Management of Biological Invasions (2016) Volume 7, Issue 1: 51–57
DOI: http://dx.doi.org/10.3391/mbi.2016.7.1.07

Open Access

Proceedings of the 5th International Invasive Sea Squirt Conference (October 29–31, 2014, Woods Hole, USA)

Research Article

Distribution and diversity of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39° and 47° north latitude (New Jersey to Newfoundland)

Mary R. Carman[1]*, Philip D. Colarusso[2], Eric P. Nelson[2], David W. Grunden[3], Melisa C. Wong[4], Cynthia McKenzie[5], Kyle Matheson[5], Jeff Davidson[6], Sophia Fox[7], Hilary A. Neckles[8], Holly Bayley[7], Stephen Schott[9], Jennifer A. Dijkstra[10] and Sarah Stewart-Clark[11]

[1]*Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA*
[2]*US Environmental Protection Agency, Boston, Massachusetts 02109, USA*
[3]*Oak Bluffs Shellfish Department, Oak Bluffs, Massachusetts 02557, USA*
[4]*Fisheries and Oceans Canada, Dartmouth, Nova Scotia B2Y 4A2, Canada*
[5]*Fisheries and Oceans Canada, St. John's, Newfoundland and Labrador A1C 5X1, Canada*
[6]*Atlantic Veterinary College, Charlottetown, Prince Edward Island C1A 4P3, Canada*
[7]*US National Park Service, Cape Cod National Seashore, Massachusetts 02667, USA*
[8]*USGS Patuxent Wildlife Research Center, Augusta, Maine 04330, USA*
[9]*Cornell University Cooperative Extension, Southold, New York 11971, USA*
[10]*University of New Hampshire, Durham, New Hampshire 03824, USA*
[11]*Dalhousie University, Truro, Nova Scotia B2N 5E3, Canada*

**Corresponding author*
E-mail: mcarman@whoi.edu

Received: 21 August 2015 / Accepted: 4 December 2015 / Published online: 8 February 2016

Handling editor: Stephan Bullard

Abstract

Seagrass meadows are ecologically important habitats that are declining globally at an accelerating rate due to natural and anthropogenic stressors. Their decline is a serious concern as this habitat provides many ecosystem services. Eelgrass (*Zostera marina*) is the dominant seagrass species in the western North Atlantic. It has recently been established that invasive tunicate species possibly threaten the health of eelgrass beds. Colonization of eelgrass leaves by tunicates can inhibit eelgrass growth and may cause shoot mortality. To document the distribution and diversity of tunicate species that attach to eelgrass in the western North Atlantic, we surveyed twenty-one eelgrass sites from New Jersey to Newfoundland. Eight species of tunicates were found to be colonizing eelgrass, of which 6 are considered invasive. *Botrylloides violaceus* and *Botryllus schlosseri* were most commonly attached to eelgrass, with *B. schlosseri* having the largest latitudinal range of any species. Tunicate faunas attached to eelgrass were less diverse north of Gloucester, Massachusetts, where individual survey sites exhibited two species at most and only 4 of the 8 species observed in this study. Percent tunicate cover on eelgrass tended to fall within the 1–25 range, with occasional coverage up to >75–100. Density of eelgrass was highly variable among sites, ranging from <1 to 820 shoots/m^2. The solitary tunicate *Ciona intestinalis* was only found on eelgrass at the highest latitude sampled, in Newfoundland, where it is a new invader. The tunicates observed in this study, both solitary and colonial, are viable when attached to eelgrass and pose a potential threat to overgrow and weaken seagrass shoots and reduce the sustainability of seagrass meadows.

Key words: eelgrass, *Zostera marina*, tunicates, Ascidiacea, invasive species

Introduction

Seagrasses are submerged marine angiosperms that inhabit many of the world's coastlines. *Zostera marina* (Linnaeus, 1753), called eelgrass, is the dominant seagrass species in New England and Atlantic Canada, where it grows in lower intertidal and shallow subtidal waters. Eelgrass meadows provide many important ecological functions and services, including coastal protection, maintenance of fisheries and biodiversity, provision of food, purification of water, and sequestration

of carbon (Barbier et al. 2011). Seagrasses are subject to a number of stressors and as a result their distribution is declining globally at an accelerating rate (Short et al. 2007; Waycott et al. 2009). Recently, overgrowth of eelgrass leaves by species of tunicates has been identified as a probable additional stress on eelgrass (Wong and Vercaemer 2012).

