The Effect of Species Richness, Resource Availability, and Community Composition on Larval Recruitment in Marine Fouling Communities

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Abstract　 Larval recruitment into the invertebrate communities that occupy marine hard substrata (marine fouling communities) has received considerable attention in the ecological literature.　While many studies have focused on the effect of a single factor on determining recruitment, few have attempted to assess the relative biological importance of multiple factors simultaneously. This study compares recruitment into naturally assembled fouling communities which differ by three factors: the area of their substrate that is initially open to larval recruitment (resource availability), the kinds of species they contain (community composition), and the total number of species they contain (species richness).　I found that while species richness and resource availability had no detectable effect on larval recruitment, species composition of the resident community was highly important.　Specifically, the anemone *Metridium senile* appears to be of profound (and varying) effect on larval recruitment.　In the early stages of the experiment, when all treatments contained unoccupied substrate available for recruitment, treatments with *Metridium* present received less recruitment; in later stages, communities with *Metridium* received greater recruitment.

Introduction

Larval recruitment into the invertebrate communities that occupy marine hard substrata (marine fouling communities) has received considerable attention in the ecological literature (see Stachowicz and Byrnes 2006 for a review). While many studies have focused on the effect of a single factor on determining recruitment, few have attempted to asses the relative biological importance of these factors by comparing multiple factors simultaneously. Varying a community parameter such as richness may affect recruitment in a tightly controlled laboratory experiment, but does this effect actually play a role in natural communities under natural conditions? This study compares recruitment into fouling communities which differ by three factors: the area of their substrate which is initially open to larval recruitment (resource availability), the kind of species they contain (community composition), and the total number of species they contain (species richness).

These particular factors may act on larval recruitment in a number of ways. First, larvae may actively choose to settle at a given place (Walters 1992) based on one or more factors. Active larval choice has been demonstrated in response to chemical signals produced by resident species (Zimmer-Faust and Tamburii 1994) and substrate shape (Keough & Downes 1982). Because the amount of unoccupied substrate available at a potential recruitment site should affect a recruit's chance of growing into a reproductive adult, I expect that regions with greater amounts of unoccupied substrate will be actively chosen by the larvae of some species.

Second, larvae may experience different levels of pre- or post- settlement mortality because of a factor. Many common fouling species are filter feeders, and it is likely that adults of these species ingest and kill waterborne larvae. This could reduce settlement in regions immediately surrounding high volume filter feeders. Established recruits may also be threatened by specific neighboring species; after settlement, overgrowth of recent recruits by fast growing species is commonly observed (e.g. Bullard et al. 2004).

Finally, it is possible that larvae are influenced in their settlement by the species richness (total number of unique species) in a given region. This factor could act through active larval choice: larvae may be repelled by diverse groups because more diverse groups better occupy available substrate over time, leaving fewer resources available to recruits (sensu Stachowicz *et al.* 2002). Species richness could also be an important factor because of pre- or post- settlement

mortality: more diverse groups may more consistently overgrow recruits, or more efficiently filter larvae from the water column.

Larval recruitment can be a crucial factor in determining the dynamics of a fouling community: established larval recruits either cause or benefit from the mortality of adult individuals by consuming their resources, and only species which successfully recruit and grow will have the chance to provide the next generation of larvae. Understanding the community factors which affect recruitment is crucial in understanding the ecology of fouling communities.

Larval recruitment into fouling communities is also interesting from the perspective of invasion by non-native species. Factors which determine larval recruitment into a community may also be important in determining the community's "invasibility," or susceptibility to invasion by non-native species. In order for a non-native to successfully invade a community, its larvae must successfully settle, recruit, and grow to reproductive age. The mechanisms underlying invasibility appear to be increasingly important in light of recent major marine invasions.

