New Series.—Vol. X., No. 3—issued October, 1914.
[Price Two Shillings, net.]

Journal

OF THE

MARINE BIOLOGICAL ASSOCIATION

OF

THE UNITED KINGDOM.

THE PLYMOUTH LABORATORY.

PLYMOUTH:

PRINTED FOR THE MARINE BIOLOGICAL ASSOCIATION BY W. BRENDON & SON, LTD.,

AND

PUBLISHED BY THE ASSOCIATION AT ITS OFFICES ON THE CITADEL HILL.

SENT FREE BY POST TO ALL MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION:
ANNUAL SUBSCRIPTION FOR MEMBERSHIP, ONE GUINEA.

Agents in London:—Messrs. DULAU & Co., LTD., 37 Soho Square, W.

On the Culture of the Plankton Diatom *Thalassiosira gravida* Cleve, in Artificial Sea-water.

By

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In a former paper,* written in conjunction with my colleague Mr. E. W. Nelson, the conditions under which a rapid and continuous growth of marine plankton diatoms can be obtained in laboratory cultures were discussed. It was pointed out that when natural sea-water is used as the basis of the culture media we are dealing with a solution of a very complex and variable character, the exact nature of which it is extremely difficult to determine, and that the ideal to be aimed at is to find a culture medium with artificially prepared sea-water as its basis, such that the absence or diminution in quantity of any one of its constituents would have a profound effect upon the growth of diatoms in it. A reference was made (*loc. cit.*, p. 446)† to some experiments with artificial sea-water, which, whilst pointing to the probability of successful work being possible on these lines, were in themselves too uncertain to be satisfactory.

Experiments in this direction have been continued at intervals during the past three years, and although the problem has not been completely solved the results obtained seem to be of sufficient interest and importance to warrant publication in their present incomplete form, more particularly because points remaining to be cleared up probably require a knowledge of the chemistry of organic compounds to which I cannot lay claim.

Stated in general terms the most interesting result so far obtained is that in the artificial sea-water tried, made by dissolving Kahlbaum's pure chemicals in doubly distilled water, little or no growth of diatom (*Thalassiosira gravida* Cleve) takes place, but if to this artificial sea-water as little as 1 per cent of natural sea-water is added vigorous and large cultures are obtained, and with an addition of about 4 per cent of

* Allen, E. J., and Nelson, E. W. "On the Artificial Culture of Marine Plankton Organisms," *Journ. Mar. Biol. Assoc.*, VIII, 1910. Also in *Quart. Journ. Micr. Sci.*, Vol. LV, 1910. The two papers are identical.

† *Q.J.M.S.*, Vol. LV, p. 393.

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natural sea-water from the Laboratory tanks better cultures result than have so far been got in any medium which has natural instead of artificial sea-water as a basis.

THE DIATOM CULTURE USED.

A culture of the diatom *Thalassiosira gravida* Cleve, isolated some years ago,* which has been kept since then by successive inoculations in fresh culture medium, has been used almost entirely for these experiments. This species is especially useful owing to the fact that in healthy cultures the cells hang together in long chains, whereas when the culture is unhealthy or becoming exhausted the chains break up. This is a most useful guide when watching the progress of an experiment.

The Purity of the Culture.—The culture contains no other diatom except *T. gravida* and no other organisms except bacteria. It would of course be preferable, if it were possible, to remove all the bacteria, so as to deal with a perfectly pure culture of the diatom. Many attempts have been made to attain this end, but so far without complete success, though it has been possible to carry the process of purification so far that only one species of bacterium capable of forming colonies on a peptone-agar plate† was at all abundant. The method adopted for purifying the culture was that of differential poisoning, a suitable poison being added to a number of culture flasks in a series of gradually diminishing strengths, in the hope that one strength might be found which would kill the bacteria without killing the diatom.

A measure of success was obtained with Copper sulphate in this way. In the most successful case a solution of the salt was added to 100 c.c. of culture medium containing *Thalassiosira gravida* in such proportion that

* Allen and Nelson, *loc. cit.*, p. 460. [*Q.J.M.S.*, p. 412.] The species was then thought to be a variety of *Thalassiosira decipiens.* Subsequent examination by Mr. Nelson has convinced him that it is really *Th. gravida.* The extreme delicacy of the siliceous skeleton of these diatoms makes the determination of species founded chiefly on valve structure very difficult. The species was formerly thought to be a variety of *Thalassiosira decipiens* Grun. since the only markings that were observed were characteristic of this species, although no markings at all could be resolved with the great majority of valves. Examination of the present cultures by Mr. Nelson with more perfect apparatus has shown the typical *Th. gravida* Cleve valve structure to which species this form is now referred. It is not unlikely that the older cultures were a mixture of *Th. decipiens* and *gravida* from which the *decipiens* have died out.

† It should be remembered that possibly the presence of some bacteria in the cultures is necessary for their success, though Miquel (*Le Diatomiste*, I, 1890-3, pp. 153–6) states definitely that he obtained cultures of fresh water diatoms which were entirely free from bacteria, and Richter (*Ber. deut. bot. Gesell*, XXI, 1903 and later papers) also succeeded in obtaining such bacteria-free cultures on solid culture media.

the 100 c.c. contained ·001 grams of $CuSO_4\ 5H_2O$. After an interval of twelve minutes a fresh flask containing 100 c.c. of culture medium was inoculated with 1 c.c. from the first one. In the second flask a very fine growth of diatoms appeared, which was much more healthy and vigorous than untreated cultures, and contained far fewer bacteria, as shown by peptone-agar plates.

Still better results were got, however, by a method which was first recommended to me by Mr. D. J. Matthews, who had made use of it for destroying bacteria in aquarium water. This consists in passing an electric current through the sea-water between carbon poles, until a considerable formation of hypochlorous acid has taken place and the water smells strongly of chlorine. The following description of an experiment will show how the method was applied in the case of the diatom cultures.

Experiment 449.—2½ litres of sea-water from the Laboratory tanks, which had been treated with animal charcoal and filtered through a Berkefeld filter, were put in a sterilized square glass jar, and an electric current varying from 1·7 to 1·5 ampères was passed through it for three minutes, two carbon plates* (sterilized by heating) being used as poles, the plates being constantly moved as the current was passing. The electrolysed water then smelt strongly of chlorine. It was allowed to stand for one hour, and then 50 c.c. of it was added to a flask (x), which contained 50 c.c. of unelectrolysed Berkefeld water,† to which had been added a quantity of *Thalassiosira gravida* from the culture which was to be cleansed.

Sixteen flasks (a–q) had previously been made ready, each containing about 75 c.c. of sterile culture medium (outside sea-water treated with Miquel's solutions and boiled). After the electrolysed water had been in contact with the *Thalassiosira* for thirty-one seconds about ½ c.c. from flask x was added to flask a, and similar amounts were added to the remaining flasks b, c, d, etc., at intervals of about ten seconds for the first two minutes, and then at longer intervals until the last flask q was inoculated after the *Thalassiosira* had been in contact with the electrolysed water for four minutes.

In this way a series of culture flasks was obtained inoculated with *Thalassiosira* which had been in contact with electrolysed water for varying times. The flasks were placed in a suitable position before a north window and the diatoms allowed to develop. At the end of a week the first flasks in the series (a, b, c, etc.) showed good growth, the later ones

* The size of each plate was 120 × 44 × 6 mm.

† See Allen and Nelson, *loc. cit.*, p. 432 [*Q.J.M.S.*, p. 375].

(*m–q*) showing little or none. At the end of three weeks the result was quite different, for whilst the early flasks showed only moderate growths and were already beginning to go off, a sure sign of contamination, two amongst the later ones (*m* and *o*) showed very fine growths of a rich brown colour and forming very long chains. The culture in flask *o* was one of the best and most vigorous that I have obtained during the whole course of my experiments, and sub-cultures from it remained excellent for many months.

The following table shows for the last few flasks of the series the times that the *Thalassiosira* remained in the electrolysed water, and the kind of growth that was obtained :—

Flask.	Time during which *Thalassiosira* was in electrolysed water.	Result culture.
l.	2 min. 28 secs.	Moderate culture, not persisting very long.
m.	2 min. 43 secs.	Very good culture, with long chains, second best of series.
n.	3 min. 0 secs.	No growth of *Thalassiosira*.
o.	3 min. 22 secs.	Very fine culture, best of series, dark brown colour and very long chains. Remained good for a long time and gave a long series of good sub-cultures.
p.	3 min. 40 secs.	No growth of *Thalassiosira*.
q.	4 min. 0 secs.	No growth of *Thalassiosira*.

(Flasks *a–k* all gave moderate growths which did not persist, with the exception of flask *h* (1 min. 44 secs.), which had no growth.)

Peptone-agar plates inoculated with 1 c.c. from flask *o* showed bacteria of two kinds only, a few large yellow colonies, and many minute, slow-growing colonies. They were of quite a different character from plates made from ordinary cultures of *Thalassiosira*, which were always crowded with yellow colonies, mixed with a large number of large milk-white colonies which liquefied the agar, both kinds of colonies developing very rapidly.

After some experience it becomes easy to distinguish a clean culture of *T. gravida* from one which is much contaminated by bacteria, by the character and progress of the growth. In a clean culture, at any rate during the summer months when the light conditions are favourable, the growth is much more rapid and vigorous, the tendency to form long

chains is very great, especially at first, the colour is a deep rich brown, and healthy growth in a flask will go on for months. In a contaminated culture, on the other hand, growth is slower and only quite short chains are seen, the colour is a much lighter brown, and the culture does not continue to grow in a healthy way, generally forming auxospores and often dying off altogether in the course of two or three weeks.

All the main conclusions detailed in this paper have been confirmed with clean and healthy cultures. Experiments with contaminated cultures are not, however, without value, since they sometimes emphasize the differences between culture media that it is desired to compare, a contaminated culture often failing to grow at all in an unfavourable medium, whereas a clean culture might give a growth, less in amount, it is true, but not much different in character from the growth in the control culture in a favourable medium.

THE ARTIFICIAL WATER.

The artificial sea-water used in the experiments was made by dissolving Kahlbaum's pure chemicals in ordinary distilled water made in a copper still which had been redistilled in all-glass apparatus after being treated with bichromate of potash and sulphuric acid, to destroy volatile organic matter. This double distilled water contained at most 0·01 mg. of ammonia per litre.*

The composition of the water was based on the analysis of sea-water published by Dittmar in the "Challenger" Reports.† The figures given by Dittmar are :—

	Per 100 parts halogen.
Cl	99·848
Br	·3402
SO_3	11·576
CO_2	·2742
CaO	3·026
MgO	11·212
K_2O	2·405
Na_2O	74·462

Dividing these figures by the respective molecular or atomic weights, and treating those for Cl and Br together as chlorine, we get after

* In connection with the preparation of the artificial sea-water I received constant help and advice from my colleague, Mr. D. J. Matthews. Without his ready assistance in connection with all chemical questions this investigation could hardly have been carried out.

† "Challenger" Report, Chemistry, Vol. I, p. 203.

reducing Na_2O to 100, the following figures, which give the relative number of molecules or atoms :—

Na_2O	100
K_2O	2·130
MgO	23·104
CaO	4·499
CO_2	0·519
SO_3	12·048
Cl	234·54

which gives the following molecular proportions for the bases and radicals separately :—

Na	100·0
K	2·13
Mg	11·55
Ca	2·25
CO_3	0·259
SO_4	6·024
Cl	117·27

If we use solutions of salts containing a gram molecular weight per litre, since 1 c.c. of each solution contains the same number of molecules, the relative number of c.cs., keeping the proportional amounts of the bases, the CO_3 and the SO_4 as above, and making the remainder chlorine, will be :—

NaCl	99·58
KCl	2·13
$CaCl_2$	2·25
$MgCl_2$	5·53
$MgSO_4$	6·02
Na_2CO_3	0·26

Since these figures give the number of molecules of *Na* somewhat too high, it was thought better to use 0·26 c.c. of sodium bicarbonate ($NaHCO_3$) instead of the normal carbonate, and this has been done throughout.

In making up artificial sea-waters it has been found most convenient to prepare first of all gram molecular solutions of each of the above salts and then to mix these in the proportions indicated. These molecular solutions are easily prepared and the strengths of the chlorides compared and corrected by titrating them with silver nitrate.

The following details of the preparation of the molecular solutions may be of assistance to future workers :—

Msol. NaCl. Kahlbaum's "Sodium chloride for Analysis." 58·5 grams dissolved in double-distilled water, and brought to 1000 c.c. at 15°C.

Msol. KCl. Kahlbaum's "Potassium chloride." 74·5 grams dissolved in double-distilled water and brought to 1000 c.c. at 15°C.

Msol. $CaCl_2$. Kahlbaum's "Calcium chloride cryst. for Analysis." About 300 grams were dissolved in about 1 litre of double distilled water. On titration with silver nitrate solution 2 c.c. of the above $CaCl_2$ solution required 30·3 c.c. of $AgNO_3$. 2 c.c. of Msol. KCl required 8·34 c.c. $AgN0_3$, so that 2 c.c. of Msol. $K_2C\,_2$ would require 16·68 c.c. $AgNO_3$. The $CaCl_2$ solution is therefore too strong in the proportion $\frac{30\cdot3}{16\cdot68}=1\cdot8166$. In order to get the Msol. $CaCl_2$ 1000 c.c. of the strong solution prepared must be diluted to 1816·6 c.c. This was done and the final solution again titrated against the Msol. KCl.

Msol. $MgCl_2$. Kahlbaum's "Magnesium Chloride for Analysis." As in the case of $CaCl_2$ a strong solution was first prepared, titrated with $AgNO_3$ and diluted with double-distilled water to the required extent, Msol. KCl being used as standard.

Msol. $MgSO_4$. Kahlbaum's "Magnesium Sulphate for Analysis." Crystallized magnesium sulphate has the formula $MgSO_4\ 7H_2O$, the molecular weight of which is 246·4. To make the molecular solution 246·4 grams of the salt were dissolved in double-distilled water and brought to 1000 c.c. at 15°C.

Msol. $NaHCO_3$. Kahlbaum's "Sodium Bicarbonate for Analysis." 84 grams dissolved in double-distilled water and brought to 1000 c.c.

In order to prevent the growth of moulds in the stock solutions these were all brought to the boil and kept in sterilized glass-stoppered bottles, the stoppers being tied down with a cap of parchment paper which was taken directly out of boiling water. When any of the solution was used,

the parchment cap was removed and placed in boiling water, the bottle was carefully opened and the amount of solution required poured out, the stopper being quickly replaced and tied down. These precautions are important, as the growth of mould in the solutions may have an important influence on the diatom cultures.

It has generally been found most convenient to make up the sodium chloride solution, of which large quantities are required, as it is wanted, and not to store it.

In the last table above the relative amounts (c.cs. of M solutions) of the different salts required to prepare the artificial sea-water are given. There remains to consider the actual salinity of the water which we are to employ, which is generally expressed as the weight in grams of the total salts contained in 1000 grams of the water. The salinity of natural sea-water in the western portion of the English Channel generally varies from about 35·5 to 35·0 per thousand, the water being generally lower in salinity near the coast. In laboratory experiments the water in the flasks becomes progressively more concentrated owing to evaporation, and a low salinity has therefore been adopted for the artificial sea-water used, namely, 35·0 per thousand.

The following table gives the composition of an artificial sea-water having a salinity of 35 per thousand, and with the salts in the relative proportions obtained above from Dittmar's analysis. The composition is stated (1) as the number of cubic centimetres of gram molecular solution contained in 1000 c.c. of the artificial water, and (2) as the number of grams of each salt contained in 1000 c.c.

	c.cs. of M. solution contained in 1 litre.	Grams per litre.
$NaCl$	480·80	28·13
KCl	10·28	0·77
$CaCl_2$	10·86	1·20
$MgCl_2$	26·70	2·55
$MgSO_4$	29·06	3·50
$NaHCO_3$	1·25*	0·11

To make up a litre of artificial sea-water the simplest procedure is therefore to weigh out 28·13 grams of sodium chloride, dissolve it in about half a litre of double-distilled water placed in a 1 litre flask, add the re-

* 2·6 c.c. was the amount generally used, as the increased alkalinity is favourable to diatom growth. See below.

quisite number of cubic centimetres of M solutions of the other salts (KCl 10·28, $CaCl_2$ 10·86, etc.) and then make the whole up to exactly 1 litre by adding double-distilled water.

Water prepared according to the figures given in this table was titrated for me by Mr. Matthews against the standard water supplied by the International Council, and was found to have a salinity of 35 per thousand.

Alkalinity.—The alkalinity has also been compared with that of sea-water from outside the Plymouth Breakwater by Sorensen's method, and was found to be very close to it, the artificial water being slightly less alkaline. It was found experimentally that better growths of diatoms were obtained when the alkalinity was increased somewhat,* the best result being obtained when an extra 1·33 c.c. of Msol. $NaHCO_3$ per litre was added, making a total of 2·6 c.c. of the molecular solution of this salt.

DIATOM CULTURES IN ARTIFICIAL SEA-WATER.

As was to be expected, it is not possible to obtain cultures of diatoms in the artificial sea-water prepared as described in the last section as it stands. The water must be first treated with nutritive solutions, and for this purpose the modifications of Miquel's solutions described in our former paper† have been used. Two solutions are employed as follows :—

Solution A.

Potassium nitrate 20·2 grm.	$=2M\ KNO_3$.
Distilled water to 100 c.c.	

Solution B.

Sodium phosphate ($Na_2HPO_4 12H_2O$)	4 grm.
Calcium chloride ($CaCl_2 6H_2O$)	4 ,,
Ferric chloride (melted)	2 c.c.
Hydrochloric acid (pure, concentrated)	2 c.c.
Distilled water	80 c.c.

To each 1000 c.c. of artificial water add 2 c.c. solution A and 1 c.c. solution B. Sterilize by bringing to the boil. When cool decant‡ off the clear

* Cf. Allen and Nelson, *loc. cit.*, p. 452 [*Q.J.M.S.*, p. 401]. The figure here given was derived from later experiments.

† Allen and Nelson, *loc. cit.*, p. 428 [*Q.J.M.S.*, p. 370]. For details as to the preparation of Solution B that paper or Miquel's original account should be studied.

‡ Filter papers should not be used to filter off the precipitate. They appear to contain some substance which inhibits the growth of the cultures. The cultures were made in wide-mouthed spherical glass flasks covered with glass capsules. Cotton wool plugs were not used, as these were found to be injurious to the growth of the diatoms.

liquid from the precipitate which is formed on the addition of solution B. The clear liquid is referred to in what follows under the name "artificial miqueled water."

It was found, however, that even after the addition of these two solutions very slight growth, if any, took place on inoculating with a small quantity (say one drop) of healthy *Thalassiosira gravida* culture. This was the case even after the addition of potassium bromide and iodide, or of Miquel's own solution A, which contains these two salts. If, on the other hand, the artificial miqueled water was inoculated with *a considerable quantity* of a culture of *Thalassiosira* in which natural sea-water had formed the basis of the culture medium, so that a considerable quantity of this natural water was transferred to the artificial medium, then the latter would develop a fine healthy growth. Experiences of this kind led me to suspect that the irregularities which had previously been met with in trying to make cultures in artificial media* might be due to varying amounts of natural sea-water introduced when inoculating. Definite experiments were therefore undertaken in order to ascertain whether the addition of natural sea-water to the artificial miqueled water would make it effective as a culture medium, and if so what proportion of natural sea-water was essential. *In all cases the culture medium was boiled after the addition of the natural sea-water and then allowed to cool before inoculation.*

As a result of these experiments it was shown that an addition of even 1 per cent of natural sea-water to the artificial miqueled water was sufficient to give very heavy cultures after inoculation with only one drop of *Thalassiosira* culture, and that with an addition of 4 per cent of natural sea-water better cultures were obtained than in any other culture medium known to me. This result has now been obtained so many times that it is in my opinion quite definitely established. If the proportion of natural sea-water added is reduced below 1 per cent smaller growths are obtained, and it is somewhat difficult to decide whether there is a definite minimum below which no growth takes place. An addition of 0·3 per cent of natural sea-water in one satisfactory experiment produced quite a heavy growth, whereas without this addition only a small growth was obtained. It has often been observed that whilst flasks containing 75 c.c. of artificial miqueled water show distinct signs of diatom growth when inoculated with one or two drops of a culture of *Thalassiosira* in natural sea-water, such flasks inoculated with one or two drops of a culture which has artificial sea-water as the basis of the culture medium show practi-

* Allen and Nelson, *loc. cit.*, p. 447 [*Q.J.M.S.*, p. 394].

cally no signs of growth at all, and remain quite clear. Since the above conclusions were reached it has been my practice in critical experiments always to inoculate from a culture in the artificial medium, so as to reduce the amount of natural water carried over on inoculation to a minimum.

From what has been said it seems clear that there is in natural sea-water some substance (or substances) not contained in the artificial water treated with Miquel's solutions, minute traces of which are essential to the growth of *Thalassiosira*. That the quantity present in the culture flasks after the addition of even 4 per cent of natural sea-water must be extremely minute is obvious from the fact that all substances which are present in natural sea-water in quantities beyond a mere trace are contained in the artificial culture medium. It becomes a matter of great interest and perhaps also of great importance to endeavour to find out what this substance may be, of which such exceedingly minute traces make all the difference between practically no growth at all and a vigorous and continued development of the diatoms, for the growths once started may go on increasing rapidly and healthily for several months.

The addition of many substances, both organic and inorganic, to the artificial miqueled water has been tried, generally in several concentrations, but up to the present no definite chemical compound has been found which can take the place of the 1 per cent of natural sea-water.

Of inorganic substances the following have been tried in different concentrations without result : Potassium bromide, potassium iodide (alone and with bromide), gold chloride, potassium nitrite, aluminium chloride, strontium chloride, lithium chloride and lithium carbonate.

It may be suggested that silica is the missing substance, but this seems precluded from the fact that all the experiments have been carried out in glass vessels, and the amount of silica which would go into solution from the glass would certainly be greater than that contained in the added 1 per cent of natural sea-water. Richter * has shown that diatoms grown in glass vessels obtain the silica they require from the glass.

In the course of the experiments it was found that the addition to the artificial miqueled water of a small percentage of sea-water from the tanks of the Plymouth Laboratory gave distinctly better cultures than the addition of the same percentage of natural sea-water brought in from outside. This comparison has been repeated a great many times, and the difference has been so marked and constant that I am compelled to regard it as firmly established. Different samples of sea-water brought

* Richter, O., *Verh. d. Gesell. deut. Naturf. u. Ärzte.*, Breslau, II, 1904, and *S.B.K. Akad. Wiss. Wien.*, CXV, 1906.

in from outside also appear to give somewhat different effects, and, although the experiments have not given sufficiently uniform results to justify a definite statement, I am left with the impression that on the whole samples of water taken from Plymouth Sound, when added to the artificial medium, give better growths than are obtained with samples from the English Channel in the neighbourhood of the Eddystone.

Now the tanks at the Plymouth Laboratory are worked on a closed system of circulation, the same water being circulated over and over again, so that the principal difference between the water taken from them and that obtained from outside consists in the greater abundance in the tank water of organic compounds, which result from the metabolism of living organisms. Is it the presence of some organic substance that is necessary for the growth of the diatoms ? A very large number of experiments have been made with a view to obtaining some light upon this question, and some of these will now be referred to.

Ulva infusion. A small piece of green seaweed (about 1 square cm. of *Ulva latissima*) was boiled for about five minutes in a flask containing 75 c.c. of artificial miqueled sea-water, and was then removed with a sterile platinum needle. In this way a weak organic infusion was obtained. When cold the flask was inoculated with one or two drops of *Thalassiosira* from a culture in artificial water. In this organic infusion a good growth was obtained, nearly equal to that in the control in artificial miquel plus 4 per cent of tank water. This experiment was repeated a number of times with a similar result.

Though it is most probable that the result is due to some organic compound the experiment is, of course, not conclusive, as an inorganic salt may have been dissolved from the ulva. In any circumstances we obtain no hint as to the nature of the organic substance, and the result remains indefinite.

It may be pointed out that Miquel * in his account of his original experiments on diatom cultures, insists upon the value of the addition of some organic infusion or maceration to his culture solutions.

Ulva Extract. A piece of *Ulva latissima* was washed in several changes of artificial sea-water and then an extract was made in absolute alcohol at a temperature of 58° C. The alcohol was evaporated to dryness on a water-bath. 75 c.c. of artificial miqueled sea-water was then boiled in small portions at a time in the vessel containing the extract, so that all soluble parts of the extract were dissolved. The water was then returned to a culture flask, which, when cold, was inoculated with *Thalas-*

* *Le Diatomiste*, I, 1890–3, p. 95.

siosira, as described in the experiment with ulva infusion. No growth was obtained in the flask.

Ulva Ash. A piece of ulva measuring about 5 cm. by 3 cm. was washed in several changes of double-distilled water. It was then put in a porcelain crucible, dried and heated over a bunsen burner till it was reduced to a white ash. The ash was added to a flask containing 75 c.c. of artificial miqueled sea-water, which was boiled, allowed to cool and inoculated with *Thalassiosira*, as in the two previous experiments. The result of the experiment was again negative.

