

Research Article

Distribution of the non-indigenous colonial ascidian *Didemnum vexillum* (Kott, 2002) in the Bay of Fundy and on offshore banks, eastern Canada

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Abstract

The invasive colonial ascidian *Didemnum vexillum* (Kott, 2002) was initially discovered in the fall of 2013 near Parrsboro, Nova Scotia. A rapid response survey was conducted in April 2014 to confirm the presence of the species and to determine its distribution near the original detection site. Subsequent surveys between May and August 2014 examined the dredge contents collected during sea scallop stock assessment surveys on German Bank, northern Browns Bank, eastern Georges Bank and in the Bay of Fundy region. The presence of *D. vexillum* was confirmed at 22 of 30 stations sampled in four areas of the Minas Basin and Minas Channel region in the northern Bay of Fundy during the rapid response survey. The scallop surveys confirmed the presence of *D. vexillum* at 9 of 829 stations sampled in the Bay of Fundy region, including 7 in the Minas Basin, 1 off Digby Gut and another off the coast of Yarmouth. Due to the presence of a native species, *D. albidum*, in the region, a PCR assay was developed to distinguish *D. vexillum* from all other species in the region. Once the PCR assay was validated this assay was used to confirm all positive identifications in this study. Colonies overgrew rocks, bivalve shells and seaweeds or were retrieved as large dislodged fragments. They were in an overwintering state in April, but healthy, and observed to grow into dense mats in summer. In other regions of the world, *D. vexillum* has been reported to foul shellfish and aquaculture gear, smother benthic organisms such as the sea scallop (*Placopecten magellanicus*), and overgrow substrates, suggesting this new colonial invasive ascidian poses a potential threat to Eastern Canada aquaculture and commercial benthic fisheries.

Key words: *Didemnum vexillum*, Bay of Fundy, Eastern Canada, colonial ascidian, fouling, scallop fishery, invasive species

Introduction

The colonial ascidian *Didemnum vexillum* (Kott, 2002) can rapidly colonize natural and artificial hard substrates and overgrow other benthic organisms such as bivalves (Valentine et al. 2007a, b). In the last two decades, non-indigenous populations of *D. vexillum* have been reported from many regions worldwide. Originally from the northwest Pacific ocean (Stefaniak et al. 2012), the species has been reported in temperate waters off New Zealand, Europe, and both coasts of North America (Bullard et al. 2007; Valentine et al. 2007a; Lambert 2009; Griffith et al. 2009;

Tagliapietra et al. 2012). In Canada, *D. vexillum* has been present in British Columbia since 2003 (Daniel and Therriault 2007), mainly on artificial structures such as aquaculture cages (Lambert 2009). In New England, where it was introduced in the 1980s, *D. vexillum* is commonly found on both natural (e.g., gravel, eel grass) and artificial substrates (e.g., docks, pontoons) in coastal marine habitats (Bullard et al. 2007; Carman et al. 2014; Valentine et al. 2007a). It has also been reported on gravel substrata offshore on the Northern Edge of Georges Bank near the Canadian/US boundary (Valentine et al. 2007b) where it occupied 50 to 90% of available space over a 230 km² area

