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Management in Practice

The development of a rapid response plan to control the spread of the solitary invasive tunicate, *Ciona intestinalis* (Linnaeus, 1767), in Newfoundland and Labrador, Canada

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Abstract

The vase tunicate, *Ciona intestinalis*, was first confirmed in Newfoundland and Labrador (NL) waters in September 2012. The Department of Fisheries and Oceans (DFO) Aquatic Invasive Species (AIS) monitoring program, in collaboration with the Department of Ocean Sciences at Memorial University of Newfoundland (MUN), detected an isolated infestation of vase tunicate in Little Bay, Placentia Bay. The solitary tunicate was attached to wharf structures, eel grass, and some vessels in the area. Early detection of AIS is one of the primary goals of the DFO AIS monitoring program. This early detection, with the species currently confined to a small area of Placentia Bay, provided a unique opportunity for mitigation activities. This study details the various stages of a rapid response plan, its development through responses to two colonial tunicates, *Botryllus schlosseri* and *Botrylloides violaceus,* and its application to control the spread of solitary tunicate, *C. intestinalis*. Pre-invasion planning and the response plan include key phases of communication, detection and demarcation, containment and risk assessment, mitigation implementation and evaluation. Mitigation trials in Little Bay, Placentia Bay (2013 and 2014) have included floating dock removal, permanent structure cleaning, and recreational and commercial vessel cleaning with application of antifouling paint. Mitigated and unmitigated harbours have been monitored to evaluate the effectiveness of the control efforts. As of 2015, surveys of the mitigated area have only detected very small numbers of *C. intestinalis*, which were removed. A rapid response plan based on experience, good communication, strong partnerships, and common goals has allowed NL to respond to a high impact AIS tunicate in an effective manner. The new Aquatic Invasive Species Regulations in the Canadian *Fisheries Act* will provide authority for response, but monitoring, vigilance, prior planning, collaboration between stakeholders and rapid action are the real tools for an effective control plan.

Key words: rapid response framework, marine pest control, controlling invasive spread, biofouling, antifouling

Introduction

When the non-indigenous solitary tunicate *Ciona intestinalis* (Linnaeus, 1767) was first discovered in September 2012 on a wharf in Placentia Bay, Newfoundland and Labrador (NL) (Sargent et al. 2013), it provided DFO an opportunity to implement and test a rapid response plan to a new invasion. The stages of the process, formal and informal, included high risk harbour studies, taxonomic and rapid assessment training, surveys, communication planning, response activities, and mitigation and control to prevent the spread of high risk invaders. These stages formed the framework for a regional rapid response plan, which was adapted from a proposed Canadian Rapid Response Framework (Locke and Hanson 2009a,c) developed through a Canadian Science advisory process (DFO 2012; Locke et al. 2011).

At the onset of this process, Newfoundland and Labrador (NL) had few reported AIS (Hooper 1975; US Navy 1951) either due to its isolation as an island, harsh climate, or lack of baseline information. This absence of widespread AIS was a unique opportunity to develop and test the full range of a rapid response plan from early detection to mitigation and control. The arrival of *C. intestinalis* in Placentia Bay, a high risk invasive tunicate, was an ideal test of the Newfoundland and Labrador Rapid Response Plan.

Although new to Newfoundland insular waters, *C. intestinalis* has colonized mussel farms in Nova Scotia (NS) since 1997 (Cayer et al. 1999) and in 2004 began to replace clubbed tunicate, *Styela clava* (Herdman, 1881), as the dominant invasive tunicate in Prince Edward Island (PEI) estuaries (Ramsay et al. 2008). This is in part, because of the high reproduction rates of *C. intestinalis* when water temperatures are suitable (> 8°C) (Carver et al. 2003, 2006). For example, in PEI, settlement plates deployed in May accumulate an average of 429 individuals/100 cm^2 by October (Ramsay et al. 2009). It has created significant fouling problems for the mussel aquaculture industry, which has increased costs of production and processing (Thompson and MacNair 2004). It also has increased potential food resource competition between species, particularly in PEI (Daigle and Herbinger 2009). Research on the ecology of *C. intestinalis* in Newfoundland waters suggests that there is one major recruitment peak per season (Reid unpublished data). This is the same as populations in PEI (Ramsay et al. 2009), but contrasts most populations in NS, which experience two generations per year (Carver et al. 2003; Howes et al. 2007; Vercaemer et al. 2011). Studies on *C. intestinalis* in NS, indicated that individuals produce competent gametes when water temperatures exceed 8 °C (Carver et al. 2003). *Ciona intestinalis* was considered high risk for Newfoundland largely due to its demonstrated economic and environmental impact elsewhere in Atlantic Canada. A national DFO tunicate risk assessment concluded that the ecological risk posed by *C. intestinalis* was high on the Atlantic coast (Therriault and Herborg 2008a, b). Research is ongoing to further investigate reproduction, growth, and overwintering of *C. intestinalis* in Newfoundland coastal ecosystems (Reid unpubl. data).

Monitoring programs have recorded spread of *C. intestinalis* along the shores of NS and PEI and increased densities in previously inhabited locations (Locke et al. 2009b; Ramsay et al. 2008; Sephton et al. 2011). Planktonic dispersal of *C. intestinalis* larvae was thought to be quite limited and natural dispersal between estuaries may require multiple generations with several intermediate steps to spread naturally (Kanary et al. 2011). Recent research on post-metamorphic substrate attachment of *C. intestinalis* in NL suggests that potential non-anthropogenic dispersal may be less limited than previously understood (Reid et al. 2016). However, large range expansions still tend to be the result of anthropogenic assistance such as fouled boat hulls or gear. Although eradication elsewhere in Atlantic Canada was eventually considered impossible, focus switched to restricting dispersal, containing current infested areas, and monitoring effects of control and quarantine (Locke et al. 2009b). In PEI, a management plan was set up in consultation with the PEI Introductions and Transfers (I&T) committee that stipulated product from tunicate-infested areas could only be transferred to tunicate-infested areas, ie. “like-to like” (Locke et al. 2009b). In tidal waters of Canada, release of live aquatic organisms into fish habitat or into a rearing facility is prohibited by regulation unless authorized by a license. The provincially-based I&T committee is responsible for the review and approval of requests to move aquatic organisms. The license is granted under specific conditions which minimize potential risks associated with movement. Various research on control options for non-native tunicates which have become invasive continues and includes, but is not limited to vinegar sprays, immersion in hydrated lime, pressure washing, hot fresh water, natural predators, bubble streams, and suspended particles (Arens et al. 2011; Bullard et al. 2010; Carver et al. 2003; Clancey and Hinton 2003; McLaughlin et al. 2013; Lowen et al. 2016; Vickerson et al. 2011).

