

## Variation in the ability of *Didemnum* sp. to invade established communities

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### Abstract

Over the past 30 years southern New England, USA has been invaded by several species of ascidians, including *Botrylloides violaceus*, *Diplosoma listerianum*, *Styela clava*, and *Asciidiella aspersa*. These species have become dominate in coastal embayments and marinas but are usually absent from more open water coastal areas. A colonial ascidian, *Didemnum* sp. has invaded southern New England during the past 10 years and we first observed this species in eastern Long Island Sound in 2000. It has become the dominant at several field sites while remaining in low abundance at others. We conducted an experiment at two places, a protected marina and an open coast site, to examine its ability to compete with the established fouling community. Small colonies of *Didemnum* were transplanted onto panels with communities that varied in age from one to four weeks old and these treatments along with controls with only *Didemnum* were exposed at both sites. In most treatments *Didemnum* became a dominant species in the communities at both sites but it reached higher abundances at the open coast site. Potential causes of the observed differences are predation on other species of ascidians at the open coast site reducing recruitment of these species and competition, lower tolerance for elevated temperatures at the marina site, or other environmental parameters that might affect growth rates.

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### 1. Introduction

Life-history traits have been long thought of as key factors in determining the ability of species to invade new regions and communities (e.g. Elton, 1958; Chaloupka and Domm, 1986; Rejmánek and Richardson, 1996). Presumably, attributes that increase the probability of a species being transported and becoming established in a new region are integral to its invasion potential. For many marine coastal species, constraints influencing their

transport may no longer be the issue. For example, recent research (e.g. Carlton, 1987, 1996, 1999; Ruiz et al., 1997) has documented the wholesale transport of an incredible diversity of species of almost all major life-history types via ship ballast water and hulls, aquaculture, aquaria species, and other human-mediated means. If we assume that most species have the potential to be transported to new regions, then life-history traits facilitating establishment of local, self-sustaining populations are the ones that determine the difference between invasion success and failure. Over the past 20 years we have taken this approach in studying a number of ascidian species that have become established in southern New England,

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USA (e.g. Osman and Whitlatch, 1995b, 2001; Whitlatch and Osman, 2001; Whitlatch et al., 1995; Stachowicz et al., 1999, 2002a,b).

Our studies have concentrated on four species of ascidians that over the past 30 years have invaded eastern Long Island Sound: *Botrylloides violaceus* (Oka, 1927), *Diplosoma listerianum* (Milne-Edwards, 1841), *Styela clava* (Herdman, 1881), and *Ascidella aspersa* (Müller, 1776). We found that these invaders joined a suite of long-term resident ascidians in dominating many harbor areas along the New England coast (Whitlatch and Osman, 2001), but were usually absent from more open coastal sites. In Long Island Sound this resulted from the exclusion of ascidians at more open sites by predators that consumed newly-settled recruit and juvenile life-stages (Osman and Whitlatch, 1995a, 1996, 1998, 2004). All four ascidian species produce lecithotrophic larvae whose short larval lifetimes (minutes to days) can reduce the distance that larvae are transported, facilitate recruitment back into these parental populations, and contribute to the maintenance of dense local populations in harbors and other embayments (Osman and Whitlatch, 1998). We have also found that these species grow rapidly, successfully compete for space, and often dominate the fouling communities. They invade more easily in low-diversity communities in which there are fewer competitors for any newly-opened space (Stachowicz et al., 1999, 2002a). Finally, the invaders are adversely affected by low winter water temperatures with the colonial species *D. listerianum* disappearing from sites when the mean March 1 temperature falls below  $\sim 2^{\circ}\text{C}$  (Stachowicz et al., 2002b; equivalent to mean January through March temperatures below  $\sim 4^{\circ}\text{C}$  [personal observations]).

In the summer of 2001, we first observed a new species, the colonial ascidian *Didemnum* sp. [described as *Didemnum lahillei* by Lambert (2004) and *Didemnum vestum* by Kott (2004)] at some of our study sites. Since then we have found it covering extensive areas of cobble and rocky bottom in eastern Long Island Sound as has been observed in other regions (Valentine et al., 2007-this issue). Our preliminary observations on the distribution of *Didemnum* sp. [henceforth *Didemnum*] suggested a pattern that was very different from that of the other ascidian invaders. We initially discovered *Didemnum* at the mouth of the Mystic River estuary and at several deeper, more open coast sites, not at those harbor and marina sites with high abundances of ascidians for over 30 years. Likewise, we initially observed larval recruits at open coast sites which traditionally had almost no recruitment of ascidians. Coupling these observations with the known deep water distribution of this new invader and

the likelihood that all these sites experienced cooler summer temperatures than shallow sites, we see the three most likely parameters controlling *Didemnum* distributions as: (1) lower tolerance of extreme temperatures reducing its presence at shallow, warmer sites, (2) resistance of new recruits to predation and increased survival of recruits at open coast and deeper sites, or (3) environmental differences in its competitive ability with other members of the fouling community.

Here we report on a study aimed at determining whether *Didemnum* growth and competitive ability varied between a shallow, protected site behind the Avery Point breakwater with ascidian-dominated communities and a site offshore of Pine Island at which ascidians were generally absent. Additional data are also presented regarding the possible contribution of temperature and predators to any observed differences.

## 2. Methods

### 2.1. Background data on recruitment

As part of a long-term monitoring program begun in 1991, we have been measuring larval recruitment and environmental parameters (mostly temperature) at the University of Connecticut's dock facilities at Avery Point, near the mouth of the Poquonnock River estuary ( $41^{\circ}19'15''\text{N}$ ,  $72^{\circ}2'40''\text{W}$ ), Groton, CT, USA. In 2001, this recruitment monitoring program was extended to several other sites in eastern Long Island Sound, including Niantic Bay (2000–2002), the Mystic River, Groton Long Point, Bushy Point, and Pine Island (Fig. 1). At each site we exposed four clean  $100\text{ cm}^2$  PVC panels approximately 1 m above the seafloor with their exposed surface facing the seafloor. Panels were exposed for 1 week, collected, and replaced with new clean panels. Sampling and replacement continued weekly except during winter months when sampling was bi-weekly. All panels were

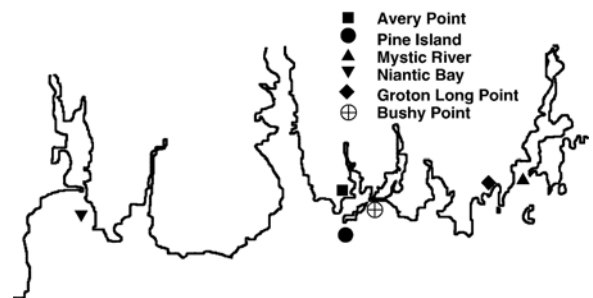


Fig. 1. Map of eastern Long Island Sound showing the locations of study sites. Experiments were conducted at the Avery Point and Pine Island sites which were approximately 1 km apart.

examined using a dissecting microscope and individuals of all macroinvertebrate species, including *Didemnum*, were counted to estimate weekly recruitment rates. Temperatures were recorded hourly at each of these sites using Onset Stowaway temperature probes.

