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Research Article

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

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Abstract

Colonization of eelgrass (*Zostera marina* L.) by tunicates can lead to reduced plant growth and survival. Several of the tunicate species that are found on eelgrass in the northwest Atlantic are highly aggressive colonizers, and range expansions are predicted in association with climate-change induced increases in seawater temperature. In 2017, we surveyed tunicates within eelgrass meadows at 33 sites from New Jersey to Newfoundland. Eight tunicate species were identified colonizing eelgrass, of which four were non-native and one was cryptogenic. The most common species (*Botrylloides violaceus* and *Botryllus schlosseri*) occurred from New York to Atlantic Canada. Tunicate faunas attached to eelgrass were less diverse north of Cape Cod, Massachusetts. Artificial substrates in the vicinity of the eelgrass meadows generally supported more tunicate species than did the eelgrass, but fewer species co-occurred in northern sites than southern sites. The latitudinal gradient in tunicate diversity corresponded to gradients of summertime sea surface temperature and traditional biogeographical zones in the northwest Atlantic, where Cape Cod represents a transition between cold-water and warm-water invertebrate faunas. Tunicate density in the eelgrass meadows was low, ranging generally from 1–25% cover of eelgrass shoots, suggesting that space availability does not currently limit tunicate colonization of eelgrass. This survey, along with our 2013 survey, provide a baseline for identifying future changes in tunicate distribution and abundance in northwest Atlantic eelgrass meadows.

Key words: regional study, *Zostera marina*, fouling organisms, Ascidiacea, introduced species

Introduction

The prevalence and distribution of non-native tunicates on a wide variety of substrates in the northwest Atlantic has been increasing (Bullard et al. 2007; Locke et al. 2007; Sargent et al. 2013; Moore et al. 2014; Carman et al. 2016). In addition, expansion to new substrates, such as the submerged vascular plant *Zostera marina* Linneaus, 1753 (eelgrass) has been documented for a number of tunicate species (Carman and Grunden 2010; Carman et al. 2014). Colonization of eelgrass by tunicates can cause reduced growth, production, and even mortality of eelgrass shoots (Wong and Vercaemer 2012; Long and Grosholz 2015).

Eelgrass is considered a foundational species that serves many critical ecological functions. It is highly productive and is widely recognized for its importance in coastal food webs and as a nursery and refuge habitat for many fish and invertebrate species (Thayer et al. 1985; Heck et al. 1995; Lazzari et al. 2003). Eelgrass stabilizes sediments and provides some shoreline protection and erosion control (Thayer et al. 1985). In addition, by sequestering large quantities of carbon, eelgrass may buffer climate change (Fourqurean et al. 2012; Colarusso et al. 2016a). Distribution and abundance of eelgrass has declined substantially in the northwest Atlantic from direct physical disturbance by dredging, dock and pier construction, and damage from boats and moorings (Burdick and Short 1999; Orth et al. 2017) and indirect effects of watershed disturbance from development that leads to reduced water clarity (Hauxwell et al. 2003; Lee et al. 2007). Colonization of eelgrass by tunicates increases stress on eelgrass plants, which could hasten losses of this important habitat.

In 2013, the first tunicate survey of northwest Atlantic eelgrass meadows was conducted to determine the extent of colonization in the region (Carman et al. 2016). In that survey, twenty-one sites between Newfoundland and New Jersey were surveyed, and a total of 8 species of tunicates were found colonizing eelgrass. Four of those 8 colonizers are considered non-native. The most common tunicates present were *Botryllus schlosseri* (Pallas, 1766) and *Botrylloides violaceus* Oka, 1927. In general, tunicate coverage of eelgrass shoots was < 25%, though it approached 75–100% in some locations, and several of the tunicate species found on eelgrass at that time (*B. schlosseri, B. violaceus, Ciona intestinalis* (Linneaus, 1767), and *Didemnum vexillum* Kott, 2002) are considered highly aggressive colonizers (Carver et al. 2006; Bullard et al. 2007; Epelbaum et al. 2009).