Species richness, discussed above as a possible influence for larval recruitment in fouling communities, has received considerable attention as a possible determinant of community invasibility (Levine and D'Antonio 2001). On the one hand, a large body of work suggests that the relationship between species richness and invasibility is negative; more species in a community decreases the community's invasibility. These studies are generally tightly controlled experiments which directly manipulate species richness (e.g. Stachowicz *et al.* 2002). Though terrestrial plant communities compose the bulk of the literature (e.g. Tilman *et al.* 2006, Levine 2000), a key experiment in marine subtidal fouling communities has also found the same relationship (Stachowicz *et al.* 2002).

On the other hand, an equally large and thorough set of research, generally composed of observational surveys across diverse habitats, finds that species richness is directly related to invasibility; more native species in a community leads to more invasion events. Again, though largely based in terrestrial plant communities (Wiser *et al.* 1998), notable work in the marine subtidal (Dunstan and Johnston 2004) has found the same positive relationship.

One explanation of the discrepancy between the contradictory richness-invasibility relationships described above suggests that richness affects invasibility, but that this effect is very small in comparison to other community parameters. Because both natives and non-natives

tend to exist in larger numbers in broadly similar environmental conditions generally favorable for life (e.g. high nutrient and energetic availability, available space), native and non-native species alike should be found in similar abundance at a given point (Levine 2000). This common dependence on other community factors is manifest in a positive correlation between native richness and non-native richness at large spatial scales.

The dichotomy in findings on the richness-invasibility relationship appears to support this explanation. Studies that find richness to be important and negatively related to invasibility are generally tightly controlled experiments with controlled levels of environmental factors, and studies which find richness unimportant (or positively related to invasibility) are large scale surveys across a range of environmental factors (Levine and D'Antonio 1999, Levine 2000). This trend holds true inside some individual research efforts which couple experiments with surveys; a negative richness-invasibility effect is clear in experiments but a positive relationship is found by larger scale surveys (Levine *et al.* 2002). Note that at least one survey coupled to an experiment has found the same negative richness–invasibility relationship as the experiment (Stachowicz 2002).

The logical approach to the problem of the richness-invasibility relationship is to test the effect of species richness across varying environmental conditions. In a review, Stachowicz and Byrnes (2006) speculate that such a test would show richness to be an important factor on invasibility primarily in situations of low resource availability, though their reasons for this are not entirely clear. They may be working under the assumption that any community which does not consume a relatively large fraction of the resources available to it must be mediated by some external environmental factor, i.e. disturbance.

Marine fouling environments are an excellent location for such an experiment. As opposed to terrestrial communities, in which available levels of energy, unoccupied substrate, and several nutrients may all limit propagule success and growth, fouling communities appear to be limited by only substrate availability (Sutherland and Karlson 1977) (evidence suggests that food supply can be limited by changes in water currents, but this appears to be effective only at the millimeter scale and below (Buss 1979)). The sessile nature of fouling communities allows for selective grooming to set species richness and compositions, as well as to set amounts of initially unoccupied substrate.

I examined the relative importance of i) species richness, ii) resource availability, and iii) community composition on larval recruitment by monitoring larval recruitment to controlled marine fouling communities. I groomed naturally assembled marine invertebrate communities to constant species compositions and constant levels of species richness and initially unoccupied substrate. Based on the literature, I expected that species richness would have a detectable effect on recruitment, but that this effect would be much smaller than that of community composition. I expected that species specific effects would be a major effect, as certain individual species (e.g. high volume filter feeders) might be of disproportionate effect on larval settlement.

Because introducing a new invasive species to the environment in which this experiment took place was not practical or ethical, I could not monitor an actual invasion by a novel species. Rather, I observed recruitment of larvae naturally present at the experimental location (these species were generally natives, though several appear to be well established invasives). As the mechanisms of invasion may differ from "normal" recruitment by the larvae of established community members, this approach does not provide direct information on invasion (Stachowicz 2006). However, the mechanisms of invasion may also be very similar to non-invasive recruitment, and understandings of the way existing communities affect larval recruitment may cast light on invasibility issues.

