1   Running head: *Effects of patch-size and complexity on diversity*

2

3   INDEPENDENT EFFECTS OF PATCH-SIZE AND STRUCTURAL COMPLEXITY ON

4   DIVERSITY OF BENTHIC MACRO-INVERTEBRATES

5

6

7   Miguel G. Matias[1],*, A.J. Underwood[1], D.F. Hochuli[2] and R.A. Coleman[1]

8

9

10   [1]Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology

11   Laboratories A11, School of Biological Sciences, The University of Sydney, NSW 2006,

12   Australia;

13

14   [2]School of Biological Sciences, The University of Sydney, NSW 2006, Australia;

15

16

17   *Corresponding author: Miguel G. Matias

18   Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology

19   Laboratories, (A11), School of Biological Sciences, The University of Sydney, NSW 2006,

20   Australia

21   Phone: +61 2 9351 4282; Fax: +61 2 9351 6713

22   E-mail: mmatias@eicc.bio.usyd.edu.au

23

24

25   Word Count (6428), Number of pages (26), Figures (2), Electronic appendices (3)

26 **ABSTRACT**

27   Despite a long history of work on relationships between area and number if species,

28 the details of mechanisms causing patterns have eluded ecologists. The general principle that

29 the number of species increases with the area sampled is often attributed to a sampling

30 artefact due to larger areas containing greater numbers of individuals. We manipulated the

31 patch-size and surface area of experimental mimics of macro-algae to test several models that

32 can explain the relationship between abundance and species richness of assemblages

33 colonizing different habitats. Our results show that patch-size and structural complexity have

34 independent effects on assemblages of macro-invertebrates. Regardless of their structural

35 complexity, larger habitats were colonised by more species. Patch-size did not have a

36 significant effect on numbers of individuals, so the increased number of species in larger

37 habitats was not simply a result of random placement associated with sampling increased

38 number of individuals. Similarly, random placement alone could not explain differences in

39 numbers of species among habitats with different structural complexity, contrary to

40 suggestions that the relationship between number of species and surface area might also be a

41 sampling artefact due to more complex habitats having larger areas and therefore sampling

42 more individuals. Future progress would benefit from manipulating properties of habitat in

43 conjunction with experimental manipulations of area.

44

45 **Keywords:** habitat, area, structural complexity, random placement, passive sampling

46   **INTRODUCTION**

47   The relationship between area and numbers of species – perhaps one of ecology's few

48   general "laws" (*sensu* Lawton 1999) – is a well-established ecological topic (Williams 1943,

49   Coleman 1981). Larger numbers of species in increasing areas have been consistently

50   demonstrated in a wide variety of habitats and organisms (see reviews by Connor and McCoy

51   1979, McGuinness 1984, Lomolino 2000), becoming a fundamental concept in studies of

52   biogeography (e.g. MacArthur and Wilson 1967) and communities (e.g. Preston 1960).

53   Studies exploring species-area relationships have also played an important role in

54   conservation biology in general (e.g. Ney-Nifle and Mangel 2000), and particularly in the

55   SLOSS debate (i.e. "Single Large or Several Small reserves"; Gilpin and Diamond 1980,

56   Higgs and Usher 1980, Wilcox and Murphy 1984).

57   Greater numbers of species in larger areas have been explained by: 1) purely

58   mathematical processes, by which sampling more individuals increases the probability of

59   finding more species (e.g. Coleman 1981); 2) the increased probability of larger patches

60   "sampling" more individuals from the population ("passive sampling" or "target area"

61   hypotheses, Simberloff 1976, Connor and McCoy 1979); 3) larger areas having a greater

62   diversity of ecological niches and associated species ("habitat diversity" hypothesis, Williams

63   1943, Ricklefs and Lovette 1999); and 4) greater rates of colonization (or immigration), thus

64   reducing the probability of extinction ("area per se" hypothesis, MacArthur and Wilson

65   1967). Most of these explanations involve extensive discussions of associated mathematical,

66   conceptual and ecological issues (see reviews by Connor and McCoy 1979, Hill et al. 1994).

67   These commonly tested hypotheses are, however, not necessarily mutually exclusive

68   explanations of species-area relationships. It is also unlikely that any of these hypotheses

69   would be supported in all types of habitats or groups of organisms. For example, the species-

70   area relationships for bryophytes and for lichens in the same areas do not support the same

71   hypothesis (e.g. Lobel et al 2006). Similarly, it has been shown that benthic assemblages at

72   different stages of colonization (i.e. early and late colonization) show patterns that support

73   different hypotheses (Anderson 1999).

74   An alternative explanation for patterns of distribution and abundance of species is

75   spatial variation in the physical structure of the environment, which is often referred to as

76   structure (or complexity) of habitats (reviewed by McCoy and Bell 1991). Structurally

77   complex habitats generally have more species because they can provide a greater diversity of

78   niches or different ways of exploiting resources (MacArthur and MacArthur 1961). Thus,

79   complexity is an important influence on the diversity of assemblages. Independently of the

80   way structure is defined or measured, such influences of complexity on diversity of

81   organisms have been observed in terrestrial (e.g. Pianka 1966) and aquatic habitats (e.g.

