Research Article

Coldwater reattachment of colonial tunicate *Didemnum vexillum* fragments to natural (eelgrass) and artificial (plastic) substrates in New England

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Abstract

The colonial tunicate *Didemnum vexillum* Kott, 2002, was introduced to New England in the 1980s and by 2000 it was widespread. This highly invasive species spreads by larval release and fragmentation. We tested the ability of *D. vexillum* fragments to reattach to natural (eelgrass *Zostera marina* (Linnaeus, 1753)) and artificial (plastic container) substrates during late fall and early winter. On average, 77% of *D. vexillum* fragments reattached to eelgrass and plastic in water temperatures between 6 and 10°C. Eelgrass appeared to facilitate *D. vexillum* reattachment success in early winter but this tendency should be further investigated.

Key words: Ascidiacea, fragmentation, invasive species, North Atlantic, Martha's Vineyard

Introduction

The non-native colonial tunicate (Ascidiacea) *Didemnum vexillum* Kott, 2002 is commonly found in New England on natural and artificial substrates in coastal marine and offshore habitats (Carman and Roscoe 2003; Pederson 2005; Bullard et al. 2007a; Dijkstra et al. 2007; Valentine et al. 2007a, b). Introduced to New England in the 1980s *D. vexillum* is a rapid global invader (Lambert 2007), probably native to Japan (Stefaniak et al. 2009; Lambert 2009; Stefaniak et al. 2012), that has been documented in New Zealand, along the east and west coasts of the US, the west coast of Canada and in Alaska and Europe (Coutts and Sinner 2004; Bullard et al. 2007a; Minchin and Sides 2006; Gittenberger 2007; Cohen et al. 2011; Tagliapietra et al. 2012) and it will likely continue to spread via multiple vectors to other locations (Herborg et al. 2009). The impact of this invasive species can be significant as it fouls boat hulls, docks, mooring lines; smothers bivalves and other sessile marine invertebrates; and causes economic hardship for shellfish aqua-culture (Coutts and Sinner 2004; Valentine et al.

2007b; Carman et al. 2010; Adams et al 2011; Switzer et al. 2011; Rolheiser et al. 2012).

D. vexillum spreads by release of larvae and by fragmentation. Fragmentation is a dispersal mechanism that occurs when pieces of colonies are removed by the colony naturally pinching-off tendrils, by human disturbance and by the effects of storm events (Lacerda et al. 2002; Boyle et al. 2006; Clarke Murray et al. 2012; Reinhardt et al. 2012). Tunicate colonies with brooded larvae (such as *D. vexillum*) that are attached to broken eelgrass can raft for miles and thus be dispersed into new habitats (Worcester 1994).

If a *D. vexillum* fragment settles on a suitable substrate, it can reattach. In several earlier experi-ments, fragments of *D. vexillum* demonstrated the ability to reattach to hard artificial substrate (PVC settling plates or plastic flow-through containers) and grow by asexual reproduction in southern New England during mid-summer (Stoner 1989; McCarthy et al. 2007; Bullard et al. 2007b; Valentine et al. 2007a; Reinhardt et al. 2012) and early fall (Morris and Carman 2012). Small fragments of *D. vexillum* tolerated suspension in the water column for weeks without reattaching,

suggesting that it can be transported great distances by tidal and storm currents (Morris and Carman 2012).

