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3 Effects of early recruits on temperate sessile marine community
4 composition depend on other species recruiting at the time

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13 ABSTRACT

14 In many environments recruitment of dispersive propagules (e.g. seeds, spores and
15 larvae) can vary from situations when particular taxa recruit in relative isolation to times when
16 they recruit simultaneously with other, functionally quite different taxa. Differences in the
17 identity and density of recruiting taxa can have important consequences on community
18 structure, but it is still not clear how the effects of individual taxa on communities are modified
19 when they recruit together with other species. Using an experimental approach we compared
20 early development of a temperate marine sessile community after the recruitment of mixtures
21 of botryllid ascidians and barnacles to that when barnacles or botryllid ascidians recruited
22 alone. Communities exposed to recruitment of botryllid ascidians in isolation differed from
23 those that received barnacles, a mixture of botryllids and barnacles or no recruitment in two
24 week old communities. These early differences were driven by higher abundances of the
25 species that were present as initial recruits in experimental treatments. After two months
26 communities also differed between barnacle and mixed recruitment treatments but not mixed
27 and botryllid or botryllid and barnacle treatments. These differences were not directly due to
28 differences in the abundances of our manipulated taxa but occurred because of two abundant
29 arborescent bryozoans, *Bugula dentata*, which occupied more space in communities that
30 initially received mixed recruitment than in those that received barnacle or no recruitment, and
31 *Zoobotryon verticillatum*, which occupied more space in communities that initially received
32 only barnacle recruitment than those that initially received botryllid or mixed recruitment.
33 These effects did not persist, and communities did not differ after six months. These results
34 suggest that, more generally, species may influence community dynamics differently when
35 they recruit alongside other species than when they recruit in relative isolation.

36 Keywords: recruitment, community structure, barnacle, ascidian, bryozoan

Populations and communities of organisms that reproduce with dispersive propagules (e.g. seeds, spores and larvae) often experience a large amount of variation in the identity, density and timing of offspring that recruit into habitats. Such variation in the density and timing of recruitment can have important effects on the population dynamics of plant, algal and marine animal populations (Reed 1990, Caley et al. 1996, Turnbull et al. 1999, Connell and Green 2000, Underwood and Keough 2001, Wright and Steinberg 2001). Differences in the identity and density of recruiting species can also have important consequences at the community level, but much less is known about the role of recruitment on community dynamics, and direct experimental evidence is limited for most systems (but see Bingham 1992, Forde and Ralmondi 2004, Fukami et al. 2005, Myers and Harms 2009, Paine and Harms 2009, Sams and Keough 2012a, b)

In a previous experiment we manipulated the densities and/or type of a single species/taxon recruiting into uniform patches of habitat during the early stages of community development, and found that differences in the recruitment of single species could cause persistent differences in the composition of some communities (Sams and Keough 2012a). However, these effects were highly variable between taxa, sites and times. Different sized recruitment “spikes” of single species are just one aspect of how recruitment can vary, however. In many habitats it is common for propagules of multiple species, often from different higher taxonomic groups, to recruit into habitats at the same time, and recruitment can vary from situations where only a single species recruits to mixtures of species/taxa recruiting alongside each other. A small number of studies have shown that different mixtures of recruiting species can cause long term divergences in community composition (Bingham 1992, Tilman 1997, Turnbull et al. 1999, Fukami et al. 2005). However, few studies have examined how the effects of individual taxa on community dynamics are altered when they recruit alongside other species in “mixed” recruitment events. Importantly, it is not clear whether the

effects of different taxa combine when they recruit together to influence communities in ways that are not necessarily predicted by their effects when recruiting alone.

A range of studies indirectly suggest that these non-additive effects can influence community composition. Different species that recruit into common patches of habitat at similar times may experience interspecific interactions that directly or indirectly alter the abundances of other species in ways that have broader effects at the community level (Menge 1995). When microspores of the major canopy forming algae *Macrocystis pyrifera* and *Pterygophora californica* recruit into the same patches of habitat, competition between them results in a decrease in the density of macroscopic recruits of *M. pyrifera* (Reed 1990). These changes in density are largely driven by competitive asymmetries in favour of *P. californica* and vary depending on the density of microscopic spores of both species present. Differences in the density of adults of these species as a result of recruitment patterns are likely to have important effects on the broader community by influencing the abundance and composition of understory species and grazers (Dayton et al. 1992, Tegner et al. 1997), and variations from mixed recruitment to non-mixed recruitment of spores could be important (though community level effects have not been tested directly).

