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Research Article

Non-indigenous tunicates in the Bay of Fundy, eastern Canada (2006–2009)

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Editor's note:
This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

Abstract

A monitoring programme was initiated in 2006 to detect invasive tunicates, especially *Ciona intestinalis*, *Botryllus schlosseri*, *Didemnum vexillum*, *Botrylloides violaceus* and *Styela clava,* in Atlantic Canada. Collectors were deployed at 11–21 monitoring stations in the southwestern New Brunswick portion of the Bay of Fundy from 2006-2009, starting in late May with some retrieved in August while others remained in the water until later in the fall. There was large variability between years and sites. *C. intestinalis* was detected through much of the southwest New Brunswick area, including Grand Manan Island, but not in the area from Dipper Harbour to Saint John. *B. schlosseri* was observed to be concentrated in the Dipper/Beaver Harbour areas and Grand Manan Island, St. Andrews Harbour, Fairhaven (Deer Island), and Harbour de Loutre (Campobello Island), with greatest settlement observed in 2009. During the study period, *B. violaceus* was first detected in 2009 and at only one location, Head Harbour, Campobello Island. *S. clava* and *D. vexillum* have not been detected from our sampling collectors to date. As the invasive colonial tunicate, *D. vexillum*, has rapidly extended its range in the northeastern United States of America and is within 2 km of Canadian waters, a rapid assessment was conducted around Deer Island and Campobello Island in September 2009 and failed to detect the species.

Key words: ascidian, *Ciona intestinalis*, *Botryllus schlosseri*, *Botrylloides violaceus*, *Styela clava*, *Didemnum vexillum*

Introduction

There has been increased attention and documentation of the number and extent of the spread of aquatic invasive species (AIS) in the past several years (Carlton and Geller 1993; Leppäkoski et al. 2002; Ricciardi and MacIsaac 2008). Aquatic invasive species (AIS) can pose threats to native plants and animals with moderate to severe ecological and economic impacts through a number of actions such as: 1) predation, 2) space competition with natural populations, and 3) alteration of habitat.

Shellfish industries in Atlantic Canada continue to be severely affected by fouling tunicates (Carver et al. 2006a, b; Locke et al. 2007; Therriault and Herborg 2008). As part of an Atlantic Canadian initiative, a monitoring program was initiated in 2006 in the Bay of Fundy to determine temporal and spatial distributions of populations of the following tunicates: *Botryllus schlosseri* (Pallas, 1776), *Botrylloides violaceus* Oka, 1927, *Ciona intestinalis* (Linnaeus, 1767), *Didemnum vexillum* Kott, 2002 and *Styela clava* Herdman, 1881. *C. intestinalis* was first documented in the Bay of Fundy in the mid 1800s (Van Name 1912, 1945) and Linkletter et al. (1977) and Logan et al. (1983) later reported *C. intestinalis* and *B. schlosseri* from the area. Carver et al. (2006a) documented *C. intestinalis* in a number of regions of Atlantic Canada including the south shores of Nova Scotia and Prince Edward Island. *B. violaceus* has been observed in adjacent areas such as Eastport, Maine (Osman and Whitlatch, 1995; as *B. diegensis* by Ritter and Forsyth, 1917; Dijkstra et al. 2007a), Nova Scotia and Prince Edward Island (Carver et al. 2006b; Locke et al.

2007, 2009). In 1998, a major infestation of *S. clava* on mussel aquaculture lines commenced in Prince Edward Island waters but the species has not been recorded in Nova Scotia or New Brunswick (Clarke and Therriault 2007).

