Biofouling Monitoring for Aquatic Invasive Species (AIS) in DFO Maritimes Region, Nova Scotia: May – December 2010.

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by

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ABSTRACT

Sephton, D., J. Ouellette-Plante and B. Vercaemer. 2013. Biofouling monitoring for aquatic invasive species (AIS) in Nova Scotia; May - December 2010. Can. Tech. Rep. Fish. Aquat. Sci. 3034

The shellfish culture industry in Atlantic Canada has been impacted by the introduction, spread and increases in populations of non-indigenous, fouling tunicates since the mid-1990's. Five species of tunicates are of present concern in the region: *Ciona intestinalis, Botryllus schlosseri, Botrylloides violaceus, Styela clava* and *Didemnum vexillum*. A surveillance and monitoring program for tunicates was initiated by the Department of Fisheries and Oceans in the spring of 2006, and continues to operate annually with the participation and support of community groups, academia and other levels of government. Geo-referenced monitoring stations were selected along the coast of Nova Scotia (NS) based on the presence of vectors (risk factors) for tunicate arrival or establishment. Monitoring collectors (plates) were deployed at 33 stations in 2010 during 3 deployment periods; first (May - August), second (August - October) and full (May – October). Water temperature, salinity and oxygen content were measured at each deployment and collection, and various surfaces were examined for tunicate fouling and the presence of other invasive species. Ten anecdotal reports of tunicate presence were received in 2010. Four additional AIS were added to the program's watch list in 2010: two tunicates; *Diplosoma listerianum* and *Ascidiella aspersa*, an amphipod; *Caprella mutica* and a bryozoan; *Membranipora membranacea*.

Styela clava, D. vexillum, D. listerianum and *A. aspersa* were not detected in 2010. *Ciona intestinalis* was found at 18 of 33 monitoring stations, and reported from 3 locations. *Botryllus schlosseri* was the most wide-spread species; present at 27 of 33 monitoring stations, and reported anecdotally at 2 locations. *Botrylloides violaceus* was found on 14 of 33 monitoring stations, and reported anecdotally at 2 locations. Five stations were free of tunicates, and they were not present at 4 locations reported anecdotally. All 3 previously mentioned tunicate species were present at 13 stations. The 3 species of tunicates reported in Nova Scotia were found in waters with 3.5 – 23.4 ^{o}C temperature, 23.9 – 32.6 salinity, and 4.58 – 11.83 mg L^{-1} dissolved oxygen.

Ciona intestinalis dominated (moderate to heavy) the biofouling community on collector plates at many stations in southwestern NS, along the south shore, in Chedabucto Bay and in coastal Cape Breton. *Botryllus schlosseri* was the dominant tunicate in the Bras D'or Lake, (moderate to very heavy), but it was found (low to moderate) throughout the province. *Botrylloides violaceus* was recorded as a low fouler at many locations, including two first records: Digby and Sober Island (eastern

shore). Only part of the north shore of Nova Scotia (Merigomish to Malagash) was tunicate free in 2010.

Caprella mutica and *M. membranacea* were present throughout Nova Scotia in 2010; at 9 and 23 of 33 monitoring stations, respectively.

RÉSUMÉ

Sephton, D., J. Ouellette-Plante and B. Vercaemer. 2013. Surveillance de biosalissures pour les espèces aquatiques envahissantes (EAE) dans la région de Maritimes de DFO, Nouvelle-Écosse :mai-décembre 2010. Can. Tech. Rep. Fish. Aquat. Sci. 3034

L'industrie de la conchyliculture dans la région de l'Atlantique a été touchée par l'apparition, la propagation et la surpopulation des tuniciers non- indigènes, depuis le milieu des années 1990. Cinq espèces de tuniciers sont préoccupantes actuellement dans la région: *Ciona intestinalis, Botryllus schlosseri, Botrylloides violaceus, Styela clava* et *Didemnum vexillum*. Un programme de surveillance et de suivi des tuniciers a été initié par le ministère des Pêches et des Océans, au printemps 2006, et continue à opérer chaque année avec la participation et le soutien des groupes communautaires, des universitaires et d'autres niveaux de gouvernement. Des stations de surveillance géo-référencées ont été sélectionnées le long de la côte de la Nouvelle-Écosse selon la présence de vecteurs (facteurs de risque) pour l'arrivée ou l'établissement de tuniciers. Des collecteurs de surveillance (plaques) ont été déployés dans 33 stations en 2010 durant 3 périodes de déploiement, première partie (Mai - Août), deuxième partie (Août - Octobre) ou saison pleine (Mai - Octobre). La température de l'eau, la salinité et la teneur en oxygène ont été mesurées à chaque déploiement et collecte et les biosalissures ont été examinées sur différentes surfaces pour détecter la présence de tuniciers et d'autres espèces envahissantes. Dix rapports anecdotiques d'observation de tuniciers ont été reçus en 2010. Quatre autres EAE ont été ajoutées à la liste de surveillance du programme en 2010: deux tuniciers; *Diplosoma listerianum* et *Ascidiella aspersa*, une crevette; *Caprella mutica* et un bryozoaire; *Membranipora membranacea*.

Styela clava, D. vexillum, D. listerianum et *A. aspersa* n'ont pas été détectés en 2010. *Ciona intestinalis* a été retrouvé à 18 des 33 stations de surveillance, et signalé dans 3 endroits. *Botryllus schlosseri* est l'espèce la plus répandues, présente à 27 des 33 stations de surveillance, et rapportée à 2 endroits. *Botrylloides violaceus* a été trouvé sur 14 des 33 stations de surveillance, et signalé dans 2 endroits. Cinq stations étaient libres de tuniciers, et ils n'étaient pas présents à 4 des endroits signalés anecdotiquement, tandis que les 3 espèces de tuniciers étaient toutes présentes dans 13 stations. Les 3 espèces de tuniciers présentes en Nouvelle-Écosse ont été trouvés dans des eaux de 3,5 – 23,4 °C de temperature, 23.9 à 32,6 de salinité, et de 4,58 à 11,83 mg L-1 d'oxygène dissous.

Ciona intestinalis domine (de façon modérée à forte) la communauté des biosalissures sur les plaques dans de nombreuses stations du sud-ouest Nouvelle-Écosse, le long de la rive sud, dans la baie Chedabucto et dans la région côtière du

Cap-Breton. *Botryllus schlosseri* était l'ascidie dominante dans le lac Bras d'Or, (modérée à très forte), mais il a été observé (faible à modérée) dans toute la province. *Botrylloides violaceus* a été observé en tant que faible biosalissure en de nombreux endroits, y compris les deux premiers endroits où il a été détecté: Digby et Sober Island (la côte d'est) . Seule une partie de la rive nord de la Nouvelle-Écosse (Merigomish à Malagash) a été libre de tuniciers en 2010.

Caprella mutica et *M. membranacea* étaient présents tout le long de la Nouvelle-Écosse en 2010; à 9 et 23 des 33 stations de surveillance, respectivement.

1.0 INTRODUCTION

Non-indigenous species (NIS) may pose great risk to native species, and biodiversity, and can negatively impact native ecosystems and their function (Sala et al. 2000). Among NIS of global concern, ascidians, more commonly known as tunicates or sea-squirts, have affected marine ecosystems in the past two decades due to their negative impacts on native species (Lambert 2001; Lutz-Collins et al. 2009) and communities (Blum et al. 2007; Dijkstra et al. 2007b). Invasive tunicates may quickly dominate marine fouling communities following their establishment in new environments (Lambert and Lambert 1998, 2003). They pose a serious threat to shellfish aquaculture operations, as they can attach to and overgrow aquaculture gear and bivalves (Carver et al. 2003; Bullard et. al. 2007). This results in increased operation and production costs (MacNair et. al. 2006) as tunicates must be removed from culture gear and from shellfish during harvesting and processing. Infestations of non-native tunicates have resulted in significant economic losses to the blue mussel, *Mytilus edulis* (Linnaeus, 1758), aquaculture industry in Prince Edward Island (PEI) and Nova Scotia (NS) since the mid 1990's (Boothroyd et al. 2002; Clarke and Therriault 2007; Howes et al. 2007; Ramsay et al. 2008).

Four species of non-native, fouling tunicates are now firmly established in Atlantic Canada. The solitary clubbed tunicate, *Styela clava* (Herdmann, 1881) has been present in PEI since 1997 (Locke et al. 2007), but has not yet been recorded in NS (Sephton et al. 2011). Another solitary tunicate species, the vase tunicate, *Ciona intestinalis* (Linneaus, 1776), may have been present in Atlantic Canadian waters since the 1850's (Stimpson 1852). Heavy infestations, however, were first noted on a mussel farm in the Lunenburg-Mahone Bay area in 1997 (Cayer et al. 1999; Carver et al. 2003), and the species is now widespread in NS (Carver et al. 2006a; Sephton et al. 2011). It has been present in PEI since 2004 (Locke et al. 2009) and is now considered the predominant threat to mussel aquaculture there (Ramsay et al. 2008). Vase tunicates have recently been reported for the first time in Placentia Bay, Newfoundland (Sargent et al. 2013). Two colonial species, the golden star tunicate *Botryllus schlosseri* (Pallas, 1766) and the violet tunicate, *Botrylloides violaceus* (Oka, 1927) have been present in PEI for about a decade (Locke et al. 2009), and in NS for a decade or more (Carver et al. 2006b). *Botryllus schlosseri* was first reported in Newfoundland in 1975 (Hooper 1975), and surveys in 2006 and 2007 revealed the presence of both colonial species (Callahan et al. 2010). A fifth species of concern, the colonial pancake batter tunicate, *Didemnum vexillum* (Kott, 2002), is present nearby on the American side of George's Bank (Bullard et al. 2007; Valentine et al. 2007) and as far north as Eastport, Maine (Martin et al. 2010). Two additional species are also now recognized as potential invaders of Atlantic Canadian waters (Locke 2009): the compound sea squirt *Diplosoma listerianum* (Milne-Edwards, 1841), present in the Gulf of Maine (Dijkstra et al. 2007a) and sporadically in the Magdalen Islands, Quebec, since 2008 (N. Simard, pers. comm.) and the European sea squirt *Ascidiella aspersa* (Muller, 1776) now present in the Gulf of Maine (Dijkstra et al. 2007a).

In response to the growing threat posed by tunicates and other invasive species to native coastal communities, fisheries and shellfish aquaculture in Atlantic Canadian waters, Fisheries and Oceans Canada developed and initiated an Aquatic Invasive Species (AIS) Biofouling Monitoring Program in the spring of 2006. A description of the DFO Maritimes Region Program, and results of monitoring from 2006 to 2009, is given in Sephton et al. (2011). Two non-tunicate biofouling species; the Japanese skeleton shrimp, *Caprella mutica* (Schurin, 1935) and the lacy-crust bryozoan, *Membranipora membranacea* (Linnaeus, 1767) were added to the program in 2009. Here we report on the monitoring for invasive biofouling species conducted in the DFO Maritimes Region, NS between May and December 2010.

2.0 MATERIALS AND METHODS

2.1. STATION SELECTION

Coastal and inland (Bras D'Or Lake) general monitoring stations were selected based on the presence of potential or existing "risk factors" for the introduction and spread of tunicate species. These included; (1) presence of shellfish or mussel processing facilities, (2) mussel or shellfish aquaculture sites, (3) important commercial port with international traffic, (4) marinas or yacht clubs with US traffic, (5) commercial fishing harbours, and (6) harbours with herring or US lobster processing facilities. Sites with air-exposure at low tide were not included. Thirty-three geo-referenced stations were monitored in 2010 (Table 1). Of these, 27 stations were monitored in 2009, one (Stn 420) was not and 5 (Caribou, Dingwall Harbour, Aspy Bay-North Harbour, LaHave River and Eel Lake) were new monitoring stations to the Program, added at the request of the shellfish aquaculture industry.

2.2. MONITORING COLLECTORS

Monitoring collectors used in 2010 are shown in Figure 1. Collectors were constructed by suspending three, 10 cm X 10 cm, lightly sanded PVC plates from a rope line. The distance from the top to the bottom plate was about 1 m. The

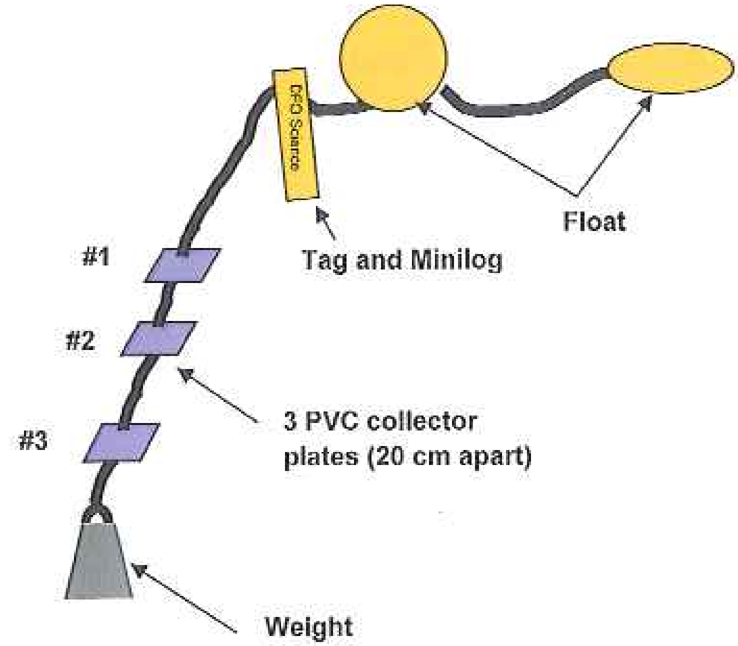

Figure 1: AIS plate collector used in biofouling monitoring in 2010.

Table 1: Aquatic Invasive Species Monitoring Stations, 2010

Stn. No.	Region	Location	Latitude °N	Longitude °W	Station Description and Deployment Structure
1	SW shore	Digby	44.63015	-65.75233	Royal Western Nova scotia Yacht club marina, large, mixed-use port, floating dock
2	SW shore	Meteghan	44.19365	-66.16688	Medium fishing and processing port, public wharf, floating dock
4	SW shore	Yarmouth Bar	43.81648	-66.14785	Medium fishing and processing port, public wharf, floating dock
6	SW shore	Wedgeport	43.71370	-65.96947	Medium fishing and processing port, public wharf, floating dock
7	SW shore	Camp Cove	43.72360	-65.84057	Medium fishing and processing port, public wharf, floating dock
108	SW shore	Eel Lake	43.82670	-65.90770	Shellfish aquaculture site, lines, buoys, oyster cages
8	SW shore	Clark's Harbour	43.44470	-65.63510	Medium fishing and processing port, public wharf, floating dock
10	SW shore	Ingomar	43.56273	-65.36245	Small fishing and processing port, public wharf, floating dock
12	S shore	Shelburne	43.75750	-65.32200	Shelburne Yacht Club marina, medium, mixed-use port, floating dock
82	S shore	Port Mouton	43.91940	-64.84340	Small fishing and processing port, public wharf
100	S shore	LaHave River	44.31160	-64.40830	LaHave River Yacht Club, marina, floating dock
19	S shore	Indian Point	44.45598	-64.30683	Indian Point Marine Farms (IPMF), shellfish aquaculture site, mussel lines

Table 1: Aquatic Invasive Species Monitoring Stations, 2010, continued.

Stn. No.	Region	Location	Latitude °N	Longitude °W	Station Description and Deployment Structure
21	S shore	Chester	44.53780	-64.23840	Medium, mixed-use port, shellfish aquaculture area, public wharf, floating dock
24	Halifax	Halifax; BIO Jetty	44.68187	-63.61187	Large, international, mixed-use port, government wharf at Bedford Institute of Oceanography, floating dock
402	Halifax	Halifax; Royal NS Yacht Squadron	44.62142	-63.58022	Royal NS Yacht Squadron marina, floating dock
41	Chedabucto	Venus Cove	45.61530	-61.39010	Venus Cove Marine Park marina, small fishing and recreational port near large, international port, public wharf, floating dock
47	Bras d'Or Lake	St. Peter's	45.66115	-60.87440	St. Peter's Lion's Club marina, entrance to Bras d'Or Lake, floating docks
51	Bras d'Or Lake	Whycocomagh	45.98640	-61.11260	Small recreational harbour, shellfish aquaculture area, public wharf, floating dock
52	Bras d'Or Lake	Orangedale	45.90070	-61.08955	Small recreational harbour, shellfish aquaculture area, public wharf, floating dock
86	Bras d'Or Lake	East Bay	46.01360	-60.38920	Aquaculture Education site, buoys
54	Bras d'Or Lake	Eskasoni	45.95570	-60.58530	Unama'ki Institute of Natural Resources, small harbour, private wharf
55	Bras d'Or Lake	Baddeck	46.09990	-60.74752	Medium recreational harbour, public wharf, floating dock
58	CB Coastal	Louisbourg	45.91798	-59.98935	Medium mixed-use port, public wharf, floating dock

Table 1: Aquatic Invasive Species Monitoring Stations, 2010, continued.

Stn. No.	Region	Location	Latitude °N	Longitude °W	Station Description and Deployment Structure
62	CB Coastal	Sydney	46.13993	-60.16775	Royal Cape Breton Yacht Club marina, near US and International cruise ship port, floating dock
63	CB Coastal	North Sydney	46.20680	-60.24900	Large, mixed-use port, near Newfoundland Ferry Terminal, public wharf, floating dock floating dock
69	CB Coastal	Dingwall	46.90320	-60.46040	Small fishing port, shellfish aquaculture area, public wharf
420	CB Coastal	Dingwall Harbour	46.90100	-60.46500	Small fishing port, shellfish aquaculture area, private wharf
71	CB Coastal	Cheticamp	46.62680	-61.01597	Large, mixed-use port, NB, PEI and Quebec boat traffic, public wharf
73	CB Coastal	Mabou	46.08573	-61.46442	Small fishing port, shellfish aquaculture area, public wharf, floating dock
77	N shore	Pictou	45.67440	-62.71180	Pictou Yacht Club marina, medium, mixed use port, floating docks
91	N shore	Caribou	45.72980	-62.65760	Small fishing port, ferry traffic to and from PEI nearby, public wharf, floating dock

Abbreviations: SW = southestern, S = south, CB = Cape Breton, N = north, NB = New Brunswick, PEI = Prince Edward Island.

line was weighted with a 250 g barrel weight to ensure that the rope hung vertically in the water column. A label with contact information for the DFO Maritimes Region AIS Monitoring Program was attached to each collector. The depth of the top collector plate was approximately 1 m.

2.3. MONITORING PROTOCOL

Twenty-five stations were monitored by staff from DFO Science Branch, Maritimes Region, Coastal Ecosystem Research Division. Additional stations were monitored by: Clean Annapolis River Project (CARP: Digby, Station 1), Eskasoni Fish and Wildlife Commission (EFWC: Whycocomagh, Station 51; Orangedale, Station 52 and Eskasoni, Station 54), Cape Breton University, Department of Biology (CBU: Louisburg, Station 58; Sydney, Station 62 and North Sydney, Station 63) and DFO, Eastern Nova Scotia Area Office, Conservation and Protection Branch (East Bay, Station 86). The Nova Scotia Department of Fisheries and Aquaculture (NSDFA) provided anecdotal information from several shellfish aquaculture sites (Table 2).

Table 2: Location of anecdotal reports in 2010.

Station No.	Region	Location	Latitude °N	Longitude °W	Station Description
424	South shore	Port L'Hebert	43.75500	- 64.95910	Shellfish lease
421	Eastern shore	Sober Island	44.84000	- 62.47000	Oyster lease
104	Eastern shore	Country Harbour	45.23220	- 61.75900	Mussel lease
36	Eastern shore	Whitehead	45.23297	- 61.15875	Mussel lease
44	Chedabucto	Petit-de-Grat	45.5071	- 60.96010	Fishing harbour
48	Bras d'Or	Gillis Cove	45.91100	- 61.05390	Oyster lease
423	Bras d'Or	Alba	45.92400	- 60.99250	Oyster lease
95	Cape Breton Coastal	Aspy Bay, South Harbour	46.88309	- 60.46551	Oyster lease
112	North shore	Merigomish	46.65000	- 62.54000	Shellfish lease
422	North shore	Malagash	45.75000	- 63.35000	Oyster lease

Two collectors (Figure 1) were deployed (hung) with the top plate approximately 1 m below the water surface at each site in late May to mid-June and collected in late October to mid-November to assess full season colonization (full deployment period) on collector plates. Collectors deployed on shellfish aquaculture leases were hung at the depth of the mussel socks: 5m at Indian Point (Station 19) and at Aspy Bay-North Harbour (Station 94) or on oyster cages at Eel Lake (Station 108). Collectors were deployed in such a way that various areas of the station were sampled. For example, 2 floating docks would be selected for collector deployment at a station with several floating docks; one near the harbor entrance and one closer to shore, and collectors would be hung on opposite sides and opposite ends of each dock. In order to determine seasonal differences in colonization at each site, two additional collectors were deployed in late May and collected in mid-August (first deployment), while two more were deployed in mid-August and collected in late October (second deployment). Dates and details of 2010 collector deployment for all stations are shown in Appendix 1.

A Garmin eTrex Unit (Garmin International, Inc., Olathe, Kansas, USA) was used to determine or verify the geo-referenced position of each monitoring station and photographs of new stations were taken in May. Temperature (^{0}C), salinity, conductivity (mS cm^{-1}) and oxygen content (% saturation and mg L^{-1}) were measured at collector depth using a YSI 85 or YSI 6600 Sonde (YSI Incorporated, Yellow Springs, Ohio, USA) at each deployment and collection. A Hydrolab-Quanta (Hach Hydromet, Loveland, Colorado, USA) instrument was used at Station 1 (Digby). The YSI 6600 Sonde also measured chlorophyll A (μg L^{-1}) and pH. VEMCO (AMIRIX Systems, Inc., Bedford, Nova Scotia, Canada) minilog-T8k temperature recorders were attached to one of the full season monitoring collectors (Figure 1) at twenty stations (see Appendix 1). A visual check of surfaces and structures adjacent to collectors was made at each station at each visit to document the presence of tunicates and non-indigenous species (Table 3).

Following the prescribed monitoring period (see Appendix 1), collectors were removed and collector ropes, tags and weights were examined in the field for the presence of tunicates, other non-indigenous species and biofouling native species. These data were recorded in the field, and later entered into a Microsoft ACCESS database for further analysis.

Individual PVC plates were cut free of each collector and placed bottom-side up in sequence (top plate = left (1), middle plate = centre (2) and bottom plate = right (3)) on a white, labelled background. The plates were photographed using a frame-mounted Canon PowerShot A260 (Canon Canada Inc., Mississauga, Ontario) digital camera inside a white photographic tent. Ideally, photographs were taken in the field, but under adverse weather conditions or when partners shipped collectors to the Bedford Institute of Oceanography (BIO), photographs were taken indoors within 24 h of collection.

Table 3: List of non-indigenous aquatic species subject to detection and monitoring in 2010. * indicates a species usually not attached to collector plates.

Group	Scientific Name	Common Name (s)
Tunicates	*Ciona intestinalis*	Vase tunicate
	Botryllus schlosseri	Golden star tunicate
	Botrylloides violaceus	Violet tunicate
	Styela clava	Clubbed tunicate
	Didemnum vexillum	Pancake batter tunicate
	Diplosoma listerianum	Compound sea squirt
	Ascidiella aspersa	European sea squirt
Bryozoans	*Membranipora membranacea*	Coffin box, lacy crust bryozoan
Crustaceans	*Caprella mutica*	Japanese skeleton shrimp
	*Carcinus maenas**	European green crab
	*Eriocheir sinensis**	Chinese mitten crab
	*Hemigrapsus sanguineus**	Asian shore crab
Algae	*Codium fragile spp. fragile**	Oyster thief, Codium, green fleece

2.4 DETERMINATION OF PRESENCE AND PERCENTAGE COVERAGE OF TUNICATES

Presence of tunicate species was determined as a positive observation on either a DFO monitoring collector (plates, rope, tag or weight) or on any submerged surface inspected at the monitoring station upon collector deployment or retrieval, or as an anecdotal report provided by a monitoring partner.

Percentage cover of each tunicate species was determined by visual examination of the bottom (under) surfaces of each plate. Categories for the percentage cover were 0 (absent); 1: < 25% coverage (low) of the under plate surface ; 2: 25–50% coverage (moderate); 3: 51–75% coverage (heavy), 4: 75-99% coverage (very heavy) and 5: >100%. A value of "1" was assigned to collectors where one to a few individual tunicates were present on surfaces of the collector other than plates (see above).