Experiments with Hemimysis. In order to test whether the products of animal metabolism could immediately supply the substance sought for, the following experiment was carried out with *Hemimysis lamornæ* Couch, a small crustacean which lives in numbers in the Laboratory tanks. In the first experiment (Exp. 404) four *Hemimysis* were passed through two changes of Berkefeld filtered water, the animals being placed on a piece of filter paper to remove surplus fluid before being placed in each change of water. They were then passed in a similar way through two changes of artificial miqueled sea-water (75 c.c. was used altogether, being divided into two portions), and finally placed in a fresh quantity of the artificial miqueled sea-water (75 c.c.). They remained healthy and active and deposited a considerable amount of fæces on the bottom of the vessel. After they had been in the water four hours the *Hemimysis* were taken out and the water placed in a culture flask and brought to the boil. A control experiment with 75 c.c. artificial miqueled sea-water to which 3 c.c. of tank water had been added was set up and brought to the boil in the same way. On the following day both flasks were inoculated with two drops of a *Thalassiosira* culture. During the first week there was a very small growth of diatoms in the flask with the water in which the *Hemimysis* had been, which died out during the next few days. This growth was similar to that which usually occurs in artificial miqueled water to which nothing has been added. The control experiment to which 3 c.c. tank water had been added gave a very fine growth from the first, which persisted for at least five months. The result of this experiment was therefore negative. In another experiment, carried out in other respects in practically the same way, the *Hemimysis* were allowed to remain living in the water for twenty-four hours before they were removed. The result was again negative.

In a third experiment five *Hemimysis* lived for nineteen hours in 75 c.c. artificial miqueled sea-water to which 3 c.c. of tank water had been added. The animals were removed, the water boiled, and when cold inoculated as

before with *Thalassiosira*. A good growth resulted, showing that the animals do not excrete substances which completely inhibit the growth of the diatoms.

Evaporated Tank Water. A number of experiments were made in which a quantity of sea-water from the Laboratory tanks was evaporated to dryness on a water bath, the residue heated to different degrees, treated with strong, pure hydrochloric acid and evaporated two or three times to get rid of the acid, and then redissolved to the original volume in double-distilled water. After being neutralized by the addition of $NaHCO_3$, 4 per cent of the resulting solution was added to artificial miqueled sea-water, the resulting culture medium being boiled, cooled and inoculated with *Thalassiosira* in the usual way.

The results of these experiments are set out in summary form in the annexed Table A. In each case proper control experiments were set up at the same time, generally one with artificial miqueled sea-water to which nothing was added, and one with the same water to which 4 per cent of tank water was added, and the controls were boiled at the same time as the other flasks of the experiment.

As is seen from the table, five series of experiments were made. In the first (Series A) the salts obtained by evaporating the tank water were heated in a porcelain dish over a bunsen burner, the heating being carried out carefully so that the flame did not actually touch the dish, which never became anywhere near red hot. In Series B the evaporation and heating were done in a platinum basin, which was raised to a dull red heat over a bunsen. In Series C the salts were again evaporated and heated in a porcelain basin and made as hot as they could be with a bunsen burner, the flame of which played directly on the outside of the dish, and was moved about so as to heat different portions in turn. In Series D the salts were heated in a hot-air oven, being kept at a temperature of 164° to 170° C. for an hour. In Series E the heating was again carried out in a hot-air oven, a temperature of from 200° to 237° C. being maintained for two hours.

In Series A, D and E, where the heating of the residue was not excessive, quite good cultures resulted. Although they did not quite come up to the controls in which 4 per cent of tank water was added, they were in every case altogether of a different order from what took place in the controls in artificial miqueled sea-water to which nothing had been added.

In the other two series, B and C, where the degree of heating was much greater, in most cases the culture was an entire failure, and in those

TABLE A, showing the results of experiments, in which 4 per cent of tank water, which had been evaporated, heated, and redissolved, was added to artificial miqueled sea-water. The number of the experiment and date of inoculation are given in each case. The cultures were inoculated with *Thalassiosira gravida*.

Evaporated Tank Water. Date of Preparation and Degree of Heating.							
A. Prepared July 11th, 1912. Heated carefully over bunsen in porcelain basin. It never became red hot.	399. D. 13. VII. 12. Good growth not up to controls.			431. D. 18. XI. 12. Good growth equal to or better than control.	433. A. 8. I. 13. Good growth, not up to controls.	455. A. 22. VIII. 13. Good growth, not up to control.	460. A. 9. IX. 13. Good growth, nearly up to control.
B. Prepared Aug. 29th, 1912. Heated over bunsen in a platinum basin to a dull red heat.	407. L. 31. VIII. 12. No growth.	407. M. 31. VIII. 12. (More alkaline than L.) No growth.	408. E. 4. IX. 12. No growth.	431. E. 18. XI. 12. Small growth, but some good chains.	433. B. 8. I. 13. Good growth, not up to A.	455. B. 22. VIII. 13. Below the control in which nothing was added to the artificial miquel.	460. B. 9. IX. 13. Some growth at first. It then went off entirely.
C. Prepared Sept. 26th, 1912. Heated over bunsen in porcelain basin. Made as hot as possible with the flame playing directly on the outside of the dish.			424. G. 18. X. 12. No growth.	431. F. 18. XI. 12. No growth.	433. C. 8. I. 13. Very small growth.	455 C. 22. VIII. 13. Below control, in which nothing was added to the artificial miquel.	460. C. 9. XI. 13. At first the worst of the series, then a fair growth, but below A, D, and E.
D. Prepared Jan. 2nd, 1913. Heated in an oven to 164°–170° C. for 1 hour.					433. D. 8. I. 13. Moderate, healthy growth, better than B.	455 D. 22. VIII. 13. Good growth not up to A.	460. D. 9. IX. 13. Good growth, nearly as good as control.
E. Prepared Jan. 4th, 1913. Heated in an oven to 200°–237° C. for 2 hours.					433. E. 8. I. 13. Moderate, healthy growth, as D.	455. E. Good growth not up to A.	460. E. 9. IX. 13. Good growth, nearly as good as control.

instances in which some growth was obtained it was distinctly below that of cultures of the former series made at the same time.

A study of Table A can, I think, leave no doubt that the general statement is justified that whatever the substance may be which occurs in tank water and the addition of which to artificial miqueled sea-water enables the latter to support a vigorous diatom growth, that substance may be dried and heated to a moderate degree without greatly impairing its efficacy, whilst if it is heated to too high a temperature its efficacy tends to be destroyed.

The experiments are consistent with the theory that the substance is an organic compound, but one of a very stable kind, which is only decomposed with difficulty.

Addition of organic substances to artificial water. Many experiments have been made by adding organic substances in a number of different concentrations to artificial miqueled sea-water, but by none of these has any marked or constant effect been produced upon the growth of *Thalassiosira.* It will be understood, of course, that such negative results are in no way conclusive, as in a case of this kind the attainment of an exactly correct degree of concentration may be essential, and when one is working quite without clue it is hardly possible to carry out a sufficiently extensive series of experiments with every substance, especially when two or three weeks must elapse before the result of any experiment becomes definite. The following substances have been tried : asparagin, calcium succinate, calcium malate, sodium salicylate, theobronine, leucine, tyrosine * (the three latter alone and together with atropine),† peptone, urea and uric acid. In all cases the result was negative.

Putrified Peptone. An isolated result which I have entirely failed to repeat in spite of many attempts may be worth putting on record as a hint for future work, but no other importance should be attached to it. Starting from the idea that the substance sought for might be one of the ultimate products of the breaking down of organic matter under the influence of bacteria, since it appears to be more abundant in the tank water of the Laboratory than in sea-water from outside, the following

* In consequence of the work of Thornton and Geoffrey Smith on Euglena (*Proceed. Roy. Soc.*, B., Vol. LXXXVIII, p. 151, 1914) special attention was given to tyrosine, and a arge number of different concentrations were tried. Entirely negative results were, however, obtained.

† The use of these three substances alone and with atropine was suggested by the work of H. C. Ross on "Auxetics." See H. C. Ross, *Induced Cell-Reproduction and Cancer*, London, J. Murray, 1910 ; *Further Researches into Induced Cell-Reproduction and Cancer*, I and II, London, J. Murray, 1911 and 1912.

experiment was carried out. 100 c.c. of a 1 per cent solution of peptone in artificial sea-water was sterilized by boiling on successive days. When cold it was inoculated by adding two drops of tank water. Under the influence of the bacteria of the tank water putrefaction set in and was allowed to continue for nineteen days. The solution was then again boiled. To 75 c.c. of artificial miqueled sea-water three drops of the putrified peptone solution were added, and the flask boiled, and when cold inoculated with two drops from a culture of *Thalassiosira* in artificial miqueled water plus 4 per cent of outside sea-water. At first the water in the culture flask became milky from the growth of bacteria, but this milkiness gradually disappeared and the diatoms commenced to grow, giving finally an excellent culture which was quite up to the control. I do not think there was any flaw in the actual carrying out of the experiment, but, as already mentioned, a number of attempts to repeat it all gave negative results.

A final point may be mentioned, which also seems to suggest some organic substance as the missing factor which the artificial miqueled sea-water must contain before it will sustain a vigorous growth of the diatoms. It has been noticed that artificial miqueled sea-water which has been kept for some weeks gives (without any addition of natural sea-water) more growth than does similar water used within a few days of being prepared. Plate-culture tests have shown that such water after a few days develops bacteria, and it is possible that the products of the metabolism of these bacteria are able to help the growth of the diatom.

The Omission of Miquel's Solutions. If 4 per cent of tank water (i.e. water from the Laboratory tanks, which are worked on a close system of circulation *) be added to artificial sea-water, made according to the formula already given, but to which neither of the Miquel solutions is added, a good growth will result after sterilization and inoculation with *Thalassiosira*. This growth may for the first week or two be quite as good as a similar culture to which the Miquel solutions have been added, but it will not continue healthy for as long as the latter, so that the total growth will be less. It is interesting to note that the mere dilution of the tank water with pure artificial sea-water produces an increase of growth, for the amount of growth obtained in say 100 c.c. of sterilized tank water is less than that obtained in a mixture of 96 c.c. of artificial sea-water with 4 c.c. of sterilized tank water. This is partly explained by a difference in alkalinity, but it also suggests that the tank water contains not only an abundance of the food sub-

* Cf. Allen and Nelson, *loc. cit.*, p. 430, *et seq.* [*Q.J.M.S.*, p. 373].

stances which the diatoms require, but also substances which in higher concentrations are detrimental to growth, whereas in low concentrations their inhibitory action is reduced or disappears.

CHANGES IN THE COMPOSITION OF THE ARTIFICIAL SEA-WATER.

A series of experiments was made to ascertain to what extent the composition of the artificial sea-water could be changed without affecting the growth of *Thalassiosira*, and it was found that, provided 4 per cent of natural sea-water were added, the various constituents of the artificial water might be varied to a surprising extent without in any way retarding the growth. Only those results are included here which were quite marked and definite. Other variations in composition were tried, but an account of these is reserved until the experiments have been repeated and extended.

Varying the Amount of Magnesium Sulphate. A series of flasks was set up, the basis of the culture medium in each being artificial sea-water prepared according to the table on p. 424, the quantity of magnesium sulphate being varied. The full amount of alkali favourable to diatom growth was added (i.e. 2·6 c.c. of $M.NaHCO_3$ per litre), together with the usual quantities of 20 per cent KNO_3 and Miquel's solution B (Na_2HPO_4; $CaCl_2$; $FeCl_3$; HCl) and 4 per cent of natural sea-water. The series contained (*a*) no magnesium sulphate, (*b*) $\frac{1}{4}$ the normal amount, (*c*) $\frac{1}{2}$ the normal, (*d*) $\frac{3}{4}$ normal, (*e*) the normal amount, i.e. 29·06 c.c. of M.sol. per litre, (*f*) $1\frac{1}{4}$ times the normal and (*g*) $1\frac{1}{2}$ times the normal. All the flasks were inoculated in the same way with *Thalassiosira gravida.* During the first month all the flasks gave excellent growths, and it was not possible to distinguish between them. At the end of three months (*a*) and (*b*) had gone off more than the others, and (*f*) and (*g*) were not quite up to (*c*), (*d*) and (*e*). A repetition of (*a*) to (*e*) again gave the same result, the cultures being particularly large and healthy. In speaking of this result, it must be remembered that although the only sulphur present in (*a*) was that introduced in the 4 per cent of natural sea-water a considerable amount of magnesium was present as magnesium chloride.

Varying the Amount of Calcium Chloride. Another series of experiments was made in every respect similar to the last, excepting that the calcium chloride in the artificial water was varied instead of the magnesium sulphate, which remained normal: (*a*) contained no calcium chloride,

(*b*) ¼ normal amount, (*c*) ½ normal amount, (*d*) ¾ normal amount, (*e*) the normal amount, i.e. 10·86 c.c. M.sol. $CaCl_2$ per litre, (*f*) 1¼ times the normal amount, (*g*) 1½ times normal.

(*a*) During the first week showed little sign of growth and was far behind the others. At the end of a month, however, there was quite a good growth, still very healthy, but the quantity was far below that in (*c*), (*d*), (*e*), (*f*) and (*g*).

(*b*) Small growth during the first week and remained always better than (*a*), but never equal to (*c*), (*d*), etc.

(*c*) Fair growth during first week and went on well, though the quantity was never up to (*d*), (*e*), etc.

(*d*) The growth was nearly equal to the normal (*e*) throughout, and at the end of a month it was not possible to distinguish between the two.

(*e*) A fine healthy growth with long chains.

(*f*) About the same as (*d*) throughout.

(*g*) About the same as (*d*) and (*f*) throughout.

A repetition of (*a*) to (*e*) gave just the same result. In connection with this series it must be noted that Miquel's B solution contains $CaCl_2$, so that the amount of Ca present in (*a*) will be that contained in the 4 per cent of natural sea-water, plus that contained in the Miquel B.

Varying the Amount of Potassium Chloride. An exactly similar series was set up in which the potassium chloride was varied from 0 to 1½ times the normal. All these gave very fine growths, of which the last two (1¼ and 1½ times normal) were the best during the first week. Subsequently it was not possible to distinguish between the amounts in the different flasks. This result was also confirmed by a second experiment.

It should be remembered that potassium was added as nitrate in this as in the other experiments (2 c.c. of a 2 M.sol. KNO_3 per litre).

Variations in Salinity. It was shown in our previous paper * that in the case of *Skeletonema costatum*, *Biddulphia mobiliensis* and *Coscinodiscus excentricus*, plankton diatoms of very similar habit and distribution to the species *Thalassiosira gravida* chiefly used in the present experiments, the salinity of the culture medium could be varied within wide limits without greatly affecting the growth of the diatoms. Thus between 35 and 40 per cent of the water could be evaporated from a culture medium having natural sea-water as its basis without seriously affecting the growth of the diatoms, whilst dilution of the culture medium up to 100 per cent

* Allen and Nelson, *loc. cit.*, p. 453 [*Q.J.M.S.*, p. 402].

also made no appreciable difference. Even when the dilution was extended to 200 per cent a fair quantity of growth took place.

The following experiment was made in order to test the same point on *Thalassiosira gravida*.

Experiment 476. Artificial sea-water was made up with the normal relative proportions of salts, but of double the normal strength. A series of dilutions was then prepared, doubly distilled water being added in the proportions stated :

	Artificial sea-water, double strenth.		Doubly distilled water added.	
	c.c.		c.c.	
A ..	100	+	0	
B ..	100	+	25	
C ..	100	+	50	
D ..	100	+	75	
E ..	100	+	100	*Normal*
F ..	100	+	125	
G ..	100	+	150	
H ..	100	+	175	
J ..	100	+	200	

The right quantities of Miquel's solutions were added to each, and 4 per cent of sea-water from the Laboratory tanks. Flasks were then inoculated with three drops each of *Thalassiosira gravida* culture. No growth took place in A and B. Excellent, healthy growths with good chain formation took place in all the others. E and F were best, and one as good as the other. G and D were excellent growths, but the quantity at any time was less than in E and F. In C, H and J, although the growths were quite good the quantity was considerably less than in E and F, that in C also being less than in H and J.

It will thus be seen that very considerable changes in the salinity of the culture medium can be made without much effect being produced on the growth of *Thalassiosira*. Dilution of the medium is less detrimental than concentration.

The experiments described in this section show how wide the variation in the chemical composition of the culture medium may be without any very marked effect being produced on the growth of the diatoms. The difficulty in growing the diatoms in artificial sea-water is clearly not due, as at one time I thought might be the case, to the fact that a very delicate balance between the amounts of the different salts is

necessary and that this balance had not been attained sufficiently exactly in preparing the solutions. It is quite clear that the artificial sea-water lacks some substance which occurs in natural sea-water, and that a very small trace of this substance is sufficient to make the difference between a considerable and continued growth of the diatoms and practically no growth at all.

GENERAL CONSIDERATIONS.

Several instances have recently been described which seem to show that in food material used to support animal life the presence of minute traces of particular organic substances is essential, if the food material is to maintain the animal body in a healthy state.

The work of Leonard Hill, M. Flack, G. Hopkins and Casimir Funk * has shown that in the outer layers of wheat and rice there is an active principle which is of essential importance to their value as food material. Young rats and mice would not live when fed exclusively upon white flour in the preparation of which the outer layers of the wheat had been removed, whilst those fed on whole meal flour did much better. Pigeons could be successfully fed on bread made of white flour to which an extract of bran and sharps had been added, but when fed on pure white bread all died. Polished rice from which the husk has been removed in the process of polishing, when used as an exclusive diet, produces the disease known as beri-beri. Cooper and Casimir Funk † were able to isolate from rice polishings a substance to which they gave the name vitamine, which effected a rapid cure when given to pigeons suffering from beri-beri. The same substance was obtained from yeast, from milk and from bran.

Hopkins ‡ has shown that young rats do not grow on an artificial diet composed of pure protein, starch, cane sugar, lard and inorganic salts, but if quite a small quantity of natural milk is added to the diet they thrive.

Thornton and Geoffrey Smith § have shown that strong growths of *Euglena viridis* in culture media prepared according to Miquel's formula are produced when in place of the organic matter used by Miquel slight traces of amido acids are added to the solutions of inorganic salts. Tyrosin in the proportion of 1 in 24,000 gave an optimal growth. The authors

* A summary of this work, as described at the meeting of the British Association in Dundee (1912), will be found in *Science Progress*, January, 1913, pp. 423–5.

† *The Lancet*, Nov. 4th, 1911, p. 1266.

‡ *Journal of Physiology*, Vol. XLIV, 1912, p. 425.

§ *Proceed. Roy. Soc.*, B., Vol. LXXXVIII, p. 151, 1914.

suggest that the amido acid acts as an auxiliary or stimulant rather than as the main source of nutrition. This view is similar to that taken by H. C. Ross in his work on *Induced Cell-Reproduction and Cancer*, to which reference has already been made (see p. 432).

It would seem that the plankton diatoms, the culture of which has been considered in the present paper, show a phenomenon of a similar character to those just mentioned. The minute trace of substance added to the culture medium in the small percentage of natural sea-water would seem to act as a catalytic agent, initiating the processes of metabolism but not being itself used up.

The experiments may also help to throw light upon what takes place in the sea. It is well known that the waters of the open ocean far from land support a much smaller proportion of plant and animal life than is to be found in coastal waters. On the other hand, in regions where a current of coastal water meets and becomes mixed with a current of ocean water conditions are produced which are specially favourable to a luxuriant growth of animal and vegetable life. This is shown in the first place in the very rich character of the plankton, and as a consequence of the abundant plankton we find a rich fauna of bottom living organisms and of fishes of different kinds. This is in agreement with the observation recorded in the present paper that a small quantity of natural sea-water of an inshore type (tank-water) mixed with a large proportion of pure artificial sea-water gives a good culture medium for the plankton diatoms. There is reason to hope therefore that culture experiments may in time throw additional light upon the general questions relating to the production of animal life in the sea, questions which are of immediate importance to a study of the productivity of the fisheries.

SUMMARY.

1. Attempts to obtain good cultures of *Thalassiosira gravida* in a purely artificial medium, made by dissolving in doubly distilled water Kahlbaum's pure chemicals in the proportions in which the salts occur in sea-water, adding nitrates, phosphates and iron according to Miquel's method and sterilizing the medium, have not succeeded.
2. If, however, a small percentage of natural sea-water (less than 1 per cent will produce a result) be added to the artificial medium and the whole sterilized excellent cultures are obtained, which are often better than any which have been got when natural sea-water forms the foundation of the culture medium.

3. The result appears to be due to some specific substance present in minute quantity in the natural sea-water which is essential to the vigorous growth of the diatoms. The nature of this substance it has not been possible to determine, but some evidence seems to suggest that it is a somewhat stable organic compound.
4. Provided the 1 per cent of natural sea-water is added, the various constituents of the artificial sea-water forming the basis of the culture medium can be varied in amount within wide limits. The salinity of the medium can also be considerably altered without serious detriment to the cultures.
5. The experiments recorded are of interest as furnishing another instance of the importance in food substances of minute traces of particular chemical compounds. They may also eventually throw light upon the nature of the conditions in the sea which are specially favourable to the production of plant life and therefore also of the animal life which that plant life sustains.

ADDENDUM.

Since the above was printed a paper has been published by Prof. W. B. Bottomley on " Some Accessory Factors in Plant Growth and Nutrition " (*Proceed. Roy. Soc.*, B., Vol. LXXXVIII, p. 237, Sept., 1914), in which it is shown that a minute trace of an organic substance, which is formed by the action of aërobic soil bacteria upon peat, acts as a powerful stimulant to the growth of plants and of nitrogen-fixing bacteria. Following the method of Cooper and Funk for obtaining " vitamines " from rice polishings, namely, by precipitating by phosphotungstic acid from an aqueous solution of the dry residue from an alcoholic extract, Bottomley has succeeded in obtaining from the bacterized peat a substance which is quite as powerful a stimulant to plant growth as the original alcoholic extract of the bacterized peat. This substance, as in the case of Funk's vitamines, can be further purified by precipitation with silver nitrate and baryta, the resulting substance being an effective growth stimulant.

A Study of the Restitution Masses formed by the Dissociated Cells of the Hydroids *Antennularia ramosa* and *A. antennina.*

By

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INTRODUCTION.

THE work described in this paper is, in the main, a repetition of that of H. V. Wilson, "On the Behaviour of the Dissociated Cells in Hydroids, Alcyonaria, and Asterias," published in October, 1911, in the *Journal of Experimental Zoology*. The results he obtained are so far-reaching in their bearing on the present theories of the organization of living matter that his work appeared well worth repeating on species closely allied to those on which he experimented. Our results largely bear out his contentions, though we were not successful in carrying the regenerative process as far as the production of new hydranths, and the histological structure of the restitution masses we obtained differed in many ways from that described in Wilson's paper. These differences are probably due to the fact that we experimented with other species of Hydroids to those used by Wilson: in other respects we have followed his technique and repeated his experiments, and up to a certain point have obtained the same results, and thus it may be considered that we have verified his very remarkable work.

The especial interest of our investigations lies in the rather anomalous fact that we have not been successful in obtaining regeneration of the complete organism from the dissociated cells. In our experiments the restitution masses, by some rearrangement or metaplastic process taking place among their conglomerated cells, formed tissue aggregates histologically reduplicating the structure of the parent organism, but in a quite irregular and apparently meaningless manner. The masses consisted of irregular convoluted tubules lined with endoderm cells, imbedded in closely packed but irregularly arranged ectoderm cells, among which many isolated endoderm cells were distinguishable, and the whole tissue aggregate was surrounded by a transparent perisarc which it had secreted. Slight contractions and alteration in shape in the cell masses often took place even after several weeks, and many of them remained alive and showed no signs of degeneration for at least fifty days, which was very much longer than the Hydroids themselves could be kept alive under similar conditions.

Before proceeding further it will be as well to briefly summarize the results obtained by H. V. Wilson. The Hydroids on which he experimented were *Eudendrium carneum* Clarke, and *Pennaria tiarella* McCrady. Proceeding as described in his paper, he squeezed pieces of the Hydroids through bolting silk of 50 and 75 meshes to the inch and then allowed the dissociated cells to form aggregate masses. The following is an abstract of some of his experiments.

Eudendrium carneum.

Experiment, July 9. A colony was squeezed, and fusion was observed under the microscope. In a few hours irregular, lobed, flattened masses about 5 mm. wide and 1 mm. thick were formed. By the next day, a perisarc surrounding the whole mass had been secreted. In 4 days outgrowths had formed in which ectoderm and endoderm could be distinguished. Some of the masses died, but others remained alive. These were isolated, and in 24 hours, one projecting outgrowth ended in a hydranth, and a day later two completely formed hydranths were developed from another mass. These hydranths have the characteristic size, shape, and colour of the normal adult polyp.

Experiment, July 14. The tissue died before mass formation.

Experiment, July 15. Flattened plasmodial masses and lumps were formed, but soon died.

Experiment, July 18. Tissue died.

Experiment, July 19. Tissue formed, but died next day.

Experiment, July 22. Tissue died next day.

Experiment, July 23. Tissue died next day.

Experiment, July 25. Small masses of tissues were formed, and secreted perisarc. They were alive 4 days after formation.

Experiment, July 27. Most of the large pieces of tissue died, but small lumps were alive 4 days later, and cœnosarcal outgrowths had sprouted.

Experiment, August 1. Small masses a fraction of a millimetre lived, showed perisarc and were alive 2 days later.

Experiment, August 2. (*a*) Most of the tissue formed was alive on August 3rd; much died by 7th. Outgrowths were formed, with vertical branches by 11th, but were sickly.

Experiment, August 2. (*b*) Many small spheroidal masses formed, and developed perisarc, but not cœnosarcal outgrowths. They were alive 5 days later.

Pennaria tiarella.