Tunicate (Ascidiacea) populations in New England and Atlantic Canada have changed over the past half century. In Massachusetts, the tunicate fauna was chiefly native species as documented in 1945 by van Name and in 1964 by Zinn and Abbott. They were minor members of fouling communities in harbors and aquaculture facilities. Several non-native tunicates were introduced to southeastern New England in the 1970s and 1980s, and by the turn of the century the tunicate fauna was dominated by non-native species (Pederson 2005; Carman et al. 2007; Carman et al. 2010). Tunicates are now major members of the fouling communities in harbors and aquaculture facilities. Similar changes have occurred in southern New England (Whitlatch and Osman 2001; Altman and Whitlatch 2007), northern New England (Dijkstra et al. 2007; Dijkstra and Harris 2007), and maritime Canada (Howes et al. 2007; Bourque et al. 2007; Locke et al. 2007; Callahan et al. 2010; Kanary et al. 2011; Sephton et al. 2011; Vercaemer et al. 2011; Ma et al. 2011; Sargent et al. 2013; Moore et al. 2014).

The number of tunicate species that colonize eelgrass may be increasing, and eelgrass may be facilitating tunicate range expansion both by providing substrate in areas where the seabed is inhospitable to tunicates and by serving as a potential dispersal mechanism (i.e. rafting of tunicates on floating plant debris). Non-native, invasive tunicates *Botrylloides violaceus* Okra, 1927, *Didemnum vexillum* Kott, 2002*, Diplosoma listerianum* (Milne-Edwards, 1841)*, Ascidiella aspersa* (D.F. Müller, 1776) and *Botryllus schlosseri* (Pallas, 1766) were recently observed attached to eelgrass at Martha's Vineyard in southeastern New England (Carman and Grunden 2010). Before 2010, *D. vexillum, D. listerianum,* and *A. aspersa* were not previously known to use eelgrass as substrate (Carman and Grunden 2010). *Botryllus schlosseri* and *Ciona intestinalis* (Linnaeus, 1767) were recently found attached to eelgrass in the Placentia Bay region on the south coast of Newfoundland (C. McKenzie et al., this issue). *A. aspersa* and *D. listerianum* were recently detected south of Lunenburg, Nova Scotia, in an area with extensive eelgrass meadows (B. Vercaemer, pers. comm.). An abundance of *B. schlosseri* and *B. violaceus* was recently observed on eelgrass washed ashore at the Prince Edward Island National Park (S. Stewart-Clark, pers obs).

The results of some recent studies suggest that invasive tunicates can have negative effects on eelgrass growth. For example, in one experiment, the attachment of *B. violaceus* and *B. schlosseri* to eelgrass leaves caused a decrease in light transmission and shoot growth rate (Wong and Vercaemer 2012). In another study, *B. violaceus, B. schlosseri*, *D. vexillum*, or *A. aspersa* on eelgrass blocked 75–80% of ambient light, resulting in reduced leaf growth rate, reduced number of leaves per shoot, reduced canopy height, and higher leaf sugar concentrations (P. Colarusso et al., unpublished data).

Due to the potential negative impacts resulting from the interaction between tunicates and eelgrass, our goal was to make the first attempt to determine the distribution and diversity of tunicates attached to eelgrass along the northwest Atlantic coast, and to document the degree of colonization of eelgrass by tunicates.

Methods

Investigators selected eelgrass meadows to survey for tunicates from July to October 2013 (Figure 1). In some cases, tunicate observations were coupled with ongoing eelgrass investigations; in others, specific surveys were conducted. Due to the large number of different investigators involved (n=15), and the facultative nature by which data were compiled from disparate research groups, it was not possible to standardize survey methods for this initial study. Data collection ranged from qualitative underwater observations (snorkel, SCUBA, wading) with no sample collections to quantitative underwater quadrat sampling. Quadrat samples (0.25 cm × 0.25 cm or 0.50 cm × 0.50 cm) were collected in different ways. At some sites, quadrats were dropped randomly within a meadow; at other sites, they were dropped along 50 to 100 m transects using either a systematic or random approach. Eelgrass and tunicate measurements were made in situ or in the lab on harvested quadrat contents. Coverage by tunicate species was measured visually using 5 categories of percent cover (0, 1–25, >25–50, >50–75, >75–100). Some investigators also documented the density of eelgrass (number of shoots/area) and canopy height (distance from the sediment to the top of the canopy, ignoring the tallest 20% of the leaves, following Duarte and Kirkman 2001).

