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

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Author contribution: MS and MK conceived and designed the experiments, analysed the data
and wrote the manuscripts. MS performed the experiments.

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

38 Populations and communities of organisms that reproduce with dispersive propagules
39 (e.g. seeds, spores and larvae) often experience a large amount of variation in the identity,
40 density and timing of offspring that recruit into habitats. Such variation in the density and
41 timing of recruitment can have important effects on the population dynamics of plant, algal and
42 marine animal populations (Reed 1990, Caley et al. 1996, Turnbull et al. 1999, Connell and
43 Green 2000, Underwood and Keough 2001, Wright and Steinberg 2001). Differences in the
44 identity and density of recruiting species can also have important consequences at the
45 community level, but much less is known about the role of recruitment on community
46 dynamics, and direct experimental evidence is limited for most systems (but see Bingham
47 1992, Forde and Ralmondi 2004, Fukami et al. 2005, Myers and Harms 2009, Paine and Harms
48 2009, Sams and Keough 2012a, b)

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

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

65 A range of studies indirectly suggest that these non-additive effects can influence
66 community composition. Different species that recruit into common patches of habitat at
67 similar times may experience interspecific interactions that directly or indirectly alter the
68 abundances of other species in ways that have broader effects at the community level (Menge
69 1995). When microspores of the major canopy forming algae *Macrocystis pyrifera* and
70 *Pterygophora californica* recruit into the same patches of habitat, competition between them
71 results in a decrease in the density of macroscopic recruits of *M. pyrifera* (Reed 1990). These
72 changes in density are largely driven by competitive asymmetries in favour of *P. californica*
73 and vary depending on the density of microscopic spores of both species present. Differences
74 in the density of adults of these species as a result of recruitment patterns are likely to have
75 important effects on the broader community by influencing the abundance and composition of
76 understory species and grazers (Dayton et al. 1992, Tegner et al. 1997), and variations from
77 mixed recruitment to non-mixed recruitment of spores could be important (though community
78 level effects have not been tested directly).

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

89 environments larval settlement patterns can also be influenced by a range of cues (Pawlik
90 1992, Steinberg et al. 2002). Mixtures of species present as recruits could produce unique
91 suites of settlement cues that influence the number and type of larvae that settle into patches at
92 a later time and contribute to community structure, though direct evidence for this is limited .
93 The density of recruits at which such community level interactions become significant is also
94 likely to be important.

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

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

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

115 b) at what densities such effects occur.

116 METHODS

117 *Study Site*

118 To test the effects of variation between mixed vs. non-mixed recruitment of distinctly
119 different taxa on community structure, we ran an experiment subtidally at Workshops Jetty
120 (37°51'39.78"S, 144°54'34.17"E) from early December 2007 until early June 2008. Workshops
121 Jetty is situated in the predominantly estuarine northern end of Port Phillip Bay, a large
122 shallow embayment in southeastern Australia. The hard artificial structures of each of the sites
123 support abundant assemblages of sessile marine animals similar to those found on subtidal
124 rocky reef and hard artificial substrata throughout temperate waters of the world (for a more
125 detailed description of the study site and assemblage refer to Sams and Keough 2007, 2012a).

126 *Experimental Design*

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

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

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

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

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

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

167 *Data Analysis*

168 To determine the structure of communities and abundance of taxa on each plate, we
169 estimated % cover (henceforth referred to as cover) of species by identifying the taxa beneath
170 100 points randomly placed over photos using the program CPCe (Claar et al. 2011). In all our
171 analysis we included the abundance of the botryllid and barnacle species that we manipulated
172 in initial recruitment treatments, as differences in their abundances due to recruitment could be
173 an important component of community differences.

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

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

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

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

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

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

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

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

230

RESULTS

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

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

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

246 Differences in overall community structure in 2-week communities were associated
247 with differences in the abundance of botryllids, barnacles and serpulid polychaetes between
248 recruitment treatments (Table 2). At this time, botryllid ascidians occupied approximately 5 to
249 10 times as much space on plates that initially received only botryllid recruitment and 4 to 8
250 times as much space on plates that initially received mixed recruitment than on plates that
251 received only barnacle recruitment or no recruitment (Figure 2, Table 2). In contrast, the
252 barnacle *Elminius modestus* occupied 2 to 3 times more space on plates that initially received
253 barnacle recruitment than those that initially received botryllid, mixed or no recruitment
254 (Figure 2, Table 2). Most of the botryllids and barnacles we observed on plates at this time
255 were juveniles, but larger than typical 2-week old individuals, and were likely to be individuals
256 that recruited during the manipulation period and formed the initial recruitment treatments.
257 Small serpulid polychaetes also occupied 2 to 3 times more space on barnacle, mixed and no
258 recruitment treatments than on only botryllid recruitment treatments at 2-weeks (Figure 2,
259 Table.2).