Among marine fouling communities, seawater temperature governs species growth rates and competitive abilities (Lord 2016), and temperature increases are expected to strongly influence changes in community composition (Hansen et al. 2006; Lord and Whitlatch 2015). Water temperatures are expected to increase 2–4 °C in the northwest Atlantic by 2100 (IPCC 2007). The 2013 eelgrass tunicate survey showed fewer tunicate

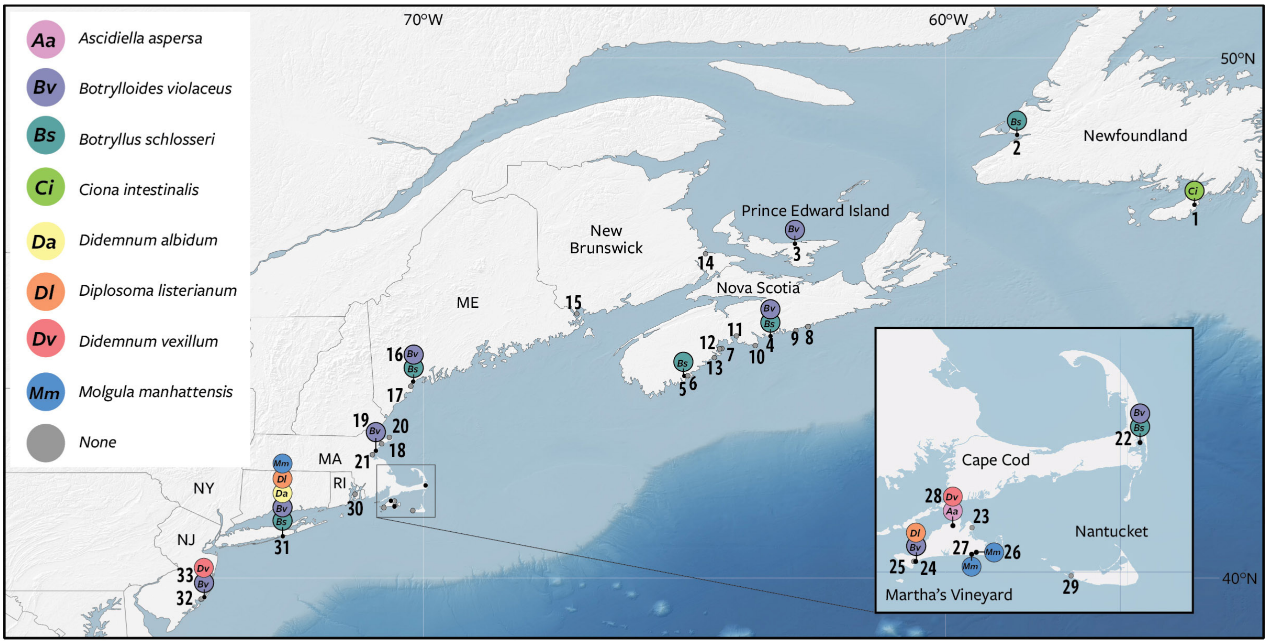

Figure 1. Map of study sites and tunicate species occurrences on eelgrass.

species in northern than in southern eelgrass meadows (Carman et al. 2016). Range expansions are predicted for non-native and native species in association with climate-induced changes in seawater temperature (Parmesan et al. 2005; Rius et al. 2014). Colder sites, often characterized by more open space available, are likely the most susceptible to future invasions. As climate change extends the ranges of potential invaders (Lord 2016), eelgrass may provide substrate for expansion of tunicate species. Thus, northern eelgrass meadows may see increases in the number of tunicate species and in tunicate overgrowth on eelgrass shoots. The purpose of this study was to improve understanding of the distribution, diversity, and abundance of tunicate species colonizing eelgrass along the northwest Atlantic coast. We revisited 11 sites that were surveyed in 2013, while adding more sites to the latitudinal gradient between New Jersey and Newfoundland. The result is an expanded baseline that can be used to predict and identify future changes in the utilization of eelgrass as substrate by tunicates.

