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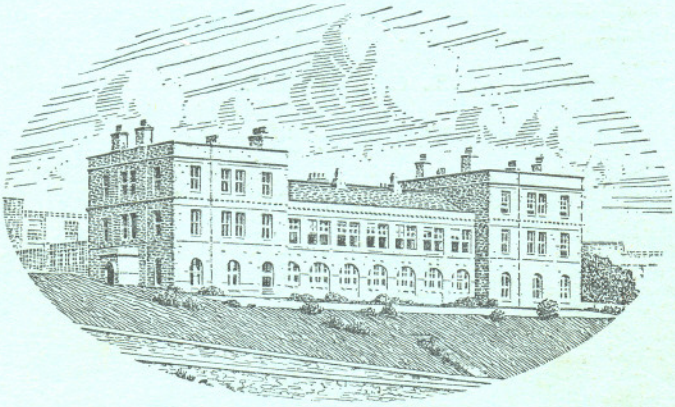

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THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY LARVAE

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. 47A, 48A) 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. 44D) 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. 51B).

Experiment 48

Dish A. In Exp. 47A 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. 47B 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. 47B. 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 BB1 and BB2. 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 BB1 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. 44D); 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. 47A).

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

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

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

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

(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. 49A). 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. 49B) or hot concentrated sulphuric acid (Exp. 48C). 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. 51D).

(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. 43A).

(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. 44A, 49A, 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. 49A, and compare Exp. 48B with Exps. 43B, 46C, 47B, 51B).

(23) Fresh Salthouse Lake (St. II) sand is much less attractive than fresh Bullhill Bank sand (Exps. 43B, 46C). 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 metamor-

phosis, 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	} 164	0	0
Meting		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

	Bullhill Bank sand (19. vi. 51) rinsed in sea water	Bullhill Bank sand (19. vi. 51), normal sterilization technique
Metd	3	0
Meting	5	4
Unmet.	0	6
Total	8	10

Conical vessel DD

Bullhill Bank sand (19. vi. 51), normal sterilization technique		
Metd	33	} +
Meting	7	
Unmet.	3	
	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	

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.

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*	

NOTES ON THE BRITISH SPECIES OF *TRIDIDEMNUM* (DIDEMNIDAE, ASCIDIACEA), WITH A REPORT OF THE OCCURRENCE OF *T. NIVEUM* (GIARD) IN THE PLYMOUTH AREA

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(Text-fig. 1)

Berrill (1950) records two species of *Tridemnum* Della Valle (1881) as occurring in British waters—*T. tenerum* (Verrill, 1871) and *T. alleni* Berrill (1947). A third species, *T. niveum* (Giard, 1872), was reported from Millport by Rankin (1900), but it is impossible to determine whether his species was *T. niveum* or some other species of *Tridemnum* or *Didemnum*. Rankin's identifications are suspect, for he describes as common at Millport species of Polyclinidae and Didemnidae which have not been seen in British waters (or indeed, anywhere) before or since. *Tridemnum niveum* has not been reported since from Millport. The synonymy and distinctness of these three species are confused. Hartmeyer (1924) stated that *T. niveum* is a synonym of *T. tenerum*. This was denied by Harant & Vernières (1933), but without any reasons of weight. Berrill (1947, 1950) claims that *T. alleni* is certainly distinct from *T. tenerum*, but he allows the possibility that it may be synonymous with *T. niveum*, which apparently he had never seen.

In the Plymouth area I have frequently collected *T. tenerum* and *T. alleni*, and I have in my possession colonies of *T. tenerum* from Naples and from Scotland. Recently I collected a colony of a *Tridemnum* species from the Salstone (Salcombe, Devon); it was growing on the test of a specimen of *Polycarpa rustica* (Linnaeus), a few centimetres below the low-water mark of spring tides. I ascribed this colony to *Tridemnum niveum*, and through the kindness of Dr C. Levi I was able to obtain specimens of this species from the type locality at Roscoff, including a portion of a colony from the type collection of that laboratory, determined by Pizon. I was thus able to check that my identification was correct. From the examination of numerous colonies of these three species of *Tridemnum* I have no hesitation in stating that they are all three distinct species.

T. tenerum is found in waters from low tide down to about 200 m. It is commonly found encrusting rocks and stones and on the holdfasts of algae. It is particularly abundant where the substratum is hard mud. *T. alleni* is recorded from a little below low tide down to about 30 m., encrusting gor-

gonians, hydroids and algal holdfasts. It seems to require situations swept by strong currents or tides. *T. niveum* extends from a little below low-tide mark to about 30 m., and is especially abundant on the sargassids which inhabit the *Laminaria* zone. My specimens from Roscoff were all found growing on *Cystoseira*. Giard (1872) states that he never found it on stones or rocks. Like many other ascidians, and especially didemnids, it seems to require an organic surface for settling.

Tridemnum tenerum forms large colonies of several centimetres in extent and 3 or 4 mm. thick. The colour varies through greenish, ochre, brown or grey, and the colonies are very rarely white. The zooids are usually visible through the test; they are arranged in systems which may be obscured by the very abundance of the zooids. *T. alleni* forms much smaller colonies of the same thickness but rarely over 1 cm. in extent. The colonies are usually brilliant white because of the abundance of the spicules, and for the same reason the zooids are not visible. *T. niveum*, growing on *Cystoseira*, is limited in its extent in one direction, but in the other the colonies may extend to 60 or 70 mm.; the thickness is about 5 mm. The test is usually colourless, but the blue pigment of the abdomina combining with the white of the spicules produces the bluish white effect which led Giard to give it the specific name 'niveum' or 'snowy'. In some colonies, however, the surface of the test is streaked with brown, and one group of colonies from Roscoff was in life a dark yellow-ochre. The zooids in all my colonies are visible through the test.

The spicules of *Tridemnum tenerum* are rather irregular in form. Commonly, elongate or needle-like crystals, or plates, are scattered in the test, but these may be aggregated into irregular, or rarely regular, spherical groupings of 20–40 μ diameter; the points of these spherical spicules are usually needle-like and numerous. The spicules in most colonies are few in number and hardly obscure the transparency of the test. In *T. alleni* the spicules are very closely packed, rendering the test completely opaque. They are spherical, of 8–25 μ in diameter and geometrical in form; looking into the sphere, in successive circles of points there are generally 1, 6 and 9 points, but rarely the formula may be 1, 4, 8 or, in the smallest, 4, 8. The rays are usually obtusely pointed but may be rounded or rod-like, even in the same colony. In *T. niveum* the spicules are intermediate in abundance between those of the other two species. They are spherical and geometrical in form with formulae of 1, 6, 12; 1, 4, 8; or 1, 4. I have not found any with the formula 4, 8. They are 20–40 μ in diameter with a few slightly smaller. The points are acute. In all three species they are most abundant in, or confined to, the upper layers of the test.

The thorax of the zooids of *T. tenerum* is at least as large as, or generally larger than, the abdomen and generally separated from it by a very narrow constriction, which is, however, short. The thorax of *T. alleni* is always

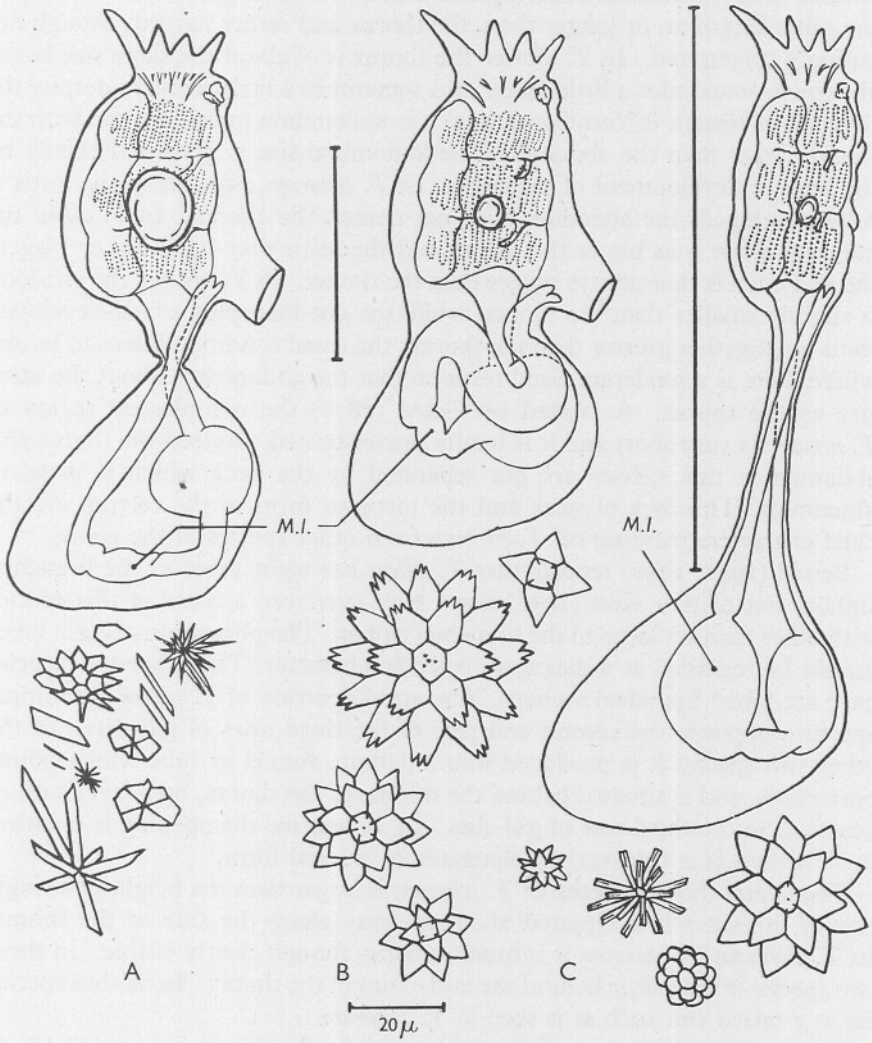

Fig. 1. Drawings from the left side of zooids of A, *T. tenerum*, B, *T. niveum*, and C, *T. alleni*, together with a group of spicules from each of the three species. Each group of spicules is from a single colony. The bottom scale line refers to the degree of magnification of the spicules. The zooids of *T. tenerum* and *T. niveum* are drawn to the same scale, but that of *T. alleni* is enlarged more, so that it may be drawn with the same thorax size; in each case the scale line represents 0·5 mm. The entire genital system and the pyloric gland are omitted, so that the course of the gut—important for identification—may be followed. The buccal lobes and tentacles of the opposite side are omitted, so that one more than half the total number of each are drawn. *M.I.* mid-intestine.

smaller than the abdomen and separated from it by a long neck which is about the same length as, or longer than, the thorax and rather narrow, though not strongly constricted. In *T. niveum* the thorax is of about the same size as the abdomen, sometimes a little larger and sometimes a little smaller; despite the drawing of Harant & Vernières (1933) it is uncommon to find a zooid with the thorax larger than the abdomen. The abdominal size is governed chiefly by the state of development of the testis. In *T. tenerum*, even when the testis is fully developed, the abdomen does not exceed the thorax. In *T. alleni* the gut-loop alone is as big as the thorax and the testis may be as big or bigger; the abdomen is thus always bigger than the thorax. In *T. niveum* the gut-loop is slightly smaller than the thorax, while the gut-loop plus a well-developed testis are together greater than the thorax; the usual condition seems to be one where there is a moderate-sized testis so that the abdomen is about the same size as the thorax. As stated by Giard (1872) the oesophageal region of *T. niveum* is very short and it is totally unconstricted. Indeed the thorax and abdomen in this species are not separated by the neck which is usual in didemnids. This lack of neck and the tortuous form of the rectum are the chief characters marking off *T. niveum* from other species of the genus.

Berrill (1947, 1950) reports that *T. alleni* has eight lobes to the branchial siphon, but neither Kott (1952) nor I have ever seen a zooid of this species with other than six lobes to the branchial siphon. The possession of eight lobes cannot be regarded as a diagnostic specific character. The other two species have six-lobed branchial siphons. The atrial aperture of *T. alleni* is a simple aperture opposite the second and part of the third rows of gill-slits. In the other two species it is produced into a definite funnel or tube which points posteriorly, and is situated behind the middle of the thorax, on a level with or posterior to the third row of gill-slits. In *T. tenerum* the opening is circular; in *T. niveum* it is transversely elongated to an oval form.

The lateral thoracic organ of *T. tenerum* is larger than the height of a single row of gill-slits; it is situated about midway along the side of the thorax. In *T. alleni* and *T. niveum* it is much smaller, though clearly visible. In these two species it lies a little behind the mid-point of the thorax. In neither species has it a raised rim such as is seen in *T. tenerum*.

A thoracic muscular retractor process may be developed in some zooids or colonies of all three species. All three commonly possess eight buccal tentacles, but large zooids of *T. tenerum* may have eight extra smaller tentacles making sixteen in all. *T. tenerum* and *T. niveum* possess nine to twelve stigmata in each half-row, with ten or eleven as the commonest number. *T. alleni* usually has seven or eight in each half-row.

The oesophagus of *T. tenerum* is more than half the length of the thorax, but less than its full length. That of *T. alleni* is longer than the thorax; that of *T. niveum* is less than half the length of the thorax. In all three it is straight and opens into a smooth globular stomach. The post-stomach of *T. tenerum*

runs posteriorly and then turns ventrally. It is separated from the horizontal mid-intestine by an oblique constriction. The mid-intestine in its turn is separated from the ascending rectum by another constriction. The rectum is slightly curved. In *T. alleni* the post-stomach is horizontal and opens after a slight constriction into the ascending, globular mid-intestine which is as large as or larger than the stomach. This passes without much break into the rectum which runs practically straight up to the atrium. In *T. niveum* the post-stomach is horizontal and is separated by an abrupt constriction from the very small mid-intestine which runs almost without constriction into the rectum. This is curved into a tight S, quite unlike the condition usual in didemnids.

Berrill (1947, 1950, p. 118) states that: 'Four or five obvious epidermal ampullae with fairly long stalks and large terminals arise from the abdominal region' in *T. tenerum*, whereas in *T. alleni* they are hardly discernible. In *T. niveum*, Lahille (1890) saw five epidermal ampullae with voluminous terminals. Giard (1872, pl. 22, fig. 1) illustrates *T. niveum* without epidermal ampullae in contradistinction to '*Didemnum cereum*' (= *T. tenerum*) which he illustrates on the same plate (his fig. 3) with large ampullae. Berrill lays great stress on this character, but I believe Van Name (1945, p. 99) is nearer the truth when he writes of *T. tenerum* that, 'at certain stages of growth clavate vascular processes extending into the test from the middle region of the body are found'. These are evidently the epidermal ampullae of Berrill. My own observations indicate a great variability in all three species in the degree of development of these ampullae. While they are never very large in *T. alleni*, they are sometimes clearly visible, and the other two species show all stages between highly developed ampullae and ampullae so small that they are only visible in sectioned material.

The number of turns the vas deferens makes around the testis has some value as a specific character in the Didemninae, although the number is never so rigidly fixed as some authors seem to imply. In *T. tenerum* the number is usually given as twelve, and this is the commonest, but zooids may be found, even in the one colony, with eleven or thirteen turns of the spire. In *T. alleni* there is similar variation between seven and nine with eight turns of spire being most common. In *T. niveum* there are equal numbers of zooids with seven and with eight turns while a very few have six or nine turns.

The larvae of the three species differ. The trunk of the larvae of *T. tenerum* and *T. niveum* is about 0·45–0·5 mm. long; that of *T. alleni* is slightly smaller, being about 0·35 mm. long. The tail of the larva of *T. niveum* is longer than that of *T. tenerum*, for it curves round the trunk even past the sensory vesicle, while that of *T. tenerum* rarely passes the branchial siphon. The tail of *T. alleni* is of the same relative length as that of *T. tenerum*. The larva of *T. alleni* has only two suckers, whereas those of the other two species possess three suckers each.

The three species may be diagnosed as follows:

T. tenerum. Colonies up to 60 mm. across, usually with few spicules. Zooids always visible through test. Spicules chiefly irregular, 20–40 μ diameter. Abdomen never larger than thorax, and separated from it by a short but pronounced constriction. Atrial siphon prolonged to a tube, behind middle of thorax. Lateral thoracic organ near middle of thorax and larger than height of one row of stigmata. About ten or eleven stigmata per half-row. Oesophagus shorter than thorax. Post-stomach behind stomach; mid-intestine horizontal; rectum little curved. Vas deferens with about twelve turns around testis.

T. alleni. Colonies up to 10 mm. across, with abundant spicules. Zooids never visible through test. Spicules geometrical, spherical, about 8–25 μ diameter, with points arranged with formulae: 1, 6, 9; 1, 4, 8; or 4, 8. Abdomen always larger than thorax and separated from it by a narrow neck longer than the thorax. Atrial aperture a simple opening opposite the middle row of gill-slits. Lateral thoracic organ behind middle of thorax and smaller than the height of a single row of stigmata. About seven or eight stigmata per half-row. Oesophagus longer than thorax. Post-stomach ventral to stomach; mid-intestine large and globular, vertical; rectum little curved. Vas deferens with about eight turns around testis. Larva with only two suckers.

T. niveum. Colonies up to 60 mm. long, with sparse spicules. Zooids usually visible through test. Spicules geometrical, spherical, about 20–40 μ diameter, with formulae: 1, 6, 12; 1, 4, 8; or 1, 4. Abdomen larger or smaller than the thorax, and not separated from it by even the slightest constriction or neck. Atrial siphon prolonged into a tube behind middle of thorax. Lateral thoracic organ behind middle of thorax and smaller than the height of a single row of stigmata. About ten stigmata per half-row. Oesophagus shorter than half the length of the thorax. Post-stomach ventral to stomach; rectum curved into a sharp S, with final curve overlying stomach. Vas deferens with about seven or eight turns around testis.

Summary

The presence of *Tridemnum niveum* (Giard) is reported from the Plymouth area. It is compared with the other two British species of *Tridemnum*, *T. tenerum* and *T. alleni*, with which it has in the past been somewhat confused, and all three are shown to be distinct species. A short diagnosis of the three species is given as an aid to identification.

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THE RESPIRATION OF SOME PLANKTONIC COPEPODS

II. THE EFFECT OF TEMPERATURE

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(Text-figs. 1, 2)

The importance of the respiratory rate of copepods in considering the food requirements of zooplankton has already been stressed (Raymont & Gauld, 1951), and some of the factors affecting respiratory rates have been indicated. Temperature has long been recognized as one of the most important factors affecting respiration, but apparently the determinations so far made on copepods have been confined to one species, *Calanus finmarchicus* (cf. Marshall, Nicholls & Orr, 1935; Clarke & Bonnet, 1939). Since, especially in neritic areas, other smaller copepods often predominate, it was felt that an investigation into the effects of temperature on some other species of copepod was necessary.

Materials and Methods

Work was confined to three species of adult planktonic copepod, *Acartia clausi* Giesbrecht,[1] *Centropages hamatus* (Lilljeborg) and *Temora longicornis* (Müller). The copepods were obtained from tow-nettings taken in Southampton Water, between Hamble Spit Buoy and Baldhead Buoy, an area within 1–2 hr. run from the laboratory. The catch was filtered through coarse bolting silk to remove ctenophores and large medusae, well diluted and kept in breffits stored in subdued light in the cold room of the laboratory at a temperature of 5–10° C. Copepods were kept for at least 24 hr., sometimes for several days, in the cold room before use, in an attempt to avoid shock, and were fed with a culture of *Chlamydomonas* unless abundant food was present in the water. Selection of the copepods for the experiments was done as far as possible by eye, although a low-power microscope was used to a limited extent, and they were transferred from the stock breffits into crystallizing dishes of filtered Plymouth 'outside' sea water, before being put into the respirometers.

[1] In a few experiments some *A. discaudata* were included.

Respiration measurements were made in Barcroft-Dixon constant pressure manometers as before (see Raymont & Gauld, 1951). Five ml. of sea water were used in each flask, which normally contained 45–55 copepods, and the flasks were shaken at a speed of 100 oscillations per minute, with an amplitude of 2·5 cm. Measurements were made either in the cold room or in the ordinary laboratory, depending on the temperature required, and in either case the flasks were immersed in a water-bath regulated to $\pm 0{\cdot}2^{\circ}$ C. of the selected temperature. Most of the experiments were conducted at temperatures ranging from 10 to 20° C., but some were carried out with *Centropages hamatus* at 6° C.

Each experiment ran for 3–4 hr. after an equilibration period of 15 min., and several readings were always made during the course of a run. Half-hourly readings were taken in the experiments at 20° C., but at the lower temperatures the manometers were read only hourly. The total change was then always converted to volume of dry gas at N.T.P.

After each experiment the copepods were fixed in formalin and the identifications checked under the binocular microscope. With a few exceptions the length of the cephalothorax of the fixed animals was also measured with a micrometer eyepiece (cf. Raymont & Gauld, 1951). Males and females were not separated in the experiments, but the number of each sex was always determined after fixation of the animals. Occasionally a few Stage V were accidentally included and sometimes one or two copepods of another species. The proportion of copepods other than adults of the selected species was always small, however. The lengths of all copepods were included in calculating the mean length for a particular run.

In two or three out of more than a hundred experiments, a nauplius or small zoea was inadvertently transferred to the experimental flask. Such animals were neglected in calculating the respiratory rates.

A very few runs showed no change in gas volume or very irregular changes and it is almost certain that leaks had occurred in the manometers. Such few runs have been disregarded.

Results

Some 127 experiments were carried out altogether, and the respiratory rates, quoted as μl. oxygen consumed per copepod per hour, of *Acartia*, *Centropages* and *Temora* are given in Tables I, II and III respectively. In each table the experiments done at each temperature, viz. 20, 17, 13, 10° C., and for *Centropages* 6° C., are grouped together and the mean rate for each group is given. The mean length (cephalothorax) of the copepods used in each experiment is also indicated. Previous work (Raymont & Gauld, 1951) showed that the respiratory rate of several species of copepods was approximately proportional to the length squared, and since the mean length of the copepods in the present investigations varied considerably from one experiment to another, a corrected

value for the respiratory rate was calculated in the following way. The overall mean length of all animals used in all experiments was first determined for the three species. These are: *Centropages*, 0·882 mm., *Acartia* 0·8585 mm., and *Temora* 0·787 mm. The respiration rate for each experiment was then multiplied by the square of this overall mean length and divided by the square of the mean length for the particular experiment.

TABLE I. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *ACARTIA CLAUSI*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
19·7	1	50	0·91	0·0935	0·083
19·7	2	50	0·92	0·122	0·107
19·7	3	51 − 1	0·92	0·121	0·106
19·7	4	50 − 2	0·92	0·0935	0·082
19·7	5	49	0·91	0·156	0·139
20·1	7	50	0·91	0·073	0·065
20·1	8	42 − 1	0·93	0·107	0·092
20·1	9	45	0·92	0·098	0·085
20·1	10	46 − 2	0·92	0·104	0·091
20·1	13	52 − 1	0·91	0·106	0·095
				Mean *0·107*	*0·0945*
17° C.:					
17·0	51	52	0·82	0·087	0·096
17·0	116	49 − 2	0·83	0·073	0·078
17·0	117	44 − 1	0·82	0·088	0·096
17·1	122	46	0·84	0·052	0·054
17·1	123	49 − 2	0·84	0·065	0·069
				Mean *0·073*	*0·079*
13° C.:					
13·1	36	48	0·85	0·075	0·077
13·1	37	53	0·87	0·072	0·071
13·1	39	49	0·82	0·0645	0·070
13·1	41	49	0·84	0·051	0·053
13·2	43	52	0·85	0·051	0·052
13·2	44	51	0·84	0·066	0·068
13·2	47	49 − 1	0·85	0·049	0·050
				Mean *0·061*	*0·062*
10° C.:					
10·7	16	41	0·85	0·062	0·064
10·7	17	46	0·79	0·069	0·082
10·4	21	41	0·85	0·132	—
10·4	22	45 − 2	0·79	0·087	0·104
10·4	23	41	0·83	0·086	0·093
10·4	24	50	0·81	0·045	0·051
10·4	25	51	0·82	0·0625	0·069
10·7	30	50	0·83	0·038	0·040
10·7	31	36 − 1	0·83	0·0535	0·057
10·7	32	50	0·83	0·043	0·047
10·4	98	53 − 1	0·90	0·042	0·039
				Mean *0·065*	*0·065*

Note. In this and the following tables any copepods dying during an experimental run are noted by a figure subtracted from the total number used.

TABLE II. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *CENTROPAGES HAMATUS*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
19·7	6	32	0·90	0·239	0·230
20·1	11	30−1	0·99	0·079	0·062
				Mean *0·159*?	*0·146*?
17° C.:					
17·0	48	58−3	0·92	0·076	0·071
17·0	50	53	1·06	0·126	0·087
17·4	57	50	—	0·0815	—
17·4	58	49−2	—	0·105	—
17·4	59	50	1·04	0·058	0·041
16·7	65	52−2	—	0·099	—
17·0	68	49	0·92	0·064	0·059
17·0	69	50	0·93	0·067	0·060
17·0	70	49−1	0·94	0·0595	0·053
17·0	118	51−1	0·83	0·108	0·122
17·0	119	46	0·83	0·104	0·117
17·0	120	53	0·84	0·085	0·092
17·0	121	47	0·83	0·109	0·121
				Mean *0·088*	*0·082*
13° C.:					
13·1	35	52	0·98	0·073	0·058
13·2	45	49	1·04	0·073	0·052
13·0	99	53	0·84	0·079	0·087
13·0	100	54	0·85	0·064	0·068
13·0	101	49	0·85	0·125	0·133
13·0	102	54−1	0·86	0·092	0·097
13·0	105	50	0·89	0·064	0·063
13·0	106	50 (?)	—	0·054	—
13·0	107	50	0·84	0·0785	0·085
13·0	108	48−2	0·86	0·115	0·119
13·0	109	51	0·86	0·106	0·111
				Mean *0·084*	*0·087*
10° C.:					
10·3	88	46	0·87	0·064	0·065
10·3	89	45−1	0·86	0·066	0·069
10·3	90	42−1	0·87	0·083	0·085
10·3	91	48	0·89	0·077	0·075
10·4	92	52	0·83	0·047	0·053
10·4	93	50	0·83	0·049	0·056
10·4	94	46	0·84	0·0535	0·059
10·4	95	52	0·84	0·071	0·077
10·4	97	51	0·81	0·058	0·069
10·4	115	68	0·84	0·040	0·044
				Mean *0·061*	*0·065*
6° C.:					
6·0	75	46	0·88	0·040	0·040
6·0	76	46	0·88	0·040	0·040
6·0	77	51	0·85	0·036	0·039
6·0	79	57	0·86	0·027	0·028
6·0	80	51	0·86	0·041	0·043
6·2	81	48	0·88	0·0255	0·025
6·2	82	49−1	0·87	0·030	0·030
6·2	83	50	0·88	0·0245	0·025
6·2	85	54	0·86	0·032	0·034
				Mean *0·033*	*0·034*

A real difficulty encountered during the course of this work was that the copepods used had to be obtained by tow-netting and that the species most commonly found varied from one haul to another. Thus in the early part of the work *Acartia* was very abundant with some *Centropages*, while towards the

TABLE III. LIST OF EXPERIMENTS AND RESPIRATORY RATES OBTAINED FOR *TEMORA LONGICORNIS*

Exact temp.	Exp. no.	No. of animals	Mean length (mm.)	Respiration rate (μl. O_2/cop./hr.)	Corrected respiration rate
20° C.:					
20·4	14	30	1·03	0·253	0·148
20·0	128	46	0·71	0·130	0·161
20·0	129	45 – 1	0·70	0·124	0·156
				Mean *0·169*	*0·155*
17° C.:					
17·0	49	50	0·95	0·143	0·098
17·4	53	52 – 2	0·89	0·089	0·070
17·4	54	51	0·91	0·085	0·064
17·4	55	57 – 1	—	0·123	—
17·4	56	48	—	0·120	—
16·7	61	50	—	0·115	—
16·7	62	50	—	0·101	—
16·7	63	49	—	0·112	—
16·7	64	55 – 1	—	0·149	—
17·0	72	41	0·75	0·164	0·182
17·0	73	46 – 1	0·78	0·116	0·119
17·0	74	40	0·81	0·120	0·114
				Mean *0·120*	*0·108*
13° C.:					
13·1	38	40 – 2	0·93	0·081	0·058
13·1	40	48	0·86	0·094	0·085
13·1	42	45 – 4	0·92	0·0945	0·069
13·0	103	47	0·73	0·1045	0·121
13·0	104	51	0·70	0·063	0·080
13·0	110	50	0·72	0·074	0·089
13·0	111	50	0·72	0·049	0·059
13·0	112	51	0·71	0·072	0·088
				Mean *0·079*	*0·081*
10° C.:					
10·3	87	53 (?)	—	0·084	—
10·4	113	59 – 2	0·71	0·051	0·062
10·4	114	62	0·71	0·059	0·073
10·0	124	48	0·72	0·071	0·085
10·0	125	44	0·72	0·099	0·119
10·0	126	47 – 1	0·73	0·063	0·074
10·0	127	47	0·72	0·062	0·075
				Mean *0·070*	*0·081*

end very few *Acartia* were found and *Temora* was most abundant. Experimental runs were made with whatever material was most readily available, and therefore at some temperatures the number of experiments on one species may have been insufficient to give a reliable mean respiratory rate. Thus for *Centropages* at 20° C. the mean (0·146 μl. O_2/cop./hr.) (Table II) must be accepted

with caution. With *Temora* (Table III) only three experiments were made at 20° C. but here the mean of the corrected respiratory rates is 0·155 μl./cop./hr. with a S.E. of $\pm$0·003 and therefore the result is significant.

Table IV. The Mean Values of the Respiratory Rates

Species	Temp. (° C.)	No. of Exps.	Mean length	Mean respiration rate	Corrected mean respiration rate
Acartia	20	10	0·92	0·107 $\pm$0·006	0·0945 $\pm$0·006
	17	5	0·83	0·073 $\pm$0·005	0·079 $\pm$0·007
	13	7	0·85	0·061 $\pm$0·006	0·062 $\pm$0·004
	10	10	0·83	0·059 $\pm$0·0055	0·065 $\pm$0·007
Centropages	20	2	—	0·159?	0·146?
	17	13	0·91	0·088 $\pm$0·006	—
		10*	—	0·086 $\pm$0·0065	0·082 $\pm$0·009
	13	11	—	0·084 $\pm$0·007	—
		10*	0·89	0·087 $\pm$0·006	0·087 $\pm$0·009
	10	10	0·85	0·061 $\pm$0·004	0·065 $\pm$0·004
	6	9	0·89	0·033 $\pm$0·002	0·034 $\pm$0·002
Temora	20	3	0·81	0·169 $\pm$0·027	0·155 $\pm$0·003
	17	12	—	0·120 $\pm$0·007	—
		6*	0·85	0·1195 $\pm$0·011	0·108 $\pm$0·016
	13	8	0·79	0·079 $\pm$0·006	0·081 $\pm$0·007
	10	7	0·72	0·070 $\pm$0·006	0·081 $\pm$0·007

* Excluding experiments for which there are no measurements.

Table IV shows the mean respiratory rates and the means of the corrected rates for each species at each of the temperatures used. The means of the corrected rates of course omit those experiments in which the copepods were not measured and for which, in consequence, no corrected respiratory rates could be calculated. Clearly in most cases the difference between the means of the uncorrected and corrected respiratory rates is very small. The most marked exception is *Temora* at 20° C. (uncorrected mean 0·169 $\pm$ 0·027; corrected mean 0·155 $\pm$ 0·003). Table III shows that of the three experiments carried out at this temperature with *Temora*, one (Exp. 14) was with copepods of large size (average 1·03 mm.) and gave a high respiration rate (0·253 μl./cop./hr.) while the two other experiments (Exps. 128 and 129) used small *Temora* (0·705 and 0·702 mm. respectively) and the respiratory rate was greatly lowered (0·130 and 0·124 μl./cop./hr.). The marked effect of size on respiration even for the same stage and species of copepod is therefore well demonstrated.

Discussion

Temora *and* Acartia

The results given in Table IV show that in general an increase in respiratory rate follows a rise of temperature for all three copepods, as with other animals. For *Temora* and *Acartia* the rise from 13 to 20° C. is steady, though the conclusions to be drawn from the experiments at 10 and 13° C. are less certain.

Thus Table IV shows that there is apparently a small rise in mean respiratory rate for *Temora* between 10 and 13° C.; but when the respiratory rates are corrected for length the means are identical at these two temperatures. For *Acartia*, the mean corrected rate at 10° C. is actually very slightly greater than at 13° C., though the difference is not significant. At these lower temperatures the relatively smaller gas-volume change probably introduces larger errors in

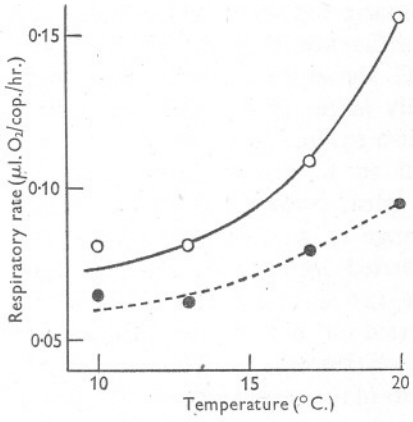

Fig. 1

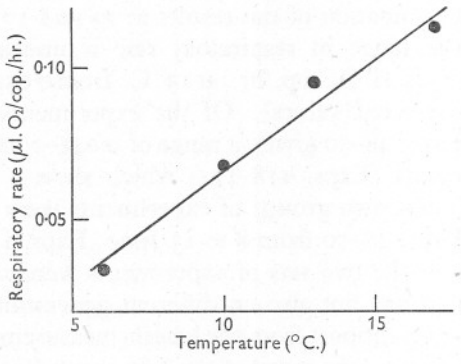

Fig. 2

Fig. 1. The relation between respiratory rate and temperature. (Respiratory rate in μl.O_2 per copepod per hour). ○ and continuous line, *Temora longicornis*; ● and pecked line, *Acartia clausi*.

Fig. 2. The relation between respiratory rate and temperature for the 2nd group of *Centropages hamatus*.

the experiments, but as the curves given in Fig. 1 indicate, it seems clear that the rise in respiratory rate with temperature for both species of copepods is slower at lower temperatures and that the curves steepen from *c.* 15–20° C. This has already been noted (cf. Zeuthen, 1947) for respiration/temperature relations for a wide variety of animals, and it agrees also with the results of Marshall *et al.* (1935) for the copepod *Calanus finmarchicus*.

Fig. 1 also indicates, however, that the respiration of the two species of copepod is probably not affected to the same extent by the same rise in temperature. Thus while for *Temora* the rate between 10 and 20° C. is almost doubled, for *Acartia* the rate increases only a little over $1\frac{1}{2}$ times.

These results may be compared with the temperature-respiration curve obtained by Marshall *et al.* (1935) for *Calanus finmarchicus*. They worked at temperatures down to 0° C., and they found that between this temperature and 10° C. the respiratory rate for male and Stage V *Calanus* was approximately doubled. In females the increase was considerably less (0·28–0·40 μl. O_2/cop./hr.). Between 10 and 20° C., however, the curves for all *Calanus* steepen,

and this agrees with the results here quoted for *Temora* and *Acartia*. Clarke & Bonnet (1939) found considerable variation in the respiratory rate of Stage V *Calanus*, but their results indicate a rise from 0·32 to 0·91 μl./cop./hr. for an increase in temperature from 5·5 to 16·8° C.

Centropages

In the experiments on *Centropages* there is a fairly steady increase from 6 to 13° C., but the rate at 17° C. (when corrected for length of animals) is slightly lower than at 13° C., though the difference is not significant. Closer examination of the results at 13 and 17° C. shows that at both temperatures the range in respiratory rate is unusually large: at 17° C. from 0·041 to 0·122 μl. O_2/cop./hr.; at 13° C., from 0·052 to 0·133 μl. O_2/cop./hr. (see Table II, corrected values). Of the experiments at 17° C. it is possible to separate Exps. 48–70 giving a range of 0·041–0·087 (mean 0·062) from the later experiments (Exps. 118–121) which show a range of 0·092–0·121 (mean 0·113). These two groups of experiments were carried out at rather different times: Exps. 48–70 from 8 to 13 June; Exps. 118–121 on 17 July, and it is probable that the two sets of experiments were carried out not only on different townettings but also on different generations of *Centropages*. This idea receives some support from the length-measurements of the two groups of experimental animals; the mean of the first group (Exps. 48–70) is 0·965, that of the second group (Exps. 118–121), 0·833 mm.

Possibly the experiments at 13° C. can also be divided into two groups. Exps. 35 and 45 were carried out on 4 and 6 June with relatively large animals (mean length 1·014 mm.) and gave a mean respiratory rate of 0·055 μl. O_2/cop./hr., whereas Exps. 99–109 conducted on 21 and 22 June were concerned with smaller copepods, possibly again of a different generation (mean length 0·856 mm.) and gave a mean rate of 0·095 μl. O_2/cop./hr.

The first group of experiments at both 17 and 13° C. appears to have been performed on similar animals (mean lengths 0·965 and 1·014 mm.) at approximately the same time, 4-13 June. The rates for this generation at 17 and 13° C. are 0·062 and 0·055 respectively. The second group of experiments was carried out later on a smaller generation (0·833 and 0·856 mm. mean lengths), and gave rates at 17 and 13° C. of 0·113 and 0·095 respectively. If either group is considered, a rise in respiratory rate with temperature is clearly indicated and the extent of the rise is of the same order (13% for 1st group; 18% for the 2nd group), but it is obvious that the two groups must be considered separately. If the experiments carried out at the other temperatures can be assigned to either group it should be possible to get a real relation between respiratory rate and temperature.

The results at 20° C. cannot be seriously considered since only two experiments (which gave widely differing rates) were conducted at that temperature. At 10° C. (Table II) nine of the ten experiments were carried out on 19–20 June,

the last (Exp. 115) about a week later. If only the first nine experiments be considered the mean of the corrected respiration rates is 0·068 (range 0·053–0·085). The mean length of the animals used in these nine experiments is 0·848 mm., and the copepods are comparable to the second group at both 17 and 13° C. Finally, the experiments at 6° C. were all conducted on 14–15 June. The average length of the copepods is 0·867 mm. and the result (mean respiration rate 0·034) should again be compared with those obtained from the second groups of animals at 17 and 13° C.

TABLE V. THE RESPIRATORY RATES OF TWO GROUPS OF *CENTROPAGES*

Temp. (° C.)	Group I (length 0·915 – 1·044; mean 0·977 mm.)	Group II (length 0·805 – 0·890; mean 0·854 mm.)
17	0·062	0·113
13	0·055 (?)	0·095
10	—	0·068
6	—	0·034

Table V shows these results for *Centropages* summarized. In Group 1 the data are obviously too scanty to permit discussion, but in Group 2 a fairly steady rise in respiratory rate follows the rise in temperature from 6 to 17° C. These experiments should be repeated on a more homogeneous brood of *Centropages*, but from Fig. 2, constructed from Group 2 data, it seems clear that once again van t' Hoff's law is not strictly followed (cf. *Temora*, *Acartia* and also *Calanus*).

Generation differences in Acartia *and* Temora

It is perhaps necessary now to re-examine the data for *Temora* and *Acartia* to see whether different generations were used in the experiments with these copepods, and whether variation between experiments might be correlated with generation differences. It must be emphasized that for all three species of copepods, if respiratory rate can be correlated with generations, it cannot be explained on differences in length of the generations since all the rates have been corrected to a standard mean length.

In some cases (e.g. *Acartia* at 17° C.) experiments were conducted several weeks apart (e.g. Exp. 51 on 8 June: Exps. 116–123 on 17–19 July). But in general for *Acartia*, the mean lengths of the copepods used (though they show some differences) and the corrected respiratory rates at any one temperature cannot clearly be separated into groups. In the experiments on *Temora* larger differences in the mean lengths and corrected respiratory rates were found than with *Acartia*, e.g. especially in the experiments at 13° C. But in general the range in respiratory rate found at each temperature is smaller than with *Centropages* and there is no obvious grouping of the experiments.

While for *Temora*, and possibly even for *Acartia*, variations attributable to

generation differences may distort the temperature/respiration curve, the evidence for such differences is not very strong and the curves given in Fig. 1 may be regarded therefore as showing a true relation between temperature and respiration for these copepods during June and July. It is still probable that different generations of these copepods at other seasons of the year might show very appreciable differences in respiratory rate, and a few preliminary experiments on *Temora* by one of us (J.E.G.R.) during the autumn give some support to this suggestion.

Differences in the respiratory rates of different generations might be suggested as an explanation of the puzzling results obtained previously with *Centropages typicus* (Raymont & Gauld, 1951). With this copepod it may be recalled that the experimental data fell clearly into two well-marked groups, one giving a mean respiratory rate of 0·29 μl. O_2/cop./hr., the second group the much lower mean of 0·18 μl./cop./hr. Differences such as time of keeping in the laboratory, feeding, and varying proportions of the sexes could not be correlated with these two groups. All the copepods used in these experiments were taken in one week (7–13 September 1949), so that the different respiration rates observed cannot be attributed to the use of different generations. But the copepods may have been taken from different populations since tow-nettings were taken both off Garroch Head, to the westward, and in Fairlie Channel, to the eastward of Cumbrae; unfortunately no records were kept of where the copepods used in any particular experiment were caught.

Sex and Respiration Rate

Marshall *et al.* (1935) have shown that male and female *Calanus finmarchicus* respire at different rates and are affected to different degrees by rising temperature. Since in the experiments here described male and female copepods were not separated, the suggestion might be made that variations in the respiratory rate, and in particular the variations seen for *Centropages* at 17 and 13° C., were due to different numbers of the sexes being used for experiments rather than different generations being employed.

The first group of experiments with *Centropages* at 17° C. (Exps. 48–70) together give a much lower proportion of females (56% ♀♀) than the second group (Exps. 118–121) in which over 90% were females. If, therefore, female *Centropages* respire more actively than males (though this is contrary to the findings of Marshall *et al.*, 1935, for *Calanus*), the higher respiratory rate in the second group of experiments at both temperatures might be attributed to the greater proportion of females. But reference to individual experiments at these temperatures shows that the suggestion is unsound. Thus at 17° C., in Exp. 48, with a great preponderance of males the respiratory rate was 0·071 μl. O_2/cop./hr., Exp. 69 with practically equal numbers was 0·060 and in Exp. 70, with three times as many females as males, it was 0·053, exactly the opposite of what would be expected.

While, therefore, differences may exist between the respiration of male and female *Centropages hamatus* the marked variation between the two groups of experiments at both 17 and 13° C. cannot be explained in this way. And indeed the possibility that instead we were really dealing with different generations of copepods is strengthened by the different proportion of the sexes used in the experiments. For example, it has already been pointed out that at 17° C. the second group of experiments (Exps. 118–121) has a much higher proportion of females (more than 90%) than the earlier first group (Exps. 48–70) with only 56% females. But since female *C. hamatus* are somewhat larger than males (cf. Sars, 1903; Marshall, 1949) the mean lengths of the copepods used in Exps. 118–121 would be expected to be greater than in Exps. 48–70. In fact the copepods used in Exps. 118–121 were definitely smaller than those used in earlier experiments, and it is therefore even more probable that they belonged to a different generation. Marshall (1949) has shown that the length of *Centropages* (Stage VI) decreases to a minimum about July and August.

Length and Respiratory Rate

Experiments on a number of species and stages of copepods have suggested that respiratory rate can be expressed as a function of the length (Raymont & Gauld, 1951) in the equation

$$\log R = 2{\cdot}19 \log L - 0{\cdot}928,$$

where R = respiratory rate at 17° C., and L = length of cephalothorax. It is of interest to see whether the present determinations on *Centropages*, *Acartia* and *Temora* will also fit this suggested relationship. Table VI gives the lengths, mean corrected respiratory rates and the respiratory rates calculated from the equation quoted above.

TABLE VI. THE CALCULATED RESPIRATORY RATES AND THE ACTUAL RATES OBTAINED

Species	Mean length (mm.)	Corrected respiration rate	Calculated respiration rate
Centropages	0·882	0·082	0·090
Acartia	0·8585	0·079	0·085
Temora	0·787	0·108	0·070

The agreement between the observed and calculated respiratory rates is reasonably good for *Centropages* and *Acartia*, but for *Temora* the calculated rate (0·070) is considerably lower than the result obtained experimentally (0·108), and therefore, in contrast to *Centropages* and *Acartia*, it would appear that the suggested respiration/length relationship is only roughly applicable to *Temora*.

Probably one of the real factors determining the respiratory rate is surface area, and the length squared may be regarded as a measure of surface. A

numerical ratio between the square of the length of a copepod and its surface area can be calculated if its cephalothorax is matched by a simple geometrical form.[1] While *Calanus*, *Acartia* and *Centropages* have elongate, oblong cephalothoraces which can be closely matched by a cylinder with a hemisphere at each end, that of *Temora* is relatively short and swollen anteriorly and can best be matched by two cones lying base to base, the longer one which forms the posterior part of the cephalothorax having its apex truncated. *Euchaeta* is more irregular in shape and the ratio quoted below was calculated by summation of a series of cylinders and parts of cones. From these forms the following ratios between the square of the length and surface area were calculated:

Species	L^2/S	Species	L^2/S
Calanus finmarchicus	1·03	*Centropages hamatus*	0·92
Euchaeta norvegica	1·13	*Acartia clausi*	1·19
Centropages typicus	0·96	*Temora longicornis*	0·72

It can be seen that in all the species except *Temora* the ratio is nearly 1, so that length squared is a fairly good measure of the surface area. That for *Temora* is much lower and its length must be multiplied by 1·2 before squaring to make it an equivalent measure of surface area. If the respiration rate of *Temora* is now calculated using 1·2 L instead of L, a value of 0·106 μl./cop./hr. is obtained which agrees very well with the experimental result.

General Conclusions

There remains the very difficult question as to how far results obtained from laboratory experiment may be applied to conditions in the sea. It is likely that the shaking of the apparatus and the concentration of some fifty small copepods in about 5 ml. of sea water for some hours may stimulate and excite the animals, so leading to a respiratory rate that is considerably higher than that under natural conditions (cf. Raymont & Gauld, 1951). But even if the rate quoted here for any given temperature is higher than in the sea, it is probable that a rise in respiration with temperature will follow in the sea just as in the laboratory. Temperatures of around 20° C. will certainly not be experienced in British waters, but a rise of 9–10° C. (say from 6 to 15° C.) between late winter and late summer is not unlikely in inshore surface waters and more striking temperature variations occur in a sea area like the Gulf of Maine. Riley (1946), for instance, quotes the mean temperature for the upper 30 m. as varying from 2·6° C. in March to 15·2° C. in September over Georges Bank (cf. also Riley, 1947, fig. 29).

[1] The surface area so calculated is not the true surface area, since the surfaces of the urosome and the appendages have been ignored; in consequence the ratios given above have no absolute significance. But the calculated surface area probably bears a reasonably constant relationship to the real surface area so that the ratios quoted are valid for comparative purposes, as they are used here.

Estimates of the food required by planktonic copepods have been made by Marshall *et al.* (1935), Clarke & Bonnet (1939), Riley (1947) & Harvey (1950). These workers based their calculations on the respiratory rate of *Calanus finmarchicus* measured by Clarke & Bonnet (1939) or Marshall *et al.* (1935), and tried to relate these requirements to the amount of available food in the sea. Measurements of the respiration rate of other species (Raymont & Gauld, 1951, and the present results) suggest that such estimates must be modified in at least two directions.

First, it has been shown that the respiratory rate of copepods is approximately proportional to the square of the length, probably because its magnitude is determined by the surface area of the animals. Now all the estimates referred to above express the food requirements as a percentage of the weight of the animal, giving values ranging from 2 to 8% at different temperatures. Since the weight is roughly proportional to the cube of the length while respiratory rate, and so metabolic food requirements, are proportional to the square of the length, the metabolic food requirements expressed as a percentage of the weight will be inversely proportional to the length. In consequence, estimates of the food requirements of *Calanus* cannot be directly applied to other species or to mixed populations, and to estimate the food requirements of a population of copepods one must know not only the total weight of the population but also the size distribution of the copepods making up that population.

Secondly, in discussing seasonal variations in the respiratory food requirements, these authors have assumed that temperature is the only factor affecting the respiratory rate. The measurements of the respiratory rate of *Centropages* described here apparently show that different generations of the same species may have different respiratory rates (apart from differences related to size differences of generations at the same temperature). If such generation differences should prove to be more wide-spread, then in addition to an increase in food requirements during summer and a decrease in winter produced by seasonal changes in temperature, further changes, at present unknown, in the food requirements of different generations will have to be taken into account.

Summary

The respiratory rates of three species of planktonic copepods, *Acartia clausi*, *Centropages hamatus* and *Temora longicornis*, were measured at four different temperatures.

The relationship between respiratory rate and temperature was found to be similar to that previously found for *Calanus*, although the slope of the curves differed in the different species.

The observations on *Centropages* at 13 and 17° C. can be divided into two groups and it is suggested that the differences are due to the use of copepods from two different generations.

The relationship between the respiratory rates and lengths of *Acartia* and *Centropages* agreed very well with that previously found for other species. That for *Temora* was rather different: the difference is probably due to the distinct difference in the shape of the body of *Temora* from those of the other species.

The application of these measurements to estimates of the food requirements of the copepods is discussed.

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DIURNAL VARIATIONS IN THE GRAZING OF PLANKTONIC COPEPODS

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Marine Station, Millport

(Text-figs. 1–3)

Wimpenny (1938) has shown that the percentage of copepods taken in the sea with food in the gut tends to be higher at night than by day. Most planktonic animals perform diurnal vertical migrations, of greater or less extent. Wimpenny's observations may well be a consequence of such migrations.

On the other hand, during investigations into grazing rate of planktonic copepods (Fuller, 1937; Gauld, 1951) some of the measurements have suggested that there is a diurnal variation in the rate at which the copepods collect their food, even under laboratory conditions when migration is impossible, and that they filter the water more quickly at night than by day. The variation in the percentage of copepods containing food may be caused simply by changes in the rate of feeding, this being controlled either by the amount of light in their environment or by some internal rhythm affecting their behaviour.

An investigation of diurnal variations in the feeding behaviour of *Calanus finmarchicus* (Gunn.) was undertaken to investigate (i) its behaviour in nature, and (ii) the possible existence of alternations of feeding and resting periods under laboratory conditions. This species was chosen because it is the dominant copepod species in the Clyde sea area, is readily obtainable in reasonable numbers at all times of the year, and has already been the subject of investigation of feeding rate.

Field Observations

Methods

Calanus were obtained from 10-min. hauls of 50-cm. coarse tow-nets (26 meshes to the inch) taken at 4-hourly intervals through 24 hr. Three nets were fished at once, 25 fathoms apart on a trawl warp, to the end of which was attached a cable depressor (Barnes, 1951). Rigged in this way, the uppermost net fished 2–3 m., the second 35–40 m. and the lowest *c.* 75–80 m. from the surface. There was no means of closing the nets before hauling, but the percentage of the fishing time spent by the lower nets in the upper strata was small and unlikely to have interfered greatly with the results. The hauls were taken at a station just outside Tarbert, Loch Fyne. At this station deep water

(170 m.) was available only half a mile from the harbour where the research vessel could be tied up in harbour, and examination of the animals begun within 20 min. of their capture.