Another species of major concern for many regions of the world including Canada's east coast is the colonial ascidian *D. vexillum* Kott, 2002 (Kott 2002, 2004; Lambert 2009). Major concerns associated with *D. vexillum* invasions include ecological traits it shares with other invasive species: high growth rates, ability to spread by fragmentation, tolerance to a wide range of environmental conditions, lack of predators, and ability to alter the abundance and composition of benthic habitat (Dijkstra et al. 2007b; Daley and Scavia 2008; Collie et al. 2009). *D. vexillum* has been documented as an aggressive and invasive colonial ascidian that is spreading along the New England coast (Bullard et al. 2007a; Dijkstra et al. 2007b; Lengyel et al. 2009; Osman and Whitlatch 2007). It was first documented in the Damariscotta River, Maine in 1993 and on the American portion of Georges Bank in 2002 (Bullard et al. 2007a; Valentine et al. 2007). *D. vexillum* is now present at a number of locations in Maine, specifically Eastport (#27), which is within 2 km of our study area (Figure 2) (Daniel and Therriault 2007; L. Harris, University of New Hampshire, pers. comm.). Its congener *Didemnum albidum* (Verrill, 1871) is listed as occurring in the Bay of Fundy in Linkletter et al. (1977) and Van Name (1945) as well as earlier publications by Verrill cited by Van Name, and therefore is considered to be native.

We present results from the Bay of Fundy portion of the Atlantic Canadian monitoring programme from 2006-2009 as well as from a rapid assessment conducted in September 2009 for *D. vexillum* in Canadian waters in close proximity to Eastport, Maine (Martin et al. 2010).

Material and methods

Collectors were suspended at 11 locations in southwest New Brunswick during 2006, 20 locations in 2007 and 21 locations in 2008 and 2009. They were placed either on floating docks, beside wharves or on buoys or rafts at aquaculture sites (Figure 1; Table 1).

Collectors were fabricated in two sections with the upper portion containing a 23 cm inverted plastic flower pot saucer with four 100 mm Petri dishes attached to the underside (three Petri dishes in 2009) and the lower section comprising a series of three grey square PVC plates (10 cm × 10 cm) horizontally attached 15 cm below the flower pot saucer and suspended at 7.5 cm intervals on a one meter weighted long rope to keep the collector line vertical. All collector lines were suspended 1 m below the surface and in sufficient depths of water so that the collectors were above bottom even at exceptionally low tides. Two collectors were set out at each site in the spring during the month of May; one collector remained suspended from spring through fall and was designated as "full season" deployment, while the other collector was retrieved in August and referred to as the "early season" retrieval. When the early season collector was removed, it was replaced by a "late season" collector for retrieval in October or November. In 2009, duplicate collector strings were deployed.

At the time of retrieval, all settling plates (Petri dishes and PVC plates) were detached from the ropes and either examined microscopically as soon after collection as possible or preserved in a 10% formalin solution in seawater. Digital photos of the PVC plates and Petri dishes were taken soon after collection. Plates were examined using Nikon SMZ1000 dissecting microscopes. The undersides of the settling plates and Petri dishes were scanned with the dissecting microscope and the approximate location and estimate of any area covered by non-indigenous tunicates was drawn onto representative diagrams. Presence of other organisms was noted.

In 2006, results from microscopic analysis were assigned into 5 categories reflecting the estimate of area covered by the species of interest: 0 - absence, <25% - minimal presence, 25–50%- moderate presence, 51–75% - abundant and >75% - very abundant. In 2007–2009, Image J version 1.37 analysis software [ImageJ 1.37 Wayne Rasbund, National Institute of Health, USA http://rsb.info.nih.gov/ijJava1.60_23 (32 bit)] was used to determine the percent cover from digital photos taken of each collector plate as well as measurements from the visual results of microscopic analysis for confirmation of species identification. Results were compiled from the "full season" collectors except when one of the "full season" collector strings was lost, then the "late season" string was analysed.

A rapid assessment for *D. vexillum* occurred on September 24th and 25th, 2009 using three

