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THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

THE 1951 EXPERIMENTS

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Introduction

An investigation into the settlement and metamorphosis of *Ophelia bicornis* Savigny larvae was begun in 1946 and has been continued during the breeding season of each succeeding year. The results for the years 1946–50 inclusive have already been published (Wilson, 1948, 1951,[1] and 1952). In the last of these three papers it was shown that size and shape of sand grains are less important factors than had at first seemed probable, and that it appears that the larvae react mainly to some other factor or factors. The nature of the latter was still largely speculative, but the evidence obtained pointed to the possibility that the larvae are influenced by minute amounts of organic materials, dead or alive, present on the surface of the sand grains, and that they detect them only on contact. Some of this organic material, particularly that on the grains of most Salthouse Lake[2] sands, is repellent to them, and in such sands *Ophelia* larvae do not settle. They settle in sands, of suitable grade, which are not repellent, or only slightly so. The repellent property appears to be largely, but not entirely, correlated with floatability of the sand grains on water, for repellent sands generally float readily when sprinkled dry on to the surface of water, while most non-repellent sands at once break through the surface film and sink. This phenomenon and related matters are discussed in a preceding paper (Wilson, 1952).

Throughout almost all the forty experiments with settling *Ophelia* larvae so far recorded in detail, it has been usual to wash and to sterilize—by heating for a few minutes in tap water on a water-bath at about 100° C.—the various sands to be tested. In any one experiment all were treated alike in this way. In many sand samples so treated very good settlements had been obtained; in others smaller settlements or none at all. Results had been very consistent, especially the contrast between Bullhill Bank sand in which larvae readily metamorphosed and Salthouse Lake (Sts. I and II) sands, in which only a very

[1] This paper was delivered orally at a colloquium held in Paris in February 1950, and the results given in it are abstracts from experiments recorded in greater detail in the 1952 paper, and are not additional to the latter.

[2] For a full description of the locality in the Exe estuary, with which this investigation is so largely concerned, see the preceding 1952 paper, and for a photograph see the 1951 paper.

few larvae would do so, except after special treatments. These experiments were at first carried out in small flat-bottomed dishes in which the tested sands, one kind to each dish, completely or almost completely covered the bottom, and later were carried out in conical vessels in which a smaller quantity of sand could be effective. It is important to note that cylinders of black paper ensured that during the daytime the vessels were illuminated only from above, the larvae (which are negatively phototactic for at least part of the time) being thereby guided, if not driven, downwards towards the sands. From definitely repellent sands they would subsequently almost all come away and swim up to attach themselves to the surface film of the water. Some would often come away even from sands which gave good settlements. Some experiments in which two or more sands were tested in the same dish showed that larvae would settle and metamorphose in greatest numbers in their own sterilized Bullhill sand in preference to others. While, however, there remained any suspicion of substances emanating from the Bullhill or other sands inducing or inhibiting metamorphosis at a distance (as for Jägerstein's *Protodrilus*), experiments with more than one sand in the same dish could not be employed generally, for they would involve uncertainties from which experiments with only one sand-sample per dish were free. But when it had become quite certain that *Ophelia* larvae are indifferent to the presence of a sand except when in actual contact with it, there remained no objection to testing different sands together in the same dish; indeed there are certain advantages, such as the elimination of the error due to variations in the numbers of larvae put into separate dishes. Because directional movements of the larvae are influenced by light it is advisable to keep in the dark all dishes that contain more than one sand, during the time allowed the larvae to settle. Experience has shown that at room temperatures in June and July larvae were generally ready to settle on the fifth day after fertilization and should be given 2 days in which to do so. This makes it possible to plan and work as many experiments as possible during the 6 weeks or so when good larvae can be obtained.

Towards the end of the 1950 season some experiments in the use of unsterilized sands gave particularly interesting results. It was found (using conical vessels) that not only did fresh, unwashed, unsterilized Bullhill Bank sand induce heavier settlements and readier metamorphosis than the same sand after the normal sterilization technique, but that fresh unwashed Salthouse Lake (Sts. I and II) sand itself induced heavy settlements and ready metamorphosis, particularly Station I sand. The latter was, however, inferior to fresh Bullhill Bank sand and Station II sand was worse (Exp. 37, Wilson, 1952). Experiments in which larvae could choose between these sands in the same dish (Exps. 39 and 40, Wilson, 1952) confirmed the superiority of fresh Bullhill sand over fresh Salthouse Lake sands as a medium for bringing about settlement, but the discovery that sands from both localities when fresh, unwashed and unsterilized bring about a heavier settlement than when washed and sterilized was so striking as to necessitate further investigation. This and other problems not completely elucidated by the end of the 1950 season were taken as starting-points for a new series of tests in 1951, and it is these latter which are recorded in the present paper.

The 1951 Experiments

The principles on which experiments are devised and the general methods used are fully explained in a previous paper (Wilson, 1952). Only those details which differed from earlier practice need be mentioned here.

Almost all the experiments in 1951 were carried out in Pyrex crystallizing dishes, 7 cm. diameter and 5 cm. high. They were a little more than half filled with filtered sea water. Small quantities of the sands to be tested were pipetted into heaps of about 0·75 cm. diameter at the base and a few centimetres apart. The maximum number of heaps in any one dish was five. Unless stated to the contrary, the sands tested were unsterilized. After adding swimming larvae the dishes were immediately covered and kept in darkness until they were examined 2 days later. For counting, the whole of a heap of sand was removed with a pipette to the counting dish. Any metamorphosing or metamorphosed worms left behind sticking to the glass under the heap were recorded and the numbers added to those found in the sand itself. The quantities of sand being small, complete counts of the larvae present could usually be obtained for each, though occasionally when they were very numerous and time pressed the system of awarding plus signs (see Wilson, 1952, p. 65) was adopted where it would not introduce error. Before the heaps were removed the general condition of each dish was noted, with records of larvae still swimming, or on the surface film, or on the glass bottom. Unless otherwise stated it may be assumed that all stages counted were normal and healthy.

Exps. 41 and 42 were carried out in filtered Outside Water, but all succeeding experiments were in filtered Celtic Sea water. This water was kindly collected by Mr G. A. Steven on a cruise to the Celtic Sea early in July 1951, and was stored at a low temperature until used.

Experiment 41[1]

At the suggestion of Dr H. W. Harvey the effect was tested of treating sands with alumina and kieselguhr for comparison with the results obtained with activated charcoal. The alumina and kieselguhr were, of course, washed out with tap water, and the sands rinsed in sea water before testing. Unfortunately in this experiment a few lumps of kieselguhr or alumina remained in the sand. The result (Table II) is almost negative so far as any favourable action of the alumina and kieselguhr is concerned.