The survival of some species may also be enhanced when they recruit with other species in ways that could potentially have broader effects on the composition of communities. For example, germlings of the alga *Ascophyllum nodosum* survive desiccation better in the presence of germlings of the alga *Fucus vesiculosus* than when they occur alone (Choi and Norton 2005). *A. nodosum* has a strong influence on community structure on many shores (Jenkins et al. 1999) and mixed recruitment with *F. vesiculosus* may produce different community dynamics from when there is monospecific recruitment. Additionally, when barnacles belonging to the genus *Cthamalus* recruit in the presence of predatory whelks, changes in their morphology occur that positively affect the abundance of encrusting algae, also leading to changes in community structure (Raimondi et. al. 2000). In aquatic

environments larval settlement patterns can also be influenced by a range of cues (Pawlik 1992, Steinberg et al. 2002). Mixtures of species present as recruits could produce unique suites of settlement cues that influence the number and type of larvae that settle into patches at a later time and contribute to community structure, though direct evidence for this is limited . The density of recruits at which such community level interactions become significant is also likely to be important.

For many systems it is not known whether the biological interactions outlined above occur during the early stages of recruitment when individual densities can be quite low relative to resources and if they do, whether they have a persistent influence on community development. The influence that recruitment of mixtures of species has on communities may be no different from recruitment of a single taxon if one taxon in a particular mixture has a dominant and predictable effect on subsequent community development. Variations between single to multi taxa recruitment may also have little influence on communities if strong predictable post-recruitment processes determine community structure, where species interactions are weak or where subsequent species arrivals override the influence of previously established recruits (e.g. Mook 1981, Dean and Connell 1987, Forde and Ralmondi 2004, Bram et al. 2005, Sams and Keough 2012a).

Here we manipulated the identity and density of early recruits of barnacles and a common colonial ascidian into a temperate sessile marine invertebrate community, so that they occur as either “non-mixed” (containing recruits of only the colonial ascidian or barnacle) or “mixed” (containing both barnacles and colonial ascidian recruits together) recruitment treatments, and followed the development of communities over six months. Our aim was to test:

- a) if the effects of species recruiting in mixtures combine to alter community composition in ways that do not necessarily reflect their individual effects in “non-mixed” recruitment events; and

b) at what densities such effects occur.

METHODS

Study Site

To test the effects of variation between mixed vs. non-mixed recruitment of distinctly different taxa on community structure, we ran an experiment subtidally at Workshops Jetty (37°51'39.78"S, 144°54'34.17"E) from early December 2007 until early June 2008. Workshops Jetty is situated in the predominantly estuarine northern end of Port Phillip Bay, a large shallow embayment in southeastern Australia. The hard artificial structures of each of the sites support abundant assemblages of sessile marine animals similar to those found on subtidal rocky reef and hard artificial substrata throughout temperate waters of the world (for a more detailed description of the study site and assemblage refer to Sams and Keough 2007, 2012a).

Experimental Design

Perspex settlement plates (10 cm x 10 cm) were attached to PVC backing panels (50 cm x 50 cm) with stainless steel bolts in an evenly spaced 4 x 4 array and suspended from horizontal pier pylons so they faced downwards at a depth of ≈ 2 m below the low water mark. These settlement plates act as hard substrate that sessile organisms readily recruit onto and they support diverse sessile communities that can be easily manipulated experimentally. Plates were left in the water for two weeks at Williamstown, allowing enough time for recruits to settle onto plates in high enough abundance to do experimental manipulations whilst ensuring that they were recently settled.

