

## Research Article

## Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013

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### Abstract

Research and monitoring efforts led to the discovery of four new non-indigenous tunicates and one new non-indigenous anemone in Nova Scotia in 2012 and 2013. In this report, we summarize and interpret the circumstances leading up to and actions taken following the first Nova Scotian records of the high impact invasive tunicate *Didemnum vexillum* Kott, 2002 as well as the tunicates *Styela clava* Herdman, 1881, *Ascidrella aspersa* (Müller, 1776), and *Diplosoma listerianum* (Milne-Edwards, 1841) and the anemone *Diadumene lineata* (Verrill, 1869). While dockside research and monitoring were responsible for detections of four species, the possibility that *Didemnum vexillum* was present was first reported by a member of the public, through Fisheries and Oceans Canada's Aquatic Invasive Species Monitoring program.

**Key words:** *Styela clava*, *Ascidrella aspersa*, *Diplosoma listerianum*, *Diadumene lineata*, invasive species, Canada, monitoring

### Introduction

Introductions and range expansions of non-indigenous marine invertebrates, especially tunicates, have caused substantial ecological impacts to fouling communities and economic impacts on bivalve aquaculture in the northeastern United States and Atlantic Canada (Blum et al. 2007; Dijkstra et al. 2007; Carman et al. 2010; Thompson and MacNair 2004; Lutz-Collins et al. 2009; McKindsey et al. 2007). Hull fouling is often considered the likeliest vector for the introduction of many non-native fouling species (Coutts et al. 2003), and Nova Scotia is particularly susceptible. Halifax, Nova Scotia was the top shipping arrival port in Atlantic Canada in 2000 and 2002 with 1215 (of 2061) and 836 (of 1225) international arrivals, respectively. Another 12 Nova Scotia ports received at least 3 visits in either of those years, including Port Hawkesbury with 80 in 2000 (Kelly 2004). Halifax and many other locations around the province, such as Lunenburg, also have high

recreational boating traffic in the summer months. In addition, southern Nova Scotia and the Bay of Fundy are adjacent to and influenced by the prevailing oceanographic currents from the United States. They would, therefore, receive the greatest propagule pressure from established populations of non-indigenous species in eastern waters of the United States undergoing northward range expansions by natural processes.

In 2006, Fisheries and Oceans Canada (DFO) initiated a monitoring program to study the spread of aquatic invasive species (AIS) in Atlantic Canada and to detect and help mitigate new invasions. In Nova Scotia, a key aspect of the AIS Monitoring program targeted fishing harbours, marinas, and aquaculture leases for deployment of collector arrays to determine the presence of two invasive tunicate species of concern: *Didemnum vexillum* Kott, 2002 and *Styela clava* Herdman, 1881 and the extent of three other species already present in the province: *Ciona intestinalis* (Linnaeus, 1767), *Botryllus schlosseri* (Pallas, 1766), and *Botrylloides violaceus* Oka,

1927 (see Sephton et al. 2011 for details). In 2009, Locke published a “watch list” of 17 invasive tunicates likely to successfully invade Atlantic Canada (hereafter referred to as the Atlantic Canada watch list) but, until 2012, there were no detections of new non-indigenous tunicates in Nova Scotia.

## Methods

In 2012, several new research projects aimed at monitoring the effect of AIS on the structure and function of native communities increased the overall monitoring effort in Nova Scotia. During 2012 and 2013, there were two AIS monitoring efforts in Nova Scotia, summarized here as AIS Monitoring and AIS Research.

### *AIS Monitoring*

The DFO Maritimes Region AIS Monitoring program in Nova Scotia and southern New Brunswick deployed collector arrays in marinas, public wharves, and near aquaculture leases across Nova Scotia (Sephton et al. 2011). Annually since 2006 in late May/early June, four collector arrays, each consisting of three downward-facing 10 cm × 10 cm sanded PVC plates, are deployed from floating docks at approximately 1 m depth. In mid-August, two of these arrays are retrieved and replaced with new ones. All four remaining arrays are retrieved in late October. Upon retrieval, arrays are examined visually for presence and percent cover of invasive species and photographed for reference. In 2012 and 2013, arrays were deployed at 70 and 75 targeted monitoring stations, respectively, along approximately 7,000 km of coastline. At some stations, an inverted plastic saucer housing three Petri dishes was added to the collector arrays to increase material choice and surface area for invertebrate settlement.