82   Hovel and Lipcius 2001) .

83   The effects of these two attributes of habitat – patch-size and structural complexity –

84   have rarely been distinguished in manipulative studies, possibly because effects of increasing

85   area and habitat heterogeneity can be extremely difficult to separate (e.g. Ricklefs and

86   Lovette 1999, McGuinness 2000). There are additional difficulties associated with

87   manipulative experiments at large and ecologically relevant spatial scales to test explanations

88   for species-area curves (McGuinness 2000). At finer scales, the effects of structural

89   complexity and surface area are also often confounded because surface area generally

90   increases with greater surface complexity (Johnson et al. 2003). Several studies have reported

91   independent effects of structural complexity and patch-size on individual species. For

92   example, survival of crabs has been shown to be correlated with increased structural

93   complexity (i.e. shoot density), regardless of patch-size of seagrass (Hovel and Lipcius 2001).

94   Species sometimes respond to local structural complexity rather than to the overall patch-size

95   of habitat, although there have been relatively few examples of responses of entire

96   assemblages (but see Taniguchi et al. 2003).

97   The aim of this study is therefore to test three general models that can potentially

98   explain effects of patch-size of and structural complexity on diversity of benthic assemblages

99   (Table 1). Predictions from these models were tested by manipulating patch-size and surface

100   area of artificial mimics of macro-algae. Larger habitats provided greater overall surface area

101   without changing the structural complexity of components of habitat per unit area. Effects of

102   surface area were investigated by manipulating the density and length of fronds, to modify

103   the surface area provided by the habitat (e.g. Sirota and Hovel 2006). Here, surface area was

104   used as an estimate of structural complexity because it is well-correlated with fractal

105   complexity (e.g. Johnson et al 2003; Kostylev et al 2005) and is a good descriptor of structure

106   of artificial habitats. Habitats with greater surface areas are often colonized by more

107   individuals (Attrill et al 2001).

108   These artificial habitats are colonized by diverse assemblages of molluscs from a

109   range of families, different life-history traits, feeding modes, reproduction, development,

110   mobility and dispersal (Beesley et al. 1998). These organisms are quite small, ranging from

111   0.5 - 3 mm in size, and have been found to respond consistently in accordance with

112   predictions based on models usually tested at larger scales (e.g. habitat heterogeneity, Matias

113   et al. 2007). Note, however, that the width of artificial habitats is more than 200 times their

114   average body lengths (i.e. < 1mm, Matias unpublished work). This is relevant because,

115   although, many gastropods colonize an area by passive advection through the water-column

116   (Beesley et al 1998), it has been shown that they actively crawl and show preferences for

117   particular types of habitats (Olabarria et al. 2002). Previous work manipulating the structure

118   of habitat at finer scales has shown that assemblages of molluscs respond to differences in

119   structure of habitat at the scale of < 0.15 m (e.g. Matias et al. 2007), which reinforces the idea

120   that, for the small snails studied here, a patch of heterogeneous habitat of 200 cm^2 can

121   properly be considered to be a landscape (*sensu* Wiens 1990, Fahrig & Merriam 1985;

122   Steffan-Dewenter et al. 2002).

123   **METHODS**

124   *Study site*

125   This study was done on intertidal rock-platforms at the Cape Banks Marine Research

126   Area, on the northern headland of Botany Bay (NSW, Australia), in July-September, 2007.

127   Experiments were done in two locations with similar orientation and exposure to waves on

128   gently sloping low-shore rock platforms or large boulders, 0.3 to 0.6 m above mean low

129   water. Artificial habitats were interspersed amongst meadows of algal turf dominated by

130   *Corallina officinalis* L. and were attached to the rock with stainless steel screws and rubber

131   washers. Assemblages associated with coralline habitats vary with slope (Akioka et al. 1999),

132   height on shore (Kelaher et al. 2003) and show small-scale patchiness (Olabarria and

133   Chapman 2001). Artificial habitats of each treatment were scattered at random over areas that

134   were previously chosen because they had similar characteristics.

135   *Design of the experiment*

136   Assemblages colonizing artificial turfs respond to changes in density and length of

137   fronds (Kelaher 2003a, b). Three types of artificial turf (Grassman Pty Ltd., NSW, Australia)

138   with different densities and length of fronds were selected (A, B, C in Table 2). These

139   artificial turfs were chosen because they differ in length (A < B < C) and also in density of

140   fronds (A > B > C), which maximized the structural differences needed to test our hypotheses

141   about different types of habitats. Previous studies have shown that assemblages of molluscs

142   colonizing artificial habitats respond negatively to greater densities of fronds (Kelaher 2003)

143   and positively to longer fronds (Kelaher 2002). Therefore, the assemblages that colonize our

144   artificial habitats were affected by the combined effect of density and length of fronds.

6

145   Experimental sub-habitats were squares of turf (5 x 5 cm^2), cut as described in previous

146   experimental work (Matias et al. 2007). Experimental habitats were of three sizes: small

147   (made up of four units); intermediate (eight units) and large (twelve units) with areas of 100,

148   200 and 300 cm^2, respectively. Previous experiments showed that artificial habitats of these

149   sizes are appropriate to test our hypotheses (Matias et al. 2007). Each unit of turf was glued

150   to squared pieces of rubber and attached to wire mesh with minimal distances between units

151   (see detailed design in Appendix A; Matias et al. 2007)

152   Artificial habitats were retrieved 60 - 65 days after being deployed. Previous studies

153   have demonstrated that artificial turfs are rapidly colonized by numerous invertebrates after

154   14 days of deployment (Olabarria 2002, Kelaher 2005). After 50 days of deployment there

155   are significant differences between assemblages colonizing habitats with different structural

156   diversity (Matias et al 2007). The diversity and abundance of invertebrates in artificial turfs at

157   2, 4 and 12 months after deployment are not different from those in natural turfs (i.e.