Management of vectors associated with mussel aquaculture has attained partial containment of *S. clava* and *C. intestinalis* in PEI largely because they only reproduce sexually and have limited dispersal (Locke et al. 2009b). However, I&T permits cannot address other vectors such as recreational or commercial vessel traffic (Acosta and Forrest 2009; Darbyson et al. 2009; Locke et al. 2009b; McKenzie et al. 2014). Moreover, containment of colonial tunicates is more difficult due to asexual reproduction requiring only fragments of colonies. Successful eradication or management of AIS is challenging and rare, but successful attempts have several characteristics in common. They include, but are not limited to early detection and accurate species identification, pre-existing authority to initiate action, limited potential for natural dispersion, political or public support, and resources for follow-up monitoring to confirm effectiveness of the program (Locke and Hanson 2009a).

This study details the various stages of a rapid response plan, its development through responses to two colonial tunicates, *Botryllus schlosseri* (Pallas, 1766) and *Botrylloides violaceus* Oka, 1927, and its application to control the spread of the solitary tunicate *C. intestinalis*. Pre-invasion planning and the response plan include key phases of communication, detection and demarcation, containment and risk assessment, mitigation implementation and evaluation.

The Newfoundland and Labrador Rapid Response Plan for Non-indigenous Species

Non-indigenous tunicates have invaded Atlantic Canada for decades (Locke et al. 2007; 2009b), but it was not until December 2006 that the first non-indigenous tunicate, *B. schlosseri* was identified in Newfoundland on a recreational vessel hull in Argentia Harbour, Placentia Bay. This detection was part of the first aquatic invasive species (AIS) high risk harbour survey (Callahan et al. 2010) conducted by Fisheries and Oceans Canada (DFO) in partnership with the Department of Ocean Sciences, Memorial University of Newfoundland (MUN) and the Provincial Department of Fisheries and Aquaculture (DFA). Although this species was reported in Argentia by the US Navy in 1945 (US Navy 1951) and on the west coast of Newfoundland in the 1970s (Hooper 1975), there was no indication that these introductions had fully established. This early detection subsequently required a rapid response plan or as stated at the time "Now what do we do?"

The NL rapid response plan for non-indigenous species is based on and modified from Locke and Hanson (2009c) and discussions with other researchers, the aquaculture industry, and managers dealing with AIS issues. The development of the response plan used for *C. intestinalis* detection and mitigation began with the discovery of *B. schlosseri* and *B. violaceus* in 2007 and mitigation attempts (in 2008 and 2009) in Belleoram Harbour, Fortune Bay on the south coast of Newfoundland and in Foxtrap Marina, Conception Bay in 2010 (Figure 1 A,B) (Deibel et al. 2014). The regional response activities for *B. violaceus* were presented as a case study for the Canadian Rapid Response Framework in 2010 (DFO 2012). In this report, we outline the pre-invasion planning stages for the response plan developed for colonial tunicates, summarized in Table 2, and then we describe the implementation of the response plan for the solitary tunicate, *C. intestinalis* in Placentia Bay, summarized in Table 3.

Key elements of preparation – regulation, communication and collaboration

The lack of AIS regulations within the Canadian *Fisheries Act* had been identified as a vital gap and source of frustration for both federal and provincial governments in trying to control or manage high impact (biological and economic) invasive species. The only available method of regulation prior to 2015 was through the DFO I&T requirements for movement of and release of marine "fish". Although DFO Science had the responsibility for monitoring, assessing risk, and research, no lead was clearly identified for management and response, at that time. Therefore, mitigation and control activities were deemed experimental research in consultation with DFO Policy and Economics branch and the Newfoundland and Labrador Aquatic Invasive Species Advisory Committee (NLAISAC).

The principle lesson learned following the detection of the first invasive tunicate in Newfoundland in 2006 was the importance of communication. Communication with active field teams, scientists, stakeholders, funding agencies, and the public through community awareness and stewardship activities may be the one key element of an effective rapid response plan. The NLAISAC was created to meet this communication need and was modelled on a similar committee in Prince Edward Island (PEI). This committee is co-chaired by a member of the aquaculture industry and government (DFO) and includes industry representatives, scientists, managers, and field personnel from both the federal and provincial governments and MUN. The inaugural meeting of the NLAISAC took place in St. John's, NL in 2007 during the annual regional aquaculture industry conference. The terms of reference for the committee were based on the overarching need for communication. Within this umbrella were critically important points that included: 1) identification of a point of contact within each organization; 2) a process for deriving consensus on priorities for monitoring and research; 3) a communication strategy for member organizations, stakeholders, and the public; and 4) identifying sources of funding for communication, monitoring, research, and response as part of a collaborative effort.

