

Research Article

Observations of recruitment and colonization by tunicates and associated invertebrates using giant one-meter² recruitment plates at Woods Hole, Massachusetts

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Abstract

Large recruitment plates measuring 1 × 1 m were deployed over an 18-month period from September 2013 to March 2015 for the purpose of documenting recruitment and colonization processes of marine invertebrate species at Woods Hole, Massachusetts. Each side of two plates was subdivided into 16 subareas (25 × 25 cm), and an observational strategy was developed whereby, at approximately two-week intervals, a different subarea was cleaned. Using this approach, we were able to photographically document species recruitment and growth interactions. Water temperature records from the site show that steady warming and cooling between 3 and 20° C changed at a mean rate of 0.2 ° C d⁻¹. However, temperature changes during the coolest and warmest parts of the temperature cycle were highly variable. In 2014, between the first and last occurrence of 0° C, temperatures were ≤0° C 15 percent of the time, but in 2015 temperatures were ≤0° C 93 percent of the time. In 2014, between the first and last occurrence of 21° C, temperatures were ≥21° C 88 percent of the time, and this warm period correlated with the disappearance of the hydroid *Ectopleura crocea*, the solitary tunicates *Asciidiella aspersa* and *Ciona intestinalis*, and the 2013 generation of *Botrylloides violaceus*. In Woods Hole, large plates provided enough space to accommodate both fast- and slow-colonizing species, resulting in the establishment of a diverse assemblage that was observed over a long time period. The most successful colonizing species had relatively long reproductive and recruitment periods, grew rapidly, repelled settlement onto their surfaces by larvae of any species, defended themselves against overgrowth by any species, overwintered, and lived a long time. Of the three dominant species observed in this study, the colonial tunicates *Didemnum vexillum* and *Botrylloides violaceus* had these qualities; the encrusting colonial bryozoan *Schizoporella unicornis* had all but one, it grew more slowly than the others. Barnacles constituted the only biological substrate that was effectively colonized by other species, both by larval recruitment and overgrowth. In Woods Hole, after a substrate had become fully colonized, there was very little opportunity for new recruitment or colony growth until new substrate opened after the death of colonies and individuals and the disappearance of biogenic structures such as amphipod tubes. An understanding of colonization processes utilized by invasive species allows prediction of their potential effects on ecosystems in areas where they are not yet present.

Key words: *Didemnum vexillum*, *Botrylloides violaceus*, *Schizoporella unicornis*, *Ectopleura crocea*, colonial tunicates, solitary tunicates, invasive species

Introduction

Previous experiments using small-size substrates at the study site in Woods Hole were directed toward studying the invasive colonial ascidian *Didemnum vexillum* Kott, 2002 to determine the timing of larval recruitment and development, colony response to low water temperatures in winter, and the ability of colony fragments to re-

attach to hard substrates after weeks of suspension in the water column. The experiments used small (12 × 12 cm, 144 cm²), horizontally-oriented, recruitment plates (Valentine et al. 2009) or small plastic containers (base = 38.5 cm²) (Morris and Carman 2012) deployed from a dock. The small recruitment plates were large enough for collecting information on the behavior of small colonies of *D. vexillum*. However, because

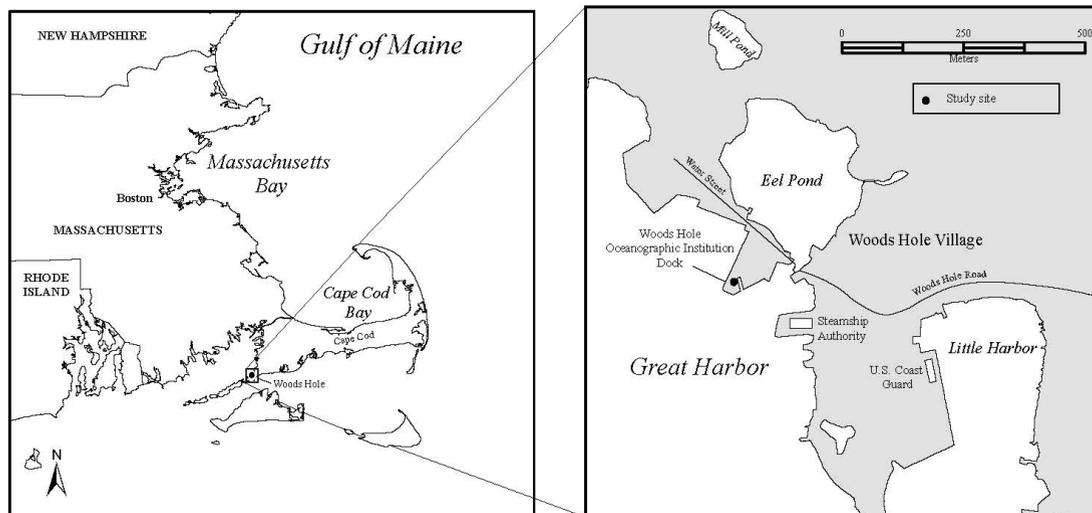


Figure 1. Maps showing the location of the study site at the dock of the Woods Hole Oceanographic Institution in Woods Hole, Massachusetts.

of the very aggressive growth and dominance of this species, they were too small to document the occurrences and interactions of associated species. Osman (1977) reached a similar conclusion on the inadequacy of small recruitment plates in this regard during a long-term recruitment study near Woods Hole. We decided to use 1×1 m recruitment plates in the expectation that a large sampling area would allow us to document the recruitment, longevity, and inter- and intraspecific interactions of a range of species, including *D. vexillum*, that are common constituents of the colonizing assemblage at the study site. We developed a methodology for observing and photographically sampling subareas of the plates that we pursued over two winters and one summer from early September 2013 to late March 2015. Our results are based on qualitative field observations that revealed patterns of species behavior. There is great potential to quantify species interactions using large plates.

Methods

Study site

The study site is located in an open area of the dock at the Woods Hole Oceanographic Institution (41.524° N, 70.672° W) in Great Harbor, Woods Hole, Massachusetts (Figure 1). The opening (~ 3.6 m on a side) is used for testing equipment and is serviced by a stationary crane. Water

depth is approximately 16 m. The study site is bounded on 4 sides by pilings of cement and steel which are colonized by attached epifauna, including mussels, barnacles, bryozoans, tunicates, sponges, hydroids, and anemones, among others. The National Oceanic and Atmospheric Administration (NOAA) maintains a tide gauge station (8447930) and water temperature logger (1.8 m below mean low water) at the dock (NOAA 2016). The mean tidal range is 0.5 m, and maximum tidal currents range up to 1 m s^{-1} . The temperature record used here (hourly mean of observations at six-minute intervals) extended from September 1, 2013 through June 1, 2015, a period of 639 days. The water column at the site is fully mixed to at least 10 m water depth (Valentine et al. 2009).

Configuration and handling of the one-meter² recruitment plates

Recruitment plates were 1×1 m sheets of medium gray PVC with a thickness of 6.35 mm (Figures 2, 3). Two plates were used in this study. The size of the large plates required they be deployed vertically for ease of deployment and retrieval and to minimize resistance to tidal currents at the study site. A hole was drilled into the upper left and lower left corners of the plate. A stainless steel shackle with a swivel was fitted into each hole. A rope from the upper left swivel extended up through the water to the dock, and a

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

Figure 2. Diagram showing the division of the surface of a 1 m² recruitment plate into sixteen subareas, each measuring 25 × 25 cm (625 cm²). During a hypothetical experiment, eight white areas are cleaned sequentially through time, while eight shaded areas remain uncleaned. Subareas are distributed so that each cleaned subarea is bounded by one cleaned and two uncleaned subareas. See the text for an explanation of the observational strategy used in this study.

rope from the lower left swivel extended downward to a heavy weight (14 kg). When deployed, the vertical plate aligned with the tidal current direction. Plate movement in the water was analogous to a flag on a pole responding to wind currents. At retrieval, the plate was raised out of the water by the lifting rope using an overhead crane. The weight was unhooked, and the plate was lowered into a horizontal position and submerged in a plastic tray 5.5 cm deep holding circulating seawater provided by a submersible pump that hung from the dock. The plate was exposed to air for 30 to 45 s during transfer from the water to the tray. Four stainless steel bolts protruded 1.5 cm from near the corners of each side of the plate and acted as legs to prevent the crushing of invertebrates attached to the down-facing side of the plate when it lay horizontally in the tray. The water in the tray covered the fauna attached to the upper surface of the plate. Upon placement in the water-filled tray, a 1 × 1 m wooden frame (subdivided into 16, 25 × 25 cm subareas by wires stretched across the frame) was placed around the plate. Photographs were made of each 25 × 25 cm subarea on both sides of the plate, and of species interactions and recruits. The entire process required approximately one hour to complete for each plate. There is an overhead crane at the study site, but as the plate when fully covered by attached organisms weighed

only ~40 kg without the weight, a smaller lifting device would suffice. Lifting and manipulating the large plate by hand would be difficult.

