METHODS

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Appropriate experimental design to evaluate preferences for microhabitat: an example of preferences by species of microgastropods

Abstract The intertidal microgastropods *Eatoniella atropurpurea*, *Amphithalamus incidata* and *Eatonina rubrilabiata* are principally found at mid- to low-shore levels in coralline turf and in patches of sediment close to algal turf on rocky shores. All three species are more abundant in coralline turf (i.e. algal beds composed primarily of *Corallina officinalis* Linnaeus, often containing patches of sediment) than in sediment, although the latter two are also quite abundant in patches of sediment. *Eatoniella atropurpurea* seem to live on branches of coralline algae, whereas *Eatonina rubrilabiata* and *A. incidata* seem to inhabit the sediment. In this study, we investigated whether or not behaviour of these animals accounts for the patterns observed in the field. Particularly, this study deals with choices amongst three microhabitats (coralline algae, coralline algae plus sediment and sediment) by these three species of microgastropods. Laboratory experiments were designed to examine preferences. The designs of such experiments are complex because demonstrating preference necessitates demonstrating different behaviour when confronted with choices from that showed when no choice is available. This study describes an appropriate series of hypotheses about preferences and experimental designs to test them. Preferences were indicated by differences in occupancy of the microhabitats when presented with a mixture and when each type is presented alone. In such experiments, snails did show preferences, except for *A. incidata* which did not show a pattern. Thus, the behaviour could, at least partially, account for the patterns of distribution in the field. The designs of experiments used here may be of value in other studies of preferences among habitats.

Keywords Behavioural choice · Experimental design · Microhabitat · Microgastropods · Preference

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Introduction

Patterns of distribution and abundance of intertidal animals are frequently explained by the behaviour of the species under consideration, or of those with which they interact (Chapman 2000). When animals are associated with a particular habitat or microhabitat, it is often assumed that they "prefer" to be there, or that they have actively "selected" that habitat and rejected the others that are available (Bennett 1993; Allainé et al. 1994). Moreover, the quality of the habitat where organisms live can affect their subsequent survival or reproductive success (Levins 1968; Patridge 1978; Cowie 1985; Hunt 1996). Animals are thus expected to "prefer" habitats in which their survival and/or reproductive success are great. Selection of habitat should co-evolve with quality of those habitats (Levins 1968).

There are, however, three broad classes of processes (related or not to selection of habitat) which can explain patterns of distribution. One is differential settlement/recruitment: larval or juvenile individuals could recruit in greater numbers to one habitat than to others (Moore and Lopez 1972; Dayton 1975). A second alternative is that populations distribute randomly throughout the available habitats, but differential mortality leads to the reduction of numbers in unfavourable habitats (e.g. Levins 1968; Russo 1987). Another possibility could be that, after widespread recruitment, adults choose the favoured habitats (Underwood and Denley 1984; Crowe and Underwood 1998). None of these processes is, however, necessarily exclusive and it could be that a combination of them explains the patterns of distribution of organisms.

Before deciding that animals are showing "preference or choice of habitat", it is necessary to define such choice. Preference for or choice of habitat means that animals have actively selected one (or more) habitat(s) from a variety of potential habitats encountered (Rosenzweig 1981; Manly et al. 1993). Moreover, selection of habitat can occur at a variety of spatial scales. We define habitat as the place where an animal lives that can often be char-

acterised by some particular physical or biological features. At some spatial scale smaller than habitat, each microhabitat differs in environmental factors that influence the allocation of time and energy by an individual within particular portions of its habitat (Morris 1987). Selection of habitat thus involves responses at a series of hierarchical levels and behaviours are potentially hierarchical in nature (Urban et al. 1987).