## 2.2. Competition experiment

The main experiment was conducted at two of the field sites. One site was at the Avery Point Breakwater and the other was near Pine Island <1 km from the Breakwater site. The Avery Point site is shallower, protected by a breakwater, has an established fouling community dominated by ascidians, and has few predators. The Pine Island site is open, unprotected, deeper, has few ascidians, and has an abundance of predators (see Osman and Whitlatch, 2004 for a more complete description of these sites). The main experiment was conducted using 100 cm<sup>2</sup> panels that were composed of 25 removable sub-panels each 4 cm<sup>2</sup> (Stachowicz et al., 2002a). In mid-July 2004, a set of 10 panels was exposed at the University of Connecticut's dock at Avery Point. New sets of 10 panels were exposed at weekly intervals so that at the end of one month (18 August 2004) there were 4 sets of 10 panels with communities that were 1, 2, 3, and 4 weeks old. In addition, in early August fragments of *Didemnum* colonies were collected from several sites and pooled. Fragments from this pool were transplanted onto individual 4 cm<sup>2</sup> sub-panels and suspended from the Avery Point dock in order to let the fragments attach and grow.

On 18 August 2004, we brought all the panels into the laboratory. The center sub-panel of each of each panel was removed and replaced by a randomly-chosen sub-panel with a *Didemnum* colony attached to it. In addition, we

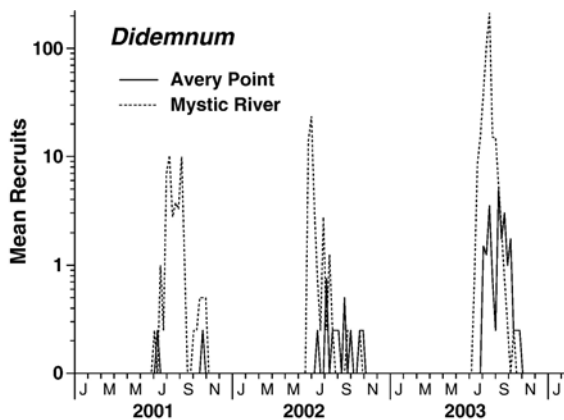


Fig. 2. Temporal variations in weekly recruitment of *Didemnum* sp. at the Avery Point and Mystic River field sites. Single recruits were found at the Groton Long Point and Bushy Point sites in 2003.

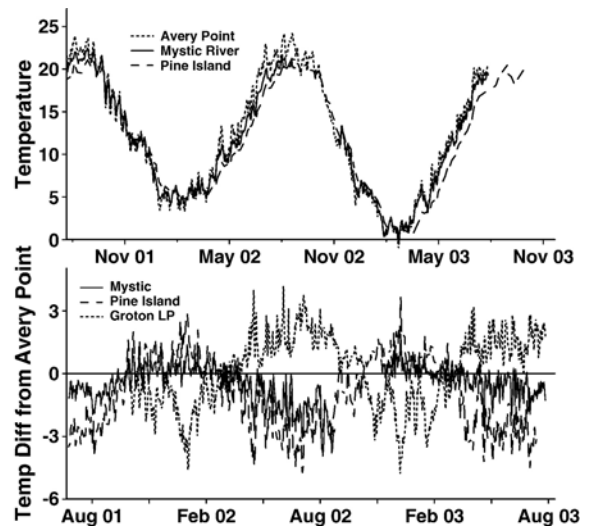


Fig. 3. Seasonal variation in seawater temperature among field sites. The top graph shows the variation at three sites and the bottom graph displays the difference in temperature between the Avery Point site and three other field sites.

replaced center sub-panels on 10 clean, unexposed panels with *Didemnum* colonies. This resulted in 5 treatments each with *Didemnum* in their center: a control and natural communities of 1–4 weeks old. Five panels of each type were chosen randomly and assigned to one of two sites: the Avery Point dock site and the Pine Island site (Fig. 1). Panels at each site were randomly assigned to racks (Osman, 1982) that held them approximately 1 m above the bottom at Pine Island and 1 m below the floating dock at Avery point. Four racks were used at each site. Each rack held 8 panels and we included an additional 7 clean panels with no *Didemnum* to both monitor natural background recruitment and examine community development at each site without the presence of *Didemnum*. We also exposed a set of four additional control panels inside 1-cm<sup>2</sup> Vexar mesh cages at each site to test for any effects of large predators (e.g., fish, crabs, sea stars).

Prior to deployment, all panels were photographed to establish initial conditions for each treatment. Panels were sampled after 1, 2, 4, 5, 7 and 10 weeks of exposure with the 10-week sampling conducted on 25 October 2004 at the end of the summer growing season. At each sampling time, divers photographed the panels *in situ* and the panels were never returned to the laboratory. The digital photographs of the panels were analyzed using NIH Image J software. Two analyses were done for each panel. First, the abundance of *Didemnum* was measured by digitizing the area it covered on each panel. Secondly, a grid of evenly-spaced points at 1 cm intervals was projected onto each digital photograph and the abundance

of each species was estimated as the number of points covering it. The grid was randomly placed for each analysis. If more than one species was under a point, all species were counted. In addition some species extended beyond the panel surface and points touching these were counted as well. Because of the size of the panels (100 cm<sup>2</sup>), each grid point represented both 1 cm<sup>2</sup> of cover and 1% of the panel surface covered.

All data were analyzed using analysis of variance (ANOVA), either as one-way analyses of site differences for each treatment or as two-way analyses of treatment  $\times$  site effects. In the two-way analysis *a posteriori* multiple comparisons of all pairs of means were done using the Tukey–Kramer HSD test. When variances were not homogeneous, data were log-transformed. The experiment involved the repeated analysis of the same experimental panels over time. Our main goal was to characterize the progression over time in differences among treatment in the abundances of the dominant taxa, especially *Didemnum*. To compare overall treat-

ment effects, we used a repeated measures ANOVA using the Residual Maximum Likelihood (REML) method (JMP v. 5.1) with panels as a random variable nested within treatment. We excluded data for the initial sampling period (pre-transplant data) in these analyses.

We also analyzed the differences in the communities that developed on the panels using Multidimensional Scaling (Primer 5.0). Abundance data for each panel were used and Bray–Curtis similarity matrices were calculated for all panels in each sampling period. These matrices were then analyzed using multidimensional scaling (MDS).

