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# Factors influencing the recruitment and abundance of *Didemnum* in Narragansett Bay, Rhode Island

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The non-indigenous colonial tunicate *Didemnum* sp. A has been observed in Narragansett Bay, Rhode Island, since 2000. We compared weekly recruitment of the species and environmental parameters (i.e. temperature, salinity, chlorophyll *a*, dissolved oxygen, pH, and nutrient concentrations) over a 6-month period among three sites: (i) the University of Rhode Island Graduate School of Oceanography dock (GSO), (ii) the Department of Environmental Management pier at Fort Wetherill (FW), and (iii) the Prudence Island T-wharf. At the GSO and FW, divers surveyed the sites for percentage cover of *Didemnum*. To assess the spread of *Didemnum* in the bay and what factors may predict the tunicate's presence, we also surveyed intertidal sites in October and November 2005, noting *Didemnum* presence, salinity, number of boats and moorings, and distances to major ports at each site. GSO had the highest percentage cover of adults and the highest recruitment of the tunicate ($p < 0.01$), reaching average peak values of 319 individuals per 100 $cm^2$ in September 2005. Temperature and salinity demonstrated the best correlation with recruitment, and higher boat and mooring numbers may be a reliable predictor of tunicate presence. Further monitoring is needed to assess the potential spread of *Didemnum* throughout Narragansett Bay.

**Keywords:** *Didemnum*, distribution, Narragansett Bay, non-indigenous tunicate, recruitment, salinity, substratum, temperature.

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## Introduction

*Didemnum* sp. A (hereafter referred to as *Didemnum*) is a non-indigenous colonial tunicate that has been observed in Narragansett Bay, Rhode Island since 2000. It was detected first in Newport (Pederson *et al*., 2001), then on the University of Rhode Island (URI) Graduate School of Oceanography (GSO) dock (C. Deacutis, pers. comm.). The species is a strong spatial competitor, colonizes substratum rapidly, and prefers hard-bottom and gravel habitats and artificial substrata (Bullard *et al*., 2007a). In dominating new habitats, it reduces the abundance of previously established species (Bak *et al*., 1996; Lambert and Lambert, 2003; Bullard *et al*., 2007a). *Didemnum* may also inhibit settlement of scallops on Georges Bank, and it frequently overgrows adult *Mytilus edulis* and other species of shellfish (Bullard *et al*., 2007a; Valentine *et al*., 2007b). Colonies of the tunicate have few organisms willing or able to settle on them, and they have no known predators to control population growth (Bullard *et al*., 2007a; USGS, 2007).

Our objective was to describe the factors that contribute to the success of *Didemnum* and to characterize areas prone to invasion by the tunicate. To do so, we examined the seasonal recruitment of *Didemnum* and the extent of its distribution in Narragansett Bay. We correlated these with water quality factors and characteristics of the sites, respectively. Although the effects of temperature on *Didemnum* have been studied before (e.g. Osman and Whitlatch, 2007; Valentine *et al*., 2007a), we explored other factors that may affect *Didemnum* abundance, additional to temperature.

## Material and methods

### Recruitment

Artificial substrata were deployed for recruitment studies at three stations: (i) the GSO dock, (ii) the Rhode Island Department of Environmental Management pier at Fort Wetherill (FW) State Park, and (iii) the T-wharf dock on southern Prudence Island (SP) (Figure 1). At each station, four grey, $10 \times 10$ $cm^2$, roughened polyvinyl chloride (PVC) panels were suspended horizontally, facing the seabed, to capture larval recruits. The panels were hung from piers, because there were no floating docks at the sites; their depth, averaging ~1 m below the water surface, varied according to tide. Each week from May to October 2005, the panels were removed and replaced with clean panels. National Estuarine Research Reserve personnel maintained SP panels and transported them to URI for analysis. Sessile animals were identified and counted under a dissecting microscope in the laboratory. A two-way analysis of variance (ANOVA) compared *Didemnum* recruitment among the sites.

