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DISCRIMINATION, PREVIOUS EXPERIENCE AND SUBSTRATE SELECTION BY THE AMPHIPOD *COROPHIUM*

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INTRODUCTION

The experiments reported below were undertaken to give more information about why animals prefer one habitat or substrate to another. They are a continuation of work already reported on substrate selection by *Corophium volutator* (Meadows, 1964*a*, *b*, *c*).

Consider an experiment in which animals may choose between two substrates, *A* and *B*, and in which at equilibrium 75 % are found in *A* and 25 % in *B*. *A* is the animal's typical habitat or substrate (mud or muddy sand in the case of *Corophium volutator*) and *B* is an atypical substrate (coarser sand, for instance) in which animals are not normally found. The 25 % in *B* would then be defined as being less discriminating in their choice than those found in *A*. Let the animal be a species that is mobile and can move at will between *A* and *B*; then if the ratio 75/25 is caused only by differences between substrate *A* and *B*, and if the experimental animals are an unbiased sample from the population as a whole, either (i) 75 % of the population always prefer *A* and 25 % *B*, or (ii) all animals in the population prefer *A* for 75 % of their time and *B* for 25 %.

(i) and (ii) may be differentiated by offering animals a choice of *A* and *B*, and then re-offering the *A*/*B* choice to the animals which chose *A* and to the animals which chose *B*. The experiment is described below (Table 2).

Another approach is to ask whether animals can be conditioned into preferring *B* substrates by keeping them in *B* for some while before giving them an *A*/*B* choice. Mechanisms of this kind might be influencing substrate preferences in the field, for on occasion populations of *Corophium volutator* are found in atypical *B* substrates. The hypothesis was tested by experiment (Table 3).

However, before the results of these experiments can be assessed, it is necessary to know how repeatable the ratios in *A*/*B* choice experiments are, and what factors influence them. The first series of experiments (Table 1) were designed to answer these questions.

MATERIAL AND METHODS

Animals used in the first series (Table 1) were collected from mud below M.T.L. near Menai Bridge Pier, Anglesey. Animals used in later series were collected from mud at about M.T.L. on a shore 5 miles east of Greenock on the Clyde estuary, Renfrewshire.

The experimental procedure for conducting two-choice preference experiments was exactly as described previously (Meadows, 1964*a*). Choice dishes used in the first series of experiments (Table 1) were of the same size as those previously used. In later experiments the dishes were slightly larger (diameter 14·5 cm., depth 7·0 cm.).

Growth and mortality experiments (p. 556). Glass funnels (diameter 13 cm., depth 9 cm. not including stem) were stoppered with glass wool and with rubber tubing held by a clip. They were supported on metal rings under a constant light source (500 lux at the water surface) in a 12 hr. light 12 hr. dark regime. Temperature was maintained at 10° C ± 2. Sand from Fairlie Sands, Ayrshire, was sieved to give fractions of (i) 500–420 μ, (ii) 350–250 μ, (iii) 210–150 μ, (iv) 110–40 μ (muddy sand), and (v) < 40 μ (mud).

250 ml. sand was placed in each funnel and covered with sea water to within about 0·5 cm. of the funnel top.

Six funnels were set up for each fraction. A single animal was placed in each funnel; two of the funnels received large animals, two medium animals and two small animals. Large animals measured between 5·0 and 7·0 mm. (telson to rostrum), medium animals between 3·5 and 5·0 mm., and small animals under 3·5 mm. As animals died, they were measured and replaced by previously measured living animals of the same size group.

Water in the funnels was changed twice weekly, and the experiment was run for 3 months.

RESULTS

Size, light and repeatability

A group of animals was divided into small and large individuals. The small and large groups were each divided into two subgroups; each subgroup was offered the *A*/*B*

Table 1. *Size, light and the choice experiment. Grain size: coarse sand* < 150 μ, > 100 μ; *fine sand* < 44 μ. *Sum of four experiments. Two choice dishes per experiment*

	Small animals		Large animals	
	Constant illumination	Total darkness	Constant illumination	Total darkness
% of buried animals in fine sand	73	90	83	92
% of buried animals in coarse sand	27	10	17	8
Total % buried	99	91	90	59
% not buried	1	9	10	41
Number of animals	266	282	258	266

Animals classified as not buried were swimming, walking, or immobile on the substrate surface.

choice, one subgroup under a constant light source and the other in total darkness. Light intensity at the water surface of the 'light' dishes was 500 lux. After 3 hr. the numbers buried in the two substrates, and the numbers not buried, were noted. The experiment was conducted 5 times. A new population of animals was collected for each experiment. There was no difference in the mean size of small or of large animals between experiments. Summing over experiments, the mean size of small animals was 2·204 ± 0·9115 mm., and of large animals was 4·046 ± 1·197 mm. (size as telson to rostrum length, standard deviations quoted after the means). The difference in the means is 28 standard deviations from zero, so there is a less than 0·1 % chance that the means are the same, $P < 0{\cdot}001$ (Bailey, 1959).