In parallel with collector-based monitoring, DFO conducted rapid assessments in targeted areas using a combination of dockside, underwater, and boat-sampling techniques. Dockside techniques used were underwater video and visual surveys of docks, ropes, and underwater structures as well as water sampling for genetic analysis. Underwater techniques used were SCUBA and snorkel surveys of docks and underwater structures. Boat-sampling techniques used were visual inspection and underwater video of small buoys, channel markers, and their associated ropes and chains. Different combinations of these techniques

were used in different situations to suit the purpose of the investigation: monitoring, rapid response, or population delineation.

The AIS monitoring program also informed harbour authorities, marina managers, fishermen, aquaculture site staff, the inhabitants of coastal communities, and the general public about non-indigenous species and when and how to report suspect specimens.

### *AIS Research*

AIS Research in 2012 examined recruitment and fouling community succession in harbours with and without tunicates. There were two experimental designs:

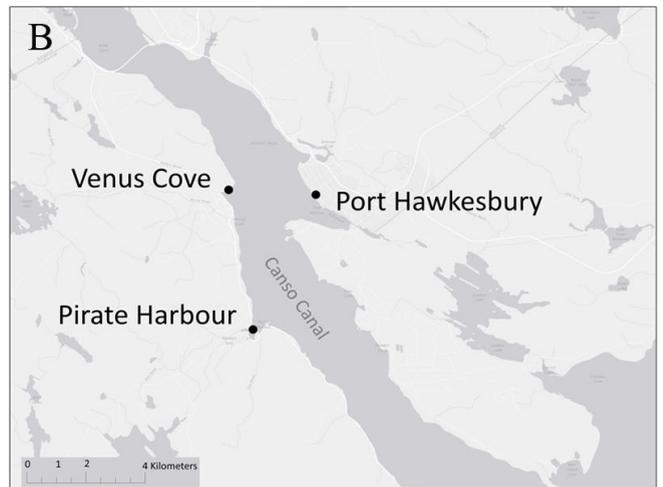
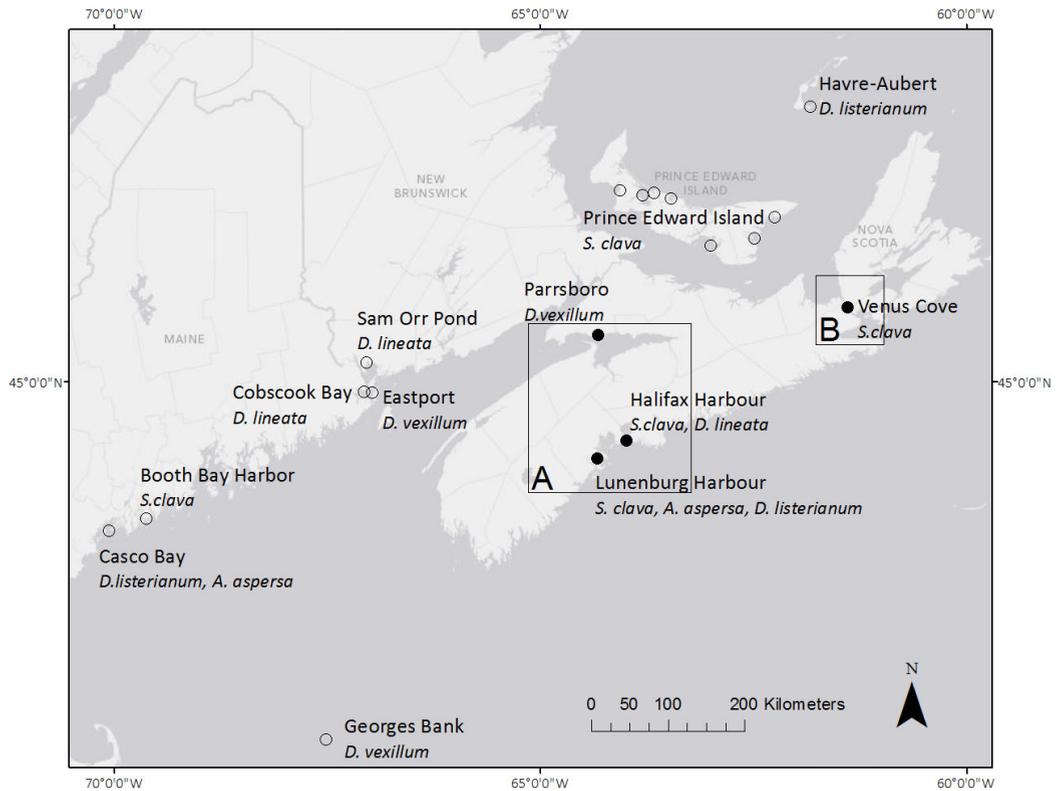
1) Twenty sanded PVC collector plates (14.6 cm × 14.6 cm) were deployed horizontally from floating docks at 1 m depth at 16 sites in Nova Scotia from late May to late October. Every two weeks, non-indigenous tunicate settlers were counted and removed from 10 collector plates per site. All plates were then photographed for further analysis (i.e., percent-cover time series) and returned to the water.