Sampling strategy and analysis

The four phases of colonization by sessile invertebrates described by Keogh and Downes (1982) are a) larval development and dispersal, b) testing by larvae for suitable substrate on which to settle, c) larval settlement (attachment and metamorphosis), and d) survival as a larva or juvenile until identified by an observer, at which time organisms become recruits. We view the colonization of substrates to be a step-wise process that begins with the four phases described above followed by e) the occupation of a substrate area through growth, f) growth and larval-settlement interactions with other living species, and g) a reproductive phase. In addition, some species “colonize” a substrate by building structures, e.g. tubes. Here, *recruits* are identifiable juvenile organisms and small colonies that have settled and survived during the period between observations. Growth inhibitions called *standoffs* develop when neither of two colonial species can overgrow the other. Species in a standoff commonly develop ridges of tissue at the interface between the neighboring colonies. Another kind of standoff occurs when the growth of a colonial species is inhibited by movements of other organisms; for example the moving appendages of barnacles, bryozoans, and hydroids, the movements of gastropods, and the flow of water by the siphonal currents of solitary tunicates. Some colonial species compete with other species by *overgrowing* and killing them or by *partially overgrowing* them and inhibiting their growth. The term *overwintering* refers to an individual or colony that ceases growth when water temperatures cool and survives to resume growth when temperatures warm. A *regressing* colony shows signs of tissue deterioration that can occur at any time of the year, depending on the life-history of the species.

The large plates afforded the opportunity to observe settlement and recruitment of species, seasonal occurrences and longevity of species, and species interactions through time over a single surface. In order to record the timing of recruitment, the plate surfaces needed to be photographed and cleaned periodically. We used two plates, A and B, and the two sides of each plate were labeled Front and Back for purposes of identification. We used photographic images to qualitatively assess colonization processes. It

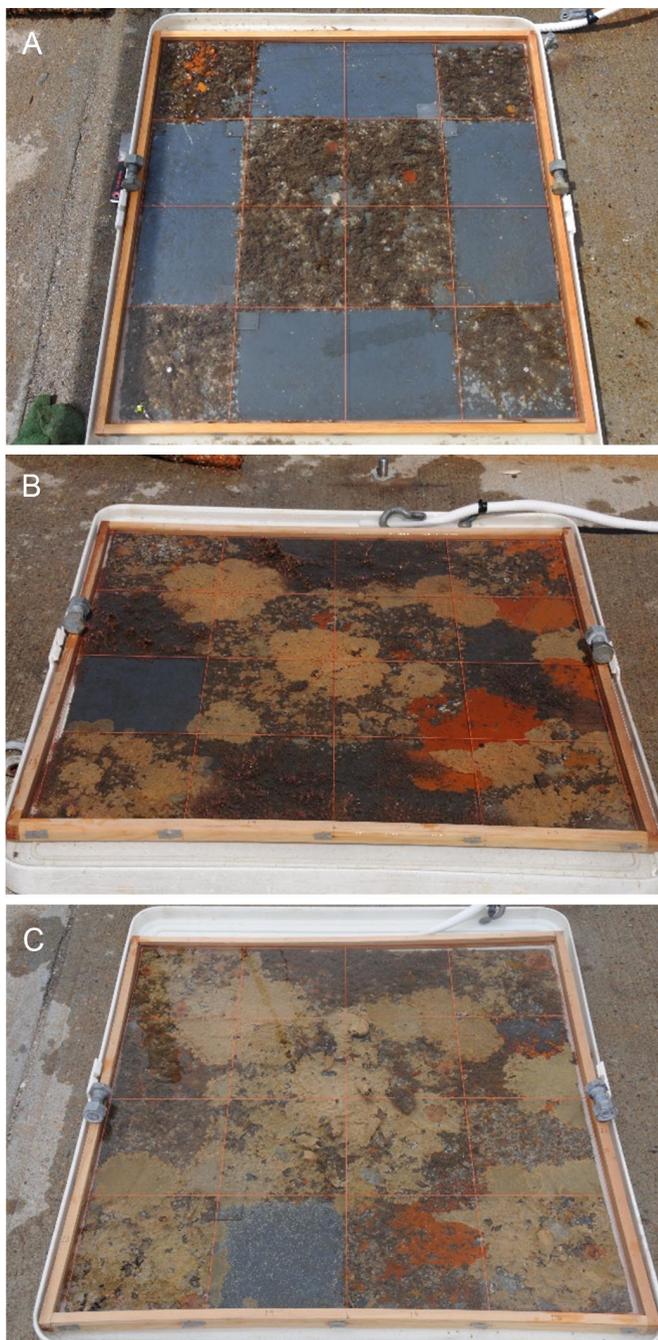


Figure 3. Views of 1 × 1 m PVC recruitment plate AF (plate A, front side). Plate is submerged in holding tray; white hose (upper right) delivers seawater. A grid of orange wires lies on the plate and divides it into sixteen, 25 × 25 cm subareas for photography. See Figure 2 for layout of subareas. Two plates (A and B) were deployed on September 4, 2013 to begin the experiment. Compare subareas of the plate over time to see colonization (photographs by D. Blackwood).

A. Plate AF removed from water on April 25, 2014 after 233 days of submergence, showing eight subareas cleaned on this date. Colonized areas are covered by brown tubes of the amphipod *Jassa marmorata*. Red patches are growing colonies of *Botrylloides violaceus* that overwintered from 2013. White patches in center are overwintering, non-growing colonies of *Didemnum vexillum*.

B. Plate AF removed from water on June 30, 2014, 299 days after initial submergence, 66 days after cleaning 8 subareas (Figure 2A), and 14 days after subarea 9 was cleaned for the second time (Table 1). Image shows growth of red *B. violaceus* and pale orange *D. vexillum* colonies that overwintered from 2013. First recruits of *D. vexillum* were recorded on this date. Dark areas are *Ectopleura crocea* colonies covering parts of the eight cleaned subareas shown in Fig. 2A. Note growth of *D. vexillum* and *B. violaceus* into cleaned subareas.

C. Plate AF removed from water on July 28, 2014, 327 days after initial submergence, 94 days after cleaning 8 subareas (Fig. 2A), and 14 days after subarea 14 was cleaned for the second time. Image shows rapid growth of pale yellow *D. vexillum* and regression of red *B. violaceus* colonies that recruited in 2013. Dark areas are dead polyps of *E. crocea*.

is beyond the scope of this initial study using large recruitment plates to provide a quantitative analysis of species recruitment, growth rates, and competition.

On September 4, 2013, in preparation for observations of recruitment in early 2014, both sides of the two plates (4 m²) were thoroughly cleaned (scraped and scrubbed) and suspended

from the dock at a depth of 1 to 2 m below mean low water (Table 1, observation 0) for the purpose of establishing an assemblage of attached species that would recruit in late fall and overwinter on the plates. By April 2014, both sides of the plates were colonized by attached species. The fauna on one side of each plate (A Back, B Front) was observed until November 5, 2014. The other side

Table 1. Strategy for documenting colonization processes using large recruitment plates at Woods Hole, Massachusetts. Two vertically-oriented 1 × 1 m recruitment plates (A, B) were cleaned and deployed on September 4, 2013, observation 0. Subarea cleaning and observation at approximately two-week intervals began at observation 5. The sampling strategy for one side of each plate (A Front, B Back) is shown below. See the text for a full explanation of the sampling strategy. Figure 2 shows the arrangement of cleaned and uncleaned subareas on plates AF and BB. Cleaning of individual subareas ended on November 5, 2014 with subarea 14 on plate BB. Twenty-two observations continued to March 26, 2015 and are plotted on the temperature curve in Figure 4. Abbreviations: *c*, subarea cleaned (shaded); Days, time between observations; Obs., observation number (plates removed from water and photographed).