As Wimpenny (1937) has pointed out, the gut contents of *Calanus* are usually very easily observed, and in these investigations were estimated on the living animals as soon as possible after capture. Thirty to forty *Calanus* were transferred from the catch to a Petri dish and most of the water withdrawn so that the animals were compelled to lie on their sides and for the most part motionless. They were then examined under a low-power microscope and the amount of food in the gut recorded. This method of examination was adopted because it was found that in fixed catches, a variable and sometimes important fraction of the animals defaecated when formalin was added to the catch, and it was thought desirable to reduce as much as possible the interval between capture and examination. The number of animals examined varied from haul to haul, but an attempt was made to examine at least fifty, and, if time and numbers permitted, 100 of both Stage V and adult female *Calanus*. Occasionally Stage IV was sufficiently numerous for significant numbers to be counted in addition to the larger stages. After this had been done the catches were fixed with formalin and brought back to the laboratory. In some of the hauls the total numbers of *Calanus* or the numbers of one or other stage were too small for counts to be made quickly on board ship and supplementary counts of fixed material were made in the laboratory.

I am indebted to Skipper R. E. Souter and the crew of M.F.V. *Calanus* for their willing co-operation in taking the 24-hr. stations which comprise the field observations to be recorded here.

Results

Before anything is said about the gut contents of the catches, some remarks must be made about the vertical distribution of the animals. Since closing nets were not used the catches of the bottom net may have been made partly in the upper waters, but the differences in distribution to which attention are to be drawn are too marked to be attributed entirely to this cause, and in any case concern primarily the top net. The tables do not give the total numbers of animals caught (which were never estimated), but on any one occasion the catches of the top net were either of more or less the same size throughout the 24 hr. or else there was a marked difference between catches made in daylight and darkness so that the general picture of diurnal changes in distribution given Tables I and II is a true one.

It can be seen from the tables that only on seven of the thirteen occasions on which hauls were taken, were the catches of the top net distinctly greater in darkness than in daylight, while on 17 April and 11 September 1950, large catches of *Calanus* were taken at the surface throughout the 24 hr. On the four remaining occasions the catches varied rather irregularly and cannot be

looked on as clear evidence for or against vertical movements. It is remarkable that clear evidence of migration was obtained only on two occasions in 1950 (10 July and 21 November), and even on 10 July the vertical movements were not so clear cut as those of 1951, while in 1951 surface hauls in daylight were always blank except in April and May. The presence of adult and Stage V *Calanus* in the surface water during daylight in spring and early summer has already been recorded for the Clyde (Brook, 1886; Marshall & Orr, 1927; Nicholls, 1933; Marshall, Nicholls and Orr, 1934), but it seems that a much larger percentage of the population than usual was present in the surface waters in daylight on 10 April, and also on 11 September 1950, when exceptionally large catches were taken in daylight, although the day was not unusually dull.

Adult Females

The details of the observations on adult females are given in Table I, in which the numbers examined from each haul and the percentage of these which contained food is given. If a haul was taken and no *Calanus* seen this is given as $(0)_0$; if a haul was not taken or was lost, it is indicated by a dash. The times are given by the 24-hr. clock and are always Greenwich mean time.

The data of Table I are summarized in Table II, from which it can be seen that the percentage of adult female *Calanus* found to contain food was practically identical in daylight and in darkness. These figures quite clearly furnish no evidence of diurnal rhythm in feeding.

On the other hand, adult female *Calanus* were caught in all twenty-two surface hauls taken in the dark, but only in twenty-seven out of the forty-eight daylight hauls.[1] In consequence, more adult females were found containing food by night (1275) than by day (1041), in spite of the fact that there were more than twice as many daylight hauls as night hauls. These observations then agree with those of Wimpenny (1938) that the feeding of *Calanus* takes place mostly at night, but they also show that the reason for this is that by day *Calanus* is usually absent from the surface water where their food is principally found.

The numbers of female *Calanus* containing food in the deeper nets was nearly always less than in the surface net, the lowest net of the three showing the smallest percentage containing food. Of the adult females taken on all occasions, 68% of those in the middle net and 46·5% of those in the lowest net contained food compared with 86% in the surface net. The percentage of animals containing food varied considerably from one series of hauls to another—the mean for the bottom net from 16% (9. i. 51) to 75% (12. v. 51), that of the mid net from 30% (9. i. 51) to 86% (28. ii. 50)—but in any given series there is no obvious pattern in the occurrence of food in the guts unless

[1] The hauls at 16.00 and 08.00 hr. were always taken in daylight, those at 20.00 and 04.00 hr. in daylight from May to August, and in darkness in the other months.

there is clear evidence of vertical migration, when more of the copepods in the lower nets tended to have food in the gut at midnight and 4 o'clock than they had before midnight.

TABLE I. FEMALES: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

Time (hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
28. ii. 50							
Top	83_{6}	89_{249}	94_{54}	75_{8}	98_{100}	90_{21}	69_{13}
Mid	100_{7}	91_{100}	75_{98}	88_{100}	89_{100}	87_{23}	83_{12}
Bottom	69_{88}	61_{100}	85_{102}	84_{74}	81_{100}	57_{100}	82_{100}
17. iv. 50							
Top	99_{100}	89_{100}	91_{100}	96_{100}	92_{100}	98_{67}	86_{100}
Mid	72_{100}	69_{100}	61_{100}	75_{100}	48_{50}	65_{79}	64_{98}
Bottom	52_{100}	24_{100}	45_{100}	28_{100}	56_{95}	36_{100}	51_{100}
29. v. 50							
Top	100_{5}	100_{2}	100_{4}	100_{4}	100_{1}	100_{3}	$(0)_{0}$
Mid	48_{50}	72_{50}	62_{50}	96_{50}	64_{50}	76_{50}	84_{50}
Bottom	34_{50}	54_{50}	64_{50}	64_{50}	56_{50}	64_{50}	44_{50}
10. vii. 50							
Top	29_{14}	$(0)_{0}$	67_{6}	83_{18}	$(0)_{0}$	50_{2}	0_{2}
Mid	0_{8}	40_{10}	32_{50}	91_{70}	43_{100}	48_{50}	$12{\cdot}5_{8}$
Bottom	39_{100}	40_{83}	69_{100}	84_{51}	79_{100}	26_{100}	20_{20}
11. ix. 50							
Top	78_{100}	91_{100}	78_{100}	88_{100}	73_{100}	74_{100}	92_{100}
Mid	57_{100}	64_{100}	68_{100}	77_{100}	82_{100}	71_{100}	70_{100}
Bottom	21_{100}	24_{100}	40_{100}	36_{100}	29_{100}	—	30_{100}
21. xi. 50	No females seen.						
9. i. 51							
Top	$(0)_{0}$	$(0)_{0}$	84_{19}	68_{19}	55_{9}	100_{1}	$(0)_{0}$
Mid	0_{3}	2_{100}	34_{83}	48_{101}	77_{22}	36_{11}	0_{19}
Bottom	0_{14}	0_{100}	15_{100}	12_{106}	16_{31}	18_{100}	0_{3}
14. ii. 51							
Top	$(0)_{0}$	—	99_{100}	99_{71}	99_{110}	$(0)_{0}$	$(0)_{0}$
Mid	$(0)_{0}$	—	85_{100}	94_{109}	83_{41}	44_{9}	13_{15}
Bottom	17_{17}	—	13_{100}	33_{100}	18_{100}	82_{82}	4_{52}
14. iii. 51							
Top	100_{3}	$(0)_{0}$	80_{5}	100_{5}	82_{43}	100_{1}	100_{3}
Mid	80_{6}	25_{4}	0_{5}	86_{35}	68_{71}	74_{23}	60_{5}
Bottom	63_{94}	47_{100}	36_{44}	50_{44}	33_{100}	0_{3}	40_{20}
5. iv. 51	Very few: top, 78_{18}; mid, 63_{11}; bottom, 72_{82}						
7. v. 51*							
Top	98_{62}	—	90_{41}	85_{100}	—	86_{100}	—
Mid	—	—	—	—	—	—	—
Bottom	76_{100}	—	72_{100}	87_{100}	—	67_{100}	—
4. 7. 51							
Top	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$	100_{60}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
Mid	17_{6}	0_{3}	20_{25}	96_{50}	82_{90}	$(0)_{0}$	$(0)_{0}$
Bottom	30_{50}	44_{50}	42_{50}	84_{50}	96_{50}	48_{50}	8_{50}
15. viii. 51							
Top	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$	94_{50}	$(0)_{0}$	$(0)_{0}$	$(0)_{0}$
Mid	40_{35}	62_{50}	62_{50}	100_{50}	76_{60}	76_{50}	94_{50}
Bottom	16_{50}	44_{25}	18_{50}	78_{50}	84_{50}	4_{50}	38_{50}

* The series on 17 May 1951 was taken off L. Ranza and is incomplete: only two nets were used and the times of the hauls were 13.00, 19.00, 22.00 and 07.30 hr.

TABLE II

	No. examined	No. with food	Percentage with food
Daylight	1190	1041	87·5
Darkness	1514	1275	84
Total	2704	2316	86

The decrease in the numbers of *Calanus* with food in the gut in deeper water and its dependence on the stratification of the food organisms was more clearly demonstrated on 11 September 1950 when a more detailed series of hauls was taken at six depths, and water samples taken at roughly corre-

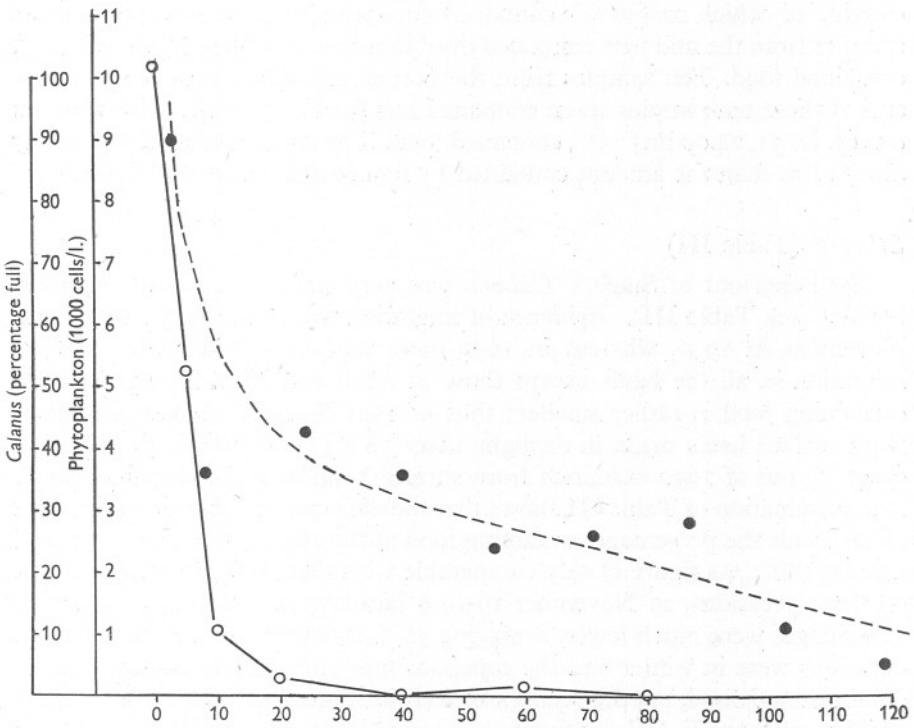

Fig. 1. Percentage of *Calanus* with food and phytoplankton concentration. Symbols: ● and broken line, percentage of *Calanus* with food; ○ and full line, phytoplankton concentration. Horizontal scale: depth in metres (0–120).

sponding depths to show the distribution of the phytoplankton. The results are illustrated in Fig. 1 in which the phytoplankton (1000's of cells/litre) and the percentage of *Calanus* with food are both graphed against depth. It can

be seen that the phytoplankton was concentrated in a narrow stratum about 10 m. deep, in which 90% of the *Calanus* were full; below this stratum the percentage fell from *c.* 40% at 10 m. to 5% at 100 m.

Adult Males

Male *Calanus* were not found in most of the hauls in sufficient numbers to obtain a clear picture of their behaviour. In addition they are relatively opaque and any food they may contain is less easy to see, so possibly small quantities of food, particularly in the anterior part of the gut, were overlooked.

Of the 1555 adult males examined from all the catches 63% were in the lowest net, and 62% of them were empty. On only one occasion were more than twenty-five taken in a surface net (18. iv. 50, 08.00 hr.) when 38 were caught, of which 22 (58%) contained food; on the same occasion three samples from the mid nets contained over 50 males, of which 45, 73 and 44% contained food. Ten samples from the bottom net contained over 50 males and of these ten samples seven contained less than 40% males with food; in one (5. iv. 51, 24.00 hr.) 73% contained food. The amount of food was nearly always less than the amount contained by female and Stage V *Calanus*.

Stage V (Table III)

The behaviour of Stage V *Calanus* was very similar to that of the adult females (see Table III). Evidence of migration was found only in July and November in 1950, whereas in 1951 there was clear evidence of vertical migration in all the hauls except those in April and May. The percentage containing food is rather smaller; thus of 1541 Stage V *Calanus* examined from surface hauls made in daylight 1200 (78%) contained food, and 1181 (72·5%) out of 1630 examined from surface hauls at night contained food. But examination of Table III shows that the data can be subdivided: in most of the hauls the percentage containing food at the surface was over 70% and averaged 81%, a figure closely comparable with that for adult females. But on three occasions, 21 November 1950, 9 January and 15 August 1951, the percentages were much lower, averaging 50% containing food. Two of these occasions were in winter and the copepods may simply have been unable to obtain enough food, but this seems less likely in August, and since from August to January Stage V *Calanus* form an overwintering population it is possible that the small percentage containing food is due to a real change in behaviour.

On 11 September 1950, the percentages containing food were also abnormally low at night, averaging only 31% in agreement with the other observations in autumn. But on this occasion *Calanus* were present at the surface in considerable numbers during the day and of those taken by day at the surface 82% contained food, a percentage comparable to those found in spring and summer catches. The behaviour shown by the *Calanus* here is exactly the

opposite of what would normally be expected because most of them were apparently feeding *by day* and not at night.

TABLE III. STAGE V: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

Time (hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
28. ii. 50	Very few: top, 60_5; mid, 38_{63}; bottom, 17_{30}						
17. iv. 50							
Top	93_{100}	78_{100}	74_{100}	90_{100}	87_{100}	91_{80}	81_{100}
Mid	49_{100}	66_{100}	56_{89}	65_{100}	70_{100}	59_{100}	68_{100}
Bottom	41_{100}	25_{100}	26_{100}	49_{100}	48_{100}	33_{100}	34_{100}
29. v. 50							
Top	100_{31}	100_{31}	100_{10}	100_{17}	87_{23}	100_{26}	100_{21}
Mid	24_{50}	32_{50}	46_{50}	58_{50}	50_{50}	48_{50}	66_{50}
Bottom	26_{50}	36_{50}	52_{50}	32_{50}	30_{50}	48_{50}	30_{50}
10. vii. 50							
Top	53_{36}	100_{18}	93_{14}	98_{136}	0_2	81_{26}	89_{62}
Mid	21_{100}	25_{100}	31_{100}	46_{50}	28_{100}	35_{100}	18_{50}
Bottom	27_{100}	43_{97}	17_{100}	18_{50}	37_{100}	14_{100}	8_{50}
11. ix. 50							
Top	99_{100}	92_{100}	20_{100}	36_{100}	38_{100}	49_{100}	88_{100}
Mid	74_{100}	83_{100}	18_{100}	16_{100}	17_{100}	26_{100}	73_{100}
Bottom	10_{100}	13_{100}	9_{100}	6_{150}	19_{100}	—	17_{100}
21. xi. 50							
Top	$(0)_0$	$(0)_0$	18_{100}	47_{58}	52_{100}	0_2	$(0)_0$
Mid	10_{10}	4_{100}	10_{100}	13_{100}	14_{100}	0_{60}	0_7
Bottom	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}	0_{100}
9. i. 51							
Top	$(0)_0$	$(0)_0$	89_{76}	58_{107}	51_{102}	100_1	$(0)_0$
Mid	0_3	2_{100}	36_{83}	48_{101}	77_{22}	36_{11}	0_{19}
Bottom	0_{14}	0_{100}	15_{100}	12_{106}	16_{31}	18_{100}	0_3
14. ii. 51	Very few: top, 100_8; mid, 0_4; bottom, 0_{30}						
14. iii. 51							
Top	75_8	100_1	$87{\cdot}5_{32}$	87_{93}	95_{97}	100_3	84_{50}
Mid	10_{10}	79_{34}	79_{100}	97_{88}	91_{100}	97_{93}	89_{63}
Bottom	80_{90}	20_{100}	14_{100}	52_{100}	36_{88}	20_{15}	68_{89}
5. iv. 51							
Top	60_{10}	78_{53}	$(0)_2$	87_{100}	95_{100}	63_8	100_4
Mid	98_{100}	96_{115}	96_{100}	95_{100}	96_{100}	80_{50}	75_{100}
Bottom	92_{100}	59_{100}	82_{100}	92_{100}	93_{100}	77_{100}	83_{100}
17. v. 51 (see footnote, Table I)							
Top	100_{16}	—	100_{37}	100_{12}	—	100_{59}	—
Mid	—	—	—	—	—	—	—
Bottom	71_{100}	—	64_{100}	51_{100}	—	44_{100}	—
4. vii. 51							
Top	44_9	$(0)_0$	$(0)_0$	100_{50}	$(0)_0$	$(0)_0$	$(0)_0$
Mid	2_{50}	0_{17}	12_{50}	100_{50}	87_{100}	67_6	$(0)_0$
Bottom	8_{50}	8_{100}	22_{50}	36_{50}	43_{50}	13_{50}	3_{50}
15. viii. 51							
Top	$(0)_0$	$(0)_0$	$(0)_0$	38_{50}	$(0)_0$	$(0)_0$	$(0)_0$
Mid	32_{50}	20_{50}	42_{60}	92_{50}	6_{50}	18_{50}	20_{25}
Bottom	12_{50}	8_{50}	20_{50}	22_{50}	16_{50}	0_{50}	12_{50}

The percentage containing food in the lower nets, as with adult females, was less than at the surface, averaging 46% in the middle net and 29% in the lowest net.

Stage IV (Table IV)

On a few occasions, for instance on 29 May 1950, fair numbers of Stage IV were caught. When the numbers were sufficiently large to give significant counts, most of those in the surface water (averaging 91 % for all the samples in Table IV) and a smaller proportion of those from the deeper layers (38 % in the middle net and 23 % in the lowest net from Table IV) contained food, so that the behaviour appears to be closely similar to that of adult female and Stage V *Calanus*.

TABLE IV. STAGE IV: PERCENTAGE WITH FOOD

(The figures in suffix give the numbers examined.)

Time (hr.)...	12.00	16.00	20.00	24.00	04.00	08.00	12.00
29. v. 50							
Top	95_{102}	98_{112}	97_{110}	99_{104}	98_{109}	100_{100}	100_{100}
Mid	69_{62}	47_{90}	54_{50}	29_{50}	26_{50}	17_{50}	34_{50}
Bottom	12_{50}	13_{50}	28_{50}	13_{50}	16_{50}	25_{50}	28_{25}
11. vii. 50							
Top	91_{100}	98_{100}	84_{50}	93_{100}	100_{12}	98_{100}	76_{100}
Mid	22_{100}	18_{100}	19_{100}	26_{50}	21_{100}	23_{100}	20_{50}
Bottom	24_{100}	8_{100}	27_{100}	20_{50}	22_{100}	7_{100}	16_{50}
17. v. 51 (see footnote, Table I)							
Top	100_{14}	—	100_{34}	100_{9}	—	100_{24}	—
Mid	—	—	—	—	—	—	—
Bottom	75_{8}	—	100_{9}	9_{21}	—	63_{8}	—
4. vii. 51							
Top	(0)	(0)	(0)	100_{10}	(0)	(0)	(0)
Mid	11_{36}	12_{17}	12_{25}	90_{10}	91_{100}	15_{20}	(0)
Bottom	6_{50}	0_{30}	15_{20}	75_{20}	30_{23}	40_{20}	0_{9}
15. viii. 51							
Top	18_{50}	(0)	(0)	33_{15}	(0)	(0)	(0)
Mid	42_{7}	24_{25}	30_{10}	46_{50}	72_{40}	30_{50}	0_{9}
Bottom	12_{25}	16_{50}	16_{25}	6_{34}	6_{50}	15_{20}	21_{34}

Amount of Food

The amount of food in the gut varied from small quantities to a mass completely filling the mid-gut. An attempt was made to record the different amounts of food which the copepods contained. For example a *Calanus* with food throughout the length of its mid-gut was recorded as 'full', but a 'full' *Calanus* in April when diatoms were abundant obviously contained more food than a 'full' *Calanus* in winter, when phytoplankton was scarce; similarly, a 'full' adult female contained more food than a 'full' Stage V. These and other difficulties in maintaining a constant standard in a subjective estimate of this kind made the value of the estimates rather doubtful. The following general statements, however, are probably a fair summary of the observations.

The amount of food contained in the guts of the copepods with food was greatest in spring (April, May) when nearly all would be packed with food. During the summer the *Calanus* were not quite so well filled, but in winter

the quantity was distinctly less, and small even in many animals at the surface. The amount of food in individuals captured in the lowest net was nearly always less than that found at the surface: for instance copepods with packed guts were rarely seen in the deepest catches. The *Calanus* in the middle net were in an intermediate condition; when food was abundant, most would contain a large amount of food, but at other times the amount might be distinctly less than at the surface. As would be expected from their sizes, females contained in general rather more than Stage V and these distinctly more than Stage IV; males contained much less for their size than any of the other stages and in general contained distinctly less than Stage V.

Calanus finmarchicus *and* C. helgolandicus

Rees (1949) has drawn attention to the existence of two possible subspecies of *C. finmarchicus* and has suggested that they differ in temperature tolerance. Marshall & Orr (1952) have recorded the presence of *C. helgolandicus* in the Clyde, occasionally in fair numbers, and have shown that the two forms differ in the time of spawning. It would be interesting to see whether its feeding behaviour is also different. The catches were accordingly examined to see if any *C. helgolandicus* were present, but, except on one occasion, 11 September 1950, this form was absent altogether or made up an extremely small fraction of the catch. So far as could be seen from these small numbers the feeding behaviour of the two forms was identical. It has already been seen that the conditions in September 1950 were unusual and the presence of *C. helgolandicus* might explain the abnormality, but the numbers of *C. helgolandicus* do not vary very much from one haul to another, and since they did not rise above 32 % of any catch, they are too few in number to account for the large changes in feeding behaviour observed. Further, the percentage of *C. helgolandicus* containing food did not differ significantly from that of *C. finmarchicus*.

Laboratory Observations

Methods

In the laboratory the existence of a possible periodicity in grazing rate was investigated, as in the field observations by direct examination of the gut contents. The copepods were kept in a suspension of a food organism, usually a species of *Chlamydomonas* (cf. Gauld, 1951), and examined from time to time, usually at intervals of 4 hr., and the amount of food recorded.

Results

Preliminary Investigations

Before the other experiments and any field observations were started, a number of experiments were made to see how long a copepod would take to fill its gut when it began to feed and how long it would remain full after it had ceased feeding. The former were done by keeping copepods in sterile sea

water for some hours, usually overnight, to make sure they were empty. They were then put into suspensions of *Chlamydomonas* and examined at intervals of 10–15 min. to see how full they were. It proved very difficult to get any satisfactory observations. The transference of the copepods from a beaker to a watch-glass for examination disturbed them, and when they were returned to the beakers some time might elapse before they settled down, during which they darted about the beaker rapidly and normal feeding would not take place. The time taken to settle down varied quite erratically. However, in a moderately thick suspension of *Chlamydomonas*, comparable to those used in the experiments, *Calanus* can fill its gut in 10–15 min. at most and in thicker suspensions in a very few minutes indeed. It is unlikely that the natural phytoplankton on which the *Calanus* examined in the field were feeding would ever be as rich as that used in the experiments except possibly at the height of the spring maximum, but even in suspensions comparable in density to natural phytoplankton *Calanus* can fill its gut in less than 30 min.

To find out how long food could remain in the gut the exact opposite procedure was followed. A number of *Calanus* were kept for some time in a fairly thick suspension of *Chlamydomonas* until they were full. They were then transferred into sterile sea water and examined as before at short intervals. Although a very small quantity of food matter may remain for several hours in the gut of a copepod which is swimming in sterile sea water, the bulk of the food will be digested and the undigestible residue passed out in about 1–2 hr.

Twenty-four-Hour Experiments

Observation of the amount of food in the gut gave no clear evidence of a consistent feeding rhythm, either for adult female or for Stage V *Calanus*. A typical series of observations is illustrated in Fig. 2. Each horizontal line illustrates the behaviour of an individual copepod and the vertical columns correspond to the hour of the observations. The quantity of food in the gut is given in an arbitrary scale—a column blacked to its full height means that the gut was quite full; three-quarters its height means that the anterior part of the gut was full but the posterior half was empty (usually because a faecal pellet had been passed out recently); half the height that the gut was only partly full, and one-quarter that only a small quantity of food was present. A white column means that the gut was empty.

It can be seen from the diagram that most of the adult females were feeding continuously throughout the 24 hr., the few drops in the columns were almost certainly due to the ejection of a faecal pellet just before examination. They were not, of course, perfectly consistent, as numbers 2 and 7 show. Stage V's (Fig. 3) seemed to be much less voracious and were much less predictable in their behaviour. Although feeding was not continuous and long gaps apparently intervened between meals, no consistent rhythm of feeding and resting can be deduced from these data. One point, however, emerges very plainly.

Calanus can spend a long time in a dense suspension of food without feeding —some observations were carried on over several days, and although the

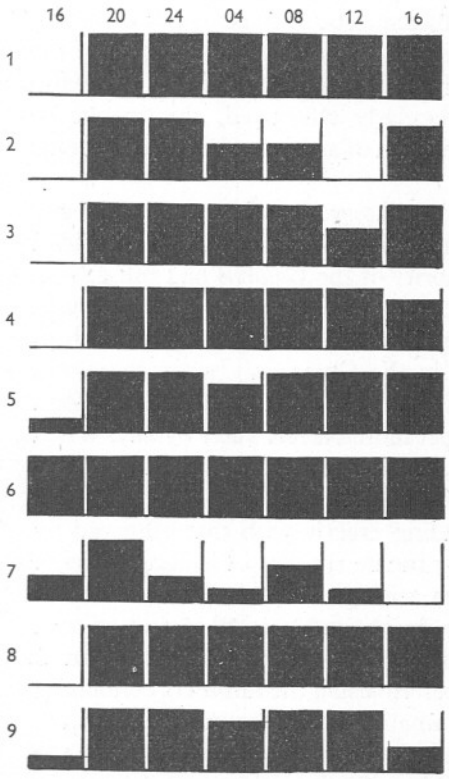

Fig. 2. Feeding of adult female *Calanus*. For explanation see text.

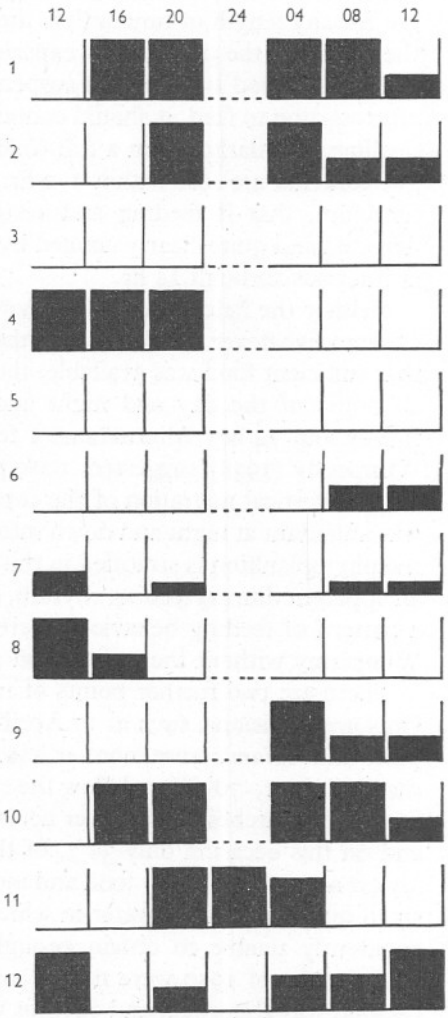

Fig. 3. Feeding of Stage V *Calanus*. For explanation see text. A broken line in 24 hr. column indicates that no observations were made at midnight.

copepod appeared to be perfectly healthy and was seen, at least on some occasions, swimming normally, no food was ever observed in the gut.

Discussion

A copepod cannot be feeding, i.e. filtering water through its maxillae and ingesting the material accumulating on the maxillae, and yet have an empty gut for any length of time unless the water is nearly or absolutely barren. As the results of the preliminary experiments show, the gut fills up quite rapidly when a copepod is put into a suspension of food and, within an hour at most after starting to feed, it should contain sufficient food to make it clear that it is feeding. Similarly, when a full *Calanus* is put into barren water most of the gut contents are lost within 1–2 hr. of the cessation of feeding. It is plain, therefore, that if feeding and resting regularly alternated, this would be detected and quite clearly defined by estimation of amount of the gut contents at intervals through 24 hr.

Neither the field observations nor the laboratory experiments provide any evidence whatever of a diurnal rhythm of this kind. On the contrary, provided that sufficient food was available, the majority of the *Calanus* had full guts at all hours of the day and night and apparently were feeding continuously throughout 24 hr. Alternation of feeding and non-feeding periods, such as Wimpenny (1938) suggested, took place in the Clyde only when there was marked vertical migration of the copepods, into the surface water where food was abundant at night and down into deepet more barren water by day. When the phytoplankton is stratified in this way, diurnal vertical migration produces an apparent diurnal feeding rhythm, as was seen on many occasions in 1951—a pattern of feeding behaviour corresponding exactly with that observed by Wimpenny without the postulation of any innate rhythm in feeding activity.

There are two further points of interest arising out of these observations. On some occasions, such as 17 April 1950, no obvious vertical migration took place and *Calanus* were more or less evenly distributed at least as far down as the lowest net, 70–80 m. below the surface. As usual the numbers containing food in the catches of the lower nets were smaller than those in the upper net, and on this occasion only 39% of the *Calanus* (females and Stage V) in the lowest net contained any food and most of these (29% of the total) only a very small quantity. In the water in which they were caught these copepods were apparently unable to obtain enough food to maintain themselves. If the observations of 1950 were not all made on occasions when the behaviour of *Calanus* was abnormal, and obvious vertical migrations do not take place over long periods, it may yet be possible that although the population was not migrating up to, and down from, the rich surface layers all together as it does when the copepods display normal migratory behaviour, it was in a continuous state of flux, some *Calanus* migrating upwards to feed and others downwards out of the rich water at all times of the day. What stimuli would induce these movements in the individual *Calanus* is difficult to say unless they are hunger and repletion. In the absence of such movement, a considerable

fraction of the population apparently remained in deep water where the copepods in time would starve.

Secondly, on most occasions in 1951 vertical migration was clearly marked and the copepods spent the daylight hours in deep water, below the strata where food is abundant. In previous work (Gauld, 1951) I was able to show that *Calanus* was able to filter about 80 ml. in 24 hr., and the amount of food required by *Calanus* in 24 hr. has been estimated as 0·002–0·013 mg. (dry weight) (Marshall *et al.*, 1935). If the vertical migration is taking place, this amount of food must be collected not in 24 hr., but during the hours of darkness only, i.e. in 8–12 hr., depending on the season, and must be present not in 80 ml. but in 30–40 ml. of sea water.

Summary

Records were made of the presence or absence of food in the guts of *Calanus finmarchicus* caught in three different depths, at intervals of 4 hr. through 24 hr.

It was found that 80–100% of the *Calanus* caught at the surface were full of food at all hours of the day.

The number of *Calanus* containing food in deeper water was distinctly less. This may be correlated with the abundance of its food close to the surface.

Diurnal vertical migrations took place on some occasions when samples were taken, but not on all.

In the absence of vertical migration *Calanus* was abundant at the surface and feeding continuously at all hours of the 24 hr. Where vertical migration took place feeding was mostly at the surface and was restricted to the hours of darkness, i.e. in summer to a period distinctly less than 8 hr. round midnight.

Laboratory observations confirm the absence of any feeding rhythm.

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NOTE ON THE ABSORPTION OF ORGANIC PHOSPHORUS COMPOUNDS BY *NITZSCHIA CLOSTERIUM* IN THE DARK

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It has been observed by Chu (1946) that the marine diatom *Nitzschia closterium*, in bacteria-free culture, grows in the light with inositol hexaphosphate as phosphorus source, and with glycerophosphate.

When this diatom is grown with inorganic phosphate as phosphorus source, after having used all the phosphate in the medium, further divisions take place, the cells becoming phosphorus-deficient. Then, on adding phosphate, this is rapidly absorbed by the deficient cells both in the dark and in light (Ketchum, 1939).

Experiments have been made to find whether phosphorus-deficient cells will also increase their phosphorus content in the dark rapidly, if supplied with inositol hexaphosphate or with glycerophosphate.

Absorption of Inositol Hexaphosphate

A bacteria-free culture of *N. closterium*, obtained by subculturing after successive growths in media containing penicillin and streptomycin (Spencer, 1952), was grown in artificial light. Two days after the phosphate in the medium had been used by the diatom cells, equal volumes were transferred to centrifuge tubes. To each tube was added a solution containing 31 μg. P as the sodium salt of inositol hexaphosphate together with inorganic phosphate present as impurity.

One set of tubes was centrifuged at once and the liquid drained from the deposits of diatom cells, whose phosphorus contents were determined.

A second set of tubes was stored for 3 hr. in darkness, before centrifuging and determining the phosphorus content of the cells.

A third set of tubes was illuminated for 3 hr.

	Micrograms P in cells	
	Exp. I	Exp. II
Cells separated by centrifuging immediately after addition of organic phosphorus*	13·5	19
After 3 hr. storage in darkness†	35	42
After 3 hr. illumination	40	45

* The centrifugate contained 10 μg. phosphate-P.
† The centrifugate then contained 1·5 μg. phosphate-P.

Hence the increase in cellular P after 3 hr. darkness was twice as much as could have been supplied by the inorganic phosphate in solution, and indicates that 10–12 μ of organic P had been absorbed. The experiment does not indicate whether the organic phosphate was absorbed as such or converted by extracellular enzyme action to inorganic phosphate before absorption. However, a subsequent experiment using cells which were not phosphorus-deficient indicated very little, if any, conversion of inositol hexaphosphate to inorganic phosphate during 3 hr. storage in the dark.

Absorption of Glycerophosphate

A similar experiment was made in which (synthetic) glycerophosphate containing 45 μg was added to each tube containing equal volumes of phosphorus-deficient *Nitzschia* cells in suspension.

	Micrograms P in cells
Cells separated by centrifuging immediately after addition of glycerophosphate*	7·5
After 3 hr. storage in darkness	18
After 3 hr. illumination	20

* The centrifugate contained 3·3 μg. inorganic phosphate-P derived from impurity in the glycerophosphate.

This experiment indicates absorption of glycerophosphate as rapidly as of inositol hexaphosphate.

The sodium salt of inositol hexaphosphate was kindly supplied by Messrs Ciba Ltd.

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SYNTHESIS OF ORGANIC NITROGEN AND CHLOROPHYLL BY *NITZSCHIA CLOSTERIUM*

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(Text-figs. 1–4)

When cells of *Chlorella* are grown in a medium containing a limiting quantity of nitrate or ammonia as nitrogen source, photosynthesis and cell division continue after all the available nitrogen in the medium has been abstracted by the growing plants. The cells become nitrogen-deficient. When kept in darkness with added nitrate these nitrogen-deficient cells synthesize a considerable quantity of organic nitrogen (Ketchum, 1939) and use more oxygen in respiration than when kept in darkness without added nitrogen (Myers & Cramer, 1948, p. 106). Growing in darkness with glucose as a carbon source, they use more oxygen when supplied with nitrate than when supplied with ammonium, and even more carbon dioxide is evolved (Cramer & Myers, 1948, fig. 1), indicating a greater break-down of carbohydrate to supply the necessary energy for synthesis of organic nitrogen compounds from nitrate than from ammonium.

These observations of nitrogen metabolism in *Chlorella* have suggested that diatoms, and phytoplankton generally, may become nitrogen-deficient when the nitrogen source is reduced to very low concentrations, such as occur in the sea during summer, and in consequence are able to absorb and build up organic nitrogen during the night (Harvey, 1945, p. 133). In order to obtain more information concerning nitrogen metabolism in phytoplankton the following experiments were made.

The marine diatom *Nitzschia closterium* var. *minutissima*, free from bacteria, was inoculated into autoclaved sea water of 26‰ salinity enriched with phosphate, iron, manganese and a limiting quantity of nitrate or nitrite or ammonia. The cultures were aerated and illuminated continuously by fluorescent lamps. Under these conditions, after a short lag period depending upon the physiological state of the inoculum, exponential growth proceeds, slowing after the nitrogen source in solution has been absorbed by the cells and stopping some 2 days later, when the cells attain a 'stationary state', being fully deficient in nitrogen.

When these 'stationary' deficient cells were stored in darkness for periods up to 120 hr., there was no significant change in cell numbers, optical density (turbidity) of the culture, content of cellular nitrogen or of chlorophyll. On the other hand, when illumination was continued a slow decrease in chlorophyll occurred.

In order to follow the rate at which organic nitrogen is synthesized by nitrogen-deficient *Nitzschia* cells in the dark when supplied with nitrate, ammonia or nitrite, or mixtures of these, four experiments have been made. Samples of the culture after adding a nitrogen source and storing in the dark were withdrawn at intervals and centrifuged, the deposit washed with sea water, and the cellular nitrogen determined by a micro-Kjeldahl method (Harvey, 1951). The results are shown in Table I and Figs. 1 and 2 (*Exps. 1–4*).

TABLE I. SYNTHESIS OF ORGANIC NITROGEN BY NITROGEN-DEFICIENT *NITZSCHIA* IN THE DARK

Nitrogen added at start of storage period (μg./ml.)	Increase as percentage of initial quantity of organic nitrogen present in 1 ml. of culture after storage in dark		
	Exp. 1 Cells in 1 ml. culture contained 0·75 μg. N and had been 3–4 days in stationary phase		
	After 24 hr.	After 48 hr.	After 120 hr.
4 μg. NH_4-N	230	240	—
4 μg. NO_3-N	125	140	140
4 μg. NO_2-N	Not significant	17	67*
4 μg. NO_3+4 μg. NO_2-N	125	125	125
	Exp. 2 Cells in 1 ml. culture contained 2·0 μg. N, 4 days in stationary phase		
	After 24 hr.	After 48 hr.	After 120 hr.
8 μg. NH_4-N	127	127	120
8 μg. NO_3-N	70	81	85
8 μg. NH_4-N+8 μg. NO_3-N	130	129	—
	Exp. 3 Cells in 1 ml. contained 3·0 μg. N, 6 days in stationary phase		
	After 24 hr.	After 48 hr.	
8 μg. NH_4-H	147	143	—
8 μg. NO_3-N	73	73	—
4 μg. NO_2-N	10	13	—
4 μg. NO_3-N+4 μg. NO_2-N	73	76	—
	Exp. 4 Cells in 1 ml. contained 2·0 μg N, 36 hr. in stationary phase		
	After 19 hr.	After 43 hr.	After 96 hr.
4 μg. NH_4-N	162	166	—
4 μg. NO_3-N	100	138	150
4 μg. NH_4-N+4 μg. NO_3-N	145	167	—

* Analysis of the culture medium showed a loss in nitrite-N similar to the gain in organic-N by the diatom cells.

In order to determine which source was utilized when nitrogen-deficient *Nitzschia* was stored in darkness after the addition of both nitrate and ammonium, the following experiment was made.

Exp. 5. Nitzschia was grown with nitrate as nitrogen source. After 3 days in the stationary state, the culture was divided, additions made and the two suspensions stored 48 hr. in darkness. The ammonia in each (cells included) was then determined by microdiffusion (Conway, 1940).

Addition per ml. culture	Decrease in NH_4-N after 48 hr. in dark, per ml. culture (μg.)
4 μg. NH_4-N	3·92
4 μg. NH_4-H + 4 μg. NO_3-N	3·64

The experiment was repeated using a culture of N-deficient cells which had been grown with ammonium as the limiting nitrogen source.

Addition per ml. of culture	Decrease in NH_4-N after 48 hr. in dark, per ml. of culture (μg.)
4 μg. NH_4-N	3·80
4 μg. NH_4-N + 4 μg. NO_3-N	3·64

The experiment shows preferential synthesis from ammonia in darkness. It had been found previously (Harvey, 1940) that a mixed community of marine diatoms utilized ammonia in preference to nitrate when grown in light.

In *Exps.* 1 and 3 there was only a 17 and a 13 % increase in cellular nitrogen after 48 hr. dark-storage with added nitrite. It seemed possible that this small increase was not due to synthesis from nitrite, as such, but from ammonia or nitrate to which some of the added nitrite had been converted in the external medium, by oxidation or by reduction due to an extracellular enzyme. In order to explore this possibility the following experiment was made.

Exp. 6. *Nitzschia* was grown with nitrite as a limiting nitrogen source until the cells were in the stationary phase. The cells in 1 ml. culture then contained 2 μg. nitrogen. The culture was divided, to one portion nitrite only was added, to the other portion both nitrite and ammonium sulphate. After storage for 48 hr. in darkness, the nitrite in both portions was estimated with the following result:

Addition per ml. of culture	Decrease in nitrite concentration after dark storage (%)
2 μg. NO_2-N only	25
2 μg. NO_2-N + 2 μg. NH_4-N	2·5

Since there was no significant change in concentration where ammonium was also added, it seems unlikely that nitrite is oxidized or reduced in the medium before being slowly absorbed and utilized in darkness by the nitrogen-deficient cells, unless nitrite-oxidizing or reducing bacteria had entered the culture during the 48 hr. and had been inhibited by ammonia.

These dark-storage experiments point to the following conclusions:

(i) The presence of nitrate had little or no effect on organic nitrogen synthesis from ammonia in the dark, ammonia being utilized in preference to nitrate (*Exp.* 5).

(ii) The presence of nitrite had little or no effect on the utilization of nitrate in the dark (*Exps.* 1 and 3).

(iii) The quantity of organic nitrogen synthesized in the dark, by the same number of nitrogen-deficient diatom cells, from ammonia-N was greater than from nitrate-N. This is presumably because more organic matter is lost by

respiration when nitrate is utilized in order to provide the energy necessary for its reduction, and less remains for the synthesis of organic nitrogen (*Exps.* 1–4, Figs. 1 and 2).

(iv) Synthesis of organic nitrogen took place in the dark from either nitrate or ammonium by cells which could utilize nitrite only very slowly during a period of 48 hr. or more (*Exps.* 1 and 3, Fig. 1).

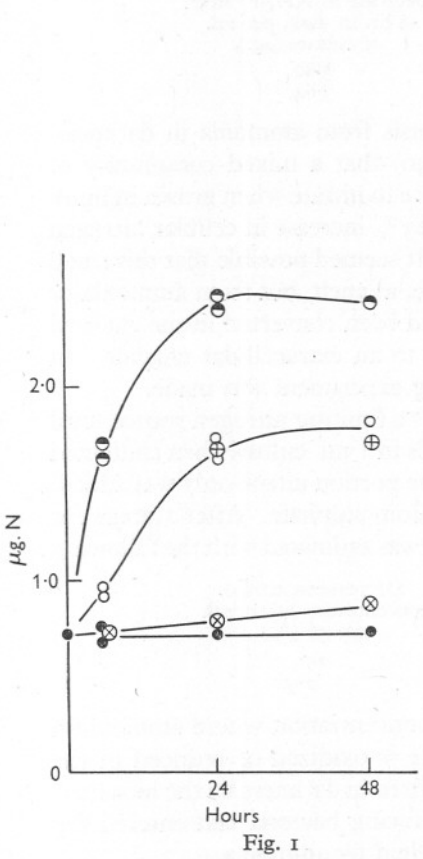

Fig. 1

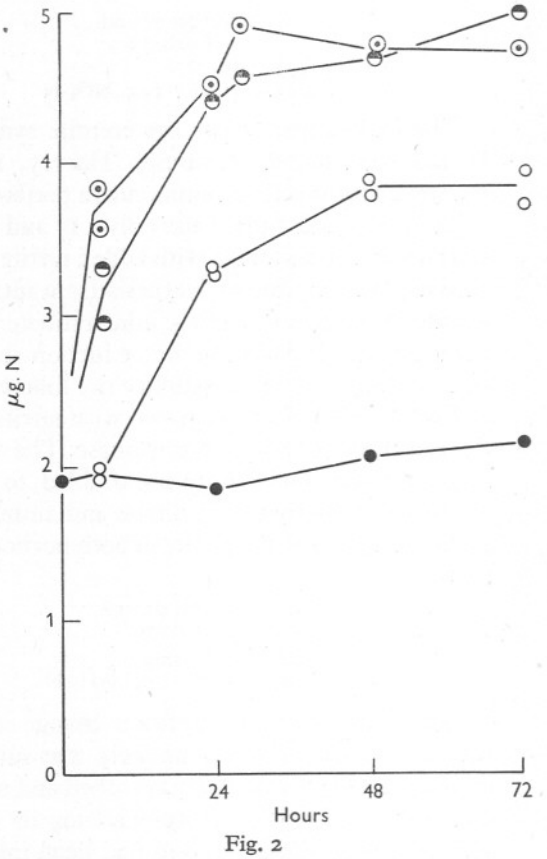

Fig. 2

Fig. 1. Micrograms organic nitrogen found in the cells contained in a cubic centimetre of nitrogen-deficient culture when stored in the dark after additions of nitrate, ammonia and nitrite (Exp. 1). ◓, 4μg. NH_4-N; ○, 4μg. NO_3-N; ⊕, 4μg. NO_3-N+4μg. NO_2; ⊗, 4μg. NO_2-N; all added before storage in dark; ●, no addition.

Fig. 2. Micrograms of organic nitrogen found in the cells contained in a cubic centimetre of nitrogen-deficient culture when stored in the dark after additions of ammonia and nitrate. ◓, 8μg. NH_4-N; ⊙, 8μg. NH_4-N+8μg. NO_3-N; ○, 8μg. NO_3-N; all added before storage in dark; ●, no addition.

A large number of observations have been made on the growth or division rate of this diatom at 17° C. at a light intensity which was such that a variation of ±30% had little effect. The cells were saturated with respect to light.

After an initial lag, exponential growth proceeded as rapidly with nitrite or nitrate as with ammonium as nitrogen source—the number of cells doubling in 9–10½ hr. (C. P. Spencer, private communication).

When deficient cells were stored in the dark with added ammonium, their nitrogen content doubled or more than doubled in 10 hr. (Figs. 1 and 2). The experiments provide no evidence that light enhances the rate of synthesis from ammonium nitrogen.

When deficient cells were stored in the dark with added nitrate, the rate of synthesis was much less. In Exp. 1, where the most rapid dark-synthesis was observed, some 18 hr. were required for the cells to double their nitrogen content. This suggests that light or some early product of photosynthesis may enhance the rate of synthesis from nitrate.

When deficient cells were stored in the dark with nitrite (*Exps. 1, 3* and *6*) very little or no synthesis took place in 10 hr.

This shows that some transient product of photosynthesis, or light energy, enhances the synthesis of organic nitrogen from nitrite very considerably.

When deficient cells are supplied with nitrite and illuminated, growth in numbers does not start until after a lag period of several hours. In order to ascertain the effect of light during this period, an experiment was made in which deficient *Nitzschia* with added nitrite were stored both in darkness and in the light of a north window. The nitrite remaining was estimated.

After 5 hr. in the dark there was a barely significant decrease in nitrite in the culture, whereas after 5 hr. illumination a third of the nitrite-N had disappeared.

Why is nitrate absorbed and reduced to amino-nitrogen quickly in darkness while nitrite is not reduced, although nitrite is the most probable first step in the series of reactions by which nitrate nitrogen is converted to protein-nitrogen?

Besides nitrogen-deficient *Nitzschia* in darkness, neither the pulp of wheat roots (Burström, 1946) nor the crown-gall tissue of sunflower (Ricker & Gutsche, 1948) can utilize nitrite, whereas they can utilize nitrate.

As stated by Burström, for wheat roots, this is inexplicable if free nitrite is produced as the first step in the reduction of nitrate. He suggests that nitrate is first absorbed becoming firmly bound to cytoplasm, and then reduced to nitrite firmly bound to plasm colloid, no nitrite ions being set free.

Although this explanation can account for nitrite not being utilized by wheat roots or sunflower tissue, it cannot wholly account for deficient *Nitzschia* not using nitrite in the dark yet using it in the light.

For utilization of nitrite in the light it is necessary to postulate that some early transitory product of photosynthesis either directly reduces the nitrite ions to oxime- or amino-nitrogen, or, alternatively, combines with the nitrite ions and allows reduction to proceed by enzyme action.

If this postulate is correct, then it is possible that during reduction of nitrate in the dark (when respiration is increased) a similar transitory product

is set free which enables nitrite, formed as an intermediate, to be further reduced to amino-nitrogen. Such would account for the behaviour of *Nitzschia*, wheat root and sunflower gall tissue, and bring the observed phenomena into line.

These speculations assume that nitrite is the first intermediate when nitrate is utilized. There is no direct evidence for this. Bonner (1950, p. 225), reviewing nitrate reduction by plants, writes 'it is entirely possible that in some cases the reduction of nitrate may proceed through other reactions than the series of steps to be expected on the basis of inorganic chemistry'. Thus there is the possibility that in darkness *Nitzschia*, wheat roots and sunflower gall tissue do not reduce added nitrate via nitrite, and that nitrite is only utilized when it can combine or react with early products of photosynthesis.

Solubility of Organic Nitrogen Compounds Synthesized in Darkness

The following experiment shows that three-quarters of the dark synthesized nitrogen is soluble, compared with one-fifth of the total nitrogen in nitrogen-deficient cells.

Exp. 7. A culture of *Nitzschia* which had been stationary for 36 hr. was divided into four portions, to which additions were made as shown below. After 43 hr. in darkness, duplicate samples from each were centrifuged and the nitrogen in the cells determined. Also duplicate samples were centrifuged, resuspended in distilled water, heated for a few minutes in a boiling water-bath, again centrifuged and the nitrogen in the leached cells was determined.

Addition per ml. of culture	Micrograms nitrogen in cells per ml. of culture: Cells untreated	After leaching cells in hot water	Soluble N (by difference)
No addition	1·96, 1·97: mean 1·96	1·59, 1·63: mean 1·61	0·35
4 μg. NH_3-N	5·15, 5·30: mean 5·22	2·57, 2·91: mean 2·74	2·48
With 4 μg. NO_3-N	4·64, 4·70: mean 4·67	2·48, 1·98: mean 2·25	2·42
4 μg. NH_3-N and 4 μg. NO_3-N added	5·34, 5·27: mean 5·30	2·55, 2·53: mean 2·54	2·76

It is seen that the increase in organic nitrogen due to synthesis from ammonia or nitrate is largely in the form of water-soluble organic matter, while from the nitrogen-deficient control cells only 18% dissolved.