[1] The experiment numbers are consecutive with those already published.

Experiment 42

Dish A. It has been shown (Wilson, 1952, Table II) that the mean grain size of Bullhill Bank sand decreases with depth, but sands from various depths had not previously been tested. The grading of the sands tested in this dish are given in Table I. The result (Table III) shows that by far the heaviest settlement occurred in the surface sand, but it is doubtful if this was related to grade; it seems more likely that other factors were involved.

Dish B. The sand collected for me at Goulven, near Roscoff (see my 1952 paper, p. 63), in which *O. bicornis* occurs in small numbers, is very much coarser than that of the Bullhill Bank (1952, Table IV). That from La Jolla, California (kindly collected by Dr R. Phillips Dales) is finer and of angular particles. It is the home of *Thoracophelia mucronata* which lives in it in great abundance (Fox, Crane & McConnaughey, 1948). In neither was there any settlement of note, but as both sands had been collected a year previously this result is scarcely significant in view of the findings with stored Bullhill Bank sands (see Exp. 47C, below).

Experiment 43

Dish A. Certain previous experiments had given some indication, short of final proof, that large grains of Bullhill Bank sand are made less attractive by mixing for a time with small grains of Salthouse Lake sand, the latter being removed by sieving before the former are tested. All sievings, both before and after mixing, were made with acetone-cleaned 60- and 86-mesh bolting silks in sea water, the sands not having been dried at any stage. The control sand was sieved to an extent equal with the mixed sands. The result shown here (Table IV) again gives some indication that contact with Salthouse grains has an adverse effect on Bullhill sand when mixed together in sea water. The results for the mixture in distilled water bears a different interpretation (see Exp. 44D below).

Dish B. The effect of exposing, on the laboratory roof, Salthouse Lake sands to the weather was tested. Small quantities of the sands were contained in funnels lined with filter-paper; rain water could drain away. They were wet with rain when removed, but had previously been baked in the sun. They were rinsed in sea water before testing. The exposure made the sands even less acceptable to the larvae than was the unexposed sand.

Experiment 44

Dish A. A comparison was made between natural Bullhill Bank sand and the same sand mixed with an equal volume of Salthouse Lake (St. II) sand. The mixture was less acceptable than the natural Bullhill sand (Table V).

Dish B. A comparison was made between Bullhill Bank sand, previously washed and dried, and the same sand shaken for 7 min. on acetone-cleaned bolting silk (200-mesh). The unshaken sand showed an unusually high floatability (see Wilson, 1952, p. 94). Neither sand produced a settlement: it is shown below (Exps. 47 A, 48 A) that under these free-choice conditions larvae do not readily settle in sand which has previously been dried.

Dish C. Exposing Bullhill Bank sand to the weather, on the laboratory roof, made it less attractive to the larvae.

Dish D. Soaking Bullhill Bank sand in distilled water, or sterilizing it, made it, under these free-choice conditions, unacceptable to the larvae. However, the same sand, soaked in distilled water and completely covering the bottom of the vessel (in this instance a glass tube surrounded by a collar of black paper and lit from above), induced a very good settlement. It is interesting to note that the metamorphosis rate appears to have been less than in the control sand in the dark.

Experiment 45

This was a repetition of some of the tests of dishes B and D of the previous experiment. A conical vessel containing Bullhill Bank sand, sterilized by the normal technique, was added. This vessel was lit by light from above during the daytime. The larvae came from the fertilization which supplied Exp. 44; but were 3 days older and showed more tendency to stick to glass surfaces and other objects than they had 3 days previously.

Dish B. Both the washed and dried Bullhill Bank sand and the same sand after friction on silk showed (Table VI) a greater settlement than previously. It should be noted, however, that there were a number of metamorphosed or metamorphosing larvae on the clean glass between the sand heaps and some of these larvae may have crawled out of the heaps. On the other hand, in the clean finger-bowl containing larvae from the same fertilization some larvae on the bottom (but not on the surface film) were metamorphosing and some had completed the process, although the great majority were still unmetamorphosed. Thus some of the young worms in the sand heaps might even have crawled in. There seems no doubt that older larvae metamorphose more readily than do younger ones and are probably not so selective. It may be significant that there were fewer in the sand agitated on silk than in the other heap.

Dish D. Normal sterilization technique, under free-choice conditions, again retained fewer larvae than sand which had only been rinsed in sea water, but in the conical vessel containing sterilized Bullhill Bank sand a very good settlement was obtained. The unusually small number of larvae in the sand which had only been rinsed (compared with Exp. 44 D) may have been due to less swimming and exploratory activity on the part of the older larvae. A fair number of unmetamorphosed larvae were on the surface film, and some attached themselves to the bottom of the dish. Only a few larvae were swimming freely when the dish was examined. Of those on the bottom most were in various metamorphosis stages.

Throughout this experiment all stages were normal and very healthy. The weather had been very warm for several days and the temperature throughout had been decidedly high. Their relatively advanced age may have been partly responsible for the readiness with which larvae metamorphosed on a clean glass surface.

Experiment 46

Dishes A 1 *and A* 2 *and Conical Vessel AA.* In a previous treatment (Exp. 41) Salthouse Lake sand had not been completely freed from large particles of alumina. The experiment was repeated here, care being taken to remove, by sedimentation, all large particles before mixing with the sand, in tap water. After 3–4 hr. all the alumina was completely washed away and the sand rinsed in sea water. Two dishes were set up, one with a small heap in the centre, the other had in addition a heap of Bullhill sand. Some of the treated sand was also tested in a conical vessel.

The treatment with alumina does not seem (Table VII) to have made the Salthouse Lake sand more favourable under free-choice conditions in the dark, but in a conical vessel lit from above a heavy settlement and high rate of metamorphosis took place. But a settlement almost as good occurred in Salthouse Lake sand not treated with alumina (see conical vessel CC 1 below). At best the alumina made the sand slightly more favourable.

A recount of dishes A 1 and A 2 on 22 July 1951 showed no significant change; the larvae had not when older gone into the alumina-treated Salthouse Lake sand, even when there was no other sand in the dish.

Dish B and Conical Vessel BB. Lead-free ballotini retained on an 86-mesh bolting silk sieve (see Wilson, 1952, p. 96) and then cleaned with strong sulphuric acid were here compared with natural Bullhill Bank sand. Although they induced some settlement they were nothing like as favourable as the Bullhill sand. In the conical vessel BB, however, they brought about a heavy settlement. In the ballotini the larvae while fairly healthy were not as healthy as in the Bullhill sand.

