Rapid assessment and biofouling monitoring of Halifax Harbour and Bedford Basin following the detection of three new invasive ascidians in Nova Scotia

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Table of Contents

ABSTRACT

Vercaemer B. and Sephton D., 2014. Rapid assessment and biofouling monitoring of Halifax Harbour and Bedford Basin following the detection of three new invasive ascidians in Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 3063: v + 28 p.

Rapid assessment and demarcation are the first steps of the Rapid Response protocol DFO is currently developing for aquatic invasive species. Monitoring of the Halifax Harbour and Bedford Basin was intensified after the detection of three new invasive ascidians in Nova Scotia in 2012. Collector-based, small-buoy and dive surveys of targeted areas were conducted in the fall of 2012 and in 2013 to complement the annual, province-wide DFO Aquatic Invasive Species (AIS) biofouling monitoring. These surveys serve as a baseline assessment of the presence/absence of invasive tunicates and other AIS in the Halifax Harbour and Bedford Basin area. We confirmed the presence and establishment of a relatively small *Styela clava* population but did not detect *Diplosoma listerianum* or *Ascidiella aspersa*. The latter two species were detected in the Lunenburg area, *A. aspersa* being widely distributed in Lunenburg Harbour. This close monitoring of the spread of these newly detected species, and others, and the delineation of the area of infestation is ongoing.

RÉSUMÉ

Vercaemer B. and Sephton D., 2014. Rapid assessment and early monitoring of Halifax Harbour and Bedford Basin following the detection of three new invasive ascidians in Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 3063: v + 28 p.

L'évaluation rapide et la démarcation sont les premières étapes du protocole d'intervention rapide que le MPO élabore actuellement pour les espèces aquatiques envahissantes (EAE). La surveillance du port de Halifax et du bassin de Bedford a été intensifiée après la détection de trois nouvelles ascidies envahissantes en Nouvelle-Écosse en 2012. Des inspections de plaques de collection déployées dans la région, une inspection sous-marine des zones ciblées et une inspection des petites bouées ont été menées à l'automne 2012 et en 2013 pour compléter le suivi annuel du MPO de l'encrassement biologique à l'échelle de la province. Ces suivis ont servi à une évaluation de base de la présence / absence de tuniciers envahissants et d'autres EAE dans le port d'Halifax et le bassin de Bedford. Nous avons confirmé la présence et l'établissement d'une population relativement modeste de *Styela clava* mais n'avons pas pu détecter *Diplosoma listerianum* ou *Ascidiella aspersa*, deux autres espèces détectées dans la région de Lunenburg, la seconde étant largement distribuée dans le port de Lunenburg. Cette évaluation est en cours et un suivi étroit de la propagation et la délimitation de la zone d'infestation de ces espèces nouvellement détectées, et d'autres, vont se poursuivre.

Introduction

The introduction, establishment and spread of non-indigenous species (NIS) (i.e. species that establish self-sustaining populations outside their native range) in the aquatic environment are often unintended consequences of human activities (Carlton 1989). Of the many pathways (shipping, boating, aquaculture, live bait and food fish, canal and water diversions, etc.) by which aquatic NIS can be introduced and spread, commercial shipping is considered to be one of the most significant. Commercial shipping includes vectors such as ballast tank (water and sediment), bilge water and fouling of hull, sea-chest and water intakes, anchor, chains and propeller shafts. From those, ballast water and hull "niche fouling" are considered to be the most important. Oil and gas drilling platforms, warships and barges are also important vectors for introduction and transport of NIS.

Once an introduced NIS has established and spread, it may cause significant damage to coastal ecosystems and to the economies that depend upon those ecosystems. These damaging species are deemed invasive. One example of an aquatic invasive species is the European green crab *Carcinus maenas*, which was first recorded in the early 1950's in Nova Scotia (NS) and is considered among the world's worst aquatic invaders (Davis et al. 1998, Audet et al. 2003, IMO 2006). It is a voracious predator of bivalves, competes with and displaces native crabs, damages eel grass beds and can completely alter intertidal ecosystems. More recently, NIS such as the coffin box or lacy crust bryozoan *Membranipora membranacea* and particularly tunicates (the solitary vase tunicate *Ciona intestinalis,* clubbed tunicate *Styela clava* and European sea-squirt *Ascidiella aspersa*, the colonial golden star tunicate *Botryllus schlosseri* and violet tunicate *Botrylloides violaceus)* have been infesting NS and Prince Edward Island (PEI) (Carver et al. 2003, Howes et al. 2007, Locke et al. 2007, Ramsay et al. 2008, Daigle and Herbinger 2009). These dominant biofouling species are threatening shellfish aquaculture operations by smothering species and increasing labour costs.

The area between Halifax and Lunenburg has been a hotspot in the establishment of invasive tunicates in NS (e.g., *Ciona intestinalis*, see Clancey and Hinton 2003, Clancey and MacLachlan 2004). Halifax is a major port in Atlantic Canada and a multi-activity harbour that has both international commercial shipping and recreational boating as well as a shipyard and a military naval base. As a result, there are many public and private structures such as permanent docks, pontoons or floating docks, buoys, anchor lines and channel markers that provide attachment space for NIS larvae. Altered environments like these could be more prone to NIS establishment (Cohen and Carlton 1998) so the abundance of artificial substrates may contribute to the increased settlement of NIS. Most international vessel arrivals in Halifax are coming from the northeastern US and Western Europe with bulkers and tankers accounting for the majority of ballast discharge. In 2002, 96% of the 869 vessel arrivals in Halifax were in full or partial ballast (Kelly 2004). Ten percent of the zooplankton sampled from ballast waters of 63 vessels between 2007–2009 contained species not established in Atlantic Canada (DiBacco et al. 2011). Hull fouling is also an important vector of invasions for Halifax: a video analysis of the hull of 20 vessels detected on average 3.2 NIS not present in the region (Sylvester et al. 2011).

The Halifax Harbour and the Bedford Basin are connected by a 3 km long, 0.5 km wide channel ("The Narrows") which is highly frequented by commercial vessels and recreational boats. The Bedford Institute of Oceanography is located at the junction of the Bedford Basin with the Narrows and was the site of the initial detection of a few juvenile *S. clava* individuals on PVC plates in 2012 (Moore et al. 2014). This detection represented the first report outside the last known range of neighbouring PEI and north eastern US. *S. clava,* native to the northwest Pacific, is a widespread, global invader, found off Australia and New Zealand, on both coasts of North America, and Europe (Davis et al. 2007). It has recently invaded the Mediterranean Sea where it was so far absent (Davis and Davis 2008). This ascidian is found on artificial substrata in harbours, marinas, and on aquaculture infrastructure (Clarke and Therriault 2007) throughout its range, which is still expanding with new, almost yearly detections. *S. clava* is a great nuisance to mussel farmers in particular because it tends to form very dense clumps fouling ropes and buoys and the cultured mussels themselves, with increasing economic impacts (Colautti 2006, Ramsay et al. 2008).

