J. Exp. Biol. (1964), **41**, 499–511
With 1 *Text-figure*
Printed in Great Britain

EXPERIMENTS ON SUBSTRATE SELECTION BY *COROPHIUM* SPECIES: FILMS AND BACTERIA ON SAND PARTICLES

By P. S. MEADOWS*
Marine Science Laboratories, Menai Bridge, Anglesey

(*Received* 20 *December* 1963)

INTRODUCTION

The mechanism by which larvae of sedentary marine invertebrates select surfaces on which to settle has received much attention recently (Knight-Jones, 1953; Wilson, 1953, 1958; Ryland, 1959; Crisp & Williams, 1960; de Silva, 1962). However, the way in which mobile benthic invertebrates with no larval phase might select suitable substrates in or on which to live has only been exhaustively analysed by Wieser (1956). This paper represents part of an experimental study of the factors affecting substrate selection by two such mobile species.

Corophium volutator (Pallas) and *C. arenarium* Crawford are amphipods which live in soft sand or mud. They usually form U-shaped tubes which extend to a maximum of 10 cm. below the surface. *C. volutator* is found in fine mud (Hart, 1930; Thamdrup, 1935), while *C. arenarium* is characteristic of more sandy substrates (Crawford, 1937; Watkin, 1941; Stock & De Vos, 1960; Gee, 1961). *C. arenarium* is a newly described species (Crawford, 1937; Stock, 1952).

Some of the factors which might influence animals about to burrow are the level of incident illumination, substrate particle size, depth of substrate, and the primary film on the individual sand particles of the substrate. In this paper only the influence of primary films will be considered.

The organic or primary film begins on newly immersed surfaces, 'with the formation of a slime film which is produced by bacteria and diatoms. The bacteria attach and grow rapidly. Algae and diatoms are uncommon during the first 2 or 3 days, but then may develop rapidly, so that several thousand per square centimetre may be present within a week' (Redfield & Deevy, 1952). These and other micro-organisms, such as fungi and flagellates, attach to all surfaces in the sea (ZoBell, 1946; Ferguson Wood, 1950; Ayers & Turner, 1952; Vishniac, 1956). Furthermore, organic molecules which are in solution in sea water (Anderson, Gehringer & Cohen, 1956; Vallentyne, 1957) will be adsorbed on to surfaces (Harvey, 1941; ZoBell, 1943; Bader, Hood & Smith, 1960). Primary films will consist therefore of one or more of the following: (*a*) adsorbed organic substances, (*b*) micro-organisms, (*c*) microbial extracellular metabolytes and slimes.

The free-choice experiments described below rely on the following behaviour patterns. *Corophium* alight on substrates at random. They show no sign of being attracted from a distance. After alighting, animals crawl over the substrate and manipulate particles on the surface. They then either burrow or swim off; both these responses

* Present address: Department of Zoology, The University, Glasgow, W. 2.

are apparently quite deliberate. Those substrates on or in which they remain are termed attractive, those from which they swim off, unattractive; animals occasionally burrow into a substrate that is normally unattractive, but usually remain in it for only a few minutes. In this way greater numbers accumulate in the attractive substrate than in the unattractive one. Wieser (1956) and Crisp & Meadows (1963) discuss this type of behaviour in greater detail. The terms 'attractive', 'unattractive', and 'prefer', as used in this paper, have no anthropomorphic implications.

MATERIALS AND METHODS

Corophium volutator were obtained from fine mud below M.T.L. near Menai Bridge pier, Anglesey. *C. arenarium* were collected at about M.T.L. on Red Wharf Bay, Anglesey, where a freshwater rivulet flows across the beach.

The dishes in which the two species were offered choices of different substrates were prepared as follows. Lengths of Tufnol, rectangular in cross-section, were cut so that they divided the circular bottom of a crystallizing dish into four quarters. Each compartment thus formed was 1·2 cm. deep, and had a surface area of 25 $cm.^2$. The crystallizing dishes had a diameter of 12 cm. and a depth of 6·5 cm. Sand or mud was added to the level of the top of the Tufnol partitions, its surface was smoothed, and sea water was run into a depth of 3·5 cm. All dishes were surrounded with black paper to exclude stray light. Each experiment was run in triplicate. Between twenty and forty animals were added to each dish, and the dishes were rotated on separate turntables under sources of constant illumination. Light intensity at the water surface varied between 450 and 650 lux, but was constant during a given experiment. Water temperatures ranged from 17 to 19° C. Typically, all animals burrowed within 15 min. of being placed in the dishes. During this time the number buried increased asymptotically to a maximum, while a few explored the substrate surface. All experiments were run for about 3 hr. At the end of the experiment the different sands were pipetted from the compartments to separate dishes. The number and sizes of animals in each sand were subsequently noted.