After the two week collection period, initial recruitment was manipulated by removing panels from the water, submerging them in large tubs of seawater and removing individuals on plates to create desired recruitment patterns. Treatments consisted of individual plates that received initial recruitment of colonial ascidians belonging to the family Botryllidae (a

combination of *Botryllus schlosseri* and *Botrylloides leachii*, henceforth referred to as botryllids), barnacles (a combination of *Amphibalanus variegatus* and *Balanus trigonus*, and *Elminius modestus* henceforth referred to as barnacles), a mixture of both barnacles and botryllids or received no recruitment of these species (Table 1). Both of these taxa commonly recruit into communities of various ages, particularly during early stages, and so have the capacity to influence community development. Often they recruit together, but at other times they recruit into patches when the other does not (Sams, *unpublished data*, Keough, *unpublished data*). The early recruits of some botryllid and barnacle species were too difficult to reliably differentiate in the field at very early recruitment at our study site, so rather than use individual species we used mixtures of closely related species for each recruitment type. For each recruitment treatment there were two densities, high and low, which were relative to the taxon being manipulated (rather than being the same across all taxa) and were based on natural recruitment densities recorded over many recruitment seasons (Keough unpublished data, Holloway and Keough 2002, Johnston and Keough 2002).

So that we could clearly determine if individual taxa had a different influence on communities when they recruited in mixed recruitment without confounding effects due to density, we used comparable total numbers of individuals between non-mixed and mixed recruitment treatments and, as much as possible, also used comparable numbers of individual taxa between non-mixed and mixed recruitment treatments (Table 1). High density mixed treatments consisted of a similar range of individual barnacles as those found in high density barnacle treatments, and individual botryllids in similar range of abundance to those found in low density botryllids treatments. Low density mixed treatments consisted of a slightly higher range of total individuals and a lower range of botryllid abundances but had a similar density of barnacles to those found in low density barnacle treatments.

Plates were sampled photographically 2-weeks, 1-, 2- and 6-months after manipulations using a standardised camera setup (Olympus C7070 with framer). Plates were removed from

panels and photographed in small tubs of seawater at the surface of the jetty before being re-attached and re-submerged under the jetty.

Data Analysis

To determine the structure of communities and abundance of taxa on each plate, we estimated % cover (henceforth referred to as cover) of species by identifying the taxa beneath 100 points randomly placed over photos using the program CPCe (Claar et al. 2011). In all our analysis we included the abundance of the botryllid and barnacle species that we manipulated in initial recruitment treatments, as differences in their abundances due to recruitment could be an important component of community differences.

Originally this experiment was designed as a repeated measures analysis that ran for 6-months, with plates being repeatedly sampled through time. Unfortunately, two panels and some plates were lost between the 2-month and 6-month samples leading to a loss of replicates and an unbalanced design. Rather than discard those replicates that did not make it past 2-months, we ran two separate main analyses: the first a partly-nested PERMANOVA/ANOVA of plates sampled from 2-weeks to 2-months and the second a two-way PERMANOVA/ANOVA comparing Recruitment by Density at the end of the experiment. Specific details of analysis are given below and details of replicate numbers of plates are shown in Table 1.

To test for the effects of non-mixed vs. mixed recruitment on overall community structure, we analysed the cover data of all taxa using the PERMANOVA add-on for Primer 6 (Anderson et al. 2008). Differences between treatments were analysed by PERMANOVA for each experiment using permutation of residuals under a reduced model with 9999 permutations, followed by PERMANOVA pairwise comparisons if significant differences were detected amongst treatments. For the first part (2-week, 1- and 2-month samples) of this analysis, factors consisted of Recruitment (Fixed at three levels: botryllids, barnacles and

mixed barnacles and botryllids), Density (fixed at two levels: high and low), plate (random with five levels and nested within Recruitment x Density), and Time (fixed at three levels: 2-weeks, 1-month and 2-months, and reflecting the three measurements made for each plate). For the second part (6-month sample) of this analysis, factors consisted of Recruitment (Fixed at three levels: botryllids, barnacles and mixed barnacles and botryllids) and Density (fixed at two levels: High and Low). Controls were excluded from these analyses because the design of the experiment was not orthogonal when they were present and because our most important question was about differences in effects of particular taxa.

To test for the effects of non-mixed vs. mixed recruitment patterns on individual taxa, we first analysed cover data using the equivalent partly-nested ANOVA on data from 2-weeks to 2-months and a separate two-way ANOVA on the 6-month data using the same factors as outlined for PERMANOVA above. Where there were significant differences between treatments, we used pairwise comparisons (Tukey's HSD) at the appropriate factor level to determine where differences occurred. We have only presented the results of ANOVAs for the major space occupying species and those species that showed significant differences in cover between treatments.