My results suggest that while richness and substrate availability are not important determinants of recruitment, community composition is of strong effect on recruit density. This effect may be owed in part to the presence or absence of a particular species, the anemone *Metridium senile*.

Methods

Experimental Apparatus I created communities of controlled richness, initially unoccupied substrate, and composition by selectively grooming sessile invertebrate communities grown on fouling panels. The panels were constructed from 10 X 15 cm black ABS plastic, with the working surface either roughened with sandpaper or naturally rough from the manufacturing process. No major (>2mm relief) topography existed on any panel. The panels had been constantly located in the water at the study site for 3-5 years prior to the start of the experiment. Some panels had been experimentally manipulated in previous studies, while others had never been physically disturbed. The sessile invertebrate communities present on a panel varied

greatly between panels. These between-panel differences in residents may be result of the varying ages of the panels – some have been in the water at the marina longer than others – or of past experiments conducted on some of the panels, or simply of natural variation.

Groups of twenty panels were attached by a stainless steel bolt passed through a hole in their center to a 2m X 1m rack constructed of 1" pvc (Fig. 1). The racks were then suspended from the floating docks such that the lower face of each panel was parallel to the surface of the water and at a constant 1m depth below the surface.

Figure 1: Fouling panels attached to the PVC rack. Each panel is 10cm X 15cm. The rack is normally suspended under the floating dock such that the surfaces of the panel which are visible in this picture face downward.

Experimental Design

In early June 2006 all panels were removed from the marina and relocated to the Telonicher marine lab. Once there, I examined the panels with the intent of grouping them by their resident species. While the exact composition of resident species on each panel varied, many panels did contain similar sets of species. I established a sorting criterion for panels based on the number and type of species they contained: panels were sorted such that all panels within a group contained at least the species listed in that group's criteria. While I would have preferred to create the criteria for sorting communities based on the ecological role of residents and their

specific effects, the panels naturally contained only certain sets of species, leading to the use of the criterion described in Table 1.

Table 1: Sorting criteria for panel grooming. Panels were sorted such that each panel in a group contained at least the species described below.

COMMUNITY A	COMMUNITY B	COMMUNITY C	COMMUNITY D
Mytilus *Metridium*	*Botrylloidies* *Halichondria (Haliclona?)*	*Mytilus* *Botrylloides* *Haliclona/chondria* *Metridium*	*Mytilus* *Botrylloides* *Haliclona/chondria* *Balanus*

By physically removing all animals which were not part of a group's shared set of species, I created a set of treatments which differed from each other, but were internally homogenous.

After grooming, four different communities – two of 2 species (low richness) and two of 4 species (high richness) – were all present at both high levels of unoccupied substrate (75% +/- 0.16% (mean +/- s.e.) of panel) and low levels of unoccupied substrate (41% +/- 0.33% of panel), creating a total of eight distinct treatments of five replicate panels each (Fig. 2).

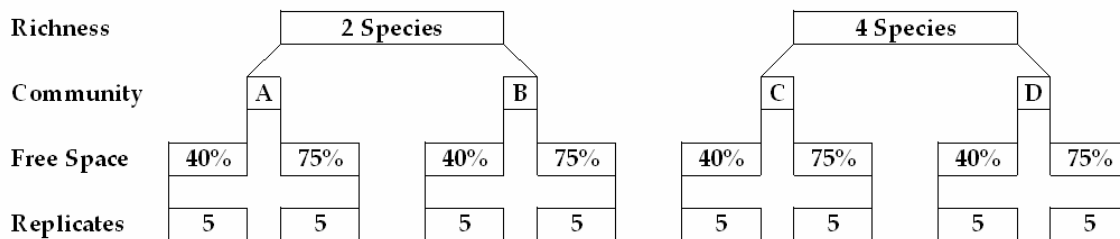| Richness		2 Species				4 Species		
Community	A		B		C		D	
Free Space	40%	75%	40%	75%	40%	75%	40%	75%
Replicates	5	5	5	5	5	5	5	5

Figure 2: Experimental design. Each treatment contained a certain set of either 2 or 4 species at either low or high levels of panel cover. For description of the species resident in each community see Table 1.

