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Research Article

Surveys for non-indigenous tunicate species in Newfoundland, Canada (2006 – 2014): a first step towards understanding impact and control

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Abstract

Fisheries and Oceans Canada initiated survey and monitoring programs in 2006 to determine presence, geographic range, and subsequent spread of non-indigenous tunicates in Atlantic Canada. Although non-indigenous tunicates have impacted aquaculture industries in Atlantic Canada for over a decade, aquaculture operations in Newfoundland and Labrador (NL) have not yet been affected. In this report, we document and explain results from biofouling collectors and various underwater techniques to survey and monitor for non-indigenous tunicates in NL between 2006 and 2014. During early surveys (2006–2007) we only observed low-impact invaders, *Botryllus schlosseri* and *Botrylloides violaceus*, at locations along the south coast of NL. *Botryllus schlosseri* became the most widely distributed species (18 locations within Placentia Bay) and evidence demonstrated spread to locations on the northeast (2011) and west (2013) coasts of NL. In 2012, we detected *Ciona intestinalis* in a single harbour in southern NL (Placentia Bay). In contrast to southern regions of Atlantic Canada, *Styela clava* and *Didemnum vexillum*, two high-impact invasive tunicates, were not observed during these surveys. Newfoundland and Labrador represents the northern range limit of non-indigenous tunicates in Atlantic Canada, but seawater temperatures are unlikely to prevent further expansion or introduction of non-indigenous tunicates throughout the province.

Key words: tunicate, biofouling, survey, non-indigenous, distribution

Introduction

Non-indigenous species, defined as organisms living outside of their native distributional range, classically spread either intentionally or accidentally by human activities. In certain cases, species become invasive after establishing themselves in new environments and have detrimental ecological or economic consequences. With escalations in global transport, focus on monitoring, risk, and research activities related to invasive species has increased, which requires accelerating documentation of introductions and range expansions of aquatic invasive species (AIS; Carlton and Geller 1993; Cohen and Carlton 1998; Mack et al. 2000). Invasive tunicates fall under the category of biofouling organisms, which include organisms that attach to underwater surfaces of vessels (e.g. hulls or niche areas such as rudders, sea chests, or propellers) and hitch a ride from one region to another (Carlton and Hodder 1995; Clarke Murray et al. 2011; Frey et al. 2014). Once transported, these biofouling species attach to local substrates (natural or artificial), reproduce, and can establish populations. Although biofouling vectors are primarily human mediated (e.g. movement of recreational and commercial vessels, slow moving barges, or transfer of gear), the likelihood of spread through natural vectors, such as rafting, drifting, or attachment to mobile organisms is an additional concern (Ruiz et al. 1997; Lewis et al. 2005; Thiel and Gutow 2005; MacFarlane et al. 2013).

In total, 7 non-indigenous tunicate species have been confirmed within the Canadian Maritimes: *Styela clava* Herdman, 1881, *Ciona intestinalis* (Linnaeus, 1767), *Botryllus schlosseri* (Pallas, 1766), *Botrylloides violaceus* Oka, 1927, *Ascidiella aspersa* (Müller, 1776), *Diplosoma listerianum* (Milne-Edwards, 1841), and most recently (2013), *Didemnum vexillum* Kott, 2002 (Ramsay et al. 2009; Martin et al. 2011; Sephton et al. 2011; Moore et al. 2014). Non-indigenous tunicates that become invasive have the ability to rapidly foul natural and man-made structures and compete with native species for food and space. On mussel lines at aquaculture sites, they compete with mussels, contributing to decreased mussel size and condition, increased mortality, and increased drop off rates from lines (Daigle and Herbinger 2009). For example, mussel aquaculture in Prince Edward Island (PEI), Canada has experienced serious impacts from the establishment and spread of invasive clubbed, *S. clava*, and vase, *C. intestinalis* tunicates (Thompson and MacNair 2004; Locke et al. 2009). The added cleaning of mussel lines and significant increases in weight from tunicates has strained aquaculture operations by increasing loss of product and requiring additional time and labor (Thompson and MacNair 2004). Although the first threat to PEI shellfish aquaculture was *S. clava*, most recently, the rapid invasion of *C. intestinalis* replaced *S. clava* as the foremost invasive tunicate species (Ramsay et al. 2009). In PEI, management responses have included regulation of aquaculture transfers and harvest dates, which have been relatively successful in controlling spread of solitary tunicates (e.g. *S. clava* and *C. intestinalis*; Locke et al. 2009). The recent discovery of *D. vexillum* within the Bay of Fundy (Canada; Moore et al. 2014) further validates the continued spread and range expansion of non-indigenous species in Atlantic Canada and the importance of sustained monitoring programs.