Experiment, July 26. Cell fusion and aggregation commenced at once. Small masses formed in an hour, and fused into tissue. In about 4 hours masses 1 mm. in diameter have formed. Next day perisarc formed, and in 3 days outgrowths were developed, but at the same time many of the larger masses died. In 5 days hydranths appeared on the outgrowths with characteristic tentacles.

Experiment, August 3. In this experiment only stem material was used. Fusion was rapid, and in about an hour a cake was formed. Next day perisarc appeared, and outgrowths commenced. Another mass from this culture in two days developed a hydranth. In 5 days all masses of this experiment except 4 were dead; the survivors developed outgrowths and were then preserved.

Many other valuable observations are included in Wilson's paper, but these experiments are the only ones with which we are immediately concerned. The paper also contains a full account of the literature on the subject of the behaviour and the regenerative properties of dissociated somatic cells of various species of animals, and accordingly a review of this literature will not be repeated here.

It is noteworthy that in Wilson's experiments the restitution masses which did not develop so far as to produce hydranths in every case died within a few days, while in our experiments, though none of the restitution masses produced hydranths, yet many of them remained alive for at least 60 days. Wilson does not state how long the masses which gave rise to hydranths in his experiments remained alive.

METHODS AND TECHNIQUE.

The species used by Wilson at Beaufort N.C., U.S.A., were not available at Plymouth. The species on which most of our experiments were carried out were *Antennularia ramosa* and *Antennularia antennina*. Species of Tubularia, Plumularia, and Clava were also tried, but did not give satisfactory results; though many of these produced restitution masses from their dissociated cells, yet these masses did not remain alive for more than a few days, and accordingly *Antennularia ramosa* or *A. antennina* were used in all our later experiments.

All material was obtained from Plymouth Sound, inside the Breakwater.

The method of obtaining the isolated cells was the same as that employed by Wilson. Squares of bolting silk of 50, 75, and 180 meshes to the inch were thoroughly washed and finally rinsed out in boiling water. A good sized colony of Antennularia was then cut up into small pieces about a quarter of an inch long and these pieces were laid in a heap in the middle of a square of bolting silk, which was then folded over so as to make a small bag containing the fragments of the Hydroid. This bag was then squeezed with a pair of wooden forceps into a watch-glass containing a little sea-water. With a quite moderate degree of pressure the body cells of the Hydroid are forced out of the cut ends of their protecting tubes of perisarc and then through the meshes of the bolting silk, and by this process become separated into isolated cells or small cell aggregates which collect as an even layer at the bottom of the watch-glass. It is necessary that a sufficient amount of material should be used to form a complete layer of isolated cells at the bottom of the watch-glass about 1 mm. thick, if the formation of restitution masses that will show any degree of subsequent development is required.

The watch-glasses containing the isolated cells were slightly shaken and rotated so as to bring the cells together as much as possible, and then when they had aggregated to some little degree the watch-glass was immersed in a finger-bowl of sea-water. It was found advisable to place

the finger-bowls in troughs of running water in order to keep them cool and at a more or less constant temperature; before this was done a very large mortality among the restitution masses occurred even under the most favourable conditions, and it would seem that the temperature of the laboratory, which is heated by hot water, was too high for these unless some artificial method of cooling was employed.

Two kinds of water were used in the experiments: (1) that brought from outside the Plymouth Breakwater, and (2) water circulating in the Laboratory tanks, treated with animal charcoal and passed through a Berkefeld filter as described by Allen and Nelson (see *Journal of Marine Biological Association*, Vol. VIII, p. 432). It proved, however, immaterial which kind of water was used. For the first day or two the water of the cultures was changed frequently with a view to keep down the infusoria and flagellates as much as possible; but it is impossible to banish them altogether, and as soon as a perisarc was well established round the masses they were immune to attacks of protozoa. After this time the culture water was not changed oftener than once a week. Possibly the flagellates developed more quickly in the Berkefeld than in the outside water.

It was found advisable to utilize the colonies of Antennularia immediately after they were brought in, as by this means more vigorous restitution masses were obtained. If the colonies were kept over night in the ordinary tank water, in the filtered Berkefeld water, in water collected from outside the Breakwater, satisfactory results were not obtained. In one experiment, however, excellent results were obtained from a colony of *A. antennina*, which had lain for some weeks in a laboratory tank. The comparative greater longevity and vitality under Laboratory conditions of the restitution masses compared to the original colonies is very curious and difficult to explain.

The changes in shape and other general external developments of the restitution masses were noted by frequently drawing under a camera lucida: for this purpose the watch-glasses containing the cultures were simply removed from the finger-bowls and placed on the stage of the microscope; after drawing they were returned to the finger-bowls without disturbing the cultures.

When required for histological examination, the restitution masses were fixed in Flemming's fluid (strong formula). Ten minutes fixation was found to be long enough for a moderate sized mass, say about the size of a grain of barley; if fixed for longer periods the cells showed a tendency to become "osmicated" and stained badly. After fixing they

were washed for a few minutes in water, passed quickly up through the alcohols to 70%, and then washed for some hours in 70% alcohol, containing a little hydrogen peroxide. After dehydration they were embedded in paraffin and cut into sections 5μ thick. Heidenheim's Iron Alum Hæmatoxylin, followed by Lichtgrün F.S. in 70% alcohol proved a satisfactory stain for general purposes.

Small restitution masses which were difficult to handle were sectionized after previously mounting on a piece of amyloid liver, the mass being made to adhere to the surface of the liver by means of a little albumen, which was subsequently coagulated by alcohol.

THE NORMAL TISSUES WHICH, AFTER DISSOCIATION, GIVE RISE TO THE RESTITUTION MASSES.

Antennularia ramosa is one of the Plumulariidæ. It consists of shoots which, springing from a single trunk at a certain height, divide and subdivide : the stems are thick and their branchlets are long and tapering, having their internodes of equal length. The branchlets are closely set and arranged in whorls where they come off the parent stem. The Hydrothecæ are small and campanulate in shape. Nematocysts are present. The Gonothecæ are pear-shaped and single ; and have a subterminal aperture facing towards the stem. In healthy specimens the perisarc is transparent and colourless, and the coenosarc is of a light yellowish green tinge.

Antennularia antennina consists of clustered stems, simple or slightly branched, springing from a sponge-like mass of interlacing fibres. The branchlets are short, swollen at the base, arranged in a whorl on each articulation of the stem. They are divided by oblique joints into internodes, which are alternately larger and smaller, the former bearing the hydrothecæ. The hydrothecæ are small and campanulate in shape. The Gonothecæ are produced singly in the axils of the branchlets ; they are oval, with a subterminal aperture looking towards the main stem. The perisarc is transparent and colourless, and the cœnosarc of a somewhat brighter yellow colour than in the case of *Antennularia ramosa*.

The cœnosarc of both species is hollow, and consists of a tube of cellular tissue in the walls of which a number of smaller tubes run in the direction of the long axis of the stem. These smaller tubes are the direct continuations of the enteric cavities of the individual hydranths, and are lined with cells of a type similar to those forming the hydranths. The whole arrangement is suggestive of that in a young dicotyledonous

plant having a hollow stem, the enteric cavities lined with the endoderm cells of the individual hydranths corresponding to the vascular bundles of the plant.

A view of a cross section through a stem of *Antennularia ramosa* is shown in Fig. 1. Externally it is limited by the structureless perisarc,

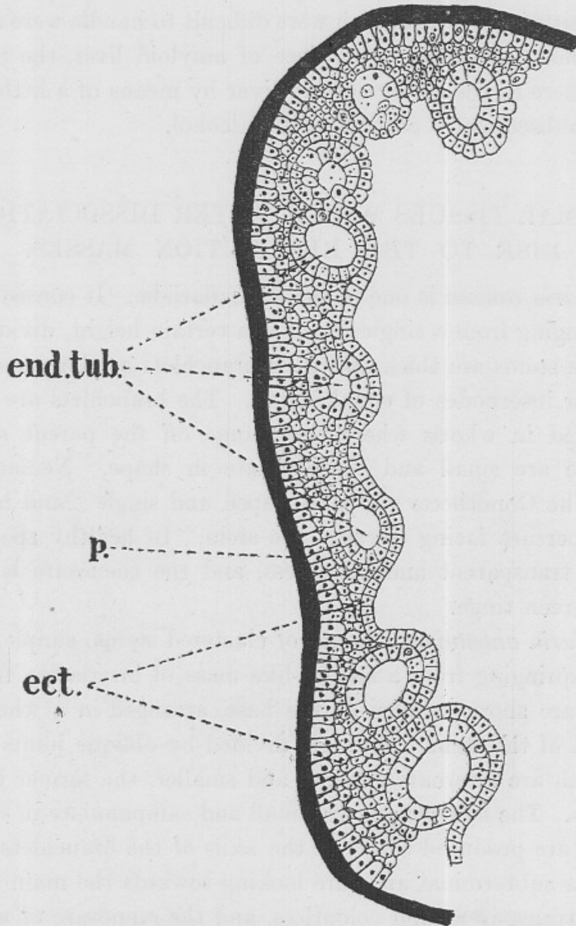

FIG. 1. ×175.—Section of portion of normal cœnosarc, showing cœnosarcal continuations of tubules continous with the enteron of individual polyps. *End. tub.*, endoderm tubules; *p.*, perisarc; *ect.*, ectoderm cells.

within this is a somewhat indefinitely arranged mass of slightly elongated cells with small but sharply staining nuclei; of these the cells in immediate contact with the perisarc are larger than the others, which appear to be tightly packed together. At regular intervals within this

cell mass tubules lined with large columnar endoderm cells can be seen, and these tubules are covered on the side where they project somewhat into the hollow cavity of the stem with a single layer of small cubical cells, which form a complete inner lining to the hollow stem. In longitudinal sections the tubules can be traced up into the individual polyps, and it can readily be seen that their cells are directly continuous with the endoderm cells lining the enteric cavity of the polyps. Similarly the smaller cells in which these tubules are embedded in the cœnosarc can be seen to be directly continuous with the ectoderm cells of the polyps. Neither in sections of the cœnosarc nor of the polyps were we able to distinguish any structure or structureless layer corresponding to the mesoglæa.

In Fig. 2 a tubule with surrounding ectoderm cells is shown under a higher power of magnification. It will be noticed that the endoderm

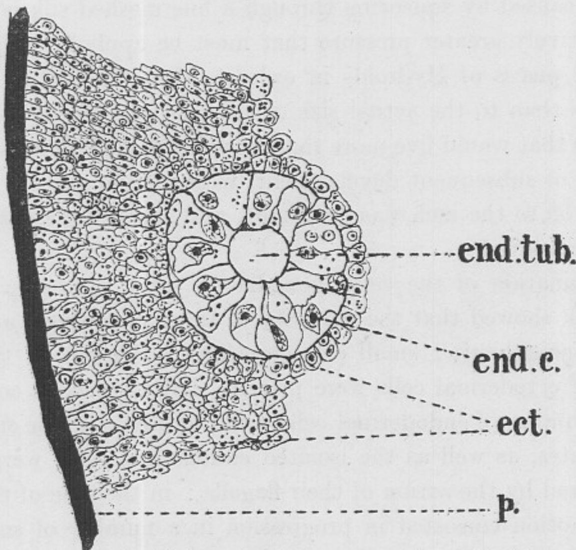

FIG. 2. ×500.—A normal tubule under higher power of magnification. *Ect.*, ectoderm cells; *end. c.*, endoderm cells; *end. tub.*, endoderm tubules; *p.*, perisarc.

cells are distinctly columnar, with broad bases, and that they are considerably larger than the ectoderm cells. Their nuclei are relatively large, and usually situated near the base of the cell: the nuclear membrane is sharp and well defined and the nucleoli are remarkably distinct; strands of chromatin are present, radiating from the nucleolus towards the nuclear membrane. The cytoplasm is distinctly granular, and frequently darkly staining vacuoles, presumably food vacuoles, were seen. Flagella do not appear to be present on these cœnosarcal endoderm cells.

The smaller ectoderm cells are slightly elongated, often with pointed ends, with the exception of those forming the layer lining the hollow of the cœnosarc, which are almost cubical in shape. The nuclei are small, and the nucleoli relatively large and distinct.

Other structures such as the germ cells, nematocysts, etc., are not described here as they appear to merely play the part of foreign bodies in the restitution masses, and do not enter into their development.

THE PROCESS OF FORMATION OF RESTITUTION MASSES.

The cells that are obtained after squeezing through bolting silk of 50 meshes to the inch are, many of them, comparatively little damaged; but if a finer silk is employed, such as that having 180 meshes to the inch, the majority of the cells are crushed and broken. It would appear that the injury caused by squeezing through a fine meshed silk is due rather to the relatively greater pressure that must be applied to the bag containing the pieces of Hydroids in order to drive the cells through the fine meshes than to the actual size of the meshes themselves. Restitution masses that would live more than a few days, and which would show any degree of subsequent development, were not obtained when a finer mesh than 50 to the inch was employed, and accordingly this was most generally employed.

An examination of the cells immediately after squeezing through a 50-mesh silk showed that the majority of them were single and isolated from their neighbours; small cell aggregates consisting at the most of six or eight ectodermal cells were present, and aggregates consisting of a smaller number of endodermal cells could be seen. These endodermal-cell aggregates, as well as the isolated endodermal cells, were in active motion caused by the action of their flagella: in the case of the isolated cells this motion consisted in progression in a number of small spirals due to the fact that the flagella are only attached to one side of the cell.

In addition to the comparatively uninjured cells and cell aggregates, a good deal of granular debris was present, and minute rounded bodies which were presumably small protoplasmic masses produced by the disintegration of cells which had been actually crushed in the squeezing process. Many nematocysts, some with their threads ejected, could also be seen, and ova were often present. In some cases small pieces of the tentacles accidentally were forced through the meshes of the silk intact; but these were usually visible to the naked eye, or under a low power of magnification, and when seen were removed with fine-pointed forceps.

If such pieces of tentacle were not removed, and became included in the restitution masses, it was noticed that they soon degenerate and

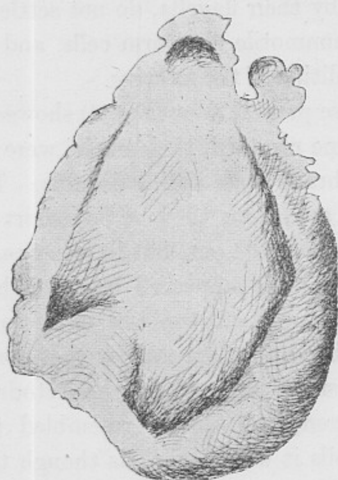

FIG. 3. ×16.—A restitution mass 48 hours old, showing curling up of edges.

never show any sign of regeneration, and in this our observations agree with Wilson's.

Preparations of the freshly squeezed cells were made by fixing on a

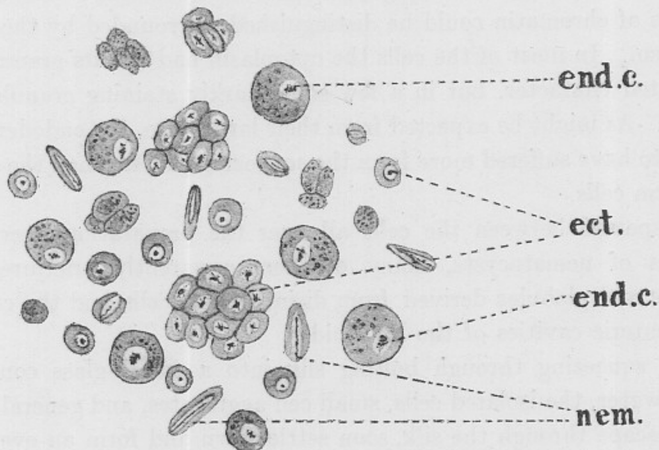

FIG. 4. ×500.—Isolated cells, after squeezing through bolting silk of 50 mesh. *Ect.*, ectoderm cells; *end. c.*, endoderm cells; *nem.*, nematocyst.

slide with Acetic Sublimate Solution, and subsequent staining with Heidenheim's Iron Hæmatoxylin, followed by Lichtgrün F.S. (Fig. 4). In such preparations both the ectodermal and endodermal cells were easily recognizable, though the proportion of endodermal cells was

comparatively small compared to the number seen in unfixed preparations. This was probably due to the fact that the endodermal cells, being kept in motion by their flagella, do not settle down on the surface of the glass like the nonmotile ectoderm cells, and consequently do not adhere to it on the addition of the fixative.

Examination of these preparations (Fig. 4) showed that the ectodermal cells had mostly become rounded, their nuclei were somewhat indistinct, and no nuclear membrane was distinguishable. The nucleoli in some cases stained darkly, and from them a few short radiating strands of chromatin could often be made out, but in other cases the whole nuclear structure stained faintly and appeared as a roughly spherical mass of indeterminate structure. The cytoplasm of these cells was clear, the periphery often staining somewhat darker than the more central part. Where occurring in small aggregates, the ectodermal cells appeared comparatively uninjured, and closely resembled the normal. In the case of the isolated cells it would seem as though the pressure to which they had been exposed had burst the nuclear membrane and caused a fusion of the nucleoplasm and cytoplasm.

The larger endodermal cells in these fixed preparations had lost their columnar shape and become rounded, and their flagella were not seen. The nuclei were indistinct and appeared as a light area in which a few granules of chromatin could be distinguished, surrounded by the darker cytoplasm. In most of the cells the cytoplasm had lost its granular and vacuolated character, but in a few some darkly staining granules were present. As might be expected from their larger size, the endoderm cells appear to have suffered more from the squeezing process than the smaller endoderm cells.

Interspersed between the cells all over the preparations were large numbers of nematocysts, many of them apparently uninjured, and granules and globules derived from disintegrated cells and the contents of the enteric cavities of the Hydroids.

After squeezing through bolting silk into a watch-glass containing a little water, the isolated cells, small cell aggregates, and general debris, which escape through the silk, soon settle down and form an even layer of a greyish yellow colour over the bottom of the watch-glass. Within two or three hours this layer shows a tendency to subdivide into a number of small nodules, and after the lapse of another hour these nodules are usually distinct elevated aggregations, often connected with one another by fine strands which gradually become thinner and contract until they are absorbed into the nodules from which they radiated. If left undis-

turbed, there seems to be no tendency for these nodules to change their position, but if they are disturbed by shaking or rotating the watch-glass so that they are brought into contact with one another they mutually adhere, and in the course of some hours may give rise to one or more large restitution masses in which no trace of the smaller nodules originally formed can be distinguished.

Similarly if the watch-glass, immediately after the cells have been squeezed into it, be rotated so that all the cells form a compact heap in the centre, the restitution masses may be formed as one or more thick flat cakes with rounded edges without the preliminary formation of the smaller nodules described above.

THE MORPHOLOGY AND DURATION OF LIFE OF THE RESTITUTION MASSES.

The after history of a restitution mass depends very much on its original size when first formed, and this again depends on whether the dissociated cells were shaken together or allowed to form the small nodular masses already described.

When one of these larger masses of tissue is first formed it consists

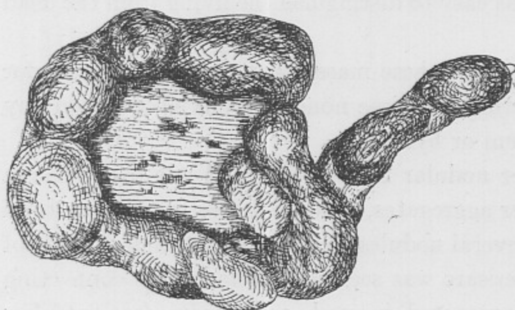

Fig. 5. ×16.—A restitution mass 8 days old, showing curling up and nodulation of edges.

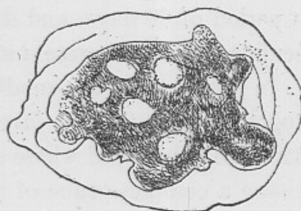

Fig. 6. ×16.—A restitution mass 34 days old, showing well-marked shrinkage away from the perisarc and fenestrated appearance of the cell mass.

of a flat cake of tissue of irregular shape, sometimes adherent to the glass. During the first 12 hours after its formation, a considerable amount of alteration in shape occurs, the edges of the mass turn up away from the glass, and a good deal of retraction takes place and consequently the tissue becomes stronger and more compact. After from 12 to 18 hours a delicate, transparent, colourless membrane is secreted round the mass, completely enclosing it and forming a tough protecting layer : this layer

is evidently similar to the perisarc of the normal animal. Once this perisarc has been secreted, the restitution mass appears to be immune to the attacks of flagellates or bacteria, which are unable to penetrate it. From this stage onwards the external changes that occur take place slowly. The turned-up edges become thicker and more nodulated at the expense of the central part, and sometimes may project in the form of spherical or ovoid nodules connected at the base with the main mass by a comparatively small isthmus of tissue. (In Fig. 3 a mass, 48 hours old, is shown, and in Fig. 5 one of 8 days.) There is later a slow but continuous shrinkage of the restitution mass away from its perisarc, leaving a clear space between the two (Fig. 6), after from three to four weeks irregularly circular spaces begin to show in the cell mass, which then presents a somewhat sponge-like fenestrated appearance (Fig. 6). Later changes are extremely slow, and consist of a further slight shrinkage, and increase in size of the spaces in the tissue. During all this time the restitution mass retains its yellowish colour and definite outlines, and sections show that the cells are healthy and undegenerated : in the case of masses that die, the yellowish colour is rapidly lost, and they appear as dirty white, soft, floccular bodies, which soon fall a prey to bacteria and other parasites ; it is thus easy to distinguish the living from the dead masses by the eye.

At the time of writing some of these masses have been kept alive for a period of 60 days, and during this time none of them have shown any tendency to regenerate a stem or hydranth.

In the case of the smaller nodular masses that had not been shaken together so as to form larger aggregates, a perisarc was secreted in from 12 to 18 hours, and where several nodules were joined by their strands of tissue a complete tube of perisarc was secreted around these connecting strands. The appearance presented in such cases was often peculiar and somewhat suggested an attempt at the formation of hydranths which had aborted through not being able to burst the surrounding perisarc ; but observations made from the earliest stages, when the nodules arose from simple aggregations of the cells show that there is no justification for such a view, and this was borne out by the internal structure of these nodules as shown in sections of fixed preparations. When the process of contraction of the restitution masses had proceeded a little further, the connecting strands of tissue between individual nodules were often completely retracted, thus leaving the nodules merely connected by empty tubes of transparent perisarc.

THE HISTOLOGY OF THE RESTITUTION MASSES.

Our observations on the histology of the young restitution masses agree closely with those of Wilson, so that it is not necessary for us to describe the younger stages in great detail.

A section of a young restitution mass from 18 to 24 hours old shows that a perisarc has been secreted, and that it is still in close contact with the cell mass. The central cells are irregular, and show no trace of stratification : the ectoderm and endoderm cells can be recognized, and present a similar appearance to that described as seen in preparations of the freshly squeezed cells, with the exception that the endoderm elements were even less definite, contained no granules and were distinguishable in relatively small numbers : a few of the cells retained their definite outlines, but others were less distinct and appeared to join up with their neighbours by means of pseudopodia-like processes. It would seem probable, as Wilson suggests, that the structure throughout is that of a cellular syncytium, and that even where the cells appear distinct they are united by protoplasmic strands. He also remarks that the endoderm cells form only a small fraction of the syncytium, though they composed a very large part of the mass when fusion began. This he explains by considering that the endoderm cells undergo a transformation which effectually precludes their recognition later, and we would suggest that the majority of these cells take on a plasmodial character, and so by forming a protoplasmic reticulum unite and draw together the other elements of the mass.

The peripheral cells in contact with the perisarc in these young restitution masses take on an epithelial character quite early, as might be expected from the fact that they have secreted the perisarc. They are distinguishable as a layer of cells resembling those forming the normal ectoderm, with flattened bases in even contact with the perisarc, and they are recognizable several days before any other rearrangement of the cells is apparent in the mass.

A comparison between sections of early and later stages shows that the nematocysts included in the masses gradually disappear and take no part in the further development. A similar observation has been made by Wilson.

Sections after 6 days (Fig. 7) show that the cells are much more definite, the individual cell walls show clearly and the nuclei of the ectoderm cells stain distinctly ; the mass has largely lost its plasmodial indefinite character, much of the cell debris has disappeared and the nematocysts

are not present or are not recognizable, and have probably been dissolved away. Some irregular darkly staining masses suggestive of endodermal cells are present, but they are somewhat indefinite. The more distinct ectoderm cells are often arranged in whorls or rows, and

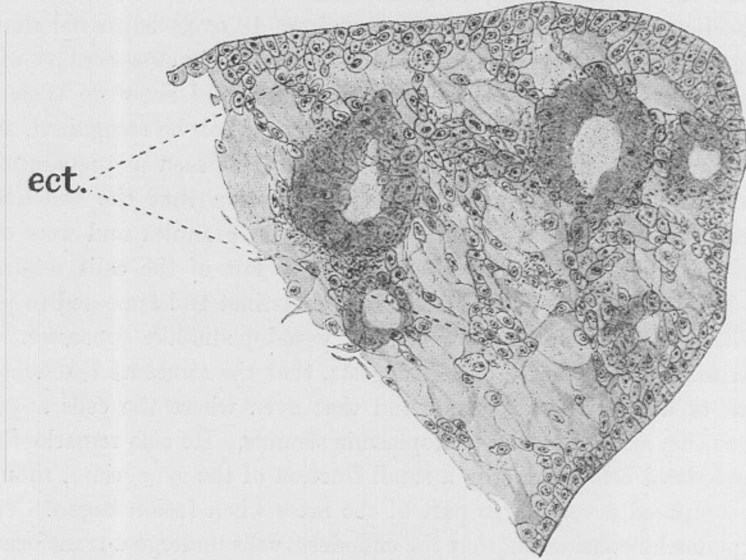

FIG. 7. ×260.—Section through part of a restitution mass 6 days old, showing indefinite arrangement of cells. The ectodermal cells are fairly well differentiated; but the endodermal elements, though showing a tendency towards tubule formation, are not well defined. The perisarc is not shown. *Ect.*, ectoderm cells.

the external layer which secreted the perisarc is well defined. The impression conveyed by examination of sections at this stage is that some process of rearrangement has been initiated among the cells, but there is little to show what may be expected to be the result of this rearrangement. No mitoses were observed.