Materials and methods

Eelgrass meadows were surveyed for tunicates at 33 sites between June and October 2017 (Figure 1, Supplementary material Table S1). In many cases, the tunicate surveys capitalized on existing eelgrass research and monitoring projects underway in the region. Methods for quantifying tunicates were comparable among sites but were not standardized. Estimates of tunicate cover on eelgrass were made by recording systematic underwater observations (snorkel, SCUBA, or wading) or evaluating replicate quadrats (0.25 m × 0.25 m) positioned randomly within meadows or along 50- to 100-m transects across meadows. Eelgrass and tunicate measurements were made in situ or in the lab on harvested quadrat contents. Cover of eelgrass by each tunicate species present was estimated visually within 5 classes (0, 1–25, > 25–50, > 50–75, > 75–100 percent cover). Some investigators also documented eelgrass density (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). In addition, the tunicate faunas on the artificial substrates nearest to the surveyed eelgrass meadows were documented at 9 sites where both eelgrass and artificial substrate types were present (Table S1). General spatial patterns of average monthly sea-surface temperatures in 2017 were examined from satellite data collected at 1-km resolution by the NASA Earth Observing System Moderate Resolution Imaging Spectroradiometer (MODIS; NASA 2019).

Study site characteristics

Eelgrass meadows were surveyed for tunicates at 33 sites (Table S1). An additional 2 sites that formed part of the 2013 survey were visited in 2017 (intertidal zone off Town Neck Beach, Sandwich, MA; Lagoon Pond on Martha's Vineyard, MA; Carman et al. 2016) but eelgrass was no longer present, therefore these sites are not included in this compilation. Where measured, the average density of eelgrass ranged from 46 to 1030 shoots/m^2 and the average canopy height ranged from 18 to 84 cm.

<u>Newfoundland</u>

<u>Little Bay (site #1)</u>

This site is a small protected, enclosed embayment located within Mortier Bay, which is on the western side of Placentia Bay, with a tidal range between 1 and 1.5 m and a fetch of 0.40 km in the prevailing wind direction (SW). Surveys were conducted along a shallow transect (2 m deep), mid-depth transect (3 m deep), and deeper transect (3–4 m deep). In Little Bay, eelgrass is generally found within a narrow band (< 50 m wide) along the shoreline in water depths ranging from 0 to 6–7 m. It is also found throughout Mortier Bay in similar, shallow subtidal habitats.

<u>Little Port Harmon (site #2)</u>

This site is located in a channel that connects a tidal pond to St. George's Bay in southwestern Newfoundland. The channel remains connected to the ocean throughout the tidal cycle; the maximum spring tide is 1.6 m above absolute low. The sampled eelgrass meadow ranges from 0 to 5 m deep; there are additional patches of eelgrass throughout the intertidal/subtidal areas of Port Harmon.

<u>Prince Edward Island</u>

<u>Savage Harbour (site #3)</u>

This site is an enclosed embayment connected by a narrow channel to the Gulf of St. Lawrence, on the north shore of Prince Edward Island. The embayment has a surface area of approximately 415 hectares (ha), a maximum depth of 3.65 m, and a tidal range of 0.9 m. There are patches of eelgrass throughout the embayment.

Nova Scotia

Lower Three Fathom Harbour (site #4)

This site is a shallow lagoon of approximately 39 ha, located on the eastern shore of Nova Scotia northeast of Halifax. The seagrass bed is located approximately 0.8 km from a narrow opening to the Atlantic Ocean. The 8 ha eelgrass bed is patchy, in soft sediment with a gravel underlayer. Water depth at low tide is approximately 0.5 m with a tidal range of 1 m.

Port l'Hebert (site #5)

This site is near the head of Port l'Hebert Bay on the south shore of Nova Scotia and is located in a migratory bird sanctuary. The 490 ha seagrass bed is in very shallow water, in muddy sediments. Eelgrass occurs intertidally to about 0.2 m at low tide, with a tidal range of 1.5 m.

Port Joli (site #6)

This site is near the head of Port Joli Bay on the south shore of Nova Scotia and is located in a migratory bird sanctuary. The eelgrass bed is about 164 ha and inhabits both sandy and muddy sediments. Eelgrass in both sediment types were surveyed for tunicates. The bed is exposed to about 0.2 m water at low tide and has a tidal range of about 1.5 m.

Sacrifice Island (site #7)

This site is located off of Back Harbor, Lunenburg, on the south shore of Nova Scotia. The eelgrass bed is approximately 3 ha and ranges in depth from 1.5 to 6 m at low tide. Both shallow (1.5 m) and deep (5 m) portions of the bed were surveyed.

