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Research Article

Early detection of the non-indigenous colonial ascidian *Diplosoma listerianum* in eastern Canada and its implications for monitoring

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Abstract

In eastern Canada, the initial suspected discovery of the non-indigenous colonial ascidian *Diplosoma listerianum* in Quebec was followed by species identification and increased awareness for its detection in the neighbouring provinces of Nova Scotia and Prince Edward Island. A phylogenetic analysis was conducted based on sequences generated from the 18S ribosomal DNA gene isolated from tissues of two Canadian samples (Quebec and Nova Scotia) and samples from several species belonging to the *Diplosoma* genus, which confirmed the species identity of the eastern Canadian specimens. Colonies of *D. listerianum* found in Quebec (2008) and Nova Scotia (2012) are currently the only tissue records of this species in eastern Canada. Water samples of concentrated plankton collected in Quebec (2010 and 2011) and in Prince Edward Island (2011) tested PCR positive for *D. listerianum* DNA, but colonies were not observed concurrently. As a case study for early detection, this paper documents how *D. listerianum* was detected in eastern Canada by different groups (government monitoring, academic research) and with different methods (dive surveys, genetic analysis of water samples, collector plates). This case study suggests that the co-operation between groups and the breadth of the methods used contributed to the early detection of this species in three eastern Canadian provinces. In particular, we show that (i) partnerships between government, academia, and industry, (ii) a national capacity for species-specific molecular detection tools, and (iii) the recognition that the effectiveness and limitations of different monitoring methods may be site-specific can have positive early detection and monitoring outcomes.

Key words: early detection, monitoring, ascidians, *Diplosoma listerianum*, eastern Canada, invasive species

Introduction

The introduction and spread of aquatic invasive species (AIS), such as some ascidian species (subphylum Tunicata, class Ascidiacea), is a global ecological and economic concern (Lambert 2007; Zhan et al. 2015). Ecologically, the introduction of invasive ascidians can alter the biodiversity of benthic communities by competing with indigenous species for space and food, through predation, and by carrying exotic diseases that harm indigenous species (Harms and Anger 1983; Blum et al. 2007; Dijkstra et al. 2007; Lutz-Collins et al. 2009). Economically, invasive ascidians can damage the real and/or perceived value of marine resources and elevate costs associated with processing aquaculture products (Davis and Davis 2009, 2010), for example, by fouling bivalve aquaculture products and equipment (Lutz-Collins et al. 2009; Rocha et al. 2009).

Non-indigenous ascidians may be introduced to non-infested areas by fouled ship and boat hulls and aquaculture equipment (Ramsay et al. 2008; Davis and Davis 2009) but may only survive and reproduce if biotic and environmental conditions are suitable. This is of concern given that invasive ascidians are generally associated with broad environmental tolerance, rapid growth and sexual maturity, and high fecundity (Lambert 2002). Following establishment,

regional spread to non-infested areas may occur via recreational boats (Clarke Murray et al. 2011; Lacoursière-Roussel et al. 2012) and commercial activities (Coutts et al. 2003; Coutts and Taylor 2004; Davidson et al. 2009; Frey et al. 2014). In Canada, public awareness, monitoring, and rapid response programmes have been established to detect invasive ascidians and other AIS incursions at early stages of the invasion process to minimise their secondary spread.

In the last decade, populations of four invasive ascidian species have established and spread widely in eastern Canada: *Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri* (Pallas, 1766), *Ciona intestinalis* (Linnaeus, 1767), and *Styela clava* Herdman, 1881 (Thompson and McNair 2004; Ramsay et al. 2008, 2009; Locke et al. 2009; Sephton et al. 2011; Simard et al. 2013; Deibel et al. 2014). Using a screening procedure based on shipping and climate zone filters, Locke (2009) identified a "watch list" of 17 ascidian species considered to be most likely to successfully invade eastern Canada. *Ascidiella aspersa* (Müller, 1776), *Didemnum vexillum* Kott, 2002, and *Diplosoma listerianum* (Milne-Edwards, 1841) were included on this list prior to their discovery in eastern Canada (Simard et al. 2013; Moore et al. 2014). Of these species, this paper focuses on the early detection of *D. listerianum* in eastern Canada.