158   *Corallina* spp; Kelaher 2003), which suggests that they are consistent with the natural

159   assemblages. Thus, the time of deployment used here is to test hypotheses about differences

160   in patch-size and structure of habitats.

161   Fauna and epiphytes may easily be dislodged from the units of turf when habitats are

162   removed from the shore. To prevent this, artificial habitats were retrieved using a grid of 50 x

163   50 mm squared plastic corers (similar to an ice cube tray), which isolated units so that they

164   could be sampled separately, but simultaneously. The grid of corers was carefully placed over

165   the artificial habitat and then pressed firmly down to enclose the whole patch. The screws

166   were then undone, so that each sub-habitat in the artificial habitat was in a separate corer and

167   each corer was emptied into a separate plastic bag, guaranteeing that the epiphytes and fauna

168   associated with each sample were completely recovered. All units were labelled and

169   preserved in 7 % formalin. Three units were randomly selected from each habitat. Each unit

170   was then washed in a 500 μm sieve and all invertebrates sorted and counted under a binocular

171   microscope at 16 x magnification. All molluscs were identified to the finest possible

172   taxonomic resolution, either species or morphospecies. Each replicate was derived from

173   pooling data from three units.

174   The relationship between numbers of individuals and numbers of species (hypothesis 1,

175   Table 1) was tested by examining the correlations between numbers of species and numbers

176   of individuals in every habitat ($n = 54$). In addition, the slopes of the relationships between

177   numbers of species and numbers of individuals in habitats of same structural complexity (i.e.

178     $n = 18$ habitats in each of 3 complexities) were tested for heterogeneity of slopes (Underwood

179   1997). Hypotheses 2, 3, and 5 were tested by comparing habitats with different areas and

180   types of components, using a three-way analysis of variance (Table 1). *Type* was a fixed

181   comparison among habitats with different type of sub-habitats (A, B, or C); *Patch-size* was a

182   fixed comparison between artificial habitats of different area (100, 200 and 300 cm^2);

183   *Location* was random with two levels; there were 3 replicate habitats of every combination of

184   Type and Size and Location. All analyses were preceded by Cochran' test, which detects the

185   type of heterogeneity of variances that can compromise analysis of variance. Numbers of

186   individuals had heterogeneous variances and were transformed using sqrt(x+1), which is

187   appropriate for this type of data and removes the relationships between means and variances

188   for Poisson-type variables (Sokal and Rohlf 1996, Underwood 1997, Quinn and Keough

189   2001). Other transformations of data were used (e.g. Log (x+1)), in response to comments by

190   reviewers, but these did not change the outcome of any analyses.

191   There were no *a priori* hypotheses about which model would best describe the species-

192   area relationship, therefore any model (e.g. linear, power function, exponential) provides a

193   valid test of our hypothesis. We tested the hypothesis that more species should be found in

194   habitats with larger surface area (hypothesis 4, Table 1) using a linear model on

195   untransformed numbers of species and surface area (cm^2) for: i) all 54 habitats (3 replicates

196   of 9 combinations of Type and Size in each of 2 locations); ii) the 18 habitats of the same

197   structural complexity for each of the 3 types (i.e. 3 replicate habitats for each of 3 sizes in the

198   2 locations) and iii) the 18 habitats of the same size for each of the 3 types (i.e. 3 replicate

199   habitats for each of 3 types in the 2 locations). These analyses were also done with log/log

200   transformation and yielded the same similar results.

201   **RESULTS**

202   Habitats with greater numbers of individuals had more species ($r = 0.86$, $P < 0.001$,

203   52 df). This pattern was significant ($P < 0.001$, 16 df) for each type of structure: type A, $r =$

204   0.71; type B, $r = 0.96$; type C, $r = 0.89$. Differences among slopes were significant (test for

205   homogeneity of slopes, $F_{2, 48} = 3.5$; $P < 0.05$; Fig 1). Although there was a clear positive

206   relationship between numbers of species and numbers of individuals (i.e. rejecting hypothesis

207   1), the slopes of this relationship were not the same for habitats of different structural

208   complexity.

209   Patch-size did not affect the numbers of individuals ($F_{2,40} = 5.9$; $P > 0.05$; see

210   ANOVA table in Appendix B and means in Appendix C). Although there were significant

211   differences in numbers of individuals between locations, there was no interactive effect with

212   any of the main factors. This result does not reject the null hypothesis of no differences in

213   numbers of individuals per unit area among habitats of different sizes (hypothesis 2).

214   There was a consistent increase in number of species with increasing area of habitats

215   (Fig. 2a). Larger habitats had more and more species per unit (Fig. 2a, SNK tests, $P < 0.05$).

