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EXPERIMENTS ON SUBSTRATE SELECTION BY *COROPHIUM VOLUTATOR* (PALLAS): DEPTH SELECTION AND POPULATION DENSITY

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INTRODUCTION

The results reported below are part of an investigation of the factors affecting substrate selection by *Corophium* species; they are concerned with depth selection by *Corophium volutator* (Pallas).

Although depth must be one of the more obvious attributes of sands and muds, there appears to be no critical work on the depth preferences of any sand-dwelling or mud-dwelling animal, and there is only one report of substrate depth influencing the distribution of a species (Chapman & Newell, 1949).

I have already outlined the background to this work (see introduction in Meadows, 1964). Suffice to say here that depth is one of the many factors which might influence *Corophium* as it selects substrates in which to burrow. Illumination, particle size, and the nature of films on substrate particles, may also play their part.

MATERIAL

Corophium volutator were obtained from mud flats in front of the Marine Science Laboratories, Menai Bridge, Anglesey, and were used only once. Until required they were maintained in dishes containing static sea water and some of their natural mud. Animals were always used within two days of collection.

Mud for all experiments was collected from the animals' natural habitat, and always used within 3 days. On reaching the laboratory it was immediately sieved through a 72 mesh sieve (pore diameter 211 μ, B.S. 410/43) to remove animals and the coarser particles, and allowed to stand for 24 hr.

THE ABILITY OF ANIMALS TO BURROW IN DIFFERENT DEPTHS OF MUD

On shores around Anglesey *C. volutator* occurs in large numbers only where its characteristic substrate is more than about 1 cm. deep. The following experiment shows that animals will burrow in muds shallower than this if offered no alternative.

Five 1 l. glass beakers were filled with mud to depths of 0·5, 1·0, 2·0, 4·0, and 8·0 cm. Sea water was run into each until the muds were covered by 6 cm. of water. All dishes were surrounded by black paper to exclude stray light, and an Anglepoise lamp

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was adjusted over each to give a light intensity of *ca.* 500 lux at the water surface. Twenty-five animals were introduced into each dish. After 3 hr. the experiment was terminated. By this time all animals, except one in the 0·5 cm. beaker, had burrowed. The experiment was repeated, with essentially the same result.

THE SELECTION OF DEEPER MUDS WHEN OFFERED A CHOICE

Animals burrow in any depth of mud when no alternative is available. In the field, however, they are rarely found in very shallow mud. This implies that if deeper muds are available animals burrow in them to the exclusion of shallow ones. Experiments were designed to test this hypothesis.

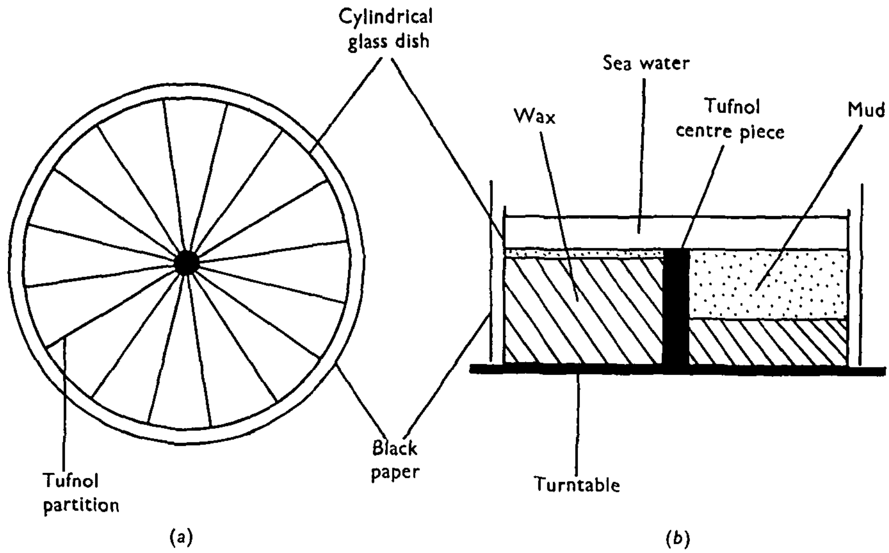

Fig. 1. Diagram of apparatus in which animals were offered different depths of mud. (*a*) Viewed from above, (*b*) viewed from the side. (*b*) passes through a 0·5 cm. deep and a 5·0 cm. deep segment.