Dish C and Conical Vessels CC 1 *and CC* 2. These were further tests of Salthouse Lake sands exposed to the weather on the laboratory roof. In this experiment the sands were removed for testing during a spell of dry sunny weather and stored in sea water overnight. The weathered sands were chosen by fewer larvae than the unweathered, and even in a conical vessel experiment the settlement and metamorphosis rate in the weathered sand was less than in the unweathered. Once again natural Bullhill sand collected most larvae.

Experiment 47

Dish A. This experiment was designed to see if under free-choice conditions Bullhill Bank sand was improved by treating with activated charcoal in tap water, and also to test the effect of drying (in a warm oven) Bullhill Bank sand after previous washing. It is seen (Table VIII) that hardly any larvae selected the latter whereas activated charcoal treatment (on sand *not* previously dried) had no effect on their choice.

Dish B. This experiment examines the effect on settlement of treating Salthouse Lake (St. II) sand with alumina and with activated charcoal. The sand was not dried before treatment; it had been kept in its natural moist state in a stoppered jar. The result leaves no doubt that activated charcoal treatment makes the sand very acceptable to the larvae whereas alumina makes it only slightly more so. It is interesting to note that there was a heavier settlement in the fresh Salthouse Lake (St. II) sand in this dish than in the dried Bullhill Bank sand in dish A.

Dish C. This is a comparison of Bullhill Bank sands collected in different years. The sands had been kept in glass-stoppered jars in their natural semi-dry state ever since collection; they had not been washed or dried. There is no doubt that the sand collected not very long before the test was the most effective, but it is interesting to note that the sand of 1947 was better than the samples of 1948 and 1949. A sample from the year 1950 was tested later (Exp. 51 B).

Experiment 48

Dish A. In Exp. 47 A the Bullhill Bank sand treated with activated charcoal had been washed in tap water but not dried. It was shown here (Table IX) that if the sand be washed and dried (in a warm oven) before treatment the activated charcoal improves it compared with dried untreated sand, but it remains much inferior to the natural sand which has not been washed and dried.

Dish B. In Exp. 47 B the Salthouse Lake (St. II) sand treated with alumina and with activated charcoal had not been dried after washing in tap water. The effect of drying (in a warm oven) after washing was tested here. It is seen that while alumina had little, if any, effect, activated charcoal so much improved the dried sand that it gave a similar result to charcoal-treated sand which had not been dried. The latter, in this experiment, did not produce as heavy a settlement as in Exp. 47 B. In all dishes in Exp. 47 a rather large number of larvae were inserted, and in setting up the present experiment fewer larvae had been used in all three dishes; this almost certainly explains the lower figures for settlement in comparable sands in the two experiments.

Included in the present dish was another test of lead-free ballotini. They proved better than the washed and dried Salthouse Lake sand, but the larvae in them were not as healthy as in the sand heaps, where all stages were very healthy.

Dish C. This was designed to test the effect of treating Bullhill Bank sands with hot concentrated H_2SO_4. It is seen that whereas the recently collected sand was less favourable after treatment than before, the 1948 sand was, if anything, slightly improved. All stages were very healthy in the untreated sands and in nearly as good condition in those which had been treated.

Experiment 49

Dish A. Here were tested mixtures of recently collected Bullhill sand with Bullhill sand stored since 1948 and with recently collected Salthouse Lake (St. II) sand, both before and after drying. The mixture with the 1948 sand had even more larvae in it than the 1951 sand alone (Table X), but many of these were unmetamorphosed and the metamorphosis rate was not as good as in the 1951 sand. As usual, the more significant figures are probably those for the metamorphosing and metamorphosed larvae, ignoring those for the unmetamorphosed. The 1948 sand here did better than in Exp. 47C, but there is always the possibility that samples of sand removed from a large jar, in which it has been stored undisturbed for a long time, vary in properties if bacterial or other growths or chemical actions have been taking place.

Dish B and Conical Vessels BB 1 *and BB* 2. This was mainly to test the effect of treating the Bullhill Bank sand, collected in 1948, with activated charcoal after washing with tap water (not dried). The results shows that under free-choice conditions the treatment brought about a slight improvement. There was a very marked improvement in the conical vessel tests. It must be noted, however, that the figures for the untreated sand in conical vessel BB 1 are not quite complete; a few more larvae of all stages were not counted (owing to lack of time). It was obvious, however, that there were not enough to double the figures, and the total for all stages would have been well under one hundred.

Experiment 50

In this experiment the bottom of each crystallizing dish was completely covered with Bullhill Bank sand, one with sand collected about a month previously, the other with the sand collected in 1948, which under free-choice conditions in Exp. 47C was not attractive to the larvae. A very large number of larvae were put into each dish and the dishes were kept in the dark for the usual 2 days allowed for settlement. Dish I, containing the recent sand, was examined first (Table XI). Almost all the larvae had disappeared into the sand and only a very few were still swimming and there were none on the surface film. Small portions of the sand were removed from all parts of the dish, and during a 20-min. search of these portions a number of larvae were found, almost all of them metamorphosed. In the dish containing the 1948 sand there were, in contrast, a large number of larvae swimming freely and a considerable number, unmetamorphosed, attached to the surface film. A 20-min. search of small quantities of sand from all parts of this dish yielded relatively few larvae, and most of these were unmetamorphosed. The experiment was continued for another 2 days, by which time most of the larvae in the dish with 1948 sand had settled and metamorphosed.

This result with dishes in which the bottom is completely covered with sand supports the contention that the larvae find their sand by chance contact. In these dishes they could not easily miss it, as they can when only small heaps are employed. Under free-choice conditions, with small heaps of sand there were, after 2 days in the dark, always a large number of unmetamorphosed larvae swimming freely or attached to the surface film. It is noteworthy that with the completely suitable sand the larvae settled straight away, whilst with the not so suitable sand there was some delay.

Experiment 51

This was the last experiment carried out in 1951. Unfortunately the larvae used were, for some unknown reason (not temperature), slow in their development and the results are therefore not as definite as they might otherwise have been. This is so especially for dish A, the examination of which began at 10 a.m. on the second day. When it was found that the larvae were still at a rather early stage of development the examination of the other dishes was postponed for a few hours, but it was not possible to leave them until the next day.