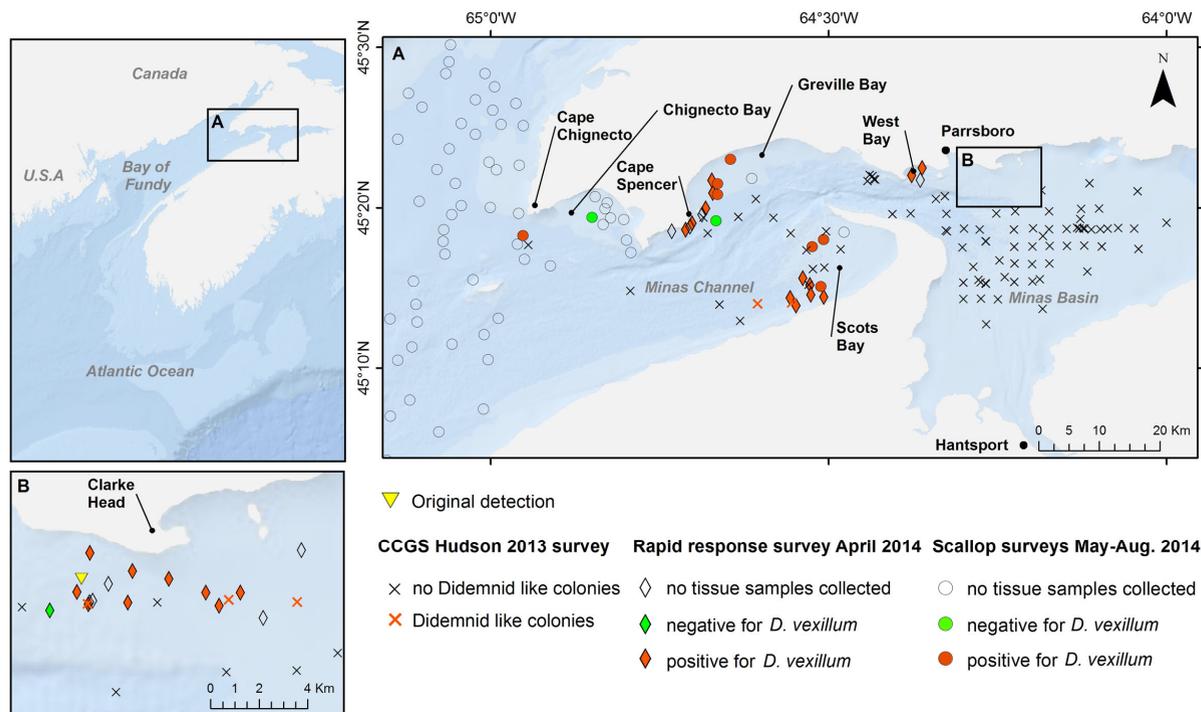


Figure 1. Sample sites and distribution of *Didemnum vexillum* in the Minas Channel and Minas Basin, Nova Scotia, including the initial detection location (Moore et al. 2014). CCGS Hudson 2013 survey sampling locations (B. Law, pers. comm.). Rapid response survey (April 2014). Scallop stock assessment surveys (May–August 2014). Open symbols represent stations with no tissue collected. Green and red symbols represent stations from which collected tissue tested negative and positive for *D. vexillum* via molecular screening protocols, respectively.

(Bullard et al. 2007; Valentine et al. 2007b). This species is a notorious global rapid invader (Lambert 2007, 2009) and will likely continue to spread via natural and anthropogenic vectors (Herborg et al. 2009). Furthermore, to date *D. vexillum* has been demonstrated particularly difficult to eradicate without effective response tools (e.g. New Zealand, Coutts and Forrest 2007; Wales, Sambrook et al. 2014).

There is considerable suitable habitat for *D. vexillum* throughout eastern Canadian waters based on the species' temperature and salinity tolerances, including the Bay of Fundy, the southwestern waters of Nova Scotia (NS) and locations around Cape Breton Island (Therriault and Herborg 2007). At least one morphologically similar native species, *D. albidum* (Verrill, 1871), is present in the area and complicates the identification of *D. vexillum*. The northernmost location of *D. vexillum* in the northwest Atlantic was Eastport, Maine until 2013 when the species was confirmed to occur in the Bay of Fundy off Parrsboro, NS

(Moore et al. 2014; Figure 1). Following this initial detection, the Canadian rapid response framework (Locke et al. 2011) was implemented by the Department of Fisheries and Oceans (DFO) Aquatic Invasive Species (AIS) Monitoring Program. A rapid response survey of the Minas Basin off Parrsboro was conducted in April 2014, and further sampling to delineate its distribution was undertaken from May to August 2014 during scallop stock assessment surveys throughout the Bay of Fundy as well as regions of the Scotian Shelf including, German Bank, northern Browns Bank and eastern Georges Bank.

Several PCR based assays have been developed to assist in monitoring for invasive species in environmental water samples and species identification of tunicate tissue samples in Atlantic Canada. These assays have been used to assist DFO in monitoring for *Diplosoma listerianum* (Willis et al. 2011) and *Botryllus schlosseri*, *Ciona intestinalis*, *Styela clava* and *Botrylloides violaceus* in the region (Stewart-Clark et al. 2009).

Since *D. albidum* is a native species in the region, and not a fouling concern, it is imperative that scientists be able to confidently distinguish between these two species. As a result, a PCR based species-specific assay was designed based on a section of the Cytochrome Oxidase 1(COI) gene that had variation between *D. vexillum* and *D. albidum*.

Methods

Rapid response survey - April 2014

The Aquatic Invasive Species (AIS) group at BIO (Bedford Institute of Oceanography) conducted a rapid response survey in the Minas Basin and Minas Channel region in the upper Bay of Fundy to collect samples in order to confirm the presence of *D. vexillum* beyond the area of its initial discovery (Figure 1). Prior to the rapid response survey, bottom images obtained from a CCGS Hudson scientific cruise conducted in June 2013 in the Minas Basin showed didemnid-like colonies at an approximate depth of 20 m depth off Clarke Head near Parrsboro and in Scots Bay, south of the Minas Channel in the upper Bay of Fundy (Figure 1). These areas were targeted for investigation during the rapid response survey along with two additional areas off West Bay and Cape Spencer, north of Minas Channel, where anecdotal reports described the presence of a bottom growth resembling didemnid colonies (Figure 1).

