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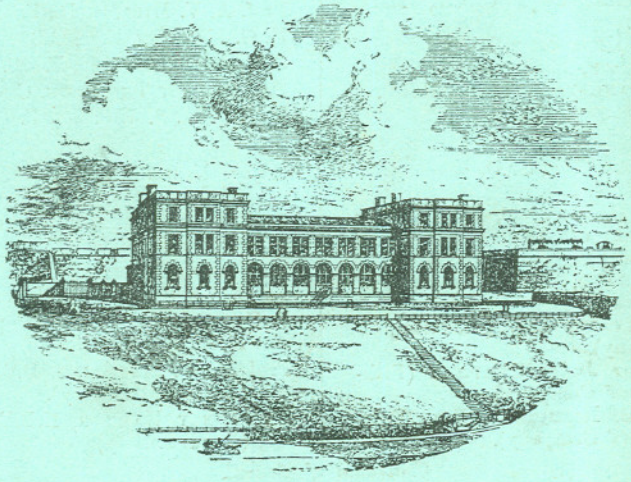

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On the Rate of Growth of *Cardium edule*. Part I. Experimental Observations.

By

J. H. Orton, D.Sc.,

Senior Naturalist at the Plymouth Laboratory.

With 12 Figures in the Text.

CONTENTS

INTRODUCTION

In continuation of the writer's work* on the rate of growth in Invertebrates generally a definite experiment was begun during 1919 to obtain detailed information on the rate of growth of the common cockle, *Cardium edule*. The plan of the experiment was to mark a representative collection of cockles and keep the same individuals under close monthly observation. As the cockle beds near Plymouth are regularly fished, it was regarded as essential that the marked cockles should be kept in a box, which could be closed by a lid in order that there should be no doubt about recovering the same cockles during the period of the experiment. Accordingly a box, 6 feet by 3 feet 8 inches by 1 foot, with an entire boarded bottom, but with the sides and top made of strong perforated zinc, with holes not more than 6 mm. in diameter, was designed and made, and fixed to stakes driven into the bed of the river (see Fig. 1, A and B). A full description of the experimental box is given on page 277. The box was entirely successful, and enabled the growth of the marked cockles to be observed. The experiment was begun under the difficult post-war conditions in August, 1919, and monthly observations were made until October, 1920, when continuous work on the problem had to be abandoned owing to the necessity of taking up more urgent work, but occasional observations were afterwards made to 1923 as opportunity arose. The essential plan of the work was to observe the rate of growth of a constant population of Cardium in the sea ; to find out the significance of the concentric grooves or rings on the shells of some mollusc—of which Cardium is a convenient type—since these grooves are commonly regarded as marking a cessation of growth in the winter period ; to observe the rate of growth of small and medium sized individuals from the time of marking the shells with a filemark, through summer and winter periods, while at the same time collecting as much information as possible about the general environmental conditions ; and to obtain data on the growth of the shell in *Cardium edule* to compare with results from all other regions where this species is found.

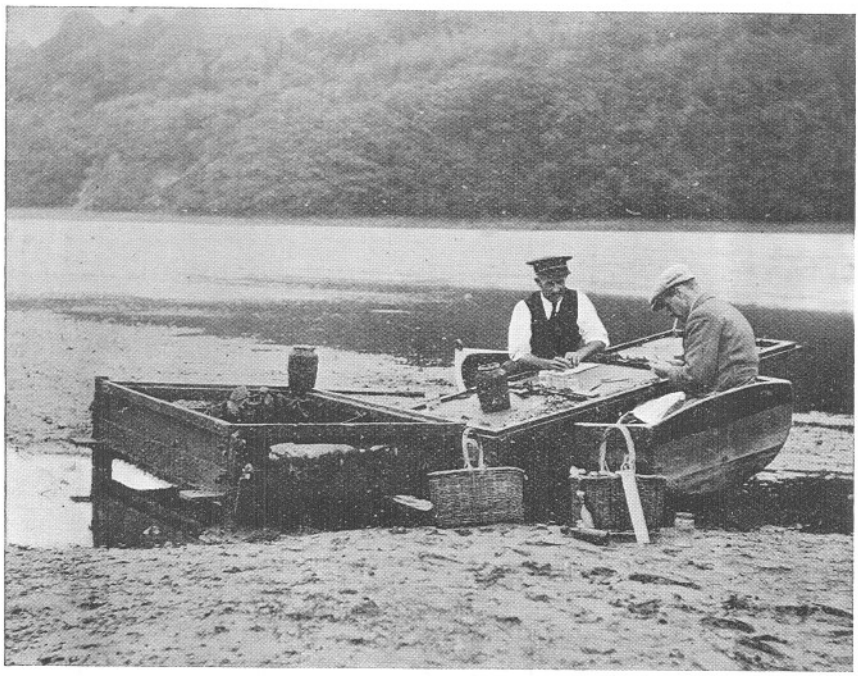
The Site of the Experimental Box.

The site of the box was chosen on the lower part of the cockle beds in the middle of the main stream of the River Yealm, about half a mile north-east of Steer Point. The exact tide-level of the site has not been accurately

* Contribution to an Evaluation of the Sea, *Journ. Mar. Biol. Assoc.*, Vol. X, No. 2, p. 312, 1914.

FIG. 1.

A



B

Photos A. J. Smith.

VIEWS OF THE EXPERIMENTAL BOX IN THE BED OF THE RIVER YEALM.

A. View of the open box showing details of the construction and the stream and mud flat in the background.

B. Showing the closed box and details of construction; the lid of the box is protected with wire-netting.

determined; it is estimated as being about 3 feet above low-water level, ordinary springs. The bed of the river where the box was fixed is of sandy gravel, and is only about 30 yards wide at this point before rising on each side into mud-banks. The box did not dry in neap tides but remained exposed at good spring tides for three to four hours, when rain had not recently fallen heavily. After recent heavy rains the box did not dry at tides rather less than ordinary spring tides, as the conformation of the estuary renders it difficult for a spate to make its way quickly out to sea. Fresh water was found running close by the box at low water even in summer in the absence of recent rains, hence cockles on the beds and in the box would experience great changes of salinity from 0 to well above 30 per mille. The cockles in the stream, which never dried, would undoubtedly experience more fresh water than those in the part of the bed which dried (see Fig. 1, p. 241) and more than those in the box. There would, however, be practically no difference in the time of exposure of cockles in the bed and in the box, as the tide at the turn rises to and submerges the beds and the box in from ten to fifteen minutes; while working on the beds it was imperative to cease work and pack up in haste as soon as the tide reached the bottom of the box.

The perforations in the zinc were large enough to permit a good circulation of water through the box, and to keep them clear of mud, which, however, accumulated in the bottom of the box to the level of the holes (4 inches) in about three months, and remained until cleared out. Besides the mud which settled in the box the young or larvæ of various animals found entrance and grew to adult or a large size; for example, Nereis, Nephthys, Arenicola, Syndosmya, Scrobicularia, Mytilus, and especially the common shore-crab, *Carcinus mœnas*, and a fair amount of the spat of *Cardium edule* itself, as it was hoped would be the case. The common shore-crab is a great enemy of small cockles particularly, as was unfortunately not realised in the early stages of the experiment, and about a dozen crabs at a time found sufficient food in the confined space of the box 6 feet by nearly 4 feet to grow in a short time to medium size. *Balanus balanoides*—and a few *B. perforata*—grew in profusion on the top of the inside of the box, as also did Campanularia, Bowerbankia and Alcyonidium, while a fair number of Balanus, a little Fucus and Ulva, and Porphyra at times in abundance, grew on the outside of the box.

THE MAIN EXPERIMENT.

Material and Methods.

The box was fixed in the river, and the experiment begun on August 16, 1919. The beds had previously been explored and a sample of tiny cockles with clear shells, that is, without rings of any kind, were accumulated, along with a sample of medium sized cockles with one well-marked —and presumed—winter ring. Each cockle was marked with a file-mark in one, two, or three places, viz. anteriorly, posteriorly, and in the median ventral line in each case, so as to cut the growing edge of the shell. By this means it would be possible to measure the subsequent growth in either length or height, or both; lengths were, however, measured mostly during the experiment, owing to the limited amount of time available at low water to obtain all the information about the bed necessary at each visit. The beds were visited monthly from August, 1919, to October, 1920—except for January—and the marked cockles taken out and measured at each visit, while the growth of similar individuals on the beds was also noted and collections made and preserved —as controls on the marked individuals—for detailed measurements later. After the first few months, when mud had accumulated in the box, the cockles had to be found by searching in the mud with one's fingers; in this way a few each time were liable to escape detection, and little ones were very difficult to find. It was soon found that the mere fact of taking the cockles out of the box, handling them, and keeping them on the improvised field-bench for an hour, a little more or less, was enough to cause the cockles to form a distinct ring on the shells (see Figs. 3 and 6, pp. 251 and 259). From these rings, which I have called "disturbance rings," it was usually possible, in the smaller individuals, to measure the amount the cockle had grown even since the last monthly visit to the box.

The Measurement of the Shell.

The chief dimension of the shell adopted for an expression of the growth is that of the length, and is the maximum distance between tangents to the anterior and posterior borders of the shell, that is to say, the maximum dimension in an antero-posterior line. The height and width of shells have also been measured for the determination of arithmetical ratios between all dimensions, but for the preliminary discussion on the rate of growth it will be sufficient to take length as a criterion. It is essential, however, to bear in mind that a small increment in length in the larger cockles (ca. 30 mm. long) indicates a relatively large increase

in total growth. The weights of shells at different sizes will also be discussed later.

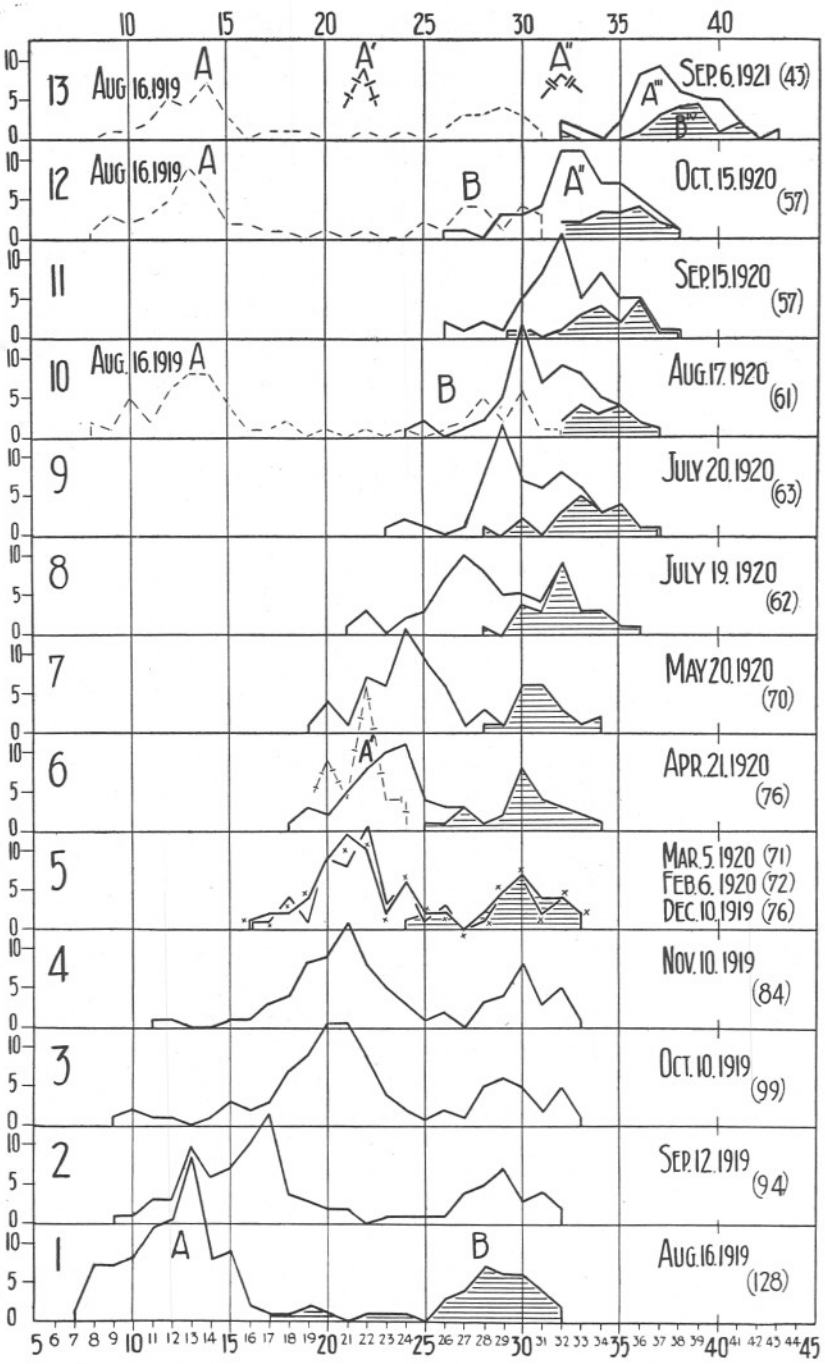
The measurement of length, height, and width was taken with steel Starrett right-angled calipers scaled to half a millimetre, and was read to the nearest $\frac{1}{10}$th of a millimetre by eye-estimation. These readings are probably correct to $\frac{1}{5}$th of a millimetre, but in the preliminary discussion the cockles are all considered in millimetre groups; for example, all cockles measuring 10·0 to 10·9 mm. are plotted in the 10 mm. group, and so on. The original size of the marked shells, and the size of disturbance and winter rings, was taken by fine Stanley dividers and read off on an ivory Stanley scale, engine—divided in $\frac{1}{5}$th of a millimetre. Those readings are probably reliable to only 0·4 millimetre, and have all been taken by the writer himself.

The Rate of Growth of the Marked Cardium.

The rate of growth of the marked Cardium is well shown in the series of graphs 1 to 13 in Fig. 2, p. 246, and Table I, p. 248, which give the history of the cockles surviving at each monthly visit to the box from August, 1919, to October, 1920 (except for January), and the visit on September 6, 1921. The original lengths of the cockles are shown (in mm. groups) in the bottom graph, No. 1, and the successive monthly size-composition of the whole population in the thick-lined graphs 2 to 12; while in graph 13, on the right, is shown the final size-composition of this group of cockles when they were preserved on September 6, 1921. In graph No. 1 the individuals are shown in two groups, A and B. Group A, of lengths upwards to 17 mm., were practically all clear-shelled, and may now be regarded as the spat of 1919; group B, of lengths 18 to 32 mm., shown by the shaded area, may now be stated to be mainly in their second summer, that is, spatted in 1918.

Only one of the next larger group of Cardium, about 38 mm., was marked; but as this died soon after marking, that individual need not be further considered. In graph 1 it may be observed that the groups A and B form two well-defined peaks, between which is a wide distinct bay. If the peaks A and B be followed in graphs 1 to 12 their gradual approach and the decrease of the intervening bay is readily seen. In graph 12, and still more clearly in graph 13, the peaks are seen to become practically merged, but the shaded area still shows that the older cockles have a higher modal value than the younger ones. The approach of the peaks of the A and B groups proves that the younger cockles are catching up in growth dimensions to the older ones, and, finally, as shown in graph 13, some of the younger individuals actually outstrip all the older ones in length, and it may be added, also in all dimensions. The size-

FIG. 2.


10 15 20 25 30 35 40
13 AUG 16.1919 A A' A'' A''' B'''
SEP. 6. 1921 (43)
12 AUG 16.1919 A B A''
OCT. 15. 1920 (57)
11
SEP. 15. 1920 (57)
10 AUG. 16. 1919 A B
AUG. 17. 1920 (61)
9
JULY 20. 1920 (63)
8
JULY 19. 1920 (62)
7
MAY 20. 1920 (70)
6 A'
APR. 21. 1920 (76)
5
MAR. 5. 1920 (71)
FEB. 6. 1920 (72)
DEC. 10. 1919 (76)
4
NOV. 10. 1919 (84)
3
OCT. 10. 1919 (99)
2
SEP. 12. 1919 (94)
1 A B
AUG. 16. 1919 (128)
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

EXPLANATION OF FIG. 2.

FIG. 2.—Graphs showing the successive lengths (in mm. groups) of *Cardium edule* from mainly monthly measurements of the same individuals which were marked and kept in an experimental box at the River Yealm (Aug., 1919, to Sept., 1921).

The graphs 1 to 13 are to be read from below upwards. Graph 1 shows two groups, A and B (shaded). The A group are the spat of 1919, and are not yet one summer old; the B group are mainly spat of 1918, and are nearly two summers old. The thick-lined graphs 2 to 13 show the history of the two groups after August 16, 1919; the B group being shaded in graphs, 1 and 5 to 13.

In graphs 10, 12 and 13 the original sizes at the date of marking (of the surviving individuals at the dates given, namely, Aug. and Oct., 1920, and Sept., 1921) are shown in the broken-line graph on the left-hand side, and lettered Aug. 16, 1919, A B. In graph 13 the peaks of the graphs of the first and second winter rings of the A group (only) are shown at A′ and A″. The final sizes of both groups on Sept. 6, 1921, are shown in graph 13 at A‴ and B^{iv}.

The figures in the brackets on the right-hand side following the dates in the graphs give the number of individuals measured on these dates.

---

composition of the A and B groups is shown throughout (except in graphs 2 to 4) by the shading of the latter group; where individuals of the A and B group are of the same mm. group, the sum of the individuals in that mm. group is plotted for the outline of the graph, so that the shading in the graph is only an analysis to show the history of the two original groups.

Individuals of the younger group begin to climb—metaphorically—on to the older group about April, and—as is easily seen in graphs 8 to 13—more and more cockles attain the same size as the older cockles until most millimetre groups have, on September 6, 1921, representatives of both the original A and B groups.

It will be remembered that it was possible in all the marked individuals to find the original size by the persistent filemark, with the assistance also of a strong disturbance ring formed at the time of marking (see Fig. 3, p. 251). In graphs 10, 12, and 13 the original sizes at marking—of the cockles surviving on the date of examination—are shown separately in the thin broken-line graph on the left-hand side of the same horizontal scales as are used for the increased sizes at the dates mentioned. It is possible, therefore, in these cases, to see at a glance the actual sizes of both the A and B groups in August, 1919, and on the dates given alongside the graphs.

In graphs 6 and 13 are also plotted the peaks of the winter ring sizes—at younger ages—of the population surviving on April 21, 1920, and September 6, 1921. The first winter ring is shown by a broken-line graph with single short lines at right angles to the graph, and the second winter ring by pairs of short lines at right angles to the graph. This convention is adopted throughout this paper to denote sizes at the first and second winter rings.

It is instructive to follow the monthly shift to the right of the peaks

TABLE I.

SHOWING THE NUMBERS OF CARDIUM AT EACH MM. GROUP PRESENT IN THE BOX AT EACH EXAMINATION. THE MARKED INDIVIDUALS AND THE SPAT SETTLING IN THE BOX IN EACH YEAR, 1919, 1920, AND 1921, ARE EACH SHOWN WITH A DIFFERENT TYPE* OF FIGURE.