The principle adopted was to offer animals a number of depths of mud in the same dish, and to observe how many burrowed in each. The apparatus (Fig. 1) consisted of a cylindrical glass dish, diameter 34 cm., depth 14 cm., divided into 16 equal sectors by Tufnol partitions. Molten wax was poured into the sectors to give four of depth 0·5 cm., four of depth 2·0 cm., four of 5·0 cm., and four of 9·0 cm. The depths were in random order within groups of four sectors, each group containing each of the four depths.

Before an experiment was to be run the sectors were filled with mud to the level of the top of the Tufnol partitions and sea water run in to a depth of *ca.* 4 cm. The dish was surrounded with black paper to exclude stray light and placed on a revolving turntable under a light source. Illumination at the water surface was always constant at *ca.* 500 lux, and uniform over all sectors during a given experiment.

At this point animals were introduced and the experiment was left running for the required time. At the end of the experiment the sea water was siphoned off and the muds were scooped out of each sector into separate dishes. The numbers and lengths of animals in the mud from each sector were subsequently noted. Animal length was measured from the tip of the rostrum to the tip of the telson.

High-density experiments

In the first series of experiments *ca.* 200 animals were pipetted into the dish. This resulted in a density of *ca.* 0·4 animals/cm.^{2} of substrate surface, which was slightly lower than that of field populations (*ca.* 1 animal/cm.^{2}). These were termed 'high-density experiments'.

Table 1. *Depth selection at high and low densities*

	% in the depths of mud indicated at the end of each column				Total no. of animals buried
	0·5 cm.	2·0 cm.	5·0 cm.	9·0 cm.	
(*a*) High density	12	22	27	39	190
(*b*) Low density (mean of 7 expts.)	3	29	33	34	17

In view of the very quick detection of any chemical differences between substrates (Meadows, 1964) preliminary experiments were run for 12 hr.; there was some indication of an avoidance of the 0·5 cm. mud, but the results were inconclusive. When the experiment was allowed to run for 7 days, however, there was a clear-cut avoidance of the shallowest mud while the greatest numbers (39%) were found in the deepest mud (Table 1). It seemed at first as if *Corophium* preferred deeper muds to burrow in when these were available. This is not necessarily true. There are two possible explanations of the observed distribution: (*a*) animals genuinely select deeper muds; (*b*) animals require a minimum volume of mud before they will remain buried. If (*b*) were so, shallower muds, having a smaller volume per unit surface area, would support fewer animals. On the other hand animals might require a minimum surface area for their burrow entrances. If this were so, fewer animals would burrow in the deeper muds than might be expected from the volume of mud available.

It is impossible to separate such effects with the experimental procedure as it stands. However, by repeating the experiments at a low population density, any influence of territorial behaviour (Crisp, 1961) dependent on surface area or mud volume would be at a minimum.

Low-density experiments

The same procedure was adopted as in the 'high-density' experiments, except that only 15–20 animals were introduced into the dish. Because of the low numbers the experiment was repeated seven times. As previously, the dish was left for 7 days before scooping out the muds. The same pattern of selection emerged as in the 'high-density' experiments (Table 1), apart from one interesting feature: considerably fewer animals were found in the shallowest mud (3% as opposed to 12%).

The possible reason for this becomes clearer if the results from both high- and low-density experiments are plotted as animals/unit surface area (Fig. 2) and as animals/unit volume (Fig. 3). For comparison, both figures include density levels in the field and from the 'no choice' experiments. It is evident from these figures that both 'no