By definition, monitoring is the gathering of data using standardized methods intended to deliver information on species characteristics and their changes over time, while surveys are the collection of information to provide an instantaneous view of a certain area at a specific time (Lehtiniemi et al. 2015). Furthermore, the combinations of monitoring, surveys, and subsequent research are necessary fundamentals for marine environmental management (Lehtiniemi et al. 2015). Throughout Atlantic Canada, the Department of Fisheries and Oceans Canada (DFO) has initiated zonal AIS monitoring programs (e.g. Martin et al. 2011; Sephton et al. 2011; Simard et al. 2013), including this study in Newfoundland and Labrador (NL). These programs use survey and monitoring strategies as the first step to understand the spread and effects of AIS and to guide projects on non-indigenous tunicates through 1) early detection, 2) rapid response and mitigation, and 3) prevention. Information provided by these strategies have informed risk assessments and provided foundations for research on species ecology, prevention practices, and mitigation methods (McKenzie et al. 2016). Prior to initiation of the DFO AIS monitoring program in NL (2006), records existed of only one non-indigenous tunicate species (*B. schlosseri*), which was observed on the west coast of NL in the 1970s (Hooper 1975; Ma et al. 2010) and Argentia in the 1940s (Placentia Bay; US Navy 1951). Since formation of the AIS monitoring program, established populations of two additional non-indigenous tunicates (*B. violaceus* and *C. intestinalis*) have been detected in NL (Callahan et al. 2010; Sargent et al. 2013). This document summarizes DFO AIS surveys and monitoring conducted to determine distribution and abundance of non-indigenous tunicates (2006–2014) throughout insular NL. Furthermore, we will discuss the range and abundance of invasive tunicates and subsequent actions in the context of rapid response, mitigation, and focused research.

Materials and methods

Province-wide AIS surveys were conducted throughout insular NL led by the Department of Fisheries and Oceans Canada in partnership with the Department of Ocean Sciences, Memorial University of Newfoundland (MUN). Survey locations (Appendix 1) were targeted to focus on marine sites defined as high risk for introduction and spread of non-indigenous tunicates (Baines 2007; Lambert and Lambert 1998). Such locations included marinas, public wharves, aquaculture and potential aquaculture sites, and other locations determined to have relatively high volumes of vessel traffic (e.g. ferry terminals or commercial ports). Moreover, survey locations were selected in close proximity to structures with high biofouling potential, such as floating docks and man-made wharves (Airoldi et al. 2015).

A combination of methods was used (2006 – 2014) to assess the distribution and spread of non-indigenous tunicates in NL, including biofouling collectors, SCUBA surveys, underwater video, and shoreline observations. The number of survey locations varied year to year (Table 1), because of travel distance between locations (up to 1000 km) and sites identified by industry or stakeholders as areas of concern. Between 2006 and 2009, surveys were conducted primarily using biofouling collectors suspended at 37 different sites in NL. These surveys encompassed the entire island of NL and included 12 harbours, 2 ferry terminals, 8 mussel aquaculture sites, and 15 sites designated as potential candidates for future aquaculture mussel seed sites. Following initial surveys, six locations found to have established populations of non-indigenous tunicates were targeted for monitoring (e.g. annually or bi-annually). This monitoring included the extent of tunicate cover, species growth, and secondary spread using a combination of collector plates and underwater surveys conducted between 2010 and 2014 (Foxtrap and Arnolds Cove, *B. schlosseri*; Belleoram, *B. violaceus*; and Little Bay, Burin, and Marystown, *C. intestinalis*). At three of these sites, experimental mitigations were conducted to evaluate control methods to prevent the spread of the non-indigenous tunicate (Belleoram, *B. violaceus* [2008 and 2009]; Foxtrap, *B. schlosseri* [2011]; Little Bay, *C. intestinalis* [2013, 2014]; Deibel et al. 2014; McKenzie et al. 2016).

Collectors at harbour and ferry sites consisted of three horizontally oriented, lightly sanded settling PVC plates (10 x 10 cm) suspended from the surface (e.g. attached to wharves, buoys, etc.) in a vertical series at 37.5 cm intervals. For another research project on the west coast of NL, PVC plates were spaced by only 10 cm. Collectors were placed in shaded areas with relatively high biofouling growth, positioned ~ 1.0 m below the surface at low tide, and weighted at the bottom to ensure they remained vertical in the water column. Collectors deployed at aquaculture and potential aquaculture sites varied slightly from collectors at harbour sites to better survey the biofouling potential of mussel mooring lines used at NL aquaculture sites in concurrence with a mussel seed research project. These collectors were hung at the same depth as mussel lines and consisted of six PVC plates (10 × 10 cm) at depths of 1.5, 3.5, 4.0, 4.5, 5.0, and 5.5 m. The three shallowest PVC plates were oriented horizontally (i.e. plate surface was parallel to water surface) in the water column whereas the deepest three PVC plates were positioned vertically (i.e. plate surface was perpendicular to water surface). Mussel spat collection ropes (4 ropes in total of 0.64 cm polyrope held in place at either end by two bucket lids) were attached to each collector between plates at 1.5 and 3.5 m depth. Temperature recorders (Vemco Minilog II-T or Hobo data logger) were attached opportunistically to collectors in several locations to record temperature during deployment.

One to three collector lines were suspended at each harbour site, while three collectors were deployed at each aquaculture and potential aquaculture site. Plates were generally deployed in late spring/early summer (~June) and retrieved in the fall (between November and December) each year. In years following 2006, new plates were often redeployed during site visits in the fall of the year and remained in the water over winter. In some scenarios, additional site visits were possible and allowed plates to be analyzed mid-season (~August) to supplement analyses on full-season plates retrieved in the fall. Collectors were in the water at least during the warmer months of the year and deployed at minimum 2–3 months and in many cases closer to one year.