Dish A. This was intended as a test of different mesh sizes of relatively fresh Bullhill Bank sand. Sieving was done in sea water through bolting silks which had been cleaned with acetone. As it has been shown that greater friction with silk than that involved in simple sieving has only a little effect on the acceptability of the sands used it seems practicable to use silk sieves for such tests. The results (Table XII) appear to confirm this conclusion, and they also seem to show that the smallest mesh sizes do not attract as many larvae as does the natural sand, or as do the larger-sized grains. Unfortunately, for the reason already stated, the experiment is inconclusive. On the whole the larvae were healthy, but in the >60-mesh sand there were a number in poor condition.

Dish B. Salthouse Lake (St. II) sand washed and soaked in aquarium water for several days is apparently not much altered in property so far as the larvae are concerned. On the other hand, the same sand kept with some living adult *Ophelia* and exposed to their excretions and extruded genital products was made even less attractive than before. All larval stages in this dish were healthy.

Dish C. This shows that Bullhill Bank sand collected in 1950 was, like those of earlier years (see Exp. 47), less acceptable to the larvae than that collected more recently. It may be noted that of the twenty-seven unmetamorphosed larvae recorded for the 1950 sand the majority swam away readily when disturbed, but in the 1951 sand the unmetamorphosed larvae had attached themselves more strongly to the grains and were probably beginning to metamorphose. In this dish all stages were very healthy.

Dish D. This was designed to investigate the effect of washing fresh Bullhill Bank sand in tap water, of treating it with soluble reagents (alcohol and formalin) which would kill living matter and which could then be removed by washing (in sea water). A sample of sand dried and heated to redness (which in early experiments had given good settlements) was also tested. The results show that washing in tap water for several minutes had little effect (unlike prolonged soaking in distilled water—see Exp. 44 D); the alcohol and formalin treatments made the sand disliked, while the sand heated to redness produced only a small settlement under these free-choice conditions and, as often in a heated sand, the larvae tended to be in poor condition and there were some dead. In all the other sands all stages were very healthy.

Dish E. This small dish had the bottom completely covered with lead-free ballotini of sizes between 60 and 86 mesh, the largest obtainable. During 2 days in the dark there was a good settlement. Although many larvae were still swimming freely, or were attached to the surface film, a considerably larger number were observed among the ballotini. Once again a dish with the bottom completely covered with 'sand' produced a bigger settlement than would have taken place in a small heap in the middle of an area of clean glass. All stages were healthy and normal.

The 1951 Results

It is now possible to extract from the experiments a series of data which can be arranged fairly logically. Except when otherwise stated, this series is based on the 1951 'free-choice' experiments, that is to say the experiments in which the sands were presented in small heaps on the bottoms of clean glass crystallizing dishes in the dark and in which the larvae were not forced into contact with them by negative phototaxis. This method has revealed that the larvae are capable of a finer discrimination between sands than was evident in most earlier experiments. Sands which induce settlement and metamorphosis will be referred to as 'attractive', and it will become apparent there are varying degrees of attractiveness.

(1) Sand from the surface of the Bullhill Bank, where *Ophelia bicornis* adults are very common, is very attractive to the larvae of that species, provided that

it has been collected within recent weeks and kept in its natural moist state. On contact with it they quickly settle and metamorphose.

(2) Larvae find the sand by chance contact, they are not attracted to it from a distance by olfactory or other senses. This should be remembered when a sand is described as being attractive. It is only attractive to them while they are in contact with it.

(3) Nothing dissolves out of the sand to induce the metamorphosis of larvae not in actual contact with it (the 1951 and all previous experiments).

(4) Washing for a few minutes in tap water, followed by sea water, has little effect on fresh Bullhill Bank sand, although it does seem to make it a little less attractive (Exp. 51 D).

(5) The attractiveness of fresh Bullhill Bank sand is destroyed by soaking for several days in distilled water, but not by soaking in sea water (Exps. 43 A, 44 D). If, however, the sand which has been soaked in distilled water completely covers the bottom of a vessel lit from above during the daytime, larvae settle in it and metamorphose readily (Exp. 44 D), though probably not as readily as they would do in fresh sand under like conditions.

(6) The attractiveness of fresh Bullhill Bank sand is greatly reduced, or destroyed altogether, by heating in tap water to about 100° C. (Exps. 44 D, 45 D). This was the normal sterilization technique adopted for almost all experiments in previous years. However, in a conical vessel lit from above during the daytime (as in earlier experiments) sand so treated will bring about a heavy settlement with a high rate of metamorphosis (Exp. 45 DD), though an earlier experiment (Exp. 37, Wilson, 1952) showed that the attractiveness of sterilized sand is less than that of fresh sand even in conical vessel experiments.

(7) The attractiveness of fresh Bullhill Bank sand is greatly reduced or destroyed altogether by drying in a warm oven after washing in tap water (Exps. 44 B, 45 B, 47 A, 48 A).

(8) The attractiveness of Bullhill Bank sand which has been reduced by previous washing and drying seems to be still further reduced, though only to a slight extent, by friction dry on silk (Exps. 44 B, 45 B).

(9) There is little or no difference in attractiveness between fresh Bullhill Bank sand and the same sand agitated on silk in sea water (Exp. 51 A).

(10) The attractiveness of Bullhill Bank sand is reduced or destroyed by soaking in sea water to which absolute alcohol or neutral formalin has been added (Exp. 51 D).

(11) The attractiveness of fresh Bullhill Bank sand washed quickly in distilled water and then treated with hot concentrated sulphuric acid is reduced but not entirely destroyed (Exp. 48 C) as it is when soaked for a long period in distilled water alone (Exps. 43 A, 44 D).

(12) Fresh Bullhill Bank sand which has been washed and dried has its attractiveness partially restored by treating in tap water with activated charcoal (Exp. 48 A).

(13) Fresh Bullhill Bank sand which has merely been washed before treatment with activated charcoal in tap water has an attractiveness similar to that of the untreated sand (Exp. 47 A).

(14) The attractiveness of fresh Bullhill Bank sand is destroyed by exposure to the weather (Exp. 44 C).

(15) The surface sand of the Bullhill Bank is considerably more attractive than that from deeper down (Exp. 42 A).

(16) There is some indication that the smallest grains of fresh Bullhill Bank sand are not as attractive as are the larger ones (Exp. 51 A).

(17) The attractiveness of Bullhill Bank sand is greatly reduced by prolonged storage (Exps. 47 C, 48 C, 49 A and B, 50, 51 C).

(18) The attractiveness of a mixture of equal volumes of fresh Bullhill Bank sand and of Bullhill Bank sand stored for several years is similar to that of fresh Bullhill Bank sand alone, and is greater than that of the stored sand alone (Exp. 49 A). This may indicate that while storage of the sand, without drying, makes it less likeable by the larvae it does not render it repellent to them. A similar conclusion seems to be suggested by the result of Exp. 50.