For each experiment only the total numbers choosing each type of substrate have been tabulated. The number of animals which were found in a particular substrate in each of the two or three dishes of an experiment were always the same within the limits of random fluctuation; the application of χ^2 showed that these numbers could be regarded as belonging to the same statistical population. The significance of the choice ratios obtained in different experiments was assessed by the χ^2 test with Yates's correction for continuity (Fisher, 1958).

Each species was offered only sand or mud from its own habitat, except in the reciprocal choice experiments.

Unless otherwise stated, all sands were washed six times in sea water before being placed in the choice dishes. Muds could not be washed because they take many hours to settle again.

The finest mud referred to below is mud from the habitat of *C. volutator* which has passed through a 350-mesh sieve—44 μ aperture; 350 sand is, similarly, sand which has passed through a 150-mesh sieve—aperture 104 μ, but which is retained by the 350 sieve.

Sand from the habitat of *C. arenarium* was always filtered through a 30-mesh sieve—aperture 500 μ, and washed on a 72-mesh sieve—aperture 211 μ, before use.

The sieves were supplied by Endecotts (Filters) Ltd., London, England (patent no. 667924, manufactured to British Standard 410/43).

Lengths of animals burrowing in different substrates were often noted. There was never any significant difference between the lengths of animals which burrowed in either of the two choices offered.

Table 1. *Reciprocal choice experiments with* Corophium volutator *and* Corophium arenarium

Species	Percentage in mud from *C. volutator* habitat	Percentage in sand from *C. arenarium* habitat	No. of animals in experiment	χ^2	*P*
C. volutator	74	26	42	10·5	< 0·05 > 0·001
C. arenarium	25	75	63	14·3	< 0·001

RESULTS

Influence of light on choice

Preliminary experiments showed that animals preferred a given substrate equally well whether the dishes were in total darkness or under a high level of illumination. In other experiments animals remained randomly dispersed in a circular dish, lit from above, whose bottom was quartered with black and white squares. Vision, then, plays little part in substrate selection; *Corophium* probably utilizes tactile and chemical senses when recognizing substrates.

Specific substrate recognition by the two species

C. volutator lives in mud, *C. arenarium* in sand. They are closely related species, yet are found in markedly different substrates. Reciprocal-choice experiments, in which each species is offered its own substrate with that of the other species, might help to explain why this is so. Such an approach has been successfully adopted in studying the influence of substrates on the settlement of various polyzoan, cirripede, and polychaete larvae (Crisp & Ryland, 1960; Crisp & Meadows, 1962; Gee & Knight-Jones, 1962; de Silva, 1962).

In reciprocal-choice experiments each species preferred its own substrate (Table 1). However, not all animals burrowed in their own substrate; those that did not may represent the less discriminating individuals which in nature would occur in the less typical habitats.

From these results one might expect that sand particles making up the substrate in which, say, *C. arenarium* lives, have a covering film which is in some way characteristic (cf. Wilson's (1958) results with *Ophelia bicornis*). This seems unlikely, however, for when offered sand from their own habitat and sand of the same particle size from an area of Red Wharf Bay where no *C. arenarium* are found, animals burrowed indiscriminately in the two substrates.

Anaerobic and aerobic substrates

C. volutator inhabits a mud which is very fine and which is black below the surface, while *C. arenarium* lives in a grey coarse sand. It seemed possible, therefore, that both species were capable of detecting differences in the anaerobic or aerobic conditions of substrates they encountered in the sea. This hypothesis was tested by offering to both species substrates previously maintained under anaerobic and aerobic conditions.

Table 2. *Choice experiments with aerobic and anaerobic mud and sand*

(*a*) *Corophium volutator*

Type of mud or sand used	Percentage in mud or sand previously maintained under: Aerobic conditions for 7 days	Anaerobic conditions for 7 days	No. of animals in experiment	χ^2	*P*
Finest mud	18	82	84	33·5	< 0·001
350 sand	42	58	96	2·35	< 0·2 > 0·1

(*b*) *Corophium arenarium*

Percentage in sand taken from aerobic surface layers	Percentage in sand taken from anaerobic subsurface layers	No. of animals in experiment	χ^2	*P*
72	28	155	29·9	< 0·001