When collectors were retrieved, PVC plates were fixed in 10% formalin buffered with sodium borate. For laboratory analyses, plates were rinsed with fresh water to remove formalin and both sides of plates were examined under dissecting (Leica M80) and compound (Zeiss Axio AX10) microscopes. The presence and area of non-indigenous tunicates was determined and recorded. Only data from the bottom side of PVC plates are presented here, as sedimentation on top side of plates characteristically reduce recruitment, and in particular, species of interest in Atlantic Canada typically exhibit photonegative behaviors and settle in low light environments (Svane and Young 1989; Lambert 2005; Ruiz et al 2010). In 2006 and 2007, the area of each plate occupied by non-indigenous tunicates was determined by overlaying a 10 × 10 cm grid divided into 1 cm^2 quadrats. Analysis conducted from 2008 onwards was completed using image analysis software (ImageJ; public domain software by W. Rasband, National Institute of Mental Health, USA, http://imagej.nih.gov/ij/index.html). The area occupied was converted to percent cover and categorized based on the following categories (see Sephton et al. 2013): 0 (Absent), <25% (low), 25–50% (moderate), 51–75% (high), >75% (very high). All biofouling organisms were identified to the lowest taxonomic level possible, but information on species other than non-indigenous tunicates is not presented here.

To supplement biofouling collectors, SCUBA and underwater video surveys (e.g. pilings on perimeter and underneath wharf, floating docks, and transects 50–100 m perpendicular and parallel to shore) targeted sites with confirmed presence of non-indigenous tunicates on collectors and often occurred opportunistically in combination with other research projects, which greatly increased

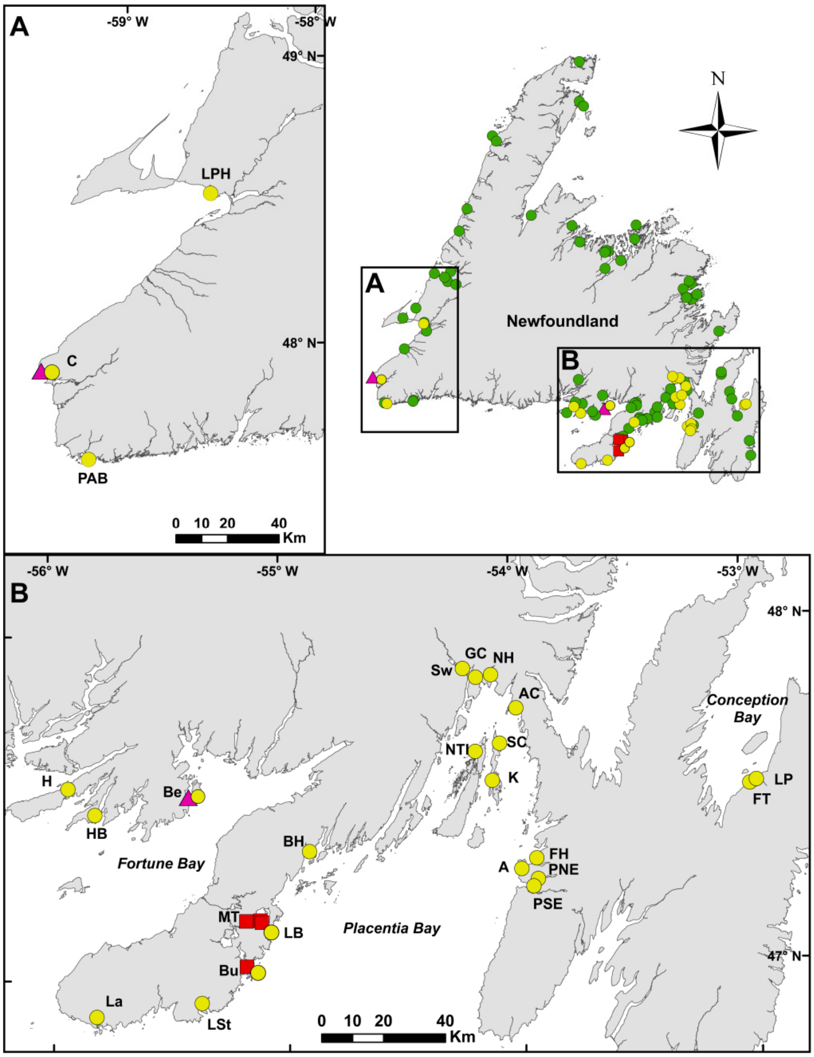

Figure 1. Distribution of survey locations for aquatic invasive tunicates in Newfoundland showing absence of tunicates (green circle) and presence of *Botryllus schlosseri* (yellow circle), *Botrylloides violaceus* (purple triangle), or *Ciona intestinalis* (red square) in (A) southwestern Newfoundland and (B) Fortune Bay, Placentia Bay, and Conception Bay. Inserts (A and B) only show positive results for invasive tunicates. No survey locations in other regions of Newfoundland were positive for invasive tunicates. Survey location names are abbreviated and listed alphabetically (A *Argentia*; AC *Arnolds Cove*; Be *Belleoram*; BH *Baine Harbour*; Bu *Burin*; C *Codroy*; FH *Fox Harbour*; FT *Foxtrap*; GC *Garden Cove*; H *Hermitage*; HB *Harbour Breton*; K *Kingwell*; La *Lamaline*; LB *Little Bay*; LP *Long Pond*; LPH *Little Port Harmon*; LSt *Little St. Lawrence*; MT *Marystown*; NH *North Harbour*; NTI *North Tilt Island*; PAB *Port aux Basques*; PNE *Placentia NE*; PSE *Placentia SE*; SC Spencer's Cove; SW *Swift Current*;).