216   These results were consistent in the two locations and among habitats of different structural

217   complexity (Appendix A), rejecting the null hypothesis of no differences in number of

218   species among habitats of different area (supporting hypothesis 3). There was an overall

219   positive relationship between surface area and number of species when all habitats were

220   pooled ($r = 0.51$, $P < 0.001$, 52 df). This relationship was significant ($P < 0.001$, 16 df) for

221   each type of habitat when pooled across the 3 sizes: type A, $r = 0.85$; type B, $r = 0.71$; type

222   C, $r = 0.78$). When habitats of the same size were examined, pooled across the 3 types, the

223   relationship between number of species and surface area was only significant for the largest

224   habitats: 100 cm^2, $r = 0.53$, $P > 0.05$); 200 cm^2, $r = 0.30$, $P > 0.05$; 300 cm^2, $r = 0.34$, $P <$

225   0.001.

226   In each location, habitats made of type C had more species than did habitats of types

227   A or B (SNK tests, $P < 0.05$; Fig. 2b; Appendix B). Number of species per unit (i.e. the mean

228   number in the 3 sub-habitats sampled per habitat) showed a similar pattern, but means could

229   not be separated using multiple comparisons (SNK tests, $P > 0.05$; Fig 2; Appendix B).

230   Similarly, there were no differences among numbers of individuals in habitats with different

231   structural complexity ($F_{2,4} = 3.7$; $P > 0.05$; Appendix B). These results do not support the

232   prediction that habitats with greater surface area should have more individuals (hypothesis 4)

233   and reject the prediction of no differences in number of species between habitats with

234   different surface area (hypothesis 5, Table 1).

235   **DISCUSSION**

236   *Species and Area*

237   The numbers of species colonizing habitats were closely associated with the numbers

238   of individuals when all habitats were analysed together, regardless of their size or structural

239   complexity. It was predicted that, if individuals were randomly allocated to patches of habitat

240   (*sensu* Coleman 1981; hypothesis 1 in Table 1), samples of equal area (i.e. 75 cm^2) taken

241   from habitats of different overall areas (100, 200 or 300 cm^2) should yield the same numbers

242   of species (e.g. Simberloff 1976). Our results showed that assemblages colonizing larger

243   habitat had more species, regardless of the structural complexity of habitat. This clearly

244   demonstrates that the random placement hypothesis alone cannot explain differences in

245   numbers of species among habitats of different sizes. Moreover, neither patch-size nor

246   surface area affected numbers of individuals per unit area, which rejects the hypothesis that

247   numbers of individuals would differ among habitats of different sizes (hypotheses 2 and 4).

248   In general, for a particular patch-size, habitats with more individuals had more species, but

249   this cannot be generalized to explain the greater number of species in larger habitats. These

250   results indicate that random placement of individuals can occur at the scale of habitat (i.e.

251   patches with more individuals have more species), but this does not explain the increased

252   number of species in larger habitats.

253   Random placement may be a good model to explain number of species at coarser

254   scales but not for finer areas (Plotkin et al. 2000). At finer scales, processes that might

255   influence the probabilities of finding different species are spatial aggregation (Hill et al.

256   1994) and intraspecific competition among individuals at finer scales (Plotkin et al. 2000).

257   Moreover, intertidal gastropods show great variability in abundances at fine scales as a result

258   of variability of patches of habitat (e.g. Olabarria and Chapman 2001) and dispersal after

259   settlement (e.g. Underwood 2004). Assemblages colonizing artificial habitats (i.e. plastic

260   scourers) have been shown to vary significantly among patches 20 cm apart (Chapman and

261   Underwood 2008), which suggests that small-scale variability in abundance is likely to

262   influence the probabilities of finding individuals in each habitat independently of the patch-

263   sizes used in this experiment.

264   An alternative to investigate random placement is to use rarefaction, which assumes

265   that individuals are randomly distributed (Magurran 2004) and are randomly sampled (Gotelli

266   and Colwell 2001). These assumptions of independence are unrealistic for many benthic

267   assemblages. Random patterns are rarely observed; most species exhibit some sort of spatial

268   aggregation (e.g. Chapman and Underwood 1996). Rarefaction could have led to biased

269   estimation of numbers of species in smaller habitats (Clarke and Warwick 2001). The same

270   would be true when using species-per-individual ratios to correct for unequal numbers of

271   individuals, because this assumes that species richness increases linearly with abundance,

272   which has been shown not to be true for benthic assemblages (Gray 1997). Patterns of

273   abundance in these assemblages are rarely this extreme and therefore the species-per-

274   abundance ratio would have distorted patterns of number of species.

275   More species in larger habitats could potentially be explained by differences in

276   numbers of microhabitats between habitats of different sizes. For example, smaller habitats

277   generally have a greater perimeter-to-area ratio and therefore more edges. Any resource being

278   concentrated near edges would support increased abundances of any species reliant on it

279   (Ries and Sisk 2004). If the numbers of individuals differ between middles and edges of

280   patches, sampling the patches as in the present study would represent smaller and larger

281   patches differently. Randomly sampling 3 units from each patch would, on average, sample

282   more of the edge units in smaller patches than in larger ones. This would matter if sampling

283   of larger patches included microhabitats that do not exist in smaller patches (Anderson 1999)

284   or where the probability of larval settlement differs between edges and middle areas of

285   patches (Mullineaux and Butman 1990). Alternatively, individuals in assemblages in larger

286   patches might colonise edges preferentially, but have a reduced overall number of species

287   when compared to assemblages in smaller patches (Anderson 1999). These processes could

288   cause the number of species per unit area to be underestimated in larger habitats. Previous

289   studies on assemblages of microgastropods have shown no differences between the numbers

290   of individuals colonizing edges when compared to interiors of natural patches of turfs

291   (Olabarria 2002), but these possibilities are being investigated (Matias, unpublished data).