No. of mm. Group.	1919.					1920.									1921.
	Aug.	Sept.	Oct.	Nov.	Dec.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Sept. 6
5	0	–	–	*1*+0		*1*									
6	0	–	–	– –		*1*	*1*							**1**	
7	1	–	–	– –		–	–				**1**		**1**	–	
8	7	0	–	– –		–	*1*				–			–	
9	7	1	0	– –		–	–				–			1	**1**
10	8	1	3	*1*+0	*1*	–	–				–			**2**	
11	12	3	*0*+1	*0*+1	*1*	*1*	–				–			**1**	
12	13	3	*3*+1	*3*+1	*0*	*0*	–	*1*	*1*		–	**1**	**1**	**0**	
13	21	9	*0*+0	*2*+0	*3*	*1*	*2*	*1*	*1*		–	–	**0**	**2**	
14	8	6	*5*+1	*1*+0	*2*	*2*	*1*	*0*	*0*		–	–	**1**	**0**	
15	9	7	*0*+3	*1*+1	*1*+0	*1*+0	*2*+0	*1*	*1*		–	–	**2**	**1**	
16	2	10	*2*+2	*4*+1	*3*+1	*3*+1	*2*+1	*1*	*1*		–	–	**0**	**1**	
17	1	14	3	*2*+3	*3*+2	*2*+1	*4*+1	*1*+0	*0*+0		–	–	**0**	**0**	
18	1	4	7	4	*1*+2	*1*+3	*1*+4	*3*+1	*3*+0		–	–	**1**	**1**	
19	2	3	9	8	4	4	1	*3*+3	*2*+1	*1*	–	–	–	**2**	

20	1	2	13	9	9	9	9	*1*+2	*3*+4	*1*+0	–	–	–	–	
21	0	2	13	13	12	10	8	*1*+5	*1*+1	*1*+1	–	–	–	**1**+ *1*	
22	1	0	9	8	10	10	13	8	*0*+7	*3*+3	*1*+ 0	–	–	–	
23	1	1	4	5	2	3	3	10	*1*+6	*2*+0	*0*+ 1	–	–	–	–
24	1	1	2	3	6	6	6	11	13	*2*+2	*0*+ 2	*1*+ 1	–	–	–
25	0	1	1	1	2	2	1	4	9	*1*+3	*2*+ 1	*0*+ 2	*1*+ 0	–	–
26	3	1	2	2	2	2	3	3	6	7	*3*+ 0	*1*+ 0	*1*+ 2	–	1
27	4	4	1	0	0	0	0	3	1	10	*2*+ 1	*1*+ 1	*0*+ 1	–	1
28	7	5	5	3	2	1	1	1	3	8	*0*+ 8	*3*+ 2	*1*+ 2	*1*+ 0	
29	6	7	6	4	5	5	5	2	1	5	*1*+14	*1*+ 5	*2*+ 1	*0*+ 3	
30	6	3	5	8	7	7	7	8	6	5	7	*1*+14	*1*+ 5	*2*+ 3	
31	4	4	2	3	4	2	2	4	6	4	6	7	*0*+ 8	*1*+ 4	
32	1	2	5	5	4	4	4	3	3	6	8	9	*2*+13	*1*+11	
33	–	0	1	1	2	2	2	2	1	3	6	8	5	*1*+11	
34	–	–	0	0	0	0	0	1	2	3	3	5	8	7	–
35	–	–	–	–	–	–	–	0	0	1	4	4	5	7	–
36	–	–	–	–	–	–	–	–	–	1	1	2	5	5	–
37	–	–	–	–	–	–	–	–	–	0	1	1	1	3	–
38	1	–	–	–	–	–	–	–	–	–	0	0	1	1	–
39	–	–	–	–	–	–	–	–	–	–	–	–	0	0	–
40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

3	–	–
2	–	–
1+*1*+	0	
3+*0*+	0	
1+*0*+	0	
0+*0*+	0	
2+*0*	2	
1+*0*+	1	
–	–	0
–	–	2
–	*3*+	9
–	*1*+	10
–	*0*+	7
–	*1*+	5
–	*0*+	5
–	*1*+	2
–	0+	0
–	0+	1

* Marked cockles are shown in figures thus : 5 ; 1919 spat settled in the box thus : *5* ; 1920 spat settled in box thus : **5** ; and 1921 spat thus : **5**.

A and B, and increase in length as seen at a glance from Fig. 2, and shown in the following table :—

TABLE II.

	Peak A.			Peak B.			
Date.	Position.	Monthly shift.	Total shift.	Date.	Position.	Monthly shift.	Total shift.
1919.	mm.	mm.	mm.	1919.	mm.	mm.	mm.
Aug. 16	13·0	—	—	Aug. 16	28·0	—	—
Sept. 12	17·0	4·0	4·0	Sept. 12	29·0	1·0	1·0
Oct. 10	20·5	3·5	7·5	Oct. 10	29·5	0·5	1·5
Nov. 10	21·0	0·5	8·0	Nov. 10	30·0	0·5	2·0
Dec. 10	21·0	0·0	8·0	Dec. 10	30·0	0·0	2·0
1920.				1920.			
Feb. 6	21·0	0·0	8·0	Feb. 6	30·0	0·0	2·0
Mar. 5	22·0	1·0	9·0	Mar. 5	30·0	0·0	2·0
April 21	24·0	2·0	11·0	April 21	30·0	0·0	2·0
May 20	24·0	0·0	11·0	May 20	30·5	0·5	2·5
June 19	27·0	3·0	14·0	June 19	32·0	1·5	4·0
July 20	29·0	2·0	16·0	July 20	33·0	1·0	5·0
Aug. 17	30·0	1·0	17·0	Aug. 17	33·5	0·5	5·5
Sept. 15	32·0	2·0	19·0	Sept. 15	34·0	0·5	6·0
Oct. 15	32·5	0·5	19·5	Oct. 15	34·5	0·5	6·5
1921.				1921.			
Sept. 6	36·5	—	23·5	Sept. 6	38·5	—	10·5

From Table II it is seen that the modal size in mm. groups of the marked Cardium are as follows :—

Group.	End of 1st Summer.	End of 2nd Summer.	End of 3rd Summer.	End of 4th Summer.
A.	21·0 (1919)	32·5 (1920)	36·5 (to Sept. 6, 1921 only)	—
B.	20·5 (1918)*	30·0 (1919)	34·5 (1920)	38·5 to Sept. 6, 1921, only

The A group were spatted in 1919 and the B group in 1918, and the subsequent difference in modal size at the same age is apparently due to a difference in environmental conditions at different ages after birth. During 1921, it may be observed, the B group and the A group both increased their modal value 4 mm., the former growing from 34·5 to 38·5 and latter from 32·5 to 36·5 mm. In their second year of growth, however, the B group only increased to 30 from 20·5 mm. (in 1919), while the A group in their second summer, in 1920, grew from 21·0 to 32·5 mm. It is clear, therefore, that there are good and bad years for the growth of Cardium, as there are in other bivalves and in fishes. The cause of this annual variation will be discussed later.

* See Fig. 10, p. 271.

FIG 3.

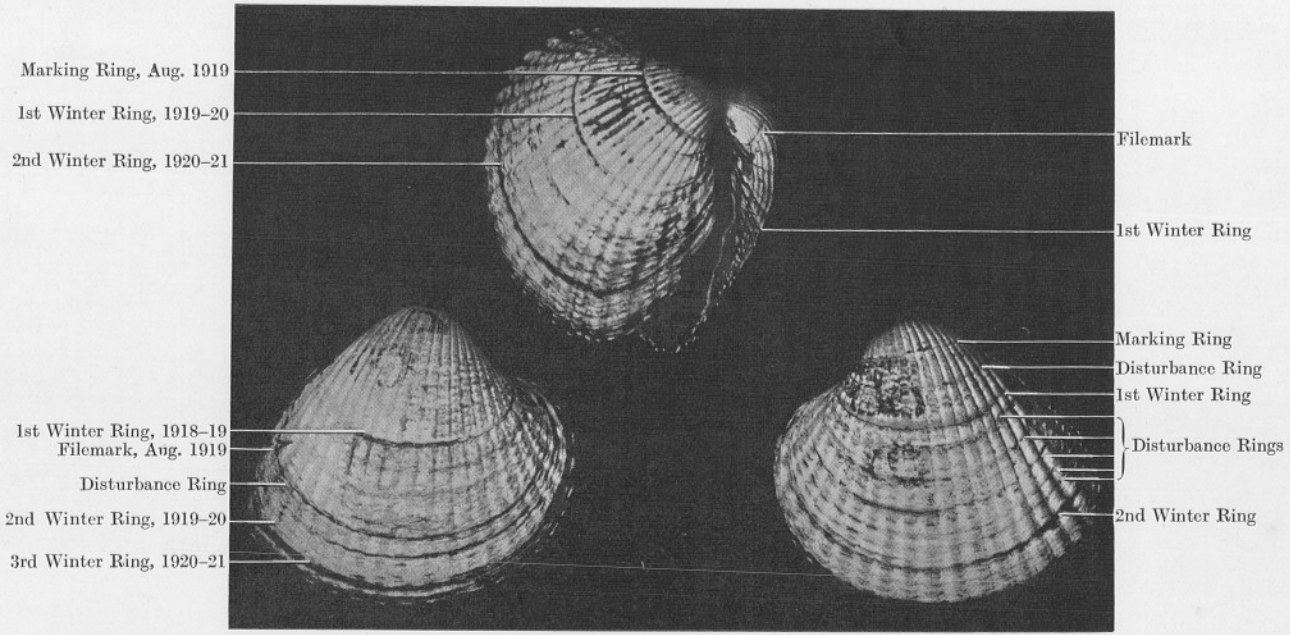


Photo A. J. Smith.

FIG. 3.—Photos of representative examples of *Cardium edule* marked with filemarks on Aug. 16, 1919, and grown in the experimental box until preserved on Sept. 6, 1921, the shell on the left is that of a B individual, the other two being typical A examples ($\times$ Ca. $\frac{1}{2}$.).

In the upper individual the filemark is well shown on the right near the umbo, and the disturbance ring due to the file-marking is shown very distinctly on the opposite portion of the shell. On this shell in this photo the next two well-marked rings are winter rings, and the disturbance rings are only indistinctly seen.

In the cockle on the right-hand side the filemark is not shown, but the first ring near the umbo is the disturbance ring due to file-marking, and is followed by another disturbance ring before the winter ring of 1919–20 is formed. Before the next winter ring is formed three distinct disturbance rings and two indistinct ones were produced on the shell.

In the individual on the left the filemark is on the left of the shell near the second ring from the umbo. The first ring from the umbo is the winter ring of 1918–19, and growth to the second ring was made on the beds. The second ring from the umbo is a disturbance ring, caused by the marking, and is followed closely by the winter ring of 1919–20; the line pointing to this winter ring has been abraded, and falls 3 mm. short of the ring. The ring near the border of the shell is the winter ring of 1920–21. This is one of the cockles which did not grow well in the box, and is actually smaller than the individual a year younger shown on the right.

A. On the Growth of Cardium Spatted in the Box in 1919.

Before passing on to a consideration of the growth of Cardium *in situ* on the beds, it will be convenient to follow the growth of the cockles which settled and grew naturally in the box. It has been mentioned that the maximum diameter of the holes in the zinc plates built into the box was 6 mm., therefore it was possible for tiny Cardium about this size to be washed into the box, assuming that a disturbance of the bottom in the neighbourhood threw up cockle spat into a tidal current strong enough to wash them on to the box. It is conceivable that fish such as flounders, mullet, or bass foraging in the locality, or high winds, might so disturb the beds, but the probability is, no doubt, small.

Tiny cockles did, however, settle in the box, and although most of these probably settled as post-larvæ it is possible that individuals of a length not greater than 6 mm. might also have been washed in.

The number of spat which settled in the box in 1919 is small, and their history is shown in the series of graphs, 1 to 6 in Fig. 4, p. 254. On October 10, 1919, ten young Cardium were found in the box, ranging from 7 to 16 mm. in length, with a modal value of 14·3 mm. On November 10, a month later, the modal value had moved to 16·6 mm., where it remained until March 5, 1920, when the number of spat found was 16 plus 2 dead ones. Growth began soon afterwards, and by October 15, 1920, the modal value of these cockles had risen to 30·5 mm. Owing to the formation of a disturbance ring in October, 1919, it is possible to show the size at that time of those individuals which survived. (See the left-hand side of graphs 5 and 6.) These cockles, grown in the box since August, 1919, grew to a mean length of 28 mm. by August, 1920, and to 30·5 mm. by October 15, 1920. In the group of graphs shown on line 6 in Fig. 4 are shown the sizes of this surviving group respectively on October, 1919, at the winter rings of 1919–20, 1920–21—which were found to be well marked—and also when the individuals were finally preserved on September 6, 1921 : the mean corresponding lengths at these times are 16, 17·3 31, and 36·3 mm. respectively. Since these spat settled in August, 1919, they must be regarded as late, for Cardium begins to breed on the Yealm in March to April, as will be shown later. In Fig. 2, p. 246, are shown the sizes of the early 1919 spat—marked and grown in the box—at the winter ring of 1919–20. The modal length of this year-group is 22·2 mm., that is, 4·9 mm. more than that of the late spat grown in the box after August, 1919. The modal value of the early 1919 spat at the second winter ring (1920–21) is, however, 32·2 mm., while that of the late spat for the same year is 31·0 mm., a fact, of which many illustrations will be noticed, demonstrating how the younger individuals gradually approach in size to older ones. On September 6, 1921, the approach of the late

spat is still nearer, as they had then a modal value of 36·3 mm. (see Fig 4, graph 6), while the younger spat (see Table I and Fig. 2) had a mean modal length of only 36·5 mm.

FIG. 4.

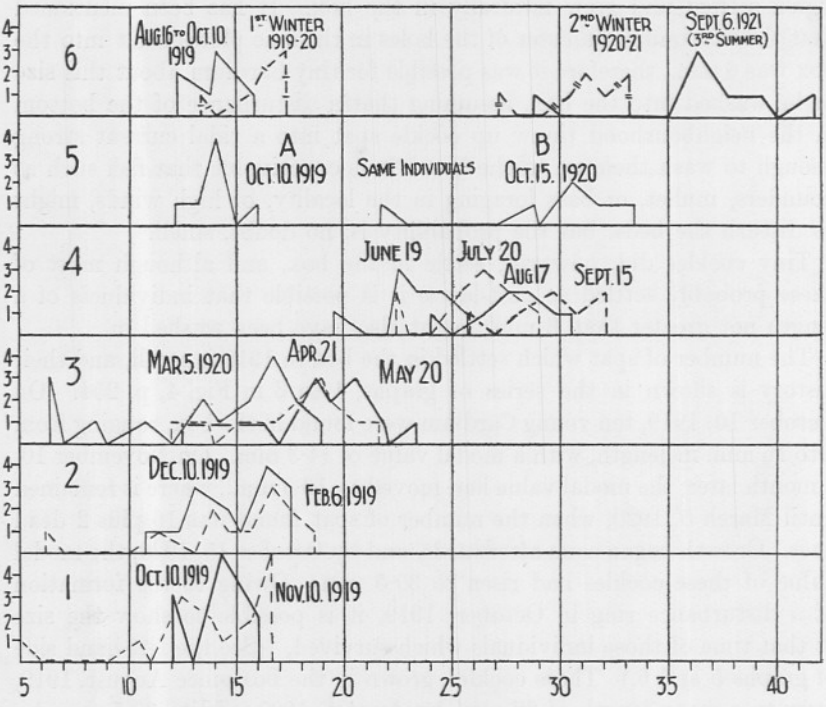


FIG. 4.—Graphs showing the successive monthly growth of *Cardium edule*, which settled and grew in the experimental box from Aug. 16, 1919, to Sept. 6, 1921.

The graphs, 1 to 6, are to be read from below upwards. The graphs show the lengths at the dates given, except in parts of graphs 5 and 6.

In graph 5 the left-hand graph shows the lengths of the same individuals as are shown on the right, but on Oct. 10, 1919, a year earlier.

The series of graphs on the graph line, No. 6, are all of the same individuals at different ages; on the left is given a graph of the lengths on Oct. 10, 1919, followed by graphs of the lengths at the first and second winter rings, while on the right is given a graph of the lengths when the individuals were preserved on Sept. 6, 1921.

B. SPAT SETTLED AND GROWN IN THE BOX IN 1920.

In July, 1920, the first spat for the year settling in the box was found at a length of 7·5 mm.; this one grew to 12·1 mm. by August 17, but then died. On September 15 six new spat were found in the box ranging from 7 to 18·0 mm., and by October 15 attained 11·3 to 22·4 mm., and a little growth followed in October to November. The winter rings of the survivors of these spat measured from 7·2 to 24·5 mm., with a mean about 17·5 mm. (see Fig. 5). It is unfortunate that the numbers of these

spat are so few, but they are nevertheless of value in showing the approximate range of size of cockles at the end of a good year for growth. By September 6, 1921, this batch, although only in their second summer,

FIG. 5.

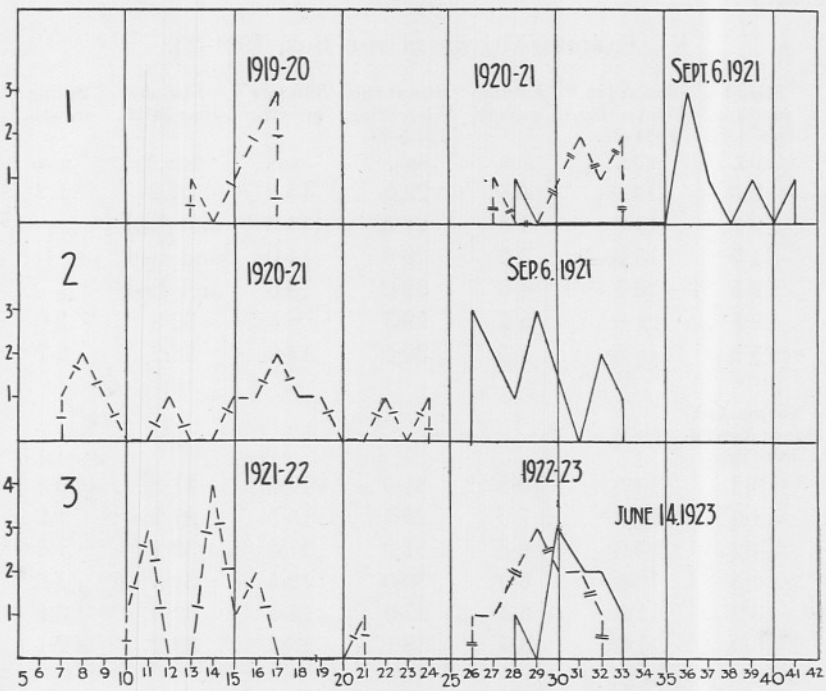


FIG. 5.—Graphs showing the history of growth of spat of *Cardium edule* which settled, grew, and survived in the experimental box during and from the summers of 1919, 1920, and 1921.

Graph 1 shows the lengths of the first and second winter rings, and at Sept. 6, 1921, of the individuals which settled in the box in 1919; similarly graph 2 shows the length of the winter rings of those which settled in the box during 1920, and at Sept. 6, 1921, and graph 3 the first and second winter rings, and size at June 14, 1923, of those which settled in the box in 1921.

which, however, was an exceptionally good one for shell-growth, had attained a mean length of about 29 mm. (see Fig. 5, graph 2), and would certainly have reached a length of 32 to 33 mm. on the average by the end of the summer, with individuals as long as 35 to 36 mm. or more at the end of 1921, if they had been allowed to live, as is shown by examination of samples from the beds.

C. Spat Settled and Grown in the Box, 1921–23.

On October 6, 1921, a few spat were marked and left in the box, and a few unmarked tiny individuals were isolated in the box in a bottle.

These spat were all collected on the same day on the beds, and were undoubtedly late spat of the 1921 brood. The box was not visited again until June 14, 1923, when everything was cleared out, and the cockles remaining were recovered and measured, and showed the following history :—

CARDIUM GROWN IN THE BOX, 1921–23.