Table 1. Number of stations surveyed using collectors or underwater techniques (e.g. SCUBA and underwater video) in Newfoundland, Canada between 2006 and 2014.

	Number of locations			Presence of species (# of sites)		
Year	Total	Collectors	Underwater surveys	*B. schlosseri*	*B. violaceus*	*C. intestinalis*
2006	21	21	1	1	0	0
2007	35	21	17	5	1	0
2008	23	17	7	1	1	0
2009	6	6	0	1	0	0
2010	7	2	6	1	1	0
2011	7	1	6	3	1	0
2012	30	4	29	18	1	2
2013	27	5	27	5	1	2
2014	29	16	29	4	2	3

Table 2. Summary of locations where non-indigenous tunicates were observed on collectors between 2007 and 2014 (Site type: M - Marina, C - Coastal).

		Coordinates				Coverage range (%)
Location	Region	Latitude	Longitude	Site Type	Year	*B. schlosseri*
Foxtrap	Conception Bay	47.513	-52.998	M	2011	0
					2013	< 1 – 100
Belleoram	Fortune Bay	47.527	-55.409	M	2008	0
					2010	0
					2012	0
Hermitage	Hermitage Bay	47.556	-55.926	M	2008	0
Argentia	Placentia Bay	47.292	-53.990	M	2007	0
Arnolds Cove	Placentia Bay	47.759	-53.989	M	2009	0 – 2
					2010	0 – 1
Burin	Placentia Bay	47.031	-55.174	M	2013	0

the overall scope of this survey and monitoring program. For example, in collaboration with other research, 2012 SCUBA surveys were focused throughout Placentia Bay on a combination of marina and coastal eelgrass (*Zostera marina* Linneaus, 1753) ecosystems, whereas during 2013 and 2014, underwater surveys examined harbours dispersed across the entire island of NL. Underwater surveys (often including video and still image transects) examined underwater structures, including pilings, floating docks, submerged materials (e.g. ropes and buoys), benthos, boat hulls, and nearby slipways. Samples of tunicates or any unknown specimen were preserved in 10% buffered formalin and ethanol for further lab analysis and species identification. When SCUBA was unavailable, a MicroVideo™ camera was mounted on an extendable pole to video vertical (1–3 m) and horizontal (up to ~ 20 m) transects along structures. Overall, we surveyed 70 coastal locations using SCUBA or underwater video (including public wharves and natural coastal sites) in addition to sites surveyed solely with biofouling collectors.

Results

Between 2006 and 2014, general surveys and targeted monitoring confirmed the presence of three non-indigenous tunicate species at marina and harbour locations in NL (*B. schlosseri*, *B. violaceus*, and *C. intestinalis*). No tunicates were observed at aquaculture or potential aquaculture sites. Generally, the rate of occurrence of non-indigenous tunicates was low, but the number of tunicate species and occurrence increased year to year (Table 1). For example, in the first survey year (2006) *B. schlosseri* was the only non-indigenous tunicate found and only at one site. In 2007, *B. violaceus* was confirmed in Fortune Bay. In 2012 (a particularly warm year), *C. intestinalis* was detected in southwest Placentia Bay, which also coincided with the greatest number of observations of *B. schlosseri* (18 sites; Table 1). Throughout all surveys, *B. schlosseri* was the dominant tunicate found in NL with the most occurrences (18 of 22 positive sites) within Placentia Bay. *B. schlosseri* was confirmed at one site within adjacent Fortune and Hermitage Bays, and more recently in Conception Bay (2011) and on the southwest coast of NL (2013; Figure 1).

Botryllus schlosseri

B. schlosseri was first detected on a recreational boat hull during a dive survey in Argentia, Placentia Bay on December 7, 2006. Interestingly, *B. schlosseri* was not observed on any collectors retrieved from Argentia or elsewhere in July 2007. In 2007, this tunicate was only detected using SCUBA in northern regions of Placentia Bay (Arnolds Cove, Garden Cove, and North Harbour) and at one site in Hermitage Bay (Hermitage; Figure 1). Colonies of various sizes were found on assorted substrates including kelp (*Laminaria* sp.), mussels, wharf pilings, benthic substrates, and various regions of fishing and recreational vessels (e.g. hull, propeller, and rotor). The number of survey sites decreased between 2008 and 2011, but targeted monitoring in Arnolds Cove revealed low amounts of growth on PVC plates (< 2% coverage) in both 2009 and 2010 and no growth on plates in 2011 (Table 2). Surveys verified *B. schlosseri* at one new site (Baine Harbour) in southwestern Placentia Bay in 2008 and for the first time in Conception Bay in 2011 at Foxtrap marina in northeastern NL (Table 3). Follow-up SCUBA surveys in Foxtrap during late