The rapid response survey was conducted on 10 and 11 April 2014. A small scallop dredge ($1 \times w \times h = 1 \times 1 \times 0.5$ m) was used to survey a total of 30 stations in the four targeted areas (Figure 1). All stations were sampled by dredging the bottom (10–40 m depth) for 5–10 min at minimal speed (~2–3.5 kn). Tow contents were photographed and substrates with attached didemnid-like colonies and detached tissue fragments were sampled and preserved in 100% ethanol for molecular screening. Multiple tissue samples were preserved when many colonies were collected in a given tow. To reduce the chances of our dredge activities spreading *D. vexillum* to other rapid assessment locations, the dredge was cleaned on station immediately after it was recovered and emptied.

Offshore and inshore surveys - May to August 2014

Dredge samples collected during DFO surveys of Scallop Production Areas (SPA) between May and August of 2014 were analyzed to determine

the geographic extent of *D. vexillum*. An AIS observer sampled didemnid-like tissues from scallop dredge contents collected on Georges, Browns and German Banks and inshore throughout the Bay of Fundy (Figure 2A). A total of 236 scallop tows were completed on offshore banks between May 22 and June 6, and 593 tows were completed during the inshore surveys in the upper, middle and outer Bay of Fundy between June 16 and August 28. Offshore surveys used a New Bedford scallop dredge ($1 \times w \times h = 7.5 \times 8 \times 1$ ft). Standardized tows were performed for 8 min on average at a minimum speed of 2.5–3.5 kn. The offshore surveys dredged a total area of 0.83 km². For inshore surveys, sampling gear consisted in a series of Miracle bucket dredges ($1 \times w \times h = 2 \times 2 \times 1$ ft) in a nine-gang configuration and tows were performed for 8 min on average at minimum speed (2.5–3.5 kn). The inshore survey dredged a total area of 2.49 km². Didemnid-like encrusted material and colony fragments were photographed and logged. Tissue from colonies resembling *D. vexillum* was sampled and preserved in 100% ethanol, which was drained and replenished within 48–72 hours, for subsequent molecular screening.

Molecular screening

Assay development

NCBI Primer-Blast was used to generate multiple primer sets for *D. vexillum*. These primers were then evaluated for specificity to ensure that no false positives would occur if the native *D. albidum* was present. Specificity against local *D. albidum* samples was considered in initial primer design by aligning COI sequences generated from native samples of *D. albidum* from across Nova Scotia with known *D. vexillum* sequences previously sequenced by our lab and from sequences located in GenBank. After computational analysis to determine propensity for primer dimers of self complementarity of primers, one primer set was chosen for lab validation: (DIDVEXF 5'-AGCTTTTGACTTTTACCACCAGC-3' DIDVEXR 5'-GCAGCTGCTAATACGGGTAA-3'). To validate the primer set, the primers were run in a polymerase chain reaction with DNA from both locally collected *D. albidum* samples collected from Nova Scotia, Canada (Halifax Harbour, Liverpool Harbour, Yarmouth Harbour and Shelburne) and *D. vexillum* samples that were collected on a global scale (Canada, United States, France, Ireland). PCR was run in 25 µl volumes with 12.5 µl Amplitaq Gold 360 Master mix (Life