After 6 days of grooming in the wet lab, the panels were digitally photographed and returned to the marina. The panels remained on the racks in the marina at all times except for sampling.

Sampling Panels were sampled every 9-11 days. At each sampling interval, all panels were relocated to the marine lab, photographed, and returned to the marina within 11 hours. Relocation typically lasted less than 90 minutes, during which time the panels were stored in plastic bins filled with seawater from the study site. Panels were occasionally noted to be of different species composition than they had originally been groomed to (either because of

intrusion by mobile species or the death of intended residents) but were not re-groomed to their original state for logistical reasons.

The resulting photographs were examined on computer monitors. Each picture was examined for the presence of larval recruits. Once located, larval recruits were identified to genus and counted, and a tag was placed over their location on the picture. Counts of these tags provided information on the number and kind of recruits which settled at each sampling interval. For simplicity of labeling, all recruits which landed between days 0 (start of experiment) and 10 were called Cohort 1; all new recruits between days 10 and 20 were called Cohort 2, and all new recruits between days 20 and 30 were Cohort 3.

Statistics   I analyzed recruitment density (dependent variable) against species richness, initial unoccupied substrate, and community composition (independent variables) in a three-way orthogonal ANOVA. Because recruitment was split into three cohorts, I repeated this ANOVA analysis for Cohort 1, Cohort 2, and Cohort 3.

I also conducted a Classification and Regression Tree analysis on the same dependent and independent variables. A CART attempts to split the data into the most internally homogenous and externally heterogeneous groups possible by using whatever independent variable best separates the data.   The first independent variable used to split the data is the variable which explains most of the variation in the data set, while secondary and tertiary splits employ variables which explain less variance. This analysis is useful because it clearly reveals the magnitude of each independent variable's effect on recruitment density.

Experimental Location   The study site was located at Woodley Island Marina, located inside of Humboldt Bay, Northern California, USA (Coordinates: 40°48'26"N, 124°09'45"W). Environmental conditions near the marina are seasonally variable, ranging from brackish conditions at peak freshwater input in spring to saline in summer and late autumn. Turbidity is typically very high. Tidally driven water exchange provides the dock with consistent water flow.

Results

ANOVA Recruitment density into Cohort 1 differed significantly with community composition. Unoccupied substrate and richness also had some effect on recruit density, and their effects were interactive (there was a significant unoccupied substrate:richness interaction term).   Recruitment density into Cohort 2 showed no significant effects from any term.

Recruitment density into Cohort 3 was significantly affected by only the community composition term.

Table 2: A repeated orthogonal three-way ANOVA for recruit density into each cohort.

Effect		Df	Mean Sq	F Value	Pr(F)
Cohort 1	Richness	1	0.00	0.26	0.62
	Community	**2**	**0.16**	**12.05**	**1.18E-04**
	Unoccupied Substrate	1	0.05	3.74	0.06
	Unoccupied Substrate:Richness	**1**	**0.11**	**8.07**	**0.01**
	Community:Unoccupied Substrate	2	0.01	1.01	0.37
	Residuals	33	0.01		
Cohort 2	Richness	1	0.00	0.00	0.96
	Community	2	0.02	0.88	0.42
	Unoccupied Substrate	1	0.08	3.39	0.07
	Unoccupied Substrate:Richness	1	0.00	0.06	0.81
	Community:Unoccupied Substrate	2	0.01	0.48	0.62
	Residuals	33	0.02		
Cohort 3	Richness	1	0.00	0.45	0.51
	Community	**2**	**0.04**	**4.08**	**0.03**
	Unoccupied Substrate	1	0.00	0.01	0.93
	Unoccupied Substrate:Richness	1	0.00	0.13	0.72
	Community:Unoccupied Substrate	2	0.01	1.01	0.38
	Residuals	33	0.01		