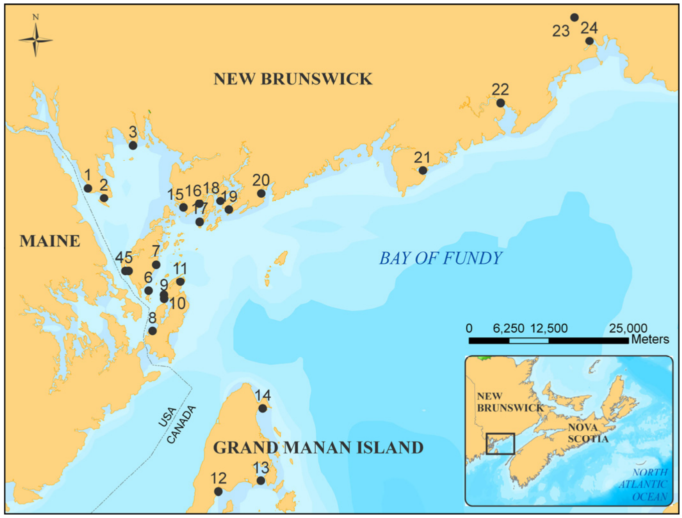

Figure 1. Locations of tunicate collectors in southwestern New Brunswick, Canada (2006-2009). See Table 1 for details.

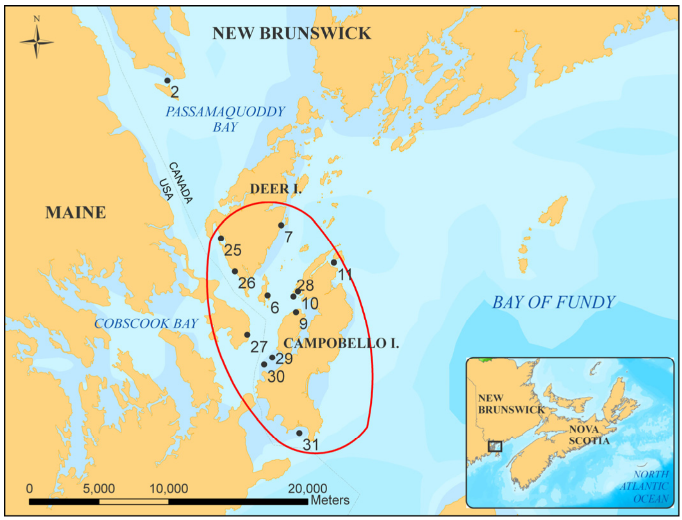

Figure 2. Locations (in red circle) sampled for *Didemnum vexillum* in 2009. See Table 1 for details.

boats investigating sites at Indian Island and a number of locations off Deer Island (Clam Cove, Cummings Cove and Leonardville), and Campobello Island (Head Harbour, Wilson's Beach, Curry Cove, Harbour de Loutre, Welshpool, Friar's Head and Lower Duck Pond) (Table 1, Figure 2). Two pairs of SCUBA divers searched the bottom at low tide at Clam Cove, Indian Island and Cummings Cove for *D. vexillum*, and collected samples of tunicates. Underwater surveillance videos around wharves and floating docks were made using a Micro Video submersible colour tube camera, MVC2120-WP (Micro Video Products, Bobcaygeon, Ontario, Canada) using Cyberlink (Cyberlink Power Director Version 5.00.2812DE, CyberLink Corp.) and Microsoft Windows Movie Maker software (Microsoft Windows Movie Maker, Version 1.1.2427.00, Microsoft Corp.). Bottom dragging was undertaken at five locations with an 18 inch wide Grand Manan "Miracle Gear" scallop drag with 4" rings for five to eight minutes. Salinity, temperature and fluorescence profiles were measured with a Sea-Bird Model 25 (Sea-Bird Electronics, Inc. 13431 NE 20th Street, Bellevue, Washington 98005 USA; http://www.seabird.com) at each site. Ascidian or "tunicate-like" samples, retrieved from scallop drags or diving, were put in Ziploc bags with menthol crystals and kept cool on ice for the return trip to the laboratory.

In the laboratory, live samples were examined, sorted and identified with a Nikon dissecting

Table 1. Collector sites for tunicates in southwestern New Brunswick, Canada listed by areas from west to east. *Ciona intestinalis* and *Botryllus schlosseri* show mean percent cover; *Botrylloides violaceus* was detected as one small colony on one plate in 2009. Fd, Floating dock, Aq, Aquaculture site. Sites 25-31 were only sampled during the *Didemnum* rapid assessment.