In the first experiment, *C. volutator* was offered finest mud previously maintained under aerobic conditions in open trays, and finest mud previously stored anaerobically under liquid paraffin in a closed flask. When removed from the flask the latter smelt strongly of sulphide. Both muds were offered to *C. volutator* in the usual way. Greater numbers burrowed in the anaerobic than in the aerobic mud (Table 2*a*); but the muds could not be washed in sea water before the experiment because they take so long to settle again. The observed preferences might therefore have been associated either with the anaerobic conditions around sand particles in the mud or with a change in the nature of the surface film on particles. This difficulty could be overcome, however, by using 350 sand rather than finest mud, as 350 sand can be washed well, yet settles within minutes. It is fortunate then, that although preferring finest mud to 350 sand, *C. volutator* will burrow readily in 350 sand if finest mud is not available. The experiment was therefore repeated using 350 sand: two aliquots were maintained as before, one under anaerobic and the other under aerobic conditions, both for 7 days. To remove any dissolved material in the interstitial water, the sands were rinsed 10 times with sea water before being placed in the experimental dishes; this ensured that only molecules adsorbed on, and micro-organisms attached to, sand grains would remain. *C. volutator* still preferred the sand previously maintained under anaerobic conditions, although the choice ratio was barely significant (Table 2*a*).

Similar experiments were conducted with *C. arenarium*. Aerobic surface sand, and anaerobic subsurface sand (from below 5 cm. depth), were collected from areas of Red Wharf Bay where *C. arenarium* abounds. These were washed 10 times with sea water, and then offered to *C. arenarium* as described above. Significantly greater numbers burrowed in the aerobic sand than in the anaerobic sand (Table 2*b*). This preference could only have been due to differences in the nature of the surface film on particles, as both samples were of identical particle size, and both were washed well before the experiment.

In experiments with *C. volutator* the mud was maintained under anaerobic and aerobic conditions in the laboratory, while in those conducted with *C. arenarium* naturally occurring aerobic and anaerobic sands were used; thus the experiments with the two species are not strictly comparable. With this reservation, however, it does seem as if *C. volutator* prefers anaerobic, and *C. arenarium* aerobic, sands.

The different biochemical activities of anaerobic and aerobic micro-organisms will affect the nature of films on the surfaces of sand grains, and it may well be the differences in these films which the two species can detect.

Treatments which remove or alter the primary film on sand grains

Primary films influence substrate selection by many marine larvae (Wilson, 1958; Crisp & Ryland, 1960; Meadows & Williams, 1963). The presence of these films on sand particles may be necessary before *Corophium* will burrow.

C. volutator was therefore offered a choice of 350 sand and the same sand previously treated to remove or alter the primary film on sand grains. The treatment consisted of either boiling the sand in sea water for 15 min., or boiling the sand with concentrated nitric acid for 12 hr. The acid-cleaned sand was subsequently washed for 48 hr. with many changes of distilled water. The latter would have lost all vestiges of a primary film, while the sand boiled in sea water would have retained only a very modified one. Before being placed in the choice dishes all sands were as usual washed six times with sea water. During this short period of washing (*c*. 5 min.), some bacteria and organic substances in solution would undoubtedly have been adsorbed onto the surfaces of sand grains. This adsorption would have continued during the 3 hr. of the choice experiment. In spite of this, *C. volutator* distinguished the untreated sand from both the boiled and the acid-cleaned (Table 3). Experiments with *C. arenarium* paralleled these results.

Table 3. *Boiled and acid-cleaned sand. Experiments with* Corophium volutator

Treatment	Percentage in treated sand	Percentage in untreated sand	No. of animals in experiment	χ^2	*P*
Boiling with sea water	27	73	70	13·7	$< 0·001$
Boiling with acid	9	91	53	33·3	$< 0·001$

It seems, then, that if the primary film is removed or altered, sands are rendered unattractive. The beginnings of a new film, which must have accrued during the choice experiments, did not render the sand attractive again.

The development of the primary film

The results of these experiments strongly suggest that when selecting substrates in the sea, *C. volutator* and *C. arenarium* are influenced in their choice by the nature of the primary film which covers sand grains. Films and their constituent micro-organisms develop quickly on the surface of newly immersed objects (Redfield & Deevy, 1952). Within 24 hr., for instance, $1\cdot9 \times 10^6$ bacteria/in.^{2} were found on glass slides immersed at La Jolla, California, while after 96 hr. the numbers had reached 8×10^7 (ZoBell, 1946, p. 194). One would expect that, after having been immersed in sea water for some days, previously cleaned sand particles would have acquired on their surfaces rich films containing many micro-organisms. Yet *C. arenarium* would not burrow in acid-cleaned sand which had been soaked in sea water for 7 days. Other experiments using sand previously boiled in sea water and sand previously soaked in distilled water (see below) also failed. Perhaps *Corophium*, in common with *Cumacea vulgaris* (Wieser, 1956), requires a more complete or thicker film containing greater numbers of micro-organisms than would have developed during the period of the experiments.