Seven days later development has proceeded much further, many cells definitely of the endodermal type are present, and they contain numbers of small granules in their cytoplasm. These cells are often arranged so as to form distinct tubules, each having a definite lumen and closely resembling in structure the cœnosarcal part of the enteron of an individual polyp. In other places the endodermal cells are arranged in rows, in irregular masses, or singly, embedded among the ectodermal cells. Where formed, the tubules are always in any one section cut transversely, longitudinally, and at intermediate angles, hence they must be irregularly coiled and crossed within the mass. At this stage the ectodermal cells

are sharp and distinct, approximating the normal in size but slightly larger; they have a tendency to be fusiform in shape with sharply pointed extremities, and are often joined end to end. Spaces between the cells are frequent, but they are occasionally found arranged in compact whorls or masses. The outline of the cells is well defined, the cytoplasm clear but slightly vacuolated, the nuclear membrane and nucleolus distinct.

Sections of restitution masses at the end of 3 weeks (Fig. 8) showed a still more definite arrangement of convoluted endodermal

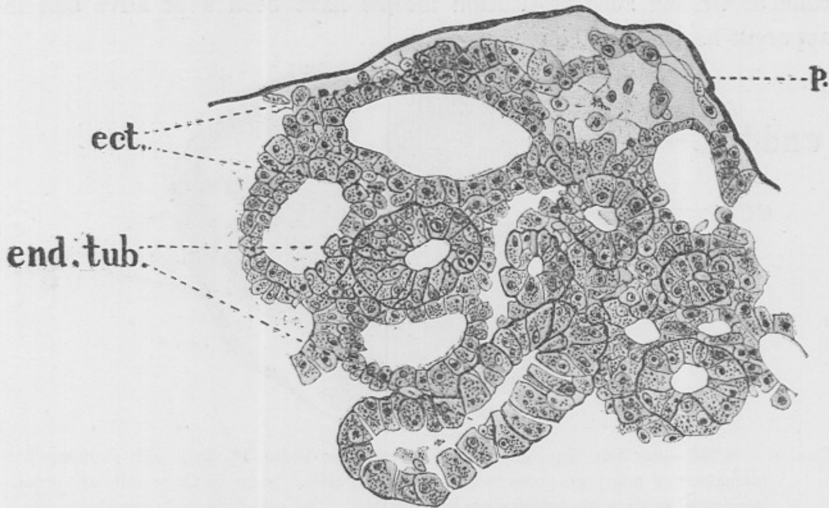

Fig. 8. ×260.—Section through part of a restitution mass 20 days old, showing formation of definite endodermal tubules; all the cells in the mass are sharp and well defined, and the plasmodial character noticeable in earlier stages is lost. *Ect.*, ectoderm cells; *end. tub.*, endoderm tubules; *p.*, perisarc.

tubules. The cells forming these tubules were regularly arranged and closely resembled those lining the enteric cavity of the normal polyp with the exception that no flagella were seen. The cytoplasm of these cells was crowded with large granules, which were often so plentiful as to partially obscure the nucleus. The ectoderm cells were present in even smaller numbers than in earlier stages, but were very definite in structure. Occasional large spaces, corresponding to the spaces producing the fenestrated appearance described as occurring in older masses, were seen among the cells, and other areas in which the cells had degenerated and left merely some granular debris were observed.

At the end of 5 weeks (Fig. 9) the endodermal cells forming the tubules had become crowded with darkly staining spherical granules of

varying size, often totally obscuring the nucleus, and the ectoderm cells often partially or completely surrounded the tubules in the form of a well-defined single cell layer. In some cases the cells of the tubules had apparently undergone autolysis, and a space containing a little granular cell debris was left surrounded by the ectodermal layer.

Later the stages show little change or further degeneration. Sections after 50 days show that a large proportion of the endodermal cells are crowded with granules, and many of the tubules have disappeared; on the other hand, the ectoderm cells are quite undegenerated. At the time of writing such restitution masses have been kept alive and in apparent health for 60 days.

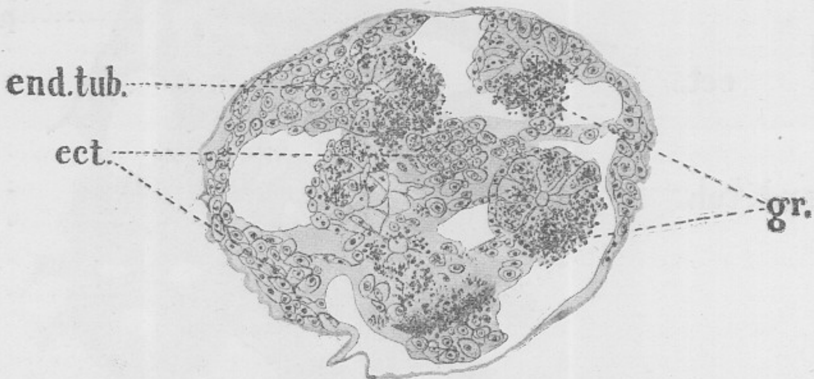

FIG. 9. ×260.—Section through a small restitution mass 35 days old, showing the formation of many granules in the endoderm cells. Some of these cells are breaking down, so that the tubules are less distinct. The ectoderm cells remain healthy. *Ect.*, ectoderm cells; *end. tub.*, endoderm tubules; *gr.*, cytoplasmic granules.

No signs of mitosis or any form of cell division was observed in any stage.

We would suggest that the changes that take place may be explained as follows:—

The endoderm cells are considerably more damaged in the process of squeezing through the bolting silk than the smaller and tougher ectoderm cells. They lose the majority of their cytoplasmic granules, which are probably in the nature of a digestive ferment, and the nuclear membrane is usually ruptured, causing certain changes in the nuclear structure. As the cells begin to form aggregates the endoderm cells become diffuse and join with each other by means of protoplasmic processes to form a plasmodium, in the midst of which the comparatively uninjured ectoderm cells are embedded. Certain ectoderm cells make their way towards the periphery of the mass, or are left there by the

contraction of the plasmodium away from them, and secrete a perisarc within 12 or 18 hours. This resumption of function in so short a time and the localization of the secreting power so that secretion of perisarc takes place only on the outside of the mass, is somewhat remarkable, considering that all the normal relationships between the cells must have been completely upset. Contraction of the plasmodium of endoderm cells still continues, eventually causing the mass to contract away from the perisarc, and it is noteworthy that when this occurs no fresh perisarc is secreted by the peripheral cells. If, however, a small piece of the perisarc is removed, it is rapidly re-secreted by the cells in the neighbourhood, and the gap is healed. From this it would seem possible that the secretion of perisarc is a direct reaction of the ectoderm cells when in contact with sea-water, and that it is not produced when they are in contact with the fluid filling the space between the shrunken mass and the perisarc, and that accordingly this fluid has a different constitution to sea-water.

During the retraction of the plasmodium, the endodermal cells gather together their ramifying processes and again become differentiated, and so very slowly resume their normal form. Of the manner in which many of them become collected so as to form definite tubules, we are unable to offer any explanation. The appearance of sections at a period when the earliest stages of tubule formation are apparent suggests that the plasmodial masses, which will later develop into endodermal tubules, become arranged and segregated before they have differentiated into recognizable endoderm cells, but this is the merest surmise. In cases like this, where individual cells cannot be watched through their modifications and development, the value of the evidence of sections of different masses is always doubtful. Considering that no sign of cell division was ever seen in our experiments it would seem strongly probable that a certain amount of actual migration and rearrangement of the cells within the masses must occur, but no clue is given as to the form or mode of action of the forces causing this rearrangement. As in the case of Wilson's experiments, however, it is difficult to imagine any "form regulation" force coming into play after isolation and subsequent agglomeration of the individual cells forming the original organism.

We consider that the granules found in the cytoplasm of the endodermal cells in the older restitution masses are probably in the nature of the pro-enzyme of the digestive ferment. Since the tubules are closed, and there is no food stimulus, the granules are not discharged, but accumulate in the cell up to a certain limit; when this is reached the cell ruptures and autolysis ensues. From the fact that these granules are formed, it

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follows that a certain amount of active metabolism is going on within the mass, and from the disappearance of the cell debris, nematocysts, ova, etc., which are included in the restitution masses when first formed, it would seem at least possible that these may be absorbed and used up in the metabolic processes of the living cells. Similarly the living cells may feed on those which degenerate.

SYNOPSIS OF EXPERIMENTS.

Numbers omitted belong to experiments not dealt with in this paper.

Experiment 1.—A colony of *Antennularia antennina* dredged on 5th December, 1911, was allowed to stand through the night in "outside" water, and at 10 a.m. on 6th was squeezed into watch-glasses containing a little Berkefeld water, through 50 mesh bolting silk.

Aggregation commenced about 12 noon, and the watch-glasses were then placed in finger-bowls of Berkefeld water. Under the microscope no direct motion of the cells towards one another was noticeable. The cells gravitated downwards and adhered together to form small spherical nodules. These were shaken towards the centre of the watch-glass and left for the night.

On the morning of the 7th nearly all the stuff had united to form irregularly shaped plates from 1 to 2 mm. in length connected by narrow strands. In various spots there were club-headed vertical upgrowths from the plates. The whole aggregate, which was greenish yellow in colour, was surrounded with a tough perisarc, no space appearing between it and the contents.

By the 8th considerable contraction of the interior mass was noticed. There was a clear space between the perisarc and the contained matter, and clear spots were seen here and there in the plates. The contents of the club-headed upgrowths also contracted.

On the 9th these cultures were found swarming with Infusoria and Flagellates, and they were fixed in Corr. Sub. It was thought then that Protozoa would injure the culture ; experience proved that they are not harmful.

Experiment 3.—Colonies of *Antennularia antennina* collected on 12th December, 1911, and kept 24 hours in Berkefeld water. Squeezed through 180 mesh bolting silk, 11 a.m., December. 13th. By 3 p.m. small spherical masses had formed. On the 14th no change had occurred, the small spheres forming a film over the bottom of the watch-glass. Microscopical examination showed cells with a broken outline ; and small

fragments, which might be portions of larger cells. This may very possibly result from pressure through such a small mesh as 180. This experiment gave no further results.

Experiment 4.—14th December. Squeezed a colony of *Antennularia antennina* that had lain for some weeks in a wooden tank in the Laboratory, through 50 mesh. No attempt at aggregation. Very probably the material had deteriorated.

Experiment 5.—19th December, 1911. Colonies of *Antennularia antennina* brought in from the Sound. Very dirty, and placed for the night in Berkefeld water. On 20th noon squeezed through 50-mesh bolting silk into watch-glasses. By 4.30 p.m. small masses had formed, mostly vertical to the bottom of the glass, but so far the cells not very coherent. By noon on the 21st numerous small masses surrounded with perisarc adherent to the glass.

Many of these masses gradually died off, without showing any marked change in shape. The contents, however, gradually contracted away from the perisarc, and finally died. Two small masses were alive on 20th January, 1912, 34 days from the commencement of the experiment. These were fixed for sectioning.

Experiment 8.—28th December. Colony of *Antennularia ramosa* squeezed through 50 mesh at 5 p.m. into outside water. By 11 a.m., 29th, many large masses formed, and adhering to the glass. On 30th the perisarc clearly defined, and the interior plasm slightly contracted away from it. These masses lived until 5th January, 1912, when they died.

Experiment 10.—12th January, 1912, 4 p.m. A very fine colony of *Antennularia ramosa* squeezed through 50 mesh into watch-glasses which were placed in bowls of outside water. A larger amount of material was used in this and subsequent cultures.

By 13th three large masses, between 3 and 4 mm. in length, had formed. They had not adhered to the glass, and the edges were rounded and turned over, somewhat resembling the helix of the human ear. The surface smooth, showing that perisarc had formed, and colour the characteristic yellowish green.

As time went on there was considerable contraction, and the edges became thicker. The cells appeared to migrate from the centre towards the edges, so leaving a thin central nearly clear plate, surrounded by thickened ridges. There was no sign of proliferation or budding of any kind, and by the 24th all three had lost the yellow colour, and looked grey and unhealthy. They were then fixed.

Experiment 13.—17th January, 1912, 4. p.m. Flourishing colony of *Antennularia ramosa* squeezed through 50 mesh and 180 mesh into watch-glasses. Allowed to settle for 3 hours, and then transferred to bowls of Berkefeld water.

18th, 10 a.m. All the 50-mesh cultures show typical lobate masses, but of smaller size than in Exp. 10. The masses from 180 mesh are smaller and lighter coloured. Pieces of tentacle and theca were observed, and removed. Small masses from each culture placed in finger-bowls containing about 450 c.c. Berkefeld water.

19th. All masses from 50 mesh have contracted greatly, and increased in length vertically. They are generally conical. In places the perisarc not formed. Colour, a healthy yellow. All adhered to the glass.

The culture from 180 mesh differs from above. Only small spherical masses have resulted, generally adhering to the glass. Perisarc has not yet been formed.

20th. Most of the masses have contracted further, but the majority are grey and unhealthy looking.

It may be noted that the Laboratory was particularly warm at this time, and the cultures were affected thereby.

24th. Most of the masses were dead, one or two remained alive until 31st. They showed no great change except contraction of the interior protoplasm away from the perisarc and slight attempts to form knobs or proliferations. These did not advance far, and the plasm soon contracted away from the surrounding perisarc. Certain of the masses were fixed for examination.

Experiment 15.—25th January, 1912. *Antennularia ramosa* squeezed through 50 mesh and *Antennularia antennina* through 180 mesh. Allowed to settle through the day.

26th. The 180-mesh culture has simply formed a film over the bottom of the glass—no masses have formed.

Numerous yellowish masses in the 50-mesh culture. Certain of these were transferred to finger-bowls of " outside " water. Generally they were not healthy in appearance, and a good deal of foreign matter was mixed with them.

28th. One mass remains healthy. Several lobes appear on it. The perisarc has formed, but is rougher than usual. A great many Infusoria in the cultures.

29th. The lobes have contracted into the main mass, which has also further contracted.

1st February. Still healthy in appearance. The contents have further contracted away from the perisarc. Fixed for examination.

Experiment 17.—31st January, 1912, noon. Squeezed colonies of *Antennularia ramosa* through 50 mesh, and placed in finger-bowls of Berkefeld water, 4 p.m. The temperature of the Laboratory is so high that these bowls were placed in a trough of running water.

1st February. Lobate masses, 1 to 2 mm. in length, surrounded by perisarc have formed.

2nd. The lobes have further contracted, and most of the masses are attached to the glass.

2nd to 11th. Very little change observable except slight contraction by which a space was left between the contents and the perisarc. Clearer spaces appeared also in the body of the mass.

20th. One mass now 20 days old and thoroughly healthy in appearance fixed.

21st. Several small masses still alive. In the largest of them contraction of the contents at various points has resulted in a markedly spongy appearance, as shown in the figure. This is observable in a less degree in other smaller masses.

9th March. All remained alive up to this date, and without any apparent change. From this date onward the contents appear to be gradually degenerating—in one or two of the masses the enclosing perisarc is almost empty.

21st. The spongy appearance of the large mass is gradually changing, and the contents appear to be concentrating in the centre. This culture is now 51 days old, and has still a healthy yellow colour. Similar concentration has taken place in one or two of the smaller masses which were fixed for examination.

Experiment 23.—1st March, 1912, 11 a.m. Squeezed fine colonies of *Antennularia ramosa* through 50 mesh. Aggregation of cells commenced almost immediately.

3rd. Of the three cultures made on the 1st, two are not healthy. Spherical masses have formed, but they look soft and flocculent, and the perisarc is not clear and smooth.

The third culture, however, has resulted in a healthy lobed mass, not attached to the glass—deep yellow in colour and with a smooth perisarc. The edges of this mass are folded over into knobbed ridges, round a thinner central plate. There is a nearly vertical cylindrical mass at one end.

13th. The edges have curled over more, and ten knobs on them are more accentuated. The centre plate thinner, and at points clear spaces appear.

15th. Cut off the end of the vertical projection.

16th. Perisarc had reformed round the cut end and the incised piece.

SUMMARY AND CONCLUSION.

1. The Hydroids experimented on were *Antennularia ramosa*, and *A. antennina*.
2. These were cut in pieces and pressed through bolting silk, with the result that isolated cells and small cell aggregates were obtained, which soon aggregated together to form compact masses.
3. These restitution masses secreted a perisarc within from 12 to 18 hours.
4. Various changes in shape, and general retraction of the mass away from the perisarc occurred later, but even up to 60 days there was no sign of the regeneration of the hydranths.
5. The restitution masses consisted of ectoderm and endoderm cells, and in addition such structures as nematocysts, ova, and broken down cells, all of which were subsequently absorbed and played no part in the future development. The ectoderm cells were relatively little damaged, and were embedded in a plasmodial mass formed by the endoderm cells.
6. A definite layer of ectoderm cells is formed on the surface, and these cells secrete the perisarc.
7. Gradual aggregation and segregation of the endoderm cells from the plasmodial mass takes place ; and they form very definite tubules similar in structure to the cœnosarcal tubules continuous with the enteric cavities of the normal hydranths. These tubules are embedded in a mass of ectoderm cells, they are convoluted and ramify in all directions. Many granules develop in the cytoplasm of these cells, and after about a month many of them have degenerated.
8. The ectodermal cells show no signs of degeneration, and the masses containing them have been kept alive for 60 days at the time of writing.
9. In none of the experiments was there any sign of the occurrence of cell division.

In conclusion, we can say that, experimenting on different species of Hydroids to those employed by Wilson, we have confirmed his results up to the stage of development at which the restitution mass is formed and the perisarc secreted. Beyond that our results differ ; in the species used by Wilson the restitution masses soon gave rise to hydranths, and practically complete new Hydroids were regenerated ; in the species of Antennularia used by us development of the restitution masses was much slower ; they never regenerated hydranths, but gave rise to tumour-like masses of convoluted tubules lined with endodermal cells embedded in masses of irregularly arranged ectoderm cells. These masses remained alive for at least 60 days.

Our experiments have resulted in the production of masses that are certainly abnormal and pathological, but nevertheless we would submit that the segregation and rearrangement of the cells after isolation, and the comparatively long duration of life of the tumour-like masses to which they give rise are facts of considerable theoretical interest.

PLYMOUTH,
March 28th, 1912.

On F_2 *Echinus* Hybrids.

By

H. M. Fuchs.

An investigation on inheritance in hybrids between the three English species of *Echinus* was carried out in the Marine Biological Laboratory, Plymouth, during 1909–1912 by C. Shearer, W. de Morgan, and H. M. Fuchs. In a paper published in the *Phil. Trans. Royal Soc.*, Ser. B, Vol. CCIV., p. 255, the results of this work were described in detail. At the time of publication, *E. miliaris* had been raised from the egg to maturity in the laboratory, in the course of one year, and a second generation had been obtained from these individuals, but none of the hybrid urchins had as yet reached maturity. This year, however, some of the hybrids have become sexually mature, and from them a second hybrid generation has been raised.

The urchins which have formed ripe genital products are four individuals of the cross *E. esculentus* ♀ X *E. acutus* ♂ (referred to below as *EA*), derived from fertilizations made in 1912. The largest of these urchins now measures 6 cm. in diameter, exclusive of the spines. On May 11th, 1914, two of these hybrids laid eggs in the tank in which they were kept. Naturally these eggs could not be used for experimental purposes, since they were deposited in the sea-water of the aquarium circulation, and therefore not under sterile conditions. On June 6th I induced three of the four to deposit genital products without cutting them open, under conditions which excluded the possible presence of foreign eggs or spermatozoa. It is hardly necessary to mention here that, as in all the previous work on *Echinus* hybrids, the fact of the complete absence of such sperm was made certain by controls of unfertilized eggs, none of which segmented. Two of the three hybrids from which genital products were obtained proved to be females and one a male. The sperm from the latter gave 100% fertilizations with the eggs of the former, yielding healthy larvæ.

From this it is seen that hybrids between the species *E. esculentus* and *E. acutus* are perfectly fertile and that a healthy F_2 generation can be obtained from them. When a larger number of these F_1 hybrids have been

reared, an examination of the characters of the fully grown urchins should decide whether the intermediate forms between the two species, which are found in the sea and which are quite fertile, are to be considered as hybrids or as extreme variants of one of the two species.

Besides making the cultures described above, I fertilized *E. miliaris* eggs with the sperm of the *EA* male, and used *E. miliaris* sperm to fertilize *EA* eggs. This was done in order to see whether the inheritance of the late larval characters (posterior epaulettes and green pigment) in these crosses would be the same as when pure *E. esculentus* or *E. acutus* was crossed with *E. miliaris*. Now, twenty-one cultures,* derived from fifteen fertilizations, have shown that the inheritance of these larval characters has this year been the same as it was in 1912 : the *E. esculentus* or *E. acutus* characters are developed in the hybrids in both reciprocal crosses with *E. miliaris*. It was found that the two reciprocal combinations of *EA* X *miliaris* likewise gave this result. From the cross *EA* ♀ X *miliaris* ♂ large numbers of vigorous fully formed plutei developed, and a number of these "triple-hybrids" have already passed through metamorphosis.

Unfortunately the F_2 generation obtained from the *E. esculentus* X *E. acutus* hybrids can give no information as to the inheritance of the late larval characters, since the latter are alike in the two species. It is the F_2 generation from hybrids between *E. esculentus* or *E. acutus* and *E. miliaris* that will give this valuable information, but none of these hybrids have as yet reached maturity. A small number of *E. miliaris* ♀ X *E. acutus* ♂ hybrids (of which the largest measured $2\frac{1}{4}$ cm. in diameter, exclusive of spines), from fertilizations made in May, 1912, were alive and healthy this summer. After having tried unsuccessfully to induce these to deposit eggs or sperm, I cut them open on June 6th of this year. They contained, however, only small and quite immature gonads.

As it must be some time before more *E. acutus* (or *E. esculentus*) X *E. miliaris* hybrids will have grown large enough to be mature, I wish to record these results up to date. The success in bringing the *EA* hybrids to maturity has been largely due to the care taken by Mr. A. J. Smith, head assistant at the Plymouth Laboratory, in attending to the cultures after metamorphosis. The investigation was made with the assistance of a grant from the Royal Society.

* Some of these cultures were reared at Plymouth, others were transported as blastulæ to the Imperial College, London, and raised there in water which came from Lowestoft.

The Trematode Parasites of Fishes from the English Channel.

By
William Nicoll, M.A., D.Sc., M.D.

With Figures 1-6 in the Text.

In continuation of my researches on the entozoa of British marine fishes I spent two months (August and September, 1909) at the Plymouth Marine Biological Station. By the courtesy of the Government Grant Committee of the Royal Society, a table was placed at my disposal and all expenses in connection with the investigation were defrayed.

Hitherto few observations have been made upon entozoa from fishes of the south coast. The area, however, is of considerable interest from a faunistic point of view, for it contains several species of fish which are uncommon or unknown on other parts of our coast. In addition it is richer in species than either the east or the west coast. The influx of Mediterranean forms adds further interest.

During the course of these two months 419 fish belonging to 70 species were examined. Later, further consignments were sent to me in London. These comprised an additional 56 fish with an additional 9 species. The total number with which this investigation deals is therefore 475 fish and 79 different species. Amongst these, Acanthopterygian fishes figured most largely. The various groups were represented as follows :—

	Species.	Fishes.
Acanthopterygii	32	213
Pharyngognathi	6	50
Anacanthini	20	109
Physostomi	5	21
Lophobranchii	4	31
Elasmobranchii	12	51
Total . . .	79	475

Amongst these 79 species there were 37 which I had not previously had an opportunity of examining, and the majority of them afforded interesting new records.

Judging by those figures this is probably the largest and most representative investigation which has hitherto been made on this subject. A comparison with the numbers dealt with in my previous reports will perhaps be of interest. For St. Andrews, Millport, and Aberdeen the corresponding figures are as follows :—

	St. Andrews.		Millport.		Aberdeen.		Total.	
	Species.	Fishes.	Species.	Fishes.	Species.	Fishes.	Species.	Fishes
Acanthopterygii .	16	74	11	34	9	30	24	138
Pharyngognathi .	0	0	2	7	0	0	2	7
Anacanthini .	14	65	11	41	13	46	23	152
Physostomi .	6	50	3	8	3	4	8	62
Lophobranchii* .	2	2	2	2	0	0	3	4
Elasmobranchii .	3	5	2	2	0	0	5	7
Total .	41	196	31	94	25	80	65	370

From this it is evident that the material examined at Plymouth was richer, not only in the gross total examined, but also in the variety of specimens, than the corresponding material from the other three localities combined. Of the individual groups only the Anacanthini and Physostomi were not so well represented at Plymouth as in these other localities.