Nitrogen source added	Increase in insoluble, and in water-soluble, nitrogen in cells per ml. of culture, due to synthesis in dark (μg.): Insoluble N	Soluble N
Ammonia	1·13	2·13 or 65%
Nitrate	0·64	2·07 or 76%
Both	0·93	2·41 or 72%

Chlorophyll Synthesis

During the course of the foregoing experiments it was noticed that the nitrogen-deficient cells became more heavily pigmented during storage in darkness with added nitrate or ammonia, and also that during the growth of a culture the cells became less heavily pigmented before growth in numbers ceased. These visual observations of the cells indicated that pigment synthesis was limited by the supply of available nitrogen to the plant, and that synthesis takes place in the dark if available nitrogen is added to the medium and synthesized by the cells.

These two aspects of pigment synthesis were examined in the following manner.

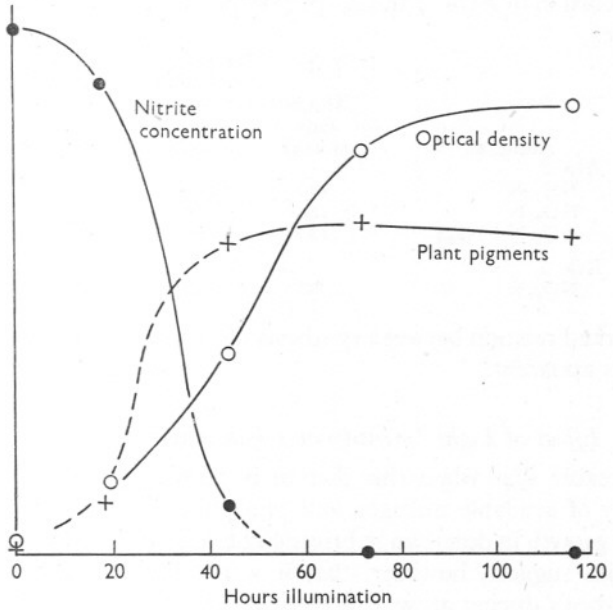

Fig. 3. Diagram showing the decrease in nitrite in the water, increase in optical density and in plant pigments in diatoms contained in unit volume of culture, of *Nitzschia* in 75 % sea water enriched with 3 mg. phosphate-P, 0·1 mg. iron, 0·02 mg. manganese and 2 mg. nitrate-N per litre (Exp. 8).

In *Exp. 8* a culture was grown with a limiting concentration of nitrite. At intervals samples were removed whose nitrite concentration and optical density were determined. The optical density provides a measure of the cell numbers. In the method of determination used the relation was almost linear during the earlier growth, but towards the end of growth cell numbers increased slightly more quickly than the optical density increased. Samples were also separated by centrifuging and the cells' pigments extracted with

boiling methanol. The yellow-green extracts were matched against a series of colour standards (Harvey, 1934; Riley, 1941). Since the tint was in all extracts a very exact match, the proportion of yellow pigments to chlorophyll in each was approximately the same.

The determinations (Fig. 3) show that pigment synthesis ceased after some 45 hr. illumination at about the time when all the nitrite in the medium had been utilized by the cells, which continued growth, making about one division thereafter.

After 120 hr. illumination, samples of the culture were stored in darkness with and without the addition of nitrate. After 96 hr. dark storage the pigment content of the cells with added nitrate had increased by 70%, while that of the cells without added nitrate remained the same.

At the conclusion of *Exps. 3* and *4*, (p. 478) plant pigments were determined in the cultures.

Addition	Hours dark storage	Percentage increase after dark storage due to the addition: In N	Percentage increase after dark storage due to the addition: In plant pigments
Exp. 2			
NH_4-N	120	120	75
NO_3-N	120	85	50
NO_3-N + Na_2S	120	60	25
Exp. 4			
NO_3-N	96	150	100

A well-marked relation between synthesis of nitrogen and of plant pigments in the dark is apparent.

Effect of Light Intensity on Chlorophyll Formation

It is noticeable that when this diatom is grown in media containing an ample supply of available nitrogen and phosphorus, the cells are more pigmented after growth in dim than in bright light. Visual observations with other marine species suggest, however, that it is not light alone which controls pigment synthesis during growth in culture.

Several experiments were made employing the following methods of estimation.

Light intensity was measured with a photometer calibrated in terms of mean noon daylight. The diatom cultures were illuminated with white light from fluorescent lamps. This light differs from daylight in being composed of a number of wave-length bands dispersed throughout the spectrum, although it is similar in respect to the mean intensities of each spectral colour. Since the sensitivity of the photometer varies throughout the spectrum, determination of the intensity of this white light only approximates to the values recorded as mean noon daylight, since this has a different composition in detail. However, in terms of overall intensity or light energy the recorded values should be a

good approximation. The greater light intensity used in the experiments (12,000 lux) is similar to that in a north window on a moderately bright day.

Chlorophyll was measured either by determining the optical density of the yellow-green methyl alcohol extract in red light or with a spectro-photometer at the wave-length for maximum absorbtion near 655 μ.

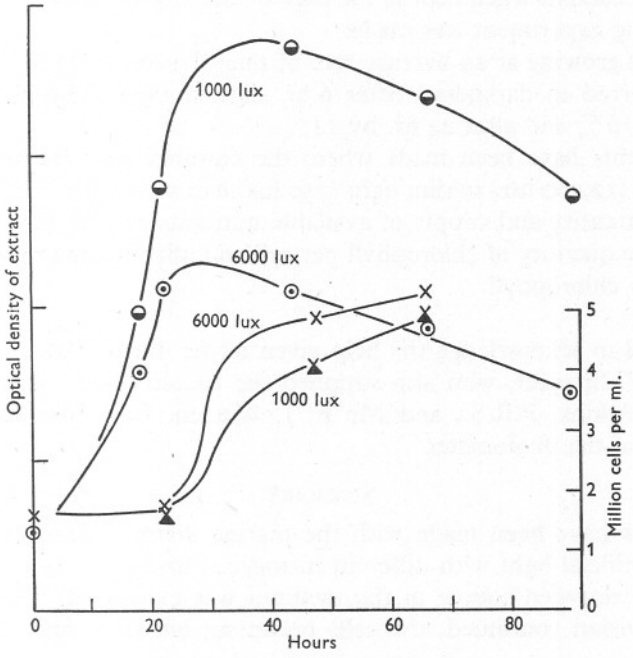

Fig. 4. Diagram showing chlorophyll formation and destruction in a culture with limiting supply of available nitrogen, illuminated at 1000 and at 6000 lux (Exp. 9).

In *Exp.* 9 sea water was diluted to 26‰ salinity and pasteurized, enriched with 3 μg./ml. phosphate-P, 2 μg. ammonium-N per ml., iron and manganese, and was inseminated with *Nitzschia*, and divided into two Roux flasks.

One was illuminated at 1000 lux and the other at 6000 lux.

The course of chlorophyll synthesis and proliferation of the cells is shown in Fig. 4. I am indebted to Dr C. P. Spencer for haemocytometer counts of the cell numbers.

The inseminum was an old culture, and over 24 hr. elapsed before cell numbers increased; meanwhile a considerable synthesis of chlorophyll had taken place.

After 45 hr. more than twice as much chlorophyll had been synthesized per cell in those illuminated at 1000 lux as in those at 6000 lux.

Thereafter the chlorophyll in both cultures decreased, due to destruction by light, at a rate of half per cent per hour.

After 90 hr. illumination the cultures were stored in darkness for 6 days; no change in chlorophyll content occurred.

In order to find whether cells growing exponentially in moderate illumination in media rich in available nitrogen and phosphorus would increase their chlorophyll content when kept in the dark or transferred to dim illumination, the following experiment was made.

A culture growing at an average rate of one division in 10 hr. in 8000 lux was transferred to darkness. After 6 hr. dark-storage chlorophyll had increased by 10% and after 24 hr. by 14%.

Experiments have been made where the cultures were transferred from bright light (12,000 lux) to dim light (750 lux) and vice versa. They indicated that light intensity and supply of available nutrients are not the only factors affecting the quantity of chlorophyll per cell and affecting the ratio of yellow pigments to chlorophyll.

I am glad to acknowledge the help given to me during these experiments by Dr C. P. Spencer, who also supplied the bacteria-free cultures. To Dr W. R. G. Atkins, F.R.S., and Mr F. J. Warren, I am also indebted for calibration of the photometer.

Summary

Experiments have been made with the marine diatom *Nitzschia closterium* grown in artificial light with different nitrogen sources.

After the nitrogen source in the medium was exhausted, photosynthesis and cell division continued, the cells becoming nitrogen- and chlorophyll-deficient.

On adding a nitrogen source to deficient cells and storing in darkness, synthesis of organic nitrogen, mostly water soluble, proceeded rapidly from ammonium, less rapidly from nitrate and very slowly from nitrite, with which it grows in light as rapidly as with nitrate or ammonium nitrogen.

Possible reasons why nitrate but not nitrite is reduced in the dark are discussed.

Chlorophyll and yellow pigments were synthesized in the dark by nitrogen-deficient cells in quantity related to the organic nitrogen synthesized.

Chlorophyll was synthesized in small amount by non-deficient cells which had been growing rapidly before transfer to darkness.

Cells contained less chlorophyll when grown in moderately bright than when grown in dim light.

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SEASONAL VARIATIONS IN THE PHOSPHATE AND SILICATE CONTENT OF SEA WATER

PART VI. 1948 COMPARED WITH THE 1923–25 PERIOD

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The Plymouth Laboratory

(Text-figs. 1–4)

The fifth paper of this series (1930) was the author's last, after which the work was handed over to L. H. N. Cooper. It was resumed by the author in 1948 to compare the early years with the post-war, since the determinations had lapsed. Measurements of the extinction coefficient of the water were also resumed, and it was desirable to obtain the chemical data at precisely the same time.

The phosphate results are, as before, expressed as P_2O_5 in mg./m.^{3}, since for crop calculations this old unit is convenient; to convert to mg. P, which is more rational, multiply by 0·437; to bring to milligram atoms multiply by 0·0141. The analyses have not been corrected for salt error, since the early results were not, and the factor for this correction has not as yet been agreed upon.

The silicate results are given as mg./m.^{3} of SiO_2, but though the standard used in Plymouth was checked for me by Dr E. J. King in Canada and agreed with his (King & Lucas, 1928), yet a different value had to be assigned to the silica factor of the picric acid solution. All values have accordingly been multiplied by 1·44.

The phosphate results for E 1, surface, from 11 February 1948 to 1 February 1949 are shown in Fig. 1 plotted against the dates. When one compares the phosphate with the corresponding values for 1923 and 1925 it is obvious that the winter concentrations are greater in the earlier years. But when considering fishery yields it is not enough to compare the winter maxima, the summer minimum must be taken into account and the difference fixes the minimum value of the plant production—minimum because some is used over again during the year. The years 1925 and 1948 were alike in having a large spring outburst, so that most of the phosphate was used up by April or May. In Fig. 2 one sees the concentration of phosphate at each depth. The maximum consumption for 1923 was reached by 10 July, and was 29·6 mg./m.^{3}. (This for calculation was rounded to 30 mg. and led to the value 1400 tons wet weight of phytoplankton per square kilometre down to 70 m.) Now taking

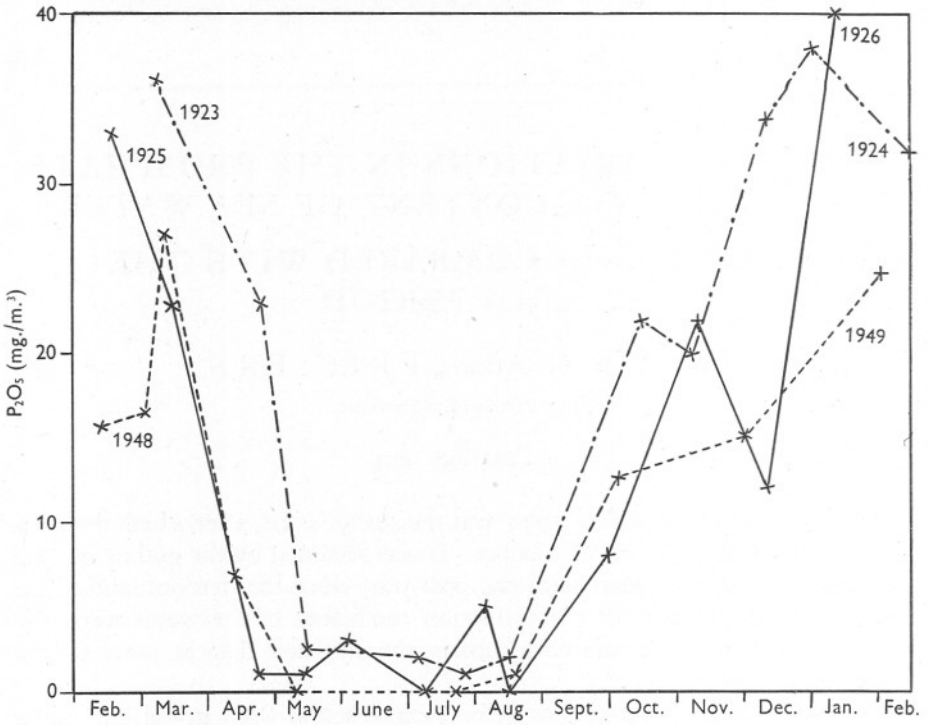

Fig. 1. Phosphate at Station E1 (50° 02′ N., 04° 22′ W.), surface. Dash and dot, 1923; full line, 1925; broken line, 1948; each year continued into the early part of the next.

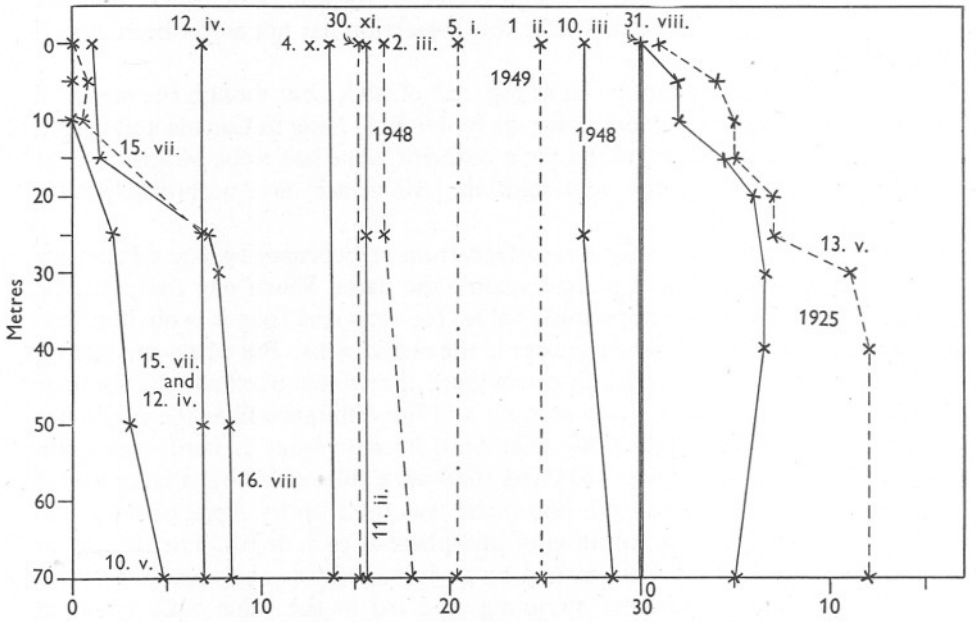

Fig. 2. Phosphate in water column at dates shown. Left-hand frame for 1948, some lines broken for clarity, and 1949, two only, dashes with dots. Right-hand frame for 1925.

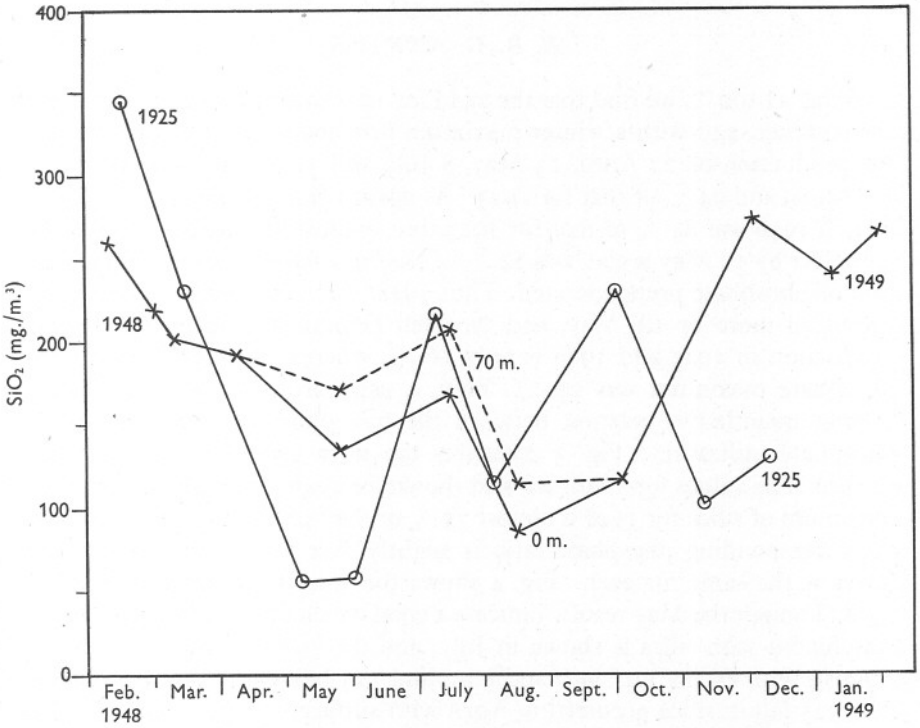

Fig. 3. Silicate at Station E 1, surface. Full line 1948 to 1949, surface and bottom in winter; broken line 70 m. when distinguishable. Full line 1925, surface.

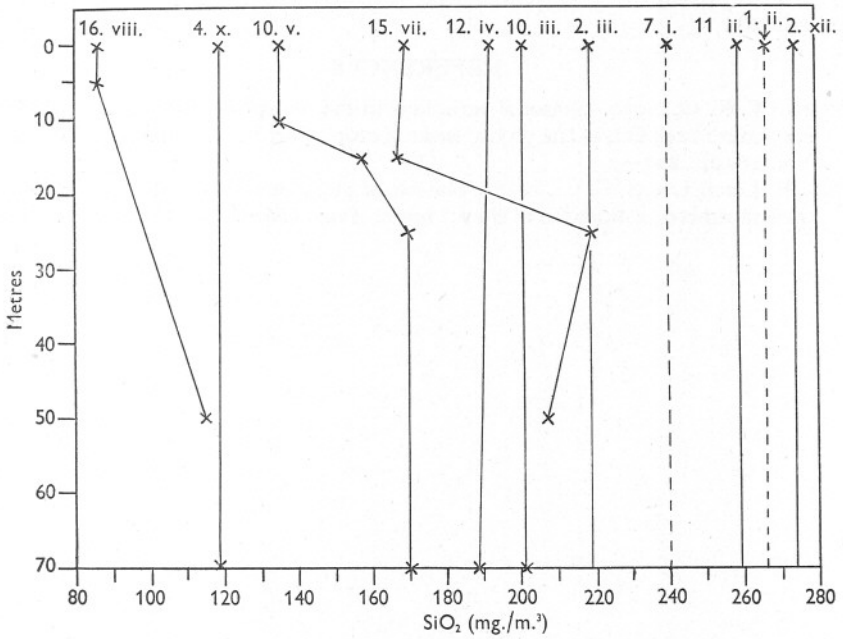

Fig. 4. Silicate in water column at dates shown, 1948, with two dates in 1949, broken line.

29·6 mg. as 100 % we find that the yield for 1925 increased a little throughout the summer, and with a winter maximum just under 92 % of that for 1923, the production by 22 April, 13 May, 8 July and 31 August was respectively 80, 80, 89 and 94 % of that for 1923. As against this the winter maximum in March 1948 was 84 % of that for 1923, but because the utilization was more complete by 10 May it also was 84 % of the 1923 value after which regeneration of phosphate preponderated. Thus 1948, with less phosphate than 1925, produced more up till May, and then fell behind. The difference between production in 1923 and 1948 is only 16 %, whereas the decrease in winter phosphate maximum was 25 %. Thus it can hardly be said that there is a strict quantitative relation between the free phosphate supplies and net phosphate utilization. Fig. 3 compares the silica cycle for 1925 and 1948, surface. The values for 70 m. are also shown for 1948, dotted line. The winter maximum of silica for 1948 is almost 75 % of that for 1925 and very curiously the corresponding phosphate ratio is slightly over 75 %—the ratio may be taken as the same for each. Fig. 4 shows the silica variation with depth for 1948. Though the May results indicate a good development of phytoplankton, enrichment with silica is shown in July, and the year's silica minimum is in August, with 86 mg./m.^{3} at 0 and 5 m. and 115 below the thermocline. But the 1925 fall in silica occurred in April with surface 58 mg., which by 3 June had become uniform down to 70 m. It should be possible to get an idea as to whether diatoms or non-siliceous algae had been mainly responsible for a plankton outburst by a comparison of the relative decreases in phosphate and silicate.

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THE SEASONAL VARIATION IN THE COPPER CONTENT OF SEA WATER

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(Text-fig. 1)

In previous papers (1932, 1933) the author showed that sea water taken from the English Channel contained about 10 mg./m.^{3} of copper, and obtained agreement between electro-deposition with subsequent colorimetric analysis and colorimetric analysis carried out on sea water concentrated to one-fifth of its volume. For extraction of the copper complex, carbon tetrachloride was found to be the most convenient solvent. The changes throughout the year have, however, only recently been determined. It is of interest to see that water from Station E 1, surface, taken 23 March 1931, gave 10 mg./m.^{3} by direct colorimetry and 9·7 with electro-deposition, whereas the extraction method curves for 1948 and 1949, read off for the same date, showed respectively 9·4 and 11·4 mg./m.^{3}.

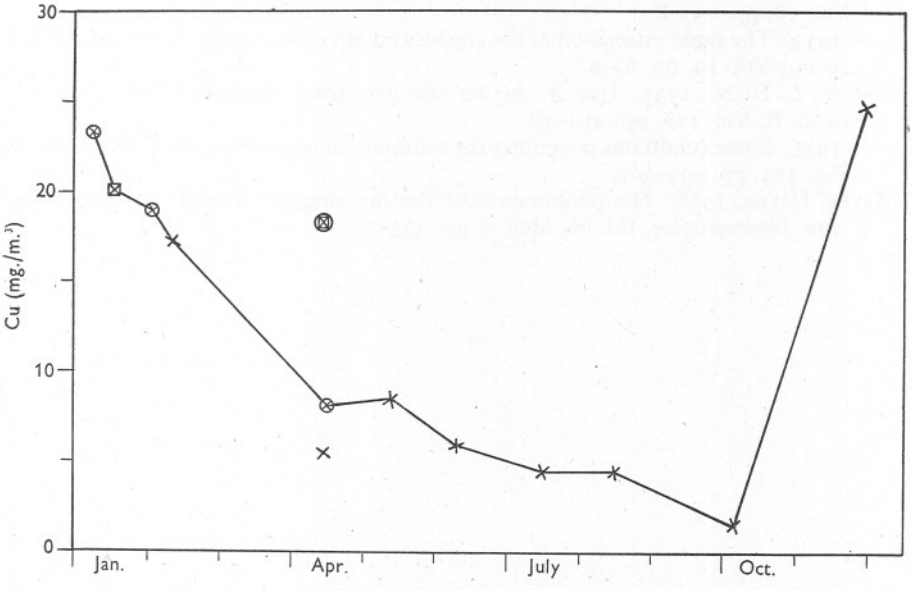

Fig. 1. The abscissae show months, the ordinates copper in milligrams per cubic metre of sea water. Results for surface water at International Hydrographic Station E 1 (50° 02′ N., 04° 22′ W.) are denoted by crosses for 1948, crosses in circle for 1949—with one in a circle and a square also for 50 m., and by a cross in a square for 1950, one only, in January.

Early attempts to examine deep water were given up on account of the solubility of the metal bottle, but in 1949 a plastic-lined bottle was temporarily satisfactory and, on 13 April, 50 m. gave 18·3 mg. Cu as against 8·1 for the surface, sampled with a wooden bucket. Copper is thus a minor constituent which is taken up by the phytoplankton. The results for the surface water during 1948 and 1949, together with one observation for January 1950, have been combined in a single curve, as shown in Fig. 1, in which, after an autumn minimum of 1·5 mg./m^3, a winter maximum 24·8 is reached. The colour given by the high values was a rather browner tint than expected, so presence of iron was suspected. It had, however, been shown in the 1932 paper that it required 1·32 mg./l. ferric iron to equal in depth of colour 0·01 mg./l. copper. Moreover, Cooper (1935, 1937) showed that no more than 2·2 mg./l. ferrous iron could remain in solution in sea water and that the amount as ferric in solution was far beyond colorimetric detection. Ferrous iron could thus have introduced no serious error in the copper analyses. Miscellaneous analyses by Meyer (1938) showed 6–15·5 mg./m.^{3} copper in the Baltic, 9–26 mg./m.^{3} in Kiel harbour, 10 mg./m.^{3} in the North Sea, 8–11 mg./m.^{3} in the Bay of Biscay, with 12 mg./m.^{3} in the Canary Stream.

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SEASONAL CHANGES IN THE PHYTOPLANKTON DURING THE YEAR 1951–52 AS INDICATED BY SPECTROPHOTOMETRIC CHLOROPHYLL ESTIMATIONS

By W. R. G. Atkins, F.R.S.
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The Plymouth Laboratory

(With Text-figs. 1–8)

In a previous paper (Atkins & Parke, 1951) the phytoplankton was studied by the collodion filtration of 20 l. samples, the extraction of the filter disk with 80% aqueous acetone and the comparison of the extracts in a Kober colorimeter against each other and a commercial chlorophyll preparation. The interference of pigments other than chlorophyll was eliminated or much reduced by the use of a Schott RG1 filter, which we subsequently found had previously been thus used by Rohde (1948). There was, however, frequently a delay in the filtration of our large samples and an increase in accuracy was desirable.

For the present work we were fortunate in having a Unicam spectrophotometer with 4·0 cm. rectangular glass vessel for which 10 ml. of extract sufficed. This was prepared usually the day after the water had been collected, and 1 or 2 l. of water were sufficient, according to season. Unfortunately, the 4 cm. vessels got broken, so for a time 1·0 cm. vessels were used and the results obtained for March and April were necessarily less accurate. The measurements of percentage transmission, as adjusted against a blank with solvent, showed a strong absorption in the red and an even stronger one in the blue-violet. Results were, however, based on the minimum transmission in the red, since the shorter wave-absorption band was frequently masked by a general absorption occasioned by carotins and xanthophylls. The accuracy of the spectrophotometer was checked at 6563 and 4861 Å. and found to be exact, using the hydrogen lamp.

The Spectral Absorption Curves

In Fig. 1 the maximum absorption percentage of the red absorption band at 635 mμ is plotted against the concentration of the commercial chlorophyll in mg./l. The relation is strictly rectilinear up to 4·0 mg./l., and by 8·0 has fallen to 31·8% against 35·8 for rectilinearity. It would appear from the position of

the band maximum that the preparation contained both chlorophyll *a* and *b*. Fig. 2 shows the absorption curves of two Chlorophyceae, in pure culture, for which we are indebted to Dr M. Parke. That for *Chlorella* I (by F. Gross) shows the chlorophyll absorption bands with minimum transmissions at 655 and 420 mμ, but for *Chlamydomonas* III (by Mrs Foyn), a solution of greater

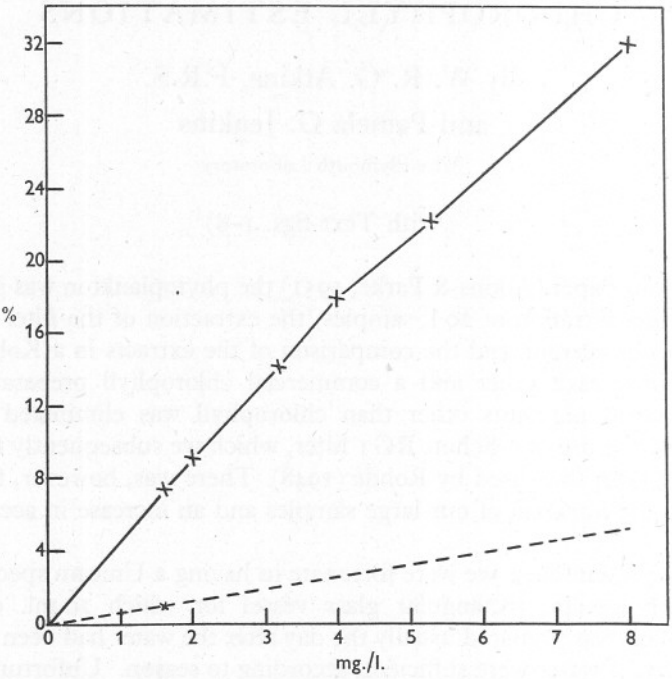

Fig. 1. Percentage absorption at 635 mμ (the position of minimum transmission in the red) for an 80 % aqueous acetone solution of a dry commercial chlorophyll the concentrations of which are given on the abscissae in mg./l., equivalent to 10 mg./m.^{3} when using 10 ml. plankton extract from 1 l. of sea water.

density also has a minimum at 655 mμ but shows complete absorption before the 420 mμ band has been reached. The same is shown by a strong solution, 320 mg./l. of the commercial chlorophyll, which gave maximum absorption at 640 mμ.

In Fig. 3 is shown an extract of *Hemiselmis rufescens*, with sharp absorption maxima at 655 and 435 mμ, also the commercial chlorophyll, 16 mg./l., showing maxima at 635 and 410 mμ. The characteristic buff colour of the alga is due to the presence of a water-soluble crimson, which remains on the collodion or paper and is not dissolved by the aqueous acetone. The wider bands exhibited by the chlorophyll solution are probably due to admixture of the two forms.

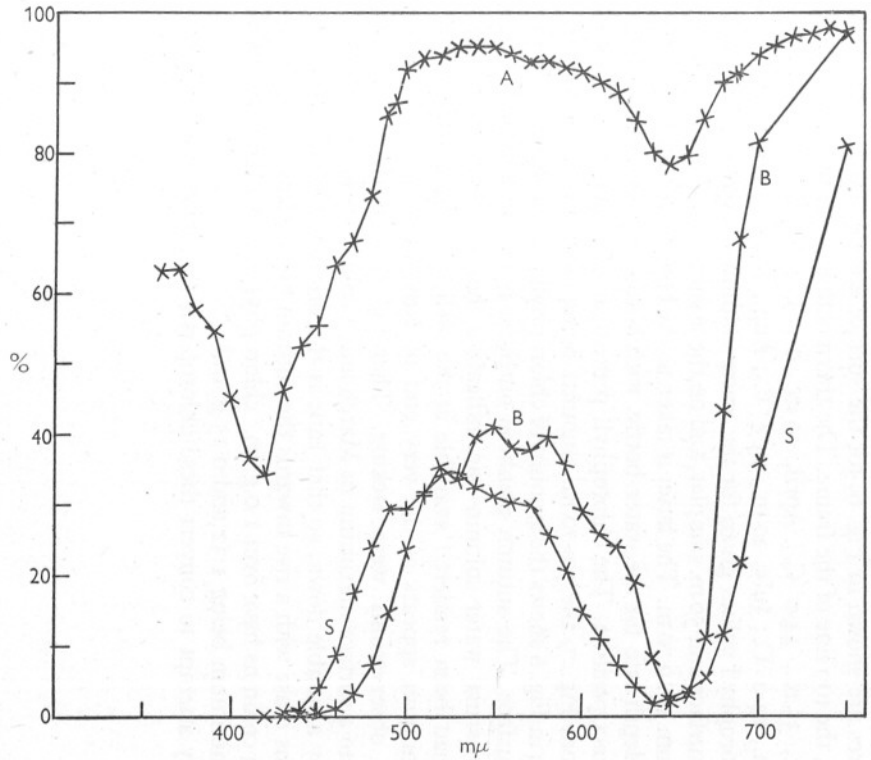

Fig. 2. Percentage transmissions from: S, a stock solution of a dry commercial chlorophyll in acetone, 90 %, with water; A, an 80 % acetone extract of a culture of *Chlorella* I; and B, the same of *Chlamydomonas* III. The abscissae in this and the three following figures show wave-lengths in millimicrons.

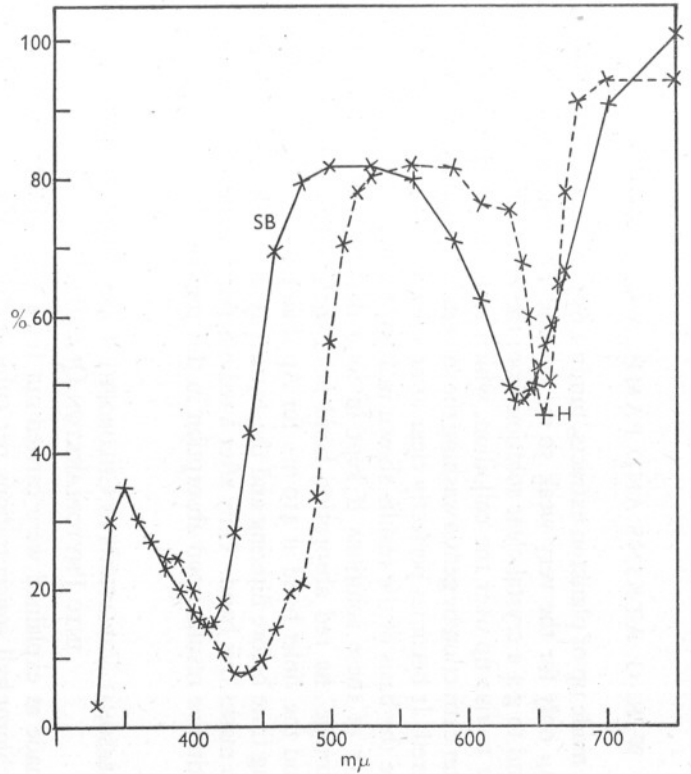

Fig. 3. Percentage transmissions from: SB, an S/20 dilution of the chlorophyll; H, an 80 % acetone extract of a growth of *Hemiselmis rufescens* Parke, developed from water of station E 1, 50 m., 7 January 1952 with a very little *Nitzschia closterium*.

Fig. 4 is made up of plankton extracts, minima 655 and 435 mμ for surface, and 650 mμ only for the very weak 50 m. sample. To avoid turbidity from fine clay and to get a crystal clear solution from the extract it is best to allow the solvent to rise up over the collodion, which rests on the side of a small beaker. After extraction by gentle washing the solvent is drained back into a tube and stoppered. It becomes perfectly clear after a few hours in the dark. Such extracts are the basis of the results shown in Figs. 6 and 7. Fig. 5 illustrates the stability of these solutions if kept in total darkness; after more than 3 years' storage the red absorption band is in each clearly a maximum at 650 mμ, and the violet band at 410 mμ for April and June. The July sample stood a long time before filtering and the extract is obviously of a yellowish tint, which masks this band. Only after a tedious purification is it possible to base quantitative results upon absorption in this region.

The Seasonal Variations in Chlorophyll and their Conversion into Phytoplankton Quantities

Extracts made as explained were carried out for a year, and the results are shown in chlorophyll concentrations per cubic metre in Fig. 6, surface and 50 m., and at intermediate depths when a thermocline existed. These desirable samples were unfortunately not taken in the isothermal March water. The sea temperatures are shown in Fig. 6, for the surface above, and for the bottom just below, the top line of the frame. The thermoclines were found as follows: September, 35 m., 14·0° C.; April, 10 m., 9·7° C.; May, 10 m., 11·7° C.; June, 15 m., 14·0° C.; July, 20 m., 15·8° C.; Aug., 20 m., 15·6° C.

The chlorophyll values given for the water column are based on the mean values for surface and 50 m. samples, and on the assumption that the water was uniform from 50 to 70 m. The latter is taken as the bottom at station E 1, but is actually a depth safe for the water-bottle, with bottom 72–74 m. according to tide and exact position. The chlorophyll present at intermediate depths was read off from Fig. 7, the 50–70 m. amounts being calculated as before. The broken line in Fig. 6 shows the amount of chlorophyll in the column per square metre of surface. The autumn plankton outburst is in late September in an almost isothermal water column—an indication that the deep water mineral nutrients had been rendered available at the well illuminated depths. The winter minimum appears at the very end of November, possibly because December observations were lacking. There is then a rise, steep after February, to a surface maximum in March and a column maximum in April, followed by a tumble down, so that June is the minimum for the year. The observations cease with a rise towards the autumn high. Thus the September and March columns have over 1·0 g./m.^{2} chlorophyll, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}.

One may attempt to convert these chlorophyll quantities into weights of

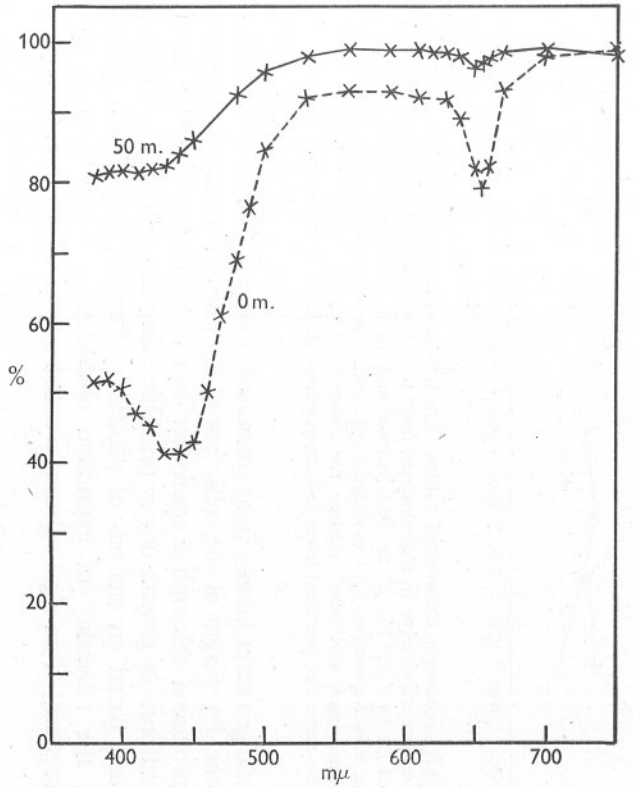

Fig. 4. Percentage transmissions given by extracts from plankton, collodion filtered from 0 and 50 m. 2 l. samples of water from station E1, 11 March 1952.

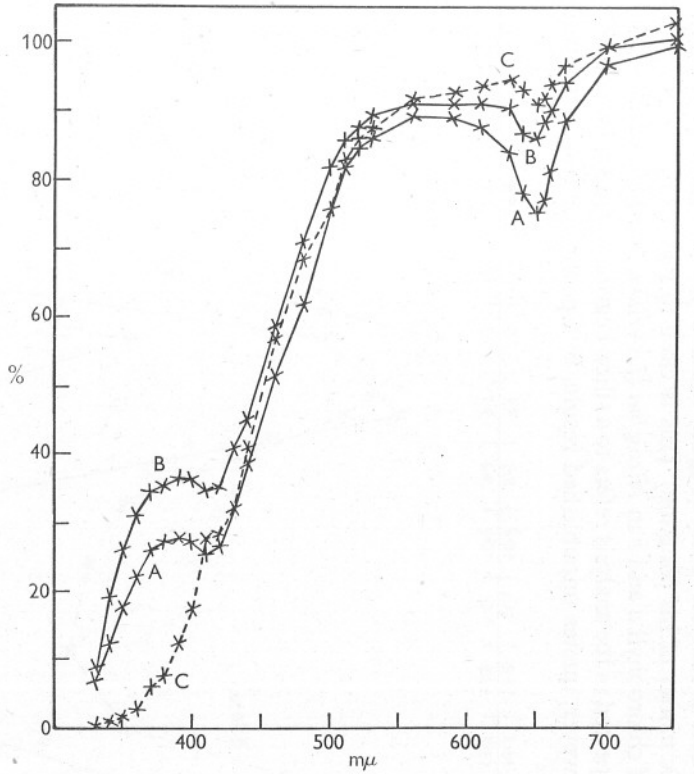

Fig. 5. Percentage transmissions given by extracts qualitatively similar to those of Fig. 4, but from about 20 l. samples through paper using larger volumes of aqueous acetone. Those from station E1, surface, were for April (A), June (B) and July (C), 1949. There was delay in filtering and samples were stored in the dark for over 3 years.

phytoplankton as follows. Riley (1941*a*) found 2·91 % of chlorophyll in the dry organic matter of his plankton. This is close to Pace's (1941) 2·32 % for the sum of chlorophyll *a* and *b* in *Nitzschia closterium*, in which a lower value is to be expected as the analysis refers to a silica-bearing organism only. Strain (1951), however, quotes unpublished results by Spoehr and Milner showing

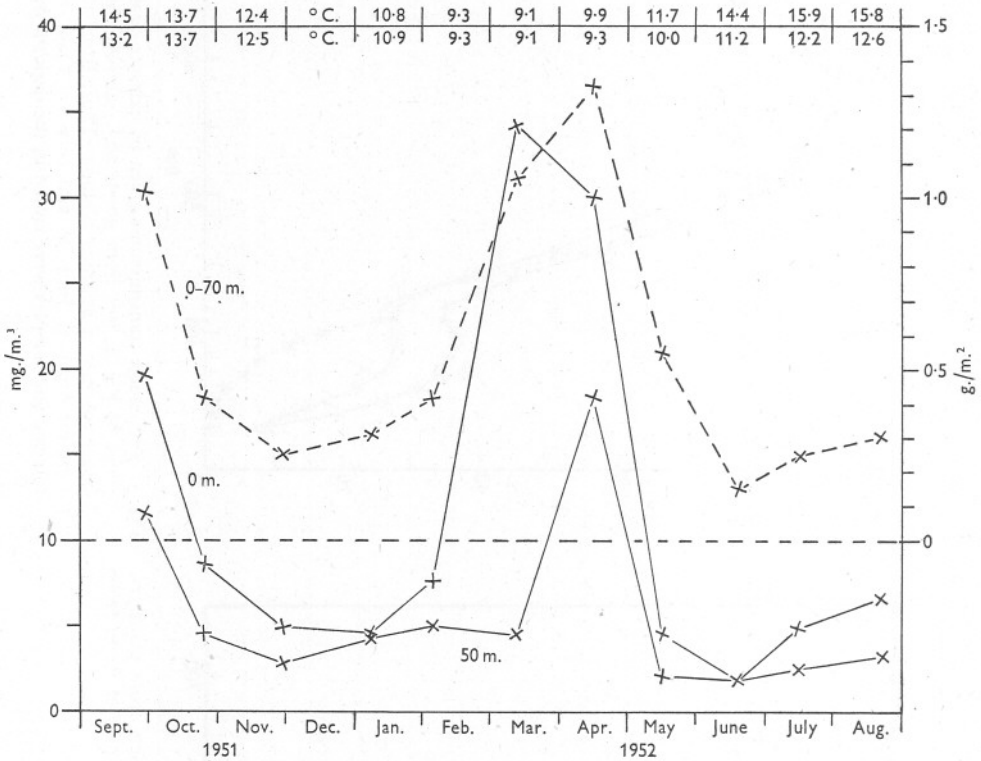

Fig. 6. Monthly variation in chlorophyll values. The left-hand ordinates (for continuous line graphs) denote chlorophyll in mg. obtained from the phytoplankton of 1 m.³ of sea water at station E 1 (50° 02′ N., 4° 22′ W.), surface and 50 m. The abscissae show dates of sampling from September 1951 to August 1952 inclusive. The right-hand ordinates (for broken line graph and base) indicate the chlorophyll in the water column, 0–70 m., per square metre, as obtained from data represented in this figure and Fig. 7.

that *Chlorella* grown in intense light contained chlorophyll equivalent to only 0·03 % of the dry weight of the cells; but in light of low intensity, the same organism produced chlorophyll equivalent to 6 % of the dry weight. Under normal conditions of growth one is probably fairly near the truth in taking Riley's value, based on analysis of plankton. Furthermore, Rohde, when discussing the reliability of extinction coefficient measurements in the quantitative study of an algal culture, concludes with 'if possible, therefore,

one should carry out counts of the cell or colony concentration and determinations of the chlorophyll content in parallel with the photometric determinations'. Taking 2·91% of chlorophyll gives a factor 34·5 by which the weight of dry matter may be found from the pigment. To convert to wet weight the dry weight is assumed to be 20% (Atkins, 1923), as was done in the calculation of the phytoplankton crop from phosphate consumption. When one adds

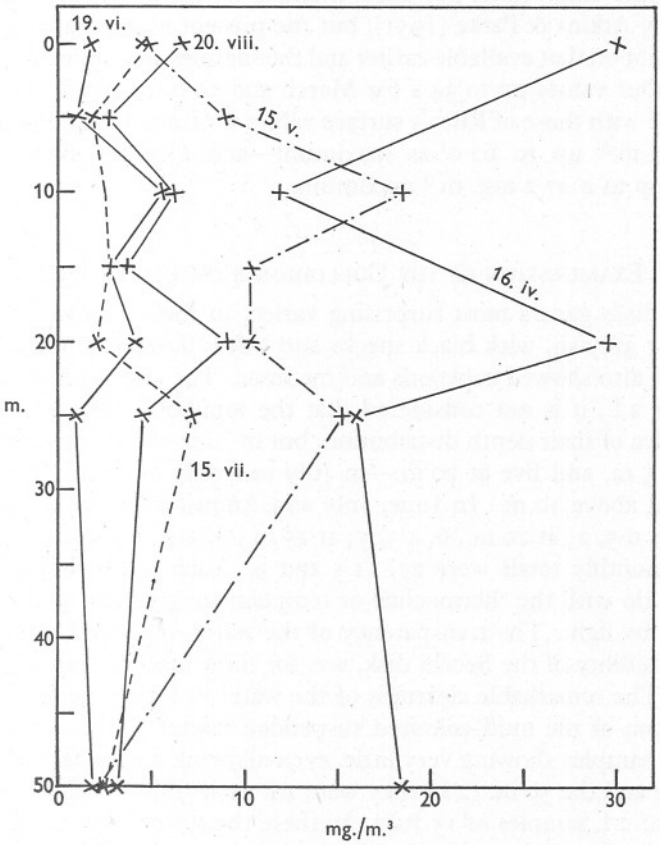

Fig. 7. Variation in chlorophyll values (as mg./m.³) with depth of sampling (metres) on five dates in 1952.

together the wet weight of phytoplankton as found for the 11 months, and for the missing December observation takes the mean of November and January, one arrives at 1·09 g. as the content in the column. This may be compared with the value found from phosphate in 1923, 1·4 g.; and in the less rich water observed more recently Atkins has found for 1948 a consumption, indicating a production, of 84% of the 1923 value, namely 1·17. The actual quantity of phosphate available in 1948 was only 75% of the 1923 value. Were the

utilization less efficient than in 1948 and proportional to the amount available in winter (for which see the discussion in the accompanying paper on phosphate), the sum of the phytoplankton produced as a minimum value would be 1·05 g. This is close enough to the yearly total of the monthly standing crop to suggest that this must be eaten almost as fast as it is produced.

The surface chlorophyll per cubic metre is on the whole greater than that recorded by Atkins & Parke (1951), but the present method appears to be an improvement on that available earlier and the samples were more expeditiously handled. Our values up to 34·2 for March and 30·0 for April 1952 are quite comparable with those of Riley's surface values for Long Island Sound (1941*a*) —17·4 mg./m.^{3} up to 62·0 as maximum—and Georges Bank (1941*b*)— 5·6 mean up to a 27·2 mg./m.^{3} maximum.

Examination of the Collodion 4 cm Filter Disks

The filter disks gave a most surprising variety in their intensity. The colour was usually greyish, with black specks and a few fibres visible with a hand-lens, which also showed copepods and medusae. The volume filtered being so small, 1 or 2 l., it is not considered that the numbers observed can give an accurate idea of their depth distribution, but in June one medusa per litre was found at 15 m. and five at 20 m.—in July one only at 15 m. No copepods were found above 10 m. In June, July and August at 10 m. we got 1, 0, 1; at 15 m., 7, 0·5, 2; at 20 m., 6, 4·5, 5; at 25 m., 8, 3·5, 0·5; and at 50 m., 5, 3, 0·5. The monthly totals were 27, 11·5 and 9. Such a distribution can have nothing to do with the thermocline or temperature gradient, and is probably influenced by light. The transparency of the water, indicated roughly by the depth of visibility of the Secchi disk, was for these months respectively 23, 18 and 11 m. The remarkable clearness of the water in June is perhaps related to the collection of the mud-coloured suspended matter in the surface sample, the deeper samples showing very little, even allowing for the fact that save for the surface and the 50 m. (2 l.) they were 1 l. each (Fig. 8). Fig. 8 also shows the series of 2 l. samples of 15 July. In these the surface and 50 m. are rather similar, and 25 m. shows up as darkest in the photograph. Visually it was not so but had a yellowish tint, as had also 50 m. to a lesser extent.

The Botanical Composition of the Phytoplankton at Station E 1

This station has been visited for many years and records of its phytoplankton have been published in the *Bulletin Planktonique* up to 1912 and summarized later by Ostenfeld (1931) but the following observations may be of interest. E 1 is 10 miles S.W. of the Eddystone and the list published by Harvey,

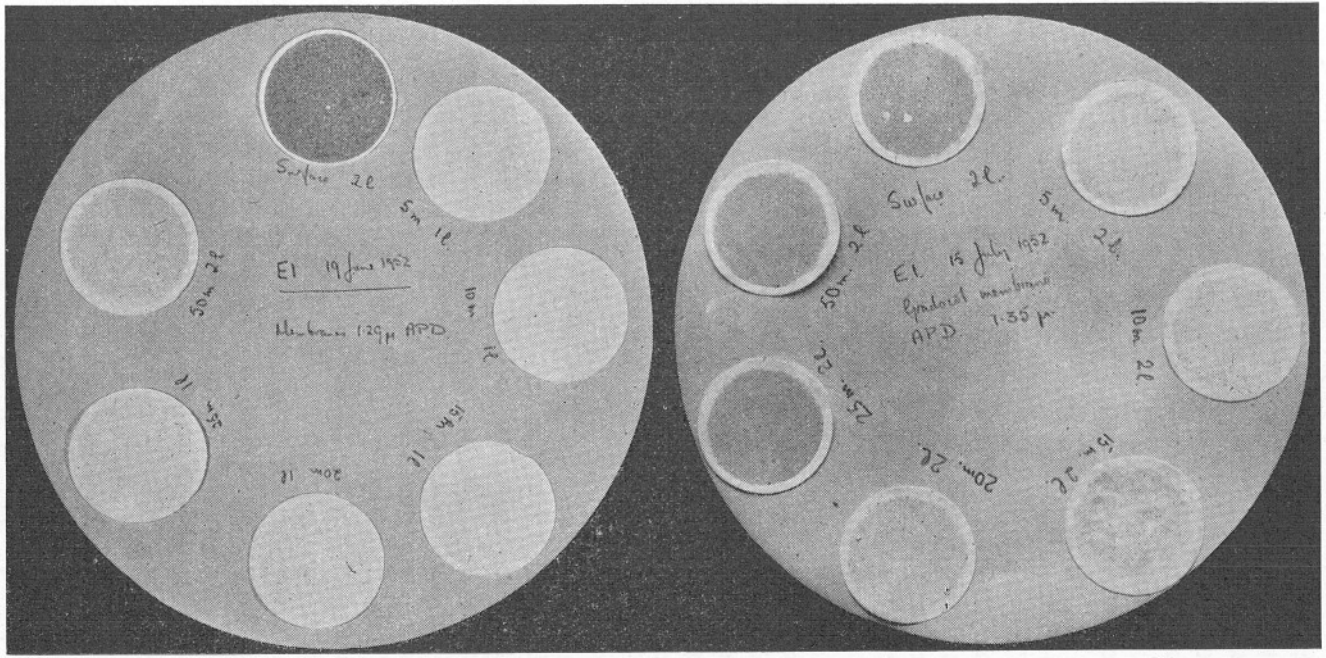

Fig. 8. View of the collodion filter disks with collected matter. Series for 19 June (left) and 15 July 1952 (right).