The following univariate figures describe the effects found by the ANOVA. Each graph compares recruitment into the different treatments of a given factor for the three cohorts. Statistical significance estimates are from t-tests.
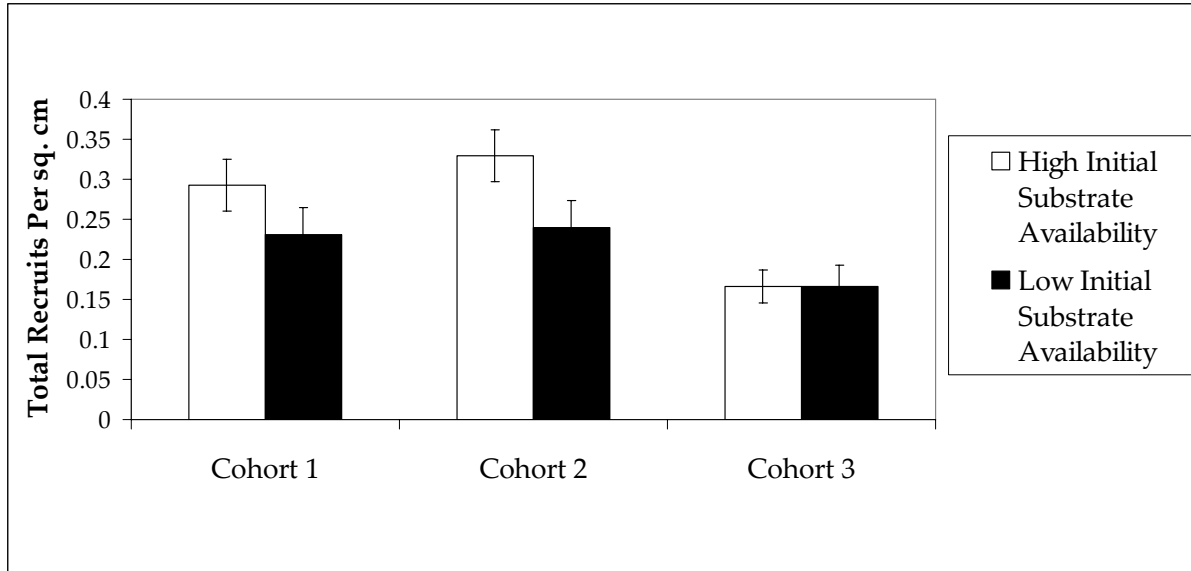
Figure 3: Recruitment density does not vary significantly between varying levels of initially unoccupied substrate for any sampling interval. Cohort 1 consists of all larvae which recruited to the panels between days 0 and 10 (measured from the start of the experiment); Cohort 2 contains all larvae which recruited between days 10 and 20; Cohort 3 contains all larvae which recruited between days 20 and 30.