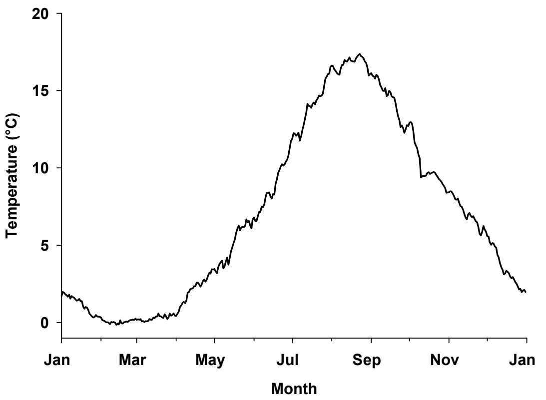

Figure 2. Average daily seawater temperatures from Placentia Bay between 2007 and 2014. Data from Argentia (Dec 2006 - Nov 2007), Arnolds Cove (Sept 2007 - Aug 2008; July 2009 - Mar 2010), Little Bay (Jan 2013 - Oct 2014), Marystown (May - Sept 2014), North Harbour (Oct 2008 - July 2012), Placentia NE (May 2013 - Nov 2014), Swift Current (July - Nov 2014).

November determined that *B. schlosseri* was restricted to one side of the marina on fouled floating wharves and *Laminaria* sp. attached to the floating wharves. A small colony of *B. schlosseri* was observed growing on the hull of only one vessel. Supplementary PVC plates deployed during November determined that recruitment was no longer occurring at this time of year (i.e. no growth on plates), which led to removal of floating wharves from the marina for the winter as part of an experimental mitigation. In 2012, underwater surveys of marinas and coastal sites observed *B. schlosseri* across all regions of Placentia Bay (Table 3, Figure 1) and documented fouling of eelgrass blades at numerous coastal sites. Furthermore, *B. schlosseri* was confirmed in Fortune Bay (Harbour Breton and Belleoram; Table 2, Figure 1). In Foxtrap (Conception Bay), targeted monitoring following mitigation trials demonstrated high recruitment of *B. schlosseri* on PVC plates retrieved in October (up to 100% coverage), but similar to 2011, the tunicate remained primarily on one side of Foxtrap marina. In 2013, *B. schlosseri* was detected at three sites on the southwest coast of NL (Codroy, Little Port Harmon, and Port aux Basques; Table 2, Figure 1). Collectors placed in southwestern NL during 2014 showed coverage of up to 76%, although coverage was very low (< 1 %) on collectors at the southernmost location (Port aux Basques; Table 2). Temperature loggers recorded temperatures from 2007 to 2014 throughout Placentia Bay, although locations and length of deployment varied year to year. Monthly average seawater temperatures in Placentia Bay ranged from 0.1ºC (February) to 16.7ºC (August; Figure 2). In Foxtrap (northeastern NL), monthly average seawater temperatures ranged from -0.1ºC (February) to 17.9 ºC (August) between 2012 and 2013.

Botrylloides violaceus

B. violaceus was first recorded in Belleoram harbour (Fortune Bay) during a dive survey on October 23, 2007 (Table 2, Figure 1). Small patches of *B. violaceus* were found on all fishing boats at the public wharf (two of which were considered heavily fouled) wharf pilings, and bottom substrate (rocks and boulders). Follow-up surveys determined that growth of *B. violaceus* was patchy, but widespread, on wharves. Patches were scattered across areas of the wharf greater than 10 m across (with ~ 10% coverage), but absent from other regions of the wharf. More specifically, coverage was generally the greatest at the head of the wharf structure. Fouling by this tunicate was greater on the wharf structure than bottom substrates and ballast rocks located under the wharf. Following the first experimental mitigation, which used encapsulation methods (March 2008, see Deibel et al. 2014), subsequent underwater surveys in June did not detect *B. violaceus* on PVC plates or previously affected areas of the wharf. Small colonies were found and subsequently removed from a small, formerly-treated boat, but no expansion of *B. violaceus* in Belleoram was observed. Re-growth of *B. violaceus* was observed by October 2008 on previously treated sections of the wharf. A second experimental mitigation in 2009 occurred prior to construction to expand the wharf structure. However, *B. violaceus* was observed on collectors retrieved in October 2010 (up to 37% coverage on mid-season [July] deployment), and colonies were observed on the new sections of the wharf and other pilings and rocks (Table 2). Furthermore, collectors recovered in 2012 exhibited up to moderate to high coverage (up to ~50%) of *B. violaceus* (Table 2). When divers returned in fall 2014 to survey Belleoram, there was almost no *B. violaceus* anywhere on or near the wharf structure. In 2013 and 2014, *B. violaceus* was observed during underwater surveys and on collectors in southwest NL (Codroy; Figure 1). Water temperature loggers recorded seawater temperatures in Belleoram for most of 2008, 2011,

Table 3. Summary of underwater survey locations where non-indigenous tunicates were observed between 2007 and 2014 (Site type: M - Marina, C - Coastal, F - Ferry terminal).