Table 4. *Dried sand. Experiments with* Corophium arenarium

Percentage in sand previously dried in air at 17° C. for 18 hr.	Percentage in untreated sand	No. of animals in experiment	χ^2	P
2	98	136	122·3	< 0·001

Substrates which have been dried

Wieser (1956) has shown that *Cumacea vulgaris* Hart will not burrow into its usual sandy substrate if this has been previously dried. *Corophium* behaves similarly; when offered untreated sand and sand which had been previously dried at 17° C. for 18 hr., animals only burrowed in the untreated sand (Table 4).

Wieser, discussing his results, states that having entered sand previously dried at room temperature animals leave it sooner than they would the untreated. This is certainly so of *Corophium*. However, he goes on to suggest that drying removes something of nutritive value; there is therefore less food available on the surface of dried than on the surface of untreated sand grains. *Cumacea* finish eating this smaller amount of food sooner than they would the larger amount present on untreated sand grains. As soon as they have finished they leave. This behaviour leads to greater numbers being found in the untreated sand than in the previously dried sand.

I take issue with Wieser over these conclusions. It is unlikely that drying sand particles will remove appreciable amounts of nutritive organic material from their surfaces. On the other hand, the nature of the film on sand particles certainly would be altered; for example, many micro-organisms would be killed. Wieser realizes this, but wrongly equates it with 'almost total depletion of nutritive material'. It seems more likely that when they quickly leave previously dried sand, both *Corophium arenarium* and *Cumacea vulgaris* are responding to a changed film, not to a lack of food.

The action of fixatives

Sea water–formaldehyde and sea water–alcohol solutions will fix both the primary film and its constituent micro-organisms. *Corophium arenarium* found this change unattractive, for they avoided sand which had been previously soaked in either of these fixatives (Table 5).

Influence of reduced salinity on substrates

As low salinities kill or inactivate many species of marine micro-organism (Lipman, 1926; ZoBell, 1941; Braarud, 1951, 1961; Kain & Fogg, 1958*a*, *b*; Curl & McLeod, 1961; Hayes, 1963), animals were offered sands which had been soaked for various lengths of time in distilled water; these they avoided. This effect was evident even when the sand was soaked for only 10 min. Within this time the distilled water washings gave no precipitate with silver nitrate solution. Sands became more unattractive the longer they were soaked in distilled water (Table 6). On the other hand, *C. arenarium* found sand which had been previously soaked in 25 % sea water for an hour equally attractive to sand soaked in 100 % sea water. The change in the primary film which renders sands unattractive must therefore occur at relatively low salinities.

Table 5. *Sand soaked in fixatives for 1 hr. Experiments with* Corophium arenarium

Fixative	Percentage in sand previously soaked in fixative	Percentage in sand previously soaked in sea water*	No. of animals in experiment	χ^2	*P*
25 % formalin, 75 % sea water	11	89	148	90·9	< 0·001
10 % ethyl alcohol, 90 % sea water	24	76	117	31·6	< 0·001

* Sea water adjusted to same strength as that in the fixative solution.

Table 6. *Sand soaked in distilled water. Experiments with* Corophium arenarium

Time sand soaked prior to experiment	Percentage in sand previously soaked in distilled water	Percentage in sand previously soaked in sea water	No. of animals in experiment	χ^2	*P*
10 min.	43	57	129	2·8	< 0·1 > 0·05
1 hr.	38	62	154	8·9	< 0·05 > 0·001
7 days	13	87	105	55	< 0·001

$\chi^2 = 25·7$, $P = < 0·001$, with 2 degrees of freedom, on a 2×3 contingency table of the numbers of animals in the two choices in each of the three experiments.

The adsorption of bacteria on sand grains

Distilled water which had been used to wash sand was very opaque. Microscopic examination at × 1000 magnification showed large numbers of motile bacteria plus a small amount of unidentifiable debris. These observations suggest that distilled water induces many bacteria to leave the surfaces of sand grains. This phenomenon has not been described before. Some at least of the bacteria which released their hold were capable of forming colonies when plated on agar (Floodgate, personal communication).

It seemed possible that this desorption of bacteria from sand grains was in some way dependent on the ionic or osmotic strength of the solution; the precipitation and adsorption of proteins, for instance, is markedly influenced by the ionic composition and strength of the ambient solution (Colowick & Kaplan, 1955). Perhaps, then, solutions of the same ionic strength or osmotic pressure as sea water could replace sea water, without affecting the adsorption of bacteria or the attractive properties of the sand. The experiments described below, however, show that this is not so.