Rapid Assessment surveys have been conducted in other regions of the world on floating docks (Pederson et al. 2005; Lu et al. 2007) and on navigational buoys (Conn et al. 2003, 2006, 2009; Conn and Conn, 2004, 2007; Lim et al. 2009) as routine inspection or ecological surveys. Following the detection of three new invasive tunicates in Nova Scotia in 2012: *S. clava, A. aspersa* and *Diplosoma listerianum* (Moore et al. 2014), Rapid Assessments were conducted in both the Lunenburg Harbour (Vercaemer et al. 2012) and the Halifax Harbour–Bedford Basin areas. In both locations, these new species were detected on PVC settlement plates at monitoring and/or research stations and the short life span of their larval stage indicate probable nearby source populations . Here we report on the Rapid Assessment conducted in the Halifax Harbor–Bedford Basin area based on collector, small-buoy and dive surveys.

1. Materials and Methods

1.1. Collector-based surveys

In 2012, six collectors were deployed on floating docks at the Bedford Institute of Oceanography (BIO), a sentinel station monitored since 2006 as part of the DFO AIS Biofouling Monitoring Program (Sephton et al., 2011, 2014). Following the standard protocol (Sephton et al., 2011, 2014), two collectors were deployed at 1m depth during three periods: first deployment (early June to mid-August), second deployment (mid-August to late October), and full deployment (early June to late October). The deployment of collectors for shorter soak times facilitated the detection of seasonal settlement patterns. Collectors are made of an upper section consisting of an inverted saucer housing three Petri dishes attached to its underside, and a lower section comprised of three 10 cm by 10 cm PVC plates. Following the detection of *S. clava* at BIO in early October, 2012 (Moore et al., 2014), four additional collectors were deployed in mid-October at BIO as well as at nine marinas and yacht clubs in the Halifax Harbour – Bedford Basin area (Figure 1 and Table 1). Some of these locations (AYC, BBYC, and RNSYS) were previous AIS monitoring stations as recreational boating is an important pathway for the spread of NIS (Table 2). These collectors were retrieved mid-November, 2012 (4 weeks soak). Pontoons and nearby submerged structures were examined using an underwater viewer at deployment and retrieval times.

In 2013, all 10 stations were monitored according to the standard AIS biofouling monitoring protocol (i.e. two collectors per each of three deployment periods). Water temperature was recorded at collector deployment and retrieval. Dates of collector deployments and retrievals during 2012 and 2013 are given in Appendix A. Nearby structures were also examined for the presence of AIS using an underwater viewer.

Presence and prevalence (percentage cover) of biofouling species (see Appendix B) were recorded for all collectors upon retrieval. Percentage cover for each fouling species was determined by visual examination of the bottom surface (underside) of each plate. Categories for the percentage cover were: 0 (absent); 1: < 25% coverage (low); 2: 25–50% coverage (moderate); 3: 51–75% coverage (heavy), 4: 75–99% coverage (very heavy) and 5: >100% (total).

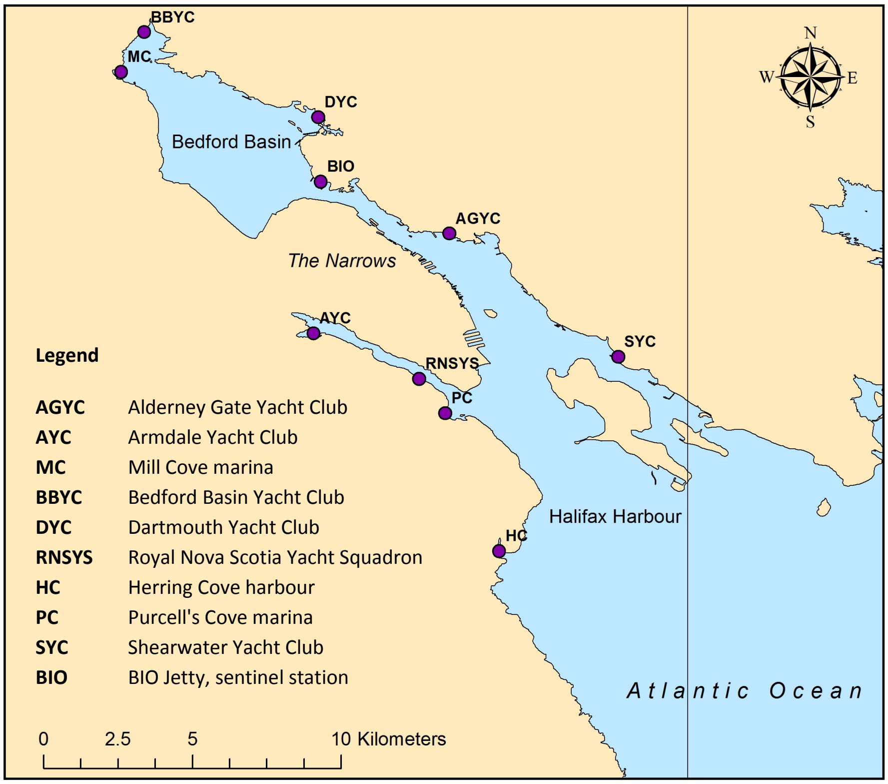

Figure 1. Location of the stations for collector-based monitoring surveys in the Halifax Harbour–Bedford Basin area in 2012 and 2013 (see Table 1. for stations ID).

Table 1. Station list for the collector-based surveys in the Halifax Harbour–Bedford Basin area in 2012 and 2013.

Location	ID	Type	Latitude	Longitude
Alderney Gate Yacht Club, Dartmouth	AGYC	Floating docks - marina	44.665234	-63.572023
Armdale Yacht Club , Halifax	AYC	Floating docks - marina	44.635147	-63.613114
Mill Cove, Bedford	MC	Floating docks - marina	44.714073	-63.671222
Bedford Basin Yacht Club, Bedford	BBYC	Floating docks - marina	44.725973	-63.664248
Dartmouth Yacht Club, Dartmouth	DYC	Floating docks - marina	44.700309	-63.611709
Royal Nova Scotia Yacht Squadron, Halifax	RNSYS	Floating docks - marina	44.621449	-63.581185
Herring Cove harbour, Herring Cove	HC	Breakwater - Fishing/recreational	44.569433	-63.556981
Purcell's Cove marina, Purcell's Cove	PC	Floating docks - marina	44.611039	-63.57331
Shearwater Yacht Club, Shearwater	SYC	Floating docks - marina	44.628061	-63.521082
BIO Jetty Darmouth, Sentinel station	BIO	Floating docks - mixed usage	44.680951	-63.610861

1.2. Buoy and water sampling surveys

Two surveys were conducted in the Bedford Basin, one on Oct. 26, 2012 and one on Nov. 21, 2012 with a total of 44 stations (Appendix C) carefully examined for the presence of *S. clava* and other invasive biofouling species. The stations consisted of buoys, tire bumpers, channel markers, pontoons, wharves, pilings or breakwaters and associated ropes and chains. Water temperature was recorded throughout the survey. Water samples (150 L filtered through a 63 µm mesh sieve) were taken in triplicate at selected stations where *S. clava* was present (or suspected). These samples were either frozen immediately for PCR-based assays, or for preserved in 10% formalin for microscopic examination and detection of *S. clava* larvae. Specimens were collected and frozen for examination and measurement.