There are, however, often problems with the definition of preference and the experimental designs to test for preferences in many studies (see discussions in Liszka and Underwood 1990; Crowe and Underwood 1998). The problem with this type of experiment, as in other experiments of choice, is that preference (i.e. discrimination among types when confronted by a choice) can be confounded with "accessibility" (i.e. the ease with which a microhabitat can be found or be occupied). Thus, when snails are presented with a given number of microhabitats, some types may have more snails occupying them, not because these types are preferred, but because they are more accessible. For example, it may be more difficult to move out from (or to move into) some habitats, perhaps because of different topographic complexity.

To avoid confounding preference (an active behaviour) from other causes of greater numbers being found in some microhabitats, it is also necessary to determine the pattern of occupancy of each type of microhabitat when available alone (i.e. when there is no choice and therefore no preference). This is exactly analogous to analyses of preference for particular prey by predators (Rapport and Turner 1970; Liszka and Underwood 1990). If the snails choose a certain microhabitat (i.e. show a preference) they should select more of that microhabitat when presented with a mixture than would be the case when each of the various choices is presented alone. Experiments to determine patterns of occupancy of microhabitats and to demonstrate preferences are therefore, of necessity, quite complex. The relevant hypotheses need to be carefully defined. The experiments require a careful comparison of possible choices with situations where there is no choice. This paper describes an approach to the design of such experiments, in analyses of behaviour of intertidal microgastropods in relation to microhabitats.

Sufficient examples are presented to demonstrate that the experimental design can distinguish among different patterns of preference (including no preference) for habitat. Also, some experiments were done twice to demonstrate that the design generates repeatable outcomes.

Eatoniella atropurpurea (Frauenfeld, 1867), *Eatonina rubrilabiata* Ponder and Yoo (1980) and *Amphithalamus incidata* (Frauenfeld, 1867) are microgastropods (size <2 mm length) found at mid- to low-shore levels in coralline turf and in patches of sediment close to algal turf (Olabarria and Chapman 2001a). *Eatoniella atropurpurea* seem to live on branches of the algae, whereas *Eatonina rubrilabiata* and *A. incidata* seem to inhabit the algae and the sediment (Olabarria, personal observa-

tion). In laboratory experiments (Olabarria and Chapman 2001b), all species showed different patterns of survival and/or growth in sediment and algal turf. Here, we investigated the choice of microhabitat by these three species of microgastropods. Because they are small and cryptic, the logistics of doing experiments on growth, survival or behaviour of individual animals, are very difficult in the field. So, we used experiments in a laboratory. Such experiments usually use a "choice chamber" into which individuals are released to move freely among different types of microhabitat (e.g. Barnes and Greenwood 1978; Carefoot 1979; Morton 1980; Johns and Mann 1987; Durante and Chia 1991; Henrikson 1993; Herler et al. 1999; Jones and Boulding 1999).

As with all laboratory experiments, it is never clear if the results are applicable to the real world (see Connell 1974). We are concerned about these difficulties, but tried to make the conditions as realistic as possible by transferring the animals to the laboratory along with patches of natural habitat, creating the minimal possible disturbance (Della Santina and Naylor 1994).

Experiments were designed to test the hypotheses that adults of *Eatoniella atropurpurea*, *A. incidata* and *Eatonina rubrilabiata* had preferences among three different types of microhabitats (sediment, coralline turf with sediment and coralline turf without sediment) and hence to determine whether the patterns of distribution in the field can be due to such preferences. Using data from the field (Olabarria and Chapman 2001a) and from other laboratory experiments (Olabarria and Chapman 2001b) we made the predictions that: (1) *Eatoniella atropurpurea* would choose coralline algae as a microhabitat, whether or not sediment was present in the algae; (2) *A. incidata* and *Eatonina rubrilabiata* would choose sediment, whether or not it occurred with coralline algae.