(19) The attractiveness of Bullhill Bank sand which has been reduced by prolonged storage is possibly to some extent restored by treatment with activated charcoal (Exp. 49 B) or hot concentrated sulphuric acid (Exp. 48 C). Previous experiments have shown that under conical vessel conditions the former treatment may be expected to improve favourable as well as unfavourable sands (Wilson, 1952).

(20) Bullhill Bank sand which has been heated to 900–1000° C., and which in conical vessel experiments brings about a heavy settlement and a good rate of metamorphosis (Wilson, 1952), is under free-choice conditions only slightly attractive (Exp. 51 D).

(21) The attractiveness of fresh > 60-mesh Bullhill Bank grains is reduced after having been mixed for several days in sea water with fresh < 86-mesh Salthouse Lake (St. II) grains (Exp. 43 A).

(22) The attractiveness of a mixture of equal volumes of fresh Bullhill Bank sand and fresh Salthouse Lake (St. II) sand is less than that of fresh Bullhill Bank sand alone (Exps. 44 A, 49 A, and compare paragraph 18 above). If the Salthouse sand be washed and dried before mixing with the fresh Bullhill sand the mixture is almost completely unattractive, indicating that the drying of the Salthouse sand has made it repellent (Exp. 49 A, and compare Exp. 48 B with Exps. 43 B, 46 C, 47 B, 51 B).

(23) Fresh Salthouse Lake (St. II) sand is much less attractive than fresh Bullhill Bank sand (Exps. 43 B, 46 C). This relation is not altered by previously washing both sands with tap water (Exp. 41). [N.B. Fresh Salthouse Lake (St. II) sand is silty.]

(24) The slight attractiveness of Salthouse Lake (St. II) sand washed in tap water is not significantly altered by treatment in tap water with alumina

or with kieselguhr (Exps. 41, 46A1 and A2), though there is some evidence that after alumina the attractiveness may be slightly increased (Exp. 47B and compare Exp. 46AA with Exp. 46CC1). Treatment with alumina of the same sand after previous drying had no significant effect (Exp. 48B).

(25) Salthouse Lake (St. II) sand which has not been dried is made strongly attractive by treatment, in tap water, with activated charcoal (Exp. 47B). The same sand made strongly repellent by previous drying (see paragraph 22 above) is also made strongly attractive, and apparently to about the same extent, by activated charcoal treatment (Exp. 48B).

(26) The exposure of Salthouse Lake (St. II) sand to the weather for several weeks lessens its natural slight attractiveness (Exps. 43B, 46C and compare Exp. 46CC2 with Exp. 46CC1). [N.B. Such exposure involves drying, as well as washing by rain.]

(27) The slight attractiveness of fresh Salthouse Lake (St. II) sand is hardly altered by soaking for several days in aquarium water (Exp. 51B). Its attractiveness is, however, reduced, after contact (in aquarium water for several days) with *Ophelia* adults, their excretions and genital products (Exp. 51B).

(28) Coarse gravel, inhabited by *Ophelia bicornis* to the extent of 12–15 per sq.m. collected one year previously from Goulven, near Roscoff, and washed in tap water before testing, is almost completely unattractive (Exp. 42B, and compare paragraph 17 above).

(29) Fine, gritty sand from La Jolla, California, inhabited by the allied *Thoracophelia mucronata*, collected one year previously and washed in tap water before testing, is almost completely unattractive (Exp. 42B and compare paragraph 17 above).

(30) Acid-cleaned, lead-free ballotini > 86-mesh (mainly < 60-mesh) are moderately attractive (Exp. 46B) and are more attractive than Salthouse Lake (St. II) sand which has been dried (Exp. 48B). In a conical vessel, and when completely covering the bottom of a dish, these ballotini induce a good settlement and a good rate of metamorphosis (Exps. 46BB, 51E).

(31) Some sands which are relatively unattractive under free-choice conditions will, in conical or flat-bottomed vessels, where the bottom is completely covered, induce good and sometimes heavy settlements and good metamorphosis rates, although likely to be delayed when compared with a strongly attractive sand under the same conditions (Exps. 44D, 45DD, 46AA, CC1 and CC2, 50, and many experiments in previous years).

Discussion

From the results it appears that sands fall roughly into three classes according to the reactions of the larvae towards them. There are, first, *attractive* sands which induce heavy settlements, with almost immediate metamorphosis, in both free-choice and conical vessel or ordinary dish experiments. Secondly there are sands which under free-choice conditions retain very few larvae and in which metamorphosis does not take place readily. Such sands are fairly attractive in conical vessels, or in dish experiments where the bottom is completely covered by them and which during the day are lit from above. Although all the larvae in the vessels or dishes do not enter them, these sands under such conditions usually induce good settlements and most larvae metamorphose reasonably soon after entering, but they are not as attractive as are sands of the first class. These second-class sands can be regarded as *neutral*, being neither particularly attractive nor particularly repellent to larvae in contact with them. In the third or last class are sands in which few or no larvae will settle and metamorphose under either experimental conditions; such sands can be regarded as being *repellent* to the larvae. As is only to be expected, the classes grade into one another and there are many sands which occupy intermediate positions. The relative positions of several sands are compared in Tables XIII–XV. These are based primarily on the free-choice experiments of 1951, but some results from conical vessel and ordinary dish experiments of earlier years are inserted in italics. This is done by assuming that Bullhill Bank sand sterilized in the normal way is neutral. With it are then compared results with other sands in the same experiments. It is easy to see whether a sand is better or worse and to obtain a fair idea of how far it departs from neutrality.

It will be observed (Table XIII) that the only fully attractive sand is fresh Bullhill Bank surface sand and that its attractiveness is lessened if it be subject to almost any treatment. Brief washings in tap water, followed or not by activated charcoal treatment in tap water for half an hour, and agitating on silk in sea water, have little or no effect on attractiveness, but normal sterilization, drying (after washing) at low heat, and soaking for a prolonged period in distilled water all render the sand neutral. Sand which has merely been stored (naturally moist) for several years occupies a variable intermediate position. Certain treatments, particularly activated charcoal treatment, impart some degree of attractiveness to Bullhill Bank sands which would otherwise be neutral. Some of these are treatments which could be expected to free the sand from adhering organic matter.