Location	Region	Site type	Coordinates		Year	Species present
			Latitude	Longitude		
Foxtrap	Conception Bay	M	47.513	-52.998	2011	*B. schlosseri*
					2012	*B. schlosseri*
					2013	*B. schlosseri*
Long Pond	Conception Bay	M	47.522	-52.969	2012	*B. schlosseri*
Belleoram	Fortune Bay	M	47.527	-55.409	2007	*B. violaceus*
					2008	*B. violaceus*
					2010	*B. violaceus*
					2011	*B. violaceus*
					2012	*B. schlosseri, B. violaceus*
					2014	*B. violaceus*
Harbour Breton	Fortune Bay	M	47.479	-55.812	2007	None
					2012	*B. schlosseri*
Hermitage	Hermitage Bay	M	47.556	-55.926	2007	*B. schlosseri*
					2010	*B. schlosseri*
					2012	*B. schlosseri*
Argentia	Placentia Bay	F	47.292	-53.990	2006	*B. schlosseri*
					2011	None
Arnolds Cove	Placentia Bay	M	47.759	-53.989	2007	*B. schlosseri*
					2011	*B. schlosseri*
					2012	*B. schlosseri*
Baine Harbour	Placentia Bay	M	47.361	-54.895	2008	*B. schlosseri*
					2012	*B. schlosseri*
Burin	Placentia Bay	M	47.031	-55.174	2007	None
					2012	*B. schlosseri, C. intestinalis*
					2013	*C. intestinalis*
					2014	*C. intestinalis*
Fox Harbour	Placentia Bay	M/C	47.321	-53.924	2012	*B. schlosseri*
Garden Cove	Placentia Bay	M	47.852	-54.158	2007	*B. schlosseri*
Kingwell	Placentia Bay	C	47.551	-54.102	2012	*B. schlosseri*
Lamaline	Placentia Bay	M	46.866	-55.795	2012	*B. schlosseri*
Little Bay (A)	Placentia Bay	M	47.164	-55.112	2012	*B. schlosseri, C. intestinalis*
					2013	*B. schlosseri, C. intestinalis*
					2014	*B. schlosseri, C. intestinalis*
Little Bay (B)	Placentia Bay	C	47.158	-55.106	2012	*C. intestinalis*
					2013	None
					2014	*C. intestinalis*
Little St. Lawrence	Placentia Bay	C	46.927	-55.368	2012	*B. schlosseri*
Long Harbour	Placentia Bay	C	47.441	-53.792	2007	*B. schlosseri*
					2012	*B. schlosseri*
Marystown	Placentia Bay	M	47.163	-55.172	2012	*C. intestinalis*
					2013	*C. intestinalis*
					2014	*C. intestinalis*
North Harbour	Placentia Bay	M	47.858	-54.093	2007	*B. schlosseri*
North Tilt Island	Placentia Bay	C	47.637	-54.171	2012	*B. schlosseri*
Placentia NE	Placentia Bay	C	47.261	-53.921	2012	*B. schlosseri*
					2013	*B. schlosseri*
					2014	*B. schlosseri*
Placentia SE	Placentia Bay	C	47.240	-53.941	2012	*B. schlosseri*
Spencers Cove	Placentia Bay	M	47.658	-54.065	2012	*B. schlosseri*
Swift Current	Placentia Bay	C	47.879	-54.213	2007	None
					2012	*B. schlosseri*
Codroy	Southwest coast	M	47.880	-59.399	2013	*B. schlosseri, B. violaceus*
					2014	*B. schlosseri, B. violaceus*
Little Port Harmon	Southwest coast	M	48.514	-58.538	2013	*B. schlosseri*
					2014	*B. schlosseri*
Port aux Basques	Southwest coast	M	47.576	-59.140	2013	*B. schlosseri*
					2014	*B. schlosseri*

and 2012, and monthly average water temperatures ranged from 1.0 ºC (February) to 16.4 ºC (August). The highest monthly average temperatures were observed in 2012.

Ciona intestinalis

On September 19, 2012 *C. intestinalis* was discovered in relatively low abundances and isolated patches during a dive survey at the public wharf in Burin (SW Placentia Bay). More extensive rapid assessment surveys in October 2012 discovered *C. intestinalis* in high abundances in Little Bay (Site A), approximately 25 km from Burin, on the underside of floating wharves and fixed wharf structures, boats, ropes, buoys, and natural substrates, such as kelp and nearby eelgrass meadows (i.e. Site B, which was ~1 km from Site A). Surveys in nearby (<5 km) Marystown also documented *C. intestinalis* on wharf structures and one vessel, but divers described abundances as low compared to observations in Little Bay. No specimens were observed on eelgrass adjacent to the Marystown wharf. More comprehensive SCUBA surveys in April 2013 determined largest abundances were in Little Bay with approximately 75% of all *C. intestinalis* growth on the underside of floating docks, but PVC plates deployed in September 2012 had no growth of *C. intestinalis* by April 2013. Following experimental mitigation in April 2013, which included removal of floating docks and cleaning growth off any substrate, divers surveyed the site again in September and only observed very few individuals fouling wharf pilings and the keel of one boat. All individuals were removed by SCUBA divers. In contrast to 2012, no individuals were found during 2013 surveys in nearby eelgrass meadows at Little Bay (i.e. Site B).

In April 2014, we observed relatively low abundances of *C. intestinalis* in Little Bay (Site A), Burin, and Marystown. Most specimens in Little Bay appeared physically damaged and not alive (potential evidence of predation or ice scour), but all observed individuals were removed. We did not detect any recruits on PVC collectors (<1 % coverage) until August 2014 on PVC plates. No *C. intestinalis* was observed on the Little Bay wharf throughout 2014, but a couple of specimens were found on one boat and were removed. In contrast to previous years, 2014 SCUBA surveys detected greatest abundances in Burin on wharf structures and adjacent boats. At the Marystown wharf, a large number of specimens were observed attached to blue mussels fouling a non-active vessel, which were removed. PVC plates retrieved during October 2014 from an eelgrass meadow in Little Bay (Site B) exhibited high coverage (up to 80%) of *C. intestinalis.* Between 2013 and 2014, monthly seawater temperatures at locations where *C. intestinalis* was present ranged from -0.4 ºC (February) to 17.2 ºC (August).

