



The influence of resident adults on larval settlement: experiments with four species of ascidians

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Received 28 April 1994; revision received 9 January 1995; accepted 10 February 1995

Abstract

Residents within any community can affect the larval settlement of both their own and other species. In marine sessile communities resident adults can affect larval settlement by preying on settling larvae, removing or adding space for the larvae to colonize, or stimulating or prohibiting larval settlement on available substratum nearby. To examine those processes by which residents affect settlement, we exposed experimental substrata with three densities of adults of a single species at a site in eastern Long Island Sound, USA for a 24-h period. Four species of common ascidians, *Botryllus schlosseri* (Pallas), *Botrylloides diegensis* Ritter and Forsyth, *Diplosoma macdonaldi* Herdman, and *Molgula manhattensis* (De Kay), were used in 11 separate experiments. Few individuals of any species settling attached to the surfaces of these species and this resulted in the main effect of these residents being the usurpation of space and the restricting of settlement to unoccupied areas. A model is also presented to explain the apparent aggregated settlement of several species in open areas adjacent to the resident ascidians. From this model we suggest that the aggregated settlement can result from limited larval mobility such that some larvae that contact and reject the resident species as settlement sites may subsequently contact open surfaces of the same substratum and increase settlement densities there over those observed on control substrata. Finally, settlement data for several species indicate that *Molgula* may influence settlement by preying on larvae.

Keywords: Larval settlement; Recruitment; Benthic communities; Sessile invertebrate; Ascidian; Bryozoan

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1. Introduction

Residents within a community can affect the recruitment dynamics of both their own and other species. The nature of this influence has long been recognized as a key component of succession theory (Connell & Slatyer, 1977) as well as the more recent concern regarding the ability of recruitment to control the local distribution and dynamics of populations (Roughgarden et al., 1985, 1988; Gaines & Roughgarden, 1987; Menge, 1991). Within marine sessile communities composed mostly of attached invertebrates and associated fauna that often have limited mobility there are four principal means by which the resident species can influence recruitment. Firstly, they can prey on settling larvae (e.g. Mileikovsky, 1974; Cowden et al., 1984; Young & Gotelli, 1988; Bingham & Walters, 1989; Osman et al., 1989; Young, 1989; Stoner, 1990). Secondly, they can both remove and/or add available space for the settling larvae to colonize, depending on whether the recruiting larvae can attach to and survive on the external surfaces of the resident species (e.g. Stebbing, 1972; Moyse & Hui, 1981; Jensen & Morse, 1984). Thirdly, residents can stimulate or prohibit larvae from settling on available substratum nearby (e.g. Grosberg, 1981; Kent & Day, 1983; Havenhand & Svane, 1989; Bingham & Young, 1991). Finally, residents can increase post-settlement mortality by preying on or overgrowing newly attached individuals (e.g. Grosberg, 1981; Osman et al., 1989, 1992; Stoner, 1990; Hurlbut, 1991a,b; Dalby & Young, 1992) or they can decrease mortality by providing structure for attachment or camouflage from predators (Osman & Haugsness, 1981; Osman, 1987).

The present study focuses on resident-settler interactions and examines how a variety of dominant resident sessile species influence larval settlement dynamics within a New England shallow-water sessile community. The effects of post-settlement interactions in modifying settlement patterns into different patterns of recruitment was the subject of a separate series of experiments and is addressed in a companion paper (Osman & Whitlatch, this issue). The goal in each experiment was to determine the effects of individual resident species on all species of larvae settling at a single point in time. Our overall objective was to determine how resident species influenced observed larval settlement patterns and not to assess the contribution of specific mechanisms to those patterns (e.g. chemical cues, hydrodynamic alterations). Using the results of these studies, we examined whether any consistent patterns of settlement existed which could be used to estimate the relative importance of substratum removal or addition, settlement enhancement or prohibition, or larval predation on settlement dynamics. The principal effect of the resident species that we examined seems to have been the removal of habitable space and the apparent aggregation of settlers in the remaining unoccupied space.

2. Methods

The study was conducted in the eastern end of Long Island Sound near the mouth of the Poquonock River. Experiments were deployed on a moored raft

behind a breakwater near Avery Point, Groton, Connecticut. As reported previously (Osman et al., 1992), the sessile community on the raft, as well as on rocks and pilings in its vicinity, was dominated by ascidians, including the colonial ascidians *Botryllus schlosseri* (Pallas), *Botrylloides diegensis* Ritter and Forsyth, and *Diplosoma macdonaldi* Herdman, and the solitary ascidians *Molgula manhattensis* (DeKay), *Styela clava* Herdman, and *Ciona intestinalis* (L.). Other common species included the bryozoans *Cryptosula pallasiana* (Moll), *Bowerbankia gracilis* Leidy, and *Bugula turrita* (DeSar), the serpulid polychaetes *Hydroides dianthus* (Verrill) and *Spirorbis* spp., the barnacles *Balanus amphitrite* Darwin, *Semibalanus balanoides* (L.), and *Balanus eburneus* Gould, the sponges *Halichondria* sp. and *Leucosolenia* sp., and the hydroid *Obelia* sp.

A total of 11 settlement experiments were conducted during the summer of 1991 using one of four dominant ascidians as test species. Four experiments were conducted using *Diplosoma*, three using *Botryllus*, two using *Botrylloides*, and two using *Molgula*. The choice of test species was determined by which species were dominant at the time of the experiment. The general design of each experiment was to compare larval settlement on equal-sized substrata which systematically varied in the areal coverage of a resident test species. Three treatments were used in each experiment: (1) control substrata with no cover of a test species, (2) low cover substrata with 30–50% cover of a test species, and (3) high cover substrata with 70–90% cover of a test species. Two methods were used to produce these treatments. For all but the *Molgula* experiment, 10 × 10 cm PVC panels were deployed at the field site 1–3 months prior to the beginning of an experiment. Substrata were examined weekly and all species except the chosen test species (usually the most abundant species) were removed. This process was continued until most substrata had >70% cover of the desired species. At this time all substrata, including controls, were carefully cleaned of all other species and assigned to one of the treatments. Excess individuals or colonies of the test species were haphazardly removed until the assigned density (in a nominally random distribution) was attained. For the *Molgula* experiments, adult *Molgula* were collected in the vicinity of the study site and cleaned. A small amount of glue was used to attach individuals by their basal surfaces onto clean 100 cm² substrata in densities of 0, 10, and 20. All panels were held for 24 hours in a flow-through, filtered (<5 μm) seawater system before beginning an experiment.

Eight replicate panels were used for each treatment in each experiment. These panels were randomly assigned to positions on panel racks suspended from the moored raft. Panel racks were similar in design to those used by Osman (1982) and Osman et al. (1989). When in the field the racks held the panels approximately 1 m below the water surface (1–2 m above the bottom). All panels were oriented horizontally with the test surface facing the seafloor. In each experiment, panels were exposed in the field for a 24-h period and then returned to the laboratory. All newly settled individuals of all species were identified and counted under dissecting microscopes. Individuals attached to the panel surface were differentiated from those on the surfaces of test species.