Total recruitment density for all larvae did not differ significantly between any richness treatments for any cohort (Fig. 4).
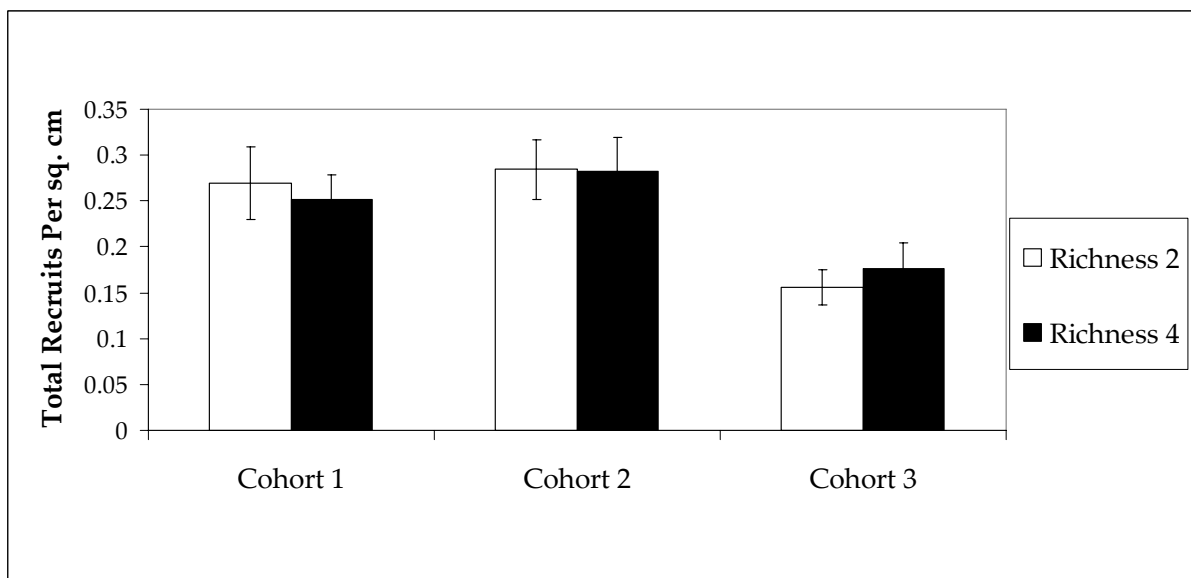
Figure 4: Recruitment density for treatments of Richness 2 and Richness 4.

Total Recruitment density for all recruits did vary with different community compositions. In Cohort 1, communities A and C received significantly less recruitment than communities B and D, (t-test, df = 39, p<0.01), while in Cohort 3, communities A and C received significantly more recruitment than communities B and D (df = 39, p<0.05) (Fig. 5).

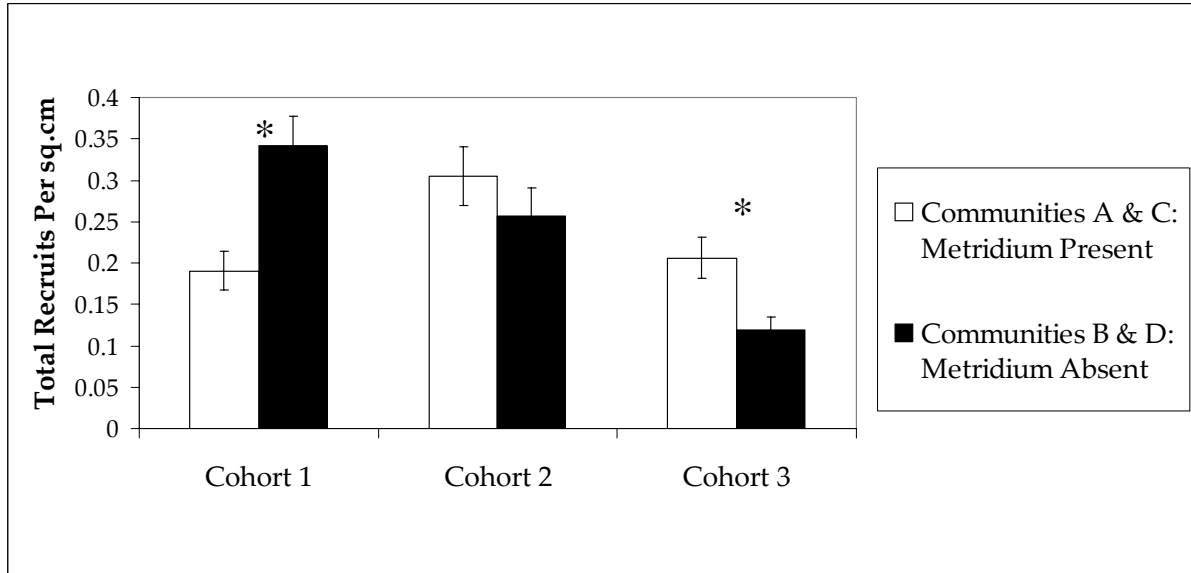Figure 5: Community composition affects recruitment density in Cohorts 1 and 3. A star indicates significant differences between treatments in a cohort.