1.3. Dive surveys

On Oct. 3, 2012, following the detection of *S. clava* individuals at BIO on PVC plates, a small scale rapid assessment, including snorkelling inspection around pontoons and wharves, was performed at the BIO site (water temperature 15^{o}C). The survey did not reveal additional individuals settled on other submerged structures. Further dive surveys took place in the spring of 2013 to target specific sites: Wright's Cove on May 1 and Dartmouth Yacht Club (DYC) and Wright's Cove on June 27. Natural (bottom and rocky outcrops) and artificial (chains, moorings) substrates were surveyed. Level of infestation was evaluated *in situ* and specimens were collected and preserved or frozen.

2. Results and Discussion

This rapid assessment was performed following the initial detection of a few juvenile *S. clava* individuals on experimental and monitoring PVC plates at BIO in (Moore et al. 2014). The detection in October 2012 represented the first report of *S. clava* in Nova Scotian waters, and in Maritime waters outside of PEI where this species has been present since 2008 (Locke 2009) and restricted to the island so far. The stations established for the collector-based survey, the buoy surveys and the dive surveys at BIO, Wright's Cove and the entrance to the Dartmouth Yacht Club covered widespread areas of the 24 km x 4 km Halifax Harbour–Bedford Basin area. These survey tools targeted a variety of artificial and natural submerged structures where larvae could attach and grow.

2.1. Presence of *Styela clava* in Halifax Harbour – Bedford Basin

2006 - 2011 surveys

Several stations used in this study have been monitored previously (Sephton et al. 2011, 2014), and *S. clava* was not detected on biofouling monitoring collectors at any of these stations between 2006 and 2011 (Table 2).

Table 2. Locations of AIS biofouling monitoring, and detections of *S. clava* in Halifax Harbour-Bedford Basin; 2006-2011. ND = not detected.

	Year					
Location	2006	2007	2008	2009	2010	2011
AYC	ND	-	-	-	-	-
BBYC	ND	-	-	-	-	-
RNSYS	ND	-	-	-	ND	-
BIO (sentinel)	ND	ND	ND	ND	ND	ND

2012 surveys

<u>Biofouling Collectors</u>

Styela clava was present on biofouling monitoring collectors deployed at the BIO sentinel station in 2012 (Figure 2). Collectors deployed in October and retrieved in November 2012 showed no settlement of *S. clava* at any location in the area. Water temperature was above 10^{o}C (unpublished data) and although *S. clava* larvae have been observed in water samples at temperature as low at 11^{o}C in PEI (Bourque et al. 2007), they may not be able to settle or successfully metamorphose below 11^{o}C. It is also possible that the six week soak time was insufficient for successful development of biofilm and tunicate settlement, although small individuals of other biofouling species were noted.

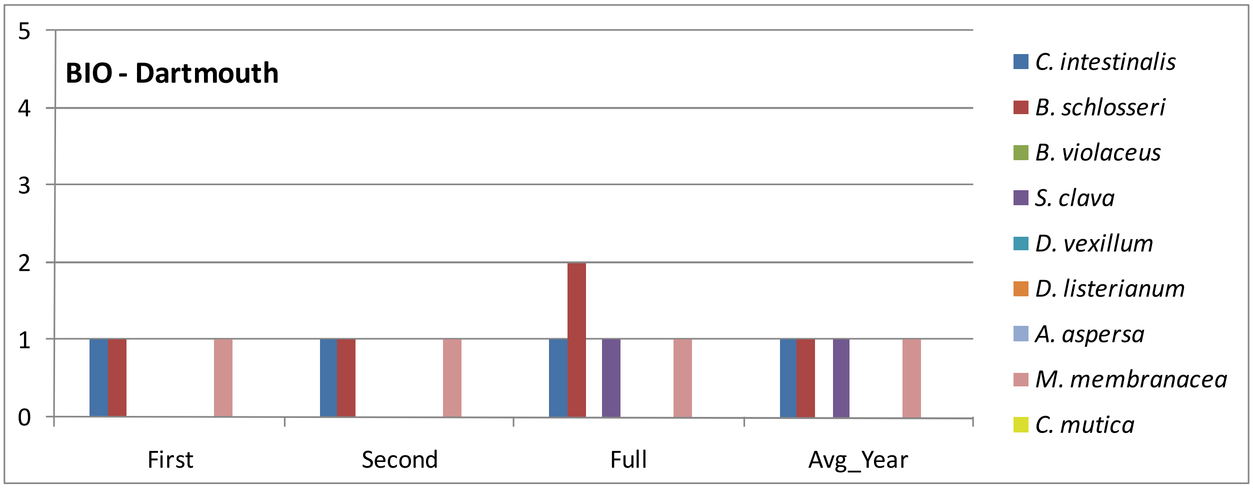

Figure 2. Average percentage cover category for nine targeted NIS at the BIO station for each deployment period (first, second and full) and average for the year 2012.

Buoy Survey

During the October-November buoy survey, *S. clava* was found at 7 of 44 stations (Figure 3 and Appendix C). These stations were all located in the southeast corner of the Bedford Basin, in three separate areas; BIO, at the entrance to and within Wright's Cove, and at the Dartmouth Yacht Club. Water samples were obtained on Oct. 26 at BIO (two stations), in Wright's Cove (one station) and in front of the DYC (three stations) where *S. clava* individuals were present or suspected to be present (Appendix C). All six water samples tested negative for *S. clava* using the STYCLAV18S PCR assay (Dr. S. Stewart-Clark, Dalhousie University). Larvae were likely rare or even absent this late in the season.