The average percentage cover category for each species of tunicate at each station was determined separately for each deployment period (first, second and full). An average cover value for a deployment period was calculated as the sum of all the

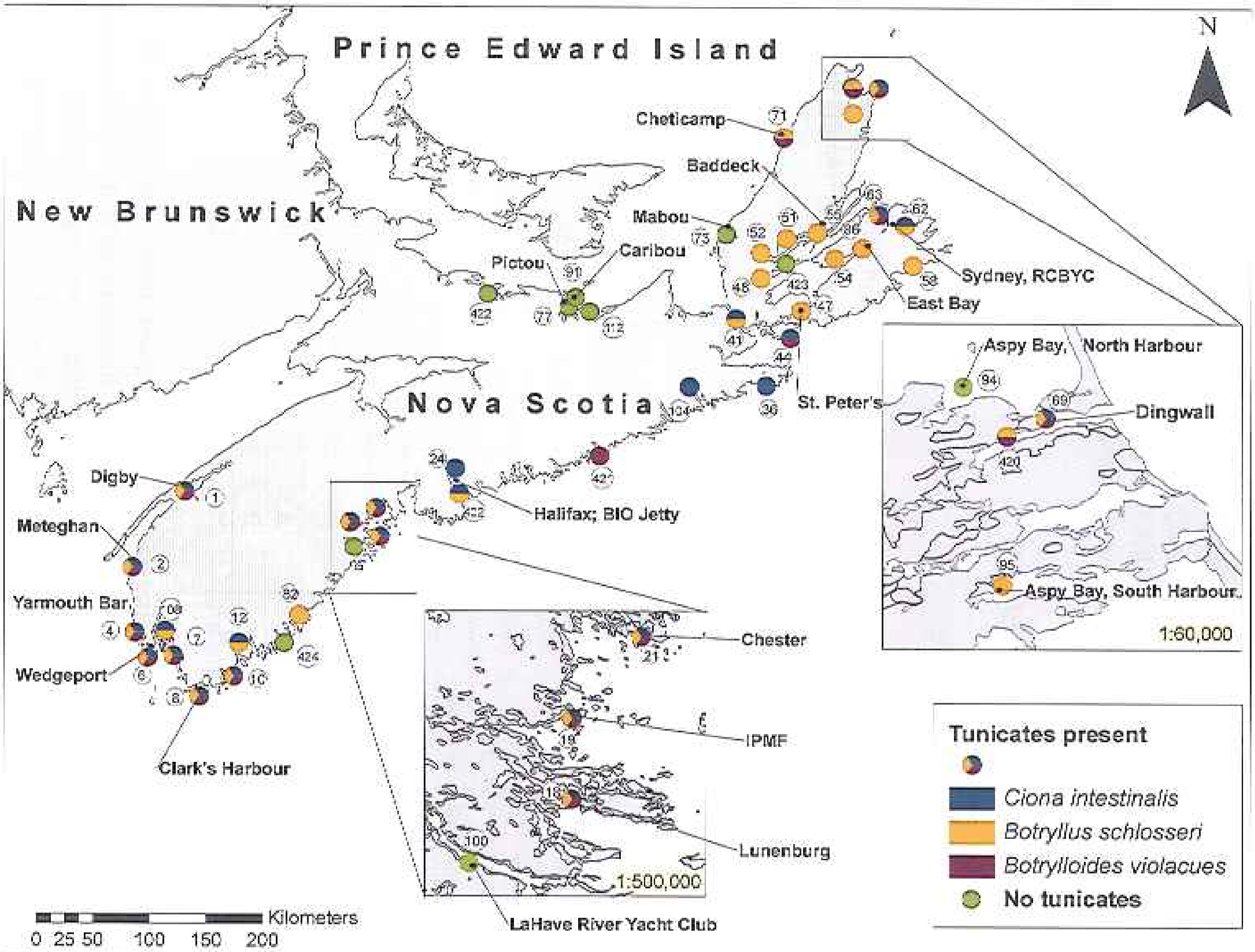

Figure 2: Presence of *Ciona intestinalis*, *Botryllus schlosseri* and *Botrylloides violaceus* in Nova Scotia in 2010.

category values divided by the total number of plates recovered (i.e. six plates for two collectors, or three plates if only one collector was recovered in a deployment period). The year average percentage cover category was determined as the sum of all category values divided by the total number of plates recovered in all deployment periods (i.e. maximimum of 18 plates for six monitoring collectors).

3.0 RESULTS

3.1 GENERAL OCCURRENCE

General results for the presence of tunicate species monitored in 2010 are given in Table 4, and the locations where *C. intestinalis*, *B. schlosseri*, and *B. violaceus* were present are given in Figure 2.

Table 4: Prevalence of aquatic invasive species (monitoring records or anecdotal reports) in Nova Scotia, May – December 2010.

Species	Monitoring Sites (33)	Anecdotal Reports (10)	Total Records (43)
Ciona intestinalis	18	3	21
Botryllus schlosseri	27	2	29
Botrylloides violaceus	14	2	16
Styela clava	0	0	0
Didemnum vexillum	0	0	0
Diplosoma listerianum	0	0	0
Ascidiella aspersa	0	0	0
No Tunicates	5	4	9
Caprella mutica	9	0	9
Membranipora membranacea	23	0	23

Botryllus schlosseri was the most frequently recorded tunicate; present at 27 (82%) monitoring stations and reported at 2 additional locations (67% of total records). *Ciona intestinalis* was found at 18 (54%) monitoring stations, and reported from 3 additional sites (49% of records). *Botrylloides violaceus* was the least frequently recorded tunicate; present at 14 monitoring stations (42%) and reported from 2 additional locations (37% of records). *Styela clava. D. vexillum*, *D.listerianum* and *A. aspersa* were not detected in 2010. *Ciona intestinalis* was formally reported as present at Country Harbour, although this species has been present in this area since 2006 or

2007 (B. Hancock, pers. comm.) and *Botrylloides violaceus* was reported for the first time in Digby and at Sober Island. Tunicates were not present at five stations: Pictou, Mabou, Caribou, Aspy Bay-North Harbour and LaHave River and were absent from another four locations, reported anecdotally: Merigomish, Malagash, Alba and Port L'Hebert. Tunicates were present on sites with every "risk- factor" selected; from aquaculture leases, to private marinas, to public fishing wharves. In terms of geographic location, only the North shore of Nova Scotia (Figure 2) remained free of invasive tunicates in 2010.

3.2 ENVIRONMENTAL RANGES

The ranges of temperature, salinity and dissolved oxygen concentration at sites where tunicates were present during the study period are shown in Table 5, YSI values are given in Appendix 2, and Minilog plots are given in Appendix 3.

Table 5: Ranges of values for temperature, salinity, and dissolved oxygen at stations where invasive tunicate species were present, May – December 2010.

Species	**YSI Temperature Range (°C)**	**Minilog Temperature Range (°C)**	**YSI Salinity Range**	**YSI Oxygen Range (mg L⁻¹)**
Ciona intestinalis	6.3 – 21.6	3.5 - 26.9	23.9 - 32.6	4.58 -11.83
Botryllus schlosseri	6.4 - 23.4	3.5 - 26.9	17.2 - 32.6	4.58 -11.83
Botrylloides violaceus	6.4 – 22.5	7.8 - 23.6	23.9 - 32.6	4.58 -11.83
No Tunicates	9.1 – 21.6	10.2 – 23.9	25.7 - 29.2	4.58 -11.83

The YSI values represent 1m depth measurements taken at each of the three station visits, and do not reflect the true tolerance range for tunicate species. However, data from Minilog recording thermistors are hourly values recorded throughout the study period and give a more accurate picture of temperature maxima and minima, as well as an indication of daily and seasonal variation. *Ciona intestinalis* was present at sites with late-summer temperatures as high 26.9°C. Of the two colonial species observed, *B. violaceus* was present in waters as warm as 23.6°C, while *B. schlosseri* was present in warmer waters up to 26.9°C. *Botryllus schlosseri* tolerated a wider salinity range than the other species and was present in brackish waters as low as 17.2. Tunicates tolerated a wide range of oxygen concentrations, from 4.58 to 11.83 mg L^{-1}.

Chlorophyll and pH values recorded during station visits (Appendix 2) were variable, and too infrequent to allow meaningful comparison.

Examination of Minilog water temperature plots (Appendix 3) showed a general pattern of warming from late May through early July, with cycles of minor cooling (2 - 4°C drops), and warming throughout the summer. Warmest temperatures were recorded either in mid-July or in early September, coinciding with the arrival of Hurricane Earl (downgraded to a tropical storm) on September 4, 2010. A gradual cooling trend was evident through September, with a brief warming and mixing event in early October, followed by a steady decline in temperature. Short-term (daily) cycles of heating and cooling were also evident. Digby was the coldest, least variable station (lowest daily variation), with a season high of 15.7°C on September 4, followed by Yarmouth Bar with a high of 17.4°C. Coastal stations in southwestern NS (Wedgeport and Clark's Harbour) showed a wider range of temperature values, reflecting higher wind and wave exposure. South shore coastal stations (Shelburne, Port Mouton, Lunenburg and Indian Point), Halifax, and Venus Cove (Chedabucto Bay) were characterized by higher (21 – 27°C) mid-summer temperatures, and greater short-term variations in temperature. Stations in the Bras D'Or Lake (St. Peter's, Eskasoni, Baddeck) showed steady warming to a mid-July peak, with steady, and moderate temperatures (20-22°C) leading to a second peak in early September (24-25°C). Variations in temperature inside the Lake were lower compared with coastal sites. Other sites in Cape Breton (Cheticamp, North Sydney, and Aspy Bay-North Harbour) showed temperature trends similar to those seen in the Bras D'Or Lake, but temperatures were cooler (22.7°C maximum) in Aspy Bay-North Harbour, at the extreme north eastern tip of Cape Breton. Caribou, on the north shore, showed moderate temperature variations, with mid-summer temperature values between 17 and 21°C, and a high of 23.9°C on September 4, 2010.

Minilog thermistors remained in the water over the winter at two mussel farms: Aspy Bay-North Harbour and Indian Point Marine Farms (IPMF). Winter temperature dropped to -1.4°C in Aspy Bay-North Harbour on January 26, 2010, with temperatures remaining at about 0°C (+/- 0.5°C) or lower between January 22 and March 4, 2010, a period of 42 days. Summer maximum temperature was 22.7°C on August 18, 2010. Winter minimum temperature at IPMF was -0.5°C on January 22, 2010 on Site 1, the most sheltered location, and remained below 0.5°C for a 19-day period between January 24 and February 11, 2010. Water temperature dropped to a low of -0.9°C on January 29 at Site 2, a more exposed location, and remained at 0.5°C or below for a 19-day period between January 25 and February 12, 2010. Summer maximum temperatures at IPMF were reached on September 9 at Site 1 (21.9°C), and August 31, 2010 at Site 2 (21.6°C).

3.3 TUNICATE COVERAGES (DEGREE OF INFESTATION)

There was variation (i.e. "patchiness"): (1) among individual plates on a collector, (2) between duplicate collectors at a site during a deployment period, and (3) among deployment periods (see Section 3.4), and this variation also was evident in the intensity of biofouling on other structures at the stations. The overall average coverage on monitoring collectors gave a comparable approximation of the level of infestation observed on fouled structures at each station.

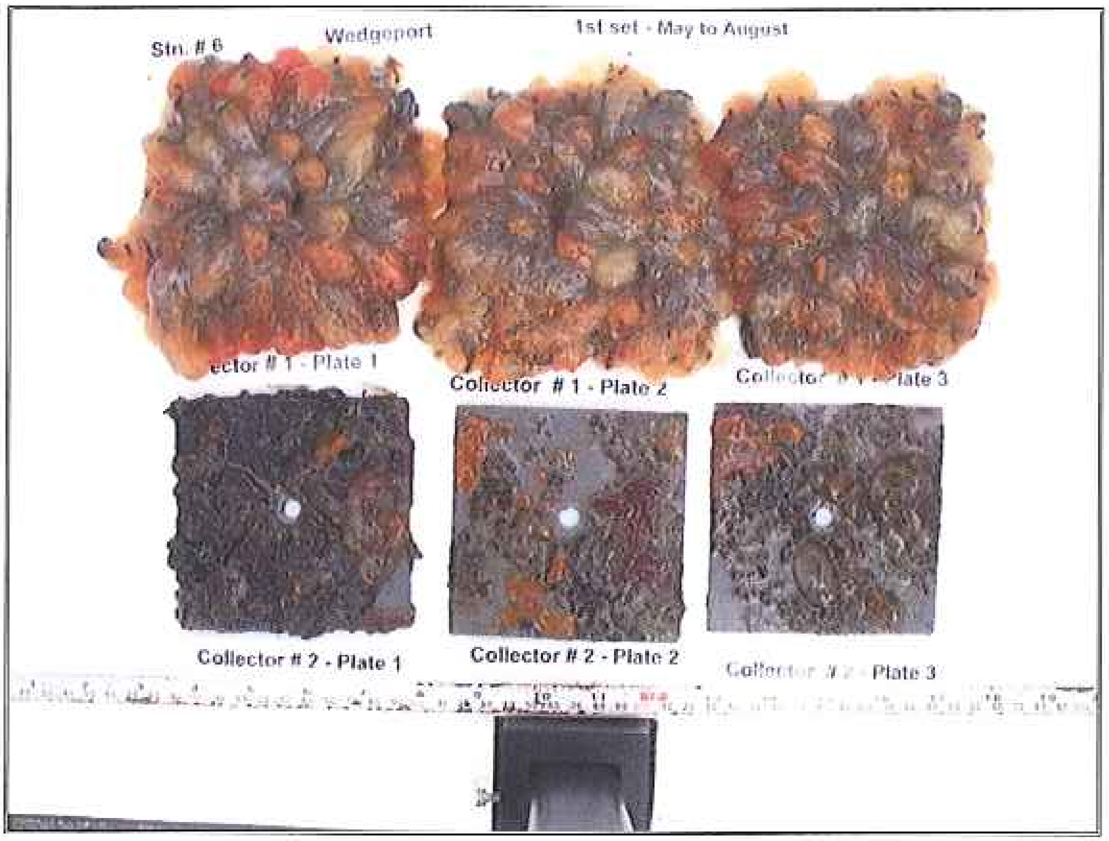

Figure 3. Photograph of first deployment collector plates at Wedgeport in 2010, showing differences between plates on the same collector and on collectors deployed duing the same period.

Photographs of plate surfaces for the first deployment period at Wedgeport (Figure 3) are typical of many stations where different tunicate species dominated different collectors (i.e. *C. intestinalis* on Collector #1, and colonial tunicates on Collector #2) deployed during the same period. Variation in species coverage was often also evident among plates of the same collector: here the top plate (Plate 1) of Collector 2 was heavily fouled by *B. schlosseri*, with low to moderate coverages of *B. schlosseri* and *B. violaceus* on the middle plate (Plate 2), and low to moderate coverages of *C. intestinalis*, *B. schlosseri* and *B. violaceus* on the bottom plate (Plate 3).

The average percentage coverages (over all plates and collectors) recorded at all stations monitored in 2010 are shown for *C. intestinalis* (Figure 4), *B. schlosseri* (Figure 5) and *B. violaceus* (Figure 6).

3.3.1 *Ciona intestinalis*

Average annual coverage of *C. intestinalis* was heaviest along the south shore (Stations 18, 19 and 21) and the southwestern tip of NS, and in the Sydney-North Sydney area (Figure 4). Coverage between Shelburne and Meteghan can best be described as "patchy", with very high coverages at Ingomar (Station 10), interspersed with much lower coverages at other stations. Low coverages were observed in Halifax Harbour (Stations 24 and 402) and in the Chedabucto Bay area (Stations 41). Ciona intestinalis was not present along the north shore (Merigomish to Malagash) or in the Bras d'Or Lake (Stations 48, 51, 52, 54 and 86). While C. intestinalis was present at the public wharf in Dingwall (Station 69), in very low numbers, it was not found at a private wharf in the same harbour (Station 420) or nearby in Aspy Bay-North Harbour (Station 94) or Aspy Bay-South Harbour (Station 95). *Ciona intestinalis* was reported anecdotally from Petit de Grat, Isle Madame, and at Whitehead and Country Harbour on the eastern shore.

3.3.2 *Botryllus schlosseri*

Average annual coverage by *B. schlosseri* was low at stations in southwestern Nova Scotia and along the south shore, but moderate in Lunenburg and Chester (Figure 5). This species was not detected along the eastern and north shores, but was low to moderate along coastal Cape Breton. Highest coverages were noted in the Bras D'Or Lake, with a very high infestation at St. Peter's (Station 47) at the southern entrance to the Lake. Again, coverage was "patchy" among stations in some areas, for example on the south shore: LaHave River (absent) to Chester (moderate). *Botryllus schlosseri* was reported anecdotally as present in Aspy Bay-South Harbour, and Gillis Cove, Cape Breton.

3.3.3 *Botrylloides violaceus*

Low average annual coverages of *B. violaceus* were observed at stations in southwestern Nova Scotia, along the south shore, and at several stations along the Cape Breton coast (Figure 6). Violet tunicates were reported for the first time at Digby. Moderate coverages were noted at North Sydney (Station 63) and Dingwall (Station 69). An oyster lease on the eastern shore, Sober Island, is to our knowledge the first record for this species in that area. As was observed with *C. intestinalis* and *B. schlosseri*,

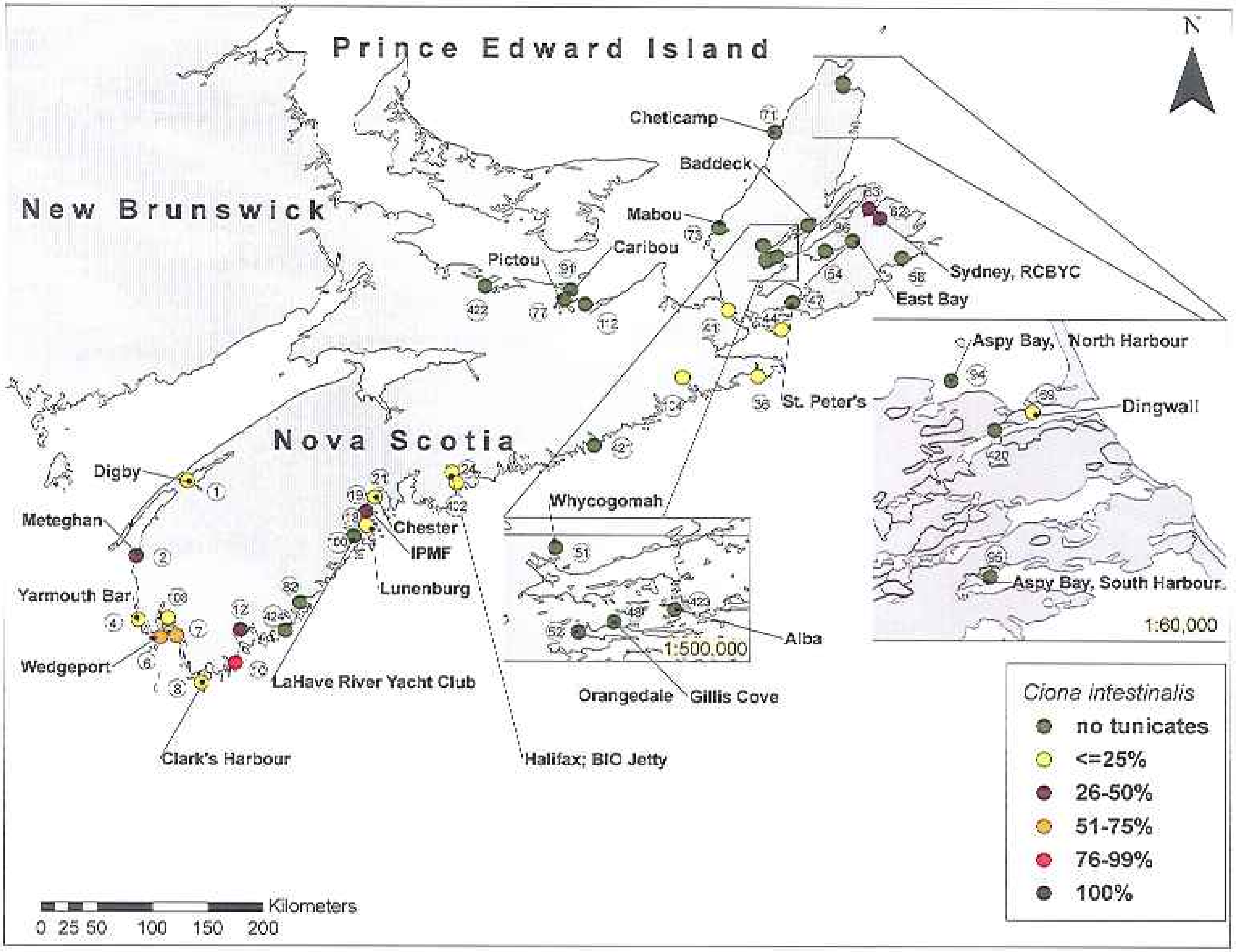

Figure 4. Average annual percentage coverage of *C. intestinalis* on monitoring collectors in 2010. Anecdotal observations (see Table 2) are given as absent or present (< 25%).

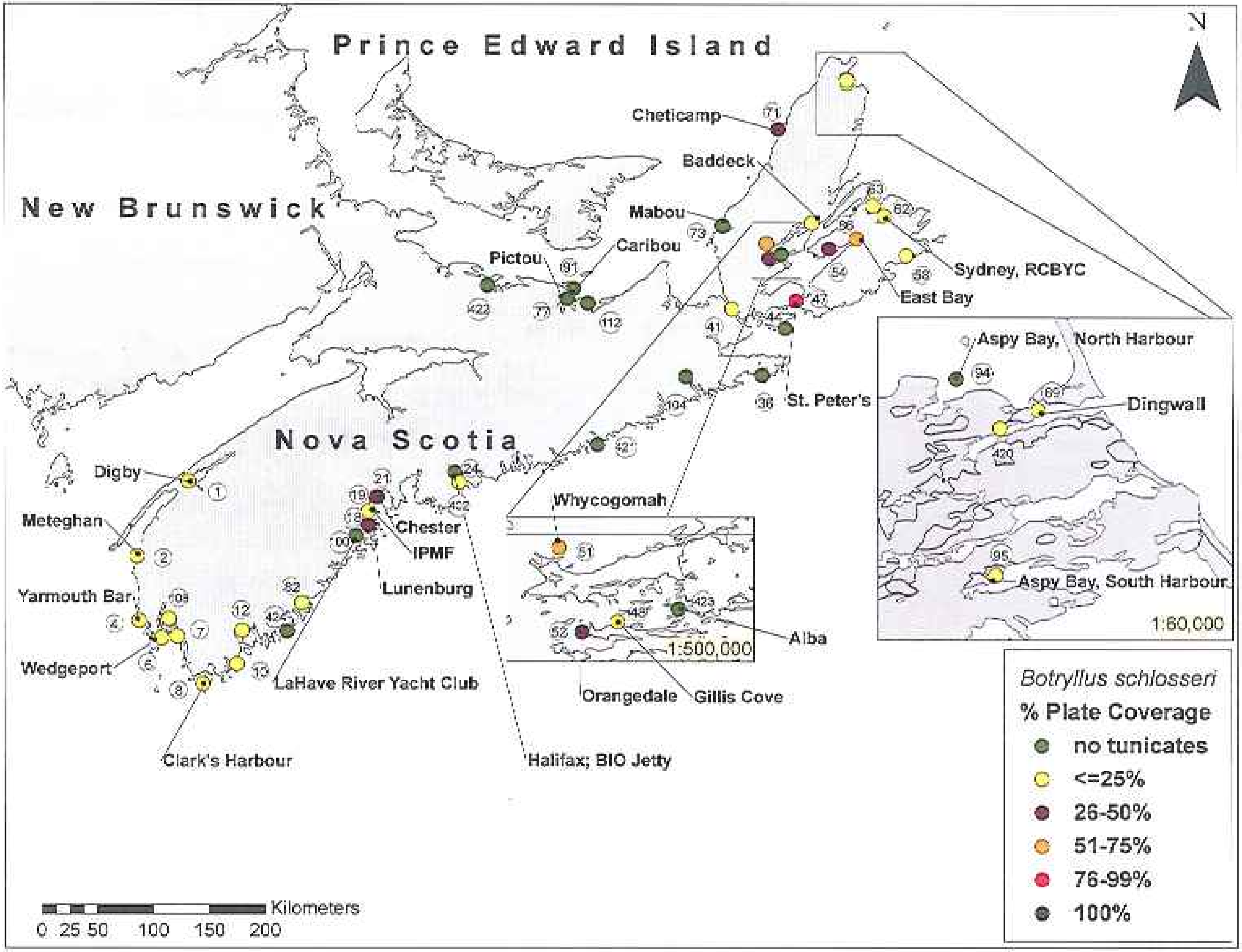

Figure 5. Average annual percentage coverage of *B. schlosseri* on monitoring collectors in 2010. Anecdotal observations (see Table 2) are given as absent or present (< 25%).

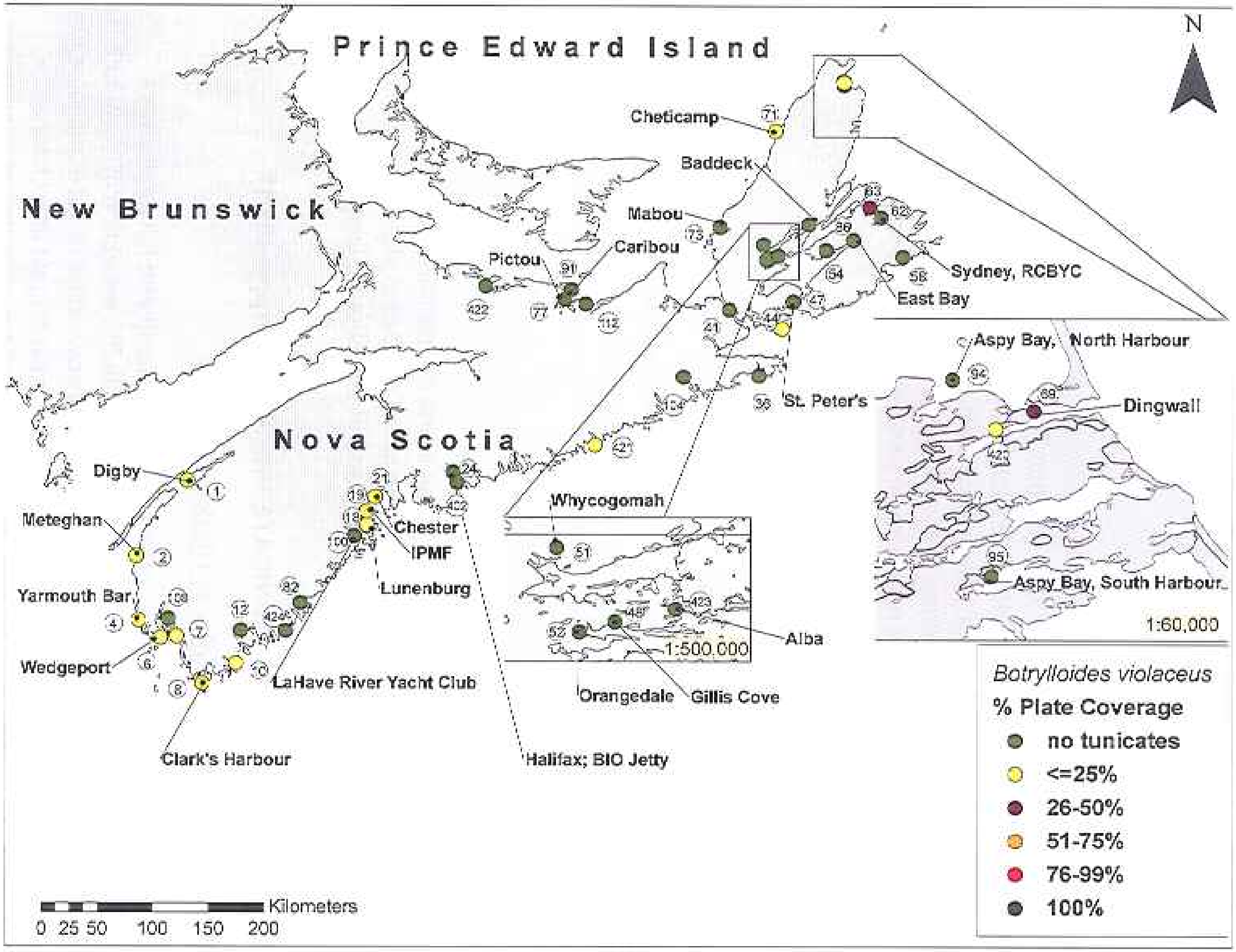

Figure 6. Average annual percentage coverage of *B. violaceus* on monitoring collectors in 2010. Anecdotal observations (see Table 2) are given as absent or present (< 25%).

stations with low coverages were interdispersed among stations that were free of *B. violaceus* (Figure 6). *Botrylloides violaceus* was reported anecdotally as present in Petit-de-Grat, Isle Madame.

