The Journal of the Marine Biological Association of the United Kingdom Vol 32 No 1 1953

THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

THE 1952 EXPERIMENTS

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INTRODUCTION

In respect of their influence on the settlement reactions of *Ophelia bicornis* larvae, sands can be classed as attractive, neutral, or repellent (Wilson, 1953: mainly on the basis of the 1951 experiments). There are varying degrees of attractiveness and repellence, and the relative effect of sands on larval reactions can be tabulated. From the tables 'it should be possible to forecast with fair accuracy the settlements which will be obtained in mixtures of various sands' (Wilson, 1953, p. 427). Part of the 1952 experiments were devoted to investigating this possibility.

Earlier experiments had indicated, though not finally proved, that some of the repellent property of Salthouse Lake sand could be transferred, by contact under water, to Bullhill Bank sand. This was demonstrated by mixing large grains of the latter with small grains of the former and separating by sieving. In the first experiments along these lines (Wilson, 1952) the sands were not fresh and were sometimes dried for sieving, and all were subsequently sterilized. In 1951 a similar experiment (43 A) with fresh sands, mixed in sea water, again seemed to show partial transference of a repellent factor, presumably organic in nature. The technique used, however, was not entirely satisfactory, for it involved interference with the natural grade of the sand. Also the sand whose properties it was intended to alter was not itself completely free from organic impurities. In the 1952 experiments these objections were met by using acid-cleaned sands of normal grade sewn into fine-meshed bolting-silk envelopes. The envelopes were partially buried in the fresh natural sands from which it was hoped to abstract the repellent factor, and possibly also an attractive one.

A suspicion that these factors included one or more substances of organic nature led to some tests with sands incubated in nutritive media. In this connexion I am much indebted to Dr C. P. Spencer, not only for practical assistance with the incubation but also for much stimulating discussion on the whole problem involved. The incubation experiments in the present series are largely the result of his suggestions.

The 1952 experiments cover also a number of other points, as will be apparent below. It was not practical during the short breeding season to investigate only one aspect at a time, and each experiment was compounded, as usual, with more than one object in view. To enable a larger number of tests than before to be dealt with in one day, actual counts of the settled and settling larvae were avoided as much as possible. The assessment of the relative attractiveness of the sand samples had to be speeded up, for it had become necessary to test most sands both by the free-choice method and by the conical vessel method. The former method alone does not distinguish between neutral and repellent sands; the latter distinguishes imperfectly between neutral and attractive sands. Therefore both methods are required. The maximum number of samples which can be dealt with in one day if the larvae be counted, as hitherto, is about twelve. For many results, however, exact counts are not necessary, obvious differences being apparent on inspection. In a few minutes such differences can be recorded in words, when to obtain figures would take much longer. Only when the differences are not great are words unsatisfactory, but in the type of experiments used in this work close figures are also of little significance. By drawing up a scale of impressions of increasing number, expressed in words, three times as many sand samples could be disposed of in one day as when counts were made. The scale of words is shown in Table II and is used throughout Tables III–XI. When two samples are shown to differ one from another only by adjacent words in Table II, then the difference between them is scarcely significant, but if the words are far apart then the difference is real. By spreading out all the sand samples, of any one experiment, in dishes alongside one another it was relatively simple to compare them for larval content, and grade them accordingly.

Notes on Technique

General techniques differed little from those of previous years (Wilson, 1952, 1953). The flat-bottomed Pyrex crystallizing dishes (about 7 cm. diam.), in which little heaps of the sands were tested under free-choice conditions, were all cleaned in hot strong sulphuric acid. The conical vessels were cleaned only in hot water containing Lissapol C and scrubbed with a bottle brush, as were also the finger bowls in which the fertilizations were made. The earlier experiments (Exps. 52–56) were in water from off Millport in the Firth of Clyde, the later ones (Exps. 57–59) in a mixture of the Clyde water and water from the English Channel near the international hydrographical station E 1, or in E 1 water only (Exp. 60). All sea water was passed through a Berkefeld filter before use.

Sands which are stated to be acid-cleaned were first washed in fresh water and then heated in fuming concentrated sulphuric acid for at least 30 min., cooled and washed in glass-distilled water and stored in acid-cleaned bottles in distilled water. Bolting silks were cleaned in acetone. New pieces were always used and different pieces for each kind of sand. Bolting-silk envelopes were sewn with white cotton, also cleaned in acetone. These envelopes were made by folding the silk and sewing bent-over edges along three sides, sand being inserted before the last side was sewn up. They were flat and measured roughly 15×10 mm. The sand inside the envelopes spread out into a thin layer and was in close proximity to the sand on which the envelope lay and which was sprinkled over it. The dishes containing these envelopes were kept in a good light but shielded from the sun. Daily, as a rule, the envelopes were turned over with forceps sterilized in a bunsen flame, fresh sand being shifted over on top of them. To remove the sand inside they were cut open with sterilized scissors after all grains on the outside had been carefully shaken off. The silk of envelopes exposed to Salthouse Lake sand was often blackened, but not so that exposed to Bullhill Bank sand. Occasionally, in all dishes, the silks showed pink patches after several days.

The flat-bottomed dishes in which larvae had a free choice of the various sand offered to them in little heaps were, as usual, kept in the dark undisturbed during the two days allowed for settlement. The conical vessels were, as usual, surrounded by black paper so as to be lit only from above, and they were decanted after about 24 hr. to wash away all larvae which were not settling. All larvae washed away by the decanting and re-filling process were examined to make sure that none were metamorphosing. It was confirmed that sands could be much disturbed without washing away metamorphosed or metamorphosing larvae, which always clung tightly to the sand grains.

The Experiments

The 1952 results (Tables III–XI) are expressed as grades of abundance (Table II), with few exceptions. Only larvae actually in the sand samples are mentioned in these tables. The unsettled larvae decanted from the conical vessels are not of special interest; neither are those in the free-choice dishes which were still swimming, or were lightly attached to the surface film or to the clear glass bottom between the sand heaps. There were always many such larvae and they were specially numerous when all the heaps were of neutral or repellent sands. They were always unmetamorphosed, except for a few metamorphosed worms that occasionally crawled out of overcrowded heaps on to the glass between them. Sometimes in heaps of fresh Bullhill Bank sand so many larvae had settled and metamorphosed that their activities caused a spreading of the sand until the base of the heap was extended to almost twice its proper diameter.

Each experiment had a control not recorded in the tables. This was a small flat-bottomed dish, the bottom completely covered with fresh Bullhill Bank sand, placed in the dark with the free-choice dishes. Invariably most of the larvae put into this dish were found to have metamorphosed, few or none being on the surface film, or still swimming.