In deploying the panels no effort was made to exclude predators. Known benthic predators of newly-settled individuals (e.g. Osman et al., 1990, 1992) were

not observed at the study site and suspending the substrata from a raft which had no contact with the bottom provided additional protection from these predators. Fish predators such as the cunner, *Tautoglabrus adspersus*, prey on juvenile *Molgula*, but not on *Botryllus* or *Botrylloides* (Osman et al., 1990). We saw no evidence of predator-removal of any of the test species from the panels during the course of the experiments. Also, in previous studies (Osman et al., 1990, 1992) no evidence of fish predation on newly-settled individuals of any species was observed. Nevertheless, we cannot rule out the possibility that fish predation could contribute to observed differences in settlement. However, since all treatments were exposed equally to fish predators, any settlement differences should have resulted from the direct or indirect influence of the test species.

2.1. Analysis

Our primary goal was to test whether resident adults of any test species affected larval settlement. Therefore, the effects of each of the four resident species of adults on larval settlement was examined separately for each settling taxa. Two types of comparisons were made. Firstly, in order to examine the overall effect of each resident species on settlement onto the substrata on which they occurred, we compared total settlement for each taxa (panel and adult surfaces combined) among the three treatments. Secondly, settlement onto adult surfaces was contrasted with settlement onto panel surfaces by analyzing the density of settlement (numbers/cm²) on both surface types. In the latter analyses data were grouped by treatment and surface type, resulting in five groups of surfaces (panel and species surfaces on high cover substrata, panel and species surfaces on low cover substrata, and control panel surfaces). Settlement densities were computed using estimates of the mean percent cover of residents (0, 40, or 80%) and open space (100, 60, or 20%) in the treatments (control, low, or high cover).

The contrast of the results of the two analyses allows us to determine whether any differences in larval behavior towards panel and adult surfaces has any overall effect on settlement onto a substratum. For example, if we assume that larvae do not recognize any difference between the panel surface and the surface of the test species, then without any direct effect of the test species (including no addition to the total area of the substrate), we would expect settlement to be proportional to the areal extent of each substrate type (Fig. 1A). This would result in equal settlement densities on all five surfaces (Fig. 1B) and the total number of settlers (the sum of those on the panel surface and on the test species) being the same on all substrata. However, if the larvae do not recognize the test species as a substratum or cannot successfully attach to it, we would expect no differences in settlement density on panel surfaces (Fig. 1D), but significantly higher total settlement on the control substrata relative to the treatment panels (Fig. 1C). Only differences from these patterns would indicate the involvement of other, more active, processes such as aggregation of settlers or predation on larvae.

Because each test species was utilized in 2 to 4 discrete experiments, a two-way mixed-model ANOVA design was used for both analyses of total settlement (3

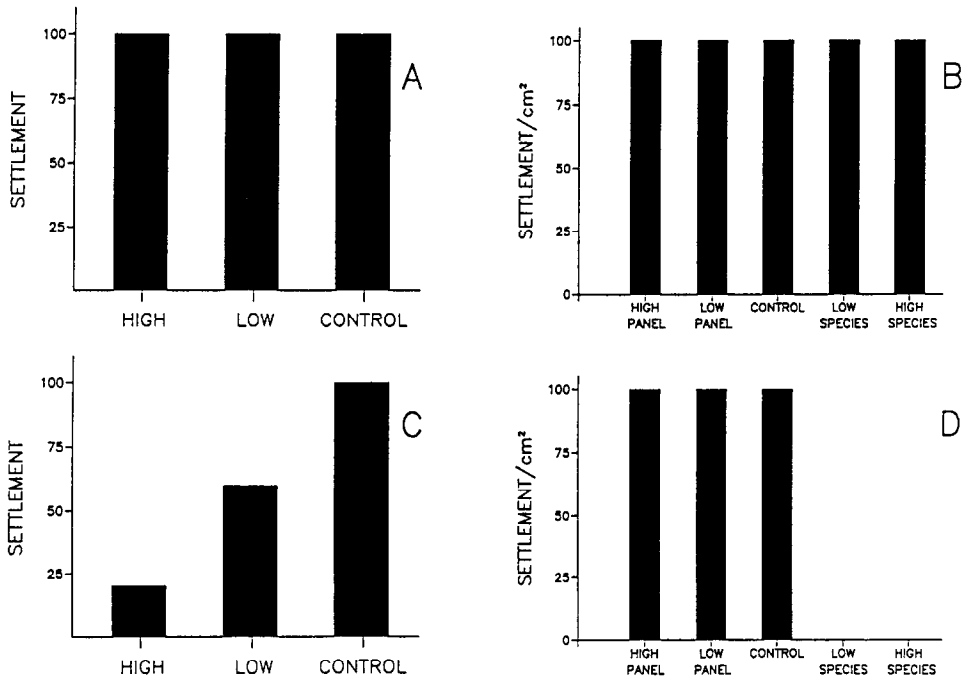


Fig. 1. Expected total settlement (A and C) and settlement density (B and D) patterns for the experiments. It was assumed that the resident test species occupies 40% of low-density panels and 80% of high-density panels. In A and B it was also assumed that settling larvae did not distinguish between substrate types and in C and D it was assumed that larvae only settled onto panel surfaces.

panel treatments \times 2–4 experiments) and settlement density (5 groups of panel surfaces \times 2–4 experiments). Treatments or groups were analyzed as fixed effects and experiments (= dates with uncontrolled fluctuations in larval abundances) as random effects. Because of heterogeneity in variances, the data were log-transformed for analyses. In the analyses of settlement density, three a priori contrast analyses were used. In the first contrast we compared mean settlement density between panel and species surfaces on the high and low cover panels (control panels were not included). In the second contrast we compared mean settlement density on control surfaces to panel surfaces on the high and low cover panels. Finally, we compared mean settlement densities on control panels to species surfaces on the high and low cover panels.

Four basic assumptions were made in analyzing the experiments. Firstly, it was assumed that larval settlement of each species was independent of all other species settling at the time of an individual experiment. Because settlement was measured over a 24-h period, settlement densities were relatively low and the opportunity for larvae to interact with each other on the substrate surface was small relative to contact with the test species.

Secondly, it was assumed that the effect of a resident species on larval

settlement was independent of any temporal variation in the density of settling larvae. With the principal difference among any set of experiments being the density of settling larvae, the two-way ANOVA's allowed us to test for significant interactions between settlement density (experiments) and the effect of the test species (treatments).

Thirdly, in order to contrast the effects of the different resident species on the larval settlement of a particular species, we assumed that no temporal differences existed in larval behavior, adult behavior, or the manner in which the test adults affected the local environment on a substratum. It was logistically impossible to conduct all experiments at the same time and even though environmental conditions at the study site changed somewhat throughout the summer; we reasoned that the basic relationships between settling larvae and resident adults should remain constant.