### Environmental data

A YSI 6920 sonde at the GSO and a YSI 6600 sonde at FW were used to measure water temperature, salinity, pH, and dissolved oxygen (%DO) data. The sondes, suspended ~1 m below the water surface, collected data every 15 min. Each week, a surface water sample was collected for analysis of chlorophyll *a* (Chl *a*) and nutrients ($NO_2$+$NO_3$). To analyse for Chl *a* concentration,

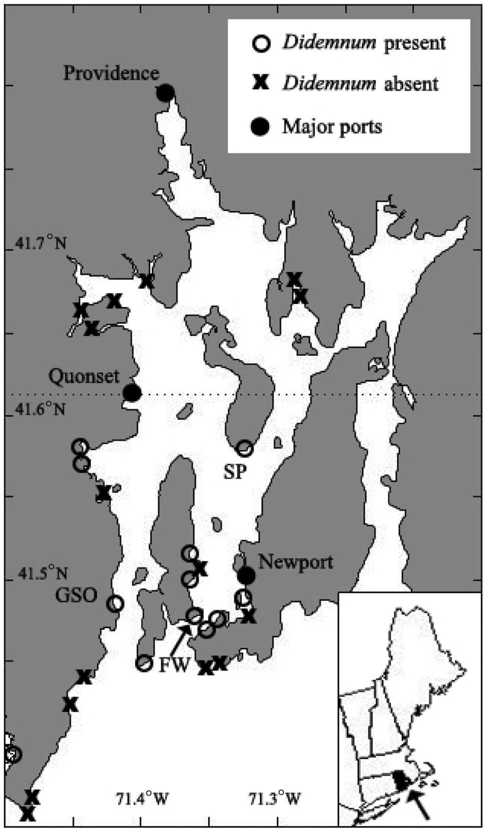


**Figure 1.** Narragansett Bay, with major ports and sites where *Didemnum* is present. The inset shows the location of the bay, in the context of the New England region of the US, as indicated by the arrow. The recruitment study sites (GSO, FW, and SP) are designated as sites where the tunicate is present. The additional sites indicated are those where the tunicate was found during the intertidal survey. The dotted line indicates the known northern boundary of *Didemnum* presence in the bay. The average salinity of the sites north of the dotted line is 22 psu and south of it, 28.9 psu.

10 ml of each water sample were vacuum-filtered on a 25 mm GF/F filter and treated with a 1% $MgCO_3$ buffer. The samples were then kept at 20°C until they were extracted with 90% acetone and analysed on a Turner Designs fluorometer (Oviatt and Hindle, 1994). Water samples were filtered by pressure through 0.4 μm 47 mm polycarbonate filters and analysed on an Automated Analyser for nutrients (Oviatt and Hindle, 1994). At SP, a YSI sonde, also fixed at 1 m and maintained by the National Estuarine Research Reserve site on Prudence Island, collected temperature, salinity, pH, Chl *a*, and dissolved oxygen data every 15 min. Monthly nutrient data were also collected from that site. We used multiple regression analysis to compare weekly averages of all factors except $NO_2+NO_3$, which was compared as a monthly average, with *Didemnum* recruitment for each site.

## Scuba survey

Scuba divers used an underwater camera and photoquadrat frame (Preskitt *et al.*, 2004) to photograph transects at each of GSO and FW, with five 0.2 $m^2$ quadrats per transect, in August and September 2005. Because of the remote location of the Prudence Island T-wharf and the limited time of the volunteer divers, we did not survey the SP pier. Two replicate transects at each site were 10.4 m long, and the divers sampled quadrats every 2.08 m (standard distance between every other GSO piling) at 3.7 m depth. An image-analysis program was used to measure percentage cover of the tunicate in the photographs. Total area of the image, cropped to the quadrat dimensions, was calculated by the program. Percentage cover of the tunicate was calculated for each quadrat as (area of tunicate/total area) × 100. The percentage cover between months and between sites was compared with a Student's *t*-test.