In these four series of investigations I have thus examined a total of 845 fish belonging to 102 different species, giving an average of a little over 8 fish of every species. Some specimens have naturally received more attention than others, and those most exhaustively dealt with have been the sprat (*Clupea sprattus*), the common dab (*Pleuronectes limanda*), *Lepadogaster gouanii*, *Ammodytes tobianus*, the mackerel (*Scomber scombrus*), and the butter fish (*Centronotus gunnellus*). Other fishes which have received a large measure of attention have been the horse-mackerel, the sea bream, the whiting, and the John Dory.

From these four localities the aggregate figures are :—

	Species.	Fishes.
Acanthopterygii	37	351
Pharyngognathi	5	50
Anacanthini	31	261
Physostomi	11	90
Lophobranchii*	5	35
Elasmobranchii	13	58
Total . . .	102	845

* The Sun-fish (*Mola mola*) is included here.

These 102 species represent practically all the marine fishes commonly occurring in British seas. Little more than 20 others have ever been recorded from the British coasts, and the majority of those only as isolated individuals.

Apart from these investigations only four species have been recorded in British waters as hosts of trematode parasites, namely, *Brama raii*, *Phycis blennoides*, *Raia radiata*, and *Trygon pastinacea*.

In addition to the four above-mentioned localities, the trematode parasites of marine fishes have been pretty exhaustively dealt with on the Northumberland coast by Miss Lebour and on the Lancashire coast by Johnstone and A. Scott. From these investigations a fairly comprehensive idea may be obtained of the trematode fauna inhabiting our marine fishes. It seems desirable, however, that further investigations should be made in such areas as the Bristol Channel, the north-west coast of Scotland, or the Hebrides, and the southern part of the North Sea (e.g. off Lowestoft). In particular it would be interesting to obtain information as to the trematode fauna of fishes from the coast of Ireland, a region still practically untouched.

Of the 475 fishes examined at Plymouth, 380 (80%) were infected with parasitic worms: 56% were infected with Trematodes, 44% with Cestodes, 48% with Nematodes, and 2% with Echinorhynchs.

It is interesting to compare these figures with those obtained in other areas. The comparison is shown in the following table:—

	Trematodes.	Cestodes.	Nematodes.	Echinorhynchs.	Total.
St. Andrews .	75%	54%	67%	8%	83%
Millport .	70%	46%	76%	13%	80%
Aberdeen .	51%	57%	58%	4%	91%
Plymouth .	56%	44%	48%	2%	80%
Total . .	60%	47%	56%	5%	81%

This table shows that although the percentage of infected fishes in the Plymouth area is only slightly less than that in the other areas, yet the variety of parasites in each fish is considerably less. It will be seen that the Plymouth figures are less in every case than those of other areas with the single exception of the incidence of Trematodes in the Aberdeen fishes.

In the present report only the trematode parasites are dealt with. Over 50 different species were collected, and these represent about three-fifths of the total number of Trematodes known to occur in British

marine fishes. The most interesting of these have already been described in a previous paper (Nicoll, 1913*a*).

At the end of this report a list is given of the fishes examined at Plymouth, with the trematode parasites which were obtained from them.

I have to thank Dr. E. J. Allen, Director of the Plymouth Marine Laboratory and his assistant, Mr. A. J. Smith, for their unfailing courtesy and help.

LIST OF SPECIES DEALT WITH IN THIS REPORT.

DIGENEA.

DISTOMATA PROSOSTOMATA.

Family **ALLOCREADIIDAE.**

Sub-Family ALLOCREADIINAE.

Genus **Podocotyle** (Dujardin).

1. *P. atomon* (Rud.).
2. *P. reflexa* (Crepl.).
3. *P. syngnathi* Nicoll.
4. *P. atherinae* sp. inq.

Genus **Lebouria** Nicoll.

5. *L. varia* Nicoll.
6. *L. alacris* (Looss).

Genus **Peracreadium** Nicoll.

7. *P. genu* (Olsson).
8. *P. commune* (Olsson).

Genus **Cainocreadium** Nicoll.

9. *C. labracis* (Dujardin).

Genus **Helicometra** Odhner.

10. *H. pulchella* (Rud.).

Sub-Family STEPHANOCHASMINAE.

Genus **Stephanochasmus** Looss.

11. *S. pristis* (Deslongch).
12. *S. caducus* Looss, var. *lusci*.
13. *S. cesticillus* (Molin).

Sub-Family Lepocreadiinae.

Genus **Lepidapedon** Stafford.

14. *L. rachion* (Cobbold).

Genus **Pharyngora** Lebour.

15. *P. bacillaris* (Molin).

Genus **Lepidauchen** Nicoll.

16. *L. stenostoma* Nicoll.

Family **FELLODISTOMIDAE.**

Sub-Family Fellodistominae.

Genus **Steringotrema** Odhner.

17. *S. cluthense* (Nicoll).
18. *S. divergens* (Rud.).
19. *S. pagelli* (v. Ben.).

Genus **Bacciger** n.g.

20. *B. bacciger* (Rud.).

Sub-Family Haplocladinae.

Genus **Tergestia** Stossich.

21. *T. laticollis* (Rud.).

Family **ZOOGONIDAE.**

Sub-Family Zoogoninae.

Genus **Zoogonoides** Odhner.

22. *Z. viviparus* (Olsson).

Genus **Zoonogenus** Nicoll.

23. *Z. vividus* Nicoll.

Family **MONORCHIDAE.**

Sub-Family Monorchinae.

Genus **Monorchis** (Monticelli).

24. *M. monorchis* (Stossich).

Family **HAPLOPORIDAE.**

Genus **Haploporus** Looss.

25. *H. benedeni* (Stossich).

Genus **Saccocoelium** Looss.

26. *S. obesum* Looss.

Family **AZYGIIDAE.**

Genus **Ptychogonimus** Lühe.

27. *P. megastomus* (Rud.).

Family **HEMIURIDAE.**

Sub-Family HEMIURINAE.

Genus **Hemiurus** Rud.

28. *H. communis* Odhner.
29. *H. ocreatus* (Rud.).

Sub-Family DINURINAE.

Genus **Lecithocladium** Lühe.

30. *L. excisum* (Rud.).

Sub-Family STERRHURINAE.

Genus **Lecithochirium** Lühe.

31. *L. rufoviride* (Rud.).

Genus **Synaptobothrium** (v. Linstow).

32. *S. caudiporum* (Rud.).

Sub-Family LECITHASTERINAE.

Genus **Lecithaster** Lühe.

33. *L. gibbosus* (Rud.).

Sub-Family SYNCOELIINAE.

Genus **Derogenes** Lühe.

34. *D. varicus* (Müller).

Genus **Hemipera** Nicoll.

35. *H. ovocaudata* Nicoll.

Genus **Derogenoides** Nicoll.

36. *D. ovacutus* Nicoll.

Family **(BUNODERIDAE).**

Genus **Bunodera** Railliet.

37. *B. nodulosa* (Zeder).

Family (**ACANTHOCHASMIDAE**).

Genus **Acanthochasmus** Looss.

38. *A. imbutiformis* (Molin).

DISTOMATA GASTEROSTOMATA.

Family **BUCEPHALIDAE**.

Sub-Family Bucephalinae.

Genus **Bucephalus** Baer.

39. *B. minimus* (Stossich)

Genus **Bucephalopsis** (Diesing).

40. *B. gracilescens* (Rud.).

Genus **Rhipidocotyle** Diesing.

41. *R. minima* (Wagener)
42. *R. viperae* (v. Ben.).

Sub-Family Prosorhynchinae.

Genus **Prosorhynchus** (Odhner).

43. *P. crucibulum* (Rud.).
44. *P. aculeatus* Odhner.
45. *P. triglae* sp. inq.
46. *P. squamatus* Odhner.

MONOGENEA.

Genus **Microcotyle** v. Ben. & Hesse.

47. *M. draconis* Briot.

Genus **Axine** Abildgaard.

48. *A. belones* Abildg.

Genus **Octobothrium** F. S. Leuckart.

49. *O. merlangi* (Kuhn).

Genus **Octocotyle** Diesing.

50. *O. scombri* Kuhn.

Genus **Pseudocotyle** v. Ben. & Hesse.

51. *P. squatinae* v. Ben. & Hesse.

Genus **Calicotyle** Diesing.

52. *C. kroyeri* Diesing.

DIGENEA.

DISTOMATA PROSOSTOMATA.

Family **ALLOCREADIIDAE.**

Sub-Family ALLOCREADIINAE.

Genus PODOCOTYLE (Dujardin).

Podocotyle atomon (Rud.).

Odhner, 1905, pp. 320–6.
Lebour, 1908, pp. 26–27.

This parasite was obtained from ten species of fish, namely, *Gobius ruthensparri*, *Centronotus gunnellus*, *Cottus bubalis*, *Cyclogaster montagui*, *Gastraea spinachia*, *Gadus merlangus*, *Pleuronectes flesus*, *Zeugopterus norvegicus*, *Nerophis aequoreus*, and *Anguilla vulgaris*. This is the first record of its occurrence in the pipe-fish (*Nerophis*).

Podocotyle reflexa (Creplin).

Odhner, 1905, p. 326.

This species was obtained from the intestine of *Gastraea spinachia* and *Onos mustela* on several occasions. It is distinguished from the previous species by its much longer cirrus-pouch and the interrupted arrangement of its yolk glands. The limits fixed by Odhner for the size of this species are too narrow, as mature specimens little over 1 mm. in length were found in *Onos mustela*. It is extremely difficult to differentiate such small specimens from *P. atomon*.

Podocotyle syngnathi Nicoll.

Nicoll, 1913a, pp. 238–40.

This species was frequently found in the pipe-fishes, *Syngnathus acus*, *Siphonostoma typhle* and *Nerophis aequoreus*.

(Podocotyle) atherinae sp. inq. (Fig. 1).

A single specimen of a species of "*Podocotyle*" was obtained from the anterior part of the intestine of *Atherina presbyter*. I am unable to refer it to any known species, and it is doubtful even if it can be included in the genus *Podocotyle*. It is a small, somewhat flattened form measuring 1·1 mm. in length by ·49 mm. in greatest breadth, and it is of a dark grey colour in life. The outline is roughly oval with a slightly attenuated neck.

The oral sucker is globular with a diameter of ·13 mm. The ventral sucker is transversely oval and measures ·21 × ·28 mm. The latter is

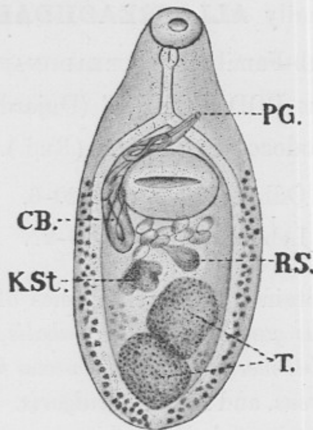

FIG. 1.—(*Podocotyle*) *atherinae*. Ventral view × 50. C.B. Cirrus-pouch; K.St. Ovary; P.G. Genital aperture; R.S. Receptaculum seminis; T. Testes. *G. Roberts del.*

situated ·46 mm. from the anterior end. The pharynx is contiguous with the oral sucker and has a diameter of ·07 mm. The œsophagus is ·11 mm. long, and the wide diverticula extend nearly to the posterior end of the body.

The genital aperture is on the left side a little in front of the level of the intestinal bifurcation. The cirrus-pouch is long and slender, and reaches to the level of the ovary. It contains a long, convoluted vesicula seminalis. The ductus is simple. The testes lie near the posterior end of the body. The distance between the posterior testis and the tip of the tail being ·13 mm. They are contiguous and oblique, the anterior being on the left. Their outline is irregularly globular, and their margins are entire. The posterior testis lies closely apposed to the right intestinal diverticulum, while the anterior one is apposed to the left diverticulum. Their greatest diameter is about ·17 mm.

The ovary lies to the right of the anterior testis and a little in advance of it, the two being almost contiguous. It is a distinctly trilobate body, the lobes being directed backwards, and its greatest diameter is ·13 mm. It is separated from the ventral sucker by a space of ·14 mm. The medium-sized, pear-shaped receptaculum seminis lies immediately in front of the anterior testis. The yolk glands are moderately developed. They are almost entirely marginal, their anterior limit being the level of the ventral sucker. They fill up a considerable part of the post-testicular space, but do not unite. Behind the ovary a few follicles are found on the inner side of the right intestinal diverticulum, while on the left a few are found internal to the diverticulum on the level of the posterior testis. The scanty ova measure ·069–·072 × ·036 mm., and are provided with a minute knob-like process at their anopercular pole.

In referring this form to the genus *Podocotyle* rather than to any of the other genera of the sub-family one is influenced by the characteristic shape of the ovary, the position of the genital aperture and the length of the cirrus-pouch. The position of the testes is the chief contradictory feature, and in this respect the species bears a closer resemblance to *Lebouria*. It might be suggested that it is an abnormal specimen of *P. atomon* in which the testes have become displaced, but as I have no previous experience of such an abnormality I am very doubtful if this could be the case.

Genus LEBOURIA Nicoll.

Lebouria alacris (Looss).

Nicoll, 1910, pp. 332–4.

This species was frequently obtained from the smaller Labridae (*Ctenolabrus rupestris*, *Centrolabrus exoletus* and *Crenilabrus melops*). A single specimen was also found in the intestine of *Labrus berggylta*.

Lebouria varia Nicoll.

Nicoll, 1910, pp. 329–32.

This species was only met with in the dragonet (*Callionymus lyra*), in which it is fairly common.

Genus PERACREADIUM Nicoll.

Peracreadium commune (Olsson).

Nicoll, 1910, pp. 328–9.

Only three specimens of this species were met with in *Labrus berggylta* and *Crenilabrus melops*. They agree with my previous description, except

that the ventral sucker is somewhat larger and more globular than in the Clyde specimens. It is thus not a feature to distinguish this species from *P. genu.*

Peracreadium genu (Rud.).

Nicoll, 1910, pp. 326–8.

This was obtained twice from the intestine of *Labrus berggylta*, but never from any of the other *Labridae* (over 30 were examined). A single immature specimen, however, occurred in the intestine of a shanny (*Blennius pholis*). It was only ·4 mm. long. The testes were oblique, and the ovary on the right side of the anterior testis. The cirrus-pouch reached almost to the ovary. The yolk glands were not visible, and there were no ova. It seemed impossible to determine whether this specimen should be regarded as *P. genu* or *P. commune,* but in any case it must be regarded as an adventitious parasite of the shanny.

Genus CAINOCREADIUM Nicoll.

Cainocreadium labracis (Dujardin).

Johnstone, 1908, pp. 44–53.

Half a dozen specimens of this species were taken from the intestine of the only bass (*Labrax lupus*) examined.

Genus HELICOMETRA Odhner.

Helicometra pulchella (Rud.).

This was by far the commonest member of the *Allocreadiidae* met with. As a parasite of littoral fishes it largely replaces *Podocotyle atomon*, which is predominant on the east coast. At Plymouth it was met with in twelve different hosts : *Serranus cabrilla, Trigla pini, Gobius paganellus, Blennius pholis, Blennius gattorugine, Lepadogaster gouanii, Labrus mixtus, Labrus berggylta, Ctenolabrus rupestris, Zeugopterus punctatus, Anguilla vulgarus* and *Conger conger*. Ninety specimens of these fishes were examined, and the parasite was met with thirty-three times (i.e. 3 in 8). Its chief hosts are the goby and the blennies. In these it occurred three times in five.

A fairly full description, partly based on the material collected at Plymouth, has already been given (Nicoll, 1910, pp. 335–40). The distribution of this species is rather noteworthy. It has been recorded from the Mediterranean, from the English Channel and from the west coast of Scotland. It has never been recorded from the North Sea.

H. pulchella (Rud.) of Odhner (1902) from Northern fishes, is probably a distinct species.

Sub-Family Stephanochasminae.

Genus STEPHANOCHASMUS Looss.

Stephanochasmus caducus Looss var. *lusci.*

Numerous young specimens of a parasite which I can only with some doubt identify as this species were taken on two occasions from the duodenum and pyloric caeca of *Gadus luscus*. A single immature specimen was also found in the caeca of *Gadus minutus*.

They measure 1·5–3 mm. in length, and most of those over 2 mm. contained ova. The cephalic spines are arranged in two rows of 25 each, and those of the anterior row are shorter than those of the posterior row, ·019 mm. and ·021 mm. respectively. In a 3 mm. species the oral sucker has a diameter of ·12 mm. and the ventral ·14 mm. The latter is situated ·63 mm. from the anterior end. The prepharynx is ·21 mm. long, and the pharynx measures $\cdot 1 \times \cdot 08$ mm.

The cirrus-pouch extends ·49 mm. behind the ventral sucker. The vagina joins it behind the sucker, and the genital sinus is ·2 mm. long. The ovary, testes and yolk glands are situated as described by Looss, but the yolk glands extend forward a short distance in front of the end of the cirrus-pouch. The few ova measure $\cdot 066 \times \cdot 036$ mm.

The chief respects in which these specimens differ from Looss's description (1901, p. 603) are the number and size of the cephalic spines, the position of the ventral sucker, the inequality of the suckers and the greater extent of the yolk glands. It seems possible to ascribe the first two of these to difference in age and size of the specimens (Looss's description was from specimens over 4 mm. long). The other two features, together with the difference in number of the cephalic spines, do not seem of sufficient importance to warrant establishing a new species, but it seems advisable to regard this form as a distinct variety.

It is interesting to note that the specimen obtained by Miss Lebour (1908, p. 36) from the whiting (*Gadus merlangus*) does not entirely agree with Looss's description of *S. caducus*. The suckers are nearly twice as great as those of Looss's form. The yolk glands are more extensive and the eggs are larger. It is possible that this may represent a third variety of the same species.

Stephanochasmus cesticillus (Molin).

Looss, 1901, pp. 598–9.

Four specimens of this parasite were collected from the stomach and intestine of *Zeus faber*. This is the first and only time this parasite has

been recorded from British waters, and it is the only occasion on which it has been met with in this host.

Sub-Family LEPOCREADIINAE.

Genus LEPIDAPEDON Stafford.

Lepidapedon rachion (Cobbold).

Odhner, 1905, pp. 332–7 (*Lepodora rachiaea*).

Lebour, 1908, pp. 29–30.

This was found frequently in the intestine of the pollack (*Gadus pollachius*) once in considerable numbers. It was not met with in any of the other Gadoids, of which nearly 40 were examined.

Lepidauchen stenostoma Nicoll.

Nicoll, 1913a, pp. 240–1.

Two specimens were obtained from the intestine of *Labrus berggylta*. This species has already been fully described.

Genus PHARYNGORA Lebour.

Pharyngora bacillaris (Molin).

Nicoll, 1910, pp. 341–7.

This parasite was met with very frequently in the intestine of *Scomber scombrus*, *Gadus merlangus*, *Capros aper* and *Cyclopterus lumpus*. Only one specimen of the last-mentioned fish was examined (22nd April, 1910), and it contained several thousand immature specimens ranging in length from ·6 mm. to 1·8 mm. I have previously recorded the occurrence of this parasite in the lumpsucker caught in St. Andrews Bay (1909, p. 22, *Distomum* sp.). In that case, too, the parasites were all immature, though very much fewer in number. In *Capros aper*, also, although I have found the parasite in moderate numbers on four occasions, they have always been immature. Only in the mackerel and whiting have fully mature specimens been obtained. The mackerel is undoubtedly the commonest host of this parasite.

Family **FELLODISTOMIDAE**.

Sub-Family FELLODISTOMINAE.

Genus STERINGOTREMA Odhner.

Steringotrema cluthense (Nicoll).

Nicoll, 1909, pp. 472–5.

This species was the commonest member of the family Fellodistomidae

found on the south coast, and indeed the only one met with in Pleuronectid fishes. It was found in five out of thirteen specimens of *Pleuronectes limanda* and *P. microcephalus*. It has already been recorded in the

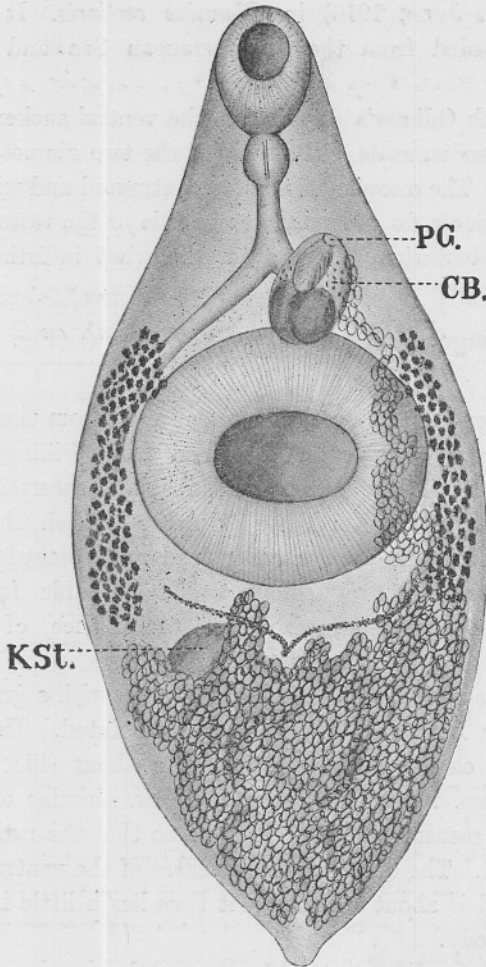

FIG. 2.—*Steringotrema pagelli*. Ventral view ×33. C.B. Cirrus-pouch; K.St. Ovary; P.G. Genital aperture. *M. Rhodes del.*

latter host from the Firth of Clyde, but it is worthy of note that it has not been met with in the North Sea, although over 100 specimens of these two hosts have been examined. It has already been fully described.

Steringotrema divergens (Rud.).

Odhner, 1911, p. 103.

This species was met with in fairly large numbers on two occasions (15th and 24th June, 1910) in *Blennius ocellaris*. It has hitherto only been recorded from the Mediterranean Sea and only in this host.

Compared with Odhner's description the ventral sucker in my specimens is less anisodiametric. The ratio of the two diameters being 6 : 7 instead of 6 : 8. The œsophagus is more contracted and wider, while the intestinal diverticula do not reach the middle of the testes. The ovary shows slight indentation, giving it a somewhat indistinctly trilobate appearance.

Steringotrema pagelli (van Beneden) (Fig. 2).

Odhner, 1911, p. 102.

A few specimens of this species were collected from the intestine of a sea-bream (*Sparus centrodontus*) obtained from Billingsgate Market, London (19th June, 1912). It was not possible to ascertain whether the fish was captured in the North Sea or in the English Channel. When collected the specimens were already dead and somewhat macerated. They were of a dull grey colour and remarkable for their great thickness and the unusual size and prominence of the ventral sucker.

The specimens measure 3·6–4·1 mm. in length with a greatest breadth of 1·6–1·8 mm. Both ends of the body are pointed. The oral sucker, which in every case is elongated, measures about ·48 × ·39 mm. in a specimen 3·8 mm. long. The ventral sucker, circular or transversely oval in outline, measures 1·05 × 1·16 mm., so that the ratio is not quite as much as 3 : 1. The distance of the centre of the ventral sucker from the anterior end is about 1·8 mm. ; it thus lies a little in front of the middle of the body.

The pharynx has a diameter of ·17 mm., and the œsophagus is somewhat longer. The intestinal diverticula diverge widely. Their ends are obscured by the great mass of ova.

The ovary and testes are also almost completely hidden by the uterus. The cirrus-pouch is a long bulbous structure lying immediately in front of the ventral sucker, which it touches. The aperture is on the level of the intestinal bifurcation and about midway between it and the left margin of the body. The vesicula seminalis is of comparatively large size.

The yolk glands are situated at the sides of the ventral sucker and extend a short distance both in front of and behind it. Their extent is more limited than in *S. cluthense*. The uterus is very firmly packed and fills almost the whole of the post-acetabular region. The ova have thick brown shells and measure ·057–·063 × ·033–·037 mm. Their measurements are considerably larger than those found by Odhner for the same species.

Genus BACCIGER n.g.

Bacciger bacciger (Rud., Stoss., 1889) (Fig. 3).

On two occasions a single specimen of a small distome was found in the stomach of *Atherina presbyter*. The fish were received in London

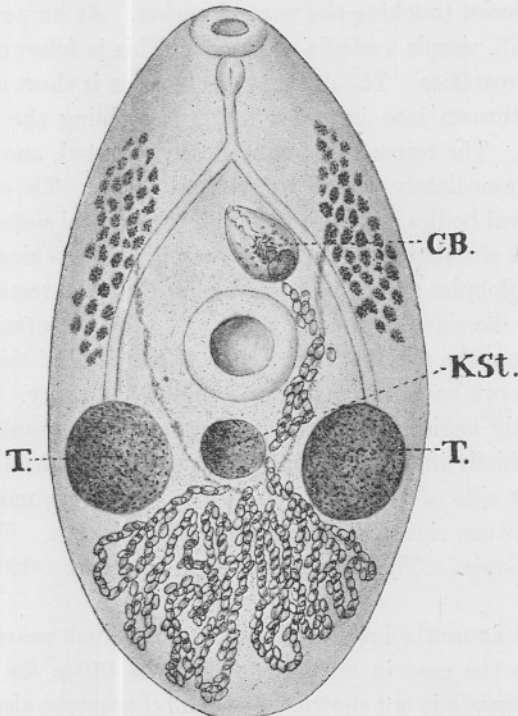

Fig. 3.—*Bacciger bacciger*. Ventral view × 100. C.B. Cirrus-pouch; K.St. Ovary; T. Testes. *G. Roberts del.*

from Plymouth, and on that account the viscera were somewhat decomposed and the parasites badly preserved. From what could be made out of their anatomy, however, they appear to be either identical with or very closely related to *Distomum baccigerum* (Rud.) Stossich from *Atherina hepsetus*.