The colonial ascidian *D. listerianum* (order Enterogona, family Didemnidae) is a sessile marine filter-feeder that has a semi-cosmopolitan distribution (Goodbody 2003; Marins et al. 2010; Mackenzie 2011; Pérez-Portela et al. 2013). In North America, this species is considered cryptogenic on the west, southeast (south of North Carolina), and Gulf of Mexico coasts (Rocha and Kremer 2005; Carman et al. 2011). *D. listerianum* was first observed in the northeast United States in 1993 and is considered non-indigenous from Maine to Massachusetts (Harris and Tyrrell 2001; Agius 2007; Dijkstra et al. 2007; Carman and Grunden 2010). There have been no historic observations of *D. listerianum* in any records from eastern Canada (see review in Ma [2012]) and thus it is considered non-indigenous in eastern Canada. *D. listerianum* colonies are thin, translucent, and fleshy with hermaphroditic zooids (2 mm in length) arranged around multiple common cloacae. Larvae are brooded and released into the water column upon maturity. The larva is non-feeding with a relatively short life-span of a few hours before settlement and metamorphosis (Lane 1973; Mackenzie 2011). Colonies of *D. listerianum* exhibit reduced surface area and mortality under experimental low salinity conditions of 20 and 27 psu but not under ambient conditions of 34 psu (Gröner et al. 2011) and colonies disappear when early March seawater temperatures are below ca. 2 °C (Osman and Whitlatch 2007).

D. listerianum is an AIS that has been targeted for monitoring in eastern Canada, which involves partnerships among government, academia, and industry. Although the first records of *D. listerianum* in the provinces of Quebec and Nova Scotia have been previously reported by Simard et al. (2013) and Moore et al. (2014), respectively, the objective of this paper is to synthesise the available information leading to its initial detection and monitoring efforts following its discovery in eastern Canada into one report, including the first report of this species in the province of Prince Edward Island (PEI). As a case study, this paper provides insights into the early detection of an AIS and to document the known geographic distribution during the early stages of its invasion of eastern Canada. Also, this paper describes the methods used to identify the species of the specimens, and a phylogenetic analysis to further confirm species identity of the Canadian specimens.

Materials and methods

Detection via monitoring programme

The federal Department of Fisheries and Oceans (DFO) implemented an AIS monitoring programme in eastern Canada in 2006. The programme's objectives are (a) to detect new AIS as early as possible, to document their spread, and to identify potential vectors of primary and secondary spread, and (b) to minimise the secondary spread of these species through awareness and rapid response. Between the years 2006 and 2015, the programme monitored approximately 350 sites in the Gulf of St. Lawrence, insular Newfoundland, and the Maritime Provinces (Martin et al. 2011; Sephton et al. 2011; Simard et al. 2013; McKenzie et al. 2016); however, only a subset of these sites was monitored annually. Monitoring efforts at high-risk AIS introduction sites, such as marinas, commercial ports, fishing ports, and aquaculture sites, are targeted by DFO with the co-operation of industry partners and provinces. Region-specific methodologies are outlined below.

Quebec

The primary AIS monitoring tools used by DFO in Quebec were: (i) employing divers to conduct underwater surveys (hereafter referred to as dive surveys), (ii) sampling for plankton in the water column for genetic analysis (water samples), and (iii) deploying settlement plates (collector plates).