Unless stated to the contrary, the sands mentioned in the tables were fresh, unwashed and unsterilized. They had been collected on the dates mentioned and had been kept since collection in clean stoppered jars. The Bullhill Bank sands were all from the surface of the middle of the Bank at places where *O. bicornis* adults were abundant. The Salthouse Lake sands were all from Station II (see Wilson, 1952, pp. 59 and 61), where *Arenicola marina* and other species were plentiful (for list of species see Holme, 1949) but *Ophelia bicornis* absent. Table I gives the percentages by weight of the various mesh sizes of these two sands, for comparison with the sands of previous years. The washings, normal sterilization (heating in a glass vessel immersed in a bath of boiling water for a few minutes) and treatments with activated charcoal mentioned were all carried out in tap water, as had been usual. The mixtures were all made by measuring, in the stem of a pipette, equal volumes in water of the two sands used.

The experiments are numbered consecutively with those already published.

Experiment 52 (Table III)

Series A consists of tests of sands put into medium (0·5 % peptone and 0·2 % glucose in sea water) 3–4 hr. after collection and incubated in a dark oven at 22° C. for 6 days. One sample of Bullhill Bank sand was inoculated with a very few grains of Salthouse Lake sand. At the end of this period the medium, which had become either yellow (Bullhill Bank sand) or greenish grey (Salthouse Lake sand) with a strong odour, was poured off and the sands well washed with clean sea water before testing. Microscopic examination of portions of the washed sands showed large numbers of minute organisms sticking to the sand grains, or free and in motion in the interstices.

Table III shows that incubation of the sands lessened their attractiveness for the larvae, compared with the unincubated controls, both under free-choice conditions and in the conical vessels. Salthouse Lake sand, both without and with incubation, was less attractive than Bullhill Bank sand correspondingly treated. Inoculation of Bullhill Bank sand with grains from Salthouse Lake, thereby introducing bacteria and micro-organisms from the latter place, had no noticeable effect. The hypothesis that Salthouse Lake contains species, culturable in the medium, which are repulsive to the larvae and which are absent from the Bullhill Bank is unsupported by this inoculation experiment. The culturable species, or their metabolic products, normally present in the Bullhill Bank sand, are attractive, or at least not repulsive, in normal abundance. When more abundant they may possibly be offensive to the larvae.

Series B and *Series C* were tests of mixtures. In the first the mixing in equal proportions of a fully attractive sand with one that is neutral (see Wilson, 1953, pp. 425–7 and Table XIII) induced, as was expected, a good settlement under free-choice conditions and an even better settlement in a conical vessel.

The second mixture, of a fully attractive with a fully repellent sand (Wilson, 1953, Table XIV), produced very little settlement under either condition, this result again being the one anticipated. The repellent property of the sterilized Salthouse Lake sand had a stronger influence on larval reactions than the attractive property associated with Bullhill Bank sand.

Experiment 53 (Table IV)

For *Series A* small portions of the acid-cleaned sands removed from bolting-silk envelopes after 5 days in sea water, with or without contact with fresh unwashed sands, were examined microscopically. That which had been in sea water only showed some very minute organisms, which may have been bacteria, and was otherwise very clean. The other sands were also relatively clean but, in addition to what may have been bacteria, a number of ciliates, minute diatoms and other living plant cells were seen. They were more abundant in the sand which had been in contact with the Salthouse Lake sand than with that from the Bullhill Bank.

The result (Table IV), compared with the two controls, shows a marked increase in attractiveness of the acid-cleaned sand which had been amid fresh Bullhill Bank sand. That which had been with Salthouse Lake sand showed no such increase; instead there appears to have been a slight decrease in attractiveness, this being most marked in the conical vessel test.

Series B, C and *D* are further tests of mixtures. In the first the formalin-treated sand appears to have been a little repellent and not neutral as a similarly treated sample mentioned in a previous paper (Wilson, 1953, Table XIII). No doubt different samples vary a little in their properties. The mixture in this instance induced somewhat smaller settlements than had been expected on the basis of formalin-soaked sand being neutral, but this result is in agreement with one component of the mixture having a slightly repellent action. The second mixture was to test a suspicion, based on earlier results (Wilson, 1952, pp. 103 and 155), that boiling Bullhill Bank sand for a prolonged period in distilled water makes it a little less favourable than normal sterilization, and therefore on the repellent side of neutral. In this test the boiled sand acted as a neutral sand and the result is closely similar to that of Exp. 52A with normally sterilized sand. Again, different samples could vary according to their condition before boiling. The third mixture of a fully attractive sand with that of a repellent sand converted to attractiveness by the use of activated charcoal gave, under both conditions, the heavy settlements which had been anticipated.

Experiment 54 (Table V)

This was devoted to testing a number of mixtures of sands whose relative powers to attract or repel were fairly well known. The settlements obtained in all four mixtures were much as expected. In *Series A* and *B* the mixing of neutral with fully repellent sands produced strongly repellent mixtures. If boiling the Salthouse Lake sand made it more repellent than normal sterilization the fact is not shown by these fifty-fifty mixtures. In *Series C* the mixture of a fully attractive sand with a neutral or nearly neutral sand (in Wilson, 1953, Table XV, the oolitic sand is perhaps placed too far towards the repellent side of neutral) was favourable to settlement, but was not fully attractive. In *Series* D a fully repellent with a fairly attractive sand gave a mixture which was repellent, though not fully repellent.

Experiment 55 (Table VI)

Part of this experiment was devoted to tests of acid-cleaned Bullhill Bank sand inoculated with a few grains of fresh Bullhill Bank sand and fresh Salthouse Lake sand and incubated at 22° C. in a series of media containing less nutrients than the medium used for Exp. 52A. Sea water was enriched with 2·5 mg. per litre ammonium/nitrogen and this was further enriched with (*a*) 2·5, (*b*) 5, and (*c*) 10 mg. per litre of glucose to give a series of increasing concentrations. The results, though interesting, are too indefinite to be worthy of detailed consideration. So far as they go they suggest that inoculation of the acid-cleaned sand by either of the fresh sands, followed by incubation, slightly increases its attractiveness, and this may vary a little according to the concentration of the medium and hence probably of the number of micro-organisms present.

The remainder of this experiment, here designated *Series A* and *B* in Table VI gave definite results. *Series A* followed closely on the lines of Exp. 53A, using this time acid-cleaned Salthouse Lake sand which was of finer grade than the acid-cleaned Bullhill Bank sand used before (see Table I). Once again the sand which had been amid fresh Bullhill Bank sand became distinctly attractive, whereas that which had lain amid fresh Salthouse Lake sand did not, showing little difference from the controls.