Taylor's Head (site #8)

This site is located on the eastern shore of Nova Scotia and is in a provincial park. The eelgrass bed is extensive and ranges from 1.5 to 5 m deep at low tide. Both shallow (1.6 m) and deep (4.3 m) portions of the bed were surveyed.

Cable Island (site #9)

This site is located outside of Ship Harbor on the eastern shore of Nova Scotia. It is about 16 ha and ranges from 0.5 to 3.5 m deep. Both shallow (0.7 m) and deep (2.7 m) portions of the eelgrass bed were surveyed.

Inner Sambro Island (site #10)

This site is located at the mouth of Sambro Harbor and is influenced by oceanic conditions. It is subjected to a strong current and sediments are sandy. The eelgrass bed ranges from 2 to 6 m deep. Both shallow (2 m) and deep (5 m) portions of the bed were surveyed. The bed size is approximately 2.3 ha.

Croucher Island (site #11)

This site is located in St. Margaret's Bay, on the south shore of Nova Scotia. It has a small eelgrass bed of about 0.75 ha. Sediments are comprised of sandy-mud with some cobbles and small gravel. Depth ranges from 1.5 to 6 m deep. The narrow bed is located on a steep shoreline. Both shallow (2 m) and deep (5 m) portions of the bed were surveyed.

Mason's Island (site #12)

This site is located in Back Harbor, Lunenburg, on the south shore of Nova Scotia. The eelgrass bed is very shallow, close to intertidal at low tide and 2 m at high tide. The bed is quite extensive but total areal coverage is unknown. Surveying occurred at 1.5 m depth.

La Have Islands (site #13)

This site has an eelgrass bed that is located on the landward side of Cape La Have Island, in the La Have Island region on the south shore of Nova Scotia. The eelgrass bed is subjected to strong tidal currents. Depth ranges from 1 to 2 m, and the area surveyed was 1 m deep at low tide. The bed is extensive but total areal coverage is unknown.

New Brunswick

Shediac (site #14)

This site is in a sheltered part of an estuarine embayment of about 20 square km that is part of the Northumberland Strait. It is located on the eastern coast of New Brunswick, at Shediac. The sampled eelgrass meadow is about 25 ha with a depth of about 1 m at low tide and is relatively sheltered from the open sea. In the sampled area, eelgrass was common and extends throughout the meadow. The maximum tidal range in the bay is 1.4 m.

St. Andrews (site #15)

This site is located in southern New Brunswick, along an exposed shore in the northwestern region of Passamaquoddy Bay. The bay is connected to the Bay of Fundy and has a maximum tidal range of about 8 m. Eelgrass was absent outside of a relatively small patch (0.12 ha) at a water depth of < 1 m at low tide.

Maine

Broad Cove, Casco Bay (site #16)

This site is a shallow, 250 ha embayment along the western shore of Casco Bay near Cumberland Foreside and has a tidal range of about 4 m. Eelgrass extends continuously from the low intertidal zone to depths of about 3 m below mean low water and covers about 80 ha of the cove.

East End Beach, Portland Harbor (site #17)

This site extends from < 1 m to approximately 3 m (mean low water). The meadow is in a relatively sheltered harbor and encompasses approximately

12 ha. Tidal range in Portland Harbor is approximately 4 m. Bottom sediments are generally silty mud.

Massachusetts

Beverly (site #18)

This site is located on the northern shore of Salem Sound. The 97 ha patchy eelgrass meadow extends seaward between West Beach and Misery Island. The offshore edge of the meadow is blocked from direct exposure to the open ocean by Misery Island. The meadow is approximately 3 m deep (mean low water) with an approximate tidal range of 2.7 m. The bottom is sandy with areas of underlying clay and pockets of silty sand.

Nahant (site #19)

This site covers most of a cove on the southwestern side of the peninsula and is exposed to the ocean. The eelgrass meadow at Nahant is approximately 1.5 ha and occurs at water depths from 1 to 5 m (mean low water); tidal range is approximately 3.5 m.

Niles Beach, Gloucester (site #20)

This site parallels a sandy beach in Gloucester Harbor. The eelgrass meadow lies in a depth range of 1 to 5.5 m (mean low water) and is exposed to the sea to the southwest. This is a large meadow of approximately 21 ha and is very patchy throughout most of this area. Tidal range in Gloucester Harbor is approximately 3.5 m.