Size at marking, Oct. 6|21.	Size at 1st Winter ring, 1921–22.	Autumn growth	Size at 2nd Winter ring, 1922–23	Summer growth.	Size on June 14|23.	Spring growth.
mm.	mm.	mm.	mm.	mm.	mm.	mm.
9.0	14.0	5.0	29.0	15.0	30.2	1.2
9·7	14·0	4·3	28·5	14·5	and died	—
11·5	15·0	3·5	29·0	14·0	and died	—
12·5	16·5	4·0	32·0	15·5	and died	—
12·5	21·0	8·5	29·5	8·5	32·5	3·0
15·0	16·5	1·5	30·5	14·0	33·2	2·7
Not marked. Size at disturbance ring.						
3·2	14·0	10·8	31·0	17·0	31·8	0·8
4·0	11·0	7·0	26·5	15·5	27·7	1·2
4·2	14·0	9·8	31·0	17·0	32·2	1·2
4·5	10·5	6·0	30·0	19·5	31·6	1·6
5·2	11·5	6·3	27·0	15·5	30·8	3·8
8·0	11·5	3·5	28·1	16·6	30·2	2·1

This interesting experiment again shows that late spat may attain commonly a length of about 29·0 mm. at the end of their second summer (see Fig. 5, No. 3), and that small spat of the same year as larger ones may catch up or even *surpass in size the larger ones in the second or third summer*. The mortality in the winter is interesting in resembling previous experience. On August 18, 1923, the box was again visited to examine a few Cardium marked and left in a bottle in the box (to ensure recovery of the same individuals) on June 14, 1923, with the following result :—

Length at mark. June 14.23.	Length Aug. 18.23.	Increase in length.	Average weekly growth.
mm.	mm.	mm.	mm.
5.6	14.6	9.0	1.0
5·0	14·8	9·8	1·1
8·3	15·9	7·6	0·8
13·6	23·2	9·6	1·1

In addition to the marked Cardium, two small individuals were again found in the box, having either settled or been washed in when tiny, and found to have the lengths 17·2 and 13·6 mm. If these spat settled as post-larvæ, as seems most probable, their age is only nine weeks, but if they were washed into the box when less than 6 mm., a month must be added to give their *maximum* age as will be shown later (see discussion on age of spat, p. 273).

D. Spat Settled and Grown in the Box, 1923–25.

On August 18, 1923, a few spat were marked and left in the box and recovered at a visit on March 2, 1926. Only three individuals were found in the box on this date ; two were marked and one had no mark, and had settled in the box in 1923. Their histories* are given as follows :—

At mark, Aug. 18, 1923.	1st winter ring, 1923–24.	2nd winter ring, 1924–25.	3rd winter ring, 1925–26.
mm.	mm.	mm.	mm.†
4·5	19·2	31·5	38·6 × 33·7 × 27·2
19·0	25·0	33·2	38·2 × 33·5 × 27·5
Settled in box	16·8	30·6	37·6 × 34·0 × 29·7

These few individuals again afford evidence of small spat overtaking in size larger individuals of the same spat-year, and show that in the period 1923–24 cockles with two summers' growth attained to 30 to 33 mm. in length in the box.

On Disturbance and Winter Rings.

With the data so far obtained on the growth of marked individuals and those spatted in the box, it will be possible to show the average growth of cockles of different sizes and ages during the summer, but it is advisable first to consider the growth of individuals taken from the beds at different times—as controls on the experimental results. In examining samples from the beds, however, it is necessary to know what interpretation to place on the marks or rings on the shells, and the information obtained on the subject may be discussed here.

It was found during the course of the experiments that practically all cockles in their first, and a fair proportion in their second, summer showed rings on the shell after each time they were taken out of the box for measurement. On June 19, 1920, a special experiment was designed

* At the same time three Scrobicularia were taken from the box with lengths as follows at the respective winter rings : (A) 1st winter ring, 18·2 (?) ; 2nd, 34·2 ; 3rd, 41·6 mm. (B) 1st winter ring, 7·4 ; 2nd, 35·7 mm. (C) 1st winter ring, 9·6 (?), disturbance ring (?), 31·0 ; 2nd winter ring, 34·7 mm.

† These measurements are respectively of length, height or depth, and width of the shell.

to obtain shells showing such monthly rings, which may appropriately be called "disturbance rings." Seven tiny Cardium with clean unmarked shells were collected from the beds on June 19, and placed in a large bottle in the box; the same result would have been obtained if the cockles had been placed in the mud in the box, but the individuals would have been found with difficulty owing to their smallness. They were taken out and measured on July 20 and August 17 at intervals of a month, and finally preserved on September 15, 1920. A series of graphs to show their sizes at the different date of examination is given in Fig. 11, p. 274, and a photograph of the shells is given in Fig. 6A, p. 259. On most of the shells three distinct disturbance rings were formed, although not all are shown clearly in the photograph; on one shell, however, no growth occurred after August 17, and therefore only two rings were formed. By comparing the sizes at the dates of examination with those at the disturbance rings, it is clear that the rings are produced in one or two days after the time of the disturbance, due merely to taking the shells out of the box and measuring them. This was further proved on October 6, 1921, by placing small cockles in a marked bottle, and finding good rings on the shells only on October 18, twelve days later, when a few mms.' new growth had been put on after the disturbance rings had been formed. It is worth while describing in some detail what was done with the cockles to cause them to make a disturbance ring on the shell. On visiting the box the lid was unscrewed and slung over the small punt, and used as a field-bench. The surface of the mud was then searched by hand for all the Cardium which could be found; the cockles were washed as found and placed on the bench. The cockles from the bottle were found by using the lid of the box to sieve off the mud with douches of water. In this way all the cockles were prepared for measurement in about half an hour. If the sun were shining, or a wind blowing, the cockles would be exposed to unusual conditions during the period they were on the bench, for a varying period of about a quarter of an hour to an hour and a half. As the

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EXPLANATION OF FIG. 6.

FIG. 6.—Photos of *Cardium edule*, showing disturbance and/or winter rings on the shells.

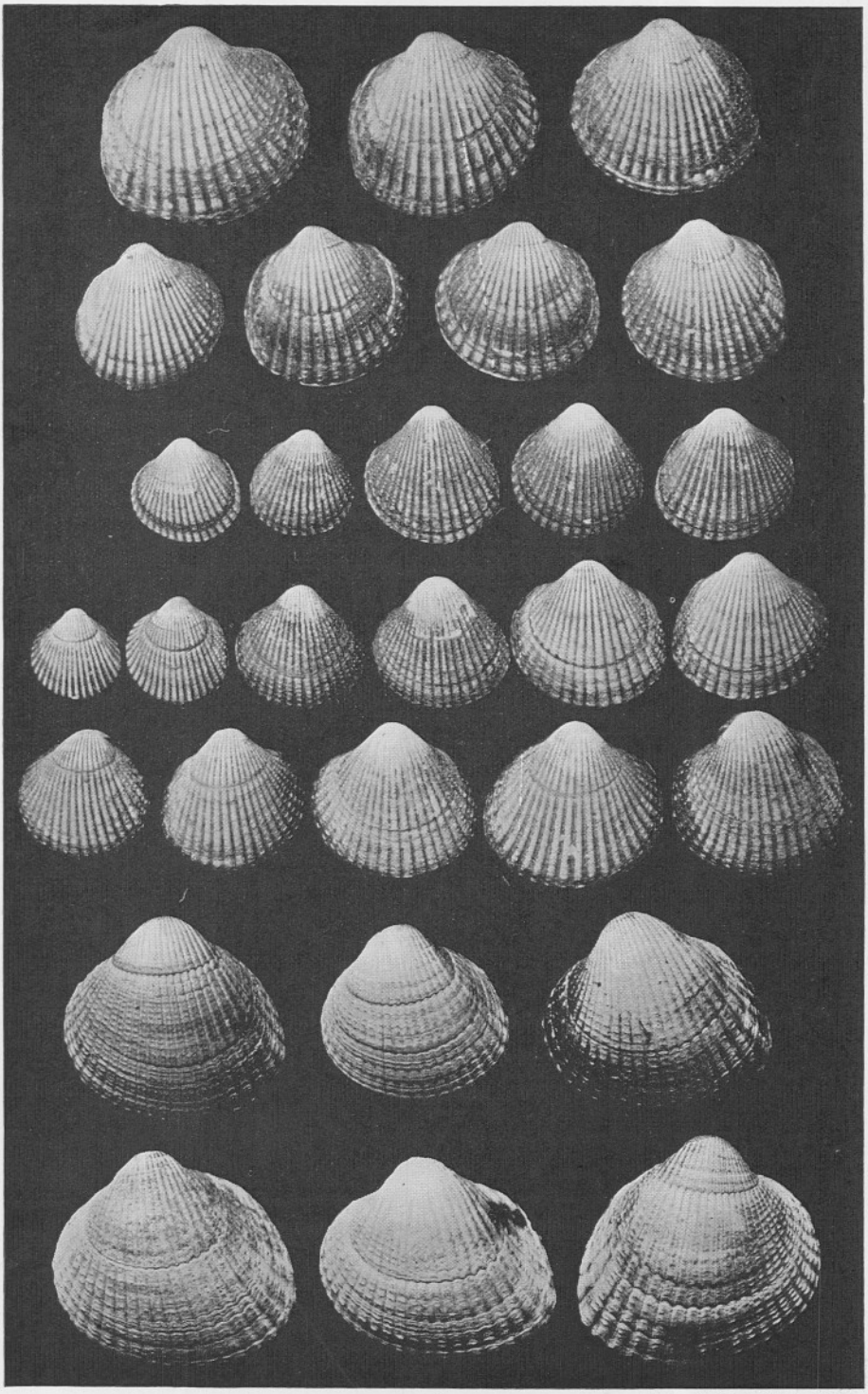
A. The upper seven individuals show mostly three monthly disturbance rings due merely to being taken out of the experimental box to be measured. (× Ca. $\frac{3}{2}$.)

B. The three middle rows are samples of *Cardium edule* from the bed of the River Yealm, illustrating the growth of individuals at different successive periods in their second summer in 1920 from variable sizes at the end of the first summer of growth. (× Ca. $\frac{2}{3}$.)

The upper row were taken from the beds on April 21, the middle row on June 19, and the bottom row on August 17. The winter ring is in all cases well defined and varies in size from 8 to 23 mms. The increase in growth during 1920 is well shown, as is also the apparently larger growth by the smaller individuals of each sample.

C. The lower six shells were taken from shifting beds of sand in Padstow Estuary, and show concentric rings which may be either disturbance or winter rings, but are indistinguishable. (× Ca. $\frac{4}{3}$.)

FIG. 6.



Composite Photo A. J. Smith.

cockles were measured they were generally put straight back in the mud or in the bottle in the box. The tiny cockles from the bottles were generally treated more expeditiously than the larger ones. Therefore, the actual amount of disturbance from normal conditions which produced a ring on the shell is very small. The disturbance rings, which were most clearly defined and indistinguishable from or better marked than winter rings, were those produced after the shells were marked with a file (see Fig. 3, p. 251). In these cases both small and large individuals (to 30 mm.) put on a well-defined ring.

In connection with the formation of disturbance rings it is interesting to know—as was found by repeated experiments—that cockles did not move about much when put back in the box. On several occasions the marked cockles were all put back and recovered from one half of the box, while the spat grown in the box were put back and entirely recovered a month later from the other half; on two occasions two and three individuals were found to have crossed the middle line of the box.

It is, therefore, established that a slight disturbance of a living cockle will cause small individuals, up to about 20 mm., almost always to form a disturbance ring on the shell, and may even cause a ring to be formed on the shell of larger individuals up to about 32 mm.

When a cockle is disturbed from its secure position in the beds it probably has some difficulty in again establishing itself in a comfortable position, and one favourable for the proper discharge of the functions of feeding, respiring, and evacuating, and as it has been shown that small individuals may be growing shell at the rate of 1 to $1\frac{1}{2}$ mm. per week, a slight disturbance extending over one or two days is enough to upset the normal deposition of calcareous material at the edge of the shell. It would seem that a thinner deposition of calcareous material is produced at the edge of the shell during the disturbance, and the groove so produced when the normal thicker deposition recommences retains a puckered deposit of brown conchyolin to mark the groove definitely. The depth of the groove is probably determined by the period of cessation of a deposit of a normal thickness; in this way it seems reasonable to conclude that the greater disturbance of the economy of the cockles due to filing their shells required a longer period of recovery than the mere taking of individuals out of their nidus in the beds, and that in the former case a reduced deposition of shell material occurred for a longer period, and so produced the deeper groove. There would seem to be little doubt that the same explanation holds for the formation of winter rings, and that therefore the mode of formation of rings is the same in disturbance and winter rings. In mild winters winter rings often become extended, or a ring or series of rings may be formed, followed by or interspersed with further normal depositions before the final winter ring is produced.

The writer has no doubt from experience that disturbance and winter rings are found in other bivalves and univalves in a similar way to that described for Cardium. There is a difference between most disturbance rings and winter rings in large cockles in that the winter rings have deeper grooves, but a heavy disturbance ring could not be differentiated with certainty from a winter ring. In large cockles, however, slight disturbances are not reflected on the shell in the same ready way as in the smaller specimens, therefore as big disturbances sufficient to produce rings on the older cockles are rare, disturbance rings are rarer on the larger than on the smaller bivalves. The same is true of the smaller cockles, except that it becomes still more difficult to distinguish between a deep disturbance ring and a winter ring. In cockles which have passed their first winter while small, i.e. below about 15 mm. in length, the first winter ring even when not well defined by a groove is often raised above the continuation of the general contour of the older shell parts, as, indeed, is often the case with the older winter rings.

This feature of the shell resembles the similar one where the protoconch passes into the growth of the adult shell, and is, no doubt, due to the same cause, namely, a different orientation, or rather a fresh orientation, of the shell secreting area of the mantle edge.

Where the first summer shell is not raised from the general contour of the older part of the shell, it is often, but not by any means in all cases, impossible to distinguish between a disturbance and a winter ring, and experience in producing disturbance rings, as has been described above, is helpful and probably necessary in all studies of bivalve shells in interpreting the growth-history from the shell markings.

Whilst inspecting the cockle beds at Padstow, the writer found a number of shells in the large banks of soft shifting sand, which are common in that estuary. These shells show a series of rings (see Fig. 6 c), which cannot be interpreted conclusively as winter rings, and are considered by the writer as disturbance and winter rings indistinguishable from one another. In these beds there is a great probability of cockles becoming buried temporarily in the shifting sand, and as it has been shown by experiment that disturbance rings may be produced in a few days by very little change from the normal, there is every probability of the production in buried cockles of disturbance rings as pronounced as winter rings.

Disturbance Rings in *Mytilus Edulis*.

Disturbance rings can be induced in the shell of the common mussel by the same method as was used in the case of *Cardium edule*, as is shown by the following experiments, which may be conveniently mentioned here.

FIG. 7.

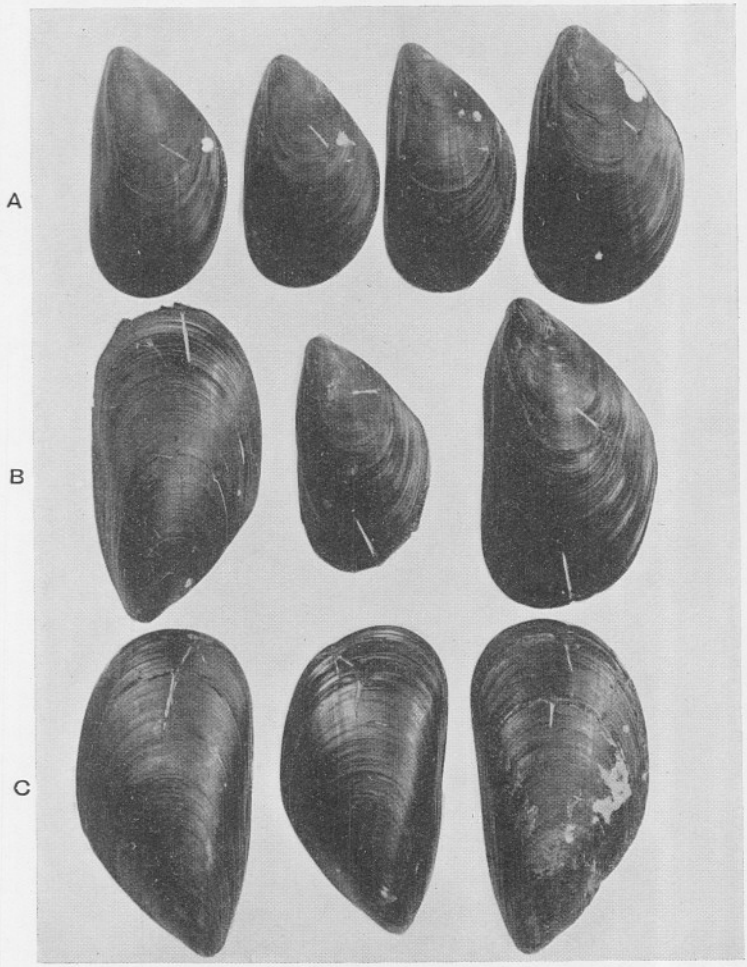


Photo A. J. Smith.

FIG. 7.—Photograph of mussels (*Mytilus edulis*) showing the formation of disturbance rings after the shells had been marked with a file, and the animals put back in the sea to grow ($\times\frac{3}{4}$).

A. Four shells marked October 13, 1923, and put in the sea at once, and again taken out and preserved on June 23, 1924. The disturbance rings are not shown so well in this photo as on the shells.

B. Three shells of the same sample as the four noted above. These were re-marked on June 26, 1924, put back in the sea, and recovered and preserved a month later, on July 26, 1924. The disturbance rings of October, 1923, are well shown on two of these shells, but those of June, 1924, although as distinct on the shells as on the three shells shown immediately below them, are not clear in the photo.

C. Three shells marked July 9, 1923, put back in the sea, and again taken out and marked on September 11, 1923; and finally recovered and preserved on October 16, 1923. The two disturbance rings shown on these shells were formed in the summer period, and are shown in the photo probably as clearly as is possible by this means.

On July 9, 1923, a number of mussels were marked with a file on the edge of the shell and put into an iron-wire meshed cage in the lower part of the oyster beds in the River Yealm, more than a mile below the site of the Cardium box.

These mussels nearly all put on a distinct disturbance ring at the file-mark (see Fig. 7 C, p. 263). On September 11, 1923, a number of the mussels were again marked with a second filemark, and again most of the mussels were found to have put on a second disturbance ring (as is seen from Fig. 7 C), when the mussels were taken out of the cage and preserved on October 16, 1923.

On October 13, 1923, a sample of mussels from the Promenade Pier, Plymouth, were filemarked and put in another compartment in the same Yealm cage. On June 23, 1924, on examining the fifty-six survivors, it was found that forty-two showed a distinct disturbance ring, while the remainder showed either an indistinct or faint one. Winter rings were also shown in addition to the disturbance rings on only a proportion of the shells, and when present were no more distinct than the disturbance rings (see Fig. 7 A, p. 263).

On June 26, 1924, the mussels were all again freshly marked with a file, and again put in the cage. On July 26, when the mussels were re-examined, a new disturbance ring was found on most of the mussels at the position of the new filemark (see Fig, 7 B, p. 263).

In both these experiments the mussels were transplanted from an intertidal level to a demersal one, and doubtless the severity of the change is reflected in the heavy mortality which occurred; but in the case of the second marking with the file, after the mussels had become acclimatised to the new situation, the cause inducing the disturbance ring was not severe. So that in the case of mussels as in that of Cardium, a relatively small change from the normal can result in the formation of a ring on the shell.

The production of rings on the shells of the mussel and the cockle are, however, different problems, although in both cases it has been shown that a disturbance may give rise to a ring. The mussel, at Plymouth, does not always put on even a winter ring in mild winters, as has also been found to be the case in certain situations with Patella.

The Rate of Growth of Cardium on the Beds adjacent to the Experimental Box.

With the information obtained on the rate of growth of Cardium in the experimental box, and with the acquired information of the significance of the concentric grooves or rings on the shells, it is possible to examine individuals from the beds themselves with a fair certainty of reading their growth-history in their shells.