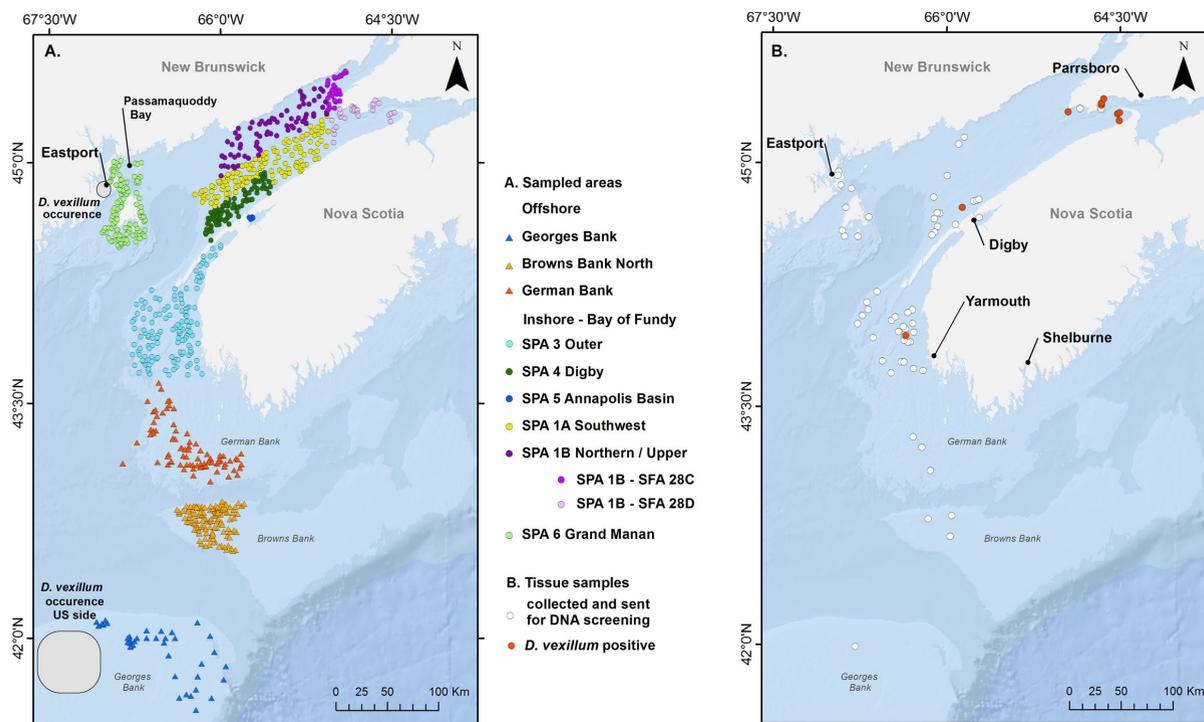


Figure 2. A. Location of closest known US occurrences of *Didemnum vexillum* (greyed areas on Georges Bank and Eastport, Maine) and location of scallop stock assessment survey sites (May-August 2014) on Georges Bank, Browns Bank North, German Bank and in the Bay of Fundy (SPAs). The SPA 1B, Upper Bay of Fundy, is further subdivided into SPA28C and 28D. B. Location of samples collected for molecular analyses (white circles) and those that tested positive for *D. vexillum* (red circles).

Technologies), 9.5 μ l Qiagen Nuclease free water, 400 pmol forward primer, 400 pmol reverse primer and 1 μ l of DNA. PCR was run using the following thermocycling protocol: 3 minutes at 92°C followed by 34 cycles consisting of denaturation at 94°C for 1 minute, a primer annealing period of 1 minute at 60°C and a 3 minute elongation period at 72°C. The reaction was completed with a 5 minute polymerization extension period at 72°C. PCR amplicons were separated using 1 % agarose gels via electrophoresis. Gels were prepared using Electrophoresis Grade Agarose, SYBR Safe DNA Gel Stain (Invitrogen) and were run at 135 volt for 1 hour. Gels were visualized under ultraviolet light and images were photographed and viewed using Quantity One Software and a Gel Documentation (Gel Doc) System (BioRad). The primer sets successfully amplified all samples of *D. vexillum* and no amplification occurred when *D. albidum* was present in the sample. Dilutions of extracted DNA were then used to evaluate the lower

detection limit of the primer set and successful amplification occurred in samples with DNA present at levels as low as 0.1ng/ μ l. Because this assay is specific for *D. vexillum*, a negative result indicates that the sample is not *D. vexillum*, rather some other unidentified species.

Assay screening of samples collected in the field

Tissue collected in the field was fixed in ethanol and submitted to the Aquaculture Genomics Laboratory at Dalhousie University. Total DNA was extracted from samples using Qiagen DNeasy extraction kits. DNA samples were analyzed using a NanoDrop2000 and were then added to PCR reactions containing the primer set designed in the above study. Each DNA sample was run in duplicate using the following thermocycling protocol: 3 minutes at 92°C, followed by 34 cycles consisting of denaturation at 94°C for 1 minute, a primer annealing period of 1 minute at 60°C and a 3 minute elongation period at 72°C.

Table 1. Rapid response dredge survey, April 2014: Station number, water depth, location, number of samples collected and molecular screening results for *D. vexillum*.