292   *Species and complexity*

293   The relationship between number of individuals and numbers of species in habitats of

294   Type B had a steeper slope than in habitats Type A and C (Fig. 1), which suggests that

295   turnover of species does not match the overall differences in diversity between types of

296   habitat. This result is somewhat unexpected because, structurally, Type B has characteristics

297   intermediate between those of Types A and C. This is analogous with some patterns observed

298   in ecological boundaries (or ecotones sensu Smith et al. 1997), where areas of transition are

299   colonized by species from two or more different types of adjoining habitats, resulting in a

300   greater turnover of species. From this, one possible explanation of this result is that habitats

301   of Type B are colonized by species that are found in habitats of Type A and C, as a result of

302   its intermediate structure or its being a "transition" habitat. There were, however, 11 species

303   that were exclusive to B and C, as opposed to only 3 species shared between Types B and A,

304   which suggests that, although, Type B has an intermediate structure between Types A and C,

305   it shares more species with habitats of Type C. This result suggests that the relationship

306   between structural complexity and number of species may not be linear (Kelaher 2003), and

307   has implications for future studies using artificial habitats, because which types of habitat are

308   chosen for experiments may affect the outcomes.

309   More species were found in Type C habitats, which had the largest surface area, but

310   the numbers of individuals colonizing artificial habitats were not affected by differences in

311   surface area. This suggests that increased numbers of species cannot be a result of random

312   placement associated with sampling increased numbers of individuals, contrary to

313   suggestions that the relationship between surface area and number of species might be a

314   sampling artefact as result of increased sample-size (Attrill et al. 2000). Furthermore, if

315   surface area alone explained the numbers of individuals and numbers of species, there should

316   be no differences between samples of equal amount of habitat (i.e. equal number of fronds

317   per unit area) taken from habitats of different sizes. As indicated above, there were more

318   species in larger habitats than in samples of equal size taken from smaller habitats, rejecting

319   the null hypothesis of no differences in numbers of species in samples of the same surface

320   area.

321   The relationship between surface area and number of species was only significant in

322   larger habitats; surface area alone did not explain differences in number of species in small

323   and intermediate sized habitats (100 and 200 cm^2). This result is not consistent with the lack

324   of statistical interactions between differences among types of habitat and differences among

325   sizes of habitats in the numbers of species and of individuals. Surface area may be a better

326   predictor of structure of these assemblages in larger habitats (i.e. 300 cm^2). This is not

327   surprising given the hierarchical nature of structure of habitats whereby assemblages respond

328   to changes in structure of habitats at different scales (e.g. patch-size, Kotliar and Wiens

329   1990). It is also further evidence that measurements of structure of habitat may quantify

330   attributes in ways that are different from the ways organisms respond to them. Understanding

331   the effects of structure of habitats at different scales must include discussions of the

332   appropriateness of the measures used at different scales.

333   Comparisons between habitats with different surface areas are confounded by other

334   attributes of habitat, such as density and length of fronds (e.g. Attrill et al. 2000, Kelaher

335   2003a, b, Sirota and Hovel 2006). The physical structure of habitat depends not just on the

336   shape of structural components, but also on the available space associated with these

337   structures. Therefore, several indices should be used jointly to capture different aspects of

338   structure of habitat (e.g. fractal geometry, Warfe et al. 2008). Structural complexity (as

339   measured by fractal geometry) can influence the number of species, even after correcting for

340   the increased surface availability on more complex surfaces (Johnson et al. 2003). These

341   assemblages are responding to differences in structure of the habitats (as measured by surface

342   area), although what causes these differences cannot be attributed to a sampling artefact. No

14

343   previous work has critically examined which indices of structural complexity might better

344   describe the structure of these complex, turf-like habitats.

345   *Conclusions*

346   The use of experimental micro- and mesoscosms to test conceptual models is quite

347   common across the ecological literature (e.g. Kneitel and Chase 2004, Srivastava 2006).

348   These experimental systems are all, by definition, small habitats, which enable appropriate

349   replication, but also provide the versatility to test relevant hypotheses that are often difficult

350   to test at larger scales (see review by Srivastava et al. 2004). The artificial habitats used here

351   share most of these advantages, such as rapid colonization, tractability and size of

352   experimental units, and, unlike laboratory experiments, are deployed across the natural

353   habitats, which they mimic. This study clearly showed that patch-size and structural

354   complexity are independent attributes of habitats and accentuate the need for proper

355   experimental manipulations to determine the relative contributions of such attributes.

356   If habitats are manipulated at the appropriate scales at which organisms respond to the

357   environment, the information obtained from these studies can be extremely valuable to the

358   interpretation of observational studies at larger scales, which are difficult to manipulate.

359   Understanding how these different attributes of habitats influence assemblages is essential for

360   predicting their responses to reductions of patch-size (Bender et al 1998, Bell et al. 2001) at a

361   time when the loss and modification natural habitats are considered to be major threats to

362   global biodiversity (Gray 1997, Pimm and Raven 2000). Reduced natural variation in

363   structural complexity of habitats may also affect the numbers of species (e.g. "habitat"

364   homogenization, McKinney and Lockwood 1999). In order to predict and explain species'

365   responses to such dramatic changes in natural habitats, it is essential to develop an

366   understanding of why species inhabit such habitats in the first place.