Map ref.	Site	Coordinates		Type of site	*C. intestinalis*				*B. schlosseri*				*B. violaceus*			
		Latitude, N	Longitude, W		2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2008	2009
1	St. Andrews Biological Station	45°04.940′	67°05.084′	Fd	10	9.7	54.8	4.5	0	0	0	0	0	0	0	0
2	St Andrews Harbour	45°04.059′	67°03.185′	Fd	20	5.3	37.3	5.7	25	6.1	0.3	12.9	0	0	0	0
3	Hog Island	45°08.510′	66°59.571′	Aq		3.8	0.1	1.5		0	0	0		0	0	0
4	Fairhaven Aqua	44°57.840′	67°00.856′	Aq	10	0.3	0	3.5	0	6.7	0	0	0	0	0	0
5	Fairhaven	44°57.841′	67°00.472′	Fd		7.7	15.3	34.5		14.7	4.2	8.0		0	0	0
6	Indian Island	44°56.091′	66°58.147′	Aq			3.7	16.7			0	0			0	0
7	Leonardville	44°58.295′	66°57.160′	Fd		8.6	35.6	16.0		0	0	1.6		0	0	0
8	Roosevelt Provincial Park	44°52.643′	66°57.797′	Fd		3.0				0.0				0		
9	Harbour de Loutre	44°55.390′	66°56.320′	Fd	3	0.6	24.7	5.8	15	26.8	3.6	30.9	0	0	0	0
10	Wilsons Beach	44°55.696′	66°56.337′	Fd			0.3	4.1			0	2.8			0	0
11	Head Harbour	44°56.793′	66°54.328′	Fd	0	0.0	47.2	16.1	0	0	0.6	19.0	0	0	0	0.1
12	Seal Cove	44°38.845′	66°50.477′	Fd	0	1.0	1.8	1.1	5	12.3	12.3	17.8	0	0	0	0
13	Ingalls Head	44°39.654′	66°45.394′	Fd		0	1.3	2.3		4.3	27.4	26.1		0	0	0
14	North Head	44°45.788′	66°44.941′	Fd	0	0.7	3.4	4.6	0	21.4	10.3	5.3	0	0	0	0
15	Letete	45°03.080′	66°53.736′	Fd		0.1	9.0	0.5		0	0	0		0	0	0
16	Back Bay	45°03.367′	66°51.828′	Fd		6.0	0.4	10.1		2.2	0	1.1		0	0	0
17	Charlie Cove	45°01.828′	66°51.841′	Aq	15	12.6	34.5	7.7	0	0	0	0	0	0	0	0
18	Lime Kiln Bay	45°03.564′	66°49.299′	Aq	52		43.5	50.1	0		0	0	0		0	0
19	Wallace Cove	45°02.798′	66°48.313′	Fd		6.2	51.0	14.0		0	0	0		0	0	0
20	Beaver Harbour	45°04.130′	66°44.388′	Fd		6.7	1.4	39.6		13.3	3.9	53.5		0	0	0
21	Dipper Harbour	45°05.666′	66°25.007′	Fd	0	0	0	0	50	16.2	1.8	42.3	0	0	0	0
22	Musquash	45°11.201′	66°15.448′	Fd			0	0			0	0			0	0
23	Kennebecasis	45°18.257′	66°06.246′	Fd		0				0				0		
24	Saint John Harbour	45°16.203′	66°04.551′	Fd	0	0			0	0			0	0		
25	Clam Cove	44°57.840′	67°00.469′													
26	Cummings Cove	44°56.550	66°59.750′													
27	Eastport	44°54.048′	66°59.163′													
28	Curry Cove	44°55.500′	66°56.587′													
29	Welshpool	44°53.376′	66°57.444′													
30	Friar's Head	44°52.968′	66°58.310′													
31	Lower Duck Pond	44°50.172′	66°56.454′													

microscope. Specimens of interest were preserved in 10% formalin in seawater and treated according to Lambert's method (http://woodshole.er.usgs.gov/project-pages/stellwagen/didemnum/htm/page41.htm). *Didemnum* sp. samples were also preserved in 95% ethanol for later examination of spicules, as an aid to identification.