Salthouse Lake (Sts. I and II) sands (Table XIV) when fresh are not quite neutral but are almost so. It is practically certain that different samples vary in the slight degree of attractiveness they show when fresh and that St. II sand is always less attractive than St. I. When these sands are dried at a low temperature, or undergo normal sterilization technique, or are variously treated, they become strongly repellent. As exposure to the weather will also make them repellent it is possible that sometimes in nature they can be obnoxious to the larvae. Certain treatments, again particularly activated charcoal and those which can be expected to remove organic matter, will make the sand better from the larval point of view. In this connexion it is interesting to note (Table XV) that the perfectly clean ballotini seem to occupy a position half-way between neutral and fully attractive sands, and they might be even more attractive if the constituents of the glass were completely insoluble so that all poisoning effects were eliminated, and if the lead-free ballotini were not so close to the lower limit of grade which the larvae can penetrate.

Of the three mixtures (see Exps. 44A and 49A) tested under free-choice conditions it is interesting to note (Table XV) that a fifty–fifty mixture of a fully attractive with a partially attractive sand (stored Bullhill Bank sand) is just as effective as a fully attractive sand alone. A mixture of a nearly neutral, slightly attractive sand (fresh Salthouse Lake, St. II) with a fully attractive sand is moderately attractive, while a mixture of a fully attractive sand with a fully repellent sand (dried Salthouse Lake, St. II) is either neutral or somewhat repellent. From the tables it should be possible to forecast with fair accuracy the settlements which will be obtained in mixtures of various sands.

Perhaps the most significant results are those which show that sterilization of a fresh sand, or any treatment which could be expected to kill living organisms, have most effect in rendering a sand less attractive that it was before. Fully attractive sands seem to become neutral, while sands that are nearly neutral when fresh become repellent. On the sand grains there is a flora and fauna of bacteria and other micro-organisms, and it is possible that these when dead are objectionable to the *Ophelia* larvae, but are attractive, or at any rate not repellent, when alive. The sand grains are likely also to carry coatings of organic materials which are changed in chemical and physical character by sterilization and other treatments. Bullhill Bank sands may differ from those of the Salthouse Lake (Sts. I and II) not only in the number and species of micro-organisms present but also in the quantity and quality of the non-living organic matter adhering to the sand grains.

It may be significant that treatments (such as hot concentrated sulphuric acid) which would remove organic matter make repellent sands more favourable and they then attract a settlement similar to that of the clean ballotini. From this it would appear that larvae will settle in some force in perfectly clean sands of suitable grade, but more strongly still if the right kind and quantity of living organisms or organic coatings, or both, are present. In fresh Bullhill Bank sands the right kinds and quantities are present, but the unfavourable Salthouse Lake sands have the wrong kinds or quantities.

The effect on larval settlement of sterilization has been shown not only by my own experiments in 1950 and 1951, but also by some work on the settlement of the polychaete *Pygospio elegans* Clap. carried out by Smidt (1951). He summarizes (p. 62) his own results as follows. 'A natural substratum (sand and mud) stimulates metamorphosis, while the lack of a substratum will impede it. Further, pure sterile sand and naturally occurring pebbles have an impeding effect. As naturally occurring Wadden sand is always mixed with some mud particles and organic particles, it is possibly the presence of these which is of importance for the settling and metamorphosis of the larvae. Larvae kept without any substratum had not yet metamorphosed after two months.' His sterile sand had been strongly heated and treated with acid. The sands were presented in wine glasses with pointed bottoms (equivalent to my conical vessels made from funnels). The percentage settlement obtained in the sterile sand was very small and was probably considerably less than *Ophelia* larvae give in acid-cleaned sands. In this connexion it should be remembered that *Ophelia* when adult lives in much cleaner sands that does *Pygospio*, and this difference may be a reflexion of that habit. Smidt does not carry his observations further and does not comment on the possible role of bacteria or of adhering organic matter.

It is, of course, known that on solid surfaces bacterial and other films are often necessary before any great settlement of larger sedentary species occurs (for a brief summary of the literature see Wilson, 1952); it would not be surprising, therefore, if much the same sort of thing were to prove true of species settling in sands and muds.

Some of my earlier conclusions (Wilson, 1952) concerning adsorbed organic matter on sand grains and its connexion, among other things, with floatation properties of the dried grains, receive support from some recent work which was unknown to me until after my own paper had been written. Turmel (1950), from observations concerning variation in the rate of percolation of water in dune sands of different kinds, and from observations on floatability of the sands when sprinkled dry on to water, came to a similar conclusion. He maintains that the grains of floatable (non-wettable) dune sands are covered with an extremely thin film of organic matter. In experiments similar to my own (Wilson, 1952) he showed that after treatment with the fat solvents alcohol, ether, carbon disulphide and tetrabenthene, grains of floatable sands did not become more readily wetted, but after treatment with hydrogen peroxide they became completely wettable. He considered that his experiments demonstrated that non-wettability was due to a thin coating of organic matter. The nature of this organic film, however, had still to be determined.

Summary

Further tests on the settlement reactions of *Ophelia bicornis* larvae have shown that both Bullhill Bank and Salthouse Lake sands are less favourable to the larvae after sterilization than they are when fresh and untreated. The effect of a large variety of treatments of both sands has been investigated under conditions where the larvae were free to choose between two or more samples of sands presented together in the same dish. Results from these experiments,

taken in conjunction with results obtained using conical vessels, show that sands may be classed as *attractive*, *neutral* or *repellent* with intermediate grading between these three main categories. It is concluded that organic material, living or dead, on the sand grains plays an important role in rendering a sand attractive or repellent to the larvae.

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TABLE I. BULLHILL BANK SAND COLLECTED 19 JUNE 1951

(From the middle of the bank; *Ophelia* present in abundance.)
Percentage mesh sizes by weight, after washing away silt.

Depth	(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
Surface	0·0	1·4	29·6	61·0	7·3	0·7	Slight	3·24	30–40
6 in. down	0·1	0·3	13·9	66·7	16·3	2·8	A little	2·93	*ca* 1
12 in. down	1·1	1·8	23·3	53·5	18·0	2·4	A little	3·07	*ca* 1
18 in. down (this layer wet)	0·2	0·6	20·3	65·1	11·3	2·3	A little	3·06	*ca* 1

TABLE II. EXPERIMENT 41

(Begun 25. vi. 51 with larvae from a fertilization of 20. vi. 51.)
Result on 27. vi. 51.

	Bullhill Bank sand (19. vi. 51) washed in tap water (1)	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water (2)	Sand as (2) mixed with alumina since 21. iii. 51 (3)	Sand as (2) mixed with kieselguhr since 21. iii. 51 (4)
Metd	24	0	0	0
Meting	17	1	0	1
Unmet.	5	13	34	18
Total	46	14	34	19

Metd = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed; *d* = dead.