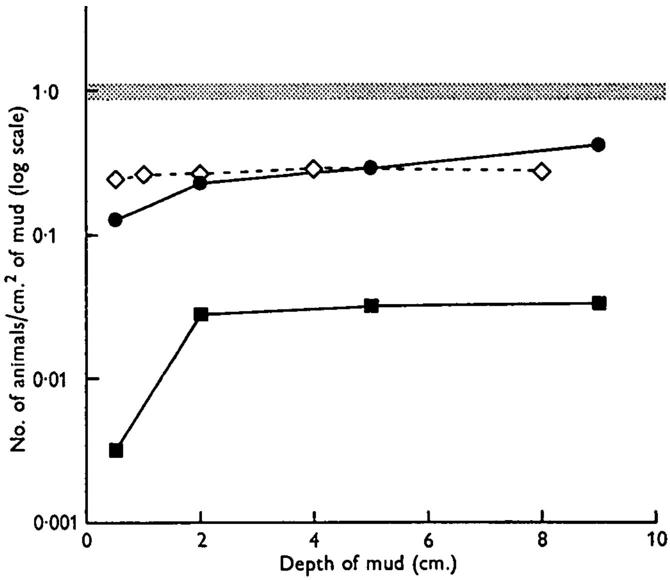

Fig. 2. Relationship between population density (no. of animals/cm.² of mud surface), and depth of mud. ◇---◇, 'No choice' experiments; ●—●, 'high-density' experiments; ■—■, 'low-density' experiments. ▦, Represents the field population density per unit surface area of mud.

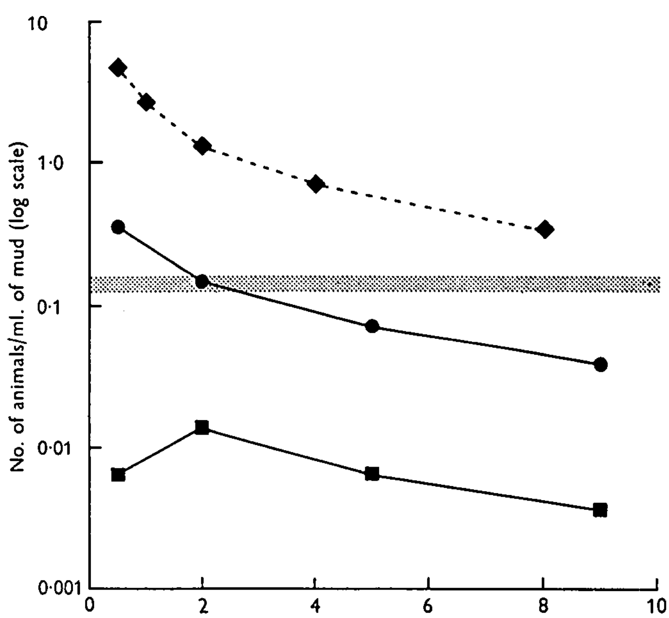

Fig. 3. Relationship between population density (no. of animals/ml. of mud) and depth of mud. ◆---◆, 'No choice' experiments; ●—●, 'high-density' experiments; ■—■, 'low-density' experiments. ▦, Represents field population density per unit volume of mud.

choice' and 'high-density multi-choice' experiments approach densities observed in the field whether expressed per unit surface area or per unit volume; the 'low-density multi-choice' experiments, on the other hand, are at much lower level of density. Comparing each set of experiments in turn:

(*a*) No choice. In Fig. 2, points lie along a straight line parallel to the *x*-axis; that is, all of the 25 animals per dish burrowed. In Fig. 3 the same data plotted per unit volume result in a curve of negative and decreasing slope, although the same number of animals burrowed in each dish. This curve, then, is purely a reflexion of increase in mud volume with increasing depth.

(*b*) High-density multi-choice experiments. In Fig. 2 the curve is slight but with positive slope: more animals per unit surface area are found in the deeper muds. However, as in the no choice experiments, when expressed as numbers per unit volume, a curve of negative and decreasing slope results. The increase in total numbers in the deeper muds is not enough to offset the effect of increase in volume with depth.

(*c*) Low-density multi-choice experiments. In Fig. 2 the curve reproduces the data given in Table 1. The first point in Fig. 3, however (density in 0·5 cm. mud), lies well below the number one is led to expect from the high-density curve. It seems likely, then, that in the high-density experiments the surface density of animals in the deeper muds is great enough to persuade some animals to choose the shallowest mud in which to burrow.

Conclusion

From the results of the high- and low-density series of experiments it appears that although animals inherently avoid very shallow muds, they can be forced to burrow in them if the surface density in deeper muds reaches a high enough level. A pattern of territorial behaviour (Crisp, 1961) which only showed itself at high population densities, would account for the difference in the distribution of animals between the high- and low-density experiments.