Station number	Depth (m)	Latitude (DD)	Longitude (DD)	Number of samples collected (N)	Positive <i>D. vexillum</i> (N)
2	17.5	45.3672	-64.2291	6	6
3	16.1	45.3687	-64.2228	1	1
4	19.0	45.3618	-64.2120	0	NA
5	17.2	45.3654	-64.2009	1	1
6	10.0	45.3744	-64.3707	1	1
7	11.7	45.3657	-64.3648	0	NA
8	16.5	45.3648	-64.3809	0	NA
9	19.4	45.3649	-64.2723	1	0
10	19.5	45.3647	-64.2702	0	NA
11	16.1	45.3624	-64.2915	1	1
12	13.3	45.3705	-64.2565	1	1
13	7.3	45.3732	-64.2789	1	1
14	4.6	45.3741	-64.2598	2	2
15	19.5	45.3595	-64.2781	6	6
16	18.2	45.3644	-64.2625	2	2
17	18.1	45.3662	-64.2277	3	3
18	12.9	45.3698	-64.2531	2	2
19	3.6	45.3762	-64.3020	2	2
20	30.5	45.2335	-64.5522	1	1
21	27.5	45.2393	-64.5315	3	2
22	25.4	45.2507	-64.5079	1	1
23	21.9	45.2350	-64.5255	1	1
24	32.2	45.2295	-64.5775	1	1
25	31.3	45.2722	-64.5242	0	NA
26	20.9	45.3241	-64.6942	1	1
27	30.1	45.3343	-64.6804	1	1
28	27.9	45.3404	-64.6792	1	1
29	15.5	45.3521	-64.6852	0	NA
30	35.1	45.3221	-64.6891	1	1
31	29.3	45.3120	-64.7081	0	NA

Table 2. Offshore and inshore scallop stock assessment surveys, May-August, 2014: Location, number of tows per cruise, mean tow length, total surface area dredged, mean bottom temperature, number of tissue samples collected and molecular screening results for *D. vexillum*.

Location	Number of tows	Mean tow length (m)	Total area dredged (m ²)	Mean bottom temp. (°C)	Number of samples collected (N)	Positive <i>D. vexillum</i> (N)
Georges Bank	39	637	136553	7.09	1	0
Browns Bank North	117	639	410903	6.05	3	0
German Bank	80	645	283895	6.24	3	0
Offshore banks Total	236				7	0
Outer Bay of Fundy ¹						
-SPA 3	135	771	572468	7.94	27	1
Middle and Upper Bay of Fundy						
-SPA 4 Digby	83	764	348766	9.35	11	1
-SPA 5 Annapolis Basin	5	730	20075	10.60	1	0
-SPA 1A Southwest	79	776	337172	9.35	0	0
-SPA 1B Northern/Upper	183	765	769973	12.43	13	7
-SPA 6 Grand Manan Bank2	108	729	433007	10.17	9	0
Inshore Bay of Fundy Total	593				61	9

¹incl. St Mary's Bay²incl. Campobello Is.

The reaction was completed with a 5 minute polymerization extension period at 72°C. PCR amplicons were separated using 1 % agarose gels via electrophoresis. Gels were prepared using Electrophoresis Grade Agarose, SYBR Safe DNA

Gel Stain (Invitrogen) and were run at 135 volt for 1 hour. Gels were visualized under ultraviolet light and images were photographed and viewed using Quantity One Software and a Gel Documentation (Gel Doc) System (BioRad).

Results

Rapid Response dredge survey - April 2014

Didemnid-like tissues were collected from 23 of 30 stations dredged at depths of 10 to 30 m from four areas including Minas Basin (off Parrsboro), Minas Channel (Cape Sharp near West Bay), Greville Bay (Spencer's Island near Cape Spencer) and Scots Bay (Figure 1, Table 1). The tissues sampled were attached to cobbles and boulders, seaweeds, epibiotic bryozoans, dead and live bivalve shells or as free floating fragments ranging in size from a few cm² to ~200 cm². Colonies and fragments were healthy, overwintering colonies. Forty of 41 tissue samples collected from these 23 stations were confirmed as *D. vexillum* via molecular screening (Table 1). All positive amplicons were sequenced to ensure the assay was detecting *D. vexillum* and not *D. albidum*.

Offshore and inshore scallop dredge surveys - May to August 2014

Dredge contents from offshore surveys (Figure 2A) were examined *in situ*, and didemnid-like tissues were sampled from rocks, seaweeds, epibiotic bryozoans and bivalve shells. Georges Bank (39 tows), Browns Bank (117 tows) and German Bank (80 tows) provided seven didemnid-like tissue samples, none of which was confirmed as *D. vexillum* by molecular analysis (Table 2). Because the assay is specific to *D. vexillum*, negative samples could not be identified to species. *Didemnum vexillum* covers a portion of the US side of Georges Bank adjacent to the US/Canada

boundary (Figure 2A). Bottom temperature varied between 6 and 7°C for the duration of the surveys, May 22 to June 6 (Table 2).