Because we weren't able to compare recruitment to controls in the factorial PERMANOVA/ANOVA outlined above, we ran further planned comparisons of recruitment treatments to controls based on the outcome of these analyses. We did this to determine if assemblages that different due to recruitment manipulations were also different from those that received "natural" or unmanipulated recruitment. When the results of the analyses outlined in the paragraphs above had no interactions but significant main effects, we ran planned comparisons within the main effect, comparing, for example, each type of recruitment against the control. Where there were significant Recruitment x Density interactions, we compared all low density treatments with controls. Where there was a significant Recruitment x Density x Time interaction, we compared only those treatments that had significantly higher abundances

than others with controls within the same time period. Where there was no significant effect, all recruitment treatments were pooled and compared to controls.

Because recruitment of our manipulated species was highly variable across plates and some replicates were lost between two and six months, our replicate numbers were uneven between some treatments levels (Table 1), so we analysed our data with Type III sum of squares, which is robust to uneven sample sizes in both traditional ANOVA and PERMANOVA, and also restricted models in PERMANOVA which is more suitable to smaller sample sizes (Quinn and Keough 2002, Anderson et al. 2008 pp 68-72).

Principal co-ordinates ordination (PCO) based on the Bray-Curtis dissimilarity matrix of % cover of taxa were also used to visualize differences in overall community structure through time. To increase the clarity of PCO plots we have only shown a single object representing the centroid for each treatment at each time sampled on the basis of the Bray-Curtis measure of % cover of taxa for each treatment at each time. This is considered one of the most suitable visual complements to PERMANOVA output (Anderson et al. 2008).

RESULTS

The communities that developed on experimental surfaces comprised a range of bryozoans, ascidians, barnacles, serpulid polychaetes and tube building amphipods. The composition of this assemblage changed considerably through time, with space initially being dominated by the colonial ascidian *Botryllus schlosseri* and a mixture of barnacles (*Balanus trigonus*, *Amphibalanus variegatus* and *Elminius modestus*), while arborescent bryozoans (*Bugula dentata*, *Bugula neritina* and particularly *Zoobotryon verticillatum*) became more dominant space occupiers during by 1- and 2- months, until the colonial ascidians *Diplosoma listerianum* and tube building amphipods became dominant space occupiers at 6 months.

Within the first 2-months of community development, treatments that received different initial recruitment differed in overall community structure, but those differences diminished

with time and density of recruits had no effect at any time (Figure 1, Table 2). At 2-weeks, plates that received mixed recruitment had a different overall community structure from those that received botryllids (Table 2). Plates that initially received only botryllid recruitment also had a different community structure from plates that initially received only barnacle recruitment, but barnacle and mixed treatments did not differ.

Differences in overall community structure in 2-week communities were associated with differences in the abundance of botryllids, barnacles and serpulid polychaetes between recruitment treatments (Table 2). At this time, botryllid ascidians occupied approximately 5 to 10 times as much space on plates that initially received only botryllid recruitment and 4 to 8 times as much space on plates that initially received mixed recruitment than on plates that received only barnacle recruitment or no recruitment (Figure 2, Table 2). In contrast, the barnacle *Elminius modestus* occupied 2 to 3 times more space on plates that initially received barnacle recruitment than those that initially received botryllid, mixed or no recruitment (Figure 2, Table 2). Most of the botryllids and barnacles we observed on plates at this time were juveniles, but larger than typical 2-week old individuals, and were likely to be individuals that recruited during the manipulation period and formed the initial recruitment treatments. Small serpulid polychaetes also occupied 2 to 3 times more space on barnacle, mixed and no recruitment treatments than on only botryllid recruitment treatments at 2-weeks (Figure 2, Table.2).

At 1-month, there were no differences in overall community structure between any recruitment treatments, and community composition had changed considerably (Table 2). A few individual taxa, however, were affected. The arborescent bryozoan *Bugula dentata* occupied twice as much space on mixed treatments as on barnacle treatments but had similar abundances on mixed and botryllid treatments (Figure 2, Table 2). The amount of space occupied by *B. dentata* was also higher on mixed treatments than on no-recruitment controls (Figure 2, Appendix A Table A1). The bryozoan *Bugula neritina* occupied 2 to 4 times more

space on plates that initially received only low barnacle recruitment treatments than on low mixed recruitment treatment and no recruitment treatments, but did not differ between other treatments (Table 2, Appendix A Figure A1 and Table A2). *Balanus trigonus* occupied half as much space on plates that initially received low barnacle recruitment than those that received high barnacle recruitment but there were no differences between other treatments (Table 2, Appendix A Figure A1 and Table A2). The barnacle *Elminius modestus* had a higher abundance on plates that received low initial recruitment of barnacles than on plates that received low mixed recruitment but not from other treatments (Table 2, Appendix A Figure A1 and Table A2). The solitary ascidian *Styela clava* also occupied more space on plates that initially received low botryllid recruitment than high botryllid, barnacle and mixed (at both densities) recruitment and no recruitment but no other treatments differed, and it occupied very little space overall (Table 2, Appendix A Figure A1 and Table A2).