Series B is a test of another mixture. In a previous paper (Wilson, 1953) it is shown that soaking fresh Bullhill Bank sand in distilled water can destroy its attractiveness under free-choice conditions, though a good settlement can be obtained when it completely covers the bottom of the vessel containing the larvae. It was rated as a neutral sand. In the present test there was a small settlement in the free-choice dish, although the sand had soaked for 3 days in distilled water. This suggests that this particular sample was a little on the attractive side of neutral, but the settlements obtained in the mixture with fully attractive fresh Bullhill Bank sand were of the type associated with a mixture of the latter with a neutral, or nearly neutral sand. They were, indeed, what had been expected.

Experiment 56 (Table VII)

For *Series A* three conical flasks each containing a small quantity (barely sufficient to cover the bottom if spread out) of acid-cleaned Bullhill Bank sand in filtered sea water were exposed to light on a window bench shielded from the sun. To one of these flasks a few grains (less than 1 %) of fresh Bullhill Bank sand were added, and to another a similar quantity of fresh Salthouse Lake sand. The flasks were gently shaken almost every day for 10 days, the sand in each flask piling up into a small heap in the middle. On the tenth day small samples from each were removed to be tested with larvae in the usual manner. In addition, a few grains of fresh Bullhill Bank sand and of fresh Salthouse Lake sand were mixed with small samples of the control sand to which nothing had been added previously. These mixed samples served as additional controls intended to distinguish between the effect of adding a few grains of the fresh sands just before the test and adding a few grains several days previously.

The settlements obtained with these various sands are set out in Table VII. The addition of a few grains of fresh sand just before testing with larvae seems to have made but little difference, except perhaps that the Salthouse Lake grains may have improved it slightly. It seems certain, however, that sands to which fresh grains had been added 10 days before testing were more attractive than any of the controls, and that the Salthouse Lake grains had had the greater effect.

The original sands in their flasks on the window bench were kept for nearly another 2 weeks, but some more acid-cleaned sand was added (on 1 July 1952) to the control flask because insufficient for another experiment remained. The tests were then repeated in an identical manner as part of Exp. 57 but only in a free-choice dish. The results were similar to those of the present experiment. There was no further increase in attractiveness of those sands to which grains of fresh sand had originally been added but these sands were still the most attractive.

Series B duplicates *Series A* except that the flasks were kept in a dark cupboard instead of on a window bench, and were exposed to light only for a few moments daily while being shaken. Table VII shows that the resulting settlements obtained were closely similar to those in sands exposed to the light. This *Series B* was also repeated (with the same sands kept longer) at a later date (as part of Exp. 58—free-choice dish only) and like the repetition of *Series A* gave settlements which differed hardly at all from the earlier ones.

It is not clear what these results indicate. If the improvement, with time, after inoculation is due to multiplication or metabolism of micro-organisms, then the species involved do not depend on light. The failure of the attractive factor to increase beyond a certain point, even with further time, might be due to early exhaustion of necessary nutrient materials from the filtered sea water used. Other explanations are possible. For instance organic matter on the fresh grains which were added may take time to transfer to the acid-cleaned sand.

Exp. 56 included tests of two more mixtures. The first (*Series C*) is a mixture of two neutral, or almost neutral sands, and gave, as anticipated, a small settlement under free-choice conditions and a good one in a conical vessel. It should be compared with Exp. 54C where the same oolitic sand was mixed with an attractive sand. The second mixture (*Series D*) is similar to that tested in Exp. 52C, except that here the Salthouse Lake sand was dried after washing and before normal sterilization. There seems to be little difference, both are strongly repellent, but in this experiment the larvae, which were a day older than usual, were a little more ready to settle than they generally are. The result, however, is only slightly different from that expected. Settlements in a fifty-fifty mixture of a fully attractive with a fully repellent sand are likely to vary around the neutral point according to the condition and age of the larvae. The 5-day-old larvae of Exp. 52C found such a mixture rather repellent, the 6-day-old larvae of the present series found it somewhat attractive. The antagonism of the two factors, one attractive, the other repellent, is clearly demonstrated.

The larvae in the clean finger bowl which supplied the experiment showed a precocious tendency to metamorphose without sand of any sort. Thus they displayed an unusual early elongation of the body, loss of prototrochal cilia and elongation of the bristles of the third setiger. Some larvae metamorphosed on the clean glass unusually early. These larvae were markedly more advanced in their development than were those of the same age in the bowl which had supplied larvae to Exp. 55. Account should be taken of this feature in assessing the results of the experiment.

Experiment 57 (Table VIII)

On two previous occasions (Exps. 53 and 55) an attractive factor from fresh Bullhill Bank sand was transferred to acid-cleaned sands consisting largely of quartz particles. In *Series A* of this experiment oolitic sand, which is almost entirely calcareous with but very few quartz particles, was used in place of an acid-cleaned sand. No attempt was made to clean it. Three samples were sewn up in 100-mesh bolting-silk envelopes. One, the control, was placed in sea water only, the other two in sea water amid fresh Bullhill Bank and fresh Salthouse Lake sands respectively. These fresh sands were changed after 11 days when fresher samples were available. After a total of 16 days the sands were removed from the envelopes and agitated in sea water on sieves of 86-mesh bolting silk. This was to ensure the removal of any particles of the fresh sands which might have worked their way through the 100-mesh silk envelopes to become mixed with the oolitic sands inside. The control sand was, of course, similarly agitated on 86-mesh silk: it was then divided into three portions, to two of which a few grains of the Bullhill Bank and Salthouse Lake sands were added. The oolitic sand which had been amid Salthouse Lake sand had a grey tinge and its silk envelope was blackish, as was much of the Salthouse Lake sand itself.

The result is shown in Table VIII. The settlements in the free-choice dish are considered first. There was little difference between any of the controls; the addition of a few fresh grains had had little or no effect and in all three the settlement was small. There was a significantly better settlement in the sand which had been with the Bullhill Bank sand, though the numbers of larvae settling were not large. Most of the larvae in the dish had gathered on the surface film or were still swimming freely, or were on the glass of the bottom, unmetamorphosed. The oolitic sand had apparently been made only a little more attractive by contact with Bullhill Bank sand, but none the less this increase in attractiveness was definite. On the other hand, the oolitic grains which had been amid Salthouse Lake sand had failed to induce any larva to metamorphose.

In the results with conical vessels there is no distinction between the controls and the oolitic sand which had been with Bullhill Bank sand. All gave settlements characteristic of neutral, or near neutral, sands. Only in the oolitic sand which had been with Salthouse Lake sand was there a difference to be noted. Here there were markedly fewer metamorphosing or metamorphosed larvae than in any of the other sands and there were rather more unmetamorphosed larvae present: the sand, in fact, appears to have been slightly repellent.