Material and methods

Collection of snails

Samples of *Eatoniella atropurpurea*, *Eatonina rubrilabiata* and *A. incidata* were collected from algal turf at the low-shore level on one shore (described in Olabarria and Chapman 2001a) in the Cape Banks Scientific Marine Research Area, New South Wales, in March 2001 (first experiment). Further samples of *Eatoniella atropurpurea* and *Eatonina rubrilabiata* from the same place were collected in November 2001 (second experiment). Patches of algal turf were taken to the laboratory and kept in aerated aquaria with a continuous supply of fresh seawater for 4 days before animals were picked from them. Sediment from patches close to the algal turf was also taken to the laboratory. Only adult snails were sorted because differential behaviour may occur depending on different age-classes (see for example Carefoot 1979; Worthington and Fairweather 1989; Jones and Boulding 1999). We defined adult snails as those with shell lengths of $0.80 \le x \le 1.40$ mm.

Collection of habitats

The coralline turf was collected using a diamond corer of 6 cm diameter (~ 5 cm deep) from a patch of low-shore coralline turf. All samples were taken to the laboratory and sediment removed by washing with running water. The sediment was sieved through
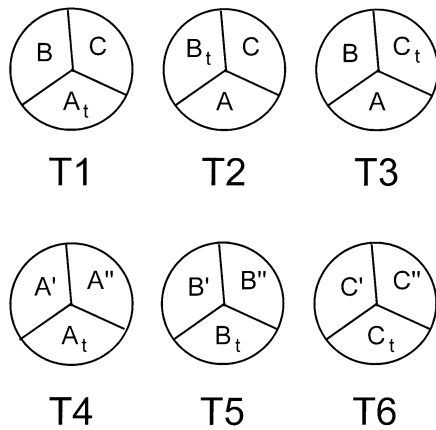
Fig. 1 Treatments (T_1–T_6) used in the experiments. *A* is coralline turf without sediment, *B* is coralline turf with sediment and *C* is sediment; *t* indicates the segment where the snails were placed at the beginning of the experiment

60 μm mesh. The sediment and coralline algae were then examined under a microscope and every snail was removed, so that known numbers of each species could be placed into the different microhabitats. Subsequently, the cores were divided into three equal sections (using a plastic template), each containing an experimental microhabitat and two other microhabitats: *A* was coralline turf from which sediment had been removed; *B* was coralline turf, with its associated sediment and *C* was sediment from patches close to the algal turf where coralline algae had previously been scraped off and the sediment removed. Equivalent amounts of this sediment (without snails) as found naturally were placed in experimental treatments *B* and *C*.

Cores were kept in transparent pots in the laboratory and snails were placed in each core. The experiments were run under 12 h light: 12 h dark with continuous water-flow. Experiments ran for 3 days, because previous observations in the laboratory showed that snails moved quickly in a few hours, so it is not unreasonable to think that they would demonstrate any choice of a microhabitat within 3 days. The temperature varied with ambient temperature and had a mean of 22.7°C (SD=1.0) and the mean salinity was 33.8 ‰ (SD=1.6). After 3 days, snails in each of the three segments of each core were counted; snails outside the three microhabitats (e.g. on the bottom of a pot, walls of the core, etc.) were ignored.

Design of experiments

Similar densities of each species to those found in the field were used in the experiments. A chosen number of individuals was placed into one experimental microhabitat (i.e. one of the three segments of a core) in each treatment. The numbers of snails used were chosen on the basis that, if all snails moved into a single microhabitat, their density would not be greater than that found at the field (*Eatoniella atropurpurea* =15, *Eatonina rubrilabiata* =6 and *A. incidata* =8).

Preference by the three species of microgastropods was tested using the following experimental design (see treatments in Fig. 1; *n*=5 cores of each treatment in the first experiment; *n*=7 in the second experiment).

The experiment compares the proportions of individuals in each of the microhabitats when presented together (treatments 1–3) or alone (treatments 4–6). At the end of each experiment in each treatment, we calculated the proportion of all snails found that were in the various microhabitats, including the one in which they were initially placed (shown with subscript *t* for each treatment in Fig. 1).