## Intertidal survey

We chose marina, fishing access, and boat ramp sites (24 total) along the coastline of Narragansett Bay and Rhode Island Sound to survey for the presence of *Didemnum* from October to November 2005. At each site, we recorded the presence or absence of *Didemnum*, Global Positioning System (GPS) coordinates, salinity, and substratum information. We surveyed just above and below the waterline at each site between an hour before and an hour after low tide. To calculate the distance from the three main ports in Narragansett Bay (Providence, Quonset Point, and Newport), we used GPS data collected on-site with a Garmin GPSMAP 76S. To count potential vectors of *Didemnum* spread, we used high resolution aerial photography on Google Earth, taken within the past 3 years by MASS GIS, for boat and mooring counts (Commonwealth of Massachusetts, EOEA). We also used a Student's *t*-test (assuming unequal variance) to compare salinity, distance from ports, and number of both recreational and commercial boats between sites where *Didemnum* was present and where it was absent. Logistic regression demonstrated the significance of each of the variables in predicting presence or absence of *Didemnum*.

# Results

## Recruitment and environmental data

The GSO site had the largest number of *Didemnum* recruits throughout the study relative to the other two sites (Figure 2). An ANOVA revealed a statistically significant difference among all three sites ($p < 0.01$). The environmental variables revealing significant correlation with *Didemnum* recruitment in a multiple regression analysis were temperature ($r = 0.57$ at the GSO), salinity ($r = 0.68$ and $r = 0.60$ at FW and GSO, respectively), and

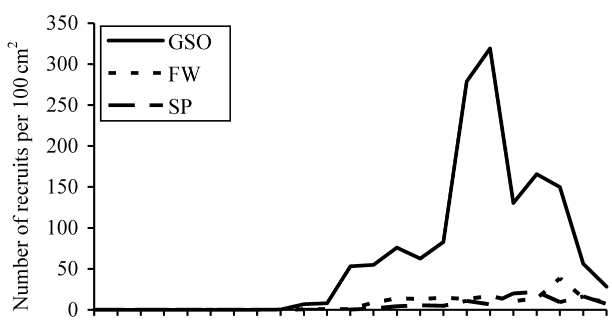


**Figure 2.** Weekly average recruitment of *Didemnum* in 2005. GSO had the highest abundance of larval recruits and numbers peaked on 15 September. FW had a peak on 6 October and Prudence Island a peak on 29 September. Both FW and Prudence Island had significantly fewer recruits than did the GSO. The dates of the study extended from 19 May to 21 October.

**Table 1.** Multiple linear regression coefficients for the environmental variables and *Didemnum* recruitment at each site.

| Parameter | GSO | FW | Prudence Island |
|---|---|---|---|
| Temperature (°C) | (+) 0.57 (<0.01)* | (+) 0.49 (0.02) | (+) 0.29 (0.20) |
| Salinity (psu) | (+) 0.60 (<0.01)* | (+) 0.68 (<0.01)* | (+) 0.19 (0.41) |
| Chl *a* ($\mu g\ l^{-1}$) | 0.00 (0.99) | (+) 0.50 (<0.01)* | (+) 0.35 (0.12) |
| DO (% saturation) | (−) 0.39 (0.11) | (−) 0.33 (0.20) | (+) 0.10 (0.66) |
| pH | (−) 0.67 (<0.01)* | (+) 0.28 (0.20) | (+) 0.57 (<0.01)* |
| $NO_3+NO_2$ ($\mu M$) | (+) 0.18 (0.41) | (+) 0.11 (0.63) | (+) 0.28 (0.64) |

Values of *p* are in parentheses; significant values are marked with an asterisk.