2) A six-plate collector array was deployed every two weeks from floating docks at between 1 and 2 m depth at four sites in Nova Scotia starting in early June and ending in late October. After one month of exposure, collector arrays were retrieved and the plates were photographed for further analysis.

AIS Research in 2013 was on a smaller spatial scale, taking place on one dock in Halifax Harbour, but was more intense in terms of plates deployed (n = 50) and sampling intensity. It involved deploying collector plates at 1 m depth from the floating dock starting in mid-July. Plates were photographed weekly until late November.

## Results

The combined monitoring and research programs detected three new non-indigenous tunicates in Nova Scotia in 2012: *Styela clava*, *Asciidiella aspersa* (Müller, 1776), and *Diplosoma listerianum* (Milne-Edwards, 1841). In 2013, a further two non-indigenous invertebrate species were identified: the tunicate *Didemnum vexillum* and the anemone *Diadumene lineata* (Verrill, 1869) (Figure 1 and Table 1). The following outlines methods that led to the species discoveries, actions taken post-discovery, the species' current status and extent in Nova Scotia as well as implications for future monitoring.



**Figure 1.** Map of central Atlantic Canada centred on Nova Scotia with confirmed locations of first records (solid circles) and nearest known records (open circles) of five non-indigenous invertebrate species: *Styela clava*, *Asciidiella aspersa*, *Diplosoma listerianum*, *Didemnum vexillum* and *Diadumene lineata*. A) Map of the Minas Basin and the South Shore of Nova Scotia. General locations of follow-up surveys are indicated with an x. B) Map of the portion of the Canso Canal where *S. clava* was observed.

**Table 1.** Summary table of the initial observation and identification of AIS in coastal Nova Scotian waters, including the exposed southern coast of Nova Scotia and Bay of Fundy