In *Series B* a new type of experiment was undertaken. A quantity of fresh Bullhill Bank sand was thoroughly shaken up with about three times its own volume of filtered sea water. After allowing all heavy particles to settle some of the water was carefully poured off into a clean dish. This was then examined to make sure that no sand grains, not even the smallest, were present. Only a little silt was to be seen. A small quantity of acid-cleaned Bullhill Bank sand was then added and the dish stood in the light, shielded from the sun, for 16 days. In a similar manner water in which fresh Salthouse Lake (St. II) sand had been shaken was used to soak another small quantity of acid-cleaned Bullhill Bank sand. In the Salthouse Lake water silt was plentiful but no sand grains were present. A control sample of the acid-cleaned sand was soaked in filtered sea water in the same manner. The experiment also includes a control of the same acid-cleaned sand stored in distilled water.

Table VIII, recording the results, shows that the sand soaked in water in which Bullhill Bank sand had been shaken induced much better settlements than did any of the others. The sand from water in which Salthouse Lake sand had been shaken also showed an improvement over the control, but not so great. This improvement contrasts with the opposite effect observed when acid-cleaned or oolitic sands are buried amid the Salthouse Lake sand itself. The control sample soaked in clean filtered sea water seems to have been slightly more attractive than the sample stored in distilled water.

In order to make reasonably certain that the presence of a few small sand grains, which might pass through 100-mesh silk, would not significantly affect the results obtained with sand samples enclosed in bolting-silk envelopes buried amid other sands, a test was made, in *Series C*, of acid-cleaned Bullhill Bank sand to which small quantities of fresh sands were added. The amounts added were appreciably larger than any which might be expected to pass through the meshes of the silk, and were 1 % or less of the whole sample. Although the conical vessel control was accidentally lost the results so far as they go confirm previous findings (Exps. 56A, B and 57A) that any effect is likely to be small.

As already mentioned (p. 215), Exp. 57 included a re-trial of the sands used in Exp. 56A.

Experiment 58 (Table IX)

An attempt was made to transfer the attractive factor in fresh Bullhill Bank sand, and the repellent factor in fresh Salthouse Lake (St. II) sand, to some artificial substance of as pure a composition as possible. I am indebted to Mr F. A. J. Armstrong for suggesting fused alumina, a reagent prepared by the Thermal Syndicate Ltd. The label on the bottle gave as the batch analysis; alumina 99·7%, carbon 0·0013%, alkalies 0·09%. The alumina is in the form of granular particles. By sieving through bolting silks it was possible to obtain a 'sand' similar in grade to that of the Bullhill Bank. The graded alumina (all of which was coarser than 86-mesh and most of it coarser than 60-mesh) was thoroughly washed in several changes of distilled water, to reduce yet further the soluble impurities and clean the large grains of adhering minute particles. The resulting 'sand' was clean and white. For *Series A* small samples were placed, in 100-mesh bolting-silk envelopes, amid fresh Bullhill Bank and Salthouse Lake sands, and there was the usual control in an envelope in sea water only. After 7 days the contents of the envelopes were removed and examined microscopically. No sand grains had penetrated the envelope which had been amid Bullhill Bank sand, but there were a very few quartz grains and some silt mixed with the alumina from the Salthouse Lake sand. Therefore all three samples were sieved separately, in filtered sea water, on 60-mesh silk about a third of each sample being lost through the meshes. This ensured the removal of all foreign sand grains and silt which had penetrated the 100-mesh silk. The sands were again examined to confirm this. No foreign grains among those of alumina could be seen, but various minute living organisms were observed on the grains, especially those which had been amid Salthouse Lake sand. The alumina grains which had been with the latter were tinged pale yellow.

The results indicate that an attractive factor from the Bullhill Bank sand had been transferred to the alumina, but they barely show any such transference of a repellent factor from the Salthouse Lake sand. They are not, however, completely negative in that respect, and taken in conjunction with earlier tests on similar lines (Exps. 53A, 55A and 57A) provide additional evidence, though slight, that such transference is possible.

In *Series B* some tests are made with activated charcoal. It is not at all clear how this reagent reacts with repellent sands to make them attractive. The grains of such sands are always darkened with adhering specks of the carbon which may do no more than stick to and cover up matter objectional to the larvae. The granular activated charcoal used in this experiment was the kind intended for gas absorption. The other kind was that used for decolorizing, the same batch having been used in all previous experiments with this substance. It is a very fine powder, and only a very small quantity of very small grains was obtained by washing away a large quantity of the still finer powder. There was sufficient for only a very small heap in the free-choice dish and not enough for a conical vessel as well. These very fine carbon particles attracted a large number of larvae, but the proportion to metamorphose was small, much smaller than amid the granular carbon or amid the same but still finer carbon particles mixed with the acid-cleaned sand. This suggests that while the activated charcoal is liked for its own sake the larvae require, among other factors, a substratum of reasonable solidity if metamorphosis is to proceed readily.

Exp. 58 also contained a re-trial of the sands used in Exp. 56B (see page 215).

Experiment 59 (Table X)

Some earlier tests, included in Exp. 55, had indicated that inoculation of an acid-cleaned sand with a few grains of a fresh sand, followed by incubation (in the dark at 22° C.) in a medium, increases slightly the attractiveness of the sand. The test for inoculation with fresh Bullhill Bank sand was repeated here, the concentration of medium which gave the best result in Exp. 55 being used. This was sea water enriched with 2·5 mg. per litre ammonium/nitrogen and 5 mg. per litre of glucose.

There was some difficulty in obtaining satisfactory larvae for this experiment. All the fertilizations intended for it failed and earlier fertilizations had to be used. The larvae put into the free-choice dish were 7 days old; those into the conical vessels were 6 days old and were from the fertilization which had supplied Exp. 58.

The result in the free-choice dish agrees with the earlier results that inoculation with fresh sand grains followed by incubation increases the attractiveness of the acid-cleaned sand. The incubated control which had not been inoculated was also made more attractive in comparison with some acid-cleaned sand which had been merely stored in distilled water until tested. Settlements in the conical vessels with younger larvae were all very similar and any slight distinctions are in line with the dish results.

Lack of suitable larvae prevented other tests, which had been planned, from being made.