Results and discussion

The first year of study in 2006 indicated that floating docks were the preferred structures for tunicate recruitment (LeGresley et al. 2008). Consequently, in the 2007–2009 seasons, monitoring effort was focused on deployment of collectors from a greater number of floating docks. Deployment on aquaculture sites seemed to have an inherent risk of collector loss as the cages and associated structures were occasionally cleaned or moved to new locations. It was also found that, for the Bay of Fundy, collectors that were deployed in sheltered locations (relative to water movements and currents) tended to have higher settlement of tunicates. Additionally settlement may have been affected by proximity to organisms (such as kelp, sea stars and sea urchins), and weather patterns.

Although more than 600 settling plates were analyzed, only *C. intestinalis*, *B. schlosseri* and *B. violaceus* (of the five species of interest) were observed. Often, these tunicates were present at low densities and rarely exceeded 25% coverage of the settling plates (Table 1). Patterns were quite variable among sites, however. During the 2006–2009 sampling period, the plates from Lime Kiln Bay consistently demonstrated relatively high levels of abundance for *C. intestinalis* with 43–52% cover. However, at Wallace Cove, Leonardville, Charlie Cove, Head Harbour and St. Andrews Biological Station there were varying levels of abundance in coverage on the individual settlement plates

(Table 1, Figure 1). *B. schlosseri* cover also varied considerably between sampling locations and in 2006 the only location where more than 50% coverage on the plates was observed was at Dipper Harbour. *B. violaceus* was found only once during the study.

C. intestinalis was detected at all but the four easternmost stations [Kennebecasis Yacht Club (#23) and Saint John (#24), Musquash (#22) and Dipper Harbours (#21)]. There generally was minimal settlement and growth on the collectors between May and August and the greatest growth occurred between late August and early fall, when temperatures are highest. At many of the sampling locations, *C. intestinalis* settlement was as much as five times greater in 2008 than during 2006, 2007 and 2009 (Table 1). This greater growth may have been triggered by a change in water temperature regime in 2008. A 20-year temperature time series for Lime Kiln Bay shows that maximum temperatures typically occur in August, but in 2008 the warmer temperatures were detected in September (Figure 3). This warm September may have allowed a longer period for growth of *C. intestinalis* in 2008.

B. schlosseri was detected at four sites in 2006 and twelve in 2009, with significant spread in Dipper Harbour, Beaver Harbour and the three Grand Manan sites (Seal Cove, Ingalls Head and North Head) (Table 1). The percent *B. schlosseri* cover on each of the plates nearly doubled between the years 2007 and 2009 with the *B. schlosseri* settlement in 2009 at Beaver Harbour and Dipper Harbour averaging 53.5% and 43.2% cover respectively and 100% cover on some plates.

For the first time during our sampling, a small colony of <20 *B. violaceus* zooids was observed in 2009 at Head Harbour, Campobello Island (Figure 1; Table 1). *B. violaceus* has been documented as abundant from Rhode Island to Maine in the northeast US (Pederson et al. 2005), but there had not been any previous documentation of presence in the Bay of Fundy although it is recorded from the Atlantic coast of southern Nova Scotia (Carver et al. 2006b). The species is known elsewhere to foul and overgrow nets used for finfish aquaculture, which may present problems for this industry in the Bay of Fundy in the future. Additional monitoring and study to assess its spread and potential impact in the Bay of Fundy is recommended.