Thus, preference by *Eatoniella atropurpurea* for coralline turf without or with sediment (*A* and *B*) would be reflected by a greater proportion of these snails in these microhabitats at the end of the experiment, compared with what is expected by chance if no preference is expressed. The chance occurrence in parts of a core at the end of the experiment is estimated from the treatments where there is no choice (4–6; see Fig. 1). Preference for *A* and *B* means that the following hypotheses must be accepted:

$$a)H_1 : \frac{n_{At1}}{N_1}, \frac{n_{Bt2}}{N_2}, \frac{n_{At4}}{N_4}, \frac{n_{Bt5}}{N_5} > \frac{n_{Ct3}}{N_3} \tag{1}$$

where, at the end of the experiment, n_{Ati} or n_{Bti} is the number of snails in the segment of the core (*A* or *B*) where they started (identified with subscript *t* in Fig. 1); N_i is the total number of snails recovered from that core at the end of experiment (see Fig. 1), *i*=1.....5, indicates the treatment.

So, if snails really prefer microhabitats *A* and *B* (coralline algae or coralline algae with sediment), the proportions in these microhabitats (A_t in treatments 1 and 4; B_t in treatments 2 and 5) should be greater than in treatment 3 (in C_t). In treatment 3, snails should move to the preferred microhabitats and therefore out of C_t. A number of possibilities exist for differences among treatments 1–4. For example, snails may move to random positions in treatments 4 or 5, resulting in n_{At4}/N_4 and $n_{Bt5}/N_5 < n_{At1}/N_1$ and n_{Bt2}/N_2. Alternatively, if they are in a preferred microhabitat, snails may not move out of their starting segment resulting in n_{At4}/N_4, n_{Bt5}/N_5, n_{At1}/N_1 and n_{Bt2}/N_2 all being similar. So, preference requires treatment 3 to show a smaller proportion of snails in C_{t3}. Treatment 6 is irrelevant to this hypothesis.

$$b)H_2 : \frac{n_{Ct3}}{N_3} < \frac{n_{Ct6}}{N6} \tag{2}$$

because snails are more likely to move from C_t in treatment 3 into preferred microhabitats (*A* and *B*), than into non-preferred microhabitats (*C'* and *C''*) in treatment 6.

Following the same logic, preference by *Eatonina rubrilabiata* and *A. incidata* for coralline turf with sediment and sediment alone (*B* and *C*) will be reflected in greater proportions of these snails in these microhabitats at the end of experiment. Thus, the hypotheses in this case are:

$$a)H_1 : \frac{n_{Bt2}}{N_2}, \frac{n_{Cl3}}{N_3}, \frac{n_{Bt5}}{N_5}, \frac{n_{Cr6}}{N_6} > \frac{n_{At1}}{N_1} \tag{3}$$

$$b)H_2 : \frac{n_{At1}}{N1} < \frac{n_{At4}}{N_4} \tag{4}$$

for identical reasoning as above. These hypotheses were tested by analyses of variance which, when designs are balanced (as here), are robust to violations of assumptions of normality and homoscedasticity. Where the assumptions are a cause of concern, the proportional data can be transformed to arc-sines or GLM used with binomial residuals.
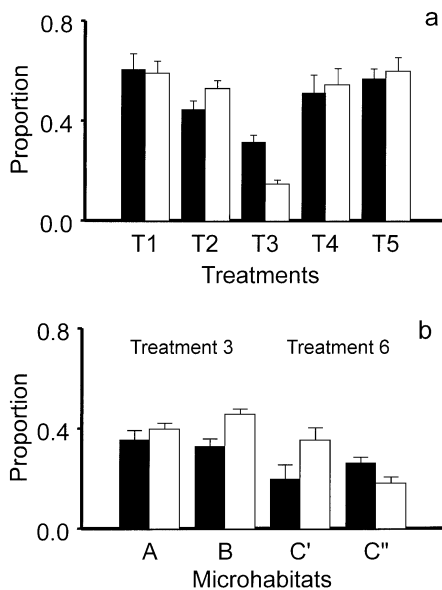
Results

Of the 870 snails put in the five replicates of the various treatments in experiment 1, 847 (97%) were recovered after 3 days. The others were on the bottoms of containers, the sides of cores, etc. In experiment 2, of the 882 individuals put into treatments, 875 (99%) were recovered in segments of the experimental cores.