Experiment 60 (Table XI)

In Exp. 51 (Wilson, 1953) an attempt to test the effect on settlement of different grades of fresh Bullhill Bank sand was only partially successful owing to the unusually slow rate of development of the particular culture of larvae used. The experiment is repeated here with a larger number of grades. These were obtained by sieving fresh Bullhill Bank sand through bolting silks of the usual mesh-sizes used in these investigations (see Wilson, 1952, p. 59), the sieving being done in filtered sea water. The ungraded control sand was agitated on 100-mesh silk in sea water for a time equivalent to that needed for sieving the other grades. Only a very few of the smallest grains were lost. The heaps in the free-choice dish were all of the same size and were much smaller than usual. Hence the unusually small number of larvae settling in even the most attractive grades. Conical vessel tests were not used in this experiment.

The result supports earlier conclusions that grade size is not without influence on settlement. The grade in which the greatest number of larvae settled is that which until about 1950 comprised about 50% of the surface sand of the Bullhill Bank (see Wilson, 1952, p. 59 and Table II, p. 127). The settlement in the control ungraded natural sand was closely similar to that amid grains of 60–86-mesh sizes, the sizes which now form the greater bulk of the surface sand of the Bullhill Bank (Table I), and therefore of the control. Relatively large, or very small, grains attracted very few larvae or none at all.

Other Experiments

A number of other experiments were attempted towards the end of the breeding season late in July, but all were spoilt by the condition of the larvae. The fertilizations made in the latter half of July appeared to be excellent for the first 2 or 3 days. Afterwards the larvae went prematurely to the bottom and many stuck together in small and large clusters. This clumping has been shown to take place most readily when the water is in some way unsuitable for healthy development (Wilson, 1951). These fertilizations during the latter half of July were made in water from hydrographical station E 1 off Plymouth, after the Clyde water available earlier had all been used up. In spite of the condition of the larvae several experiments were set up. The results they gave were, as far as they went, in line with previous findings. Thus another attempt to transfer to oolitic sand the attractive factor from fresh Bullhill Bank sand seems to have been successful. Some tests based on filtering water in which fresh sands had been shaken gave promising, though imperfect, results. These are, however, sufficiently interesting to merit further attention at a later date.

Analysis of Sands for Nitrogen Content

Dr C. P. Spencer kindly undertook the analysis of surface samples of Bullhill Bank and Salthouse Lake (St. II) sands collected on 9 May 1952. The sands were first thoroughly washed in clean sea water, from the laboratory circulation, to remove silt. They were then sieved, in sea water, through 26-mesh bolting silk to remove large mineral particles and any larger living organisms which might be present. The Salthouse Lake sand was further sieved through 40-mesh bolting silk, for it was found that a few small molluscs and worms had passed the larger mesh. Samples of the sand were then digested with the digestion mixture of Harvey (1951) and distilled with a Markham micro-stream distillation apparatus (Markham, 1942). The figures given below are the mean of triplicate estimations. The blank estimations were done on separate samples: Bullhill Bank sand, 490 μg. N/g. wet weight; Salthouse Lake (St. II) sand, 1760 μg. N/g. wet weight.

In view of the thorough washing, the nitrogen found must have been present on the sand grains in an insoluble form and is therefore probably organically bound. If this assumption is made, the nitrogen determination estimates organic matter, and so the Salthouse Lake sand, at least after washing, contained some three times as much organic matter as that from the Bullhill Bank.

The striking effect on settlement of treating sands with activated charcoal has frequently been demonstrated, repellent sands being made attractive by its use. I am much indebted to Mr F. A. J. Armstrong for making analyses in an attempt to find out whether the activated charcoal removed organic nitrogen compounds from the sand. The attempt was unsuccessful because the high nitrogen content of the activated charcoal masked any possible changes in the nitrogen content of the sand.

Discussion

In assessing the results of experiments such as these, and especially when comparing one experiment with another, it is essential to remember that they are based on the reactions of living organisms of which no two are ever quite alike. There is much accumulated evidence, not always set out in these papers, to show that different cultures of larvae vary in the readiness with which they will metamorphose under relatively unfavourable conditions, such as those of a clean glass vessel without sand. Some cultures remain free-swimming, with no tendency to metamorphose, longer than others of the same age, or younger, even when beside them on the same table under, practically identical conditions of temperature and lighting. Thus quite apart from variation in the numbers of larvae used from experiment to experiment, the results are to some extent affected by the characteristics of the particular batch of larvae used, as well as by their age when this is different. But in any one experiment very seldom has more than one fertilization of larvae been used, and all comparisons are made between larvae of the same age and with the same mixture of characteristics. Within each experiment results are thus strictly comparable. These remarks are necessitated by the long series of experiments which have now been published and which if compared without these points in mind may occasionally appear to be contradictory. For instance, as pointed out above (p. 216), in Exp. 56 the particular larvae used showed a precocious tendency to metamorphose without sand of any sort. This may well explain why more of them settled in the acid-cleaned sand straight from storage in distilled water than was usual for larvae of their age. Though this peculiarity has to be remembered when comparing this particular experiment with any other, the comparisons between the different sands in the experiment are not in the least invalidated. On the whole, atypical results due to variable characteristics have been infrequent. Most results, indeed, have been consistently repeatable.

The results with mixtures may now be considered. Before the present series of experiments was started, a table was drawn up (using information gained in 1951) listing the mixtures it was proposed to test and forecasting the intensity of the settlements expected in them under both free-choice and conical vessel conditions. Twelve mixtures were proposed and, as already noted in the descriptions of the actual experiments, the results with them showed very little variation from those expected. Such small variations as occurred, chiefly in Exp. 53 B and C, can be attributed to one component of each mixture not having been quite of the same neutral or repellent property as a similar sand previously assessed. Such variation between sands of different batches is only to be expected. The experiments, however, show that if the relative power to attract or repel of two sands be known then it is possible to forecast the intensity of settlement which will be given by 5-day-old larvae in a mixture of equal volumes. A mixture consisting of two attractive sands, or of an attractive with a neutral, remains attractive. A repellent sand with another repellent, or with a neutral, gives a repellent mixture. Neutral with neutral remains neutral. Attractive sands mixed with repellent sands produce results varying on either side of neutral in accordance with which factor, attractive or repellent, is dominant. Only when the opposing factors are equal will such a mixture be neutral.

That the attractive and repellent factors are distinct, and at least to some extent independent of the mineralogical characters of the sand grains which carry them, seems to be shown by several experiments in which they were transferred to sands consisting mainly of quartz grains (after drastic cleaning in hot concentrated sulphuric acid), to a mainly calcareous oolitic sand, and to a purely artificial sand of fused alumina. The attractive factor was transferred quite readily, though no sand so treated became nearly as strongly attractive as fresh Bullhill Bank sand itself. Before 1952 no experiment had shown transference of an attractive factor. Indeed the existence of such a factor was almost unsuspected before the results of the 1951 experiments had been considered. Evidently, the attractive factor is present only in fresh sand, particularly that from the Bullhill Bank, and that almost any treatment including sterilization, drying, subjection to hot concentrated sulphuric acid and red heat destroys it.