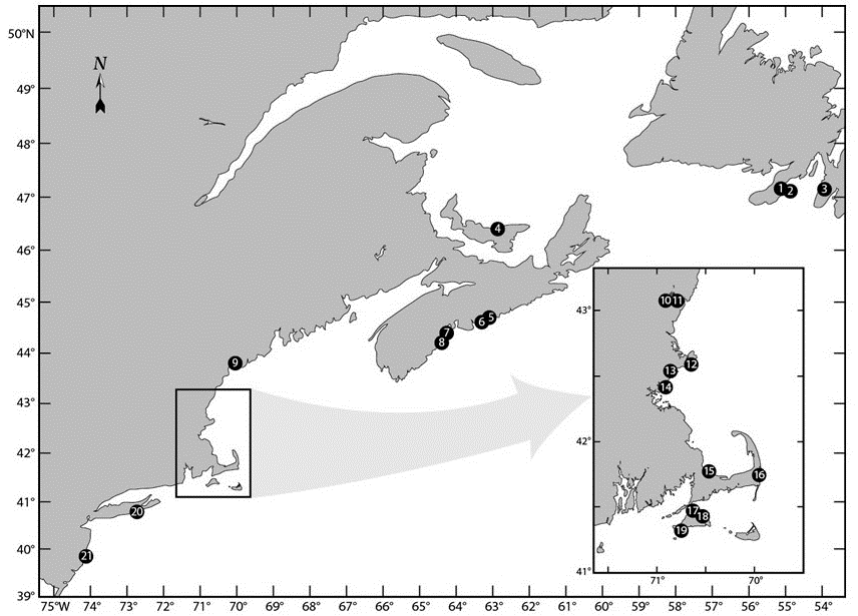

Figure 1. Map showing sites surveyed for tunicates on eelgrass. Site numbers correspond to those in supplementary material Table S1.

Study site characteristics

New Jersey

Barnegat Bay forms a long, narrow basin that is separated from the ocean by a series of barrier islands. The bay side of the barrier beach was inspected along two north-south transects.

New York

Moriches Bay is an enclosed embayment located behind a barrier beach along Long Island's south shore. The eelgrass meadow is located approximately 3 km east of Moriches Inlet in Westhampton Dunes, New York. The meadow is shallow (<2 m deep), with a tidal range of <1 m.

Massachusetts

Stonewall Pond

Stonewall Pond is a shallow coastal pond in the Menemsha Pond system located on the southwest coast of Martha's Vineyard. It has restricted access to the sea and lies in an area of low anthropogenic impact. Water depths are <3 m, and the tidal range is <1 m.

Farm Pond

Farm Pond is a 33-acre pond at Oak Bluffs on the northeast coast of Martha's Vineyard. It has restricted access to the sea. The eelgrass meadow lies at water depths up to 1.5 m in a tidal range of <1 m.

Lagoon Pond

Lagoon Pond is a large pond on the north coast of Martha's Vineyard with water depths up to 8 m and a tidal range <1 m. It has restricted access to the sea and contains a large eelgrass meadow.

Little Pleasant Bay

Little Pleasant Bay lies at the upper end of the Pleasant Bay estuary, the largest coastal embayment on Cape Cod. The mean tidal range is approximately 1.5 m. Observations were collected along each of 3 transects. Water depth was 0.64 m below mean low water (MLW) at the shallow transect, 0.84 m at the mid-depth transect, and 1.35 m at the deep transect.

Sandwich

The study site is at Town Beach, which is open to Cape Cod Bay with a tidal range of 3 m. The area surveyed was an intertidal pool with a depth of <1 m at low tide.

Nahant

The eelgrass meadow occurs in a depth range from 1 to 5 m below MLW. The meadow covers

most of a cove on the south side of the peninsula and is exposed to the sea to the southwest.

Salem

A small eelgrass meadow in Salem Sound lies at a depth of 2 m below MLW. The site is protected in all directions.

Gloucester

The eelgrass meadow lies in a depth range of 1 to 5.5 m below MLW and parallels a sandy beach (Niles Beach) in Gloucester Harbor. It is exposed to the sea to the southwest.