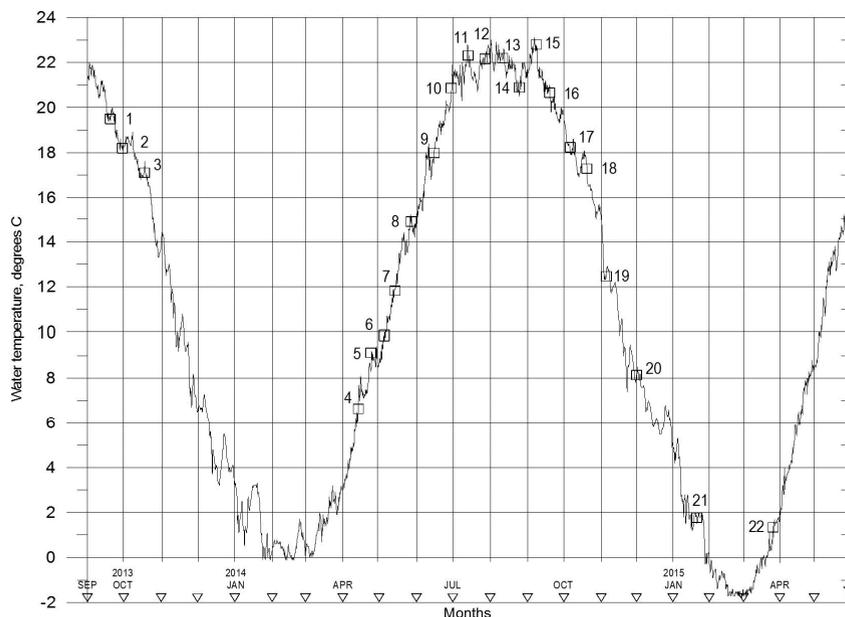
Obs.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Year	2013					2014											2015							
Date	Sep 4	Sep 20	Sep 30	Oct 18	Apr 14	Apr 25	May 5	May 14	May 28	Jun 16	Jun 30	Jul 14	Jul 28	Aug 11	Aug 25	Sep 8	Sep 19	Oct 6	Oct 20	Nov 5	Dec 1	Jan 21	Mar 26	
Days	0	16	10	18	178	11	10	9	14	19	14	14	14	14	14	14	14	11	17	14	16	26	51	64
Plate AF, subarea																								
2	<i>c</i>					<i>c</i>																		
3	<i>c</i>					<i>c</i>	<i>c</i>																	
5	<i>c</i>					<i>c</i>		<i>c</i>																
8	<i>c</i>					<i>c</i>			<i>c</i>															
9	<i>c</i>					<i>c</i>				<i>c</i>														
12	<i>c</i>					<i>c</i>					<i>c</i>													
14	<i>c</i>					<i>c</i>						<i>c</i>												
15	<i>c</i>					<i>c</i>							<i>c</i>											
1	<i>c</i>																							
4	<i>c</i>																							
6	<i>c</i>																							
7	<i>c</i>																							
10	<i>c</i>																							
11	<i>c</i>																							
13	<i>c</i>																							
16	<i>c</i>																							
Plate BB, subarea																								
2	<i>c</i>					<i>c</i>									<i>c</i>									
3	<i>c</i>					<i>c</i>										<i>c</i>								
5	<i>c</i>					<i>c</i>											<i>c</i>							
8	<i>c</i>					<i>c</i>												<i>c</i>						
9	<i>c</i>					<i>c</i>													<i>c</i>					
12	<i>c</i>					<i>c</i>														<i>c</i>				
14	<i>c</i>					<i>c</i>															<i>c</i>			
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16	<i>c</i>																							

of each plate (A Front, B Back) was divided by the removable grid into sixteen, 25 × 25 cm subareas for photographic documentation of colonization processes (Figure 2). Eight subareas (2, 3, 5, 8, 9, 12, 14, 15) on A Front and B Back were selected to record recruitment and growth interactions during the experiment. They were cleaned on April 25, 2014 (Table 1, observation 5). Each of the 8 cleaned subareas was bounded by one cleaned subarea and two uncleaned subareas that were colonized beginning in September 2013. The other 8 subareas (1, 4, 6, 7, 10, 11, 13, 16) on A Front and B Back were not

cleaned. Potential sources of larvae for recruitment beginning in April 2014 included the heavily colonized dock pilings on all sides of the study site, the 8 colonized subareas on one side of each plate (AF, BB) that were not cleaned, and the colonized reverse side of each plate (AB, BF).

At subsequent observations of the plates, beginning with plate AF, one subarea that had been cleaned on April 25, 2014 (observation 5) was re-cleaned (except for subarea 2 that was cleaned on April 25 but not again). For example, subarea 3 was cleaned at observation 5 and re-cleaned on the next visit (observation 6) but not

Figure 4. Water temperature record from September 1, 2013 to June 1, 2015 at the study site located at the dock of the Woods Hole Oceanographic Institution in Woods Hole, MA. Numbered boxes indicate observations when large recruitment plates were retrieved from the water and photographed (see Tables 1, 3). Hourly data is from National Oceanic and Atmospheric station 8447930 located at the study site (NOAA 2016). The record has been smoothed by plotting every 10th hourly data point. Triangle symbols represent the first day of a month.



again (Table 1). After a subarea had been cleaned, it was checked for recruitment on the following visit. As a further example, subarea 9 on plate AF was cleaned at observation 5 and checked for recruitment at observation 6; it was next cleaned at observation 9 and checked for recruitment at observation 10. This strategy allowed recruitment to be observed twice in subarea 9 and colonization to be documented from observation 6 to 9 and from observation 10 to the end of the experiment. A total of 14 subareas (7 on plate AF followed by 7 on plate BB) were cleaned from May 5 to November 5, 2014. On each visit, observations were photographically recorded in specific subareas, as described above, and in other parts of the plates as needed.

After the initial deployment of the plates on September 4, 2013, a total of 22 observations of the recruitment plates were made during the experiment. The plates were inspected twice in September and once in October of 2013, and again on April 14, 2014 to determine the status of overwintering species (Table 1). Fifteen observations were made at approximately 14-day intervals from mid-April to early November 2014. In the description of results that follows, the reader should note that the dates and water temperatures associated with recruitment and colony growth events are those recorded at the end of an observation period. For example, the

first observed recruitment of *D. vexillum* in 2014 was on June 30 when the water temperature was 21.0° C. The first recruit actually settled sometime between June 16 and June 30 when temperatures increased from 18.3 to 21.0° C. It is not possible to more accurately determine the timing of such events without making observations at shorter time intervals.

Results

Water temperature

The annual water temperature cycle from late 2013 to mid-2015 at Woods Hole plotted as an approximately symmetrical curve with small superimposed temperature fluctuations caused by local atmospheric variability (Figure 4). A pattern of slow, seasonal rise and fall of hourly water temperatures characterized most of the year. Water temperatures between 3 and 20° C increased and decreased at a steady pace. Analysis of the one warming period (~April to ~July, 88 d) and the two cooling periods (~Oct to ~Jan, 108 d each) determined that temperatures increased at a mean rate of 0.19° C d⁻¹ and decreased at an almost identical rate of 0.16° C d⁻¹.

Water temperatures were more variable during the coolest and warmest parts of the year (Table 2). During the coolest part of the year in 2014,

Table 2. Timing and variability of water temperature during coolest and warmest periods of the annual temperature cycles in 2014 and 2015. Time periods are determined by the first and last observations of temperature values that define a range. For example, during the development of the cool period in 2014, hourly temperature values $\leq 0.0^\circ\text{C}$ (the defined range) occurred from January 24 to March 6, a total of 42 days, during which 983 out of a possible 1008 hourly observations were recorded. During the time period, water temperature varied considerably, as only 148 (15 percent) of the temperature observations were $\leq 0.0^\circ\text{C}$ (Figure 4). Both maximum and mean temperatures were $>0.0^\circ\text{C}$. Hourly temperature data is from National Oceanic and Atmospheric Administration station 8447030 located at the study site (NOAA 2016). The number of hourly temperature observations is up to 3 percent less than expected due to gaps in the data.