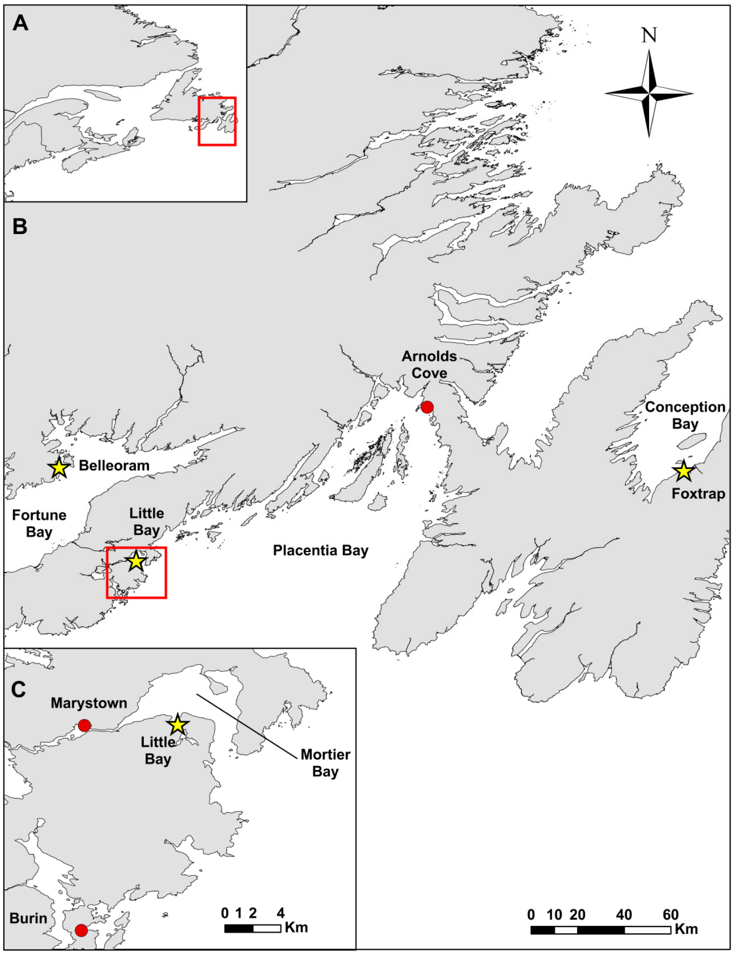

Figure 1. Maps of Atlantic Canada (A), eastern Newfoundland (B) showing sites of 4 rapid response activities (Belleoram, *B. violaceus*; Arnolds Cove, *B. schlosseri;* Foxtrap, *B. schlosseri*; and Little Bay, *Ciona intestinalis*) and Mortier Bay (C) showing the distribution of *C. intestinalis* at mitigated (star) and unmitigated (circle) sites.

Table 1. List of species of concern in Newfoundland and Labrador.

Newfoundland and Labrador species of concern	
Carpet tunicate	*Didemnum vexillum* Kott, 2002
European green crab	*Carcinus maenas* (Linnaenus, 1758)
Vase tunicate	*Ciona intestinalis* (Linnaeus, 1767)
Clubbed tunicate	*Styela clava* Herdman, 1881
Violet tunicate	*Botrylloides violaceus* Oka, 1927
Golden star tunicate	*Botryllus schlosseri* (Pallas, 1766)
Coffin box bryozoan	*Membranipora membranacea* (Linnaeus, 1767)
Oyster thief seaweed	*Codium fragile* spp. *fragile* (Suringar) Hariot, 1889

Some rapid response plans have separate scientific and stakeholder groups (Locke and Hanson 2009c), but in this case, the committee combines science, management, and industry in a network of collaboration. This has worked well as the monitoring and research needs are directly communicated to scientists by managers and industry. Equally, public sector program managers and industry are clearly involved in the early planning stages of monitoring, research, and mitigation experiments so that priorities and goals are achievable and targeted. Building on the strengths and priorities of each organization, roles and responsibilities were identified and joint communication, research and mitigation projects have been developed and completed. The NLAISAC has hosted four AIS workshops to provide a forum for information, awareness and priority setting. This strong communication and advisory network has been vital to the collaborative rapid response to AIS in NL.

Detection and demarcation phases

Early detection of AIS of concern and appropriate response increase the likelihood that a response will be effective and cause less collateral damage (Wotten and Hewitt 2004). Monitoring and surveys can include a wide range of activities based on the AIS of concern and their habitat (Lehtiniemi et al. 2015). In June 2012, the NLAISAC developed a list of species of concern (Table 1) or a "trigger list" for rapid response and action if these species were detected in NL waters. Species on this list were selected based on species risk assessments, known invasiveness, and impact in other regions, particularly the Maritimes region of Canada. The species list of concern provided guidance for monitoring and research in the NL Region. A Canadian Marine Invasive Screening Tool (CMIST) (DFO 2015b) was recently developed to provide a ranking system and screen invertebrate invasive species to provide a comparative level of risk for introduction or spread within Canada.

Since 2006 over 100 harbours, marinas, and coastal locations have been surveyed in Newfoundland and Labrador for the presence of non-indigenous and invasive species (Callahan et al. 2010; McKenzie et al. 2016). The monitoring protocol is standardized with the other DFO AIS Atlantic Zone regions (Maritimes, Gulf, Quebec and Newfoundland and Labrador) using PVC collection plates, SCUBA, underwater videography, wharf, floating docks, and boat hull surveys (Martin et al. 2011; Sephton et al. 2011), and rapid assessment surveys (Martin et al. 2010). Partnerships with other DFO and DFA field teams, aquaculture site workers, fish harvesters, First Nation's fisheries guardians, recreational boaters, and harbour authorities have led to a wide network of informed and aware personnel throughout NL. A communication program based on the theme of Recognize, Remove, and Report has provided fact sheets and maps for the identification of AIS, a website, toll free numbers and email contacts for reporting new or unknown species.

Accurate identification of the invasive species is a critical component of early detection and it is vital that field personnel are trained to recognize species of concern. Rapid response frameworks (Locke and Hanson 2009c) and non-indigenous species monitoring recommendations (Ojaveer et al. 2014; Lehtiniemi et al. 2015) highlight the importance of this skill but also the decreasing number of persons with this taxonomic expertise. Fortunately, as part of the early training program within DFO, tunicate identification workshops in 2007 (PEI) and 2009 (Quebec) were conducted by the world's foremost experts in tunicate taxonomy for field personnel and researchers as part of the DFO AIS monitoring program.