Long Island, Boston Harbor (site #21)

This site is on the south side of Long Island in Boston Harbor near the mouth of Boston Harbor. The eelgrass is growing on the lee side of the island. The meadow covers a narrow depth range from approximately 1.5 to 3 m (mean low tide) and covers approximately 4 ha. Tidal range is approximately 3.5 m.

Little Pleasant Bay, Cape Cod (site #22)

This site lies at the upper end of the Pleasant Bay estuary which connects to the Atlantic Ocean through a barrier beach. It is the largest coastal embayment on Cape Cod. Eelgrass occurs throughout most of the Little Pleasant Bay. The mean tidal range is approximately 1.5 m. Surveys were conducted along 3 transects parallel to the shoreline. Water depths (mean low water) were 1.35 m at the deepest transect, 0.84 m at the mid-depth transect, and 0.64 m at the shallow transect.

Farm Pond, Martha's Vineyard (site #23)

This site is a 13 ha pond at Oak Bluffs on the northeast coast of Martha's Vineyard. It has restricted access to the sea via a culvert. The eelgrass meadow (5.3 ha) lies at water depths up to 1.5 m in a tidal range of < 1 m. Maximum water depth is 1.5 m.

Stonewall Pond, Martha's Vineyard (site #24)

This site is a shallow coastal pond in the backwaters of the Menemsha Pond system located at Aquinnah on the southwest coast of Martha's Vineyard. It is connected to Vineyard Sound via Nashaquitsa Pond and Menemsha Pond and lies in an area of low anthropogenic impact. Water depths are < 3 m, and the tidal range is < 1 m. Eelgrass occurs throughout the pond.

Nashaquitsa Pond, Martha's Vineyard (site #25)

This site is a shallow, protected coastal pond between Stonewall Pond and Menemsha Pond located on the southwest coast of Martha's Vineyard. Water depth is approximately 1–2 m (mean low water) throughout the pond. Eelgrass occurs in small patches at numerous locations within the pond.

Edgartown Great Pond, Martha's Vineyard (sites #26, 27)

These 2 sites are located in Great Pond, a 360 ha brackish coastal pond located on the south shore of Martha's Vineyard. It is separated from the Atlantic Ocean by a barrier beach that is artificially breached 3–4 times per year. Water depth varies, with the deepest areas about 3 m deep. Slough Cove (site #26) is narrow and shallow, with freshwater input from groundwater at the head of the cove. Mid-Beach (site #27) is in a large open basin located just north of the barrier beach, thus it tends to be more saline than Slough Cove. Eelgrass occurs throughout most of the pond.

Lake Tashmoo (site # 28)

This site is a 109 ha elongate coastal pond on the north shore of Martha's Vineyard with access to Vineyard Sound via a narrow channel. Water depth is up to 3 m and the tidal range is < 1 m. Eelgrass occurs in patches throughout the pond.

Jackson Point, Nantucket (site #29)

This site is a shallow coastal area along the west coast of Nantucket that is open to Nantucket Sound on the northwest. Water depths are < 3 m, and the tidal range is < 1 m. Eelgrass occurs throughout the area.

Rhode Island

Prudence Island, Narragansett Bay (site #30)

This site is located on the southwest side of the island, which is mid-bay in Narragansett Bay. The eelgrass meadow is approximately 6 ha in size and occurs in shallow water to a depth of approximately 2.5 m. Tidal range in Narragansett Bay is 1.2 m. There is a pile-supported pier and large rock outcroppings on the north and western side of the meadow that supports extensive tunicate growth.

New York

Moriches Bay, Long Island (site #31)

This site is an embayment on Long Island's south shore and is located behind a barrier beach that separates it from the Atlantic Ocean. The eelgrass meadow is located approximately 3 km east of Moriches Inlet in Westhampton Dunes. The meadow is shallow, at depths < 2 m, with a tidal range of < 1 m.

New Jersey

Middle Sedge and Ham Island, Barnegat Bay (sites #32, 33)

These sites are located in the southern part of Barnegat Bay, which is a long, narrow tidal basin that is separated from the Atlantic Ocean by a series of barrier beaches. It is shallow (1.5 m average depth) with a limited tidal range (~ 0.2–1 m). Middle Sedge (site #32) had a water depth of 1.1 m with the eelgrass bed approximately 1 ha in size, while Ham Island (site #33), located about 8 km northeast of Middle Sedge, had a similar water depth, but lies within an expansive eelgrass mosaic in this region of Barnegat Bay.