Eelgrass *Zostera marina* (Linnaeus, 1753) is a valuable coastal species that often co-occurs with *D. vexillum*. Eelgrass is a protected species in New England and elsewhere that serves many important functions, including providing habitat for shellfish and fish. *D. vexillum* and other tunicates including *Botryllus schlosseri* (Pallas, 1776), *Botrylloides violaceus* Okra, 1927, *Diplosoma listerianum* (Milne-Edwards, 1841) and *Molgula manhattensis* (Dekay, 1843) were recently documented attached to live and detached pieces of eelgrass in Lake Tashmoo on Martha's Vineyard (Carman and Grunden 2010). Larvae of *B. violaceus* settle on eelgrass (Worcester 1994) and larvae of other tunicates, including *D. vexillum*, probably also settle on eelgrass. It is unknown however if fragments of *D. vexillum* will reattach to eelgrass especially in late fall and early winter when *D. vexillum* colonies begin to show signs of slow growth and regression (Valentine et al. 2007a). We also wondered if the presence of eelgrass would facilitate *D. vexillum* fragment reattachment to solid surfaces. The water temperature range for *D. vexillum* fragment reattachment has been 16 to 22°C (Morris and Carman 2012). The purpose of this study was to determine experimentally whether *D. vexillum* can reattach to eelgrass and plastic substrates in water temperatures lower than 16°C.

Methods

During late fall 2012, healthy-looking colonies of *D. vexillum* were collected from cultured blue mussels and aquaculture gear in Lagoon Pond at Oak Bluffs on Martha's Vineyard, Massachusetts. Colonies were cut into pieces (about 4 cm^2) and placed in 7 labeled, flow-through plastic containers. The containers were made from covered Petri dishes, 9cm in diameter and 1.5cm deep, with 5 holes about 0.5cm in diameter in the lid and bottom of the container so that there were 10 holes per container. Blades of green and brown eelgrass were collected nearby from drift on the shore and 2 blades were placed in half of the containers (4 of the 7 sample containers for the first trial) containing *D. vexillum*. Controls included 5 flow through plastic containers (covered containers were 9cm in diameter and 5cm deep with 5 holes about 0.5cm in diameter in the lid and bottom of the container so that there were 10 holes per container) with a single adult mussel that had previously attached *D. vexillum*. In addition there were 2 identical containers with just 2 blades of eelgrass inside. Mussels with previously attached *D. vexillum* were used as controls because they served as a source for *D. vexillum* fragments for the experiment.

All containers were randomly placed on a horizontal line secured to the Martha's Vineyard Shellfish Group (MVSG) dock on Lagoon Pond (41.45°N, 70.6°W) and maintained at between 1.0m and 1.5m water depth. Water temperature and salinity were measured at the beginning and end of the each of the 3 trials. Tidal range in the area was less than 1m.

At the end of November after 15 days in the water, during which water temperature cooled 2-3°C, containers were retrieved from the dock and examined in the laboratory at MVSG. Reattachment success was evaluated by using a plastic pipette to gently squirt the presumably reattached fragment with ambient seawater followed by submerging the fragment in a tub of seawater. If the fragment moved or floated, it was considered not attached. If a fragment was deemed attached, the substrate type was noted (container, eelgrass, or container and eelgrass) and any new growth was measured using a transparent cm-grid. This process was repeated twice, using freshly harvested *D. vexillum* fragments and with larger numbers of samples in November (n=39) and December (n=24), so that there were 3 sets of samples. The number of fragments used in the 3 trials varied because we had initially expected fragments to not reattach but when they did, we continued the experiment and collected new fragments for a second and third trial at colder water temperatures. The number of days that sample trials were in the water varied because of weather conditions. After inspection of the first 2 sets of samples, some reattached fragments were returned to the water and allowed to continue to grow.

Results

Water temperatures ranged between 3 and 10°C and salinity remained at 31 throughout the experiment period (Nov 15 to Jan 8).