3.3.4 No Tunicates Present

Tunicates were absent from five monitoring stations in 2010: LaHave River Yacht Club (Station 100) on the south shore, Mabou (Station 73), on the Gulf coast of Cape Breton, Aspy Bay-North Harbour (Station 94) and Pictou (Station 77) and Caribou (Station 91), on the north shore (Figure 2). Four anecdotal reports highlighted the absence of tunicates on oyster aquaculture leases in the following locations: Alba, in Cape Breton, Malagash and Merigomish, on the north shore, and Port L'Hebert, on the south shore (Figure 2).

3.4 OTHER BIOFOULING ORGANISMS

3.4.1 *Membranipora membranacea*

The lacy crust bryozoan, *M. membrancea*, was present on monitoring collectors deployed at 23 of 33 monitoring sites throughout the province (Figure 7). Sites included marinas, small to large fishing harbours, and mussel farms, and there was no clear regional pattern of occurrence.

3.4.2 *Caprella mutica*

The Japanese skeleton shrimp, *C. mutica,* was found on monitoring collectors deployed at 9 of 33 monitoring stations (Figure 8). It was found at several stations in southwest NS, Lunenburg, and Halifax on mainland NS, and at Louisbourg, Dingwall and Aspy Bay-North Harbour in Cape Breton.

3.5 SEASONAL VARIATION IN TUNICATE PRESENCE (Appendix 1)

3.5.1 Southwestern shore (Digby to Clark's Harbour)

Vase tunicates, *C. intestinalis*, were present on first, second and full deployment collectors at all stations on the southwestern shore, with the exception of Yarmouth Bar (Figure 9C). It is important to note, however, that low coverages of vase tunicates were noted on fouling surfaces (buoys, floating docks, ropes) in August and October. Coverage by *C. intestinalis* was heaviest on first deployment collectors at Wedgeport (Figure 9D) and Camp Cove (Figure 9E), the result of early season settlement, and on full deployment collectors at Digby (Figure 9A) and Meteghan (Figure 9B),

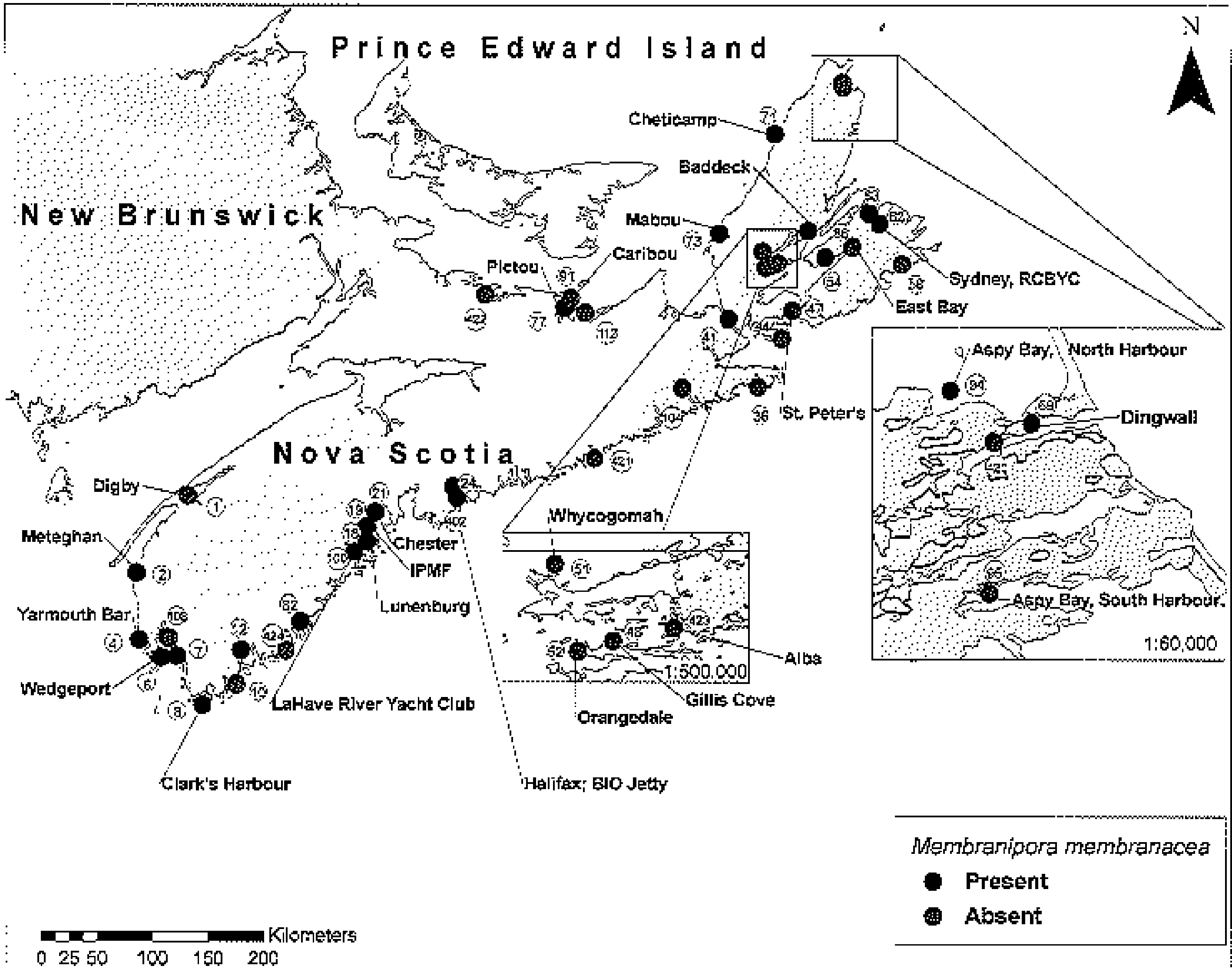

Figure 7: Presence/absence of *M. membranacea* in Nova Scotia in 2010.

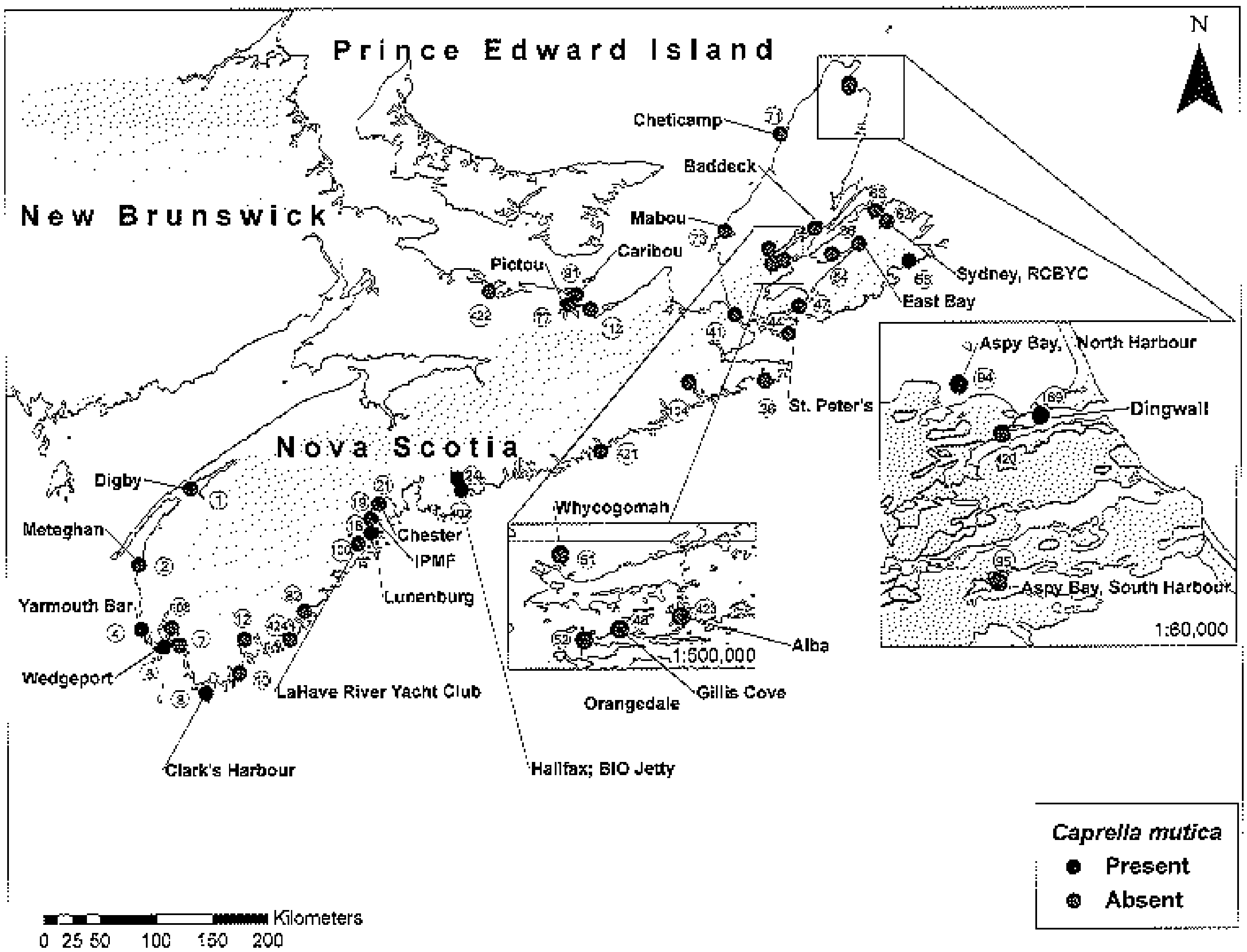

Figure 8: Presence/absence of *C. mutica* in Nova Scotia in 2010.

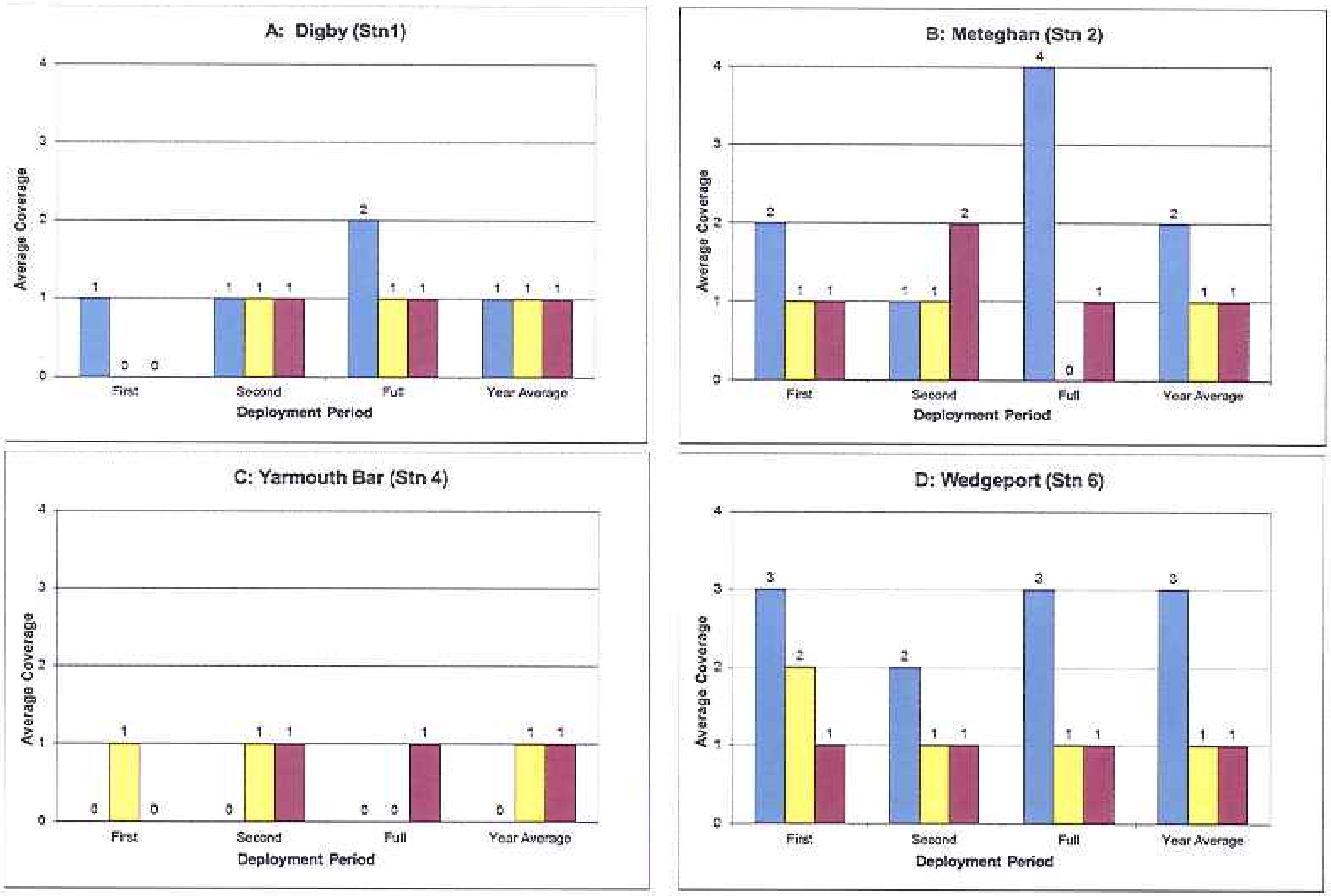

Figure 9A - D: Seasonal and year average coverage of tunicates on the southwestern shore; Digby (A), Meteghan (B), Yarmouth Bar (C), and Wedgeport (D) in 2010, for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

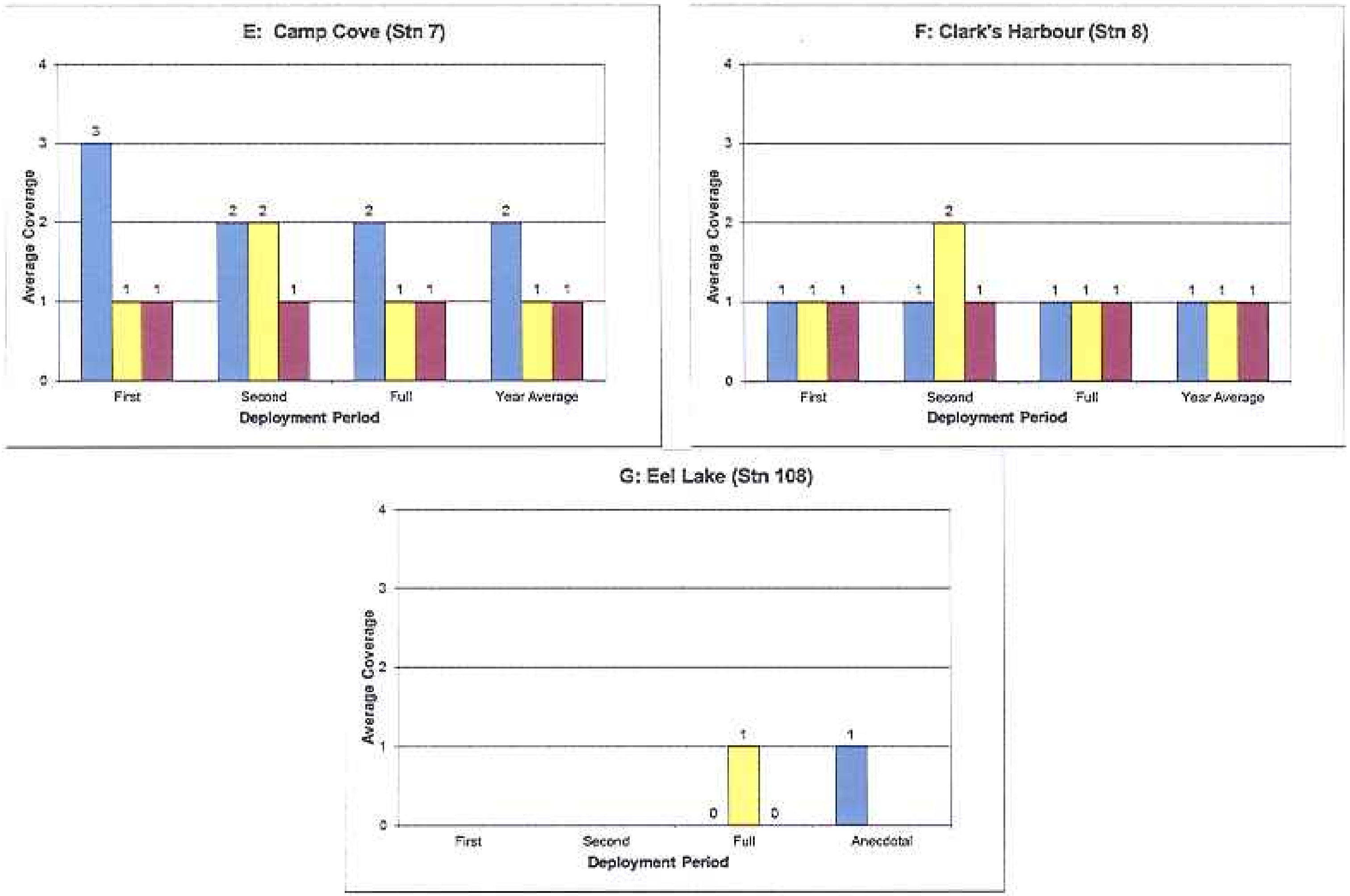

Figure 9 E - G: Seasonal and year average coverage of tunicates on the southwestern shore, continued; Clark's Harbour (F) and Eel Lake (G) in 2010, for *C. intestinalis* ( ), *B. schlosseri* ( ), and *B. violaceus* ( ).

indicative of settlement and growth throughout the entire deployment period. Vase tunicates were generally present from May through October in this region, with year average coverages heaviest at Wedgeport (heavy: 51-75%), moderate at Meteghan and Camp Cove (25-50%), and low at Digby and Clark's Harbour (<25%).

Golden star tunicate, *B. schlosseri*, were present on first, second and full deployment collectors at all stations, with the exception of first deployment collectors at Digby, where water temperatures were below 15°C during the first deployment period (Appendix 3), and full deployment collectors at Yarmouth Bar and Meteghan, where plates were totally covered by vase tunicates. The species was reported on buoys and oyster cages at Eel Lake. In general, coverages were low at most stations, but moderate coverages were noted on first deployment collectors at Wedgeport, and second deployment collectors at Camp Cove and Clark's Harbour, which may reflect higher settlement and growth as the water warmed. Water temperatures at Digby were still below 15°C (Appendix 3) when second set collectors were deployed, which may explain the absence of *B. schlosseri*, often described as a warm-water species, on first deployment collectors.

Violet tunicate, *B. violaceus*, coverages were generally low during all deployment periods, and this species was absent during the first deployment period at Digby and Yarmouth Bar, the two coldest stations. Moderate coverages were noted during the second deployment period at Meteghan only.

Vase tunicate, *C. intestinalis*, was the dominant tunicate in this region, often settling on plates earlier in the season before settlement by golden star and violet tunicates. Colonial tunicates were frequently observed growing on top of vase tunicates, which occupied most of the plate surfaces on second and full deployment collectors.

3.5.2 South shore (Ingomar to Chester)

The vase tunicate, *C. intestinalis*, was present at all stations in this region with the exception of Port Mouton (Figure 10C), and during the first deployment period at Shelburne (Figure 10B). Coverage was highest at Ingomar (Figure 10A), where collectors were completely fouled by *C. intestinalis* during all deployment periods. Low fouling was noted during the first deployment period at Lunenburg (Figure 10D) and Indian Point (Figures 10E and 10F), with moderate fouling at Chester (Figure 10G). Fouling was heavy at Indian Point, and very heavy (>75%) at Shelburne, during the second deployment period. They were present in low to moderate coverages in all deployment periods at Lunenburg (Figure 10D) and Chester (Figure 10G).

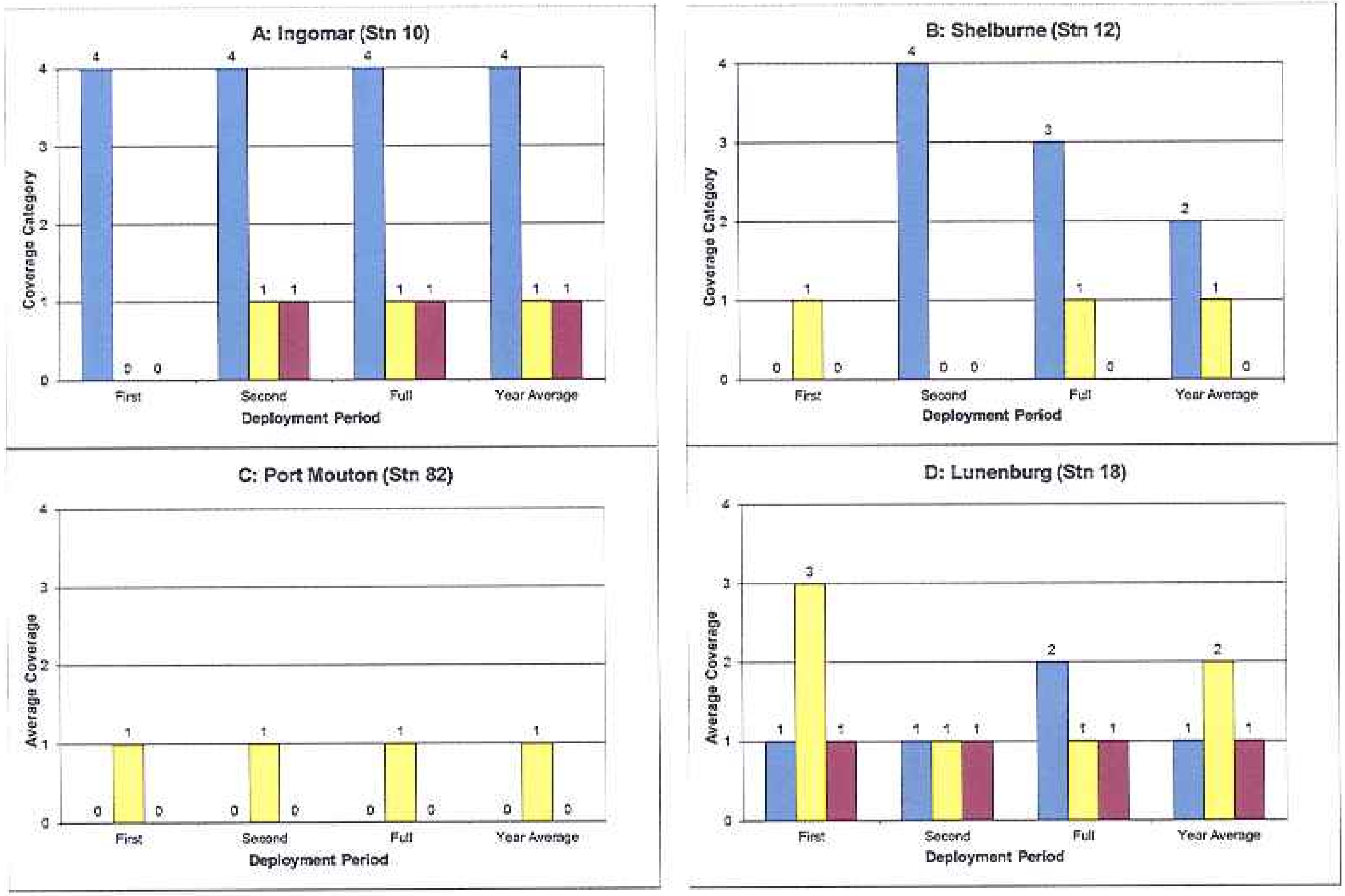

Figure 10 A - D: Seasonal and year average coverage of tunicates on the south shore; Ingomar (A), Shelburne (B), Port Mouton (C) and Lunenburg (D) in 2010, for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

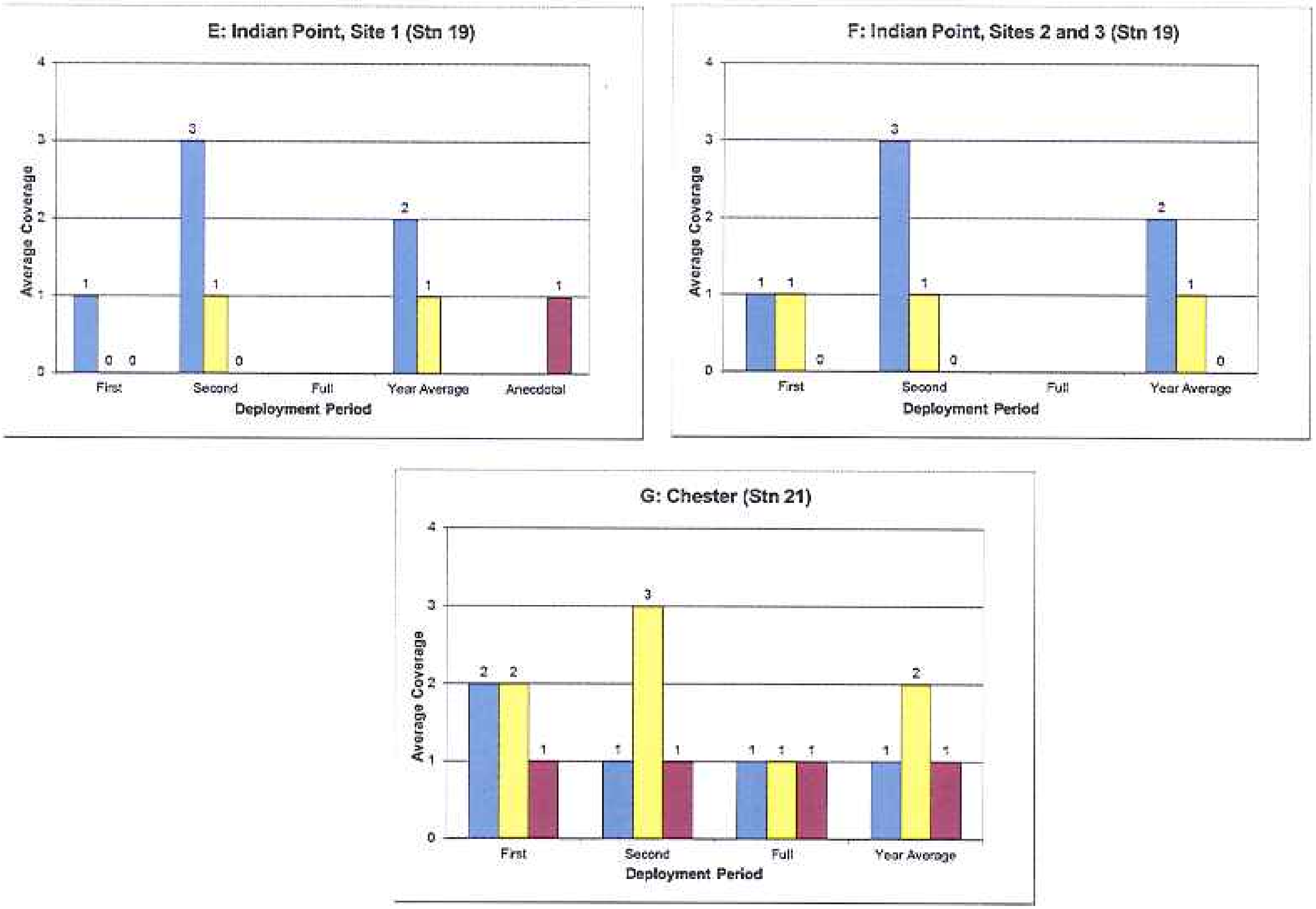

Figure 10E – G: Seasonal and year average coverage of tunicates on the south shore, continued; Indian Point (E, F) and Chester (G) in 2010, for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

Coverage by *B. schlosseri*, golden star tunicate, was heavy during the early deployment period at Lunenburg (Figure 10D), and during the second deployment period at Chester (Figure 10G), and these stations showed the highest (25-50%) average annual coverages. Golden star tunicates were present in low coverage throughout the year at Port Mouton (Figure 10C), and at Sites 2 and 3, Indian Point (Figure 10F). They were absent from collectors at Site 1, Indian Point (Figure 10E) during the first deployment period, and from Shelburne (Figure 10B), during the second deployment, when plates were covered in vase tunicates.