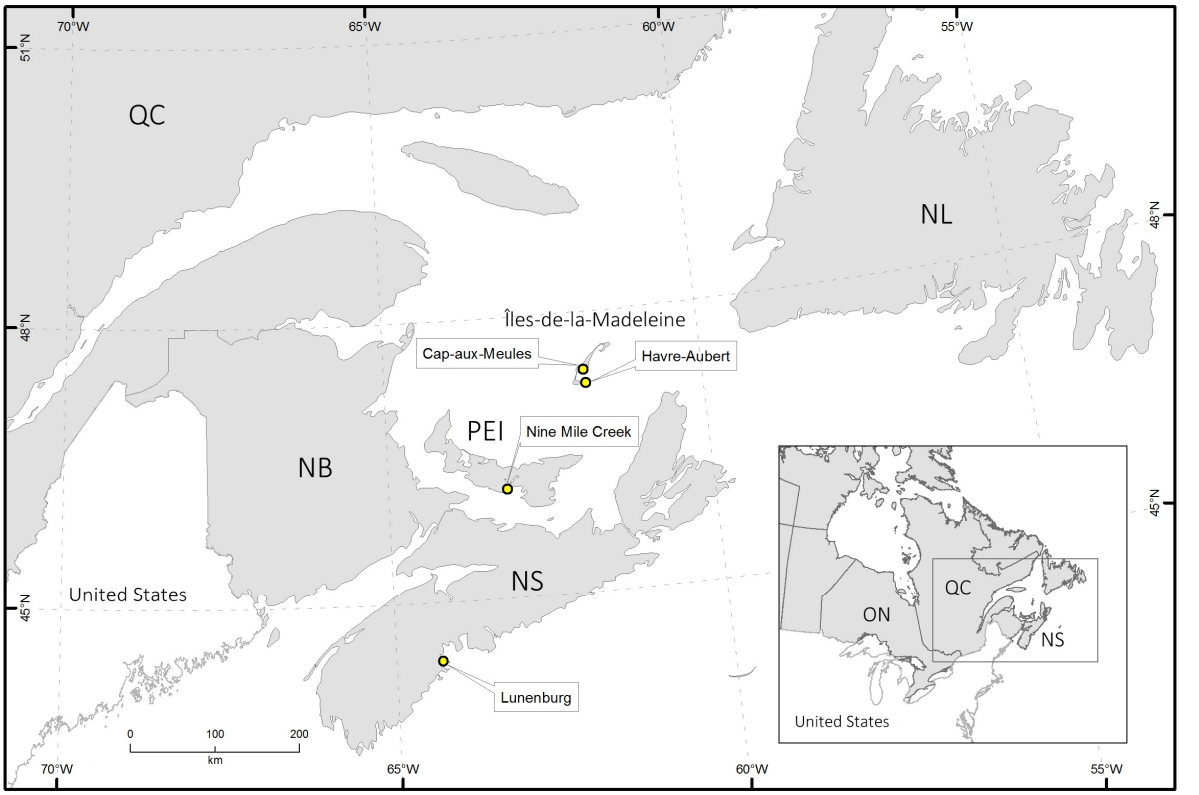

Figure 1. Map of eastern Canada and the currently known distribution of *Diplosoma listerianum* based on collection of colonies (Simard et al. 2013; Moore et al. 2014) and analysis of water samples for *D. listerianum* DNA (present study). NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; ON = Ontario; PEI = Prince Edward Island; QC = Quebec.

As part of the programme, a total of 39 sites in Quebec were monitored between 2006 and 2015 (Simard et al. 2013). Dive surveys were conducted at marinas, fishing docks, and aquaculture sites (Simard et al. 2013). At each site (depth < 3 m) that was monitored, two divers searched for AIS present on the sea bottom and artificial structures (e.g., floating and permanent docks, aquaculture socks, ropes, and buoys) for approximately two hours. All AIS were noted, photographed, and geographically referenced. Specimens of unknown species were collected, preserved in ethanol, and transported to the laboratory for identification.

In Îles-de-la-Madeleine, Quebec, water samples were collected at three sites (10s of metres apart) in a marina in Havre-Aubert and three sites—one site in a marina, one in a fishing port, and one in a commercial port—in Cap-aux-Meules (Figure 1) in August (2010–2014) and October (2010–2012). At each site, a water sample was collected by pumping 150 L of seawater through a 64-micron mesh. The inflow hose connected to the pump was lowered through the water column to ensure that samples contained water from various depths between 1–3 m below the water surface. For each sample, concentrated plankton accumulated on the mesh was then transferred into DNA-free tubes. The samples were kept on ice during the transport from the field to the laboratory, and then stored at −80 °C until they were analysed. A PCR-based assay targeting *D. listerianum* was used to analyse the water samples (Willis et al. 2011).

Collector plates were arranged in a sampling device consisting of three Petri dish and three roughened PVC plates (10 cm × 10 cm; see Simard et al. 2013 for methodology). For each of the years between 2007 and 2015, collector plates were either anchored or attached to existing structures at sites deemed to be of high-risk for AIS introductions, including Havre-Aubert and Cap-aux-Meules, for about five months from June to October.

In addition to the AIS monitoring programme, a separate DFO study on biodiversity consisted of dive surveys to characterise benthic fouling communities on dock pilings at targeted ports in Îles-de-la-Madeleine (N. Simard, unpublished data). Samples

of fouling benthic species were preserved in a formaldehyde solution and transported to the laboratory for identification.