In contrast to a month earlier, 2 months into community development, communities that initially received recruitment of barnacles had a different overall structure from those that received mixed initial recruitment (Table 2). There were no differences in community structure between any of the other recruitment treatments at this time. Differences in community structure were associated with differences in the abundance of two species of bryozoan. *Bugula dentata* occupied over 3 times as much space on plates that initially received a mixture of botryllid and barnacle recruitment than on those that initially received only botryllids, only barnacles or no recruitment (Figure 3, Table 3, Appendix A Table A1). In contrast the bryozoan *Zoobotryon verticillatum* occupied more than 5 times as much space on plates that initially received barnacle recruits than those that initially received mixed, botryllid or no recruitment (Figure 3, Table 2, Appendix A Table A1). No other taxa differed in abundance between treatments at 2 months.

Many of the major space occupiers such as didemnid ascidians, *Diplosoma listerianum*, and *Watersipora subtorquata* were not influenced by initial recruitment patterns and showed

no differences between recruitment treatments during the early stages of community development (Table 2, Appendix A Figures A1 and A2 and Table A1).

Over the initial 2 months of community development, tube-building amphipods and the solitary colonial ascidian *Ciona intestinalis* occupied more space on plates that initially received higher density recruitment regardless of the type of species present as recruits (Table 2). These differences may also have contributed to differences in overall community structure. Botryllid ascidians also had a significant Density x Time interaction, but this interaction occurred between different treatments sampled at different times, which was not of particular interest to our main question.

At 6 months, any differences in community structure caused by initial recruitment had disappeared (Table 2). Space was dominated by the colonial ascidian *Diplosoma listerianum* and tube building amphipods, which occupied a mean of 39.5 ± 5.7 % and 13.96 ± 2.7 % of space per plates respectively. *D. listerianum* in particular dominated space by overgrowing other taxa on plates, but its cover varied from a maximum of 88 % and a minimum of 1 % of space across plates. Neither of these taxa was affected by initial recruitment treatments. Small serpulid polychaetes were the only taxon that showed any difference in abundance as a result of initial recruitment patterns at 6 months, occupying more space on treatments that received high initial mixed recruitment than on other recruitment treatments (Figure 2, Table 2).

Because there was no strong recruitment by density interactions at any of the times sampled for most species, we pooled recruitment treatments over density and compared them to control plates that received no initial recruitment. Comparison of recruitment treatments with controls showed similar patterns of community differences through time as comparisons without controls, and for brevity are shown in Appendix A, Tables A1 and A2. Four taxa did show an R x D x T interaction (all at 1 month samples), so we compared treatments with significantly higher abundances of those species with controls at the relevant time period.

These differences which were not associated with changes in overall community composition and again, for brevity, the analyses are shown in Appendix A.

DISCUSSION

This study shows that when different taxa recruit together as mixtures during the initial stages of community development they can exert different effects on communities compared to when they recruit in isolation. In this particular study, initial recruitment did not exert strong long term-effects that persisted into 6-month old communities, but did influence community structure during earlier stages of community development as well as the abundances of individual species.

At 2-weeks, communities differed from each other in ways that are largely attributable to the growth and development of initial recruits established in respective experimental treatments. Some further recruitment of new individuals of manipulated species may have occurred in the two weeks after manipulations and caused differences in communities, but the individuals we observed at this time were mostly juveniles that were larger than individuals that recruit in a two week window. As most individuals were juveniles, differences in two week communities may not be particularly important for population and community dynamics if they do not persist in this system.