Violet tunicates, *B. violaceus*, were absent from Shelburne and Port Mouton, and present in low coverage at some point during the year at the other stations in this region. At Ingomar, where coverage by vase tunicates was very high, colonial tunicates were observed growing on vase tunicates, instead of on plate surfaces.

Tunicates were absent from the LaHave River Yacht Club (Station 100), where collectors were heavily fouled by the blue mussel, *Mytilus edulis*.

3.5.3 Halifax

Ciona intestinalis was present at both stations monitored in this area. It was the only tunicate present at the BIO Jetty (Figure 11A), where its coverage was low to moderate, and the only tunicate present at the Royal Nova Scotia Yacht Squadron marina (Figure 11b) during the early deployment period. Golden star tunicates were present at RNSYC in low coverages during the second deployment period only. *Botrylloides violaceus* was absent from these two stations.

3.5.4 Eastern shore (Ship Harbour to Whitehead)) and Chedabucto Bay (Venus Cove, Petit-de-Grat)

Only one station was monitored from these regions in 2010: Venus Cove, in Chedabucto Bay. *Ciona intestinalis* and *B. schlosseri* were present with low coverage during the second and full deployment periods (Figure 12), where water temperatures were lower water early in the season than in nearby Cape Breton. *Ciona intestinalis* were reported anecdotally as present at Petit-de-Grat, and on two mussel aquaculture sites; Country Harbour and Whitehead.

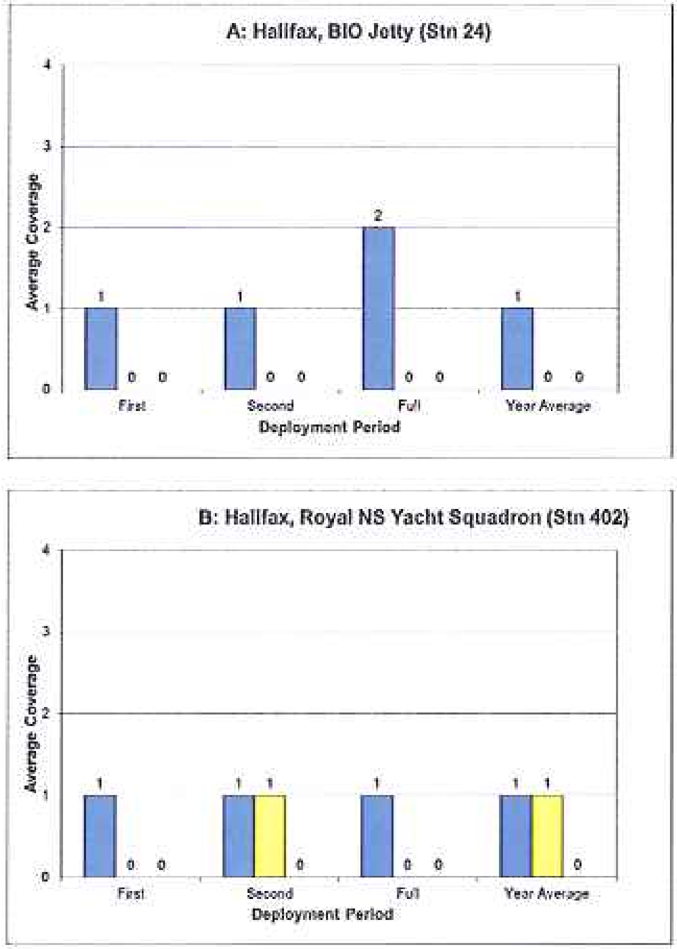

Figure 11: Seasonal and year average coverage of tunicates in Halifax: BIO Jetty (A) and RNSYS (B) in 2010, for *C. intestinalis*, ( ) and *B. schlosseri* ( ).

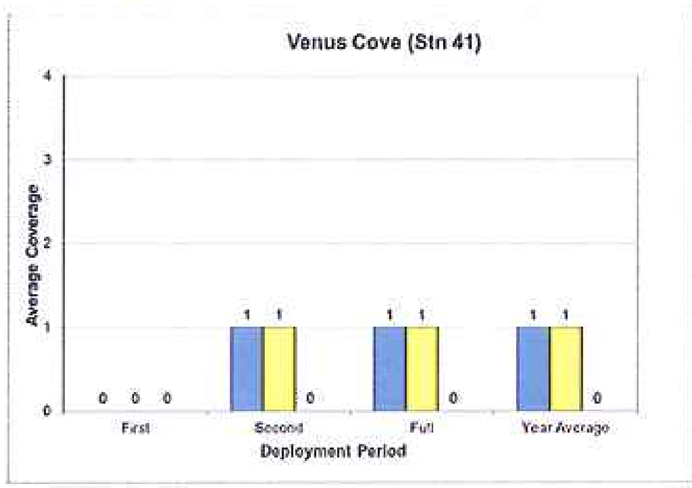

Figure 12: Seasonal and year average coverage of tunicates at Venus Cove in 2010, for *C. intestinalis* ( ) and *B. schlosseri* ( ).

3.5.5 Cape Breton Coastal

Ciona intestinalis was not found at Louisbourg (Figure 13A), Dingwall Harbour (Figure 13E) and Cheticamp (Figure 13F) and coverages were low at Dingwall (Figure 13D). Coverages were heavy during first and full deployments at Sydney (Figure 13B) and first deployment at North Sydney (Figure 13C).

Botryllus schlosseri showed low coverages at all stations, except for very heavy coverages during second deployment at Cheticamp (Figure 13 F). This tunicate was present on first deployment collectors at Louisbourg, but the second and full deployment collectors were lost here. It was reported anecdotally at Aspy Bay-South Harbour.

Botrylloides violaceus was not found at Louisbourg and Sydney, and heavy coverage was noted late in the season at North Sydney (Figure 13C), and coverage was low to moderate at Cheticamp. Coverage was moderate on full season collectors at Dingwall.

Tunicates were absent from two stations monitored in this region: Aspy Bay-North Harbour (Station 94) and Mabou (Station 73). Tunicate fouling was heaviest in North Sydney, with an early dominance by *Ciona intestinalis* followed by second deployment dominance by *Botrylloides violaceus*. *Botryllus schlosseri* was dominant at Cheticamp.

3.5.6 Bras d'Or Lake

Botryllus schlosseri was the only fouling tunicate present at monitoring stations in the Bras d'Or Lake in 2010. The species was also reported anecdotally from an oyster farm at Gillis Cove. Coverage by B. *schlosseri* was highest during the first deployment at Eskasoni (Figure 14C), and higher during the second deployment period at St. Peter's (Figure 14A), Whycocomagh (Figure 14E) and Orangedale (Figure 14F). Full deployment collectors were most heavily fouled at St. Peter's (Figure 14A), Whycocomagh (Figure 14E) and Orangedale (Figure 14F), reflective of their longer deployment period and yearly averages were heavy (51-75%) at these locations. No tunicates were present on a shellfish lease at Alba.

3.5.7 North shore (Merigomish to Malagash)

Tunicates were not present on monitoring collectors deployed at stations in Pictou (Station 77) and Caribou (Station 91), and were not reported from oyster farms at Malagash and Merigomish.

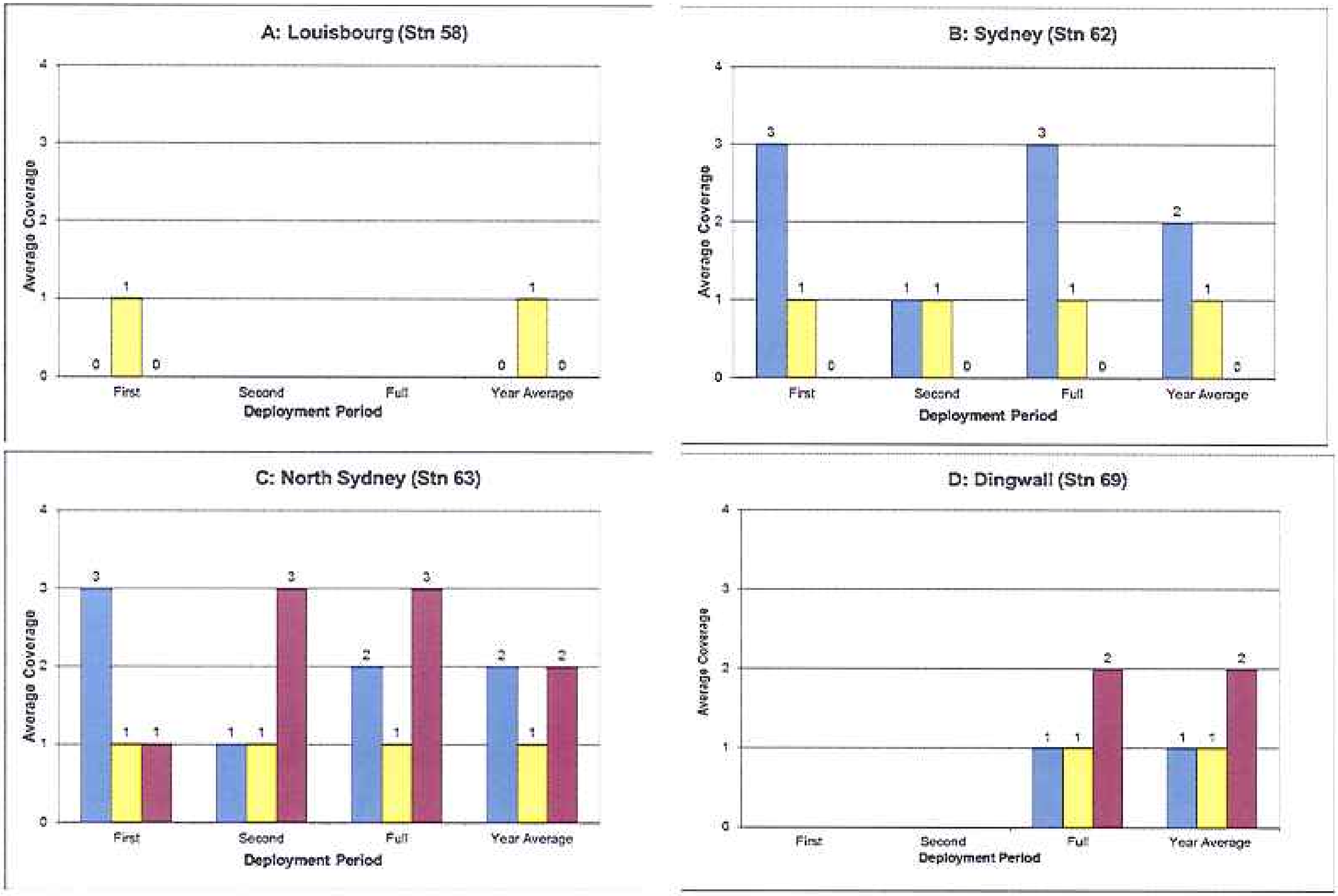

Figure 13A – D: Seasonal and year average coverages of tunicates on the Cape Breton coast; Louisbourg (A), Sydney (B), North Sydney (C) and Dingwall (D) n in 2010 for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

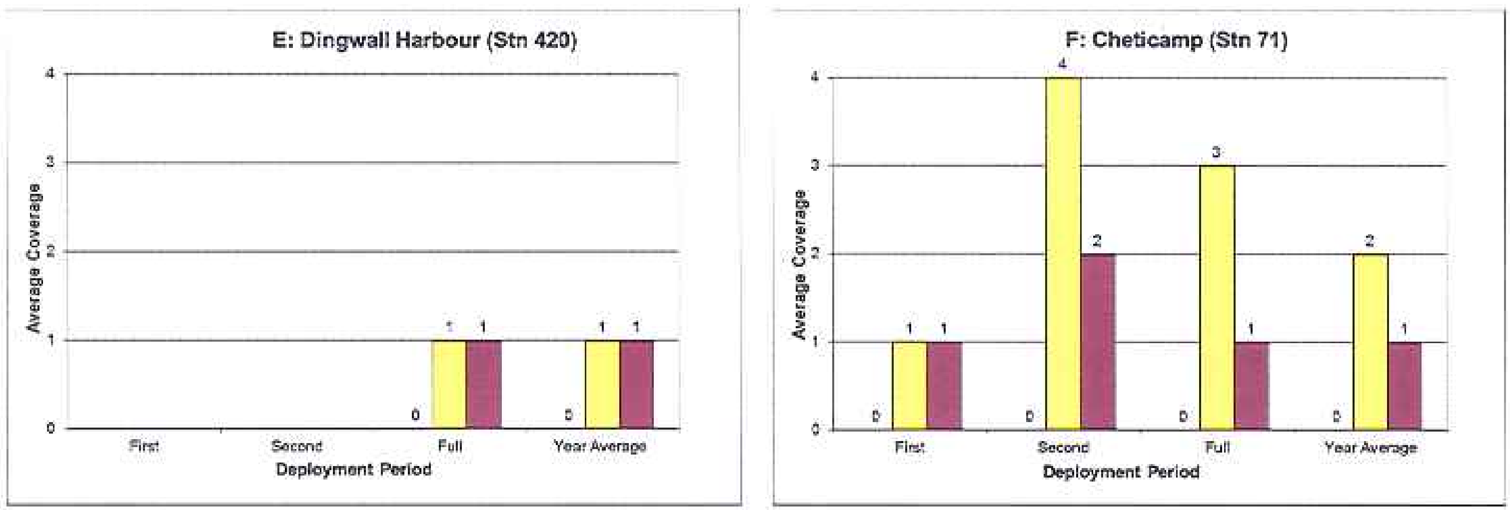

Figure 13 E – F. Seasonal and year average coverage of tunicates on the Cape Breton coast continued; Dingwall Harbour (E) and Cheticamp (F) in 2010, for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

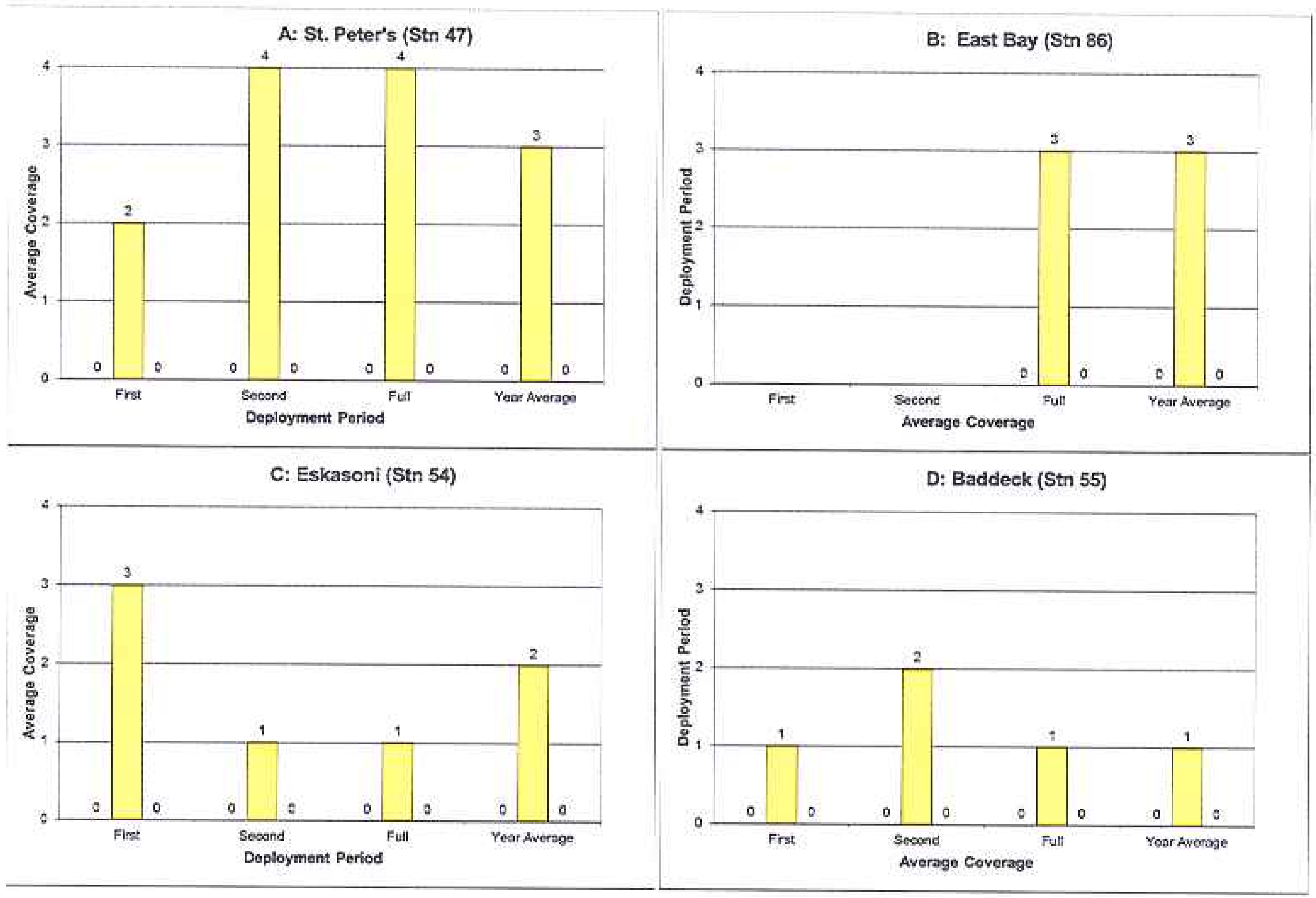

Figure 14 A - D: Seasonal and year average coverage of tunicates in the Bras D'Or Lake; St. Peter's (A), East Bay (B), Eskasoni (C) and Baddeck (D) in 2010 for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

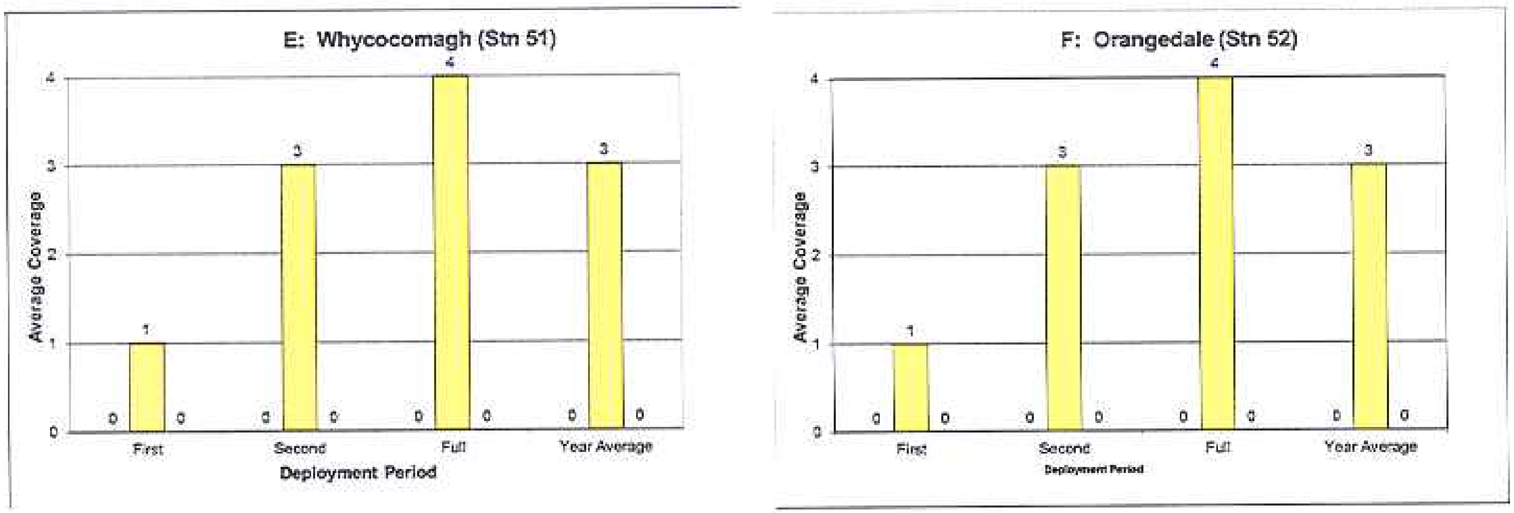

Figure 14 E – F: Seasonal and year average coverage of tunicates in the Bras D'Or Lake, continued; Whycocmagh (E) and Orangedale (F) in 2010 for *C. intestinalis* ( ), *B. schlosseri* ( ) and *B. violaceus* ( ).

4.0 DISCUSSION

4.1 TUNICATE ESTABLISHMENT AND SPREAD

The results of ongoing DFO monitoring in Nova Scotia from 2006 to 2009 (Sephton et al. 2011) and in 2010 indicate that populations of *Ciona intestinalis*, *Botryllus schlosseri* and *Botrylloides violaceus* are (1) well established in many locations, and (2) spreading to new areas.

The successful survival, establishment and spread of non-indigenous species, beyond the existence of a suitable vector for introduction, lies in the presence of a suitable environment (Locke 2009). Temperature and salinity tolerances of *C. intestinalis*, *B.* schlosseri and *B. violaceus* recorded in 2010 were comparable with earlier values (Sephton et al. 2011), well within the range of tolerances given for these species (Carver et al. 2006 a,b; Epelbaum et al. 2009; Vercaemer et al. 2011), and consistent with the environmental conditions of coastal Nova Scotia in general. The trend in warmer summer (and winter) water temperatures observed in many locations in Nova Scotia since 2006 may be conducive to the spread of established AIS such as *B. violaceus*, and heavier infestations of tunicates at several stations. Stachowicz et al. (2003) found a positive correlation between the timing of introduction and total recruitmen of introduced sea squirts and warmer winter and summer water temperatures, respectively.

Minilog temperature data indicated that 2010 summer water temperatures were comparable with temperatures observed in 2008, and 1-2°C higher than in 2009 (Sephton, unpub. data; Vercaemer et. al. 2011). Long term monitoring and research (Vercaemer et al. 2011) at IPMF (Station 19) has tracked changes in environmental temperature and subsequent recruitment of *C. intestinalis* larvae and degree of infestation at this site. Air temperature data indicated that the winters, and following summers, of 2004, 2005 and 2007 were cooler, and low infestations of *C. intestinalis* were noted, while water temperatures during the winters and summers of 2008, 2009 and 2010 were warmer, with much higher infestations of vase tunicates during those summers (Vercaemer et al. 2011). Minilog data from the winter of 2010 showed that water temperature at Indian Point dropped to a low of only -0.5°C (Lease #1) and -0.9°C (Lease #2) and remained there for a period of only 19 days, while maximum summer water temperature exceeded 21°C. Winters on the south shore of NS have historically been characterized by extended (4-8 weeks) of water temperatures well below 0°C (Darnell, pers. obs.). Indeed, the heaviest infestation of *C. intestinalis* ever recorded at this site was evident in 2011, (Sephton and Vercaemer, pers. obs.) to the extent that mussel harvesting operations were greatly reduced.