TERRITORIAL BEHAVIOUR AND GREGARIOUSNESS

It was suggested above that in the low-density series of experiments any confusing influence of territorial behaviour would be at a minimum. However as means are available to test this assumption, it was worth while to submit data to appropriate statistical analysis.

The method chosen was that used by Crisp (1961) to analyse the territorial behaviour of cirripede cyprids at settlement. During the low-density experiments, records had been kept of the numbers of animals found in each of the four sectors at each depth. At a given depth, if the distribution of animals between the sectors is random, it will fit the Poisson distribution, which is given by successive values of the term

$$\frac{e^{-m}m^x}{x!},$$

for $x = 0, 1, 2$, and so on, where m = mean number of animals per sector, and x = the different values for the number of animals per sector (Fisher, 1958). If the distribution of animals is over-dispersed, the counts will be more uniform; this implies some type of territorial behaviour.

When the data for the different depths were submitted to this treatment it was found that in all depths animals were slightly under-dispersed; in fact, far from there being any territorial tendency, there was some indication of gregariousness. However, when tested by χ^2, only in the deepest mud were the values significantly different from the expected Poisson distribution (Table 2). The proportions of animals burrowing in the three deeper muds (29, 33, 34%, see Table 1) do not differ markedly; why, then, the deepest mud should be in any way different from the others with respect to gregariousness remains obscure.

Table 2. *Low-density experiments*

Significance of the difference between the observed numbers of animals per sector and the numbers to be expected from the Poisson distribution.

Depth of mud (cm.)	χ^2	Degrees of freedom	P
0·5	No values because of the small numbers of animals		
2·0	2·938	3	< 0·5 > 0·3
5·0	0·285	3	< 0·98 > 0·95
9·0	15·958	3	< 0·01 > 0·001

$\Sigma\chi^2 = 19{\cdot}181$; Σ degrees of freedom $= 9$; $0{\cdot}05 > P > 0{\cdot}02$.

RATE OF EXPLORATION OF SUBSTRATES WHICH VARY IN DEPTH

During the low-density experiments records were kept of the numbers of pairs of holes appearing in each segment as the experiment progressed (an individual almost always maintains two openings, and two only, to its burrow). When the values thus obtained were plotted against time (Fig. 4) it became obvious that more animals explored the deeper muds from a very early stage.

The final burrow density (no. of pairs of holes/cm.^{2} of substrate surface) in the different depths at first glance agrees well with the observed numbers of animals in the same depths. But a more detailed comparison (Table 3) shows that at the end of the experiment under 60% of the burrows in the deeper muds were inhabited, and that although there were fewer burrows in the 0·5 cm. mud than elsewhere, an even smaller proportion of these were occupied—22%.

The values for the number of burrows occupied, although lower than might be expected, correlates well with observed behaviour patterns of *Corophium* (author's unpublished observations). Animals do not remain in one burrow for long—1–2 days in these experiments—but soon emerge to explore the substrate surface again. Eventually they either form a new burrow, or discover the opening to another burrow and enter; if it is empty they stay, if there is already an occupant a fight ensues. The original occupant, irrespective of its size, usually manages to throw the intruder out. The uninhabited burrows, then, are explained by the tendency for animals to emerge periodically and burrow elsewhere (cf. behaviour of *Pontoporeia affinis*, p. 16 in Segerstråle, 1960).

These behaviour patterns may also explain why hardly any new burrows appear in the 0·5 cm. mud after the 1st day (Fig. 4). Animals in the dish certainly continue to explore, for the numbers of burrows appearing in the three deeper muds continue to increase (Fig. 4, upper three curves). This suggests that animals exploring the substrate surface appreciate the shallowness of the 0·5 cm. mud without burrowing in it. This may be just possible, for the lengths of the 2nd antennae exceed 0·5 cm. in length amongst larger animals, and individuals, before they burrow, can often be seen to probe the substrate with their prehensile 2nd antennae.