Finally, we assumed that all substrata within an experiment were exposed to an equal number of larvae contacting them. Since panels were positioned randomly on the field racks, differences in larval availability within and among treatments were minimized.

3. Results

During the course of the experiments, seven taxa settled in sufficient abundance to be analyzed. These included the ascidians *Botryllus schlosseri*, *Botrylloides diegensis*, and *Diplosoma macdonaldi*, the bryozoans *Bugula turrita* and *Cryptosula pallasiana*, serpulid polychaetes of the genus *Spirorbis*, and barnacles of the genus *Balanus*. Of the seven taxa, *Diplosoma* had consistently high abundances in all experiments with mean abundances in all treatments greater than one individual per panel. *Botryllus* had similarly high abundances in all but the *Diplosoma* experiments. For the remaining taxa, mean settlement abundances were often less than one individual per substratum in more than one treatment. Although such low abundances make detecting treatment effects difficult, they nonetheless represent actual settlement densities and the effects that adults are likely to have on observed settlement at the study site.

3.1. Effects of adults on overall settlement and settlement densities

The most consistent pattern observed for *Diplosoma* settlement was the significantly lower settlement densities on the surfaces of all four species of resident adults (Fig. 2). However, settlement densities on adjacent panel surfaces varied among the experiments. In experiments with resident *Botryllus* and *Botrylloides* colonies, settlement densities on panel surfaces in all treatments were not significantly different. In the *Molgula* experiments settlement density was higher on panel surfaces adjacent *Molgula* than on control panels and in the *Diplosoma* experiments densities were significantly higher on control panel surfaces. These patterns resulted in two patterns of overall settlement among the

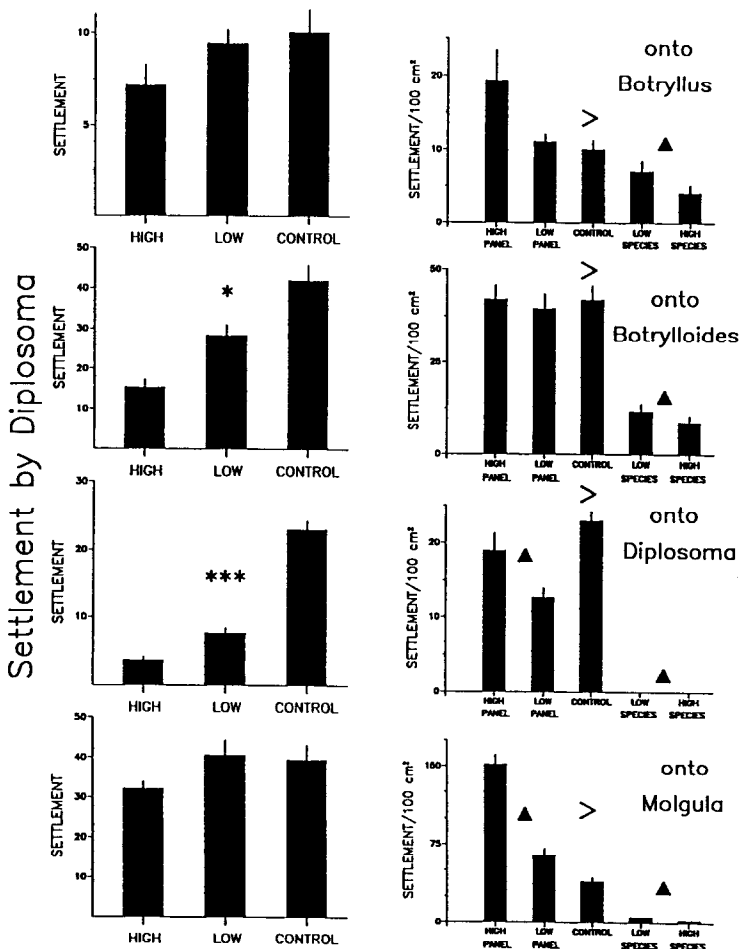


Fig. 2. Settlement patterns of *Diplosoma* in the four sets of experiments. Pairs of graphs are shown for each experiment with the left-hand graph contrasting total settlement onto each of the treatments and the right-hand graph comparing settlement density among the five substrate groups (panel surfaces on high and low cover treatments, species surfaces on high and low cover treatments, and control panel surfaces). Means are for all experiments conducted with each resident test species. Statistical analyses were based on a two-way ANOVA design (experiment \times treatment or experiment \times substrate group) with data log-transformed but untransformed means and standard errors are shown in the figure. Significant differences in total settlement are indicated as: *, $p < 0.05$, **, $p < 0.01$, and ***, $p < 0.001$. For the analyses of settlement density, significant results of the three a priori contrast analyses are also shown. (>) indicates significantly lower or greater mean settlement on panel than species surfaces in the high and low density treatments and \blacktriangle indicates a significant difference in mean settlement on control surfaces when compared to panel surfaces and/or species surfaces in the high and low density treatments. Mean settlement and settlement densities are for the five substrate groups: panel surfaces in the control, high-, and low-density treatments and surfaces of test species in the high- and low-density treatments. Patterns can be compared to the expected patterns in Fig. 1.

treatments. *Diplosoma* settlement onto resident *Botryllus* was sufficiently high that when combined with settlement onto adjacent panel surfaces, the total settlement was not different from controls. Significantly higher settlement densities on panel surfaces adjacent *Molgula* resulted in a similar lack of effect of this species on total settlement. Finally, the lack of *Diplosoma* settlement onto *Botrylloides* and *Diplosoma* adults coupled with no increases in settlement on adjacent panel surfaces resulted in a pattern of decreasing overall settlement as the percent cover of the resident species increased.

The pattern of *Botryllus* settlement (Fig. 3) was similar to that of *Diplosoma*, with *Botryllus* larvae settling in even lower abundances onto adults of all of the four test species. No *Botryllus* were ever observed on colonies of its own species or of *Diplosoma* and only an occasional individual was found settling on