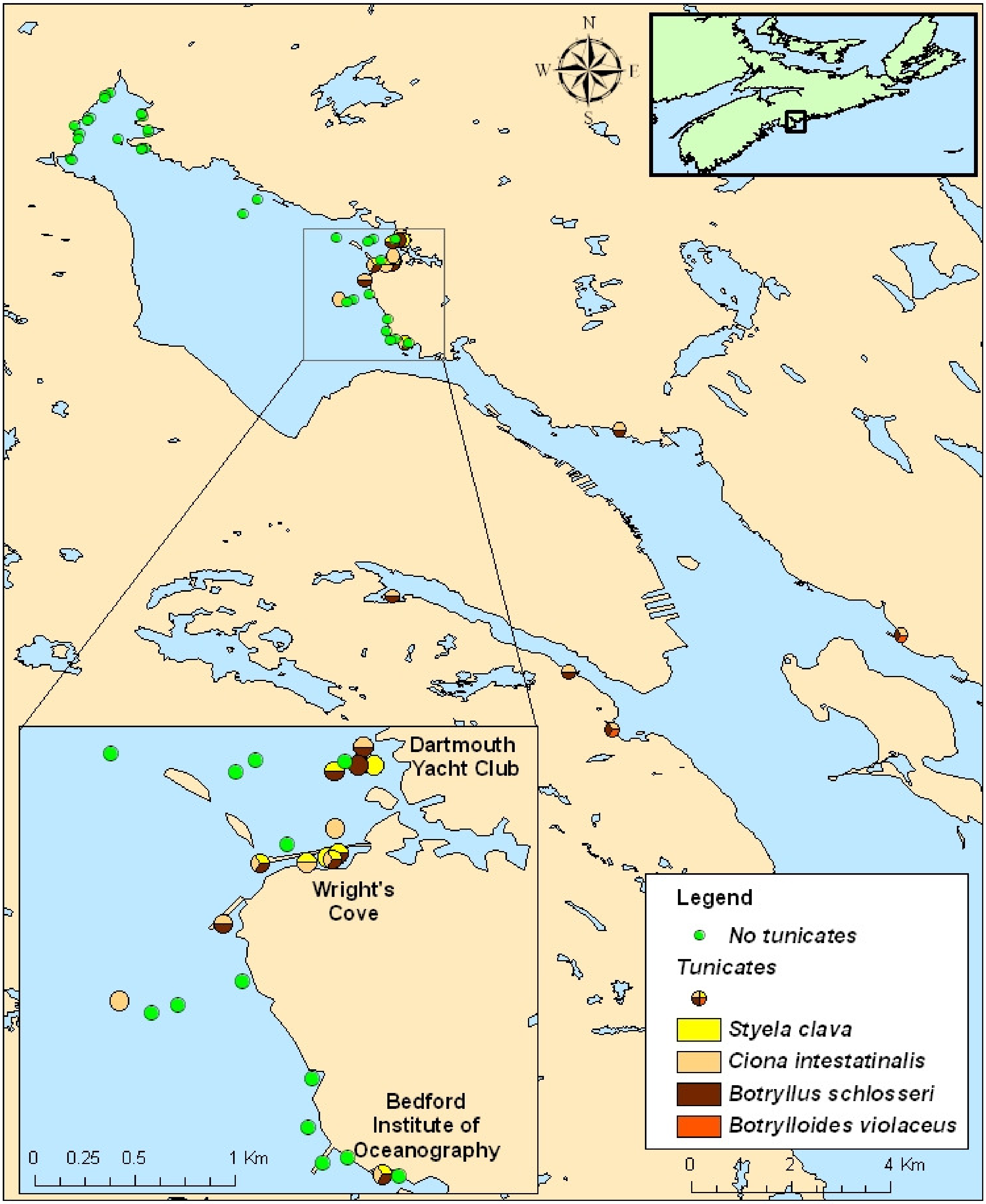

Figure 3. Buoy survey as part of the Rapid Assessment of Halifax Harbour-Bedford Basin Oct. - Nov. 2012.

2013 surveys

Biofouling Collectors

During the Halifax Harbour–Bedford Basin collector-based survey in 2013, *S. clava* was found only at the BIO station (Figures 4 and 5). It was also detected at DYC on anchor lines and on the undersides of floating docks in an area where monitoring plates had been deployed.

Dive surveys

Hundreds of *S. clava* specimens were observed during the dive surveys in a small area in Wright's Cove and at the entrance of the DYC (Figure 6). In both cases, a few large individuals were generally found on rocks at the bottom while smaller individuals were found on the ropes or snuggled between the chain links of moorings and channel marker buoys.

2.2. *S. clava* population assessment in NS

The buoy and dive surveys of the Halifax Harbour–Bedford Basin area in the fall of 2012 and spring 2013 indicated a *S. clava* population that seemed to be limited to Wright's Cove, to the entrance of the DYC (plastic breakwater, ropes and chains of channel marker buoys) and to the few individuals found at BIO during this period (Figure 3). No individuals were observed at any other station in the Bedford Basin or at the stations surveyed at the entrance of the Halifax Harbour in the fall of 2012 or in 2013.

About 500 individuals were collected during the surveys and almost 60% of the individuals retrieved from the buoys originated from a single channel marker chain at the entrance of the DYC and were very small (2–18 mm in length) (Figure 7). The rest of the population included several large, mature (>85 mm) individuals from Wright's Cove. Wright's Cove could be a breeding area from which dispersal may have occurred, with larvae spreading north (towards DYC) and south (towards BIO) (Figure 7). Since *S. clava* individuals can live up to three years (Clarke and Therriault 2007), the few individuals sampled in Wright's Cove could have been present as early as 2010 or 2011. With a low (0.03) Sørensen-Dice's Similarity Index (QS)[1], the Halifax Harbour species community is highly different from the community in ballast waters or fouling vessel hulls (Sylvester et al. 2011). This implies that that the invasion risk is high for this harbour (Sylvester et al. 2011). The detection of *S. clava* in the harbour could be one of the initial points of

[1] QS= 2J/(H+P) where *H* and *P* are the number of species in samples from hull and port communities, respectively, and *J* is the number of species shared by the two communities.

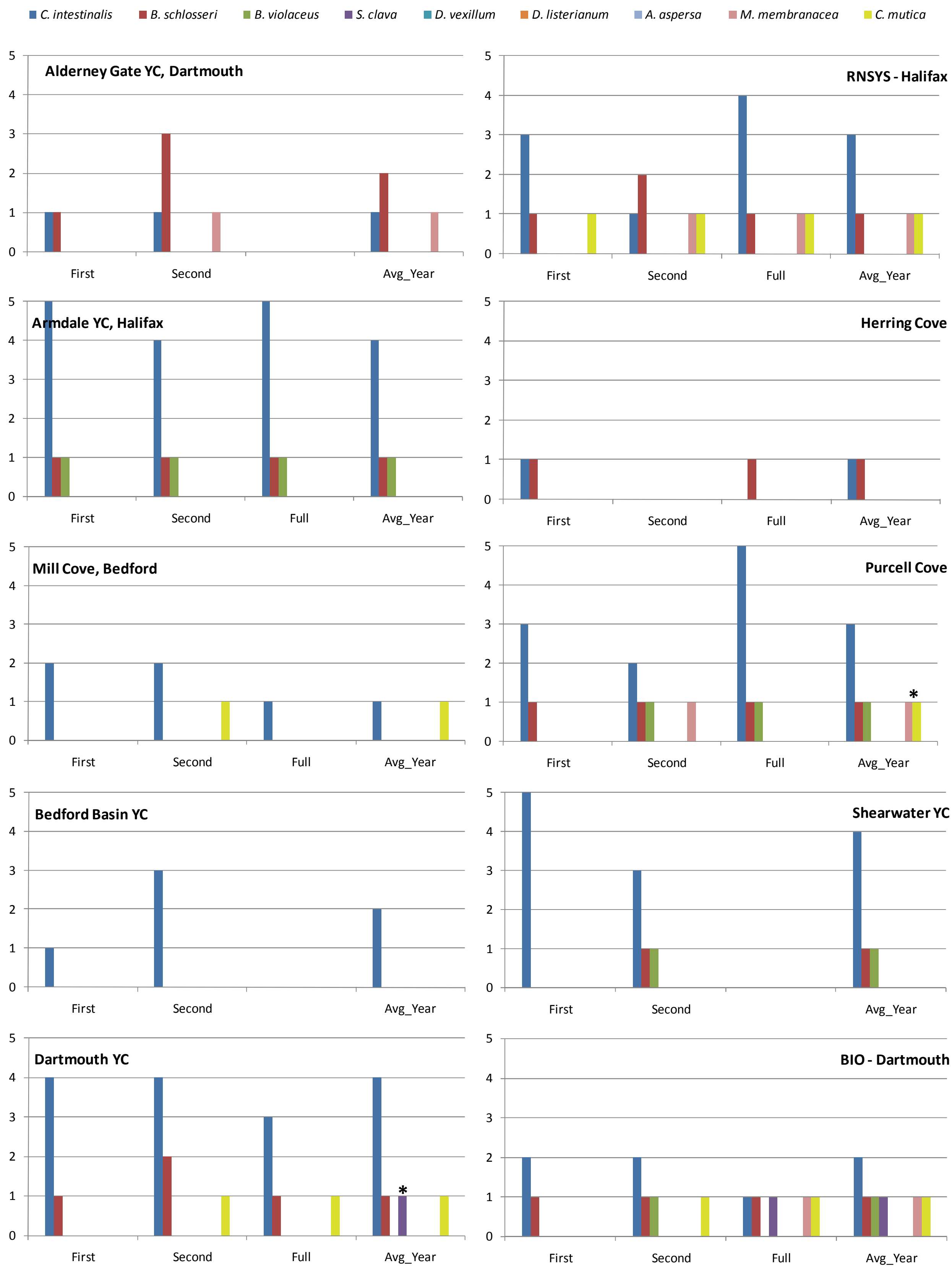

Figure 4. Average percentage cover category for nine targeted NIS at the 10 surveyed stations for each deployment period (first, second and full) and average for the year 2013. * indicates presence of the species on nearby structures.