Chl *a* ($r = 0.50$ at FW; Table 1). The temperature at which the tunicate first appeared at each site was 18.4°C at FW, 19.7°C at GSO, and 22.5°C at SP. At the GSO, there was a peak in *Didemnum* recruitment concurrent with the peak of surface water temperature (Figure 3). There was also a concurrent peak in *Didemnum* recruitment with salinity at FW (Figure 4). There was a significant correlation between *Didemnum* and pH at two of the sites; however, it was negative at the GSO ($r = 0.67$) and positive at SP ($r = 0.57$). Problems with the pH probes in these sondes resulted in an incomplete dataset for each station, and it is not clear if these correlations would truly be significant if the missing data were present. There was no significant correlation between either percentage DO or $NO_2+NO_3$ and recruitment.

### Scuba survey

The scuba transects demonstrated a greater percentage cover of *Didemnum* at the GSO site (Figure 5). A *t*-test indicated that there was a significant difference in percentage cover of the tunicate between the sites ($p < 0.05$). There was no difference between months at each station.

### Intertidal survey

Of the 24 sites surveyed, nine demonstrated presence of the tunicate (Figure 1). A logistic regression analysis indicated that the tunicate was more likely to be found near sites with large numbers of recreational and commercial boats, moorings, and docks ($p < 0.05$). None of the sites north of Quonset Point had the tunicate present. A *t*-test revealed significant differences in number of boats and moorings ($p < 0.01$) and distance from Newport Harbor ($p < 0.05$) between invaded sites and non-invaded sites.

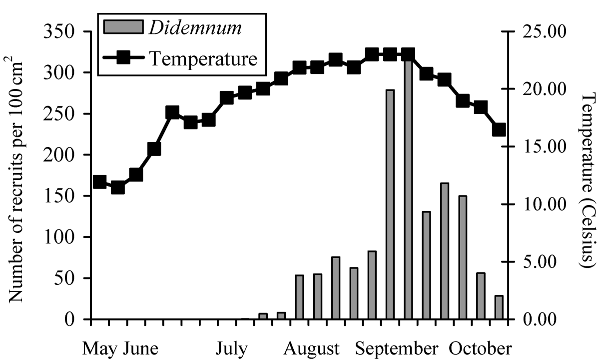


**Figure 3.** Temperature and recruitment of *Didemnum* at GSO. Peak recruitment was simultaneous with the peak in summer temperature.

## Discussion

There were significant differences in recruitment between the three sites in Narragansett Bay, the GSO site having the highest average *Didemnum* recruitment. This site also had a large, established population of the tunicate, as suggested by the scuba transect data. We assumed that the high percentage cover of adult colonies resulted in subsequently high rates of recruitment. As lecithotrophic didemnid larvae do not travel far in the plankton (Olson, 1985; Marshall and Keough, 2003), we speculated that if there were enough suitable substratum, the larvae would settle nearby, creating more colonies. However, the abundances of adult populations may not have accurately reflected abundances of larval recruitment, because *Didemnum* uses asexual budding in addition to larval recruitment to create more colonies (Bullard *et al.*, 2007b). Moreover, currents in some areas may have been faster, carrying larvae farther from adult colonies.

We suggest several characteristics that are likely to explain the success of *Didemnum* colonization at the GSO site. The GSO dock orientation, perpendicular to prevailing north–south tidal flow, increased the potential for larvae to settle on the substratum, because larvae will move with the prevailing current (Olson, 1985). Furthermore, there was an abundance of available substratum at the GSO, including plastic pipes, shell fragments, mussel beds, and wooden dock pilings, which the tunicate could colonize readily. Finally, there was little competition with macroalgae in shaded areas of the dock, where *Didemnum* thrived.

The site has also been invaded previously by two other colonial tunicates, *Botrylloides violaceous* and *Botryllus schlosseri*. Auker (2006) determined that the GSO site had more abundant *B. violaceous* and *B. schlosseri* recruits than either the FW or the SP site in 2005. These results appear to support the invasion–meltdown theory, which states that previous invaders may facilitate further invasions by other species (Simberloff and Von Holle, 1999).