During the course of the experiments described above samples of Cardium of different sizes were frequently examined and the recent growth compared with that of the individuals in the box. It was seen that the shell-growth was very similar in the two situations, but nevertheless monthly samples were collected during 1920, and others later, and preserved for more detailed examination, accurate measurement and comparison with the marked individuals in the box. It was found from examining the experimental Cardium that a distinct winter ring was formed, and as the history of these shells was known, it was possible in these cases to distinguish the winter ring from others in nearly all cases. In cockles only one winter old, the ring became recognisable in March to April; but in individuals two winters old, the last winter ring did not become well defined, i.e. the spring growth did not become evident, before April to July. Armed with this information, with a knowledge and experience of disturbance and winter rings, and a knowledge of the range of growth in different years, it became possible to read the correct history of growth, as shown in the shell of nearly 100% of the cockles from the beds, but an insignificant number of shells which could not be read were not recorded.

Samples from the Beds in 1920.

The sizes of monthly samples from the beds in 1920, and showing only one winter ring (that of 1919–20), are given in the series of graphs 1 to 9 in Fig. 8, p. 267. In considering samples from the beds it is essential to know the modal value of the sizes of the winter ring, in order to interpret correctly the sizes at the time of sampling, since it has been shown that smaller individuals on the average increase in *length* at a greater rate than larger ones. Accordingly in each case in the graphs 1 to 9 in Fig. 8, the winter rings are shown on the left-hand side on the same line of ordinates as the graph of sizes at the time of sampling. All the graphs showing sizes (as lengths) in mm. groups at the first winter ring are given as broken lines with single short lines at right angles to the graph line, thus: —|—|—; in a similar way graphs of sizes at the second winter ring are shown by a broken line with *pairs* of parallel short lines at right angles to the main graph line, and so on. The sizes at the time of sampling are in *all* graphs shown by a thick or thin continuous line.

From the samples collected from the beds during 1920, the individuals with two (winter) rings as well as one ring and no ring were separated and measured, as shown in Figs. 8, 9, and 10. Special search had to be made for the 0-group, which are not easy to find. A simple comparison of the graphs in Fig. 8, with those in Fig. 2 (of the sizes of Cardium

FIG. 8.

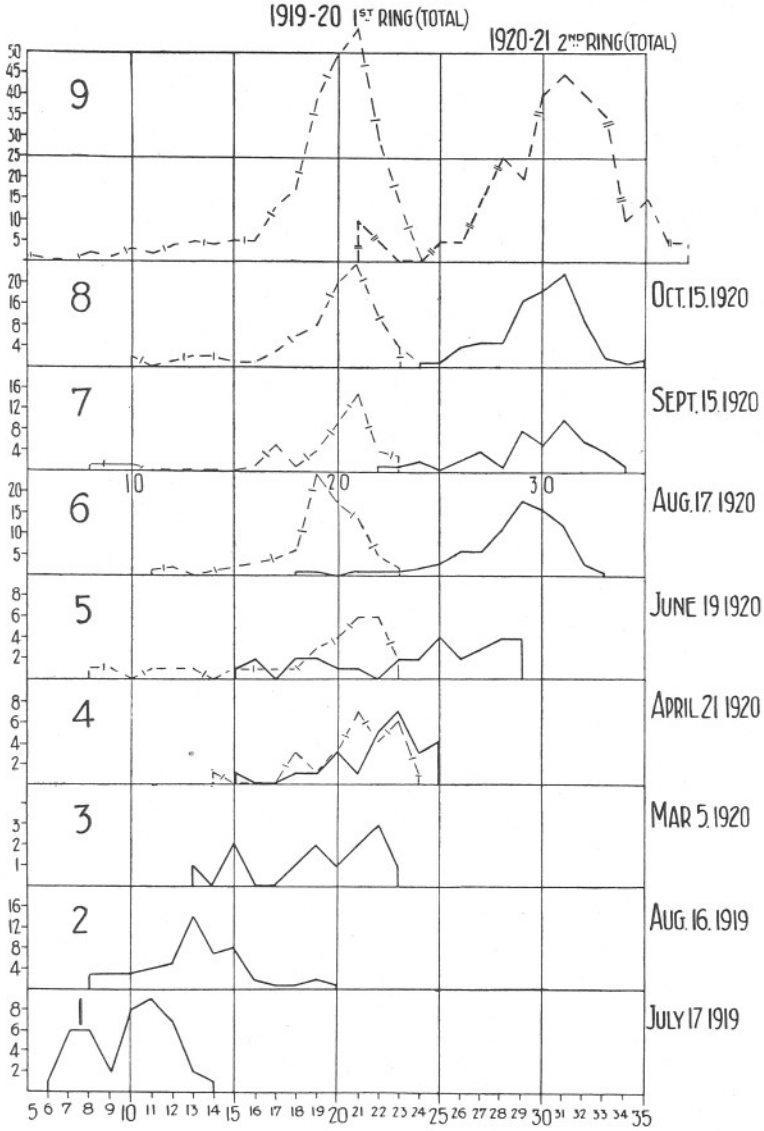


FIG. 8.—Graphs of samples of *Cardium edule* taken from the bed of the River Yealm adjacent to the experimental box, for comparison with individuals of similar age grown in the experimental box. The samples taken during 1920 are composed of individuals with one winter ring for comparison with the A group of Cardium shown in Fig. 2. The sizes at the winter ring of the individuals in each sample are shown by the conventional broken-line graph, with short lines at right angles to the main graph-line.

The two graphs at the top of the figure (in space 9) show the size-composition in mm. groups of all individuals at the first winter ring in 1919–20, and of these with two winter rings after the winter of 1920–21 respectively. Owing to the smallness of the samples taken with two winter rings after the winter of 1920–21, it was deemed advisable to add here the individuals of the appropriate age from the box, in order to present a representative graph of individuals living in the period 1919–21.

grown in the box) both of which show the spat of 1919, can be given in the following tabular form :—

Date.	Partially grown in the box.		Grown on the beds.	
	Modal size.	Maximum size.	Modal size.	Maximum size.
Winter ring, 1919–20	22	24	21	24
1920, Mar. 5	22	24	22	23
April 21	24	26	23	25
June 19	27	31	28–29	29
Aug. 17	30	34	29–30	33
Sept. 15	32	35	31	34
Oct. 15	32–33	37	31	35

In August, September, and October the modal value of the sizes at the date of sampling is in each case, it may be noted, 10 millimetres

FIG. 9.

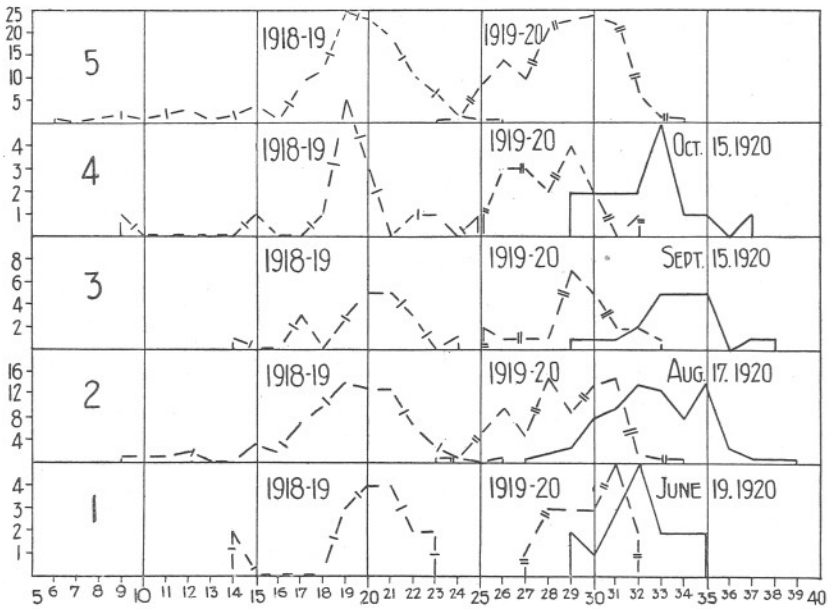


FIG. 9.—Graphs of samples of *Cardium edule*, with two winter rings, from the bed of the River Yealm adjacent to the experimental box taken at successive periods during 1920 for comparison with the B group of individuals grown in the box.

In each sample the sizes of the first and second winter rings are shown conventionally by the broken-line graphs with one short line and a pair of short lines respectively at right angles to the graph line. The sizes at the dates of sampling are shown by the continuous line graphs on the right in each case.

At the top of the figure in space 5 are brought together all the individuals taken from the beds with a first winter ring laid down in 1918–19, and all those with two winter rings for the winters of 1918–19 and 1919–20, for comparison with the smaller samples shown in spaces 1 to 4 of the figure.

greater than that of the winter ring sizes. The modal size of the winter ring in the August sample was, however, 19 mm. against 21 mm. in the September and October samples; but an examination of the individual records of the August sample shows that those cockles with a winter ring of 20 to 21 mm. had attained in August a size mostly of 29 to 31 mm., and were very similar in size to those grown in the box. On the whole, the results shown in the preceding table and in graphs 3 and 8 indicate that there was little difference in growth at these sizes in the box and on the beds. A similar comparison of samples with two winter rings at various dates in 1920 can be drawn up from Figs. 2 and 9. A preliminary glance at Fig. 9, p. 268 (showing cockles from the beds with two winter rings in 1920) will show that the difference in length between the modal sizes of the first (1918–19) and second (1919–20) winter rings, is, with the exception of the September sample, about 10 mm., and that on the whole cockles which grew to 19–21 mm. in 1918 increased to 29–31 mm. in 1919. The modal value at the winter rings must, however, be considered in following the growth *during* 1920, as shown in the following table :—

Partially grown in the box.				Grown on the beds.			
Modal size of 2nd winter ring (1919–20).	Date.	Sizes at date given. Modal length.	Max. length.	Modal size of 2nd winter ring (1919–20).	Date.	Sizes at date given. Modal length.	Max. length.
30	June 19	32	36	31	June 19	32	35
30	Aug. 17	32–35	37	28–31	Aug. 17	32–35	39
30	Sept. 15	34–36	38	29–30	Sept. 15	33–35	38
30	Oct. 15	34–36	38	29	Oct. 15	33	37

The comparison of cockles with two winter rings from the experimental box and from the beds, as given in the table above, brings out the fact that there was very little difference in growth in the two situations; there is, however, an indication of slightly greater growth in the box in this case, as in others, to be noticed. A comparison of the complete growth-history of the Cardium in the box with a sample grown on the beds can be shown in the graphs in Fig. 10, p. 271. The original A group put in the box is shown on the top line of graphs, and compared in the second line of graphs with a sample of two-ring cockles taken from the beds on September 6, 1921, on the same day as those from the box were preserved.

The two lower graphs in the same figure compare the history of the B group originally put in the box with a sample of three (winter) ringed cockles taken from the beds on the same day as the B group were preserved. The modal and maximum sizes in mm. groups at the several

corresponding winter rings and the final sizes, can be best summarised in the following tabular form :—

Spat-year, 1919.	1st winter ring. Mode.	Max.	2nd winter ring. Mode.	Max.	3rd winter ring. Mode.	Max.	Sept. 6, 1921. Mode.	Max.
Box (A group)	22	24	32–33	37	—	—	36–37	43
Beds	21	24	28–31	34	—	—	33–35	40
1918.								
Box (B group)	—	—	30	33	34–36	38	37–39	41
Beds	19–21	26	28–31	34	33–36	37	35–38	40

It is again seen from this comparison that the growth on the beds and in the box was very similar, but that there is once more an indication of rather better growth in the box. The results from the two situations may therefore be regarded as comparable, if it be remembered that growth in the box may be slightly greater on the average than on the adjacent beds.

The Breeding Period of *Cardium edule*.

Cardium edule is known to begin to breed early in the year (Johnstone),* in March to April, and during the summer. The writer has already shown† that the species matures at an early age, and during the course of the experiments observation on breeding were made by tow-netting over or near the beds, and by investigating the condition of the gonad from time to time. Artificial fertilisation was also attempted, but the ovarian egg of Cardium, like that of many bivalves, is not suitable for this kind of experiment without a special technique, which has not yet been devised.

The egg of *Cardium edule* is pelagic, or rather floats freely in the water at the mercy of tidal currents; it has an ovum 80 μ in diameter, surrounded by a large, thin, clear, hydrostatic and micropylar envelope about 180 μ in diameter, in which the egg develops to a *trochosphere* and finally a shelled veliger. The period of the pelagic stage has not been determined, but on the analogy of the oyster,‡ this period may be estimated at two to four weeks.

On October 13, 1919, an artificial fertilisation yielded a few revolving larvæ in the egg-envelope, and it may be presumed along with the evidence of the fall of spat that Cardium was still breeding at this time.

On April 21, 1920, a tow-netting taken below the cockle beds yielded a few pelagic eggs of *Cardium edule* in segmentation stages, and the

* *L.M.B.C. Memoir*, II, Cardium, 1899.
† *Journ. Mar. Biol. Assoc.*, Vol. XII, p. 352, 1920.
‡ *Journ. Mar. Biol. Assoc.*, Vol. XIV, No. 1, 1926.

FIG. 10.

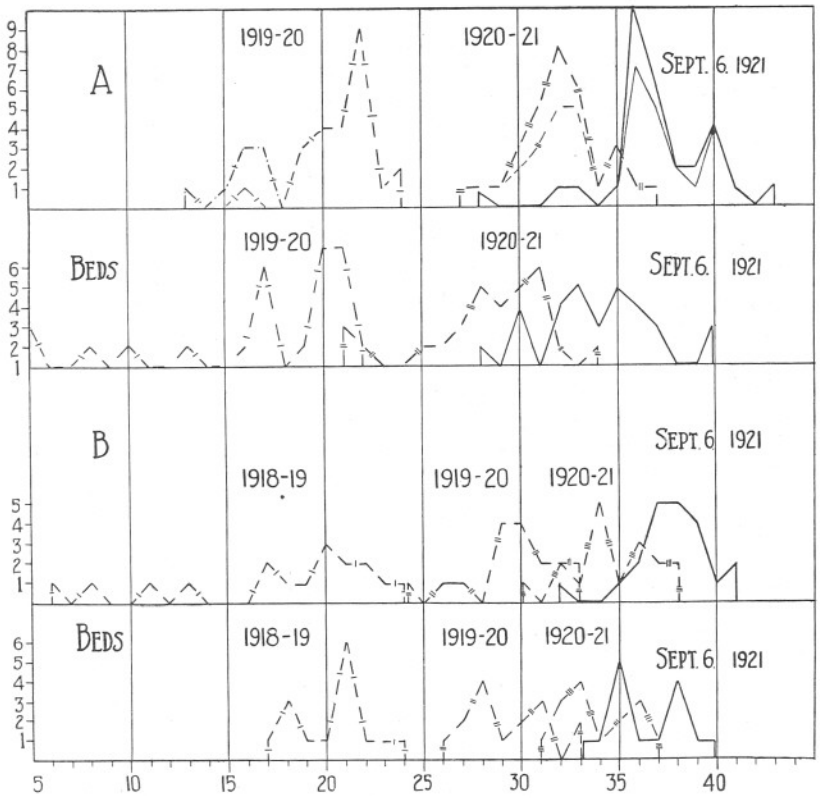


FIG. 10.—Graphs of individuals grown in the box and on the beds of the River Yealm comparing—

(1) The growth of the A group put in the box with a sample of similar individuals grown on the beds, and

(2) The growth of the B group put in the box with a sample of similar individuals grown on the beds.

A shows the size-composition of the first and second winter rings, and on Sept. 6, 1921 of the A group of individuals put in the box on Aug. 16, 1919.

B shows the size-composition of the first, second, and third winter rings, and on Sept 6, 1921, of the B group of individuals put in the box on Aug. 16, 1919.

In the graph of the A group are also shown the sizes of the individuals—at the corresponding times—which settled and grew in the box. These are indicated in the graph of the lengths at the first winter ring by the part of the broken-line graph which has dots, namely, from 13 to 17 mm. ; in the graph of the lengths at the second winter ring these individuals are plotted between the thin and the thick-lined graphs with pairs of short lines at right angles to the graph line ; while in graphs of lengths on Sept. 6, 1921 (continuous line graphs), the individuals grown in the box are again shown between the thick and the thin line graphs.

The history of the spat grown in the box and shown above illustrates markedly how small individuals of the same year group may grow so rapidly as to exceed in size larger individuals of the same year group.

following day a sample of small and large adults were examined microscopically and an unsuccessful artificial fertilisation tried. The sizes (lengths) of the smaller ones and the conditions of the gonad were as follows :—

Length in mms.	Condition of gonad.	Sex.
15·3	∞ ripe sperm with sperm morulæ	♂
18·9	∞ ripe eggs with some unripe	♀
19·5	some ripe eggs ; many unripe	♀
19·6	some ripe sperm	♂
20·2	∞ ripe eggs ; many unripe	♀
20·5	ripe sperm with sperm morulæ	♂
20·6	,, ,, ,, ,,	♂
21·0	∞ ripe eggs ; fewer unripe	♀
22·2	ripe sperm with sperm morulæ	♂
22·5	,, ,, ,, ,,	♂
23·7	,, ,, ,, ,,	♂
25·0	∞ ripe eggs ; many unripe	♀
29·2	,, ,,	♀

On May 20, 1920, two of the spat which settled in the box in 1919, and therefore less than one year old, were examined ; one, 23·0 mm., had ripe eggs with the egg-envelope developed, but with the nucleus still visible, the other, 18·2 mm., yielded a few ripe sperm.

On July 27, 1920, tiny cockles, 17, 21, 20, and 18 mm. were found to have ripe and active sperm ; one, 15 mm., had ripening eggs up to 28 μ ; one, 18 mm., with eggs up to 30 μ, and probably larger in the gonadial tubes, while another, 21·5 mm., had almost ripe eggs, 70 to 80 μ in diameter with egg-membranes. An artificial fertilisation of the last pair did not succeed in producing embryos.

On September 16, 1920, a sample of tiny spat, 14·6 to 20·5 mm., were examined and found to be mostly immature ; but one, 19·2, had mature ova, and one, 18·2, mature sperm, and this pair gave one swimming larva on artificial fertilisation. A successful fertilisation on October 7, 1920, yielded a few larvæ, which developed into trochospheres, and later a bivalve shell, while still within the egg-membrane (see a similar development in the estuarine forms, *Syndosmya alba* and *Cardium fasciatum*, Orton, *Nature*, Vol. 114, p. 244, 1924). There would seem to be no reason why Cardium was not spawning on the beds at this time as in October, 1919.

Cardium edule has therefore an extended breeding period, beginning in April or March and extending to October or later. It is probable from the condition of the gonad that Cardium spawns continuously, shedding batches of ova at intervals throughout the summer. The significance of

such an extended breeding period in connection with the problem of growth lies in the probability of a continual fall of spat throughout the summer and neighbouring periods, and the consequent possible large range in size of the individuals of one particular year group, which may vary from a few millimetres to as much as 27 or 28 mm.

The Age of Young Spat with Experimental Observations.

Various experiments were made to obtain the just settled spat of a known age from tow-nettings put inside the box in a large bottle covered with coarse bolting silk and lashed to the side of the box (see Fig. 1, p. 241). It was found that little or no circulation occurred in covered bottles immersed in water, so tow-nettings were left in the bottles uncovered in the hope that advanced larvæ might settle on the bottom of the bottle and grow *in situ*. In this way two spat, respectively 6·8 and 2·4 mm. long, were obtained on October 15, 1920, from bottles left with tow-nettings a month earlier, on September 15. It is impossible to state dogmatically that these spat were a maximum age of one month, as it will be remembered that the perforations in the zinc plates ranged from 5·8 to 6·0 mm. in diameter, so that it would be possible for a tiny cockle, about 6 mm. long, to be washed into the box. There is, however, as will be shown, nothing improbable in a spat attaining a size of 6 mm. in a month, and it is as certain as is possible in an experiment of this kind that the spat did grow in a month. A design to use a smaller box, with smaller perforations in the zinc, inside the big one—to confirm the result—could not be carried out later. There is, however, less doubt that the 2·4 mm. spat grew in the bottle than the 6·8 mm. one from the tow-netting taken on September 15.