367

374   **LITERATURE CITED**

375   Akioka, H., M. Baba, T. Masaki, and H. W. Johansen. 1999. Rocky shore turfs dominated by

376   *Corallina* (Corallinales, Rhodophyta) in northern Japan. Phycological Research 47:199–206.

377   Allen, T. F. H., and T. B. Starr. 1982. Hierarchy - Perspectives for ecological complexity.

378   The University of Chicago Press, Chicago.

379   Anderson, M. J. 1999. Effects of patch size on colonisation in estuaries: revisiting the

380   species-area relationship. Oecologia 118:87-98.

381   Attrill, M. J., J. A. Strong, and A. A. Rowden. 2000. Are macroinvertebrate communities

382   influenced by seagrass structural complexity? Ecography 23:114-121.

383   Beesley, P. L., G. J. B. Ross, and A. Wells. 1998. Mollusca: the southern synthesis. Fauna of

384   Australia. CSIRO, Melbourne.

385   Bell, S. S., R. A. Brooks, B. D. Robbins, M. S. Fonseca, and M. O. Hall. 2001. Faunal

386   response to fragmentation in seagrass habitats: implications for seagrass conservation.

387   Biological Conservation 100:115-123.

388   Bender, D. J., T. A. Contreras, and L. Fahrig. 1998. Habitat loss and population decline: A

389   meta-analysis of the patch size effect. Ecology 79:517-533.

390   Chapman, M. G., and A. J. Underwood. 1996. Influences of tidal conditions, temperature and

391   desiccation on patterns of aggregation of the high-shore periwinkle, *Littorina unifasciata*, in

392   New South Wales, Australia. Journal of Experimental Marine Biology and Ecology 196:213-

393   237.

394   Chapman, M. G., and A. J. Underwood. 2008. Scales of variation of gastropod densities over

395   multiple spatial scales: comparison of common and rare species. Marine Ecology-Progress

396   Series 354:147-160.

397   Clarke, K. R., and R. M. Warwick. 2001. Change in marine communities: an approach to

398   statistical analysis and interpretation. PRIMER-E, Plymouth.

399   Coleman, B. D. 1981. On random placement and species-area relations. Mathematical

400   Biosciences 54:191-215.

401   Connor, E. F., and E. D. McCoy. 1979. Statistics and biology of the species-area relationship.

402   American Naturalist 113:791-833.

403   Gilpin, M. E. and J. M. Diamond. 1980. Subdivision of nature reserves and the maintenance

404   of species-diversity. Nature 285:567-568.

405   Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in

406   the measurement and comparison of species richness. Ecology Letters 4:379-391.

407   Gray, J. S. 1997. Marine biodiversity: Patterns, threats and conservation needs. Biodiversity

408   and Conservation 6:153-175.

409   Higgs, A. J. and M. B. Usher. 1980. Should nature reserves be large or small. Nature

410   285:568-569.

411   Hill, J. L., P. J. Curran, and G. M. Foody. 1994. The effect of sampling on the species-area

412   curve. Global Ecology and Biogeography Letters 4:97-106.

413   Hovel, K. A., and R. N. Lipcius. 2001. Habitat fragmentation in a seagrass landscape: Patch

414   size and complexity control blue crab survival. Ecology 82:1814-1829.

415   Johnson, M. P., N. J. Frost, M. W. J. Mosley, M. F. Roberts, and S. J. Hawkins. 2003. The

416   area-independent effects of habitat complexity on biodiversity vary between regions. Ecology

417   Letters 6:126-132.

418   Kelaher, B. P. 2003a. Changes in habitat complexity negatively affect diverse gastropod

419   assemblages in coralline algal turf. Oecologia 135:431-441.

420   Kelaher, B. P. 2003b. Effects of frond length on diverse gastropod assemblages in coralline

421   turf. Journal of the Marine Biological Association of the United Kingdom 83:159-163.

422   Kelaher, B. P., A. J. Underwood, and M. G. Chapman. 2003. Experimental transplantations

423   of coralline algal turf to demonstrate causes of differences in macrofauna at different tidal

424   heights. Journal of Experimental Marine Biology and Ecology 282:23-41.

425   Kneitel, J. M. and J. M. Chase. 2004. Disturbance, predator, and resource interactions alter

426   container community composition. Ecology 85:2088-2093.

427   Kotliar, N. B., and J. A. Wiens. 1990. Multiple scales of patchiness and patch structure - a

428   hierarchical framework for the study of heterogeneity. Oikos 59:253-260.

429   Lawton, J. H. 1999. Are there general laws in ecology? Oikos 84:177-192.

430   Lobel, S., T. Snall, and H. Rydin. 2006. Species richness patterns and metapopulation

431   processes - evidence from epiphyte communities in boreo-nemoral forests. Ecography

432   29:169-182.

433   Lomolino, M. V. 2000. Ecology's most general, yet protean pattern: the species-area

434   relationship. Journal of Biogeography 27:17-26.

435   MacArthur, R. H., and J. W. MacArthur. 1961. On bird species diversity. Ecology 42:594–

436   598.