Discussion

Survey and monitoring programs for non-indigenous species provide baseline information to assess change, which is necessary to deliver advice for environmental management decisions (Lehtiniemi et al. 2015). Non-indigenous tunicates have historically been reported throughout southern regions of Atlantic Canada (Carver et al. 2006 a; b) including population outbreaks at mussel aquaculture sites in PEI (*S. clava*) and Nova Scotia (*C. intestinalis*) since 1997 and 1998, respectively (Cayer et al. 1999; Clarke and Therriault 2007). Generally, DFO AIS monitoring programs between 2006 and 2009 observed trends of expanded ranges, heavier infestations, and increased tunicate biodiversity at stations in these regions of Atlantic Canada (Martin et al. 2011; Sephton et al. 2011). Although *B. schlosseri* was the only non-indigenous tunicate historically reported within NL (Placentia Bay, US Navy 1951; West coast, Hooper 1975), this study documented established populations of *B. schlosseri*, *B. violaceus*, and *C. intestinalis* concentrated primarily on the south coast and range expansions to northeast and southwest coasts of Newfoundland, respectively.

Botryllus schlosseri was the lone non-indigenous tunicate confirmed during initial surveys (2006) and only on the hull of a recreational boat in Argentia (Placentia Bay), which was the same location as historical reports of this species (US Navy 1951). Interestingly, *B. schlosseri* is now absent from historical west coast locations (Deibel et al. 2014). These results demonstrated a contrasting situation to the widespread and relatively long-term presence of non-indigenous tunicates in more southern regions of Atlantic Canada. However, the presence of *B. schlosseri* on only one recreational boat hull and its absence on collectors in Argentia the subsequent year suggested either a small population at this location or that *B. schlosseri* is present elsewhere in the area. In 2007, surveys confirmed *B. schlosseri* at four additional locations in northern regions of Placentia Bay, which corroborated a more widespread population and supports the suggestion that the original introduction of *B. schlosseri* may have occurred several decades ago (Deibel et al. 2014).

The lack of baseline information on the presence and distribution of non-indigenous tunicates in NL prompted surveys covering vast expanses of coastlines, which targeted public harbours and aquaculture sites. Monitoring only selected sites at this stage would have narrowed the comprehensive geographic distribution of the surveys and ineffectively targeted non-indigenous tunicates (Lehtiniemi et al. 2015). Although survey locations and strategies varied across years, we observed evidence of local and regional population expansion with all non-indigenous tunicate species in NL (*B. schlosseri*, *B. violaceus*, and *C. intestinalis*). Following most recent surveys (2014), *B. schlosseri* was observed at the most locations (24), and by 2013, new, discontinuous populations were detected in Conception Bay, Hermitage Bay, and the southwest coast. In Placentia Bay during 2012, *B. schlosseri* was found fouling natural substrates including eelgrass at numerous locations. Such findings have led to collaborations with a latitudinal study (New Jersey, USA to NL, Canada) examining distribution and diversity of invasive tunicates on eelgrass (Carman et al. 2016). These locations in Placentia Bay have little to no vessel traffic, which indicates that natural dispersal has occurred from presumable sites of introduction (e.g. public harbours) leading to relatively continuous populations within Placentia Bay. Planktonic tunicate larvae generally have limited dispersal potential (Svane and Havenhand 1993; Lambert and Lambert 1998; Shanks 2002), however colonies rafting on natural substrates (e.g. eelgrass) can travel long distances (Worcester 1994; Carman and Grunden 2010). While continuous populations within Placentia Bay likely resulted from combinations of natural dispersal and point to point anthropogenic transport, discontinuous populations elsewhere in NL (e.g. Conception Bay) are expected consequences of human-mediated movements.

Botrylloides violaceus remained restricted to Belleoram harbour, but coverage on collectors and various substrates (wharf, floating docks, and rocks) increased substantially between 2007 and 2010. Interestingly, this expansion also coincided with the enlargement of the fixed wharf in 2009 (Deibel et al. 2014). Such alterations create new, bare substrates, which allows occupation by non-indigenous species better adapted to compete for bare space (i.e. rapid reproduction and growth) than native biofouling organisms (Byers 2002; Altman and Whitlatch 2007; Tyrrell and Byers 2007). Early detection of such relatively isolated populations created opportunities for rapid response and experimental mitigation trials by DFO in 2008 and 2009, which used encapsulation methods to remove *B. violaceus* in Belleoram harbour (McKenzie et al. 2016). Early results were encouraging, but the use of plastic wrap to encapsulate structures also provided new exposed substrate, ideal for tunicate larvae and fragments to settle and grow unimpeded (Deibel et al. 2014).