A tiny spat, 6·5 mm., was also taken inside the box on September 12, 1919, one month after the box was laid down in the river, while on October 10, 1919, two months after the box was put out, ten spat which had settled in the box were found varying in size from 12 to 16 mm. An experiment was carried out at the same time to find out how quickly the smallest cockles were growing. On September 12, a number of spat 6 to 10 mm., and eight spat below 6 mm., were put in a bottle in the box, and found on October 10, a month later, to have grown an average amount of 3 mm., while some individuals had increased nearly 6 mm. in length (see Fig. 11, p. 274) at this late period of the year. These spat continued to grow a little even into December.

In 1920 a slight new growth was observable both in the cockles in the box and on the beds on March 5, but no new spat were found in the box until July 20, when a tiny individual, 7·5 mm. long, was taken. It is probable that others similar were present, but remained undiscovered

in the mud. This spat grew to 12·1 mm. by August 17, but then died. On September 15 six new spat, having grown to a larger size, were found in the box, ranging from 7 to 18 mm. The history of the growth of seven tiny spat from June to September has already been described (see also Fig. 11 below); in this case, individuals 4·8 to 9·2 mm., grew to 14·7 to 20·6 mm. in three months in spite of monthly disturbances. It is probable that if these spat had been left undisturbed they would have added to their length in the same time a few millimetres more.

FIG. 11.

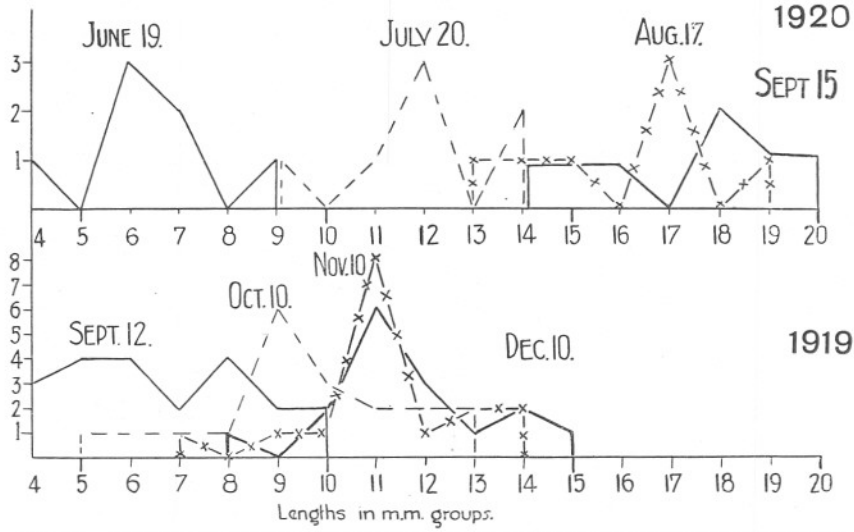


FIG. 11.—Graphs showing successive monthly increase in lengths of small cockles which formed monthly disturbance rings on the shell.

The upper series are graphs of the successive sizes of the seven Cardium photographed for Fig. 6 A and grown in 1920.

The lower series gives monthly increases in length of small individuals towards the end of the growing season, showing that growth continued to November in 1919.

There is good evidence, therefore, for the statement that a post-larval cockle may grow to a length of 6 mm. in its first month of growth, and that afterwards it may add to its length at the rate of 4 to 6 mm. per month until October, and that growth afterwards will vary with the peculiar environmental conditions of that year. Thus if Cardium spawns in April and a month be allowed at this time of the year for the attainment of the post-larval stage, the April spawn might, by June, have developed to tiny cockles, 6 mm. in length. If the subsequent monthly increments in length in July to November be respectively 6, 5, 4, 4, 2, 1, ending in December, then the spat of that year would range upwards to 28 mm. in length, therefore spat might occur on the beds as follows: June, 6 mm.; July, 12 mm.; August, 17 mm.; September, 21 mm.;

October, 25 mm. ; November, 27 mm. ; December, 28 mm. Such spat occurred at the end of the warm year of 1921, and there can be no doubt that a continuously warm summer is favourable for shell-growth in most estuarine shell-bearing animals.

The O-Group of Cardium and Length at the First Winter Ring.

By using the information on the rate of growth of the tiny Cardium recorded above, it is possible to understand and assess the collections of spat made from time to time on the beds. On July 17, 1919, a collection of spat, none of which showed rings on the shell, ranged in size from 6 to 14 mm., and a month later a similar collection ranged to 17 mm. ; but it is probable that larger spat were present on the beds, as only a limited time was available that day for searching for spat. These August cockles grew in the box, as we have seen, to 22 to 24 mm. by the end of the year, and similar sizes were found on the beds. In 1920, spat were found with clean shells up to 15 mm. in August and 20 mm. in October, and the 1920 spat in the box also grew to 20 mm. by October, and 24·5 mm. by the end of the growing year. On the beds spat 24·2 mm. were found at the end of the same growing year. On September 6, 1921 a collection of clean shelled spat for that year showed already individuals ranging to 25 mm. with a good proportion of shells at 19 to 20 mm., so that by the end of 1921, shells would be common at 23 to 24 mm., and might easily range to 27 or 28 mm., as was subsequently found to be the case. In 1918, spat grew commonly to a size of 21 mm., as is shown by the occurrence of shells clean to the winter ring, and occasional individuals attained 26 mm. Thus from the examination of shells during the period of the experiment, it is found that in the first year of growth on the River Yealm, *Cardium edule* will attain a length commonly of 19 to 22 mm., with a small proportion of individuals of 25 mm., and in years of very good growth, as in 1921, of a length of even 27 or 28 mm.

The Length of Cardium of the Second Winter Ring.

The record of the growth of the marked cockles in the box during 1920, with the examination of samples in the same period from the beds, enables us to obtain unimpeachable information of growth in the second summer. The year 1920 as well as the year 1921 was good for growth of shell-material in most bivalves, so that the observations for these years will probably give records of growth close to the maximum possible for this locality. In that case they must not be regarded as average results. Cockles in the box grew from a common size of 22 mm. to 32 to 33 mm. in 1920, and from a maximum of 24 mm. to a maximum of 37 mm. On the beds individuals grew from a common size of 21 to 31 mm.

in the same time, and from a maximum of 24 to 35 mm. In 1919 the second summer cockles marked and put in the box grew by the end of their second summer to a common size of 30 and a maximum of 33 mm., while on the beds individuals of similar age grew to the same size, but were grouped about 28 to 31 mm. The year 1919 was probably near an average year. Thus in their second summer of growth (1919 and 1920), with a maximum growth-period of two summers, Cardium may attain commonly a length of 28 to 31 mm., and a possible maximum of 35 mm.

The Length of Cardium at the Third Winter Ring.

The B group of cockles in the box shown in Figs. 2 and 10 continued to grow until September 6, 1921, and at the size they had then attained would not increase much more in length in their fourth summer of growth. The modal lengths at each winter ring have already been shown on page 270 and in Fig. 10 B. At the third winter ring these individuals had a modal length of 34 to 36 mm. with a maximum of 38, and those on the beds a mode at 33 to 36 with a maximum at 37. Thus in the three summers, 1918, 1919, and 1920 Cardium grew commonly to a length of only 33 to 36 with a maximum near 38 mm., and this result may be regarded as near the average condition. In contrast with these years of growth, it has been noted that already in September, 1921, the A group of cockles spatted in 1919 had reached a modal length of 36 to 37 with a maximum of 43 mm., and that on the beds similar Cardium had a modal value of 33 to 35 with a maximum of 40 mm., although these cockles had not then finished their third summer of growth ! These interesting facts prove that good years for growth affect younger individuals more than older ones, and in such a way that the younger ones may commonly attain the size of individuals one year older than themselves. This aspect of the work will be dealt with in more detail later.

After a series of years of good growth, therefore, Cardium may attain in its third summer of growth a size commonly of 36 to 37 mm. with a maximum of 43 mm. or more.

The Length of Cardium at the Fourth Winter Ring.

It is now obvious that the length of Cardium at ages greater than three summers must be subject to a wide range of fluctuation dependent on the environmental influences of the early years of growth. Towards the end of their fourth summer of growth, on September 6, 1921, the B group of Cardium in the box attained commonly a length of 37 to 39 mm. with a maximum of 41 mm., while individuals on the beds growing in the same period, but unfortunately estimated from a small sample,

showed the commonest lengths at 35 to 38 mm. and a maximum of 40 mm. and as little increase in length would be expected in this size of cockle after September, the common sizes for these four years of growth may be placed at 35 to 39 mm. with a maximum about, but probably greater than, 41 mm. In contrast to these individuals the A individuals one year younger had a modal size of 36 and a maximum of 43 mm. at the same time (see Fig. 11 for graphical comparison), a result which again illustrates the greater influence of environmental conditions on the younger individuals.

Thus while in average years common lengths of Cardium at the end of the fourth year range from 35 to 39 and a maximum of about 41 mm., yet after a series of good growth-years the lengths may range several mm. higher with a maximum round about 45 to 46 mm. The results so far obtained may be summarised to show the common and extreme length at the first, second, third, and fourth winter rings :—

No. of winter ring.	Common lengths.	Maximum lengths.	Common increase in length.	Approx. ratio of yearly increment to preceding yearly increment.
	mm.	mm.	mm.	
1	19–22	24–28	—	—
2	28–32	35–37	9–10	50%
3	33–36	38–43	4–5	50%
4	35–39	41–45	2–3	50%

In establishing the figures given above for the common lengths at each age, it may be repeated that the actual growth on the beds has been followed and substantiated throughout with exact observations on the growth of a constant population of Cardium. The only occasions when personal judgment has been exercised are when deciding whether slight rings on the shells are to be interpreted as disturbance or winter rings, and in these cases the experience gained during the course of the work has probably permitted but few errors.

DESCRIPTION OF THE EXPERIMENTAL BOX.

The experimental box was made 6 feet by 3 feet 8 inches by 11½ inches deep in overall measurements. The bottom of the box was completely boarded with boardings 6 to 8 inches by about 1 inch thick, placed between the long sides of the box for strength (see Fig. 1, p. 241, for many details of the construction). The two end bottom boards were allowed to protrude about 6 inches on each side, in order to form a projection, which could be used for attaching the box to stakes driven into the bed of the river at the corners of the box. Strong 2½-inch square

battens were also fixed on the under side of the bottom along the length of the box and about 4 inches from the edge, and also allowed to project from the short ends of the box near the corners in order to form a fork with the projecting bottom boards, into which the head of the bed-stakes could be fitted and fastened. The sides and removable lid of the box were made of perforated zinc plates* (ca. 1·8 mm. thick) with sufficient woodwork to form a frame to which the plates could be attached. At

FIG. 12.

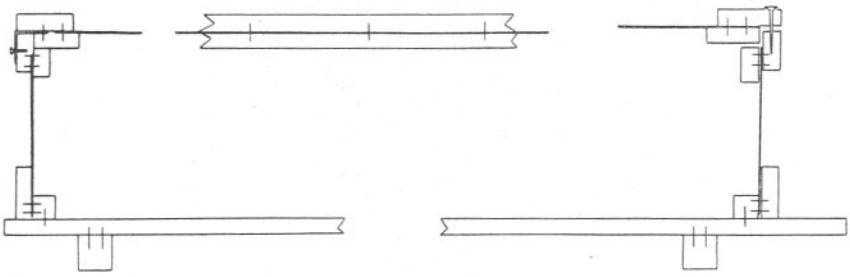


FIG. 12.—Diagrammatic sections of the box across the longer axis. The thick line represents sections of the zinc plates. On the left the section is shown passing through the clamp seen in Fig. 1 B. At the top on the left is shown a section of the lid passing through the clamp. On the right at the bottom the section is drawn through the box near the end, but not through the vertical batten at the corner of the box; at the top of the section on the right the lid construction is shown where one of the screws is nearly screwed home to keep the lid tight. In the upper part of the middle of the diagram the strengthening battens in the lid (which are well shown in Fig. 1 A) are drawn in section.

the corners of the sides of the box, 2½-inch square blocks of wood were used as a basis of the framework, to which 4-inch battens along the bottom and top of the sides were secured (see section of box in Fig. 12, above). The zinc plates in the sides were secured to the framework so formed by battens placed inside the sides of the box at the top and in the angle at the bottom (see Fig. 12). The upper batten on the inside of the sides of the box was fixed, so as to leave the opposing batten on the outside 1 inch higher, and so leave a ledge on the edge of the box, on to which the lid could fit snugly. The zinc plate on the lid was mounted with a pair of opposing 4-inch battens at each of the four edges, these battens being strongly nailed together with the border of the zinc plate between. The outer batten at the edge of the lid projected 1 inch beyond its opposite fellow, in order to rest on the outer batten at the top side of the box when the lid was in place. To prevent sagging of the lid two strong pairs of opposing 4-inch battens were carried across at one-third of the length from each end, and fastened to the bordering battens of the lid (see section, Fig. 12, above). Strong

* Supplied by J. Staniar and Co., Manchester Wire Works, Manchester.

angle irons were fastened in the upper and lower corners of the sides of the box (see Fig. 1, A and B) and on the outside corners of the lid.

The lid fitted so snugly on to the top of the box that nothing above microscopic size could enter the box where it fitted. In addition to its fitting into a ledge, the lid was secured to the sides of the box (a) by six brass screws, two on each long side and one on each short side, screwed through the lid into the outer top-side battens, and (b) by four iron lugs fixed between the bordering battens of the lid and screwed into the sides of the top-side battens (see Figs. 1, A and B, and Fig. 12). Stakes were driven 2 to 3 feet into the bed of the river at the corners of the box in the fork made by the projecting ends of the battens as described, and long nails were driven through the battens into the stakes. In addition tarred rope was wound over the stakes and battens and stapled and tied to ensure security. When the box was fixed it rested on the bed of the estuary, but during the ensuing winter the bed was washed from under the box at the upstream end, and the pit so formed gradually increased in size so that eventually there became a clear space under the box. It is interesting to note that a bank formed on the down stream side of the box showing that the resultant stream by the box was strongest down stream (see Fig. 1). The perforated zinc remained sound until 1923, but at that time the woodwork was heavily infected with and weakened by Teredo. Early in 1926 the woodwork in the bottom was found broken and collapsed; and in places where mud had rested against the zinc, erosion had produced holes a few inches in diameter, but the zinc in the lid at that time remained sound and serviceable.

Acknowledgments.

The writer wishes to express his gratitude to Mr. J. Kingcome, of Steer Point, Devon, who courteously provided the small boat and materially assisted in other ways at each monthly visit to the neighbouring cockle beds. I am also indebted in this work especially to Mr. Wm. Searle, who cheerfully assisted in bad weather and in other trying circumstances. Mr. Ronald Winckworth willingly examined the material once during my unavoidable absence, and to Mr. A. J. Smith I am greatly indebted for the care and patience exercised in obtaining the very difficult photographs which illustrate the work done.

The Comparative Behaviour of Native Oysters (*Ostrea edulis*) and Portuguese Oysters (*Ostrea* (*Gryphea*) *angulata*) in certain Lethal Solutions of T.N.T. (Trinitrotoluene).

By

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With Analyses by the Government Chemist.

With 2 Figures in the Text.

CONTENTS.

INTRODUCTION.

In connection with the cause of the unusual mortality of oysters reported on the oyster beds in the Thames Estuary in 1920 (see Min. Agric. and Fisheries Report, Fishery Investigations II, 6, Nos. 3 and 4, 1923–24, London), it is of interest to compare the simultaneous effects of known solutions of T.N.T. (Trinitrotoluene) in sea-water on both Portuguese and native oysters. In order to obtain some information on this problem the experiments described in the following pages have been carried

out at the request of the Fisheries Department of the Ministry of Agriculture and Fisheries and with the help of the Government Chemist and his assistants.

The Comparative Physiological Condition of Portuguese and Native Oysters.

Owing to the difference in habits and constitution presented by the two species to be compared certain difficulties arise—in addition to those incidental to experiments on oysters (see Report l.c., p. 178)—in beginning the experiment with comparable material. The chief physiological differences in the two kinds of oysters are: (1) Portuguese oysters are mainly imported into England and mostly laid between tide marks, and are therefore accustomed to wide variations in temperature, whereas the native oyster lives mainly below low-water mark, and is accustomed to a relatively lower range of temperature. (2) The Portuguese oyster shoots its sexual products, eggs and sperm, directly into the sea, whereas the female functioning native oyster retains its eggs inside the shell, and incubates them there to the fully developed larval stage. In English waters the Portuguese oyster spawns with difficulty in a normal summer, whereas the native spawns naturally. (3) In addition, in the native oyster, a rapid change of sex occurs immediately after an individual spawns as a female; whereas the conditions of sex-change, if any, are not known in the Portuguese oyster.

The Material used for the Experiments.

In order to reduce the difficulties detailed above to a minimum a mixture of English-grown and a few imported Portuguese oysters were dredged along with native oysters from the beds in the River Blackwater, and kept on the shore in Mersea Creek at dead low-water springs for two months (June 8 to Aug. 6, 1925). From the survivors of these a sample of sixty-seven Portuguese and sixty-two native were sent to Plymouth and kept in the tanks under circulation for six weeks before beginning the experiments (Sept. 21–22) now to be described.

Of the oysters sent to Plymouth one native died on August 18 and one Portuguese on September 18, the rest remaining apparently sound to the beginning of the experiments.

The T.N.T. used for the Experiments.

The T.N.T. used for the experiments was obtained from the lump used in the cage experiment in the sea at Whitstable (see O.M.I. Report l.c., p. 81). This block of T.N.T. had been kept in sea-water since 1921,

but was found by the Government Chemist this year to be practically unchanged T.N.T. It was, however, porous and very sodden with water. Before using it, it was well scrubbed to remove any deposit of altered T.N.T. from the surface, and kept in running tap-water for a period altogether of about twelve hours. It was then split in two halves, which were used for the experiment in Tank 5 ; some fragments were used for experiments in bell-jars and others were analysed at the Government Laboratory.

Experiment I.

Two native (N1 and N2) and four Portuguese oysters (P1 to P4) put in 5000 ccs. of Channel sea-water in a bell-jar with a good air-jet with fragments of T.N.T. on September 21st, 5.30 p.m.

Sept. 22, 1 p.m.	Oysters closed ; water yellow but clear.
,, 22, 5 p.m.	All well except one Portuguese, P1, with slow closing reaction.
,, 23, 11 a.m.	Water deeper yellow, almost orange coloured ; P1 as yesterday.
3 p.m.	P2 and P3 with slow closing reaction, but both can still close.
,, 24, 11 a.m.	P1 to P3 gaping, but all can almost close after repeated compulsory closing.
3 p.m.	N1 and N2 now gaping slightly and unable to remain closed ; the smallest Portuguese, P4, the only one left tightly closed.
6 p.m.	All gaping.
,, 25, 10 a.m.	Ditto.
noon.	Ditto, removed, well washed, put in running water.
,, 26, 11 a.m.	Ditto, P1 to P3 dead, removed.
,, 27, noon.	N1 and N2 and P4 gaping with no reaction.
,, 28, 10.30 a.m.	Ditto, but P4 can now remain closed on pressure.
4.30 p.m.	N1 and N2 gaping widely, dead, removed. P4 now with distinct reaction and recovering.
,, 29, 11.45 a.m.	P4 recovered ; put back in T.N.T. solution (see Experiment II).
Oct. 1.	P4 dead or dying, removed. Experiment finished.