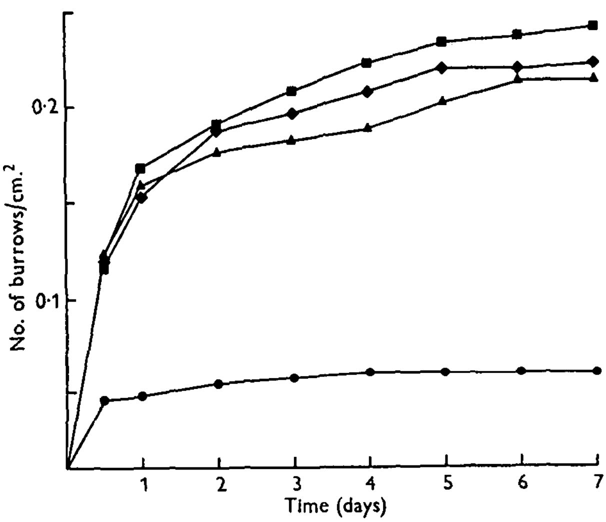

Fig. 4. Numbers of burrows appearing in the different depths of mud during the 'low-density' experiments. ■—■, Burrows in 9 cm. mud; ◆—◆, burrows in 5 cm. mud; ▲—▲, burrows in 2 cm. mud; ●—● burrows in 0·5 cm. mud.

Table 3. *Low-density experiments*

Number of burrows occupied after 7 days.

	Depth of mud (cm.)			
	0·5	2·0	5·0	9·0
No. of burrows/cm.$^2 \times 10^{-2}$	5·96	21·3	22·2	24·1
No. of animals/cm.$^2 \times 10^{-2}$	1·3	11·4	13·0	13·3
% of burrows which are occupied	22	54	59	55

On the other hand the following sequence of events, based on the behaviour patterns described above, will adequately explain why no new burrows are found in the 0·5 cm. mud after the 1st day or so. On introduction into the dish, animals alight and explore the surface at random (Meadows, 1964). Shortly after—within minutes—most have burrowed. Those in the deeper muds remain there, while nearly all in the shallow 0·5 cm. mud soon emerge leaving behind their empty burrows. These latter individuals continue exploring, and are joined in time by some from the deeper muds who periodically come out of their burrows (see above). Exploring individuals on encountering a burrow enter it; if empty but too shallow they emerge (burrows in the 0·5 cm. mud). If occupied, they are thrown out, but examine other holes, or burrow to form new ones. In this way some 40% of the burrows in the deeper depths, and over 75% of those in the shallowest mud, are unoccupied at the end of 7 days (Table 3).

If these conditions are reproduced in the sea, it seems that at low population densities 40–80% of burrows may be unoccupied.

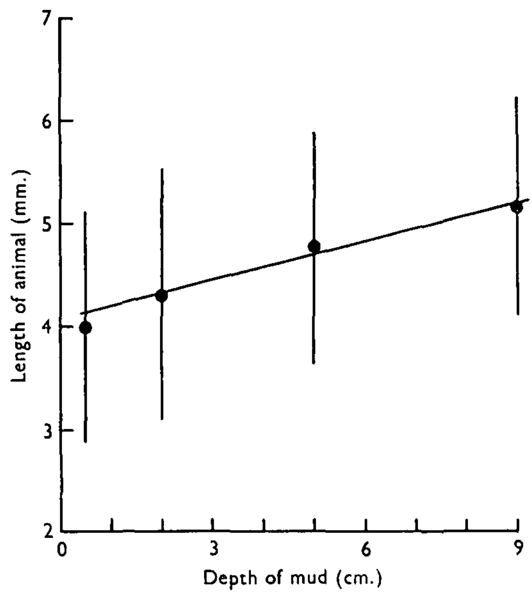

Fig. 5. Relationship between length of animal and depth of mud. $y = 0{\cdot}123x + 4{\cdot}094$. Line fitted by least squares regression. 0·123, the value for the regression coefficient, is 3·50 standard errors away from zero, therefore $P < 0{\cdot}001$. Vertical lines extend to one standard deviation on each side of the means, ●.

Table 4. *Regression of animal size on substrate depth*

Analysis of variance.