The body is flat and of oval outline. The cuticule is unarmed. The length of the body (slightly pressed) is ·95 mm., and the maximum breadth in the middle, ·52 mm. The sub-terminal oral sucker has a diameter of ·105 mm., and the ventral sucker, situated a little in front of the middle of the body, measures ·15 mm. There is a small pharynx, contiguous in the oral sucker. The œsophagus is two or three times as long as the pharynx, the intestinal bifurcation taking place about midway between the pharynx and the genital aperture. The intestinal diverticula are very narrow, they extend into the posterior part of the body, but their termination is obscured by the uterus.

The genital aperture is median, and lies about ·15 mm. in front of the ventral sucker. There is a small, stout cirrus-pouch lying entirely in front of but almost touching the ventral sucker. At its posterior end it contains a small, simple vesicula seminalis. This is followed by a short inflated pars prostatica. The ductus ejaculatorius is short and wide and its walls are thrown into irregular folds, resembling the condition in *Steringophorus*. The testes are symmetrically situated, one on each side of the body, immediately behind the ventral sucker. They are globular or elongated oval bodies a little larger than the ventral sucker.

The ovary is situated between the testes, directly behind the ventral sucker. It is globular and somewhat smaller than the testes. The yolk glands occupy the sides of the body in front of the ventral sucker, and they extend from the middle of the sucker to the level of the œsophagus. The yolk ducts run backwards and unite behind the ovary. The uterus is confined entirely behind the testes. It forms a large number of narrow convolutions chiefly in a longitudinal direction. The initial convolutions are on the left side of the body, where the eggs are almost colourless. Towards the other side the colour gradually deepens. The vagina is apparently simple. The numerous eggs measure ·020–·024 × ·014–·017 mm.

In Stossich's figure the intestinal bifurcation is much nearer the ventral sucker than is the case in my specimens ; the testes are much larger and the cirrus-pouch is not shown. The genital aperture also is described as being immediately behind the pharynx. How far these discrepancies are due to errors of observation it is impossible to say, as I have had no opportunity of examining Stossich's original material.

This species undoubtedly represents the type of a distinct genus of the family FELLODISTOMIDAE and the sub-family FELLODISTOMINAE. Its general build rather suggests an affinity with the *Monorchidae*, but the structure of the cirrus-pouch and vagina excludes it from that family.

Sub-Family Haplocladinae.

Genus TERGESTIA Stossich.

Tergestia laticollis (Molin).

Odhner, 1911, p. 111–13; Nicoll, 1913b, pp. 192–3.

This species was found frequently in the intestine of the horse-mackerel (*Trachurus trachurus*). It occurs occasionally in fairly large numbers, though as a rule only a few specimens are present in one host. It appears to be an exclusive parasite of this host.

Family **ZOOGONIDAE**.

Sub-Family Zoogoninae.

Genus ZOOGONOIDES Odhner.

Zoogonoides viviparus (Olsson).

Odhner, 1902a, p. 62; Nicoll, 1907, p. 83.

This very common parasite has already been recorded from eight species of British fishes. An additional five have here to be noted, namely, *Zeus faber*, *Blennius gattorugine*, *B. ocellaris*, *Solea vulgaris*, *S. variegata*. It was also found in *Callionymus lyra*, *Pleuronectes limanda*, *P. microcephalus* and *P. platessa*. The chief hosts of this parasite are undoubtedly *Callionymus lyra*, *Pleuronectes spp.* and *Solea spp.* It is rather curious that it has never once been recorded from Gadoid fishes or from the Labridae. It is worth remarking that the specimens I obtained from the cat-fish (*Anarrhichas lupus*) at St. Andrews belong to this species and not to the more recently discovered *Z. subaequiporus* Odhner, from the same host.

Genus ZOONOGENUS Nicoll.

Zoonogenus vividus Nicoll.

Nicoll, 1912, pp. 200–2.

A species which I have already described was met with frequently in the intestine of *Sparus centrodontus*. As I have previously remarked, it is an extremely delicate species and exceedingly difficult to preserve in a satisfactory state. An additional six bream obtained from Billingsgate Market were examined in June, 1912, but they were not infected with the parasite.

Family **MONORCHIDAE.**

Sub-Family MONORCHINAE.

Genus MONORCHIS Monticelli.

Monorchis monorchis (Stossich).

Looss, 1902b, pp. 117–18.

A couple of specimens of this parasite were found in the intestine of *Blennius gattorugine.*

Family **HAPLOPORIDAE.**

Genus SACCOCOELIUM Looss.

Saccocoelium obesum Looss.

Looss, 1902a, pp. 140–1.

A few specimens of this parasite were found in the intestine of a grey mullet (*Mugil chelo*).

Genus HAPLOPORUS Looss.

Haploporus benedeni (Stossich).

Looss, 1902a, pp. 136–8.

A few specimens were obtained from the intestine of a grey mullet (*Mugil chelo*) along with specimens of *Saccocoelium obesum.*

Family **AZYGIIDAE.**

Genus PTYCHOGONIMUS Lühe.

Ptychogonimus megastomus (Rud.).

Jacoby, 1899, pp. 16–24 ; Jägerskiöld, 1900, pp. 68–74.

This parasite was obtained from the stomach of four out of six specimens of *Mustelus vulgaris.* It usually occurred in moderate numbers.

Family **HEMIURIDAE.**

Sub-Family HEMIURINAE.

Genus HEMIURUS (Rud.).

Hemiurus communis Odhner.

Odhner, 1905, p. 351 ; Lebour, 1908, p. 46.

This exceedingly common and widespread fish parasite was met with in sixteen different species of fish, namely, *Sparus centrodontus, Capros aper, Lophius piscatorius, Cottus bubalis, Trigla pini, T. gurnardus, Gobius paganellus, Lepadogaster gouanii, Gadus luscus, G. merlangus, G. minutus, G. pollachius, Ammodytes lanceolatus, Molva molva, Zeugop-*

terus punctatus and *Nerophis aequoreus*. The species is now known to occur in thirty species of British marine fishes, and it is, with the exception of *Derogenes varicus*, the most widely distributed of all British fish parasites. In the above list the bream (*Sparus centrodontus*) from which the parasites were obtained were bought in the London market, so that their actual origin is unknown. None of the bream examined at Plymouth harboured the parasite.

Although so widely distributed, the parasite shows a distinct preference for Gadoid fishes, of which eleven species have been found to harbour it. I have myself examined over 120 Gadoids and found the parasite in 34% of them. Amongst the total number of other fishes which I have examined it has been present in less than 5%. Next to the Gadoids, *Cottus bubalis* and *Hippoglossus vulgaris* are probably the most frequent hosts.

Hemiurus ocreatus (Rud.).

=H. LUHEI Odhner.

Odhner, 1905, p. 352 ; Nicoll, 1909, pp. 21–2.

By far the commonest host of this parasite was found to be the pilchard (*Clupea pilchardus*). It was also met with on one occasion in each of the following hosts : *Trachurus trachurus*, *Capros aper*, *Scomber scombrus*, *Gadus merlangus*, and *G. pollachius*.

Sub-Family (Dinurinae).

Genus LECITHOCLADIUM Lühe.

Lecithocladium excisum (Rud.).

Looss, 1907, pp. 131–2.

This species was found only in the stomach of the mackerel (*Scomber scombrus*). It occurred in three out of eight specimens examined.

Sub-Family Sterrhurinae.

Genus LECITHOCHIRIUM Lühe.

Lecithochirium rufoviride (Rud.).

Looss, 1907, p. 147.

This was found in the stomach of the common eel (*Anguilla vulgaris*), the conger (*Conger conger*) and the angler (*Lophius piscatorius*). It is an extremely common parasite of the first two fishes, but has not previously been recorded from *Lophius*. Looss regards the conger as the only authentic host of the parasite, but there is no doubt that the single

specimen I have obtained from *Lophius* really belongs to this species. It is about 5 mm. in length and has suckers measuring respectively ·65 mm. and ·78 mm. in diameter.

The encysted stage of this parasite was met with frequently in the shanny (*Blennius pholis*). It occurred in fairly large opaque brown cysts measuring ·7–1·4 mm. in diameter. They were attached to various abdominal viscera, but chiefly the intestine and the liver. They were commonest in the intestinal wall, either loosely attached or firmly embedded, and in more than one case free larvæ were found actually in the intestine. The larvæ when freed from the cyst were about 2 mm. in length (*ecsoma retracted*), and they had suckers measuring ·28 mm. and ·4 mm. respectively in diameter. The genital organs were well developed and fairly numerous eggs were present in many. These measured ·015 × ·009 mm.

The occurrence of the larvæ of *Lecithochirium gravidum* encysted in pipe-fishes has already been recorded by Looss (1907, p. 148).

Genus SYNAPTOBOTHRIUM von Linstow.

Synaptobothrium caudiporum (Rud.).

Looss, 1907, pp. 150–2.

This parasite has not hitherto been recorded from British waters. It occurred in the stomach of three out of five specimens of *Trigla hirundo* and once in *Zeus faber* and *Lophius piscatorius*. The specimens are considerably larger than those examined by Looss, reaching a length of 4 mm. (unpressed specimens) or 5 mm. (pressed specimens). The vesicula seminalis is confined entirely in front of the ventral sucker, while the metraterm may reach the centre of the sucker. The eggs have the characteristic shape described by Looss.

Encysted larvæ of this specimen were found along with those of *Lecithochirium rufoviride* in the liver and intestinal wall of a small specimen of *Labrus berggylta*, and two cysts were found in the intestinal wall of *Crenilabrus melops*.

Sub-Family Lecithasterinae.

Genus LECITHASTER Lühe.

Lecithaster gibbosus (Rud.).

Looss, 1907, p. 164 ; Odhner, 1905, pp. 356–8 ; Nicoll, 1909, pp. 18–20.

This parasite occurred in seven different hosts, namely, *Serranus cabrilla*, *Trachurus trachurus*, *Zeus faber*, *Trachinus vipera*, *Trigla pini*,

Gadus merlangus, and *Zeugopterus norvegicus*. Though fairly widespread, it is by no means a common parasite, and its numbers in any particular host rarely exceed two or three.

Sub-Family Syncoeliinae.

Genus DEROGENES Lühe.

Derogenes varicus (O. F. Müller).

Odhner, 1905, pp. 360–4 ; Johnstone, 1907, pp. 188–92 ; Lebour, 1908, pp. 45–6.

By far the commonest of marine fish parasites, this species was found in the stomach of twenty-eight different hosts, namely, *Mullus barbatus*, *Sparus centrodontus*, *Trachurus trachurus*, *Capros aper*, *Zeus faber*, *Trachinus vipera*, *T. draco*, *Lophius piscatorius*, *Cottus bubalis*, *Agonus cataphractus*, *Callionymus lyra*, *Trigla pini*, *T. gurnardus*, *T. hirundo*, *Cyclopterus lumpus*, *Blennius ocellaris*, *Gadus luscus*, *G. minutus*, *G. merlangus*, *G. pollachius*, *Molva molva*, *Onos tricirratus*, *Bothus maximus*, *Pleuronectes flesus*, *P. limanda*, *Solea vulgaris*, *Salmo trutta*, and *Conger conger*.

Genus HEMIPERA Nicoll.

Hemipera ovocaudata Nicoll.

Nicoll, 1913a, pp. 242–3.

This species was found a few times in the stomach of *Lepadogaster gouanii*.

Genus DEROGENOIDES Nicoll.

Derogenoides ovacutus Nicoll.

Nicoll, 1913a, pp. 243–6.

This parasite was met with only once in the stomach of a weever, *Trachinus draco*.

Family **BUNODERIDAE.**

Genus BUNODERA Railliet.

Bunodera nodulosa (Zeder).

Looss, 1894, pp. 33–41.

A few specimens of this species were obtained from the intestine of a trout (*Salmo trutta*) from the River Yealm.

Family **ACANTHOCHASMIDAE.**

Genus ACANTHOCHASMUS Looss.

Acanthochasmus imbutiformis (Molin).

Looss, 1901, pp. 632–3; Johnstone, 1906, pp. 177–9.

About thirty specimens of this parasite were found in the intestine of *Labrax lupus*.

What appears to be the larval stage of this parasite was found encysted in the gills of the pipe-fish, *Siphonostoma typhle*. A single cyst containing a living larva was also found on one occasion amongst the stomach contents of a whiting (*Gadus merlangus*).

The cysts in the gills of *Siphonostoma* are oval and measure ·3–·65 mm. in length. The oral sucker is slightly larger than the ventral, and is surrounded by eighteen cephalic spines. The cyst in the whiting had a diameter of ·38 mm. and the cercaria a length of 1·5 mm. The oral sucker measured ·15 mm. and the ventral ·17 mm. The cephalic spines numbered eighteen and measured ·056 mm. in length.

GASTEROSTOMATA.

Of this sub-order eight different species were collected, only one of which appears to be hitherto undescribed. A remarkable feature of this group is the great variation in the anatomical topography which may occur, even within specific limits. This variation affects chiefly the position of the genital glands and of the mouth. The size of the excretory vesicle also varies considerably. On the other hand, the position of the yolk glands and the size of the cirrus-pouch are fairly constant.

The variation is particularly well illustrated in the case of *Prosorhynchus crucibulum* (Rud.), in which as regards the position of the genital glands no two descriptions have yet agreed. It has been pointed out (Nicoll, 1910) that these discrepancies are due to the extreme variation in the position of the genital glands in this species. A similar, but less extensive, variation is found in *Prosorhynchus aculeatus* Odhner.

Within generic limits a still wider variation may be observed. In illustration it is sufficient to compare the condition in *Prosorhynchus squamatus* Odhner with that in *P. aculeatus*. In the former the ovary and testes lie almost directly one behind the other, along the right margin of the middle part of the body. In *P. aculeatus*, on the other hand, they are disposed in a triangle in the posterior part of the body, the ovary being in front and the testes lying one on each side of the body.

In the prosostomate distomes we are accustomed to regard the relative position of the genital glands as constant within narrow limits for the same species, and any such difference as exists between *P. squamatus* and *P. aculeatus* would be sufficient to warrant generic separation. In the Gasterostomata, however, it is evident that one cannot regard this feature as a satisfactory basis of classification, and recourse must be had to others of a more constant nature. Odhner has already (1905) denoted the chief of these, namely, the structure of the copulatory organs, the structure of the head and the disposition of the yolk glands.

Apart from the situation of the genital glands and the configuration of the uterus, the species included in the genus *Prosorhynchus* appear to form a homogeneous group. The same, however, cannot be said with regard to the remaining species of Gasterostomes, included by Odhner under the genus *Gasterostomum* (=*Bucephalus*).

They all agree in having the yolk glands arranged in two distinct groups, which are usually marginal in position, and, so far as is known, the structure of the copulatory organs does not vary very much from the type found in *Bucephalus polymorphus* (=*Gasterostomum fimbriatum*). It is in the structure of the anterior end that we meet with the most pronounced features of difference. Three main types may be recognised. (1) The anterior end may be provided with a simple sucker as in *Gasterostomum gracilescens* and *G. tergestinum*. This sucker closely resembles the ventral sucker of the prosostomate distomes, and is regarded by Odhner as the primitive type of head structure in the Gasterostomes. (2) From the sucker muscular prolongations may grow out in the form of tentacles or fimbriae as in *G. fimbriatum* and *G. minimum* Stossich. (3) The sucker may degenerate in musculature, become very shallow and be surmounted by a contractile fan-shaped hood as in *G. triglae* and *G. viperae*. It is apparent that some generic separation of these three groups is desirable, and it is only on the structure of the anterior end that this is practicable. Each of these three groups has already, in earlier literature, been regarded as of generic or at least subgeneric importance. The synonymy is slightly complicated.

The monotypical genus *Gasterostomum* was founded on *G. fimbriatum*, and as this has been shown to be the adult of the earlier known *Bucephalus polymorphus* the name *Gasterostomum fimbriatum* must be regarded as *nomen nudum* and the genus *Gasterostomum* as a synonym of *Bucephalus*.

In 1855 Diesing erected the sub-genus *Bucephalopsis* for the larval form *B. haimeanus*, and this has been shown to be the larva of *Gasterostomum gracilescens* (Rud.). This species is undoubtedly the type of a

distinct genus, and on that account I propose to raise the sub-genus *Bucephalopsis* to generic rank with *B. gracilescens* (Rud., 1819) as type.

A complication enters here, however, for in 1858 Diesing erected the genus *Rhipidocotyle* for the reception of the two species *G. gracilescens* and *G. minimum* Wagener (*nec.* Stossich) without designating the type. Stiles & Hassall (1908) have tentatively taken *G. gracilescens* as the type of this genus, but, as I shall show, *G. minimum* Wagener is undoubtedly the real type of this genus.

I am, unfortunately, not personally familiar with Wagener's original specimens, nor have I had an opportunity of examining any Gasterostomes from the type host, *Trigla microlepidota*, but a careful study of Wagener's original figure (1852) has suggested to me that *G. minimum* Wagener is identified with *G. triglae* van Beneden, 1870, and with the form which I have described under that name (1909). The most characteristic feature of this species is the highly contractile fan-shaped structure which surmounts the anterior sucker. To both Wagener and van Beneden this structure must have appeared in a very contracted condition and so have escaped observation. Diesing, however, must have been familiar with it and have been influenced by it in his choice of a generic name, hence the highly descriptive combination *Rhipidocotyle* (ῥῖπίς, ῥῖπῖδος=a fan, κοτύλη=a cup). No more appropriate term could have been invented. At the same time it is remarkable that Diesing should have included in the same genus *G. gracilescens*, which possesses no such fan-shaped structure.

There is nothing in the remaining anatomy of either of these species which to my mind bears the slightest resemblance to a fan, and on that account I am convinced that it was the fan-shaped cephalic hood which Diesing regarded as the distinctive feature of this genus. It will thus be necessary to revive the old generic term which Odhner (1905, p. 296, note 3) somewhat cavalierly consigned to the "lumber room of useless names."

As Gasterostomum has become a *nomen nudum* the family name Gasterostomidae must be replaced by Bucephalidae, and this family will now include the genera *Bucephalus* Baer, 1827, *Bucephalopsis* (Diesing, 1855), *Rhipidocotyle* Diesing, 1858, and *Prosorhynchus* Odhner, 1905. As Odhner has already suggested, a further separation of these genera appears advisable. I propose to separate them into two sub-families, *Bucephalinae*, n. subfam. and *Prosorhynchinae*, n. subfam.

The definitions of these sub-families are identical with the definitions given by Odhner (1905, pp. 296–7) for the genera *Gasterostomum* and

Prosorhynchus respectively. Bucephalinae includes the genera *Bucephalus*, as type, *Bucephalopsis* and *Rhipidocotyle*.

The definition of these three genera may be summed up briefly as follows :—

Bucephalus Baer, 1827.

Bucephalinae in which the anterior end is provided with a muscular sucker around which are a number of muscular retractile tentacles or fimbriae. Type : *B. polymorphus* Baer, 1827 (=*Gasterostomum fimbriatum* v. Siebold, 1848) *B. minimus* (Stossich) (*Gasterostomum minimum* Stossich, 1887, *nec.* Wagener, 1852) may also be included in this genus.

Gasterostomum gorgon Linton, 1905, may be provisionally included here, but will probably require to be regarded as the type of a distinct genus. The " Gasterostomum sp." depicted by Linton (1910, Fig. 225) from *Sphyraena barracuda* may possibly belong to this genus.

Bucephalopsis Diesing, 1855.

Bucephalinae in which the anterior end is provided with a simple globular muscular sucker. Type : *B. gracilescens* (Rud., 1819). *G. tergestinum* Stossich, 1883, should be included here, and probably a number of American forms.

Rhipidocotyle Diesing, 1858.

Bucephalinae in which the anterior end is provided with a feebly developed, shallow sucker surmounted by a fan-shaped hood. When this is contracted the anterior end appears square. Type : *R. minima* (Wagener, 1852), Nicoll, 1914 (=*Gasterostomum triglae* van Ben., 1870, Nicoll, 1909), includes also *R. viperae* (van Ben., 1870), Nicoll, 1914, and probably a number of species from American fishes (Linton, 1910, Figs. 217, 222, 223).

Family **BUCEPHALIDAE.**

Sub-Family Bucephalinae.

Genus BUCEPHALOPSIS (Diesing).

Bucephalopsis gracilescens (Rud.).

Lebour, 1908, pp. 18–21.

This common parasite of *Lophius piscatorius* was found in four out of five specimens of that fish. It occurred in the intestine and pyloric caeca, usually in large numbers.

Genus BUCEPHALUS Baer.

Bucephalus minimus (Stossich).

=*Gasterostomum minimum* Stossich, 1887, p. 96.

About two dozen specimens of a small Gasterostome were found in the intestine of *Labrax lupus*. They correspond in most respects with Stossich's description of *Gasterostomum minimum* from the same host, but the position of the testes and the extent of the uterus are different.

It is a small plump form reaching a length of a little over 1 mm. The outline is oval and the maximum breadth is about half the length. The anterior sucker is terminal and is surrounded by a circle of six highly contractile tentacles. When extended these tentacles may be long and almost filiform. When completely retracted they are almost impossible to discern. In a semi-contracted state they appear as small, fleshy, knob-like protuberances.

The ovary lies on the right side of the pharynx, which is situated about the middle of the body. The testes lie directly behind the pharynx. They are oblique and overlap each other considerably. The uterus fills up a large part of the body, extending forward as far as the level of the anterior sucker. The ova measure ·022–·024 × ·013–·015 mm.

Genus RHIPIDOCOTYLE Diesing.

Rhipidocotyle minima (Wagener).

=*Gasterotomum triglae* (van Ben.), Nicoll.

This species was met with in the intestine of the gurnards, *Trigla pini*, *T. gurnardus*, and *T. hirundo*. In the last-named it occurred also in the pyloric caeca. The parasite was not found in the half-dozen specimens of *Trigla lyra* which were examined.

The only note which may be added to my previous description (Nicoll, 1909, pp. 23–4) is that in this species the excretory vesicle is of great length and extends a considerable distance in front of the ventral sucker. It even reaches further forward than the fundus of the stomach.

Several specimens of what appeared to be this species were met with in the intestine of *Trachinus vipera*. They agree in every particular except that the pharynx is situated further back. It is constantly behind the middle of the body, and may even be found as far back as the anterior end of the cirrus-pouch. Its relation to the other organs is consequently very variable. Sometimes it is on the level of the anterior testis. The only other noticeable feature is that the anterior sucker and the pharynx are less unequal in diameter than is the case in typical

examples of *G. triglae*. In consequence of the great variation which undoubtedly occurs it is not thought advisable to regard this as a distinct species.

Rhipidocotyle viperae (van Ben.) (Fig. 4).

I am identifying as this species a few Gasterostomes which were obtained from the intestine of *Trachinus draco*. It is a form which resembles *G. triglae* in general appearance, but which differs from it in

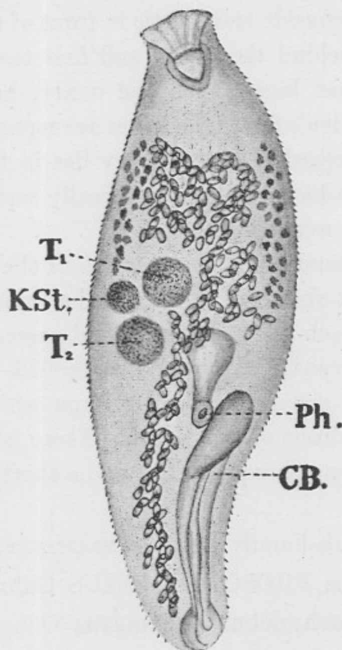

Fig. 4.—*Rhipidocotyle viperae*. Ventral view ×85. C.B. Cirrus-pouch; K.St. Ovary; Ph. Pharynx; T. Testes. *G. Roberts del.*

having the genital glands arranged differently and in having a long and slender cirrus-pouch which extends forward to near the middle of the body.

The length of mature specimens is ·7–1·2 mm., and the maximum breadth in the middle of the body is about ·4 mm. The anterior end is square-cut and the posterior end pointed. The whole surface of the body is beset with minute spines.

At the anterior end there is a shallow sucker measuring, in the largest specimen, ·13 mm. in diameter. Its musculature is very feebly developed. Surmounting the sucker is a five-rayed fan-shaped structure, closely

resembling the corresponding structure in *R. minimum*. This is not represented in van Beneden's figure.

The pharynx (ventral sucker) is situated ·8 mm. from the anterior end of the body. Its diameter is about ·055 mm. The short intestinal sac is obscured by the uterus.

The genital glands lie close together on the right side a short distance in front of the mouth. The ovary is about ·6 mm. from the anterior end of the body and lies close up against the side. It is globular and has a diameter of ·1 mm. The testes are contiguous to it and to each other, the first testis lying alongside and a little in front of the ovary, the second testis lying directly behind the ovary and first testis. Both testes are globular and somewhat larger than the ovary, having a diameter of ·12 mm. In van Beneden's figure the testes are separated by the pharynx, and what might be regarded as the ovary lies in front. It is possible, however, that that problematic structure really represents the intestinal sac, in which case the ovary is not shown.