Although tunicates generally select shaded areas to settle (Svane and Dolmer 1995), including introduced species in Atlantic Canada of interest to this study (e.g. *C. intestinalis* and *B. schlosseri*; Svane and Young 1989; Lambert 2005), some tunicate species are exceptions to this rule and prefer well-lit environments (Ruis et al. 2010). Subsequently, distribution of tunicates can be further influenced by post-settlement mortality (Young and Chia 1984). Other monitoring programs in eastern Canada indicated that undersides of floating docks are ideal for tunicate recruitment (LeGresley et al. 2008) as they provide shaded environments, constant contact with seawater, and escape from local benthic predators or competitors. Although populations of *C. intestinalis* were already widespread in other provinces of Atlantic Canada (Martin et al. 2011; Sephton et al. 2011), it was the most recent non-indigenous tunicate detected in coastal NL waters (2012) and the highest concentrations were on undersides of two floating docks in Little Bay harbour. Once more, early detection and the fact most *C. intestinalis* were isolated to floating docks facilitated an effective rapid response effort. This led to removal and cleaning of floating docks during mitigation trials and subsequent foundation for ongoing research on post-mitigation population dynamics of this species in Little Bay, including growth rates, development, and settlement patterns (Reid et al. unpublished data). While numbers of *C. intestinalis* in Little Bay have remained low since mitigation trials began in 2013, SCUBA surveys have described increases in numbers and distribution on a nearby public wharf in Burin, which did not undergo similar mitigation trials until May 2015 (McKenzie et al. 2016).

Newfoundland and Labrador represents the northern invasion front in the northwest Atlantic for non-indigenous tunicates as these species continue to expand geographically within Atlantic Canada (Martin et al. 2011; Sephton et al. 2011). Environmental parameters, including cold water temperatures, may limit further expansion, but following our initial surveys, related research documented constraints of water temperature on life cycle events for *B. schlosseri* in Placentia Bay and revealed relatively high survival of overwintering colonies (Ma 2012; Deibel et al. 2014). Therefore, although non-indigenous tunicates remain absent from most regions of NL, further range expansion is possible. Surveys are increasingly essential to document irregular range expansions symptomatic of organisms hitchhiking through human-mediated transport. For example, although non-indigenous tunicates have been predominantly detected on the south coast of NL, in 2011 and 2013, *B. schlosseri* was detected >100 km from previous recorded locations attached to public wharves on the northeast (Foxtrap, Conception Bay) and southwest (Codroy) coasts of NL, respectively. Furthermore, in 2013, *B. violaceus* was also detected in Codroy, 100s of km west of the only previous sighting in NL. Such discontinuous range expansions have led to the development of ongoing research on the population dynamics of non-indigenous tunicates across a ~270 km latitudinal gradient on the west coast of NL.

In Atlantic Canada, NL has a relatively high number of small craft harbours encircling the island (Therriault and Herborg 2008), which represent high risks for introduction and spread of biofouling communities (Clarke Murray et al. 2011). Although *B. schlosseri* is customarily perceived as a nuisance species, *C. intestinalis* has caused havoc within the aquaculture industry in PEI (Cayer et al. 1999). *C. intestinalis* is currently relatively isolated within the area of Mortier Bay, but this area experiences relatively high vessel traffic to all regions of NL which increases the risk of human-mediated transport throughout NL. Risk assessment models have indicated that favorable environmental conditions exist along the southwest and south coasts of NL and are at risk for establishment and spread of *C. intestinalis* and other non-indigenous tunicates currently in Atlantic Canada (Therriault and Herborg 2008). The presence of *B. schlosseri* on the northeast coast of NL, in combination with its high overwintering survival and short generation times indicates further spread by non-indigenous tunicates to more northern regions of NL may occur (Deibel et al. 2014). Seawater temperatures observed across survey locations were well within the ranges of growth and reproduction for all non-indigenous tunicates detected in NL (Carver et al. 2006a; Therriault and Herborg 2008). Therefore, the threat of non-indigenous tunicates to nearby shellfish aquaculture operations, particularly within Placentia Bay, is of concern.

Survey and monitoring programs have discovered non-indigenous tunicates new to Atlantic Canada, including the high impact, invasive species, *D. vexillum* within the Bay of Fundy in 2013 (Moore et al. 2014). Vessel traffic between NL and the rest of Atlantic Canada fosters apprehension for further introductions and northward expansion of non-indigenous tunicates. *D. vexillum* can grow in seawater temperatures between -2 and 24 ºC (Lambert 2009), and therefore is another threat to NL waters. Although not all coastal regions of NL may be suitable for large, invasive populations of all tunicates species (Therriault and Herborg 2008), changes in climate may make insular NL waters more susceptible to invasion. Through early detection or range expansions, the NL DFO AIS survey and monitoring program has been the first step in an aquatic invasive species response plan for prevention, early detection, rapid response, mitigation trials, and research on the ecology of these species at their current northwest Atlantic range limit (McKenzie et al. 2016). Results from past projects have continued to promote research on the vulnerability of early-life stages to treatments (e.g. ultrasound generation, suspended sediments, and bubble streams) to prevent biofouling and minimize risks of introduction (Lowen et al. 2016). Survey and monitoring programs not only provide baseline data and early detection, but further encourage diversification and expansion of research, which ultimately expands the scope of available strategies and science advice for prevention and management.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. List of locations surveyed for non-indigenous tunicates between 2006 and 2014.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2016/Supplements/MBI_2016_McKenzie_etal_1_Supplement.xls