Figure 5. *S. clava* individuals on PVC plates (BIO) in 2013. Photographs: B. Vercaemer.

Figure 6. S*tyela clava* individuals underwater at DYC location (left) and retrieved from chain and ropes during dive surveys (right). Photographs: B. Vercaemer.

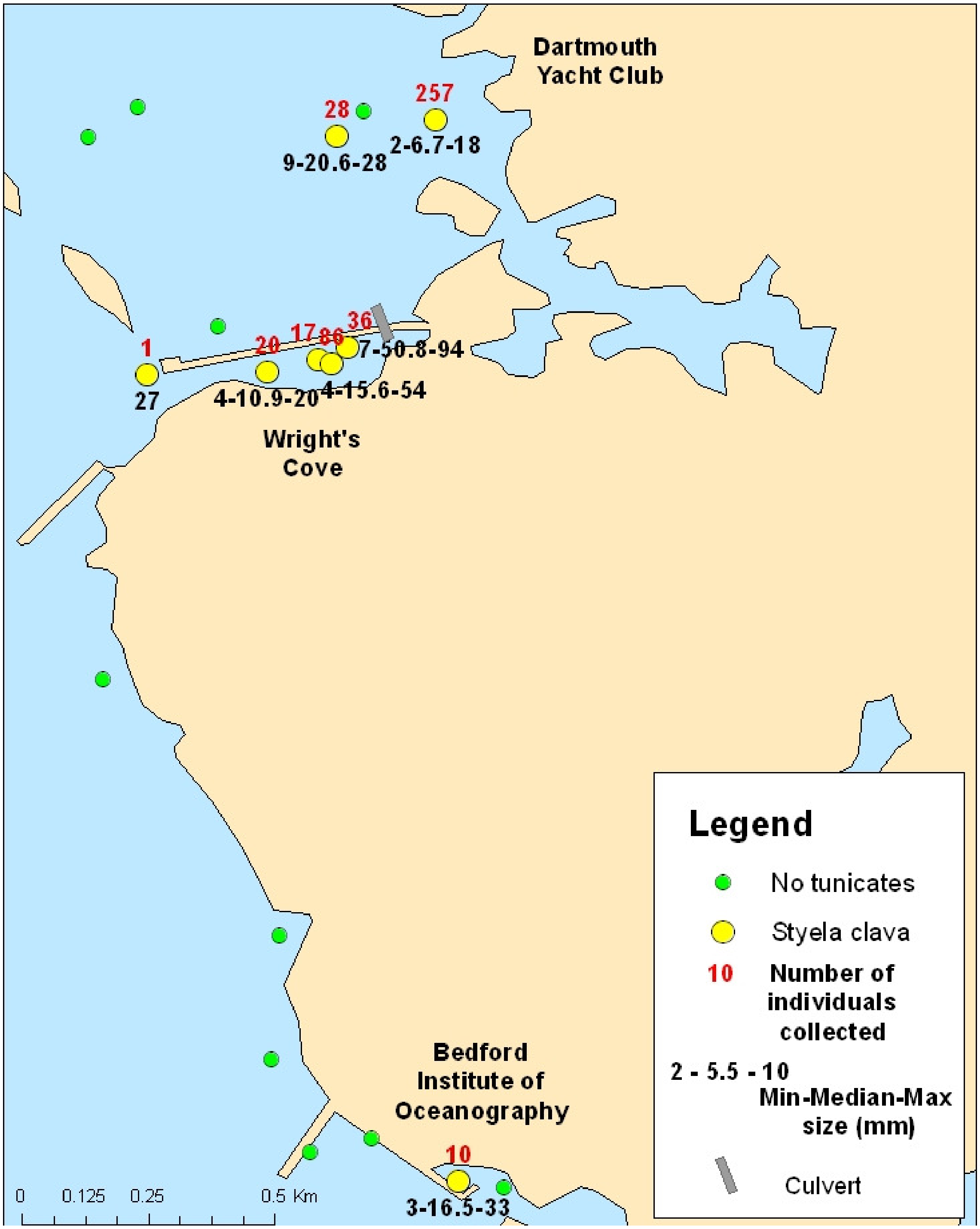

Figure 7. Characteristics of *S. clava* populations found on the buoys surveyed in Oct-Nov. 2012 The Bedford Institute of Oceanography (BIO) represents the original detection.

introduction for the species, which in turn suggests that hull fouling of commercial ships may be the likely vector.

Hull fouling (including sea-chest fouling) is considered as a stronger subvector compared to ballast water for the introduction of NIS (Hewitt at al. 2009, Sylvester et al. 2011, Frey et al 2014). Most of the foreign ship arrivals to the Halifax Harbour - Bedford Basin area come from the north eastern US and Western Europe where *S. clava* is present. Wright's Cove, in particular, has a private commercial and international wharf for shipping gypsum and other construction materials. In a 2007–2009 study of 20 transoceanic hull vessels in Halifax Harbour, Sylvester et al. (2011) found an average of 3.2 non-established NIS and 0.2 established NIS per vessel out of an average of 34 fouling species per vessel hull (including niche areas such as seachests). Although fouled recreational boats from PEI cannot be dismissed as a potential vector of *S. clava* introduction in the area, Darbyson et al. (2009) showed that the northeastern region of NS was the most at risk, not the south shore. Small recreational boats are, however, considered a risk factor for secondary spread of AIS (Locke et al. 2009).

Styela clava was also detected in Lunenburg Harbour on monitoring and research plates in October 2012 and since, in 2013 and in 2014 (Vercaemer et al. 2012; Moore et al. 2014). It has also been reported anecdotally in Pirate Harbour, Chedabucto Bay (S. Sorowka, St. Francis-Xavier University, pers. comm.). Although no individuals were detected on the AIS monitoring plates deployed at the Venus Cove station nearby in 2013, some individuals were observed on the underside of the wharf in August 2013. Furthermore, another anecdotal report (N. Filip, St. F-X University, pers. comm.) from the fall of 2013 confirmed that *S. clava* had spread in Chedabucto Bay with observations of individuals under floating docks of the Strait of Canso Yacht Club, Port Hawkesbury. *S. clava* is now reported on collector plates in 2014 at both stations (Venus Cove and Port Hawkesbury). While Lunenburg Harbour is mostly a fishing and recreational boating area, Chedabucto Bay, like Halifax Harbour, receives international traffic (at the Strait SuperPort of Mulgrave, Port Hawkesbury and Port Hastings). Chedabucto Bay with its high levels of commercial shipping (453 international ship arrivals in 2007; Lacoursiere-Roussel et al. 2012) is considered another hotspot for tunicate invasions (Lacoursiere-Roussel et al. 2012). A third historical hotspot of invasive biofouling organisms is the area around southwest Nova Scotia (Clancey and Hinton 2003, Clancey and MacLachlan 2004). An extensive Rapid Assessment conducted in September 2013 between Yarmouth and Shelburne did not detect *S. clava* in the area (Sephton et al., in prep).