Water temperature range, $^\circ\text{C}$	Time period defined by first and last observations of temperature values that define the range		Hourly temperature observations recorded during the time period			Temperature variability within the time period, $^\circ\text{C}$		
	Dates	Days	Total	Within the temperature range		Min	Max	Mean
				No.	Pct of total			
2014 cool period								
≤ 0	24 Jan – 6 Mar	42	983	148	15	-0.5	1.8	0.4
2014 warm period								
≥ 21	29 Jun – 19 Sep	83	1952	1723	88	20.3	23.3	21.7
≥ 22	2 Jul – 9 Sep	70	1639	683	42	20.3	23.3	21.8
≥ 23	13 Jul – 7 Sep	57	1342	28	2	20.5	23.3	21.9
2015 cool period								
≤ 0	27 Jan – 21 Mar	54	1251	1168	93	-1.8	0.4	-1.0
≤ -1	6 Feb – 10 Mar	33	770	649	84	-1.8	-0.6	-1.4

between the first and last occurrences of 0°C (42 d), temperatures fluctuated between -0.5 and 1.8°C , with a mean of 0.4°C and were $\leq 0^\circ\text{C}$ only 15 percent of the time. In 2015, between the first and last occurrences of 0°C (54 d), temperatures fluctuated between -1.8 and 0.4°C , with a mean of -1.0°C , and were $\leq 0^\circ\text{C}$ 93 percent of the time. Thus, the cool period in 2015 was longer, colder, and its temperatures less variable than in 2014. Temperatures were recorded in only one warm period during the experiment, and variability in that record may not be representative of other years. During the warmest part of 2014, hourly temperatures between the first and last occurrences of 21°C (83 d) were $\geq 21^\circ\text{C}$ 88 percent of the time and averaged 21.7°C . By contrast, temperatures between the first and last occurrences of 23°C (57 d) were $\geq 23^\circ\text{C}$ only 2 percent of the time and averaged 21.9°C . The maximum temperature in the warm period was 23.3°C .

Occurrence and recruitment of major colonizing species

The following species were major constituents of the assemblage that developed on the plates in Woods Hole from September 2013 to March 2015. The presence of settled, recently-metamorphosed recruits was used to determine the recruitment dates and temperatures for the colonial tunicates

D. vexillum and *Botrylloides violaceus* Oka, 1927, and the colonial bryozoan *Schizoporella unicornis* (Johnston, 1874). Barnacle species recruited to the plates throughout the study period. They played a role in colonization of the plates, but they could not be identified to species for this experiment. Early post-settlement recruits were not observed for the colonial hydroid *Ectopleura crocea* (Agassiz, 1862), so its recruitment dates were based on the first occurrence of small colonies. The occurrence of the amphipod *Jassa marmorata* Holmes, 1905 was based on the presence of individuals and their tubes. In the following descriptions, observation dates are followed by temperature values that represent the mean water temperature recorded on that day.

Didemnum vexillum (invasive colonial tunicate)

After recruitment plates were cleaned and submerged on September 4, 2013 (21.7°C), newly-recruited *D. vexillum* colonies were recorded during three observations from September 20 to October 18 (19.7 to 17.2°C), the last observation in 2013 (Table 3). At the next observation of the plates on April 14, 2014 (6.9°C) small *D. vexillum* colonies were present that had recruited in late 2013 and overwintered. They had increased in size since they were last observed in 2013. However, they were not growing, as they had not increased in size by April 25, 2014 (8.9°C), 11 days later.

Table 3. Recruitment and growth of major colonizing species. Record of species' colonization of two 1 × 1 m recruitment plates from September 4, 2013 to March 26, 2015. Observations 1 to 22 are plotted on the temperature curve of Figure 4. Note gap in recruitment of *D. vexillum*, *B. violaceus*, and *S. unicornis* in early 2014. See text for explanation of individual species' recruitment and growth patterns and Table 1 for explanation of observation strategy. Abbreviations in column headings – 1st row: Jm, *Jassa marmorata*; Bar, barnacle species. 2nd row: C, colonies; I, individuals; R, recruits; T, tubes. 3rd row: Chg., change in temperature from last observation, except for the 178-day gap ending on April 14, 2014; 13, time period of observations in years (e.g. 13 = 2013). Abbreviations in body of table – g, colonies growing; g-, colonies regressing; g/g-, some colonies growing, others regressing; ng, colonies not growing; ng/g-, some colonies not growing, others regressing; np¹, colonies not present, died; np², colonies not present and remaining stems presumed dormant; ow/g, colonies overwintered, growth since last observation in 2013; p, present; r, recruitment; r+, peak recruitment.

Obs.	Date	Days	Water temp on date, °C		<i>Didemnum vexillum</i>				<i>Botrylloides violaceus</i>				<i>Schizoporella unicornis</i>				<i>E. crocea</i>		Jm		Bar
			Mean	Chg.	R	R	C	C	R	R	C	C	R	R	C	C	R	C	T	I	R
			13	14	13-15	14-15	13	14	13-14	14-15	13	14-15	13-15	14-15	14	14-15					
0	4 Sep 13	0	21.7																		
1	20 Sep	16	19.7	-2.0	r		g			r		g								r	
2	30 Sep	10	18.2	-1.5	r		g			r		g									
3	18 Oct	18	17.2	-1.0	r		g			r		g							p	r	
4	14 Apr 14	178	6.9				ow/g					ow/g						p	p		
5	25 Apr	11	8.9	+2.0			ng					g						p	p		
6	5 May	10	9.7	+0.8			g					g						p	p	r	
7	14 May	9	12.0	+2.3			g					g						p	p	r	
8	28 May	14	14.8	+2.8			g					g			r	g		p	p		
9	16 Jun	19	18.3	+3.5			g		r	g	g		r	g	g	r	g	p	p		
10	30 Jun	14	21.0	+2.7		r	g	g	r+	g/g-	g		r	g	g	r	g	p	p		
11	14 Jul	14	22.1	+1.1	r+	g	g		r+	g-	g		r	g	g					r	
12	28 Jul	14	22.3	+0.1	r+	g	g		r	g-	g		r	g	g		np ²			r	
13	11 Aug	14	22.0	-0.3	r	g	g		r	g-	g		r	g	g					r	
14	25 Aug	14	20.9	-1.1	r	g/g-	g		r	np ¹	g		r	g	g					r	
15	8 Sep	14	22.5	+1.6	r	g/g-	g		r		g		r	g/g-	g					r	
16	19 Sep	11	20.6	-1.9	r	g/g-	g		r		g		r	g/g-	g					r	
17	6 Oct	17	18.0	-2.6	r	g/g-	g		r		g		r	g/g-	g					r	
18	20 Oct	14	16.8	-1.2	r	g	g		r		g		r	g/g-	g		g			r	
19	5 Nov	16	12.6	-4.2		g/g-	g-		r		g		r	ng/g-	ng/g-		g			r	
20	1 Dec	26	8.2	-4.4		g/g-	g-		r		g		r	ng/g-	g-		g			r	
21	21 Jan 15	51	1.8	-6.4		g/g-	g-				g		r	ng	g-		g	p	p	r	
22	26 Mar	65	1.1	-0.7		ng	ng				ng		r	ng	g-		g	p	p	r	

The non-growing colonies were thin and white and initially were misidentified as *Didemnum albidum* (Verrill, 1871), a native species. The overwintering *D. vexillum* colonies began to grow by May 5 (9.7° C) and gradually increased in thickness and changed color from white to shades of pale orange-yellow.

In 2014, recruitment of *D. vexillum* colonies was observed on June 30 (21.0° C) and continued for sixteen weeks to October 20 (16.8° C). Peak recruitment was observed on July 14 (22.1° C) and July 28 (22.3° C). Colonies were not inspected for larval content. Potential sources of early recruits were *D. vexillum* colonies from 2013 that had overwintered on the recruitment plates and on the adjacent dock pilings. Larvae that settled later in the year could have been produced

by the overwintering colonies just mentioned and by the colonies that recruited early in 2014 to the plates and dock pilings. Thus, by June 30, 2014 two generations of *D. vexillum* colonies occupied the plates, those that recruited in 2013 and those that recruited in 2014 (Table 3). Some of the colonies that overwintered from 2013 and began growing by May 5, 2014 began to regress by August 25 (20.9° C) while others continued to grow until at least January 21, 2015 (1.8° C). The tunics of the regressing colonies were pale white and exhibited dark areas that represented cloacal canals clogged by fecal pellets (Valentine et al. 2007). Colonies that recruited in 2014 had a shorter growing season than those that recruited in 2013 and were smaller. They showed the first signs of regression by November 5 (12.6° C),

Table 4. Recruitment and growth interactions between and within species. Abbreviations in column headings – 3rd row: Aa, *A. aspersa*; Ac, *A. constellatum*; Ag, *A. glabrum*; Bar, barnacle spp; Bs, *B. schlosseri*; Bv, *B. violaceus*; Ci, *C. intestinalis*; Dl, *D. listerianum*; Dv, *D. vexillum*; Ec, *E. crocea*; Jm, *J. marmorata*; Sc, *S. clava*; Su, *S. unicornis*. 4th row: C, colonies; I, individuals; R, recruits; T, tubes. Abbreviations in body of table – nr, no recruitment; ntc, no tubes constructed; og, complete overgrowth; og-, partial overgrowth; r, recruitment; so, standoff; --, data inadequate or no data; ¹ partial overgrowth of overwintering *S. unicornis*; ² recruitment to overwintering *S. unicornis*.