After the first set of samples were in the water for 15 days, 100% of the *D. vexillum* fragments were reattached in sample containers with and without eelgrass (Figures 1, 2). During this period water temperatures ranged from 8 to 10°C
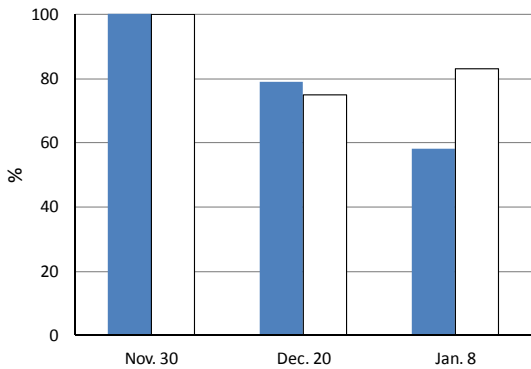
Figure 1. Percent of *D. vexillum* fragments that were reattached in the first set of samples (Nov 15 to Nov 30), second set of samples (Nov 30 to Dec 20), and third set of samples (Dec 20 to Jan 8), in containers without eelgrass and containers with eelgrass. Blue bars are containers without eelgrass and white bars are containers with eelgrass.
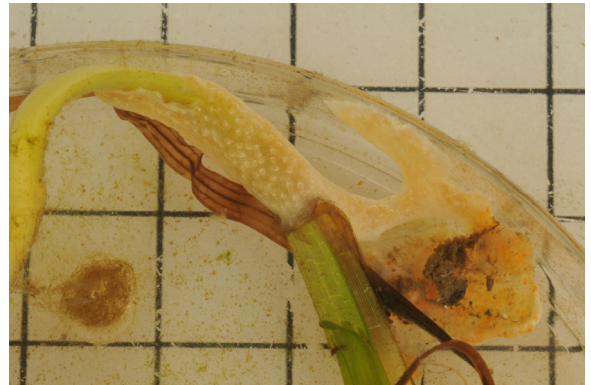

Figure 2. In containers with eelgrass, the percent of *D. vexillum* fragments that reattached to container only, container and eelgrass, or eelgrass only in the first set of samples (Nov 15 to Nov 30), second set of samples (Nov 30 to Dec 20), and third set of samples (Dec 20 to Jan 8). White bars are container and eelgrass, green bars are eelgrass only and blue bars are container only.

Figure 3. Fragment of *D. vexillum* reattached to and growing on the inside of the plastic flow-through container and on green eelgrass; December 20, 2012; cm grid in background (Photo credit: D. Blackwood).
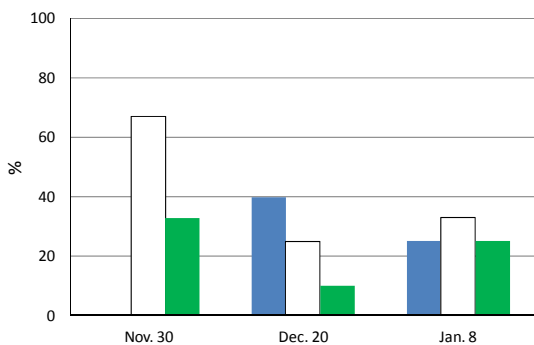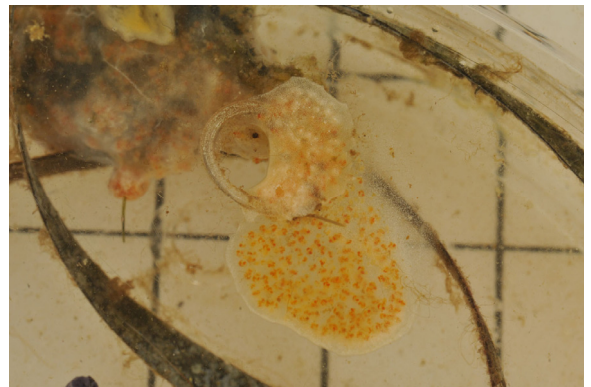

Figure 4. Fragment of *D. vexillum* reattached to and growing on the inside and outside of the plastic flow-through container (through a hole in the container); December 20, 2012; cm grid in background (Photo credit: D. Blackwood).

Figure 5. Fragment of *D. vexillum* reattached to and growing on brown eelgrass; January 8, 2013; cm grid in background (Photo credit: D. Blackwood).

and new growth occurred in all of the samples. The average amount of new growth was 1.5cm^2 and the greatest amount of new growth was 4cm^2. Four of the reattached fragments were returned to the water and allowed to continue growing.