### 2.3. Predation experiments

Two types of experiments were conducted to test the effects of predators on *Didemnum*. First, a field experiment was conducted at the Pine Island site that exposed panels with newly settled recruits (<1 week old) to different levels of predation. Three treatments were used (see Osman and Whitlatch, 2004 for a fuller description): (1) artificial

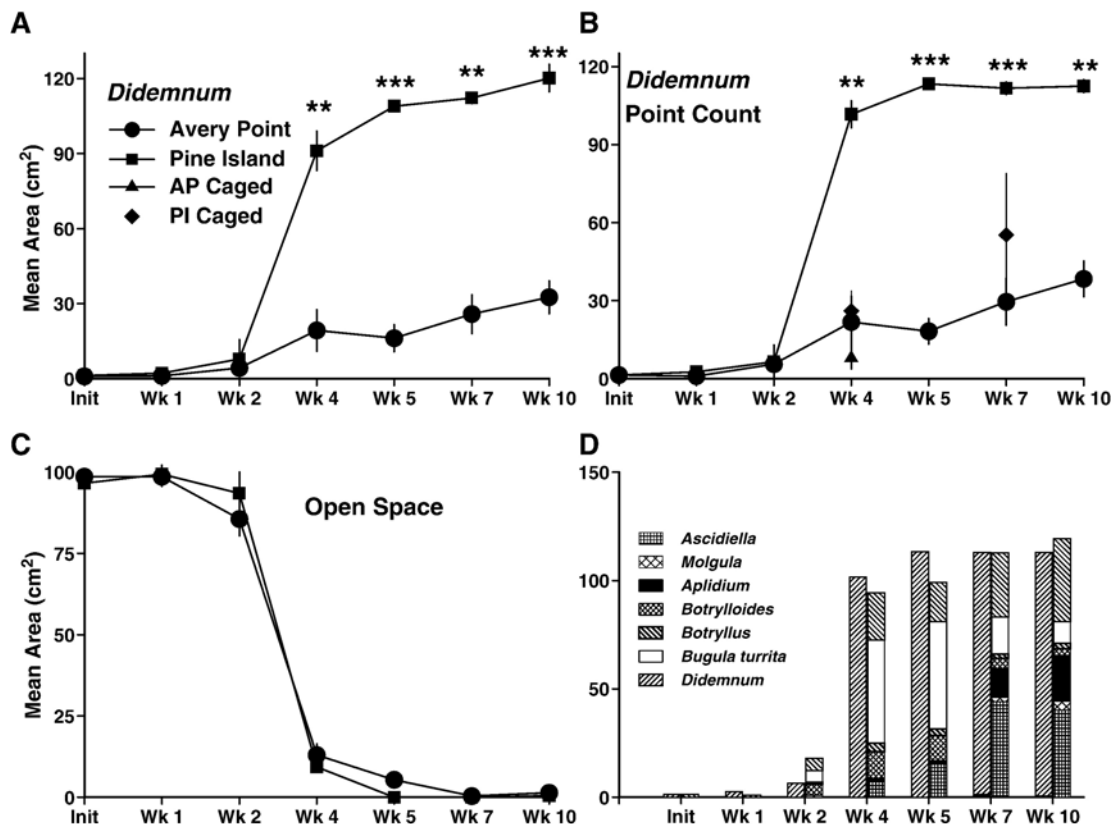


Fig. 4. Differences in *Didemnum* sp. mean abundance on Control and Caged panels at the Avery Point and Pine Island field sites. *Didemnum* sp. abundances (cm<sup>2</sup>) based on (A) area measured by digitizing photographs and (B) by using point counts are both shown. The mean abundance of open space remaining (C) is also contrasted between the two sites. Finally the changes in the mean abundances of dominant species (D) are contrasted between the two sites over the term of the experiment. The left-hand bar in each pair represents the Pine Island site and the right-hand bar is the Avery Point site. For A–C significant differences between sites at each sampling period are represented by: \* —  $p < 0.05$ , \*\* —  $p < 0.01$ , \*\*\* —  $p < 0.001$ .

pilings in contact with the seafloor that exposed attached panels to all predators, (2) panels on caged pilings (1 cm<sup>2</sup> mesh, that excluded all large benthic and fish predators), and (3) panels on racks suspended above the bottom to exclude or reduce access by both large and small benthic predators. Three experiments were conducted in August 2004. The first experiment ran for 3 days and the second and third for 11 days each. Secondly, we conducted two experiments in which paired 7.5 cm<sup>2</sup> panels with known numbers of newly-recruited *Didemnum* were exposed to a single predator, either *Mitrella lunata* or *Anachis* spp. Each experiment was run for 2 d using small chambers enclosing a panel with a single individual gastropod or no gastropod in controls. All chambers were suspended at the Avery Point site, approximately 1 m below the water surface.

### 3. Results

#### 3.1. Background monitoring

Prior to September 2000, we measured weekly recruitment only at the Avery Point site, but *Didem-*

*num* never recruited at this site during 10 years of study. In June 2001, we began monitoring recruitment at Groton Long Point, Bushy Point, and the Mystic River (Fig. 1). In this year we observed *Didemnum* recruits at all sites, but only single recruits during one or two sampling periods were found at all but the Mystic River site. The recruitment season was fairly similar in all three years with the first recruits seen in early to mid-July and the last recruits collected in late October to early November. Mean recruitment in the Mystic River peaked at 10 individuals panel<sup>-1</sup> wk<sup>-1</sup> in August 2001 (Fig. 2) and increased to >23 individuals panel<sup>-1</sup> wk<sup>-1</sup> in July 2002 and >200 panel<sup>-1</sup> wk<sup>-1</sup> in August 2003 while never exceeding 1 individual panel<sup>-1</sup> wk<sup>-1</sup> at the Groton Long Point and Bushy Point sites and 4 individuals panel<sup>-1</sup> wk<sup>-1</sup> at Avery Point.

Although all of the sites showed similar seasonal and annual fluctuations in temperature, there were consistent differences among them. Fig. 3 shows the daily average temperatures at the Pine Island site and daily differences between Avery Point and Pine Island, Mystic River, and Groton Long Point. We have the longest continuous