Control to Experiments I and II.

Similar oysters placed in a bell-jar under the same conditions as in Experiments I and II remained well throughout the period of both experiments. The only point of interest arising out of the control being that on or before September 29th one or more of the Portuguese oysters

shed a few eggs which did not segment. On October 3 the control oysters were put in Tank 8.

Experiment II.

The T.N.T. water from Experiment I was partially decanted and partially filtered into a clean bell-jar, and in it were placed N1 and N2 and P1 and P2 oysters from the control tank (8) on September 25 at 3.45 p.m. with again a good air-jet.

Sept. 26, 11 a.m.	N1 gaping with poor reaction ; others closed.
,, 27, noon.	Ditto.
,, 28, 10.30 a.m.	N1 gaping with slight reaction ; N2 closed. P1 gaping and cannot remain closed. P2 closed.
,, 29, 11.45 a.m.	N2 and P2 closed. N1 dead, removed. P1 gaping widely, but with a trace of reaction. P4 from Experiment 1 put in this water.
,, 30, 10.45 a.m.	P1 dead, removed. P2 gaping with slight reaction. N2 and P4 closed.
Oct. 1.	P4 gaping, removed as dead or dying.
,, 2.	No examination.
,, 3.	P2 and N2 dead, removed. T.N.T. concentration 1 in 140,000. Experiment finished.

Summary and Remarks on Experiments I and II.

The T.N.T. in solution gradually increased in strength in Experiment I to a maximum at the end of the experiment, but the concentration was not determined. The same water was used for Experiment II, and had at the end of that experiment a concentration of 1 in 140,000 (approximately 7·1 parts per million) (see p. 293, Government Chemist's report).

In Experiment I all the oysters gaped badly in three days, two of the four Portuguese gaping in advance of the natives. The smallest Portuguese survived all the others, recovering after being in the T.N.T. nearly four days, but succumbed afterwards in about one day when put back into the T.N.T. solution.

In Experiment II N1 gaped in one day, P1 on the 3rd, P2 on the 5th, and N2 on the 6th or 7th day. All were dead or dying on the 7th or 8th day.

In these experiments no marked difference is observable in the susceptibility to T.N.T. at the concentration used, since first one kind and then the other showed the gaping effect first and last. The widely gaping small Portuguese is interesting in showing that recovery is possible at

this stage, but a slightly longer period in the T.N.T. would probably have prevented recovery.

The temperature of the water during the experiments was mainly close on 57° F., the salinity and alkalinity normal or sub-normal; the pH at the end of Experiment I was courteously determined by Mr. N. J. Berrill as 7·95 to 8·00.

Experiment III in Tank 5.

Thirty-one* Portuguese (P1 to P31) and thirty-one* native oysters (N1 to N31) were established in Tank 5; the circulation was cut off on September 22nd, two good air jets added and two lumps of T.N.T. placed alongside the air jets. End of experiment, October 6th.

The results of the experiment are shown in the following table, and in Figs. 1 and 2, p. 286 and p. 287.

TABLE I.

Date, Sept.–Oct.	25	26	27	28	29	30	1	3	4	5	6
Days in T.N.T.	3	4	5	6	7	8	9	11†	12	13	14
Portuguese gaping	0	1	2	6	9	9	11	12	8	5	0
Native gaping	1	1	5	5	6	9	13	5	5	1	0
Portuguese dead or dying	0	0	0	0	1	4	8	17	22	26	31
Natives dead or dying	0	1	1	1	1	1	4	21	24	30	31
Totals affected (additive)—											
Portuguese	0	1	2	6	10	13	19	29	30	31	31
Natives	1	2	6	6	7	10	17	26	29	31	31
Parts per million T.N.T. in solution	2·3 (approx.)		3·3					4			5
Salinity in parts per 1000	36·36‡		36·66				36·80			36·92	

Control to Experiment III (Tank 8).

In the control experiment in Tank 8 with thirty natives and twenty-nine Portuguese oysters, begun under exactly similar conditions as held for Tank 5 (except for absence of T.N.T.) there was one death, a native; the remaining oysters were all well at the end of the experiment and—except for one Portuguese which died about November 9th—as late as December 12th, as were also two Crepidula, a few anemones, worms, and simple ascidians. The temperature and salinity in Tank 8 varied in almost exactly the same way as in Tank 5.

* One brood Portuguese and one brood native included.

† On October 3rd when the maximum number of oysters was found gaping or dead, the pH was found to be 8·00 (courteously determined by Mr. C. F. A. Pantin).

‡ The salinity of the tank water is ordinarily high, and rose in the experimental tanks (5 and 8) to 36·92‰ which is a very high figure and abnormal in our seas.

FIG 1.

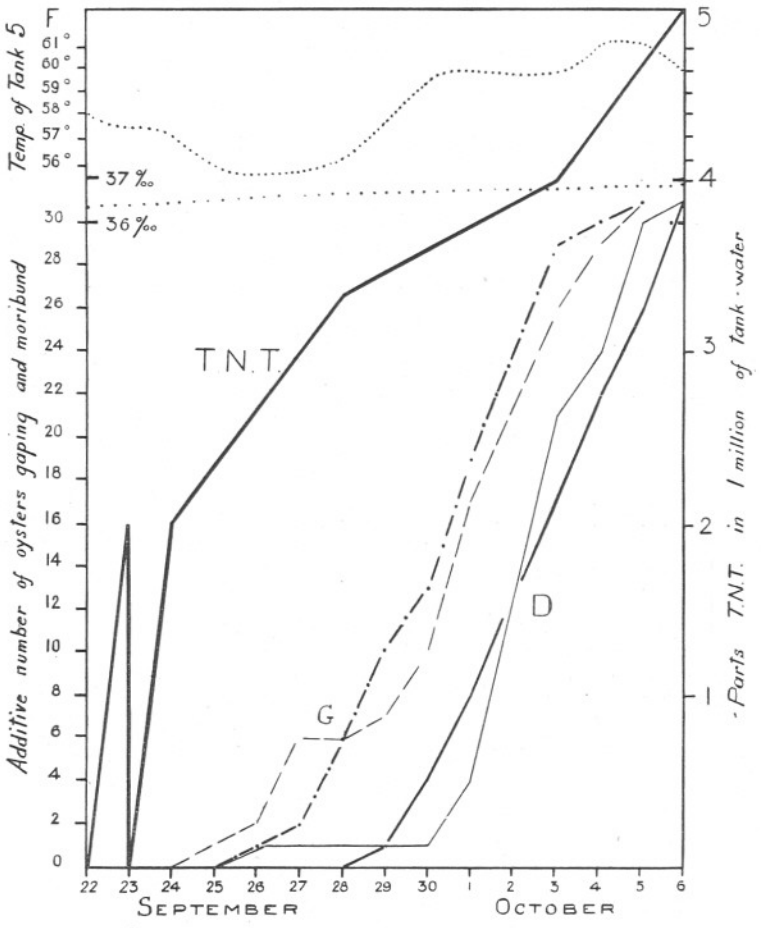

FIG. 1.—Graphs showing the additive numbers of native and Portuguese oysters (G) gaping and (D) dead or moribund, along with the increase in concentration of T.N.T. in Tank 5 and the variation in temperature and salinity.

The thick continuous line with the letters T.N.T. adjacent shows the increase in concentration of the poison.

The discontinuous lines which cross at G give the additive numbers of oysters gaping on the dates denoted; the thicker line represents Portuguese and the thinner one the native gapers.

The continuous lines which cross at D give the additive number of oysters taken from the tanks dead or dying; the thicker one again represents the Portuguese and the thinner the native oysters.

The dotted curve gives the temperature variation, and the dotted (almost) straight line the salinity variation.

Summary of Experiment III.

A glance at Fig. 1, p. 286, shows that although the earliest oysters to be affected were natives, these did not succumb so soon as the earliest affected Portuguese. Later after about eight days in the T.N.T. the natives began to die rapidly after gaping, but did not on the whole gape so soon as the Portuguese. The lag-period between the first obvious effect of the poison, gaping, and death, was at first longer in the natives than the Portuguese, and afterwards, shorter ; the same lag-period being fairly constant in the Portuguese throughout the experiment.

A glance at Fig. 2, below, shows that the magnitude of the daily rate of gaping alternated in the two species ; and that the maximum was first reached by the natives. The daily rate of mortality was at first higher in

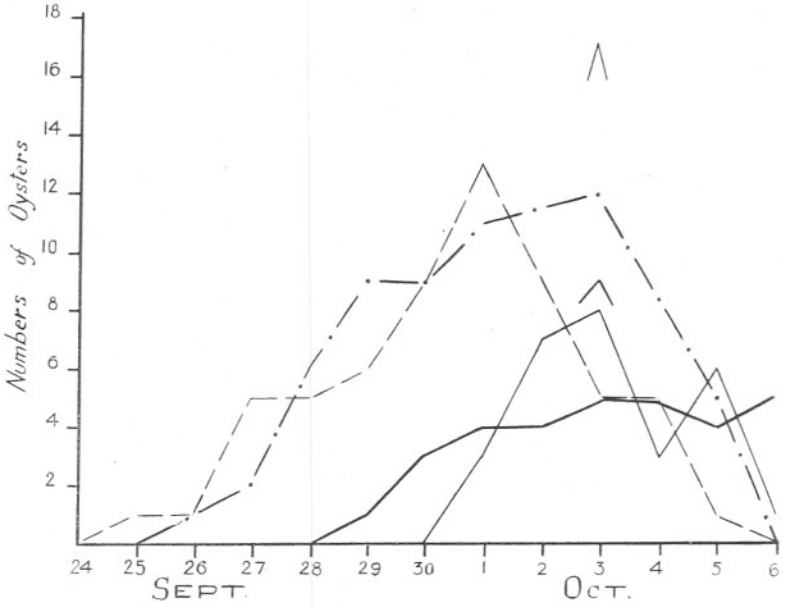

Fig. 2.—Graphs showing the daily number of gaping oysters (discontinuous lines) and daily number of moribund oysters (continuous lines) in Tank 5. The thick lines denote Portuguese oysters and the thin ones natives.

The isolated peaks give the sum of the mortality for the two days,* October 1st to 3rd, which is distributed between the 2nd and 3rd October in the main graph to maintain uniformity.

the Portuguese, but a sudden death of a number of natives took the mortality rate of these afterwards to a maximum, after which the rate fluctuated, while that of the Portuguese remained fairly steady to the end of the experiment.

* The tanks could not be examined on Oct. 2nd.

Table II shows—by the numbers of the oysters—the order of gaping, while the sequence from above downwards shows the order of death (or moribundity). It is observed that there is a distinct grouping of males amongst the early dying Portuguese and a tendency for the smaller native oysters to group amongst the later dying natives.

Table III gives the history of the oysters from gaping to the condition of being moribund. The recovery of N7 and longevity of N2, 3, 5, 6 and F1 and F4 are remarkable.

TABLE II.

Showing by the sequence from above downwards the order of death or moribundity in Portuguese oysters (of size and sex shown) and native oysters (size only shown). The serial numbers of both Portuguese and native oysters give the order of gaping. Usually many oysters gaped on one day, as also many died on one day. The length —antero-posterior measurement—,the height—dorso-ventral measurement—and the 1925 approximate growth or shoot measured dorso-ventrally in the median line, are given in millimetres.

Natives.					Portuguese.					
Date.	No.	Length.	Height.	1925 growth.	Date.	No.	Length.	Height.	1925 growth.	Sex.
Sept. 26	1	58	69	13	Sept. 29	2	66	85	30	Male.
Oct. 1	4	64	71	12	,, 30	3	56	76	?	,,
,, 1	8	61	66	8	,, 30	8	69	91	5?	,,
,, 1	9	61	67	6	,, 30	10	52	79	7	,,
,, 3	2	49	57	2?*	Oct. 1	7	50	90	4–31?	,,
,, 3	3	68	57	8	,, 1	6	47	57	17	One male and
,, 3	5	68	72	8	,, 1	6*a*	43	56	10	one female.
,, 3	6	71	66	9	,, 1	5	57	74	5	Spawning female
,, 3	10	76	77	10	,, 3	14	47	103	13	Male.
,, 3	11	73	83	5–22	,, 3	11	57	71	17	Spent female.
,, 3	12	64	64	12	,, 3	12	42	82	13	,, ,,
,, 3	13	57	58	13	,, 3	1	60	70	9–19	Female.
,, 3	14	67	75	10	,, 3	9	52	115	?	,,
,, 3	15	56	66	7	,, 3	13	54	62	6	,,
,, 3	16	62	60	3	,, 3	15	59	67	22	,,
,, 3	17	57	59	7	,, 3	16	39	71	6	,,
,, 3	18	64	63	7	,, 3	19	57	58	15	,,
,, 3	19	75	87	9	,, 4	21	51	80	7	,,
,, 3	20	69	75	8	,, 4	22	59	69	16	,,
,, 3	21	58	56	10	,, 4	25	48	65	2?	,,
,, 3	22	62	65	18	,, 4	27	54	84	15	,,
,, 4	23	56	62	14	,, 4	28	54	105	9+?	,,
,, 4	25	56	55	13	,, 5	20	51	87	5	,,
,, 4	26	52	54	9	,, 5	23	62	103	9	,,
,, 5	24	61	72	7	,, 5	24	64	90	5+?	Male.
,, 5	27	54	54	3+?	,, 5	26	44	86	5	Female.
,, 5	28	44	50	8	,, 6	4	85	108	14	,,
,, 5	29	56	61	5+?	,, 6	17	67	76	10	,,
,, 5	30	66	69	3	,, 6	18	51	72	20	,,
,, 5	20*a*	18	23	–	,, 6	29	36	97	8	,,
,, 6	7	54	56	7	,, 6	30	48	112	7+?	,,

* Dumpy.

Remarks on Experiments I to III.

Each oyster was numbered on the day of first gaping. Oysters were removed from the experimental vessels when they gaped widely, and showed no reaction when repeatedly forcibly closed; hence although there was no certainty that the oysters removed were actually dead,

TABLE III.

HISTORY OF THE EXPERIMENTAL OYSTERS IN TANK 5 WITH REGARD TO GAPING AND REMOVAL.

Date, Sept.	25	26	27	28	29	30	Oct. 1	3	4	5	6
Serial numbers of Portuguese Oysters gaping in the tank daily at the dates given above	0	1	1 2	1 2 3 4 5 6	1 3 4 5 6 7–10	1 4 5 6 7 9 11–13	1 4 9 11–18	4 17 18 20–28	4 17 18 20 23 24 26	4 17 18 29 30	30
Portuguese Oysters removed daily, dead or moribund at the dates given					2	3 8 10	5 7 6 6*a*	1 9 11–16 19	21 22 25 27 28	20 23 24 26	4 17 18 29 30
Natives gaping in Tank 5 daily at the dates given	1	2	2 3 4 5 6	2 3 4 5 6 7	2 3 4 5 6 8	2 3 4 5 6 8–10	2 3 5 6 10–18	23 24 25 26 27	21*a*† 24 27 28 29	7	7
Natives removed moribund at the dates given		1					4 8 9	2 3 5 6 10–22	23 25 26	21*a*† 24 27 28 29 30	7

these would certainly be moribund and very near death, if not actually dead. It was necessary to remove oysters at a stage near death, in order to prevent unnecessary fouling of the water. To reduce the fouling of the water in the experimental tank, debris was frequently siphoned off and the air jets maintained in good condition; but so long as sickly oysters were present in the tank, it was impossible to ensure water as clean as that in the control. The fact that the water on October 3rd had a pH of 8·00‡ after the heaviest effect of the T.N.T. on the oysters indicates that the water was maintained in fairly good condition.

The instant at which an oyster gaped in the T.N.T. would depend, among other factors, on how much the bivalve had "tasted" the water. Mitchell has shown (1914)§ that American oysters can remain closed, without taking oxygen, for a week without harm, and Orton‖ (1923)

* Brood on No. 6. † Spat on No. 21.

‡ I am indebted to Mr. C. F. A. Pantin for this determination.

§ The Oxygen requirements of Shellfish. Bull. U.S. Bureau of Fisheries, XXXII, 1912. (1914).

‖ O.M.I. Report loc. cit., p. 64, with full details in MSS. of Report.

showed that native oysters can live for one to three weeks on a very small quantity of oxygen. Therefore, oysters need not open for upwards of a week for the purpose of taking in oxygen. Nevertheless oysters gaped in Experiment I in from two to four days, and must therefore have taken in T.N.T. water before that time in the act of tasting the water. There may be a difference between native and Portuguese oysters in this respect which might affect the result of the experiments, but it is doubtful if the difference would be great. Experiments on style-formation prove that although the native oyster may not appear to be open, it does mostly taste the water within a period of a few hours from being immersed in it ; and Portuguese oysters probably behave similarly. All the oysters in the experiments therefore probably obtained an early dose of T.N.T., and the more cautious ones would take in at first a smaller dose than the others, and probably be able in this way to delay the acquisition of such a dose as would cause gaping.

Portuguese oysters have, it was found, a slower natural closing reaction than natives, showing that there is a distinct difference in the control of the closing muscles in the two forms. It is possible that the difference in control of the closing muscles is closely correlated with the tendency of Portuguese oysters to lose control of their adductor muscles earlier than natives in T.N.T., and with the experimental observation that when a native gapes in T.N.T. it is nearer death than the Portuguese.

On examining the oysters as they were removed from the experimental tank it was found that all the oysters, except one native and the spawning Portuguese females, were well fished. Generally the tissues of the natives were far more flabby and dead-looking than those of the Portuguese, while the mantle in the natives was mostly well shrunken, though still adhering to the shell, but only about half retracted from the border in the Portuguese. In a few cases, notably N7 and P30, gaping oysters showed a contraction of the heart, if the ventricle were pricked with a needle after removal ; but there can be no doubt that such oysters, if not dead, were in the last stages of vitality, and would soon have died if left a little longer in the T.N.T. In the sea, it may be remarked, a widely gaping oyster would have little chance of avoiding death by enemies.

On the morning of September 23rd, the day after the experiment was begun in Tank 5, it was found that one or more male and several female Portuguese oysters had spawned in the water. In this water, which had a concentration of two parts T.N.T. in a million, active sperm was found buzzing around the eggs, which, however, did not segment. The whole of the water in the tank was removed, the oysters and tank well washed and fresh tank water run in to restart the experiment. On the following morning, September 24th, six Portuguese oysters were found to be

extruding eggs in greater or less quantity (P11 and P12 continued to spawn intermittently for several days), and later four other females shot a portion of their eggs. On this day when the concentration of T.N.T. may be assumed to have been about the same as that on the previous day (about two parts T.N.T. per million), upwards of 5 to 10% of eggs were found segmenting, and afterwards developed in smaller numbers to a swimming stage, but then died.

The following histories of spawning females are worthy of note as showing that although the slightly spawning females withstood the T.N.T. on the whole, as well as the non-spawners, the two more fully spending females were amongst the first females to die.

No. of Oyster.	Date of first Spawning.	Date of gaping.	Date Moribund.	Remarks.
P5	24.9	28.9	1.10	Very little spent.
P11	25.9	29.9	3.10	Spent.
P12	24.9	30.9	3.10	Half spent.
P20	24.9	2–3.10	5.10	Very little spent.
P21	24.9	2–3.10	4.10	Ditto.
P22	24.9	2–3.10	6.10	Ditto.
P29	24.9	4.10	6.10	Ditto.

The fully and half-spent females, it is to be noted, did not die earlier than most of the males (see Table II). After nine days in the T.N.T. eight Portuguese oysters died, and of these six were males, one a spawning female, and one a ripening female. The physiological condition in relation to sex is therefore an important factor in the experiments. One male lived, however, until the last day but one of the experiment, and was found then to have sperm, which showed mobility in small numbers in Channel water, but none in T.N.T. water.