2.3. Other fouling NIS

Three tunicate species, *C. intestinalis , B. schlosseri* and *B. violaceus*, the bryozoan *M. membranipora* and the amphipod C*aprella mutica* have been detected sporadically throughout the harbour and at nearby locations during AIS monitoring since 2006 (Appendix D). In 2012, both *C. intestinalis* and *B. schlosseri* were present on monitoring plates at the BIO sentinel station in 2012 (Figure 2). In the fall of 2012, *C. intestinalis* was present at 7 of 10 stations, *B. schlosseri* at 6 of 10 stations, with an anecdotal report from the Armdale Yacht Club (Drouin, pers. observ.) and *B. violaceus* was present at DYC, RNSYS and SYC (Table 3). These stations represented different areas of the harbour, with tunicates absent only in the Bedford Basin and Herring Cove areas. During the fall buoy survey (Figure 4), *C. intestinalis* and *B. schlosseri* were present at stations (buoys) at the entrance of the Bedford Basin (around BIO and DYC) and in the middle of the Harbour. Water temperature throughout the Bedford Basin averaged 9.8^{o}C during the buoy survey. No tunicates were detected further north in the Bedford Basin (Upper Basin) where salinities were low (25 psu) at that time of year. However *C. intestinalis* and *B. schlosseri* species have been detected on collectors with PVC plates in the past in the northern part of the basin (Sephton et al. 2011).

Table 3: Tunicate species present on monitoring collectors deployed in Halifax Harbour and Bedford Basin, fall 2012. P: present, ND: not detected.

Location	**ID**	***C. intestinalis***	***B. schlosseri***	***B. violaceus***
Alderney Gate Yacht Club	AGYC	P	P	ND
Armdale Yacht Club	AYC	P	ND	ND
Mill Cove	MC	ND	ND	ND
Bedford Basin Yacht Club	BBYC	ND	ND	NP
Dartmouth Yacht Club	DYC	P	P	P
RNSYS	RNSYS	P	P	P
Herring Cove	HC	ND	ND	ND
Purcell's Cove	PC	P	P	ND
Shearwater Yacht Club	SYC	P	P	P
BIO Jetty Sentinel station	BIO	P	P	ND

In 2013 *C. intestinalis* was present at all 10 stations monitored and *B. schlosseri* at 8 of 10 stations. The latter was not observed at the Bedford Yacht Club and Mill's Cove, the two further inside stations where freshwater influx is most important in spring and fall. *B.violaceus* was present at only 4 stations (Purcell's Cove, Armdale, BIO and Shearwater), representing different areas of the harbour. *Caprella mutica* and *M. membranacea* were sporadically observed on collector plates in various parts of the harbour and always at low levels (Figure 4). *C. intestinalis* is by far the dominant fouling species in the area with level of infestation reaching 4 or 5 at several stations (Figure 4). The infestation for these above species is similar in 2014, with the exception of an increase in the prevalence of *B. violaceus* (not reported here).

There was no detection of either *A. aspersa* or *D. listerianum* in the Halifax Harbour–Bedford Basin in 2012, 2013 or 2014, species present in Lunenburg Harbour in 2012 and 2013 and in 2012, respectively. Similarly the invasive red algae *Heterosiphonia japonica* (Savoie and Saunders 2013) detected in Mahone Bay near Lunenburg in 2012, was not detected in the Halifax Harbour–Bedford Basin in 2012, 2013 or 2014. However, the small orange-striped green sea anemone *Diadumene lineata* detected in 2013 at the BIO station on a research collector plate was also found on mussels on the sides of floats at both BIO and the DYC in 2014 (Moore et al. 2014). *Didemnum vexillum*, a highly invasive colonial tunicate, was also not observed in either the Halifax Harbour–Bedford Basin area or in Lunenburg as of 2014; the first record of this species in eastern Canada is in the Minas Basin and Minas Channel, upper Bay of Fundy (Moore et al. 2014).

Concluding remarks

A Rapid Assessment of the Halifax Harbour and Bedford Basin revealed a *S. clava* population that seems, both in the fall of 2012 and in 2013, to be limited to a small specific area of the Bedford Basin although many potential structures and habitats suitable for colonization were surveyed. However, it should be noted that the inability to detect *S. clava* at other sites does not necessarily mean that they are not present at those sites but rather, if they were present, that they could not be detected by the surveying tools used. Many *S. clava* individuals (< 1 year old) have been collected again at BIO in 2014 on submerged structures (e.g. mesocosm). A few individuals have been anecdotally reported as well in the fall 2014, south of BIO, by the MacDonald Bridge in the Narrows on the Dartmouth side.

As there can be significant lag times in detection, the actual date of introduction of *S. clava* in the Bedford Basin is unknown. The origin of the small population detected in the Bedford Basin is also unknown and only further genetic studies would indicate the most likely donor region. The relationship between invasions and shipping activity is not clear. In a study of 16 major shipping ports, Ruiz et al. (2013) found no relation between the number of vessel arrivals or the volume of ballast water discharge and the number of NIS. They attributed this result to the "high variation among vessels in the abundance and diversity of organisms associated with vessels' hulls and ballast tanks." To determine this variation for the port of Halifax, we would need to better characterize shipping activity: the geographic origin of vessels, the voyage conditions (which will affect survivorship of fouling and ballast biota) and the type of vessels and their operating characteristics (e.g., vessel speed, port residence time, antifouling practices).

This rapid assessment also confirmed the presence of *C. intestinalis, B. schlosseri* and *B. violaceus*, one bryozoan *M. membranipora*, and one amphipod *C. mutica* but could not detect the colonial tunicate *D. listerianum* or solitary tunicate *A. aspersa.* The latter was confirmed to be firmly established in the Lunenburg area (Vercaemer et al. 2012), which is connected to Halifax Harbour via high recreational vessel traffic. As this species seems to be highly invasive, at least in the Lunenburg area, it is important to be vigilant and continue the monitoring of the Halifax Harbour–Bedford Basin for its early detection. *C. intestinalis* could represent a strong competitor for substrate availability as it is the dominant fouling species in both the Halifax Harbour and the Bedford Basin. However, the present preliminary survey needs to be further validated in particular for the spread of *S. clava* in the Bedford Basin and the demarcation process is planned to continue, including snorkelling, dive or video surveys north and south of Wright's Cove and DYC in 2014 and further south in the Narrows and beyond.

