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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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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

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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. nodusum* 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

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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

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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

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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

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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

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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

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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

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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 A1and 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

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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

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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.

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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

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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 bryzoan *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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501   on local abundance of a marine alga. Ecology **82**:2200-2215.
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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

22

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
Diplosoma listerianum	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
Ciona intestinalis	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
Styela clava	**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
Amphibalanus variegatus	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
Balanus trigonus	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
Elminius modestus	**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
Bugula dentata	**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
Bugula neritina	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
Watersipora subtorquata	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
Zoobotryon verticillatum	**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

Hydroides ezoensis	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
Ciona intestinalis	H>L
Styela clava	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
Bugula dentata	B=M=Ba; M>Ba M=B B=Ba; M>Ba M=B B=Ba
Bugula neritina	BL=BH=ML=MH=BaL=BaH; BaL>ML BaL=BaH=MH=BL=BH ML=MH=BaH=BL=BH; BL=BH=ML=MH=BaL=BaH
Zoobotryon verticillatum	B=M=Ba; B=M=Ba; Ba>M Ba>B M=B
Amphipod tubes	H>L
Balanus trigonus	BL=BH=ML=MH=BaL=BaH; BaH>BaL BaH>BL BaH=BH=MH=ML BaL=BL; BL=BH=ML=MH=BaL=BaH
Elminius modestus	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