Eatoniella atropurpurea

It was proposed that *Eatoniella atropurpurea* should demonstrate a preference for *Corallina*, with or without

Table 1 Analyses of proportions of *Eatoniella atropurpurea* in experimental treatments (data are in Fig. 2); *n*=5 cores in experiment 1, initially with 15 snails; *n*=7 cores in experiment 2, initially with 15 snails

	Experiment 1				Experiment 2			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*
1. Hypothesis 1. A_{t1}, B_{t2}, A_{t4}, B_{t5} > C_{t3} (more in preferred microhabitats)								
Among treatments	4	0.065	5.48	<0.005	4	0.251	16.40	<0.00001
Residual	20	0.012			30	0.015		
Contrast 1	A_{t1}, B_{t2} vs A_{t4}, B_{t5}				A_{t1}, B_{t2} vs A_{t4}, B_{t5}			
Means	0.525		0.541		0.559		0.570	
	F=0.09; 1, 20 *df*; P>0.75				F=0.06; 1, 30 *df*; P >0.80			
Contrast 2	A_{t1}, B_{t2}, A_{t4}, B_{t5} vs C_{t3}				A_{t1}, B_{t2}, A_{t4}, B_{t5} vs C_{t3}			
Means	0.533 >		0.315		0.564 >		0.145	
	F=68.71; 1, 20 *df*; P<10^{-7}				F=65.54; 1, 30 *df*; P<10^{-7}			
2. Hypothesis 2. C_{t3} > C_{t6} (fewer in non-preferred microhabitats where there is a choice)								
Among treatments	1	0.124	26.78	<0.001	1	0.362	25.30	<0.0001
Residual	8	0.005			12	0.014		
Means	C_{t3}	C_{t6}			C_{t3}	C_{t6}		
	0.315 <	0.538			0.145 <	0.467		

Fig. 2 Mean (SE) proportion of *Eatoniella atropurpurea* in experiments 1 and 2 (*n*=5; *n*=7 cores, respectively); **a** proportion of individuals remaining in treatments in the microhabitats where they were initially placed; **b** proportion of individuals in microhabitats *A*, *B* in treatment 3 and *C'* and *C''* in treatment 6

sediment (conditions *A* and *B* in Fig. 1). In each experiment, there were significant differences among the proportions of animals in different microhabitats (Table 1).

To test the first hypothesis (see Materials and methods), *a priori* determined contrasts were used (as in Table 1), which demonstrated that greater mean proportions of the animals were recovered of those initially placed in segments with *Corallina* (A_t in treatments 1 and 4, B_t in treatments 2 and 5; Fig. 2) than in segments without *Corallina* (C_t in treatment 3). This is entirely consistent with the hypothesis.

There were greater proportions of snails in segments with sediment and no *Corallina* where other segments also had no *Corallina* (C_t in treatment 6) than where choices of the hypothetically preferred habitat were available (C_t in treatment 3; see Table 1). All analyses supported the hypotheses derived from the model that *Eatoniella atropurpurea* prefer *Corallina*, with or without sediment.
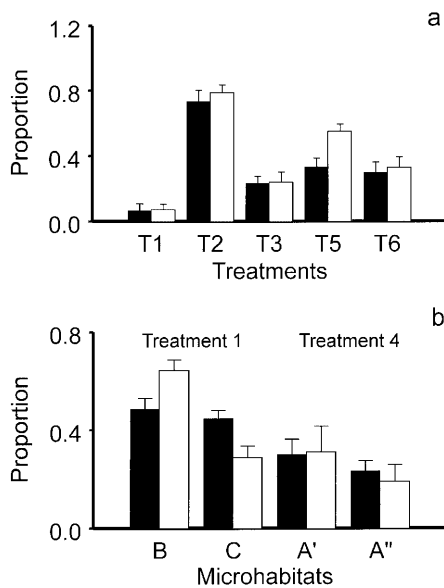
Eatonina rubrilabiata