Cooper, Lebour & Russell (1936) concerns station L4, depth 50 m. and 14 miles nearer shore, about $5\frac{1}{2}$ miles from Plymouth breakwater. We had no plankton hauls and no time to count the algae in the filter disks, but a qualitative examination was made as follows. Samples of the water, 100 or 250 ml., were placed in conical flasks enriched with Miquel's solution and exposed in a south window in winter or a north window in late spring and summer. Some, but probably not all, of the phytoplankton multiplied rapidly, and periodical examinations were made. The plus signs in Tables I and II are an attempt to indicate the relative amounts found in the earlier stages of the cultures; +, present; + +, more frequent; + + +, plentiful; + + + +, very plentiful. Though sampling was begun in September it was only, unfortunately, on 8 February 1952 that the enriched samples were exposed in the window; before that the Winchester bottles had diffuse light in a south room. The earlier dates are probably too sparsely represented. No attempt has been made as yet to represent the depth distribution.

Table I shows the Bacillariophyceae found, twenty species, of which *Nitzschia closterium*, *Melosira Borreri* and *Navicula* sp. were the most regular in occurrence. As compared with the L4 list of 1935 it is remarkable that *Nitzschia closterium* then appeared in negligible amount in March, April and September. *N. delicatissima* and *N. seriata* appear on both lists. *Paralia sulcata*, common at L4, was only found once at E1. *Guinardia flaccida*, which occurred regularly at L4, was absent from E1, as were also over sixteen other species. Found at E1, but not mentioned for L4, were *Fragilaria* sp., *Grammatophora marina*, *Melosira* sp., *Rhizosolenia alata* f. *genuina* and *R. hebetata* f. *semispina*.

Table II lists the Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae and Miscellaneous found as for Table I. None of the Chlorophyceae are recorded in the L4 list, yet in our cultures they were the striking feature in most of the April—August samples, save the 0 m. samples, the 5 m. for April and the 50 m. sample for July, which were brown with diatoms; also, the 20 m. July sample had a very minute bacterium (with one flagellum visible) which produced a pink colour, modified to buff by a Cryptomonad. This organism became conspicuous in the 10 m. sample later. In several of the series 20 m. was noticeably behind the others in development.

Of the Chrysophyceae, *Phaeocystis* was recorded from L4 and has often been found at E1, but it was absent from our samples. Two others, however, were present at E1 though not at L4: *Coccolithophora* sp., very plentiful in July and August samples, and a member of the Chrysomonadaceae.

The Cryptophyceae afforded two records, in July a species of the Cryptomonadaceae and most remarkably from 50 m., of 7 January, we obtained an almost pure growth of *Hemiselmis rufescens* Parke, which was identified by its appearance and the scarlet pigment insoluble in 80% acetone which was left on the collodion. Its chlorophyll with possibly other pigments gave the curve

Table I. Algae identified in chemically enriched samples.

Bacillariophyceae:	27. ix. 51	24. x. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
Asterionella japonica Cleve & Möller*	.	.	.	.	++	.	++	.	.	++
Cerataulina Bergonii H. Pérag.†	.	.	.	.	.	.	.	.	.	+
Chaetoceros sp.	.	.	++	.	.	.	.	.	+++++	.
Coscinodiscus sp.	+	.	.	.	++	.	.	.	.	.
Fragilaria sp.	.	.	.	.	.	++	+	.	+	.
F. oceanica Cleve	.	.	.	.	.	.	.	.	.	++
Grammatophora marina Kütz.	.	.	.	.	.	.	.	.	.	+
Leptocylindricus danicus Cleve	.	.	.	.	.	.	.	.	+++	++
Melosira borreri Greville	++	++	.	.	++	++++	+++	++	.	+
Navicula sp.	.	.	.	++	+++	++++	++++	++++	+++	++
Nitzschia closterium Ehr.	+++	+++	++++	+++	+++	++++	+	+++	++++	+++
N. delicatissima Cleve*	.	.	.	.	.	.	.	.	.	++++
N. seriata Cleve†	.	.	.	.	.	.	.	.	.	++
Paralia sulcata Ehr.‡	.	.	.	++	.	.	.	.	.	.
Rhizosolenia alata Brightw. f. *genuina* Gran.‡	.	.	.	.	.	.	.	.	+	+++
R. f. *indica* Péragallo	.	.	.	.	.	.	.	.	.	+
R. hebetata (Bail.) f. *semispina* Hensen‡	.	.	.	.	.	.	.	.	.	++
Skeletonema costatum Greville‡	.	.	.	.	++	.	+	.	++	+++
Thalassiosira gravida Cleve	.	.	++	.	+++	++	.	.	.	.
Thalassiothrix (*Thalassionema*) *Nitzschiodes* Grun.‡	.	.	.	.	.	.	.	.	.	+

* For southern part of North Sea or Flemish area.
† Indicates species similarly listed for eastern part.
‡ Indicates species listed by Ostenfeld (1931) as common in western part of English Channel.

September, October, January and February samples enriched and exposed in window, 8 February 1952; the same holds for Table II.

TABLE II. ALGAE IDENTIFIED IN CHEMICALLY ENRICHED SAMPLES.

	27. ix. 51	7. i. 52	5. ii. 52	11. iii. 52	16. iv. 52	15. v. 52	19. vi. 52	15. vii. 52	20. viii. 52
CHLOROPHYCEAE:									
Chlamydomonas sp.	.	.	.	++	.	.	.	.	.
Chorella sp.	.	.	.	++++	++++	++++	+++	++	+
Endoderma sp.	.	.	.	+++	++++	+	+++	.	.
Halosphaera viridis Schmitz	+	.	.	.	.	.	.	.	.
Platymonas tetrahele West.	.	.	.	.	.	.	.	++	.
Pyramimonas sp. Schmarda	.	.	.	+++	.	++	.	.	.
Stichococcus bacillaris Naeg.	.	.	.	+++	++++	+++	+++	++	.
Ulothrix subflaccida Wille	++	.	+	.	.	.	.	.	.
CHRYSOPHYCEAE:									
Chrysomonadaceae sp.	.	.	.	.	.	.	.	++	.
Coccolithophora sp.	.	.	.	.	.	.	.	++++	++++
CRYPTOPHYCEAE:									
Cryptomonadaceae sp.	.	.	.	.	.	.	.	++	.
Hemiselmis rufescens Parke	.	++++	.	.	.	.	.	.	.
DINOPHYCEAE:									
Prorocentrum micans Ehr.	.	.	.	.	.	++++	.	.	.
MISCELLANEOUS:									
Flagellates, colourless	.	.	.	.	.	++	.	++++	++++
Amoebae	.	.	.	.	.	.	+++	.	++
Ciliates, colourless	.	.	.	.	.	++	++++	++	++
Bacterium, pink	.	.	.	.	.	.	.	++++	.

shown in Fig. 3. Of the seven Dinophyceae in the L4 list only *Prorocentrum micans* was observed in the E1 cultures.

We desire to express our indebtedness to Miss D. Ballantine, Dr M. V. Lebour, Dr T. J. Hart and Dr M. Parke for much help in the identifications, also our thanks to Mr F. A. J. Armstrong for filtering the samples and for temperature observations and to Mr A. E. Stoate and Mr A. Mattacola for the photographs. Finally, for these and many other sea-water samples and Secchi disk observations we have pleasure in thanking Lieut.-Comdr. C. A. Hoodless, D.S.C., R.N.R., and the crew of the R.V. *Sabella*.

Summary

Chlorophyll from the phytoplankton at station E1 was examined from September 1951 till August 1952, from 0 to 50 m. The minimum was in June, 1·8 mg./m.^{3}, and the maximum 34·2 in March, both surface samples. The maximum 50 m. sample contained 18·4 mg./m.^{3} in April. The winter minima were 4·6 and 4·3, surface and bottom, in November. Converted to water column (70 m.) values, over 1·0 g./m.^{2} is obtained for the wet weight of phytoplankton for September and March, the April maximum and the June minimum being 1·32 and 0·15 g./m.^{2}. A comparison with the production obtained from phosphate analyses suggests that the phytoplankton crop each month is rapidly devoured.

Exact proportionality was found between concentration and absorption in a chlorophyll band up to 4·0 mg./l. and a moderate error up to 8·0 mg./l. The spectral absorption curves have been given for pure cultures of phytoplankton and for cells filtered out of sea water. The chlorophyll in such extracts, made with 80% acetone, is stable when kept in total darkness.

The collodion disks containing suspended clay and the algal cells may show a surprising variation in their colour intensity, from dark grey to a very faint tint, even though obtained from sea water about 20 miles from land and over 70 m. in depth. The surface may be far darker than 5, 10 or 15 m. samples.

The botanical composition of the water was studied by allowing the algae to multiply in diffuse light after enriching the water chemically. Eight species of Chlorophyceae, and one species each of the Chrysophyceae and Dinophyceae were recorded. Of the Cryptophyceae one winter sample from 50 m. gave a nearly pure growth of *Hemiselmis rufescens* Parke. The diatoms *Melosira borreri*, *Nitzschia closterium* and *Navicula* sp. occurred commonly.

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THE PRESERVATION OF FISHING NETS, TRAWL TWINES AND FIBRE ROPES FOR USE IN SEA WATER

By W. R. G. Atkins, F.R.I.C., F.R.S. and F. J. Warren
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This paper is a continuation of our publication, in 1941, of exposure tests interrupted by the war. As no sea-water basin was available till recently the work was delayed, and in the post-war confusion several mixtures intended for trial unfortunately got mislaid. During the war, however, one of us started water-absorption tests which have proved interesting and appear to be more useful for work in the sea than the British Standards Institute tests, which extend only to 6 hr. immersion; this is far too short a period for some purposes.

TABLE I. WATER ABSORPTION TESTS ON SISAL ROPE IN SEA WATER

The numbers represent percentage absorbed.

February 1943	Immersion period (hr.)					Diameter (cm.)	
	0·5	5	24	48	96	Initial	Final
Untreated	53	53	54	58	59	8·2	8·6
Emulsion treated	.	28	55	52	.	9·4	10·2
Waterproofed	.	12	26	38*	.	8·7	9·8
Above repeated after 22 days air-drying, till constant							
Untreated	.	.	.	38	64	8·6	8·8
Emulsion treated	.	.	.	48	54	9·8	9·9
Waterproofed	.	.	.	39	42	10·2	10·2

Percentages here are calculated on the dry weight after immersion.
* 72 hr. All 6 ft. with ends bound with hemp string.

WATER ABSORPTION TESTS

Table I shows the absorption of water by sisal rope—the only fibre then available—untreated and after commercial treatments with emulsion and waterproofing. The emulsion treated was indistinguishable from the untreated after 1 day, whereas the waterproofing still showed a lesser uptake after 3 days. But with a second immersion the differences varied irregularly.

Table II records the percentage uptake of Cuprinol and of tar by manila, sisal and coir ropes 3 in. in circumference, also the uptake with commercially waterproofed manila and sisal. Dry weights of preservative are also shown and the weight of preservative remaining after drying and slow draining, calculated as a percentage of the wet weight after treatment. There is little difference

between the final water uptake of manila and sisal, but the 'waterproofed' ropes ultimately absorb more water than the untreated, and the same holds for preservatives. Coir takes up more than twice as much water as the other two. But the treatment of manila, waterproofed manila and sisal with the preservatives fails to make the ropes heavy in water, for the water absorption is reduced by approximately the weight of the preservative taken up. There

TABLE II

Sample no.	Treatment, May 1950	Pre-servative wet wt. (%)	Pre-servative dry wt. (%)	Pre-servative residue (%)	Sea water absorbed (%)		Water + preservative % on untreated
					1 day	15 days	
1	Manila 3 in. dry	.	.	.	.	.	.
2	Do. untreated	.	.	.	35	45	45
3	Do. Cuprinol	11	3	28	31	41	44
4	Do. tar	21	17	84	14	29	46
5	Manila 3 in. water-proofed, dry	.	.	.	.	.	.
6	Do. untreated	.	.	.	26	48	48
7	Do. Cuprinol	13	6	45	28	44	50
8	Do. tar	21	18	84	16	34	51
9	Sisal 3 in. dry	.	.	.	.	.	.
10	Do. untreated	.	.	.	37	44	44
11	Do. Cuprinol	16	7	42	34	40	46
12	Do. tar	26	23	87	15	26	49
13	Sisal 3 in. water-proofed, dry	.	.	.	.	.	.
14	Do. untreated	.	.	.	47	55	55
15	Do. Cuprinol	22	11	50	43	52	63
16	Do. tar	38	33	87	17	31	64
17	Coir 3 in. dry	.	.	.	.	.	.
18	Do. untreated	.	.	.	88	117	117
19	Do. Cuprinol	49	31	63	77	89	120
20	Do. tar	79	72	90	49	75	147

is some increase with waterproofed sisal when tarred. In the foregoing the ends of all ropes were whipped as usual with hemp twine, but were not sealed, as this is not customary in fishing vessels. Samples shown as 'untreated' means no further treatment after receipt from the factory. The tar treatment was cold immersion for 48 hr. (31 May 1950) by courtesy of the Plymouth branch of the Gourock Ropework Co. The netting was allowed to drain for 1 day. Cuprinol samples were immersed for 18 hr. (30 May 1950) and weighed wet after 4 hr. draining; they were then air dried till constant in weight after 47 days, and placed in sea water 18 July 1950, till saturated.

Table III shows the results of tap water absorption tests on the untreated dry controls of Table II. Duplicate tests were made in April 1952, the ends being also sealed, with Berry's bitumastic rubber compound. The B.S.I. test (1946, no. 908) specifies for waterproofing 1 hr. immersion, wiping dry and immersion for 5 hr. more. Though a 6 hr. test shows benefit from water-proofing and a decreasing effect is shown for 1, 2 and perhaps 3 days, with

longer immersion no difference is apparent. With so much water and preservative held in the interstices it is obvious that exact percentage duplications are not to be expected, but the absorptions of the untreated fibres agree reasonably well in the various tables.

TABLE III. WATER ABSORPTION TESTS ON ROPES

(B.S.I. 1946 No. 908 specification but continued longer. April, 1952. The numbers represent percentage absorbed.)

Sample no.	Sample	Immersion period (hr.) 1	6	24	48	72	120	144
1	Manila 3 in.	28	35	41	45	49	51	51
5	Do. waterproofed	8	18	29	38	43	49	49
9	Sisal 3 in.	39	40	44	45	48	49	48
13	Do. waterproofed	12	19	29	40	47	53	52
17	Coir	90	86	108	110	120	126	120

DURABILITY TESTS

It is difficult to devise an exposure test which shall be of real value as indication of usefulness on nets, trawl twines and ropes. One can only arrange the methods in order under the conditions of test selected or imposed by nature. Tests on ropes in Plymouth Sound, on the old Hoe pier, now destroyed, were valuable, as the polluted water was a good sample of harbour conditions and pollution in fishing gear. The water in the basin at Pier Cellars is far purer. At times, however, fouling occurs from drifting seaweed. Though samples cannot there be stolen, they can be washed away in heavy weather. The tests tend to become a measure of the solubility of the preservative, as against a life for the untreated specimen far longer than under harbour conditions.

In the present series the best of the earlier treatments, see Atkins & Purser (1936), copper naphthenate (Cuprinol), and the quite good treatment—save for ropes—recommended by Dr Olie were tested against a few others. Olie's method (see Atkins, 1936) is cheap and easily applied and many millions of sand bags were thus preserved during the war.

The specimens were suspended in the basin so as to be out of the water for 4–6 hr. every tide. In spite of what seemed secure fixing most of one set of trawl twines and one entire set of ropes were washed away.

The specimens were inspected once a fortnight or once a month and were tested by hand.

On cotton netting aluminium stearate in petrol did surprisingly well in duplicate tests, and ranked with the copper soaps, the best of which lasted 9 months, but Olie's treatment was so near as to have advantages on general grounds. On the trawl twines the British Columbia grade of Cuprinol was easily the best, and lasted 16 months. Among the ropes the four Cuprinol

samples, including two diluted with Coalite oil, low-temperature distillation, an American ammoniacal copper and novenate copper in petrol are still under test after 16 months.

TABLE IV. DURABILITY OF SAMPLES IMMERSED IN SEA-WATER BASIN, NEAR CAWSAND, PLYMOUTH SOUND

Sample no.		Cotton netting (%)	Trawl twines (%)	Sisal rope (%)
1	Untreated: kept in dark in laboratory	•	•	•
2	immersed	100	100*	100
3	Cuprinol: standard grade	167	121	++
4	British Columbia grade	162	183	++
5	Copper N.H. 8	128	138	++
6	Aluminium stearate: 1 lb. to 1 gal. benzole	134	100*	100
7	1 lb. to 1 gal. Coalite oil	128	106*	123
8	1 lb. to 1 gal. petrol	162	100*	100
9	Novenate copper: 1 lb. to 1 gal. benzole	162	121	129
10	1 lb. to 1 gal. Coalite oil	145	106	127
11	1 lb. to 1 gal. petrol	150	106	++
12	Cuprinol: with equal volume Coalite oil	133	121	++
13	1 vol. with 3 vol. Coalite oil	150	106	++
14	Olie's treatment	150	106	123
	Life of untreated in days	164	265	382

Samples immersed in Pier Cellars tidal basin 21 February 1951.
Cotton 14 S/6-ply, white, 2½ in. mesh. Mean of duplicate sets.
Trawl twine, manila, 3-ply, 150 yd/lb. Duplicate set washed away 9 January 1952; only specimens showing result of duplicate tests have * mark. Sisal rope was ⅞ in. six thread.
One set of ropes washed away, 26 February 1952, before any failed; sound after 493 days marked ++.

We are indebted to the courtesy of the Gourock Ropework Co. and Messrs Hawkins and Tipson for ropes; to Messrs Cuprinol, the Coalite Low Temperature Carbonization Co., Messrs Boake Roberts and the Nuodex Products Co., New Jersey, U.S.A., for preservatives; also to Mr A. E. Stoate for much help throughout the work.

SUMMARY

A trade waterproofing of sisal rope had still some effect after 3 days in sea water, the untreated being four-fifths saturated in 1 hr.

Treating manila, sisal and coir rope with Cuprinol or tar increases the wet weight at the most only slightly as less water is taken up than without preservative.

Water equilibrium is not attained till long after the B.S.I. specification test period for waterproofing sisal rope, but some effect of waterproofing may be distinguished up to perhaps 3 days.

On cotton netting copper naphthenate preservatives were most durable in a clean sea-water basin, but Dr Olie's method with cutch and ammoniacal copper sulphate ran them close and is cheaper. The best lasted 9 months as against untreated 5½ months.

On trawl twines the British Columbia grade of Cuprinol, a proprietary mixture containing copper naphthenate, was the best of those tried, and the twines lasted 16 months as against almost 9 for untreated manila.

On thin sisal rope six copper preparations are still under test.

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OBSERVATIONS ON *NUCULA TURGIDA* MARSHALL AND *N. MOOREI* WINCKWORTH

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(Plate I and Text-figs. 1–8)

CONTENTS

INTRODUCTION

In 1950 a study of the specific differences and growth rates of the British species of *Nucula* (Mollusca, Protobranchiata) was commenced. Winckworth (1932) lists five species which, with the exception of *N. turgida* and *N. moorei*, can be readily distinguished from each other. The present observations on over 2500 specimens of *N. turgida* and *N. moorei* show that the diagnostic characters differentiating the two species are not valid, and as no new differences have been found it is proposed that they should be recombined as one species, *Nucula turgida* Marshall 1875, this being the first available name.

Until 1930, *N. turgida* and *N. moorei* were considered to be one species under the name *N. nitida* Sowerby. Then Winckworth (1930) pointed out that the name *N. nitida* was preoccupied and proposed *N. nitidosa* instead. Two papers by Leckenby & Marshall (1875) and Marshall (1893) had been overlooked in which a dark unrayed form of *N. nitida* was described as variety *turgida* and a form having coloured rays as variety *radiata*.[1] Jeffreys (1879) described another variety under the name *ventrosa* from the Mediterranean (*Porcupine* Expedition, 1870); this was considered by Marshall (1893), however, to be his variety *turgida*. After examination of Marshall's specimens from the Dogger Bank and from the work of Moore (1931 *a*, *b*) on the faeces of the rayed and unrayed forms, Winckworth (1931) considered the two

[1] Not to be confused with *N. radiata* Hanley which is now known as *N. hanleyi* Winckworth.

varieties (*turgida* and *radiata*) distinct and renamed them *N. turgida* Marshall, and *N. moorei* Winckworth. The two species were diagnosed as follows:

Nucula turgida

Shell glossy, with olive-green periostracum, somewhat inflated, anterior margin distinctly prominent, posterior margin evenly rounded; growth lines form distinct wrinkles on the anterior third and the extreme posterior. Faecal pellets subcircular in section, with seven grooves.

Nucula moorei.

Shell usually more glossy, with a pale green periostracum, streaked with coloured rays (usually obvious but exceptionally they may be only just visible), anterior margin almost straight, posterior margin arched; growth lines much finer and wrinkles slight. Faecal pellets quadrate oval in section, with five grooves.

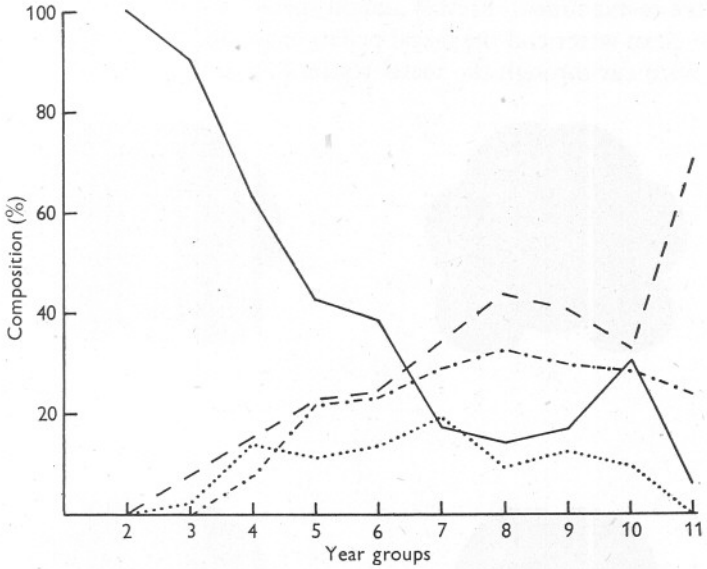
N. nitida is a widely distributed species ranging from Sweden to the Mediterranean. Forbes & Hanley (1853) and Jeffreys (1865) state that it can be taken in sand and sandy silt at low spring tides and at depths ranging from the latter mark to 86 fathoms (157 m.) Both radiate (*N. moorei*) and non-radiate (*N. turgida*) forms are present together throughout the Clyde Sea Area, but there is no record of them being taken at low-water mark. In a survey of the sublittoral fauna in 1949 and 1950 they were taken from sandy silt at 10 m. to fine mud at 180 m. The densest populations occurred in muddy silt (80–90 m. depth) in company with *Corbula gibba*, *Abra alba*, *Cuspidaria cuspidata* and *Nucula sulcata*. The material for the present investigation was obtained from the fauna survey and additional large samples from the Cumbrae Deep off the east coast of Bute and from the Minnard Narrows, Loch Fyne.

Shell Markings

The colour of the shell has been variously described as pale green, olive-green and yellow. The ground colour of the periostracum of specimens from the Clyde Sea Area is yellow, on which purple-grey markings may be superimposed. These markings can completely mask the yellow ground giving the non-radiate dark form which Marshall called the variety *turgida*. The purple-grey colour is not always laid down continuously and banded forms are found with the bands running parallel to the lines of growth. It can also take the form of radiations at right angles to the lines of growth. These latter forms vary from those that are heavily radiate to those in which the radiations are hardly visible (Plate I, figs. 1, 2). These markings, also, may not be laid down continuously, being often limited to bands parallel to the lines of growth.

Examination of the specimens obtained showed that it was possible to group them into (1) those with markings, and (2) those without markings. Group (1) was then further divided into (*a*) those in which radiate markings could be seen with the naked eye, and (*b*) those in which the markings were

not obviously radiate. On microscopic examination group (*b*) was again divided into (i) those that were probably radiate, and (ii) those which were probably non-radiate (Pl. I, fig. 2). The group that was probably non-radiate included the dark non-radiate form and the banded non-radiate form. In a large sample of over 1400 specimens dredged from the Cumbrae Deep 35·3% were found to be non-radiate, 12·4% probably non-radiate, 29·5%



Text-fig. 1. Percentage composition of the year groups. —, non-radiate; ···, probably non-radiate; —·—, radiate; — —, probably radiate.

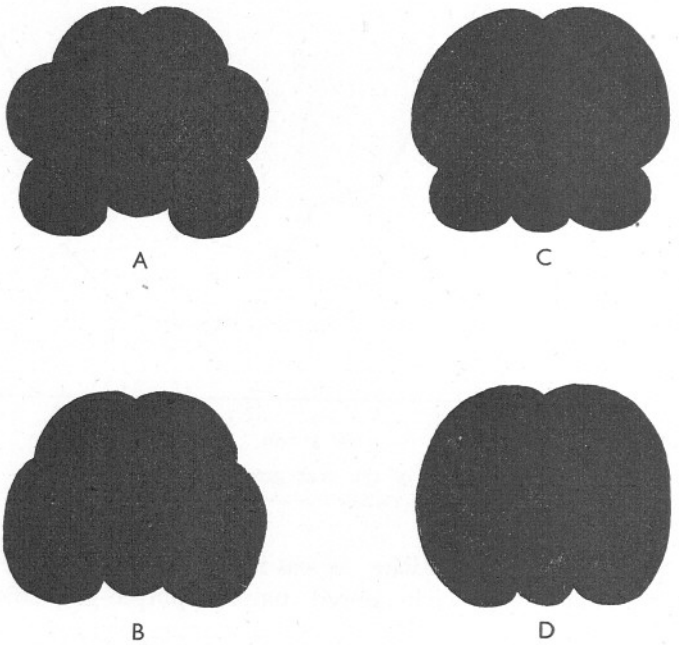
probably radiate and 22·8% radiate. It was found possible to make a graded series ranging from those which showed complete purple-grey coloration to those without markings.

This sample shows that the purple-grey markings are not visible until the animal is approximately 3 mm. long (in its third year) and that the first clearly radiate specimen is 3·6 mm. long. This is shown in Text-fig. 7 in which individual length is plotted against numbers in the four groups. In larger shells with purple-grey radiations there is an area in the region of the umbo corresponding to early shell growth that is not marked. Text-fig. 1, which plots the percentage composition for each year group of this population, shows clearly that the number of shells with markings increases with age.

Examination of the growth lines shows a considerable amount of variation in both radiate and non-radiate forms. In both, distinct wrinkles may be formed on various parts of the shell, but this feature could not be used for classification.

Faeces

Moore (1931 *a*, *b*) points out that the form of the faecal pellets is of value in the specific identification of various molluscan families. He describes their form in the British species of *Nucula* and differentiates the non-radiate form from the radiate form by this means. He states that the former has seven grooves and the latter five grooves (Text-fig. 2, A and B). The faeces of the two forms were re-examined. Freshly caught specimens were placed separately in vessels of clean water and the faecal pellets examined. In addition, transverse sections were cut through the rectal region (Table I; Pl. I, fig. 3).



Text-fig. 2. Faecal pellet types (end view); A, non-radiate form (Moore); B, radiate form (Moore); C, and D, other forms.

It will be seen from Table I that the present observations do not agree with those of Moore. Of fifty-six specimens examined, fifty-one had the non-radiate type of faeces (seven grooves) and only three had the radiate type (five grooves). Only one of these three corresponds to an animal with radiate shell markings and one to a shell that was probably radiate. Pl. I, fig. 3, is a photomicrograph of a transverse section of the rectum of an animal with a radiate type of shell. This shows a faecal pellet with seven ridges which Moore states is found in the non-radiate form. In addition, two other variations in the form of the faecal pellets were noted (Text-fig. 2, C and D).

TABLE I

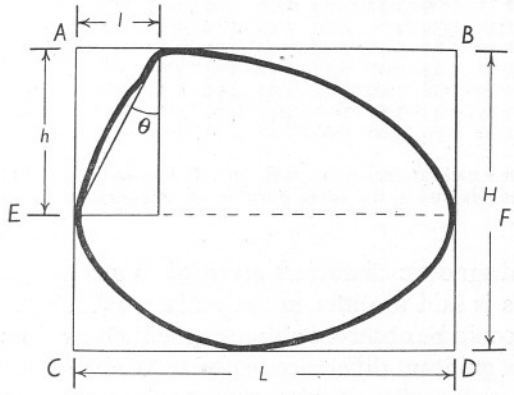
Shell markings	Number examined	Faecal pellets: Radiate form	Faecal pellets: Non-radiate form	Faecal pellets: Other forms
Radiate	14	1	12	1*
Probably radiate	7	1	6	—
Non-radiate	20	1	18	1†
Probably non-radiate	15	—	15	—
Totals	56	3	51	2

* See Text-fig. 2C. † See Text-fig. 2D.

Although the two forms as described by Moore exist, it is clear from this examination that they cannot be used as a means of differentiating the two species.

SHELL MEASUREMENTS

Measurements shown in Text-fig. 3 were taken for all British species of *Nucula*. The shell was placed on a grid (*ABCD*) divided into squares of side 1 mm. so that a line through the points *E* and *F* was parallel to the lines *AB*



Text-fig. 3. Diagram to show the shell measurements taken. For explanation of the symbols see text.

and *CD*. The measurements were taken with the aid of a travelling microscope the magnification being ×8. The measurement *W*, the greatest width of the shell (pair of valves), was also taken. The measurements *h* and *l* were taken so that the angle θ could be calculated, as it was found that this angle differed in the different species of *Nucula*. In addition, neglecting the curvature of the shell margins, the values *l* and $L-l$ and *h* and $H-h$ define the outline of the shell and therefore are likely to be the best values from which discriminant functions can be calculated. Comparisons have been made mainly from the measurements of definitely radiate and non-radiate forms, particularly those from the large sample taken from the Cumbrae Deep.

Comparison of the radiate and non-radiate forms in which mean measurements for height (H) and width (W) were plotted against length (L) show that, except for high shell measurements where numbers are low and the variations in shell shape are the greatest, there is no significant difference between the two forms. Further comparisons of the mean shell measurements of each year group indicate even more strongly that there is no difference between the radiate and non-radiate forms even in the older age groups (Table II, Text-figs. 4 and 5). In addition, it is seen that the variations themselves show identical ranges. It would be possible to obtain identical curves even though the angle θ differed in the two forms. From Table II it is seen that the shell angle does not so differ.

TABLE II

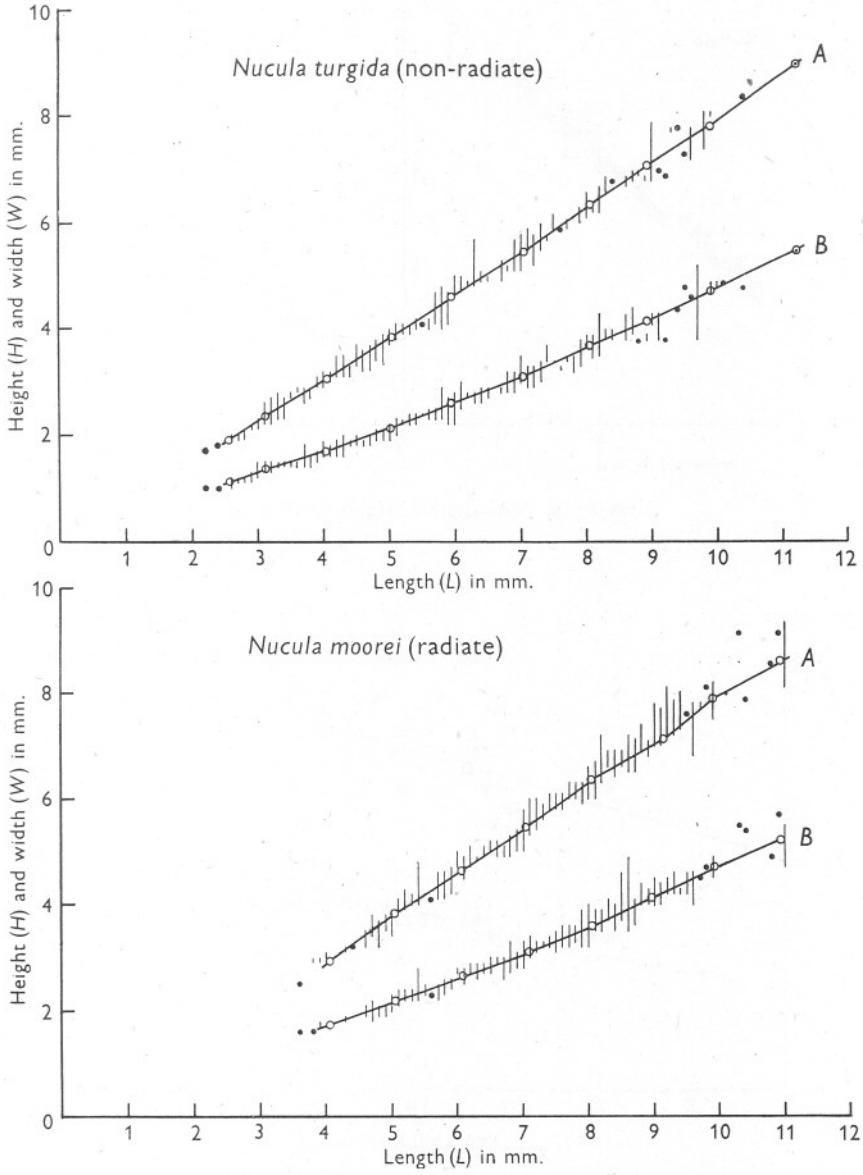
Year group	L		l		H		h		W		θ		Nos./yr. group	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	2·55	—	0·70	—	1·90	—	1·01	—	1·10	—	34° 43′	—	11	—
2	3·11	—	0·83	—	2·33	—	1·21	—	1·33	—	34° 27′	—	81	—
3	4·04	4·05	0·98	0·98	3·05	2·94	1·65	1·69	1·68	1·74	30° 43′	30° 07′	109	13
4	5·02	5·04	1·11	1·09	3·82	3·84	2·06	2·06	2·12	2·17	28° 19′	28° 53′	107	54
5	5·97	6·08	1·32	1·35	4·60	4·63	2·49	2·54	2·58	2·65	28° 04′	28° 01′	99	61
6	7·04	7·05	1·56	1·63	5·46	5·47	3·04	3·04	3·09	3·10	27° 10′	28° 12′	47	78
7	8·05	8·04	1·87	1·83	6·35	6·33	3·61	3·55	3·69	3·63	27° 23′	27° 16′	35	82
8	8·92	8·94	1·99	1·98	7·09	7·12	4·00	4·06	4·14	4·12	26° 27′	26° 00′	23	41
9	9·88	9·90	2·15	2·23	7·81	7·91	4·42	4·63	4·72	4·73	29° 00′	25° 41′	13	12*
10	11·20	10·93	2·30	2·60	9·00	8·60	5·10	4·73	5·50	5·20	24° 17′	28° 48′	1	4*

Comparison of shell measurements (in mm.) of (A) non-radiate and (B) radiate forms.
* Discrepancies due to the small number of specimens in the year groups.

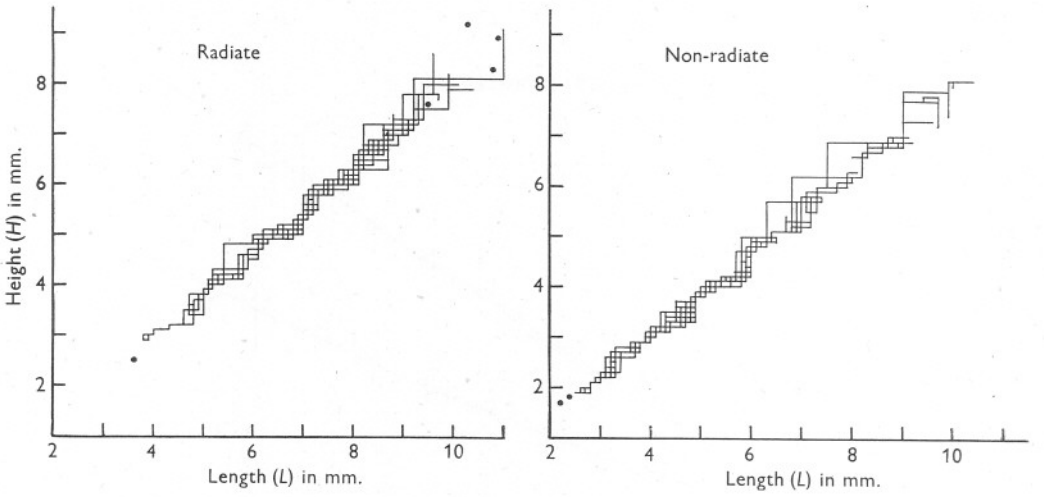
In the list of diagnostic characters given by Winckworth the curvature of the shell margins is said to differ in the radiate and non-radiate forms. No such distinction could be observed although the shell outlines were compared.

As there is no significant difference between the shell measurements and no difference in the shell outline of the two forms, it is not necessary to calculate discriminant functions. Values of t calculated for L, H, W, l and h are 1·59, 0·47, 0·97, 0·87, and 1·34 respectively. All these are considerably lower than the value 2·447 for t (corresponding to a probability $P = 0{\cdot}05$) given in Fisher & Yates' (1948) tables. Therefore the means of the values in the radiate and non-radiate groups do not differ significantly from one another.

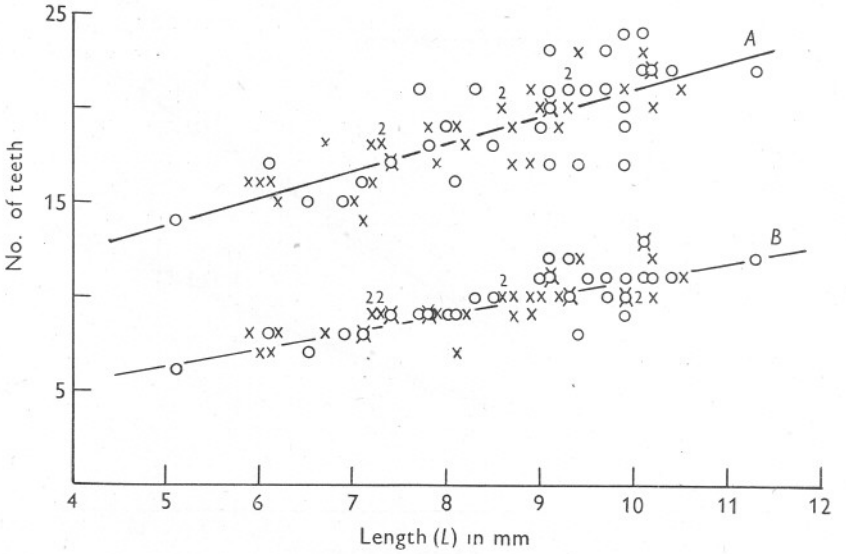
In addition to the measurements listed above the numbers of hinge teeth in the two forms were compared (Text-fig. 6). Although there is a considerable variation in the numbers of teeth in each form for a particular shell length, comparison of the numbers of anterior and posterior teeth at different shell lengths in the radiate and non-radiate forms shows that no distinction can be made by using this character. Both show a similar increase in the number of teeth with increasing shell length.



Text-fig. 4. Comparison of mean values of height (*H*) and width (*W*) with length (*L*) for each year group and the range of values for height and width with length. *A*, height/length; *B*, width/length.



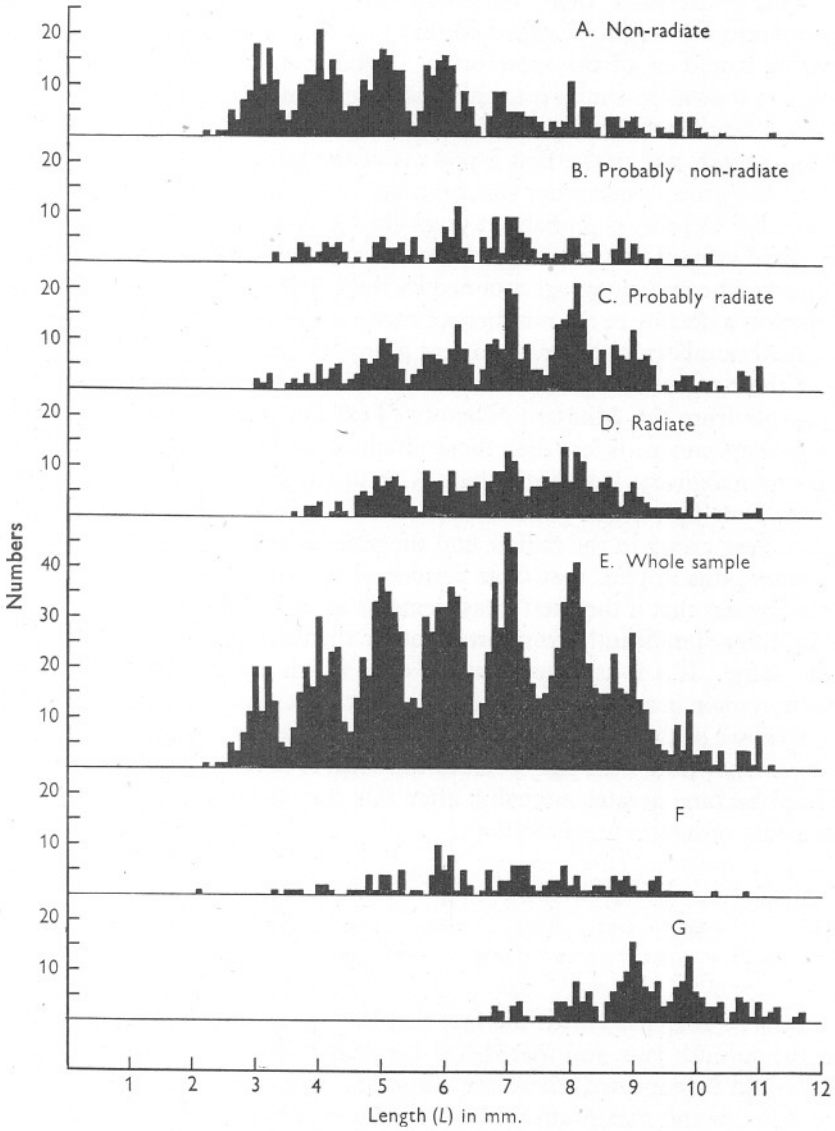
Text-fig. 5. Variation of height with length.



Text-fig. 6. Comparison of the numbers of anterior (*A*) and posterior (*B*) teeth at different shell lengths in the radiate and non-radiate forms. ×, radiate; ○, non-radiate; the numerals indicate the number of coincident readings.

Age and Growth

It can be seen that the samples fall into distinct groups (Text-fig. 7); these are presumably year groups though actual proof is lacking. The range in shell length of each group is identical in the four colour groups examined. The



Text-fig. 7. Population histogram of three samples. A–E, Cumbrae Deep, 31. x. 50.; F, Cumbrae Deep, 17. vi. 51.; G, Minnard Narrows, 27. vii. 51.

undulations of the ranges of variations in Text-fig. 4 also indicate the year groups and again show no difference between the radiate and non-radiate forms. Lebour (1938) reports that the egg diameter of *N. nitida* is 90 μ and that of *N. nucleus*, a species that grows to a similar size, 100 μ. Both species have a very short larval life, but no measurements of the larvae are given. A drawing by Bernard (1896) of a prodissoconch of *N. nucleus* measures 180 μ long, therefore it might be expected that the size of the newly settled spat of *N. nitida* would be of the same order. Lebour states that *N. turgida* from Plymouth spawns in winter and that she successfully reared fertilized eggs to the larval stage in February. If the spawning times at Millport are the same and the growth rate in the first 2 years is of the same order as the following, the smallest group (maximum size 2·7 mm.) of the Cumbrae Deep haul taken at the end of October is probably 2 years old. Although no successful fertilizations were carried out, ripe sperm and ova were present from October to February. The maximum age attained by the Clyde specimens is 10–11 years, but there is a decline in the numbers of each year group after the seventh year. The small numbers in the first two year groups is due to the fact that the mesh size of the net was too great to retain the smaller shells; this also applies to the sample from the Minnard Narrows (Text-fig. 7). The maximum size of the Clyde specimens is less than those obtained by Marshall from the Dogger Bank, the maximum length of which is recorded as from 13·0 to 13·75 mm.

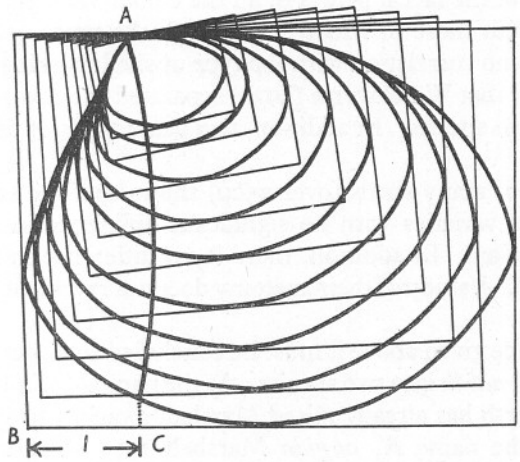
From Text-figs. 4 and 7 it is seen that the shapes of the population curves for each year group in the radiate and the non-radiate forms correspond with each other; this implies that their periods of spawning are the same.

It is obvious that if the shell measurements at various ages in the two groups do not differ significantly from one another then the rates of growth of each are the same. If it is assumed that the cells which lay down the purple-grey pigment remain in a constant position on the mantle edge then the rays indicate the curvature of the growth. Thus the successive yearly increments of growth will be as shown in Text-fig. 8. Until the sixth or seventh year, increments to the shell become greater annually, after this they decrease. Thus the annual increments of height are as follows:

Year groups...	1	2	3	4	5	6	7	8	9
Height	1·90	2·33	3·05	3·82	4·60	5·46	6·35	7·09	7·81
Increments		0·43	0·72	0·77	0·78	0·86	**0·89**	0·74	0·72

This may be correlated with the fact that the age groups decline in numbers after the seventh year and that this is the peak followed by senescence.

As would be expected, there are variations in the shape of the shells, and the minimum and maximum values from the mean shell values increase with age. From Text-figs. 4 and 5 it has been seen that the minimum and maximum values are of the same order in both the radiate and non-radiate types.



Text-fig. 8. Shell growth calculated from the mean shell measurements of each year group. *AC* is a ray which passes from the umbo to a point on the shell margin where *BC* is the measurement *l*.

Discussion and Conclusions

Observations have been made on a large number of specimens of the two alleged species *N. turgida* and *N. moorei*. In particular, those characters listed by Winckworth as being diagnostic have been studied. These are of three types: the colour of the shell, the shape of the faeces, and the shape of the shell.

The basic colour is yellow. This may or may not have purple-grey markings. *N. moorei* is differentiated from *N. turgida* by having these markings in the form of rays. *N. turgida* includes the unmarked form and those shells in which the markings are not in the form of rays. It was found that the shells could be arranged in a continuous series from a form that was so heavily marked that the radiations have merged into one another to give a dark unrayed shell through forms with varying degrees of radiate markings to those that have none at all. The shell markings are not necessarily laid down continuously, and shells with coloured bands parallel to the lines of growth are found. A study of a large sample shows that the purple-grey colour does not appear until the end of the second year and that the percentage of unmarked forms decreases with age. These facts indicate that there are not two species but one which exhibits a continuous series of colour varieties. The increase in the number of specimens which show this coloration as their age increases suggests that the purple-grey colour is a waste product produced by the metabolic activities of the animal. Comfort (1951) points out that 'the shell pigments of the lower bivalves are secreted into the shell as a means of disposal, being derived from the diet or unmanageable metabolic residues'.

Examination of the faecal pellets of all the colour varieties gave results that did not agree with those of Moore. Although the types listed by him were found there was no correlation with the type of shell markings. It was mainly on this character that Winckworth (1931) separated the two colour varieties of *N. nitida* into two species. In addition, two other types of faecal pellets were noted.

Examination of many shells (over 2500) shows that the shape is the same in all the colour varieties with no significant difference in any of the shell measurements taken. In addition, there is no difference in growth rate and spawning period. A study of their anatomy does not reveal differences between the colour types.

On the evidence given above it must be concluded that there are no grounds for separating *N. nitida* into two species. As the name *N. nitida* is preoccupied, and as Winckworth has already raised Marshall's variety to specific rank, it is proposed that the name *N. turgida* Marshall 1875 should be applied and *N. moorei* a synonym of it.

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Summary

In the course of a study of the British Nuculidae the specific characters of a large number of specimens of *N. turgida* and *N. moorei* were re-examined. It was found that no distinction could be made between the two.

Examination of variation in shell colour showed that it was not possible to differentiate into two distinct types. There was a continuous graded series from a dark purple-grey in which the basic yellow colour was almost completely masked, through a series with decreasing numbers of purple-grey radiations, to shells in which the markings could not be differentiated as rays, and finally to shells without purple coloration. The purple-grey colour is not necessarily laid down continuously, and it does not develop until the end of the second year. As the shells get older an increasing percentage of them become coloured.

Although the two types of faecal pellets described by Moore were found they could not be associated with the radiate and non-radiate shell types. The five grooved faeces that he claimed were typical of the radiate variety, were

found in only three of the fifty-six specimens examined. Only one of them was from a definitely radiate shell.

Shell measurements and growth rates were studied, and no feature was found on which two forms could be separated. Samples show that reproduction takes place at the same time in the rayed and unrayed shells.