The yolk glands extend along the margins of the body from the level of the ovary to a point about ·25 mm. from the anterior end. The uterus fills up the space between the yolk glands and passes backwards between the testes and the pharynx. The ova measure ·036–·037 × ·018–·021 mm.

The cirrus-pouch is a long slender structure which extends forwards to a short distance in front of the mouth. There is a fairly large simple vesicula seminalis, a long pars prostatica and a short ductus.

Sub-Family Prosorhynchinae.

Genus PROSORHYNCHUS Odhner.

Prosorhynchus squamatus Odhner.

Odhner, 1905, pp. 297–304 ; Lebour, 1908, pp. 21–3.

This was found on two occasions in the duodenum of *Cottus bubalis*, the first time in large numbers, the second time as a single specimen.

Prosorhynchus aculeatus Odhner.

Nicoll, 1910, pp. 350–2.

This common parasite of the conger was found in three out of four specimens of the fish.

Prosorhynchus crucibulum (Molin).

Nicoll, 1910, pp. 352–4.

This was found in association with *P. aculeatus* and quite as frequently.

Prosorhynchus triglae sp. inq. (Fig. 5).

Single specimens of this species were found on two occasions, once in the intestine and once in the stomach of *Trigla gurnardus*. Both specimens were quite immature, so that it is impossible to give a complete description of the species, which appears to be hitherto unrecorded. It measures 2·2–2·4 mm. in length and ·75 mm. in maximum breadth. At the anterior end there is a wedge-shaped rostellum resembling that in

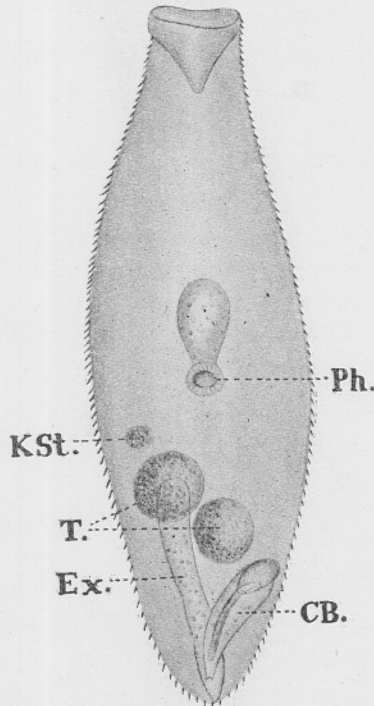

FIG. 5.—*Prosorhynchus triglae.* Ventral view × 40. C.B. Cirrus-pouch ; Ex. Excretory vesicle ; K.St. Ovary ; Ph. Pharynx ; T. Testes. *G. Roberts del.*

P. crucibulum (diameter ·3 mm.). The small ventral sucker (pharynx) is situated nearly in the middle of the body (diameter ·1 mm.).

The intestinal sac extends forwards and is not of very large size. The excretory vesicle extends about one-half or two-thirds the distance between the posterior end of the body and the pharynx.

The cirrus-pouch is about half the length of the excretory vesicle. The testes are fairly large, close together and nearly tandem. The posterior (left) testis is contiguous with the cirrus-pouch in one and displaced to the left in the other.

The small ovary lies immediately in front of the anterior testis on the right side. The yolk glands were not distinct.

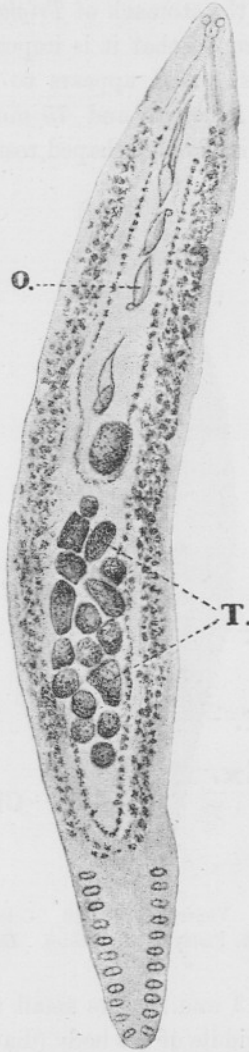

Fig. 6.—*Microcotyle draconis.* Ventral view × 25 (Specimen from Aberdeen). O. Ova; T. Testes. *M. Rhodes del.*

MONOGENEA.

The number of ectotrematodes collected was not very large, and they were all, with perhaps one exception, common and well-known species. *Axine belones*, *Octobothrium merlangi* and *Octocotyle scombri* were found on the gills of *Belone vulgaris*, *Gadus merlangus* and *Scomber scombrus*

respectively. *Pseudocotyle squatinae* was found on the skin of *Rhina squatina*, and *Calicotyle Kroyeri* was met with in the cloaca of the three rays, *Raja circularis*, *R. maculata* and *R. clavata*. A species of *Microcotyle* was obtained from the gills of *Trachinus draco*. Two specimens only were present, and they were not quite mature. Their posterior end was provided with seven suckers on each side. Some similar specimens were collected from the same host at Aberdeen (Fig. 6). They were larger and apparently fully mature. In them the posterior end had eleven pairs of suckers. I am unable to decide whether these two forms are identical or not, and whether both are identical with the *Microcotyle draconis* obtained by Briot (1904) from the same host. As Briot's work is not accessible to me I am unable to make any comparison of these forms. The probability is that my specimens are identical with those of Briot.

Descriptions of *Calicotyle Kroyeri* and *Octobothrium merlangi* are to be found by Lebour (1908, pp. 49 and 50). A description of *Axine belones* will be found by Scott (1911, p. 69).

LIST OF FISHES EXAMINED AT PLYMOUTH, WITH THE TREMATODE PARASITES COLLECTED FROM THEM.

1. *Labrax lupus.*

Cainocreadium labracis.	Intestine.
Acanthochasmus imbutiformis.	Intestine.
Bucephalus minimus.	Intestine.

2. *Serranus cabrilla.*

Helicometra pulchella.	Intestine.
Lecithaster gibbosus.	Rectum.

3. *Mullus barbatus.*

Derogenes varicus.	Stomach.

4. *Sparus centrodontus.*

Steringotrema pagelli.	Intestine.
Zoonogenus vividus.	Rectum.
Derogenes varicus.	Stomach.
Hemiurus communis.	Stomach.

5. *Trachurus trachurus.*

Tergestia laticollis.	Rectum.
Derogenes varicus.	Stomach.
Lecithaster gibbosus.	Intestine.
Hemiurus communis.	Intestine.

6. *Capros aper.*

Pharyngora bacillaris.	Intestine.
Derogenes varicus.	Stomach.
Hemiurus communis.	Stomach.
Hemiurus ocreatus.	Stomach.

7. *Scomber scombrus.*

Pharyngora bacillaris.	Intestine.
Hemiurus ocreatus.	Stomach.
Lecithocladium excisum.	Stomach.
Octocotyle scombri.	Gills.

8. *Zeus faber.*

Stephanochasmus cesticillus.	Intestine.
Zoogonoides viviparus.	Rectum.
Derogenes varicus.	Stomach.
Lecithaster gibbosus.	Rectum.
Synaptobothrium caudiporum.	Stomach.

9. *Trachinus draco.*

Derogenoides ovacutus.	Stomach.
Derogenes varicus.	Stomach.
Rhipidocotyle viperae.	Intestine.
Microcotyle draconis (?).	Gills.

10. *Trachinus vipera.*

Derogenes varicus.	Intestine.
Lecithaster gibbosus.	Intestine.
Rhipidocotyle minimum.	Intestine.

11. *Lophius piscatorius.*

Derogenes varicus.	Œsophagus.
Hemiurus communis.	Stomach.
Lecithochirium rufoviride.	Stomach.
Synaptobothrium caudiporum.	Stomach.
Bucephalopsis gracilescens.	Intestine and coeca.

12. *Cottus bubalis.*

Podocotyle atomon.	Intestine.
Derogenes varicus.	Stomach.
Hemiurus communis.	Stomach and intestine
Prosorhynchus squamatus.	Duodenum.

13. *Agonus cataphractus.*

Derogenes varicus.	Caeca and intestine.

Host / Parasite	Location
14. *Callionymus lyra.*	
Lebouria varia.	Intestine.
Zoogonoides viviparus.	Rectum.
Derogenes varicus.	Mouth.
15. *Trigla pini.*	
Helicometra pulchella.	Caeca.
Derogenes varicus.	Stomach.
Lecithaster gibbosus.	Stomach.
Hemiurus communis.	Stomach.
Rhipidocotyle minima.	Intestine.
Phyllocotyle gurnardi.	Gills.
16. *Trigla gurnardus.*	
Derogenes varicus.	Stomach.
Hemiurus communis.	Stomach.
Rhipidocotyle minima.	Intestine.
Prosorhynchus triglae.	Stomach and intestine.
17. *Trigla hirundo.*	
Derogenes varicus.	Stomach and mouth.
Synaptobothrium caudiporum.	Stomach and mouth.
Rhopidocotyle minima.	Intestine and caeca.
18. *Cyclopterus lumpus.*	
Pharyngora bacillaris.	Intestine and caeca.
Derogenes varicus.	Stomach.
19. *Cyclogaster montagui.*	
Podocotyle atomon.	Intestine.
20. *Gobius ruthensparri.*	
Podocotyle atomon.	Intestine.
21. *Gobius paganellus.*	
Helicometra pulchella.	Intestine and stomach.
Hemiurus communis.	Stomach.
22. *Blennius gattorugine.*	
Helicometra pulchella.	Intestine and stomach.
Zoogonoides viviparus.	Rectum and intestine.
Monorchis monorchis.	Stomach.
23. *Blennius ocellaris.*	
Steringotrema divergens.	Duodenum.
Zoogonoides viviparus.	Rectum.
Derogenes varicus.	Mouth.

24. *Blennius pholis.*

Peracreadium genu.	Intestine.
Helicometra pulchella.	Intestine.
Lecithochirium rufoviride (larva).	Encysted in viscera.
Synaptobothrium caudiporum (larva).	Encysted in viscera.

25. *Centronotus gunnellus.*

Podocotyle atomon.	Intestine.

26. *Mugil chelo.*

Haploporus benedeni.	Intestine.
Saccocoelium obesum.	Intestine.

27. *Atherina presbyter.*

(Podocotyle) atherinae.	Intestine.
Bacciger bacciger.	Stomach.

28. *Gastraea spinachia.*

Podocotyle atomon.	Intestine.
Podocotyle reflexa.	Intestine.

29. *Lepadogaster gouanii.*

Helicometra pulchella.	Intestine.
Hemipera ovocaudata.	Stomach.
Hemiurus communis.	Rectum.

30. *Labrus berggylta.*

Peracreadium genu.	Rectum.
Peracreadium commune.	Intestine.
Lebouria alacris.	Intestine.
Helicometra pulchella.	Intestine.
Lepidauchen stenostoma.	Intestine.
Synaptobothrium caudiporum (larva).	Encysted in intestinal wall.

31. *Labrus mixtus.*

Helicometra pulchella.	Intestine.

32. *Crenilabrus melops.*

Peracreadium commune.	Intestine.
Lebouria alacris.	Intestine.
Synaptobothrium caudiporum (larva).	Encysted in intestinal wall.

33.	*Centrolabrus rupestris.*	
	Lebouria alacris.	Intestine.
	Helicometra pulchella.	Intestine.
34.	*Centrolabrus exoletus.*	
	Lebouria alacris.	Intestine.
35.	*Gadus luscus.*	
	Stephanochasmus caducus.	Intestine and coeca.
	Derogenes varicus.	Stomach.
	Hemiurus communis.	Stomach.
36.	*Gadus minutus.*	
	Stephanochasmus caducus.	Caeca.
	Derogenes varicus.	Stomach.
	Hemiurus communis.	Stomach.
37.	*Gadus merlangus.*	
	Podocotyle atomon.	Caeca.
	Stephanochasmus pristis.	Duodenum.
	Pharyngora bacillaris.	Caeca.
	Derogenes varicus.	Stomach.
	Lecithaster gibbosus.	Intestine.
	Hemiurus communis.	Stomach.
	Hemiurus ocreatus.	Stomach.
	Octobothrium merlangi.	Gills.
38.	*Gadus pollachius.*	
	Lepipapedon rachion.	Intestine.
	Derogenes varicus.	Stomach.
	Hemiurus communis.	Stomach.
	Hemiurus ocreatus.	Stomach.
39.	*Molva molva.*	
	Derogenes varicus.	Stomach.
	Hemiurus communis.	Stomach.
40.	*Onos mustela.*	
	Podocotyle reflexa.	Intestine, caeca and stomach.
41.	*Onos tricirratus.*	
	Derogenes varicus.	Stomach.
42.	*Ammodytes lanceolatus.*	
	Hemiurus communis.	Stomach.

Host / Parasite	Location
43. *Bothus maximus.*	
Derogenes varicus.	Stomach.
44. *Pleuronectes flesus.*	
Podocotyle atomon.	Intestine.
Derogenes varicus.	Intestine.
45. *Pleuronectes limanda.*	
Steringotrema cluthense.	Duodenum.
Zoogonoides viviparus.	Rectum and intestine.
Derogenes varicus.	Stomach and intestine.
46. *Pleuronectes microcephalus.*	
Steringotrema cluthense.	Duodenum.
Zoogonoides viviparus.	Rectum and intestine.
47. *Pleuronectes platessa.*	
Zoogonoides viviparus.	Rectum.
48. *Zeugopterus punctatus.*	
Helicometra pulchella.	Intestine.
Hemiurus communis.	Stomach.
49. *Zeugopterus norvegicus.*	
Podocotyle atomon.	Rectum.
Lecithaster gibbosus.	Intestine.
50. *Solea vulgaris.*	
Zoogonoides viviparus.	Intestine.
Derogenes varicus.	Stomach and intestine.
51. *Solea variegata.*	
Zoogonoides viviparus.	Rectum and intestine.
52. *Salmo trutta.*	
Bunodera nodulosa.	Intestine.
Derogenes varicus.	Stomach.
53. *Clupea pilchardus.*	
Hemiurus ocreatus.	Stomach.
54. *Belone vulgaris.*	
Axine belones.	Gills.
55. *Anguilla vulgaris.*	
Podocotyle atomon.	Intestine.
Helicometra pulchella.	Intestine.
Lecithochirium rufoviride.	Stomach.

56. *Conger conger.*

Helicometra pulchella.	Intestine.
Derogenes varicus.	Stomach.
Lecithochirium rufoviride.	Stomach.
Prosorhynchus aculeatus.	Intestine.
Prosorhynchus crucibulum.	Intestine.

57. *Syngnathus acus.*

Podocotyle syngnathi.	Intestine.

58. *Nerophis aequoreus.*

Podocotyle atomon.	Intestine.
Podocotyle syngnathi.	Intestine.
Hemiurus communis.	Intestine.

59. *Siphonostoma typhle.*

Podocotyle syngnathi.	Intestine.
Acanthochasmus imbutiformis (larva).	Encysted in gills.

60. *Raja circularis.*

Calicotyle kroyeri.	Rectum.

61. *Raja maculata.*

Calicotyle kroyeri.	Cloaca.

62. *Raja clavata.*

Calicotyle kroyeri.	Rectum.

63. *Mustelus vulgaris.*

Ptychogonimus megastomus.	Stomach.

64. *Rhina squatina.*

Pseudocotyle squatinae.	Skin.

REFERENCES.

1. van Beneden, P. J. 1871. "Les Poissons des Côtes de Belgique, leurs parasites et leurs commensaux" in *Mem. Acad. Roy. d. sc. de Belg., Bruxelles*, XXXVIII, pp. 1–100.

2. Diesing, K. M. 1855. "Revision der Cercarieen" in *Sitzungsber d. k. Akad. d. Wissenschaft, Math. Naturwissensch. kl.*, XV, pp. 377–40.

3. Diesing, K. M. 1858. "Revision der Myzhelminthen. Abt. Trematoden," *ibid.*, XXXII, pp. 207–90.

4. Jacoby, S. 1899. "Beiträge zur Kenntnis einiger Distomen," *Archiv f. Naturgeschichte*, LXVI, pp. 1–30.

5. Jägerskiöld, L. A. 1900. "Ein neuer Typus von Copulationsorgan bei Distomum megastomum" in *Centralbl. f. Bakt. und Parasitenkunde*, Abt. 1, XXVII, pp. 68–74.

6. Johnstone, J. 1906. "Internal Parasites and diseased conditions of fishes" in *Trans. Biol. Soc. Liverpool*, XX, pp. 151–85.

7. Johnstone, J. 1907. "Internal Parasites and diseased conditions of fishes" in *Trans. Biol. Soc. Liverpool*, XXI, pp. 170–203.

8. Johnstone, J. 1908. "Redescription of a Trematode Parasite, *Allocreadium labracis* (Dujardin) from the Bass" in *Trans. Biol. Soc. Liverpool*, XXII, pp. 44–53.

9. Lebour, M. V. 1908. "Fish Trematodes of the Northumberland Coast" in *Northumberland Sea Fisheries Report* for 1907, pp. 11–67.

10. Linton, E. 1910. "Helminth fauna of the Dry Tortugas. II Trematodes" in *Publication No.* 133 *Carnegie Institute of Washington*, 1910, pp. 11–98.

11. Looss, A. 1894. "Die Distomen unserer Fische und Frösche," *Bibliotheca zoologica*, 1894, Heft 16, pp. 1–296.

12. Looss, A. 1901. "Ueber die Fasciolidengenera Stephanochasmus, Acanthochasmus und einige andere" in *Centralbl. f. Bakt. und Parasitenkunde*, Abt. 1, XXIX, pp. 595–606, 628–34, 654–61.

13. Looss, A. 1902a. "Die Distomen unterfamilie der *Haploporinae*" in *Arch. de Parasitologie*, VI, pp. 129–43.

14. Looss, A. 1902b. "Zur Kenntnis der Trematoden-fauna des Triester Hafens II, Ueber *Monorchis* Montic. und Haplosplanchnus n.g." in *Centralbl. f. Bakt. und Parasitenkunde*, Abt. 1, Orig. XXXII, pp. 115–22.

15. Looss, A. 1907. "Beiträge zur Systematik der Distomen. Zur Kenntnis der Familie Hemiuridae" in *Zoolog. Jahrbücher*, Abt. f. Syst., XXVI, pp. 63–180.

16. Nicoll, W. 1907. "A Contribution towards a knowledge of the Entozoa of British Marine Fishes, Part I," in *Ann. Mag. Nat. Hist.*, Ser. 7, Vol. XIX, pp. 66–94.

17. Nicoll, W. 1909. "A contribution towards a knowledge of the Entozoa of British Marine Fishes, Part II," in *Ann. Mag. Nat. Hist.*, Ser. 8, IV, pp. 1–25.

18. Nicoll, W. 1910. "On the Entozoa of Fishes from the Firth of Clyde" in *Parasitology*, III, pp. 322–59.

19. Nicoll, W. 1912. "On two new Trematode Parasites from British Food Fishes" in *Parasitology*, V, pp. 197–202.

20. Nicoll, W. 1913a. "New Trematode Parasites from Fishes of the English Channel" in *Parasitology*, V, pp. 238–46.

21. Nicoll, W. 1913b. "Trematode Parasites from Food Fishes of the North Sea" in *Parasitology*, VI, pp. 188–94.

22. Odhner, T. 1902a. "Mitteilungen zur Kenntnis der Distomen," I, in *Centralbl. f. Bakt. und Parasitenkunde*, Abt. 1, Orig. XXXI, pp. 58–69.

23. Odhner, T. 1902b. "Mitteilungen zur Kenntnis der Distomen," II, in *Centralbl. f. Bakt. und Parasitenkunde*, Abt. 1, Orig. XXXI, pp. 152–62.

24. Odhner, T. 1905. "Die Trematoden des arktischen Gebietes" in *Fauna Arctica*, IV, pp. 291–372.

25. Odhner, T. 1911. "Zum naturlichen System der digenen Trematoden," III, in *Zool. Anzeig.*, XXXVIII, pp. 97–117.

26. Scott, T. 1911. "Notes on some Trematode Parasites of Fishes" in *28th Ann. Rept. Fishery Board Scotland*, pp. 68–72.

27. Stossich, M. 1889. "Brani di elmintologia tergestina," VI, in *Boll. Soc. Adriat. sc. Nat. Trieste*, XI, pp. 1–8.

28. Wagener, G. R. 1852. "Enthelminthica No. 3; Ueber *Distoma dimorphum* Diesing, *Distoma marginatum* Rud." in *Arch. f. Anat., Physiol. und Wissensch. Med., Berlin*, 1852, pp. 555–69.

On a Hermaphrodite Specimen of Amphioxus with Notes on Experiments in Rearing Amphioxus.

By

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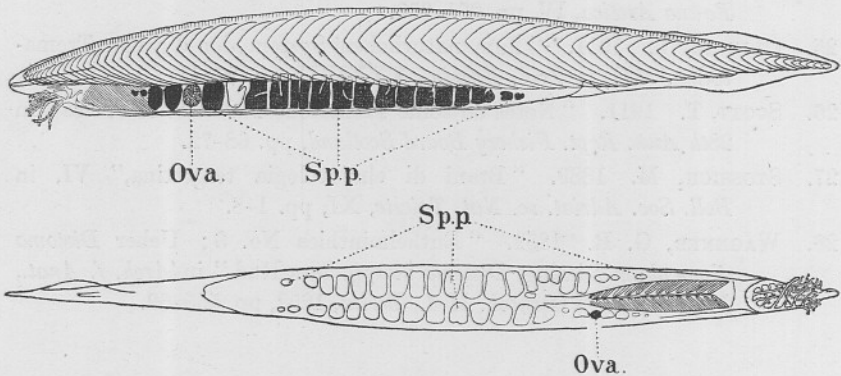
With Figures 1–5 in the Text.

A SPECIMEN of *Amphioxus lanceolatus* containing both sperm and eggs was observed in June this year whilst experiments were being conducted on obtaining the larvæ of this animal. The individual in which this phenomenon was observed is predominantly male, and was actually seen to discharge a large amount of living spermatozoa, but of the forty-three



FIGS. 1 and 2.—*Views of a hermaphrodite specimen of Amphioxus from the left side and the ventral region respectively. Ova are present in one gonadial pouch only, while all the remaining pouches contain sperm. (Drawn from the whole animal stained in Delafield's hæmatoxylin and cleared in cedar-wood oil, × about 2½.) Sp.p. Gonadial pouches full of sperm.

large gonadial pouches present in the animal one only contains eggs and the remainder sperm. On the right side of the body are twenty-two well-developed gonadial pouches, two of which became nearly empty by the discharging of spermatozoa; the remainder were full of sperm when the animal was preserved. On the left are nineteen pouches full of sperm and one is nearly empty, but one pouch, the fifth from the anterior end,

* I am indebted to Mrs. Orton for the drawings for Figs. 1 and 2, and for assistance with that for Fig. 3; and also to Mr. E. Ford for the lettering of the Figures.

is full of eggs (see Figs. 1 and 2). The relation of the pouches on the right side to those on the left appears (as may be seen from Fig. 2) to be normal. The smaller pouches have been reduced somewhat in size from the loss of sperm referred to above. A few other rudimentary microscopic pouches are present in the posterior region of the body.

FIG. 3.

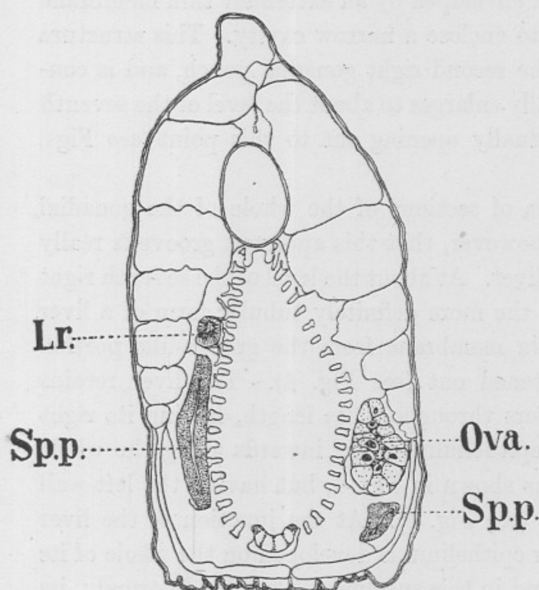


FIG. 4.

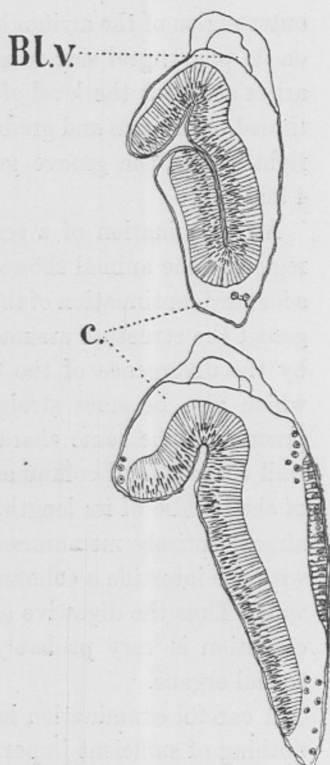


FIG. 5.

FIG. 3.—Transverse section through a hermaphrodite specimen of *Amphioxus lanceolatus* showing gonadial pouches with sperm or ova and the anterior end of the abnormal liver. Ova are present in one pouch only.
Sp.p. Gonadial pouches filled with sperm.
Lr. Anterior prolongation of liver.

FIG. 4.—Transverse section of liver at the level of the sixth right gonadial pouch (semi-diagrammatic).
Bl.v. Blood vessel in cœlomic space surrounding the liver.
C. Cavity of liver.