It would seem that male Portuguese oysters are more susceptible to T.N.T. poison than females, and also that the larger natives gape sooner than the medium-sized ones, but further experiments would be necessary to verify the observation. The last Portuguese to die in Experiment II, it should be noted, was a male.

No Portuguese oysters spawned in the control tank until after the experiment, but a few eggs were discharged by one or more females in the control bell-jar experiment, and none were discharged from four ripe females in Experiment I, and none from two ripe females in Experiment II.

T.N.T. only caused spawning in one experiment, and it is possible that in this case one oyster in Tank 5 was on the point of spawning

when the T.N.T. was put in the tank, and that either the T.N.T. or the effects of the preparation of the tank, had the effect of making it spawn at once, in the same way as the uncomfortable conditions consequent upon being taken out of water will cause ripe sea-urchins and other marine animals to spawn. The spawning of one individual, especially a male, may very well have started a number of others, male and female, to spawn, as such an occurrence is a not uncommon phenomenon amongst marine animals.*

On the 6th day after putting the T.N.T. in Tank 5 two slipper-limpets (*Crepidula fornicata*), which were attached to Portuguese oysters, were found detached, and were removed with a third on the following day as dead or dying. Two Crepidula lived in the control tank to date (Dec. 12) in a similar situation.

A few *Ascidiella* and *Ciona* (blubber) died in Tank 5 by the 11th day. when similar forms were doing well in Tank 8. Some small anemones also died in Tank 5, while other similar ones were still alive in Tank 8 on Dec. 12.

CONCLUSIONS.

In concentrations of T.N.T. rising rapidly to five to seven parts per million in unchanged sea-water, there is little difference in the susceptibility of the Portuguese and the native oyster to the poison. Subject to confirmation by further experiments—since the early gaping Portuguese were mostly either males or spawning females—it is indicated that Portuguese oysters gape on the average earlier than natives, but do not die so soon after gaping as the natives, especially as the poison becomes more concentrated.

In the same way it is indicated that male and spawning female Portuguese oysters die sooner in T.N.T. than ripening females, and that on the average large native oysters die sooner in T.N.T. than medium-sized natives.

If further information is desired on the comparative effects of T.N.T. on Portuguese and native oysters, it is suggested that more valuable results could be obtained by subjecting the experimental oysters to T.N.T. at a concentration of one to two parts per million in constantly renewed fresh sea-water. In this way a closer approach to the ideal experiment (see O.M.I. Report, p. 179) would be obtained, and the conditions and results would approximate more closely to those which might occur in the sea.

* H. M. Fox, Proc. Camb. Phil. Soc. Biology, I, 2, p. 71, 1924.

The Government Chemist's Report on the Results of the Examination of Samples of Sea-water for T.N.T. and Salinity.

(Copy.)

GOVERNMENT LABORATORY,
CLEMENTS' INN PASSAGE,
STRAND, LONDON, W.C. 2.

14*th October*, 1925.

The T.N.T. was determined by Dr. Brady's method, with certain modifications (see Fishery Investigations II, VI, No. 4, 1924, "An account of Investigations into the cause or causes of the unusual Mortality among Oysters in English Oyster Beds during 1920 and 1921," Part II).

The salinity was determined according to the International Method by Titration against Copenhagen Normal Water.

Lab. No.	Particulars of Sample.		T.N.T.	Salinity (parts per 1000).
83	No. 1, Tank 5	23.9.25	1 in 500,000	36·38
85	No. 2 ,, ,,	28.9.25 11 a.m.	1 in 300,000	36·66
86	No. 3 ,, ,,	3.10.25 12.30 p.m.	1 in 250,000	36·80
88	No. 4 ,, ,,	6.10.25 3.30 p.m.	1 in 200,000	36·92
87	Experiment II	3.10.25 12.45 p.m.	1 in 140,000	35·59
2854	Tank circulation	22.9.25 4 p.m., temp. 14·4° C.		36·36
2855	Control (to) Experiment II, bell-jar	3.10.25 1.5 p.m., temp. 15·5° C.		35·37
2856	Control Tank, 8	3.10.25 1.5 p.m., temp. 14·9° C.		36·92
2857	Tank 8	6.10.25 3.30 p.m.		36·98

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Structure and Physiology of the Organs of Feeding and Digestion in *Ostrea edulis.*

By

C. M. Yonge, B.Sc., Ph.D.,

Temporary Assistant Naturalist at the Plymouth Laboratory.

With 42 Figures in the Text.

CONTENTS.

1. INTRODUCTION.

This research is the outcome of a recommendation made by Dr. J. H. Orton in his report (1923) on the cause of the mortality of oysters during 1920 and 1921, in which he pointed out the necessity of our obtaining more precise information regarding the physiology of the oyster, both for its own sake and for its possible economic applications. I have endeavoured, therefore, to give as complete an account as possible of the structure and function of the food collecting and digestive organs in the oyster—larval, "spat," and adult—in the hope of so determining the optimum conditions for feeding and digestion, and, consequently, for growth and "fattening." In view of the fact that no complete account of the anatomy and histology of these organs exists, it has been necessary to devote considerable time to this aspect of the work, since a sound knowledge of the structure of any organ is essential if the function is to be determined. The research covers a great deal of ground, so that it has been impossible in the time available to investigate in detail every problem that has been encountered or to perform all the experiments that have suggested themselves, but no problem of the first importance has been neglected, while it is hoped in the near future to carry out further investigations into those aspects of the work which have been found the most important.

The work on the adult oysters was carried out at the Plymouth Laboratory, the oysters being obtained from the River Yealm, and the work on the larval and " spat " oysters at the Fisheries Experimental Station at Conway during July and August, 1925. I wish to express my gratitude for their kindness and help to the Director and members of the Staff, especially Dr. J. H. Orton, of the Plymouth Laboratory, and to Dr. Dodgson, Mr. H. P. Sherwood, and the other members of the Staff at Conway.

2. ANATOMY AND HISTOLOGY OF THE ALIMENTARY SYSTEM.

A. ADULT OYSTERS.

1. Anatomy.

The arrangement of the food collecting and digestive organs in the oyster can best be described by reference to Fig. 1 in which an oyster is shown lying on the lower (left) shell valve with the right fold of the mantle cut away. The surface of the mantle (L.M.F.) is transversely ridged, and is bounded by a thickened margin bearing two rows of small tentacles. The mantle cavity is divided into inhalent (I.C.) and exhalent chambers (E.C.), the former being some four times the larger, and containing the gills (G.), which consist of four demibranchs, the inner ones being broader than the outer ones, the inner one on the left (under) side being the broadest of all. The outer demibranchs are attached directly to the mantle, the inner ones being attached to the mantle on the outer side and to one another on the inside. In the oyster the two pairs of demibranchs are not separated by a protruding foot or visceral mass. The gills extend in a semicircle from the junction between the right and left mantle folds, which forms the division between the inhalent and exhalent chambers (D.B.C.), to the labial palps (L.P.). The latter consist of triangular flaps attached by a broad base and arranged in two pairs, one on each side of the mouth (M.). The inner, opposing surfaces are ridged (see Fig. 2), the outer surfaces being smooth. The palps enclose the gills for a short distance, the outer and inner demibranchs of each side lying between the corresponding pairs of palps, the inner demibranchs arising slightly nearer the mouth than the outer, and immediately behind the most distal fold on the palp surface. Unlike the majority of Lamellibranchs, the inner and outer palps of the two sides are united to one another in the region of the mouth (M.), which lies in the middle line in the groove formed by the continuation of the grooves between the two

sets of palps. The outer palps are united for about a quarter of their length, so that the mouth is entirely enclosed.

The mouth is a narrow horizontal slit and leads into a short œsophagus (O.) which has the the same shape in cross section and passes backwards and downwards into the stomach (S.). This is an irregular sac-shaped organ which is surrounded on all sides by the brown mass of the digestive

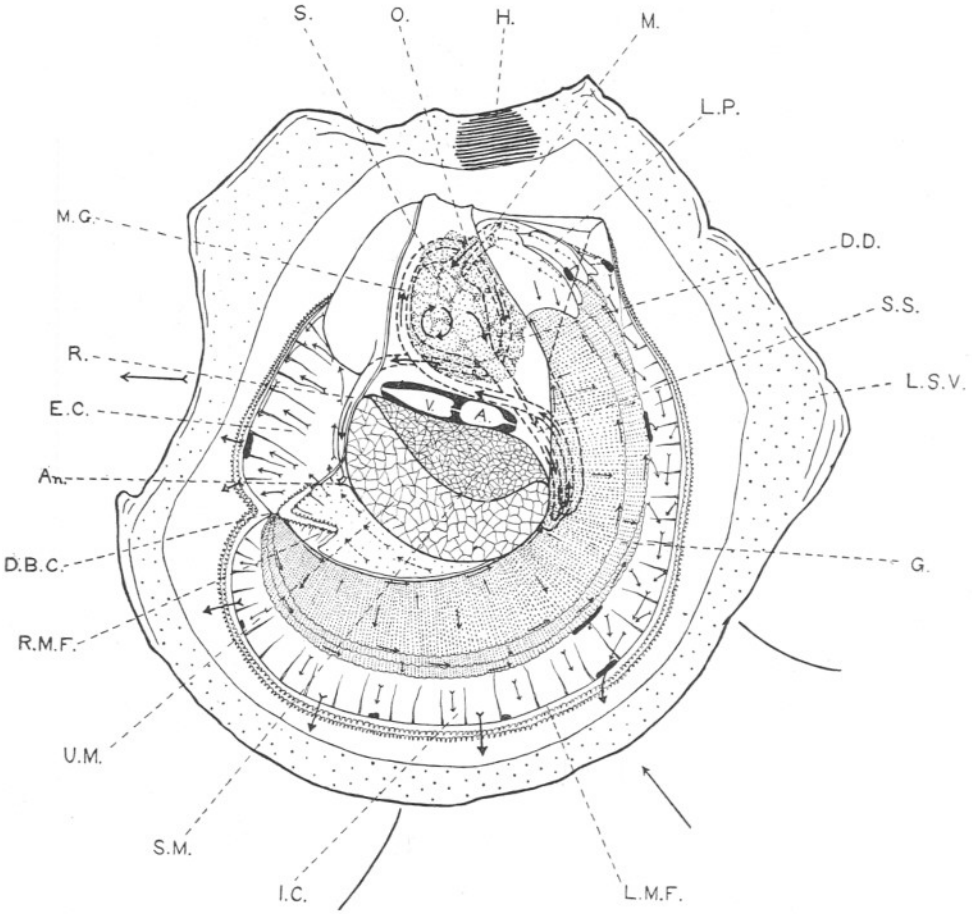

FIG. 1.—*Ostrea edulis*, right shell valve and mantle fold removed. × 1. A., auricle; An., anus; D.B.C., division between inhalent and exhalent chambers; D.D., digestive diverticula; E.C., exhalent chamber; G., gills; H., hinge; I.C., inhalent chamber; L.M.F., left mantle fold; L.P., labial palps; L.S.V., left shell valve; M., mouth; M.G., mid-gut; O., œsophagus; R., rectum; R.M.F., right mantle fold; S., stomach; S.M., adductor muscle, portion with striated fibres; S.S., style-sac; U.M., adductor muscle, portion with smooth fibres; V., ventricle. Large arrows external to shell denote direction of ingoing and outgoing currents, within shell plain arrows denote direction of ingoing currents and feathered arrows direction of outgoing currents, broken arrows (except in gut) denote currents on under surfaces.

diverticula (D.D.), while internally the walls are thrown into a series of ridges and folds so that the exact shape of the stomach in the living animal is difficult to determine when it is opened for inspection. In order to obtain a clear idea of the anatomy, casts of the stomach were made by injecting, by way of the œsophagus, a warm, concentrated solution of gelatin. This was allowed to cool and solidify, the tissues were then dissected away, and the cast hardened in formalin and stained lightly with hæmatoxylin. Gutheil (1912) used plaster for making casts of the stomach

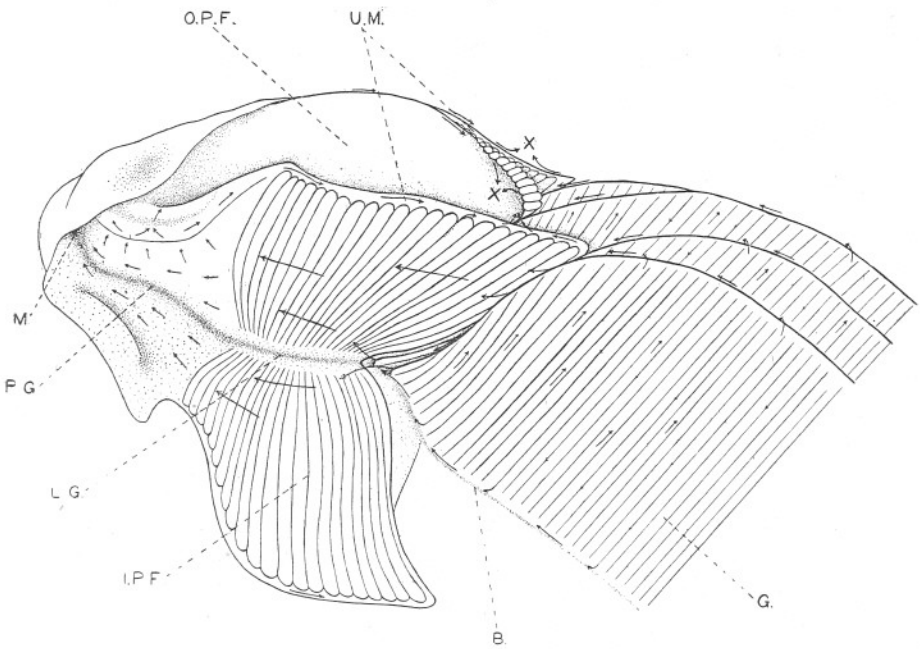

FIG. 2.—Junction of palps and gills, right palps opened out so as to expose inner, ridged surfaces. × 8. B., base of gill demibranch; G., gill; I.P.F., inner palp face; L.G., lateral oral groove; M., mouth; O.P.F., outer palp face; P.G., proximal oral groove; U.M., upper margin of palps; X., point where material is rejected from palps.

of Anodonta, but I have found the gelatin method much more satisfactory, and by its use have been able to demonstrate in detail the anatomy of the stomach—a much more complex and important organ in the Lamellibranchs than it has usually been considered—and its associated organs. Figs. 3 and 4 are drawings of a cast, the former from the ventral aspect and the latter from the dorsal aspect. The most conspicuous structure in the stomach is the long, grooved food sorting cæcum (F.C.), which extends backwards beneath the floor of the stomach, and is connected

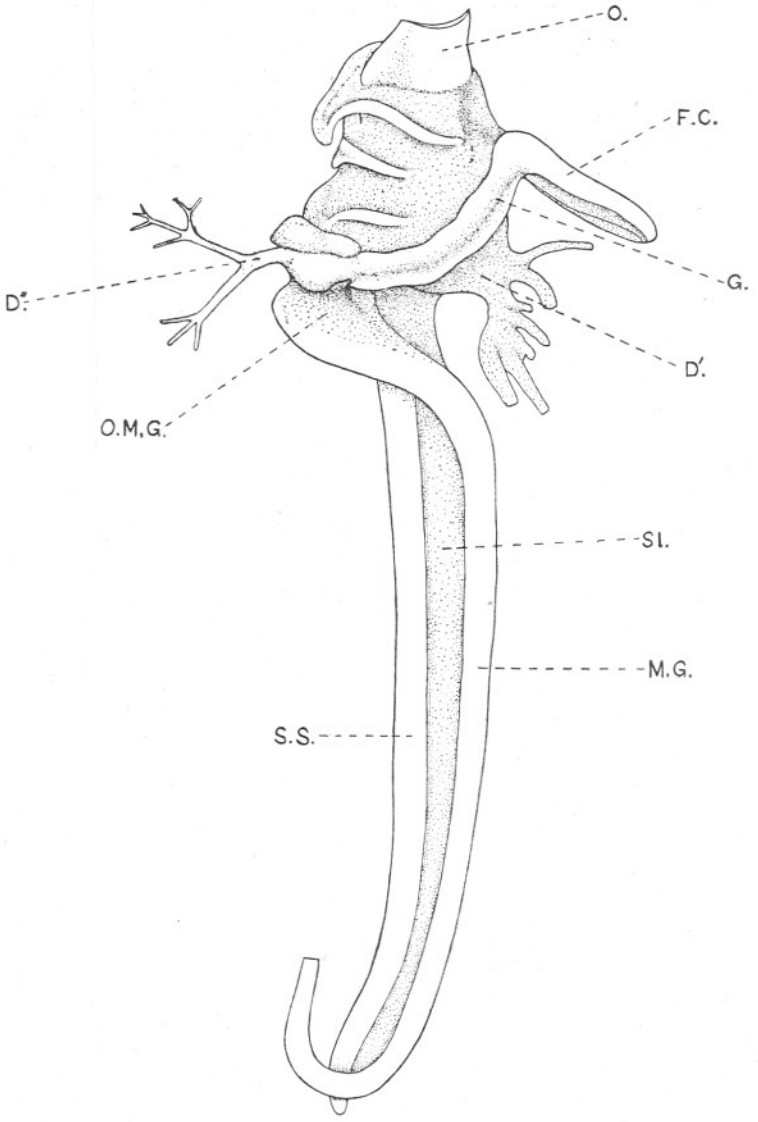

FIG. 3.—Gelatin cast of stomach with style-sac and first part of mid-gut and portion of œsophagus, from ventral aspect. × 4. D′., larger, left duct of digestive diverticula; D″., smaller, right duct of same; F.C., food sorting cæcum; G., ventral groove; M.G., mid-gut; O., œsophagus; O.M.G., opening of mid-gut; S.S., style-sac; Sl., slit connecting mid-gut and style-sac.

with the opening of the mid-gut (O.M.G.) by means of a deep groove (G.), which runs across the floor of the stomach. On opposite sides open the two ducts which lead into the digestive diverticula, that on the left side (D′.) being the larger and dividing into a greater number of smaller

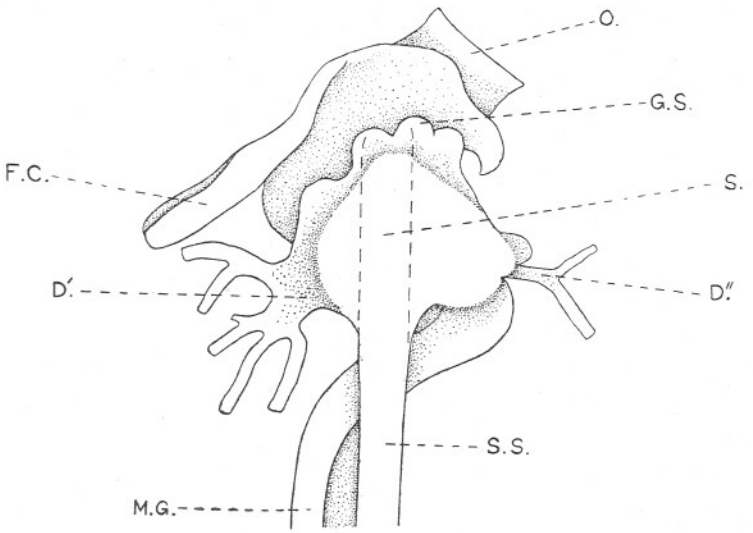

FIG. 4.—Stomach cast from dorsal aspect. × 4. G.S., gastric shield S., style. Other lettering as in Fig. 3.

ducts than the one on the right (D″.). On the dorsal wall of the stomach is borne the gastric shield (G.S.), a cuticular structure of somewhat irregular shape (see Fig. 5) consisting of two broad lobes united by a narrow neck, the larger of the lobes being thin and smooth, while the smaller is thicker and bears a number of teeth, which are also shown in Fig. 4. It is against this shield that the crystalline style bears, and the dotted line in Fig. 4 shows the position of the style as it projects into the stomach from the style-sac (S.S.), and bears against the gastric shield on the opposite wall. The cavities of the style-sac and mid-gut are united by a narrow slit (Sl.), and pass downwards and slightly forwards from the stomach, as shown in Fig. 1.