Following the identification of a "trigger species" the next step is a survey to determine the extent of the infestation or the demarcation phase (Locke and Hanson 2009c). A biological field team investigates potential vectors of spread for the species of concern, studies surrounding environmental parameters, and consults local knowledge. This local knowledge has been particularly important in identifying likely pathways, which again emphasized the importance of pre-invasion communication with all possible stakeholders and should be used in new areas where little previous research or monitoring has been conducted.

Containment and risk assessment phase

Once the extent of the infestation was determined by the demarcation phase, criteria for determining the need for containment, methods of containment, and requested restrictions of use are determined (see also Locke et al. 2009b). Voluntary containment involving vessel traffic, wharf and dock usage were attempted at three locations through communication and stakeholder awareness (Table 2). The seasonal timing, late fall, of the discovery of *B. schlosseri* and *B. violaceus* was fortunate as vessel movement was much reduced and were in the process of being removed from the water. The removal of small vessels and even floating docks from harbours in NL is a common practice in response to anticipated winter ice conditions.

Once information on the extent of the invasion and related biological data was collected, and realistic containment was in place, the next step was an assessment of the risk and possible control options. Risk assessment and planning occurred during the winter when risk of natural dispersal was low due to the reduction in viable gametes and colony size over the winter months (Deibel et al. 2014). When considering mitigation

Table 2. Rapid response phases for colonial invasive tunicates in Newfoundland and Labrador.

Phase	*B. schlosseri*	*B. schlosseri*	*B. violaceus*
Detection	Argentia, Placentia Bay	Foxtrap Marina, Conception Bay	Belleoram, Fortune Bay
Demarcation	Several locations in Placentia Bay	Confined to small area of marina, first location in Conception Bay, no vessels affected	Confined to small area of Belleoram Harbour, Fortune Bay
Containment	Stakeholder awareness activities	Stakeholder awareness and restriction of gear transfer between bays	Stakeholder awareness Requested boat owners to leave vessels in place.
Risk Assessment	Wide spread in Placentia Bay	First detection outside Placentia Bay, mitigation attempt recommended	Confined location and risk to nearby aquaculture sites, mitigation attempt strongly recommended and supported
Mitigation	No mitigation, public awareness focus	Removal of infested floating docks (15) Removal of infested kelp on breakwater	Vessel wrapping, pylon and crossbeam wrapping, infested rock and debris removal, "vacuum" benthic sediment
Evaluation	Spread further in Placentia Bay now also found on eelgrass	No re-infestation Year 1 post-mitigation, Year 2 present again on floating docks	Initially successful but re-infested during the fall and became more wide spread in subsequent years.

Table 3. Rapid response phases and timeline for the solitary invasive tunicate, *Ciona intestinalis*, in Placentia Bay, Newfoundland and Labrador.

Phase	*Activity*	*Date*	*Responsibility*
Detection	*C. intestinalis* (Burin)	September 2012	DFO Science
Demarcation	Little Bay infestation on floating docks, wharf, vessels and mooring ropes	October 2012	DFO AIS /MUN
Containment	Communication with boat owners and harbour managers	October 2012	NLAISAC
Risk Assessment	Relatively confined area, limited amount of boat traffic, high risk and potential impact on the economy and environment, success potential increased due to reproductive strategies of the species Rapid and comprehensive mitigation was recommended and supported	October 2012 -April 2013	NLAISAC
Mitigation	Floating dock removal, wharf cleaning, mooring line and gear removal, vessel cleaning and application of antifouling paint on vessels and floating docks	June 2013 Water temp < 8°C	DFO/MUN/ DFA/NAIA
Evaluation	Evaluation examination dives detected very few (< 10) *C. intestinalis* on harbour infrastructure or vessels in the months and years following the mitigation, these were removed.	June 2013 -September 2015	DFO/MUN/ DFA/NAIA

activities there are four general options which include: 1) attempt eradication or more likely contain the problem to a given area, 2) suppress the population to slow its spread, 3) develop management strategies to keep the species abundance below an economic or ecological threshold, or 4) learn to adapt with the problems caused by the species (Myers et al. 2000).

Following the detection of *B. schlosseri* in Argentia, several SCUBA surveys were conducted at nearby harbours, marinas, and mussel aquaculture sites in northern and eastern Placentia Bay. Arnold's Cove harbour was of particular concern as it had relatively high vessel traffic and was the home port for mussel aquaculture support vessels. Rapid assessment surveys found *B. schlosseri* in 3 additional harbours in Placentia Bay, including Arnold's Cove. When *B. schlosseri* was detected in Foxtrap Marina, it was the first detection of this species outside of Placentia Bay. There was support for trying to eradicate or control this spread within a new bay in Newfoundland. The rapid response demarcation survey found that it was growing on three of the more exposed floating docks and kelp (*Laminaria* sp.) attached to docks in the marina, but it was not found on any vessels during the follow up survey. During the risk assessment it was discovered that a common practice is to bring rope and gear from Placentia Bay (Arnold's Cove) to hold lobsters in Foxtrap Marina and this was believed to be a possible vector (Table 2).