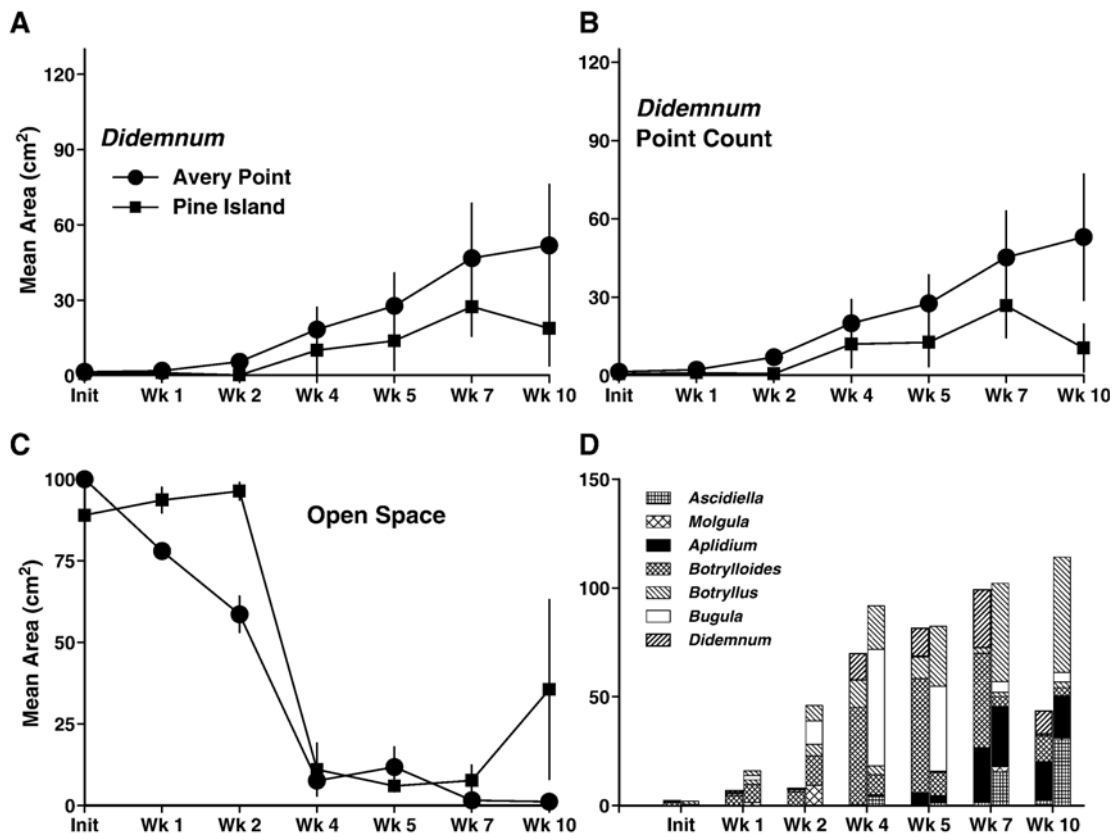


Fig. 5. Differences in *Didemnum* sp. mean abundance on panels that started with 1-week-old communities at the Avery Point and Pine Island field sites. The description of the individual graphs is the same as in Fig. 4.



record for Pine Island. This more open coast and deeper site exhibited fairly consistent summer high temperatures between 20 and 21 °C and winter low temperatures of 2–5 °C in the warmer winters of 2001 and 2002 and 0–1 °C in the colder 2003 and 2004 winters. The Mystic River site was fairly similar but slightly warmer in the summers. Temperatures at both of these sites were less variable than the other two sites and consistently lower than Avery Point in summer and higher in the winter. Groton Long Point was the shallowest and most protected site and it showed the opposite pattern being ~3 °C colder than Avery Point in the winter and ~3 °C higher in the summer.

### 3.2. Competition experiment

The transplanted *Didemnum* colonies had an initial mean size of  $2.38 \pm 0.10 \text{ cm}^2$  and ranged in size from 0.09 to  $2.83 \text{ cm}^2$ . Of the 58 transplanted *Didemnum* colonies, only one (on a Pine Island control panel) disappeared after one week of exposure. An additional 8 colonies failed to grow or actually regressed in size

(Pine Island: one control, three 1-week, one 4-week, Avery Point: one control, two 3-week) over the first 2 weeks. It was assumed that colonies that showed no initial growth were damaged during transplanting and these 8 panels were excluded from all analyses. All but one of these transplants were smaller than the mean and they had an initial size of  $0.24 \pm 0.07 \text{ cm}^2$ .

The most dramatic differences between the Avery Point protected site and the open coast Pine Island site was between the control treatments (Fig. 4). By the end of 4 weeks very little open space remained on either set of panels. At Pine Island the occupied space was completely covered by *Didemnum* and it maintained complete coverage of the panel surface until the end of the experiment. In contrast, at Avery Point *Didemnum* covered approximately  $25 \text{ cm}^2$  of control panel surfaces after 4 weeks and this increased to approximately  $30 \text{ cm}^2$  by the end of the experiment. The remaining space was dominated by *B. violaceus*, *A. aspersa*, *Bugula turrita* (Desor, 1848), and eventually *Aplidium* sp.

No differences in *Didemnum* growth between sites were found for the 1-week treatment (Fig. 5). The

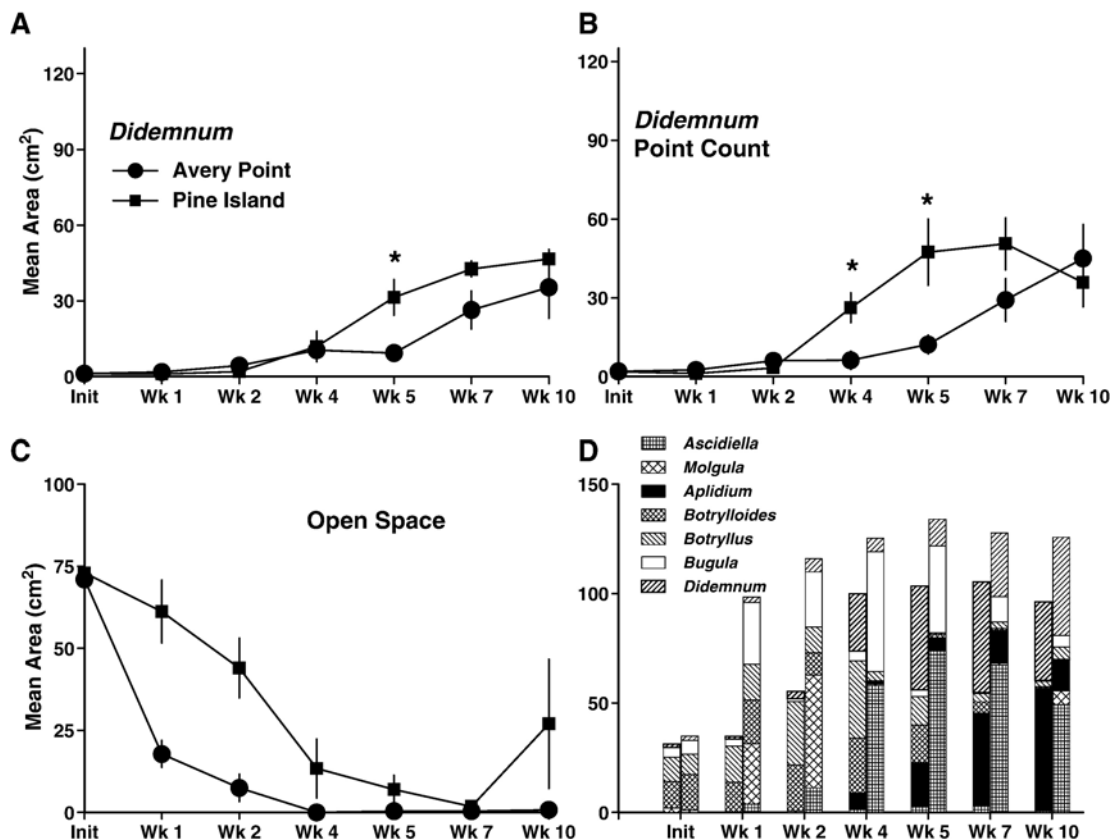


Fig. 6. Differences in *Didemnum* sp. mean abundance on panels that started with 2-week-old communities at the Avery Point and Pine Island field sites. The description of the individual graphs is the same as in Fig. 4.

decline in available space on these panels was very similar to the control panels in that open space declined from 100 cm<sup>2</sup> to <10 cm<sup>2</sup> by week 4. The major difference between the sites was the early loss of almost all solitary ascidians and *B. turruta* at Pine Island, resulting in higher abundances of *B. violaceus*, *Botryllus schlosseri* (Binney, 1870), and *Aplidium* sp. These differences had no apparent effect on *Didemnum* abundance, which steadily increased at both sites.