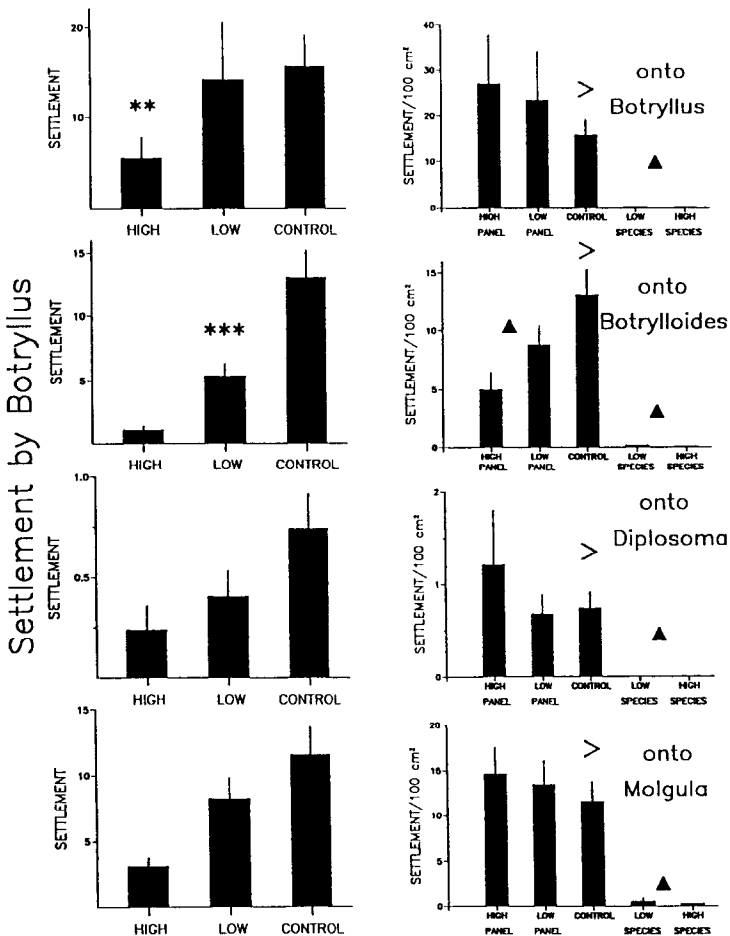


Fig. 3. Settlement patterns of *Botryllus schlosseri* in the four sets of experiments. Description the same as Fig. 2.

Botrylloides or *Molgula*. In all experiments, densities of settling *Botryllus* were significantly less than on both control and adjacent panel surfaces. However, in all but the *Botrylloides* experiments, settlement densities were not significantly different among the panel surfaces of the different treatments. In the *Botrylloides* experiments settlement density on panel surfaces was significantly higher on control than on treatment panels. These patterns resulted in decreasing total settlement from the control panels to the high-density substrata, with differences between control and treatment panels being significant in only the *Botryllus* and *Botrylloides* experiments.

The third species of ascidian, *Botrylloides*, settled in fairly low abundances in all experiments, making it difficult to detect any possible differences in settlement (Fig. 4). In general the density of *Botrylloides* settlers was lower on species

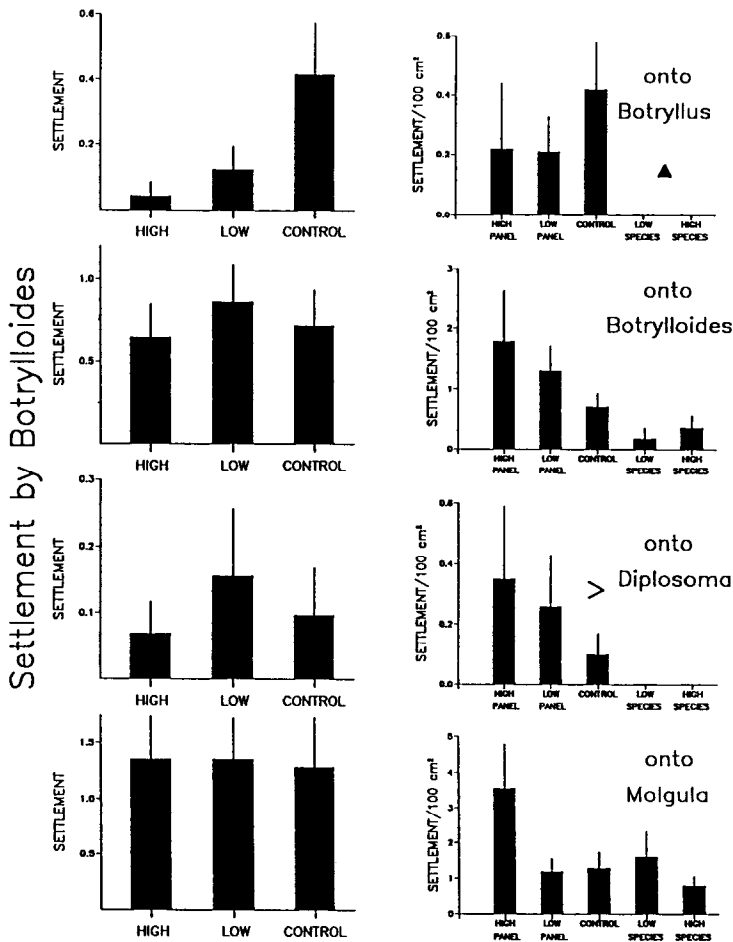


Fig. 4. Settlement patterns of *Botrylloides diegensis* in the four sets of experiments. Description the same as Fig. 2.

surfaces, but significant differences from control panels were found only in the *Botryllus* experiments and significant differences from adjacent panel surfaces were seen only in the *Diplosoma* experiment. No significant differences in total settlement were found in any of the experiments.

Of the two bryozoan species, *Bugula* settled in higher abundances, but still much lower when compared to *Botryllus* and *Diplosoma*. Settlement patterns were fairly similar to those seen with the ascidians (Fig. 5). Again, settlement onto resident adults was low with no settlement onto *Diplosoma*. Significantly lower densities from those found on control and/or adjacent panel surfaces were found in all but the *Molgula* experiments. *Bugula* settlement densities onto panel surfaces adjacent *Botryllus* and *Diplosoma* were higher than found on control panels (significantly with *Diplosoma*). Total settlement was also significantly

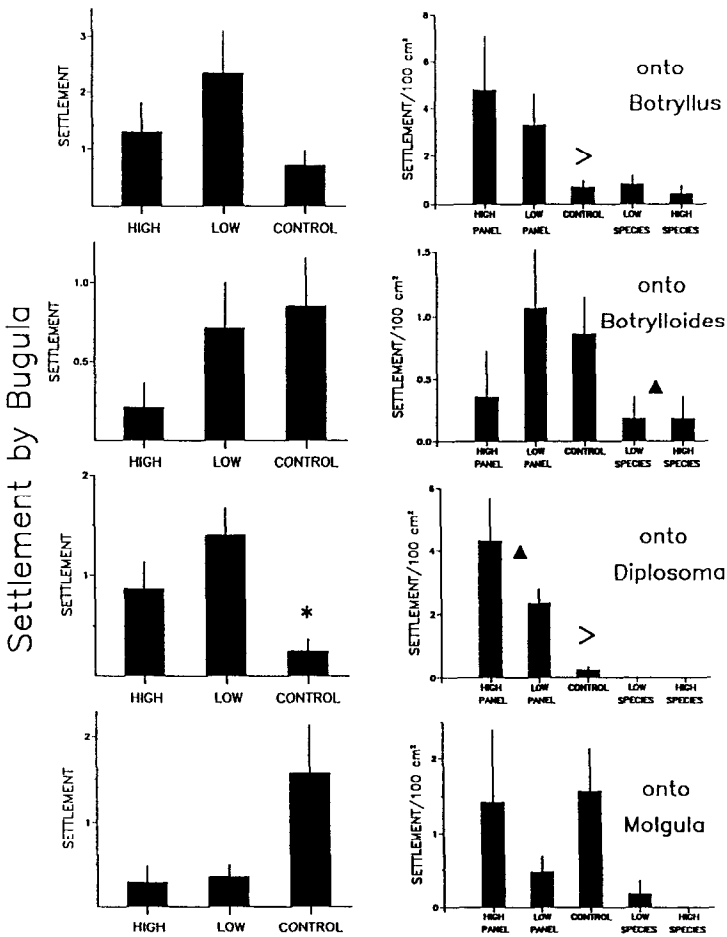


Fig. 5. Settlement patterns of *Bugula turrita* in the four sets of experiments. Description the same as Fig. 2.

higher on panels with *Diplosoma* than on control panels, even though no *Bugula* settled onto *Diplosoma*.