A sample of sand collected from where *C. arenarium* abounds was filtered through a 30-mesh sieve to remove shell particles and animals, and was then washed 10 times with sea water. After dividing the sand into portions of 20 ml., each portion was rinsed three times with 30 ml. of sea water, then quickly four times with 30 ml. of a given test solution; all these washings were discarded. Over the next 90 min. each aliquot of sand was rinsed five times, each rinse being with 30 ml. of the same solution; these five rinses were subsequently transferred to a glass container. The optical densities of all the solutions so obtained were measured with a Spekker photoelectric absorptiometer and no. 1 filter, coupled with a d'Arsonval galvanometer. Out of a total of five series of experiments, in each of which all solutions were tested, haemocytometer counts of bacterial concentrations were taken in two.

Solutions tested in this way included those of the more common ions in sea water, all of which were made up to an ionic strength of 0·7—equivalent to the ionic strength of sea water. Two non-electrolytes were similarly tested: sucrose and glycerol. Solutions of these were prepared at a concentration of 1·0 M, that is, at the same osmotic pressure as sea water (Harvey, 1955).

Microscopic observation showed that in the more opaque solutions larger numbers of bacteria were present. This was confirmed when the Spekker readings were plotted against haemocytometer values (Fig. 1). The linear relationship thus obtained proved highly significant.

In repeated experiments distilled water always proved the most opaque, closely followed by solutions of the non-electrolytes glycerol and sucrose. Hence these had induced the greatest numbers of bacteria to desorb. Of the electrolytes tested only the Na_2SO_4 solution was very opaque; the remaining solutions were relatively clear, although all were more dense than the sea-water washings (Table 7). An analysis of the variation amongst the optical densities of the solutions showed that the observed differences were statistically significant (Table 8).

It seems, therefore, that a lack of ions induces the desorption of many bacteria, while smaller numbers are released in the presence of solutions containing only two ionic species. Even in sea water—a composite mixture of many ions—some bacteria become detached. Whether the bacteria release their hold actively or do so on account of the break up of the primary film is problematical, although their presence as separate entities rather than clumps contraindicates the latter possibility. The presence or absence of ions having different valencies, besides influencing the zeta potential of the sand grain surface (Glasstone & Lewis, 1960), will probably alter the zeta potential of the bacterial cell surface itself; this, in its turn, has been implicated in the aggregation and dispersion of certain types of bacteria (Arkwright, 1920; Joffe & Mudd, 1935; Moyer, 1936). It is perhaps in this way that bacteria are induced to release their hold of sand-grain surfaces.

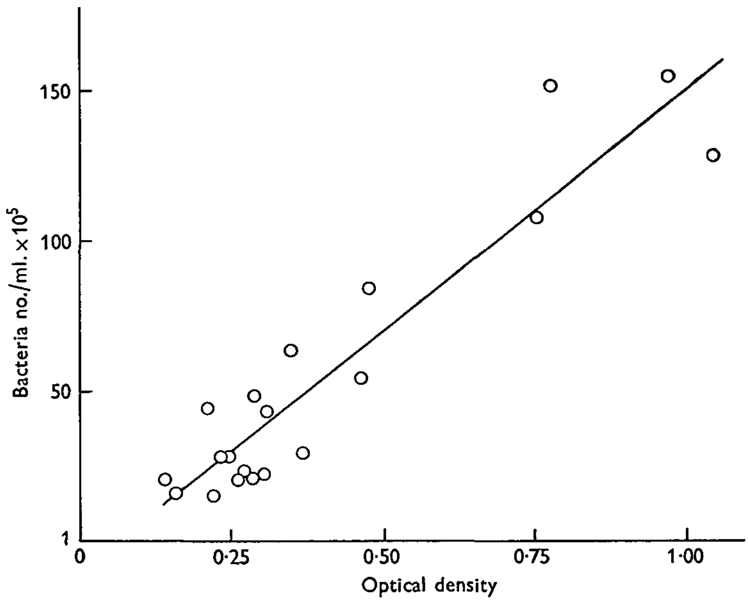

Fig. 1. Relation between numbers of bacteria in a solution and its optical density. Regression analysis: $y = 158{\cdot}3x - 9{\cdot}107$, Student's $t = 11{\cdot}2$, $P = < 0{\cdot}001$.

Table 7. *Optical densities of solutions of electrolytes and non-electrolytes which have been shaken with sand*

Test solution	Mean relative optical density	Test solution	Mean relative optical density
Distilled water	3·49	NaCl	1·60
Glycerol	2·89	$MgSO_4$	1·53
Sucrose	2·24	KCl	1·53
Na_2SO_4	2·18	$CaCl_2$	1·50
$MgCl_2$	1·61	Sea water	1·00

$$\text{Mean relative optical density} = \sum \frac{\text{absolute optical density of solution}}{\text{absolute optical density of sea water}} \Big/ N$$

where $N = 5$, the number of times the experiment was repeated.