Prince Edward Island

As part of DFO's (Gulf Region) monitoring programme in PEI, AIS were monitored at 39 sites between 2006 and 2015. Nine Mile Creek, PEI, is an area of concern for the presence of AIS since it is a commercially important mussel-producing site on the southern shore of the island. For this reason, DFO received requests from the internal Introductions and Transfers sub-committee to conduct rapid assessment surveys within this area. Rapid assessments were conducted in Nine Mile Creek in 2009 and yearly from 2011 to 2015. Crop, seed, and anchor lines of mussel leases were inspected visually by divers and by lifting mussel socks onto boats. Floating docks and permanent structures were examined by filming underwater with a video camera and analysing the video footage. Water samples were collected from four sites in Nine Mile Creek on October 19, 2011, and from five sites on August 22, 2012, using the same methods as those used in Quebec.

Nova Scotia

Approximately 120 sites were monitored by DFO (Maritimes and Gulf Regions) for AIS in Nova Scotia from 2006 to 2015 (D. Sephton and R. Bernier, personal communication). On November 1, 2012, the programme collected three water samples at the same location where colonies of *D. listerianum* were discovered by Moore et al. (2014) in Lunenburg, Nova Scotia (Figure 1). Each water sample was collected by pumping 300 L of seawater. The inflow hose of the pump was slowly lowered to just above the seafloor (4.6 m below the water surface) and then returned to the surface. The methods of storing and analysing the water samples were the same as those collected in Quebec and PEI.

Genetic identification of Canadian samples

PCR positive tissue samples of different species belonging to the *Diplosoma* genus were analysed by amplifying and sequencing a 522 bp section of the 18S ribosomal DNA (rDNA) gene in both directions using the universal CASIS CAS2 primer set developed by Le Roux et al. (1999). To ensure that the species identity of Canadian samples (GenBank accession number: HM641906.1) was correct, samples of several species belonging to the *Diplosoma* genus were also sequenced in the 18S rDNA region. Samples of *Diplosoma aggregatum* Hirose and Hirose, 2009 (HM641903.1), *D. listerianum* (HM641905.1), *Diplosoma gumavirens* Hirose E., Oka and Hirose M., 2009 (HM641900.1), *Diplosoma ooru* Hirose and Suetsugu, 2005 (HM641902.1), *Diplosoma variostigmatum* Hirose and Oka, 2008 (HM641901.1), and *Diplosoma watanabei* Hirose E., Oka and Hirose M., 2009 (HM641904.1) were obtained from Ishigaki Island, Okinawa Prefecture, Japan, in 2007–2008, and identified by E. Hirose. The identities of generated sequences were confirmed by BLAST analysis of the GenBank database (Altschul et al. 1997). Multiple sequence alignments were done using the Clustal W application in MEGA (Larkin et al. 2007; Tamura et al. 2007) with these Canadian and Japanese samples and with the 18S rDNA sequences of *D. ooru* (AB211100.1), *Diplosoma simile* (Sluiter, 1909) (AB211104.1), *Diplosoma simileguwa* Oka, Suetsugu and Hirose, 2005 (AB211108.1), *Diplosoma* sp. (AB211120.1), and *Diplosoma virens* (Hartmeyer, 1909) (AB211114.1). Neighbour-joining (NJ) Kimura 2-parameter model with gaps and missing data handled by complete deletion and maximum parsimony (MP) phylogenetic trees were constructed using the 18S rDNA sequences with MEGA version 4.1 (Tamura et al. 2007; Kumar et al. 2008). The validity of constructed NJ and MP tree topologies was evaluated by using the bootstrap (re-sampled 1000 times) test of phylogeny (Felsenstein 1985). *Echinorhinus cookei* Pietschmann, 1928 (M91181.1) was used as an outgroup species for the 18S rDNA tree.

Results

Colonies of Diplosoma listerianum

D. listerianum colonies were detected by a diver in the Havre-Aubert marina (47.2360ºN, 61.8343ºW), Îles-de-la-Madeleine, Quebec, on October 23, 2008 (Simard et al. 2013). Based on morphology, the identification of these specimens was confirmed by G. Lambert (Marine Biological Consultants, United States) and M. R. Carmen (Woods Hole Oceanographic Institute, United States). Specimens were adult colonies growing on mussels (*Mytilus* sp.) on marina docks at depths between 1 and 2 m. A single *D. listerianum* colony was also collected during the biodiversity study in Havre-Aubert on September 11, 2008. This specimen was initially misidentified as *Aplidium constellatum* (Verrill, 1871) and then correctly identified as *D. listerianum* by G. Lambert on October 5, 2009. No colonies have been observed in subsequent dive surveys (2009–2015) in Îles-de-la-Madeleine. Additionally, colonies of *D. listerianum* were not observed on any of the collector plates deployed as part of DFO's AIS monitoring programme (2006–

Table 1. Known records of *Diplosoma listerianum* in eastern Canada.