In contrast, the community differences that were found at 2-months occurred between mixed, barnacle and control treatments were driven by indirect biological interactions between manipulated taxa and two abundant bryozoans that subsequently recruited to plates. Communities that initially received only barnacle recruitment had a significantly higher abundance of *Zoobotryon verticillatum* than those that had botryllid recruits present as either non-mixed or mixed recruitment. Larvae of many marine invertebrates, including bryozoans, are capable of actively selecting suitable habitat to settle upon, and can avoid settling near stronger competitors (Todd and Keough 1994, Matson et al. 2010). Little is known about the

larval settlement behaviour of *Z. verticillatum* specifically, but it is possible that the presence of botryllids acts as a negative settlement cue. Bryozoan recruits are often outcompeted by ascidians for space and mortality can be higher for them in the presence of botryllids (Osman et al. 1992, Osman and Whitlatch 1998). *Z. verticillatum* that settled on plates with higher cover of botryllids may have been overgrown and experienced higher mortality in the presence of botryllids, though *Z. verticillatum* is very fast growing and may grow out of competition quickly. Approximately 40 % of space was free after one and two months, suggesting that space was not limited at these times. Alternatively, barnacles may have enhanced survival, growth, and/or settlement of *Z. verticillatum*. Barnacle tests increase the structural complexity of plates, which can enhance the settlement and post-settlement survival of bryozoans (Walters and Wethey 1996, Allen et al. 2008), and may have similarly influenced *Z. verticillatum*.

Communities that initially received mixed recruitment had higher abundances of *Bugula dentata* at 2-months than plates that only had botryllids, barnacles or no recruitment. Notably, the mixture of botryllids and barnacles as recruits had a stronger positive effect on survival, growth or settlement of *B. dentata* than when each species was present in isolation. The exact reasons behind why *B. dentata* occurred in highest abundance on these plates are not clear, and may be complex. The combination of botryllids and barnacles may enhance growth, survival or settlement of this species. Barnacle tests also have the potential to enhance settlement of *Bugula neritina*, a close relative of *B. dentata* (Walters and Wethey 1996, Allen et al. 2008). However, *B. dentata* did not recruit in higher abundances on plates with higher densities of barnacles and no botryllids, which would be predicted if barnacles enhanced settlement. One potential explanation for the observed increase in *B. dentata* abundance on mixed recruitment plates could be that higher abundance of *Z. verticillatum* on barnacle recruitment plates inhibited/prevented *B. dentata* from settling or decreased growth and survival, but the presence of barnacles and absence of *Z. verticillatum* on mixed plates had positive effects on its settlement growth and/or survival.

Although influencing short term community dynamics, variation of mixed and non-mixed recruitment did not have a strong persistent influence on community structure and at 6-months there were no differences in the structure of communities that initially received botryllid, barnacle, mixed or no recruitment. These findings are similar to those of previous experiments at Williamstown in which initial recruitment of different taxa at different densities did not have a strong effect on community structure over longer periods of time, although did influence communities over shorter time periods (Sams and Keough 2012a). In this particular experiment, the lack of effect of different initial recruitment patterns appears to be driven by the mortality and decrease in abundance of species present as initial recruits over time, a lack of long lasting effects on subsequently recruiting species by initially colonising recruits as well as the dominance of space and overgrowth of taxa by the colonial ascidian *Diplosoma listerianum* and, to a lesser extent, other didemnid ascidians at later community stages. *D. listerianum* is a competitive dominant in similar environments in other parts of the world, and these effects on communities may not be unique to this study (Osman et al. 2010, Edwards and Stachowicz 2011). Like botryllids and barnacles, *D. listerianum* and other didemnids can occur in quite high abundances as recruits during early community development (e.g. Sams and Keough 2007, 2012a). It is possible that initial differences in recruitment of more spatially dominant taxa like *D. listerianum* might have stronger and more persistent effects on community dynamics than the barnacles and botryllids manipulated in this experiment. We were unable to manipulate densities of *D. listerianum* or didemnid recruits as they did not occur in high enough abundance during times when experiments were set up.

While differences in community composition caused by variation between mixed and non-mixed recruitment events did not necessarily exert strong long term-effects on communities in this experiment, they could still have important consequences on the dynamics of short lived species that rapidly colonise new patches of habitat and then disappear from communities. For example we found that changes between non-mixed to mixed recruitment of

botryllids and barnacles can alter the density of two species of arborescent bryozoan, *Zoobotryon verticillatum* and *Bugula dentata*. Changes in the density of conspecifics have been shown to alter the fecundity, biomass and growth of another arborescent bryozoan *Bugula neritina* (Allen et al. 2008, Gooley et al. 2010). In other sites, or for different species, where initial recruitment patterns exert a more long lasting effect on community structure, such variations between mixed and non-mixed recruitment of taxa may have even stronger and more persistent effects on communities. Consequently, it is important to consider them when trying to predict the effects of recruitment patterns on communities at different stages of development in a variety of systems.