Warming temperatures may create new opportunities for the establishment of colonial tunicates which grow well at warmer temperatures (Chadwick-Furman and Weissman 1995) and are also tolerant of low salinities (Epelbaum et al. 2009). *Botryllus schlosseri*, present throughout coastal NS, is now dominant and widespread in the warm, brackish waters of the Bras d'Or Lake, while the low salinities (below 20) may have prevented the establishment of *B. violaceus* (Epelbaum et al. 2009). In St. Peter's Bay, PEI, Paetzold et al. (2012) noted that the biomass of *B. schlosseri* on mussel socks was 4-fold higher in 2010, where water temperature reached 20°C in mid-summer compared to 2009, where temperatures were 4-5°C lower. Increases in the coverage of *B. schlosseri* at Lunenburg and Eskasoni, and of *B. violaceus* in North Sydney in 2010 may also reflect this warming trend.

While the long-standing year-to-year presence of *C. intestinalis*, *B. schlosseri* and *B. violaceus* in southwestern NS and along the south shore, and *B. schlosseri* in the Bras d'Or Lake, may denote their initial points of introduction in ports via commercial shipping and subsequent establishment (Lacoursière-Roussel et. al. 2012a, b), their secondary spread to adjacent bays, harbours, and marinas indicates human-mediated, stepping-stone type introductions (Lacoursière-Roussel, 2012a; Sephton et al. 2011), most likely as "hitchhikers" on the hulls of small recreational or fishing vessels, or on fishing gear (Lambert and Lambert, 1998, 2003; Lacoursière-Roussel et al. 2012a, b). Once introduced to a new harbour or bay where environmental conditions are favorable, tunicates may spawn, attach to natural or man-made surfaces, grow and successfully overwinter, resulting in an established population. Lacoursière-Roussel et al. (2012b) found that potential for spread via recreational vessels was high in NS, where about half of the boats examined were fouled by colonial tunicates, and that those boats moved among multiple marinas during summer months. Specifically, they found that the ports of Yarmouth and Sydney, and marinas in Mahone Bay and Chester (south shore), and in the Bras d'Or Lake were the busiest in Nova Scotia in 2009, with multiple opportunities for the introduction and spread of new, or presently established tunicate species. Given that tunicates have a very low potential for natural spread (larval drift and rafting), it is crucial, that ongoing public outreach to fishers and recreational boaters, and education to raise awareness of the threats posed by non-native tunicates in coastal communities, continue as a preventative measure to their continuing spread. Citizen groups should be established to help with ongoing monitoring, given their success in other regions (Cohen et al. 2011; Salem Sound Coastwatch 2013).

4.1.1. *Ciona intestinalis*

Heavy infestations of *C. intestinalis* resulting in losses to shellfish aquaculture were first reported on the south shore of Nova Scotia in 1997 (Cayer et al. 1999). Information from the shellfish aquaculture industry shore (Carver et al. 2003; Carver et al. 2006a; Clancey and Hinton, 2003; Clancey and MacLachlan, 2004) and from DFO monitoring efforts since 2006 indicate that vase tunicates are well established in southwestern NS, along the south shore, in Chedabucto Bay and on Isle Madame. Sephton et al. (2011) report that this species has spread (present at 39% of all monitoring stations in 2006, rising to about 50% of all stations in 2007- 2010), and was present at 64% of NS Marinas in 2009 (Lacoursière-Roussel et al. 2012a). However, the number and locations of stations monitored/surveyed in each year differed, which could account for some of the year-to-year differences. Information from sentinel stations (Table 6; key stations monitored in all, or most years, since 2006) shows that, despite some inter-annual variability, *C. intestinalis* is present at about 50-66% of sentinel stations, with increasing coverage noted at three stations. *Ciona intestinalis* is now present in Digby, in Halifax Harbour, from Country Harbour to Canso, and in Dingwall, North Sydney and Sydney in Cape Breton. It has not established in the Bras d'Or Lake, probably due to low salinities, although a few individuals (or small populations) have been present sporadically in St. Peter's, at the southern entrance of the Lake close to Isle Madame, and also in Sydney. Both locations represent entry points for recreational boats and yachts from PEI, other regions of NS, and the US eastern seaboard, all important AIS vectors. *Ciona intestinalis* was not observed on the eastern shore from Halifax to Country Harbour, or from Merigomish to Malagash on the north shore in 2010. The north shore is probably more at risk of invasion by *C. intestinalis*, given their presence at Havre Boucher in 2008 and 2009 (Sephton et al. 2011), and in Ballantyne's Cove, St. George's Bay in 2009 (A. Lacoursière-Roussel, per. comm), the close proximity to PEI, and volume of vessel traffic from this area (Darbyson et al. 2009). A Rapid Assessment to determine the presence of tunicates was conducted at Caribou in August 2010. While no tunicates were found, it is important to maintain monitoring efforts in this area given the risk of introduction of their introduction.

4.1.2 *Botryllus schlosseri*

Botryllus schlosseri, present in Nova Scotia since the 1980's (Carver et al. 2006b), is now the most widely distributed tunicate; present at 69% of monitoring stations between 2006 and 2009 (Sephton et al. 2011) and at 88% of sentinel stations in 2010 (Table 6). Golden star tunicates are established in all regions of Nova Scotia, with the exception of part of the north shore, and from Ship Harbor to Liscombe on the eastern shore.

Table 6. Average prevalence of *Ciona intestinalis*, *Botryllus schlosseri* and *Botrylloides violaceus* at sentinel stations in Nova Scotia in 2006 through 2010. Nm = not monitored, P = present. Categories for the percentage cover were 0 absent), 1: <25% (low), 2: 25-50% (moderate), 3: 51-75% (heavy), 4: 75-99% (very heavy) and 5: >100% cover. Red and green lines indicate increasing and decreasing abundance trends, respectively.

Region	Sentinel Station	*Ciona intestinalis*					*Botryllus schlosseri*					*Botrylloides violaceus*				
		2006	2007	2008	2009	2010	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010
SW Shore	Digby	1	0	1	1	1	1	1	1	1	1	0	0	0	0	1
	Meteghan	2	1	2	2	2	1	1	1	1	1	0	1	1	1	1
	Yarmouth Bar	1	0	0	1	1	1	0	0	1	1	0	0	1	1	1
	Wedgeport	1	1	1	2	3	1	2	2	1	1	1	1	2	1	1
	Camp Cove	1	3	3	3	2	1	1	1	1	1	1	1	1	1	1
	Clark's Harbour	nm	3	1	3	1	nm	1	1	1	1	nm	1	1	1	1
S Shore	Shelburne	nm	3	2	1	2	nm	1	1	1	1	nm	1	0	0	0
	Lunenburg	1	4	4	2	1	1	1	1	1	2	1	1	1	1	1
	Indian Point	2	4	4	4	2	1	1	2	1	1	0	1	1	1	1
	Chester	3	1	1	1	1	1	1	2	3	2	0	1	1	1	1
Halifax	Halifax BIO	1	1	1	2	0	0	0	1	1	0	0	0	0	0	0
E Shore	Ship Harbour	nm	0	nm	0	0	nm	0	nm	0	0	nm	0	nm	0	0
Chedabucto	Venus Cove	nm	nm	1	2	1	nm	nm	1	1	1	nm	nm	0	0	0
	Petit de Grat	0	nm	4	3	P	1	nm	1	1	P	1	nm	1	1	P
Bras D'Or	St Peters	0	1	1	1	0	1	2	3	1	3	1	0	1	0	0
	Whycocomagh	0	0	0	0	0	0	0	4	3	3	0	0	0	0	0
	Eskasoni	0	0	0	0	0	1	1	0	1	2	0	0	0	0	0
	Baddeck	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
CB Coast	Louisbourg	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0
	Sydney	1	1	0	1	2	0	1	1	2	1	0	0	0	0	0
	North Sydney	nm	1	1	2	2	nm	1	1	1	1	nm	1	0	1	2
	Cheticamp	0	0	1	0	0	1	2	2	2	2	1	1	1	1	1
	Mabou	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N Shore	Pictou	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Presence	10	12	15	16	14	14	16	19	22	21	6	10	11	12	12
	# Stations	19	22	23	24	24	19	22	23	24	24	19	22	23	24	24
	%	52	54	65	66	58	73	72	82	92	88	31	45	47	50	50

Botryllus schlosseri was found on settlement collectors in 2009 at Cribbon's Point and Ballantyne's Cove in St. George's Bay on the north shore (low during first deployment; heavy during second deployment), and in Musquodoboit Harbour on the eastern shore in 2009 (Lacoursière-Roussel et al. 2012 b). While this species dominates with heavy coverages in the Bras D'Or Lake (specifically between 2008 and 2010, Table 6), and is now widespread in Nova Scotia as a low to moderate infesting AIS (87% of NS marinas in 2009; Lacoursière- Roussel et al. 2012 b), it is generally regarded as a nuisance species (Ramsay et al, 2008; Paetzold et al. 2012) with less impact than *C. intestinalis*. Arens et al. (2011) and Paetzold et al. (2012) determined that fouling by *B. schlosseri* on mussel socks in St. Peter's Bay, PEI had no effect on mussel productivity, growth, length, abundance of condition index), despite an aquaculture industry perception that fouling by colonial tunicates was detrimental to mussel crops.

4.1.2 *Botrylloides violaceus*

Botrylloides violaceus, present since the 1990's (Carver et al. 2006b), is also now established in many areas, including the southwestern and south shores, on Isle Madame, at North Sydney, Dingwall and Cheticamp (Sephton et al. 2011). This species has spread to a few new locations each year since 2006; from 19% of all monitoring stations in 2006, to 38%, 50% and 42% of stations in 2008, 2009 and 2010 respectively, and present at 33% of marinas surveyed in 2009 by Lacoursière-Roussel et al. (2012a). It was present at 50% of sentinel stations in 2010 (Table 6) and reported for the first at two locations; Digby, in the Bay of Fundy, and Sober Island on the eastern shore in 2010. Generally, this species occurs in low coverage, but heavier coverages were noted in North Sydney and Dingwall in 2010. As is the case for *B. schlosseri*, *B. violaceus* is regarded as a nuisance species, and its fouling of blue mussels does not impact productivity or growth (Arens et al. 2011; Paetzold et al. 2012)

4.1.3 Absence of *Styela clava*, *Didemnum vexillum*, *Diplosoma listerianum* and *Ascidiella aspersa*

Four additional tunicate species, either present in nearby eastern Canadian (PEI: *S. clava*), or in northeastern US coast waters (*D. vexillum*, *D. listerianum* and *A. aspersa*), were not detected in NS in 2010. Given the volume of commercial shipping and recreational boating vectors from these regions (see Darbyson et al 2009; Locke 2009; Lacoursière-Roussel, 2012a) and the similarities between donor regions and NS waters, it is possible that these species will successfully establish following their introduction (Locke 2009).

Populations of *S. clava* have declined in PEI in recent years, concurrent with the arrival of *C. intestinalis* (Ramsay et al. 2008). The concerted efforts of government and the shellfish culture industry described by Locke et al. (2009) may have prevented its arrival in Nova Scotia through the transfer of infected shellfish. Indeed, the spread of exotic tunicates in coastal waters through bivalve aquaculture is likely not as important as other vectors (McKindsey et al. 2007), as stock transfers are limited and carefully managed through Introduction and Transfer Committees (Locke et al. 2009). *Styela clava* may be introduced attached to drifting materials or on boat hulls, as recreational and commercial boats regularly travel from PEI to Nova Scotia (Darbyson et al. 2009; Locke et al. 2009). Another solitary tunicate, *A. aspersa*, native to Europe and the Mediterranean, and present in New England since the 1980's, has not yet been found in Canadian waters (Mackenzie, 2011b), but it remains on our monitoring list as a potential invader.

Of the colonial tunicate species, *D. vexillum*, present on the US seaboard as far north as Eastport, ME, is not yet present in Atlantic Canadian waters. It has not been detected in the Bay of Fundy, either through biofouling monitoring efforts (LeGresley et al. 2008, Martin et al. 2010a) or during annual Rapid Assessments conducted since 2009 (Martin et al. 2010b; Sephton and Vercaemer, pers. obs.). It has not been found in Nova Scotian waters to date during annual biofouling monitoring (Sephton et al. 2011), or during the Rapid Assessment conducted in Yarmouth Bar in 2010 (pers. obs.). The shell-gravel bottom of the US side of George's Bank is heavily covered with *D. vexillum* (Valentine et al. 2007), so the commercial scallop fleet is a potential, yet unintentional, vector for its introduction to southwestern Nova Scotia. Once dislodged from the bottom when benthic sediments are disturbed by scallop dragging, *D. vexillum* may enter non-infested areas on the Canadian side of the Bank as floating fragments in ocean currents (Lengyel et al. 2009), and reattach on hard substrates (Bullard et al. 2007), or be introduced attached to scallop shells or caught in fishing gear. This species also threatens the scallop fishery directly through habitat loss, making the ocean bottom unsuitable and inaccessible for settling scallops (Morris et al. 2009).

Diplosoma listerianum, native to Europe (Mackenzie, 2011a), and present sporadically in the Gulf of Maine for about 20 years (Dijkstra et al. 2007a), was reported in the Magdalen Islands at Havre Aubert, QC in 2008 (Simard, pers. comm.). A Rapid Assessment was conducted in 2009, and no sign of the tunicate was found, nor has it been recorded elsewhere in Atlantic Canada since. This species remains on our watch list, as it has potential for rapid establishment and dominance in new habitats (Vance et al. 2009).

4.2 SPATIAL AND TEMPORAL VARIATION IN TUNICATE DISTRIBUTIONS

Tunicate distributions in Nova Scotia have previously been described as discontinuous or "patchy" in space and "sporadic" in time (Sephton et al. 2011) and these characteristics were again evident in 2010.

4.2.1 Spatial Variations

Small scale "patchiness" was evident by variable coverage by one or more of the three dominant tunicate species on separate plates of the same collector (Figure 3). This small-scale patchiness may reflect the limited natural dispersion ability of tunicates as gametes or larval stages (Lambert and Lambert 1998; Lambert 2005). Differences between collectors hung during the same deployment period, most often within several meters of one another or on alternate sides of the same floating dock, are representative of limited larval dispersal at scales of less than 10 m (Osman and Whitlatch 1995), or small differences in environmental variables such as light or current. Deploying duplicate collectors and calculating an average coverage value for each deployment period, as well as visually examining alternate surfaces, allowed the determination of a more meaningful estimate of tunicate coverage at monitoring stations. The importance of the visual search was emphasized in situations where one duplicate collector was lost, or where tunicate presence was so low that settlement did not occur on monitoring collectors, but tunicates were indeed present (ie *C. intestinalis* at Yarmouth Bar). Visual inspections may also reveal the presence of other invasive species, such as *Codium fragile* and other algae, *Caprella mutica, Membranipora membranacea*, and *Carcinus maenas* and other crab species.

Large scale "patchiness" was evident across larger areas in 2010, where differences in the degree of tunicate coverage were noted from one station to the next, while environmental conditions were very similar. Examples of this type of "patchiness" were noted on the southwestern shore, where heavy coverage by *C. intestinalis* were noted at Wedgeport, Camp Cove and Ingomar, while stations nearby at Eel Lake and Clark's harbour had low coverages. These observations may also be related to low intensity of a vector for secondary spread from an infested to a non-infested area, and reduced propagule pressure (Charlton 1996).

4.2.1 Temporal Variations

While *C. intestinalis. B. schlosseri* and *B. violaceus* were present as low to moderate foulers on plates throughout the year at many stations, there were some temperature-related seasonal patterns in tunicate presence.

Dominance by *C. intestinalis* was noted as heavy coverages during the first deployment period in Meteghan, Wedgeport, and Camp Cove (southwest NS), Chester (south shore) and at Sydney and North Sydney. We observed moderate to substantial numbers of overwintered vase tunicates in southwest NS and along the south shore in late May, evident potential for reproduction and early settlement on monitoring plates once water temperatures reached 8°C, regarded as the lower limit for spawning in Nova Scotian populations (Carver et al., 2006), although gamete production has been observed at 4°C (Carver et al. 2003) . The spawning period for this species in Nova Scotia begins in late May and continues through until November or early December (Vercaemer et al. 2011; Appendix 3). Larvae grow quickly following settlement (Carver et al. 2003), covering surfaces including monitoring plates and leaving little room for colonial tunicates, which explains their low coverages on plates in many locations where vase tunicates dominate, and their settlement on vase tunicates themselves (eg. Ingomar, Meteghan). Vase tunicates were noted in low coverage throughout the year in Halifax, and at colder stations in Digby, Yarmouth Bar and Clark's Harbour, and in Lunenburg, a somewhat warmer station where they co-occurred with colonial tunicates. At Venus Cove, *C. intestinalis* was not observed until the second deployment after a warming period in mid-July (~ 22°C) , and their coverages were highest during this period at Indian Point and Shelburne, which may reflect the September peak in recruitment observed by Vercaemer et al. (2011) along the south shore.

Settlement and growth by colonial tunicates when waters are warmer was reflected by the first deployment absence of *B. schlosseri* in Digby, at RNSYS (Halifax) and Venus Cove, and *B. violaceus* in Digby and Yarmouth Bar. This trend was clearly evident at Cheticamp, when both colonial species were most abundant during the second deployment period. Coverage by *B. schlosseri* was much higher during late summer to fall at stations in the Bras D'Or Lake (St. Peter's, Baddeck, Whycocomagh and Orangedale) where it was the only tunicate present. At Chester, *B. schlosseri* replaced *C. intestinalis* as the dominant species during the second deployment period, and there were more golden star tunicates present during this period in Camp Cove, Sydney and Cheticamp. *B. violaceus* was heaviest on second set collectors at North Sydney, where it replaced *C. intestinalis* as the dominant tunicate.

Anomalies in seasonal patterns of settlement, such as heaviest coverage of *B. schlosseri* on first deployment collectors, and lower coverages on second and full deployment plates at Eskasoni (Fig. 14C), may also reflect intense settlement at very small scales, which does not necessarily reflect the overall pattern of settlement at a location. Deployment of additional collectors may address this shortcoming of the present monitoring protocol, although this may not be logistically feasible.

Inter-annual "patchiness" in tunicate presences were also noted at sentinel stations (Table 6). The absence of a species in subsequent year(s) following its appearance (ie introduction) can signal that (1) it has not survived through the winter in the new environment, or (2) that its density is "patchy" or so low that it is not detected through the monitoring protocol used. For example, *Ciona intestinalis* was not found on monitoring plates at Yarmouth Bar in 2007, 2008 or 2010, or at St. Peter's in 2007 and 2010. Similarly, *B. schlosseri* was not found on monitoring plates at BIO in 2006, 2007 and 2009, but this species is well established, in very low abundances, throughout Halifax Harbour (Sephton, pers. obs.). Monitoring protocol in areas where tunicates have not been observed, or where infestations are very low, should be adjusted to include additional "search time" to examine fixed structures, the deployment of additional monitoring collectors, or the use of modified collectors with shading saucers and additional fouling surfaces (2006 collector: Sephton et al. 2011). There have been instances, such as the occurrence *D. listerianum* at Havre Aubert, QC in 2008, where tunicates have been present one summer and absent the following year. However, negative reports in 2 consecutive years are required before a water body is considered "tunicate free" and shellfish transfers can resume in PEI (Locke et al. 2009).

4.3 DISTRIBUTION OF NON-TUNICATE BIOFOULERS

4.3.1 *Membranipora membranacea*

Membranipora membranacea, was first reported in the Gulf of Maine in 1987 (Berman et al. 1992). This encrusting bryozoan was probably introduced from Europe (Schwaninger 1999), and first reported in the Mahone Bay and St. Margaret's Bay area in the early 1990's (Scheibling et al. 1999). This species alters benthic habitat by encrusting on the blades of kelp, causing them to become brittle and break with wave action, resulting in mass defoliation of native kelp beds (Berman et al. 1992; Saunders and Metaxas 2008; Scheibling and Gagnon 2006; Scheibling et al. 1999). With the destruction of kelp beds, colonization and establishment by other algal species, including the invasive alga *C. fragile*, may proceed. The presence of *Codium fragile*, which is of a lower nutritional quality than native kelp, results in changes in benthic community structure (Schmidt and Scheibling 2007).

Consistent recording of the presence of *M. membranacea* began in 2010, and although the bryozoan was noted in many locations in 2009, a reliable comparison between those years cannot be made. This species was present at 23 of 33 monitoring sites, in all regions of the province, in 2010. Population outbreaks of this species have been associated with warmer water temperatures (Saunders and Metaxas 2007, 2008; Scheibling and Gagnon 2009), and given the warming water trend observed in many areas of Nova Scotia in the last several years, the infestation is likely to continue and spread. Colonies of *M. membranipora* can overwinter in Nova Scotia on a number of kelp species (Saunders and Metaxus 2009), and these colonies can provide larvae for first settlement in May or June (Saunders and Metaxus 2007).

To date, our monitoring for *C. fragile* has been confined to visual observations at a few biofouling monitoring sites, and anecdotal reports. It was noted as present at 2 locations: Camp Cove and Sober Island, in 2010. Given the widespread presence of *M. membranacea*, which facilitates the removal of native kelps and their replacement by species such as *C. fragile*, future monitoring efforts should place increased emphasis on the detection of the alga at locations with, and without, *M. membranacea*.

4.3.1 *Caprella mutica*

Caprella mutica, native to north-eastern Asia (Turcotte and Ste-Marie, 2009), was first reported in Atlantic Canadian waters in 1998 Prince Edward Island (Locke et al. 2007), where it is now well established. Since that time, the presence of Japanese skeleton shrimp has been noted anecdotally on many shellfish aquaculture sites, and at biofouling monitoring locations throughout Nova Scotia (Sephton, pers. comm.), primarily on artificial structures. *C. mutica* was observed at 9 of 33 monitoring stations in 2010, located throughout the province. Temperature and salinity are the key factors that limit its spatial distribution, but with a temperature tolerance between -1.8 to 25°C, and a salinity tolerance between 11 and 35, (Turcotte and Ste-Marie, 2009), this highly productive species is likely to survive throughout Atlantic Canada.

Its effect on natural ecosystems remain largely unknown, although it has been confirmed that a decrease in mussel (*Mytilus edulis*) spat collection and growth is associated with the appearance of *C. mutica* (Ashton 2006; B. Sainte-Marie and C. Turcotte, pers. obs). In the laboratory, *C. mutica* is an aggressive competitor towards native caprellid species (Shucksmith et al. 2009). Its primary method of dispersal is attached to boat hulls or in ballast water, and although it does not have a planktonic larval stage, it can move actively by crawling or swimming (Caine 1980).

4.4 NEXT STEPS IN AIS MONITORING IN NOVA SCOTIA

Given the number of established AIS in Nova Scotia, and the potential for introduction of new AIS through shipping and boating from other regions, and the additional of new species to the "watch list" annually, it is imperative that DFO's monitoring of AIS continue in Atlantic Canada. Several areas for improvement have been identified during the course of the present work:

1. <u>Increased monitoring of non- and low-infested regions</u>

   Areas such as the north and part of the eastern shores of NS remained free of tunicates until the summer of 2013, and infestations are low, or restricted to *B. schlosseri* only. DFO Gulf Region has monitored stations on the north shore since 2011, but communication and co-ordination between DFO Gulf and DFO Maritimes Region will continue to ensure that a sufficient number of key stations are monitored, given the threat to shellfish aquaculture in this area.

   Increased monitoring activity will be initiated along the eastern shore, specifically in the Ship Harbour area, site of an active and viable mussel aquaculture site.

2. <u>Increased efforts to detect *Didemnum vexillum* in southwest NS and along the south shore</u>

   There have been sporadic, unconfirmed reports *D. vexillum* in this area, but to date, no samples that would allow confirmation of its presence have been collected, and the species has never been found on monitoring plates. A native didemnid, *D. albidum*, is very similar in appearance to *D. vexillum*, making identification in the field difficult and necessitating microscopic examination to differentiate the species. Additional monitoring stations will be established in this area, and outreach at local fisheries meetings may also be attempted, where identification and collection materials are provided to scallop fishermen. A Rapid Assessment, similar to the annual Bay of Fundy Assessment, was conducted in September 2013, with a total of 28 harbours and marinas examined. *Didemnum vexillum* was not observed during the course of this assessment.

3. <u>Demarcations of AIS distributions</u>

   Additional geo-reference surveys/sightings (e.g. small buoy surveys; Vercaemer et al. 2013) will be conducted as part of AIS monitoring activities, as time permits. While new AIS monitoring stations will not be established, serendipitous searches will be conducted whenever an area is visited, and monitoring partners and the general public will be encouraged to report AIS sightings, provide GPS co-ordinates, and reference samples for identification.

4. <u>Continuing, and expanded, outreach efforts to recreational marinas, the aquaculture industry, and coastal communities in general</u>

   A number of new AIS products have been developed recently, and increased efforts will be made to distribute them to the general public. AIS Identification posters will be displayed at marinas, fishing harbours, and replace at AIS monitoring stations if required. A tri-fold, tabletop AIS booth and a Pop-up Banner have been developed with the help of DFO Communications. These products will form the basis of new AIS outreach efforts at Fisheries and Marina meetings and the Halifax International Boat Show. AIS Identification booklets, temporary tattoos, stickers and pens, will encourage coastal users to "recognize, remove and report" AIS.