After the second set of samples were in the water for 20 days, 77% of the *D. vexillum* fragments were reattached (Figure 1). In sample containers without eelgrass 79% of the fragments were reattached to the container. In sample containers with eelgrass 75% of the fragments were reattached: 40% to only the container, 10% to only eelgrass and 25% to container and eelgrass (Figure 2). During this period water temperatures

ranged from 6 to 8°C and new growth occurred in 64% of the samples. The average amount of new growth was $0.9cm^2$ and the greatest amount of new growth was $3cm^2$. Fragments reattached to eelgrass and the inside of the containers (Figure 3) and in a few samples, fragments reattached to the inside and grew through the holes to the outside of the container (Figure 4). Eight reattached fragments were returned to the water dock and allowed to continue to grow.

After the third set of samples were in the water for 19 days, 71% of the *D. vexillum* fragments were reattached (Figure 1). In sample containers without eelgrass 58% of the fragments were reattached to the container. In sample containers with eelgrass 83% of the fragments were reattached: 25% to only the container, 25% to only eelgrass and 33% to container and eelgrass (Figure 2). During this period water temperatures ranged from 3 to 6°C and new growth occurred in 29% of the samples. The average amount of new growth was $0.1cm^2$ and the greatest amount of new growth was $0.25cm^2$. Fragments were often not as robustly reattached as they were for the first 2 trials and were often only reattached by a small portion of the fragment. Fragments reattached and grew on green eelgrass (Figure 3) and on brown eelgrass (Figures 4, 5).

All but one of the samples of reattached *D. vexillum* fragments that were allowed to continue growing from the first (n = 4) and second (n = 8) trials remained attached to the same substrate. The exception was a fragment originally reattached to both container and eelgrass but on January 8 was only attached to eelgrass. The greatest amount of new growth in continuing-growth fragments was $1cm^2$ in water temperatures that ranged from 3 to 8°C.

D. vexillum colonies attached to mussels in control containers remained attached and healthy in all 5 of the control samples for each of the 3 trials (n=15 total); no new growth laterally or volumetrically was observed. During the experiment, no *D. vexillum* was observed in the 2 control samples (eelgrass only in plastic containers) for each of the 3 trials (n=6 total).

Discussion

This study demonstrated that *D. vexillum* fragments can reattach to natural and artificial substrates in water temperatures between 6 and 10°C. The water temperature at the cessation of larval release by *D. vexillum* in fall is between 9 and 11°C at a nearby site (Valentine et al. 2009). Therefore, during

our experiment containers were not examined for larval recruits because water temperatures were <10°C.

The presence of eelgrass appeared to facilitate reattachment success in the third trial but not in the first and second trials. At the conclusion of the first 2 trials (on Nov 30 and Dec 20), the percent of reattachment fragments in containers without eelgrass was similar to containers with eelgrass but on January 8 the percent of reattached fragments in containers with eelgrass (83%) was greater than in containers without eelgrass (58%). The presence of eelgrass or other natural substrate may enhance the ability of *D. vexillum* to reattach in cold water especially in early winter. However, this tendency should be further investigated.

Earlier studies documented the successful reattachment of fragments of *D. vexillum* on artificial substrates in summer and early fall in water temperatures ranging from 22 to 16°C (McCarthy et al. 2007; Bullard et al. 2007b; Valentine et al. 2007a; Morris and Carman 2012). This study extends the lower temperature range to at least 6°C, perhaps lower. During the third trial, reattachment occurred while water temperatures ranged between 3 and 6°C but we do not know exactly when the reattachment occurred. The ability of *D. vexillum* to reattach at water temperatures <6°C needs to be tested directly. The *D. vexillum* fragment reattachment success declined with declining water temperature and suggests that colder temperatures negatively influenced fragment reattachment success. However, reattached fragments that were allowed to grow remained attached as water temperatures dropped. Thus, it may be easier for a fragment to stay attached than it is for a new fragment to reattach in cold water. As well, the growth rate of reattached fragments declined with declining water temperatures.