Results, summarized in Table 1, were submitted to a factorial analysis of variance by classifying the data into five effects: small/large animals, constant illumination/total darkness, buried/not buried, in or on fine/coarse substrates, experiments 1–5.

The following conclusions are based on first- and second-order interactions which, when compared to the residual variance estimate, were significant at the 0·1 % level ($P < 0{\cdot}001$).

More animals burrowed in the light than in the dark, and the difference was more marked for large than for small animals. Fewer large animals than small burrowed. Of those animals which burrowed, more did so in the coarse, *B*, substrate in the light than in the dark, and this was more marked for small than large animals. However, there was no significant difference between the proportion of small and large animals in the coarse substrate, when constant illumination and total darkness were considered separately.

The number of animals which burrowed, and the proportion that chose the fine or coarse substrate, varied significantly between experiments.

These conclusions are interesting in relation to the biology of *Corophium*, but their importance here is to outline the limitations of the present experimental design.

First, no comparison can be made between experiments unless animals are collected at the same time and place. Secondly, if there is a wide size range in the population, the range should be equally represented in all dishes. Lastly, dishes to be compared must be run under the same level of illumination.

These considerations were taken into account in the planning of later experiments.

Choice of A and B substrates by animals which had chosen A or B previously

A population of animals was divided into four groups containing the same number of animals. Each group was given the same choice (*A*/*B*). After 3 hr. animals that had buried themselves were removed from substrates *A* and pooled, and from substrates *B* and pooled. The two pooled groups, *A* animals and *B* animals, were separately offered the same choice again (*A*/*B*).

There was no significant difference in the choice ratios between the first choice (Table 2, Expt. 1) and the second choices (Table 2, Expt. 2*a*, *b*). The same result was obtained in three repeat experimental series.

These experiments show that populations of *Corophium volutator* do not contain animals that consistently choose *B* rather than *A*. This means that there are no animals in the population that are consistently less discriminating.

Choice of A and B substrates by animals that had lived for a month in A or B

A population of about 500 animals was divided into two equal groups. The substrate in which they occurred naturally was sieved into fine, *A*, and coarse, *B*, fractions; one group was put into *A* and the other into *B*. The two groups were kept in glass dishes (diameter 24 cm., depth 12 cm.) filled 4 cm. deep with substrate, and covered with sea water. The dishes were aerated, and maintained at 10 ± 2° C. under a 12 hr. light 12 hr. dark regime for a month. There was no significant difference in the total number of deaths or moults over the month between the *A* and *B* dishes (χ^2). Moult rates remained fairly constant in both dishes, while mortality rates increased.

At the end of the month, animals from *A* and *B* dishes were offered the *A*/*B* choice. Particle sizes of the *A* and *B* substrates offered in the choice experiment were the same as those of the substrates that the animals had been living in for a month. There was no difference in the choice ratios (Table 3, Expts. 1, 2).

Previous enforced experience in a *B* substrate, therefore, does not alter animals' preferences for *A* in an *A*/*B* choice.

Table 2. *A/B choice experiment (Expt.* 1). *Animals from A and from B re-offered the A/B choice (Expt.* 2*a*, *b*). *Grain size: coarse sand* $< 350\ \mu$, $> 250\ \mu$; *fine sand* $< 250\ \mu$, $> 150\ \mu$. *Four dishes in Expt.* 1, 1 *in* 2*a and* 1 *in* 2*b*

Expt.	Sand from which animals were taken in Expt. 1	Percentage in fine sand	Percentage in coarse sand	No. of animals in experiment
1	Not applicable	87	13	239
2*a*	Fine	94	6	48
b	Coarse	78	22	31

χ_2 on a 3×2 table of animals buried in fine and coarse sand in Expts. 1, 2*a*, and 2*b* = 4·481 with 2 degrees of freedom, $P < 0.2$, > 0.1.

Table 3. *Previous experience and substrate selection. Grain size: coarse sand* $< 500\ \mu$, $> 250\ \mu$; *fine sand* $< 250\ \mu$, $> 100\ \mu$. *Three dishes per experiment. The large percentage swimming is typical of animals that have lived in the laboratory for a while*

Expt.	Sand in which animals had lived for 1 month	Percentage in fine sand	Percentage in coarse sand	Percentage swimming	No. of animals in experiment
1	Fine	36	1	63	213
2	Coarse	39	2	59	170

χ^2 On a 2×2 table of animals buried in fine and coarse sand in Expts. 1 and 2 = 0·2858 with 1° of freedom, $P < 0.7$, > 0.5.

Long-term growth and mortality in fine and coarse substrates

The experiments already described show that no animals in a population are consistently less discriminating in their choice of substrates, and that preferences cannot be altered by previous experience. It is conceivable that natural selection, perhaps through low growth rates and life expectancy in coarser substrates, may be favouring populations that prefer to live in fine-grained substrates.

The hypothesis was tested by recording the growth and mortality of animals maintained for 3 months in substrates of different particle sizes (for experimental details see material and methods section).