The repellent factor, on the other hand, is most readily demonstrated in sterilized or dried sand, particularly that from the Salthouse Lake (St. II), though hot concentrated sulphuric acid or red heat destroy it. Its transference, though imperfectly achieved both in earlier experiments and in 1952, seems to be no less real than the transference of the attractive factor. In 1952 its transference may have been complicated by the presence of the attractive factor, for both seem to be present on fresh Salthouse Lake (St. II) sand, which is normally almost neutral. Neutrality could result, as is shown by the mixture experiments, when the two factors are balanced against each other.

The manner in which transference takes place from fresh sands to acid-cleaned or other neutral sands is not clear. For the attractive factor, physical contact of the sand grains is evidently not necessary, since it was present in sea water in which fresh Bullhill Bank sand had been shaken (Exp. 57B). It seemed also to be present, though less concentrated, in water in which fresh Salthouse Lake (St. II) sand had been shaken (Exp. 57B). The latter experiment and certain inoculation experiments (Exps. 55, 56A and B) appear to show that the attractive factor is present on fresh Salthouse Lake sand and can be separated from the repellent factor which is also there. The repellent factor seems to be transferred less readily than the attractive factor, and then only to sands in close proximity if not in actual contact with it.

The experiments involving incubation of sands in media are difficult to interpret. In the first of these experiments (Exp. 52A), where fresh sands were put into a medium which produced a heavy growth of organisms, the sands became, even after thorough washing, less attractive than before. On the other hand, acid-cleaned sands inoculated with only a few grains of either of the fresh sands, and incubated in weaker media producing only a relatively small growth of organisms (Exps. 55 and 59), showed an increase in attractiveness after incubation. Even without enriching the sea water, and at room temperature both in the light and in the dark, the inoculation of acid-cleaned sand with a few grains of fresh sand of either sort, followed by a period during which a small growth could take place (Exp. 56A and B), resulted in a small increase in attractiveness. These experiments, taken by themselves, might indicate that the attractiveness or repellence of a sand depends not on two different factors but on the relative abundance of a single factor. It will be remembered that the organic nitrogen content of Salthouse Lake (St. II) sand was some three times that of Bullhill Bank sand (p. 221). None the less, other results are more easily interpreted if two factors are assumed, one of which, the attractive one, is readily destroyed by sterilization, drying and other simple treatments.

The natures of the attractive and repellent factors are still unknown, but on the evidence are probably organic or the products of organic activity. The results with sterilized sands suggest that the repellent factor may be non-living material; but the status of the attractive factor is even less evident.

Even when the nature of the factors is known the manner in which the larva perceives them, or is affected by them, has still to be sought. The experiments with activated charcoal may give a clue on how the attractive factor stimulates the larva to settle and metamorphose. This highly adsorbent substance appears to be attractive in itself (Exp. 58 B), although for maximum effect it must be present in particles of a size simulating a sand, or have sand grains mixed with it. The very property of adsorbing readily, by aiding the larva to get rid of excretions, may act favourably on the larva. The attractive factor might thus be something which removes excretory matter from the larva; though this is by no means the only possibility. It is well known that minute amounts of copper, or other substances, stimulate the larvae of certain species to metamorphose (see Wilson, 1952, pp. 51–2 for a brief discussion and references). The activated charcoal is not a pure substance and I am indebted to Mr F. A. J. Armstrong for showing that it contains an appreciable amount of copper. The decolorising charcoal, which has been used throughout these investigations, gave, on analysis, 320 parts per million of copper, while the granular charcoal used in Exp. 58 B gave 600 parts per million. From the natural sands themselves the amounts of copper extracted by heating to fuming with nitric and sulphuric acids were: Bullhill Bank, 6 parts per million; Salthouse Lake (St. II), 8 parts per million. The total copper contents, determined after opening the samples by hydrofluoric acid treatment, were 24 and 15 parts per million respectively. These figures are given without implying that they are of significance in relation to settlement in the natural sands; whether the larger quantities present in the charcoals influence metamorphosis cannot be decided on present evidence.

The experiments with activated charcoal again showed that if metamorphosis is to take place freely the particulate matter must be of a grade not too far removed from that of the natural sand itself. This is further brought out in Exp. 60 in which graded sizes of Bullhill Bank grains were tested under free-choice conditions. The grades which form the bulk of the surface sand of the Bullhill Bank attracted the largest settlements and there was a marked falling off in the numbers of larvae settling in coarser and finer grades. Sands can evidently be too fine or too coarse.

Much of the foregoing discussion is speculative, and further experiments will surely modify or refute some of the ideas put forward, but these ideas are of value in so far as they facilitate the planning of future work.

Summary

Previous conclusions that it should be possible to forecast the intensity of settlements which will be obtained in fifty-fifty mixture of two sands, provided the relative attractiveness or repellence of each component is known,

were fully confirmed. The results of these mixture experiments agree with the classification of sands as attractive, neutral or repellent.

It is shown that an attractive factor is capable of transference, in sea water, from fresh Bullhill Bank sand to acid-cleaned quartz sands, to calcareous oolitic sand and to an artificial sand of fused alumina. The attractive factor is present in water in which fresh Bullhill Bank sand had been shaken.

A similar transference of a repellent factor from fresh Salthouse Lake sand is not so clearly demonstrable. Water in which fresh Salthouse Lake sand has been shaken appears to contain the attractive factor. Thus both attractive and repellent factors may be present in Salthouse Lake sand.

Activated charcoal stimulates larvae to settle and metamorphose, but needs to be in granular form, or associated with neutral grains, to exert its full influence. The charcoal contains copper in minute proportion, but whether this has any significance for metamorphosis is undetermined.

The grade of a sand was again shown to be a settlement factor of some importance. The most attractive grain sizes appear to be those of which the bulk of the Bullhill Bank surface sand is composed.

Analysis shows that the surface sand of Salthouse Lake (St. II) contains some three times the organic nitrogen content of the surface sand of the Bullhill Bank.

TABLE I. GRADING OF SURFACE SANDS

Date	(6) >26 mesh	(5) 26–40 mesh	(4) 40–60 mesh	(3) 60–86 mesh	(2) 86–100 mesh	(1) 100–200 mesh	Silt	Mean category	Percentage floatability
				From the middle of Bullhill Bank					
10. vi. 52	0·2	1·1	25·0	61·2	11·7	0·7	Slight	(3·15)	20–30
8. vii. 52	0·2	0·9	20·2	63·7	13·8	1·1	Slight	(3·07)	20–30
				From the Salthouse Lake (St. II)					
10. vi. 52	0·3	1·1	16·0	33·9	32·0	16·7	Fair quantity	(2·54)	50–60

Categories (1) to (6) are percentage mesh sizes by weight, after washing away silt.