5. Continue to collect information on AIS life histories, and environmental tolerances and species interactions to support ongoing research

   AIS monitoring efforts have included the collection of environmental data, as well as noting the presence of AIS and native species on monitoring collectors, and at monitoring stations, throughout the monitoring period and beyond. We will continue to support ongoing AIS research, as we strive to compile more information that will enhance our understanding of the impacts of AIS in Nova Scotia waters.

5.0 CONCLUDING REMARKS

The DFO AIS Biofouling Monitoring program, ongoing in Nova Scotia since 2006, has clearly identified that five biofouling AIS: the tunicates; *Ciona intestinalis*, *Botryllus schlosseri* and *Botrylloides violaceaus*, the caprellid shrimp *Caprella mutica* and the bryozoan *Membranipora membranacea* are now firmly established in Nova Scotia's coastal waters. Along with two established non-fouling species; the alga *Codium fragile* and the European green crab *Carcinus maenas*, these invasive species have demonstrated harmful effects to native species, communities and habitats, and continue to spread to new environments each year. The nuisance impacts of these AIS, which include biofouling of recreational structures, fishing and aquaculture gear and product, and commercial and recreational boats, have been a deterrent to the use and enjoyment of coastal marinas and environments, as well as an additional financial burden to recreational boaters and marine and aquaculture industries.

Given the volume of recreational and commercial vessels from many regions of the world, the potential for the introduction and spread of new AIS, as well as the spread of established AIS in Nova Scotian waters, is of great concern for DFO biologists and managers alike, especially given recent observations of warmer winter and summer water temperatures. The biofouling monitoring program has been instrumental in the early detection of three new species of tunicates: *Styela clava*, *Ascidiella aspera* and *Diplosoma listerianum* in Nova Scotia in 2012, and *Didemnum vexillum* remains on the DFO Maritimes Region watch list. Locke (2009) has identified and additional 14 tunicate species as potential invaders to the region, and the potential for the introduction of exotic crustaceans and fish species is also increasing, so it is important that this program continues.

The process of developing Aquatic Invasive Species Regulations under the *Fisheries Act* began in 2010. These Draft Regulations are nearing completion, and have been the subject of Public Comment and Review in recent months, so their inclusion in upcoming amendments to the Fisheries Act is widely anticipated. AIS Regulations will provide a legal framework and process to prohibit the introduction and spread of AIS, while facilitating their management, mitigation and eradication. It is imperative that current AIS surveillance and monitoring efforts, led by DFO and with the support of other levels of government, First Nations, the aquaculture industry, NGO's and fishing and coastal communities, continue concurrently with the development, introduction and enforcement of these Regulations. DFO monitoring and surveillance will provide accurate and up-to-date information on the distributions of AIS to inform and strengthen the Regulations.

The strengths and value of the present AIS monitoring program lie in its potential and/or ability to:

- Facilitate surveillance, early detection and rapid response activities for new AIS, so that new infestations may be documented and managed,
- Identify habitats, and environmental conditions, where AIS are found, thus informing research on the biology of these species, and their ecological interactions in native communities and habitats,
- Monitor the establishment of new AIS, and track the spread of existing populations,
- Provide information and advice for management decisions, and
- Increase awareness of AIS within the general public and among DFO clients, and encourage reporting of new AIS occurrences.

The program makes integral contributions to DFO's strategic outcomes to maintain healthy and sustainable aquatic ecosystems, and economically prosperous Maritime sectors and fisheries, faced with the ecological and economic threats posed by AIS.

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Appendix 1: Details of monitoring and tunicate coverage on individual collectors at each station in 2010. Stations are grouped by geographical Region or Bay. Species coverage are given for top, middle and bottom plates (x,y.z), where 0 = no tunicate coverage, 1 = <25% coverage, 2 = 26-50% coverage, 3 = 51-75% coverage, 4 = 75-99% coverage and 5 = 100%+ coverage. Minilog thermistors (blue text) were deployed at several stations. (C.i. = *Ciona intestinalis,* B.s. = *Botryllus schlosseri*, B.v. = *Botrylloides violaceus*, P-A = Present, anecdotal report and O-A = Absent, anecdotal report, ** = *tunicates* present at station, but not on monitoring collector) and nf = no fouling.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
Southwestern shore	1	Digby MINILOG	CARP	First (28 May – 17 August)	81	1,1,0	0,0,0	0,0,0
				First (28 May – 17 August)	81	0,0,0	0,0,0	0,0,0
				Second (17 Aug. - 8 October)	52	0,0,0	0,0,0	0,1,0
				Second (17 Aug. - 8 October)	52	1,1,1	1,0,0	0,0,0
				Full (28 May – 8 October)	133	4,3,2	0,3,0	0,0,1
				Full (28 May – 8 October)	133	0,0,0	0,0,0	0,1,0
	2	Meteghan	DFO	First (20 May – 5 August)	77	3,3,1	1,0,0	0,0,0
				First (20 May – 5 August)	77	1,3,2	0,0,1	1,1,1
				Second (5 Aug. – 14 October)	70	1,0,0	0,0,0	3,3,3
				Second (5 Aug. – 14 October)	70	1,1,1	0,0,2	2,0,0
				Full (20 May – 14 October)	147	5,5,5	0,0,0	0,1,1
				Full (20 May – 14 October)	147	lost	lost	lost
	4	Yarmouth Bar MINILOG	DFO	First (20 May – 5 August)	77	0,0,0	0,0,1	0,0,0
				First (20 May – 5 August)	77	0,0,0	0,0,0	0,0,0
				Second (5 Aug. – 14 October)	70	0,0,0**	0,0,1	1,1,0
				Second (5 Aug. – 14 October)	70	0,0,0**	0,0,1	2,2,2
				Full (20 May – 14 October)	147	0,0,0	0,0,0	1,1,1
				Full (20 May – 14 October)	147	0,0,0	0,0,0	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
Southwestern shore				First (19 May – 4 August)	77	5,5,5	1,1,1	1,1,1
				First (19 May – 4 August)	77	1,1,2	4,3,3	1,2,1
	6	Wedgeport	DFO	Second (4 Aug. – 14 October)	71	0,1,1	2,1,1	1,0,0
		MINILOG		Second (4 Aug. – 14 October)	71	5,5,4	0,1,0	1,0,1
				Full (19 May – 14 October)	148	4,4,4	1,1,1	1,1,1
				Full (19 May – 14 October)	148	lost	lost	lost
	108	Eel Lake	DFO	Full (15 May – 20 October)	127	0,0,0**	1,1,1	0,0,0
				Full (15 May – 20 October)	127	0,0,0**	1,1,1	0,0,0
				First (19 May – 4 August)	77	2,1,4	2,1,0	1,1,1
				First (19 May – 4 August)	77	5,5,4	0,0,0	0,1,1
	7	Camp Cove	DFO	Second (4 Aug. – 15 October)	72	1,2,1	2,2,2	1,1,1
				Second (4 Aug. – 15 October)	72	3,3,2	1,1,2	0,1,1
				Full (19 May – 15 October)	149	1,1,1	1,1,0	2,1,3
				Full (19 May – 15 October)	149	4,4,4	0,0,0	0,0,1
				First (19 May – 4 August)	77	1,1,1	1,1,1	1,1,0
				First (19 May – 4 August)	77	1,0,1	1,1,1	0,0,0
	8	Clark's Harbour	DFO	Second (4 Aug. – 13 October)	70	1,1,1	2,1,2	1,2,1
		MINILOG		Second (4 Aug. – 13 October)	70	1,1,1	2,3,2	2,1,1
				Full (19 May – 13 October)	177	1,1,1	1,0,0	1,0,1
				Full (19 May – 13 October)	177	1,1,0	1,1,1	0,0,0

Appendix 1. continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
				First (19 May – 4 August)	77	5,5,5	0,0,0	0,0,0
				First (19 May – 4 August)	77	5,5,5	0,0,0	0,0,0
South shore	10	Ingomar	DFO	Second (4 Aug. – 13 October)	70	5,5,5	0,0,0	1,0,0
				Second (4 Aug. – 13 October)	70	4,5,3	1,1,1	1,1,1
				Full (19 May – 13 October)	147	1,5,4	1,1,1	1,0,1
				Full (19 May – 13 October)	147	4,4,4	0,0,0	0,0,0
				First (19 May – 4 August)	77	0,0,0	1,1,1	0,0,0
				First (19 May – 4 August)	77	0,0,0	0,0,1	0,0,0
	12	Shelburne	DFO	Second (4 Aug. – 13 October)	70	5,5,5	0,0,0	0,0,0
		MINILOG		Second (4 Aug. – 13 October)	70	5,5,5	0,0,0	0,0,0
				Full (19 May – 13 October)	147	3,4,4	1,1,1	0,0,0
				Full (19 May – 13 October)	147	2,4,3	0,0,0	0,0,0
	424	Port L'Hebert	NSDFA	Dec-10	n/a	0-A	0-A	0-A
				First (19 May – 4 August)	77	0,0,0	0,0,0	0,0,0
				First (19 May – 4 August)	77	0,0,0	1,0,0	0,0,0
	82	Port Mouton	DFO	Second (4 Aug. – 13 October)	70	0,0,0	0,0,0	0,0,0
				Second (4 Aug. – 13 October)	70	0,0,0	1,1,1	0,0,0
				Full (19 May – 13 October)	147	0,0,0	1,0,0	0,0,0
				Full (19 May – 13 October)	147	0,0,0	0,0,0	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
South shore				First (19 May – 4 August)	76	0,0,0	0,0,0	0,0,0
				First (19 May – 4 August)	76	0,0,0	0,0,0	0,0,0
	100	LaHave River	DFO	Second (4 Aug. – 13 October)	69	0,0,0	0,0,0	0,0,0
				Second (4 Aug. – 13 October)	69	0,0,0	0,0,0	0,0,0
				Full (19 May – 13 October)	145	0,0,0	0,0,0	0,0,0
				Full (19 May – 13 October)	145	0,0,0	0,0,0	0,0,0
				First (21 May – 6 August)	77	0,0,0	4,4,4	1,1,1
				First (21 May – 6 August)	77	0,1,0	2,3,3	1,1,1
	18	Lunenburg	DFO	Second (6 Aug. – 13 October)	68	1,1,2	0,1,1	1,2,2
		MINILOG		Second (6 Aug. – 13 October)	68	1,1,1	1,1,0	1,1,1
				Full (21 May – 13 October)	145	1,2,1	1,1,1	1,1,1
				Full (21 May – 13 October)	145	3,4,4	1,1,1	1,2,1
				First (12 May – 3 August)	83	0,0,0	0,0,0	0,0,0
	19	Indian Point	DFO	First (12 May – 3 August)	83	1,1,1	0,0,0	0,0,0
		(Site 1)		Second (3 Aug. – 12 October)	70	4,2,nf	1,0,nf	0,0,nf**
		MINILOG		Second (3 Aug. – 12 October)	70	4,4,2	0,0,0	0,0,0
				First (12 May – 3 August)	83	1,1,1	1,0,0	0,0,0
	19	Indian Point	DFO	First (12 May – 3 August)	83	1,2,4	0,0,0	0,0,0
		(Site 2)		Second (3 Aug. – 12 October)	70	4,1,3	0,0,1	0,0,0
		MINILOG		Second (3 Aug. – 12 October)	70	3,4,3	1,1,1	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
South shore				First (12 May – 3 August)	83	0,0,0	0,0,0	0,0,0
	19	Indian Point	DFO	First (12 May – 3 August)	83	1,1,1	1,1,1	0,0,0
		(Site 3)		Second (3 Aug. – 12 October)	70	3,2,3	0,0,0	0,0,0
		MINILOG		Second (3 Aug. – 12 October)	70	4,4,4	1,1,1	0,0,0
				First (25 May – 6 August)	73	4,4,4	0,0,0	0,0,1
				First (25 May – 6 August)	73	0,0,0	4,4,4	0,0,0
				Second (6 Aug. – 20 October)	75	0,0,1	3,2,3	1,2,1
	21	Chester	DFO	Second (6 Aug. – 20 October)	75	lost	lost	lost
				Full (25 May – 20 October)	148	0,1,1	1,1,0	2,1,1
				Full (25 May – 20 October)	148	lost	lost	lost
				First (14 May – 3 August)	81	1,1,1	0,0,0	0,0,0
				First (14 May – 3 August)	81	1,1,1	0,0,0	0,0,0
Halifax	24	Halifax; BIO Jetty	DFO	Second (3 Aug. – 29 October)	87	1,1,1	0,0,0	0,0,0
		MINILOG		Second (3 Aug. – 29 October)	87	1,1,1	0,0,0	0,0,0
				Full (14 May – 29 October)	168	1,1,	0,0,0	0,0,0
				Full (14 May – 29 October)	168	2,1,3	0,0,0	0,0,0
				First (21 May – 6 August)	77	1,1,1	0,0,0	0,0,0
				First (21 May – 6 August)	77	1,0,0	0,0,0	0,0,0
				Second (6 Aug. – 29 October)	84	1,0,1	3,0,0	0,0,0
	402	Halifax; RNSYS	DFO	Second (6 Aug. – 29 October)	84	1,0,1	1,0,0	0,0,0
				Full (21 May – 29 October)	161	1,1,1	0,0,0	0,0,0
				Full (21 May – 29 October)	161	1,0,0	0,0,0	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
Eastern	421	Sober Island	NSDFA	Dec-10	n/a	0-A	0-A	P-A
	37	Whitehead	NSDFA	Dec-10	n/a	P-A	0-A	0-A
	104	Country Harbour	NSDFA	Dec-10	n/a	P-A	0-A	0-A
				First (26 May – 10 August)	76	0,0,0	0,0,0	0,0,0
				First (26 May – 10 August)	76	0,0,0	0,0,0	0,0,0
	41	Venus Cove	DFO	Second (10 Aug. – 7 October)	58	1,1,1	0,1,1	0,0,0
		MINILOG		Second (10 Aug. – 7 October)	58	1,1,0	1,1,1	0,0,0
				Full (26 May – 7 October)	134	1,0,0	1,1,1	0,0,0
				Full (26 May – 7 October)	134	lost	lost	lost
Chedabucto	44	Petit de Grat	DFO	19-Oct-10	n/a	P-A	0-A	P-A
				First (26 May – 10 August)	76	0,0,0	3,4,1	0,0,0,
				First (26 May – 10 August)	76	0,0,0	2,2,3	0,0,0
Bras d'Or Lake	47	St. Peter's	DFO	Second (10 Aug. – 8 October)	59	0,0,0	4,4,4	0,0,0
		MINILOG		Second (10 Aug. – 8 October)	59	0,0,0	4,4,4	0,0,0
				Full (26 May – 8 October)	135	0,0,0	4,4,4	0,0,0
				Full (26 May – 8 October)	135	0,0,0	4,4,4	0,0,0
	48	Gillis Cove	NSDFA	Dec-10	n/a	0-A	P-A	0-A
				First (31 May – 3 August)	64	0,0,0	1,0,0	0,0,0
				First (31 May – 3 August)	64	0,0,0	1,0,3	0,0,0
	51	Whycocomagh	EFWC	Second (3 Aug. – 3 Nov.)	92	0,0,0	1,5,5	0,0,0
				Second (3 Aug. – 3 Nov.)	92	0,0,0	3,5,4	0,0,0
				Full (31 May – 3 November)	156	0,0,0	5,4,3	0,0,0
				Full (31 May – 3 November)	156	0,0,0	3,5,5	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
				First (31 May – 3 August)	64	0,0,0	0,0,0	0,0,0
				First (31 May – 3 August)	64	0,0,0	0,1,1	0,0,0
Bras d'Or Lake	52	Orangedale	EFWC	Second (3 Aug. – 3 November)	92	0,0,0	4,3,3	0,0,0
				Second (3 Aug. – 3 November)	92	0,0,0	3,3,3	0,0,0
				Full (31 May – 3 November)	156	0,0,0	4,4,2	0,0,0
				Full (31 May – 3 November)	156	0,0,0	4,4,4	0,0,0
				First (31 May – 3 August)	64	0,0,0	2,4,4	0,0,0
				First (31 May – 3 August)	64	0,0,0	2,3,4	0,0,0
	54	Eskasoni	EFWC	Second (3 Aug. – 3 Nov.)	92	0,0,0	1,1,1	0,0,0
		MINILOG		Second (3 Aug. – 3 Nov.)	92	0,0,0	1,1,1	0,0,0
				Full (31 May – 3 November)	156	0,0,0	1,1,1	0,0,0
				Full (31 May – 3 November)	156	0,0,0	2,2,1	0,0,0
				First (26 May – 18 August)	84	0,0,0	0,2,1	0,0,0
				First (26 May – 18 August)	84	0,0,0	0,0,0	0,0,0
	55	Baddeck	DFO	Second (18 Aug. – 8 October)	51	0,0,0	2,2,3	0,0,0
		MINILOG		Second (18 Aug. – 8 October)	51	0,0,0	1,1,0	0,0,0
				Full (26 May – 8 October)	135	0,0,0	0,0,0	0,0,0
				Full (26 May – 8 October)	135	0,0,0	0,2,0	0,0,0
	86	East Bay	DFO	Full (15 June – 2 November)	144	0,0,0	4,1,3	0,0,0
				Full (15 June – 2 November)	144	0,0,0	4,3,1	0,0,0
	423	Alba	NSDFA	Dec-10	n/a	0-A	0-A	0-A

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
Cape Breton Coastal				First (8 June – 20 August)	73	0,0,0	0,0,0	0,0,0
				First (8 June – 20 August)	73	0,0,0	1,1,1	0,0,0
	58	Louisbourg	CBU	Second (20 Aug. – 15 Oct.)	56	lost	lost	lost
				Second (20 Aug. – 15 Oct.)	56	lost	lost	lost
				Full (8 June – 15 October)	129	lost	lost	lost
				Full (8 June – 15 October)	129	lost	lost	lost
				First (8 June – 28 August)	81	5,5,5	0,0,0	0,0,0
				First (8 June – 28 August)	81	5,1,2	0,0,1	0,0,0
	62	Sydney	CBU	Second (28 Aug. – 27 October)	60	0,0,0	1,1,1	0,0,0
				Second (28 Aug. – 27 October)	60	1,0,1	1,1,1	0,0,0
				Full (8 June – 27 October)	141	2,3,1	0,0,1	0,0,0
				Full (8 June – 27 October)	141	5,5,5	1,0,0	0,0,0
				First (8 June – 28 August)	81	4,4,4	0,0,0	0,0,0
				First (8 June – 28 August)	81	4,1,0	0,1,2	1,2,3
	63	North Sydney	CBU	Second (28 Aug. – 27 Oct.)	60	1,0,0	1,2,1	2,2,3
		MINILOG		Second (28 Aug. – 27 Oct.)	60	1,0,1	1,1,0	3,4,4
				Full (8 June – 27 October)	141	4,4,1	1,1,0	3,3,4
				Full (8 June – 27 October)	141	0,0,0	1,1,1	4,3,2
	69	Dingwall	DFO	Full (27 May – 7 September)	104	0,1,1	1,1,0	1,3,1
				Full (27 May – 7 September)	104	0,0,1	0,1,1	1,2,1
				Full (27 May – 7 September)	104	0,0,0	1,4,2	1,1,2
				Full (27 May – 7 September)	104	0,0,0	2,1,1	2,3,3

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
Cape Breton Coastal	94	Aspy Bay, North Harbour	DFO	Full (29 May – 9 Sept.)	104	0,0,0	0,0,0	0,0,0
		MINILOG	DFO	Full (29 May – 9 Sept.)	104	0,0,0	0,0,0	0,0,0
	95	Aspy Bay, South Harbour	NSDFA	Dec-10	n/a	0-A	P-A	0-A
	420	Dingwall Harbour	DFO	Full (27 May – 7 September)	104	0,0,0	1,0,0	2,1,1
				Full (27 May – 7 September)	104	0,0,0	0,0,0	0,1,1
	71	Cheticamp MINILOG	DFO	First (27 May – 17 August)	82	0,0,0	1,0,0	1,0,0
				First (27 May – 17 August)	82	0,0,0	0,0,1	1,0,1
				Second (17 Aug. – 7 October)	51	0,0,0	4,3,3	2,2,1
				Second (17 Aug. – 7 October)	51	0,0,0	4,4,4	1,1,2
				Full (27 May – 7 October)	133	0,0,0	3,3,4	2,1,1
				Full (27 May – 7 October)	133	0,0,0	2,3,1	1,1,0
	73	Mabou	DFO	First (27 May – 17 August)	82	0,0,0	0,0,0	0,0,0
				First (27 May – 17 August)	82	0,0,0	0,0,0	0,0,0
				Second (17 Aug. – 7 October)	51	0,0,0	0,0,0	0,0,0
				Second (17 Aug. – 7 October)	51	0,0,0	0,0,0	0,0,0
				Full (27 May – 7 October)	133	0,0,0	0,0,0	0,0,0
				Full (27 May – 7 October)	133	0,0,0,	0,0,0	0,0,0

Appendix 1, continued.

Region	Stn No.	Location	Monitored By	Deployment Dates	Deployment Period (days)	C.i. cover	B.s. cover	B.v. cover
North shore	112	Merigomish	NSDFA	Dec-10	n/a	0-A	0-A	0-A
				First (26 May – 17 August)	83	0,0,0	0,0,0	0,0,0
				First (26 May – 17 August)	83	0,0,0	0,0,0	0,0,0
	77	Pictou	DFO	Second (17 Aug. – 7 October)	51	0,0,0	0,0,0	0,0,0
				Full (26 May – 7 October)	134	0,0,0	0,0,0	0,0,0
				Full (26 May – 7 October)	134	0,0,0	0,0,0	0,0,0
				First (26 May – 17 August)	83	0,0,0	0,0,0	0,0,0
				First (26 May – 17 August)	83	0,0,0	0,0,0	0,0,0
	91	Caribou	DFO	Second (17 Aug. – 7 October)	51	0,0,0	0,0,0	0,0,0
		MINILOG		Second (17 Aug. – 7 October)	51	0,0,0	0,0,0	0,0,0
				Full (26 May – 7 October)	134	0,0,0	0,0,0	0,0,0
				Full (26 May – 7 October)	134	0,0,0	0,0,0	0,0,0
	422	Malagash	NSDFA	Dec-10	n/a	0-A	0-A	0-A

Appendix 2: Environmental variables measured using YSI probes at monitoring stations in 2010. Substation numbers are given for Indian Point. ChlA = chlorophyll a, IPMF = Indian Point Marine Farms, RNYS = Royal Nova Scotia Yacht Squadron, Ched. = Chedabucto.

Region	Stn. No.	Location	Sample Date	Probe Depth, m	YSI Type	Temp, °C	Salinity	Oxygen, %	Oxygen, mg L^{-1}	Conductivity, mS cm^{-1}	ChlA, µg L^{-1}	pH
Southwest Shore			20-May	1.4	6600	9.03	31.99	102.1	9.6	49.33	0.32	7.84
	2	Meteghan	05-Aug	1.3	6600	13.15	32.04	97.3	8.37	49.09	2.64	7.69
			14-Oct	1.8	6600	11.65	32.63	97.8	8.65	49.98	0.97	NA
			20-May	2.1	6600	8.44	31.82	99.7	9.51	49.16	0.64	7.83
	4	Yarmouth Bar	05-Aug	1.6	6600	13.48	31.7	100.5	8.6	48.6	2.25	7.97
			14-Oct	1.8	6600	12.31	32.2	100.3	8.77	49.36	0.45	NA
	108	Eel Lake	20-Oct	NA	Pro+	11.5	NA	102.6	NA	NA	NA	NA
			19-May	1.6	6600	11.66	29.66	100.5	9.05	45.88	1.16	7.82
	6	Wedgeport	04-Aug	1.7	6600	16.03	30.52	96.9	7.94	46.9	1.8	7.85
			14-Oct	1.2	6600	12.47	30.64	94.8	8.34	47.18	1.42	NA
			19-May	2.8	6600	11.09	31.08	103.4	9.35	47.88	0.97	7.87
	7	Camp Cove	04-Aug	1.5	6600	16.66	30.43	101.6	8.22	46.76	9.27	7.58
			15-Oct	1.4	6600	12.85	31.91	95.1	8.24	48.92	2.58	NA
			19-May	0.9	6600	9.45	31.58	106	9.9	48.71	0.9	7.79
	8	Clark's Harbour	04-Aug	1.9	6600	13.7	30.82	104.9	8.99	47.38	1.67	7.67
			13-Oct	1.6	6600	12.38	31.74	95	8.32	48.71	0.58	NA

Appendix 2, continued.

Region	Stn. No.	Location	Sample Date	Probe Depth, m	YSI Type	Temp, °C	Salinity	Oxygen, %	Oxygen, mg L^{-1}	Conductivity, mS cm^{-1}	ChlA, µg L^{-1}	pH
South shore			19-May	1.1	6600	9.8	31.73	104.9	9.71	48.9	0.97	7.53
	10	Ingomar	04-Aug	1.7	6600	15.28	30.61	95.6	7.95	47.04	0.84	7.69
			13-Oct	1.5	6600	11.04	31.39	94.1	8.5	48.32	0.64	NA
			19-May	1.9	6600	9.34	30.77	106.5	10.02	47.6	2.12	6.87
	12	Shelburne	04-Aug	1.5	6600	18.42	26.1	101.1	8.12	40.7	4.31	7.13
			13-Oct	1.7	6600	12.63	31.3	102	8.91	48.09	2.58	NA
			19-May	1.6	6600	7.66	31.26	102.8	10.01	48.48	0.77	NA
	82	Port Mouton	04-Aug	1.9	6600	11	30.86	110.9	10.06	47.59	0.19	7.15
			13-Oct	1.7	6600	11.17	31.02	83.3	7.52	47.8	0.71	NA
			21-May	1.2	6600	9.55	28.69	99.2	9.41	44.66	1.09	7.69
	100	Lahave River	05-Aug	1.3	6600	18.01	25.72	106.2	8.62	40.18	2.7	7.78
			13-Oct	1.5	6600	12.43	28.2	98.8	8.84	43.78	3.41	NA
			21-May	1.6	6600	9.63	31.33	104.9	9.78	48.35	0.13	7.97
	18	Lunenburg	06-Aug	1.5	6600	14.69	30.81	99.9	8.39	47.33	0.32	8
			13-Oct	1.3	6600	11.22	31.63	92.2	8.29	48.64	0.64	NA
			12-May	5	NA	6.5	30.7	105	10.6	NA	NA	NA
	19(1)	IPMF	03-Aug	5	6600	13.89	31	110.2	9.41	NA	NA	8.14
			12-Oct	5	Pro+	13	28.6	95.6	8.36	44.35	NA	NA
			12-May	5	NA	6.4	30.7	104	10.6	NA	NA	NA
	19(2)	IPMF	03-Aug	5	6600	13.6	31.04	108.6	9.35	47.75	NA	8.13
			12-Oct	5	Pro+	13	28.46	99.2	8.34	44.32	NA	NA
			12-May	5	NA	6.5	30.59	110	9.7	NA	NA	NA
	19(3)	IPMF	03-Aug	5	6600	13.57	30.99	110.3	9.44	47.7	NA	8.09
			12-Oct	5	Pro+	12.9	29	96	8.45	44.92	NA	NA
			25-May	1.2	6600	10.58	31.13	129.5	11.83	48	0.32	7.92
	21	Chester	06-Aug	0.9	6600	18.31	30.42	105.9	8.31	46.72	NA	7.58
			20-Oct	NA	Pro+	12	NA	119.8	NA	NA	NA	NA

Appendix 2, continued.