Cryptosula, the second species of bryozoan, only settled onto *Molgula* and was not found on adults of the other three species (Fig. 6). This resulted in settlement densities on all four species of resident adults being significantly lower than on control panels and/or adjacent panel substrate. Settlement densities on panel surfaces adjacent residents were significantly higher than on control panels in the *Diplosoma* experiments and lower in the *Molgula* experiments. Although the total settlement of *Cryptosula* was higher on treatment panels than on controls in the *Diplosoma* experiments and lower in the other three sets of experiments, none of these differences were significant.

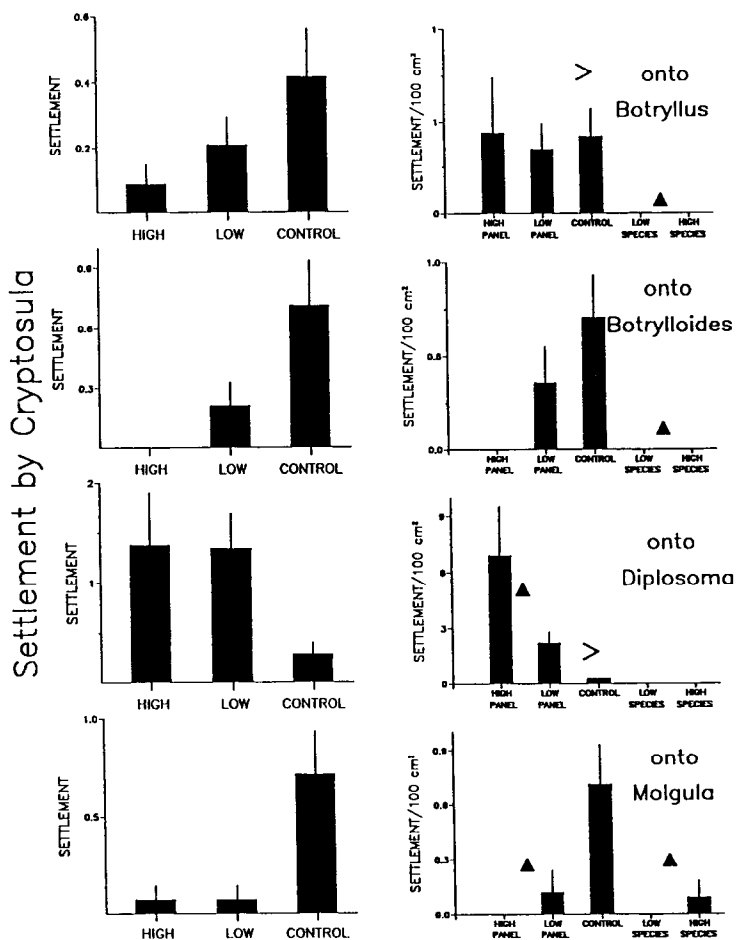


Fig. 6. Settlement patterns of *Cryptosula pallasiana* in the four sets of experiments. Description the same as Fig. 2.

Although *Balanus* (Fig. 7) and *Spirorbis* (Fig. 8) settled in very low abundances, settlement patterns were fairly similar to the other five species. *Balanus* did not settle onto any of the resident adults and *Spirorbis* settled only onto surfaces of *Botryllus*. Usually, differences in settlement densities between controls and surfaces of the resident species were not significant. However, *Balanus* did settle in higher densities on panel surfaces adjacent residents of all three colonial species. *Spirorbis* exhibited the same pattern in the *Diplosoma* experiments.

3.2. Response of larvae to different species of adults

We also examined whether larval settlement of each taxon differed as a function of the particular species resident on the substratum. To correct for

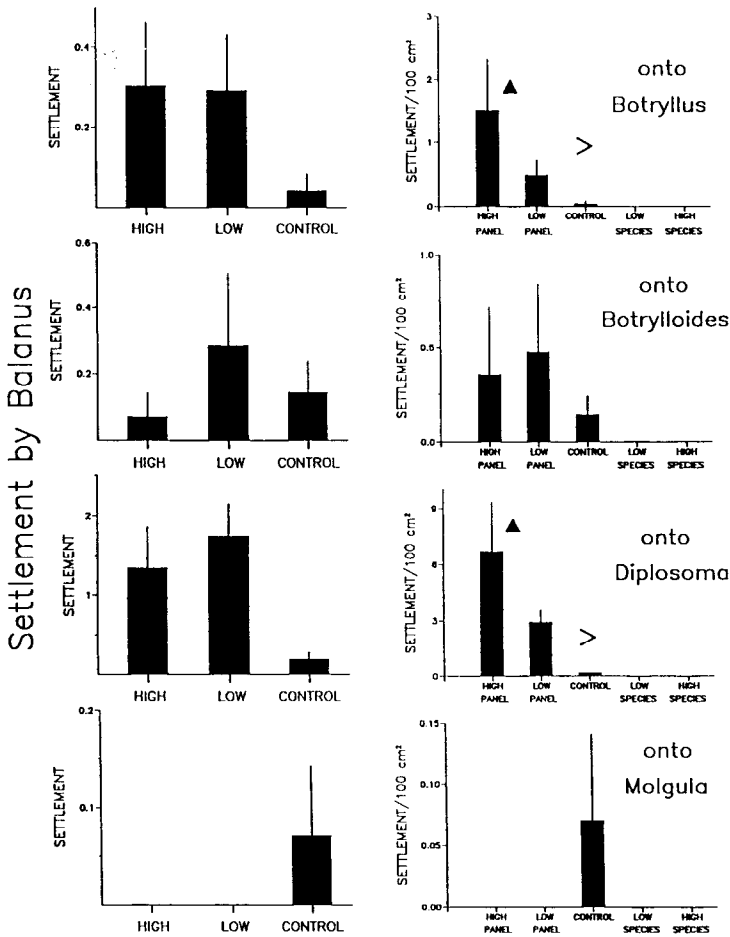


Fig. 7. Settlement patterns of *Balanus* spp. in the four sets of experiments. Description the same as Fig. 2.

