First occurrence of the invasive tunicate Didemnum vexillum in eelgrass habitat

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Received: 2 September 2009 / Accepted: 22 December 2009 / Published online: 6 January 2010

Abstract

During the late 20th century, several species of alien tunicates invaded New England marine coastal waters. In Autumn 2008, we surveyed for tunicates in Lake Tashmoo, a protected marine pond with shellfish aquaculture operations and restored bay scallop Argopecten irradians irradians habitat on Martha’s Vineyard, Massachusetts. We found the invasive tunicates Ascidia aspera, Botrylloides violaceus, Botryllus schlosseri, Didemnum vexillum, Diplosoma listerianum, Styela clava and native tunicate Molgula manhattensis attached to artificial substrates throughout Lake Tashmoo and B. violaceus, B. schlosseri, D. vexillum, D. listerianum and M. manhattensis attached to eelgrass Zostera marina in the middle of Lake Tashmoo. Tunicates were growing on the stalk and blade of in situ eelgrass, floating pieces of eelgrass (a transport and dispersal mechanism), and pieces of eelgrass in fouling communities on boat hulls and aquaculture floats. Botrylloides violaceus, B. schlosseri, D. listerianum and M. manhattensis have been previously recorded as utilizing eelgrass as substrate, but this is the first recorded occurrence of D. vexillum to utilize eelgrass as substrate. Perhaps because of lack of substrate space in Lake Tashmoo, D. vexillum spread to eelgrass. Eelgrass serves as a juvenile shellfish and fish habitat and threats to it are of concern by coastal managers and the fishing industry.

Key words: invasive species, seagrass, Ascidiaeae, Martha’s Vineyard, Massachusetts

Introduction

Several alien tunicate species were introduced into marine habitats of New England in the past 40 years, including Ascidia aspera (D. F. Müller 1776), Botrylloides violaceus Okr 1927, Didemnum vexillum Kott 2002, Diplosoma listerianum (Milne-Edwards 1841) and Styela clava (Herdan 1881 (Whitlatch and Osman 2001; Carman and Roscoe 2003; Pederson 2005; Bullard et al. 2007; Dijkstra et al. 2007; Valentine et al. 2007a). Botryllus schlosseri (Pallas 1774) was introduced in the early 1800s (J. Carlton, pers. comm.). Of particular concern is D. vexillum because it poses a threat to New England shellfisheries. For example, it encrusts sea scallops Placopecten magellanicus (Gmelin 1791) on the rocky seafloor of Georges Bank (Valentine et al. 2007b) and, along with other invasive tunicate species, cultured oysters Crassostrea virginica (Gmelin 1791), Northern bay scallops Argopecten irradians irradians (Lamarck 1819), blue mussels Mytilus edulis Linnaeus 1758, and quahogs Mercenaria mercenaria (Linnaeus 1758) in nearshore environments (Valentine et al. 2007a; Carman et al. 2009a; Carman et al. 2009b). Recent literature has focused on subtidal algal communities in North American Atlantic and Pacific waters (Fell and Lewandrowski 1981; Harris and Jones 2005; Stachowicz and Whitlatch 2005; Locke et al. 2007; Valentine et al. 2007a; Williams 2007; Carman et al. 2009a), bivalve aquaculture (Couuts and Forrest 2007; Locke et al. 2007), and wild fisheries (Lengyel et al. 2009; Morris et al. 2009). Little attention has been paid to tunicate epibiosis in eelgrass habitats.

Eelgrass meadows provide many ecosystem services including habitat for a high number of individuals and species, filter coastal waters, dissipate wave energy, and anchor sediments (Edgar and Shaw 1995). From a fisheries perspective, eelgrass is important since it serves as a protective habitat for juvenile fish and shellfish, including bay scallops. In New England, eelgrass, Zostera marina (Linnaeus 1753), is a common form of seagrass. The focus of this study was to determine tunicate distribution on artificial and natural surfaces in Lake Tashmoo, a marine pond with
expansive eelgrass meadows and an important shellfish restoration and shellfish aquaculture site on Martha’s Vineyard. Here we report on the first known occurrence of *D. vexillum* in eelgrass habitat.