Eatonina rubrilabiata also showed absolute consistency with hypotheses derived from the model of preference for microhabitats with sediment (*B* and *C* in Fig. 1). Unlike results for *Eatoniella atropurpurea*, there were significant differences between the responses of snails where confronted with choices versus the situation where there were no choices. Relatively large mean proportions were found in the segment in which they were initially placed where there were segments of the non-preferred microhabitat (i.e. A_t in treatment 1 and B_t in treatment 2; see Fig. 3). These proportions were larger that those in initial segments in treatments where all segments were the same microhabitat (B_t in treatment 5 and C_t in treatment 6; see Fig. 1). Otherwise, in both experiments, outcomes for Hypothesis 1 were as predicted (Table 2).

As for *Eatoniella atropurpurea*, *Eatonina rubrilabiata* were found in smaller proportions in the non-preferred habitat (without sediment; *A* in Fig. 1) where the alternatives were preferred microhabitats (A_t in treatment 1, where alternatives were *B* and *C*) than where alternatives were all non-preferred (A_t in treatment 4). This occurred because more snails moved into (or failed to return from) the preferred microhabitats where they were available (Table 2).

Table 2 Analyses of proportions of *Eatonina rubrilabiata* in experimental treatments (data are in Fig. 3); *n*=5 cores in experiment 1, initially with 6 snails; *n*=7 cores in experiment 2, initially with 6 snails

	Experiment 1				Experiment 2			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*
1. Hypothesis 1. B_{t2}, C_{t3}, B_{t5}, $C_{t6} > A_{t1}$ (more in preferred microhabitats)								
Among treatments	4	0.303	21.00	<0.0001	4	0.542	28.58	<0.00001
Residual	20	0.014			30	0.019		
Contrast 1	B_{t2}, C_{t3} vs B_{t5}, C_{t6}				B_{t2}, C_{t3} vs B_{t5}, C_{t6}			
Means	0.483		0.317		0.512		0.439	
	F=10.32; 1, 20 *df*; P<0.01				F=11.03; 1, 30 *df*; P<0.003			
Contrast 2	B_{t2}, C_{t3} vs A_{t1},B_{t2}				B_{t2}, C_{t3} vs A_{t1},B_{t2}			
Means	0.483 >		0.067		0.512 >		0.071	
	F=41.60; 1, 20 *df*; P<2×10⁻⁶				F=42.50; 1, 30 *df*; P<2×10⁻⁶			
2. Hypothesis 2. $A_{t1} > A_{t4}$ (fewer in non-preferred microhabitats where there is a choice)								
Among treatments	1	0.400	14.38	<0.006	1	0.643	23.71	<0.0005
Residual	8	0.028			12	0.027		
Means	A_{t1}		A_{t4}		A_{t1}		A_{t4}	
	0.067 <		0.467		0.071 <		0.267	