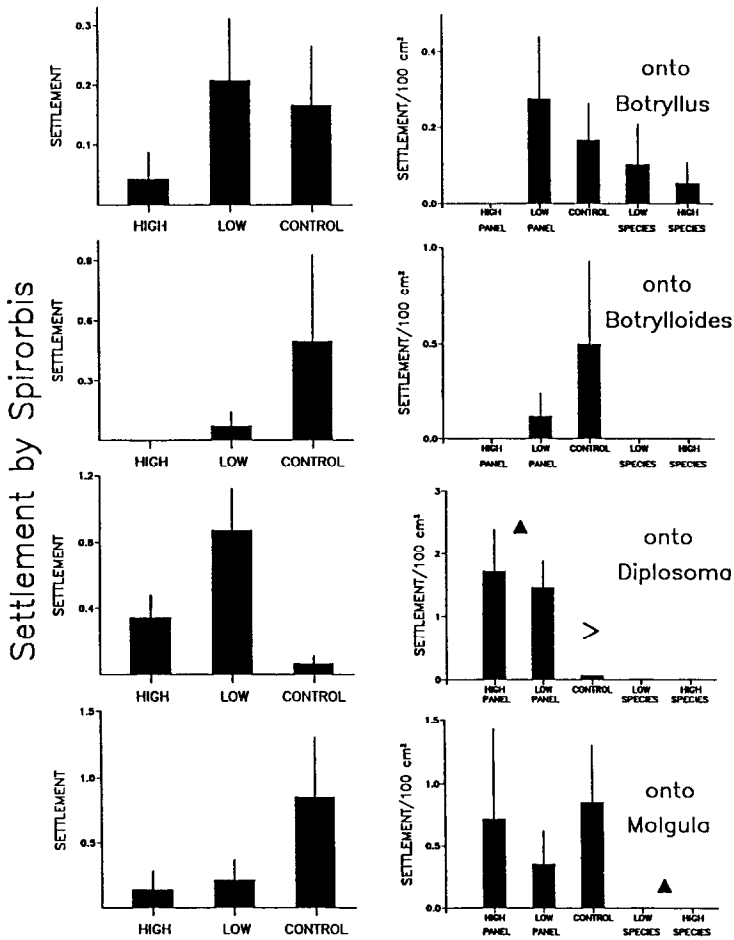


Fig. 8. Settlement patterns of *Spirorbis* spp. in the four sets of experiments. Description the same as Fig. 2.

variations in larval availability among the experiments, both the number and density of larvae settling onto panel and resident species surfaces in each experiment were converted to a proportion of the means for the controls in the same experiment. Data for settlement onto panel and resident adults were analyzed separately and differences among the four adult species in their effects on settlement were analyzed using ANOVA with data blocked by adult density. Each species of settling larvae was analyzed separately.

Although there were differences among the seven settling species in their responses to residents, two general patterns emerged (Table 1). The most consistent patterns resulted from the effects of resident *Diplosoma*. None of the species ever settled onto the surface of *Diplosoma*. This greater avoidance of *Diplosoma* or, more likely, inability to attach to its surface appears to have

Table 1

Comparison of the effects of the four species of resident adults on the settlement and settlement density of seven taxa

Settlement					Settlement density			
Species settling on panel								
<i>Diplosoma</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>
	87	65	39	26	273	118	100	71
<i>Botryllus</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Diplosoma</i>	<i>Botrylloides</i>
	46	46	35	24	120	115	98	52
<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Diplosoma</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Botryllus</i>
	264	79	62	16	515	222	208	34
<i>Cryptosula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>
	613	41	25	8	1978	109	42	14
<i>Bugula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Botrylloides</i>
	545	336	42	26	1568	889	98	83
<i>Spirorbis</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Botrylloides</i>
	600	47	25	4	1477	78	75	6
<i>Balanus</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>
	1048	541	125	0	3199	1860	291	0
Species settling on resident								
<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>
	29	14	0	0	56	25	9	0
<i>Botryllus</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>
	2	1	0	0	3	1	1	0
<i>Botrylloides</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botryllus</i>
	60	29	0	0	114	42	0	0
<i>Cryptosula</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Molgula</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Botryllus</i>
	8	0	0	0	10	0	0	0
<i>Bugula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>
	45	12	4	0	89	21	10	0
<i>Spirorbis</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Molgula</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Diplosoma</i>	<i>Molgula</i>
	17	0	0	0	32	0	0	0
<i>Balanus</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>	<i>Diplosoma</i>	<i>Botryllus</i>	<i>Botrylloides</i>	<i>Molgula</i>
	0	0	0	0	0	0	0	0

To correct for differences in larval abundances between experiments data were analyzed as a percentage of the control during each experiment. Lines connect species of resident adults which had no significant difference on the settlement or settlement density of each larval species.

resulted in most species settling in higher numbers and densities adjacent to *Diplosoma* than the other species of ascidians. Five of the seven taxa examined all settled in highest relative numbers and densities on panels with *Diplosoma*. For four taxa (*Cryptosula*, *Bugula*, *Spirorbis*, and *Balanus*) the numbers of settlers were significantly higher adjacent *Diplosoma* and for two of these (*Cryptosula* and *Spirorbis*) densities were also significantly higher. The taxa not exhibiting higher settlement adjacent *Diplosoma* were *Diplosoma*, itself, and *Botryllus*, which exhibited no differences in its settlement near the different species of residents.

The other trend was that *Molgula* and *Botryllus* generally accumulated more settlers on their surfaces than the other resident species. Relative densities of *Diplosoma* and *Bugula* were significantly higher on *Botryllus*, while *Botryllus* and *Botrylloides* settled in significantly higher densities on *Molgula*. These patterns seemed to have little effect on the pattern of settlement on adjacent panel surfaces.

Regardless of these trends, settlement on the surface of resident species was generally low. *Balanus* never settled on any of the four resident ascidians. *Spirorbis* and *Cryptosula* only settled onto *Botryllus* while *Botryllus* never settled onto any of the resident species in densities greater than 3% of those found on control panels. Several of the settling species also exhibited little or no difference in their settlement adjacent the four residents. No significant differences were found among the four resident species in their effect on the numbers or densities of *Botryllus* or *Botrylloides* settling. In addition, the densities of *Balanus* and *Bugula* settling on adjacent panel surfaces did not differ significantly among resident species.

4. Discussion

In general, the four species of resident adult ascidians tested had markedly similar effects on the settlement patterns of all seven taxa studied. In most cases settlement was highest on control substrata and decreased as the abundance of resident adults increased. The almost total lack of settlement onto the surfaces of the ascidian species contributed to this difference. Clearly, all the settling species that were investigated were able either to differentiate between panel surfaces and those biological surfaces present or were inhibited by the resident adults from attaching to their exposed surfaces. This pattern of non-settlement on residents was most evident in the presence of *Diplosoma* and, to a lesser degree, *Botrylloides* (Table 1). Both of these species have relatively thin, “unfirm” tunics which may not have been recognized as substrate.