Date	Location	Method	Reference
September 11, 2008	Havre-Aubert, Quebec	Underwater survey via SCUBA	N. Simard, unpublished data
October 23, 2008	Havre-Aubert, Quebec	Underwater survey via SCUBA	Simard et al. 2013
October 13, 2010	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
August 23, 2011	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
August 24, 2011	Cap-aux-Meules, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 12, 2011	Cap-aux-Meules, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 13, 2011	Havre-Aubert, Quebec	PCR-based assay on water sample	Present study; see Table 2
October 19, 2011	Nine Mile Creek, Prince Edward Island	PCR-based assay on water sample	Present study; see Table 2
October 2, 2012	Lunenburg, Nova Scotia	Specimen on collector plate	Moore et al. 2014

2015) in Îles-de-la-Madeleine (Simard et al. 2013; N. Simard, unpublished data).

To date, no colonies of *D. listerianum* have been observed in PEI through DFO's extensive monitoring efforts (R. Bernier, unpublished data). However, juvenile colonies (maximum age: four weeks) of *D. listerianum* were found on experimental collector plates hanging from a floating dock in Lunenburg, Nova Scotia, on October 2, 2012 (Moore et al. 2014). Identification was confirmed by G. Lambert (via digital photographs) and later by S. E. Stewart-Clark using the PCR-based assay developed by Willis et al. (2011). Following the detection of *D. listerianum*, an underwater rapid assessment was performed in Lunenburg but no specimens were found on natural nor on artificial structures in the harbour (Vercaemer et al. 2012). In addition, no colonies of *D. listerianum* were observed on any of DFO's collector plates on nearby docks in Lunenburg in the same year. All currently known records of *D. listerianum* in eastern Canada are tabulated in Table 1 (see supplementary Table S1 for more information).

Water samples

Water samples from Havre-Aubert in 2010 and 2011, Cap-aux-Meules in 2011, and Nine Mile Creek in 2011, tested positive for *D. listerianum* DNA (Tables 1 and 2). Between 2012 and 2014, water samples from all locations (Îles-de-la-Madeleine [2012–2014], Nine Mile Creek [2012], and Lunenburg [2012]) tested negative for *D. listerianum* (Tables 2 and S1).

Phylogenetic analysis

18S rDNA sequences generated from Quebec and Nova Scotia samples had 100% sequence similarity but had a consecutive 41 base deletion in the sequence compared to the sequence from the *D. listerianum* positive control sample from Japan. Phylogenetic analysis shows that the sequences generated from the Quebec and Nova Scotia locations group together with the Japanese *D. listerianum* sample in both NJ and MP phylogenetic trees (Figure 2), as confirmed using the bootstrap test, which also confirmed the species identity of the two Canadian samples as *D. listerianum*. The sequences generated from the three *D. listerianum* samples group together and separate from all other *Diplosoma* species analysed in this study. This reveals that the 18S rDNA gene is a good species-specific marker for differentiating *D. listerianum* from other species classified under the *Diplosoma* genus.

Discussion

Invasion history and implications of Diplosoma listerianum

The presence of *D. listerianum* colonies in Quebec (Mackenzie 2011; Simard et al. 2013) and Nova Scotia (Moore et al. 2014) and *D. listerianum* DNA in PEI (present study) represent the first records of this species in each of their respective provinces in eastern Canada as well as a continuing trend of biological invasions by non-indigenous ascidians. Despite extensive monitoring efforts (primarily analyses of water samples) following its discovery in each of the three provinces, there are no known established populations of *D. listerianum* in eastern Canada. However, given the number and breadth of occurrences, these sporadic observations (in space and time), as reported in this paper, suggest widespread propagule pressure at early stages of the invasion process throughout eastern Canada. The vectors and the pathways of the *D. listerianum* detected in Quebec, PEI, and Nova Scotia are unknown. Fouling of ship hulls and niche areas, aquaculture equipment, and aquaculture products are suspected vectors for the introduction of non-indigenous ascidians (Dijkstra

Table 2. Summary of the detection of *Diplosoma listerianum* using a PCR-based assay on water samples collected in Quebec, Prince Edward Island, and Nova Scotia, from 2010 to 2014 (summer = July and August; autumn = September, October, and November).