In our study area, the Petri dishes were more effective as settling substrates than the corresponding PVC plates which had only minimal

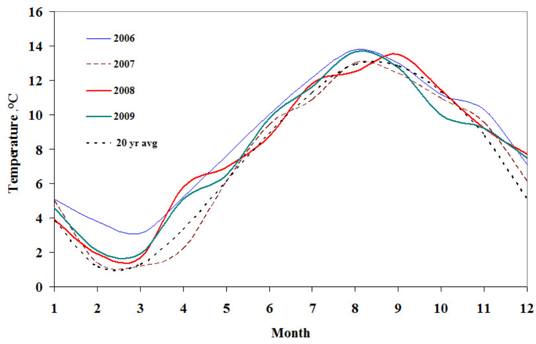

Figure 3. Average monthly temperature from Lime Kiln Bay, New Brunswick, Canada, including twenty year average temperature from 1989 to 2009. Data from Wildish et al. (1988, 1990); Martin et al. (1995, 1999, 2001, 2006, unpublished).

settlement. For example, the Wallace Cove 2008 coverage on the Petri dishes was 86.6% while the corresponding PVC plates averaged 3.6% cover giving an average of 51% for the site (Table 1). In addition, the Petri dishes seemed to be better for detecting organisms present at low densities. It is possible that the inverted flower pot saucer, to which the Petri dishes were attached, provided the tunicates with more shelter from the strong tidal currents in the Bay of Fundy, compared to the flat PVC plates.

Salinity ranged from 19.5 to 33.1 and water temperature ranged from 0.5°C–18.8°C at the sites investigated during the four year period, well within ranges for growth of the five tunicate species monitored during the study (Carver et al. 2006b; Therriault and Herborg 2008).

While *D. vexillum* was not observed on the settling plates during the four years of the study, small colonies of *D. albidum* were detected on the plates. During the rapid assessment in September 2009, *D. vexillum* was not detected at any of the Canadian monitoring sites (Martin et al. 2010). Oceanographic conditions at the sites were appropriate for *D. vexillum*, however. Salinity during the rapid assessment ranged from 32.35–32.58. Annual salinities in the region can range between 21.19 in the spring to 33.39 in late fall (Martin et al. 2006). This is well within the tolerance levels for *D. vexillum* which can tolerate wide fluctuations in salinity above 20 but is rarely found in salinities less than 20 (Lambert 2002, 2009). Surface water temperatures for the region are highest and lowest in mid-Passamaquoddy Bay (-1.0°C to 17°C) in February and September (Martin et al. 2006), and ranged from 11.5°C to 12.0°C during the assessment. Temperature ranges for *D. vexillum* are from -2°C to 24°C (Lambert 2009; Valentine et al. 2009). Osman and Whitlatch (2007) suggest the species has a preference for either cooler summer temperatures or an environmental factor that is related to temperature. Daley and Scavia (2008) suggest that temperature ranges along the northeast coast of the United States are ideal for *D. vexillum* to produce new colonies and spread. *D. vexillum* has been detected in Eastport, Maine (Dijkstra et al. 2007a; Bullard et al. 2007a). Its range has been documented to be coastally from Eastport to New York, and offshore on Georges, Stellwagen and Tillies Banks, with 230 km^2 coverage on Georges Bank with 50–90% cover in some areas (Bullard et al. 2007a; Lengyel et al. 2009; Valentine et al. 2007). Conditions in the Bay of Fundy should therefore be conducive for *D. vexillum* establishment and growth. The Bay of Fundy native *Didemnum* species, *D. albidum*, has been present for many years and is often mistaken for the invasive *D. vexillum* (Daniel and Therriault 2007).

The biology and life cycle of *Didemnum vexillum* has been widely documented (Kott 2002; Bullard et al. 2007a; Daniel and Therriault 2007; Lambert 2009). *D. vexillum* is hermaphroditic and produces tadpole larvae which only swim in the water for minutes to hours before settling suggesting that this is not a primary method of dispersal and introduction into Passamaquoddy Bay although it would facilitate maintenance of a population once established in a particular area (Osman and Whitlatch 1995, 2007). A possible means of dispersal of *D. vexillum* in our region might be asexual division through fragmentation (of rope-like tendrils) with subsequent transport by currents, waves or tides, or by transport of adult colonies through hull fouling, aquaculture (Bullard et al. 2007b; Lengyel et al. 2009; Dijkstra et al. 2007a). Experiments by Bullard et al. (2007b) have shown that many fragments can reattach in 6-12 hr with 75–80% reattaching in 30 hr. Asexual reproduction and fragments that break off seem to play an important role in the spread of *D. vexillum*. With the large tides and currents in Passamaquoddy Bay and the St. Croix estuary, flushing times have been estimated at about 6–8 days for the St. Croix estuary and 8–17 days for Passamaquoddy Bay (Ketchum and Keen 1953; Trites 1962; Trites and Garrett 1983). These conditions and the fact that only the St. Croix River (<2 km wide at Eastport) separates Canada from Eastport, ME suggest opportunities for transport, retention and settlement of *D. vexillum* segments through the region.