**Methods**

To examine the occurrence of epibionts, specifically *D. vexillum*, in eelgrass beds, we surveyed a variety of substrates in Lake Tashmoo on Martha’s Vineyard, Massachusetts, USA. Bay scalloping is a cultural rite on Martha’s Vineyard, Massachusetts and a winter fishery that provides an income to many local fishermen (Karney 1991). The study site was Lake Tashmoo, an approximate 270 acre, elongate, north to south trending, protected marine pond on the northwest side of the island Martha’s Vineyard, exposed to Vineyard Sound in the North Atlantic (Figure 1A). The island is composed of glacial tills and the seafloor of Lake Tashmoo is composed of fine-grained sediments. Tidal range is less than 1 m. Shoreline development is mostly residential though business districts are within the watersheds.

We surveyed aquaculture equipment, floats, docks, boat hulls, and seafloor using small boats, viewer boxes, boatside collections and snorkel
gear. Nine people spent 3 days surveying Lake Tashmoo in September 2008. In addition, an underwater side-mounted video camera and GPS device were used to determine the geographical distribution of tunicates on eelgrass. The camera was mounted on a collar and manually suspended about one meter above the seafloor. On October 15, the first video transects were made perpendicular to shore. On November 21, video transects were made parallel and perpendicular to shore. We used a grappling hook mounted to the camera collar to collect bottom samples to ground truth the video images. Depth was measured with the Garmin GPSMAP 178 Chartplotter sonar on the boat. Salinity measurements were taken with an Atago refractometer.

Results

*Botrylloides violaceus*, *B. schlosseri*, *D. vexillum*, *D. listerianum*, *M. manhattensis*, *S. clava* and *A. aspersa* were attached to artificial substrates including boat bottoms, dock pilings, aquaculture cages, bags and ropes (Table 1) and *B. violaceus*, *B. schlosseri*, *D. vexillum*, *D. listerianum* and *M. manhattensis* attached to live and dead eelgrass (Figures 2, 3). Tunicates were living on eelgrass at water depths of 1.5 m to 3 m at mid-tide in the mid-pond area (Figure 1B). *Didemnum vexillum* occurred on the stalk and blade of in situ eelgrass (Figure 3). *Didemnum vexillum* was also attached to live bush weed *Agardhiella subulata* (C. Argardh) Kraft and Wynne 1979, which was interspersed amongst eelgrass on the seafloor. Also commonly present on in situ eelgrass was the invasive sponge *Halichondria panicea* (Pallas 1766) and snail *Bittium* sp. *Didemnum* colonies appear to have a successful defense system against potential predators (crabs) and grazers (snails) (Figure 3). *Botrylloides violaceus*, *B. schlosseri*, *D. listerianum* and *M. manhattensis* were observed attached to rafting pieces of eelgrass. We did not find rafting *A. subulata*. Pieces of eelgrass were incorporated into *B. violaceus*, *B. schlosseri*, *D. listerianum* and *D. vexillum* colonies growing on the bottom of a dingy at the public landing on the eastern shore and an aquaculture float on the western shore.

Tunicates were not found on eelgrass near the entrance (north end) or back area (south end) of Lake Tashmoo. No tunicates were observed attached to bottom sediments. Salinity of Lake Tashmoo ranged from 30 to 33 PSU.
Table 1. Lake Tashmoo, Martha’s Vineyard, Massachusetts sites surveyed for tunicates, September 2008. Abbreviations: Aa=Ascidiella aspersa; Bv=Botrylloides violaceus; Bs=Botryllus schlosseri; Dv=Didemnum vexillum; Dl=Diplosoma listerianum; Mm=Molgula manhattensis; Sc=Styela clava. The most common species are listed first.