The assessment protocols reported here include some of tools that facilitate the crucial demarcation step of the AIS Rapid Response protocol that DFO is currently developing for AIS. Tools will vary depending on each situation and environmental conditions. For example, the invasive colonial *D. vexillum* was recently detected in the upper Bay of Fundy (Moore et al. 2014) and different tools (e.g., scallop dredging) are being used for confirmation and delineation of the spread of this species because of the highly tidal environment. The assessment of the Halifax Harbour and Bedford Basin is ongoing and close monitoring of the spread and delineation of tunicates infestations will continue while providing some early detection tools for other NIS.

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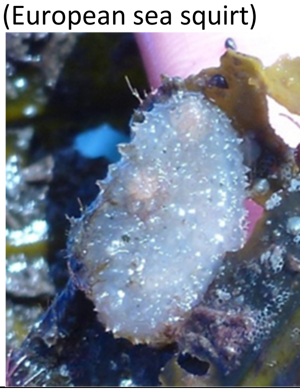
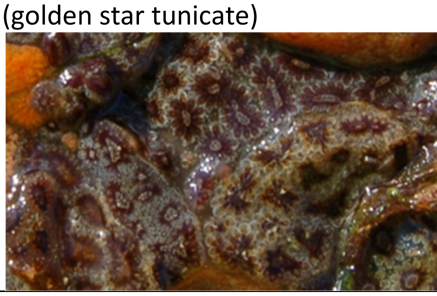
Appendix A. Deployment periods of biofouling monitoring collectors deployed in Halifax Harbour – Bedford Basin in 2012 and 2013. Deployment periods are indicated as First, Second (Sec), Full and Rapid Assessment (RA).

Location	2012 Date In	2012 Date Out	Soak (wk)	2013 Date In	2013 Date Out	Soak (wk)
BIO Jetty; AIS Sentinel Station	13 June (First) 28 Aug (Sec) 13 June (Full) 17 Oct (RA)	28 Aug (First) 19 Oct (Sec) 19 Oct (Full) 13 Nov (RA)	11 7 18 4	18 Jun (First) 20 Aug (Sec) 18 Jun (Full)	20 Aug (First) 06 Nov (Sec) 06 Nov (Full)	9 11 20
Alderney Landing Yacht Club	18 Oct (RA)	13 Nov (RA)	4	12 Jun (First) 21 Aug (Sec)	21 Aug (First) 31 Oct (Sec)	10 10
Armdale Yacht Club	18 Oct (RA)	13 Nov (RA)	4	20 Jun (First) 22 Aug (Sec)	22 Aug (First) 04 Nov (Sec)	9 11
Mill Cove	18 Oct (RA)	13 Nov (RA)	4	12 Jun (First) 21 Aug (Sec)	21 Aug (First) 25 Oct (Sec)	10 9
Bedford Basin Yacht Club	18 Oct (RA)	14 Nov (RA)	4	12 Jun (First) 21 Aug (Sec)	21 Aug (First) 25 Oct (Sec)	10 9
Dartmouth Yacht Club	18 Oct (RA)	13 Nov (RA)	4	12 Jun (First) 21 Aug (Sec)	21 Aug (First) 31 Oct (Sec)	10 10
Halifax; RNSYS	19 Oct (RA)	13 Nov (RA)	4	20 Jun (First) 22 Aug (Sec)	22 Aug (First) 04 Nov (Sec)	9 11
Herring Cove	22 Oct (RA)	13 Nov (RA)	4	20 Jun (First) 22 Aug (Sec)	22 Aug (First) 04 Nov (Sec)	9 11
Purcell's Cove	22 Oct (RA)	13 Nov (RA)	4	20 Jun (First) 22 Aug (Sec)	22 Aug (First) 04 Nov (Sec)	9 11
Shearwater Yacht Club	22 Oct (RA)	7 Nov (RA)	3	12 Jun (First) 21 Aug (Sec)	21 Aug (First) 31 Oct (Sec)	9 10

Appendix B. Characteristics of AIS monitored during the Rapid Assessment Survey.

Latin name (common name)	First occurrence in NS South Shore	Description and characters distinguishing from similar species
Solitary tunicates		
Ciona intestinalis (vase tunicate) 	1850's (also present in new England (from Northern Europe)	• Size: up to 15 cm in length • Shape/Texture: cylindrical body, unstalked with a translucent, soft and smooth tunic • Colour: varies from light greenish yellow to orange or pink • Ecology: Solitary, but may grow in very dense clumps;
Styela clava (clubbed tunicate) 	2012 (Lunenburg, Bedford basin, NS, also present in new England and present in the Gulf of St. Lawrence since 1997) (from Pacific West)	• Size: up to 18 cm in length, the stalk accounting for one third of its length • Shape/Texture: shaped like a water-fi lled wineskin, firm body, tunic wrinkled with little bumps, leathery texture, solid stalk • Colour: brown • Ecology: solitary, but may grow in very dense clumps;
Ascidiella aspersa (European sea squirt) 	2012 (Lunenburg area, NS, present in Gulf of Maine) (from Europe)	•Size: up to 5 cm, egg shaped, •Shape/Texture: egg shaped, one siphon at top, one on side separated by 1/3 of body length, firm, bumpy, grayish, semi-translucent tunic • Ecology: solitary
Colonial tunicates		
Botryllus schlosseri (golden star tunicate) 	1950's (cosmopolitan)	• Size: zooids 1 to 2 mm in length • Shape/Texture: colonies soft, smooth and fleshy, zooids that make up colonies daisy shaped • Colour: variable; orange, yellow, red, greenish-black, violet, dark grey or black • Ecology: dense, mat-like colonies of several microscopic individuals, called zooids

Botrylloides violaceus (violet tunicate) 	1990's (from Asia)	• Size: zooids 2 to 4 mm in length • Shape/Texture: colonies soft, smooth and fleshy, zooids arranged in a network of curving tracks • Colour: variable; whitish, yellow, orange, reddish-brown, violet • Ecology: dense, mat-like colonies of several microscopic individuals, called zooids
Didemnum vexillum (pancake batter tunicate) 	2013 Parrsboro, Bay of Fundy, NS Present in Maine and on US side of Georges' bank (from Japan)	• Size: zooids 1 to 2 mm in length • Shape/Texture: dense colonies that resemble pancake batter • Colour: variable; white, cream, yellow or light brown • Ecology: colonies form thin, encrusting sheets, gelatinous carpets or hanging lobes
Diplosoma listerianum (compound sea squirt) 	2008 (Iles de la Madeleine) 2012 (Lunenburg, NS) (also present in New England) (from North Europe)	• Size: zooids 2 mm in length • Shape/Texture: Dense colonies form thin, soft, gelatinous, translucent sheets that form fragile crusts which are hard to remove without tearing. Zooids are randomly grouped around large exhalent openings sometimes spotted with white dots • Colour: milky, darkish greyish/bluish
Amphipods		
Caprella mutica (Japanese skeleton shrimp) 	1990's (from East Asia)	• Size: Males up to 3.5 cm and females up to 1.5 cm in length • Shape/Texture: Long cylindrical body, Males with a long two-segmented neck, body very hairy on neck and claws; Females carry eggs in a ventral (belly) pouch, which is covered with dark red spots • Colour: variable from pale orange to red
Bryozoans		
Membranipora membranacea (coffin box, lacy crust bryozoan)	1992 (from Europe)	• Shape/Texture: colonies round, composed of many small, rectangular "cells", rough in texture • Colour: white • Ecology: dense, encrusting colonies made up of filter-feeding zooids