Inshore surveys (Figure 2A) in the outer Bay of Fundy (SPA 3) completed 135 tows of which twenty-seven contained didemnid-like tissues that were sampled and preserved. Only one of these tissue samples, from a tow located off Yarmouth, was confirmed as *D. vexillum* (Figure 2B, Tables 2 and 3). Three hundred and fifty tows were conducted in the mid and upper Bay of Fundy (SPA 1A, 1B, 4 and 5), and 25 didemnid-like tissue samples were collected for molecular analysis. Only one tow off Digby Gut (SPA 4) and seven tows in the upper Bay of Fundy (SPA 1B) contained *D. vexillum* (Figure 2B, Tables 2 and 3). In the Grand Manan Island region (SPA 6) none of the didemnid-like tissues collected from 9 of 108 dredge samples were confirmed as *D. vexillum*. This area is in very close proximity to Eastport, Maine where *D. vexillum* is present (Bullard et al. 2007) (Figure 2A). In the Upper Bay of Fundy (i.e., Cape Chignecto, Greville Bay, Scots Bay) the seven tows that collected *D. vexillum* are located in Scallop Fishing Area (SFA) 28D, in the northeast portion of SPA 1B (Figures 2A and 2B). In summary, 9 tows (1.5 %) of 593 tows conducted in Bay of Fundy region contained *D. vexillum*. These samples were collected at mean water depths of 10 to 34 m, except for the two samples off Yarmouth and off Digby located in deeper cooler waters at 62 and 66 m, respectively (Table 3). Bottom temperatures in the Bay of Fundy varied between 7.9 and 12.4 °C during the 10 weeks of the surveys (Table 2).

Table 3. Location, depth and bottom temperature of the 9 stations where *D. vexillum* was collected during the May-August 2014 scallop stock assessment surveys.

Cruise	SPA	Date	Station	Latitude start	Longitude start	Latitude end	Longitude end	Mean depth (m)	Mean temp (°C)
BI2014	3	June 22	110	43.94038	-66.3546	43.93353	-66.3499	62	8.1
BF2014	4	August 28	340	44.72512	-65.8684	44.72462	-65.8777	66	11.9
BF2014	1B	August 13	225	45.34723	-64.6640	45.35320	-64.6599	30	14.3
BF2014	1B	August 13	226	45.35850	-64.6640	45.36145	-64.6555	20	14.3
BF2014	1B	August 13	227	45.38383	-64.6451	45.38598	-64.6361	10	14.4
BF2014	1B	August 13	229	45.29300	-64.5242	45.29687	-64.5167	34	15.3
BF2014	1B	August 13	230	45.30053	-64.5071	45.30478	-64.4979	34	15.3
BF2014	1B	August 13	232	45.25177	-64.5113	45.24512	-64.5150	26	14.4
BF2014	1B	August 15	258	45.30472	-64.9519	45.30427	-64.9605	24	14.0

Discussion

Distribution of Didemnum vexillum

The rapid response survey (April 2014) and scallop stock assessment surveys (May - August 2014) confirmed the establishment of *D. vexillum* predominantly in the Minas Basin and Minas Channel areas, from Parrsboro to Cape Chignecto (East to West) and from Greville Bay to Scots Bay (North to South) (Figure 1). The expectation that healthy, overwintering colonies sampled in April would re-grow as water temperatures increased in summer was confirmed in July and August. This intra-annual expansion/regression cycle for *D. vexillum* colonies, typical of colonial tunicates in temperate waters, has been reported in coastal New England (Valentine et al. 2007a). Summer bottom temperatures in the Upper Bay of Fundy are higher than on the Canadian offshore banks, where *D. vexillum* was not observed, and the seven tows that tested positive for *D. vexillum* in the middle and upper Bay of Fundy (Table 3) were conducted at locations that ranged from 14 to 15.3°C in bottom temperature, conducive to rapid development and growth of the colonies.

The 2014 scallop assessment surveys (Figure 2B) revealed single occurrences of *D. vexillum* off Digby Gut (SPA 4) and off Yarmouth (SPA 3). Compared to the upper Bay of Fundy, summer temperatures in these deeper waters are colder (8 to 12°C) (Table 3), which may explain the lower density of the species at those locations. Therriault and Herborg (2007) suggested the area south of Yarmouth and north of German Bank, off southwestern Nova Scotia (SFA 29, not shown in Figure 2), might provide suitable habitat for *D. vexillum*. This area warrants further surveys, although no didemnid-like tissues were reported during a scallop survey here in September 2014 (A. Glass, pers. comm.). A rapid assessment survey conducted the previous year (September 2013) in harbours and marinas at depths less than 10 m from Yarmouth to Shelburne (Figure 2B) did not detect *D. vexillum* (Sephton and Vercaemer 2015). The areas covered by the May to August 2014 scallop stock assessment surveys were limited to known scallop habitats. Other areas of the Bay of Fundy identified as conducive to the establishment of *D. vexillum* should be surveyed, in particular areas characterized by gravel/sand substrates and warm temperatures, for example in the eastern part of Minas Basin and portions of Chignecto Bay.