Species	Species providing substrate for overgrowth and recruitment by species listed in the first column																
	Colonial species											Solitary species					
	Dv		Bv		Bs	Ac	Ag	Ec	Dl	Su		Bar	Aa	Ci	Sc	Jm	
	R	C	R	C	C	C	C	C	C	C	R	C	I	I	I	I	T
Dv	C	og	so	og	so	so, og-, og	so	so	og-, og	so	og	so, og ⁻¹	og-, og	og-	og-	og-	og-, og
	R	nr	nr	nr, so	nr	nr	nr	nr	--	nr	nr	nr	r, og-, og	nr	nr	nr	--
Bv	C	--	so	--	so	so	--	so	og-, og	so	og	so, og ⁻¹	og-, og	--	og-	og-	og-, og
	R	nr, so	nr	nr	nr	nr	nr	nr	--	nr	nr	nr	r, og-, og	nr	nr	nr	--
Bs	C	--	so	--	so	--	--	so	--	--	--	--	--	--	--	--	--
Ac	C	--	so	--	--	--	so	so	--	--	--	--	--	--	--	--	--
Ag	C	--	so	--	so	so	so	so	--	--	--	so, og ⁻¹	--	--	--	--	og-, og
Dl	C	--	so	--	so	--	--	--	--	so	--	--	og-, og	--	--	--	--
Su	C	--	so	--	so	--	--	so	--	--	--	so	og-, og	--	--	--	--
	R	--	nr	--	nr	nr	nr	nr	--	nr	--	nr	og-, og	nr	nr	nr	--
Bar	R	--	nr	--	nr	nr	nr	nr	nr	nr	--	nr, r ²	so	r	r	r	nr
Jm	T	--	ntc	--	ntc	--	--	--	--	--	--	--	og-, og	og-	og-	--	--

later than the 2013 colonies. Both generations of colonies ceased growth between January 21 and March 26, 2015 (1.8 to 1.1° C).

Larvae of *D. vexillum* successfully recruited to the plate substrate and to the tests of barnacles (Table 4). They did not recruit to the living tissues of itself and other colonial tunicates, solitary tunicates, and the encrusting colonial bryozoan *S. unicornis*. Growth interactions between recently-settled recruits of *D. vexillum* and *B. violaceus* resulted in standoffs. Colonies of *D. vexillum* were the most aggressive colonizers of the plates in 2014. They successfully overgrew unoccupied plate substrate, recently-settled recruits of *D. vexillum*, *B. violaceus*, and *S. unicornis*, and most, but not all barnacle tests, amphipod tubes, and hydroid stalks (except for some polyps at the ends of the stalks). Growth interactions resulted in standoffs with colonies of itself and other colonial tunicates, including *B. violaceus*, *Aplidium constellatum* (Verrill, 1871), *Aplidium glabrum* (Verrill, 1871), *Botryllus schlosseri* (Pallas, 1766), and *Diplosoma listerianum* (Milne-Edwards, 1841). Growth interactions between *D. vexillum* and *S. unicornis* resulted in standoffs, except that *D. vexillum* colonies partially overgrew the margins of regressive *S. unicornis* colonies. The species also partially overgrew the tunics of solitary

tunicates, including *Ciona intestinalis* (Linnaeus, 1767), *Styela clava* Herdman, 1881, and *Asciidiella aspersa* (Müller, 1776) but was unsuccessful in closing off their active siphons. Growth at the margins of *D. vexillum* colonies sometimes was prevented by the movements on the plates of the filter-feeding gastropod *Crepidula fornicata* (Linnaeus, 1758).

Botrylloides violaceus (invasive colonial tunicate)

After recruitment plates were cleaned on September 4, 2013 (21.7° C), newly-recruited *B. violaceus* colonies were observed from September 20 to October 18 (19.7 to 17.2° C), the last observation in 2013 (Table 3). At the next observation of the plates on April 14, 2014 (6.9° C), small *B. violaceus* colonies were present that had recruited in late 2013. They had increased in size since they were last observed in 2013, and apparently they were growing on April 14, as they had increased in size by April 25 (8.9° C), 11 days later.

In 2014, recruitment of *B. violaceus* colonies was observed on June 16 (18.3° C) and continued for twenty-four weeks to December 1, 2014 (8.2° C). Peak recruitment was observed on June 30 (21.0° C) and July 14 (22.1° C). Colonies were not inspected for larval content. Potential sources of

the early-settled larvae were *B. violaceus* colonies from 2013 that had recruited to plates and to the adjacent dock pilings. Some colonies that overwintered from 2013 began to regress (fall apart) by June 30, 2014 (21.0° C) during peak larval recruitment, and all of the 2013 colonies had disappeared by August 25 (20.9° C). Thus, larvae that settled later in 2014 were produced by colonies that recruited to the plates and dock pilings earlier in 2014. The colonies continued to grow until at least January 21, 2015 (1.8° C), but were not growing and showed no signs of regression when observed on March 26, 2015 (1.1° C).

Larvae of *B. violaceus* successfully recruited to the plate substrate and to the tests of barnacles (Table 4). Larvae did not recruit to the living tissues of itself and other colonial tunicates, solitary tunicates, and the encrusting colonial bryozoan *S. unicornis*. Growth interactions between recently-settled recruits of *B. violaceus* and *D. vexillum* resulted in standoffs. Colonies of *B. violaceus* successfully overgrew unoccupied plate substrate, recently-settled recruits of *S. unicornis* and most, but not all barnacle tests, amphipod tubes, and hydroid stalks (except for some polyps at the ends of stalks). Growth interactions with colonies of itself, other colonial tunicates, and *S. unicornis* resulted in standoffs, except that *B. violaceus* colonies partially overgrew the margins of regressive *S. unicornis* colonies. The species also partially overgrew the tunics of solitary tunicates but was unsuccessful in closing off their active siphons.

Schizoporella unicornis (invasive colonial bryozoan)

After recruitment plates were cleaned on September 4, 2013 (21.7° C), newly-recruited *S. unicornis* colonies were observed from September 20 to October 18 (19.7 to 17.2° C), the last observation in 2013 (Table 3). At the next observation of the plates on April 14, 2014 (6.9° C), small *S. unicornis* colonies were present that had recruited in late 2013 and overwintered. They had increased in size since they were last observed in 2013. However, they were not growing, as they had not increased in size by April 25, 11 days later. The non-growing colonies were a pale white color. They began to grow by May 28 (14.8° C) and gradually changed color from white to pink to pale orange.

In 2014, recruitment of *S. unicornis* colonies was observed on June 16 (18.3° C) and continued for 40 weeks to at least March 26, 2015 (1.1° C). A period of peak recruitment was not observed.

Colonies were not inspected for larval content. Potential sources of the early-settled larvae were *S. unicornis* colonies from 2013 that had overwintered on the recruitment plates and on the adjacent dock pilings. Larvae that settled later in 2014 could have been produced by the overwintering colonies just mentioned and by colonies that recruited earlier in 2014 to the plates and dock pilings. Thus, by June 16, 2014 two generations of *S. unicornis* colonies occupied the plates, those that recruited in 2013 and those that recruited in 2014 (Table 3). Some of the overwintered colonies from 2013 began to regress by September 8 (22.5° C) while others continued to grow until at least October 20 (16.8° C). All growth had ceased by January 21, 2015 (1.8° C). Colonies that recruited in 2014 had a shorter growing season than those that recruited in 2013, and they did not show the first signs of regression until November 5 (12.6° C), later than the 2013 colonies.