520   * 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
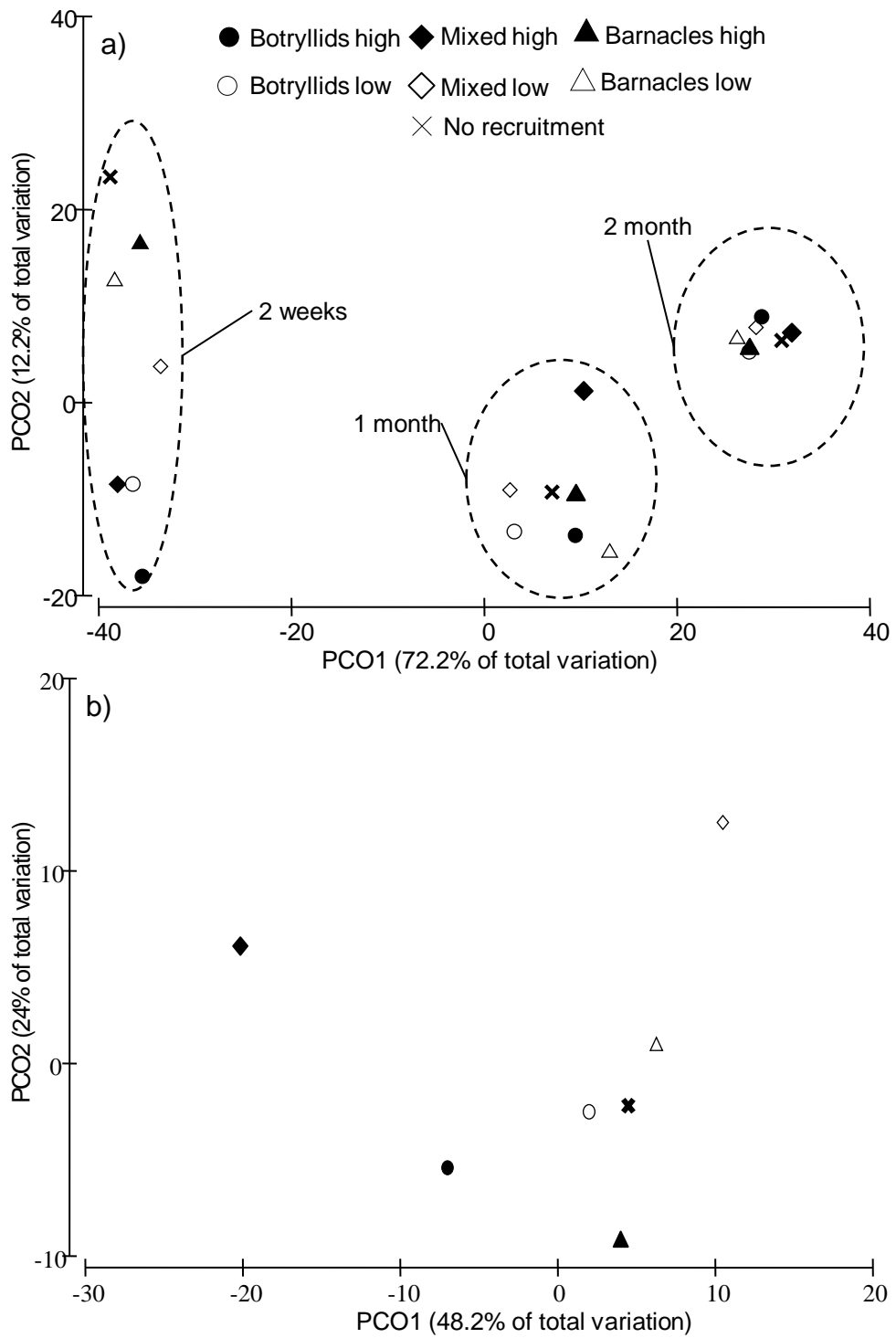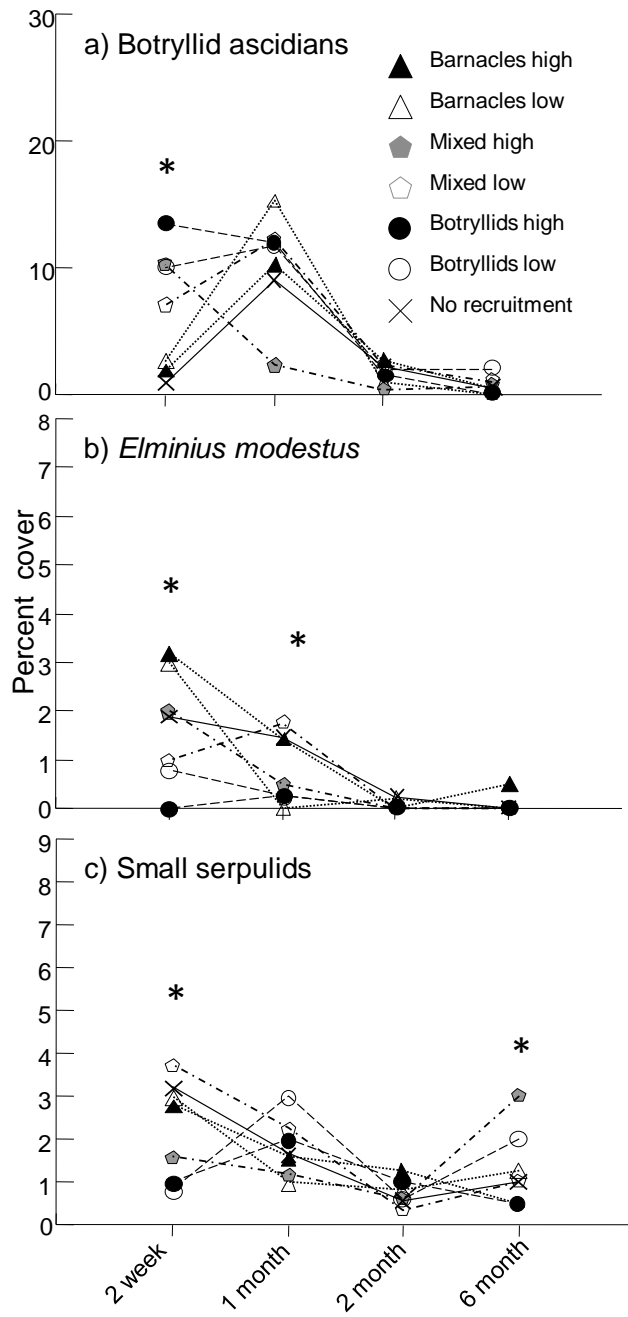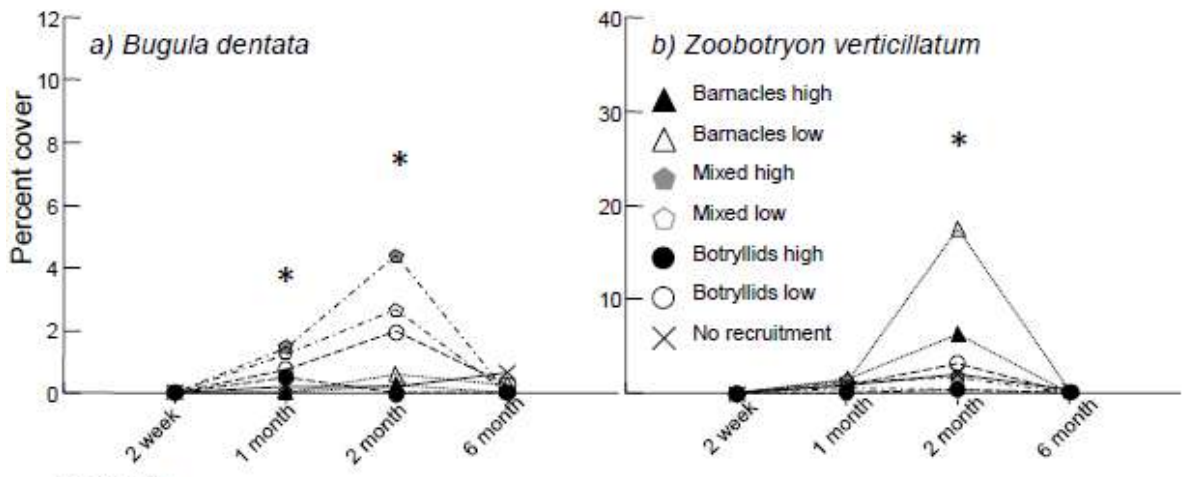
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Fig 1.

Figure 2.

Figure 3.

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