None of the 9 didemnid-like samples retrieved from SPA 6 (Grand Manan Island region) tested positive for *D. vexillum* (Figures 2A, B). Microscopic examination revealed that these were likely *D. albidum*, an indigenous species in New England (Lambert 2009) and the Bay of Fundy (Gosner 1971). *Didemnum candidum* (Savigny, 1816) is also present in this area (Daniel and Therriault 2007; Gosner 1971). A fourth species, *D. lutarium* (Van Name 1910), was abundant and widespread in New England waters north to Maine between the 1870's and early 1900's, although no didemnids with a matching description have been collected there recently (Lambert 2009). In the Eastport, Maine region, *D. vexillum* has been established since 2003 (Bullard et al. 2007), but annual DFO rapid assessment surveys (scuba surveys, video camera inspection of docks and pontoons, dredge samples) conducted since 2009 in SPA 6 (Figure 2A), in particular Passamaquoddy Bay, NB and in areas in Canada <10 km away from Eastport, Maine, have not detected the species (Martin et al. 2010; Sephton and Vercaemer 2015). Further, no *Didemnum* species have ever been detected in nearshore coastal habitats of Nova Scotia, despite ongoing DFO biofouling monitoring via settlement plate collectors deployed on floating docks since 2006 (Sephton et al. 2011, 2014), although small colonies of *D. albidum* has been detected on settlement plates along the northern (New Brunswick) shore of the Bay of Fundy (Martin et al. 2011).

Populations of *D. vexillum* may be absent, patchy or in low abundance in these shallow nearshore habitats where monitoring efforts have been concentrated. It is unlikely that current AIS monitoring collectors (PVC plates) are not effective for detecting didemnids, as *D. albidum* has been observed on plates in southwestern New Brunswick (Martin et al. 2011). Both PVC and Plexiglass settlement plates were successfully used at three sites in New England off piers dominated by *D. vexillum* (Valentine et al. 2009). Acrylic plates and plastic snow fence have been successfully used to detect the species in New Zealand and British Columbia, respectively, when suspended close to a *D. vexillum* larval source such as fouled aquaculture cage (Forrest et al. 2013, T. Therriault, pers. comm.). Similarly, two artificial substrates made of seven rolls of snow fence (0.25 m diameter and 1.25 m long each) deployed at a depth of 15 to 20 m off Parrsboro from 27 June to 6 October 2014 revealed considerable settlement (446 and 2610 cm², respectively) of *D. vexillum* colonies upon retrieval.

Spread of Didemnum vexillum

It is difficult to unequivocally identify vectors that facilitated the introduction of *D. vexillum* to the upper Bay of Fundy. We hypothesize that the dispersal of naturally or anthropogenically fragmented colonies is most likely the main mechanism for spread, not natural larval dispersal. Colonial tunicates such as didemnids reproduce sexually (*D. vexillum* at 9.3–22.6°C in New England; Valentine et al. 2009) and asexually by propagative budding (reviewed in Daniel and Therriault, 2007). Tadpole larvae swim for a maximum of a few hours before settling, thus making natural dispersal a slow process. Forrest et al. (2013) showed *D. vexillum* in New Zealand has a greater ability to establish as a result of fragmentation than through ambient larval recruitment. As demonstrated in a laboratory experiment, floating fragments of *D. vexillum* were viable and contained eggs and larvae for up to 4 weeks (Morris and Carman 2012). A re-attachment experiment in the wild recorded weak re-attachment of *D. vexillum* fragments to experimental container surfaces (plastic Petri-dish) and to eel grass blades within the container at water temperatures as low as 6°C, and stronger re-attachment at higher temperatures. Fragments of *D. vexillum* colonies could have been dispersed by currents into the Bay of Fundy and introduced into the upper Bay of Fundy by progressive fragmentation and re-attachment. However, this scenario would presume the existence of intermediate patches of colonies along the coast, but so far, only two areas not in the upper bay, off Yarmouth and Digby Gut, have been identified despite extensive sampling.