TABLE III. EXPERIMENT 42

(Begun 26. vi. 51 with larvae from a fertilization of 21. vi. 51.)

Result on 28. vi. 51.

Dish A. Bullhill Bank sands collected 19. vi. 51, *unwashed*

	Sand from surface	Sand from 6 in. below surface	Sand from 12 in. below surface	Sand from 18 in. below surface
Metd	232	60	9	34
Meting		5	2	10
Unmet.	15	11	12	8
Total	247	76	23	52

Dish B

	Bullhill Bank sand (19. vi. 51) washed in tap water	Sand from La Jolla, California, washed in tap water	Sand from Goulven, near Roscoff, washed in tap water
Metd	108	0	0
Meting	18	3	0
Unmet.	10	8	14
Total	136	11	14

TABLE IV. EXPERIMENT 43

(Begun 11. vii. 51 with larvae from a fertilization of 6. vii. 51.)

Result on 13. vii. 51.

Dish A

	Bullhill sand (19. vi. 51) > 60-mesh in sea water for several days	Bullhill sand > 60 mesh mixed in sea water with < 86-mesh S.L. (St. II) for several days	Bullhill sand > 60-mesh in distilled water for several days	Bullhill sand > 60-mesh mixed in distilled water with < 86-mesh S.L. (St. II) for several days
Metd	56	33	0	0
Meting	20	10	0	0
Unmet.	7	4	0	1
Total	83	47	0	1

Dish B

	Bullhill sand (19. vi. 51) rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) sand rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) exposed to weather for 3 weeks, rinsed in sea water	Salthouse Lake (St. I—19. vi. 51) exposed to weather for 3 weeks, rinsed in sea water
Metd	43	3	1	0
Meting	13	2	1	0
Unmet.	7	11	2	5
Total	63	16	4	5

Table V. Experiment 44

(Begun 15. vii. 51 with larvae from a fertilization of 10. vii. 51.)

Result on 17. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Mixture of about equal volumes of Bullhill (19. vi. 51) and Salthouse Lake (St. II—19. vi. 51) sands rinsed in sea water
Metd	19	8
Meting	4	5
Unmet.	3	0
Total	26	13

Dish B

	Bullhill Bank sand (19. vi. 51) washed and dried. (Floatability 70–80 %)	Bullhill Bank sand (19. vi. 51) washed and dried. Shaken on silk for 7 min. (Floatability 95 %.)
Metd	0	0
Meting	0	0
Unmet.	5	1
Total	5	1

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) exposed to weather for 3½ weeks, rinsed in sea water
Metd	4	0
Meting	0	0
Unmet.	11	4
Total	15	4

Dish D

	Bullhill Bank sand (19. vi. 51) soaked in sea water for 2 days	Bullhill Bank sand (19. vi. 51) soaked in distilled water for 2 days	Bullhill Bank sand (19. vi. 51), normal sterilization technique
Metd Meting	} 164	0 0	0 0
Unmet.	1	1	0
Total	165	1	0

As above, but in small glass-stoppered tube

Metd	17	} ++
Meting	34	
Unmet.	12	

Table VI. Experiment 45

(Begun 18. vii. 51 with larvae from a fertilization of 10. vii. 51.)

Result on 21. vi. 51.

Dish B

	Bullhill Bank sand (19. vi. 51) washed and dried. (Floatability 70–80 %)	Bullhill Bank sand (19. vi. 51) washed and dried. Shaken on silk for 8 min. (Floatability 100 %.)
Metd	11	3
Meting	3	2
Unmet.	4	4
Total	18	9

	Dish D		*Conical vessel DD*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51), normal sterilization technique	Bullhill Bank sand (19. vi. 51), normal sterilization technique
Metd	3	0	33 } +
Meting	5	4	7
Unmet.	0	6	3
Total	8	10	*c.* 90

TABLE VII. EXPERIMENT 46

(Begun 17. vii. 51 with larvae from a fertilization of 12. vii. 51.)

Result on 19. vii. 51.

	Dish A 1		*Dish A 2*	*Conical vessel AA*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) treated with alumina	Salthouse Lake (St. II—19. vi. 51) treated with alumina	Salthouse Lake (St. II—19. vi. 51) treated with alumina
Metd	14	3	1	15 }
Meting	13	1	1	8 } +++
Unmet.	4	1	1	1 }
Total	31	5	3	*c.* 150–200

	Dish B.		*Conical vessel BB.*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Lead-free ballotini >86-mesh, acid cleaned	Lead-free ballotini >86-mesh, acid cleaned
Metd	14 }	15	8 }
Meting	43 } ++	15	12 } +++
Unmet.	2 }	5	9 }
Total	*c.* 150–200	35	*c.* 150–200

	Dish C				*Conical vessel CC 1*	*Conical vessel CC 2*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Salthouse Lake (St. II—19. vi. 51) stored in sea water overnight	Salthouse Lake (St. II—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight	Salthouse Lake (St. I—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight	Salthouse Lake (St. II—19. vi. 51) stored in sea water overnight	Salthouse Lake (St. II—19. vi. 51) exposed to weather for 4 weeks, stored in sea water overnight
Metd	7 }	6	0	0	14 }	1 }
Meting	35 } +	3	0	1	5 } ++	3 } +.
Unmet.	4 }	1	2	0	5 }	16 }
Total	*c.* 100	10	2	1	*c.* 75	*c.* 40

Table VIII. Experiment 47

(Begun 23. vii. 51 with larvae from a fertilization of 18. vii. 51.)

Result on 25. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water, treated with activated charcoal (in tap water for half an hour) washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Rinsed in sea water
Metd	37	35	1
Meting	4 } ++	7 } ++	0
Umet.	1	9	7
Total	*c.* 150–200	*c.* 150–200	8

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) treated with alumina in tap water, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) treated with activated charcoal in tap water, washed in tap water, then in sea water
Metd	30	38	16
Meting	6	9	34 } ++
Unmet.	16	17	*c.* 60
Total	52	64	*c.* 300–350

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1947) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1949) rinsed in sea water
Metd	35	21	0	2
Meting	11 } +	6	3	1
Unmet.	7	11	8	6
Total	*c.* 100–150	38	11	[illegible]

TABLE IX. EXPERIMENT 48

(Begun 26. vii. 51 with larvae from a fertilization of 21. vii. 51.)