Results

Eelgrass meadows

A total of 8 tunicate species were identified colonizing eelgrass at the 33 sites sampled (Table S1). Four are non-native species, including the solitary *Ascidiella aspersa* (Müller, 1776) and colonials *Botrylloides violaceus, Didemnum vexillum,* and *Diplosoma listerianum* (Milne Edwards, 1841). Two are native species, colonial *Didemnum albidum* (Verrill, 1871) and solitary *Molgula manhattensis* (De Kay, 1843). One species is cryptogenic, the colonial *Botryllus schlosseri* and 1 species is considered native in the US and non-native in Canada, the solitary *Ciona intestinalis* (Figure 1). The species with the greatest latitudinal ranges were *B. schlosseri* (5 sites, from New York to Newfoundland) and *B. violaceus* (7 sites, from New Jersey to Prince Edward Island). The least common species were *D. albidum* and *A. aspersa* (1 site each) and *D. listerianum* (2 sites). Tunicate faunas attached to eelgrass were less diverse north of Cape Cod, Massachusetts, where most individual survey sites exhibited a single species and several sites exhibited 2 species. From Cape Cod south, most sites surveyed exhibited from 2 to 5 tunicate species. The average eelgrass density was greater north of Cape Cod (414 shoots/m^2) than south of Cape Cod (259 shoots/m^2). The average eelgrass canopy height was also greater north of Cape Cod (49 cm) than south of Cape Cod (31 cm; Table S1).

Where tunicates occurred on eelgrass, the average tunicate coverage was low; the average percent cover of tunicates on eelgrass was 1–25% (Table S1). At 19 sites, no tunicates were found attached to eelgrass. Within-site distribution of tunicates was patchy, and some eelgrass quadrats were observed with tunicate cover of 25–50% (Lower Three Fathom Harbour, NS; Moriches Bay, NY) and 50–75% (Slough Cove in Edgartown Great Pond, MA; Ham Island, NJ).

The depth distribution of tunicates attached to eelgrass ranged from the low intertidal zone to depths of about 4 m. The shallowest occurrence among the sites surveyed was at Broad Cove, in Casco Bay, Maine (Table S1, site #16), where *B. violaceus* and *B. schlosseri* were found at very low abundance in the low intertidal and shallow subtidal portion of the eelgrass bed. The deepest occurrence was at Nahant (Table S1, site #19), where *B. violaceus* was found on eelgrass growing at 4 m (MLW), which is close to the deepest edge of the eelgrass bed at that site.

Artificial substrates

Where surveyed, artificial substrates generally supported more tunicate species than eelgrass within the same site (Table S1). At some sites, the difference in tunicate diversity between substrate types was quite dramatic; for example, at Nashaquitsa Pond, MA (Table S1, site #25) 4 tunicate species were found attached to mooring lines, whereas none were found attached to eelgrass. The general latitudinal gradient in tunicate diversity on artificial substrates mirrored that on eelgrass, with fewer species co-occurring within northern sites than within southern sites.

Sea surface temperature patterns

Sea surface temperature increased predictably from the northern to southern limit of the survey during the warmer months of the year. From May through November, the coastal surface waters north of Cape Cod, MA were markedly cooler than the coastal waters south of Cape Cod (Figure 2).

Discussion

Most of the tunicate species found on eelgrass in this survey were non-native or cryptogenic species and the majority of these are very aggressive colonizers (*B. schlosseri, B. violaceus, D. vexillum, D. listerianum,* and *C. intestinalis*). The 2 native species occurred only in Edgartown Great Pond, MA (*M. manhattensis*) and Moriches Bay, NY (*D. albidum* and *M. manhattensis*). Although space often limits the settlement of tunicates in coastal habitats (Whitlatch and Osman 2009; Lord 2017) and tunicate cover on artificial substrates is often near or at 100% (Valentine et al. 2016; Lord 2016), we found cover on eelgrass shoots to be generally 1–25% throughout the northwest Atlantic meadows we surveyed. Tunicate spatial distribution in eelgrass was patchy and tunicates were not found in all eelgrass beds across the latitudinal gradient. This is consistent with earlier studies (Wong and Vercaemer 2012; Carman et al. 2016; Colarusso et al. 2016b) and suggests that space availability does not currently limit tunicate colonization in eelgrass habitat.