New Hampshire

Great Bay

A large eelgrass meadow occurs at a depth of 1.5 m below MLW. Great Bay is an inland sea, well protected from waves and prevailing storms. The bay experiences periods of very low salinity resulting from rainfall runoff.

Little Bay

A large eelgrass meadow occurs at an elevation only slightly deeper than in Great Bay. Little Bay is part of an inland sea system that includes Great Bay and also experiences seasonal periods of very low salinity.

Maine

Maquoit Bay is a shallow, protected embayment that forms the northwestern extent of the Casco Bay estuary. Observations were made in an eelgrass bed on the eastern shore of the bay, close to Mere Point, at a depth of about 1.5 m below MLW.

Nova Scotia

Lower Three Fathom Harbour

Lower Three Fathom Harbour is a lagoon with restricted access to the open ocean. A relatively small, patchy eelgrass bed occurs in sediments that are primarily silt at a depth of 1 m relative to MHT (mean high tide).

Crescent Beach

Crescent Beach is located behind a barrier beach with full hydrologic exchange. A large, continuous eelgrass meadow lies in silt and mud sediments at 2.5 m below MHT.

East Petpeswick

Eelgrass at East Petpeswick is located on an elevated platform along a channel that is exposed to strong tidal currents with full hydrologic exchange, in sand and mud sediments at 1.5 m deep (MHT).

Second Peninsula

Second Peninsula is a protected site, with very highly depositional, silt sediments at 2.5 m deep (MHT).

Prince Edward Island

Savage Harbour is an enclosed embayment on the north shore of Prince Edward Island with a surface area of approximately 415 hectacres, a maximum depth of 3.65 m, a tidal range of 0.9 m, and a temperature range of -1.5 to 24º C. The surveyed area ranged from 0.30 to 1.0 m in depth.

Newfoundland

Placentia Bay (Northeast Arm)

Placentia Bay (Northeast Arm) is a small cove within an enclosed embayment protected from open coastal waters on 3 sides. The tidal range is between 1 and 2 m.

Little Bay

Little Bay is an enclosed embayment with a tidal range between 1 and 1.5 m that is protected on all sides from open water. Surveys were conducted along one deep (3–4 m deep) and two shallow (2–3 m deep) transects.

Results

In total, 21 sites were sampled from July to October 2013 (Supplementary material Table S1) and a total of 8 species were identified: 5 non-native species, including the solitary *Ascidiella aspersa,* and colonial *Botrylloides violaceus, Botryllus schlosseri, Didemnum vexillum* and *Diplosoma listerianum*; 2 native species, including the colonial *Didemnum albidum* (Verrill, 1871), and solitary *Molgula manhattensis* (Dekay, 1843); and 1 species considered native in the US and non-native in Canada, solitary *Ciona intestinalis* (Table S1; Figure 1). The most common species were *B. schlosseri* and *B. violaceus* (13 sites each). The least common species were native species *D. albidum* (1 site) and *M. manhattensis* (2 sites).

Tunicate coverage within a meadow, within a quadrat, and on a single plant was patchy (i.e. with irregular occurrence, with some areas of high coverage and others with low to no coverage), and some of the eelgrass areas that were surveyed contained no tunicates (Table S1). It is unknown if the tunicate species observed in eelgrass meadows were present elsewhere at their respective sites, attached to other substrate types. Tunicates occurred on eelgrass at different water depths throughout the region, from intertidal to subtidal habitats. The most common percent cover of tunicates on eelgrass was 1–25%. Although uncommon, some tunicate coverage on eelgrass approached >75–100%. At the site with the highest tunicate cover (Placentia Bay (Northeast Arm), NF), a large amount of eelgrass detritus was also heavily fouled (75–100%) by *B. schlosseri*. Eelgrass density dropped substantially at this site from 2012, possibly due to bioturbation (i.e. digging for prey or burrowing for shelter) by the invasive green crab (*Carcinus maenas* Linnaeus, 1758). Canopy height of eelgrass, where measured, ranged from 19–80 cm, and tunicates were present on all range of lengths of eelgrass blades. The shortest canopy height measured was at Stonewall Pond (19 cm) where the greatest diversity of tunicates (n=7) was observed on eelgrass.

The diversity of tunicates on eelgrass beds decreased north of Gloucester, Massachusetts, with only 1 or 2 species present at survey sites. *Ciona intestinalis* was found on eelgrass and other marine plants only in Newfoundland, where it is a new invader. *Botryllus schlosseri* displayed the greatest latitudinal range; and *B. violaceus* and *B. schlosseri* were the only species growing on eelgrass in both the US and Canada. *Ascidiella aspersa, D. albidum, D. vexillum, D. listerianum* and *M. manhattensis* occurred on eelgrass only in the US (Table S1).