As the name *N. nitida* Sowerby is preoccupied it is suggested that the name *N. turgida* Marshall should now be used with *N. moorei* a synonym of it.

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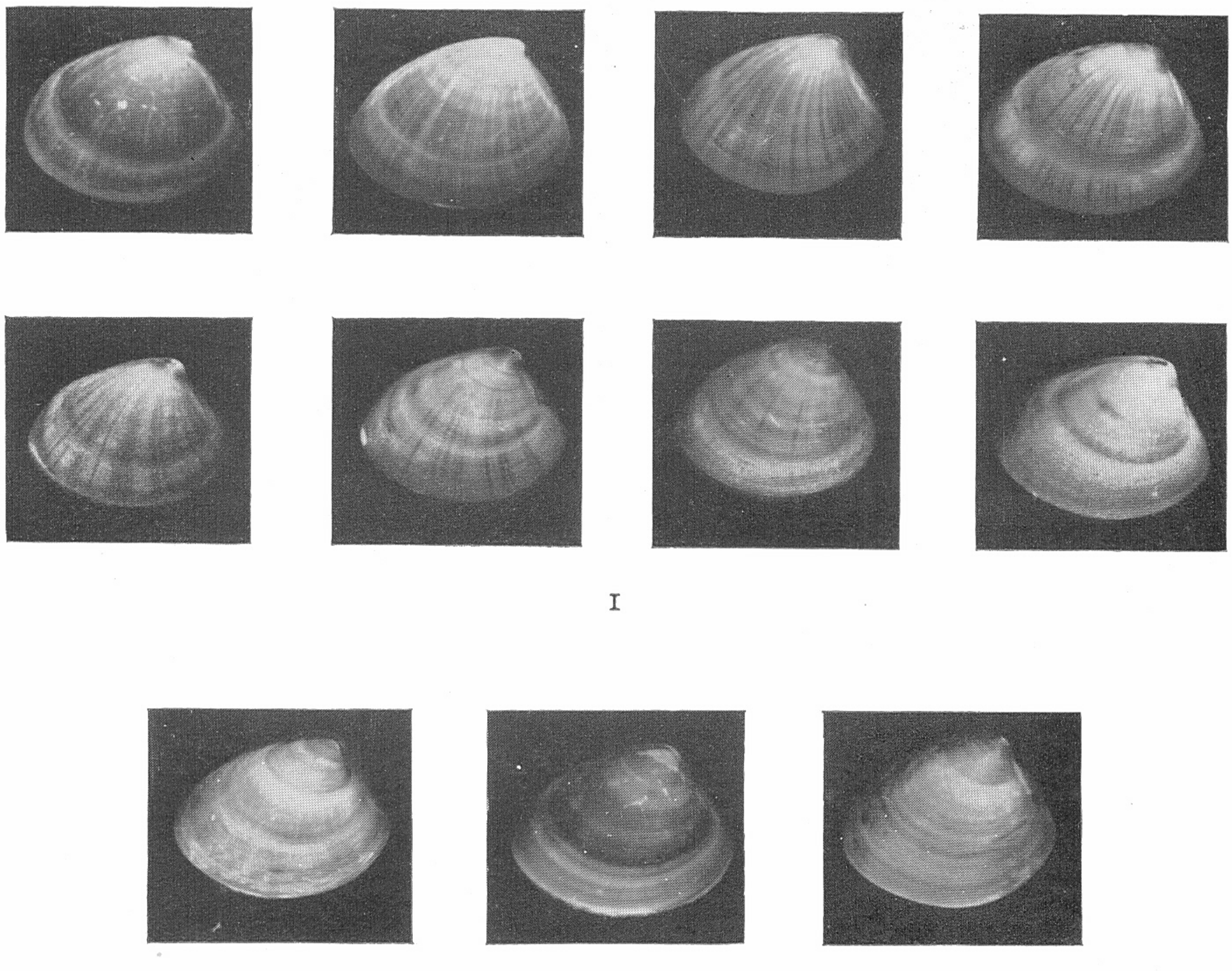
EXPLANATION OF PLATE I

Fig. 1. Series showing the change from a heavily radiate shell to a non-radiate shell. (Some show the sculpturing of the shell, this should not be confused with the purple-grey markings.)

Fig. 2. Left and centre: probably radiate shells; right probably non-radiate shell. These also show shell sculpturing.

Fig. 3. Transverse section of rectum of a radiate shell showing a seven-grooved faecal pellet.



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OBSERVATIONS ON THE SKIN PIGMENT AND AMOEBOCYTES, AND THE OCCURRENCE OF PHENOLASES IN THE COELOMIC FLUID OF *HOLOTHURIA FORSKALI* DELLE CHIAJE

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(Text-fig. 1)

Black or brown pigments are common among echinoderms and certain of them have been described as melanins—for example by Briot (1906) in unspecified holothurians; Crozier (1915) in *Holothuria captiva* and *H. surinamensis*; Verne (1926) in *H. forskali*; Cornil, Mosinger & Calen (1935*a*) in *H. forskali* and *H. tubulosa;* and Millott (1950) in *Thyone briareus*. The evidence for such statements has not always been made clear, but in certain instances, for example, in *Thyone* (Millott, 1950, and unpublished observations), other holothurians (Briot, 1906), and in the echinoid *Diadema* (Millott & Jacobson, 1951; 1952*a*), such pigments have been shown to possess the chemical and histochemical characteristics of melanin. The fact that the black skin pigment of *Holothuria forskali* also shows the characteristics of melanin has been mentioned in a preliminary note (Millott, 1952).

In *Diadema* it was also shown that the pigment arose within the amoebocytes of the coelomic fluid by a process of oxidation involving an enzyme system with the characteristics of phenolases (Millott & Jacobson, 1952*b*).

In view of the frequent association of melanin production with such enzymes, the importance of these observations is obvious, and it is therefore appropriate to extend them to other echinoderms. This is all the more so, because studies on echinoderm pigmentation have been relatively few, and most of these concern echinochrome or allied pigments, and because the origin of melanin in other forms has proved so difficult to elucidate. Despite extensive investigation, the process of melanogenesis is still a controversial matter, and there is thus good reason to extend observations on the occurrence of the pigment, and to glean all indications as to how it is produced.

As reported previously (Millott, 1952), *Holothuria forskali* with its abundant black pigment forms a very suitable object for study, and in this essentially introductory and incomplete account evidence will be produced showing that the black skin pigment has the properties of melanin and that, as in *Diadema*, its presence is coupled with the existence of a well-defined phenolase system,

distinct from the cytochrome-cytochrome oxidase system. The amoebocytes of the coelomic fluid appear to play a vital part in the formation and distribution of the pigment.

The Properties of the Skin Pigment

Unfortunately the precise chemical nature of melanin is unknown. Indeed the term would appear to refer to a group of pigments rather than to a specific substance. Specific tests are therefore non-existent, but it is permissible to identify a pigment, provisionally at least, as a melanin, if it has the following characteristics (Lison, 1936): (i) if it occurs in the form of black, brown, or yellow granules; (ii) if it shows extreme resistance to solvents; (iii) if it is decolorized by oxidizing agents; (iv) if it reduces directly ammoniacal solutions of silver nitrate.

As indicated below, the black body wall pigment of *Holothuria forskali* shows these characteristics.

Mode of Occurrence

The body-wall pigment exists in the form of black granules scattered throughout the body wall, sparsely or in irregular patches, which are most numerous in the epidermis and subepidermal connective tissue. The granules are present inside cells as well as in intercellular spaces (see p. 534).

Solubility

The following simple method was used to test the solubility of the pigment.

The densely pigmented superficial layer of the body wall was stripped off and shaken with distilled water. A dark greenish yellow pigment dissolved and the solution was decanted off. The residue was shaken with distilled water and the whole filtered. The precipitate, which formed a dark brown film on the filter-paper, was extracted repeatedly with various solvents. The final filtrate from each solvent was evaporated to dryness on a water-bath to detect small amounts of pigment which might have dissolved. All other operations were carried out at laboratory temperature.

The results are shown below.

Solvent	Remarks	Solvent	Remarks
Water	Slightly soluble	Carbon disulphide	Insoluble
Ethanol 90%	Insoluble	Pyridine	Slightly soluble
Acetone	Insoluble	1·0 N-HCl	Slightly soluble
Benzene	Insoluble	1·0 N-H_2SO_4	Insoluble
Ether	Insoluble	1·0 N-HNO_3	Insoluble
Petrol ether	Insoluble	1·0N (approx.)-NaOH	Slightly soluble
Chloroform	Insoluble		

Where the pigment proved slightly soluble, the residue obtained by evaporation to dryness was extracted repeatedly with the same solvents. Its behaviour towards all solvents was precisely the same as the original precipitate, thus showing that the latter was not a mixture of similarly coloured substances, nor changed by the action of the solvents.

In general, therefore, the behaviour of the pigment towards solvents resembles that of melanin.

Bleaching by Oxidizing Agents

Small pieces of superficial body wall measuring about 1·5 by 0·5 cm. were subjected to the action of the following oxidizing agents, known to decolorize melanin (Lison, 1936). The results are indicated below:

Agent	Remarks
Chlorine	Pigment bleached completely in 12 hr.
Bromine water	Pigment bleached completely in 12 hr.
Hydrogen peroxide (about 12 vol.)	Black pigment changed to red in 12 hr. Prolonged action of agent led to no further change
2% chromic acid	Bleached to pale brown in 10 hr.
Potassium permanganate followed by oxalic acid	Bleached completely in 1 hr. after addition of oxalic acid

The pigment thus behaves like a melanin.

The Argentaffine Reaction

Paraffin sections of the body wall, 10 μ thick, were subjected to Masson's technique for the argentaffine reaction (see Lison, 1936), and counterstained in Orange G.

The granular body-wall pigment under study readily reduced ammoniacal silver nitrate.

Thus the black pigment of the body wall of *Holothuria forskali* shows clearly the characteristics of melanin.

The Coelomic Fluid

The Amoebocytes

Suspended in the coelomic fluid are numerous amoeboid cells, which in general features resemble those previously described in a wide variety of echinoderms (see Geddes, 1879; Cuénot, 1891; McClendon, 1912; Kindred, 1921, 1924, 1926; Dawson, 1933; and Ohuye, 1938).

When examined alive, or after fixation in Bouin, their most striking feature is the large number of cytoplasmic inclusions, certain of which are especially interesting, taking the form of spheroids (Fig. 1 A and B, *sph.*), which may be colourless, yellow or brown, bearing on their surfaces, deep brown, or black granules.

The appearance of granules of black pigment in association with cytoplasmic spheroids, at once recalls the condition observed in the amoebocytes of *Diadema*, where it has been shown that melanin appears in association with similar spheroids (Millott & Jacobson, 1952*b*).

Formation and Darkening of the Coagulum

When removed from the animal and exposed to air, the coelomic fluid slowly forms a sparse white stringy clot, which, after about 12 hr. at laboratory temperatures, breaks up into isolated pale brown globules frequently flecked with black. After a further 12 hr. the entire globule turns black.

Examination by microscope shows that the clot is formed of free and compacted amoebocytes.

In coelomic fluid which has been exposed for several hours, many spheroids (both brown and colourless) lie free in the clot following disorganization of many of the amoebocytes, and it is to the brown spheroids and the associated granules that the colour of the clot is due.

As far as can be judged from appearances, the pigment granules at the surface of the spheroids tend to amalgamate, forming densely brown patches around the spheroids, from which brown colour seems to spread into the spheroid (Fig. 1A, *b.p.*). This process occurs progressively, and, though the spheroids of many amoebocytes remain colourless, most become brown on continued exposure, until after 2 days the coagulum is seen to consist of a dense network of brown strands formed by the spheroids and debris of disorganized amoebocytes, with isolated, intact and apparently healthy, amoebocytes in the interstices.

Such appearances at once recall the changes seen to occur in the coelomic fluid of *Diadema* (Millott & Jacobson, 1952*b*), where, however, the clot is much more extensive and darkens more rapidly. It is especially significant, however, that here, as in *Diadema*, the darkening involves both spheroids and the granular pigment at their surfaces.

Although it was not possible in the time available to show directly by chemical or histochemical means, as in *Diadema*, that the pigment formed in the amoebocytes is a melanin, that it probably is becomes clear from histological preparations, in which it can be seen that the black pigment of the skin (which as we have already seen has the properties of melanin) is undoubtedly derived from the coelomic amoebocytes (see below).

Such a conclusion is fully supported by the reaction of the amoebocytes to ammoniacal silver solutions, for, after using Masson's technique for the argentaffine reaction, the pigment granules and many of the associated spheroids blackened intensely.

Histological Evidence of the Derivation of Skin Pigment from the Amoebocytes

Methods

Small pieces of body wall were fixed in Bouin's fluid for 24 hr. at laboratory temperature. Sections 10 μ thick were cut in wax and stained in safranin and light green.

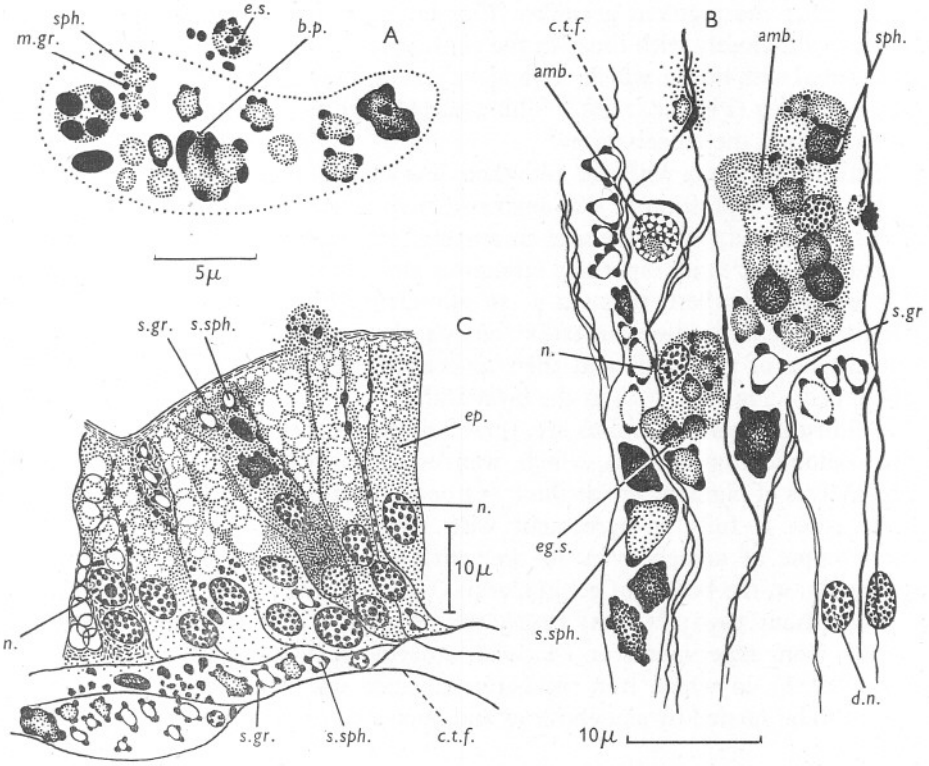

Fig. 1A. Living amoebocyte, from the coagulum formed in the coelomic fluid of *Holothuria forskali*, sketched after exposure of coelomic fluid in an open dish for 20 hr. at laboratory temperature and redrawn. B. Portion of the subepithelial zone of the body wall of *H. forskali*, showing amoebocytes and melanin deposits. From a preparation fixed in Bouin's fluid and stained in safranin and light green. C. Part of a section through the superficial region of the body wall of *H. forskali*, showing melanin deposits in epithelial and subepithelial zones. Fixed Bouin's fluid, stained by Masson's method for the argentaffine reaction (see p. 531), counterstained in Orange G.

amb. amoebocyte.
b.p. brown pigment spreading into spheroid (see p. 532).
c.t.f. connective tissue fibre.
d.n. isolated nucleus (?) from disintegrated amoebocyte.
eg.s. spheroid apparently being egested by amoebocyte.
ep. epithelial cell.
e.s. spheroid eliminated from amoebocyte with granules of pigment.
m.gr. melanin granule.
n. nucleus.
s.gr. melanin granule in body wall.
sph. spheroid in amoebocyte.
s.sph. spheroid in body wall (?) eliminated from amoebocyte.

Detailed examination of the deposits of black or brown pigment in the skin shows that the pigment granules (Fig. 1C, *s.gr.*), in size, form and colour, correspond closely with those in the amoebocytes, and further, that most are associated with bodies which, in range of size and appearance, closely resemble the spheroids (Fig. 1C, *s.sph.*). This clearly suggests that the skin pigment is derived from the amoebocytes.

Fully in harmony with this are other clear signs of participation of amoebocytes in the deposition of skin pigment, such as the constant presence in all parts of the body wall of numerous amoebocytes indistinguishable from those in the coelomic fluid, especially numerous in the loose connective tissue below the epidermis where pigment is so abundant (Fig. 1B). Again there are frequent signs of disorganization among the amoebocytes, and occasional indications of spheroids and their associated pigment being cast out from these cells (Fig. 1B, *eg.s.*) in the body wall.

All histological indications are, therefore, that the pigment is derived from the coelomic amoebocytes, which, wandering into the body wall, unburden themselves of pigment by casting it out or by disintegration. Such a tentative conclusion is fully in agreement with many previous indications of the importance of amoebocytes in the carriage of pigments and metabolites generally, in the bodies of echinoderms (Cuénot, 1891; List, 1897; Kindred, 1926; Lison, 1930; Millott, 1950), and especially with the more revealing results from experiments on *Diadema*, reported previously (Millott & Jacobson, 1952*b*), in which iron saccharate injected into the coelomic fluid was found to be carried by amoebocytes and deposited in the melanophores of the skin.

Again, the histological evidence of breakdown of the amoebocytes in the body wall, with liberation of their contained pigment, parallels the same phenomenon observed to occur in the living amoebocytes of the coagulum (p. 532).

It is interesting to note, in passing, that Cornil *et al.* (1935*b*), working on *Holothuria forskali* and *H. tubulosa*, observed the frequent association of pigment deposits in the body wall with amoebocytes and the appearance of cellular disintegration, and, further, believed that the matter eliminated was re-utilized by the amoebocytes. '...et l'on peut penser que les amas de désintégration sont susceptibles d'être réutilisés par le travail amibocytaire. L'appareil pigmentaire serait ainsi soumis de façon constante à un processus de destruction (pigmentolyse) et de néoformation (pigmentogenèse).'

Finally, the participation of coelomic amoebocytes in melanogenesis is supported by the discovery of a well-defined enzyme system in these cells, which brings about the oxidation of both mono- and poly-phenols, and is distinct from the cytochrome-cytochrome oxidase system. Such enzymes have been shown to be associated with melanogenesis in a wide variety of forms.

The Phenolase System in the Coelomic Fluid

Simple colour reactions, based on those previously employed by Raper (1932) and Pugh & Raper (1927), were used to reveal the presence of phenolases, coelomic fluid being added to buffered phenolic substrates, oxidation being indicated by darkening of the solutions. The experimental solutions were made up as follows: 5·0 c.c. coelomic fluid (freshly withdrawn and well shaken to disperse the coagulum) + 5·0 c.c. 0·06 M Sørensen buffer (mostly at pH values ranging between 6·5 and 7·0), containing 0·1–0·2 % of the phenolic substrate + a few drops of toluol or chloroform (as an antiseptic).

In all instances experiments were accompanied by controls in which the coelomic fluid had been boiled and cooled before use. Both experiments and controls, frequently inspected and gently agitated, were incubated at 24° C. The controls were especially necessary in view of the tendency of some phenolic substrates to auto-oxidize, and the fact that the coagulum itself darkens on exposure. Usually a little detergent was added both to experiments and controls (see below).

These experiments showed that the coelomic fluid possesses the power to oxidize phenols such as 'dopa', pyrocatechol, L-tyrosine, pyrogallol and cresol (mixed isomers) with glycine. The characteristic coloured oxidation products always appeared in the initially colourless solutions, those containing 'dopa' or tyrosine becoming grey or black, pyrocatechol reddish brown, pyrogallol brown, whilst those containing a mixture of cresols and glycine became scarlet or orange. The controls, with boiled coelomic fluid, showed either no change, or where the substrates tended to auto-oxidize, relatively little change.

It is noteworthy that detergents, such as 'Cetavlon' (Imperial Chemicals (Pharmaceuticals) Ltd.) and saponin, exerted a marked accelerating effect on oxidation. Without detergent, oxidation of the phenolic substrates occurred only slowly, requiring at least 12 hr. at pH 7·0 and 24° C. to bring about a distinct colour difference between experiment and control. After adding a little detergent pronounced darkening of the experimental solutions as compared with the controls could be observed within 3 hr. at the same temperature, pH, and with the same substrate concentrations.

Thus it is clear that the capacity of the coelomic fluid to oxidize phenols depends on a heat-labile factor with marked surface action. However, it is not yet clear whether this is an enzyme system or not, since such oxidations can be brought about by heat-labile factors such as copper-protein complexes that are not usually regarded as enzymes (see Bhagvat & Richter, 1938).

Two features of the reactions, however, namely their sensitivity to potassium cyanide and pH, indicate the participation of enzymes. Thus oxidation of L-tyrosine and pyrocatechol at pH 7·3 is strongly inhibited by concentrations of potassium cyanide as low as 0·0002 M, and the oxidation of tyrosine occurs

most readily in solutions buffered within the pH range 6·0–7·5 at 24° C., falling off rapidly on either side.

Owing to the limitations of the method employed, it was pointless to attempt the determination of a precise optimum; nevertheless it is clear that the range of activity corresponds with that of tyrosinase (Raper, 1928).

It now remains to be discovered whether the enzyme system indicated is to be found in the coelomic fluid, or in the suspended amoeboid cells. That it is present only in the cells can be shown by separating them by centrifuge, boiling the remaining fluid, and after cooling, reconstituting the coelomic fluid by replacing them in the fluid. The reconstituted fluid thus formed shows an undiminished power to oxidize phenolic substrates, and thus the heat-labile factor I believe to be an enzyme system, must be present in the amoebocytes, and not in the fluid.

Such experiments have a further significance in showing that there is no heat-labile factor in the fluid inhibiting oxidation, as was found in *Diadema* (Millott & Jacobson, 1951), since no increased oxidizing power resulted from the treatment.

The existence of an enzyme system in the amoebocytes capable of oxidizing phenolic substrates at once leads us to suspect as responsible, in some measure at least, the widely distributed cytochrome-cytochrome oxidase system. However, the fact that mono-phenols such as L-tyrosine are oxidized shows that this system cannot be entirely responsible. The sensitivity of the cytochrome-cytochrome oxidase system to sodium azide at pH 7·3, and to acetone, shown by Keilin (1936) and Keilin & Hartree (1938), enables us to eliminate this system as responsible in any significant measure for the effects described here, for the oxidation of polyphenols such as catechol was not affected either by 0·002 M sodium azide at pH 7·3, or by acetone.

There are thus clear indications that the coelomic amoebocytes of *Holothuria forskali* contain an enzyme system of the phenolase type commonly associated with melanogenesis.

It is now appropriate to point out certain peculiar features in the system. First, thiourea exerted but slight inhibitory effect, at a concentration of 0·001 M, on the oxidation of tyrosine at pH 6·5 and 24° C.; and no discernible effect, at a concentration of 0·0005 M, on the oxidation of pyrocatechol at pH 7·3. Phenolases are characteristically sensitive to substances binding copper ions, so that this is an unexpected property of the system. Secondly, in some preliminary experiments, it was found that irradiation with ultraviolet light exerted but a small and inconstant effect: 5 min. irradiation of coelomic fluid by means of a 'Hanover' mercury arc, sometimes increased (but only slightly) its power to oxidize L-tyrosine at pH 6·5. Irradiation for longer periods diminished it. This too was unexpected, as similar treatment of the coelomic fluid of *Diadema* was found to produce the normal effect and activate the enzyme system (Millott & Jacobson, 1952*b*). These

findings are advanced with due caution, however, as it is considered that more experiments are necessary, especially to eliminate possible heating effects.

Discussion

The foregoing investigation, though regrettably incomplete, is adequate to confirm previous indications of the occurrence of melanin in *Holothuria forskali*, and to show that the occurrence of melanin in this form is coupled with the presence of an active phenolase system. The association is noteworthy in view of many indications of the participation of such enzymes in melanogenesis in a wide variety of animal forms, but especially because of their importance in the echinoid *Diadema*. Thus in both echinoderms the enzyme system occurs in the histologically similar coelomic amoebocytes, which in both produce a black pigment with the characteristics of melanin. Further, it is most significant that the pigment appears in the amoebocytes of both, in association with cytoplasmic elements, the spheroids.

The association of granules of melanin with spheroids is remarkably constant, not only within the amoebocytes, but also in the skin (Fig. 1 c), and the ubiquity of the association suggests that the spheroids are involved in pigment formation, a suggestion which is fully supported by the histological evidence brought forward on p. 534. This is most significant, for in *Diadema*, where the same association prevails, there is evidence that the spheroids and their immediate surroundings are regions of high redox potential, a factor known to be important in melanogenesis (Figge, 1940; Dawes, 1941; Schuppli, 1950).

The enzymic activity of the coagulum formed in the coelomic fluid, in its capacity to bring about oxidation of both mono- and poly-phenols, sensitivity to cyanide, pH range and insensitivity to sodium azide and acetone, resembles that of tyrosinase, an enzyme that has been widely associated with melanogenesis. In the absence of more precise experiments, it is clearly unprofitable to speculate as to whether one or more enzymes are involved, a matter that has proved difficult to decide even with refined techniques (see Dennell, 1947; Lison, 1936; Greenstein, 1948; Lerner & Fitzpatrick, 1950; Schaaf, 1950; Kertész, 1951). It is for this reason that I have at all times preferred to refer to an 'enzyme system' rather than to use more committal terms.

The indications of the participation of cytoplasmic elements such as the spheroids in melanogenesis are interesting in relation to the controversy as to whether melanin is of nuclear or cytoplasmic origin (see Verne, 1926; Meirowsky, Freeman & Fischer, 1950; Meirowsky & Freeman, 1951; etc.), but it should be borne in mind that the relevant evidence cited in this investigation is largely histological, and can be advanced only as an indication, and the possible origin of some of the melanin at least, from the nucleus, has been by no means excluded. Clearly further work is required, but it may be

noted in passing, that it is doubtful whether the distinction is worthy of much emphasis in the light of modern conceptions of cellular metabolism.

Finally, it must be emphasized that the incomplete information obtained makes it unprofitable to attempt to formulate a conception of the process of melanogenesis in *Holothuria*, as was done with *Diadema* (Millott & Jacobson, 1952*b*), but it must be admitted that, so far, the processes seem remarkably similar, and further comparison must necessarily await further work.

My thanks are due to Dr H. G. Vevers, Dr F. W. Jacobson, and to Prof. E. Meirowsky for help in connexion with references, and to the Director and staff of the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, who kindly gave me facilities to work during a short but pleasant visit.

Summary

The black body-wall pigment of *Holothuria forskali* shows the characteristics of melanin.

From histological evidence it appears that the pigment is formed in association with the amoebocytes of the coelomic fluid, which eliminate the pigment in the body wall.

The amoebocytes contain a phenolase system, distinct from the cytochrome-cytochrome oxidase system, with the properties of tyrosinase.

The relation of these findings to those of a preceding and more complete investigation into melanogenesis in *Diadema* is discussed.

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SPONTANEOUS SQUIRTING OF AN ASCIDIAN, *PHALLUSIA MAMMILLATA* CUVIER

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(Text-figs. 1–16)

Introduction

The simple monascidian *Phallusia mammillata* Cuvier was found by the author to exhibit rhythmical spontaneous activity during long periods of observation in the laboratory (Hoyle, 1952) The movements consist principally of quick contractions of the whole body-wall together with water ejection from the branchial and atrial cavities. The water movements are of the nature of squirts similar to those which the animals give in response to a violent external stimulus, and are accompanied by siphon-rim closure as an inevitable consequence of the contraction of the body-wall musculature. The closures can be recorded without disturbing the animals unduly by a method which was described in the same paper. A slight inward movement of the test can be observed as the total volume of the animals diminishes during the squirts. The present work is a study of the frequency patterns of the spontaneous squirting of *Phallusia*, together with a brief study of the physiological mechanisms involved in its production and a consideration of the possible functional significance of the activity.

The phenomenon of spontaneous activity in ascidians has not attracted much attention. Polimanti (1911) observed spontaneous opening and closing of the siphons of *Ciona intestinalis* at high temperatures (30° C.) but does not describe spontaneous movements under more normal conditions. Several earlier workers on ascidians observed activity only in relation to the definite ejection of foreign particles, faeces or sexual products (Magnus, 1902; Fröhlich, 1903; Jordan, 1908). The phenomenon was first clearly described by Hecht (1918) working with *Ascidia atra* from the Bermuda coast. He found spontaneous contractions of the branchial siphon at intervals of about 5 min. at unspecified temperatures. He observed contractions visually in a colonial species *Ecteinascidia turbinata*. In considering the functions of the rhythm Hecht examined the possibilities that the movements may serve either to remove waste or to subserve respiration. He rejected both possibilities by argument based on his earlier observations on the ciliary current (Hecht, 1916). He finally suggested that the rhythm may be 'the degenerate remains of an activity homologous with the rhythmic pulsations of the salps', and that 'no function can be ascribed to this rhythmic occurrence'. This suggestion begs

the question of phylogenetic relationships within the Tunicata and assumes the salp activity to be a primitive locomotory mechanism. The work of Hecht was followed by that of Day (1919), who observed spontaneous contractions in amputated branchial siphons of *Ciona*. This observation indicated that the nerve ganglion is not necessarily responsible for the rhythmicity, but Day did not observe spontaneous activity in the intact animal.

The problem of the nature of the activity and its relation to the ganglion was more completely investigated by Yamaguchi (1931), who showed that in *Styela clava* the rhythmicity affects both siphons synchronously. Marked contractions occur at intervals of about 8 min. in running sea water, together with smaller contractions at irregular intervals (published record). The rhythmicity became much more marked and also more frequent after a period in filtered sea water. The interval was then reduced to about 3 min. After ganglion extirpation the siphons still showed spontaneous activity, but the co-ordination was lost and the frequency was very variable. Moreover, the frequency was now markedly increased and the amplitude reduced. Yamaguchi also recorded spontaneous activity from both amputated oral and atrial siphons. According to Yamaguchi the normal spontaneous contractions of *Styela* are of two kinds. One concerns the circular muscles and results in water expulsion. The other consists of logitudinal muscle contractions and siphon closure which does not involve water expulsion. The latter is less frequent. Yamaguchi does not give measurements of the relative frequencies, but from his published records the ratio seems to be about 1 : 6. In his paper he makes no attempt to explain the functional significance of the rhythm.

Bacq (1935), in commenting on the spontaneous activity of *Ciona*, remarked that after ganglion extirpation he found the spontaneous activity more frequent and of reduced amplitude. Ganglion extirpation results in reduced muscle tone. Miss P. Kott has recorded spontaneous activity in *Ciona intestinalis*, *Ascidia aspersa* and *Molgula hattensis*, but unfortunately she has discontinued this unpublished work (see Report of the Council of the Marine Biological Association in *Journ. Mar. Biol. Assoc.*, Vol. 30, 1951). The most recent published comment on the activity was that of Young (1950), who suggested that the automaticity may be something in the nature of a hunger contraction.

Patterns of Spontaneous Activity

In Intact Animals

About sixty normal *Phallusia* have been observed altogether at either the spring or autumn of two seasons. All these animals showed spontaneous activity. With only two exceptions the contractions of both siphons were synchronous except for small movements caused by vibrational and other stimuli. Most of these animals were kept in tanks of the Plymouth aquarium under circulation for a few hours before observation. Some, however, were

observed immediately after collection some 5 hr. previously. In the latter animals the siphons opened slowly, taking about 4 hr. to open completely. Spontaneous movement is apparent after the siphons begin to open in all instances and the contractions increase in amplitude as the siphons open more widely. The frequency of the contractions of these animals is high at first and gradually reduces in Plymouth tank sea water. Eventually a nearly constant rhythm of contraction is apparent, with spontaneous squirts at 6–9-min. intervals. All the observations were at temperatures from 11 to 14° C. Although this type of activity is most commonly recorded it changes at times to other equally regular patterns. These patterns include one in which large contractions alternate with a number of smaller ones (Fig. 10, lower record). Some of Yamaguchi's animals showed this pattern, and he described the large contractions as due to longitudinal muscles and the smaller ones to circular muscles. It is not possible to distinguish muscle types clearly in *Phallusia*, where the general muscle pattern appears to be very irregular. This type of activity is not common in *Phallusia*. Another type consists of bursts of squirts alternating with periods of rest. This type of variation recalls the familiar patterns of *Arenicola* preparations (e.g. Wells, 1949).

By far the commonest variations are simple increases or decreases of frequency. The changes to a different frequency occur fairly abruptly, sometimes very abruptly, and are often prefaced by a short burst of vigorous activity (Fig. 1 D). The new rate is almost exactly twice or one half the original one and is always maintained for a few hours. It is possible to distinguish slow, medium and fast rates of spontaneous squirting (Fig. 1, A–C). The animals were kept in 20–30 l. of sea water which was changed usually once a day. The value of intervals between squirts averaged approximately 16 : 8 : 4 min. The rate changes were not accompanied by any change in the amplitude of contraction. Sometimes, after a fresh supply of outside sea water, an animal will change to the slow rate after about 2–3 hr., maintain this for a further 4 hr., then transfer to an ultra-slow rate (intervals of about half an hour) for about 3 hr., during which time the amplitude gradually declines until the spontaneous contractions are only just perceptible. The first animal showing this behaviour was discarded in the belief that it was dying, but when another followed suit this was left overnight and was later found to have started again at the medium rate. Later, after a new supply of the same outside sea water, it repeated the performance. When the amplitude decreased, the branchial siphon declined first, the atrial later. When the amplitude increased again the branchial siphon increased before the atrial. The observations show that the decline in frequency is unlikely to be due to poor condition of the animals.

In Operated Animals

Since the squirting is rhythmical it is reasonable to suppose that there must be a pacemaker controlling the frequency. This may be situated in the

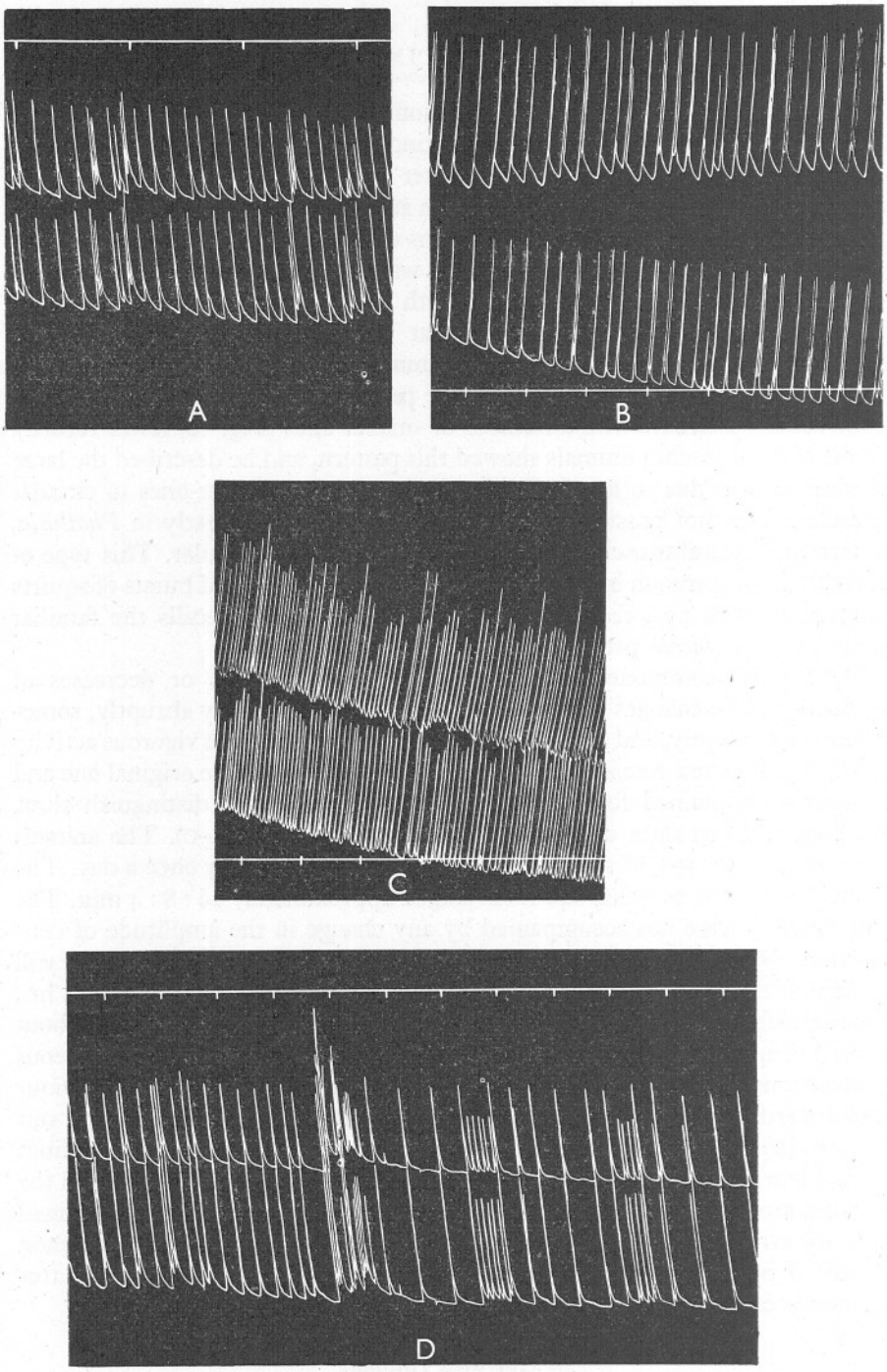

Fig. 1. A, the 'normal' 8 min. rhythm; B, the 'slow' 16 min. rhythm; C, the 'fast' 4 min. rhythm; D, spontaneous change-over from the 'medium' to the 'slow'. Time marks A–C, 1 hr.; D, 30 min. Upper record branchial siphon, lower record atrial siphon.

ganglion, in one or other of the siphons, or in some part of the body-wall. The purpose of the experiments described under this heading was to attempt to determine the site of the hypothetical pacemaker. The first experiments consisted of recording from the siphons of deganglionated animals. The method of operation to remove the ganglion has been described by Carlisle (1951). Animals in which the ganglion has been removed recover from the operation in a few hours and again show spontaneous activity. This is very variable from one animal to another. The amplitude of the contractions is invariably reduced. The atrial siphon tends to maintain the medium rate whilst the branchial siphon, although sometimes contracting at the same time as the atrial, also shows many other erratic contractions (Fig. 2). The activity is relatively constant over many hours regardless of the state of the bathing

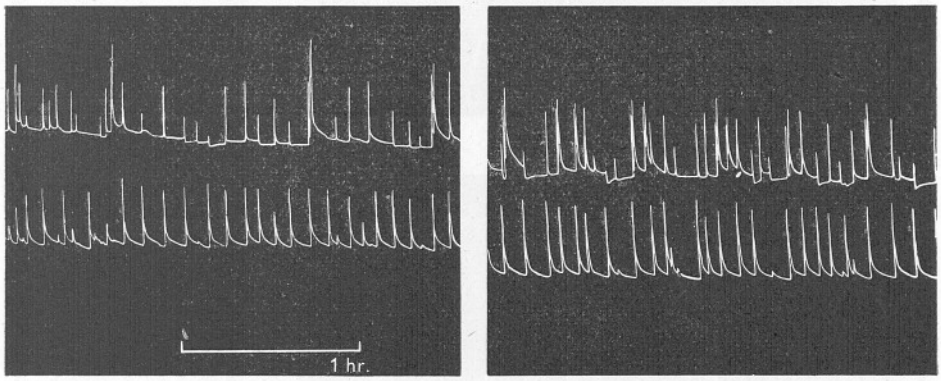

Fig. 2. Movements of the siphons of a deganglionated animal. Upper record branchial siphon, lower record atrial siphon. Filtered sea water. An interval of 10 hr. separated the two tracings. Note the occasional positive openings of the branchial siphon.

sea water and food supply, and the spontaneous changes of pattern seen in intact animals are not apparent. In other experiments the ganglion was left undamaged, but the nerves leaving it and passing to a siphon were cut close to their point of attachment to the ganglion. After this operation there is a general loss of tone as evidenced by the more sluggish responses of both the siphon-rims. There is a tendency at intervals for the denervated siphon to give a continuous series of closely spaced contractions. These summate to close that siphon and expel water from it almost completely.

Whole siphons were amputated, and after about 6 hr. records were made of the activity of both the amputated siphons and the intact ones. No movements of amputated siphons occurred. The intact siphons, after remaining closed for a few hours following the operation gradually open. When fully open the intact siphons, both atrial and branchial, resume a fairly normal rhythmicity but with considerable amplitude variations (Fig. 3). The results are in general agreement with those of Yamaguchi (1931), but whilst both he and Day (1919)

observed or recorded spontaneous movements in amputated pieces of siphon, no such movements are observed with *Phallusia* material. In this animal there is a strong body-wall attachment only at the siphon rims and contractions of the body-wall in the cut portion could not affect the siphon rim very much even if they were vigorous.

The general conclusion from all these results is that the pacemaker is not situated in either the ganglion or a particular siphon. So far only body-wall

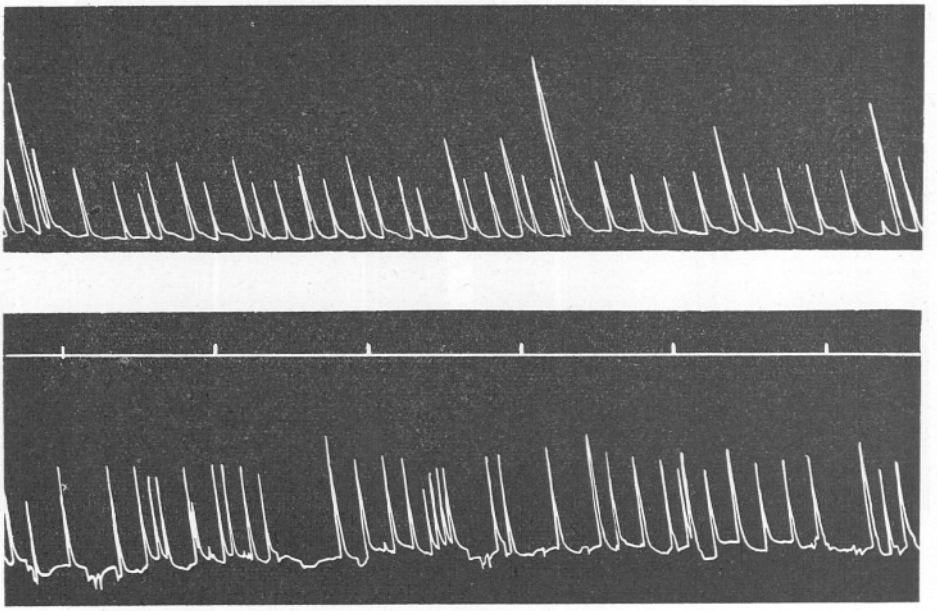

Fig. 3. Upper tracing from branchial siphon after amputation of the atrial. Lower tracing from an atrial siphon after amputation of the branchial siphon. Note the occasional positive openings of the atrial siphon. Time in hr.

muscle from the intersiphonal region has been examined for the presence of purely local spontaneous activity. A strip of muscle 3 cm. long can easily be obtained from the intersiphonal region of the left side. Such strips have been dissected out and mounted vertically with the free end attached to an isotonic lever. These strips show spontaneous slow contractions on which quicker movements are superimposed. The movements of one of these strips were recorded at the same time as those of the siphons of a deganglionated animal and are illustrated in Fig. 7. The quick movements are irregular, but occur at similar intervals to the movements of the branchial siphon of the deganglionated animal. It is just possible that the isolated body-wall strip movements are myogenic: all parts of the body-wall may have a similar

activity, but this remains to be proved. Also, since Hunter (1898) has figured cell-bodies in nerve-muscle strips of an ascidian, and since the strips used undoubtedly included some fine nerve elements, the possibility must be included that diffuse nerve elements act as local pacemakers. However, the observations suggest that rhythmicity is a natural property of the body-wall. In the whole animal this is subject to considerable co-ordination and control by the ganglion, but there is still some co-operation even in the absence of the ganglion, especially within a siphon.

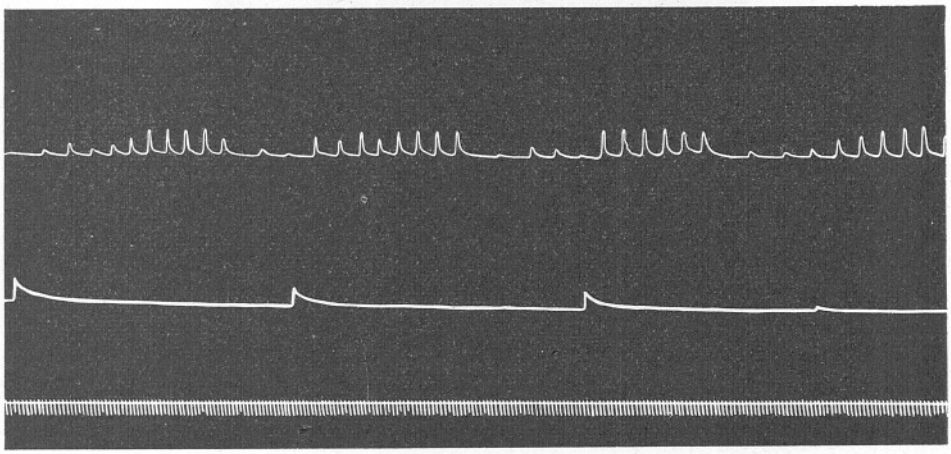

Fig. 4. Peculiar pattern of activity shown by one specimen in which the nerves to the atrial siphon had been cut. The amplitudes were quite small. Upper record branchial, lower record atrial. Time in min.

In fatigued and operated animals the spontaneity often presents peculiar patterns, but these always suggest that the basic phenomena are cyclical. One such pattern is shown in Fig. 4. The nerves to the atrial siphon had been cut, and the pattern illustrated soon developed and was maintained for several hours. The amplitude of the branchial siphon fluctuated in a periodic manner and the atrial siphon contracted at times corresponding only with the minima of the branchial siphon movements. Periodic fluctuation of the branchial siphon amplitudes is not uncommon in intact animals. The interval between the modulation peaks is then about 45 min. The mechanical response of a siphon to electrical stimulation is partially a function of the previous history of the animal, partly a function of the stage in the spontaneous activity cycle (Hoyle, 1952). A single excitation given just before a spontaneous squirt is due frequently elicits a large response. A single excitation given just after a spontaneous contraction tends to set up a series of closely spaced contractions. Both phenomena are present in operated animals. Thus, in Fig. 5, a single

shock produced the latter effect although the nerves to the atrial siphon had been cut. After a short period of electrical stimulation the frequency of spontaneous squirting is reduced. The previous rate is regained fairly quickly (Fig. 6). All these phenomena must be due to some effects of external stimuli on the basic mechanisms which determine pacemaking in the body-wall.

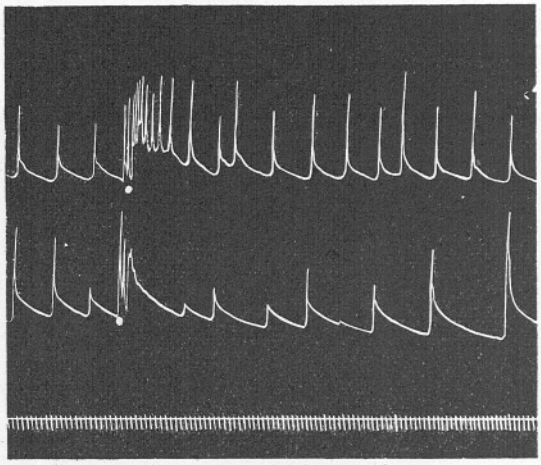

Fig. 5. The consecutive series of contractions in response to a single electrical stimulus given immediately after a spontaneous squirt evidenced in an animal with the nerves to the atrial siphon (lower trace) severed. Time in min.

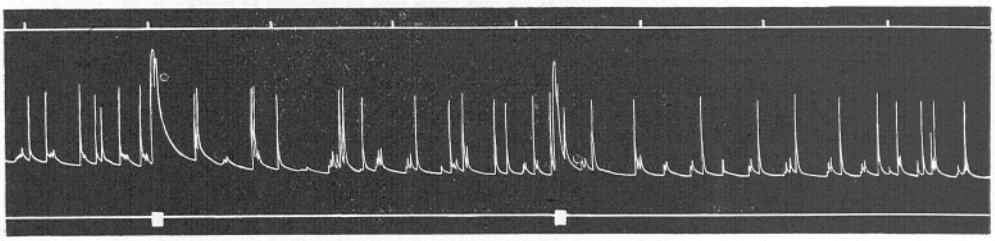

Fig. 6. The effect of a series of electrical impulses at 1 in 2 sec. intervals (indicated on the lower line) on the frequency of the spontaneous activity. Branchial siphon. Time in hr.

Volumes of Sea Water Displaced

The classical interpretation of feeding in sedentary monascidians is that they extract food particles from a continuous stream of water. This stream is maintained by the activity of cilia situated on the inside of ostia which perforate the branchial sac. The stream enters the branchial siphon, passes through the perforations and leaves via the atrial siphon (Fol, 1876; Roule, 1884; Orton, 1913). This current is supposed to be continuous, although no long-term

observations have been made. Squirting, on the other hand, produces no flow through the pharynx; the water is expelled from the whole branchial and atrial sacs through the respective siphons at the same moment. It is of considerable value to know the actual volumes displaced by the two activities and to compare them. The only published observation on the rate of the through-current with which I am familiar, and one which is often quoted, is by Hecht (1916). Hecht fitted a glass tube into the atrial siphon of *Ascidia aspersa* and then fed the animal on carmine particles. When the particles appeared in the exhalent stream he measured the time taken for them to traverse the length of the tube. By a simple calculation (not specified), he deduced that a 100 g. animal passes 173 l. of sea water per day. This means an average output of

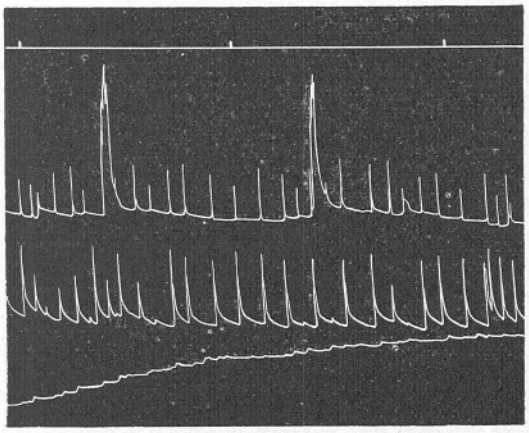

Fig. 7. Spontaneous movements of an isolated strip of body-wall from the intersiphonal region. The movements are the lowest tracing and are recorded at the same time as those of the siphons of a deganglionated animal. Upper record branchial, lower atrial. Time in hr.