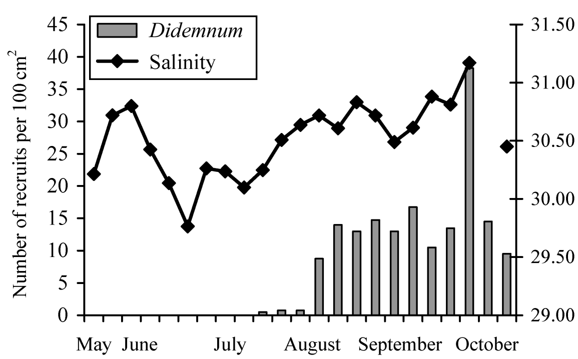


**Figure 4.** Salinity and recruitment of *Didemnum* at FW.

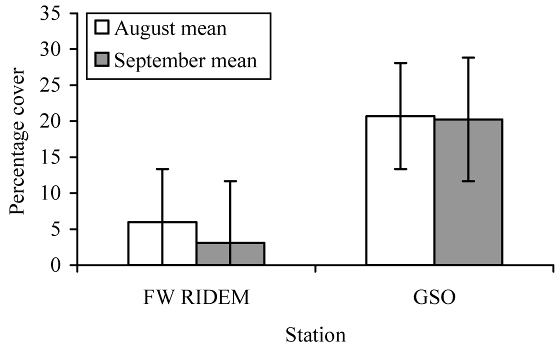


**Figure 5.** Mean percentage cover of *Didemnum*. Cover at FW RIDEM pier was significantly less than at the GSO dock ($p < 0.05$).

FW had significantly lower *Didemnum* abundance in both recruitment and percentage cover than the GSO. The recruitment panels at FW were hung next to a rock wall with abundant macroalgae. The predominant substratum on the seabed was silt, and there was little hard surface in the area on which larvae could settle. However, a few abandoned plastic-coated-wire lobster traps served as suitable habitat and were covered with the tunicate. Although no dives were conducted on SP, the tunicate did reveal a significant increase in percentage cover on panels near the end of the sampling period (Auker, 2006). However, because only two transects were conducted at each site per month for two months, the addition of dive surveys at each site is necessary to determine better the adult abundance of *Didemnum*. Overall, the data collected for this study suggest that the adult population at the GSO is greater than that at FW and may result in greater propagule pressure, resulting in further recruitment.

Salinity and temperature were the most important environmental factors influencing the tunicate's distribution and recruitment, respectively. In Narragansett Bay, the salinity ranged from 28.5 to 31.3 psu at all three recruitment sites. Salinity had a significant positive correlation to recruitment in the multiple regression analysis at both GSO and FW. *Didemnum* was not observed north of Quonset Point in the intertidal survey, most likely because the bay north of that point had, at times, lower salinities (between 15 and 25 psu) as a consequence of fresh-water input into the estuary (Figure 1). Ascidian zygotes and larvae are generally unable to withstand salinities below 20 psu (Millar, 1971). However, as we examined only the surface water for *Didemnum*, our data did not address deeper waters in the upper bay, which have higher salinity, and therefore may hold populations of *Didemnum*.

Temperature, although less critical than salinity in determining distribution of *Didemnum*, is a factor in controlling marine invertebrate sexual reproduction (Millar, 1971). *Didemnum* peaked in recruitment at the GSO at the same time as summer water temperatures peaked, suggesting a link between temperature and recruitment (Valentine *et al.*, 2007a). However, temperatures on Georges Bank range from 4°C to 15°C annually, and *Didemnum* thrives there. The tunicate population on Georges Bank does not exhibit the same growth cycles as in shallow-water systems (Valentine *et al.*, 2007a), suggesting that *Didemnum* is highly adaptive and able to thrive in wider ranges of physical factors, including temperature, than endemic species, a trait shared by other non-native tunicates (Lambert and Lambert, 2003).