| Species                      | Location       | Lat.    | Long.    | Date Identified | Earliest Known Date Present | Substrate     | Method <sup>a</sup> | Confirmation <sup>b</sup> |
|------------------------------|----------------|---------|----------|-----------------|-----------------------------|---------------|---------------------|---------------------------|
| <i>Styela clava</i>          | Lunenburg      | 44.3755 | -64.3105 | Oct 2012        | Oct 2012                    | PVC plate     | R                   | EV (specimen)             |
|                              | Halifax        | 44.6810 | -63.6109 | Oct 2012        | Oct 2012                    | PVC plate     | M, R                | EV (specimen)             |
|                              | Pirate Harbour | 45.5871 | -61.3883 | Jul 2012        | Jul 2012                    | floating dock | A                   | unconfirmed               |
|                              | Venus Cove     | 45.6153 | -61.3901 | Aug 2013        | Aug 2013                    | wharf         | M                   | EV (specimen)             |
| <i>Asciidiella aspersa</i>   | Lunenburg      | 44.3755 | -64.3105 | Oct 2012        | Jul 2012                    | PVC plate     | M, R                | EV(photo)                 |
| <i>Diplosoma listerianum</i> | Lunenburg      | 44.3755 | -64.3105 | Oct 2012        | Oct 2012                    | PVC plate     | R                   | genetic                   |
| <i>Didemnum vexillum</i>     | Parrsboro      | 45.3761 | -64.2770 | Oct 2013        | Oct 2013                    | rock          | A                   | genetic                   |
| <i>Diadumene lineata</i>     | Halifax        | 44.6810 | -63.6101 | Feb 2014        | Nov 2013                    | PVC plate     | R                   | EV (photo)                |

<sup>a</sup>Method: R = research, M = monitoring, A = amateur

<sup>b</sup>Confirmation: EV = expert visual

### *Styela clava*

*Styela clava* is a solitary, stalked, club-shaped tunicate with a brown, leathery tunic covered in tubercles. Sexually mature individuals typically range in size from 90 to 160 mm (See Millar 1970 for a full description). *S. clava* is indigenous to the NW Pacific Ocean but has invaded temperate waters worldwide including both North American coasts, the United Kingdom, Europe, Australia, and New Zealand. It has been present in New England since 1970 and was first documented in Atlantic Canada in 1998 in the Brudenell River, Prince Edward Island (Clarke and Therriault 2007). Since 1998, *S. clava* has spread to other estuaries on Prince Edward Island but had not been detected beyond that province's waters until 2012.

*Styela clava* was discovered on 2 October 2012 on collector plates in Lunenburg Harbour and in Halifax Harbour (Figure 1; Table 1). Specimens of *S. clava* were removed, preserved in ethanol, and positively identified. In both locations, only a few juvenile individuals of *S. clava* were observed, although one larger individual (36 mm in length) was present in Lunenburg Harbour (Figure 2). In response to these detections, DFO conducted surveys in Halifax Harbour Basin and in Lunenburg Harbour (Figure 1A) in November 2012 using snorkelling and small buoy inspections (Vercaemer et al. 2012; Vercaemer and Sephton 2014). Underwater video revealed moderate populations of *S. clava*, including mature individuals on ropes and chains of buoys in both locations.



**Figure 2.** *Styela clava* (length 36 mm) attached to a brick in Lunenburg Harbour, NS. Photograph by A.M. Moore.

In 2012 and 2013, there were also anecdotal reports of *S. clava* from Pirate Harbour, in the Canso Canal (S. Sorowka, Department of Biology, St. Francis Xavier University, pers. comm.) and the Strait of Canso Yacht Club (SCYC) in Port Hawkesbury, Nova Scotia (N. Filip, Department of Biology, St. Francis Xavier University, pers. comm.) (Figure 1B). Dockside inspections at Pirate Harbour uncovered no *S. clava* individuals; however, several individuals were present on the underside of floating docks at nearby Venus Cove, which has been monitored annually since 2008 (except 2012). Both the SCYC and Venus Cove were collector-based monitoring stations in 2014.

*Styela clava* has likely been present in Nova Scotia since at least late 2011. However, given the distribution and extent of current populations, the size of individuals, and the timing of detection, *S. clava* could have been present in Nova Scotia years earlier and simply gone undetected due to insufficient monitoring efforts and/or low population abundance.