During spring and summer, when tunicate larvae are colonizing open space, eelgrass is routinely creating new substrate by defoliating and growing

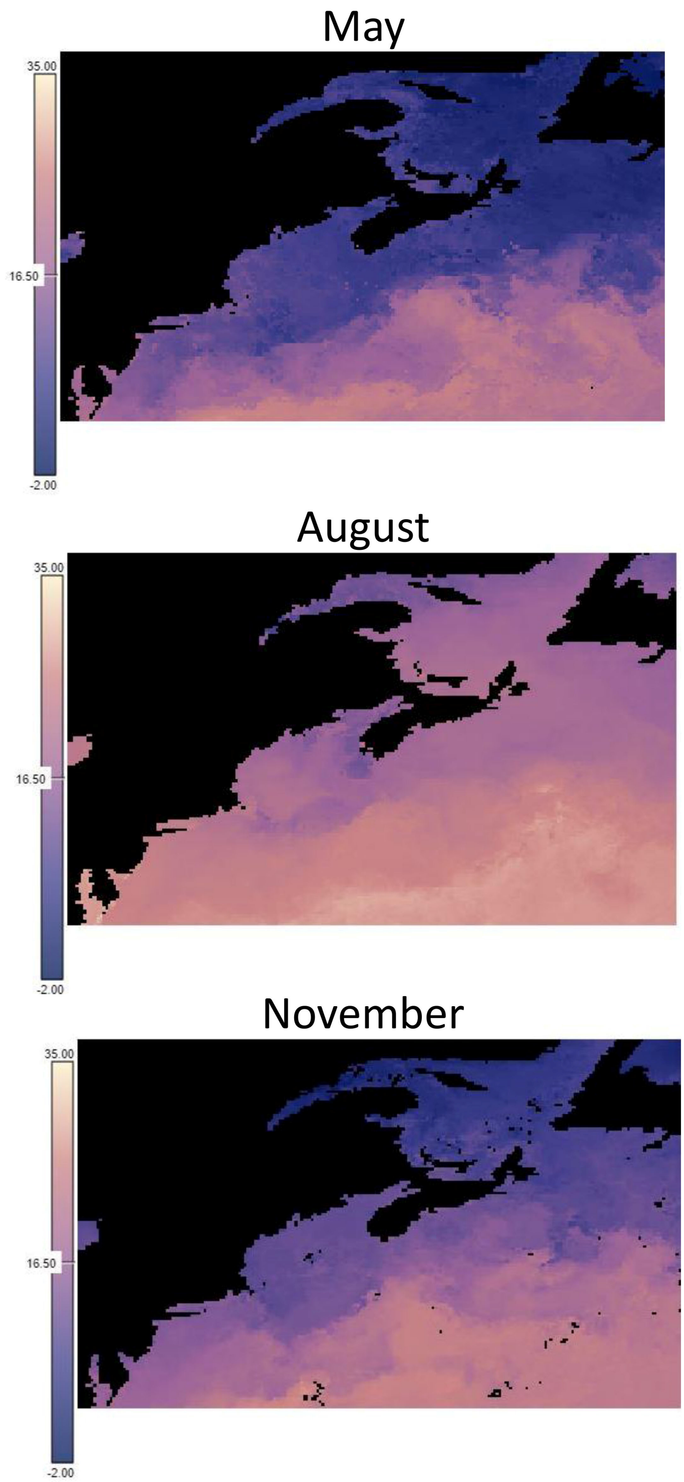

Figure 2. Average monthly sea surface temperatures in the study region during May, August, and November 2017. Scale is degrees C; data and images from NASA Earth Observations (NASA 2019).

new leaves. Defoliated eelgrass blades (with tunicate epifauna) either settle on the seafloor where the tunicates can continue to live and grow (Carman and Grunden 2010; Carman et al. 2014, 2016) or float on the water's surface providing a rafting vector for tunicate distribution to new locations (Worcester 1994). Thus, eelgrass may provide substrate for range expansion of tunicate species.