Fragmentation of *D. vexillum* may encourage growth along the incised edges of the colonies. In contrast to the observed growth of fragments in our experimental containers, we observed no growth by the *D. vexillum* colonies attached to mussels in the control samples. Disturbance at the colony edge might be a requirement for colony growth at the low temperatures experienced during this study period.

Rafting plant debris should be considered as a dispersal mechanism for *D. vexillum*. Fall is a period of natural shedding and rafting of *Zostera* leaves and a period of storms (end of hurricane season and beginning of nor'easters). The

possibility of long distance dispersal of *D. vexillum* on eelgrass by rafting occurs in fall and would seem to extend well into winter. The ability of this highly invasive species to reattach and grow in cold water and to be dispersed during fall and early winter contributes to its potential spread.

Acknowledgements

References

Adams CM, Shumway SE, Whitlatch RB, Getchis T (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigations. *Journal of the World Aquaculture Society* 42(2): 242–252, http://dx.doi.org/10.1111/j.1749-7345.2011.00460.x

Boyle M, Janiak D, Craig S (2006) Succession in a Humboldt Bay marine fouling community: The role of exotic species, larval settlement and winter storms: Proceedings of the 2004 Humboldt Bay Symposium, pp 215–233

Bullard SG, Lambert G, Carman MR, Byrnes J, Whitlatch RB, Ruiz G, Miller RJ, Harris L, Valentine PC, Collie JS, Pederson J, McNaught DC, Cohen AN, Asch RG, Dijkstra J, Heinonen K (2007a) The invasive colonial ascidian *Didemnum* sp.: current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. *Journal of Experimental Marine Biology and Ecology* 342: 99–108, http://dx.doi.org/10.1016/j.jembe.2006.10.020

Bullard SG, Sedlack B, Reinhardt JF, Litty C, Gareau K, Whitlatch RB (2007b) Fragmentation of colonial ascidians: differences in reattachment capability among species. *Journal of Experimental Marine Biology and Ecology* 342: 166–168, http://dx.doi.org/10.1016/j.jembe.2006.10.034

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

Carman MR, Roscoe LS (2003) The didemnid mystery. *Massachusetts Wildlife* 53: 2–7

Clarke Murray C, Therriault TW, Martone PT (2012) Adapted for invasion? Comparing attachment, drag and dislodgment of native and nonindigenous hull fouling species. *Biological Invasions* 14: 1651–1663, http://dx.doi.org/10.1007/s10530-012-0178-0

Cohen CS, McCann L, Davis T, Shaw L, Ruiz G (2011) Discovery and significance of the colonial tunicate *Didemnum vexillum* in Alaska. *Aquatic Invasions* 6: 263–271, http://dx.doi.org/10.3391/ai.2011.6.3.03

Coutts ADM, Sinner J (2004) An updated benefit-cost analysis of management options for *Didemnum vexillum* in Queen Charlotte Sound. Cawthron Report No. 925, Biosecurity New Zealand, Wellington, 29 pp

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

Gittenberger A (2007) Recent population expansions of non-native ascidians in The Netherlands. *Journal of Experimental Marine Biology and Ecology* 342: 122–126, http://dx.doi.org/10.1016/j.jembe.2006.10.022

Herborg LM, O'Hara P, Therriault TW (2009) Forecasting the potential distribution of the invasive tunicate *Didemnum vexillum*. *Journal of Applied Ecology* 46: 64–72, http://dx.doi.org/10.1111/j.1365-2664.2008.01568.x

Lacerda LD, Conde JE, Kjerfve B, Alvarez-León R, Alarcón C, Polanía J (2002) American Mangroves. In: Lacerda LD (ed), Mangrove Ecosystems: Function and Management, Springer-Verlag, Berlin, pp 1–62, http://dx.doi.org/10.1007/978-3-662-04713-2_1