437   MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton

438   University Press, Princeton.

439   Magurran, A. E. 2004. Measuring biological diversity. Blackwell, Oxford.

440   Matias, M. G., A. J. Underwood, and R. A. Coleman. 2007. Interactions of components of

441   habitat alter composition and variability of assemblages. Journal of Animal Ecology 76:986–

442   994.

443   McCoy, E. D., and S. S. Bell. 1991. Habitat structure: the evolution and diversification of a

444   complex topic. Pages 3-27 *in* E. D. McCoy, S. S. Bell, and H. R. Mushinsky, editors. Habitat

445   structure: The physical arrangement of objects in space. Chapman and Hall, London.

446   McGuinness, K. A. 1984. Equations and explanations in the study of species area curves.

447   Biological Reviews of the Cambridge Philosophical Society 59:423-440.

448   McGuinness, K. A. 2000. Distinguishing area and habitat heterogeneity effects: a simulation

449   test of the MacNally and Watson (1997) protocol. Austral Ecology 25:8-15.

450   McKinney, M. L., and J. L. Lockwood. 1999. Biotic homogenization: a few winners

451   replacing many losers in the next mass extinction. Trends in Ecology & Evolution 14:450-

452   453.

453   Mullineaux, L. S., and C. A. Butman. 1990. Recruitment of encrusting benthic invertebrates

454   in boundary-layer flows - a deep-water experiment on cross seamount. Limnology and

455   Oceanography 35:409-423.

456   Ney-Nifle, M., and M. Mangel. 2000. Habitat loss and changes in the species-area

457   relationship. Conservation Biology 14:893-898.

458   Olabarria, C. 2002. Role of colonization in spatio-temporal patchiness of microgastropods in

459   coralline turf habitat. Journal of Experimental Marine Biology and Ecology 274:121– 140.

460   Olabarria, C., and M. G. Chapman. 2001. Comparison of patterns of spatial variation of

461   microgastropods between two contrasting intertidal habitats. Marine Ecology Progress Series

462   220:201–211.

463 Olabarria, C., A. J. Underwood, and M. G. Chapman. 2002. Appropriate experimental design

464 to evaluate preferences for microhabitat: an example of preferences by species of

465 microgastropods. Oecologia 132:159–166.

466 Pianka, E. R. 1966. Convexity Desert Lizards and Spatial Heterogeneity. Ecology 47:1055-

467 1059.

468 Pimm, S. L. and P. Raven. 2000. Biodiversity - Extinction by numbers. Nature 403:843-845.

469 Plotkin, J. B., M. D. Potts, N. Leslie, N. Manokaran, J. LaFrankie, and P. S. Ashton. 2000.

470 Species-area curves, spatial aggregation, and habitat specialization in tropical forests. Journal

471 of Theoretical Biology 207:81-99.

472 Preston, F. W. 1960. Time and space and the variation of species. Ecology 41:611-627.

473 Quinn, G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologists.

474 Cambridge University Press, Cambridge.

475 Ricklefs, R. E., and I. J. Lovette. 1999. The roles of island area per se and habitat diversity in

476 the species-area relationships of four Lesser Antillean faunal groups. Journal of Animal

477 Ecology 68:1142-1160.

478 Ries, L., and T. D. Sisk. 2004. A predictive model of edge effects. Ecology 85:2917-2926.

479 Simberloff, D. 1976. Experimental zoogeography of islands - effects of island size. Ecology

480 57:629-648.

481 Sirota, L., and K. A. Hovel. 2006. Simulated eelgrass Zostera marina structural complexity:

482 effects of shoot length, shoot density, and surface area on the epifaunal community of San

483 Diego Bay, California, USA. Marine Ecology-Progress Series 326:115-131.

484 Smith, T. B., R. K. Wayne, D. J. Girman, and M. W. Bruford. 1997. A role for ecotones in

485 generating rainforest biodiversity. Science 276:1855-1857.

486 Sokal R. R. & F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in

487 biological research. W. H. Freeman and Co., New York.

488   Srivastava, D. S. 2006. Habitat structure, trophic structure and ecosystem function:

489   interactive effects in a bromeliad-insect community. Oecologia 149:493-504.

490   Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P.

491   Munguia, T. Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms

492   useful model systems for ecology? Trends in Ecology & Evolution 19:379-384.

493   Taniguchi, H., S. Nakano, and M. Tokeshi. 2003. Influences of habitat complexity on the

494   diversity and abundance of epiphytic invertebrates on plants. Freshwater Biology 48:718-728.

495   Underwood, A. J. 1997. Experiments in ecology: their logical design and interpretation using

496   analysis of variance. Cambridge University Press, Cambridge.

497   Underwood, A. J. 2004. Landing on one's foot: small-scale topographic features of habitat

498   and the dispersion of juvenile intertidal gastropods. Marine Ecology-Progress Series 268:173-

499   182.

500   Underwood, A. J., and M. G. Chapman. 2006. Early development of subtidal macrofaunal

501   assemblages: relationships to period and timing of colonization. Journal of Experimental

502   Marine Biology and Ecology 330:221-233.

503   Warfe, D. M., L. A. Barmuta, and S. Wotherspoon. 2008. Quantifying habitat structure:

504   surface convolution and living space for species in complex environments. Oikos 117:1764-

505   1773.

506   Wilcox, B. A. and D. D. Murphy. 1985. Conservation strategy - the effects of fragmentation

507   on extinction. American Naturalist 125:879-887.