Location	Sampling description	Autumn of 2010	Summer of 2011	Autumn of 2011	Summer of 2012	Autumn of 2012	Summer of 2013	Autumn of 2013	Summer of 2014
Havre-Aubert, Quebec	Three sites within a marina were sampled	One of three samples tested positive	One of three samples tested positive	Two of three samples tested positive	All three samples tested negative	All three samples tested negative	All three samples tested negative	No samples collected	All three samples tested negative
Cap-aux-Meules, Quebec	A marina, a fishing dock, and a commercial dock were sampled	All three samples tested negative	Of the three samples, only the sample from the fishing dock tested positive	Of the three samples, only the sample from the commercial dock tested positive	All three samples tested negative	All three samples tested negative	All three samples tested negative	No samples collected	All three samples tested negative
Nine Mile Creek, Prince Edward Island	Several sites within a mussel lease and one site at a wharf were sampled	No samples collected	No samples collected	All samples (three from the mussel lease and one from the wharf) tested positive	All samples (four from the mussel lease and one from the wharf) tested negative	No samples collected	No samples collected	No samples collected	No samples collected
Lunenburg, Nova Scotia	Three sites within the port were sampled	No samples collected	No samples collected	No samples collected	No samples collected	All three samples tested negative	No samples collected	No samples collected	No samples collected

et al. 2007). Shipping was identified as the potential vector for the transport of *D. listerianum* to the north-eastern United States, where it was first detected in Cape Cod, Massachusetts, in 1993 (Dijkstra et al. 2007).

Investigations of the intra-specific diversity and structure of genetic markers could reconstruct the invasion history of an AIS and identify the putative source population of an introduced population (Wares et al 2005). In fact, a global analysis of sequences of the mitochondrial gene cytochrome *c* oxidase subunit I suggests that *D. listerianum* is a species complex that consists of four monophyletic clades (Pérez-Portela et al. 2013). Unfortunately, the 18S rDNA sequences from this study cannot be compared with the mitochondrial sequences analysed by Pérez-Portela et al. (2013) to assess which clades are invading eastern Canada.

Invasive ascidians are associated with negative ecological and economic impacts, such as outcompeting other ascidian species for space (Schmidt and Warner 1986; Altman and Whitlatch 2007; Vance et al. 2009), fouling benthic communities, including eelgrass (Blum et al. 2007; Carman and Grunden 2010), and fouling bivalve aquaculture sites (Gittenberger 2009; Lutz-Collins et al. 2009; Rocha et al. 2009). *D. listerianum* has had notable ecological impacts in both the Gulf of Maine (United States) and the United Kingdom. In the Gulf of Maine, colonies of *D. listerianum* appeared for a short time on experimental collector plates made of Plexiglas and at low abundances relative to other non-indigenous colonial ascidian species (Dijkstra et al. 2007) but reached a maximum cover of 64% determined from SCUBA dive surveys of rocky sub-tidal sites in November of 1995 (Harris and Tyrrell 2001). Additionally, this species was more abundant on sub-tidal rocks in deeper waters (e.g., 20% cover at 20 m depth; Harris and Tyrrell 2001; Dijkstra et al. 2007). In the United Kingdom, Vance et al. (2009) documented that *D. listerianum* exhibited rapid growth and became dominant in a fouling community, quickly occupying up to about 50% of PVC recruitment panels and encrusting many species in the process. Although this species has not been found at any Canadian aquaculture sites, colonies of *D. listerianum* were previously reported on mussel lines at contaminated aquaculture sites in The Netherlands (Gittenberger 2009) and in Brazil (Rocha et al. 2009). Hence, if populations of *D. listerianum* do establish in eastern Canada, this could have ecological and economic consequences.

The results of the present study show that the application of different monitoring methods strengthened the ability to detect *D. listerianum* early in the invasion process in eastern Canada. As of 2015, dive surveys found *D. listerianum* colonies in Quebec, PCR-based assays detected DNA of this species in Quebec and PEI, and collector plates detected colonies in Nova Scotia. This shows that not all methods were always successful at every site, which suggests that the successful application of a particular method may be site-specific even for the same species. Therefore, it is important for future AIS