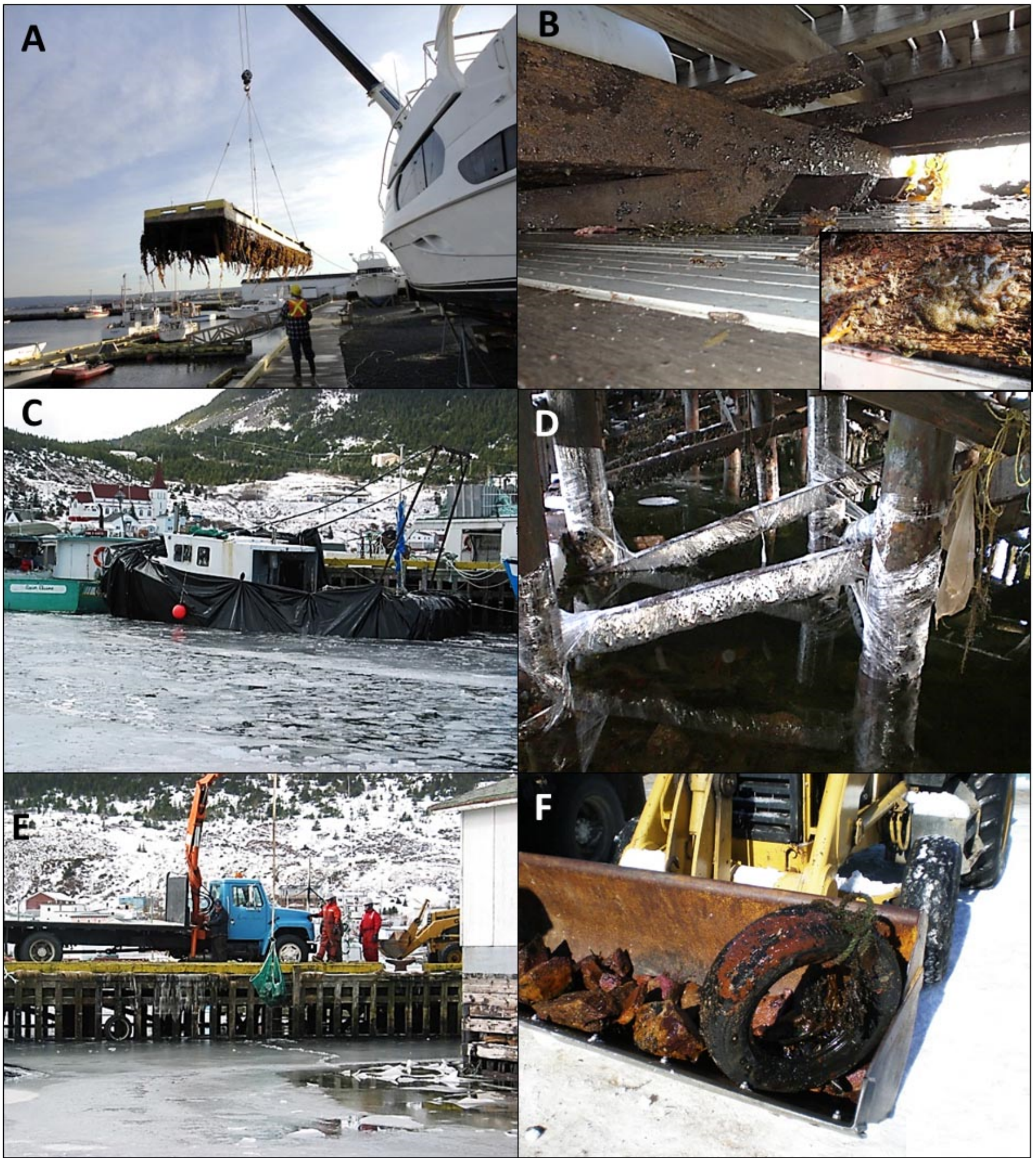

Figure 2. Photographs of mitigation of *B. schlosseri* in Foxtrap Marina (A & B) (Conception Bay) showing removal of (A) floating docks and (B) evidence of tunicates growing in niche areas on floating docks and photographs of mitigation of *B. violaceus* in Belleoram Harbour (C – F) (Fortune Bay) showing (C) wrapping used on boat, (D) clear plastic wrap used on pilings and crossbeams of wharf, and (E and F) removal of debris fouled with *B. violaceus*. Photographs by C.H. McKenzie (A,B), G. Perry (C-F).

When *B. violaceus* was detected in October 2007 on the wharf structure in Belleoram harbour, Fortune Bay, the level of concern for invasive tunicates reached a more urgent state. Not only was *B. violaceus* considered to be higher risk than *B. schlosseri* due to its rapid growth and spread (Therriault and Herborg 2008b), but the location was closer to several aquaculture sites on the south coast. SCUBA rapid assessment surveys determined that the tunicate was found in only a small protected corner of the permanent wharf and on three vessels. Due to the location, the species, early detection, and small area of infestation there was strong consensus for mitigation. The mitigation was attempted in March 2008 when the water temperature was the coldest, the tunicate had reduced in area during overwintering, and larval dispersal was highly unlikely. The mitigation activities were based on eradication attempts in New Zealand for the invasive colonial tunicate, *Didemnum vexillum* (Coutts and Forest 2007; Kott 2002; McEnnulty et al. 2001).

Mitigation and evaluation phase

The mitigation or control strategies for invasive colonial tunicates varied based on the case circumstances and the results of the risk assessments for each situation. Following the mitigation activities the sites were monitored and evaluated for the effectiveness of the mitigation. Since *B. schlosseri* was found at several areas in Placentia Bay, the decision was made not to attempt removal or control. Instead, the focus was on educating stakeholders to prevent further spread through hull fouling to other areas of NL. As recommended by the risk assessment for the control of *B. schlosseri* in Conception Bay (Table 2), arrangements were made with the local Harbour Authority to have a crane truck remove all 15 floating docks from the marina, which remained out of the water in a nearby field through the winter (Figure 2A, 2B).