The latitudinal gradient in tunicate diversity we observed corresponds to traditional biogeographical zones of the northwest Atlantic, where Cape Cod represents a transition zone between northern, cold-water invertebrates of the Acadian Biogeographic Province and southern, warm-water invertebrates of the Virginian Biogeographic Province (Hale 2010). This pattern correlates with gradients of summertime sea surface temperature, with cooler temperatures north of Cape Cod (Figure 2). Temperature affects tunicate metabolism and growth, space acquisition, and reproductive output. For example, warmer regions of the temperate zone such as Japan and the Mediterranean experience continuous spawning by *C. intestinalis*, producing up to 4 generations annually, but the cooler temperate regions such as Atlantic Canada experience 2 spawning events per year (Harris et al. 2017). Ultimately, rapid growers that can overgrow neighbors (Osman 2015) and reproduce earlier or later than others in the community have a competitive advantage (Stachowicz et al. 2002), and there is indication that some non-native species may benefit from predicted increases in global seawater temperatures. During a 12-year study of tunicate recruitment, Stachowicz et al. (2002) found that *A. aspersa*, *B. violaceus*, and *D. listerianum* recruited earlier in warmer years, whereas native tunicates showed no such relationship between water temperature and onset of recruitment. Mesocosm experiments show that tunicate growth responses to elevated temperature are greater in the cooler parts of their range (Lord and Whitlatch 2015), suggesting that *B. violaceus* will likely increase in abundance in the northern portion of our survey as seawater temperatures rise.

Warming seawater temperatures are also expected to enable the arctic-ward range expansion of invasive, temperate tunicates. For example, *A. aspersa*, *D. listerianum*, *D. vexillum*, and *Styela clava* Herdman, 1881, have recently spread from northern New England to Atlantic Canada via shipping, boating, rafting, and fragmentation (Moore et al. 2014). *Ciona intestinalis* has similarly spread poleward, from Maine (Trott 2004) to Nova Scotia in 1997 (Carver et al. 2003) to Prince Edward Island in 2004 (Ramsay et al. 2009) to Newfoundland in 2012 (Sargent et al. 2013; McKenzie et al. 2016), a range expansion of about 1,000 km in about 15 years. *Didemnum vexillum* fragments on eelgrass have the ability to reattach and grow on new substrates in temperatures as low as 6 °C (Carman et al. 2014), implying that rafting fragments drifting northward in colder temperatures could survive, asexually reproduce, and colonize. Invasive species of tunicates in North Carolina, south of our study area, that are more abundant in the colder months, such as *Polyandrocarpa anguinea* (Sluiter, 1898) and *P. zorritensis* (Van Name, 1931), may spread northward into southern New England as temperatures warm (Villalobos et al. 2017). Interestingly, although tunicate assemblages similar to those reported in our survey occur on hard substrates of coastal North Carolina and Puget Sound, tunicates were not found attached to eelgrass in those regions

(Susanna Lopez-Legentil and Jeff Gaeckle *pers. comm.*), but have been recorded elsewhere on the US west coast in California (Worcester 1994; Williams 2007; Long and Grosholz 2015). Conditions have already favored tunicate expansion to eelgrass in the northwest Atlantic. Understanding the mechanisms of thermal adaptation and responses to stressors will help improve predictions of future distribution of these species (Woodin et al. 2013).

To best conserve the critical ecosystem services that eelgrass meadows provide, natural resource managers need information on the magnitude of existing and potential threats to this species. The data reported in our survey provide a baseline for identifying changes in tunicate cover of eelgrass. Continued systematic monitoring has provided for an expanded and important understanding of tunicate populations within eelgrass beds, a valuable and severely threatened coastal habitat. Rapidly warming ocean temperatures may enhance tunicate abundance on eelgrass substrate in northern latitudes. Because of the negative impacts of tunicates on seagrasses (Wong and Vercaemer 2012; Long and Grosholz 2015; Colarusso et al. 2016b), consistent monitoring of sentinel eelgrass sites would allow increased threats to eelgrass from tunicate cover to be detected.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Locations of study sites, dates of observations of eelgrass meadow properties, and tunicate occurrences.

This material is available as part of online article from:
http://www.reabic.net/journals/mbi/2019/Supplements/MBI_2019_Carman_etal_SupplementaryTable.xlsx