TABLE II. SCALE OF WORDS USED TO EXPRESS NUMBER IN TABLES III–XI

Very few (sometimes also expressed in numbers 1–4)
Few
Several
Fair number
Good number
Many
Very many
Multitude

Table III. Experiment 52

(Begun 16. vi. 52 with larvae from a fertilization of 11. vi. 52.)
Results on 18. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52): Rinsed in sea water	Bullhill Bank sand (10. vi. 52): Incubated in medium for 6 days	Bullhill Bank sand (10. vi. 52): Incubated in medium for 6 days after inoculation with Salthouse Lake sand (10. vi. 52)	Salthouse Lake sand (10. vi. 52): Rinsed in sea water	Salthouse Lake sand (10. vi. 52): Incubated in medium for 6 days
			Settlements in Dish		
Metd	}Very many	None	None	}Several	None
Meting	}	3 or 4	2 or 3	}	2
Unmet.	Few	Several	Several	}	Several
			Settlements in Conical Vessels		
Metd	}Multitude, mainly metd.	}Many	}Many	}Fair number, mainly meting	}Several
Meting	}	}	}	}	}
Unmet.	None	Several	Several	Several	}

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	}Multitude, mainly metd.	None	}Many, mainly metd.	}Multitude, almost all metd.
Meting	}	2	}	}
Unmet.	Few	1	Few	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	}Multitude, mainly metd.	None	None	}Very few
Meting	}	None	1	}
Unmet.	Several	Several	Several	Few

TABLE IV. EXPERIMENT 53.

(Begun 17. vi. 52 with larvae from a fertilization of 12. vi. 52.)
Results on 19. vi. 52.

Series A

	Stored in distilled water	Acid-cleaned Bullhill Bank sand (9. v. 52) In bolting-silk envelope in sea water for 5 days: In clean dish	Amid Bullhill Bank sand (10. vi. 52)	Amid Salthouse Lake sand (10. vi. 52)
		Settlements in Dish		
Metd	1	} Few	} Many, mainly metd.	None
Meting	1	}	}	None
Unmet.	Few	Few	Few	Few
		Settlements in Conical Vessels		
Metd	Good number	Fair number	} Multitude, mainly metd.	Few
Meting	Good number	Fair number	}	Few
Unmet.	Good number	Fair number	Few	Few

Series B

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) soaked 1 hr. in 5% formalin in sea water	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	} Multitude, mainly metd.	None	*c.* 5	} Many, mainly metd.
Meting	}	None	2	}
Unmet.	Few	None	1	None

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Bullhill Bank sand (10. vi. 52) washed (distilled water), boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude	None	} Fair number, mainly metd.	Multitude
Meting	Few	None	}	Very few
Unmet.	1 or 2	None	Several	None

Series D

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, treated activated charcoal 2–3 hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
		Settlements in Dish		*Settlement in Conical Vessel*
Metd	Multitude	} Fair number	Multitude	Multitude
Meting	} Very few	}	Few	Very few
Unmet.	}	Fair number	Few	None

Table V. Experiment 54

(Begun 25. vi. 52 with larvae from a fertilization of 20. vi. 52.)
Results on 27. vi. 52.

Series A

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) boiled in distilled water 2–3 hr.	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	2 or 3	None	None	None
Unmet.	None	None	None	None

Series B

	Bullhill Bank sand (10. vi. 52) washed, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	1 or 2	None	None	None
Unmet.	Few	3 or 4	3 or 4	Very few

Series C

	Bullhill Bank sand (10. vi. 52) rinsed in sea water	Oolitic sand, normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	Multitude	1	Many	} Multitude
Meting	Several	None	Several	
Unmet.	None	2	Few	None

Series D

	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Salthouse Lake sand (10. vi. 52) washed, dried, activated charcoal $\frac{3}{4}$ hr., normal sterilization	Mixture of the two sands	Mixture of the two sands
	Settlements in Dish			*Settlement in Conical Vessel*
Metd	None	None	None	None
Meting	None	Fair number	None	Very few
Unmet.	1 or 2	Fair number	Few	Many

Table VI. Experiment 55

(Begun 30. vi. 52 with larvae from a fertilization of 25. vi. 52. Results on 2. vii. 52.)

Series A

Acid-cleaned Salthouse Lake sand (10. vi. 52)

	Stored in distilled water	In bolting-silk envelope in sea water for 6 days: Amid acid-cleaned Bullhill Bank sand (9. v. 52)	Amid Bullhill Bank sand (23. vi. 52)	Amid Salthouse Lake sand (23. vi. 52)
Settlements in Dish				
Metd	1 or 2	1 or 2	25–30	None
Meting	1 or 2	1 or 2	Few	2
Unmet.	Few	Several	Several	Few
Settlements in Conical Vessels				
Metd	1 or 2	3 or 4	Very many	5 or 6
Meting	Few	Few	Several	Few
Unmet	Good number	Good number	Several	Several

Series B

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Bullhill Bank sand (23. vi. 52) kept in distilled water for 3 days	Mixture of the two sands
Settlements in Dish			
Metd	Multitude	Good number	Many
Meting	Few	Few	Few
Unmet.	None	None	None
Settlements in Conical Vessels			
Metd	—	Multitude	Multitude
Meting	—	Few	Few
Unmet.	—	None	None

Table VII. Experiment 56

(Begun 1. vii. 52 with larvae from a fertilization of 25. vi. 52. Results on 3. vii. 52.)

Series A

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) In sea water in light for 10 days	(3) Sand no. 2 to which a few grains of Bullhill Bank sand (23. vi. 52) were added on 1. vii. 52	(4) On 21. vi. 52 a few grains of Bullhill Bank sand (10. vi. 52) added. In sea water in light for 10 days	(5) Sand no. 2 to which a few grains of Salthouse Lake sand (23. vi. 52) were added on 1. vii. 52	(6) On 21. vi. 52 a few grains of Salthouse Lake sand (10. vi. 52) added. In sea water in light for 10 days
			Settlements in Dish			
Metd	Few	Few	Several	Good number	Good number	Many
Meting	Several	Several	Few	Several	Several	Several
Unmet.	Several	Several	Few	Few	Few	Few
			Settlements in Conical Vessels			
Metd	Several	Many	Many	Very many	Many	Multitude
Meting	Very many	Many	Many	Many	Many	Many
Unmet.	Many	Several	Several	Very few	Few	Very few

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52), treated exactly as for Series *A* but nos. 2 (including 3 and 5), 4 and 6 kept in the dark for 10 days