There was no difference in lifespan between small, medium, and large animals; summing over fractions (i) to (v), the mean length of life in days for small animals was 22.0 ± 9.75 ($n = 11$), for medium animals was 24.46 ± 16.94 ($n = 26$), and for large animals was 19.94 ± 25.40 ($n = 33$).

Animals lived for slightly longer in the finest fraction, (v), than in the others.

Summing over small, medium, and large animals for each fraction, mean lengths of life were $20{\cdot}57 \pm 12{\cdot}23$ for fraction (i) ($n = 14$), $25{\cdot}64 \pm 28{\cdot}33$ for fraction (ii) ($n = 14$), $17{\cdot}88 \pm 6{\cdot}57$ for fraction (iii) ($n = 16$), $16{\cdot}50 \pm 19{\cdot}26$ for fraction (iv) ($n = 22$), and $60{\cdot}50 \pm 32{\cdot}65$ for fraction (v) ($n = 4$). Standard deviations are given after the means.

There was no difference in the growth rates of small, medium, and large animals. Growth rates in mm./month summed over fractions were: small animals, $0{\cdot}8078 \pm 0{\cdot}9804$; medium animals, $0{\cdot}8652 \pm 0{\cdot}6298$; large animals, $0{\cdot}9664 \pm 0{\cdot}9754$.

Table 4. *Difference between the size of grains in* Corophium *tubes and the size of grains in the surrounding substrate. Analysis of variance*

Source of variation	Sum of squares	Degrees of freedom	Variance estimate	Probability that variation is not different from residual
Tube/substrate	0·15368	1	0·15368	< 0·001
Dishes	2·67579	4	0·66894	< 0·001
Tube/substrate—dishes interaction	0·07532	4	0·01883	< 0·2 > 0·1
Residual	5·81931	491	0·01185	—
Total	8·72410	500	—	—

There was also no difference in the growth rates of animals living in the separate fractions. Growth rates, summed over small, medium, and large animals, were: fraction (i), $0{\cdot}9325 \pm 1{\cdot}232$; fraction (ii), $0{\cdot}8433 \pm 0{\cdot}6832$; fraction (iii), $0{\cdot}8063 \pm 0{\cdot}6416$; fraction (iv), $1{\cdot}054 \pm 0{\cdot}6965$; fraction (v), $0{\cdot}7750 \pm 0{\cdot}7372$. Standard deviations are given after the means.

The appearance after death of animals that were alive at the end of the experiment did not differ from the appearance of those which died during the experiment.

Some animals constructed tubes of sand (Meadows & Reid, 1966). Tubes were removed from fractions (i), (ii), and (iii) at the end of the experiment, and the size of grains from the tubes and the surrounding substrate measured. Tubes were always made up of a higher proportion of small grains than the substrate. This was apparent on microscopic examination, and significant in an analysis of variance (Table 4). It means that over a range of particle sizes, animals select the smaller grains from those available to them.

DISCUSSION

The experiments reported in Table 2 demonstrate that populations of *Corophium* do not contain individuals that are consistently less discriminating. It appears that this experiment has not been conducted with other mobile animals which show habitat preferences, other invertebrates, fish or birds, for instance; the comparison would be interesting. Metamorphosing larvae are not amenable to the experiment for obvious reasons; however, there is evidence that they do not change their discriminatory abilities with age (Crisp & Meadows, 1963; Williams, 1964). On the other hand animals, such as settling larvae, that make an irrevocable choice of the less typical of two substrates, must by definition be less discriminating. A small proportion of the larvae of many marine species do exactly this (Knight-Jones, 1953; Wilson, 1958; Ryland, 1959; Scheltema, 1961; Meadows & Williams, 1963).

Experiments in which I attempted to change a population's preference by previous experience (Table 3) are in essence the same as those conducted by Hailman and Klopfer on birds. The results are also similar, for in both instances the results were negative (Table 3; Klopfer & Hailman, 1964) or only transiently positive (Klopfer, 1963; Klopfer & Hailman, 1965).

The results of the long-term growth and mortality experiments show little difference in life expectancy or growth of animals in a range or particle sizes. Animals are also successful at burrowing into a wide range of particle sizes when no other substrate is available (Meadows, 1964*c*). These considerations suggest that animals can live satisfactorily in coarser grained substrates, and in fact they sometimes do so in the field. Substrate preferences, therefore, may be dependent on fine behavioural requirements such as the selection of smaller particles for tube building (Table 4).

The first series of experiments (Table 1) and their analysis of variance, show that carefully designed pilot experiments are necessary before detailed investigations of habitat preferences can be undertaken. This will probably apply also to experiments on larval settlement.

SUMMARY

1. Populations contain no animals that are consistently less discriminating than others in their choice of substrates.
2. Previous experience in atypical substrates does not alter preferences for typical substrates.
3. Animals live slightly longer in very fine-grained substrates than in coarser ones.
4. Their growth rates do not differ over a range of particle sizes.
5. Animals select the smaller grains in a substrate to build their tubes with.

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