Larvae of *S. unicornis* successfully recruited to the plate substrate and to the tests of barnacles (Table 4). Larvae did not recruit to the living tissues of itself or of solitary and colonial tunicates. Colonies successfully overgrew unoccupied plate substrate and most, but not all barnacle tests. Growth interactions with colonies of itself, *D. vexillum* and *B. violaceus* resulted in standoffs. Some barnacles recruited to the surface of *S. unicornis* when it was overwintering.

Ectopleura crocea (native colonial hydroid)

Here we refer to this species as *E. crocea*, but as Schuchert (2010) notes, it is very difficult to distinguish from *E. larynx* (Ellis and Solander, 1786). After recruitment plates were cleaned on September 4, 2013 (21.7° C), *E. crocea* recruits were first observed on May 28, 2014 (14.8° C) (Table 3). Recruits and large growing colonies were observed thereafter until June 30 (21.0° C). Colonies began to regress by July 14 (22.1° C), and they disappeared by July 28 (22.3° C). Potential sources of recruits were *E. crocea* colonies that had overwintered from 2013 on adjacent dock pilings. Growing colonies reappeared on the plates by October 20 (16.8° C) and were present until March 26, 2015 (1.1° C). Although colonies of *E. crocea* rapidly colonized open areas of the plates, they were not observed to recruit to or overgrow any other species. By contrast, they were partially and sometimes completely overgrown by colonies of *D. vexillum* and *B. violaceus* (Table 4).

Jassa marmorata (cryptogenic amphipod)

After recruitment plates were cleaned on September 4, 2013 (21.7° C), *J. marmorata* individuals, but no tubes, were observed on October 18 (17.2° C), at which time barnacles of various sizes covered much of the plate area (Table 3). At the next observation on April 14, 2014 (6.9° C), the plates were covered by the brown *J. marmorata* tubes attached to the plate substrate and to the barnacle substrate that was developing in October, 2013. Individual amphipods were observed in and on the tubes, which did not increase in number over a period of at least 2.5 months until June 30, 2014, after which the tubes fell off the plates. Both amphipods and their tubes were observed in small numbers on January 21, 2015 (1.8° C) and March 26 (1.1° C).

The amphipod tubes that were abundant when first observed in April 2014 were not attached to the colonies of *D. vexillum* and *B. violaceus* that recruited in 2013 (Table 4). However, their tubes partially to fully covered barnacles and parts of the tunics of the solitary tunicates *A. aspersa* and *C. intestinalis*. Colonial tunicates *D. vexillum*, *B. violaceus*, and *A. glabrum* overgrew *J. marmorata* tubes to a large extent except in a few instances where tubes occupied by amphipods remained open. Barnacle larvae did not settle and recruit to the tubes.

Barnacle species

Barnacles produce planktonic larvae that are present in the water column in Woods Hole throughout most of the year (Fish 1925; Pineda et al 2002). After recruitment plates were cleaned on September 4, 2013 (21.7° C), newly-recruited barnacles were observed on September 20 (19.7° C) and on October 18 (17.2° C) (Table 3). Observations in 2014 began on April 14 (6.9° C), and barnacle recruits were first observed on May 5 (9.7° C) and then on May 14 (12.0° C). No further recruitment was observed until July 14 (22.1° C), after which recruits were consistently recorded over an 8-month period until March 26, 2015 (1.1° C). At times many recruits appeared within a 2-week period and at other times they were few in number, possibly reflecting the reproductive cycles of several different species. Potential sources of barnacle recruits that settled in late 2013 and early 2014 were barnacles on the adjacent dock pilings. Larvae that settled later in the year could have been produced by barnacles on the plates and on the dock pilings.

Barnacles recruited almost exclusively to clean plate surfaces and were not observed on colonial tunicates and amphipod tubes, or on most solitary tunicates except for *A. aspersa*, *C. intestinalis*, and *S. clava* (Table 4). They did not recruit to the colonial bryozoan *S. unicornis* except when it was overwintering. Growth interactions between closely-spaced barnacles resulted in deformation of their test margins. Larvae of *D. vexillum* and *B. violaceus* recruited to the sides of barnacle tests. Most barnacles were overgrown by colonies of *D. vexillum*, *B. violaceus*, *D. listerianum*, and *S. unicornis*, and the tubes of *J. marmorata*.

Occurrence of minor colonizing species

The following species were minor constituents of the assemblage that recruited to the plates in Woods Hole from September 2013 to March 2015. Settled, recently-metamorphosed recruits were not observed, probably because of their scarcity, so recruitment dates and temperatures are based on the presence of partially-grown colonies and individuals. In the following descriptions, observation dates are followed by temperature values that represent the mean water temperature recorded on that day.

Aplidium constellatum (native colonial tunicate)

Colonies of *A. constellatum* were observed during a 3-month period from August 11 to October 20, 2014 (22.0 to 16.8° C) (Table 5). Regressing colonies were observed on November 5 and December 1 (12.6 to 8.2° C). Recently-settled recruits were not observed during the experiment. Growth interactions with itself, *D. vexillum*, and *A. glabrum* resulted in standoffs (Table 4). No larvae of *D. vexillum*, *B. violaceus*, *S. unicornis*, or barnacles were observed to recruit to *A. constellatum*.

Aplidium glabrum (native colonial tunicate)

Colonies of *A. glabrum* were observed during a 9-month period from June 16, 2014 to March 26, 2015 (18.3 to 1.1° C) (Table 5). Recently-settled recruits were not observed during the experiment. Growth interactions with itself and the other colonial tunicates resulted in standoffs (Table 4). The species also displayed standoffs with growing *S. unicornis*, but partially overgrew the margins of overwintering colonies of that species. *Aplidium glabrum* partially to fully overgrew the tubes of the amphipod *J. marmorata*. No larvae

Table 5. Presence of minor colonizing species. Record of species presence on two 1 × 1 m recruitment plates from September 4, 2013 to March 26, 2015. See the text for explanation of species occurrence, Table 1 for explanation of observation strategy, and Table 4 for abbreviations of species names. Abbreviations: Cap, caprellid ampipods; Chg., temperature change; Days, time between observations; Obs., observation number; p, present.

Obs.	Date	Days	Water temp on date, ° C		Colonial species				Solitary species			Cap
			Mean	Chg.	Ac	Ag	Bs	DI	Aa	Ci	Sc	
0	4 Sep 13	0	21.7									
1	20 Sep	16	19.7	-2.0				p				
2	30 Sep	10	18.2	-1.5				p				
3	18 Oct	18	17.2	-1.0				p				
4	14 Apr 14	178	6.9						p	p	p	p
5	25 Apr	11	8.9	+2.0					p	p	p	p
6	5 May	10	9.7	+0.8					p	p	p	p
7	14 May	9	12.0	+2.3					p	p	p	p
8	28 May	14	14.8	+2.8					p	p	p	p
9	16 Jun	19	18.3	+3.5		p			p	p	p	p
10	30 Jun	14	21.0	+2.7		p			p	p	p	p
11	14 Jul	14	22.1	+1.1		p	p		p	p	p	p
12	28 Jul	14	22.3	+0.1		p	p			p	p	
13	11 Aug	14	22.0	-0.3	p	p	p			p	p	
14	25 Aug	14	20.9	-1.1	p	p	p				p	
15	8 Sep	14	22.5	+1.6	p	p	p				p	
16	19 Sep	11	20.6	-1.9	p	p	p				p	
17	6 Oct	17	18.0	-2.6	p	p	p			p	p	
18	20 Oct	14	16.8	-1.2	p	p	p			p	p	p
19	5 Nov	16	12.6	-4.2	p	p	p			p	p	p
20	1 Dec	26	8.2	-4.4	p	p				p	p	p
21	21 Jan 15	51	1.8	-6.4		p				p	p	p
22	26 Mar	65	1.1	-0.7		p			p	p	p	p

of *D. vexillum*, *B. violaceus*, *S. unicornis*, or barnacles were observed to recruit to *A. glabrum*.