Regardless of mechanism, this larval substratum discrimination or resident-mediated settlement inhibition did not completely account for the observed settlement patterns. In 25% of the 28 cases examined the density of settlement onto panel surfaces did not remain constant as might be predicted, but increased significantly as the density of resident adults increased (Figs. 2–8, Table 2). An additional 25% of the cases exhibited this same density increase adjacent

Table 2

Summary of the relationship of settlement on panel surfaces adjacent residents to settlement on control panels. Apparent patterns, based on visual inspection of Figs. 2–8, and significant patterns are tabulated

	Equal	Greater	Less
<i>Apparent patterns</i>			
<i>Numbers:</i>			
<i>Botryllus</i>	2	1	4
<i>Botrylloides</i>	2	0	5
<i>Diplosoma</i>	1	4	2
<i>Molgula</i>	2	0	5
Total	7	5	16
<i>Density:</i>			
<i>Botryllus</i>	2	4	1
<i>Botrylloides</i>	3	2	2
<i>Diplosoma</i>	0	6	1
<i>Molgula</i>	3	2	2
Total	8	14	6
<i>Significant patterns</i>			
<i>Numbers:</i>			
<i>Botryllus</i>	3	1	3
<i>Botrylloides</i>	3	0	4
<i>Diplosoma</i>	1	4	2
<i>Molgula</i>	3	0	4
Total	10	5	13
<i>Density:</i>			
<i>Botryllus</i>	6	1	0
<i>Botrylloides</i>	5	1	1
<i>Diplosoma</i>	2	4	1
<i>Molgula</i>	5	1	1
Total	18	7	3

residents, even though the differences were not significant. This pattern suggests an enhancement or aggregation of settlement on open surfaces near resident adults. Nevertheless, no density-dependent mechanisms are necessary to account for these settlement patterns. For discrete habitats such as boulders, rocks, cobbles, or the panels used in this study, observed settlement patterns can simply be a consequence of settling larvae discriminating between substratum types coupled with the subsequent movement, whether passive or active, of any larvae that fail to attach. For example, equal densities on all panel surfaces would result if all larvae encountering the panel surface settled and all larvae that contacted the test species re-entered the water column and moved on without finding the panel surface. However, if larvae had limited mobility (e.g. re-entered the water column for a short time) or a localized searching behavior (e.g. Davis & Butler, 1989), then it is possible that those encountering the test species would move only a short distance before attempting to settle and a portion would encounter the panel surface. In this case, the densities on panel surfaces next to species may actually be enhanced over those found on control substrata. Thus, it is possible

that many observed non-random patterns of settlement in our experiments could result simply from larvae not recognizing a test species as substratum rather than from any active interaction such as predation or alteration of currents.

The effect of these actions on settlement can be demonstrated in a simple model where larval discrimination (or inhibition) is represented as a probability of settlement when contacting either the substratum (s_s) or a resident adult (s_a), and the mobility of larvae is represented as the probability of those larvae not attaching being lost by re-entering the water column and moving or being moved away from the substratum (m). Assuming some initial number of available larvae (L_0) the number settling onto the open substratum surface (N_s) will be:

$$N_s = L_0 \cdot (1 - a) \cdot s_s + \sum L_t \cdot (1 - a) \cdot s_s$$

and the number settling onto adults (N_a) will be:

$$N_a = L_0 \cdot a \cdot s_a + \sum L_t \cdot a \cdot s_a$$

where a = proportion of substratum covered by adults; s_a = probability of settling on adult; s_s = probability of settling on substratum; L_t = number of larvae remaining at time $t = L_{t-1} \cdot (1 - m) \cdot (((1 - s_a) \cdot a) + (1 - a) \cdot (1 - s_s))$.

If we assume that all larvae that contact the natural substratum will attach ($s_s = 1$) then:

$$L_t = L_{t-1} \cdot a \cdot (1 - s_a) \cdot (1 - m) \text{ and}$$

$$N_s = L_0 \cdot (1 - a) + \sum L_t \cdot (1 - a) .$$

Based on our experiments, the probability of settlement onto adults of any of the four species of ascidians tested is extremely small. Assuming this to be a constant (e.g. $s_a = 0.1$, Fig. 9), it is then clear that the mobility of the larvae that fail to attach (m) will control the pattern observed among the various treatments. As the mean distance that larvae move increases beyond the radius of a substratum, m will approach one and most larvae that initially contact an adult surface will be lost before having another chance to contact any other surface on the same substratum. This would result in a sharp decline in settlement onto panel surfaces from control to high-density treatments, but no difference in settlement density (Fig. 9). However, as the distance that larvae move between attempts at attaching decreases, m will approach 0, and an increasing proportion of larvae that initially contact adult surfaces will have one or more additional chances to attach to suitable sites on the substratum. This low mobility will result in a shallower gradient among the treatments in settlement, but a steep, increasing gradient in the density of settlement from control to high-density treatments (Fig. 9).

Fig. 9 represents a fairly complete range of settlement patterns that could be expected, assuming that larvae avoid settling onto adults and some percentage of larvae that contact adults make subsequent attempts to settle on the particular substrate. Most (75–90%, Table 2) of the settlement patterns that we observed on panels were within the range of those generated by the model. This suggests that

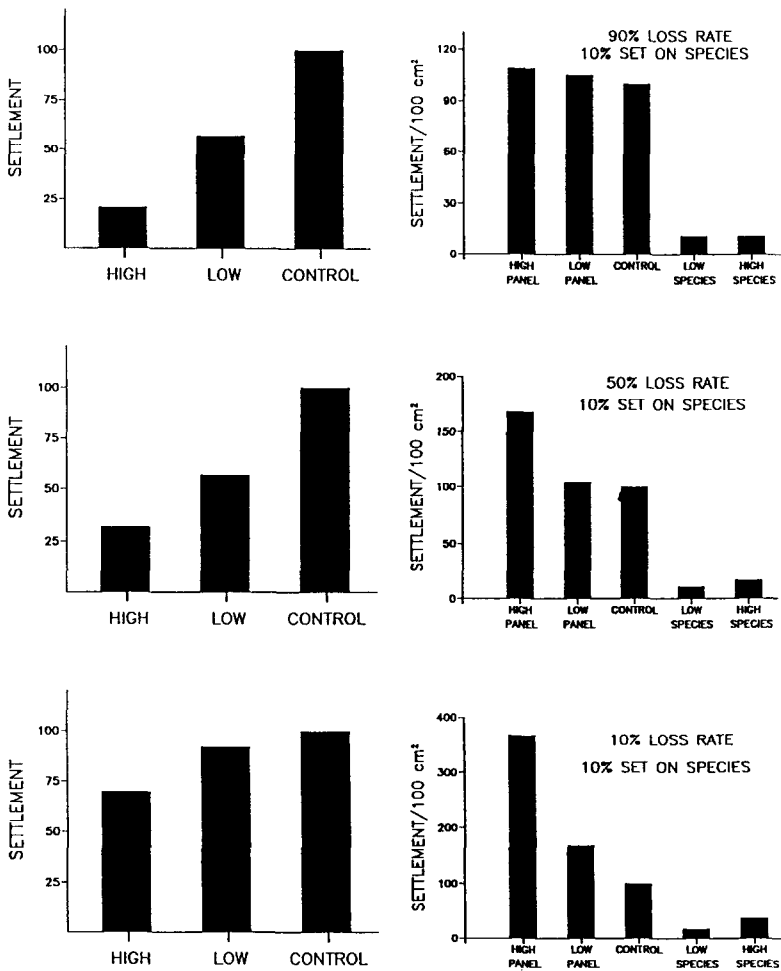


Fig. 9. Patterns of settlement and settlement density based on the settlement model. In all three cases it was assumed that all larvae that contacted panel surfaces attached and that only 10% of those that contacted surfaces of test species attached to these surfaces. Three loss rates (90, 50, and 10%) were assumed for those larvae that did not attach and larvae not lost could make another attempt at settling. It was also assumed that a resident species covered 90% of the surface of high-density panels and 50% of low-density panels.

the major and most common influence of the resident adults examined was to decrease the useable substratum available to settling larvae.