Result on 28. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Treated with activated charcoal in tap water, washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water and dried. Rinsed in sea water
Metd	57	6	0
Meting	12	12	0
Unmet.	5	14	4
Total	74	32	4

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Treated with alumina in tap water, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water and dried. Treated with activated charcoal, washed in tap water, then in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed in tap water, treated with activated charcoal, washed in tap water, then in sea water	Lead-free Ballotini >86 mesh, acid-cleaned, washed in tap water, then in sea water
Metd	0	0	10	8	0
Meting	0	2	20	32	10
Unmet.	2	3	29	17	15 *d* 12
Total	2	5	59	57	37

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in distilled water, treated with hot conc. H_2SO_4, washed in distilled water, then in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) washed in distilled water, treated with hot conc. H_2SO_4, washed in distilled water, then in sea water
Metd	51	5	2	3
Meting	8	2	2	4
Unmet.	3	10	4	18
Total	62	17	8	25

TABLE X. EXPERIMENT 49

(Begun 28. vii. 51 with larvae from a fertilization of 23. vii. 51.)

Result on 30. vii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sands of 19. vi. 51 and 1948 mixed in equal proportions, rinsed in sea water	Bullhill Bank (19. vi. 51) and Salthouse Lake (St. II—19. vi. 51) sands mixed in equal proportions, rinsed in sea water	Bullhill Bank sand (19. vi. 51) rinsed in sea water mixed with equal volume of Salthouse Lake sand (St. II—19. vi. 51), washed and dried
Metd	23	8	15	8	0
Meting	14	8	22	14	1
Unmet.	7	28	30	25	8
Total	44	44	67	47	9

	Dish B			*Conical vessel BB1*	*Conical vessel BB2*
	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) treated in tap water with activated charcoal, washed in tap water, then in sea water	Bullhill Bank sand (1948) rinsed in sea water	Bullhill Bank sand (1948) treated in tap water with activated charcoal, washed in tap w ter, then in sea water
Metd	25	2	3	9	20 } +++
Meting	24	1	9	17	18 } +++
Unmet.	4	7	18	28	21 } +++
Total	53	10	30	54	*c.* 250–350

TABLE XI. EXPERIMENT 50

(Begun 28. vii. 51 with larvae from a fertilization of 23. vii. 51.)

Result on 30. vii. 51.

	Dish I	Dish II
	Bottom of dish *completely covered* with Bullhill Bank sand (19. vi. 51), rinsed in sea water	Bottom of dish *completely covered* with Bullhill Bank sand (1948) rinsed in sea water
On surface film	None	Considerable number unmet.
In mid-water	Two or three swimming	Large number swimming
In sand (small portion)	Metd 24 Meting 6 Unmet. 1	Metd 1 Meting 4 Unmet. 8

Result on 1. viii. 51.

	Dish I	Dish II
On surface film	A very few unmet. or meting; 1 metd	A fair number unmet., a very few meting or metd
In mid-water	None	A few unmet.
In sand (small portion)	Metd 28 Meting 0 Unmet. 1 (abnormal)	Metd 20 Meting 10 Unmet. 2

Table XII. Experiment 51

(Begun 31. vii. 51 with larvae from a fertilization of 26. vii. 51.)

Result on 2. viii. 51.

Dish A

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) shaken on silk in sea water	Bullhill Bank sand (19. vi. 51) > 60-mesh sieved in sea water	Bullhill Bank sand (19. vi. 51) < 86-mesh sieved in sea water	Bullhill Bank sand (19. vi. 51) < 100-mesh sieved in sea water
Metd	0	0	0	0	0
Meting	8	13	2	0	0
Unmet.	17	17	12; *d* 1, poor cond. 5	9	5
Total	25	30	20	9	5

Dish B

	Salthouse Lake sand (St. II—19. vi. 51) rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed and soaked in aquarium water for 5 days. Well rinsed in sea water	Salthouse Lake sand (St. II—19. vi. 51) washed with aquarium water and then kept in contact with mature *Ophelia* for 5 days. Well rinsed in sea water
Metd	7	10	0
Meting	17	9	3
Unmet.	10	6	4
Total	34	25	7

Dish C

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (18. vii. 50) rinsed in sea water
Metd	3	0
Meting	5	0
Unmet.	14	27
Total	22	27

Dish D

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51) washed in tap water, then in sea water	Bullhill Bank sand (19. vi. 51) soaked in sea water and absolute alcohol, then washed in sea water	Bullhill Bank sand (19. vi. 51) soaked in sea water neutral formol, then washed in sea water	Bullhill Bank sand (1947) washed, dried and subject to heating (900–1000° C.) in 1948
Metd	8	2	0	0	2
Meting	19	20	0	1	6
Unmet.	2	8	8	7	28, *d*. 9
Total	29	30	8	8	45

Dish E, strewn with lead-free ballotini > 86-mesh

On surface film	Many, mainly unmet.: a few early meting
In mid-water	Number swimming freely
In a portion of the ballotini	Metd 9, Meting 21, Unmet. 18 } ++

Table XIII. Bullhill Bank Sands

Attractive		Neutral		Repellent
Surface sand, fresh, untreated	Deeper sand, fresh, untreated		Washed in tap water, sterilized at about 100° C.	
	Fresh, washed for a few minutes in tap water		Soaked for several days in distilled water	
		After prolonged storage		
			Washed in tap water, dried at low heat	
Fresh, treated in tap water with activated charcoal	Washed in tap water, dried, treated with activated charcoal			
		Treated with hot conc. H_2SO_4		
Fresh, agitated on silk in sea water			Fresh sand treated with formalin or absolute alcohol	
		Heated 900–1000° C.		

Table XIV. Salthouse Lake Sands

Attractive			Neutral		Repellent
			Surface sand from St. II, fresh and untreated		St. II sand washed in tap water, dried at low heat
		Surface sand from St. I, fresh and untreated			*St. II sand washed in tap water, sterilized at about 100° C.*
		St. I sand, recombined, heated 900–1000° C.			
	Fresh St. II sand treated with activated charcoal in tap water				*St. I sand, dried and recombined. Extracted with alcohol, acetone or ether*
	St. II, sand washed in tap water, dried, treated with activated charcoal				
		St. I sand, washed and dried, treated with hot conc. H_2SO_4		*St. I sand, washed and dried, treated with* H_2O_2	

Table XV. Mixtures and Various

Attractive	Neutral		Repellent
Fresh Bullhill Bank sand + Bullhill Bank sand stored several years	Fresh Bullhill Bank sand + fresh Salthouse Lake (St. II) sand	Fresh Bullhill Bank sand + Salthouse Lake (St. II) sand washed and dried	
	Lead-free ballotini, mainly 60–86 mesh sizes	*Oolitic sand from the Great Salt Lake*	