2 c.c./sec. Hecht's observations are open to some criticism. He appears to have multiplied the rate of movement of particles along the tube by its cross-sectional area in order to obtain rate of flow values. This assumes the rate to be uniform across the tube. In practice it is a function of the distance from the walls. There is no flow in the region in immediate contact with the walls. The rate increases towards the centre, where it is maximal, by a factor which is proportional to the square of the distance, i.e. the curve relating rate of flow to distance from the wall is parabolic. The Poiséille formula for the quantity of liquid, Q, flowing through a tube in unit time is

$$Q=\frac{\pi}{8}\frac{pa^4}{\eta},$$

where p = pressure drop per unit length of tube, η = viscosity of liquid, and

a = diameter of tube. When the formula is derived assuming the velocity to be uniform it becomes

$$Q = \frac{\pi}{4}\frac{pa^4}{\eta}.$$

That is, Hecht's values are probably too large by a factor of $\times 2$, since most of the particles will travel down the centre of the tube if the conditions for smooth flow are satisfied. In practice also, Hecht's higher rates are such that they give Reynold's numbers of the order of 2000. A Reynold's number of 1000 or greater is taken as an indication of the conditions for turbulent flow, which would make measurements by Hecht's method impossible. As a further check on the possible validity of these high figures, I have arranged a constant-flow device to deliver sea water containing carmine particles through a tube with the same dimensions as Hecht's and find difficulty in following the progress of any particles through the tube when the rate of flow is 2 c.c./sec.

The ciliary current of *Phallusia* has been measured in two different ways.

(1) The first method gave an intermittent record. A cannula with a wide tube and long, right-angled, narrow-bore side arm was inserted in the branchial siphon. The cannula and side arm were fixed in a horizontal position. At 2 min. following a spontaneous squirt, when the animal was quite undisturbed, the main arm was suddenly closed. The rate of water uptake was then measured by timing the movements of an aniline bubble along the side arm. With this method the greatest rate of flow observed was 4 c.c. per minute in Plymouth tank sea water at 14° C., during March 1952. The flow was not infrequently zero in the same individual. A rough average figure was 1 c.c./min.

(2) The second method was devised to give a continuous record of the rate of flow. It was similar to that used by Wells & Dales (1951) for recording the circulation of water in the tubes of Polychaete worms. A special cannula was gently inserted into an open atrial siphon. The bulb of the cannula was made just larger than the diameter of the siphon. The natural seal thus formed was capable of withstanding about 12 cm. pressure of sea water. The cannula was mounted vertically with its side arm horizontal. The wide vertical tube contained a float which was attached to a lever writing on a kymograph (Fig. 8). The side arm opened to the exterior through a short length of capillary tubing which could be by-passed for null-level determinations by a T-piece with pinchcock. The whole was covered with sea water (about 20 l.) to a level above the side arm. The device could be calibrated by calculation (see Wells & Dales), given the magnitude of the capillary, lever amplification, etc., or a constant-flow device could be inserted in place of the animal. The ciliary flow maintains a continuous depression below the null-level line, whilst the squirts are also recorded quantitatively as superimposed spikes. A typical atrial siphon record obtained by this method is shown in Fig. 10. The contractions appear slightly less regular in frequency than those recorded by the

siphon-rim closure method. Hence it is probable that the cannula constitutes a greater disturbance than the lever arms. Also, the method interferes to some extent with defaecation. However, one animal survived a week of this treatment without obvious deterioration. The method can also be used to determine branchial siphon activity by using a larger cannula and either removing or increasing the diameter of the capillary input resistance so that free access to the sea water is possible. The ciliary through-current is not then recorded.

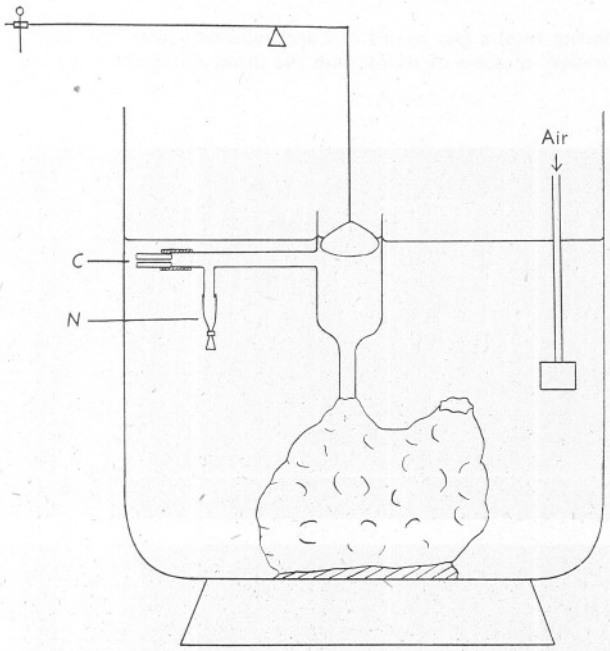

Fig. 8. Method of recording continuous flow and squirting simultaneously. *C*: capillary resistance; *N*: null-level indication pinchcock.

With the second method of recording it is seen that the branchial sac squirts about 12 c.c. and the atrial about 10 c.c. The average capacity of the branchial sac of a full-sized *Phallusia* is 17 c.c. The corresponding atrial sac holds about 14 c.c. These are necessarily rough values owing to the difficulties involved in making the determinations, but it is apparent that each sac expels some two-thirds of its contents on each squirt. A tracing from a fast record of a branchial siphon squirt is shown in Fig. 9. This was obtained by the float method. The water ejection is very rapid, occupying only $\frac{3}{5}$ sec. With the float method the continuous depression due to the ciliary current again shows it to have an average value of only 1 c.c./min. This represents a pressure in the side arm of 2 mm. water, which may be as large as the current is capable of producing.

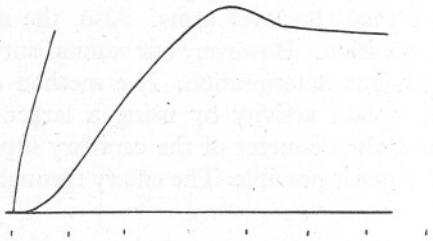

Fig. 9. Tracing from a fast record of a spontaneous squirt. The first stroke marks a calibration injection of 10 ml. with the drum stationary. Time in $\frac{1}{5}$th sec.

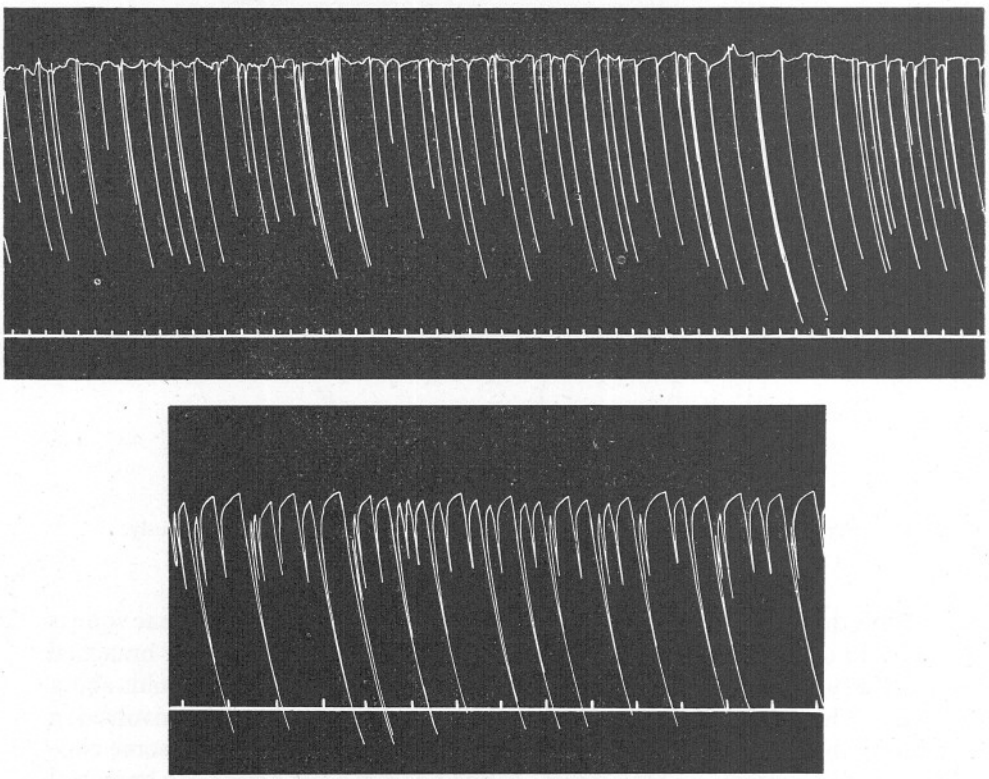

Fig. 10. Upper record: typical record of atrial siphon activity by the flow method. Time in 10 min. Lower record: variant of above showing alternate large and small contractions. Smaller diameter capillary than in upper record. Time intervals: 10 min.

Thus one might suspect that the real value might be larger than 1 c.c. However, the value was often less than 1 c.c./min., as recorded with the same method. We are therefore left with the problem of the still enormous difference between the *Phallusia* values for the through-current and the Hecht values which, even when allowance for possible errors in method are made, show the ciliary current to be some 100 times greater. Further, we must consider whether the observed *Phallusia* values would be adequate to subserve respiratory and nutritional requirements.

On the first of these points, an observation of considerable importance is that when carmine or graphite particles are fed to *Phallusia* they do not appear in the exhalant current. They are not again apparent until they are ejected as faeces. If very thick suspensions are added some of the particles are ejected from the branchial siphon at the next squirt. However, it is clear that the animals are very efficient at removing particles from the water going in between squirts. If Hecht's carmine particles were coming out of the atrial siphon almost immediately, how were they getting through the mucus film lining the branchial sac so quickly? Why were they not picked up by the feeding mechanism? Is this mechanism capable of selecting food particles and rejecting carmine? Some ascidians are known to have large slits connecting the anterior part of the pharyngeal sac directly with the atrial cavity. These slits were first described by von Kupffer (1875) and were called pharyngo-cloacal slits by Garstang (1891). Their function is uncertain, although Garstang offers the suggestion that they may serve to allow water from the contracting branchial siphon to flush through the atrial siphon and so help to remove accumulated faeces. I have observed carmine particles fed to *Ciona intestinalis* passing through such slits and passing directly out of the atrial siphon a few seconds after entering the branchial siphon. Clearly, the behaviour of these particles cannot indicate the fate of particles within the main body of the branchial sac which can presumably trap and remove them efficiently. Further, in view of the ease with which a contractile response can be elicited, it is just possible that Hecht's observations were unwittingly made on particles passing through these slits and subsequently being forcibly ejected by actual contraction of the body-wall.

The second point to be considered is the functional adequacy of the low values observed in the *Phallusia* experiments. Observations on the nutritional requirements of ascidians were made by Moore, Edie, Whitley & Dakin (1912). They found that a group of small unidentified ascidians of capacity 10 c.c. could be expected to obtain adequate oxygen and more than adequate food, on the assumption that metabolism is constant, from 150 c.c. Port Erin outside sea water per hour. If we assume a *Phallusia* to require 2–3 times this volume then the observed through-current is clearly inadequate. The water brought in by the current is not, however, the only source of food and respiratory gas. Respiration is also served by both the branchial and the atrial pumping. The

former drives about 90 c.c./hr. and the latter about 76 c.c./hr. into contact with the internal body surfaces. Also, blood circulates through the test to the periphery and respiratory exchange may occur by diffusion across this surface. In all the experiments described, a large surface of sea water has been exposed to the air so that gaseous diffusion alone should serve to keep the water fully aerated, but vigorous air currents have also been added, to ensure both aeration and constant circulation. However, when the latter was deliberately omitted, the frequency of squirting was not affected. The total volume available for feeding is the sum of the through-current and the volume driven by the branchial siphon, i.e. about 150 c.c./hr. in normal animals. If we accept the data of Moore *et al.* this is only barely adequate for an animal living on outside sea water. The economy would be severely strained if for any reason the available food concentration became further reduced. However, since the volume driven by squirting is large compared with the through-current, it is possible that squirting constitutes an integral part of the feeding mechanism and the amount squirted can be varied by changing the frequency of spontaneous squirting, a phenomenon which has been observed in *Phallusia* as described above.

The Effects of Feeding and Starvation

This interesting possibility, that the squirting is part of a controlled feeding mechanism, has been tested in the following way using both siphon-closing and flow-recording methods. Animals were left to starve in filtered sea water and then fed with mixed flagellate cultures. Alternatively, they were kept in circulation in the Plymoth tank sea water with different rates of flow. The results of the experiments were consistent and precise. After a period of starvation lasting some 15 hr. the frequency changes from the normal to the fast (Fig. 11). The addition of food restores the normal frequency in about 2 hr. After continued starvation for 2 days the frequency is ultra-fast, of the order of one every 2 min. The amplitude is considerably reduced, possibly as a consequence of the increased frequency which may produce some fatigue. Another phenomenon now appears: the siphons slowly move about and it is difficult to keep the levers on the drums. On adding food the siphons become still, and both frequency and amplitude are restored to normal in about 3 hr. (Fig. 12). Control experiments with graphite particles (aquadag S) in place of food particles failed to produce an effect (Fig. 14). In one experiment (illustrated in Fig. 13) the food was added continually and in large measure following a short period of starvation. The animal quickly produced its normal and then its slow speeds. Later, the same phenomenon which had been observed in some freshly fed animals, namely the decrease in amplitude of the contractions with the branchial siphon leading, was again recorded. Some contractile activity was still present but the amplitude was almost completely suppressed. During this time the animal was relatively insensitive

to external stimuli. After 2 hr. the food concentration was reduced by mixing with fresh sea water and the normal activity was quickly resumed.

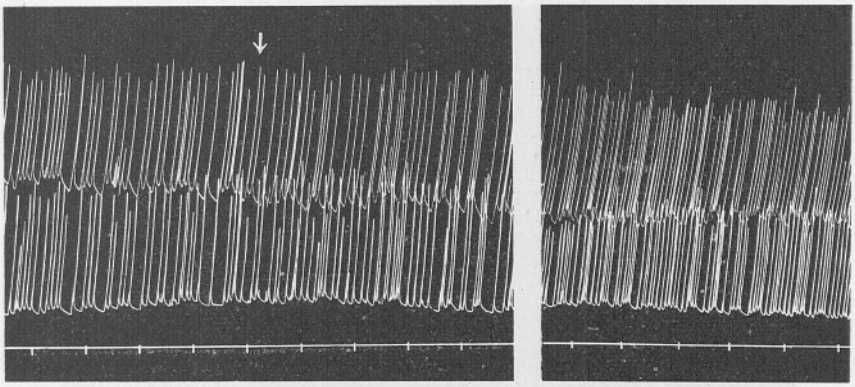

Fig. 11. Continuous records in the Plymouth tank circulation. The rate of flow was great at first and reduced at the point indicated by the arrow to a tiny trickle. An interval of 16 hr. separated the two parts of the record. Time in hr.

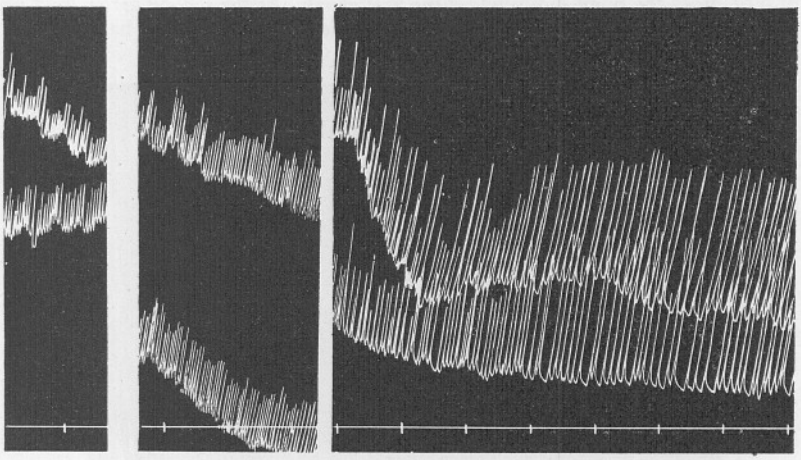

Fig. 12. Records of an animal after 48 hr. starvation in filtered sea water. Food was given to the animal towards the end of the second part of the record, and the third part is a direct continuation following lever adjustment. An interval of 10 hr. separated the first and second parts. Time in hr.

The records obtained by the flow method show similar results (Fig. 15). In addition, they show that the ciliary current is reduced (whether by aperture control in the branchial wall or change of ciliary beat cannot be decided) during starvation, and is restored remarkably quickly in the presence of food

particles. If the branchial siphon is denied free access to fresh sea water and forced to resample the same volume continually the rate of squirting goes up

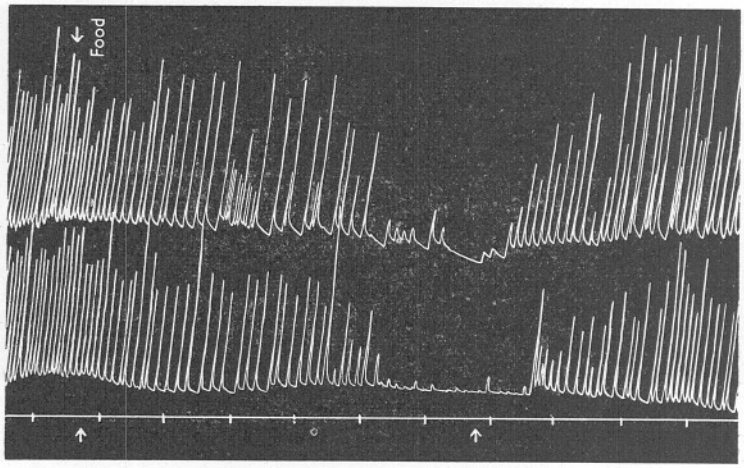

Fig. 13. Records of the activity of an animal starved for 14 hr. and given food continuously for 6 hr. from the point indicated by the first arrow. At the second arrow food was no longer given. A rich flagellate culture was used for feeding. Time in hr.

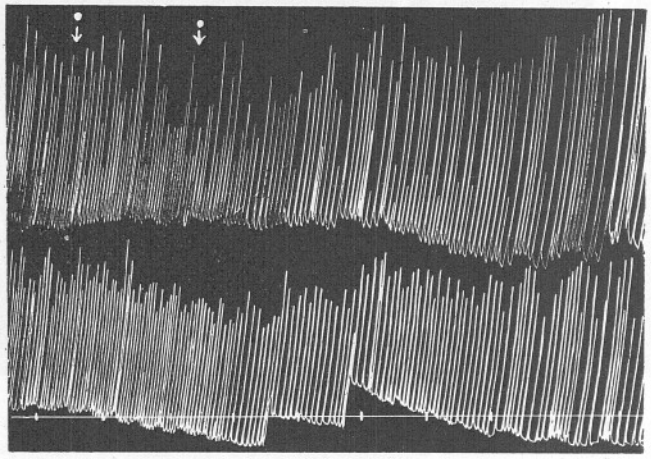

Fig. 14. Control experiments for the above (Fig. 13). The animal was fed only on graphite particles. It was given some food as well at the second arrow. Time in hr.

rapidly (Fig. 16). The ciliary current was not restricted since a suitable capillary input was used and there was no interference with the atrial squirting, hence it is unlikely that the increased rate is due to interference with re-

spiration, but is most probably due to the progressive depletion of food from the water. It is clear from all these experiments that the frequency of spontaneous squirting in *Phallusia* depends upon the concentration of food available

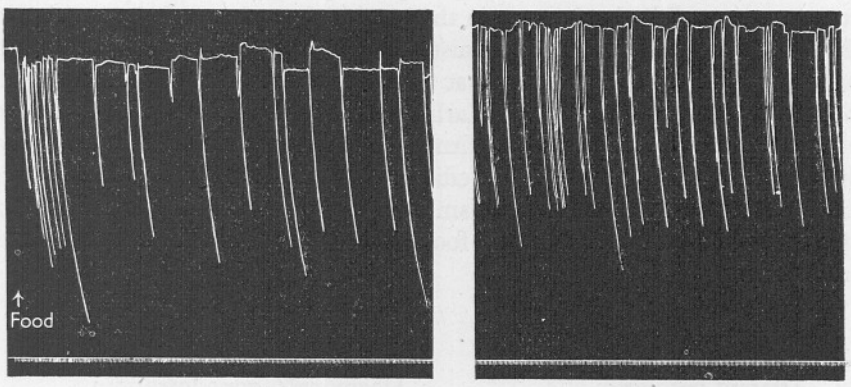

Fig. 15. Flow method recording of atrial siphon squirts immediately after feeding and 16 hr. later. The through-current is proportional to the depression below the upper edge of the record. In the left-hand figure the mean depression represents a continuous flow of 1 c.c./min.

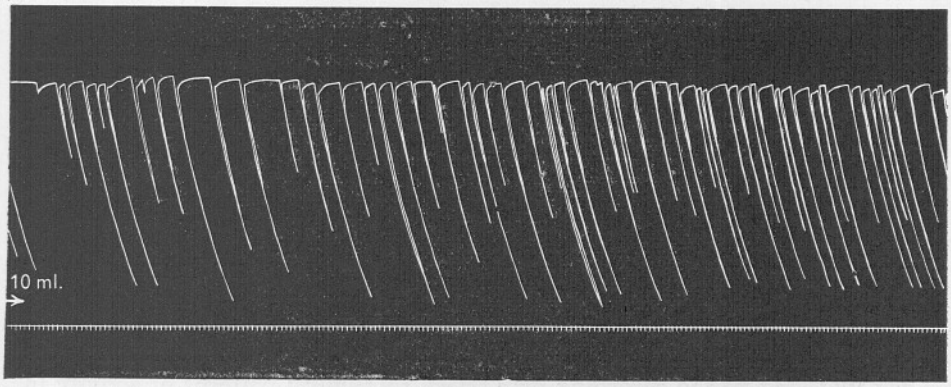

Fig. 16. Flow method recording of branchial siphon squirts showing the effect of restricting the sampling by introducing a capillary input. The arrow at the start of the record indicates a calibration depression of 10 ml. The frequency of the activity increases rapidly during the experiment. Time in min.

in the branchial siphon. The frequency is low when there is a rich supply, high when the supply is impoverished. In this connexion the recent report of Collier, Ray & Magnitzky (1950) is interesting. They find that the volume of the effluent of oysters is proportional to the concentration of some unknown soluble organic material (measured as arabinose equivalents) in the sea water.

Data on the important question of relation between feeding habits and feeding frequency and the concentration of available food over long periods are almost non-existent.

The demonstration that the rate of squirting in an ascidian depends on the concentration of food substance in the branchial sac demands an inquiry into the nature of the receptor mechanism. It has been shown that some of the particles entering the branchial sac of an ascidian enter the mouth of the ciliated pit (van Weel, 1940). Carlisle (1951) has demonstrated the same phenomenon in a salp and confirmed the observation in a monascidian, finding the particles in both the ciliated pit and the neural gland. Carlisle found a gamete-releasing mechanism in *Phallusia* in response to the presence of eggs of the same species in the food. He suggests that the reflex pathways are as follows:

Stimulus	Chemical from ingested gametes
Receptor	Neural gland
Afferent pathway	Hormonal—gonadotrophin
C.N.S.	
Efferent pathway	Neural
Effector	Gonads
Response	Release of gametes

Nervous connexions between the ciliated pit and the ganglion have been demonstrated in two forms (Metcalf, 1895; Hunter, 1898) but have been denied in others (Julin, 1881). The nature of the afferent-efferent pathways needs investigation, but there is certainly a possible receptor mechanism for the food stimulus in the ciliated pit—neural gland complex.

Discussion

The work falls into two parts for the purposes of discussion: (1) the study of the physiological mechanisms of the spontaneous activity; (2) the consideration of its functional significance.

(1) The recent work of Batham & Pantin (1950) has drawn attention to the fact that long-period recording may reveal rhythmical patterns of activity in sedentary animals which on casual observation appear to be quite passive. The sea-anemone, *Metridium senile*, was shown to exhibit periodic contractions of the parietal musculature at intervals of about 10 min. The time sequence is, as Batham & Pantin say, quite low, and they compare it to the slow rhythm of the outbursts of activity of the *Arenicola* proboscis (Wells, 1949) and mammalian uterus. The normal 6–9 min. rhythm of the spontaneous squirting of *Phallusia* is also of this order and deserves equal comparison. The nature and site of the pacemaker presents a difficult problem. The nervous system is extremely simple in anemones and it is difficult to visualize how both pacing

and the co-ordination of muscular movements as complex as those described by Batham & Pantin can be effected by it. The *Arenicola* extrovert is paced by a region of the oesophageal wall (Wells, 1937). In *Metridium* a part of the body-wall appears to act as 'leader'. Other parts of the wall follow the 'leader's' contractions. Although a leading sector may continue to set the pace for a considerable time the site of the leadership may change from time to time. One important aspect of the activity is now clear for both the *Arenicola* and *Metridium* systems: the activity is inherent. It is modified by external stimulation but is not initiated from without. The chain-reflex theory of activity does not apply to either the gross activity cycles of the polychaetes or the slow movements of the anemone. The patterns of spontaneous activity of *Phallusia* clearly associate it with these animals in this respect. The activity is incessant under normal conditions, it is not determined by the periodic fluctuations of any environmental factor or by the gross products of its activity CO_2, faeces etc. It occurs in the absence of food over long periods.

In monascidians spontaneous activity can be recorded from isolated siphons (*Ciona* and *Styela*), and from isolated muscle strips (*Phallusia*). The inherent activity may, therefore, be myogenic with considerable neurogenic control. This situation is perhaps comparable with that found in *Metridium* where one part of the wall leads the rest. The nervous system need only be involved in so far as it effects co-ordination. The quick contractile activity in *Phallusia* involves the whole musculature synchronously, although each part probably has its own activity. The co-ordination is seriously impaired in ascidians in the absence of the ganglion, which also regulates muscle tone and some reflexes.

(2) Concerning the functions of the squirting rather more can be established. The spontaneous contractions of the body-wall serve regularly to clear the exhausted contents of the branchial sac. They must, therefore, assist in keeping the branchial mechanism clean and free from accumulated waste matter. They equally regularly clear the contents of the atrial siphon and this must assist in removing faeces and sexual products. The activity brings fresh supplies of sea water to the exposed internal body-wall and siphon surfaces and must assist in respiratory exchanges. The fresh sea water brought into the branchial siphon following squirts brings with it a supply of suspended food matter. In *Phallusia* the volume of water moved in this way is appreciably larger than that circulated by the ciliary through-current. The frequency of squirting varies inversely with the concentration of available food. It is therefore probable that the principal function of the spontaneous activity in *Phallusia* is to subserve feeding. The method works as follows. Following a spontaneous squirt a new sample of sea water, with various small organisms and food detritus in suspension is sucked into the branchial siphon. The particles are swept to the walls of the sac by the internal circulation of sea water produced by the activity of cilia lining the sac and by the current being drawn

through the apertures of the sac, also produced by ciliary activity. On the walls the particles are caught in the mucus stream proceeding upwards from the endostyle and driven towards the dorsal lamina. Here the twisted cords of mucus containing the particles are driven backwards into the oesophagus. Silt and mud which has not been picked up by the mucus during the few minutes elapsing between squirts is then ejected and the cycle is repeated.

The advantages of this method of feeding are fairly clear. *Phallusia* lives intertidally in sheltered places, or commonly offshore, where the food supply is at times very rich, but subject to considerable fluctuations and often liberally mixed with mud and silt. The branchial mechanisms are particularly liable to become clogged by this estuarine dirt. Squirting helps to remove the waste which would accumulate especially rapidly in an animal with a fast through-current. It also allows a degree of control over the rate of feeding. The continuous-current method does not allow careful control of the rate of feeding when food is plentiful and perhaps being taken in at a far greater rate than can be coped with adequately. The squirting method, since its frequency can be modified, permits a range of feeding volume from zero (when the activity is inhibited after heavy feeding) to about 300 c.c./hr. during starvation. The system is economical of energy. It is now known that the quick contractions of the salps are concerned principally with feeding rather than locomotion (Fedele, 1933; Carlisle, 1950). Some, at least, of the sedentary monascidians may be regarded as feeding by a somewhat similar method of gulping or rather squirting.

The existence of spontaneous activity in several invertebrate phyla has been clearly established, and this activity is apparently always inherent. It has been demonstrated by Wells (1950) that it serves a useful purpose in the lives of certain polychaetes. It is probably equally useful in actinians, although the evidence for this is only just being brought to light, and it is hoped that the present paper will establish its usefulness to certain ascidians. This type of rhythmic oscillation of the work output of certain muscles is widespread. In addition to the groups so far discussed it has also been described in the mantle of lamellibranch molluscs (Redfield, 1917), the *Aurelia* medusa (Widmark, 1913), the sabellid retractor muscles (Wells, 1951) and the retractor pharynx muscles of *Holothuria* (Pople, 1952). Wider investigation may be expected to reveal still more instances.

The work was carried out at the Plymouth laboratory during parts of the springs of 1951 and 1952 and the autumn of 1951, whilst occupying the London University table. I wish to thank the Director and staff of the laboratory for their kindness; Dr M. Parke for supplying the flagellate cultures; and Dr G. P. Wells and Prof. P. B. Medawar, F.R.S., for reading the draft.

SUMMARY

The previous work on spontaneous activity in ascidians is briefly reviewed: there is clear evidence for the existence of spontaneous contractions of the siphons of certain monascidians but no function has been ascribed to this activity. *Phallusia mammillata* exhibits spontaneous contractions of its siphons at intervals of 6–9 min. over long periods in Plymouth tank sea water. The siphon contractions are synchronous. The frequency of the contractions may shift fairly quickly under constant conditions to a higher or lower rate which is usually twice or one half the normal rate.

A method of recording the volume of water propelled by spontaneous activity is described. The method also records the ciliary through-current at the same time as the squirts.

The frequency of the squirting is increased during a period of partial or complete starvation. It is reduced again by adding food cultures to the water.

The physiological mechanisms involved in the spontaneous activity are investigated and comparisons are made with the spontaneous activity exhibited by *Metridium senile* and *Arenicola marina*. It is finally suggested that the spontaneous squirting of certain ascidians is an integral part of their feeding mechanism.

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NERVOUS ORGANS IN THE PERICARDIAL CAVITY OF THE DECAPOD CRUSTACEA

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(Plates I–IV and Text-figs. 1–4)

The observations on nervous organs in the Stomatopoda which led to the assumption that they might have a neurosecretory function (Alexandrowicz, 1952*a*, and in press) gave an impulse to the search for similar organs in other crustaceans, the more so because during the course of the work on the innervation of the heart of these animals a peculiar arrangement of nervous elements in the pericardium had been noticed (Alexandrowicz, 1929, 1932). An examination of these previously casually observed elements has shown that they are parts of a system of fibres which are the subject of the present account, and for which the term 'pericardial organs' is proposed.

The observations were made on different species of decapod crustaceans with preparations stained in methylene blue. The pericardial organs stain readily in a solution of this dye (10–15 drops of 0·5 % solution of methylene blue in distilled water to 100 c.c. of sea water) provided they are exposed in the proper way. Some hints how this should be done are given below, as they can be better understood when the arrangement of these elements, which is not the same in various species, is known. The fixation and further treatment were the same as described previously (Alexandrowicz, 1951).

General Remarks and Nomenclature

A characteristic feature of the nerves in the pericardium is that their branches break up into fine fibrils forming neuropile-like networks. These can be distributed either in the connective tissue ensheathing bundles of thicker nerve-fibres or in plexuses spreading over membraneous parts of the pericardium. The first arrangement produces what will be called 'trunks' and the second 'plexuses' of the pericardial organs. It must be pointed out that they are not totally separated from other nerves. In fact, as we shall see, through the trunks may pass motor fibres and processes of muscle receptor cells. In some instances the pericardial organs are represented by neuropiles accompanying a nerve bundle for a short distance only, but there hardly can be any mistake about their belonging to the same system.

It should be added that the pericardium itself receives another set of fibres

not entering into this system, viz. the fibres supplying the muscles of the pericardium. Some data about these nerves were given previously (Alexandrowicz, 1932) and will not be discussed in the present paper.

The term 'neuropile' is adopted in order to indicate a very dense and intricate arrangement of the nerve endings. It may be objected that it evokes a picture of a rather three-dimensional structure, as in the central nervous system, while in the pericardium these terminations are spread in a thin stratum (even in the trunks they are on their periphery) and, moreover, the fibrils may appear as strands running in the same direction. However, no other short designation proves to fit them better and, besides, the neuropiles in the central nervous system may have various arrangements (see Hanström, 1928, p. 44).

Observations

Brachyura

Among the Brachyura the following species were investigated: *Maia squinado*, *Cancer pagurus*, *Portunus puber*, *P. depurator* and *Carcinus maenas*. The smaller species proved to be less suitable for this purpose and after it had been found that there are only minor differences in the arrangement of the organs in all the crabs investigated the work was carried on with *Maia* and *Cancer* which were obtainable in every size required.

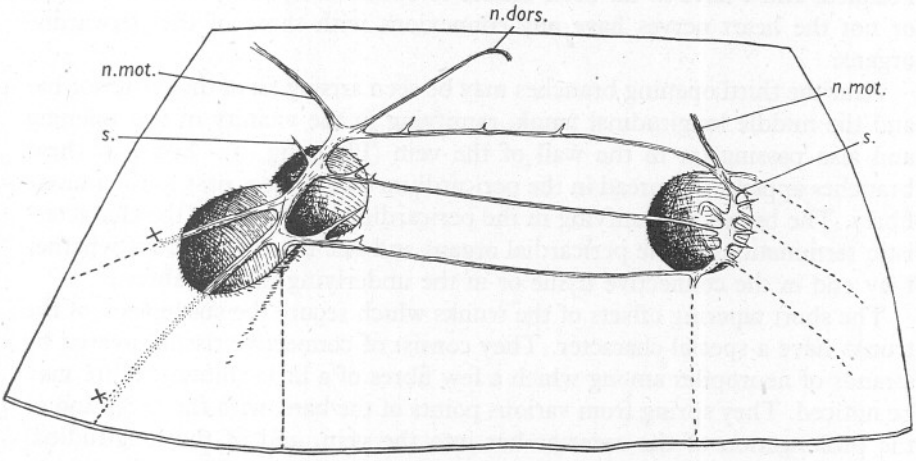
The pericardial organs in crabs are composed of thick nervous trunks anastomosing with each other according to a characteristic pattern. They lie on the inside of the lateral pericardium wall in such a position that their stoutest parts span the three openings of the branchio-cardiac veins. For facilitating the description these thickest parts will be called anterior and posterior bars, and those uniting the two bars will be called longitudinal trunks.

In *Maia*, the anterior bar has a comparatively simple shape, as seen in Text-fig. 1 and Pl. I, fig. 1, and the various specimens examined have shown no great deviation from this pattern. It crosses obliquely the first two openings of the veins and sends through the first of them a stout prolongation which runs far into the vein up to the point indicated by a cross in Text-fig. 1. There are two other prolongations, of smaller calibre: one running into the same vein but situated more dorsally and not extending so far, the other directed ventrally.

The posterior bar stretches opposite the 3rd opening which serves for the supply of blood from two veins which join just before they reach the pericardium. This bar has the form of a crescent and may have a more variable appearance owing to the anastomoses of its parts (Text-fig. 1; Pl. I, figs. 1, 2).

The longitudinal trunks, three in number, run more or less parallel to each other linking the two bars. Sometimes, but rather exceptionally, an additional trunk may be seen, or an anastomosing branch passes from one trunk to

another. All these elements of the pericardial organs, at certain points only, are attached to the pericardial wall. These are: (i) points where the nerves connected with the trunks enter or leave the pericardial cavity; (ii) attachments of the strands which secure the position of the bars and the trunks; (iii) a part of the middle longitudinal trunk which is applied to the pericardium wall. With the exception of the latter all other parts are floating free in the pericardial cavity. The anterior prolongations are also floating in the lumen of the vein, being attached only at the points indicated.



Text-fig. 1. *Maia squinado*. Semi-diagrammatic view of the pericardial organs of the right side, with part of the lateral pericardium wall showing the three openings of the branchiocardiac veins. The nerves running from the central nervous systems into the pericardial organs are drawn in dotted lines. The points at which the two anterior nerves pass into prolongations of the bar situated in the lumen of the vein are indicated by crosses. *n.mot.*, nerves running to the muscles; *n.dors.*, dorsal nerve of the heart; *s.*, strands suspending the trunks.

Connected with the trunks are: (i) nerves coming from the central nervous system; (ii) nerves arising from the trunks but not belonging to the system of the pericardial organs; (iii) strands securing the trunks.

The nerves from the central ganglia pass into the bars. The anterior one is joined by three nerves. The first and the second run into two prolongations situated in the anterior vein, the third runs along the vein of the second opening. How many nerves join the posterior bar is more difficult to determine. One can see five or even six of them approaching the bar from the ventral side and from behind. One stout nerve runs in the wall of the vein. It may be that some are composed exclusively of fibres which only pass through the bar to the muscles, others may be branches of the same segmental nerve. It may be assumed that no less than four segmental nerves reach the posterior bar, but this estimation is not certain.

The nerves coming from the central nervous system and entering the bars carry also fibres which have a different destination. These are: (i) fibres innervating the muscles situated at the dorsal edge of the thoracic epimera (*n.mot.*, Text-fig. 1, Pl. II, fig. 6); (ii) fibres running to the heart as a nerve called n. cardiacus dorsalis (Alexandrowicz, 1929, 1932) (*n.dors.*). The former are sometimes distinctly seen simply to pass through the bars without entering into relation with their fibres (*mot.*, Pl. II, fig. 5). The heart nerves, however, pass through that portion of the anterior bar in which the fibres are intermingled, and I have so far been unable to obtain adequate evidence whether or not the heart nerves have any connexions with those of the pericardial organs.

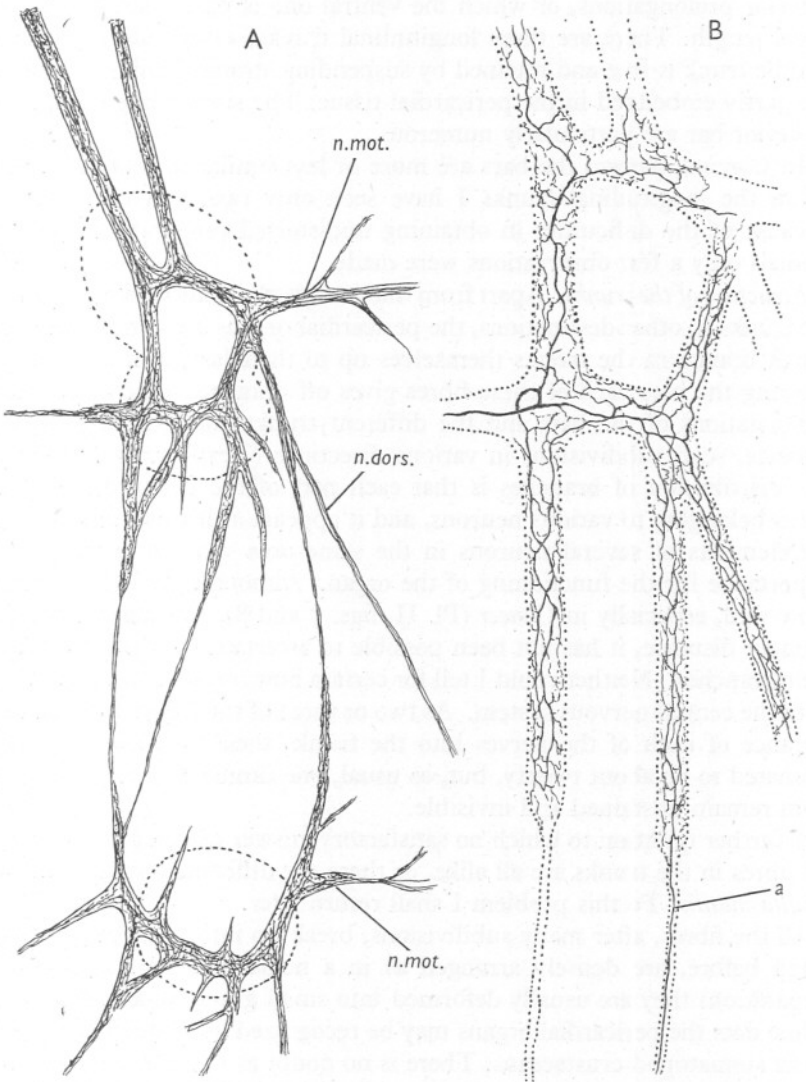
Near the third opening branches may be seen arising from the posterior bar and the middle longitudinal trunk, ramifying in the vicinity of this opening and also passing on to the wall of the vein (Pl. I, fig. 2). Some of these branches apparently spread in the pericardium wall, others pass to the muscle fibres. The branches ramifying in the pericardium do not show the characteristic terminations of the pericardial organs and one may be in doubt whether they end in the connective tissue or in the underlying muscle fibres.

The short tapering offsets of the trunks which secure the suspension of the trunks have a special character. They consist of connective tissue covered by strands of neuropiles among which a few fibres of a little stouter calibre may be noticed. They spring from various points of the bars, with the exception of the prolongation of the anterior bar into the vein, and of the longitudinal trunks, with the exception of that part of the middle one which is applied to the pericardium wall.

It may be mentioned that at the dorsal end of the anterior bar there is a nerve cell with long processes, one of which, the axon, passes through this bar towards the central ganglia (*n.c.*, Pl. II, fig. 6). This cell belongs perhaps to a special category of muscle receptor cells found in the Macrura and which will be mentioned later. In *Maia* I have noticed only one cell in this region and I was unable to ascertain where its processes end.

In other crabs the arrangement of the pericardial trunks shows some differences. In *Cancer pagurus*, instead of a single anterior bar, there are four or five short ones joined in the manner shown in Text-fig. 2A. Two thick prolongations pass through the first opening into the branchio-cardiac vein. The posterior bar is more or less similar to that of *Maia*. The longitudinal trunks are more irregularly arranged than in *Maia*, for, starting from the anterior bars, they may divide and send branches into another trunk, or two of them may unite into one (Text-fig. 2A; cf. Pl. I, fig. 4). Only the anterior and posterior portions of the longitudinal trunks are floating free, since for about half or more of their total length they are embedded in the tissue of the pericardum wall where they are deprived of the neuropile sheath.

In *Portunus puber* and *P. depurator* the arrangement of the anterior bars is



Text-fig. 2. *Cancer pagurus*. A, pericardial organ of the right side. The outlines of the openings of the veins are indicated by a dotted line. Note the different appearance of the longitudinal trunks losing their neuropile sheaths in the middle of their course (cf. Pl. I, fig. 4); *n.mot.*, motor nerves; *n.dors.*, dorsal nerve of the heart. B, ramification of one fibre entering the anterior bar; *a*, fibre running towards the posterior bar. Finer ramifications are not represented.

about the same as in *Cancer*, only the bars appear somewhat longer. The two anterior prolongations, of which the ventral one is particularly thick, are of equal length. There are three longitudinal trunks, as in *Maia*, but here the middle trunk is free and retained by suspending strands while the two others are partly embedded in the pericardial tissue. The suspending strands of the posterior bar are particularly numerous.

In *Carcinus maenas* the bars are more or less similar to those in *Portunus*. From the longitudinal trunks I have seen only two, and both were free. Because of the difficulties in obtaining undistorted preparations from these animals only a few observations were made.

Structure of the trunks. Apart from those nerve elements which pass through the trunks to other destinations, the pericardial organs are composed of fibres which branch in the trunks themselves up to their last terminations. After entering the bars each of these fibres gives off branches which pass into the prolongations of the bars and the different trunks and, running for a long distance, send subdivisions in various directions (Text-fig. 2B). A result of this distribution of branches is that each part of the pericardial organ has fibres belonging to various neurons, and it appears as if this intermingling of the elements of several neurons in the same area were of some particular importance for the functioning of the organ. Although the individual fibres show well, especially in *Cancer* (Pl. II, figs. 7 and 8), and may be traced for a longer distance, it has not been possible to ascertain the distribution of all their branches. Neither could I tell for certain how many of these fibres come from the central nervous system. As two or three of them may be found at the entrance of each of the nerves into the trunks their total number may be estimated to be about twenty, but, as usual, one cannot be sure that some of them remain unstained and invisible.

A further question to which no satisfactory answer can be given is whether the fibres in the trunks are all alike, or there are different sorts of them as in *Squilla mantis*. To this problem I shall return later.

All the fibres, after many subdivisions, break up into fine fibrils which, as stated before, are densely arranged as in a neuropile. In methylene-blue preparations they are usually deformed into small granules, and by this mass of fine dots the pericardial organs may be recognized in all decapod as well as in the stomatopod crustaceans. There is no doubt as to their nervous nature, since the transformations of the fine fibres into small beads can be often observed, but nothing can be said about the histological structure of such ostensibly deformed elements.

In the trunks which are cylindrical in shape the various fibres are so arranged that the thicker are nearer to the axis with their branches approaching the periphery at which the neuropiles form a superficial layer. This arrangement can be best observed in *Cancer*. All the fibres are held together by connective tissue which does not stain with methylene blue. In sections it

may be seen that this tissue fills the spaces between the nerve fibres, forming concentric layers around the thicker of them. The nerve fibres are not so tightly packed as in the lamellae of *Squilla*. Apart from scattered nuclei, seemingly belonging to the connective tissue and the neurolemma, there may be seen cells with granular cytoplasm showing the characteristic features of amoebocytes and which are evidently brought into the trunks by small arteries. Except for these cells no elements in the trunks could be noticed having special affinity to eosin, acid fuchsin, or dyes entering in the Azan-mixture.

The pericardial organs in crabs are easily accessible. After removing the carapace, the dorsal pericardium wall, and cutting through the dorsal ligaments of the heart, one can see the pericardial organs spanning the openings of the veins or applied to the wall near to these openings, the latter position evidently due to the adherence of blood clots to the organs and the neighbouring parts. As these organs are attached at few points it is possible to cut them out, but for staining it is better to cut out the epimeral plates leaving the organs *in situ*. The preparation, after trimming the protruding parts, should be fixed to the paraffin plate and put into methylene-blue solution. The removal of the chitin and the muscles should be made later, during washing after fixation. The chitin then detaches quite easily. Final cleaning up, as removing the last of the muscle tissue and cutting out parts of pericardium membrane obstructing the view, can be made when the preparations have been taken to xylol.

The pericardial organs can also be approached from the ventral side. It is then advisable, when removing the carapace, to leave in place the membrane covering the pericardial cavity from the dorsal side and cut out the epimeral plates with the heart attached to them. After stretching the preparation with its inside uppermost the ventral pericardium wall (pericardial septum) should be cut through on each side of the heart, preferably with the adjoining half of the ventral heart wall, and stretched so that the pericardial cavity is well exposed from the inside. This method has its advantages for the observation of the nerves passing through the pericardial trunks to the muscles and to the heart. The latter nerve, however, is elusive and there are some difficulties in tracing it up to the heart.

Eupagurus bernhardus

In *Eupagurus* the most conspicuous parts of the pericardial organs appear as two trunks curving along the sides of the heart as shown in Text-fig. 3A (cf. Pl. III, fig. 9). These trunks vary in thickness and in some places divide into strands which unite again. Trunks of smaller dimensions connected with these main trunks are of two sorts: some conveying fibres from the central nervous system, others being branches given off by the trunks. It is often difficult to distinguish one sort from another, since, at the points where they join the main trunks, fibres from the trunks may pass into the nerves of central origin to run in the same nerves in the opposite direction. They can be identified if it can be stated that farther away they leave this nerve, but more than often after seeing a portion of such mixed nerve one remains in doubt in which direction its fibres may run. Moreover, as the branches of the trunks tend to anastomose in a plexus the tracing of fibres is very uncertain.

It may be assumed that no less than five segmental nerves are sent by the

central ganglia to form the pericardial organs but possibly there are more of them. It is also uncertain whether they all belong to the thoracic segments since one of them coming from behind can be followed so far backwards that its origin in the 1st abdominal segment is probable.

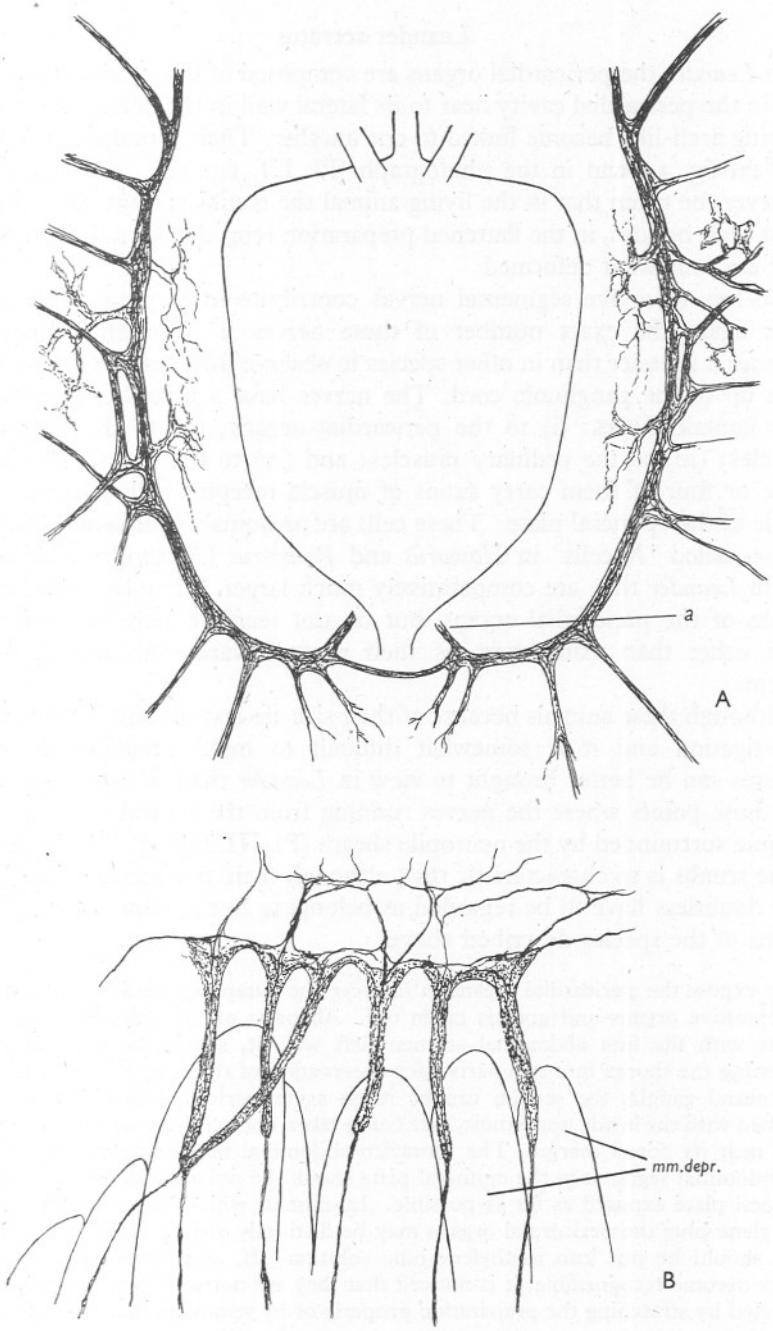
The branches arising from the trunks are of various lengths. Some at a short distance form a plexus spreading over the lateral pericardium wall which as a thin membrane covers the muscles inserting into the epimeral plate; the longer ones pass on to the farther parts of the pericardium and to the heart ligaments. An anastomosing branch links the posterior ends of the main trunks (Text-fig. 3A).