The seven taxa of larvae were all fairly similar in their settlement patterns, even though there were large taxonomic differences. Except for *Balanus* cyprids, all larvae were lecithotrophic, brooded by adults, and probably settled within a few hours of being released. They ranged in size from the small 0.1 mm diameter larvae of *Spirorbis* to the moderate-sized larvae of *Bugula* (0.2 mm), *Cryptosula* (0.2 mm), and *Balanus* (0.6 mm long) to the ascidians which varied in diameter

(*Botryllus*–0.35 mm, *Diplosoma* –0.7 mm, and *Botrylloides*–1.0 mm) but had similar lengths exceeding 2.0 mm. Given these differences in larval type and size, it is likely that both larval swimming strength and motility would also vary among the species. Regardless, there seems to be little in their settlement patterns that can be correlated with these differences.

The only patterns that differed significantly from those that could be generated by the settlement model were the significant reductions in settlement densities compared to control panels for *Cryptosula* settlement adjacent *Molgula*, *Botryllus* adjacent *Botrylloides*, and *Diplosoma* adjacent adult *Diplosoma*. In addition, *Cryptosula* and *Spirorbis* adjacent *Botrylloides*, and *Balanus* adjacent *Molgula* displayed similar, but non-significant patterns. This is the type of pattern we would expect in the presence of a species that either preys on larvae or inhibits their settlement in its vicinity. In the laboratory we have observed the ingestion of oyster larvae by *Molgula* as well as by other solitary ascidians such as *Ciona intestinalis* and *Styela clava* (Osman et al., 1989). Therefore, it is possible that *Molgula* and perhaps *Botrylloides* decreased the settlement of some taxa by preying on larvae. However, with overall low settlement rate observed for *Cryptosula*, *Spirorbis*, and *Balanus*, these results are only suggestive. In the case of *Diplosoma* it is certainly possible that the ascidian avoided settling near adults of its own species. However, *Diplosoma* is a very fast growing and somewhat loosely aggregated colony and it is also possible that some newly-settled individuals were seen as part of adult colonies and not counted.

For the most part our data support Young's (1989) conclusion that there is at present little evidence outside of the laboratory for any significant effect of larval predation by resident adults on settlement or recruitment. Predation on larvae has been observed in the field. For example, Davis & Butler (1989) reported that <5% of the larvae of the ascidian *Podoclavella moluccensis* followed in the field were consumed before they could settle and Stoner (1990) observed that 29% of the larvae of the ascidian *Diplosoma similis* were eaten. However, in 2–4 week field experiments designed to measure the effects of barnacles (Young & Gotelli, 1988) and ascidians (Young, 1989) on the recruitment dynamics of a variety of sessile invertebrates, the residents had little effect. Our experiments demonstrate strikingly similar patterns for settlement measured over a 24-h period.

Although there is some question as to how well laboratory experiments predict patterns of settlement in the field (Young and Gotelli, 1988; Young, 1989), the results of the present settlement experiments are very similar to those of earlier laboratory experiments conducted using a similar experimental design (Osman et al., 1989). In those experiments we examined the effects of encrusting ascidians (*Botryllus* and *Botrylloides*), barnacles (*Balanus*), bryozoans (*Schizoporella errata*, *Cryptosula*, and *Bugula*), and a solitary ascidian (*Ciona intestinalis*) on the settlement of the oyster *Crassostrea virginica*. In experiments with bryozoans and encrusting ascidians there was little oyster settlement on the species and elevated densities (compared to controls) on primary substrate next to the attached adults. These patterns are identical to those found in the present study. Settlement patterns in the barnacle experiments differed somewhat in that the oyster larvae

(which are attracted to CaCO_3 substrata) settled on the barnacles as well as the primary substratum. Increasing s_a in the model produces a pattern similar to that seen in the experiments. Only in the experiments with *Ciona*, which was observed preying on oyster larvae, was the settlement pattern distinct from those produced by the model. However, it was very similar to that observed for present *Cryptosula* settlement in the *Molgula* experiments.

Based on both the field and laboratory experiments, it appears that the most common effect that resident adults have on settlement is the usurpation of primary substratum. Settlement onto the exposed surfaces of most species is clearly reduced over that observed onto the primary substratum. This was seen in the field for species settling in both high and low densities as well as in the laboratory (Osman et al., 1989) where densities were high and larval movement restricted. The other common pattern was the enhancement of settlement densities on primary substrate adjacent to most species of adults. Given that most species of larvae have some ability to discriminate between types of substratum (e.g. Thorson, 1966; Crisp, 1974; Scheltema, 1974), this enhancement can result from limited movement after rejecting a resident species as a suitable substratum. For example, Davis & Butler (1989) found a 100-fold decrease in the distance larvae moved after they made first contact with a substratum. However, reduced movement does not have to result from a single mechanism. It could result from active crawling or short-distance swimming or saltation along the bottom. It could also result from passive transport resulting from re-entering the water column for a brief period.

Some species, such as barnacles, may present substratum that is indistinguishable from or selected over primary substratum. The presence of these species could further enhance settlement. It is also possible that larval predators, such as solitary ascidians, hydroids, anemones, etc. may cause significant reductions in local settlement. However, the effects of these predators on settlement may be extremely difficult to see in the field. Given that measurable longer-term effects of larval predation on recruitment have also been difficult to find (Young & Gotelli, 1988; Davis & Butler, 1989; Young, 1989), larval predation may not be a generally important process affecting settlement, recruitment, and subsequent community development.

Acknowledgements

This work was supported by a grant from the Jessie B. Cox Charitable Trust and NSF grants OCE-9101815 and OCE-9123890. We thank L. Sedgwick, P. Mitchell, A. Frese, M. Holt, R. Malatesta, and B. Lussier for their assistance in the laboratory and the field, G. Gernier for help in constructing experimental apparatus, R. Malatesta and R. Zajac for discussions and criticism along the way, and D. Breitburg, A. Davis, and an anonymous reviewer for their comments on earlier versions of this manuscript. Contribution No. 278 to the Marine Sciences Institute, University of Connecticut.

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