The mitigation trials in Belleoram harbour took place in March 2008 and continued for 7 days. The mitigation was a three part approach which depended on the substrate the invasive tunicate was attached to at the site. The three substrates were i) vessel hulls, ii) the wooden wharf structure, and iii) rocks and debris at the base of the wharf. The three infected vessels were wrapped in large plastic sheets (hay bale plastic) with the assistance of SCUBA divers and the boat owners (Figure 2C). Freshwater was introduced by a hose into the space between the hull and plastic to lower the salinity and temperature (ice particles). The vessels were wrapped for 7 days before the plastic was removed. The wooden wharf structures (pylons and crossbeams) were wrapped by commercial SCUBA teams using freezer grade pallet wrap (Figure 2D). The plastic remained on the structures for one month before being removed. The rocks and various debris (e.g. tires, water heater) were removed by commercial divers using catch bags and floats (Figure 2E, F). Finally, a vacuum tanker truck was used to "vacuum" benthic sediments to remove fragments that may have settled in the sediment.

In the seven years since the first detection of *B. schlosseri* in Placentia Bay, additional monitoring has shown it to have spread to several other areas of the bay (McKenzie et al. 2016). It has recently been found covering eelgrass in several areas (Carmen et al. 2016) and additional spread through rafting is likely.

During the first year post-mitigation, no *B. schlosseri* was detected in Foxtrap Marina. However, in the second year post-mitigation, which had particularly warm water temperatures, surveys detected *B. schlosseri* again on the floating docks at this site.

No trace of *B. violaceus* was found on the wharf structures or vessels throughout the spring and summer following the mitigation attempt. In October 2008 (7 months post-mitigation), however, *B. violaceus* was detected on wharf structures and had expanded rapidly on the cleared pylons (Table 2). Assessment of the area led to the conclusion that the dark and small niche areas of the wharf structure outside of the mitigation perimeter probably led to re-infestation at the site and wrapping was ultimately an ineffective method of control. Experimental antifouling/settling preventive measures for docks and vessels hulls are now being tested.

Rapid Response Plan in Practice– *Ciona intestinalis* in Placentia Bay, Newfoundland and Labrador

The rapid response plan used in this experimental study is not a mandated official response plan for federal and provincial public sector regulatory authorities in NL. It is an informal guideline used by the members of NLAISAC to direct and focus the unified response of the region for action against an identified and listed (Table 1) AIS of high concern, such as the post invasion process phases and timeline in the rapid response plan for *C. intestinalis* in Little Bay, Placentia Bay (Table 3).

Detection and demarcation phase

Detection of the solitary tunicate *C. intestinalis* on a wharf in Burin (Figure 1, Table 3) triggered the implementation of the rapid response plan. Specimens were preserved in ethanol for genetic analyses and deposited into the local Provincial Museum of Natural History. North of Burin in Little Bay harbour (Figure 1 C) larger populations were found on wharf structures, various kelp species, and mussels. The densest infestation was on the undersides of two floating docks (with up to 75% coverage or 352 individuals per m^2). In Little Bay, *C. intestinalis* was also attached to the permanent wharf structure, kelp, mussels, ropes, eelgrass, and several vessels at the wharf. The number and size of individuals suggested that *C. intestinalis* has been present in NL for more than one full year, which indicated that the species can overwinter in this environment (Sargent et al. 2013; Reid unpubl. data). Immediately following detection, and determining extent of distribution all members of NLAISAC were informed and a meeting date was set to discuss next phases for rapid response planning (Table 3).

Containment and risk assessment phase

While the mitigation and control plan was being developed through the risk assessment, every effort was made for boater education and vessel vector containment. Most fish harvesters clean their vessel hulls and apply antifouling paint every year. They were encouraged to continue this practice. However, one boat owner was requested to clean his boat in Little Bay after mentioning that he planned to travel across Placentia Bay to clean it. He voluntarily complied with this request. *Ciona intestinalis*, if detected early, is a good candidate for eradication or suppression because, in contrast to colonial tunicates, the solitary *C. intestinalis* only reproduces sexually. This eliminated concerns of new populations becoming established from small fragments of the colony and further supported manual removal strategies. The high economic risk associated with the discovery of *C. intestinalis* in NL caused the committee to recommend a rapid and comprehensive mitigation response (Table 3).

Based on consensus, NLAISAC decided that manual or mechanical removal was the optimal containment strategy to prevent movement of this species within Placentia Bay. During the risk assessment, vessel traffic was determined to be the most likely transmission vector, in part because of relatively limited natural dispersal of *C. intestinalis* (Kanary et al. 2011). Since demarcation dive surveys by MUN Field Services identified most *C. intestinalis* on floating docks and other man-made objects (i.e. rope), the decision was made to remove these objects.

Prolonged periods of very cold seawater temperatures in NL have been shown to be important factors in control planning for previous mitigation activities. Therefore, mitigation activities have been planned for late winter to early spring when the water temperatures are at their lowest (between <1°C and 7°C) and the invasive tunicate is reduced or in the overwintering stage. Determining the vulnerability of the invasive tunicate particularly relating to temperature, growth and reproduction can be a key factor to success (Deibel et al. 2014).

Mitigation and evaluation phase

A pre-mitigation dive survey in April 2013 by MUN Field Services indicated that there was noticeable die-back of *C. intestinalis* since the previous fall, but highest densities remained on floating docks. On June 3, 2013, the floating docks were removed and turned over (Figure 3A). Once the docks were out of the water, detailed population studies (quadrats/photographic records) were conducted to determine biomass and distribution on both floating docks (Figure 3B,C). The docks were cleaned of biofouling organisms either by scraping or by a high pressure sprayer (Figure 3D). The floating docks remained out of the water for over a year and a copper-based antifouling paint was applied before they were returned to the water. The effectiveness of the antifouling paint on the wooden dock structure will be monitored. During subsequent dives, members from MUN Field Services removed any remaining *C. intestinalis* individuals from the permanent wharf structures by hand (Figure 3E). Any fouled mooring rope was replaced with new rope (Figure 3F).

The four active fishing vessels at the harbour had all been cleaned and repainted with antifouling paint the previous fall and had no *C. intestinalis* present. However, a small boat tied to the Little Bay wharf was found heavily fouled with *C. intestinalis* and was cleaned (Figure 4A, B, C). Although there was concern that using high pressure treatments can disperse animals and gametes into nearby water, the low water temperature (< 8 °C) and the pre-treatment with steam and disinfectant reduced the dispersal risk of viable gametes (Figure 4C,D,E). Following this treatment, all obvious animals were collected from the beach and disposed of. After the boat was cleaned, a copper-based antifouling paint was applied, and it was returned to the wharf (Figure 4F,G,H).