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Substrate</th>
<th>Tunicate Species</th>
<th>Latitude, °N</th>
<th>Longitude, °W</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Town Landing</td>
<td>pilings</td>
<td>none</td>
<td>41.455947</td>
<td>70.62207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dingy bottom</td>
<td>Sc, Bv, Dv, Bs, Dl, Mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eelgrass on dingy bottom</td>
<td>Bv, Dv, Di, Mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>floating eelgrass pieces</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>East Shore</td>
<td>untreated culture bag</td>
<td>Bv, Dl</td>
<td>41.454596</td>
<td>70.621877</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bay scallops in bag</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>culture float</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>untreated culture box</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>quahog box (sandy bottom)</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>West Shore</td>
<td>untreated culture cage</td>
<td>Aa, Bv, Dv, Dl, Sc</td>
<td>41.454837</td>
<td>70.625331</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bay scallops in cage</td>
<td>Aa, Bv, Dv, Dl, Sc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>culture float</td>
<td>Aa, Bv, Dv, Dl, Sc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eelgrass on float bottom</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>floating eelgrass pieces</td>
<td>Bv, Dl</td>
<td></td>
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<td></td>
<td></td>
<td>eelgrass on float bottom</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>eelgrass</td>
<td>Bv, Dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>South Shore</td>
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<td>none</td>
<td>41.448951</td>
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<td>5</td>
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<td>41.466206</td>
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<td>6</td>
<td>Northwest Shore</td>
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<td>none</td>
<td>41.463666</td>
<td>70.631039</td>
</tr>
</tbody>
</table>

Discussion

Invasive tunicate species are common on Martha’s Vineyard (Carman et al. 2007; Carman et al. 2009a). Previous reports have documented tunicates attached to eelgrass and macro algae. Botrylloides violaceus, B. schlosseri, D. listerianum and M. manhattensis have been previously recorded as utilizing eelgrass as substrate, but this is the first recorded occurrence of D. vexillum to utilize eelgrass as substrate. Botryllus schlosseri and B. violaceus foul northwest Atlantic eelgrass (Lippsone and Lippsone 1984; Williams 2007; Locke et al. 2007). Fell and Lewandrowski (1981) listed M. manhattensis as colonizing eelgrass in lower Mystic River, Connecticut. Didemnum is abundant on Fucus serratus (Linnaeus, 1753) in Strangford Lough, Ireland (Seed et al. 1981). Diplosoma listerianum commonly occurs on eelgrass in California coastal waters (J. Stachowicz, pers. comm.) and was observed on eelgrass at Sandwich in southern Massachusetts (Carman, unpublished data) and at Cape Ann and Gloucester Harbor in northern Massachusetts (P. Colarusso, pers. comm.). Botrylloides violaceus and D. listerianum have recently colonized the invasive macro algae Codium fragile tomentosoides (van Goor) P. C. Silva 1955 in exposed and protected habitats in the Gulf of Maine (Harris and Jones 2005).

Anthropogenic surfaces are more likely to be utilized by the co-existing invasive tunicates B. violaceus, B. schlosseri, D. vexillum, D. listerianum and M. manhattensis (Tyrell and Byers 2007). It is possible that there is little artificial substrate space available in Lake Tashmoo, leading invasive tunicates to colonize natural substrate not typically inhabited. Further, Lake Tashmoo may be representative of tunicate occurrence in eelgrass habitat elsewhere. The use of eelgrass as substrate in Lake Tashmoo by D. vexillum may be indicative of other areas. Our observations are the first record of what is
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probably occurring at many locations. *Didemnum vexillum* was first observed in New England on floating docks, boulders, and boat hulls in the 1980s and 1990s, and on sea scallops in 2003 (Bullard et al. 2007).

Marine plants located near an infested source, such as a dock, are likely to be infested with tunicates (Carman et al. 2009a). *Botrylloides violaceus* has also been found in open meadows of eelgrass (Worcester 1994). In Atlantic Canada, patches of *B. violaceus* have been found on blades of eelgrass in places with no other substrate for tunicate attachment (Locke et al. 2007). In Lake Tashmoo, *D. vexillum* and *B. violaceus* were found on eelgrass near infested buoys and aquaculture floats and in open areas.