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503

Table 1. Summary of recruitment treatments and the number of replicates of each treatment type. Treatments were replicated across individual plates.

Taxa	Density	No individuals plate ⁻¹	No. replicates: 2 weeks to 2 months	No. replicates: 6 months
Botryllids	High	60-100	5	4
	Low	10-30	4	2
Barnacles	High	60-120	5	4
	Low	15-35	5	3
Botryllids and Barnacles	High	60-120*	5	3
	Low	20-40*	4	2
No recruitment	-	-	9	8

* Mixed recruitment consisted of approximately 15 - 30 individuals of botryllids and 80 - 100 individual barnacles in High treatments and 5 - 10 individuals of botryllids and 10 - 30 individual barnacles in Low treatments.

511 Table 2 Results of a) partly-nested ANOVA on the most abundant taxa or those that were influenced by recruitment treatments and partly nested
512 PERMANOVA on all taxa from 2 weeks to 2 months and b) 2-factor ANOVA on the same taxa and 2-factor PERMANOVA on all taxa at 6 months. Comparison
513 were between percent cover of taxa on treatments consisting of initial recruitment of either botryllids, barnacles or a mixture of both taxa (Recr) at high or low
514 density (Dens). Percent cover was measured at 2 weeks, 1, 2, 3 and 6 months after manipulations of recruitment. Numbers underneath main factor headings are
515 p-values, while values under MS (btwn) are the mean square error values between subjects (plates) and values under MS(within) are the mean square error
516 values within subjects (plates) for the 2 week to 2 month analysis or the mean squares error term for the analysis at 6 months. Where there were significant
517 differences in the cover of taxa or the overall structure of communities between treatments results of pairwise post-hoc comparisons are shown below. Letters
518 denote treatments types in each pairwise comparison (see key). Where there was an interaction with time semicolons separate each time periods plates were
519 sampled. Comparisons for each time period are in order from left to right.

	(a)	Recr	Dens	Time	RXD	RXT	DXT	RXDXT	MS(btwn)	MS(wthn)	(b)	Recr	Dens	RXD	MS(error)
df	2	1	2	2	4	2	4	22	44		2	1	2	11	
Botryllid ascidians	0.276	0.524	0.000	0.599	0.003	0.040	0.392	51.972	24.437		0.788	0.527	0.547	3.379	
Didemnids ascidians	0.385	0.229	0.000	0.590	0.914	0.251	0.481	14.620	12.440		0.463	0.350	0.570	21.492	
<i>Diplosoma listerianum</i>	0.982	0.992	0.000	0.546	0.102	0.857	0.426	163.127	117.672		0.899	0.408	0.891	1,119.136	
<i>Ciona intestinalis</i>	0.144	0.012	0.846	0.594	0.606	0.469	0.424	0.182	0.196		0.679	0.544	0.679	0.614	
<i>Styela clava</i>	0.010	0.144	0.068	0.069	0.033	0.139	0.002	0.076	0.076		0.592	0.153	0.960	4.545	
<i>Amphibalanus</i> <i>variegatus</i>	0.995	0.453	0.000	0.819	0.074	0.433	0.787	14.723	10.932		0.989	0.837	0.769	2.409	
<i>Balanus trigonus</i>	0.578	0.421	0.002	0.204	0.278	0.937	0.043	6.735	3.626		0.141	0.103	0.591	3.068	
<i>Elminius modestus</i>	0.002	0.860	0.000	0.364	0.000	0.917	0.023	0.993	0.796		0.136	0.153	0.136	0.045	
<i>Bugula dentata</i>	0.019	0.854	0.000	0.405	0.022	0.957	0.351	3.430	2.044		0.830	0.394	0.830	0.136	
<i>Bugula neritina</i>	0.127	0.473	0.000	0.075	0.230	0.428	0.018	4.032	3.637		0.679	0.544	0.679	0.068	
<i>Watersipora</i> <i>subtorquata</i>	0.374	0.362	0.000	0.644	0.618	0.511	0.681	5.342	4.509		0.914	0.278	0.295	7.152	
<i>Zoobotryon</i> <i>verticillatum</i>	0.004	0.153	0.000	0.197	0.000	0.091	0.127	18.872	16.646		0.679	0.544	0.679	0.068	