Source of variation	Sum of squares	Degrees of freedom	Mean square or variance estimate
Due to regression	15·8	1	15·8
About regression	152·2	118	1·29
Total	168	119	—

Snedecor's F test (variance ratio test): $F = 12{\cdot}25$, $P < 0{\cdot}001$ (0·001 represents the lowest value for P given by Fisher & Yates, 1963).

SIZE OF ANIMALS AND DEPTH OF MUD

During the low-density series of experiments the lengths of animals found in the different depths were recorded. The results showed conclusively that larger animals tend to be found in the deeper muds (Fig. 5, Table 4). This relationship was highly significant when submitted both to an analysis of variance (Davies, 1957, p. 128) and to a regression analysis (Bailey, 1959).

If these observations can be extended to natural conditions they imply that substrate depth may influence the size structure and hence age distribution of field populations; younger populations would be found in shallower muds.

THE MECHANISM BY WHICH ANIMALS APPRECIATE DEPTH

The results of the low-density experiments prove conclusively that animals prefer to live in deeper muds (Table 1). An analysis of the lengths of animals in the different muds reveals that small animals can tolerate shallower muds than can large animals (Fig. 5, Table 4). Observations on the behaviour of animals when in natural and artificial glass burrows, taken together with these conclusions, give some clues as to the mechanisms by which animals appreciate depth.

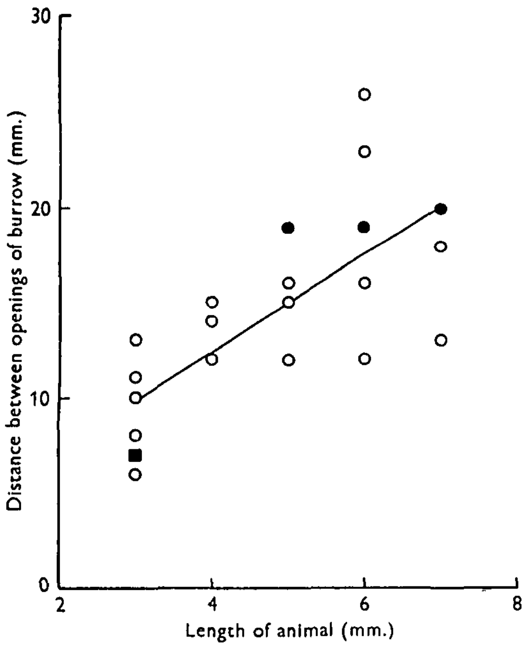

Fig. 6

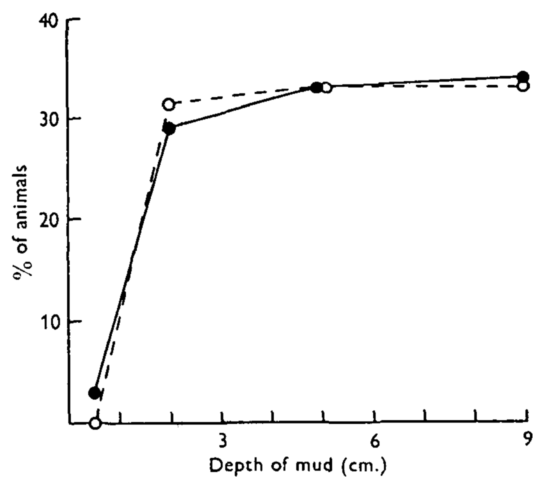

Fig. 7

Fig. 6. Relationship between the distance between burrow openings and animal length. ○, One observation; ●, two observations; ■, three observations. $y = 2{\cdot}595x + 2{\cdot}02$. Line fitted to individual points by least squares regression. 'Student's' $t = 5{\cdot}325$, with 24 degrees of freedom, $P < 0{\cdot}001$. Readings were obtained from burrows formed by animals in the 'low-density' experiments.

Fig. 7. Observed (●—●) and calculated (○---○) values for the percentage of animals found in the different depths of mud at the end of the 'low-density' experiments.

Individuals occupying burrows have a fixed sequence of movements reminiscent of those of *Arenicola* (Wells, 1950). After collecting food from one entrance of the burrow, they move backwards and forwards within the burrow, eventually turning to face the other way. This pattern is constantly repeated, with intermittent periods of no activity, until the animal either emerges from its burrow or is interrupted by an intruder. Because of this, animals will require a minimum length of burrow before they can begin their typical sequence of feeding, backward and forward movement, and reversal. Larger animals will need larger burrows, both in length and diameter, to perform the same sequence as smaller animals do in shallower burrows.