Discussion

The purpose of our study was to document the distribution and diversity of tunicate species that attach to eelgrass along the coasts of the northwest Atlantic Ocean. While our study was latitudinal in design, it also included a longitudinal range from W 55° to W 74° because of the aspect of the North American Atlantic coastline. Non-native and native species of tunicates were observed to grow on eelgrass throughout the study area (Table S1). Tunicate distribution in eelgrass was irregular, similar to patchy growth documented for other epifauna on seagrass in intertidal habitats (Barnes and Hamylton 2015).

Where measured, the average canopy height of eelgrass ranged from 19 to 80 cm.

At most sites, density of eelgrass ranged from 127 to 820 shoots/m^2 (Table S1), and there were no apparent differences in eelgrass density between study sites that could be attributed to the effect of tunicate colonization. At the site at Placentia Bay (Northeast Arm), Newfoundland, where eelgrass density was <1 shoot/m^2 and much reduced in 2013 compared to 2012, the reduction was more likely due to green crab behavior than to tunicate colonization.

The different methodologies used and the large number of investigators (n=15) in our study may have influenced our results. A future study to document site differences in tunicate colonization on eelgrass may want to use a more standard methodology and fewer investigators.

The most common species of tunicates on eelgrass were non-native species (Table S1). Of the 8 species found attached to eelgrass, 5 are considered non-native, 2 are native, and 1 is native in US but non-native in Canada. Most of the meadows surveyed exhibited 2 or 3 species, except for Stonewall Pond in Massachusetts where 7 species were recorded. Tunicate faunas attached to eelgrass were less diverse north of Gloucester, Massachusetts, where individual survey sites exhibited two species at most and only 4 of the 8 species observed in this study. Three of these four species are very aggressive colonizers (*B. violaceus, B. schlosseri, C. intestinalis*). At Gloucester and to the south, the number of species at a site ranged from 1 to 7, and 2 of them are aggressive colonizers (*B. violaceus, D. vexillum*). The low diversity north of Gloucester could be due to a decrease northward in water temperature and/or to a lack of opportunities for southern species to rapidly spread northward.

It is notable that tunicates were not found on eelgrass meadows located in Great Bay and Little Bay, New Hampshire. These sites are located in an estuarine system where salinity ranges from near marine (~30 ppt) to single digits (CICEET 2015) in response to seasonal rainfall runoff. Eelgrass beds occurring in waters that experience constant or seasonal low salinity likely will not be colonized by the tunicate species observed in this survey.

The colonial *B. violaceus* most commonly occurs in temperate climates (Callahan et al. 2010; Ma et al. 2011), which may explain why it was found along the deep (3–4 m) transect but not the

shallow (2–3 m) transect in Little Bay, Newfoundland, where water temperatures would be more extreme.

Ciona intestinalis, a documented invasive pest species at blue mussel aquaculture sites at PEI (Locke et al. 2007) and Nova Scotia (Vercaemer et al. 2011), recently appeared in Newfoundland where it attached to eelgrass and kelp (Sargent et al. 2013). In the present study, *C. intestinalis* was found on eelgrass only in Newfoundland. The species has also been documented using eelgrass as substrate in Denmark (Petersen and Svane 1995; Petersen et al. 1997). These occurrences suggest that eelgrass beds may be susceptible to colonization by *C. intestinalis* primarily in northern regions (i.e., cooler latitudes of the temperate zone).

The tunicate species observed in this study most commonly colonize hard (or firm) substrates such as rocky seabed, docks, floats, aquaculture gear, and other anthropogenic materials. Our observations show that seagrasses are also suitable substrates for attachment by these species over a wide latitudinal range. However, seagrasses provide a non-permanent substrate as they are vulnerable to damage and removal by strong currents, sedimentation, and pollution. The tunicates observed in this study, both solitary and colonial, are viable when attached to eelgrass and pose a potential threat to overgrow and weaken seagrass shoots and reduce the sustainability of seagrass meadows. In addition, the colonization of eelgrass leaves by tunicates may reduce the habitat value of eelgrass for other organisms. For example, tunicates could occupy space that would otherwise be used for attachment by larvae of the bay scallop, *Argopecten irradians* (Lamarck, 1819). Scallop larvae will not settle on *D. vexillum* (Morris et al. 2009), nor will juvenile or adult scallops attach to it (Valentine et al. 2007). Other sessile invertebrates may be similarly impacted by tunicate overgrowth. More work is needed to assess such ecosystem impacts of tunicate colonization of eelgrass.