	(1)	(2)	(3)	(4)	(5)	(6)
			Settlements in Dish			
Metd	Few	Few	Several	Many	Several	Many
Meting	Several	Few	Several	Several	Several	Several
Unmet.	Several	Few	Several	Few	Few	Few
			Settlements in Conical Vessels			
Metd	*See A 1 above*	Many	Many	Very many	Many	Very many
Meting		Many	Many	Good number	Good number	Good number
Unmet.		Good number	Several	Few	Few	Very few

Series C

	Bullhill Bank sand (23. vi. 52) normal sterilization	Oolitic sand, normal sterilization	Mixture of the two sands
		Settlements in Dish	
Metd	None	2	None
Meting	Few	Few	Very few
Unmet.	Few	Few	Few
		Settlements in Conical Vessels	
Metd	Very many	Many	Many
Meting	Many	Many	Many
Unmet.	Very few	Good number	None

Series D

	Bullhill Bank sand (23. vi. 52) rinsed in sea water	Salthouse Lake sand (10. vi. 52) washed, dried, normal sterilization	Mixture of the two sands
		Settlements in Dish	
Metd	Multitude	None	None
Meting	Good number	None	2 or 3
Unmet.	None	3 or 4	3 or 4
		Settlements in Conical Vessels	
Metd	—	Several	Many
Meting	—	Several	Many
Unmet.	—	Several	Many

TABLE VIII. EXPERIMENT 57

(Begun 14. vii. 52 with larvae from a fertilization of 9. vii. 52.)
Results on 16. vii. 52.

Series A

Oolitic sand from the Great Salt Lake

	(1) In bolting-silk envelope amid Oolitic sand in sea water for 16 days	(2) Sand no. 1 to which a few grains of Bullhill Bank sand from no. 3 were added on 14. vii. 52	(3) In bolting-silk envelope amid Bullhill Bank sand (23. vi. 52 and 8. vii. 52) in sea water for 16 days	(4) Sand no. 1 to which a few grains of Salthouse Lake sand from no. 5 were added on 14. vii. 52	(5) In bolting-silk envelope amid Salthouse Lake sand (23. vi. 52 and 8. vii. 52 in sea water for 16 days
Settlements in Dish					
Metd	3	2	11	1	None
Meting	3	5	8	1	None
Unmet.	Several	Several	Several	Several	Several
Settlements in Conical Vessels					
Metd	Good number	Good number	Good number	Good number	Few
Meting	Good number	Good number	Good number	Good number	Few
Unmet.	Several	Several	Several	Several	Good number

Series B

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	Stored in distilled water	Kept for 16 days, in the light in: Filtered sea water	Sea water in which Bullhill Bank sand (23. vi. 52) had been shaken	Sea water in which Salthouse Lake sand (23. vi. 52) had been shaken
Settlements in Dish				
Metd	None	Few	Many	Several
Meting	1 or 2	Few	Good number	Several
Unmet.	Several	Few	Many	Several
Settlements in Conical Vessels				
Metd	Several	Good number	Very many	Many
Meting	Good number	Good number	Very many	Many
Unmet.	Many	Many	Many	Severa

Series C

Acid-cleaned Bullhill Bank sand (10. vi. 52)

	(1) Stored in distilled water	(2) Sand no. 1, to which on 14. vii. 52 1%, or less, of Bullhill Bank sand (8. vii. 52) was added	(3) Sand no. 1 to which on 14. vii. 52 1%, or less, of Salthouse Lake sand (8. vii. 52) was added
Settlements in Dish			
Metd	None	None	None
Meting	None	None	None
Unmet.	Good number	Good number	Good number
Settlements in Conical Vessels			
Metd	—	1 or 2	1 or 2
Meting	—	Few	Few
Unmet.	—	Good number	Several

Table IX. Experiment 58

(Begun 15. vii. 52 with larvae from a fertilization of 10. vii. 52.)
Results on 17. vii. 52.

Series A

Fused alumina

	Stored in distilled water	In bolting-silk envelope in sea water for 7 days: In clean dish	In bolting-silk envelope in sea water for 7 days: Amid Bullhill Bank sand (8. vii. 52)	In bolting-silk envelope in sea water for 7 days: Amid Salthouse Lake sand (8. vii. 52)
		Settlements in Dish		
Metd	2	1	26	1
Meting	2	4	32	None
Unmet..	Fair number	Fair number	Many	Fair number
		Settlements in Conical Vessels		
Metd	1 or 2	Several	Several	1 or 2
Meting	Several	Fair number	Many	Several
Unmet.	Many	Many	Very many	Several

Series B

	Acid-cleaned Bullhill Bank sand (10. vi. 52): Stored in distilled water	Acid-cleaned Bullhill Bank sand (10. vi. 52): Mixed with activated charcoal powder	Small grains of activated charcoal	Granular activated charcoal
		Settlements in Dish		
Metd	None	Many	Several	Very many
Meting	None	Many	Several	Very many
Unmet.	None	Several	Very many	Good number
		Settlements in Conical Vessels		
Metd	Few	Multitude	—	Very many
Meting	Several	Multitude	—	Very many
Unmet.	Very many	Few	—	Very many

TABLE X. EXPERIMENT 59

(Begun 16. vii. 1952 with larvae from fertilizations of 9. vii. 1952 and 10. vii. 1952.)
Results on 18. vii. 1952.

Acid-cleaned Bullhill Bank sand (10. vi. 1952).

	Stored in distilled water	Incubated in medium for 5 days	Inoculated with Bullhill Bank sand (8. vii. 1952) and incubated in medium for 5 days
	Settlements in Dish		
Metd	1 or 2	Fair number	Many
Meting	3 or 4	Fair number	Many
Unmet.	Few	Good number	Fair number
	Settlements in Conical Vessels		
Metd	Few	Few	Few
Meting	Fair number	Good number	Many
Unmet.	Many	Many	Many

TABLE XI. EXPERIMENT 60

(Begun 19. vii. 1952 with larvae from a fertilization of 14. vii. 1952.)
Results on 21. vii. 1952.

Bullhill Bank sand (8. vii. 1952) graded by sieving through bolting silks in filtered sea water

	Sand shaken on 100-mesh silk in sea water	Sand grains >26-mesh sizes	Sand grains 26–40-mesh sizes	Sand grains 40–60-mesh sizes	Sand grains 60–86-mesh sizes	Sand grains passing 86-mesh	Sand grains passing 100-mesh
	Settlements in Dish						
Metd	7	None	6	12	7	3	1
Meting	3	None	1	4	1	None	None
Unmet.	Few	Few	3	Few	Few	1 or 2	1 or 2

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