Multivariate Techniques Analysis by Classification and Regression Tree (JMP 5.1 Statistical Package) shows that of all possible explanatory variables, community composition best predicts recruitment density for Cohort 1. Unoccupied substrate and richness interact within this effect to explain some remaining variability in recruitment density; however this result is not statistically strong. (Fig. 6)
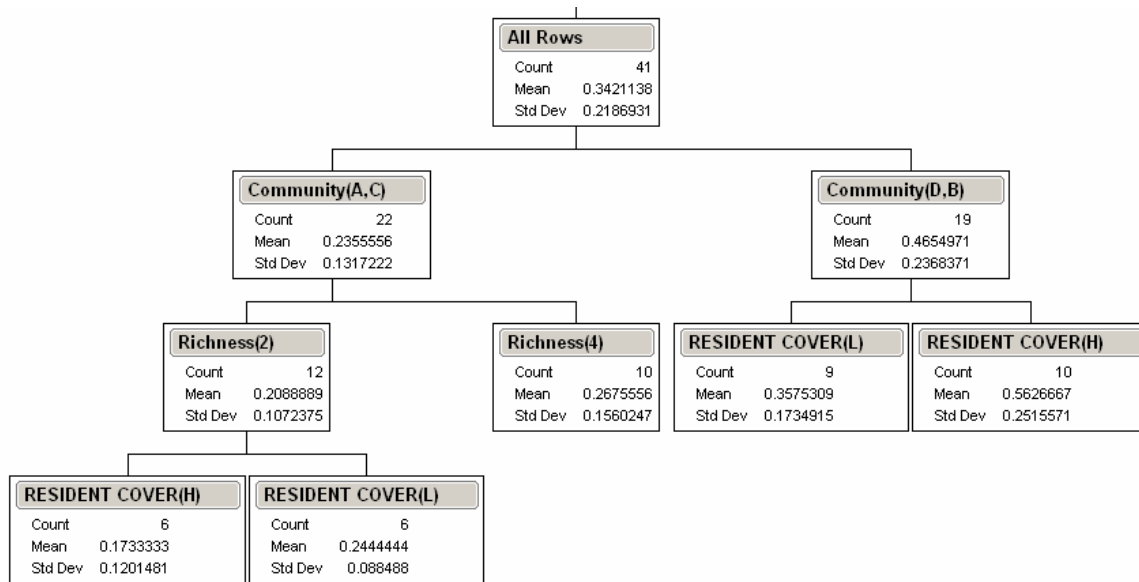
Figure 6: Classification and Regression Tree (CART) analysis of the recruitment density data for Cohort 1. This CART first split the data using community composition variable to separate communities with *Metridium* (A and C) from communities without *Metridium* (D and B). The next split in the tree indicates that Richness and Resident Cover may each have an interactive effect with Community Composition, but these trends are not as significant as the Community Composition trend.

Discussion

This project investigated three possible determinants for larval recruitment into temperate marine fouling communities: species richness, resource availability (in the form of initially unoccupied substrate), and species composition. Of the three factors tested, community composition was the only factor to have a statistically significant, independent effect on larval recruitment density in any cohort (Table 2, Fig. 6). Resource availability and Species Richness have an interaction effect in Cohort 1, but this effect is not found in other cohorts (Table 2, Fig. 6).

The interaction effect of resource availability and richness is difficult to interpret. According to the CART, the majority of the variance in recruitment can best be explained by the community composition factor, with any interaction between initially unoccupied substrate and species richness explaining much less of the total variance (Fig. 6). This is also suggested by community composition's higher Mean Sq. term in the ANOVA (Table 2). Both models predict that the community composition term is of large effect on recruitment density.