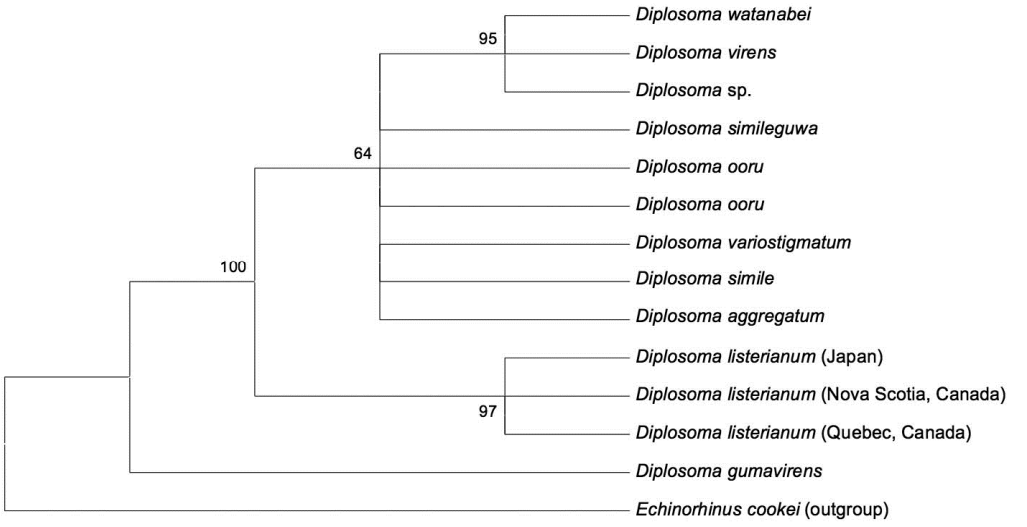

Figure 2. Condensed phylogenetic tree (50% bootstrap cut-off value) constructed from 18S rDNA sequences of *Diplosoma* species using neighbour-joining method with *Echinorhinus cookei* as an outgroup.

monitoring to have different monitoring tools available and, perhaps, to use a combination of different methods (despite being costly and time consuming) for a given site while recognising that the effectiveness and limitations of monitoring methods may be site-specific. Future research can further explore why the success of certain monitoring methods might be site-specific for the same species and how to optimise available methods to match local environmental conditions (e.g., temperature and salinity), hydrological patterns (e.g., wave action), and type of site (marina, port, aquaculture site, etc.) while reducing costs and detecting invasive species as early as possible.

Patterns associated with the early detection of invasive ascidians

In the broader context of biological invasions of eastern Canada by seven ascidian species (*A. aspersa*, *B. schlosseri*, *B. violaceus*, *C. intestinalis*, *D. listerianum*, *D. vexillum*, and *S. clava*), we reviewed the literature and summarised the first records for each species by province in Table 3 to better understand the circumstances that led to their initial discoveries. Presently, there are six non-indigenous ascidians that have been detected in Nova Scotia, five in PEI, five in Quebec, three in Newfoundland, and two in New Brunswick (total of 21 cases; Table 3). A majority of these first records were detected in the autumn (47.6%) and fewer in the summer (23.8%). The remainder of the records (28.6%) did not mention the month or season of initial discovery. Most of these first records (42.9%) were detected through government monitoring programmes, 23.8% by industry stakeholders, 19.0% by research activities, 4.8% by a member of the public, and 9.5% by unknown or uncategorised groups. In regards to the method of discovery, 28.6% were detected by anecdotal observations of specimens (mostly on hard substrates and aquaculture products), 28.6% via SCUBA, 23.8% via collector plates, 9.5% by molecular probes, and 9.5% by unknown methods.

The above summary suggests that early detection and monitoring of non-indigenous ascidians can benefit from different methods and from the involvement of different groups and stakeholders. However, this benefit for early detection should not be interpreted as an optimal approach for detecting and monitoring of non-indigenous ascidians. However, there are a couple patterns that may offer insights into the detection of non-indigenous ascidians in eastern Canada. Firstly, Table 3 indicates that detection occurs mostly in autumn (between September and October), perhaps corresponding to the maturity (i.e., larger size) of individuals that were introduced earlier in the year or to greater reproductive activity (i.e., greater density of larvae and eDNA in the water column) that may be detected by means of molecular probes. Secondly, DFO's monitoring programme has the advantage over local interest groups (e.g., recreational divers, aquaculturists, etc.) to detect AIS because the programme, in its current form, has a larger geographic coverage (national versus local

Table 3. First records of non-indigenous ascidian species by province in eastern Canada. NA = information not available.