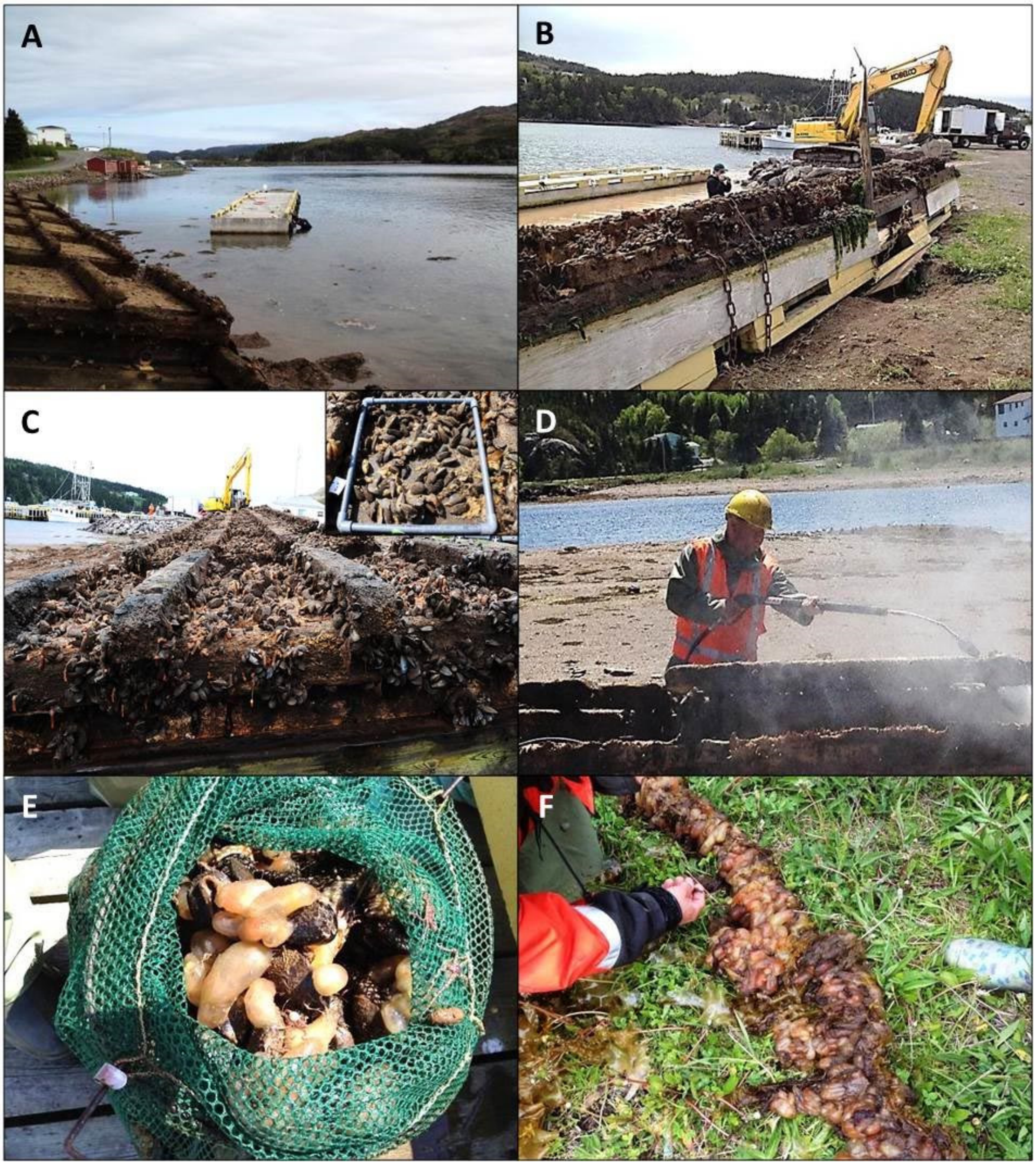

Figure 3. Photographs of mitigation of *C. intestinalis* in Little Bay (Placentia Bay) showing (A) removal of floating docks, (B & C) population study of tunicates on floating dock (insert on C showing use of quadrat to assess densities), (D) use of high-pressure spraying to remove tunicates from floating docks, (E) removal of *C. intestinalis* by hand from wharf structure, and (F) heavily fouled mooring rope that was removed. Photographs by C.H. McKenzie.

Following the initial mitigation, settlement plates were deployed and post-mitigation dive surveys have been conducted regularly between 2013 and 2015. The first post-mitigation dive occurred on June 12, 2013 and only a few individuals of *C. intestinalis* were found and removed. By October 2013, dive surveys found less than ten tunicates on the permanent wharf, but a few specimens were detected on an adjacent boat. Similar numbers of *C. intestinalis* were also observed in early 2014 in Marystown, but were removed from the wharf in 2014 as part of the expanded mitigation. During the most recent mitigation evaluation (September 2015) in Little Bay the species was