Table 8. *Analysis of variation amongst the absolute optical densities of the solutions*

Source of variation	Sum of squares	Degrees of freedom	Mean square or variance estimate
Between treatments (NaCl, sucrose, etc.)	0·88654	9	0·098504
Between the 5 series of experiments	0·58586	40	0·014646
Total	1·47240	49	—

Snedecor's F test (variance ratio test): $F = 6{\cdot}73$, $P < 0{\cdot}001$.

Distilled water induces the greatest numbers of bacteria to desorb from sand grain surfaces; it also renders sands unattractive to *C. arenarium* (Table 6). One might expect, therefore, that other solutions, such as those of glycerol, sucrose and Na_2SO_4, which resulted in loss of bacteria from sand particles, would also render sands unattractive.

Table 9. *Sand soaked in solutions of electrolytes and non-electrolytes for 1 hr. Experiments with* Corophium arenarium

Test solution	Percentage in sand previously soaked in test solution	Percentage in sand previously soaked in sea water	No. of animals in experiment	χ^2	Degrees of freedom	P
Solutions of electrolytes having an equivalent ionic strength to sea water						
NaCl	53	47	133	0·37	1	< 0·7 > 0·5
Na_2SO_4	52	48	104	0·15	1	< 0·7 > 0·5
KCl	50	50	99	0·01	1	< 0·95 > 0·90
$MgSO_4$	53	47	109	0·45	1	< 0·7 > 0·5
$MgCl_2$	42	58	272	10·4	2	< 0·01 > 0·001
$CaCl_2$	24	76	289	84·4	2	< 0·001
Solutions of non-electrolytes having an equivalent osmotic pressure to sea water						
Glycerol	56	44	109	1·55	1	< 0·25 > 0·20
Sucrose	48	52	141	0·18	1	< 0·7 > 0·5

Surprisingly, this is not so. The only solutions which altered the attractive properties of sands were solutions of $MgCl_2$ and $CaCl_2$ (Table 9). Yet with these two electrolytes only relatively small numbers of bacteria became detached. It appears that the loss of surface bacteria in itself does not result in a particular sand becoming unattractive. This is borne out by the results of treating sand with sucrose and glycerol solutions; here almost as many bacteria are desorbed as are desorbed with distilled water, but the sand remains attractive. Both Mg^{2+} and Ca^{2+} are, however, divalent, and so would be more likely to cause adsorption or precipitation of lyophilic sols than would Na^+ or K^+; perhaps they affect marine bacteria in some similar manner. But here one is hampered by the almost complete lack of physico-chemical data on the adsorption of inorganic and organic substances, and of bacteria, on to surfaces in sea water.

CONCLUSION

It is evident that any treatment which alters the primary film or kills its constituent micro-organisms renders sand unattractive to *Corophium*. On the other hand animals do not appear to notice the absence of large numbers of bacteria on sand particles, except when caused by distilled water (Tables 6 and 9).

These observations, and my inability in repeated attempts to render sand attractive once it had been boiled, soaked in distilled water, or acid cleaned, underline the complex nature of the stimuli which *Corophium* must receive before it burrows. Certainly each species prefers its own habitat, and one selects anaerobic and the other aerobic substrates; but there is no immediately definable specificity such as the gregariousness of cirripede cyprids (Knight-Jones, 1953; Crisp & Meadows, 1962, 1963), or the settlement of certain polyzoan larvae on only one species of sea weed (Ryland, 1959). The difficulties experienced in analysing the more general type of substrate selection exhibited by, say, *Corophium* (this paper) or *Nassarius obselatus* Say (Scheltema, 1961), is a result of our lack of knowledge of the nature of the primary film. Little is known of even its more obvious characteristics; an understanding of these must prelude any further advance of our insight into this type of substrate selection.

SUMMARY

1. A simple method is described for determining the substrate preferences of *Corophium volutator* (Pallas) and *Corophium arenarium* Crawford.

2. If offered a choice of its own substrate with that of the other species each prefers its own.

3. Level of illumination and colour of substrate have little effect on choice. An animal's size and hence its age has little effect on its substrate preferences.

4. *C. volutator* prefers a substrate previously maintained under anaerobic conditions, *C. arenarium* vice versa.

5. Treatments which kill, inactivate, or remove micro-organisms render sands unattractive to *Corophium*. These include boiling, acid-cleaning, drying, and soaking in fixatives or distilled water. Attempts to make these sands attractive again failed.

6. Distilled water, and solutions of the non-electrolytes sucrose and glycerol at the same osmotic pressure as sea water, induce many bacteria to desorb from sand particles; smaller numbers are desorbed in the presence of solutions of electrolytes at the same ionic strength as sea water (NaCl, Na_2SO_4, KCl, $MgSO_4$, $MgCl_2$, $CaCl_2$). Of all these, only distilled water and solutions of $MgCl_2$ and $CaCl_2$ reduce the attractive properties of sands. Hence the loss of bacteria from the surface of sand grains, though related to the ionic strength and composition of the medium, is not necessarily associated with a substrate becoming unattractive.