The panels with 2-week-old communities began with approximately 25% of their surfaces covered by *B. violaceus*, *B. schlosseri*, *Molgula manhattensis* (DeKay, 1848), and *B. turruta* (Fig. 6). Open space declined more rapidly on panels at Avery Point than at Pine Island. As in the 1-week panels, the solitary ascidians *M. manhattensis* and *A. aspersa* as well as the bryozoan *B. turruta* disappeared from the Pine Island panels which contrasted with their dominance at Avery Point. Thus, the colonial ascidians *B. violaceus*, *B. schlosseri*, and later *Aplidium* sp. were dominant at Pine Island. Although the greater availability of open space at Pine Island appears to have allowed *Didem-*

*num* abundance to increase more rapidly there than at Avery Point, we found no significant difference in its abundance between sites by the end of the experiment (Fig. 6).

Panels in the 3-week-old community treatment began with only 25% of their surfaces open and *B. violaceus*, *B. schlosseri*, *B. turruta*, and *M. manhattensis* dominating the remaining space (Fig. 7). The apparent loss or lack of growth of solitary ascidians and *B. turruta* at Pine Island resulted in open space declining more slowly at this site than at Avery Point. This may have contributed to the significantly greater increase in *Didemnum* abundance at Pine Island, at least through the first 7 weeks. By week 10 the fouling and abrasion of some Pine Island panels by drift kelp resulted in the loss of some of the fauna, including *Didemnum*, and an increase in open space. As in the previous treatment series, the community at Avery Point was dominated by solitary ascidians and *B. turruta* while colonial ascidians dominated at Pine Island. At Pine Island the early dominants *B. violaceus* and *B. schlosseri* were replaced by *Didemnum* and *Aplidium* sp.

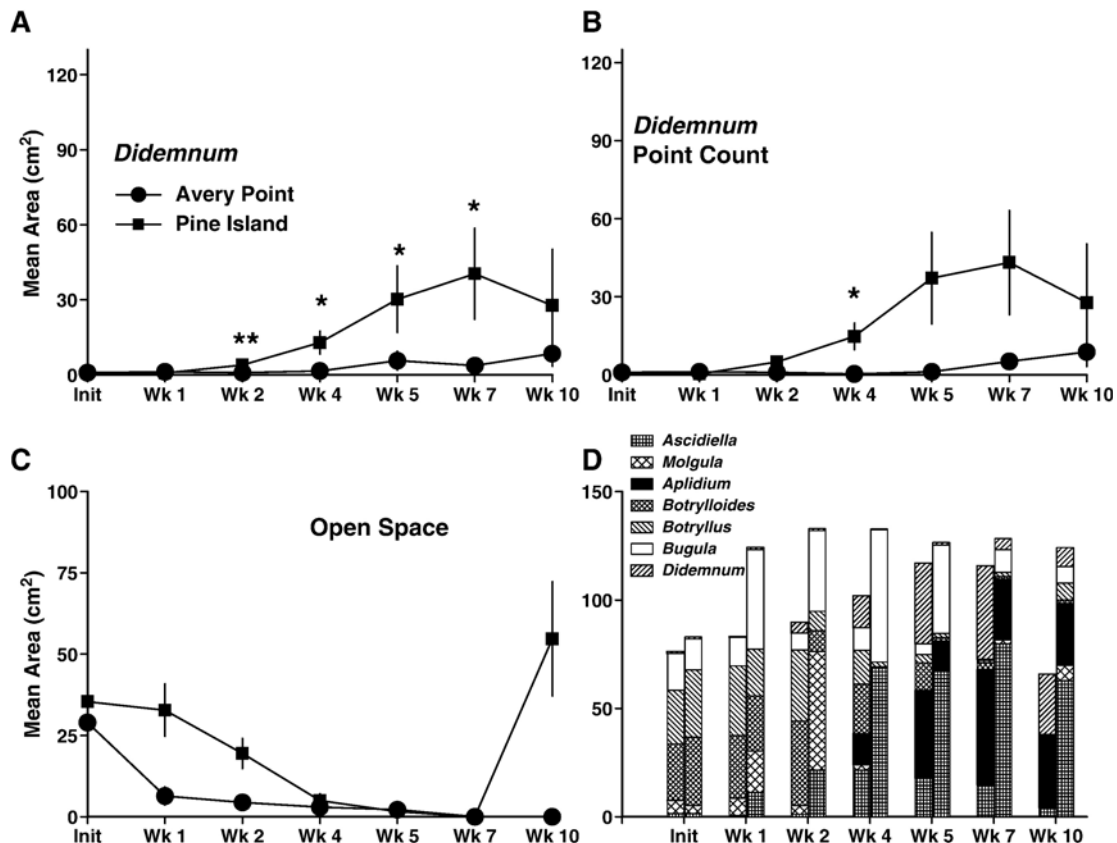


Fig. 7. Differences in *Didemnum* sp. mean abundance on panels that started with 3-week-old communities at the Avery Point and Pine Island field sites. The description of the individual graphs is the same as in Fig. 4.

Finally, the 4-week panels had a well-developed initial community on them and little open space (Fig. 8). The main differences between the sites were the increased abundances of *M. manhattensis* (early) and *B. turrita* (later) at Avery Point which appears to have allowed *Didemnum* to reach significantly greater abundances on panels at Pine Island.

In the repeated measures analyses of abundances over the course of the complete experiment we excluded initial (pre-transplant) data and data from the Blank panels. For some dominant species (*B. schlosseri*, *B. turrita*, *Aplidium* sp., and *M. manhattensis*) the iterations in the REML procedure failed to converge and we excluded these analyses.

The analysis of *Didemnum* yielded significant differences among treatments and between the two sites (Table 1). Overall, *Didemnum* was significantly more abundant at Pine Island and in the control treatment. Analyses of the dominant species on the experimental panels yielded significant differences among treatments, sites (except for encrusting bryozoans) and treatment  $\times$  site interactions. In

general, the abundances of the competing species were higher on the treatments that began with older communities and less open space. Also, the encrusting colonial ascidian, *B. violaceus* was significantly more abundant at Pine Island and the solitary ascidians (especially *A. aspera*) were significantly more abundant at Avery Point. The other colonial ascidians, *B. schlosseri* and *Aplidium* sp. showed similar patterns to *B. violaceus* (Figs. 4–8) but REML analyses of these species failed to converge and were excluded. Finally, no site differences were seen for encrusting bryozoans.