Botryllus schlosseri (cryptogenic colonial tunicate)

Colonies of *B. schlosseri* were observed during a 4-month period from July 14 to November 5, 2014 (22.1 to 12.6° C) (Table 5). Recently-settled recruits were not observed during the experiment. Growth interactions with *B. violaceus*, and *A. glabrum* resulted in standoffs, and interaction with *D. vexillum* resulted in standoffs or overgrowth by *D. vexillum* (Table 4). No larvae of *D. vexillum*, *B. violaceus*, *S. unicornis* or barnacles were observed to recruit to *B. schlosseri*.

Diplosoma listerianum (invasive colonial tunicate)

Colonies of *D. listerianum* were observed only for a short period in September and October, 2013 (19.7 to 17.2° C) (Table 5) when clean substrate was present at the start of the experiment. It did not overwinter from 2013 to 2014 and was not observed during the remainder of the experiment.

Growth interactions with itself, *D. vexillum*, and *B. violaceus* resulted in standoffs (Table 4). The species partially to fully overgrew the tests of living barnacles. No larvae of *D. vexillum*, *B. violaceus*, *S. unicornis*, or barnacles were observed to recruit to *D. listerianum*.

Ascidella aspersa (invasive solitary tunicate)

Individuals of *A. aspersa* were observed during the 3-month period from April 14 to July 14, 2014 (6.9 to 22.1° C) (Table 5). Thereafter, it was absent until March 26, 2015 (1.1° C). The species was partially overgrown by colonies of *D. vexillum* and the tubes of *J. marmorata*, but its siphons remained open (Table 4). Barnacles in low numbers recruited to the surface of *A. aspersa*. No larvae of *D. vexillum*, *B. violaceus*, or *S. unicornis* were observed to recruit to *A. aspersa*.

Ciona intestinalis (cryptogenic solitary tunicate)

Individuals of *C. intestinalis* were observed during a 4-month period from April 14 to August 11, 2014 (6.9 to 22.0° C) (Table 5). After an approximate

2-month hiatus, it was observed from October 6, 2014 to March 26, 2015 (18.0 to 1.1° C), the end of the experiment. The species was partially overgrown by colonies of *D. vexillum* and *B. violaceus* and tubes of the amphipod *J. marmorata*, but its siphons remained open (Table 4). Barnacles in low numbers recruited to the surface of *C. intestinalis*. No larvae of *D. vexillum*, *B. violaceus*, or *S. unicornis* were observed to recruit to *C. intestinalis*.

Styela clava (invasive solitary tunicate)

Individuals of *S. clava* were observed during an 11-month period from April 14, 2014 to March 26, 2015 (6.9 to 1.1° C) (Table 5). The species was partially overgrown by colonies of *D. vexillum* and *B. violaceus*, but its siphons remained open (Table 4). Barnacles in low numbers recruited to the surface of *S. clava*. No larvae of *D. vexillum*, *B. violaceus*, or *S. unicornis* were observed to recruit to *S. clava*.

Caprellid amphipods (skeleton shrimp)

Caprellids were not identified to species, but they very likely represent *Caprella mutica* Shurin, 1935, an invasive species in New England that originated from Japan (Turcotte and Sainte-Marie 2009, and references therein). Skeleton shrimp (Table 5) did not colonize the plates in terms of occupying space, but they were present in large numbers during a 3-month period from April 14 to July 14, 2014 (6.9 to 22.1° C). After an approximate 3-month hiatus, they were observed from October 20, 2014 to March 26, 2015 (16.8 to 1.1° C), the end of the experiment.

Discussion

Observations of two 1 × 1 m recruitment plates over a period of 18 months and a sampling strategy that required the periodic cleaning of 25 × 25 cm subareas of the plates showed that successful colonization by several species likely was controlled by a combination of factors, including: a) available firm substrate; b) water temperature; c) timing and duration of recruitment; d) timing and duration of growth; e) the ability of organisms to overgrow other organisms and/or to defend against overgrowth; and f) the ability of species to maintain their space by overwintering and living longer than competitors. The interplay of these factors resulted in the dominance, over varying periods of time, of two colonial tunicates (*D. vexillum*, *B. violaceus*), a colonial bryozoan

(*S. unicornis*), a colonial hydroid (*E. crocea*), a tube-building amphipod (*J. marmorata*), and barnacle species.

The recruitment plates were isolated from the seabed and were not affected by sediment movement or by the activities of species living on the seabed. Tidal currents did not exceed 1 m s⁻¹. Substrate and water temperature provided the physical framework within which recruitment and colonization occurred. The principal substrate in this study was the firm surface of the PVC plates and the tests of living and non-living barnacles. Plate surfaces became available for colonization through periodic cleaning of selected subareas of the plates, the death of colonial species (*B. violaceus*, *E. crocea*), and the falling off of amphipod tubes and dead barnacle tests.

The initial colonizers of the plates, who overwintered from 2013 to 2014, were chiefly barnacles and tube-building amphipods, and to a minor extent three colonial species, two tunicates (*D. vexillum*, *B. violaceus*) and one bryozoan (*S. unicornis*). None of these species recruited to the plates from April 25 to May 28, 2014, during which time water temperature increased from 8.9 to 14.8° C. This recruitment gap possibly began earlier in the year, but we have no observational data for that time period.

If we define the cool season as occurring between the first and last occurrences of 0° C, the differences between 2014 and 2015 in duration (days), temperature variability, and temperature minima did not seem to affect colonization by the dominant colonial tunicates *D. vexillum*, and *B. violaceus*, the colonial bryozoan *S. unicornis*, and the hydroid *E. crocea*, all of which successfully overwintered in 2015 when temperatures remained ≤-1° C for several weeks. Sustained mean temperatures above 21° C in 2014 possibly contributed to the disappearance of the colonial hydroid *E. crocea*, the tube-building amphipod *J. marmorata*, caprellid amphipods, and the less well-documented solitary tunicates, *C. intestinalis* and *A. aspersa*. Temperature and/or senescence may have contributed to the disappearance of the 2013 generation of the colonial tunicate *B. violaceus*. The disappearance of the tunicates, the hydroid, and the amphipod tubes made substrate available to recruits of other species.

Didemnum vexillum was a very successful colonizer. Its strongest qualities included rapid colony growth, overgrowth of some competitors for space (*E. crocea*, *J. marmorata* tubes, barnacle tests), and the ability to defend itself by preventing larvae from settling onto its surface

and by creating standoffs with other species. Standoffs occurred between other colonies of *D. vexillum*, other colonial tunicate species, and the colonial bryozoan *S. unicornis*. The tunics of some solitary tunicates provided substrate for *D. vexillum*, but overgrowth was usually partial and ended in a standoff around the siphons of *S. clava*, *C. intestinalis*, and *A. aspersa*. The reproductive season was relatively short, lasting from late June into October (16 weeks). Larvae were most abundant early in the reproductive season, most likely produced by colonies that overwintered from 2013, as colonies that recruited in 2014 were, at most, one month old during this period. Colonies were relatively long-lived. The colonies originating in late September 2013 survived at least 18 months, overwintered twice, and they were firmly attached to the substrate in late March 2015, at the end of the experiment. A study of *D. vexillum* larval recruitment in 2007 at the same dock (Valentine et al. 2009), using small recruitment plates (144 cm²), recorded the first occurrence of recruits in late June (19.4° C), very similar to observations of the present study. By contrast, in 2007, the last *D. vexillum* recruited to the plates in late November (9.3° C), a month later than in 2014. In 2007, the last recruits were observed until mid-December and did not bud to form colonies. The colonizing potential of late-season *D. vexillum* recruits remains an open question.

Botrylloides violaceus also was a very successful colonizer, shared many of the characteristics of *D. vexillum*, and behaved similarly with regard to rapid colony growth, overgrowth of competitors, and the ability to defend itself by standoffs and by repelling larval settlement. Larvae were most abundant early in its reproductive season, most likely produced by colonies that overwintered from 2013. The reproductive season lasted from mid-June through November 2014 (24 weeks), fifty percent longer than that of *D. vexillum*. By contrast with *D. vexillum*, colonies were shorter-lived. *Botrylloides violaceus* colonies that originated in September 2013 overwintered from 2013 to 2014 and grew and reproduced until late June 2014 after which they regressed and disappeared by late August, approximately 11 months after recruitment. A similar phenomenon, reproduction followed by regression and death, was reported for a “spring generation” of *B. violaceus* colonies in Japan (Yamaguchi 1975). At Woods Hole, colonies that originated in mid-June 2014 showed signs of growth to at least late January 2015, but had not increased in size by

the next observation in late March 2015. By that time, they had survived for 10 months and overwintered without signs of regression, and it is likely they grew well into 2015 if they behaved as did colonies that originated in late 2013 and overwintered into 2014.