#### *Ascidella aspersa*

*Ascidella aspersa* is a solitary tunicate, ovate in shape, with a clear-grey, papillate, tunic sometimes tinged pink around its two prominent siphons. It resembles *Ciona intestinalis* especially in its early growth stages (< 10 mm in length); however, its tunic is firm to the touch unlike that of *C. intestinalis*, which is soft and gelatinous. *Ascidella aspersa* is indigenous to the Mediterranean Sea and coastal waters throughout Europe and the British Isles but has become abundant in Australia and New Zealand since arriving in the early 1900s (Kott 1998). It was first detected in New England in the 1980s, presumably spread by hull fouling or ballast water, and is currently established in Massachusetts (Fofonoff et al. 2003) and present at least as far north as Casco Bay, Maine. *Ascidella aspersa* was included on the Atlantic Canada watch list in 2009.

*Ascidella aspersa* was first recognized and positively identified in Nova Scotian waters on 2 October 2012 from specimens on collector plates in Lunenburg Harbour (Figure 1; Table 1). This is the first record of *A. aspersa* in Canada. Using photographic time series from months previous, we determined that this species was actually present as early as 5 July 2012 on collector plates from three different docks in Lunenburg Harbour. Individuals were present in densities up to 20 individuals per plate, ranging in length from 10 to 40 mm by early October (Figure 3). In response to the detection (and others, see *Styela clava* and *Diplosoma listerianum*), DFO conducted a rapid assessment survey of the Lunenburg area (Figure 1A) in November 2012 using snorkelling and small buoy inspections (Vercaemer et al. 2012). The survey revealed extensive populations of *A. aspersa* on docks, pilings and buoys in Lunenburg Harbour and as far south as Lower South Cove.

*Ascidella aspersa* was likely present in 2011, perhaps before, having successfully overwintered to spawn for the first time in detectable numbers



**Figure 3.** Multiple specimens of *Ascidella aspersa* (large, ovate solitary organisms) on a collector plate from Lunenburg Harbour, NS. Photograph by A.M. Moore.

in early 2012 when it developed multiple large, dense, and widespread populations in multiple bays near Lunenburg, Nova Scotia.

#### *Diplosoma listerianum*

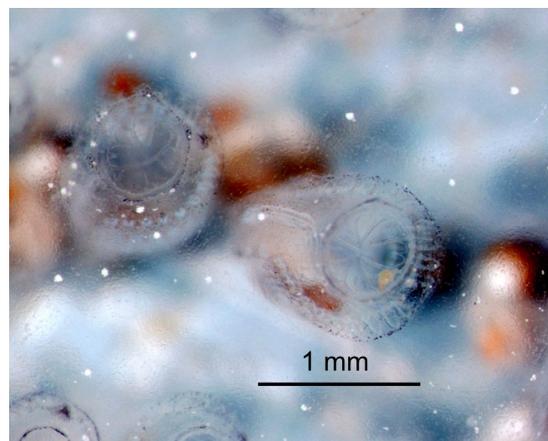
*Diplosoma listerianum* is a colonial tunicate, transparent and grayish in colour with pigmented zooids. Its tunic is gelatinous to the touch, unlike other non-indigenous colonials *Botryllus schlosseri* and *Botrylloides violaceus*, which are firmer. *Diplosoma listerianum* is cryptogenic, although it is presumed to be indigenous to northern Europe as it was first described by Millar (1952) as one of the region's more common ascidians. It was first detected in the Gulf of Maine in the Isles of Shoals in 1993 and is now considered established from Cape Cod, Massachusetts to Casco Bay, Maine, although its distribution is temporally variable (Dijkstra 2007). In Canada, *Diplosoma listerianum* was first collected in 2008 from Havre-Aubert, Quebec, which is located in the Magdalen Islands (Figure 1) (Simard et al. 2013). However, no colonies have been found since, despite targeted surveys and despite *Diplosoma listerianum* DNA being detected via targeted PCR-based assays in plankton samples from Havre-Aubert in 2010 and 2011 and Cap-aux-Meules (also in the Magdalen Islands) in 2011 (Simard et al. 2013). *Diplosoma listerianum* was included on the Atlantic Canada watch list in 2009.