		
Cnidaria		
Diadumene lineata (orange-striped green sea anemone) 	2009 southern coast of NB 2013 Dartmouth, NS (from Japan, China, Hong-Kong)	• Size: 3/4" (2 cm) tall and 1/4" (5 mm) wide on average. • Shape/Texture: smooth, cylindrical body with 50-100 tentacles • Colour: olive-green or greenish-brown with distinctive orange or tan vertical stripes • Ecology: Can be very abundant in the fouling community on the sides of floats in marinas, reproduces asexually outside of Asia, very tolerant of a wide range of temperature, salinity and air exposure conditions
Alga		
Codium fragile spp. fragile (Codium, oyster thief, dead-man finger) 	1989 Mahone Bay (from Japan)	• Size: up to 90 cm in length • Shape/Texture: Shaped like a small bush, soft and velvety as a sponge to the touch. Thick, spongy Y-shaped cylindrical branches, 3 to 10 mm in diameter • Colour: Light to dark green
Heterosiphonia japonica 	2012 Blandford area (from Asian Pacific coast)	• Size: 5 to 15 cm • Shape/Texture: Highly branched • Colour: Brownish to bright red-orange

Appendix C. Rapid Assessment Station information (0: absent, 1: present).

Location	Description	Latitude	Longitude	*S. clava*	*C. intestinalis*	*B. schlosseri*
BIO Jetty *	4th Red bumper on Main Jetty, down from finger jetty	44.68167	-63.61242	0	0	0
BIO Jetty *	6th red bumper (middle), Finger jetty	44.68144	-63.61352	0	0	0
Jetty	right of Jetty, Buoy for BIO water intake line	44.68307	-63.61419	0	0	0
BIO Boat Launch	wharf, wooden	44.68523	-63.61405	0	0	0
NE side of Basin	Private wharf, close to BIO	44.68969	-63.6172	0	0	0
mid Basin	Buoy A; one of 3, closest to NE shore, yellow	44.68861	-63.62013	0	0	0
mid Basin	Buoy B; one of 3, middle, close to NE shore, yellow	44.68825	-63.62127	0	0	0
mid Basin	Buoy C; one of 3, farthest, from NE shore, red H42	44.6888	-63.62278	0	1	0
Wright's Cove	Concrete wharves, small, next to DYC	44.6923	-63.61808	0	1	1
Wright's Cove	2 Orange Buoys at head of Cove	44.69536	-63.61296	0	0	1
Wright's Cove *	pinkish buoy, with 2 small yellow floats	44.6953	-63.61337	1	0	0
Wright's Cove	Black Buoy, head of Cove	44.6955	-63.61285	1	0	1
Wright's Cove	Orange Buoy	44.69522	-63.61313	1	1	1
Wright's Cove	Buoy beyond small wharf	44.69507	-63.61428	1	1	0
Between Wright's Cove and DYC	Breakwater, wharf, Boat, South side	44.69503	-63.61641	1	1	1
Cove next to DYC	White marker, private North side of wharf	44.69586	-63.61515	0	0	0
Cove next to DYC *	Yellow Steel Buoy (Hfx Port Authority #422	44.69662	-63.61301	0	1	0
DYC *	Red float close to breakwater #1	44.69949	-63.61127	1	0	0
DYC	Red float, middle (third)	44.69949	-63.61197	0	0	1
DYC	Red float, fifth	44.69961	-63.61256	0	0	0

	(closest to mouth of harbour)					
DYC *	Artificial Breakwater	44.6992	-63.61304	1	0	1
DYC	Buoy	44.6997	-63.61657	0	0	0
DYC	Buoy: barrel	44.69916	-63.61746	0	0	0
BIO	Mesocosm floats	44.68082	-63.61006	0	0	0
Mid Basin	red marker buoy HY2	44.70000	-63.62317	0	0	0
Mid Basin	Navy mooring 22	44.70417	-63.64017	0	0	0
Mid Basin	DND white buoy	44.70683	-63.63750	0	0	0
Mid Basin	private mooring, yellow	44.71617	-63.65767	0	0	0
Mid Basin	pale pink to white with blue hook	44.71617	-63.65833	0	0	0
Mid Basin	private pontoon and red buoy	44.71583	-63.65833	0	0	0
Upper Basin	private mooring, white, blue hook, yellow fender	44.71767	-63.66267	0	0	0
Upper Basin	pink private mooring	44.71883	-63.65733	0	0	0
Upper Basin	pink buoy D ROY with yellow fender	44.71933	-63.65733	0	0	0
Upper Basin	small white buoy/fender	44.72183	-63.65817	0	0	0
Upper Basin	white mooring with green rope	44.72217	-63.65850	0	0	0
Upper Basin	green buoy	44.72233	-63.65850	0	0	0
Upper Basin	pink buoy, corner of BBYC	44.72550	-63.66500	0	0	0
Upper Basin	white buoy with blue ring	44.72517	-63.66517	0	0	0
Upper Basin	small pontoon	44.72167	-63.66783	0	0	0
Upper Basin	pink buoy	44.72100	-63.66817	0	0	0
Upper Basin	pink buoy	44.72017	-63.67050	0	0	0
Upper Basin	pale pink/white buoy DEWIS	44.71867	-63.66967	0	0	0
Upper Basin	big pink buoy facing Dewulf Park	44.71767	-63.66983	0	0	0
Mill Cove	pontoons	44.71400	-63.67117	0	0	0

* denotes water sampling stations

Appendix D. Presence of NIS at monitoring stations in Halifax Harbor – Bedford Basin from 2006 to 2011**.** Coverage categories are defined above. NM = not monitored, NR = not reported, ND = not detected, and P = present**.**

Species	Year	BIO	Armdale	BBYC	RNSYS	Sambro
C. intestinalis	2006	1	2	3	1	NM
	2007	1	NM	NM	NM	NM
	2008	1	NM	NM	NM	NM
	2009	2	NM	NM	NM	NM
	2010	0	NM	NM	1	NM
	2011	1	NM	NM	NM	5
B. schlosseri	2006	0	2	2	0	NM
	2007	0	NM	NM	NM	NM
	2008	1	NM	NM	NM	NM
	2009	1	NM	NM	NM	NM
	2010	0	NM	NM	0	NM
	2011	1	NM	NM	NM	0
B. violaceus	2006	0	0	0	0	0
	2007	0	NM	NM	NM	NM
	2008	0	NM	NM	NM	NM
	2009	0	NM	NM	NM	NM
	2010	0	NM	NM	0	NM
	2011	0	NM	NM	NM	1
C. mutica	2006	NR	NR	NR	NR	NR
	2007	NR	NM	NM	NM	NM
	2008	NR	NM	NM	NM	NM
	2009	NR	NM	NM	NM	NM
	2010	P	NM	NM	P	NM
	2011	ND	NM	NM	NM	P
M. membranipora	2006	NR	NR	NR	NR	NR
	2007	NR	NM	NM	NM	NM
	2008	NR	NM	NM	NM	NM
	2009	P	NM	NM	NM	NM
	2010	P	NM	NM	P	NM
	2011	ND	NM	NM	NM	P