Acknowledgements

We thank Chris Heinig for tunicate data from Maine. Funding for Carman came from the Community Preservation Committee of Oak Bluffs, Massachusetts, and US EPA RARE program. We thank Page Valentine and two anonymous reviewers for constructive edits to the manuscript. Use of trade, product, or firm names does not imply endorsement by the US Government.

References

Altman S, Whitlatch RB (2007) Effects of small-scale disturbance on invasion success in marine communities. *Journal of Experimental Marine Biology and Ecology* 342: 15–29, http://dx.doi.org/10.1016/j.jembe.2006.10.011

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81: 169–193, http://dx.doi.org/10.1890/10-1510.1

Barnes RSK, Hamylton S (2015) Uniform functional structure across spatial scales in an intertidal benthic assemblage. *Marine Environmental Research* 106: 82–91, http://dx.doi.org/10.1016/j.marenvres.2015.03.006

Bourque D, Davidson J, MacNair NG, Arsenault G, LeBlanc AR, Landry T, Miron G (2007) Reproduction and life history of an invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. *Journal of Experimental Marine Biology and Ecology* 342: 78–84, http://dx.doi.org/10.1016/j.jembe.2006.10.017

Callahan AG, Deibel D, McKenzie CH, Hall JR, Rise ML (2010) Survey of harbours in Newfoundland for indigenous and non-indigenous ascidians and an analysis of their cytochrome c oxidase I gene sequences. *Aquatic Invasions* 5: 31–39, http://dx.doi.org/10.3391/ai.2010.5.1.5

Carman MR, Bullard SG, Donnelly JP (2007) Water quality, nitrogen pollution, and ascidian diversity in coastal waters of southern Massachusetts, USA. *Journal of Experimental Marine Biology and Ecology* 342: 175–178, http://dx.doi.org/10.1016/j.jembe.2006.10.037

Carman MR, Grunden DW (2010) First occurrence of the invasive tunicate *Didemnum vexillum* in eelgrass habitat. *Aquatic Invasions* 5: 23–29, http://dx.doi.org/10.3391/ai.2010.5.1.4

Carman MR, Morris JA, Karney RC, Grunden DW (2010) An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. *Journal of Applied Ichthyology* 26: 8–11, http://dx.doi.org/10.1111/j.1439-0426.2010.01495.x

CICEET, Cooperative Institute for Coastal and Estuarine Environmental Technology (2015) Great Bay real time monitoring network, http://www.greatbaydata.org/gb_buoy.html (Accessed August 13, 2015)

Dijkstra JA, Harris LG (2007) Predicting the success of invasive species in the Great Bay Estuarine Reserve: a final report to The New Hampshire Estuaries Project, 20 pp

Dijkstra J, Harris LG, Westerman E (2007) Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *J. of Experimental Marine Biology and Ecology* 342: 61–68, http://dx.doi.org/10.1016/j.jembe.2006.10.015

Duarte CM, Kirkman H (2001) Methods for measurement of seagrass abundance and depth distribution. In Short FT, Coles RG (eds), Global seagrass research methods. Elsevier Science B.V., Amsterdam, The Netherlands, pp 141–153, http://dx.doi.org/10.1016/B978-044450891-1/50008-6

Howes S, Herbinger CM, Darnell P, Vercaemer B (2007) Spatial and temporal patterns of recruitment of the tunicate *Ciona intestinalis* on a mussel farm in Nova Scotia, Canada. *Journal of Experimental Marine Biology and Ecology* 342: 85–92, http://dx.doi.org/10.1016/j.jembe.2006.10.018

Kanary L, Locke A, Watmough J, Chassé J, Bourque D (2011) Predicting larval dispersal of the vase tunicate *Ciona intestinalis* in a Prince Edward Island estuary using a matrix population model. *Aquatic Invasions* 6: 491–506, http://dx.doi.org/10.3391/ai.2011.6.4.14