*Diplosoma listerianum* was initially found in Nova Scotia in small patches on collector plates in Lunenburg Harbour on 2 October 2012 (Figure 1; Table 1). Specimens were collected and preserved in ethanol. Identification was confirmed by G. Lambert (Marine Biological Consultants, Seattle, Washington, USA) via digital photographs and later using the PCR-based assay developed by Willis et al. (2011) (S. Stewart-Clark, Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada, unpubl. data). Colonies were present on both newly deployed plates (deployed for approximately four weeks) and plates with fully developed communities (deployed for approximately four months) (Figure 4). Photographs from the beginning of the experiment were re-examined to see if *Diplosoma listerianum* could have been present earlier, but no colonies could be positively identified. In response to the detection (and others, see *Asciidiella aspersa* and *Styela clava*), DFO conducted a rapid assessment survey of the Lunenburg area (Figure 1A) in November 2012 using snorkelling and small-buoy inspections (Vercaemer et al. 2012). No additional specimens of *Diplosoma listerianum* were observed; however, visibility was poor during the survey, and colonies, with their transparent tunic, could have been overlooked.

Previous detections of *Diplosoma listerianum* in Atlantic Canada have not led to established populations (Simard et al. 2013). However, given that there have been several detections of *Diplosoma listerianum* in Atlantic Canada both visually and from DNA in water samples (Simard et al. 2013), there could be as yet undiscovered populations in Atlantic Canada.

#### *Didemnum vexillum*

*Didemnum vexillum* is a colonial ascidian, usually creamy white or tan in colour, that forms a firm gelatinous layer on natural and artificial substrates. The species sometimes has darker-coloured lines (cloacal canals of the shared excurrent system) that run between groupings of zooids (1 mm long) just beneath the surface and calcareous spicules embedded at the surface of the matrix. Originally from Japan (Stefaniak et al. 2012), the species has been reported in temperate and cold waters of both coasts of North America, in Europe, and in Australia. In Canada, *Didemnum vexillum* has been reported in British Columbia, mainly on artificial structures, such as aquaculture cages. The northernmost coastal location from which it has been reported



**Figure 4.** *Diplosoma listerianum* on a collector plate from Lunenburg Harbour, NS. Photograph by K.C.K. Ma.



**Figure 5.** *Didemnum vexillum* colonies on rocks from the upper Bay of Fundy, NS. Photograph by B. Vercaemer.



**Figure 6.** *Diadumene lineata* on a collector plate from Halifax Harbour. Photograph by A.M. Harris.

on the eastern shore of the US is Eastport, Maine, where it was first observed in 2003 (Figure 1) (Bullard et al. 2007). This species also occurs in deeper waters on Georges Bank off New England (Valentine et al. 2007) where it covered an area of approximately 230 km<sup>2</sup>. *Didemnum vexillum* was included on the Atlantic Canada watch list in 2009, and there was an intense public awareness campaign regarding its appearance and likely habitat. Rapid assessment surveys conducted in Passamaquoddy Bay, New Brunswick, and areas near Eastport, Maine, since 2009 (Martin et al. 2010), as well as in southwestern Nova Scotia in 2013, did not detect this species.

A specimen thought to be *Didemnum vexillum* was collected in October 2013 by a recreational diver off the coast of Parrsboro, in the Minas Basin, at a depth of approximately 10 m (Figure 1; Table 1). Microscopic examination of spicules and subsequent molecular identification (S. Stewart-Clark, unpublished data) confirmed the sample as *Didemnum vexillum*. In April 2014, in response to the detection, DFO conducted a rapid assessment targeting 30 stations in four areas of the Minas Basin and Minas Channel (Figure 1A). The assessment used underwater video to identify potential patches of *Didemnum vexillum* that were then sampled by scallop dredge and sent for genetic analysis. Numerous colonies were observed overgrowing rocks, bivalve shells, and seaweeds (Figure 5). Samples from 22 of the 30 stations were confirmed to be *Didemnum vexillum* by a PCR-based assay (S. Stewart-Clark, unpublished data). Colonies were in a wintering state, but were healthy and likely to grow and spread in the spring and fall. Because initial observations on the east coast of the USA documented isolated and small populations that underwent a rapid population expansion within a decade (Bullard et al. 2007), DFO has initiated an assessment to delineate the current distribution of *Didemnum vexillum* throughout the Bay of Fundy as well as regions of the southern Scotian Shelf, including Georges Bank, German Bank, and Browns Bank. This assessment will establish a baseline to monitor the populations' abundance changes and rate of range expansion.