We also analyzed the differences between the Blank and Control panels as a measure of the effects of *Didemnum* on developing communities at the two sites. Both the Blank and Control panels were clean when exposed at the start of the experiment and the only difference was the presence of the transplanted *Didemnum* on the Control panels. Open space remaining on the panels was significantly greater on the Pine Island Blank panels than on the other treatments. *Didemnum* was significantly more abundant on the Controls than Blanks

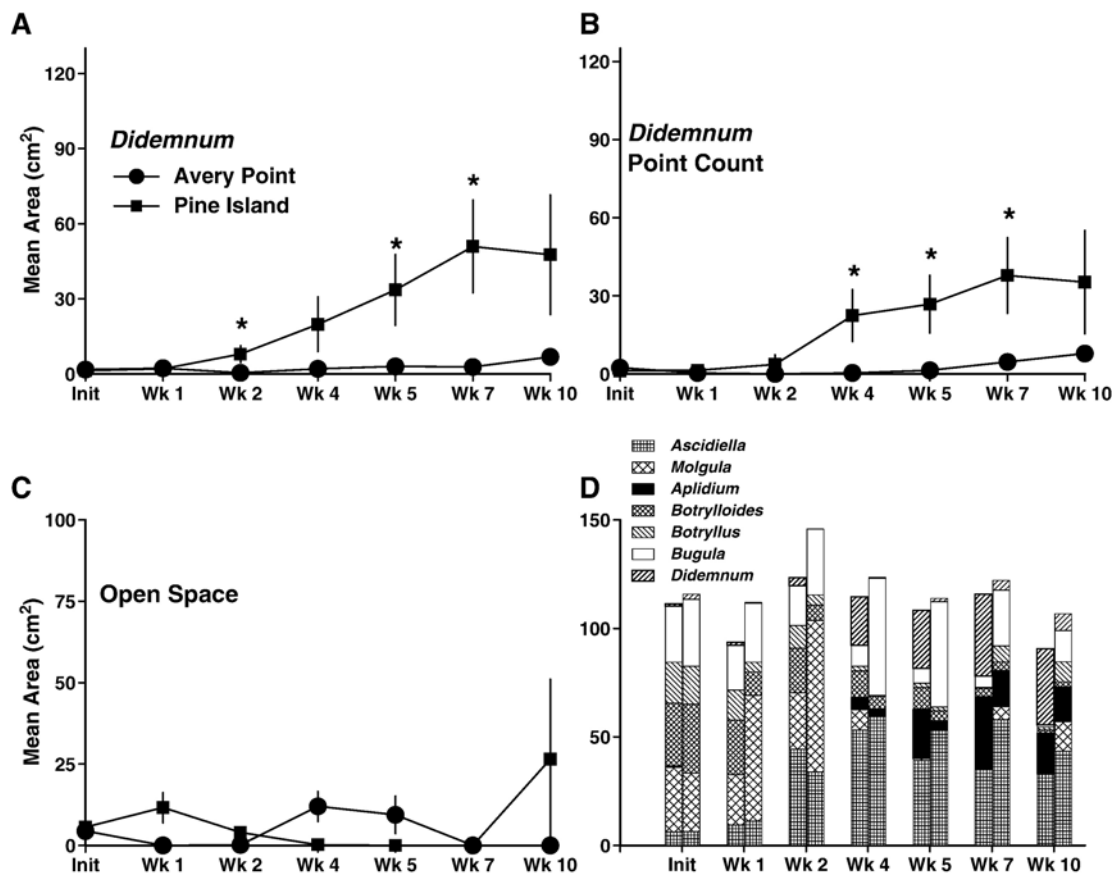


Fig. 8. Differences in *Didemnum* sp. mean abundance on panels that started with 4-week-old communities at the Avery Point and Pine Island field sites. The description of the individual graphs is the same as in Fig. 4.



at both sites with its abundance on Pine Island Controls being significantly greater than on Avery Point Controls. There was almost no recruitment onto either Blank or

Control panels at Pine Island which resulted in significantly higher abundances of all species other than *Didemnum* at Avery Point. In addition, we

Table 1  
Results of repeated-measures ANOVA for *Didemnum* sp. and other species

<i>Didemnum</i> sp.					
Source	DF	SS	MS	F	p
Trtmnt	5	14,189.55	2837.91	4.65	0.0014
Panel[Trtmnt] and Random	52	14,336.37	275.70		
Site	1	10,979.57	10,979.57	18.01	<0.0001
Trtmnt × Site	5	15,805.18	3161.04	5.19	0.0002
Error	248	151,154.10	609.49		

Treatment		Site		Treatment × Site	
Con	A	PI	A	PI Con	A
2 wk	B	AP	B	PI Cage	A
1 wk	B			AP 1 wk	B
Cage	B			PI 2 wk	B
4 wk	B			PI 4 wk	B
3 wk	B			PI 3 wk	B
				AP Con	B
				AP 2 wk	B
				PI 2 wk	B
				AP 3 wk	B
				AP Cage	B
				AP 4 wk	B

<i>Botrylloides violaceus</i>					
Source	DF	SS	MS	F	p
Trtmnt	5	7220.44	1444.09	8.81	<0.0001
Panel[Trtmnt] and Random	52	710.39	13.66		
Site	1	875.81	875.81	5.34	0.0215
Trtmnt × Site	5	4554.22	910.84	5.56	<0.0001
Error	285	46,732.78	163.28		

Treatment		Site		Treatment × Site	
1 wk	A	PI	A	PI 1 wk	A
3 wk	B	AP	B	PI 3 wk	A
4 wk	B			PI 4 wk	A
2 wk	B			PI 2 wk	B
Cage	B			AP 3 wk	B
Con	B			AP 4 wk	B
				AP 2 wk	C
				AP 1 wk	C
				AP Cage	C
				AP Con	C
				PI Con	C
				PI Cage	C

Total solitary ascidians					
Source	DF	SS	MS	F	p
Trtmnt	5	86,681.71	17,336.34	41.30	<0.0001
Panel[Trtmnt] and Random	52	9809.10	188.64		
Site	1	28,819.51	28,819.51	68.78	<0.0001
Trtmnt × Site	5	14,026.88	2805.38	6.68	<0.0001
Error	285	119,642.49	419.80		

Table 1 (continued)

Treatment			Site		Treatment × Site		
4 wk	A		AP	A	AP 4 wk	A	
3 wk		B	PI	B	AP 3 wk	A	B
2 wk		B			AP 2 wk		B
Con					PI 4 wk		B C
Cage		C			AP Con		D
1 wk		C			PI 3 wk		D
					AP Cage		C D
					AP 1 wk		D
					PI 2 wk		D
					PI 1 wk		D
					PI Cage		D
					PI Con		D

Encrusting bryozoans					
Source	DF	SS	MS	F	p
Trtmnt	5	60.50	12.10	2.80	0.0258
Panel[Trtmnt] and Random	52	122.73	2.36		
Site	1	0.33	0.33	0.07	0.7817
Trtmnt × Site	5	63.64	12.73	2.94	0.0130
Error	285	1230.71	4.32		

Treatment			Site		Treatment × Site		
3 wk	A		PI	A	PI 3 wk	A	
Cage	A	B	AP	A	AP Cage	A	B
2 wk	A	B			PI 2 wk	A	B
1 wk	A	B			PI Cage	A	B
Con	A	B			AP 1 wk	A	B
4 wk		B			AP Con	A	B
					AP 3 wk	A	B
					AP 2 wk	A	B
					PI 4 wk	A	B
					PI 1 wk		B
					AP 4 wk		B
					PI Con		B

The a posteriori tests are based on Tukey's HSD (JMP 5.0).

contrasted differences in *Didemnum* abundance among Caged, Control, and Blank panels at both sites for the week 4 sampling period, the only time for which we had samples for all three treatments at both sites. We found significant differences in *Didemnum* abundance among all three treatments at both sites with Controls > Caged > Blank panels. As in previous analyses *Didemnum* abundance was significantly greater at Pine Island than Avery Point.

The results of the MDS analyses of the panel communities at the two sites are shown in Fig. 9. Initial differences among panels prior to the beginning of the experiment were almost exclusively among treatments with no real differences between the two sites. As expected the greatest differences were between those panels with the youngest and oldest communities. By week 4 and continuing to week 7, there was almost complete differentiation of panels between the two sites.