The fibres arising from the trunks and from their branches have various destinations. Some of them run to supply the muscle fibres which in the pericardium of *Eupagurus* are more abundant than in other species investigated. Others ending in neuropiles can be regarded as the elements of the pericardial organs. Their arrangement and distribution is much less uniform than in crabs, since they are present not only in the form of the trunks but as strands extending plexus-like over the pericardium wall or accompanying branches of the nerves.

Nor in *Eupagurus* was it possible to determine the areas of expansion of individual neurons, although the nerves stain well, and this animal would perhaps be the most suitable for this kind of investigation were it not so difficult to obtain preparations in which the trunks could be observed for a sufficient distance. The details which could be noticed confirm the observations made in crabs. Thus it can be seen that one fibre gives off branches passing into different parts of the trunk (Pl. III, fig. 10). It can also be stated that the nerves coming from the central nervous system each carry more than one fibre to the pericardial organs, and that the fibres brought in by different nerves overlap each other in their terminal distribution. The presence of an anastomosis through which the fibres pass into the trunks of the opposite side shows that in the neuropiles the contralateral neurons also take part.

The structure of the trunks resembles greatly that described previously in crabs, the main difference consists in that the trunks in *Eupagurus* are not cylindrical but more flattened, and that there is less connective tissue between the fibres. Otherwise the branching of the thicker fibres resulting in forming the neuropiles appears to follow the same pattern.

In *Eupagurus* it is better to expose the pericardial organs approaching them from the ventral side. After the thorax has been opened from this side and the gonads and digestive organs removed, the preparations should be spread so that the pericardial septum is well exposed. It is advisable not to try to open the pericardial cavity before the trunks of the pericardial organs, which stain readily even when covered by this membrane, become visible. Even without taking away this membrane they show fairly well in mounted preparations. In any case good preparations are difficult to obtain, for on removing the chitinous parts the pericardial organs are easily damaged or displaced.



Text-fig. 3. A, *Eupagurus bernhardus*. Pericardial organs flanking the heart. *a*, branches running in the dorsal direction, shown as cut. B, *Leander serratus*. Pericardial organs of the right side. *mm.depr.*, musculi depressores. Other muscles in this region are not represented.

Leander serratus

In *Leander* the pericardial organs are composed of five or six trunks which run in the pericardial cavity near to its lateral wall in the dorsal direction and curving arch-like become linked to one another. Their arrangement is shown in Text-fig. 3B and in the photograph (Pl. III, fig. 11). Account should, however, be taken that in the living animal the trunks are not all in the same plane and, besides, in the flattened preparation reproduced in the photograph they are somewhat deformed.

No less than five segmental nerves contribute in forming these organs. Here again the exact number of these nerves is uncertain, although in *Leander* it is easier than in other species to observe the courses of these nerves even up to the ganglionic cord. The nerves have a mixed composition for they contain fibres: (i) to the pericardial organs; (ii) to the pericardium muscles; (iii) to the ordinary muscles; and (iv) to the heart. In addition, three or four of them carry axons of muscle receptor cells situated on the inside of the epimeral plate. These cells are presumably the same elements as the so-called '*N*-cells' in *Homarus* and *Palinurus* (Alexandrowicz, 1952*b*), but in *Leander* they are comparatively much larger. They lie quite near the trunks of the pericardial organs, but do not seem to have any relation to them other than using them as their route towards the central nervous system.

Although these animals because of their size are less suitable for this kind of investigation and it is somewhat difficult to make preparations, certain features can be better brought to view in *Leander* than in other crustaceans e.g. those points where the nerves running from the central nervous system become surrounded by the neuropile sheath (Pl. III, fig. 12). The appearance of the trunks is so characteristic that, although their arrangement is different, they doubtless have to be regarded as belonging to the same category as the organs of the species described above.

To expose the pericardial organs in *Leander* the carapace should be removed and the digestive organs and gonads taken out. All parts of the animal, excepting the thorax with the first abdominal segment left with it, should be cut away. After sectioning the thorax into two parts—for observation of the course of the nerves into the central ganglia the section can be made asymmetrically—each half has to be attached with the inside uppermost, care being taken not to injure parts of the epimeral plate near its dorsal margin. The thoracico-abdominal muscles stretching from the 1st abdominal segment to the epimeral plate should be pulled aside so as to leave the epimeral plate exposed as far as possible. In animals which had been injected with methylene blue the pericardial organs may be distinctly visible. If not, the preparations should be put into methylene-blue solution. If, after some time, when the trunks become recognizable, it is noticed that they are not well exposed, this may be corrected by stretching the preparation properly or by removing the tissues hindering the staining.

Homarus vulgaris

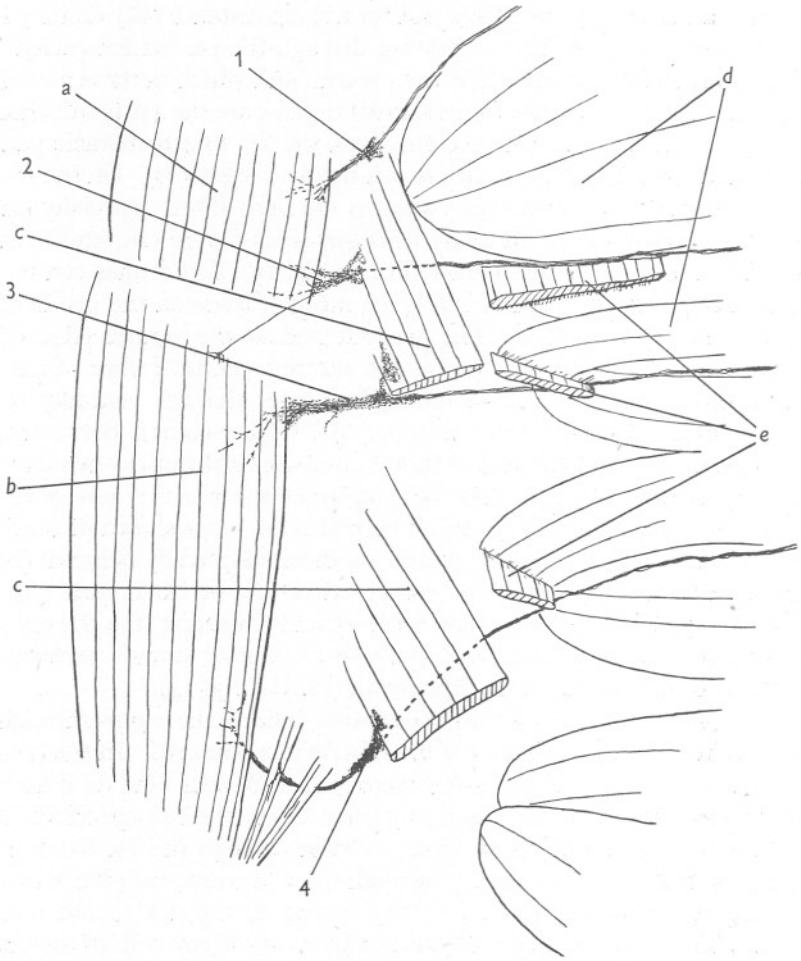
The pericardial organs in *Homarus* are less conspicuous than in the species previously described, for the fine neuropile-like terminations mostly spread in plexuses forming a very thin layer on the pericardial wall and on heart ligaments, and in but a few places and for a short distance only do they form sheaths surrounding the nerves passing through the pericardial cavity. The nerves around which such sheaths are present, and which in these parts have the appearance of the trunks of pericardial organs, are the 1st to 4th thoracic nerves (so termed because they come from the 1st to 4th thoracic ganglia, though in fact belonging to the 4th to 7th thoracic segments). On the 1st and 4th of these nerves the neuropile sheaths are very short, especially on the former where its presence in many instances seems to be doubtful. On the 2nd and 3rd they are quite distinct (Pl. IV, fig. 13). The trunks can be best identified at points where they reach the muscles lying on the inside of the epimeral plate (Text-fig. 4). The 1st and 2nd at the ventral edge of the m. contractor epimeralis, the 3rd at the anterior ventral corner of the 2nd head of the lateral thoracico-abdominal muscle, the 4th ventrally of the accessory bundles of the same muscle. All trunks stretch between these points and the edge of the dorsal thoracico-abdominal muscles which bulge into the pericardial cavity. The topography of the muscles was described previously (Alexandrowicz, 1952*b*). The names of those shown in the figure and not mentioned in the paper quoted are those adopted by Schmidt (1915).

The 2nd trunk may appear as composed of two or three parts (Pl. III, fig. 13); the 3rd, the longest of all, sends strands inserting into the epimeral plate obviously serving the same purpose as similar strands securing the suspension of the trunks in the Brachyura (Pl. III, fig. 14).

Apart from motor fibres which are of stout calibre, there pass through the trunks, as in *Leander*, the axons of the muscle receptor cells. In the description of these elements in which they were termed *N*-cells (and designated by the numbers 1–5) the course taken by their axons was not specified. Now I could observe that cell no. 3 sends its axon through the 1st trunk, no. 2 through the 2nd trunk, and no. 1 through the 3rd trunk, into the 1st to 3rd thoracic ganglion respectively, i.e. they belong to the 4th to 6th thoracic segments. Through the 4th trunk pass the axons of the two cells of the muscle receptor organs of the 7th thoracic segment. No relation has been noticed between the axons of receptor cells and of the motor nerves to the elements of the pericardial organs.

The structure of the trunks is very much the same as in other Decapoda. The differences depend on the comparatively stouter calibre of the motor fibres passing through the trunks; the neuropile sheath in the 1st trunk may look as if it it were formed only at the point where the nerve fibres start expanding on the connective tissue membrane. The quantitative relation of

the nerve elements may be expressed in such a way that in Brachyura the pericardial organs form trunks through which pass nerve fibres of other sorts, whereas in *Homarus* and *Leander* the elements of the pericardial organs are attached to the nerves passing through the pericardial cavity.



Text-fig. 4. *Homarus vulgaris*. Topography of the pericardial organs of the left side in relation to the muscles inserting into the epimeral plate. 1–4, 1st to 4th trunks of the pericardial organs. *a*, m. contractor epimeralis; *b*, m. lateralis thoraco-abdominalis (2nd head); *c*, mm. dorsales thoraco-abdominales; *d*, mm. depressores; *e*, mm. thoracales anteriores.

The small dimensions of the trunks of pericardial organs may perhaps be explained by their topographic relations. There is in *Homarus* only a narrow space between the epimeral plate and the dorsal thoracico-abdominal muscles

which pass dorsally from the pericardial septum and lie between this plate and the heart. Since the pericardial organs in the form of trunks need to float free, as seems to be essential for their function, there is no room for the longer trunks, nor presumably can they extend dorso-ventrally as in *Leander*.

The diminutive dimensions of the trunks in *Homarus* are compensated by a rich expansion of nerve fibres arising from these trunks and spreading over large areas of the membraneous tissue lining the lateral wall of the pericardium and forming the heart ligaments. The photograph (Pl. IV, fig. 16) shows such fibres starting from the 3rd trunk, but only the thicker ones are seen fairly well. Actually, strands of the finest filaments spread in plexuses between these fibres and are so densely arranged that at many places little space is left uncovered by fine granules in which the fine fibrils are disintegrating. The nerve elements of this kind appear to be particularly abundant in the vicinity of the trunks. On the dorsal and ventral wall of the pericardium they seem to be missing. This, however, does not imply that the ventral wall is deprived of nerves for, as stated before, there are nerve fibres running here to the muscles of the pericardial septum and to the arterial valves (Alexandrowicz, 1932).

Hints about dissection and staining of the nerve elements in this region have been given in the paper quoted dealing with the muscle receptors in the thorax. The technique is much as with *Leander*, i.e. to approach the nerves from the dorsal side and to turn the epimeral plate in such a way that it becomes exposed from the inside.

It is also possible to observe the trunks in their natural position. After the dorsal wall of the pericardium has been removed the dorsal thoracico-abdominal muscles must be pulled a little aside. Then, through the cleft between these muscles and the epimeral plate, the openings of the branchio-cardiac veins come into view, and stretched above them the 2nd and 3rd trunk of the pericardial organs may be seen. The identification of these trunks may, however, be uncertain, as strands of connective tissue and blood clots may stretch in a similar way. The 1st and the 4th trunk may be also found, but here the mistakes are more likely to occur. In stained preparations there is no difficulty in distinguishing the nerves from other elements.

Isopoda

In the pericardial cavity of *Ligia oceanica* I noticed two nerve trunks running in a longitudinal direction alongside the heart tube which do not show connexions with other elements innervating the heart and the muscles of the pericardium. It is therefore probable that they might be pericardial organs similar to those in decapods and stomatopods. However, no satisfactory evidence could be obtained, chiefly because of difficulties in exposing the parts of the pericardium for staining. Moreover, these trunks do not stain well, and in the animals of such a small size the character of nerve fibres is difficult to ascertain.

Palinurus vulgaris, Scyllarus arctus, Astacus fluviatilis

These species have not been examined in the course of the present work, but a mention of observations previously made may be not out of place. In all of them I noticed, when investigating the innervation of the heart, the existence of 'peculiar terminations of the nerves entering the pericardial cavity'. These were doubtless the elements of the pericardial organs, as follows from the statement: 'the thin fibrils which originate from the thicker fibres present a mass of blue points like a "punctate substance" of the neuropiles.... Similar structures can be seen on the epimeral plate and I had previously observed them in *Potamobius*' (Alexandrowicz, 1932, p. 230). In *Astacus fluviatilis* (then temporarily called *Potamobius astacus*) I had, moreover, described and pictured a peculiar arrangement of fibres around the dorsal nerves of the heart ending in irregular plates or cell-like bodies (Alexandrowicz, 1929). I called it 'apparatus nervi dorsalis', suggesting that it possibly might be concerned with the appreciation of the pressure in the pericardial cavity. In the light of the later findings it seems more probable that it belongs to the system of the pericardial organs, and as a similar body has not been found in other Decapoda it might be of interest to examine it from this angle.*

Discussion

It has been shown above that the two forms of arrangement of neuropile-like endings, viz. as sheaths round the bundles of fibres or spread in plexuses over a larger area, may predominate in one group of the Crustacea and play a minor role in another. The one extreme is represented in the Brachyura, which possess remarkably large trunks and not any, or at most few, neuropile plexuses. On the other hand, in *Homarus* the plexuses are widely spread and the trunks are greatly reduced. A question arises: has this different arrangement some bearing on the function of these organs, or is it rather accidental? The morphological evidence is not unequivocal. It may be assumed, as already suggested, that purely topographical conditions are decisive in developing one or the other form. In the crabs, in which there is a wide space in the pericardial cavity, the trunks are free to attain large dimensions; while in *Homarus*, because of the narrowness of space, the conditions for functioning of the longer trunks are unfavourable and consequently the fibres have to spread out. However, this simple and plausible explanation seems not to tally well with the fact that in the pericardial cavity of *Squilla* both sorts of neuropiles are present, and apparently there is no such lack of space pre-

* Since sending this paper to the printers I have had the opportunity at Naples of observing the pericardial organs in *Eriphia spinifrons*, *Dromia vulgaris*, *Pagurus striatus*, *Lysmata seticaudata* and *Penaeus camerote*. In each of these species the pericardial organs are arranged according to a particular pattern differing to varying degrees from those described above. In *Penaeus* the plexuses spread over the pericardium wall attain the largest dimensions of all species investigated.

venting the development of the first sort. (In *Squilla* these have been designated 'lamellae', but they are nothing else than short flattened trunks.) Thus, since the two forms of neuropiles exist in the same segments and not far from one another, it might be supposed that their functions may not be identical.

Another question which arises on comparing the pericardial organs in decapods with those in stomatopods is the origin of their nerve fibres. In *Squilla* they have been found to come from two different sources: one set is given off by nerve cells lying in the abdominal segments outside the ganglionic cord, while the second set, of obviously different kind, originates in the fore-parts of the central nervous system. Peripheral nerve cells taking part in the forming of the pericardial organs have not been found in decapods, and it is to be assumed that the corresponding elements became centralized. Unless, however, the structure of the pericardial organs has not at the same time become simplified in the Decapoda, the second set of fibres should be present in them. However, the histological examination does not afford evidence to lend sufficient weight to this assumption. There may in places be seen fibres of somewhat different appearance and, in particular, I tried to trace those in the anterior prolongation of the bar (in the Brachyura). But the fibres mix in such a way that no certainty about their special behaviour could be obtained. This, of course, does not imply that similarly looking nerves should be all of the same kind, and that the pericardial organs in the Decapoda carry no fibres of different origin which might have different physiological functions.

In approaching the problem of the function of these organs, it ought to be emphasized that exposure to the blood stream coming from the gills must be a paramount condition for their activity. The trunks are not only in these very places when the blood passes into the pericardium, but they are suspended in such a way that they can be bathed on all sides by the blood. How essential it must be for the neuropiles to be in the closest touch with the blood can be judged by the fact that in the same trunks, as in the longitudinal trunks of crabs, they do not develop at those parts where the trunks are embedded into the tissue of the pericardium, and are abundant where they are free. In other species this position of the trunks is not so easily demonstrable, but whenever it is possible to verify their relation to the pericardium they are found floating in its cavity.

As regards the neuropiles spreading over the membranes in a thin layer, their particular abundance at those places where the blood stream entering the pericardial cavity passes, gives support to the assumption about the vital importance of this kind of topographic relation.

This conclusion affords a clue towards the solution of a problem of the situation of the pericardial organs in the Stomatopoda. In these animals the heart and the pericardial cavity extend over some fifteen segments, but the organs in question are present in five abdominal segments only, and no reason

for it could previously be found. Now in stomatopods the main respiratory organs, the abdominal gills, which are five in number, are attached to the same five abdominal segments and thus the pericardial organs prove to be situated exactly where the blood from the gills passes to the heart. It is not impossible, also, that an organ of a similar histological structure which in these animals is suspended in the ventral blood sinus, described under the name of 'the transverse bar of the 6th segment' (Alexandrowicz, 1952*a*), owes its situation to the existence of the so-called thoracic gills. This matter needs further investigation, for the knowledge of the blood circulation in stomatopods is deficient and no precise data seem to have been published about the way taken by the blood passing through the thoracic gills.

In the papers dealing with the innervation of the heart of crustaceans, in which some observations relating to the nerves in the pericardial cavity were made, I expressed the supposition that they might have some sensory function. However, in view of the findings in *Squilla mantis* this supposition had to be discarded, and it was suggested that these organs release some substances into the blood. The observations made with the Decapoda provide supporting evidence for this hypothesis and, moreover, this could be confirmed by the results of experiments performed in collaboration with Mr D. B. Carlisle. They have shown that the extracts of pericardial organs, if added to the saline solution perfusing an isolated heart of *Cancer* or *Maia*, exhibit a pronounced action on the heart rhythm. It appears probable that one of the substances produced in these organs may be adrenaline or some adrenaline-like compound, as may be inferred from the fact that the extract of the organs of *Cancer* gives a distinct positive fluorescence test as well as the blood of this animal taken from the pericardial cavity (but not that from the leg arteries). However, the effect of the extract on the heart is not identical with that of adrenaline solution. Consequently, it has to be assumed that the liberated substance is either not adrenaline or the latter is mixed with some other product. Neither of the compounds so far tested, such as noradrenaline, tyramine, acetylcholine and histamine, proved to induce effects identical with those of the pericardial organs extract. The results of these experiments will be published separately.

With regard to the question which elements of the pericardial organs are responsible for their secretory activity the balance of evidence favours the view that these are the terminations of the nerve-fibres. The alternative supposition would be to ascribe this faculty to the connective tissue and consider the nerves as destined only to convey stimuli to this tissue. However, not only does it seem unlikely that connective tissue could exhibit this faculty, but the quantity of nerve elements concentrated in these organs and their predominance over other elements makes such an assumption attributing them a rather secondary role quite untenable. Therefore, all available information considered, there is justification for drawing the conclusion that the

Crustacea possess in their pericardial cavity organs which by the way of neurosecretion release substances regulating the heart-beat.

I wish to record my gratitude to Mr G. M. Spooner for his kind help in preparing the manuscript.

SUMMARY

In the pericardial cavity of the decapod Crustacea the nerve fibres end in characteristic neuropile-like networks which surround bundles of thicker fibres or spread over the pericardium wall and the heart ligaments. It is proposed to designate the whole system of these elements as 'pericardial organs'.

The arrangement of the elements constituting the pericardial organs varies in different groups of the Decapoda. In the Brachyura the bundles of fibres surrounded by neuropiles form conspicuous trunks linked with each other; the main parts of these trunks are situated at the openings of the branchio-cardiac veins into the pericardial cavity. In *Eupagurus bernhardus* two main trunks flank the heart giving off branches which spread in various directions and form at some places plexuses on the pericardium wall. In *Leander serratus* the pericardial organs are represented by several trunks running near the lateral wall of the pericardium in the dorsal direction and uniting by arch-shaped branches. In *Homarus vulgaris* there are four comparatively much shorter trunks situated at the lateral wall of the pericardial cavity, but plexuses of neuropile-like nerve fibres spread over large areas.

The trunks of the pericardial organs are suspended in the pericardial cavity in such a position that they may be bathed on all sides by the blood passing from the gills. The plexuses extending over the membranes are situated in places also exposed to the blood stream. There is evidence indicating that the pericardial organs produce substances influencing the heart-rhythm. It is assumed that these substances are liberated into the blood by a process of neurosecretion occurring at the terminations of the nerves.

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EXPLANATION OF PLATES I–IV

All photomicrographs were made from preparations stained with methylene blue, fixed in ammonium molybdate and mounted in xylol-dammar.

PLATE I

Fig. 1. *Maia squinado*. Pericardial organs of the left side (cf. Text-fig. 1). Parts of the pericardium are still attached to the trunks.

Fig. 2. *M. squinado*. Posterior part of the pericardial organs. The ramifying branches end on the muscle fibres; the destination of some of them is uncertain.

Fig. 3. *M. squinado*. Part of the posterior bar showing the intermingling of nerve fibres. The small dots are elements of neuropiles.

Fig. 4. *Cancer pagurus*. Anterior portion of the pericardial organs of the right side. The trunks span the second opening of the branchio-cardiac veins. On the left upper corner is part of the first opening into which pass the anterior prolongations of the bars. Note the absence of neuropile sheaths in the lower parts of the trunks. These parts are embedded in the tissue of the pericardium. The situation of the bars is a little distorted artificially (cf. Text-fig. 2A).

PLATE II

Fig. 5. *Maia squinado*. Part of the posterior bar. *mot.*, fibres of the motor nerves passing through the pericardial organs without branching in them.

Fig. 6. *M. squinado*. Dorsal part of the anterior bar of the right side. *n.mot.*, motor nerve; *n.dors.*, fibres of the dorsal heart nerve; *n.c.*, nerve cell.

Fig. 7. *Cancer pagurus*. Nerve fibre entering the anterior bar and ramifying in the trunks (cf. Text-fig. 2B).

Fig. 8. *C. pagurus*. Part of a trunk of the pericardial organs with fibres branching in it. Nearly all nerve elements seen in this and in the preceding figure belong to one neuron. They show well owing to the incomplete staining of the superficial layer of neuropiles (seen as small dots).

PLATE III

Fig. 9. *Eupagurus bernhardus*. Part of the main trunk of the pericardial organs near its posterior end (cf. Text-fig. 3A).

Fig. 10. *E. bernhardus*. Part of the main trunk with a nerve fibre sending ramifications into different branches of the trunk.

Fig. 11. *Leander serratus*. Pericardial organs of the right side. The position of the trunks is somewhat distorted by spreading and mounting the preparation (cf. Text-fig. 3B).

Fig. 12. *L. serratus*. Part of the pericardial organs of the left side, showing the changing of the appearance of the nerves coming from the central nervous system at the point where they pass into the trunks.

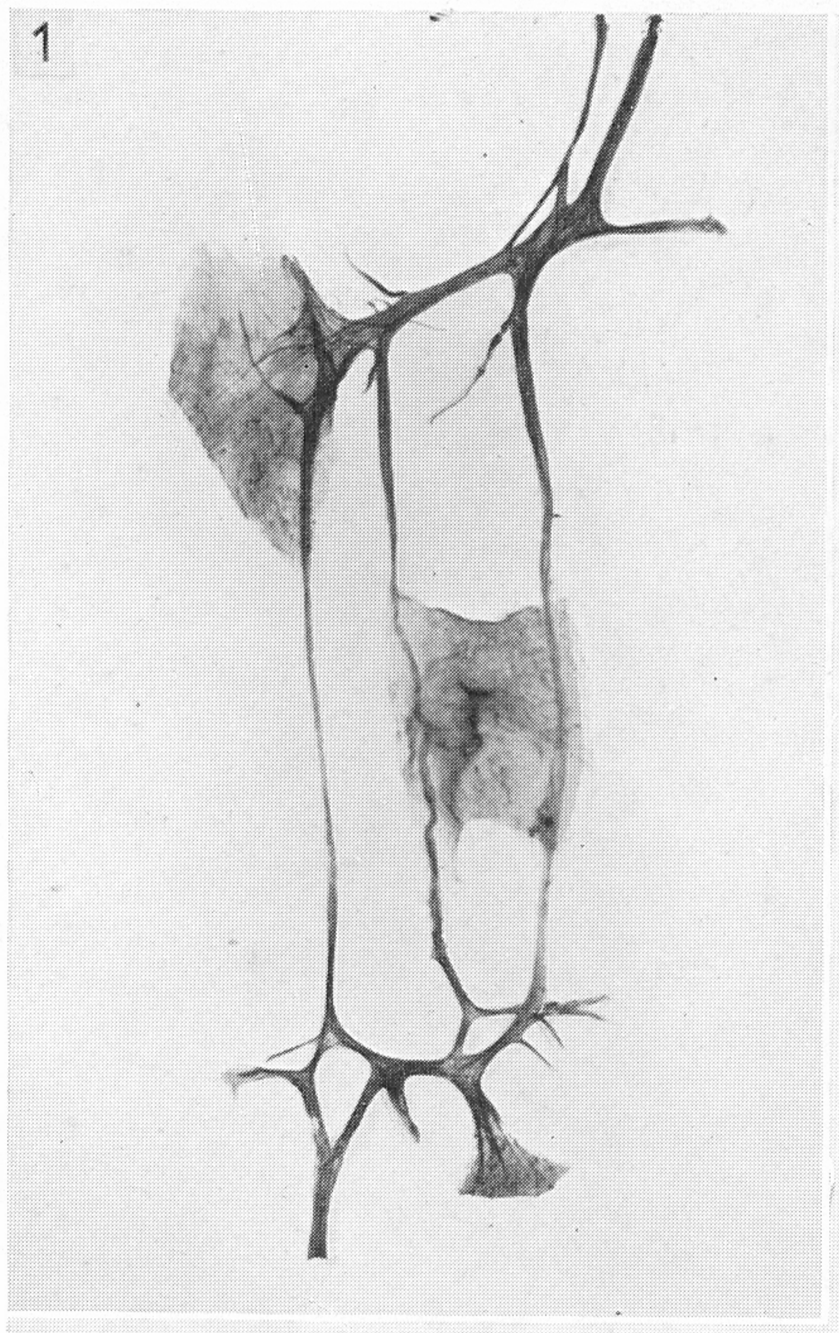
PLATE IV

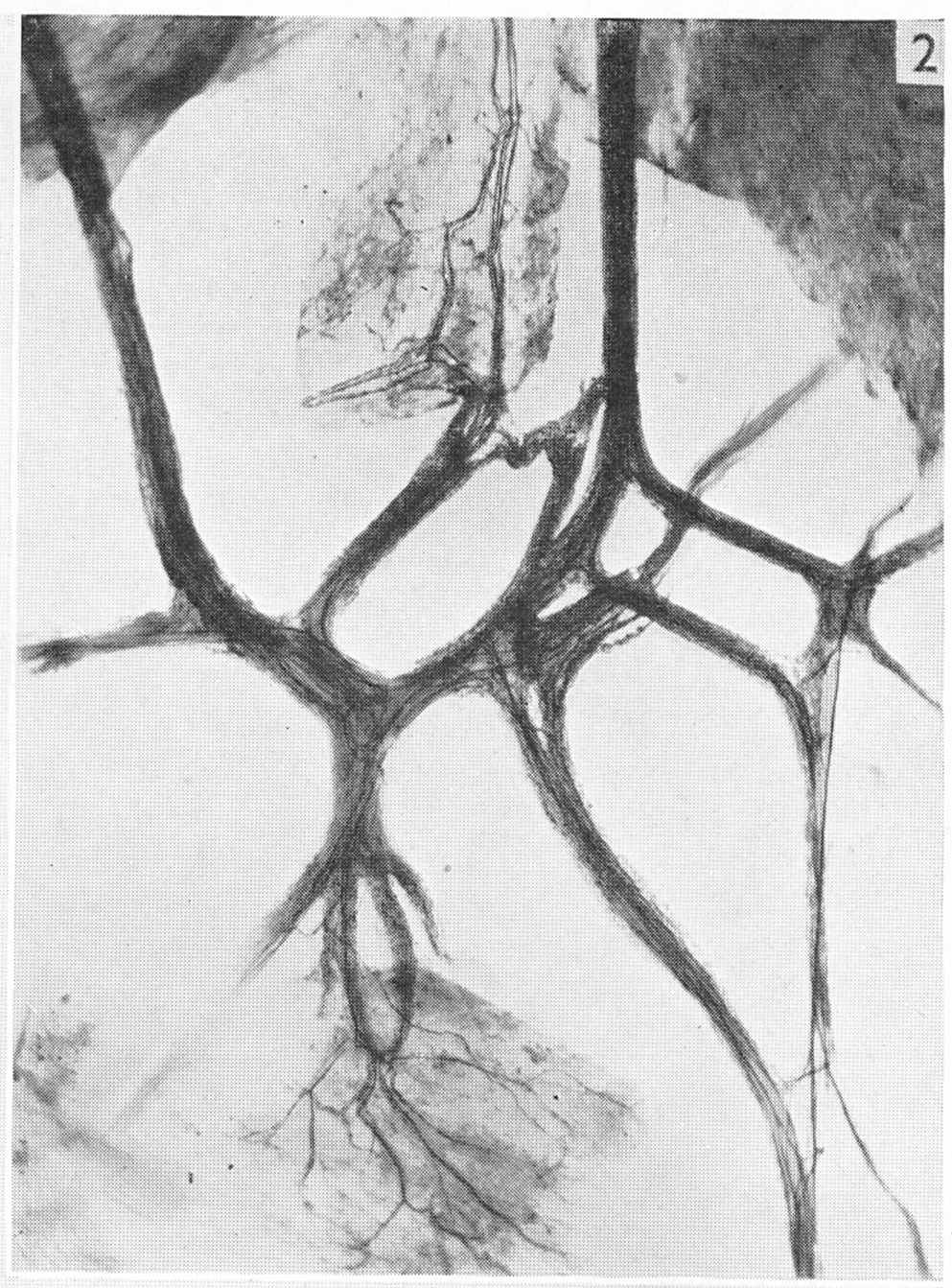
Fig. 13. *Homarus vulgaris*. Second and 3rd trunks of the pericardial organs (cf. Text-fig. 4).

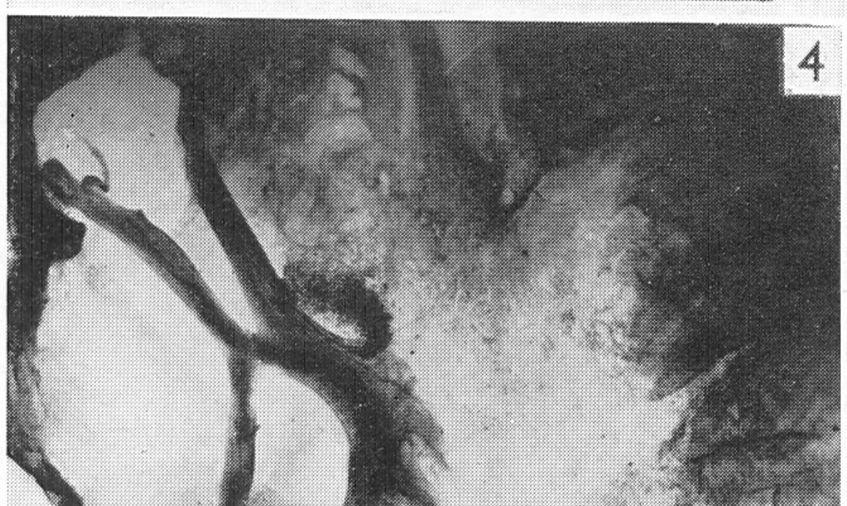
Fig. 14. *H. vulgaris*. Part of the 3rd trunk of the pericardial organ with the strands securing its suspension (on the right).

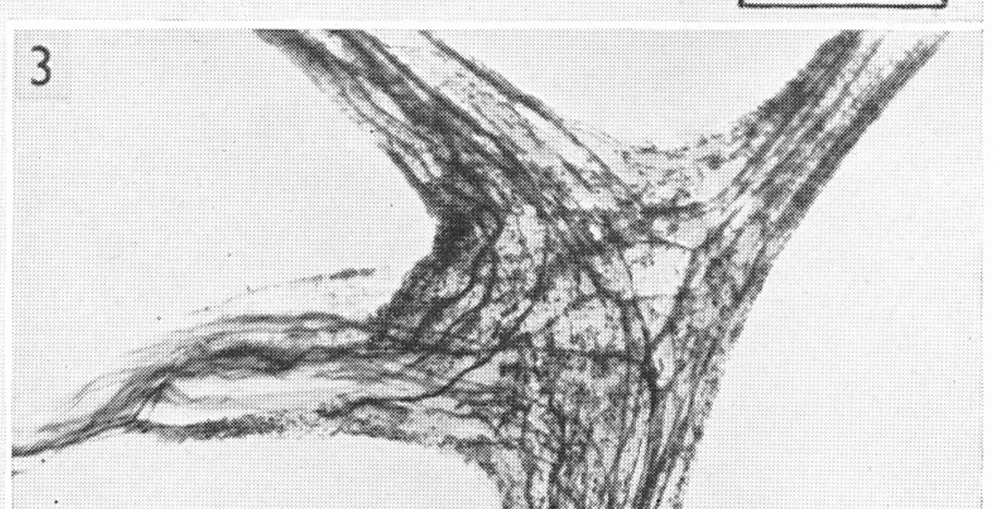
Fig. 15. *H. vulgaris*. Parts of the trunk of the pericardial organs showing the characteristic beaded appearance of finer fibres.

Fig. 16. *H. vulgaris*. Fibres arising from the trunk of the pericardial organs (lower left corner) and spreading in one plane. The dots seen in some places are elements of neuropiles stained indistinctly.









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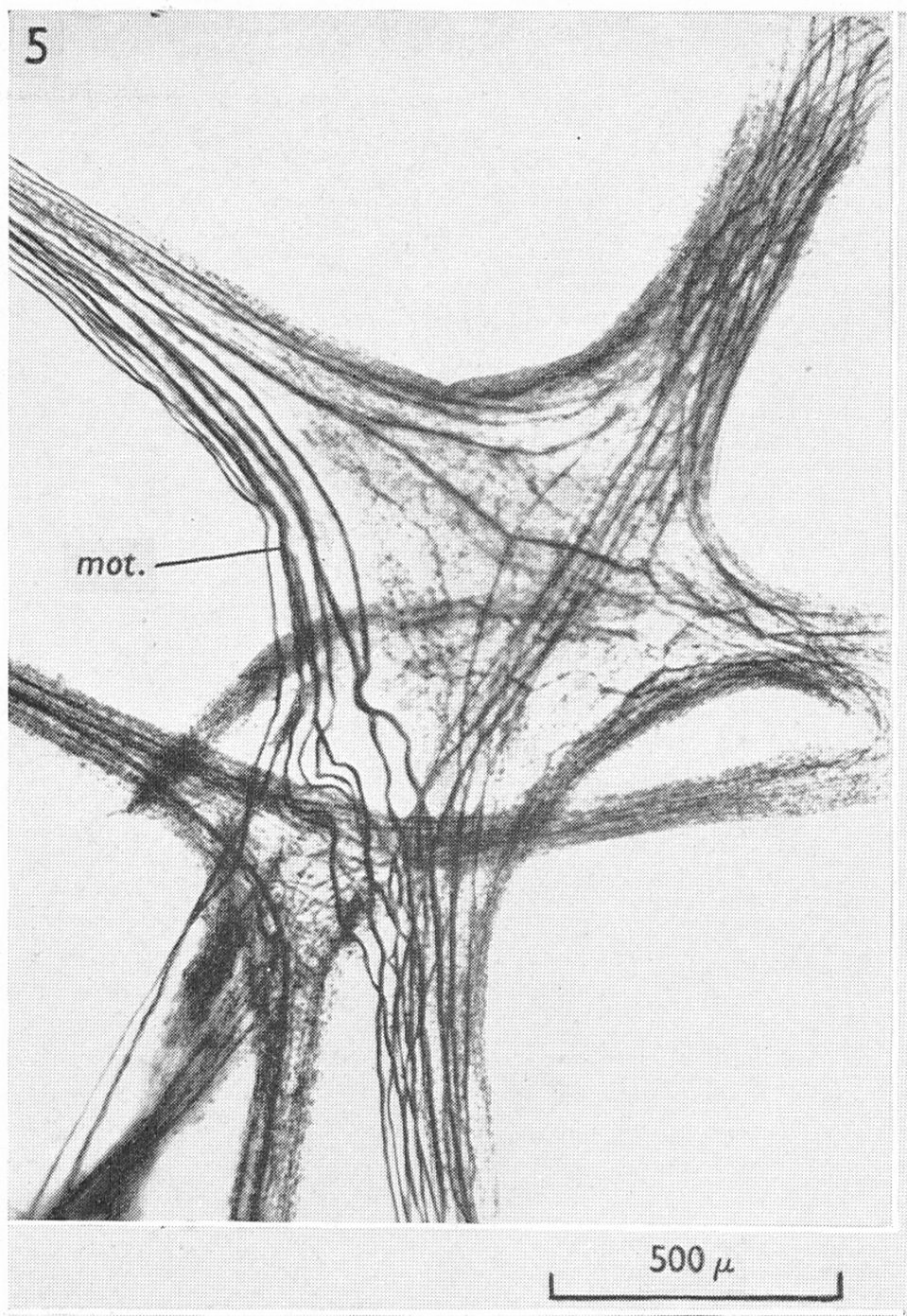