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

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

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

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

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

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

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

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

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

320 DISCUSSION

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

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

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

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

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

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

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

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

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500 Wright, J. T. and P. D. Steinberg. 2001. Effect of variable recruitment and post-recruitment herbivory
501 on local abundance of a marine alga. *Ecology* **82**:2200-2215.
502
503

504 Table 1. Summary of recruitment treatments and the number of replicates of each
 505 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

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

509
 510

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 PERMNOVA 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 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 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 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-nested ANOVA**

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 verticillatum</i>	B=M=Ba; B=M=Ba; Ba>M Ba>B M=B
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

521

List of Figures

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

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

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

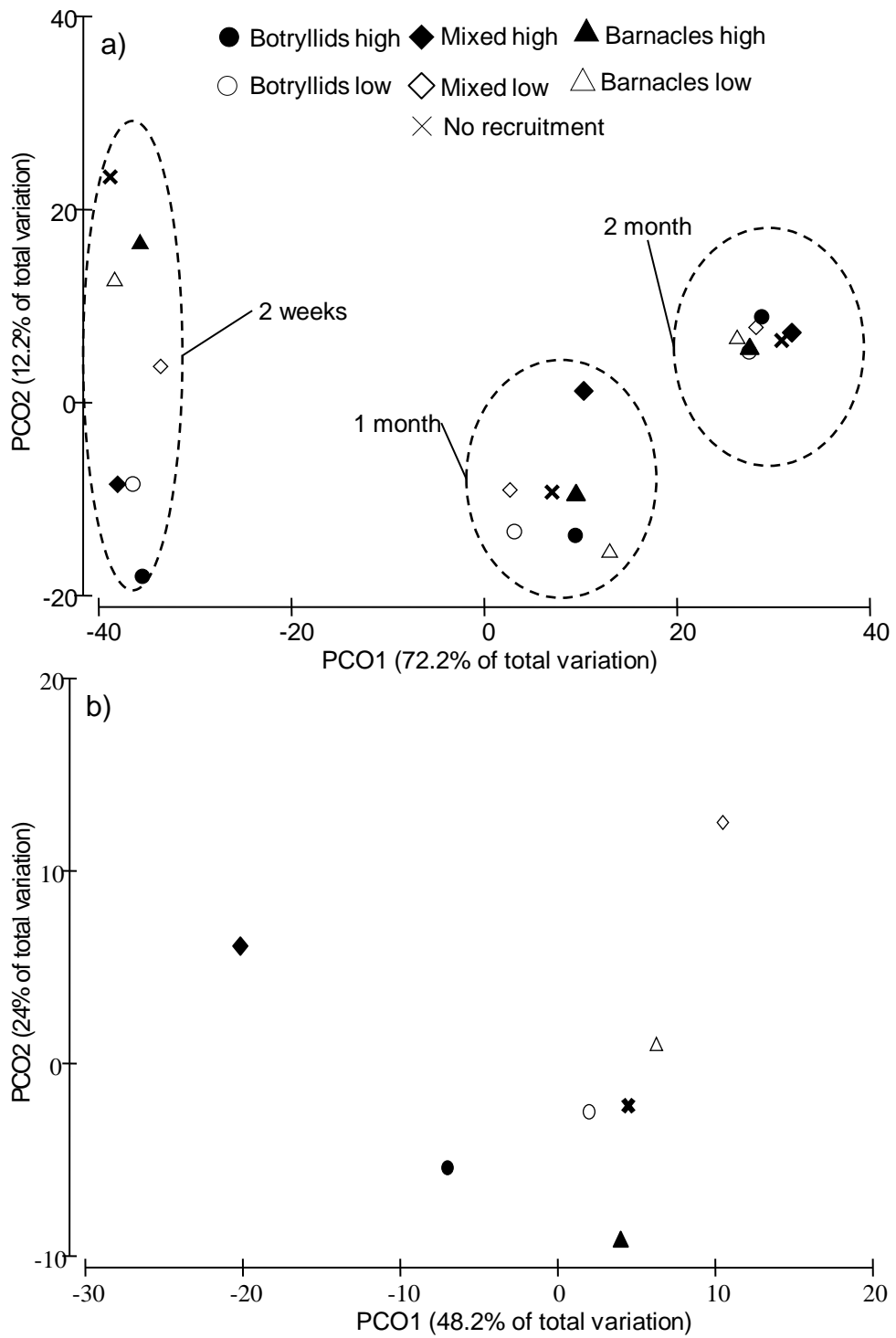


Fig 1.