Vessel traffic is a pathway that may have facilitated the introduction and secondary spread of *D. vexillum* to the Minas Basin and Minas Channel region. Colony fragments could be dislodged from fouled hulls and barges to establish viable colonies, and hull fouling has been identified as a potential vector of introduction for *D. vexillum* (Clarke Murray et al. 2012). The Minas Basin and Minas Channel, where *D. vexillum* is well established, is in close proximity to Hantsport (Figure 1), a gypsum terminal in operation until 2010 from which cargo ships transited between the Bay of Fundy and northeastern US ports. Further, the Fundy Ocean Research Center for Energy (FORCE) installed an in-stream tidal turbine 10 km west of Parrsboro in the Minas Channel in 2009, during which time equipment was delivered and installed by vessels and slow-

moving barges from outside the Bay of Fundy. Rafting is also a viable mechanism for dispersal and secondary spread of *D. vexillum* in the Bay of Fundy since we frequently found colonies attached to seaweeds or to the leafy bryozoan lemon weed (*Flustra foliacea*), the latter reported to be commonly washed ashore.

In summary, *D. vexillum* is established in the Minas Basin and Minas Channel within SFA 28D, a small portion of SPA 1B (Figures 1 and 2A) where approximately a dozen small (30 to 45') fishing vessels are active, mostly from the upper Bay of Fundy sea scallop fleet (J. Sameoto, pers. comm.). A few larger vessels (45 to 65') of the full Bay fleet also fish for scallops in the area. More intensive fishing activities by much larger fleets occur in the other SPAs of the Bay of Fundy and offshore. Fishing with scallop dredges and bottom trawls disturbs and fragments colonies, has the potential to transport fragments via fouled fishing gear and could increase secondary spread of *D. vexillum*. Such anthropogenic vectors should be assessed and considered by management in the development of preventative measures and mitigation strategies to stop or slow the further spread of *D. vexillum* (Locke and Hanson 2009; Locke et al. 2011).

It is not uncommon for ascidians to be detected at a geographic distance far from their last known occurrence. *Didemnum vexillum* was identified in Sitka, Alaska in 2010 and represents a 1000-km northward leap along the Pacific coast (Cohen et al. 2011). In this case, it is likely the species was established several years earlier through the transport of contaminated aquaculture gear or floating docks (McCann et al. 2012). However, as the central British Columbia coast has not been monitored for the presence of *D. vexillum*, other anthropogenic vectors or even natural dispersal cannot be ruled out. In the case of the Bay of Fundy, the apparent leap in distribution from Eastport, Maine to Parrsboro, NS is approximately 250 km. *Didemnum vexillum* is the only one of five recently detected non-indigenous marine species which has not been clearly associated with shipping, recreational boating activities (Moore et al. 2014) or aquaculture in Nova Scotia. In fact, the SPA 28D where *D. vexillum* is well established (Figure 1) is an area of the Bay of Fundy characterized by very low shipping and relatively low fishing activity. It is also a high-energy area characterized by high

velocity currents and sediment movements in the middle of the Minas Channel but there are coastal areas with lower currents and retentive gyres, especially along northern and southern shores of the Bay (Wu et al. 2011). We hypothesise that colony fragments drifting with tidal currents east and west of their original point of introduction (e.g. FORCE site) collect and reattach in these low-energy, retentive areas. Once established, these colonies can serve to enhance local dispersal and spread via natural or anthropogenic vectors. It would be beneficial to employ existing current and bottom shear stress models (A. Drozdowski, pers. comm.) to assess the predictability of these factors on the current distribution of *D. vexillum* across the Bay of Fundy, especially on the single occurrences of *D. vexillum* off Digby Gut and off Yarmouth.

Potential ecological impact of Didemnum vexillum

The spread of *D. vexillum* in the Bay of Fundy could cause the smothering of bottom substrata and the overgrowth of attached and sedentary benthic invertebrate species including commercial bivalves (Osman and Whitlatch 2007; Valentine et al 2007b; Janiak et al. 2013). Large colonies can cement gravel and small rocks into solid mats, possibly preventing benthic larval settlement and access to prey for various bottom feeders. The reduction of the spatial complexity of benthic habitats caused by the spread of *D. vexillum* also increases the risk of predation on shelter-seeking organisms (Bullard et al. 2007; Daniel and Therriault 2007 and refs within). It is unclear at this point how far the invasive *D. vexillum* has spread. Colonies resembling “biofilm” or “scrambled eggs” similar to the specimens collected off Parrsboro have been observed in the area possibly since 2011. Fishermen from the three scallop fleets operating in the Bay of Fundy have been asked to collect specimens to help determine the distribution of the species. The impacts of the spread of *D. vexillum* in this region are unknown, and additional surveys and vessel traffic analysis are needed to provide information about the species that will provide insights into containment, mitigation and other management options. Having access to molecular diagnostic assays to distinguish between native and invasive tunicates is critical in monitoring for invasive species in this region.

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