Locke A, Hanson JM, Ellis KM, Thompson J, Rochette R (2007) Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): potential mechanisms for invasions of Prince Edward Island estuaries. *Journal of Experimental Marine Biology and Ecology* 342: 69–77, http://dx.doi.org/10.1016/j.jembe.2006.10.016

Ma K, Deibel D, McKenzie CH (2011) Indigenous and non-indigenous ascidian tunicates of Newfoundland and Labrador. In: Proceedings of the Contributed Papers of the 27th Annual General Meeting of the Aquaculture Association of Canada: ACC Advance Special Publication Vol. 17, pp 58–63

Moore AM, Vercaemer B, DiBacco C, Sephton D, Ma KCK (2014) Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013. *BioInvasions Records* 3: 225–234, http://dx.doi.org/10.3391/bir.2014.3.4.03

Morris J, Carman MR, Hoagland KE, Green-Beach E, Karney R (2009) Impact of the invasive tunicate *Didemnum vexillum* on the recruitment of the bay scallop (*Argopecten irradians irradians*) and implications for recruitment of the sea scallop (*Placopecten magellanicus*) on Georges Bank. *Aquatic Invasions* 4: 207–211, http://dx.doi.org/10.3391/ai.2009.4.1.21

Pederson J (2005) Marine invaders in the Northeast. Rapid assessment survey of non-native and native marine species of float dock communities, August 2003. MIT Sea Grant College Program Publication No. 05-3, Cambridge, MA, 40 p

Petersen JK, Svane I (1995) Larval dispersal in the ascidian *Ciona intestinalis* (L.). Evidence for a closed population. *Journal of Experimental Marine Biology and Ecology* 186: 89–102, http://dx.doi.org/10.1016/0022-0981(94)00157-9

Petersen JK, Schou O, Thor P (1997) *In situ* growth of the ascidian *Ciona intestinalis* (L.) and the blue mussel *Mytilus edulis* in an eelgrass meadow. *Journal of Experimental Marine Biology and Ecology* 218: 1–11, http://dx.doi.org/10.1016/S0022-0981(97)00064-6

Sargent PS, Wells T, Matheson K, McKenzie CH, Deibel D (2013) First record of vase tunicate, *Ciona intestinalis* (Linnaeus, 1767) type B, in coastal Newfoundland waters. *BioInvasions Records* 2: 89–98, http://dx.doi.org/10.3391/bir.2013.2.2.01

Sephton D, Vercaemer B, Nicolas JM, Keays J (2011) Monitoring for invasive tunicates in Nova Scotia, Canada (2006-2009). *Aquatic Invasions* 6: 391–403, http://dx.doi.org/10.3391/ai.2011.6.4.04

Short FT, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. *Journal of Experimental Marine Biology and Ecology* 350: 3–20, http://dx.doi.org/10.1016/j.jembe.2007.06.012

Valentine PC, Carman MR, Blackwood D, Heffron E (2007) Ecological observations on the colonial ascidian *Didemnum* sp, in a New England tide pool habitat. *Journal of Experimental Marine Biology and Ecology* 342: 109–121, http://dx.doi.org/10.1016/j.jembe.2006.10.021

Van Name WG (1945) The North and South American ascidians. *Bulletin of the American Museum of Natural History* 84: 1–476

Vercaemer B, Sephton D, Nicolas JM, Howes S, Keays J (2011) *Ciona intestinalis* environmental control points: field and laboratory investigations. *Aquatic Invasions* 6: 477–490, http://dx.doi.org/10.3391/ai.2011.6.4.13

Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences* 106: 12377–12381, http://dx.doi.org/10.1073/pnas.0905620106

Whitlatch RB, Osman RW (2001) Geographical distributions and organism-habitat associations of shallow-water introduced marine fauna in New England. In: Pederson J (ed), Marine Bioinvasions Proceedings of the First National Conference, January 24–27, 1999, pp 61–65

Wong MC, Vercaemer B (2012) Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (*Zostera marina*). *Aquatic Invasions* 7: 315–326, http://dx.doi.org/10.3391/ai.2012.7.3.003

Zinn DJ, Abbott DP (1964) Urochordata. In: Smith RI (ed), Keys to Marine Invertebrates of the Woods Hole Region. Contribution No 11, Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts, pp 193–199

Supplementary material

The following supplementary material is available for this article:

Table S1. Eelgrass study sites, sampling methodology, and tunicate and eelgrass observations.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2016/Supplements/MBI_2016_Carman_etal_Supplement.xls