Although first recorded in 2013, *Didemnum vexillum* could have been present in Nova Scotia as early as 2011, when a recreational diver first noticed and photographed patches of a white organism on the benthos off the coast of Parrsboro. Unfortunately, samples were not obtained until 2013; consequently this initial report is unconfirmed.

### *Diadumene lineata*

*Diadumene lineata* is a small greenish anemone with distinctive vertical orange or tan stripes around its body. Native to northeastern Asia including Japan, China, and Hong Kong, *D. lineata* was first recorded on the Atlantic coast (Connecticut and Massachusetts) of North America in 1892 (Verrill 1898) and is now established as far north as Cobscook Bay, Maine (Figure 1) (Trott 2004). In Canada, *Diadumene lineata* was found in Sam Orr's Pond on the southern coast of New Brunswick on the Bay of Fundy in 2009, possibly introduced on substrates (e.g., shells) transported by the invasive alga *Codium fragile fragile* (Suringar) Hariot, 1889, which can float to new locations (Saunders et al. 2013). This species can reproduce asexually and sexually, although sexually reproducing populations of *Diadumene lineata* are rare, if not absent, outside of its native range in Asia (Ting and Geller 2000).

In Nova Scotia, *Diadumene lineata* was first detected in February 2014 during photographic analysis of collector plates deployed at a dock in Halifax Harbour (Figure 1; Table 1). The photographs were taken on 19 November 2013, and one specimen was retrieved from a collector plate on 3 July 2014 (Figure 6). In October 2014, an extensive population of *Diadumene lineata* was observed along the same dock, consisting of numerous individuals interspersed amongst mussels near the water's surface. No further attempt at determining the species' extent is underway as the species is not perceived to be a major threat to indigenous communities or aquaculture operations.

### Discussion

The Atlantic Canada watch list, using shipping and climate zone filters, included three tunicate species found in Nova Scotia in 2012 and 2013: *Asciidiella aspersa*, *Diplosoma listerianum*, and *Didemnum vexillum*. *Styela clava*, *Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides violaceus*, and *Molgula manhattensis* (De Kay, 1843) were excluded from the watch list because they already were present in Atlantic Canada. All of the new non-indigenous invertebrates reported in this study, except *Didemnum vexillum*, were found near high traffic shipping (Halifax and Port Hawkesbury) or recreational (Lunenburg) ports, where other similar non-indigenous species (i.e., *Ciona intestinalis*, *Botryllus schlosseri*, and *Botrylloides violaceus*) are already present. *Didemnum vexillum*

was found seemingly far from a major port city; however, until late 2011, the nearby town of Hantsport shipped gypsum, with 119 gypsum ships docking there in 2000, many of which arrived from the United States (Carver and Mallet 2003) where *Didemnum vexillum* is established.

Earlier first records of detections have also occurred in the high traffic port of Mahone Bay (Figure 1). Only 11 km from Lunenburg, Mahone Bay shares Lunenburg's high recreational boat traffic and is the first reported location for four other non-indigenous species that have hull fouling or ballast as a likely vector: *Codium fragile fragile*, a green alga, discovered in 1989 (Bird et al. 1993), *Membranipora membranacea* (Linnaeus, 1767), an encrusting bryozoan, found in 1992 (Scheibling et al. 1999), *Botrylloides violaceus* found in 2001 (Carver et al. 2006), and *Heterosiphonia japonica* Yendo, 1920, a red alga discovered in 2012 (Savoie and Saunders 2013).