Professor D. J. Crisp, Marine Science Laboratories, Menai Bridge, was a constant source of energy and enthusiasm during this work. Dr A. O. Christie and Dr C. P. Spencer advised me on chemical matters, and Dr G. D. Floodgate on bacterial matters. Dr L. E. R. Picken, Department of Zoology, University of Cambridge, drew my attention to some early references on the physical chemistry of bacteria. Miss J. Walley initially pointed out the Red Wharf Bay population of *Corophium arenarium.* Dr J. H. Stock, Zoological Museum, Amsterdam, kindly checked my identification of *C. arenarium.* I thank my wife for her extremely constructive criticism during the preparation of this paper.

REFERENCES

ANDERSON, W. W., GEHRINGER, J. W. & COHEN, E. (1956). Physical oceanographic, biological, and chemical data—South Atlantic coast of the United States. *United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report—Fisheries*, no. 178, 1–160.

ARKWRIGHT, J. A. (1920). Variation in bacteria in relation to agglutination by salts and by specific sera. *J. Path. Bact.* **23**, 358–60.

AYERS, J. C. & TURNER, H. J. (1952). In *Marine Fouling and its Prevention* (ch. 9). Prepared for Bureau of Ships, Navy Department, by Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. United States Naval Institute, Annapolis, Maryland.

BADER, R. G., HOOD, D. W. & SMITH, J. B. (1960). Recovery of dissolved organic matter in sea-water and organic sorption by particulate material. *Geochim. cosmoch. Acta*, **19**, 236–43.

BRAARUD, T. (1951). Salinity as an ecological factor in marine phytoplankton. *Physiol. Plant.* **4**, 28–34.

BRAARUD, T. (1961). Cultivation of marine organisms as a means of understanding environmental influences on populations. In *Oceanography* (ed. M. Sears). Baltimore, Maryland: The Horn-Shafer Co.

COLOWICK, S. P. & KAPLAN, N. O. (1955). *Methods in Enzymology*, vol. 1. New York: Academic Press.

CRAWFORD, G. I. (1937). A review of the amphipod genus *Corophium*, with notes on the British species. *J. Mar. biol. Ass. U.K.* **21**, 589–630.

CRISP, D. J. & RYLAND, J. S. (1960). Influence of filming and of surface texture on the settlement of marine organisms. *Nature, Lond.*, **185**, 119.

CRISP, D. J. & WILLIAMS, G. B. (1960). Effect of extracts from fucoids in promoting settlement of epiphytic Polyzoa. *Nature, Lond.*, **188**, 1206–7.

CRISP, D. J. & MEADOWS, P. S. (1962). The chemical basis of gregariousness in cirripedes. *Proc. Roy. Soc.* B, **156**, 500–20.

CRISP, D. J. & MEADOWS, P. S. (1963). Adsorbed layers: the stimulus to settlement in barnacles. *Proc. Roy. Soc.* B, **158**, 364–87.

CURL, H. & MCLEOD, G. C. (1961). The physiological ecology of a marine diatom, *Skeletonema costatum* (Grev.) Cleve. *J. Mar. Res.* **19**, 70–88.

FERGUSON WOOD, E. J. (1950). Investigations on underwater fouling. 1. The role of bacteria in the early stages of fouling. *Aust. J. Mar. Freshw. Res.* **1**, 85–91.

FISHER, R. A. (1958). *Statistical Methods for Research Workers*, 13th ed. London: Oliver and Boyd.

GEE, J. M. (1961). Ecological studies in South Benfleet Creek with special reference to the amphipod genus *Corophium*. *Essex Nat.* pp. 291–309.

GEE, J. M. & KNIGHT-JONES, E. W. (1962). The morphology and larval behaviour of a new species of *Spirorbis* (Serpulidae). *J. Mar. Biol. Ass. U.K.* **42**, 641–54.

GLASSTONE, S. & LEWIS, D. (1960). *Elements of Physical Chemistry*, 2nd ed. London: Macmillan and Co. Ltd.

HART, T. J. (1930). Preliminary notes on the bionomics of the amphipod *Corophium volutator* Pallas. *J. Mar. Biol. Ass. U.K.* **16**, 761–90.

HARVEY, H. W. (1941). On changes taking place in sea water during storage. *J. Mar. Biol. Ass. U.K.* **25**, 225–33.

HARVEY, H. W. (1955). *The Chemistry and Fertility of Sea Waters*. Cambridge University Press.