Species	Date	Province[1]	Method of initial discovery	Group	Reference
Ascidiella aspersa	July 5, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)
Botrylloides violaceus	2001	NS	Anecdotal observation	Industry[2]	Carver et al. (2006)
	2002	PEI	Underwater survey via SCUBA	Government	Locke et al. (2009)
	September 6, 2006	NB	Anecdotal observation; specimen on a buoy	Government	Bernier et al. (2009), Locke et al. (2009)
	October 2007	NL	Underwater survey via SCUBA	Academia	Callahan et al. (2010)
	October 10, 2010	QC	Underwater survey via SCUBA	Government	Simard et al. (2013)
Botryllus schlosseri	Before the 1980s	NB	NA	NA	Brinkhurst et al. (1975), Linkletter et al. (1977)
	Before the 1980s	NS	NA	NA	Carver et al. (2006)
	1945	NL	Specimen on collector plate	Government	US Navy (1951)
	Summer of 2001	PEI	Anecdotal observation	Industry	Locke et al. (2009)
	July 2006	QC	Anecdotal observation; specimen on dock structures	Industry[3]	Simard et al. (2013)
Ciona intestinalis[4]	October 2004	PEI	Anecdotal observation	Industry	Locke et al. (2009)
	August 3, 2006	QC	Specimen on collector plate	Government	Simard et al. (2013)
	September 19, 2012	NL	Underwater survey via SCUBA	Government	Sargent et al. (2013)
Didemnum vexillum	October 2013	NS	Underwater survey via SCUBA	Public	Moore et al. (2014)
Diplosoma listerianum	September 11, 2008	QC	Underwater survey via SCUBA	Government	Mackenzie (2011), Simard et al. (2013), N. Simard, unpublished data
	October 19, 2011	PEI	PCR-based assay on water sample	Government	Present study
	October 2, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)
Styela clava	1997	PEI	Anecdotal observation; specimen on aquaculture products	Industry	Locke et al. (2009)
	August 24, 2011	QC	PCR-based assay on water sample	Government	N. Simard, unpublished data
	October 2, 2012	NS	Specimen on collector plate	Academia	Moore et al. (2014)

[1] NB = New Brunswick; NL = Newfoundland and Labrador; NS = Nova Scotia; PEI = Prince Edward Island; QC = Quebec

[2] Claire Carver is an industry stakeholder

[3] Species was detected by Merinov

[4] Molecular and morphological evidence (Brunetti et al. [2015]) suggest that *Ciona intestinalis* is two distinct species: *Ciona robusta* (formerly *Ciona intestinalis* type A) and *Ciona intestinalis* (formerly *Ciona intestinalis* type B); global molecular analysis (Bouchemousse et al. [2016]) indicates that *Ciona intestinalis* is distributed in eastern Canada; in the present paper, we considered *Ciona intestinalis* as a cryptogenic species in eastern Canada but a non-indigenous species in Îles-de-la-Madeleine, insular Newfoundland, and Prince Edward Island based on the absence of historical records of any *Ciona* sp. on these islands

interests) and over research activities because it tends to have a larger temporal coverage (2006 to present versus the typical life-span of a research project). Greatly expanding the role of the public and industry stakeholders to report AIS has the potential advantage of larger geographic and temporal coverage matching or exceeding the scale of DFO's monitoring programme.

Conclusion

Early detection and monitoring for AIS along an extensive and complex coastline, as is the case in eastern Canada, is costly and time consuming. The continued use of molecular tools in monitoring activities and rapid assessments may assist in the early detection of invasive ascidian species (Stewart-Clark et al. 2009, 2013; Willis et al. 2011). Species-specific molecular probes, as used in this study, are thus critical to the identification of an AIS by means of water samples. Hence, a national capacity for the development of species-specific (e.g., PCR-based assays) and non-targeted (e.g., DNA [meta] barcoding) molecular detection tools is advocated. In recent years, there are several examples of citizen reporting and collaborations among government, academia, and industry that resulted in the early detection of non-indigenous ascidian species (Sargent et al. 2013; Deibel et al. 2014; Moore et al. 2014). Additionally, the conscientious reporting of unknown organisms and fouled barges by industry stakeholders to the government contributed to the initial discoveries of several non-indigenous ascidians in PEI (Locke et al. 2009). Therefore, we advocate for the continued public AIS awareness and partnership among government, academia, and industry to co-ordinate monitoring efforts and allocate resources efficiently.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Location of the studied sites (records of *Diplosoma listerianum*).

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2016/Supplements/MBI_2016_Ma_etal_Supplement.xls