Almost all panels at Avery Point cluster together largely because of the strong dominance of solitary ascidians, *B. turrita*, and *B. neritina* on them. Panels from Pine Island generally fell into three clusters: (1) those panels with a large abundance of open space, (2) those dominated by *Didemnum*, and those largely dominated by *Aplidium* sp. Those with an abundance of open space were the Blank panels and those Control and 1-week panels on which *Didemnum* transplants failed. The panels dominated by *Didemnum* were of varied treatments, but were generally Controls and those in the younger community treatments. Those dominated by *Aplidium* sp. were largely the older community treatments.

### 3.3. Predation experiments

The results of the experiments in which we transplanted newly-recruited *Didemnum* to the Pine

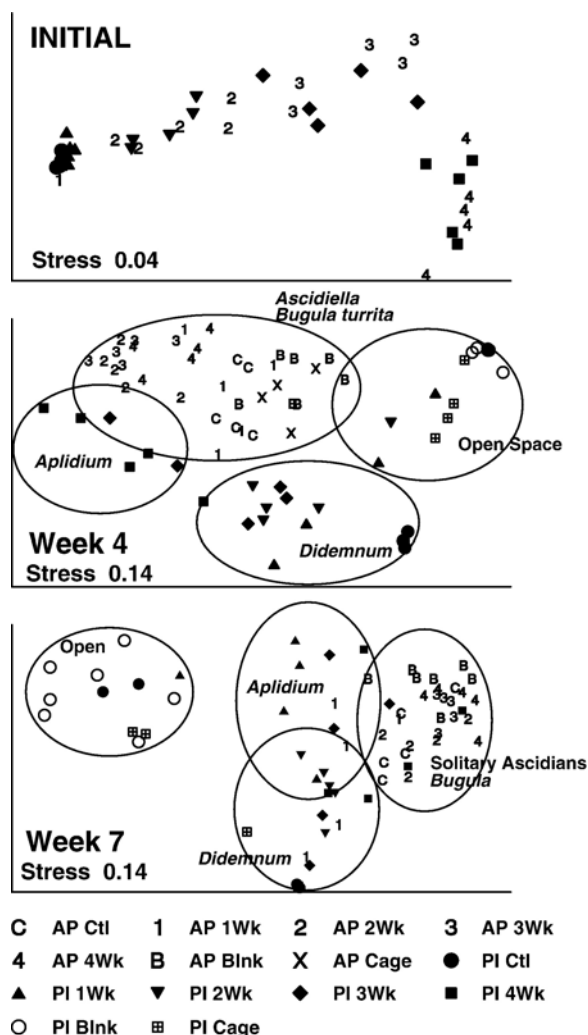


Fig. 9. Results of MDS analyses of Bray–Curtis similarities among all panel treatments at both sites. Data for the Initial (pre-transplant), Week 4, and Week 7 sampling periods are shown. Ellipses were drawn around those panels on which different taxa were dominant.

Island site differed among the 3 experiments (Table 2). In the first experiment conducted over a 3-day period there were no significant differences among the 3 treatments. However, in the 2 experiments run for a longer 11-day period, the caged piling treatment had significantly higher survivorship than the open piling and suspended rack treatments. In third experiment survivorship on open pilings was also significantly higher than on the suspended racks.

Two experiments were conducted using individuals of the two gastropod predators. In the first, both *M. lunata* and *Anachis* spp. were used and the second only *Anachis* spp. was used. Only in the *M. lunata* treatment was survivorship of *Didemnum* recruits significantly lower

than in the Control treatment without any gastropod predator.

#### 4. Discussion

The recent invasion of eastern Long Island Sound by the colonial ascidian *Didemnum* sp. has been rapid with some areas accumulating extremely high abundances similar to those observed on Georges Bank (Valentine et al., 2007-this issue). In trying to understand the process of its invasion and what environmental and ecological factors contribute to its success, we focused on the apparent differences between it and earlier ascidian invaders of this region such as *B. violaceus*, *D. listerianum*, *S. clava*, and *A. aspersa*. Initial observations suggested that *Didemnum* was much more prevalent in deeper water sites while most other ascidians (both native and invaders) were dominant in shallower embayments and marinas. Studies reported here, concentrated on defining possible processes underlying these apparent differences in *Didemnum* abundances between sites and its interaction with other sessile species within the epifaunal community, especially other ascidian species. The questions we addressed were: (1) whether its growth and abundance on experimental panels differed between two representative coastal sites, (2) whether differences in the physical environment (i.e. temperature regime) between the sites could contribute to any differences, (3) whether the age and abundance of competing epifaunal species contributed to any observed site differences in the ability of *Didemnum* to invade the community, and (4) whether predation on *Didemnum* recruits differed from that observed for other ascidians (e.g. Osman and Whitlatch, 2004).

The role of temperature in influencing *Didemnum* recruitment was suggested by previous studies (Stachowicz et al., 2002b) in which warmer winter temperatures resulted in earlier recruitment of several invasive ascidians and ultimately higher summer abundances. In particular, the colonial ascidian *D. listerianum* failed to recruit at all if mean temperatures the preceding winter fell below  $\sim 4^{\circ}\text{C}$ . Subsequent to this study, *D. listerianum* failed to recruit at any of our sites in 2003 and 2004, both years with colder winters with temperatures well below  $4^{\circ}\text{C}$ . We did find some recruitment in 2004 in Niantic Bay, CT near areas exposed to the warm water effluent of Millstone Nuclear Power Plant, Waterford, CT (unpublished data) and after the warmer winter of 2005 we observed fairly high numbers of recruits at Avery Point and Groton Long Point. The distribution of *Didemnum* at deeper sites with colder summer temperatures (and generally warmer winter temperatures) and its absence from shallow sites suggests

Table 2  
Analyses of the survival of *Didemnum* recruits in the predation experiments

Expt	Source	DF	SS	MS	<i>F</i>	p				% Survival
<i>Piling experiments</i>										
1	Trtmnt	2	0.208	0.104	3.170	0.0637	Cage	A		75.55
	Error	20	0.658	0.033			Open	A		75.42
							Rack	A		52.44
2	Trtmnt	2	1.091	0.545	10.886	0.0006	Cage	A		70.07
	Error	21	1.052	0.050			Rack		B	26.09
							Open		B	17.04
3	Trtmnt	2	2.580	1.290	27.003	0.0000	Cage	A		87.64
	Error	21	1.003	0.048			Open		B	36.00
							Rack		C	12.63
<i>Individual predator experiments</i>										
1	Treatment	2	3.749	1.875	36.718	0.0000	Control	A		92.91
	Error	29	1.481	0.051			Anachis	A		72.61
							Mitrella		B	17.41
2	Treatment	1	0.000	0.000	0.005	0.9444	Control	A		95.93
	Error	18	0.226	0.013			Anachis	A		95.42

Data were arcSine Square root transformed for analyses.

a response to temperature that is different from the other invasive ascidians that we have investigated. A laboratory study (McCarthy et al., 2007-this issue) also found that *Didemnum* may be less tolerant of warmer summer water temperatures than two other colonial ascidians, *B. violaceus* and *B. schlosseri*. Finally, from 2001 to 2003 *Didemnum* recruited almost exclusively at the Mystic River site (Fig. 2) with cooler summer temperatures compared to our other monitoring sites (Fig. 3). Although these data suggest that warm summer temperatures (or another environmental parameter correlated with temperature) may play a role in limiting the distribution of *Didemnum*, we cannot rule out other factors.