Previous threats to eelgrass caused considerable damage. For example, in the 1930s and 1980s, the slime mold *Labyrinthula* sp. greatly reduced *Z. marina* in the North Atlantic (Orth and Moore 1983; Short et al. 1987). The decline in eelgrass on Martha’s Vineyard has been dramatic since the 1980s (Wilcox 2006). Eelgrass is susceptible to the uptake of herbicides in marine antifouling paints, especially near marinas (Scarlett et al. 1999). Environmental conditions, such as global climate change, warming water temperatures, and rising sea level, negatively impact seagrass habitats (Short and Neckles 1999). In contrast, these conditions may enable invasive tunicate populations (Stachowicz et al. 2002).

The ecological effects of invasive tunicates introduced to seagrass and macroalgal beds remain unassessed, but in general, the majority of introduced epibionts have negative effects on marine flora (Williams 2007). Invasive tunicates can smother bivalves and other sessile invertebrates (Bullard et al. 2007; Rajbanshi and Pederson 2007; Coutts and Forrest 2007) and can likely smother plants. The encrusting invasive bryozoan *Membranipora membranacea* (Linnæus 1767) dramatically reduced the number of kelp during storm events (Lambert et al. 1992). The heavy weight of tunicates coupled with their rapid asexual and sexual reproduction may make them more harmful to marine plant communities than encrusting bryozoans. Further, bay scallops may become overgrown and smothered by *D. vexillum* in infested eelgrass habitats. There are no known predators of healthy *D. vexillum* (Carman et al. 2009c). In the absence of predators in eelgrass habitats, epifauna can grow unimpeded (Bologna 2007).

No macroinvertebrates were observed attached to the tunicates on eelgrass. *Bittium* sp. appeared to be grazing on the non-tunicate covered eelgrass. Colonial tunicates *Aplidium stellatum* (Verrill 1871) and *Botryllus planus* (Van Name 1902) attached to turtle grass *Thalassia testudinum* Banks ex König 1805 in Atlantic shallow water habitats were found to contain bioactive secondary metabolites that aid in prevention of attached surface fouling (Bryan et al. 2003). Tait et al. (2007) identified similar anti-fouling, microbial compounds in *D. vexillum*. The surface of *D. vexillum* has an average pH value of 3 to 4 (Bullard et al. 2007; Morris et al. 2009). Perhaps this explains why we observed no organisms attached to *D. vexillum* or *B. violaceus* in Lake Tashmoo.

The spread of *D. vexillum* has been attributed to human mediated processes, but other dispersal mechanisms may be important and deserve further attention (Daley and Scavia 2008). Rafting on eelgrass is a previously unknown dispersal mechanism for *D. vexillum*, but has been documented for other tunicates including botryllids and *Ciona intestinalis* (Linnæus 1767) (Highsmith 1985; Jackson 1986; Worcester 1994; Riisgård et al. 1995; Petersen and Svané 1995; Edlund and Koehl 1998; Thiel and Gutow 2005). Other plant material may transport tunicates in the marine environment. For example, fragments of mangrove roots transport *Ecteinascidia* (Bingham and Young 1991) and drift material can transport *Symplegma* (Dias et al. 2006).

This new use of eelgrass substrate by *D. vexillum* may be an indication of continued invasiveness of the New England coastline. Attached tunicates probably block photosynthesis, release of seed, and natural defoliation. Eelgrass serves as a juvenile shellfish and fish habitat and threats to it are of concern by coastal managers and the fishing industry. The effect of *D. vexillum* overgrowth of eelgrass, and the community dependent upon eelgrass, should be assessed further because *D. vexillum* can negatively impact habitat such as is occurring on Georges Bank (Valentine et al. 2007b). The use of eelgrass as a mechanism of transport for *D. vexillum* may be contributing to its spread. The results of our study demonstrate that *D. vexillum* is continuing to spread, occupying substrate not typically used, and posing a threat to the protected species *Z. marina*.
Acknowledgements

We thank D. Cimeno, A. Basilleres, F. Benson, H. Allen, M. M. Carman, G. Blackwood and D. Blackwood, for their field assistance. C. Stuart, University of Massachusetts, provided the snail identification. Funding support for this project came from Sailors’ Snug Harbor of Boston and The Adelaide and Charles Link Foundation. We also thank two anonymous reviewers who provided edits that improved the manuscript.

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