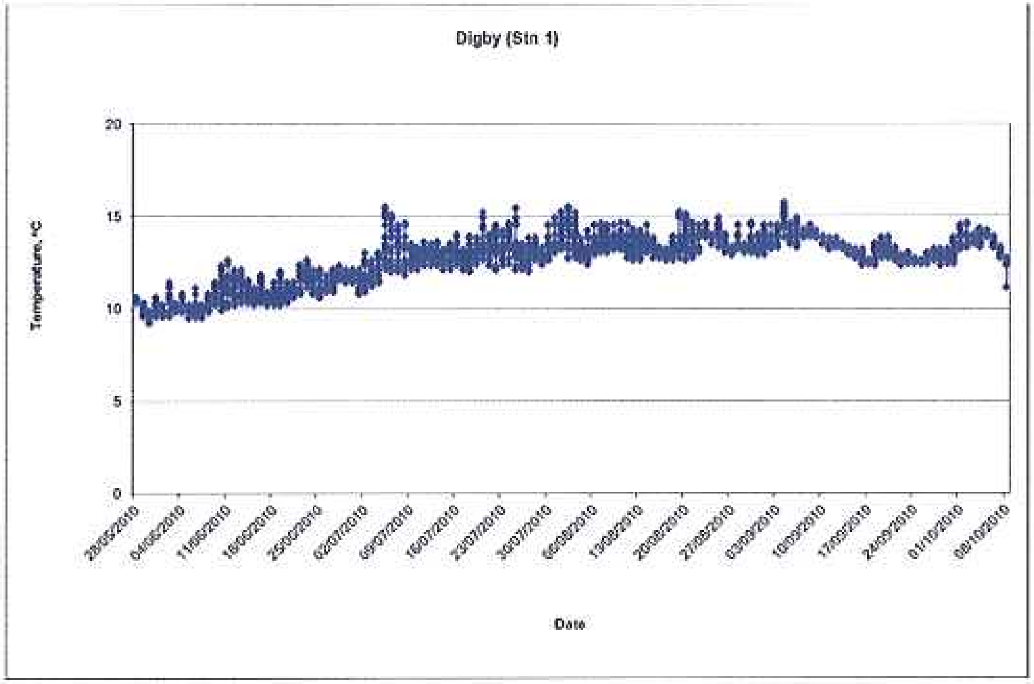
Region	Stn. No.	Location	Sample Date	Probe Depth, m	YSI Type	Temp, °C	Salinity	Oxygen, %	Oxygen, mg L^{-1}	Conductivity, mS cm^{-1}	ChlA, µg L^{-1}	pH
Halifax	24	Halifax; BIO Jetty	14-May	NA	Pro+	6.3	29.94	94	7.1	NA	NA	NA
			30-Aug	1.1	6600	19.86	30.33	103.5	7.89	46.61	3.28	7.79
			28-Oct	1.5	6600	10.57	29.78	91.4	8.43	46.12	2.06	NA
	401	Halifax; RNSYS	21-May	1.6	6600	7.58	30.91	101.2	9.9	47.99	0.58	7.66
			06-Aug	1	6600	16.42	29.09	105.8	8.68	44.99	1.8	7.98
			20-Oct	1.2	6600	10.33	30.24	92.8	8.57	46.78	2.83	NA
Eastern shore	41	Venus Cove	26-May	1.6	6600	7.43	31.06	105.8	10.38	48.22	0.52	7.83
			10-Aug	0.9	6600	18.49	30.28	104.3	8.16	46.53	0	NA
			07-Oct	1.1	6600	15.14	29.94	98.5	8.24	46.1	1.48	NA
Ched. Bay	44	Petit de Grat	12-Sep	1.33	6600	15.05	30.22	97.2	8.14	46.49	1.15	NA
Bras d'Or Lake	47	St. Peter's	26-May	1.2	6600	12.44	20.9	103.8	9.72	33.37	0.39	7.95
			10-Aug	1.2	6600	20.79	21.03	96.8	7.66	33.5	1.61	NA
			08-Oct	1.2	6600	15.16	21.4	100.1	8.82	34.03	1.61	NA
	51	Whycocomagh	31-May	NA	NA	10.9	19.23	99.5	9.56	NA	NA	NA
			03-Aug	NA	NA	21.5	18.14	103.1	8.19	NA	NA	NA
			03-Nov	NA	NA	8.8	17.96	92.1	9.46	NA	NA	NA
	52	Orangedale	31-May	NA	NA	10.8	19.46	103.9	10.19	NA	NA	NA
			03-Aug	NA	NA	23.4	17.47	107.7	8.39	NA	NA	NA
			03-Nov	NA	NA	9.8	17.22	66.8	9.79	NA	NA	NA
	54	Eskasoni	31-May	NA	NA	12.9	19.38	114.5	10.74	NA	NA	NA
			03-Aug	NA	NA	22.6	18.97	105.5	8.04	NA	NA	NA
			02-Nov	NA	NA	9.5	17.82	87.6	8.88	NA	NA	NA
	55	Baddeck	26-May	1.4	6600	11.38	22.43	105	9.96	35.62	NA	7.9
			18-Aug	1.5	6600	21.38	22.38	100.2	7.78	35.45	1.03	8.17
			08-Oct	1.1	6600	16	22.98	97.5	8.37	36.29	1.55	NA

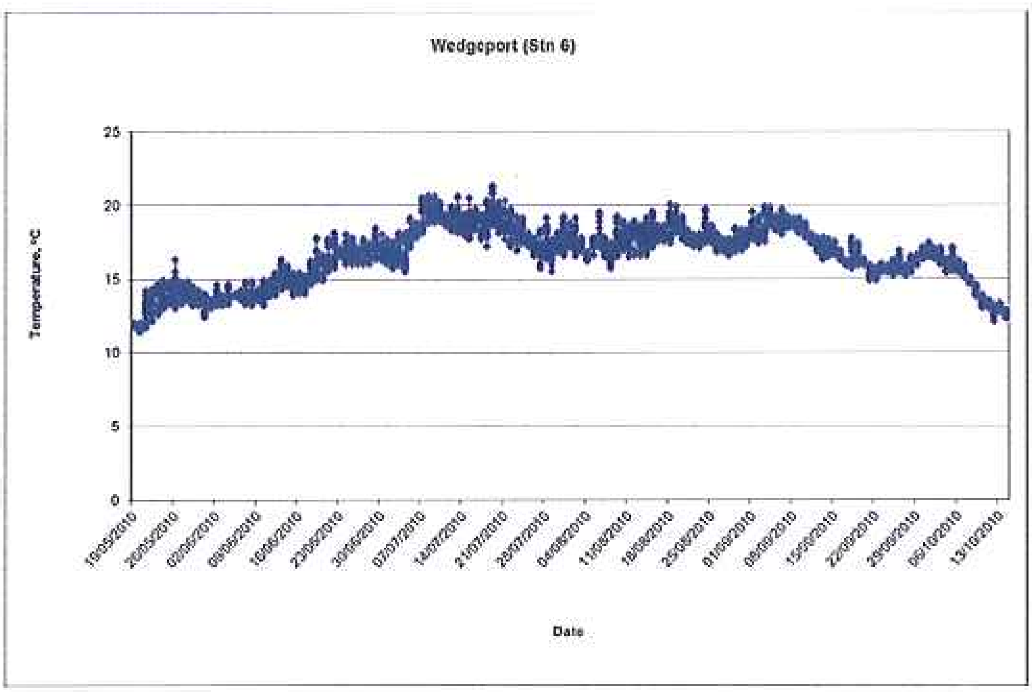
Appendix 2, continued.

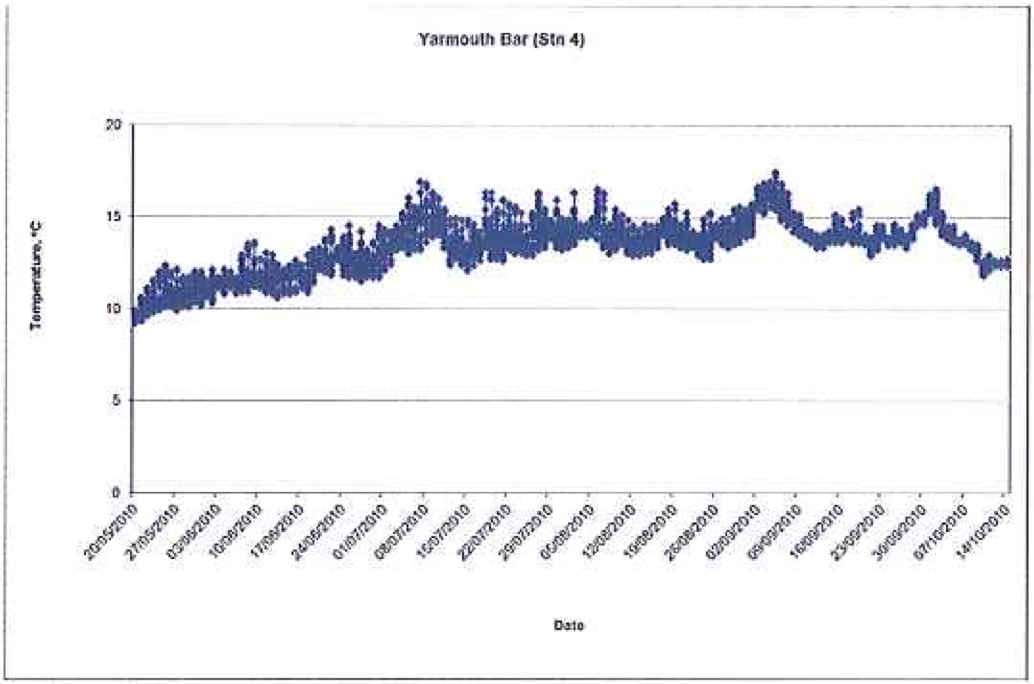
Region	Stn. No.	Location	Sample Date	Probe Depth, m	YSI Type	Temp, °C	Salinity	Oxygen, %	Oxygen, mg L^{-1}	Conductivity, mS cm^{-1}	ChlA, µg L^{-1}	pH
Cape Breton	69	Dingwall	27-May	1.3	6600	8.87	29.66	105.9	10.14	46.1	1.87	7.98
			08-Sep	1.2	6600	14.73	29.55	8.04	8.04	36.64	2	7.68
	94	North Harbour, Aspy B.	09-Sep	1.4	6600	17.17	26.22	97.3	8	40.88	NA	7.78
	95	South Harbour, Aspy B.	07-Sep	0.2	6600	20.57	23.04	97.3	7.64	36.39	2.12	7.64
	71	Cheticamp	27-May	2	6600	10.53	29.42	104.1	9.62	45.62	1.8	8.1
			17-Aug	1.6	6600	22.48	28.5	107.6	7.91	44.11	2.32	8.1
			07-Oct	2	6600	15.56	28.28	98.8	8.29	43.79	4.19	NA
	73	Mabou	27-May	1.1	6600	9.08	29.2	102.4	9.79	45.43	0.39	8.09
			17-Aug	0.9	6600	21.58	27.97	101.1	7.57	43.36	1.09	7.84
			07-Oct	1.4	6600	15.62	27.84	98.4	8.27	43.18	2.19	NA
North shore	77	Pictou	26-May	1.5	6600	11.66	28.34	98.7	8.96	44.03	0.9	7.69
			17-Aug	0.9	6600	21.02	27.56	100.8	7.65	42.78	2.45	7.89
			07-Oct	1.1	6600	16.09	27.06	95.9	8.01	42.08	3.03	NA
	91	Caribou	26-May	1.3	6600	11.52	29.11	100.2	9.08	45.11	1.42	7.76
			17-Aug	1.3	6600	18.6	29.19	90	7.08	45.03	1.55	8
			07-Oct	1.2	6600	15.39	29.12	39.7	3.32	44.96	3.41	NA

Appendix 3: Minilog plots from selected monitoring stations, 2010.

Southwestern shore (Digby to Clark's Harbour)






Yarmouth Bar (Stn 4)
Temperature, °C
0
5
10
15
20
20/05/2010
27/05/2010
03/06/2010
10/06/2010
17/06/2010
24/06/2010
01/07/2010
08/07/2010
15/07/2010
22/07/2010
29/07/2010
05/08/2010
12/08/2010
19/08/2010
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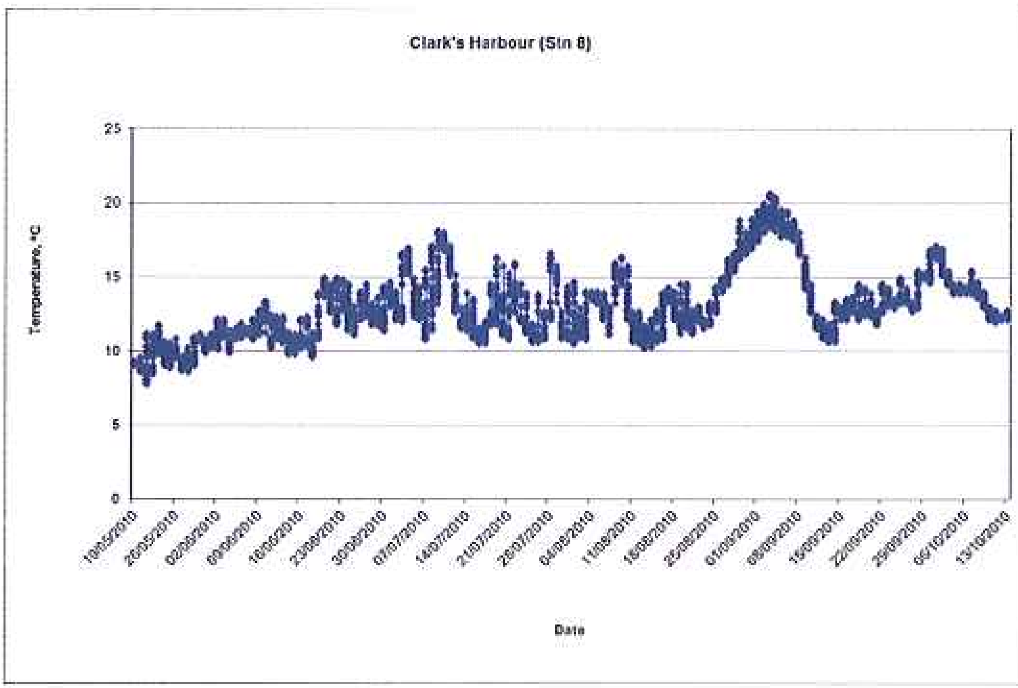
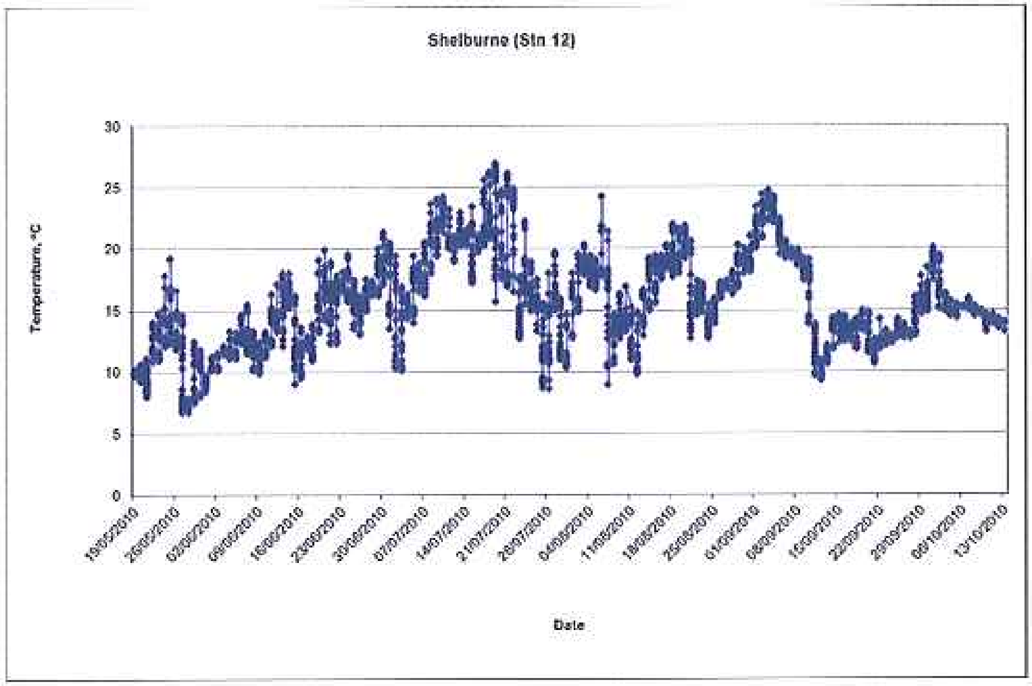
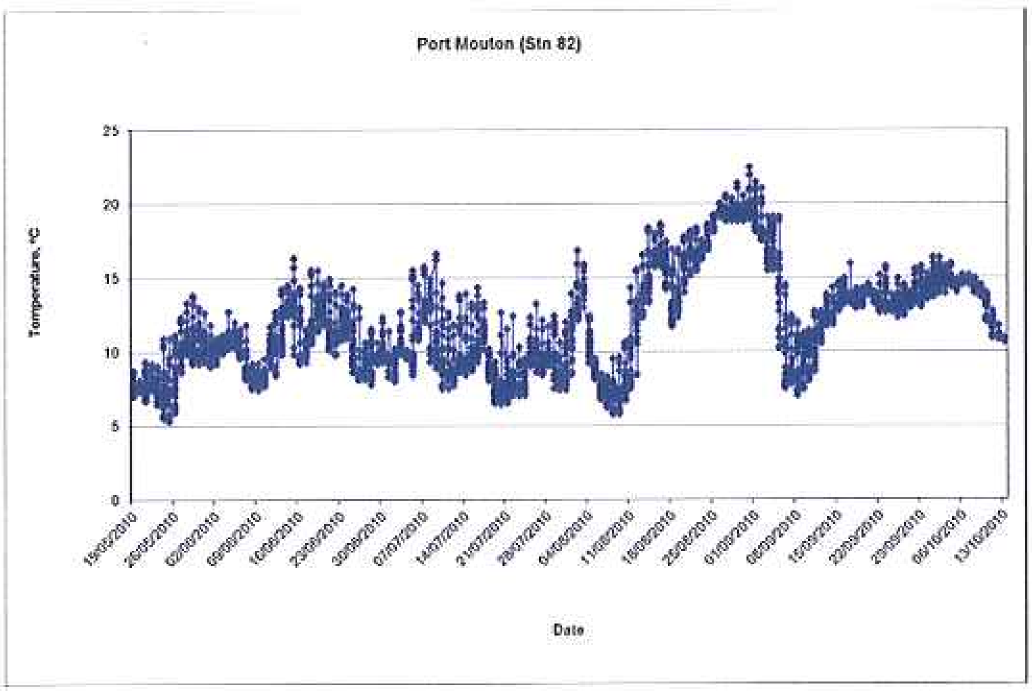
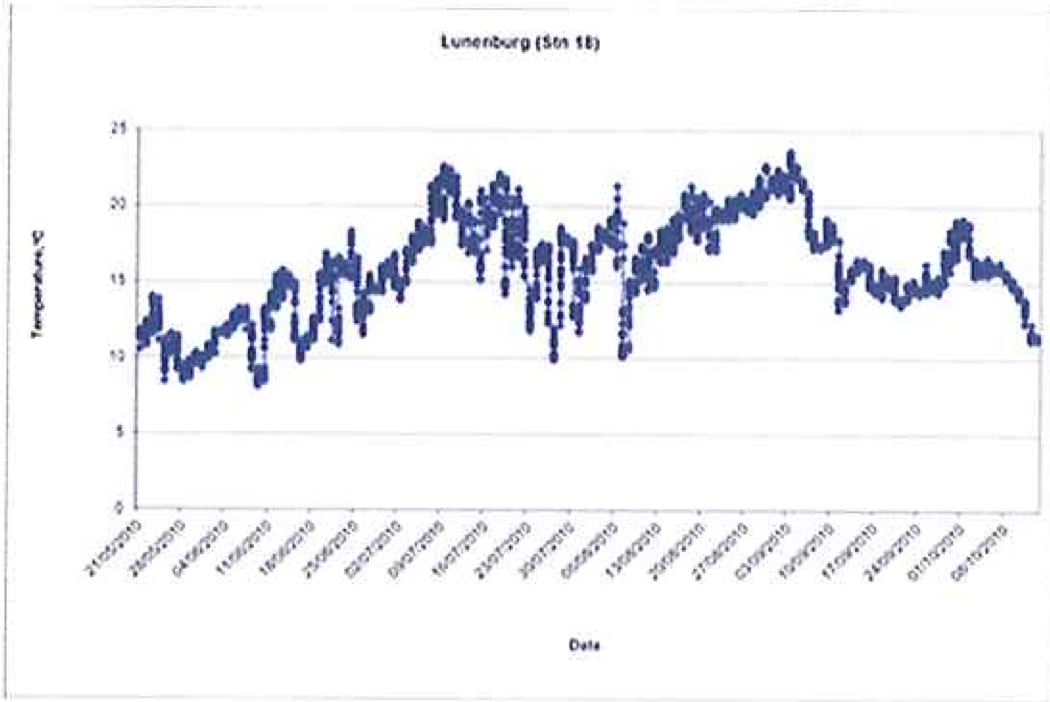
07/10/2010
14/10/2010
Date

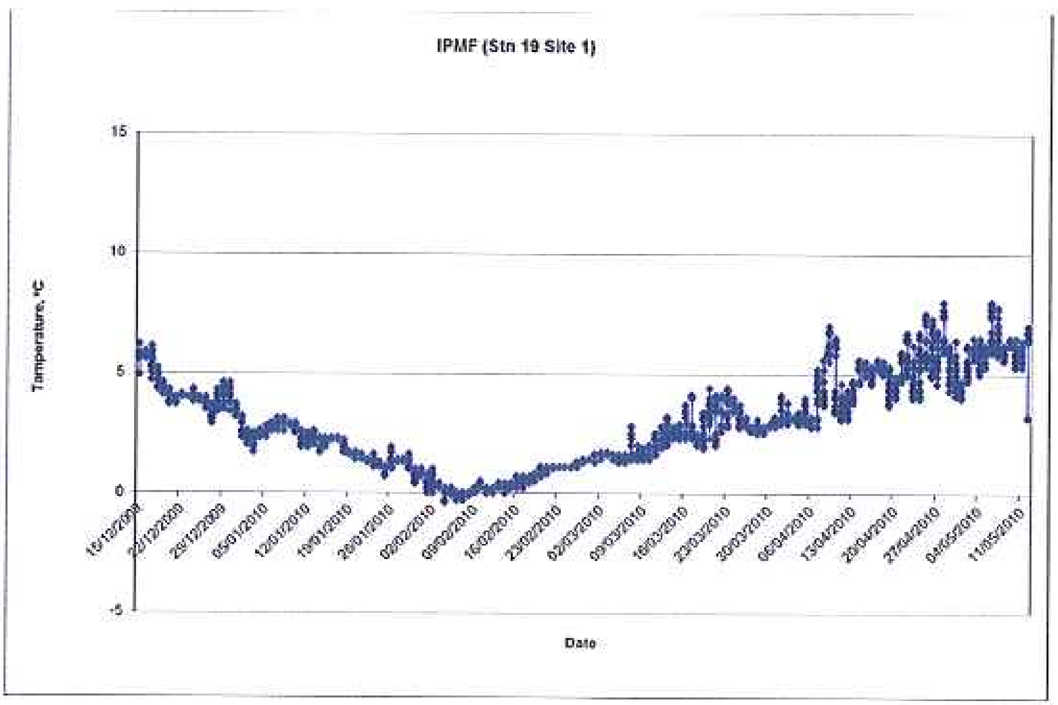

Clark's Harbour (Stn 8)
Temperature, °C
0
5
10
15
20
25
19/05/2010
26/05/2010
02/06/2010
09/06/2010
16/06/2010
23/06/2010
30/06/2010
07/07/2010
14/07/2010
21/07/2010
28/07/2010
04/08/2010
11/08/2010
18/08/2010
25/08/2010
01/09/2010
08/09/2010
15/09/2010
22/09/2010
29/09/2010
06/10/2010
13/10/2010
Date

South shore (Ingomar to Chester)