It seems likely, then, that it is not the length of animals themselves, but the length of their burrows which determines the minimum depth in which they can live. Moreover, as burrows in the field are almost always semi-circular or U-shaped, shapes close to these will be necessary before normal behaviour can be established.

If shape and size of the burrow determine the shallowest mud in which an animal can live, and burrow shape approximates to a U or semi-circle, it is possible to calculate the maximum length of animal expected for each depth of mud. If the burrows were strictly semi-circular the minimum depth for a given burrow size would be equal to half the distance between the burrow openings. However, by accepting a minimum depth of twice this figure, that is the distance separating the burrow openings, allowance is made for the U-shape of the burrow and for the finite thickness of the burrow itself. By this reasoning the largest animal which could live in the 2 cm. mud, for instance, would be the animal whose burrow openings were, at the most, 2 cm. apart. The size of this animal is obtained by substituting in the equation $y = 2\cdot595x+2\cdot02$, which relates animal length (x) to distance between burrow openings (y) (Fig. 6).

By this method the maximum length of animals to be expected in the 0·5 cm. mud is 1·15 mm., in the 2·0 cm. mud 6·9 mm., in the 5·0 cm. mud 18·5 mm., and in the 9·0 cm. mud 34 mm. There are few animals as small as 1·15 mm., which agrees well with the low number in the 0·5 cm. mud (Table 1); similarly, some animals exceed 6·9 mm. in length, which explains why slightly fewer are found in the 2·0 cm. mud than in the two deeper ones. The values for the 5·0 and 9·0 cm. muds have no meaning, for *Corophium* do not reach this size.

This argument may be carried one step further, to give the percentage of animals in the different depths of mud which are to be expected from the burrow hypothesis; these values may then be compared with the observed frequencies from the low-density experiments given in Table 1.

The expected frequencies are arrived at as follows. The equation connecting animal size with distance between burrow openings, and the size distribution of the population used in the low-density experiments, are both known. The maximum length of animal expected for each depth has already been calculated. It is possible, therefore, to read from the size-distribution graph the percentage of animals at each maximum length. This percentage is then divided equally between the depth for which it is the maximum length and all deeper muds. In this way a table can be built up showing the percentage of the total population to be expected at each depth. When compared with the observed low-density distribution, the two curves are almost identical (Fig. 7).

It seems probable, then, that shape and size limitations of the burrow, rather than animal size in itself, present an adequate explanation of why animals avoid very shallow muds, and why larger animals are found in relatively deeper muds.

SUMMARY

1. An apparatus is described for testing substrate-depth preferences of *Corophium volutator* (Pallas).

2. *Corophium* burrow in mud whatever its depth, if only one depth of mud is available.

3. If presented with muds of different depths, more animals burrow in the deeper muds. Very shallow muds, of 0·5 cm. depth, are particularly avoided.

4. Although preferring deeper muds, animals are persuaded to burrow in shallow muds when the surface population density in the deeper muds is at a high level (> 0·1 animals/cm.^{2}). This would be explained by a pattern of territorial behaviour which shows itself only at relatively high population densities.

5. Analysis of the distribution of animals at low densities indicates some tendency towards gregariousness.

6. Because of the periodic tendency for animals to vacate their burrows and burrow elsewhere, more than 50% of burrows at very low surface-population densities (*ca.* 0·01 animals/cm.^{2}) may be unoccupied.

7. Larger animals tend to be found in deeper muds. This would lead to substrate depth influencing the age structure of populations in the field.

8. Limitations imposed by the shape and size of the burrow, rather than animal size in itself, probably explain why animals avoid shallow muds and why larger animals are found in relatively deeper muds.

Prof. D. J. Crisp, Marine Science Laboratories, Menai Bridge, gave me every encouragement during this work, and advised me on certain statistical procedures. Mr G. B. Williams, The Laboratory, Plymouth, suggested some improvements in my experimental procedure. My wife kindly read and criticized the text.

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