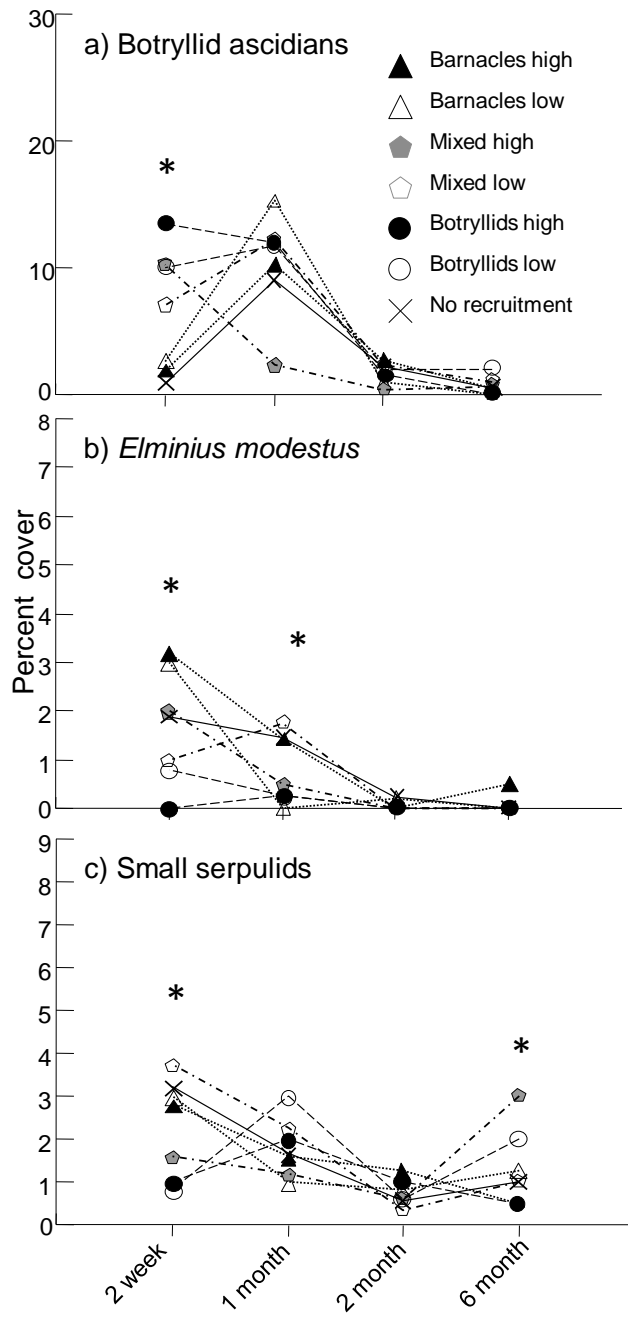


Figure 2.

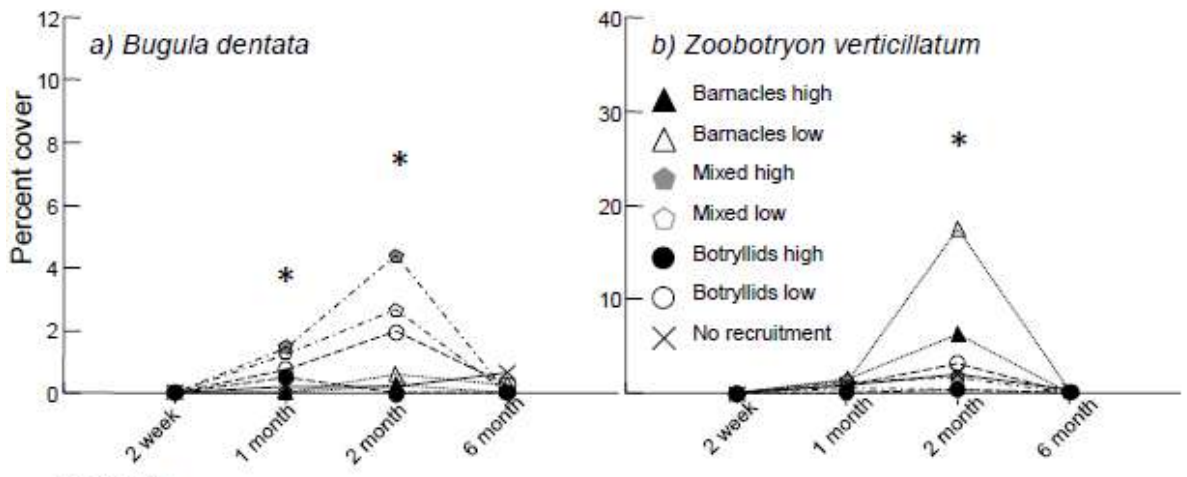


Figure 3.