It is possible that the effect of community composition may be due to the presence of the anemone *Metridium senile*. Communities A and C, which received significantly less recruitment than communities B and D in Cohort 1 and significantly more recruitment in Cohort 3, both contain *Metridium*, while communities B and D do not. The presence of *Metridium* might be important to recruits for a number of reasons: *Metridium* are large, arborescent filter feeders whose tentacles sting and entrap planktonic organisms, possibly including larvae. *Metridium* also pose a threat to recently settled recruits; during the experiment, *Metridium* were frequently observed moving around the experimental panels, smothering and killing the comparatively small recruits in their path. Finally, *Metridium* reproduce by fission, producing many small clones, which were observed to move around the panel, occupying previously available substrate and killing recruits by moving over them.

The changing effect of community composition through time (comparatively less recruit density in Cohort 1, greater recruit density in Cohort 3) may also be an effect of *Metridium* presence, because *Metridium* presence may control the amount of time in which unoccupied substrate is available for recruitment. While this study did not find that varying levels of initially unoccupied substrate had any effect on recruitment density, it does not follow that unoccupied substrate at the time of recruitment is not important for recruitment; each recruit needs a place to settle. If there are fewer recruits entering a community with *Metridium* at any given time, these communities may be colonized more slowly than colonies without *Metridium*. Consequently, communities with *Metridium* might offer much greater levels of the unoccupied substrate resource to recruits in later cohorts. Even though fewer larvae from later cohorts will be able to touch down on the panels containing *Metridium*, those that do would be more likely to find unoccupied substrate in which to settle, and thus *Metridium* panels would show greater recruitment in later cohorts. This could also explain the absence of any significant independent factors in Cohort 2, as in this interval the effect of *Metridium* on incoming larvae may have been balanced by the increased odds of success for larvae that did touch down on a panel.

While these explanations for the observed effect of species composition are compelling, it is important to note that they are only one possibility. As this study was not designed to test for the effect of *Metridium*, no definitive causal link between the observed pattern and *Metridium* presence is possible at present. However, the high likelihood of the *Metridium* effect does suggest some interesting aspects of the community composition factor.

The experimental communities created in this study are superficially similar to each other: all are dominated by sessile filter feeders of varying longevity and growth rate, and all are devoid of primary producers. Yet patterns of larval recruitment into the communities vary substantially, possibly because of the presence of a single species (*Metridium*) which is, at first approximation, functionally similar to other species in the study.   Clearly, if *Metridium* is responsible for the differing patterns of recruitment, then characterizing it as being similar to the other species in this experiment would be a major error, at least so far as recruitment is concerned. In a sense, this is what the species richness idea does. By assuming that a community can be modeled by simply summing the number of species it contains, irrespective of the particular features of each species, the species richness metric assumes that all resident species should have, on average, the same effect on recruitment. This study suggests that this is not a valid assumption.

While this study has not disproved the possibility of effects on larval recruitment from either initial resource availability or species richness, it does suggest that these factors are not as important as community composition in determining larval recruitment.   It is entirely possible that either (or both) species richness and resource availability had some effect on recruitment. Any effect, however, was so much smaller than the community composition effect as to be indistinguishable from stochasticity.

Stachowicz *et al.* (2002) showed that species richness could effect recruitment by larvae of invasive species into communities under tightly controlled laboratory experiments. However, correlational studies in natural systems have consistently failed to find the same negative relationship between species richness and invasibility (Dunstan & Johnston 2004). Invasion is a unique process and probably different from the kind of recruitment quantified in this study (Stachowicz 2006); however, if the invasive species are indeed responding to similar community parameters as the species in this study, then this discrepancy could be explained by the relative unimportance of species richness in determining the patterns of settling larvae.

The interpretations of this study could be expanded by further investigation. Understanding the mechanism by which *Metridium* affects larval recruitment and examining how specific species of recruits respond to different community parameters might allow for a greatly improved understanding of fouling ecology. However, at this juncture a broad trend is evident: the species specific attributes of resident species can be of profound effect on the recruitment of

larval invertebrates into a temperate marine fouling community, and appear to be a primary factor in determining recruit density.

Acknowledgements

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