Fig. 3 Mean (SE) proportion of *Eatoniella rubrilabiata* in experiments 1 and 2 (*n*=5; *n*=7 cores, respectively); **a** proportion of individuals remaining in treatments in the microhabitats where they were initially placed; **b** proportion of individuals in microhabitats *B*, *C* in treatment 1 and *A′* and *A″* in treatment 4
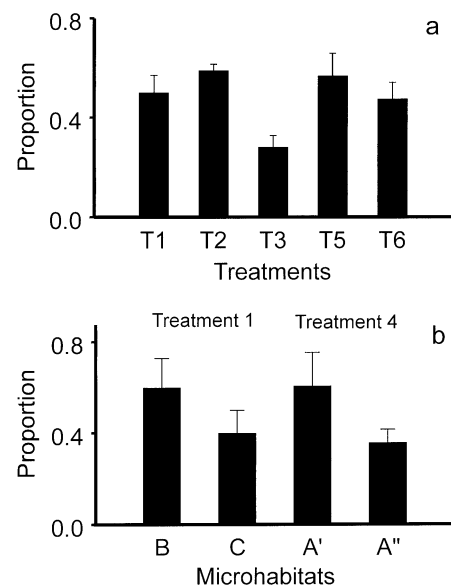
Fig. 4 Mean (SE) proportion of *Amphithalamus incidata* in experiment 1 (*n*=5 cores); **a** proportion of individuals remaining in treatments in the microhabitats where they were initially placed; **b** proportion of individuals in microhabitats *B*, *C* in treatment 1 and *A′* and *A″* in treatment 4

Amphithalamus incidata

This species demonstrated no preference for any microhabitat in experiment 1. Although there were some differences among the mean proportions in different experimental treatments (Table 3), these were not consistent with the hypotheses and, if anything, suggested preferences for microhabitats with *Corallina*, in contrast to what was found in the previous laboratory experiments (Fig. 4).

Discussion

For two of the three species, the experimental data clearly supported the hypotheses derived from the model that snails move more often into (or stay longer in) habitats in which they survive and/or grow better. *A. incidata* did not behave as predicted and did not choose the microhabitats (with sediment) in which they survived best in the laboratory (Olabarria and Chapman 2001b).

In the case of *Eatoniella atropurpurea*, the proportions of animals found in preferred habitats where there

Table 3 Analyses of proportions of *Amphithalamus incidata* in experimental treatments (data are in Fig. 4); n=5 cores in experiment 1, initially with 8 snails; this species was not included in experiment 2

	Experiment 1			
	df	MS	*F*	*P*
1. Hypothesis 1. B_{t2}, C_{t3}, B_{t5}, $C_{t6} > A_{t1}$ (more in preferred microhabitats)				
Among treatments	4	0.074	3.72	<0.025
Residual	20	0.020		
Contrast 1	B_{t2}, C_{t3} vs B_{t5}, C_{t6}			
Means	0.436		0.523	
	F=1.90; 1, 20 *df*; P>0.05			
2. Hypothesis 2. $A_{t1} > A_{t4}$ (fewer in non-preferred microhabitats where there is a choice)				
Among treatments	1	0.0008	0.03	<0.006
Residual	8	0.0231		
Contrast	A_{t1}		A_{t4}	
Means	0.500		0.518	

was a choice were not different from those where there was no choice. (A_{t1}, B_{t2} versus A_{t4}, B_{t5}; Table 1). This implies that snails in preferred microhabitats tended to stay there (or to return there after any excursion), rather than move to other areas. Most probably, the snails moved very little and so were recovered from the segments of experimental cores in which they were initially placed.

In contrast, *Eatonina rubrilabiata* had greater mean proportions in the preferred segments where there were choices (B_{t2}, C_{t3}) than where there were no choices (B_{t5}, C_{t6}; see Table 2). This was presumably the result of snails moving out of the initial segment where the rest of the core was also preferred habitat. Snails did not move as much into (or returned more from) areas of cores that were non-preferred habitat.

Despite this difference, both species demonstrated preference. Greater numbers moved to preferred microhabitat from non-preferred microhabitat where the choice was available (from C_{t3} for *Eatoniella atropurpurata* and from A_{t1} for *Eatonina rubrilabiata*; see Tables 1, 2). In both cases, this outcome was quite different from the movement and eventual occupation of microhabitat by snails where there was no choice (from C_{t6} for *Eatoniella atropurpurata* and from A_{t4} for *Eatonina rubrilabiata*).

The experimental design allowed independent assessments for each species (they were each on separate sets of cores) and allowed independent assessment of the outcomes of behaviour in the presence and in the absence of choices of microhabitat.