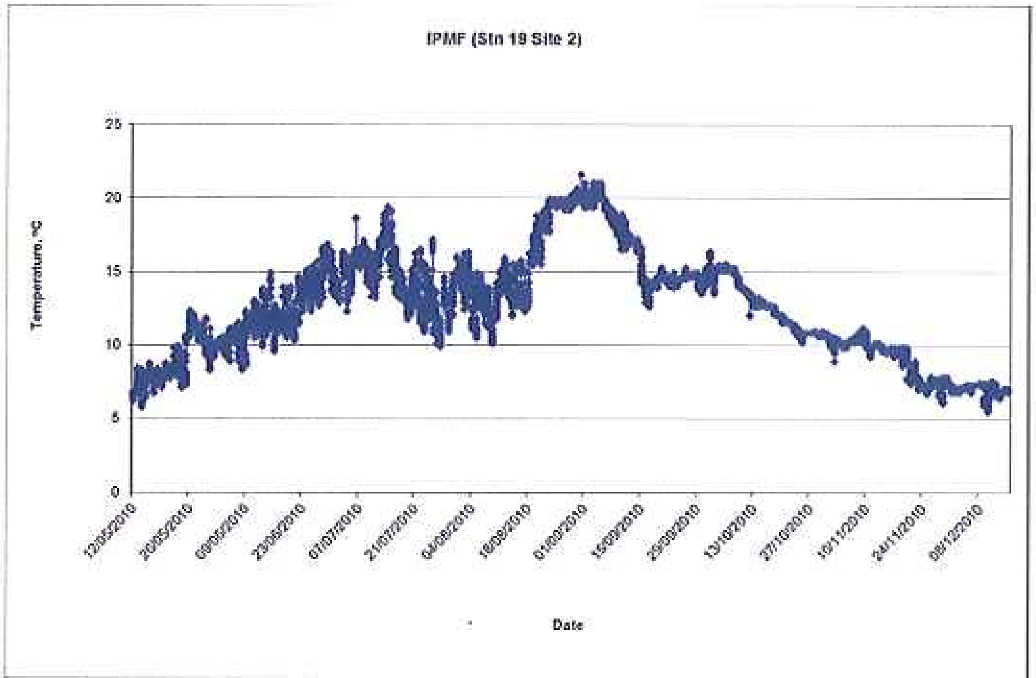


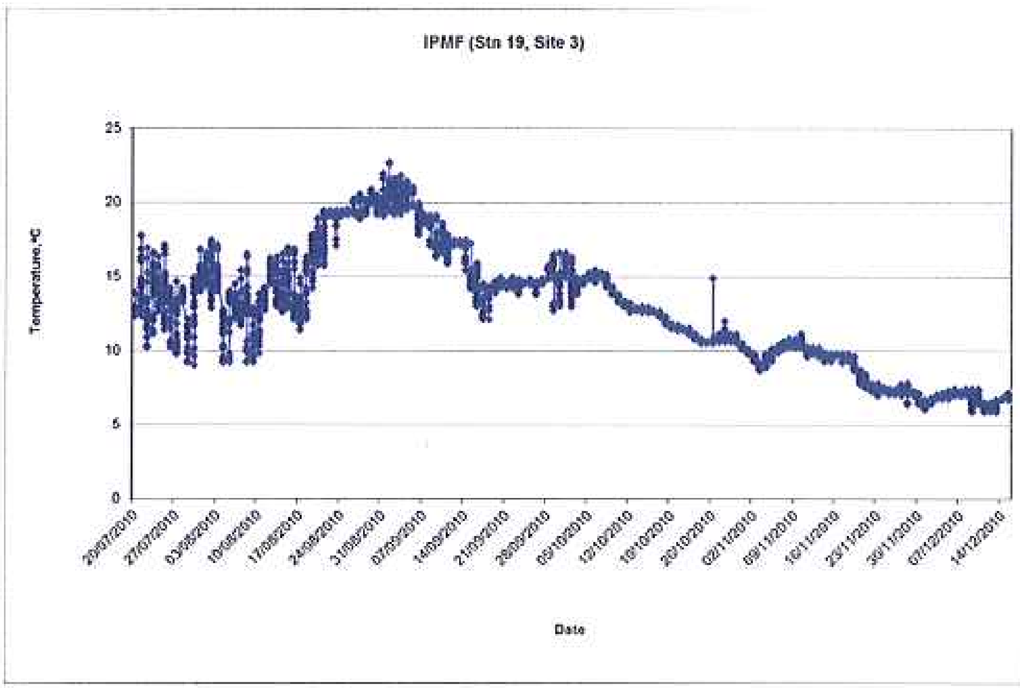

Lunenburg (Stn 18)
Temperature, °C
25
20
15
10
5
0
Date


IPMF (Stn 19 Site 1)
Temperature, °C
15
10
5
0
-5
Date

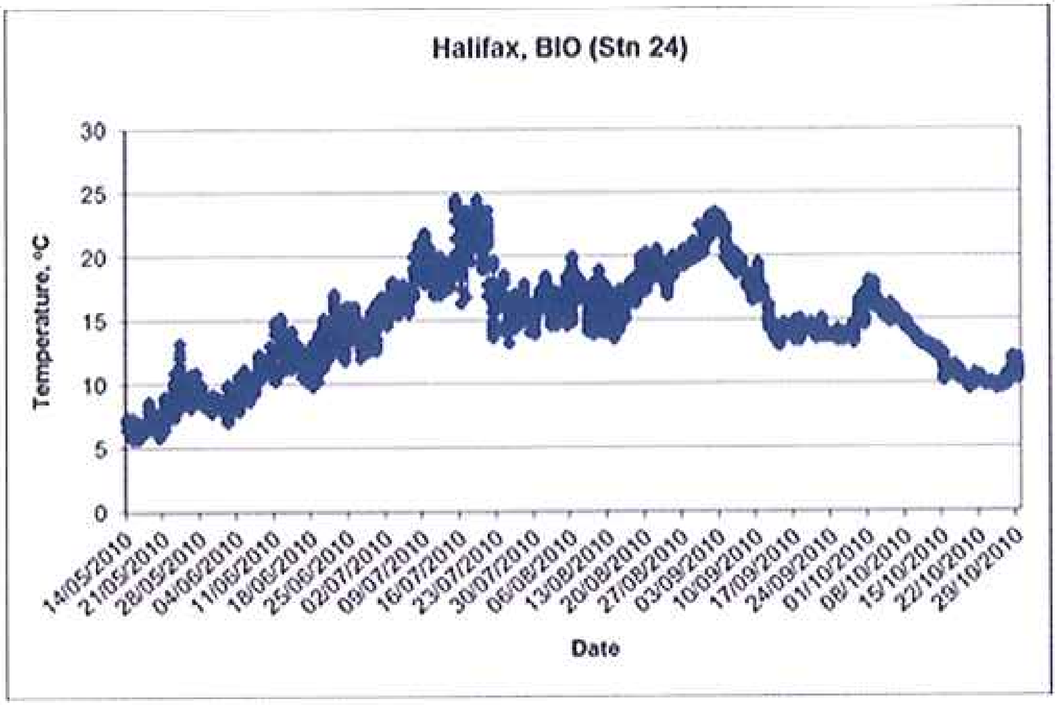

IPMF (Stn 19, Site 1)
Temperature, °C
25
20
15
10
5
0
03/08/2010
10/08/2010
17/08/2010
24/08/2010
31/08/2010
07/09/2010
14/09/2010
21/09/2010
28/09/2010
05/10/2010
12/10/2010
19/10/2010
26/10/2010
02/11/2010
09/11/2010
16/11/2010
23/11/2010
30/11/2010
07/12/2010
14/12/2010
Date


IPMF (Stn 19, Site 2)
Temperature
15
10
5
0
-5
15/12/2009
22/12/2009
29/12/2009
05/01/2010
12/01/2010
19/01/2010
26/01/2010
02/02/2010
09/02/2010
16/02/2010
23/02/2010
02/03/2010
09/03/2010
16/03/2010
23/03/2010
30/03/2010
06/04/2010
13/04/2010
20/04/2010
27/04/2010
04/05/2010
11/05/2010
Date

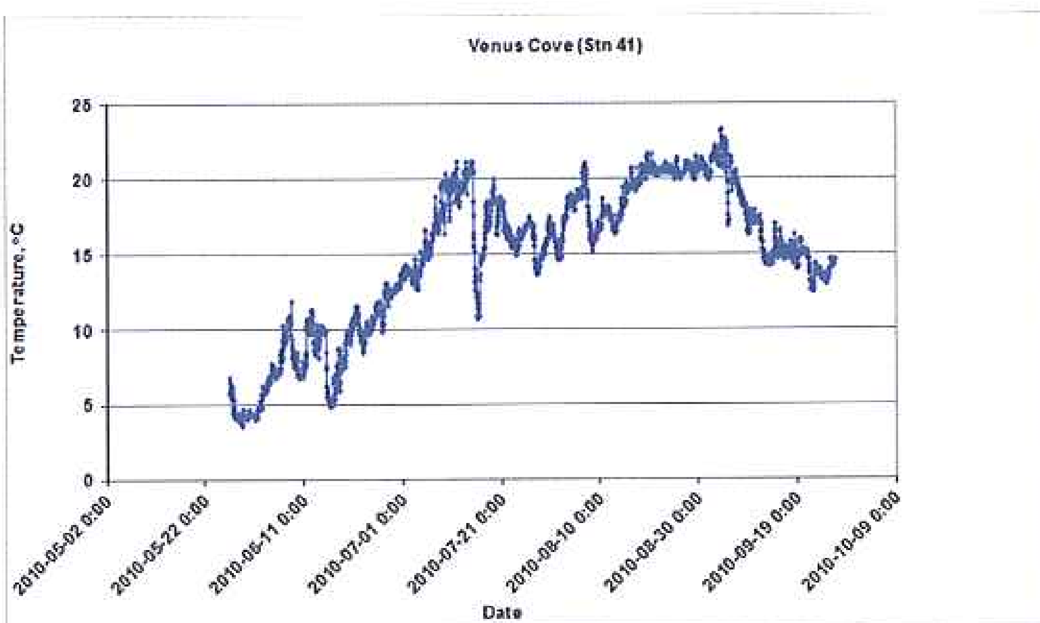

IPMF (Stn 19 Site 2)
Temperature, °C
25
20
15
10
5
0
Date


IPMF (Stn 19, Site 3)
Temperature, °C
25
20
15
10
5
0
Date

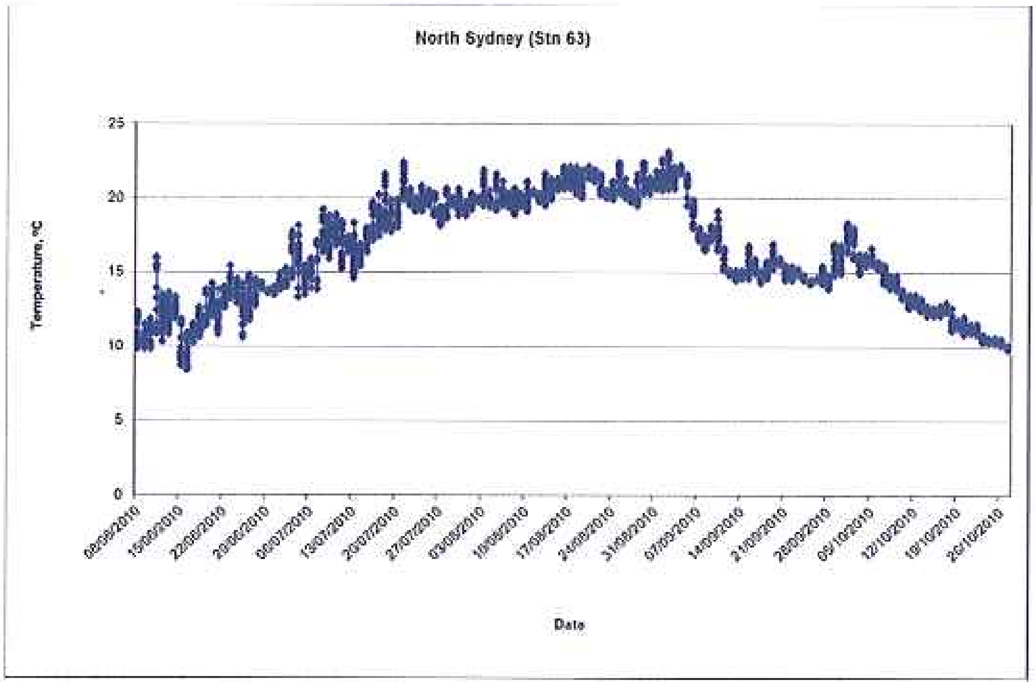
Halifax

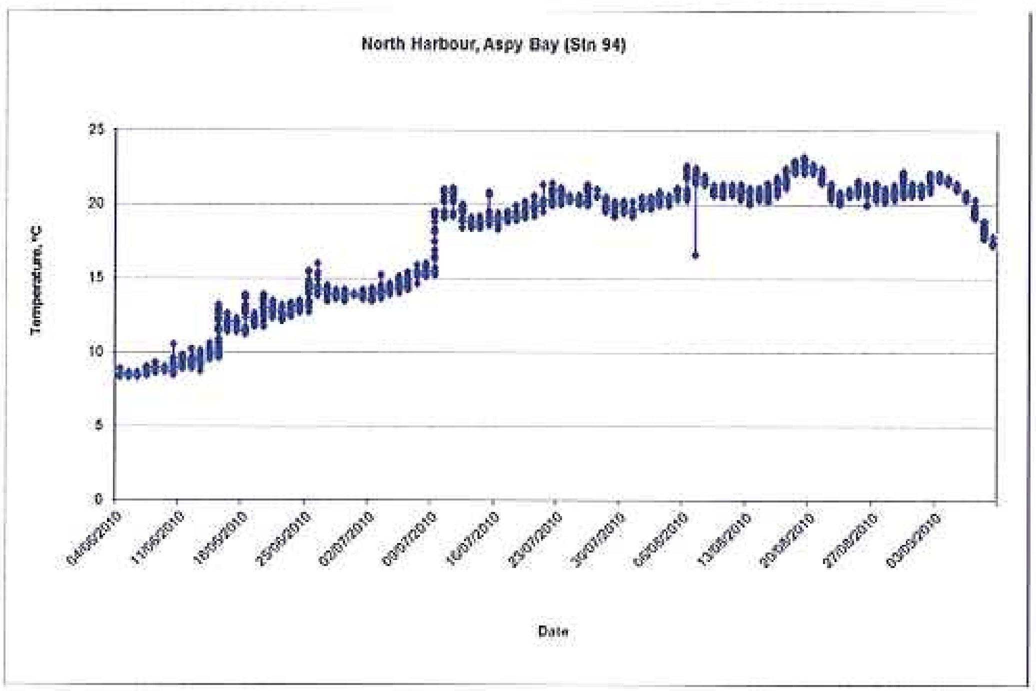


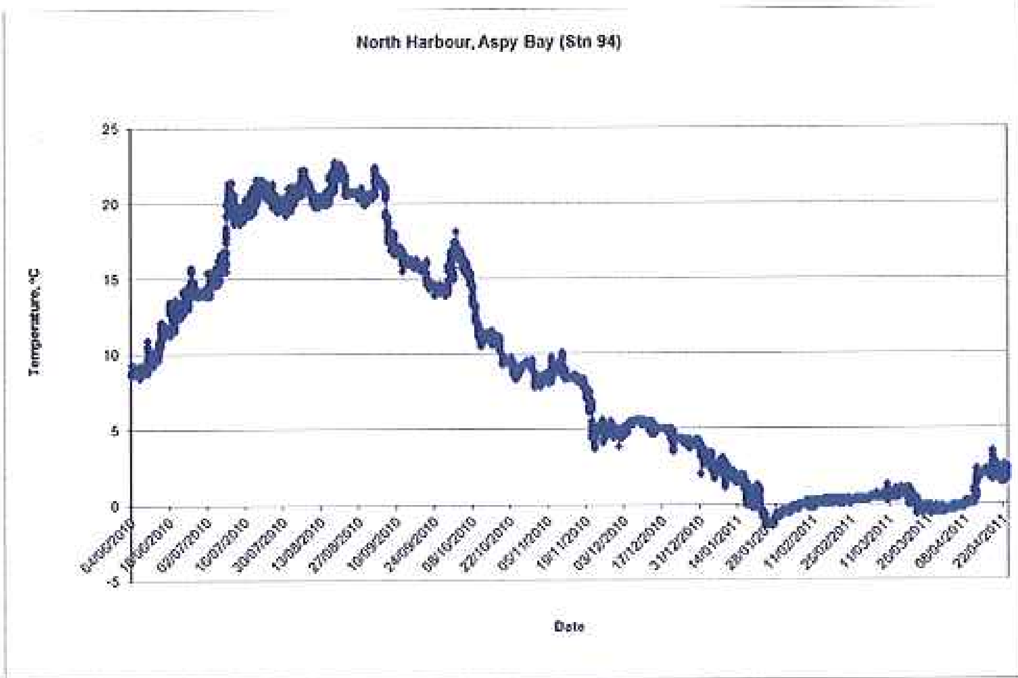
Chedabucto Bay

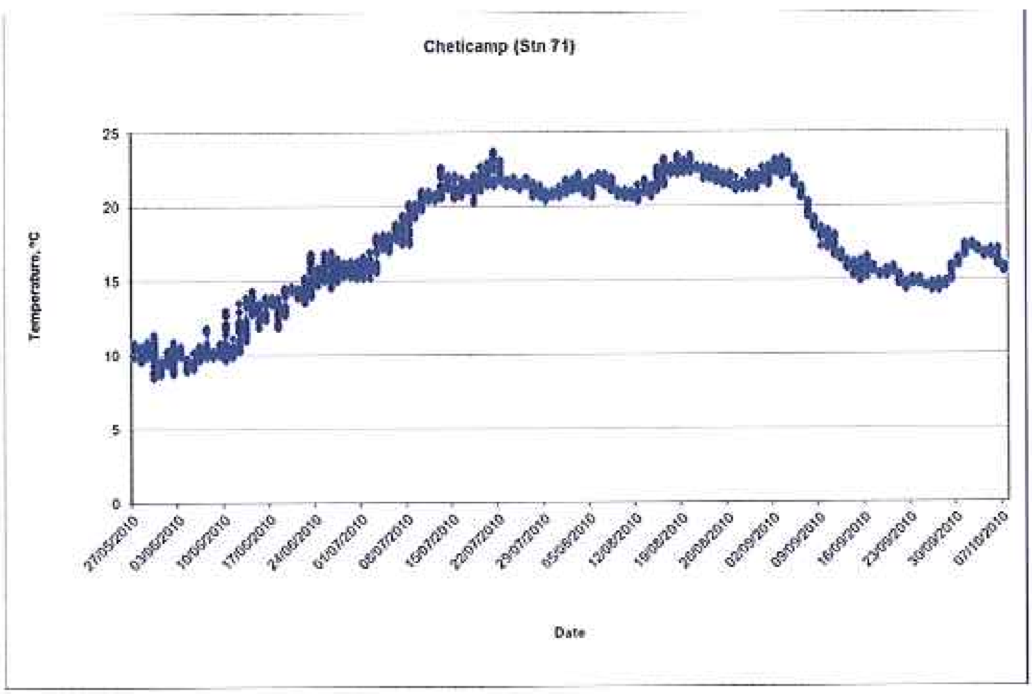


Cape Breton Coastal

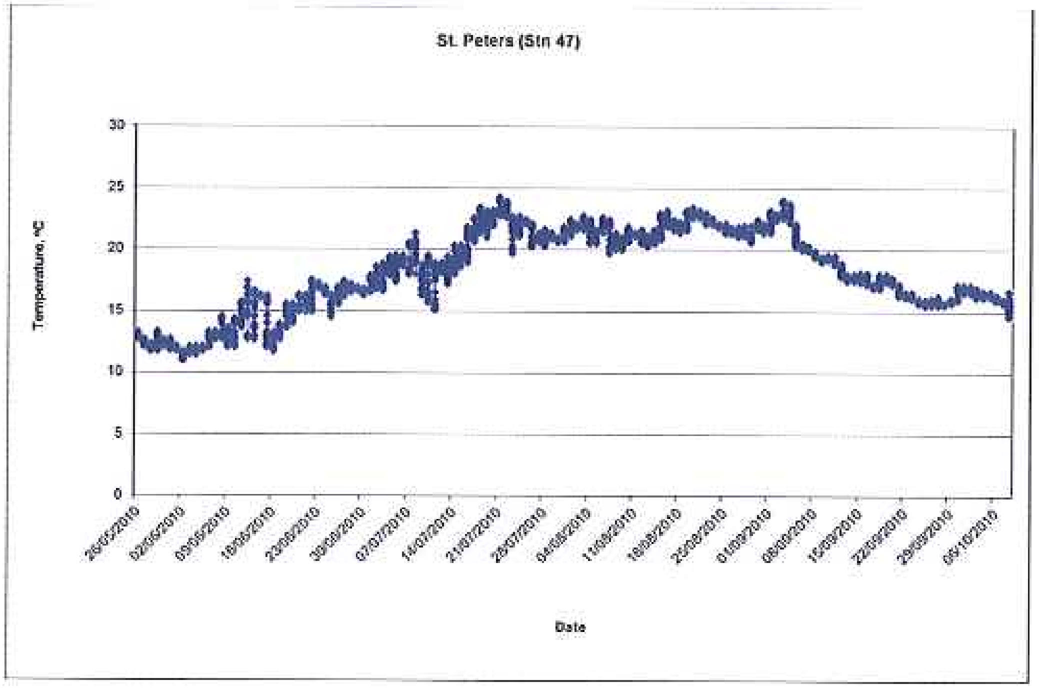


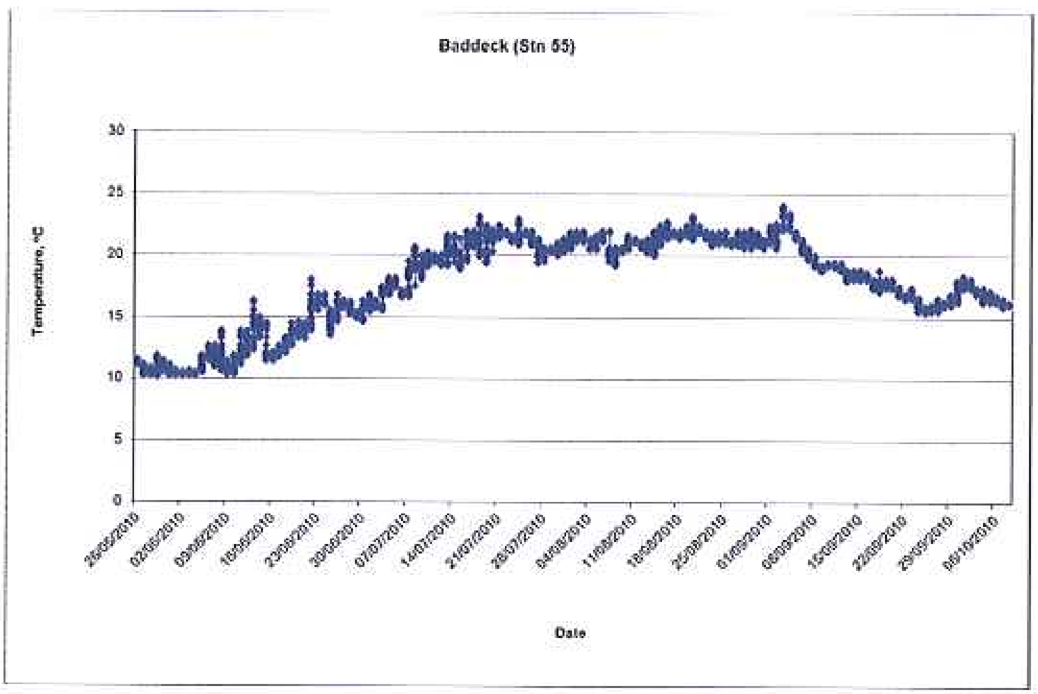


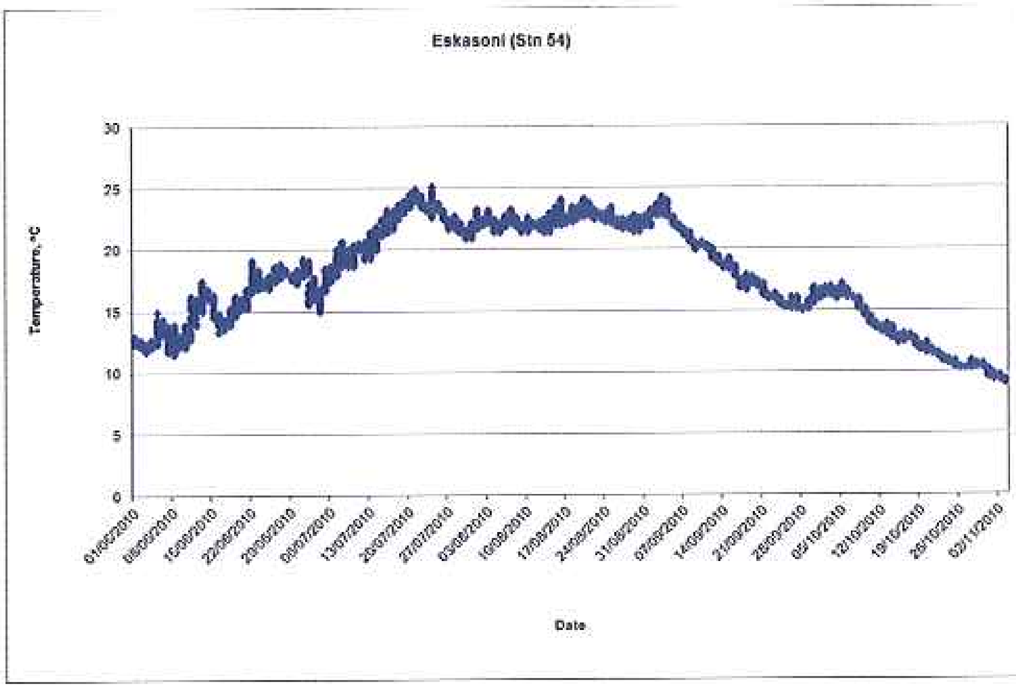

North Harbour, Aspy Bay (Stn 94)
Temperature, °C
25
20
15
10
5
0
-5
Date


Cheticamp (Stn 71)
Temperature, °C
25
20
15
10
5
0
Date

Bras d'Or Lake







North Shore (Merigomish to Malagash)