Unfortunately *D. vexillum* does not seem to be reduced by predators once established (Osman and Whitlatch 2007; Carman et al. 2009). Attempts at removal have shown that *D. vexillum* is extremely difficult to remove from structures (Daniel and Therriault 2007). A concern is that once established, *D. vexillum* could be responsible for a shift in species diversity or a displacement of native species, which would pose a threat to the local biodiversity, marine protected areas, traditional fisheries and aquaculture (Osman and Whitlatch 1995; Dijkstra et al. 2007b; Dijkstra and Harris 2009; Lengyel et al. 2009). *D. vexillum* has been observed as having a significant impact on species composition in the benthic community and outcompeting both epifaunal and macrofaunal communities. *D. vexillum* grows on a number of different substrates such as rocks, gravel, boulders, ascidians, sponges, macroalgae, anemones, bryozoans, hydroids, eelgrass, scallops, mussels, oysters and barnacles (Bullard et al. 2007a; Valentine et al. 2007; Daniel and Therriault 2007; Carmen and Grunden 2010). Much of this habitat is typical of bottom substrates in Passamaquoddy Bay (near the Eastport populations) further suggesting that *D. vexillum* could successfully invade the region. When Dijkstra and Harris (2009) conducted two studies (one from 1979–1982 and the other from 2003–2006 following the detection of *D. vexillum* in the northeast US), they found that the co-existence of species on their collector plates had shifted where the numbers of mussels that had been documented historically as abundant and dominant species on their collector panels and ropes had decreased significantly. This is cause for concern as a change in species presence/absence can result in patches of "free space" and allow or enable settlement of non-indigenous species.

A vector implicated in the transport of *D. vexillum* from Cape Cod to Maine (Carman 2007) is ship traffic (including hull fouling) movements between regions of the northeastern USA. Herborg et al. (2009) indicated in their forecasting of the spread of *D. vexillum* that slow vessels such as barges, aquaculture support vessels, recreational boats and large commercial vessels could act as vectors. There is a definite possibility in the Passamaquoddy Bay region that *D. vexillum* could be transported in a similar manner as a result of the high volume of these types of vessels traveling through the area.

Therriault and Herborg (2008) determined from results of an on-line survey of AIS by tunicate experts that the level of impact from an invasion of *D. vexillum* to the Bay of Fundy was high and the region would be favourable for the arrival, settlement, survival and capability to reproduce. Other countries, such as New Zealand, have experienced the impacts of an invasion of *D. vexillum* (Coutts and Forrest 2007). Their attempts at eradication have so far been unsuccessful although they have developed some response tools that might work in the future for different substrates. In June 2008, *D. vexillum* was detected in Wales, eradication measures were initiated in 2009 (Kleeman 2009). They tried wrapping plastic and bags to isolate, trap, smother and kill the *D. vexillum*. They later added calcium hypochlorite to the bags and wrapping products. Initially this treatment appeared to have positive results, but in late summer of 2010, colonies were again observed (Holt and Cordingley 2011).

New Zealand, Australia and other countries are establishing guidelines and quarantines for imports to prevent introductions such as *D. vexillum* (Hewitt and Campbell 2007). Biosecurity strategies and legislation are being implemented both externally and locally to aid in preventing and managing not just *D. vexillum*, but all AIS invasions.

The 2006–2009 Bay of Fundy tunicate surveys have shown a large amount of temporal and spatial variability of populations. Invasive tunicate surveys continue in the region to monitor the spread of already present species and to continue searching for *Didemnum vexillum* along the Canada/US boundary in the Bay of Fundy.

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