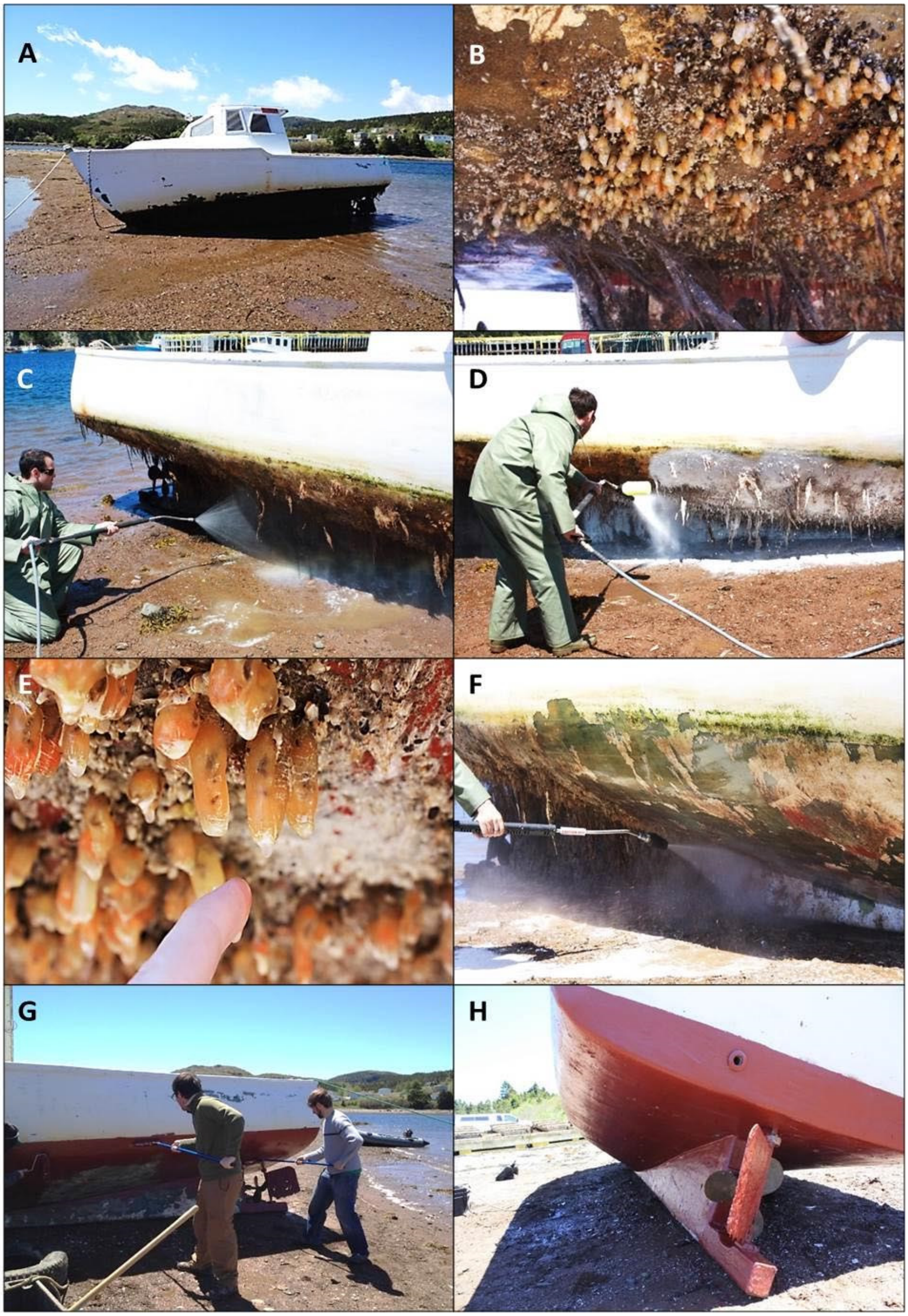

Figure 4. Photographs of process to clean *C. intestinalis* off a small vessel in Little Bay (Placentia Bay) showing (A) boat beached at low tide, (B) infestation of *C. intestinalis* on hull of boat, (C) steam treatment, (D) application of detergent disinfectant, (E) physical appearance of tunicates after steam and detergent treatments, (F) use of high-pressure spray to clean boat, (G) application of antifouling paint, and (H) boat following treatment showing area of keel that rested on the ground and could not be painted. Photographs by C.H. McKenzie.

very rare and in Marystown none were found. Throughout 2014, *C. intestinalis* populations increased in Burin where no mitigation had taken place. In May 2015 over 500 kg of biofouling material, primarily *C. intestinalis* growing among mussels, was removed from the wharf structure and nearby vessels by SCUBA divers in Burin Harbour.

Conclusions

Although a Canadian long-term monitoring program has been in place since 2006 to detect non-indigenous species and follow their spread (Martin et al. 2011; McKenzie et al. 2016; Sephton et al. 2011), Canada had lacked a comprehensive national AIS rapid response framework and the regulatory authority to enforce a response and control plan. Perhaps most importantly, no prohibitions had been in place to limit movement of AIS between provinces or into Canada. On June 17, 2015 the *Aquatic Invasive Species Regulations* under the Canadian *Fisheries Act* were posted in the *Canada Gazette* and are now in effect (DFO 2015a). The objectives of these Regulations are 1) to prevent the introduction and spread of AIS in Canadian waters; 2) to avoid costs associated with the establishment of invasive species; 3) to support management activities to control the spread of AIS once introduced into Canada; and 4) to fill regulatory gaps, and ensure a consistent national strategy for the management of AIS.

A vector based approach to prevent the introduction and spread of AIS is currently the focus of NL planning and communication. There are numerous potential vectors of concern which include biofouling of vessels and gear, release of bilge or waste waters, live transport of species, and fouling of permanent or temporary structures (i.e. wharves and docks). Best management practices are being developed in NL to present a framework to advise marine resource users on strategies (e.g. cleaning gear, antifouling coatings) to manage introduction and spread of AIS based on efficacy, cost effectiveness, and practicality. These best management practices can benefit many marine resource stakeholders, including recreational boaters, commercial fish harvesters, aquaculture personnel, researchers, and other industries.

A rapid response plan based on experience, good communication, strong partnerships, and common goals has allowed NL to respond to a high impact AIS tunicate in an effective manner. How long the control and containment lasts may depend on the continued commitment of the stakeholders and their education in best practices for vector control. The new Canadian AIS regulations will provide authority for response, but monitoring, vigilance, prior planning, collaboration between stakeholders and rapid action are the real tools for any effective control plan.

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