Schizoporella unicornis also was a successful colonizer, and like *D. vexillum* and *B. violaceus* defended itself by preventing larval settlement and by standoffs. Similar observations have been documented for this species at Beaufort, NC (Sutherland and Karlson 1977; Sutherland 1978). In Woods Hole, the species exhibited a long period of recruitment and colony growth. The reproductive season lasted from mid-June 2014 to at least late March 2015 (40 weeks), longer than that of either *D. vexillum* or *B. violaceus*. Colonies that originated in late 2013 survived to at least late March 2015 (18 months) and had overwintered twice, although by that time, they were not growing and some, but not all, colonies showed signs of regression. Sutherland (1978) reported the life span of *S. unicornis* in North Carolina to be approximately 2 years.

Ectopleura crocea was a highly successful colonizer of unoccupied space for a limited time period as it apparently was sensitive to increasing water temperatures. It recruited to newly-cleaned subareas of the plates in late May 2014 and was successful in growing large, multi-stemmed colonies. However, it was not able to recruit to areas already colonized by species that had overwintered from 2013, and it was rapidly overgrown by expanding colonies of *D. vexillum* and *B. violaceus*. By mid-July 2014 (~21–22° C), the polyps of *E. crocea* died and fell off the plates. Some colonies of *E. crocea* appeared in October 2014 (~16–17° C) when water temperatures had cooled. These colonies possibly represented re-growth of dormant stems of colonies that had recruited earlier in the year to substrate that now was mostly occupied by other species.

Jassa marmorata built tubes that “colonized” substrates in the cool part of the year. Recruitment plates that were observed to have no tubes in mid-October 2013 were almost covered in tubes by the next observation in mid-April 2014. The tubes were built on barnacle tests, on parts of solitary tunicates *A. aspersa* and *C. intestinalis*, and on unoccupied substrate, but avoided overwintering colonies of *D. vexillum* and growing colonies of *B. violaceus*. In turn, the tubes provided substrate for growing colonies of *D. vexillum*, *B. violaceus*, and *A. glabrum*, although some tubes, in which amphipods were active, were not over-

grown. The amphipods and their tubes disappeared by late June (~21° C) as water temperatures warmed, offering substrate for recruitment by other species.

Barnacle species recruited to unoccupied substrate during most of the year, sometimes in high numbers, but they were not effective in maintaining their presence over a long period. They did not recruit to the surfaces of any other species except occasionally to the tunics of *A. aspersa*, *C. intestinalis*, and *S. clava*, and to the surfaces of overwintering *S. unicornis* colonies, and they were ineffectual in overgrowing these species. Barnacles were overgrown by *D. vexillum*, *B. violaceus*, and *S. unicornis* and covered by the tubes of the amphipod *J. marmorata*. Barnacles produced many larvae in most seasons and, in the absence of competitors for space, had the potential to be successful colonizers.

The minor colonial tunicate species were represented by few individuals, but they showed consistent and interpretable patterns of presence and absence. Larval recruits were not observed for these species, but our observations suggest they require unoccupied substrate for settlement. Colonial tunicates *A. constellatum*, *A. glabrum*, and *B. schlosseri* were first observed in the warm season (mid-June to mid-August) of 2014 and persisted at least into November, with *A. glabrum* overwintering into late March 2015. These species, though minor in terms of area colonized, were successful in maintaining their presence by creating standoffs with other colonial tunicates, including the faster-growing *D. vexillum* and *B. violaceus*, and by not allowing settlement onto their surfaces by larvae of other species. In one exception, *D. vexillum* was observed to overgrow a small colony of *B. schlosseri*. In contrast to its minor role in the present study, *A. glabrum* was shown to be a major colonizer of a complex subtidal vertical rock wall substrate in Nahant, MA (Sebens 1986), although potential competitors *D. vexillum* and *B. violaceus* were not reported to be present at the site. Another colonial tunicate, *D. listerianum*, covered much of the plate surfaces in September and October 2013 at the start of the experiment but was not observed thereafter.

Of the solitary tunicates recorded during the experiment, *A. aspersa*, *C. intestinalis*, and *Styela clava* first appeared in April 2014, possibly the result of recruitment in the latter part of 2013 or the early part of 2014. Two of these species, *A. aspersa* and *C. intestinalis* likely are sensitive to warm water temperatures in Woods Hole. *Aplidium aspersa* was not present after mid-July but re-appeared in March 2015. *Ciona intestinalis*

was not present after mid-August 2014 but re-appeared in early October and persisted to late March 2015. There is some evidence that northern populations of *C. intestinalis* in Denmark do not tolerate warm temperatures well, possibly because filtration rates decrease markedly above 21° C (Petersen and Riisgard 1992). *Styela clava* apparently is less sensitive to temperature change, as it was present from mid-April 2014 to late March 2015. Solitary tunicates served as substrate for overgrowth by *D. vexillum* and *B. violaceus*, although overgrowth was incomplete and did not impede the flow of water through their inhalant and exhalant siphons.

In Woods Hole, once a substrate became fully colonized, there was very little opportunity for new recruitment until new substrate opened after the death of colonies and individuals and the disappearance of structures such as amphipod tubes. The most successful colonizer of the recruitment plates in Woods Hole was *D. vexillum* followed by *B. violaceus*. Both species had relatively long reproductive and recruitment periods, grew rapidly, repelled settlement by larvae of any species, defended themselves against overgrowth by any species, overwintered, and lived a long time. Peak recruitment for both species occurred early in their reproductive seasons. *Botrylloides violaceus* reproduced over a longer time period and overwintered without regressing, but its colonies were shorter-lived than those of *D. vexillum*. We do not yet know if some portion of *B. violaceus* colonies always dies in the warm season in Woods Hole. *Didemnum vexillum* colonies grew more rapidly, and they overgrew new recruits of *B. violaceus* and *S. unicornis*. The bryozoan *S. unicornis* was a long-lived species but was a somewhat less successful colonizer because it grew more slowly than *D. vexillum* and *B. violaceus*. However, it had a longer recruitment period, which provided more opportunity for it to recruit to newly-available substrates. In addition, the calcareous, encrusting colonies of *S. unicornis* were physically harder and more firmly attached to the substrate than those of *D. vexillum* and *B. violaceus*. When comparing these three species, it appears that *D. vexillum* and *B. violaceus* could be better adapted to rapid, long-term colonization and *S. unicornis* to slow, long-term colonization.

Among the remaining species observed, the colonial hydroid *E. crocea* was very successful in colonizing open plate substrate, but was not able to recruit to substrate occupied by other species, and its longevity was shortened apparently by warming water temperature. *Ectopleura crocea*

colonies and *J. marmorata* tubes disappeared from the plates and opened recruitment space for *D. vexillum* and *B. violaceus* during their peak recruitment periods. The colonial tunicates *A. glabrum* and *B. schlosseri*, when compared to *D. vexillum* and *B. violaceus*, grew relatively slowly, and their size was constrained by standoffs with faster-growing neighbor species. Solitary tunicates provided substrate for colonial tunicates, as their tunics did not repel attachment, but their siphonal currents did prevent complete overgrowth. Barnacle tests constituted the only biological substrate that was successfully colonized by other species.

The size of recruitment plates determines their effectiveness in providing a record of substrate colonization by attached invertebrates. Small plates (e.g. 10 × 10 cm) are suitable for documenting settlement and recruitment of larvae over short time periods but often are too small to record processes of colonization, especially in the presence of rapidly-growing invasive species such as *D. vexillum* and *B. violaceus*. Large plates (1 × 1 m) permit the design of innovative strategies for sampling and cleaning areas of the substrate so as to observe the timing and environmental conditions of species recruitment, as well as recruitment and growth interactions during the colonization process. In Woods Hole, large plates provided enough space to accommodate both fast- and slow-colonizing species, resulting in the establishment of a diverse assemblage that was observed over a long time period. Large plates potentially are suitable for monitoring the effects of climate on the growth of species and on the stability of assemblages over time. The methodology described here can be used to document the longevity of colonial species, which to date has been difficult to determine. Finally, an understanding of colonization processes utilized by invasive species allows prediction of their potential invasive success and subsequent effect on ecosystems in areas where they are not yet present.

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