FIG. 5.—Transverse section of liver at the level of the thirteenth right gonadial pouch (semi-diagrammatic).

In transverse sections the gonadial pouch bearing eggs is seen to have the primary and secondary cavities normally shown by the female

gonads (see Fig. 3), and the invaginated form of the pouch described by Legros * is also well shown in some sections (but is not shown in Fig. 3). Thus the structure of this pouch is of the normal female plan.

The transverse section shown in Fig. 3 shows also an abnormal feature of much interest. On the right side (the reader's left) in the dorsal region of the atrial cavity is a structure resembling a groove opening on to the outer region of the atrium but enveloped by an extremely thin membrane on its pharyngeal side so as to enclose a narrow cavity. This structure arises at about the level of the second right gonadial pouch, and is continued backwards and gradually enlarges to about the level of the seventh right gonad, the groove gradually opening out to this point (see Figs. 4 and 5).

An examination of a series of sections of the whole of the gonadial region of the animal shows, however, that this apparent groove is really a forward continuation of the liver. At about the level of the seventh right gonad the structure assumes the more definitely tubular form of a liver by the divergence of the thin membrane from the groove-like portion which also becomes straightened out (see Fig. 4). The liver retains practically the same characters throughout its length, having its right wall composed of columnar epithelium tucked inwards along the whole of the middle of its length, as shown in Fig. 4, but having the left wall almost entirely membranous (see Fig. 5). At the junction of the liver with the intestine a columnar epithelium is developed on the whole of its wall. Thus the digestive gland in this specimen is highly abnormal ; its condition is very probably correlated with the abnormal state of the sexual organs.

A careful examination has been made of the tissues for parasites, but nothing of sufficient importance has so far been detected to confirm the suggestion that the hermaphroditism may be due to the influence of parasites. A fair number of small nucleated spherical bodies are present in the cavity of the liver, and bodies are present in some of the abnormal intuckings of the intestinal wall which may be parasites, but the methods of staining employed up to the present have certainly not disclosed such an extensive invasion of foreign bodies in the tissues as perhaps one might expect to find in an animal whose sex is abnormally changed by an infection of parasites.

The occurrence of hermaphrodite specimens of Amphioxus has been recorded previously on only two occasions so far as I know. In 1876

* M. Legros, " Sur la Morphologie des Glandes sexuelles de *l'Amphioxus lanceolatus*," *Comptes-Rendus du Troisième Congrès Internationale de Zoologie*, Leyden, 1895.

Langerhans stated* that he had detected the tails of spermatozoa among young ovarian ova of Amphioxus, and recently in 1912 Goodrich† described a more definitely hermaphrodite specimen from Naples which closely resembles the one obtained here at Plymouth in having only one gonadial pouch producing eggs—the ninth on the left side—but with the remaining forty-nine pouches full of spermatozoa.

Goodrich has already pointed out that hermaphroditism would appear to be a rare phenomenon in Amphioxus since large numbers have been and are being carefully examined. It occurred to me, however, that possibly the hermaphroditism might be more common in Amphioxus than would appear to be in the case, for after the discharge of the gonadial products the gonadial pouches contract into a very small compass, and presumably a fresh set of gonadial products are formed subsequently. Thus it would be possible for a given individual Amphioxus to have two different sets of gonads, first of one sex and afterwards of the other. Moreover, such a change would probably not be detected unless a number of Amphioxus were reared in captivity from the young to the adult stage. In this way the chance observations referred to above of two males each with one egg-bearing pouch may possibly indicate an unsuspected change of sex in the life-history of the animal. If such a change were to occur the occurrence of egg-bearing pouches in a male might be an expression of a precocious development of female characteristics. A further indication that a change of sex would be in the direction of male to female lies in the fact that the hermaphrodite forms are both of medium size: Goodrich's specimen being about 4·7 cms. long, and mine measured 4·4 cms. in cedar-wood oil. It is very probable that the animals become mature for the first time at about this size.

On the view, however, that there is a sex-change in Amphioxus one would expect all the young individuals to be of the same sex, but the researches of three different investigators, namely, Legros (l.c.), Neidert and Leiber,‡ and Zarnik,§ show that both male and female gonads have been traced in very small individuals. Hence it seems certain that there cannot be any total sex-change in Amphioxus; but there still remains

* P. Langerhans, *Archiv f. Mikr. Anat.*, Bd. XII, 1876, p. 326.

† E. S. Goodrich, "A Case of Hermaphroditism in Amphioxus," *Anat. Anz.*, 42 Bd., 1912.

‡ L. Neidert u. A. Leiber, "Über Bau und Entwicklung der Weiblichen Geschlechtsorgane des Amphioxus," *Zool. Jahrb.*, Bd. XVIII, 1903.

§ B. Zarnik, "Uber die Geschlechtsorgane von Amphioxus," *Zool. Jahrb. Abt. Anat.*, Bd. XXI, 1905.

the possible, but hardly probable, occurrence of a sex-change in only some individuals.

It would therefore appear that these occasional hermaphrodites in Amphioxus must remain for the present unexplained like the similar and not uncommon phenomena among many fishes.* The abnormality of the liver and the intestine as well as that of the sexual organs points, however, to some deep-seated disorganisation in the economy of the animal, the cause of which I have not been able to detect.

NOTES ON EXPERIMENTS ON REARING AMPHIOXUS.

A few experiments were carried out this summer with the object of obtaining the larvæ of Amphioxus. As two of the experiments were successful, and as the larvæ of Amphioxus have apparently not been obtained at Plymouth before, these notes may be useful to future workers.

Larvæ of Amphioxus were obtained on two occasions on June 10th and June 15th by merely isolating a number of adult males and females in a small glass bowl. The adult specimens were examined under a microscope when brought in and a few mature males put into bowls in company with mature females. It is possible to distinguish the adult sexes in the living condition, and indeed the hermaphrodite specimen described in the preceding pages was identified as such while alive. It was found advantageous to feed the Amphioxus on a fairly thick culture of the diatom Nitzschia which the animals ingest in great numbers. By gorging themselves with this food the Amphioxus are probably able to extrude eggs or sperm more easily than when they are empty.

Spawning apparently occurs usually overnight, as gastrulæ were obtained in both experiments about midday. It is not easy to calculate the time of spawning from the known age of gastrulæ reared at Naples, since the rate of development of larvæ at Plymouth is undoubtedly slower than at Naples, as was shown by subsequent observations. Since, however, gastrulæ were obtained about noon it seems likely that spawning had occurred sometime about midnight. In this respect it is interesting to observe that the hermaphrodite specimen just described was

* With regard to the occasional hermaphrodites among fishes it may be remarked that it is highly important to know the size and also the age of specimens—which are usually omitted from descriptions—if the data are to be useful for investigating the life-history of the fish.

observed to spawn during the day,* but it is probable that this spawning may have been abnormal and induced by the animal having jumped out of a bowl and remained dry on the bench for a little while.

In the second experiment in which fertilised eggs were obtained the embryos had reached the blastula and gastrula stages by 1.0 p.m. At 6.0 p.m. on the same day, June 15th, the gastrulæ began to elongate, and by 9.0 p.m. two to three mesoblastic somites were developed and the larvæ were beginning to find their way out of their fertilization membranes. On June 16th at 2.5 p.m. the larvæ had developed six to eight pairs of mesoblastic somites and the head cavities. On the 17th at 2.30 p.m. the larvæ reached a stage similar to Hatschek's Fig. 64,† in which the club-shaped gland is present. At Naples this stage is reached at an age of about 36 hours, whereas the Plymouth embryos only reached the corresponding stage at an age of about 60 hours. During subsequent days the larvæ increased a little in length and were observed to be feeding, but even on the 30th of the month when the larvæ were a fortnight old only the first few gill-slits had appeared, and shortly afterwards it was unfortunately necessary to abandon the larvæ.

In preserving batches of larvæ it was observed that the individual of the latter stages became stuck to the bottom of the vessel in the head region. This circumstance seems to point to the possibility of the club-shaped gland pouring out some secretion to the exterior, since this gland is the only organ developed at this stage ; and since moreover, according to Willey,‡ " this stage of the larval development appears to be of the nature of a *resting phase*, during which the larvæ accumulates energy for future growth," it may be that a secretion of the club-shaped gland serves to attach the larva temporarily to objects during this resting stage, or to suspend the larva in the water (see Willey, l.c., p. 130). These are, however, merely suggestions, which nevertheless might well repay further investigation by naturalists who may have the opportunity of doing so.

It may further be noted that the opening of the club-shaped gland is on the left side ; hence if a secretion of this gland is used for attaching the larva temporarily to objects, then the larva would be able to feed only from the right side of the body. As is well known, the first formed gill-slits do develop on the right side of the body and afterwards shift over

* The actual time was not recorded, but it was some time between 11 a.m. and 4.30 p.m.

† B. Hatschek, " Entwicklung des Amphioxus," *Arb. Zool. Inst. Wien.*, 1881, Vol. IV.

‡ A. Willey, *Amphioxus and the Ancestry of the Vertebrates*, 1894, p. 172.

to the left after the disappearance of the club-shaped gland. It is therefore possible that the larval asymmetry of Amphioxus may be correlated with the function of the club-shaped gland, and if the suggestion here made that this gland may secrete a substance for attaching the larva temporarily to objects is found to be a fact, a simple explanation similar to that put forward by Korscheldt and Heider is offered of the curious asymmetry in the early larval development of this interesting animal.

SUMMARY.

A hermaphrodite specimen of Amphioxus has been taken at Plymouth having one gonadial pouch filled with ova and the remaining pouches filled with sperm. This specimen closely resembles a similar one taken by Goodrich at Naples.

The liver and intestine of the Plymouth specimen are abnormal, but no parasites have been identified in the tissues to account for these abnormalities.

It is, moreover, improbable that there is any normal sex-change in Amphioxus, since three independent investigators have found very small specimens of both sexes, therefore no satisfactory explanation can be given of the occurrence of hermaphroditism in the specimen.

Amphioxus have been found to spawn in June, and larvæ have been obtained from the captive specimens.

It is suggested that the club-shaped gland may secrete a substance for attaching the larva of Amphioxus to objects, and that this function may be correlated with the asymmetry shown in the early development of the Amphioxus larva.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Influence of the Position of the Cut upon Regeneration in *Gunda ulvæ*. By Dorothy Jordan Lloyd, B.Sc. (*Proc. Roy. Soc.*, B, Vol. XXVII, 1914.)

In 1889 Hallez published a paper in the *Comptes Rendus* in which he stated that any fragment from a Triclad could regenerate completely, while from a Polyclad, only those fragments could do so which contained a portion of the central nervous system. The paper quoted above on the regeneration of *Gunda ulvæ*, a marine Triclad common at Plymouth, shows that this generalization is not of universal application, since in *G. ulvæ* regeneration of the anterior end is found to be dependent, as in Polyclad, on the presence of the central nervous system.

Posterior, anterior, and lateral regeneration are considered separately. Posterior regeneration, i.e. regeneration of any structure lying behind the brain, is found to take place equally well in the presence or absence of the cerebral ganglia. Lateral regeneration, in order to be complete, requires the presence of one intact ganglion. If only part of a ganglion is present, heads regenerate but are abnormal. If both ganglia are absent lateral regeneration only takes place behind the level of the ganglia. Anterior regeneration never occurs except in the presence of at least two-thirds of both ganglia.

The paper also records the formation of heteromorphic heads from short head-pieces of *G. ulvæ* in which the cut has passed transversely across both ganglia.

D. J. L.

The Influence of Osmotic Pressure upon the Regeneration of *Gunda ulvæ*. By Dorothy Jordan Lloyd, B.Sc. (*Proc. Roy. Soc.*, B, Vol. XXVII, 1914.)

The animals used in the experiments recorded in this paper were collected from the shore near the borders of a small stream and between the tide-marks. They were therefore exposed in their natural habitat to a wide diurnal variation of osmotic pressure. Experiments made with whole animals showed that they are capable of living indefinitely in water having an osmotic pressure of more than two and less than thirty-three atmospheres. Experiments on the rate of regeneration of the posterior end only were considered. These showed that the rate of regeneration of the posterior end depends on the osmotic pressure of the medium. This has an optimum value at eighteen atmospheres, and limiting values at five and thirty-five atmospheres.

Restoration of the lost parts in *G. ulvæ* is brought about entirely by the undifferentiated parenchyma cells, which migrate to the region of the wound and form the new tissues. The growth of the new parts is always accompanied by reduction of the old ones. For values of the osmotic pressure lying between the optimum and the limiting values the migration of the parenchyma cells is retarded, and the rate of restoration is retarded to a similar degree. At the limiting values there is no migration and no restoration of lost parts.

G. ulvæ also shows the phenomena of reduction under conditions of starvation. These are (1) absorption of the genital system, (2) general reduction in size. Both of these changes are brought about by the phagocytic action of the parenchyma cells. During regeneration the same reduction processes occur as in starvation. When the restoration of lost parts is retarded, as happens on raising or lowering the osmotic pressure, reduction is retarded to the same extent.

In strongly hypotonic solutions the gut cells increase in size and become vacuolar; in strongly hypertonic solutions they diminish in size and become dense, showing that there has been actual gain or loss of water by the tissues.

D. J. L.

Hydrographical Observations in the Labrador Current in 1913. By Donald J. Matthews. (*Report on the work carried out by the ss. "Scotia," 1913. H.M. Stationery Office, London, 1914.*)

The *Scotia* was sent out by the Board of Trade in the spring of 1913, under the command of Capt. T. Robertson, to make observations on the amount of ice collected in the Labrador Current to the northwards of the liner tracks, which might prove a danger to shipping later in the year. She was fitted with apparatus for hydrographical work to a depth of 550 fathoms and for meteorological and plankton investigations. The scientific staff consisted of Mr. G. I. Taylor (meteorologist), Mr. L. R. Crawshay (biologist), and the writer.

The *Scotia* left Dundee on March 8th, but was much hindered by bad weather, and did not get clear of the Hebrides till March 23rd. She reached St. John's, Newfoundland, on April 14th, having passed a group of bergs on the Flemish Cap.

The *Scotia* left St. John's again on April 23rd, and steamed first southwards to Cape Race and then south-eastwards across the Banks to the deep water. The surface water had a temperature between 1·5° and 0°; in the deep channel under the coast a minimum of —1·5° or less was reached at 40 fathoms and extended to the bottom in 90 fathoms off St. John's; near Cape Race the lower layers were somewhat warmer. This temperature distribution, with a minimum at some intermediate depth, is characteristic of polar waters; it was not found on the Banks. Warm salt water was encountered off the south-eastern edge of the Banks.

The next run was made northwards along the edge of the Banks and then eastwards beyond the Flemish Cap, and large numbers of icebergs were sighted in spite of almost continuous fog. The Labrador Current extended seawards as far as the western edge of the Flemish Cap; eastwards of this the bergs were melting rapidly in relatively warm high salinity water. Between the Cap and the Banks the polar water was underlaid by water with a salinity of over 34, but the vertical changes were irregular.

The *Scotia* then proceeded to Bonavista Bay, where the characteristic minimum, —1·7°, was found at 70 and 100 fathoms. From this point she worked northwards through or along the edge of pack ice to about 54° N. It had been intended to proceed as far as Hamilton Inlet, but a strong northerly gale made this impossible. The pack encountered during the more northern part of the run was very heavy and in places hummocky,

while behind it were countless large bergs driving more slowly before the wind. Observations made on the edge of the pack in about 53° N. showed salinities varying between 33 and 34 down to 100 fathoms, with a temperature minimum of about —1·3° between 25 and 50 fathoms. From 100 fathoms to the bottom in 200 fathoms there was relatively unmixed Atlantic water with salinity and temperature over 34·0 and 0°.

The next run was south-eastwards to the Flemish Cap. The edge of the Labrador Current was passed in about 50½° N., 49½° W., the temperature rising suddenly from 0° to 3° and the salinity from 33·9 to 34·7. Eastwards of the Flemish Cap the salinity and temperature now (May 24th) were much higher than nineteen days earlier, and far fewer bergs were sighted.

On May 28th a buoy fastened to a sinker and drag by 1000 fathoms of piano wire was put over close to a berg on the outer edge of the Labrador Current in about 45½° N., 47° W. The berg was found to be drifting S. 52°E., 0·55 mile per hour, in a smooth sea with scarcely any wind.

The *Scotia* then proceeded to St. John's, passing no more bergs until close under the land.

The second cruise, from June 10th to July 19th, consisted of a series of diagonal courses as far as 44° W., between the parallels of Cape Race and Hamilton Inlet. The finer weather now made scientific work much easier, and a number of vertical series and current measurements were made. The outer boundary of the Labrador Current was clearly defined, at least northwards of the fiftieth parallel, by the isohaline of 34 and a change of temperature of three or four degrees. In conformity with the general rule for oceanic currents it followed the edge of the continental slope closely. Seaward of it the surface temperature and salinity increased eastwards very slowly from 4° to 7° or 8°, and from 34·0 to 34·8. Vertically the water was nearly homogeneous; the temperature fell from 6° or 7° at the surface to 4° at 50 or 100 fathoms, and then very slowly to 3° at 500 fathoms, while the salinity increased from 34·6 or 34·7 to 34·8 or 34·9. Current measurements with a buoy showed almost no motion.

The Labrador Current flowed over the continental shelf where the depths were less than 300 fathoms; it had a salinity of less than 33·5 and a temperature below 0° except where the surface layer had been heated by the sun, and the intermediate temperature minimum was well marked. In places Atlantic water with positive temperatures and salinity over 34 underlay the polar water. Off the Labrador coast the pack had shrunk considerably since the previous run, and now lay westwards of the fifty-

fourth meridian. The effect of the melting of the ice showed itself in the decreased surface salinity.

Current measurements with anchored buoys showed weak and variable currents, and in some places the pack lay in an eddy which was actually moving northwards.

From the Labrador coast the *Scotia* proceeded out to the Flemish Cap and then in to Cape Race. On the Banks the surface temperature was as high as 8°, with less than 0° at from 35 to 50 fathoms, while in the deep channel under the land —0·9° was found at 20 fathoms and —1·70° at 50 fathoms. A buoy was anchored for 26 hours in 100 fathoms off Cape Race, where there is normally a set of about one mile per hour to the south and west; on this occasion the current was found to be setting slowly northwards.

The *Scotia* entered St. John's on July 19th and left again on the homeward voyage on July 24th. A digression was first made for current measurements on the southern part of the Banks. Two complete sets of measurements with the Ekman metre, each lasting about thirteen hours, at 5 fathoms and 25 fathoms, were made in 50° W. at two points about 60 miles apart; the ship was anchored in 30 fathoms in each case. The current was found to be tidal with a slight easterly resultant, which at 5 fathoms in 43° 53′ N. reached 1·4 miles in the course of a tide; at the other positions it was less than 0·5 mile. The direction changed regularly through south to west, north, and east.

The *Scotia* then proceeded to the Flemish Cap and Cape Farewell. The East Greenland pack, with about three small bergs, was followed from 59° 6′ N., 42° 27′ W., to 62° 11′ N., 39° 42′ W. Near the edge of the pack the character of the water changed very rapidly, so that some of the ice was floating in water of nearly 35 salinity, with a temperature of 8°. From this point the *Scotia* turned homewards and reached Dundee on August 24th.

During the year 1913 the ice was as a whole held up to the northwards of the forty-third parallel, and the *Scotia* observations make it seem probable that this was due to an easterly set of water from the region off the Cabot Straits and the coast of Nova Scotia, which covered the southern half of the Newfoundland Banks.

The observations were worked up and the report on the hydrographical work written at the Laboratory of the Marine Biological Association, and I wish to express my thanks to the Council and to Dr. Allen for putting apparatus and a table at my disposal for the purpose.

D. J. M.

Report on the Distribution of the Microplankton. By L. R. Crawshay. (*Report on the work carried out by the ss. "Scotia,"* 1913. (*Pages* 68–126, *Plates* 23–35.) *H.M. Stationery Office, London.* 1914.)

THE purpose of the *Scotia* Expedition to the North Atlantic in 1913 having been to study the movement and distribution of the ice in the region of the Labrador Current, the subject of the Report on the plankton investigations concerns the distribution of the microplankton, especially the Diatoms, as regulated by the Labrador Current on the one hand, and by the warm Atlantic water on the other.

The area of investigation extended roughly between 44° and 55° N., and between about 44° W. and the coastal water to the westward.

The species dealt with are very largely neritic forms, characteristic of the polar water, and the distribution of these as contrasted with that of certain oceanic species, especially *Rhizosolenia styliformis*, was found to conform very closely with the distribution of the Labrador water as ascertained by the hydrographic observations. Among the species considered, a third class is distinguished as "intermediate" species, as including those which possess a wider hydrographic range, and of which the hydrographic relations are of chief importance in their bearing on secondary details. Such importance have the distribution of the Peridinidæ in the month of July, after the decline of the neritic Diatoms, and the distribution of *Ceratium arcticum* as compared with that of *C. longipes*.

The report is divided into two sections, dealing with the surface and vertical distribution respectively of the species considered.

The first section is illustrated by a series of charts showing the distribution, as observed over four periods between April and July, of certain of the more important species. These four periods correspond with those observed by Mr. Matthews in the construction of his charts of the physical conditions, with which the plankton charts may therefore be directly compared.

Up till the end of June, the seaward boundary of the neritic species was found to follow, north of the Grand Banks, approximately the 34·50 isohaline and an isotherm somewhat above 4°. Outside the 34·70 isohaline and the 5° isotherm they were only recorded in a single instance, in an isolated patch of 34·50 water, and the whole of this region was tenanted by an abundance of *Rhizosolenia styliformis*, in company with

other oceanic species. In the region of the Flemish Cap the neritic species were found spreading out more irregularly to the eastward, to a considerable distance outside the Cap, their distribution between the latter point and the eastern edge of the Banks being much confused with that of the oceanic species. Here also the hydrographic limits of the neritic species were about 34·50 and 5°, though the isohalines and isotherms were very irregular in this region. The southern boundary of the neritic species referable to the Labrador water could not be definitely ascertained, but so far as the investigations extended they were found to be confused with oceanic species along a line between Cape Race and the south-eastern border of the Banks from April onwards.

In the beginning of July, the seaward boundary of the neritic species, north of about 50° N., showed only a slightly more westerly position than was observed in the middle and latter part of June. The most important change during this month concerned the entire disappearance of the neritic Diatoms from the surface water of the northern half of the Banks, and for some distance outside their northern edge, the species being nowhere abundant south of 50° N. The Diatoms were found to be superseded by an abundance of Peridinidæ and other forms, notably Tintinnidæ, the species, however, including also *Ceratium arcticum*, which is generally characteristic of the true Labrador water though not confined to it. These changes occurred concurrently with a general rise in the surface temperatures in this region to 6° and over, and a fall in the salinities, at most points, below 33·00. From several vertical series of water samples which Mr. Matthews kindly obtained for me with the water bottles during the working of his stations it was found, at all points investigated on the Banks and a short way to the northward, that the Diatoms were present in an underlying body of cold water, the upper limit of their vertical range varying from 17 to 40 fathoms or more below the surface. At a station off the southern end of the Flemish Cap these conditions did not occur, and the dominating oceanic species *Rhizosolenia styliformis* ranged from 0 to 50 fathoms.

A second point of importance observed in July concerned the zone of transition between the two forms *Ceratium arcticum* on the north, and *C. longipes* on the south. This was found occurring in the latitude of Cape Race, and southwards to near the south-eastern border of the Banks, in a region where in April, as has previously been stated, the neritic Diatoms of the Labrador water were found confused with *Rhizosolenia styliformis* and other oceanic species. *C. longipes* was in fact once recorded in April in the same region, in about 45° N. and 51° W.,

and it is probable that the zone of transition between the two *Ceratium* forms was fairly constant in position on the southern half of the Banks from April onwards. There is little to suggest that the transition represents a gradual change in process between two nearly related forms of possibly the same species, prompted by changes in the physical conditions. The observations lead to the view that the origin of each is entirely distinct, that of *C. arcticum* being in the Labrador water on the north, and that of *C. longipes* being in the south and west, a southerly origin being also ascribed to the oceanic Diatoms that were found present here as early as April.

As has been stated, the limitations of the neritic Diatoms as a whole were found to conform closely with the hydrographic boundaries along the outskirts of the Labrador Current, and only at those points where the salinities and isotherms became irregular, as, especially, in the region of the Flemish Cap, did the former become confused. This point is brought out in the very low average temperatures which species show, as compared with those obtained by the International Investigations in the North European waters. For a large number of "abundant" records, *Thalassiosira Nordenskiöldii*, for example, shows an average temperature of 0·9°, as against an International Investigations average of 5·3°; in *T. gravida* the average is 1·4°, as against 6·3°; in *C. sociale* it is 0·9°, against 3·9°. The sharpness of the limitations in distribution usually occurring in this region seems to be due to the suddenness and extent of the change which at most points occurs between the polar and the Atlantic water. At the surface, wave movement was probably accountable for the fact that frequently, when intersecting the boundary of the polar water, a narrow intervening belt was traversed in which both polar and oceanic Diatoms became almost or entirely absent, the conditions being apparently intolerable to both. In the vertical direction, at positions where different layers of water were superimposed, and where little or no such mixing occurred, the vertical range of species was found so sharply defined that it was measurable within a metre or less.

I am indebted to the Council of the Marine Biological Association and to Dr. Allen for having placed all facilities at my disposal at the Plymouth Laboratory for the examination of the material and the preparation of the report.

L. R. C.

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