All of these first records support the idea that primary introductions to Nova Scotia are occurring via either shipping or boating activities (DiBacco et al. 2012). Monitoring for new non-indigenous species, including the 14 tunicates remaining on the Atlantic Canada watch list, should therefore be focused on high traffic locations, particularly those with other non-indigenous species and those with a history of first records. While it is possible that *Didemnum vexillum* could have been introduced by hull fouling or ballast in 2011, it has not yet been detected in ports in the area. Instead, its introduction into the Bay of Fundy could have been a range expansion from the United States via rafting on eelgrass (Carman and Grunden 2010) or fragmentation caused by physical disturbance (e.g., current and wave action) of natural substrates (Reinhardt et al. 2012). Because of the uncertainty around the introduction of *Didemnum vexillum*, monitoring should also continue to focus on all potential vectors with public education programs targeting boaters, fishermen, and divers who regularly observe the substrates suitable for colonization.

Monitoring a large area to detect species soon after they invade is a difficult and costly task. By collaborating and sharing information, the AIS research and monitoring programs in Nova Scotia were able to detect and delineate five new non-indigenous species in a short time that would not have been possible independently. On its own, the AIS Monitoring program would have directly detected only two of the new species: *Styela clava* in Halifax Harbour (not Lunenburg Harbour) and *Asciidiella aspersa* in Lunenburg

Harbour with indirect detections of *Styela clava* in Pirate Harbour as well as *Didemnum vexillum* via the public outreach program. Conversely, the AIS Research program would have observed all species but *Didemnum vexillum*, but would not have been able to perform follow-up inspections to delineate local populations or plan and execute subsequent monitoring. Because of the success of this collaboration, we examined the reasons for differential detection patterns between the research and monitoring programs to identify potential improvements.

The main differences between the research and monitoring programs were spatial coverage of collectors, renewal interval of settlement space, and monitoring frequency. The main AIS Research project had 20 collectors deployed along one or more docks with a total of 4,263 cm<sup>2</sup> of settlement area per site, whereas the AIS Monitoring Group deployed four collectors on one or more docks with a total of only 1,200 cm<sup>2</sup> of settlement area per station. While both groups spread collectors out to account for spatial variability of larval settlement, the AIS Research group had 3.5 times the surface area available for settlement, increasing the likelihood of detecting a species if present, especially rare species. In addition, the 2012 AIS Research projects involved bi-weekly removal of tunicates from some plates or the monthly replacement of collectors. Both of these methods facilitated the detection of rare species (indigenous or non-indigenous) by consistently providing settlement space and examining that space before dominant fouling species could obscure detections. The AIS Monitoring group visits its stations twice post-deployment, in August and October, and although two collector arrays are replaced mid-season, at stations with heavy fouling, common in high traffic marinas, new species present in small numbers could easily be prevented from settling or be overgrown and obscured on plates by the end of the season and remain undetected. Ultimately, more surface area and more frequent collections, especially in high traffic areas, would increase the likelihood of detecting new non-indigenous species early in the invasion process.

The reports leading to the detection of *Didemnum vexillum* in Parrsboro and *Styela clava* in Venus Cove are a reflection of public outreach efforts of the DFO Maritimes AIS Monitoring program. In Nova Scotia, public education is a fundamental part of the AIS Monitoring program, and outreach efforts take the form of signage, presentations, sample collection, interviews with the media, and even monitoring itself (which increases interaction

with the public). Members of the public are encouraged to learn, identify, and report suspected non-native species, which helps cover a territory that is too large to adequately monitor through collector plates or rapid assessments alone. The detection of the high-impact invader *Didemnum vexillum* by a knowledgeable recreational diver underlines the importance of continued public outreach programs to educate the general public about AIS in general, but especially for high risk species.

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