Timing of the *Didemnum* peak recruitment in Narragansett Bay was later than in other studies. Osman and Whitlatch (2007) found that recruitment peaked in August of 2001 and 2003, and in June 2002 at an open coast site in Long Island Sound. The temperature peak there was also earlier than in Narragansett Bay; daily temperature peaked in July and August (23–25.6°C) during their 2005/2006 study. Valentine *et al.* (2007a) surmised that sexual reproduction takes place simultaneously with the warmest water temperatures in shallow systems. Our GSO data and those of Osman and Whitlatch (2007) support that conclusion.

*Didemnum* recruitment did not correlate with the other factors we measured, with a few exceptions. For example, at FW there was some correlation between recruitment and Chl *a* concentration. We initially assumed that Chl *a* was an indicator of a potential food source for filter-feeders in the community, but *Didemnum*, like other ascidians, may feed on particles other than phytoplankton (Petersen, 2007). Additionally, we observed a correlation between recruitment and pH, although it was positive at SP and negative at GSO. Those stations' sondes each had pH probe failures, resulting in missing data, so the data could not be considered reliable. However, there was a complete set of pH data for FW, and there was no significant correlation between pH and recruitment there.

Potential vectors of *Didemnum* spread generally include aquaculture and hull fouling (Coutts and Forrest, 2007; Dijkstra *et al.*, 2007). Our study suggests that, because of the significant correlation between *Didemnum* presence and high boat and mooring counts, hull fouling could be a factor in its spread throughout Narragansett Bay. The bay is a major area of commercial and recreational access for ships and sea traffic, and the East Passage (the channel between Conanicut and Aquidneck Islands) is the only shipping channel in the bay (Ely, 2002). Most of the sites with *Didemnum* were in the East Passage and close to Newport Harbor, a large commercial port and the first site of discovery of the tunicate in the bay (Pederson *et al.*, 2001). There were also areas of unexpected *Didemnum* colonization in the East Passage, including Taylor Point on Conanicut Island, an area without marina access or boat ramps, but located near large, anchored commercial tankers.

Because Narragansett Bay is an economic base for commercial fishing and shellfishing (RIDEM, 1998; Ely, 2002), continued spread of this tunicate throughout the bay could have potentially serious economic consequences. However, management of the invasion of this tunicate has been restricted to small-scale attempts in locations such as New England (L. Harris, pers. comm.) and New Zealand (on *Didemnum vexillum*; Coutts and Forrest, 2007). Coutts and Forrest (2007) studied ways to eradicate the tunicate, and found that many of the techniques that they used to kill the tunicate are effective on small scales, but cannot completely eradicate it from a region. They noted that "baseline knowledge and an effective surveillance regime" are the first requirements for eradication success (Coutts and Forrest, 2007). Now that we have an initial sense of both *Didemnum* distribution in Narragansett Bay and the factors that influence its success, future monitoring programmes can be developed. From these programmes, perhaps a bay-wide management plan can be established.

In conclusion, several factors led to *Didemnum* abundance in Narragansett Bay. We contributed an example of peak *Didemnum* recruitment coinciding with peak summer water temperatures. Moreover, the steady high salinity and ample availability of hard substratum in the lower bay provide a favourable habitat for the tunicate. *Didemnum* often inhabited locations where there were stationary boats, both commercial and recreational, indicating that such vessels may be a vector in the spread of *Didemnum* throughout the bay. Several of these sites, as discovered in the intertidal survey, had an abundance of *Didemnum* colonies. Expanding a recruitment survey to these invaded areas, coupled with measurement of water currents and a clearer understanding of the larval competency period, may further increase our understanding of the larval dispersal and timing of *Didemnum*.

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