, CMU, CV(s/l)[2,5], DL, DV, GA, HM, HZ, MA, ME(s/l), MG(s/l), MO, PL, SC, SP, SS	-	-	-	-	-	AI(s/l), CG(s/l), CV(s/l)[4,5], ME(s/l), MG(s/l), OE(s/l), PM (s/l)
Virkon® [3%]	30 s	-	-	CI(juv.)	-	ME(l)	AA, BA, BS, BV, BZ, CF, CG(s/l), CI(adult)[2], CM, CMU, CV(s/l), DL, DV, GA, HM, HZ, MA, MO, ME(s), MG(s/l), PL, SC, SP, SS	-	-	ME(l)	-	-	AI(s/l), CG(s/l), CV(s/l), ME(s), MG(s/l), OE(s/l), PM(s/l)

[1]No data with these parameters; 100% mortality with other parameters (see detailed Tables 6 and 7)

[2]No data with this parameter; Not effective with other parameters (see detailed Tables 6 and 7)

[3]No data with these parameters; impacted with other parameters (see detailed Tables 9 and 10)

[4]No data with these parameters; ≥90% survival with other parameters (see detailed Tables 9 and 10)

[5]Based on qualitative results only

Table 14. Conceptualization of a process to be used by managers for selection of most appropriate treatment type(s) to maximize both mortality of the Aquatic Invasive Species (AIS) and survival of the moved shellfish species in the context of movements of marine organisms (e.g., aquaculture transfers, scientific transfers).

Step 1 Are you aiming to mitigate an AIS assemblage (or unknown AIS threat) or targeting just one AIS?	
Scenario A AIS assemblage/unknown AIS	Scenario B Species-specific (targeting just one AIS)
Step 2A Identify possible effective physical and chemical treatment options in Tables 12 and 13 that would include all AIS groups of concern (e.g., colonial tunicates, bryozoans, *Codium fragile*), which also ensure a high survival of the moved species (e.g., small *Mytilus edulis*—see "ME(s)" in Tables 12 and 13). Step 3A From treatment options identified in Step 2A, evaluate feasibility/ applicability of treatments, since they are context dependent, and determine optimal treatment(s) in your given situation. Step 4A Recommend/implement optimal treatment(s) for your given situation.	Step 2B Identify possible effective physical and chemical treatment options in Tables 12 and 13 for your AIS of interest (e.g., *Ciona intestinalis*, which is included in solitary tunicates—see "CI" in Tables 12 and 13), which also ensure a high survival of the moved species (e.g., small *Mytilus edulis*—see "ME(s)" in Tables 12 and 13). Step 3B From treatment options identified in Step 2B, find optimal treatment(s) specific for the targeted AIS species (e.g., *C. intestinalis*) in Tables 5–7, which ensure treatment effectiveness for that specific AIS and high survival of the moved species (e.g., small *Mytilus edulis*) in Tables 8–10. Step 4B From treatment options identified in Step 3B, evaluate feasibility/applicability of treatments, since they are context dependent, and determine optimal treatment(s) in your given situation. Step 5B Recommend/implement optimal treatment(s) for your given situation.

APPENDIX 1. SEARCH TERMS

1	(decontaminat* OR “hot water” OR steam* OR clean* OR disinfect* OR protocol* OR guideline* OR standard* OR trial* OR method* OR spray* OR heat* OR dry* OR prevent* OR immers* OR manage* OR antifoul* OR biofoul* OR foul OR infest* OR sun* OR treat* or treatment* OR hot OR inspect* OR airdry* or air drying OR rins* OR salinity OR pressure* OR desiccat* OR expos* OR brush* OR sodium hypochlorite OR chlorination OR bleach OR chlorine OR lime or hydrated lime OR brine OR Virkon OR acetic acid or OR citric acid OR vinegar OR fresh* OR control OR eradicate* OR biosecurity OR brush*)
2	(invasive OR non-native OR non-indigenous OR exotic OR foreign OR alien OR spread* OR invad*)
3	(aquatic OR aquaculture OR commercial* OR farm* OR harvest* OR cultur* ocean OR sea OR water OR coastal OR introduction* OR transfer* OR marine)
4	(species OR genus OR family OR class OR phylum OR organism* OR animal* OR plant* OR invertebrate* OR mollusc* OR bivalve* OR mussel* OR shell* OR oyster* OR scallop* OR barnacle* OR littorina OR gastropod* OR drill* OR snail* OR crust* or encrust* OR grape* OR crab OR amphipod* OR shrimp OR bryozoan* OR sponge* OR hydroid* OR hydrozoan* OR polychaete* OR worm* OR blister* OR epibiont* OR bore* or boring* or burrow* OR star* OR echinoderm* OR pest OR macrophyte or alga* or macroalga* OR weed* OR seaweed*)
5	(viability OR viable OR mortality OR death OR removal OR surviv* OR reproduc* OR dispersal OR “overland transport” OR vector OR effect OR OR tolerance OR resistance OR lethal* OR “acute upper lethal temperature” OR temperature OR heat OR hot OR thermal OR “critical maximum temperature” OR behav* OR response OR sensitivity)
6	1 AND 2 AND 3 AND 4 AND 5
7	(biology or biological OR size OR height OR length OR market OR develop* OR life or life stage* OR cycle OR grow* OR rate OR maxim* or minim* OR matur* or immatur* OR sex* OR spat OR seed OR adult* OR juvenile* OR small OR large)
8	3 AND 4 AND 7