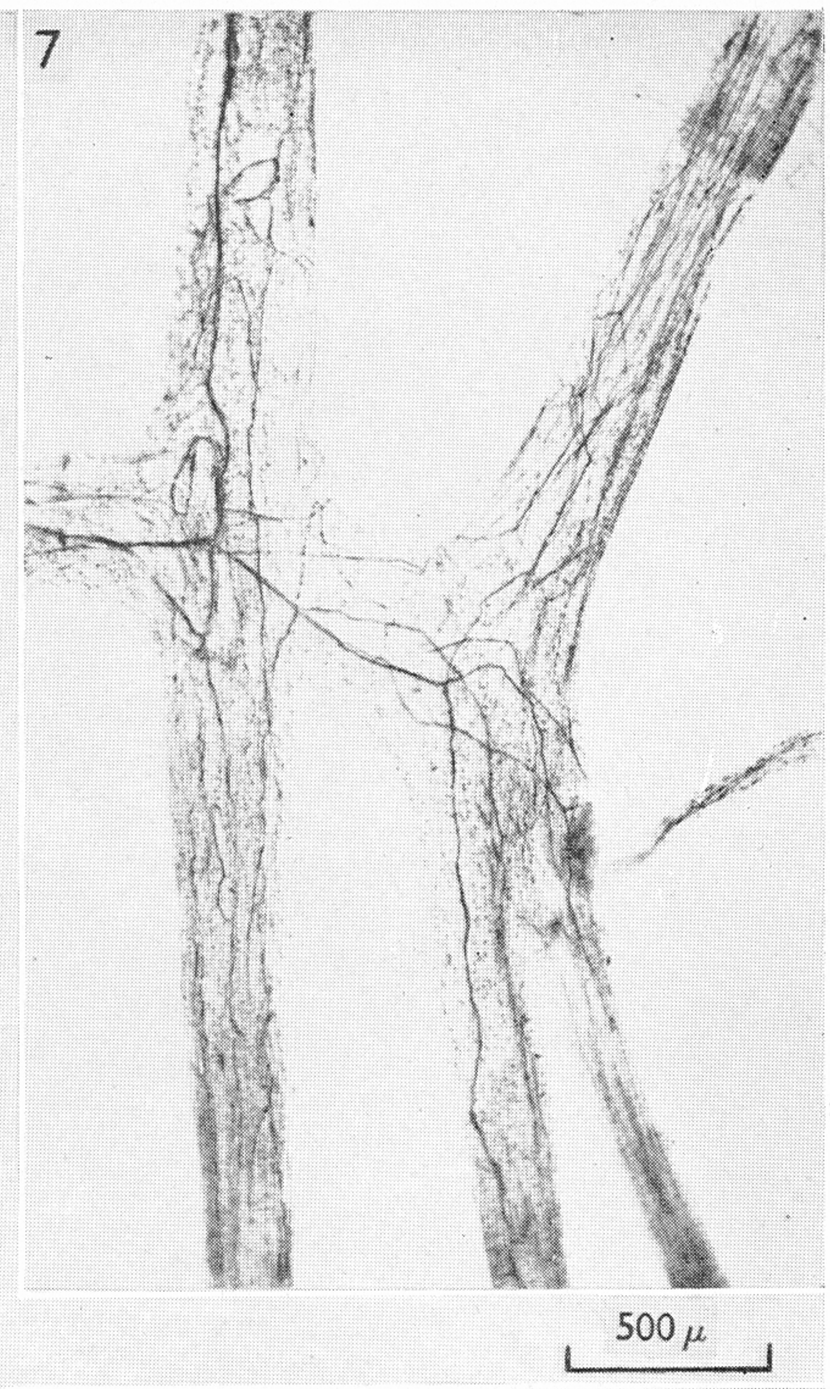

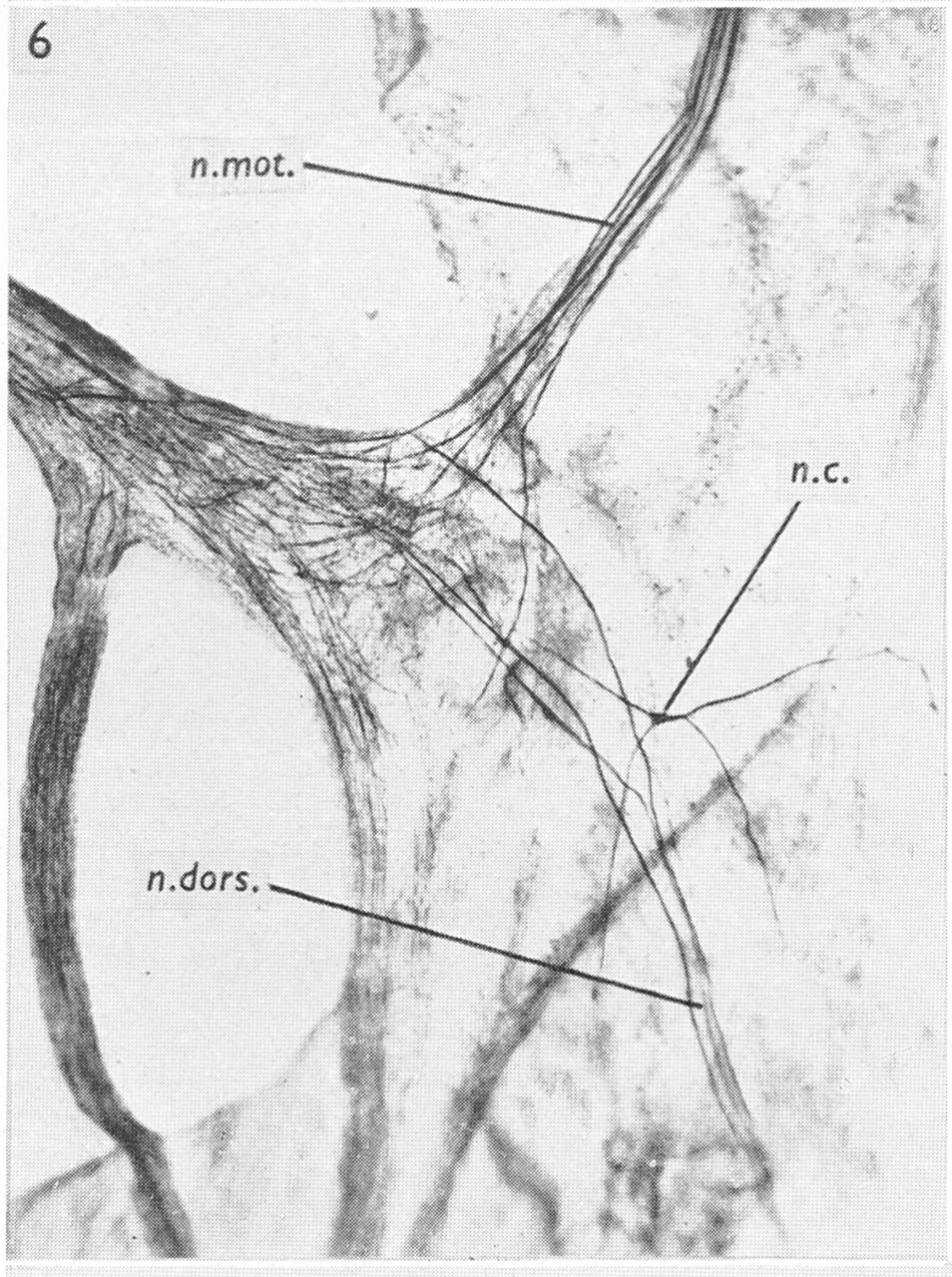

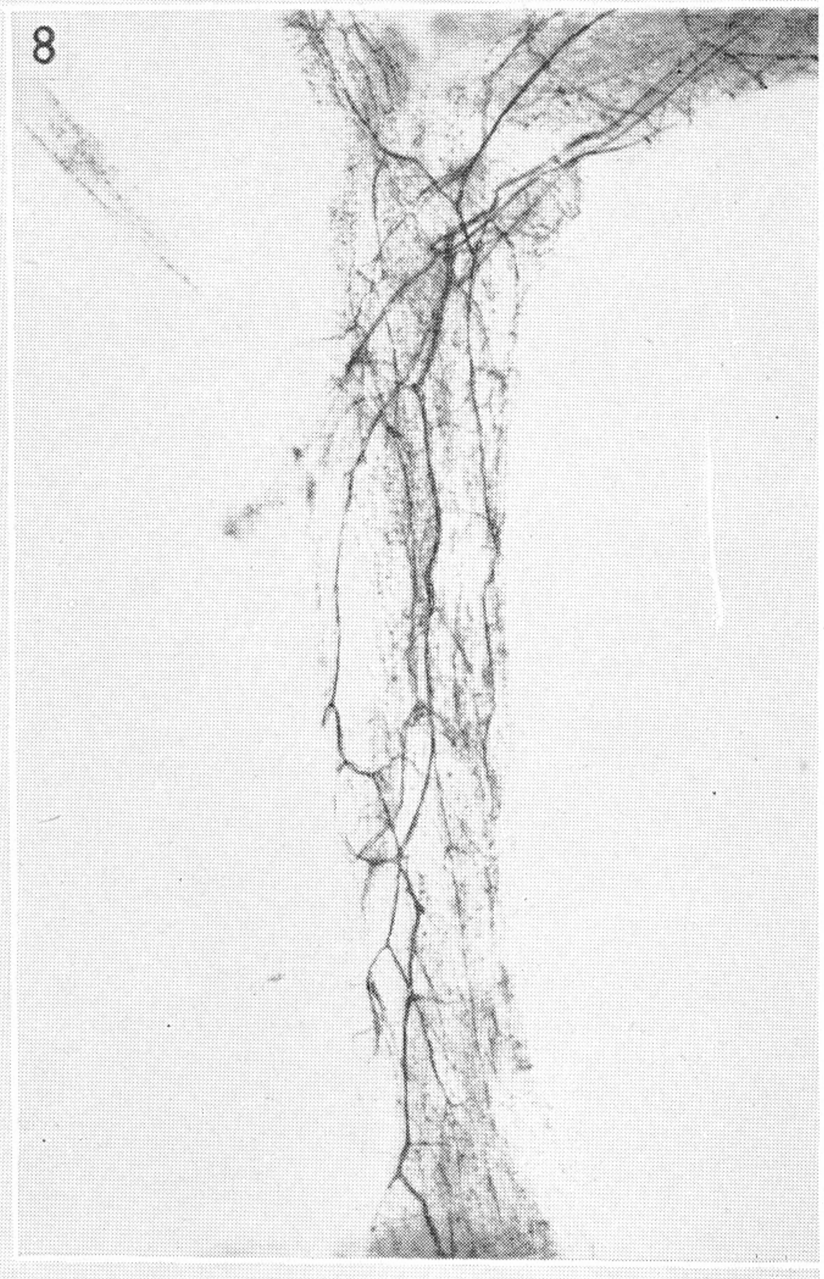

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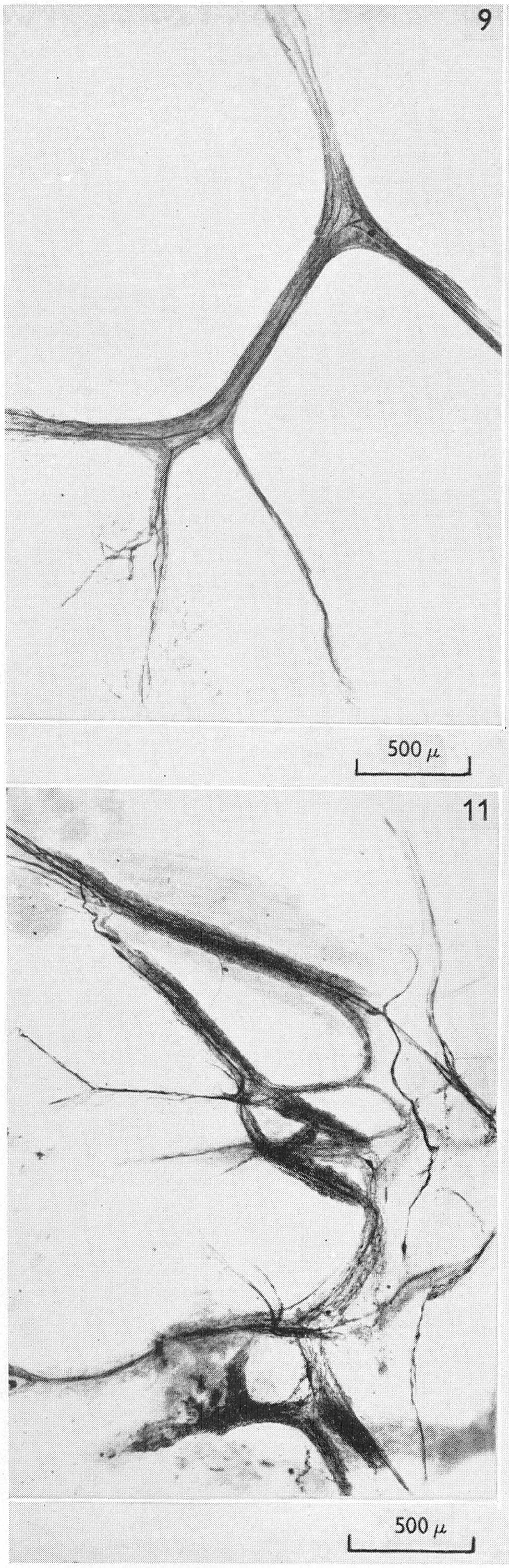
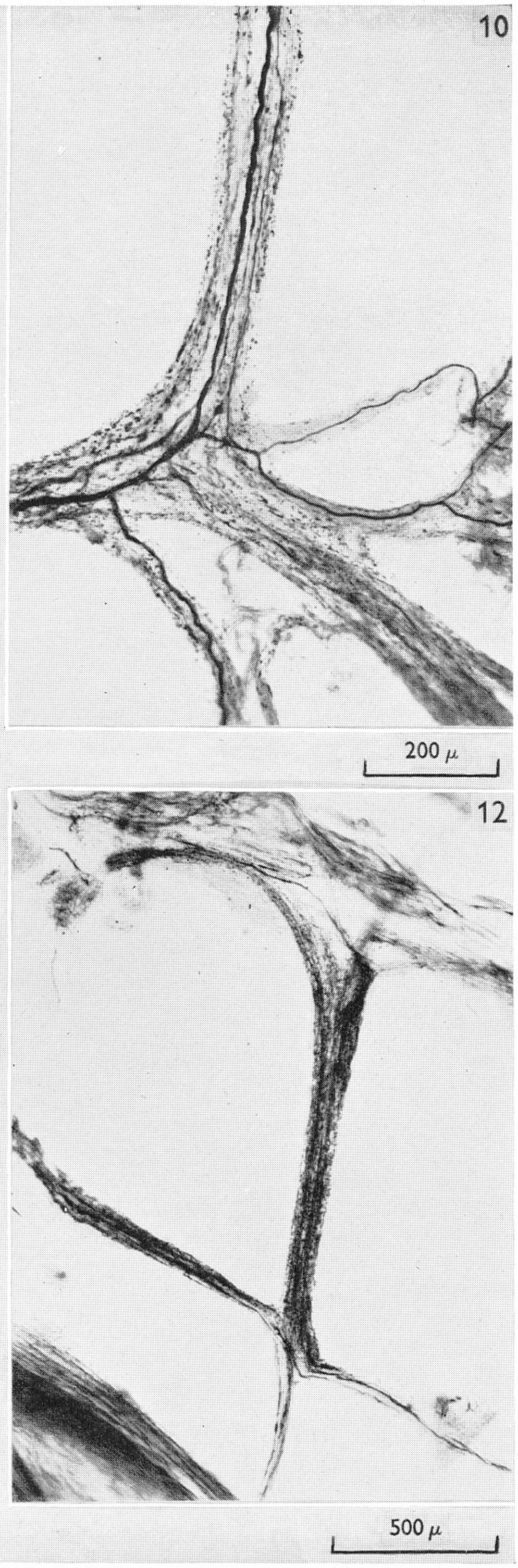
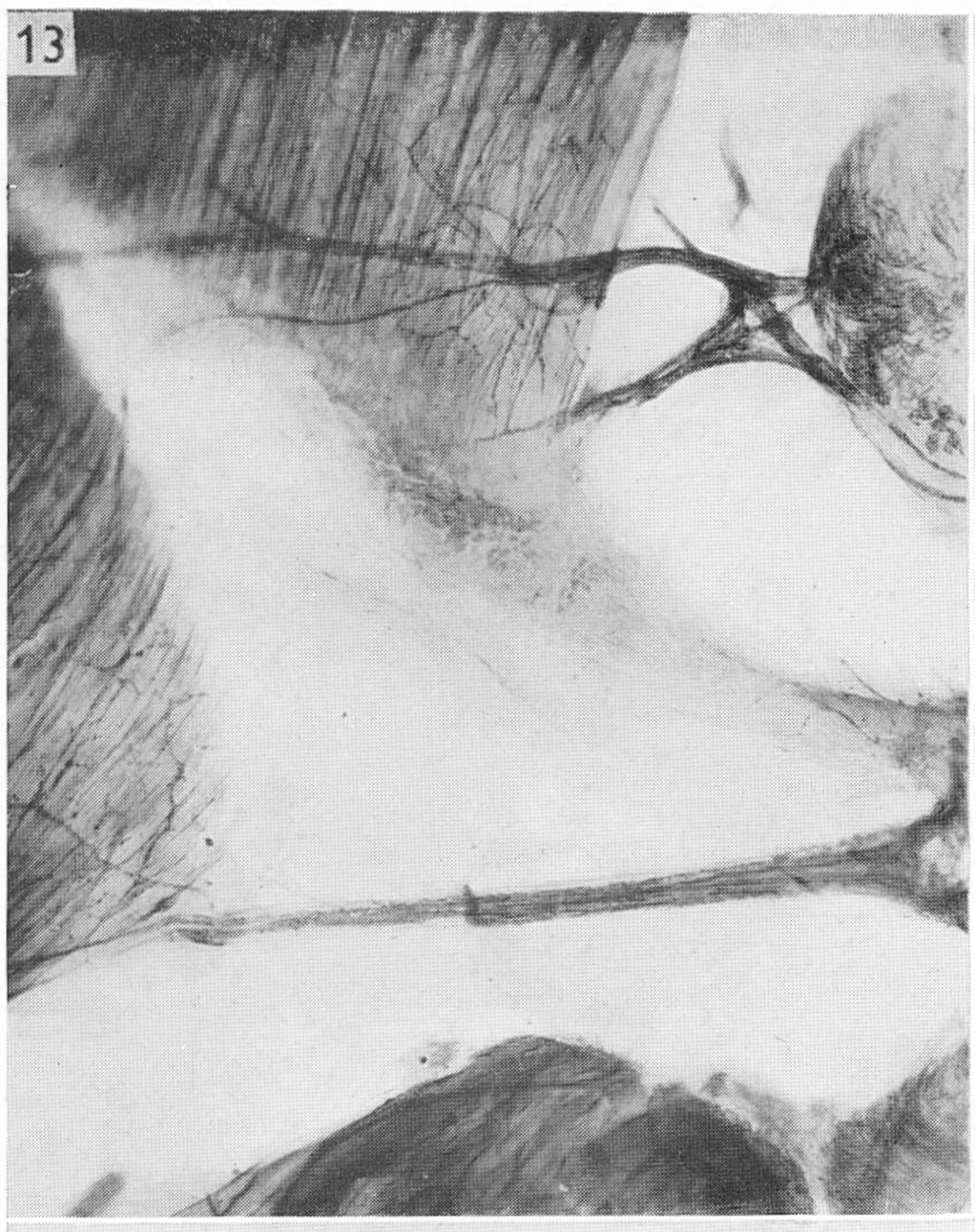
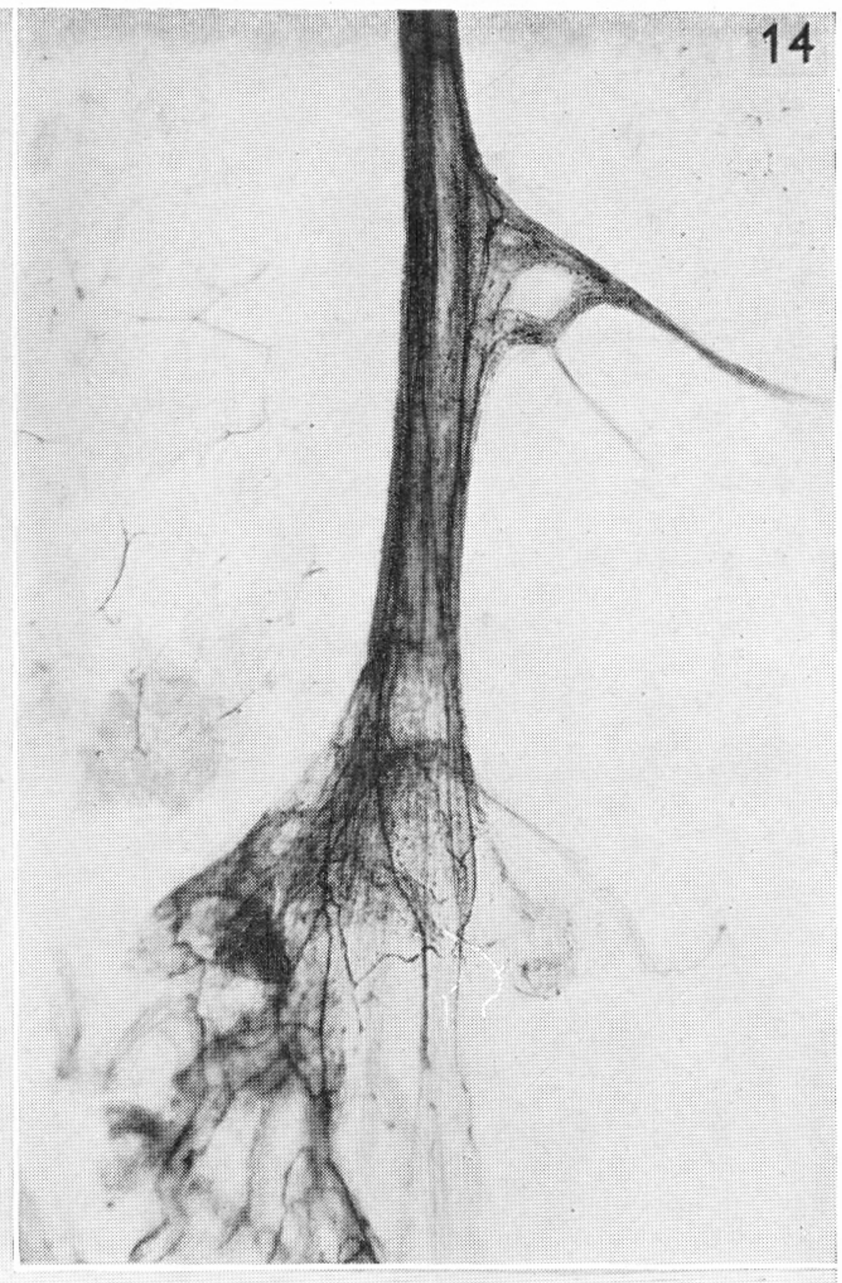
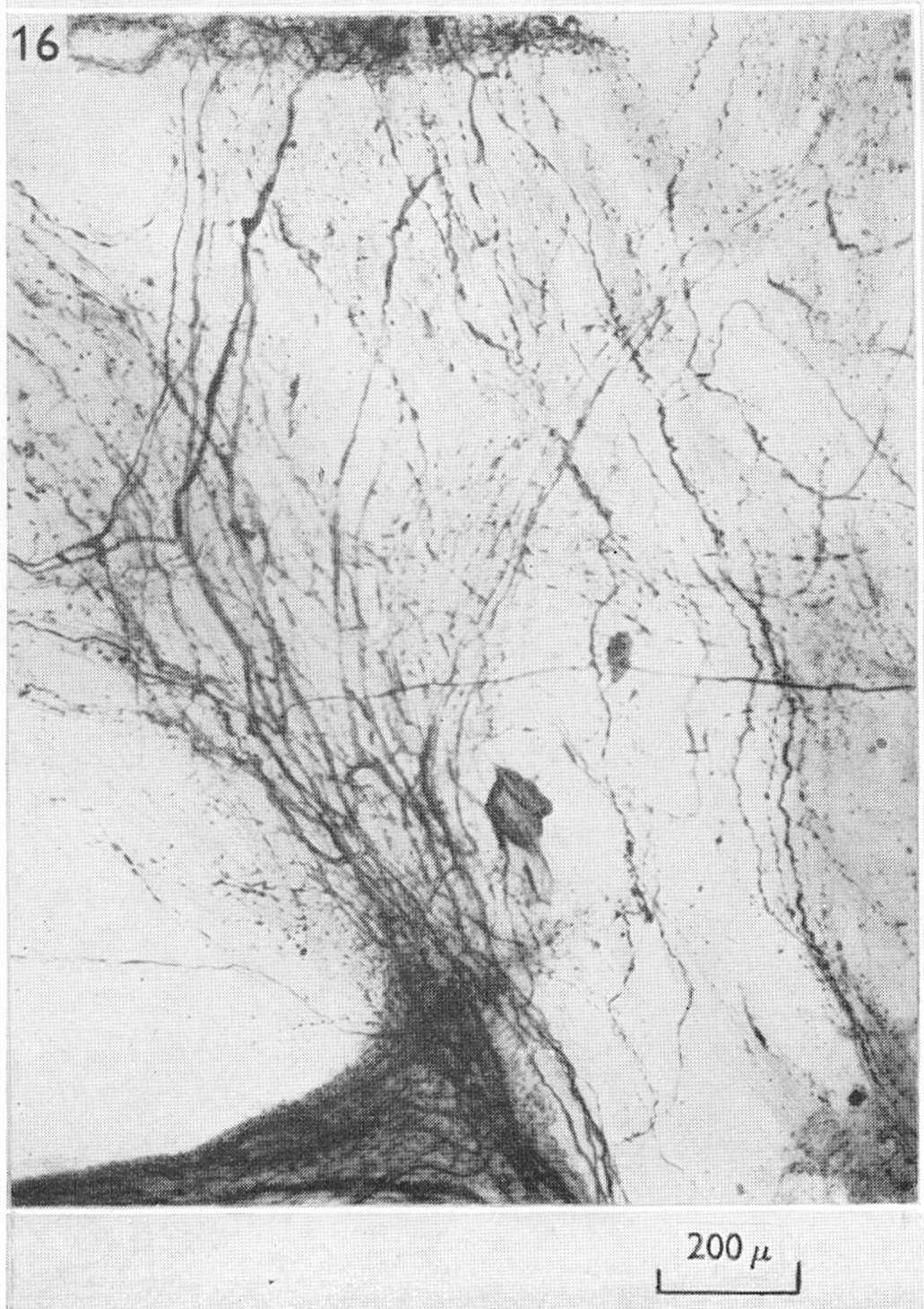


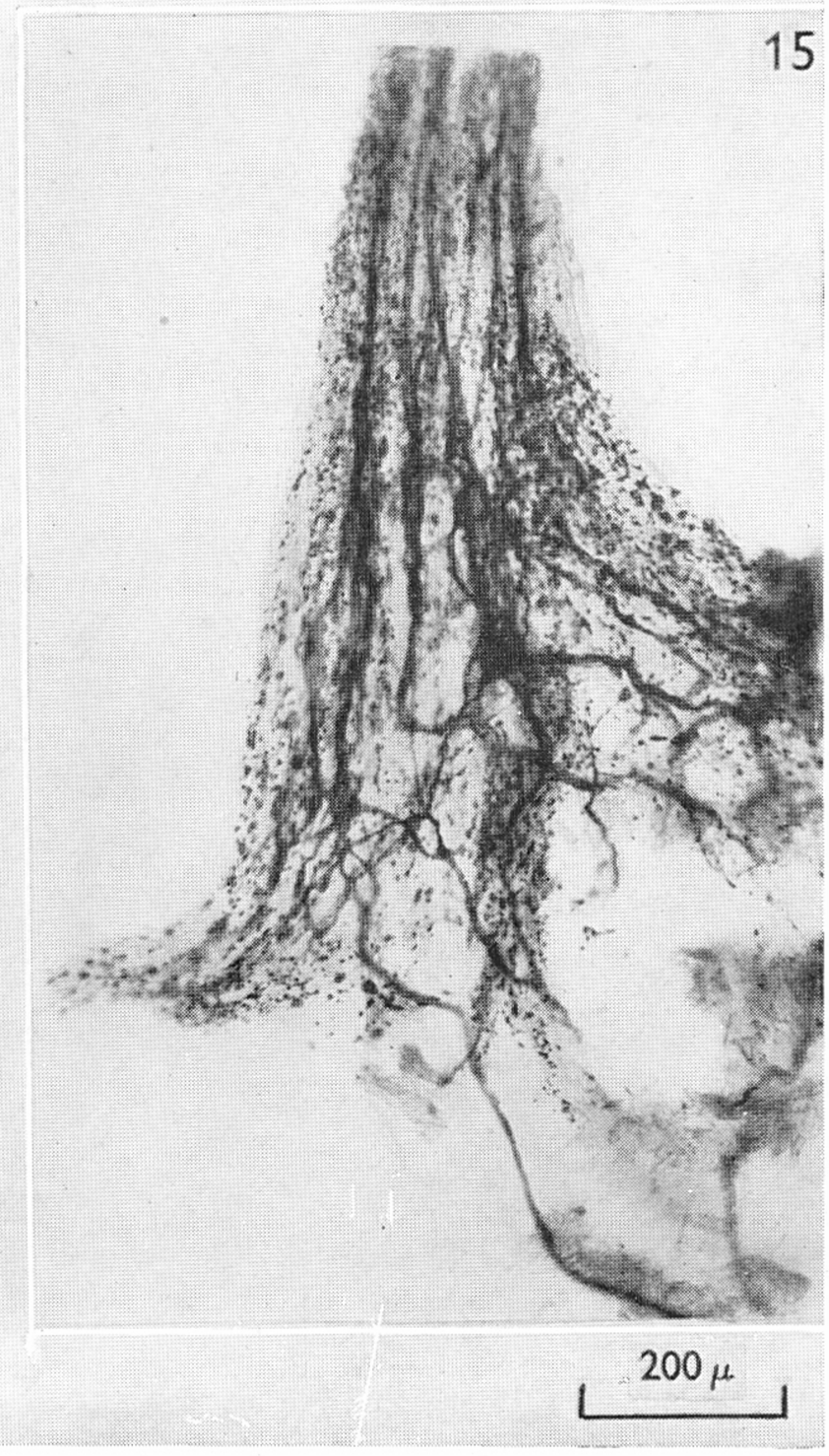


Figs. 9–12.









Figs. 13–16.

CERCARIA REESI N.SP., A NEW FURCOCERCOUS LARVA FROM PLYMOUTH

By Robert F. Hutton

From the Plymouth Laboratory

(Text-fig. 1)

A new longifurcous, pharyngeate, distome cercaria has been found to develop in the marine bivalves *Hiatella arctica* (L.) and *H. striata* Fleuriau. This larva was present in 12 of 200 specimens of *H. arctica* collected from Drake's Island during the summer months (May–August) of 1952, while the same parasite was found in 15 of 100 specimens of the limestone borer, *H. striata*, dredged from Plymouth Sound during June and July of the same year.

I first learned of this larva from Dr W. J. Rees, British Museum (Natural History), who observed it several years ago in *H. arctica* (L.). However, because of other duties both then and now, he has been unable to complete a description of this species. I am indebted to him for giving me information as to its habitat and for suggesting that I give a description of this parasite; for these reasons the present species is named in his honour. Furthermore, I am grateful to Dr W. J. Rees and Dr F. G. Rees for reading and criticizing the manuscript.

Specific nomenclature for molluscs mentioned in this paper is in accordance with Winckworth (1932 and 1951).

This work was carried out at the Plymouth Laboratory of the Marine Biological Association while the author was holding a Fulbright Scholarship.

Methods

Fully developed cercariae were obtained by isolating specimens of *H. arctica* and *H. striata* in glass tubes and allowing the cercariae to emerge. Sporocysts and developing cercariae were obtained by crushing specimens of these bivalves. Cercariae and sporocysts were studied alive and unstained, also fixed in sublimate and stained with Giemsa and with gold chloride. Measurements of cercariae were taken from naturally released living specimens slightly compressed between slide and cover glass.

Observations

Cercaria reesi n.sp. (Fig. 1)

Specific diagnosis. Marine, longifurcous, pharyngeate, distome cercaria developing within ovoid to sausage-shaped sporocysts. Nonoculate; body elongate, widest slightly posterior to centre of body; cuticle covering the

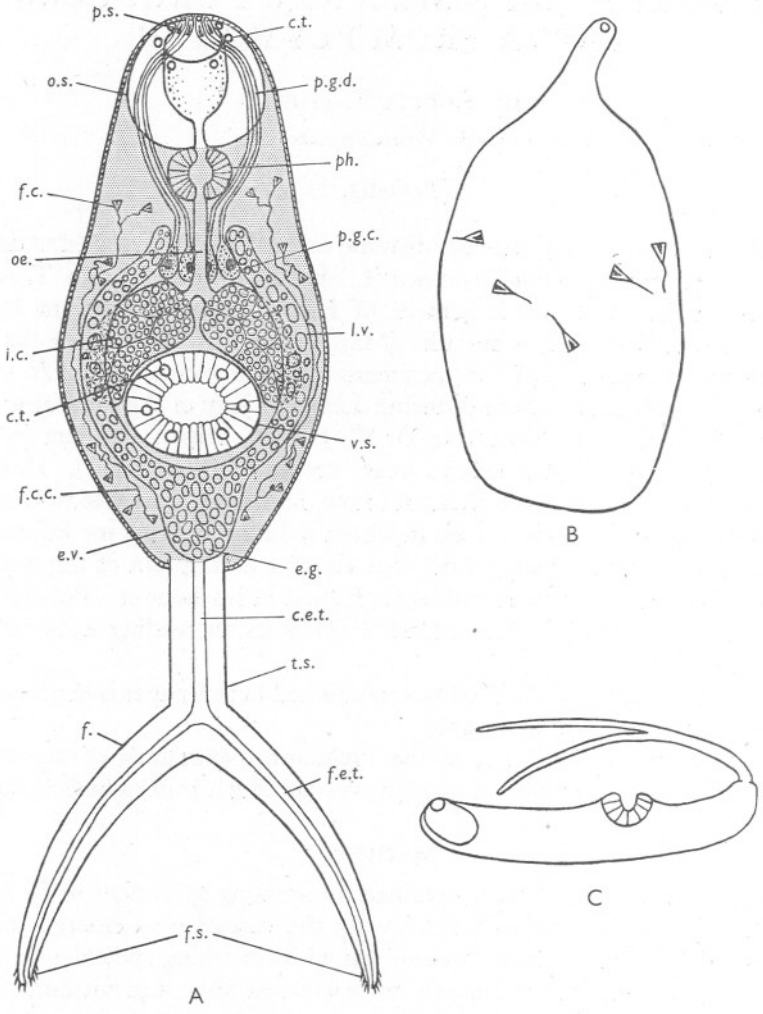


Fig. 1. A, *Cercaria reesi* n.sp. B, sporocyst. C, swimming position of cercaria. (All figures are semi-diagrammatic.) *c.e.t.* caudal excretory tube; *c.t.* cuticular tubercle; *e.g.* excretory granule; *e.v.* excretory vesicle; *f.* furca; *f.c.* flame cell; *f.c.c.* flame cell canal; *f.e.t.* furcal excretory tube; *f.s.* furcal setae; *i.c.* intestinal caecum; *l.v.* lateral branch of excretory vesicle; *oe.* oesophagus; *o.s.* oral sucker; *p.g.c.* penetration gland cell; *p.g.d.* penetration gland duct; *ph.* pharynx; *p.s.* penetrating spine; *t.s.* tail stem; *v.s.* ventral sucker.

body showing small dots arranged densely and regularly; this pattern seen in sections appears to be the surface view of fine striae passing obliquely through the thickness of the cuticle. Two pairs of penetration glands located dorsal and lateral to the posterior end of oesophagus, their ducts running forward to open to the exterior near the tip of the oral sucker; two pairs of protrusible penetrating spines located slightly behind the penetration-duct openings; oral sucker subterminal and ovoidal in shape with six semi-transparent tubercles present on its exterior surface; prepharynx absent; oesophagus of moderate length; intestinal caeca prominent, terminating lateral to the centre of the ventral sucker; ventral sucker elliptical, broader than long, with six semi-transparent tubercles present on its exterior surface. Excretory vesicle conspicuous, the lateral branches extending forward converging anterior to the intestinal caeca and terminating a short distance from the oesophagus; caudal excretory tube passes through the furcae to open to the exterior near their tips; the excretory formula is $2[(2+2)+(2+2)]=16$; tail stem attached to the body ventrally; setae on distal ends of furcae. Measurements in microns of fifty fully developed cercariae under slight pressure of cover-slip: body length 102–216 (av. 159); body width 54–100 (77); oral sucker width 30–42 (36) pharynx width 14–22 (18); ventral sucker width 30–42 (36); tail stem length 18–50 (34); tail stem width 12–26 (19); furca length 30–68 (49); and distance of ventral sucker from anterior end of body 56–92 (74). The maximum diameter of the excretory granules is $6{\cdot}8 \times 3{\cdot}0\,\mu$. Measurements in microns of fifteen living sporocysts under slight pressure of cover-slip: body length 320–800; body width 196–352.

Hosts. *Hiatella arctica* (L.) and *H. striata* Fleuriau.

Habitat. Sporocysts in digestive gland and gonad.

Locality. Drake's Island and Plymouth Sound, Plymouth, England.

Incidence of infection. 6 % in *H. arctica* and 15 % in *H. striata*.

Type material. To be deposited in British Museum.

This cercaria swims with its head in a forward direction and with its tail curved as shown in Fig. 1 C; this is also its normal resting position. The small dots on the cuticle of this cercaria are similar to those of *Cercaria fulbrighti* Hutton; in the latter larva spines develop from these areas. No significant difference was observed between measurements of cercariae from *Hiatella gallicana* and those from *H. arctica*.

Discussion

Miller (1926) includes eight marine species in his check-list of what he considers to be all the furcocercous cercariae, excluding gasterostome cercariae, known at the time. Young (1936), reporting a fork-tailed cercaria from the Bering Sea, states: 'Thus far there have been described to my knowledge twelve marine furcocercous cercariae, at least three of which are of doubtful identity.'

Our knowledge of marine furcocercous cercariae is still very limited. In so far as the present author is aware there are but thirty-five marine forms reported in the literature. Many of these accounts are so meagre that they are of little taxonomic value. The same species has probably been designated under more than one name.

The furcocercous cercariae, other than gasterostomes, reported from British marine waters are as follows:

Parasite	Author	Reported by
Cercaria dichotoma	Müller (according to La Valette St George, 1855)	Lebour, 1908, etc.
Cercaria sp.	Jones & Rothschild, 1932	Jones & Rothschild, 1932
Haplocladus sp.	Rees, 1947	Rees, 1947
Cercaria fulbrighti	Hutton, 1952	Hutton, 1952

In addition to the above list, Rothschild (1936) notes an abnormal cercaria which possesses a 'perfectly symmetrical forked tail in a species normally possessing a simple tail'.

Cercaria dichotoma Müller (according to La Valette St George, 1855) is probably the larva of *Tergestia laticollis* (Rudolphi): most certainly it is not the same species dealt with by Pelseneer (1906) and Lebour (1908), to which both workers attributed the name *Cercaria dichotoma* Müller. Dawes (1946, p. 245) gives the name *C. fissicauda* Villot to the latter cercaria, but this does not seem likely since there are major differences between Villot's cercaria and that of Pelseneer and of Lebour. Villot reports two small teeth on the anterior border of his cercaria, yet he states that he was unable to observe a 'bulbe oesophagien'. Both Pelseneer and Lebour record the presence of a pharynx in their reports, but neither mentions the presence of any anterior teeth-like structures.

Sinitsin (1911) described a marine fork-tailed cercaria from *Abra* (= *Syndosmya*) *alba* under the name *Cercaria discursata*. While this paper was in the proof stage, a publication was received in which Uzmann (1952) described a similar larva, *C. myae*, from *Mya arenaria*. Of the marine furcocercous larvae which have been described, *Cercaria discursata* and *C. myae* appear to be most like *C. reesi*. All three species possess two pairs of penetration glands anterior to the ventral sucker. Possession of these anterior penetration glands places these forms in the 'Strigea' group of Wesenberg-Lund (1934). However, *C. reesi* appears to differ from the other two larvae by possessing two pairs of anterior penetrating spines and by possessing cuticular tubercles on the oral and ventral suckers. Although I was unable to find any mention of cuticular tubercles, or of similar protuberances, in Sinitsin's description of *C. discursata*, his figure (plate III, fig. 45) shows the ventral sucker's aperture to have a fringed outline. It is possible that this shape of the ventral sucker was produced by similar structures which various authors, including myself, have termed 'cuticular tubercles'.

At the present state of our knowledge I feel justified in separating the *Hiatella* parasite from *Cercaria myae* Uzmann and *C. discursata* Sinitsin under the name *C. reesi*.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

THE FORCE EXERTED BY ACTIVE STRIATED MUSCLE DURING AND AFTER CHANGE OF LENGTH

By B. C. Abbott and X. M. Aubert

Journ. Physiol., Vol. 117, 1952, pp. 77–86

A study of the tension changes during enforced stretch of activated dogfish muscle has been made. Isolated coracohyoid muscles at 0° C. were stretched at constant speed during tetanic stimulation. Tension changes and work done were recorded. The events during stretch proved not to be merely the converse of those during shortening, for the tension rise during an imposed stretch depends on the distance moved and hardly at all on the speed of movement, in contrast to the changes observed during shortening. This was different from the findings of Levin & Wyman (1925) on the same muscle, and the discrepancies are discussed; but the results were similar to those obtained with frog muscle.

The readjustment of tension after constant speed stretches and shortenings was followed when stimulation was continued after movement had ceased. The tension moved toward but remained appreciably different from the isometric value at the final length; above after stretch, below after shortening; the differences increased as the speeds of movement were smaller. If stimulation was interrupted long enough for tension to drop to zero the normal isometric value was exerted when excitation was resumed. The evidence indicates that shortening or lengthening offset the internal structure of the muscle perhaps by altering some crystalline orientation and so affects the final tension at any length, giving this hysteresis-like effect. B.C.A.

MUSCLE RECEPTOR ORGANS IN THE ABDOMEN OF *HOMARUS VULGARIS* AND *PALINURUS VULGARIS*

By J. S. Alexandrowicz

Quart. Journ. Micr. Sci., Vol. 92, 1951, pp. 163–99

Nerve cells, connected with special muscle units which are considered to be muscle receptor organs, are described in *Homarus vulgaris* and *Palinurus vulgaris*. In each of the six abdominal segments there are two muscle receptor organs on each side, situated at the level of the superficial dorsal muscles. The

muscle components of these organs are independent of the ordinary muscles. Each of them has its own nerve cell sending several processes to the muscle, and its axon into the ganglionic cord. Differences in the structure of the muscles and in the appearance of nerve cells are described, indicating that the two muscle receptor organs on the same side are of a different kind. Apart from the nerve cell each organ is supplied by at least three kinds of nerves.

In the ganglionic cord of the embryonic lobster a tract is described composed of the axons of the receptor cells. J.S.A.

Notes on the Nervous System in the Stomatopoda. I. The System of Median Connectives

By J. S. Alexandrowicz

Pubbl. Staz. Zool. Napoli, Vol. xxiii, 1952, pp. 201–14

Unpaired nerve trunks running between the longitudinal connectives of the ganglionic chain have been found in *Squilla mantis*. The nerves arising from these trunks carry two kinds of fibres. The supplying of the alary muscles of the pericardium by one kind reveals their motor character. The fibres of the second kind give off numerous branches ending in neuropile-like networks which spread over the wall of the blood sinus and in the sheaths surrounding the nerves. In the sixth thoracic segment the nerves with the ensheathing connective tissue and the neuropile-like terminations form an organ in the shape of a bar suspended across the blood sinus between the flexor muscles. It is suggested that the second kind of nerves may have a neurosecretory function. J.S.A.

Vitamin C Reserves of British Troops in England and Scotland during the Winter and Spring, 1941–42

By W. R. G. Atkins

(Late Captain, Royal Army Medical Corps)

British Journ. of Nutrition, Vol. 5 (nos. 3 and 4), 1951, pp. 275–86

The object of the work was to study the vitamin C reserve in relation to the prevalence of gingivitis and scurvy or antecedent conditions. The general plan of investigation was drawn up by Major-General D. T. Richardson, C.B., M.C., K.H.S., Director of Hygiene. Two hundred recruits—representing the civilian population—and four hundred soldiers were examined in early winter and again in late spring. Their mouths were examined by the late Brigadier H. Stobie, Consulting Dental Surgeon to the Army, whose report showed no

connexion between incidence of gingivitis and vitamin C reserve in these 1200 men. There was no scurvy.

Recruits showed more vitamin C than soldiers, so that even in spring their reserves were greater than those of the soldiers had been 4 months previously in winter.

Both recruits and soldiers from the country were superior to those from industrial areas. Only 1 % of recruits remained unsaturated after five or six doses of synthetic vitamin C, whereas in spring soldiers of Scottish Command (Glasgow) showed 34 % unsaturated after five doses and 21 % after six. It appeared desirable to increase the vitamin C supply to troops in winter. One test with forty-three men showed that saturation was reached more rapidly in those who had the vitamin dose after breakfast rather than before it.

W.R.G.A.

The Relation between Thickness of Cloud Layers and their Transmission of Light

By W. R. G. Atkins

Quart. Journ. Roy. Met. Soc., Vol. 77, 1951, pp. 659–62

Apparent minimal cloud-transmission percentages for Bircham Newton, Norfolk, are plotted against vertical distribution of clouds observed from aircraft, and tenths of cloud cover, the latter being indicated by modifications in the line joining the points showing base and top of each cloud layer. With under 20 % transmission great cloud thickness is possible, but with over 30 % no great thickness of low cloud occurs. Percentage frequencies of minimal cloud transmission are given for a year. Making use of Hewson's calculations for reflexion, absorption and transmission of solar radiation for droplets of 40 μ (fog) and 10 μ (cloud) for various water contents it was found that the observed transmissions of morning fog at Bircham Newton in May were in accord with 1·0 g./m.^{3} water content, whereas transmission by thick cloud during a depression in May, and by layers of cloud another day were probably due to droplets of 10 μ at 0·1 g./m.^{3} concentration. W.R.G.A.

Photoelectric Measurements of the Seasonal Variations in Daylight at Plymouth from 1947 to 1949

By W. R. G. Atkins and Pamela G. Jenkins

Quart. Journ. Roy. Met. Soc., Vol. 78, 1952, pp. 70–5

Daylight received on a horizontal surface has been measured for 14 years using a Burt vacuum sodium cell and a Cambridge thread-recorder galvanometer. Mean monthly values of daily maxima, in kilolux, are tabulated, also

mean monthly values of illumination in kilolux hours. These are compared with similar measurements at Kew in 1947 and 1948, got with a selenium cell. The kilolux hours for each month are shown as a percentage of individual years, with mean values for 14 years and percentage range for variation. Annual totals were 108, 124 and 122 megalux hours for 1947, 1948 and 1949 respectively with 116 as the mean of the whole series. Between these and sunshine or rainfall there is no simple relation. Cloudless days are not the brightest owing to downward reflexion from clouds. Darkest days in each month commonly show 17–40 % as many kilolux hours as do brightest, though smoke pollution may reduce illumination to 5 %. W.R.G.A.

Muscular and Hydrostatic Action in the Sea-anemone *Metridium senile* (L.)

By E. J. Batham and C. F. A. Pantin

Journ. Exp. Biol., Vol. 27, 1950, pp. 264–89

The mechanics of muscular action and hydrostatic action are discussed in the sea-anemone *Metridium senile*. This animal exhibits a great variety of body shapes which are brought about by continual slow muscular activity. The action of most of the muscles is very slow, isotonic contraction of the parietal muscles requiring 40–60 sec. to reach its maximum and many minutes to relax. The body-wall is capable of extension of about 400 % but there are limits to extensibility in the normal animal. The mechanisms by which the animal itself increases or reduces extension by controlling its coelenteric volume are described. Pressure changes in the coelenteron which occur during activity show that both retraction and extension of the column are active processes involving a rise in pressure which enforces reciprocal extension of the opposing musculature. The relation of normal activity and shape to the coelenteric pressure is shown. This average pressure is extremely small, about 2–3 mm. of water. In a moderately filled unstimulated animal the natural muscular contractions are accompanied by a rise in pressure not generally exceeding 6–7 mm. of water. The isometric pressure which the body-wall can develop in the coelenteron has been estimated. Pressures developed during natural contractions demand muscular tensions in the body-wall ranging between 20 and 50 % of the isometric tension. An estimate is deduced from the coelenteric pressure of the isometric tension developed by the circular muscle of the column. This is about 3·5 g./cm. of body-wall transverse to the muscle. Extensive responses of the powerful retractor muscles involve much greater pressures than those against which the column muscles can operate. Development of these muscles is related

to the necessity of speed of action. Muscular action in a hydrostatic skeletal system is contrasted with that in the jointed skeletal system of vertebrates and arthropods. C.F.A.P.

Inherent Activity in the Sea-anemone *Metridium senile* (L.)

By E. J. Batham and C. F. A. Pantin

Journ. Exp. Biol., Vol. 27, 1950, pp. 290–301

Methods of observing and analysing continual muscular activity of the sea-anemone *Metridium senile* are discussed. This activity is so slow that it is rarely appreciated by the eye as movement. The activity of the column consists of a sequence of reciprocal contractions of the parietal muscles and the circular muscle coat. The activity of different parts of the body-wall may show striking co-ordination, a contraction of one part of the parietal musculature is usually followed by a contraction of the others. In other cases there may be no trace of co-ordination. Co-ordination takes place through one part of the body-wall acting as leader. The other parts of the body-wall follow this contraction with long delays. There is evidence that the delay is of local origin. One sector usually maintains leadership for long periods but from time to time the site of leadership changes. Evidence is given that the activity is inherent and continues unaltered in the absence of external stimulation. There is considerable variation in character and extent of activity in different animals and at different times. C.F.A.P.

Phases of Activity in the Sea-anemone *Metridium senile* (L.), and their Relation to External Stimuli

By E. J. Batham and C. F. A. Pantin

Journ. Exp. Biol., Vol. 27, 1950, pp. 377–99

Basic activity in the sea-anemone *Metridium senile* has been studied and analysed in detail. Under constant conditions the animal exhibits continual slow inherent activity. The pattern of this activity varies in character from time to time and these different patterns of activity have been termed phases. A change of phase may be initiated by certain stimuli such as ingestion of food. In contrast with the direct responses such as the retraction reflex, the stimulus does not directly maintain a phasic response; it merely initiates a new phase, and the activity pattern of the latter is maintained long after the initiating stimulus. Phase changes also differ from simple reflex in that the threshold for their initiation varies enormously in different animals and in the same animal at different times. Locomotion is another phasic activity which

may be initiated by various stimuli or may take place spontaneously in the absence of evident external stimuli. The alternating phases of expansion and contraction occur frequently and their relation to diurnal and other rhythms is discussed. Daily illumination can often initiate and control regular daily phases of contraction. In complete darkness and constant environmental conditions, alternating phases of expansion and contraction may still take place. It appears that a periodic stimulus such as daily illumination acts by 'setting the pace' of this inherent alternating phase change. Experiments show that continual and varying patterns of inherent activity play an important part in the behaviour of *Metridium*. Phasic activities are less easily observed than direct responses because they are so slow, but they play an essential part in behaviour patterns such as food capture, and they tend to be relative to a future possible event rather than to a past stimulus. C.F.A.P.

The Organization of the Muscular System of *Metridium senile*

By E. J. Batham and C. F. A. Pantin

Quart. Journ. Micr. Sci., Vol. 92, 1951, pp. 27–54

The muscular system of *Metridium* consists of fields of relatively short muscle fibres. These form a connected network and can shorten to about a fifth of the extended length. Deformation of the body-wall is described and is shown to be controlled in part by the contractility of the muscle-fibres, and in part by the properties of the mesogloea. A longitudinal contraction of the body-wall is accompanied by thickening of the mesogloea and buckling which throws the layer of circular muscles into folds. A natural limit to the extension of anemone tissue is reached when the muscle-layer is completely unbuckled. The function of the muscle-fields is analysed and the muscular plan of the pedal disk is compared with the tube foot of *Asterias*. A significant functional similarity is found in the operation of vertical, oblique and radial muscles bearing on the adhesive disk. Finally, the functional organization of the oral disk and tentacles is discussed. C.F.A.P.

Ultraviolettabsorption der Meeresalgen

By Richard Biebl

Ber. Deutsch. Bot. Ges., Bd. 65, 1952, pp. 37–41

In the summer of 1951, at the Marine Biological Laboratory in Plymouth, experiments were made with a Unicam photoelectric quartz spectrophotometer to determine the absorption of the green alga *Ulva lactuca*, the red algae *Porphyra umbilicalis* f. *laciniata*, *Phycodris rubens*, *Polyneura hilliae*, and

of the brown alga *Dictyota dichotoma*, by exposing their thalli to light of wave-length ranging from 400 to 800 mμ and to ultraviolet rays of 200–400 mμ.

The resulting diagrams show that the absorption of visible light corresponds to what has already been recorded in the literature. The results of the measurements on the absorption of light in the ultraviolet region, however, are new. They show characteristic absorption curves between 290 and 400 mμ for each alga, though with partly opposite trends for red algae and green algae. But typical absorption of green, red, and brown algae could be found when exposing them to light of wave-lengths ranging from 200 to 290 mμ. Such absorption curves are flat and run almost parallel.

It is of ecological interest that these findings fit the fact that algae in their natural surroundings are at best exposed to rays of 300 mμ upwards, whereas they are never reached by shorter wave lengths. R.B.

Some Experiments with the Common Hermit Crab (*Eupagurus bernhardus* Linn.), and Transparent Univalve Shells

By L. R. Brightwell

Proc. Zool. Soc. Lond., Vol. 121, 1951, pp. 279–83

Between 10 and 24 July 1950, experiments were made at the Plymouth Laboratory with glass univalve shells, as originated by the late Richard Elmhirst at Millport. The shells used were perfect replicas of *Buccinum* and the work of Mr J. G. Brett of Rugby.

Eupagurus readily accepted the shells, often leaving a natural shell for the counterfeit. The commensal worm (*Nereis fucata*) similarly accommodated itself. It invariably approached from behind, climbing over the body whorl and slipping over the lip with utmost stealth, the hermit being normally antagonistic to it. Once safe within the apical whorls the worm is accepted with indifference, and freely helps itself to food from between the pagurid's foot-jaws. The worm can be independent of the crab indefinitely, if well supplied with food.

Tests seemed to show that, of potential enemies, swimming crabs at least rely much on sight. A ravenous *Portunus puber* at once rushed at the naked abdomen of a hermit, but was baulked by the glass shell. *Eupagurus*, when hungry, will freely search the interior of the anemone *Caliactis*, as encountered attached to the borrowed shell of another hermit, or living, as it often does, quite independently.

A hermit having the terminal joint of its great claw crushed did not discard it at the breaking plane, but picked it clean of muscular tissue L.R.B.

The Structure and Function of the Basement Membrane Muscle System in *Amphiporus lactifloreus* (Nemertea)

By J. B. Cowey

Quart. Journ. Micr. Sci., Vol. 93, 1952, pp. 1–15

The body-wall of *A. lactifloreus* has the following structure from the outside inwards.

(i) A basement membrane of five to six layers immediately underlying the epithelium. Each layer consists of right-hand and left-hand geodesic fibres making a lattice, whose constituent parallelograms have a side length of from 5 to 6μ. The fibres are attached to one another where they cross; so there can be no slipping relative to one another.

(ii) A layer of circular muscle fibres running round the animal containing two systems of argyrophil fibres—one of fibres at intervals of 10μ running parallel to the muscle-fibres and the other of fibres running radially through the layer from the basement membrane to the myoseptum.

(iii) A myoseptum which is identical in structure with a single layer of the basement membrane.

(iv) A layer of longitudinal muscle, whose fibres are arranged in layers on each side of a series of longitudinal radial membranes.

Membranes identical in structure with the basement membrane invest the nerve cords, the gut, the gonads, and the proboscis.

The interrelations of argyrophil and muscle fibres in the muscle layers are described and their functioning discussed.

The system of inextensible geodesic fibres is analysed from a functional standpoint. The maximum volume enclosed by a cylindrical element (cross-section circular), of such a length that the geodesic makes one complete turn round it, varies with the value of the angle θ between the fibres and the longitudinal axis. When θ is 0° the volume is zero; it increases to a maximum when θ is 54° 44′ and decreases again to zero when θ is 90°. The length of the element under these conditions varies from zero when θ is 90° to a maximum (the length of one turn of the geodesic) when θ is 0°.

The body volume of the worm is constant. Thus it has a maximum and minimum length when its cross-section is circular and at any length between these values its cross-section becomes more or less elliptical. It is maximally elliptical when θ is 54° 44′, i.e. when the volume the system could contain, at circular cross-section, is maximal. From measurements of the ratio of major to minor axes of this maximally elliptical cross-section, the maximum and minimum lengths of the worm relative to the relaxed length and values of θ at maximum and minimum lengths are calculated. The worm is actually unable to contract till its cross-section is circular; but measurements of its

cross-sectional shape at the minimum length it can attain, permit calculation of the theoretical length and value of θ for this cross-sectional shape.

Calculated values of length and the angle θ agree well with the directly observed values. J.B.C.

The Blood System in the Serpulimorpha (Annelida, Polychaeta). I. The Anatomy of the Blood System in the Serpulidae. II. The Anatomy of the Blood System in the Sabellidae, and Comparison of Sabellidae and Serpulidae. III. Histology

By Jean Hanson

Quart. Journ. Micr. Sci., Vol. 91, 1950, pp. 111–29; pp. 369–78; Vol. 92, 1951, pp. 255–74

The anatomy and histology of the blood system in a number of serpulids and sabellids from Plymouth and Naples have been investigated: *Serpula*, *Hydroides*, *Vermiliopsis*, *Pomatoceros*, *Salmacina*, *Protula*, *Apomatus*, *Spirorbis*, *Sabella*, *Potamilla*, *Branchiomma*, *Dasychone*, *Amphiglena*, *Fabricia*, *Jasmineira*, *Dialychone* and *Myxicola*. In both families one can distinguish a central blood system in which a true circulation is probably maintained, and a peripheral blood system of predominantly blind-ending vessels which are alternately empty and full, receiving their blood from the central system and then returning it along the same channels. The central blood system is built on the same plan in both families, but the peripheral system is variable, especially amongst the sabellids. The variations are partly attributable to differences in body size. The functions of subepidermal and coelomic capillaries and the blood supply to the muscles of the body-wall are discussed. In both families all vessels possess a three-layered wall consisting of an endothelium, a skeletal coat, and a peritoneum containing muscle fibres which lie transversely to the long axis of the vessel. The structure of these three coats is described in detail. Blood cells are absent in all genera. J.H.

The Sodium and Potassium Content of Cephalopod Nerve Fibres

By R. D. Keynes and P. R. Lewis

Journ. Physiol., Vol. 114, 1951, pp. 151–82

A method is described for determining sodium and potassium simultaneously, in quantities down to 0·3 and 3 μg. respectively. The technique used was 'activation analysis', which involved irradiation of the tissue samples in a neutron pile for a week, followed by β- and γ-counts to determine the amounts of ^{42}K and ^{24}Na which had been formed.

Figures were obtained for the sodium and potassium concentrations in

freshly dissected 200 μ *Sepia* axons, and for the rate of loss of potassium and gain of sodium in axons kept in sea water at about 10° C. Estimates for a further series of axons which had conducted about 120,000 impulses before being analysed, showed that during activity there was a net gain of 3·8 $\mu\mu$mol. Na/cm.^{2}/impulse, and a net loss of a roughly equal quantity of potassium. Analyses of samples of axoplasm extruded from 500 μ squid axons gave very similar results. Measurements of the chloride content of the axoplasm samples showed a considerably smaller net gain on stimulation.

These results provide direct confirmation for the 'sodium hypothesis' of nervous conduction, put forward by Hodgkin, Huxley & Katz. R.D.K.

Colours of Marine Animals

By J. A. Colin Nicol

School Science Review, Vol. xxxiii, 1952, pp. 208–18

A general account is given of coloration in marine animals, and of the pigments and structural modifications which are responsible. Either may be responsible for the colour of a particular animal, or both may play a complementary role. The principal pigments responsible for external colours are carotenoids, porphyrins, indole pigments, melanins, quinones, purines, and various protein substances. The distribution and biological implications of some of these substances are discussed. Structural colours are due to interference effects, and may be seen in the iridescence of various animals. Morphological and physiological colour changes are distinguished, and the significance of adaptive coloration to certain species is considered. The article concludes with a series of references which provide an introduction to the bibliography dealing with the subject. J.A.C.N.

A New Subterranean Gammarid (Crustacea) from Britain

By G. M. Spooner

Proc. Zool. Soc. Lond., Vol. 121, 1952, pp. 851–9

Niphargus glenniei is a new species of the subterranean genus *Niphargus* discovered in limestone caves near Buckfastleigh, S. Devon, where it was first collected by Brig. E. A. Glennie of the Cave Research Group. A detailed description is given. The species is small, and the male sex has not yet been recognized.

The nearest possible relatives on the continent are *N. arndti*, known from four localities in Silesia, and *N. nolli*, known from two localities in the upper Rhineland, both small species in which the mandibular palp shows an unusual simplification. However, differences between *N. glenniei* and these

two species are considerable, and the resemblances may conceivably be but the effect of convergence.

Schellenberg's genus *Niphargellus* is not to be considered valid, an opinion enhanced by the discovery of *Niphargus glenniei*.

The existence of this distinct new species was indeed unexpected, in view of the poverty of the British subterranean arthropod fauna and the lack of endemic forms. G.M.S.

The Respiratory Significance of the Crown in the Polychaete Worms *Sabella* and *Myxicola*

By G. P. Wells

Proc. Roy. Soc., B., Vol. 140, 1952, pp. 70–82

The tube of *Sabella pavonina* is irrigated by means of piston-like waves travelling tailwards along the worm's body, a process which is hardly affected by decapitation. The tube of *Myxicola infundibulum* is not irrigated at all. After bisection of tubeless worms at the junction of thorax and abdomen, the oxygen consumption of both halves together is greatly depressed in *Myxicola* but is unaffected in *Sabella*. The conclusion is drawn that the body of *Myxicola* depends largely on the crown for its oxygen supply while that of *Sabella* normally derives its oxygen from the irrigation current through the tube. The results are discussed in relation to the differences in anatomy, and in the ability to survive and regenerate after injury, between the two worms. G.P.W.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888. The cost of the building and its equipment was £12,000 and, since that date, a new library and further laboratory accommodation have been added at an expenditure of over £25,000.

The Association is maintained by subscriptions and donations from private members, scientific societies and public bodies, and from universities and other educational institutions; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. XXVII (p. 761) and Vol. XXXI (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

TERMS OF MEMBERSHIP

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Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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