In the competition experiment, the greatest difference observed was the much higher abundances of *Didemnum* on the Control treatments at Pine Island than at Avery Point (Fig. 4, Table 1). The difference between the two sites resulted from extremely rapid growth of *Didemnum* at Pine Island between weeks 2 and 4 while the growth at Avery Point was significantly less. Except for this time period we found no significant differences in growth (measured as change in area covered) between the two sites. In addition, no significant differences were seen between the sites in available space. Both sites show rapid loss of open space between weeks 2 and 4. This suggests that recruits of other species along with *Didemnum* rapidly occupied the space on Control panels at Avery Point while *Didemnum* alone occupied all the space at Pine Island. Much of this difference can be explained by

the lack of recruitment of other competing species at Pine Island.

The overall results of the experiment showed that the presence of competitors can limit the abundance of *Didemnum* but its continued increase at both sites after week 4 when most space was occupied indicates an ability to overgrow or displace most other species. At Pine Island it became a dominant species in all treatments, usually covering >30% of the available space on the panels (Figs. 5–8). The communities that *Didemnum* interacted with changed with community age (treatments) as well as between the two sites. In the younger 1-week and 2-week old community treatments a significant amount of open space remained at the beginning of the experiment at both sites (Figs. 5 and 6). In addition, the young solitary ascidians generally disappeared from the panels at Pine Island, presumably from the intense predation on these species at this site (Osman and Whitlatch, 2004). Regardless of the differences in communities between the two sites there was no significant differences in *Didemnum* growth. There was also little difference between the sites in the decline of open space suggesting that the competing species and *Didemnum* together grew into and occupied the available space. In the treatments with 3-week and 4-week old communities little open space existed at the beginning of the experiment. In general, solitary and colonial ascidians were dominant in these treatments at both sites. In both treatments *Didemnum* abundances were significantly higher at Pine Island,

suggesting that it displaced the older communities more rapidly at this site.

Together the results from all the treatments demonstrate that *Didemnum* should be able to invade communities in both shallow and deep water, regardless of the age of the community. It was also able to invade equally on those panels dominated by colonial or solitary ascidian species. Its greater dominance on Control (and Caged) panels at Pine Island would seem to result from the absence of recruiting competitors than from any other difference in environmental conditions. The MDS analyses (Fig. 9) coupled with the increase in *Didemnum* in all treatments also support these conclusions. At the beginning of the study all the panels group by treatment. By the end of the study 4 groups of panels were found. Those panels at Pine Island with little or no recruitment and having started with no *Didemnum* (Blanks) or having lost their *Didemnum* transplants were dominated by unoccupied open space. *Didemnum* became extremely dominant on those panels at Pine Island initially with young communities or on Control or Caged panels with no competitors. On the remaining panels *Didemnum* shared dominance with *Aplidium* (mostly at Pine Island) or solitary ascidians and the bryozoans *B. turrita* and *B. neritina*. Although differences between the sites in the recruitment of new competitors into open space appears to have affected *Didemnum* abundance, the differences in the composition of competing communities at the two sites did not.

One phenomenon that we observed in this experiment that may have affected differences in *Didemnum* abundance between the sites was the frequency at which colonies fragmented. The potential of fragmentation by various species of the genus *Didemnum* has been noted (Stoner, 1989), but never linked to environmental conditions. In our experiments significantly more of the transplanted *Didemnum* fragmented at Avery Point than at Pine Island (Table 3). We also observed significantly more *Didemnum* colonies developing holes by the loss of patches of internal zooids as well as suffering late stage or repeated episodes of fragmentation. This development of holes and fragmentation did not result from an overgrowing species dividing the colony. Given the much greater number and diversity of predators at Pine Island (Osman and Whitlatch, 2004) it is unlikely that the differences between sites resulted from predation. In large colonies the loss was at the center or oldest part of the colony. This process opened space within the colony for other species to recruit and grow. At present, it is unclear as to what environmental conditions led to a difference in this fragmentation at the two sites.

Table 3

Nonparametric analysis of the frequency of fragmentation of transplanted colonies of *Didemnum* sp. between the two field sites

Site	Total	Number that fragmented	
Avery Point	25	11	
Pine Island	25	3	
Source	DF-log likelihood	$R^2$ (U)	
Model	1	3.33	0.11
Error	48	26.32	
C. Total	49	29.65	
<i>N</i>	50		

Tests	$\chi^2$	Prob > $\chi^2$
Likelihood ratio	6.653	0.0099
Pearson	6.349	0.0117
Fisher's exact test		
Left		0.0127 AP > PI
Right		0.9982 PI > AP
2-Tail		0.0255 different across sites

Finally, the predation experiments suggest that *Didemnum* recruits are vulnerable to at least some predators. Overall, the piling experiments indicate that one or more larger invertebrate or fish species excluded by cages may prey on *Didemnum* recruits. The greater mortality on suspended racks suggests a possible fish predator. The experiments with individual gastropod species would appear to eliminate *Anachis* spp. as a *Didemnum* recruit predator but not *M. lunata*. In the past *M. lunata* has been abundant at the Pine Island site and along with *Anachis* spp. has been implicated as a major predator on ascidian recruits at this site (Osman and Whitlatch, 2004). The high survival of *Didemnum* recruits in the cage treatment, which was open to both gastropod species, conflicts with the result of the experiments with individual predator species. A prior massive invasion of blue mussels at the site, potentially covering *M. lunata* prey, may have contributed to a decline in *M. lunata* abundances (unpublished observations) and lowered predation by this species. Regardless, the experiments do demonstrate that *Didemnum* recruits are preyed on by one or more predators (but not *Anachis* spp.). The growth and dominance of *Didemnum* at Pine Island also suggests that adult and juvenile colonies of this species, as in other colonial ascidians (Osman and Whitlatch, 2004) are not significantly reduced by predators.

Our results indicate that *Didemnum* is capable of invading epifaunal communities in southern New England in both deep and shallow water habitats. In protected shallow water embayments and marinas it may be inhibited by warmer summer water temperatures, by the greater recruitment and growth of competing species, or



by losses resulting from colony fragmentation. Its success at deeper sites may result from the low recruitment and general absence of these competing species, little loss from fragmentation and, perhaps, reduced recruit predation relative to these competitors. At present it remains clear that *Didemnum* grows extremely well in more open coast and deeper water habitats of southern New England where other invasive ascidians such as *B. violaceus*, *D. listerianum*, *A. aspersa*, and *S. clava* have been excluded, presumably by intense predation on new recruits and young juveniles. Even when confronted by these and other competitors, *Didemnum* shows the ability to overgrow them at a significantly greater rate in these open coast, deep water sites (e.g. Figs. 7 and 8). As such it represents a threat to these habitats and the species residing in them.

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