<i>Hydroides ezoensis</i>	0.368	0.490	0.000	0.609	0.575	0.846	0.069	13.241	13.793	0.700	0.596	0.870	48.833
Small serpulids	0.767	0.490	0.002	0.414	0.018	0.353	0.625	3.134	2.136	0.143	0.849	0.017	0.705
Amphipod tubes	0.199	0.041	0.000	0.849	0.907	0.280	0.955	17.275	29.826	0.938	0.575	0.833	203.583
PERMANOVA	0.02	0.51	0.000	0.61	0.00	0.79	0.33	913.86	762.63	0.93	0.58	0.3222	1743.2

*pairwise comparisons for 2 weeks to 2 month partly-nestedANOVA**

Botryllid ascidians	B=M B>Ba M>Ba; B=M=Ba; B=M=Ba
<i>Ciona intestinalis</i>	H>L
<i>Styela clava</i>	BL=BH=ML=MH=BaL=BaH; BL>BH BL>MH BL>ML BL>BaL BL>BaH BH=ML=MH= BaH=BaM; BL=BH=ML=MH=BaL=BaH
<i>Bugula dentata</i>	B=M=Ba; M>Ba M=B B=Ba; M>Ba M=B B=Ba
<i>Bugula neritina</i>	BL=BH=ML=MH=BaL=BaH; BaL>ML BaL=BaH=MH=BL=BH ML=MH=BaH=BL=BH; BL=BH=ML=MH=BaL=BaH
<i>Zoobotryon</i>	B=M=Ba; B=M=Ba; Ba>M Ba>B M=B
<i>verticillatum</i>	
Amphipod tubes	H>L
<i>Balanus trigonus</i>	BL=BH=ML=MH=BaL=BaH; BaH>BaL BaH>BL BaH=BH=MH=ML BaL=BL; BL=BH=ML=MH=BaL=BaH
<i>Elminius modestus</i>	BaH=BaL>MH=ML=BH=BL BaH=BaL=BH=BL; BaL<ML=MH=BL=BH=BaH BaL=MH=BL=BH=BaH; BaH=BaL=MH=ML=BH=BL
Small serpulids	Ba>B Ba=M B=M

* B= Botryllid treatments, M= Mixed treatments, Ba = Barnacle treatments, H=High density, L=Low density

List of Figures

Figure 1. Principal co-ordinates ordination (PCO) of distances among centroids on the basis of the Bray-Curtis measures of percent cover of taxa found colonizing settlement plates at Williamstown at a) 2 weeks, 1 month, 2 months, and b) 6 months. Plates were exposed to either high or low initial recruitment of botryllids, barnacles or a mixture botryllids and barnacles or cleared of initial recruits. Communities at 2 weeks to 2 months are shown on a separate figure since they were analysed separately (see methods). Centroids represent average distances between recruitments treatments at each time sampled within each experiment. For details of differences between treatments see statistical analysis and results for PERMANOVA in Table 2

Figure 2. Percent cover of a) botryllid colonial ascidians, b) the barnacle *Elminius modestus* and c) small serpulid polychaetes found on settlement plates at 2 weeks, 1, 2, and 6 months after exposure to initial recruitment at high or low densities of either botryllids, barnacles, a mixture of barnacles and botryllids or no initial recruitment. Asterisks (*) denote differences in cover between treatments according to partly-nested ANOVA (2 weeks to 2 months) or two-way ANOVA (6 months). For details see pairwise comparisons on Table 2

Figure 3. Percent cover of the bryozoans a) *Bugula dentata* and b) *Zoobotryon verticillatum* found on settlement plates at 2 weeks, 1, 2, and 6 months after exposure to initial recruitment at high or low densities of either botryllid, barnacles, a mixture of barnacles and botryllids or no initial recruitment. Asterisks (*) denote differences in cover between treatments according to partly-nested ANOVA (2 weeks to 2 months) or two-way ANOVA (6 months). For details see pairwise comparisons on Table 2

Fig 1.

Figure 2.

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