508   Williams, C. B. 1943. Area and number of species. Nature 152:264-267.

509

510

Table 1. Models, hypothesis and tests of effects of patch-size and surface area on number of species (S) and number of individuals (N)

Model	Hypothesis	Null	Tests of hypothesis
Random placement: Sampling more individuals increases the probability of finding more species	H1: Positive relationship between the S and N.	Ho: No relationship between S and N.	Regressions of S and N in: 1) all samples, irrespectively of patch-size or surface area; 2) habitats of the same size, irrespectively of type of surface area; 3) habitat of the same surface area, irrespectively of patch-size.
Passive sampling or ***target area:*** Larger islands "sample" more colonists (i.e. have greater immigration) than do smaller islands	H2: Differences in N (per unit area) between habitats of different sizes. H5: Differences in N (per unit area) in between habitats of with larger surface area.	Ho: No difference in N (per unit area) between habitats of different sizes. Ho: No difference in N (per unit area) between habitats with different surface area.	ANOVA of N in habitats of different sizes (i.e. 100, 200 and 300 cm^2). ANOVA of N in habitats with different surface area (i.e. A, B and C).
Area per se: Greater rates of colonization (or immigration),	H3: Greater S in larger habitats.	Ho: No differences in S in habitats of different sizes.	ANOVA of S (per unit area) found in habitats of different sizes.

22

thus reducing the probability of extinction	H4: Greater S in habitats with greater surface area.	Ho: No difference S in habitats with different surface area.	ANOVA of S (per unit area) found in habitats with different surface area (i.e. A, B and C).

Table 2. Dimensions of artificial habitats with different size and type of structural components: Area (cm^2), fronds per cm^2, length of fronds (cm) and surface area (SA) of fronds per habitat (cm^2).

Habitat	Area (cm^2)	Fronds per cm^2	Length of fronds (cm)	Surface area of fronds (cm^2)
A1	100	66.2	1	26.5
A2	200	66.2	1	53.0
A3	300	66.2	1	79.5
B1	100	22.6	2	18.5
B2	200	22.6	2	36.1
B3	300	22.6	2	54.1
C1	100	16.2	4	25.9
C2	200	16.2	4	51.8
C3	300	16.2	4	77.8

Figure legends

Figure 1. Regressions of relationships between number of species and number of individuals per habitat: black symbols are habitats made of components of type A; white symbols are habitats made of components of type B; grey symbols are habitats made of components of type C.

Figure 2. Diversity of assemblages in habitats with different: a) patch-size and b) type of structure; clear bars are the mean (+ S.E.) number of species per unit; black bars are the mean (+ S.E.) number of species per habitat. Numbers denote significant differences at $P < 0.05$.
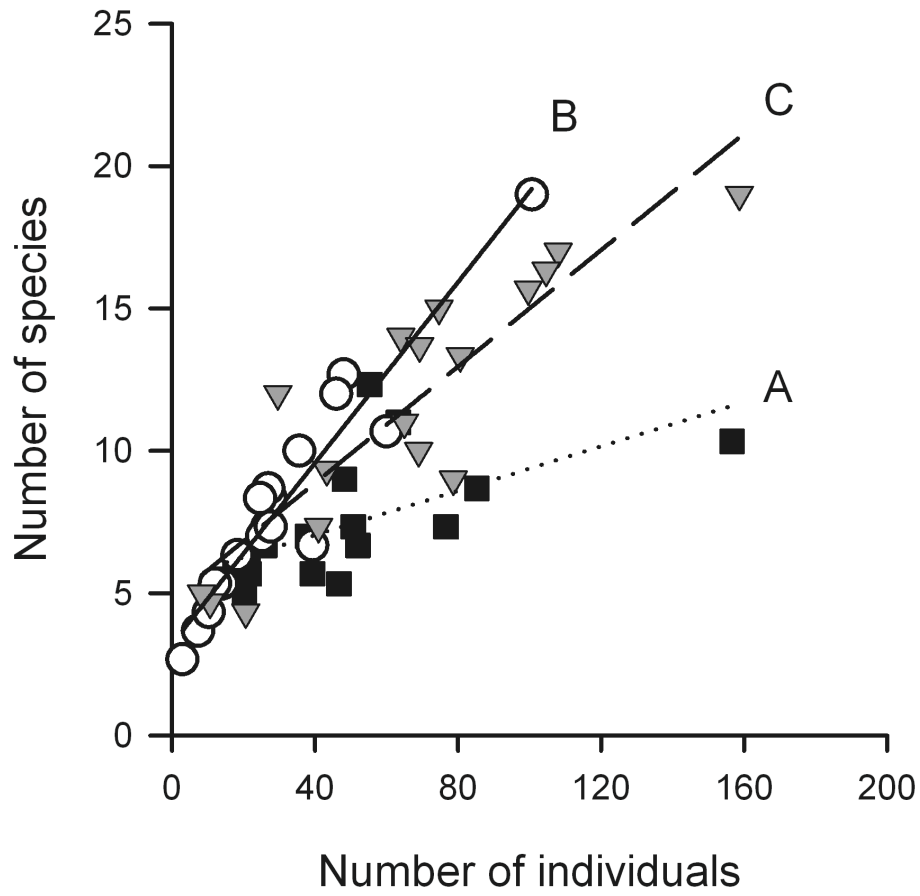
24

Figure 1

Figure 2