Lambert G (2007) Invasive sea squirts: a growing global problem. *Journal of Experimental Marine Biology and Ecology* 342: 3–4, http://dx.doi.org/10.1016/j.jembe.2006.10.009

Lambert G (2009) Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. *Aquatic Invasions* 4: 5–28, http://dx.doi.org/10.3391/ai.2009.4.1.2

McCarthy A, Osman RW, Whitlatch RB (2007) Effects of temperature on growth rates of colonial ascidians: a comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. *Journal of Experimental Marine Biology and Ecology* 342: 172–174, http://dx.doi.org/10.1016/j.jembe.2006.10.036

Minchin D, Sides E (2006) Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. *Aquatic Invasions* 1: 143–147, http://dx.doi.org/10.3391/ai.2006.1.3.8

Morris JA, Carman MR (2012) Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate *Didemnum vexillum* with implications for dispersal. *Biological Invasions* 14: 2133–2140, http://dx.doi.org/10.1007/s10530-012-0219-8

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 pp

Reinhardt JF, Gallagher KL, Stefaniak LM, Nolan R, Shaw MT, Whitlatch RB (2012) Material properties of *Didemnum vexillum* and prediction of tendril fragmentation. *Marine Biology* 159: 2875–2884, http://dx.doi.org/10.1007/s00227-012-2048-9

Rolheiser KC, Dunham A, Switzer SE, Pearce CM, Therriault TW (2012) Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. *Aquaculture* 364–365: 53–60, http://dx.doi.org/10.1016/j.aquaculture.2012.07.038

Stefaniak L, Lambert G, Gittenberger A, Zhang H, Lin S, Whitlatch RB (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions* 4: 29–44, http://dx.doi.org/10.3391/ai.2009.4.1.3

Stefaniak L, Zhang H, Gittenberger A, Smith K, Holsinger K, Lin S, Whitlatch RB (2012) Determining the native region of the putatively invasive ascidian *Didemnum vexillum* Kott, 2002. *Journal of Experimental Marine Biology and Ecology* 422–423: 64–71, http://dx.doi.org/10.1016/j.jembe.2012.04.012

Stoner DS (1989) Fragmentation: a mechanism for the stimulation of genet growth rates in an encrusting colonial ascidian. *Bulletin of Marine Science* 45: 277–287

Switzer SE, Therriault TW, Dunham A, Pearce CM (2011) Assessing potential control options for the invasive tunicate *Didemnum vexillum* in shellfish aquaculture. *Aquaculture* 318: 145–153, http://dx.doi.org/10.1016/j.aquaculture.2011.04.044

Tagliapietra D, Keppel E, Sigovini M, Lambert G (2012) First record of the colonial ascidian *Didemnum vexillum* in the Mediterranean: Lagoon of Venice (Italy). *BioInvasions Records* 1 (4): 247–254, http://dx.doi.org/10.3391/bir.2012.1.4.02

Valentine PC, Carman MR, Blackwood D, Heffron E (2007a) 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

Valentine PC, Collie JS, Reid RN, Asch RG, Guida VG, Blackwood DS (2007b) The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat-ecological observations and potential effects on groundfish and scallop fisheries. *Journal of Experimental Marine Biology and Ecology* 342: 179–181, http://dx.doi.org/10.1016/j.jembe.2006.10.038

Valentine PC, Carman MR, Dijkstra J, Blackwood DS (2009) Larval recruitment of the invasive colonial ascidian *Didemnum vexillum*, seasonal water temperatures in New England coastal and offshore waters, and implications for spread of the species. *Aquatic Invasions* 4: 153–168, http://dx.doi.org/10.3391/ai.2009.4.1.16

Worcester SE (1994) Adult rafting versus larval swimming: dispersal and recruitment. *Marine Biology* 121: 309–317, http://dx.doi.org/10.1007/BF00346739