HAYES, P. R. (1963). Studies on marine flavobacteria. *J. Gen. Microbiol.* **30**, 1–19.

JOFFE, E. W. & MUDD, S. (1935). A paradoxical relation between zeta potential and suspension stability in S and R variants of intestinal bacteria. *J. Gen. Physiol.* **18**, 599–613.

KAIN, J. M. & FOGG, G. E. (1958*a*). Studies on the growth of marine phytoplankton. I. *Asterionella japonica* Gran. *J. Mar. Biol. Ass. U.K.* **37**, 397–413.

KAIN, J. M. & FOGG, G. E. (1958*b*). Studies on the growth of marine phytoplankton. II. *Isochrysis galbana* Parke. *J. Mar. Biol. Ass. U.K.* **37**, 781–8.

KNIGHT-JONES, E. W. (1953). Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. *J. Exp. Biol.* **30**, 584–98.

LIPMAN, C. B. (1926). The concentration of sea water as affecting its bacterial population. *J. Bact.* **12**, 311–13.

MEADOWS, P. S. & WILLIAMS, G. B. (1963). Settlement of *Spirorbis borealis* Daudin larvae on surfaces bearing films of micro-organisms. *Nature, Lond.*, **198**, 610–11.

MOYER, L. S. (1936). Changes in the electrokinetic potential of bacteria at various phases of the culture cycle. *J. Bact.* **32**, 433–64.

REDFIELD, A. C. & DEEVY, E. S. (1952). In *Marine Fouling and its Prevention* (ch. 4). Prepared for Bureau of Ships, Navy Department, by Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. United States Naval Institute, Annapolis, Maryland.

RYLAND, J. S. (1959). Experiments on the selection of algal substrates by polyzoan larvae. *J. Exp. Biol.* **36**, 613–31.

SCHELTEMA, R. S. (1961). Metamorphosis of the veliger larvae of *Nassarius obselatus* (Gastropoda) in response to bottom sediment. *Biol. Bull., Woods Hole*, **120**, 92–109.

DE SILVA, P. H. D. H. (1962). Experiments on choice of substrata by *Spirorbis* larvae (Serpulidae). *J. Exp. Biol.* **39**, 483–90.

STOCK, J. H. (1952). Some notes on the taxonomy, the distribution and the ecology of four species of the Amphipod genus *Corophium* (Crustacea, Malacostraca). *Beaufortia*, no. 21, 1–10.

STOCK, J. H. & DE VOS, A. P. C. (1960). Einige wirbellose Tiergruppen des Dollart-Ems-Estuarium. *Verh. Kon. Ned. Geol. Mijnb. Gen. (Geol. Serie)*, **19**, 203–20.

THAMDRUP, H. M. (1935). Beiträge zur Ökologie der Wattenfauna auf Experimenteller Grundlage. *Medd. Komm. Havundersøg, Kbh.* (*Ser. Fiskeri*), **10**, no. 2, 1–125.

VALLENTYNE, J. R. (1957). The molecular nature of organic matter in lakes and oceans, with lesser reference to sewage and terrestrial soils. *J. Fish. Res. Bd Can.* **14**, 33–82.

VISHNIAC, H. S. (1956). On the ecology of the lower marine fungi. *Biol. Bull., Woods Hole*, **111**, 410–14.

WATKIN, E. E. (1941). The yearly life cycle of the amphipod, *Corophium volutator*. *J. Anim. Ecol.* **10**, 77–93.

WIESER, W. (1956). Factors influencing the choice of substratum in *Cumella vulgaris* Hart (Crustacea, Cumacea). *Limnol. Oceanogr.* **1**, 274–85.

WILSON, D. P. (1953). The settlement of *Ophelia bicornis* Savigny larvae. The 1951 experiments. *J. Mar. Biol. Ass. U.K.* **31**, 413–38.

WILSON, D. P. (1958). Some problems in larval ecology related to the localized distribution of bottom animals. In *Perspectives in Marine Biology*. Ed. Buzzati-Traverso. Berkeley and Los Angeles: University of California Press.

ZOBELL, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J. Mar. Res.* **4**, 42–75.

ZOBELL, C. E. (1943). The effect of solid surfaces upon bacterial activity. *J. Bact.* **46**, 39–56.

ZOBELL, C. E. (1946). *Marine Microbiology*. Waltham, Mass.: Chronica Botanica.

To accommodate an increasing number of papers submitted for publication, the *Journal of Experimental Biology* will be published in two volumes each of three parts yearly, starting with Volume 42 (1965). The subscription price will be £3 (U.S.A. $11.00) a volume including postage. Single parts will be £1. 10*s*. (U.S.A. $5.00) postage extra.