Eatoniella atropurpurea did prefer coralline algal microhabitat whether or not sediment was present. This species is really scarce in patches of sediment, whereas it is always very abundant in coralline turf (with variable amounts of trapped sediment; Olabarria and Chapman 2001a). In the laboratory, this species survived and grew best on turfing algae, regardless of the presence or absence of sediment in the algae. In the field and laboratory, this species tends to climb quickly up branches of coralline algal turf, so that many individuals can be found on a single branch (Olabarria, personal observation). Such aggregation can be explained by different models of behaviour in response to substratum (Levings and Garrity 1983), conspecific trails (e.g. Wells and Buckley 1972; Cook and Cook 1975; Chelazzi et al. 1990; Chapman 1998) or stresses (e.g. Garrity and Levings 1984; Chapman 1998). Explanations for these observations about *Eatoniella atropurpurea* must await appropriate experiments.

Even though *Eatonina rubrilabiata* survived and grew better on sediment (whether or not coralline algae were present), this species is common in sediment in coralline turf in the field (Olabarria and Chapman 2001a). Their relationship to the amount of sediment in turf has not yet been investigated in the field, but it can be predicted that the amount of sediment may vary at small spatial scales, causing small-scale heterogeneity in algal turfs.

An important aspect of the experimental design used here was the unconfounding of preference (an active behavioural choice) from any other reasons for different occupancies of different type of microhabitats (e.g. differences in accessibility). Many studies on the selection of microhabitats by snails have not used an experimental design like that used here, i.e. also determining the pattern of occupancy of each microhabitat when there is no choice (e.g. Fenchel et al. 1975; Lodge 1985; Durante and Chia 1991). In many studies, if species are more abundant in one habitat than in others, preference has been assumed (e.g. Gendron 1977; Allainé et al. 1994). A common problem is the probable or actual misuse of term "preference". It is always necessary to test (and be able to provide evidence to support) the model that behaviour of the animals can indeed account for the observed patterns.

Laboratory experiments do, however, cause problems because artificial laboratory conditions are unlikely to reflect accurately what animals do in the field (Connell 1974; Chapman 2000). Particularly, experiments in laboratories cannot reflect spatial variability in behaviour or, more importantly, interactions between spatial and temporal patterns (Chapman 2000), because environmental variation, disturbances, predators, etc. are all absent. In spite of the constraints, studies on behaviour of larger snails have been done successfully in the laboratory (e.g. Evans 1965; Cook and Cook 1975; Watanabe 1984; Cowie 1985; Tankersley 1990; Chapman 1998; Jones and Boulding 1999). We minimised potential effects of disturbance by transferring and maintaining snails in the laboratory along with natural patches of algal turf prior to the experiment. The experimental design also allowed us to use unmarked snails, thereby avoiding possible effects of handling and mark-

ing on behaviour (see Underwood 1988; Chapman and Underwood 1992).

In addition, the experiments were repeated to examine the consistency of patterns. Despite laboratory experiments being done under similar conditions, some variability in conditions cannot be avoided. Individuals are also known to show different behaviour from time to time in response to different conditions (e.g. availability of food, light, temperature). Thus, repetition of experiments is always essential in order to be sure that results are not just an artefact of some condition prevailing at one time. It would be desirable to do some components of these experiments in the field if logistic problems could be overcome, using the information gained here as a good guide.

Conservation of species is often based on conservation of their habitats or microhabitats. It is therefore important to have a mechanistic understanding of how associations between species and habitats are maintained. Improving the experimental designs used to evaluate different behavioural processes is required. The experiments described here provide a formal framework of hypotheses and experimental designs to test hypotheses about the existence and nature of preferences among microhabitats. Despite the limitations of experiments under laboratory conditions, it seems that the behaviour of these small (and poorly understood) gastropods can, at least partially, account for their small-scale patterns of distribution in the field.

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