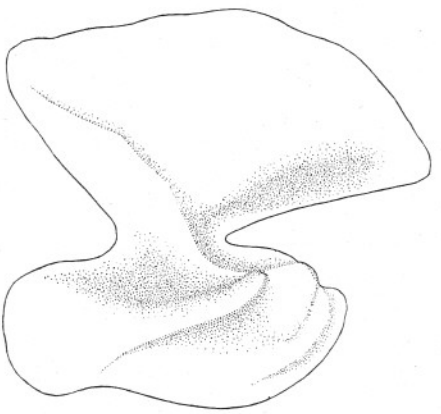

FIG. 5.—Gastric shield. × 20.

The style-sac is practically straight but the mid-gut, which opens into the stomach on the right side of the style-sac (Figs. 3 and 4), twists round to the left side immediately behind. At the distal end of the style-sac, the gut turns anteriorly and then completely back on its course, subsequently passing dorsal to the heart (Fig. 1, A. and V.) and encircling the stomach on the left side. Finally, it passes into the rectum (R.), which runs round the posterior margin of the adductor muscle (U.M.), and ends at the anus (A.), which lies at the tip of a small papilla on the posterior ventral surface of the muscle, and opens into the exhalent chamber.

II. Histology.

Material for histological examination was fixed in Bouin's fluid or in Flemming's strong fluid, sections were cut 6μ thick, unless otherwise stated, and were stained with Delafield's hæmatoxylin and erythrosin; or with iron hæmatoxylin either with acid fuchsin as counterstain or with mucicarmine for the demonstration of mucus glands; or with alum carmine and picro-nigrosin.

(*a*) *Gills.*

An excellent account of the gills of *Ostrea edulis* has been given by Ridewood (1903), the substance of whose statements is as follows: "There are 9–12 filaments to the plica. The front of the principal filament has the form of a broad ridge. The filaments adjacent to the principal filament are slightly larger than usual, and have been called *transitional filaments* by Kellogg (1892). . . . The interlamellar junctions have the form of septa. At a short distance up, the interlamellar septa occur only in relation with alternate principal filaments, but the order is not absolutely regular. Higher up still each fourth septum only persists. The bars which run across the floor of the suprabranchial cavity from descending to ascending lamella are the thickened upper edges of alternate high septa. They recur at intervals of about eight plicæ. Most of the interfilamentar junctions are bands of tissue running horizontally round the inner surface of the plica, but each third or fourth in a vertical series extends across the plica as a horizontal septum. . . . The frontal and lateral cilia are normal. There are short cilia on the interlamellar edges of the principal filaments. No intrafilamentar septum is present. There is a fair amount of muscle in the interfilamentar junctions and in the inner edge of the horizontal septa. . . ."

Many of the characteristics of the gill of Ostrea given by Ridewood, and also other points which he does not emphasize but which are of importance functionally, are shown in Fig. 6, which represents a trans-

verse section through a single lamella (i.e. one side only of a complete demibranch). A principal filament (P.F.) is figured with four filaments on either side, all being united by an interfilamentar junction (I.). (For the structure of the interlamellar junctions and the horizontal interfilamentar septa, reference must be made to Ridewood's figure.) The large size of the principal filament and the thickness of the chitinous supporting rods (C.R.) within it and the two transitional filaments (T.F.) one on either side of it are well marked. There are many strands of horizontal muscle (H.M.) in the interfilamentar junction and also a slight development (not mentioned by Ridewood) of vertical muscle (V.M.)

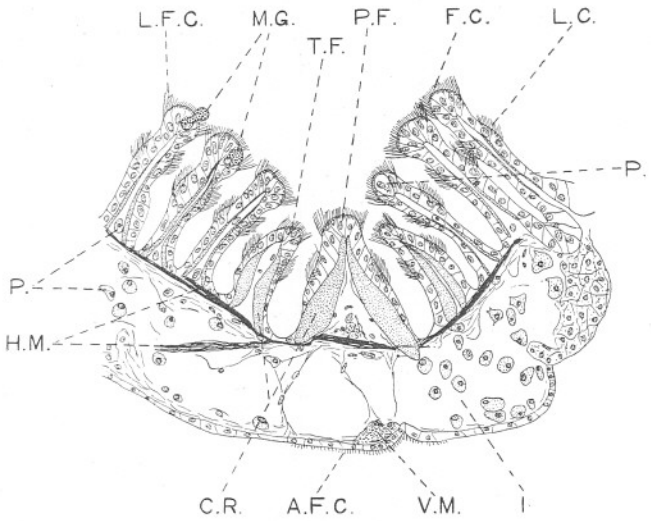

FIG. 6.—Transverse section single gill lamella through groove between plicæ. Delafield's hæmatoxylin and erythrosin. × 220. A.F.C., abfrontal cilia; C.R., chitinous supporting rod; F.C., frontal cilia; H.M., horizontal muscle; I., interfilamentar junction; L.C., lateral cilia; L.F.C., laterofrontal cilia; M.G., mucus glands; P., phagocytes; P.F., principal filament; T.F., transitional filament; V.M., vertical muscle.

in the principal filament. The abfrontal cilia (A.F.C.) noted by Ridewood are shown in the figure, and also the lateral (L.C.), frontal (F.C.), and laterofrontal (L.F.C.) cilia. The latter are not well developed in Ostrea and are difficult to distinguish in sections, but are easily seen in fresh material (see Fig. 21, p. 323). Mucus glands (M.G.) occur in the epithelium of the filaments, particularly in the frontal region. Wandering blood cells (P.) are present in large numbers within the filaments and the interfilamentar junctions, and are also frequently to be found actually between the cells of the epithelium. These cells, as will be shown later, are phagocytic, and will be referred to as phagocytes in the remainder of this account.

Along the free, lower margins of the demibranchs and along their axes there are ciliated grooves.

(*b*) *Palps.*

The folds on the inner palp surfaces arise near the attached base of the palps, and run across the face to the upper free margin, gradually increasing in height and breadth. In cross section (Fig. 7) they are seen to bend forward slightly on the proximal side (i.e. in the direction of the mouth), a tendency which is most pronounced near the free margin.

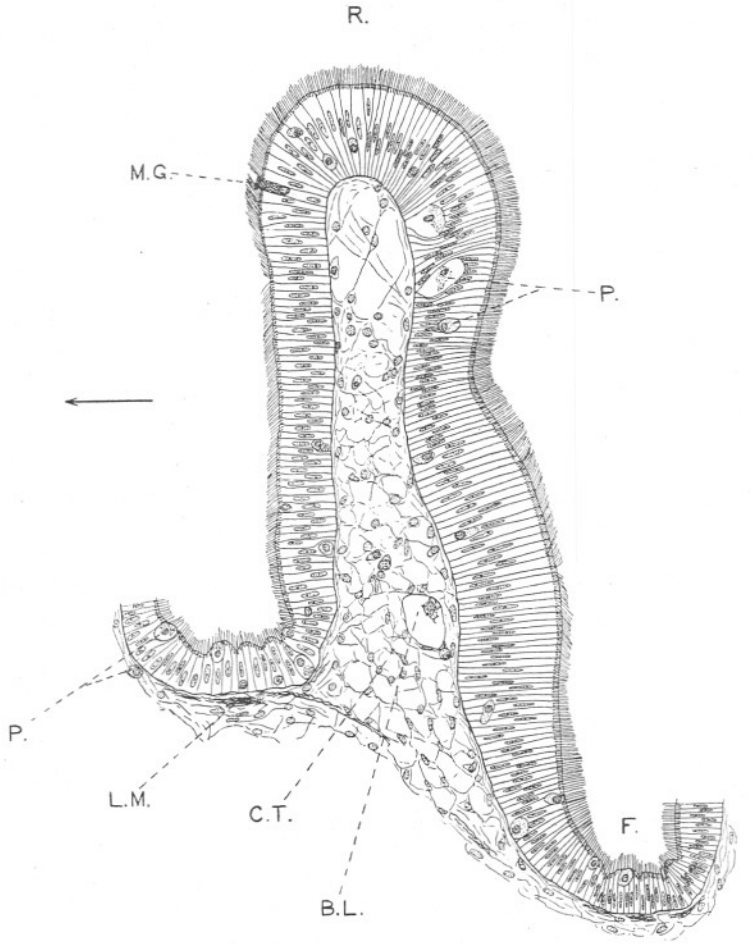

FIG. 7.—Transverse section through fold on inner palp face. Delafield's hæmatoxylin and erythrosin. × 330. B.L., blood lacuna; C.T., connective tissue; F., furrow between ridges; L.M., longitudinal muscle; M.G., mucus gland; P., phagocytes; R. summit of ridge. Arrow indicates direction of mouth.

The proximal wall of the folds is comparatively straight, but on the distal wall there is a well-developed longitudinal groove about one-third of the distance between the summit of the fold (R.) and the furrow (F.), which lies between adjacent folds. The epithelium of the folds is composed of long, regular cells with oval nuclei and bearing a thick covering of long cilia. These cilia, as can be seen by the directions in which they lie in the sections, do not all beat in the same direction. It is, however, impossible to distinguish all the different tracts except in the living tissue, an account of which is given in a later section. Unicellular mucus glands (M.G.) of the goblet type are present, almost exclusively near the summits of the folds. Siebert (1913) has also found sense cells in the epithelium of the palps in Anodonta. Between the epithelial cells are many phagocytes (P.), which are also present in the connective tissue and blood lacunæ (B.L.), some of them containing yellow or brown granules. The connective tissue is very open in character, consisting of a network of fine strands. There are longitudinal muscle fibres (L.M.) under the epithelium of the furrows, and running through the connective tissue at the base of the ridges. There are also occasional fibres running across to the smooth surface of the palp, and a feeble development of circular muscle immediately beneath the epithelium of the furrows. It is important to note that there are *no muscles within the folds* such as could cause it to contract downwards.

The epithelium of the smooth surface of the palps (Fig. 8) is very different. The cells are lower and more irregular, cilia are present but often difficult to distinguish in sections, so that some workers have denied their presence. I have often seen them in my sections of Ostrea, while experiments on the living palp demonstrate immediately their presence. Mucus glands of the usual type are extremely numerous. The contents of these cells may be granular, and stain darkly with iron hæmatoxylin or contain a mass of swollen granules or spheres which stain lightly with mucicarmine. Since intermediate stages between these two types are frequently found (this applies wherever mucus cells are found, from the mantle to the rectum), it seems probable that the granules represent an early

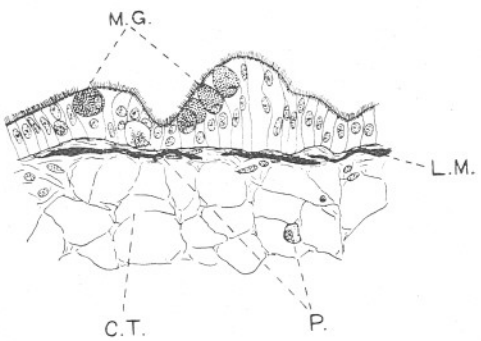

FIG. 8.—Transverse section epithelium of smooth, outer palp face. Delafield's hæmatoxylin and erythrosin. × 330. Lettering as in Fig. 7.

stage in the elaboration of the secretion. The glands invariably stain deeply with erythrosin, and have a great affinity for many stains. Beneath the basement membrane there is a well-developed layer of longitudinal muscle (L.M.). There is the usual network of connective tissue (C.T.) with darkly staining nuclei among which are many phagocytes (P.), which may also penetrate into the epithelium.

(c) *Mouth and Œsophagus.*

The epithelium of the mouth is a continuation of that of the grooves between the palps and consists of extremely long, thin cells, about four times the height of those on the folded surface of the palps. Long cilia are borne by the cells, mucus glands occur but not in large numbers, while phagocytes are present in great abundance.

The œsophagus (Fig. 9) is exceptionally large in the oyster and much compressed dorso-ventrally. The epithelium consists of narrow cells of

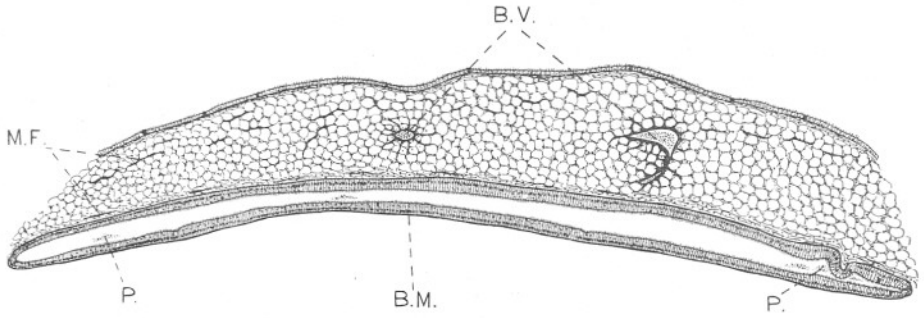

FIG. 9.—Transverse section œsophagus. Iron hæmatoxylin and acid fuchsin. × 18. B.M., basement membrane; B.V., blood vessels; M.F., muscle fibres; P., phagocytes and food matter in lumen.

much the same height as those of the mouth region, but with cilia of only about a third the length. Phagocytes are very numerous between the cells of the epithelium and also *free in the lumen* (P.), sometimes with ingested matter. Mucus glands are here very rare, but Gutheil (1912) has described and figured what he considers to be secretory cells in the œsophagus of *Anodonta cellensis* and similar cells can be distinguished in Ostrea. They stain more lightly and have rather more vacuolated protoplasm than the neighbouring cells, and have, in addition, no cilia. On the other hand, their nuclei are identical with those of the ciliated cells, and there is really very little evidence that they are secretory cells; it seems more probable that they are damaged or degenerating epithelial cells. Particles are continually passing over the epithelium, which must suffer in the process, and it is quite common, moreover, to

find dividing nuclei near the free surface of the epithelium. An exceptionally thick basement membrane (B.M.), through which phagocytes pass, surrounds the epithelium, and outside this there are thin strands of circular and longitudinal muscle fibres (M.F.). Muscle strands also occur in the vesicular connective tissue in which the œsophagus is embedded and which contains large blood-vessels (B.V.), in and out of which the phagocytes pass.

(*d*) *Stomach.*

The epithelium of the stomach is of two kinds, that composed of typical ciliated cells, which covers the greater part of the surface, and that which lies beneath the gastric shield. Fig. 10 represents a section through the junction between the two. The ciliated epithelium consists of narrow cells, a little higher than those of the œsophagus and possessing longer cilia. The border cuticle (B.C.) is particularly well developed here,

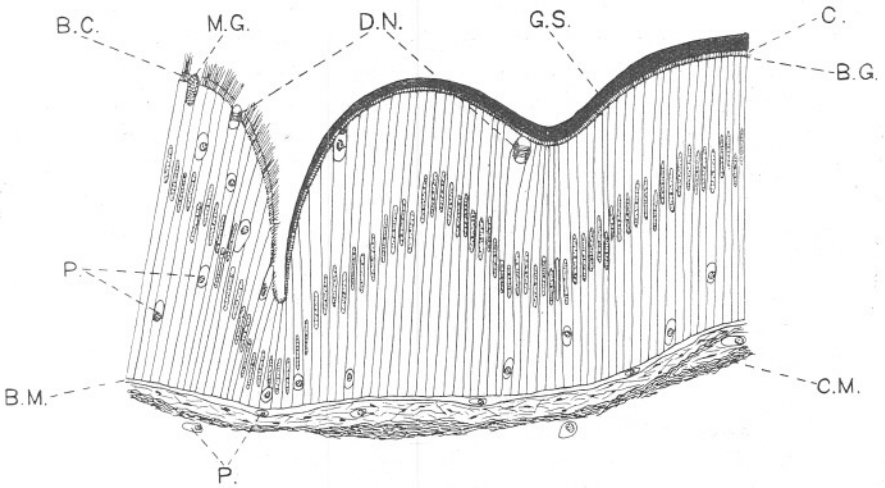

FIG. 10.—Transverse section stomach epithelium at junction between gastric shield area and ciliated epithelium. × 425. B.C., border cuticle; B.G., basal granules; B.M., basement membrane; C., cilia-like strands between edge of cells and gastric shield; C.M., circular muscle; D.N., dividing nuclei of epithelial cells; G.S., gastric shield; M.G., mucus gland; P. phagocytes.

consisting (as in all the ciliated cells) of a clear cuticular layer distal to the line of basal granules, from which the cilia arise. The distinct nature of this layer is not shown by the usual staining methods, but after staining with Prenant's three-colour process (iron hæmatoxylin, erythrosin, and light green) the border cuticle is stained by the light green and the cytoplasm by the erythrosin. Mucus glands (M.G.) are occasionally found; phagocytes (P.) are very abundant in the connective tissue, basement membrane, between the cells of the epithelium and free in the lumen.

Dividing nuclei (D.N.) of epithelial cells are frequently seen, the nuclei passing to the surface of the cells in the manner characteristic of the dividing nuclei of ciliated cells (see Ehrhard (1910), and particularly Gutheil (1911), who has described and figured in detail the process of mitosis). There is a fairly thick basement membrane (B.M.), and, beneath that, strands of muscle (C.M.) the whole being surrounded by vesicular connective tissue in which lie embedded the tubules of the digestive diverticula.

The epithelium which lies beneath the gastric shield resembles closely that of the rest of the stomach. Mucus glands are never present, and phagocytes, though invariably present, occasionally even in the substance of the gastric shield, are not so numerous. Dividing nuclei are frequent. The gastric shield (G.S.) in cross section appears as a homogeneous substance composed of indistinct horizontal strata. It stains vividly with light green, moderately deeply with erythrosin (except in Prenant's stain) and very lightly with mucicarmine. Gutheil considers that it is formed by droplets of secretion from the cells beneath, and this has been the general view with regard to its formation. In my sections, however, I have failed to find any evidence of secretion from the cells, while the substance of the shield is united to the epithelium by fine strands having the appearance of cilia (C.) and arising from basal granules (B.G.) at the edge of the cells, as shown in Fig. 10. It is possible in certain places to observe the continuation of the strands transversely through the whole substance of the shield, while, as we have seen, the shield takes up light green in the same way as the border cuticle. In view of these facts and also that the cells of the gastric shield area are in no way different from those of the rest of the epithelium with regard to either nucleus or cytoplasm, it seems very probable that the gastric shield is *not* a secretion, but is formed by the fusion of cilia, originally in response to the irritation caused by the head of the style. Nelson (1918) thinks the shield is probably in the nature of chondrin, which would appear to support this view.

(*e*) *Digestive Diverticula.*

These consist of a brownish mass of blind tubules which surround the stomach. They have been called " liver " and " hepatopancreas," but, as I have emphasized in a recent paper (1926) to which reference should be made for a detailed account of the structure and function of these organs, they are organs of assimilation and of intracellular digestion with none of the functions of a true liver or pancreas, and I suggested, therefore, that they are more suitably termed digestive diverticula.

They communicate with the stomach by way of two large ducts (Figs. 3 and 4, D′. D″.). These ducts are quite distinct in structure from the

tubules with which they communicate. They are usually circular in cross section, though the lumen is irregular owing to the variation in height of the epithelium, which is similar to that of the stomach of which it is a direct continuation. Cilia are always present, the protoplasm is not vacuolated and stains darkly with erythrosin. Mucus glands are present and also phagocytes in large numbers, both in the epithelium and in the lumen. There is a layer of circular muscle beneath the basement membrane.

The tubules (Fig. 11) are quite different. Cilia are never present in sections nor can a border cuticle be distinguished as in the case of some

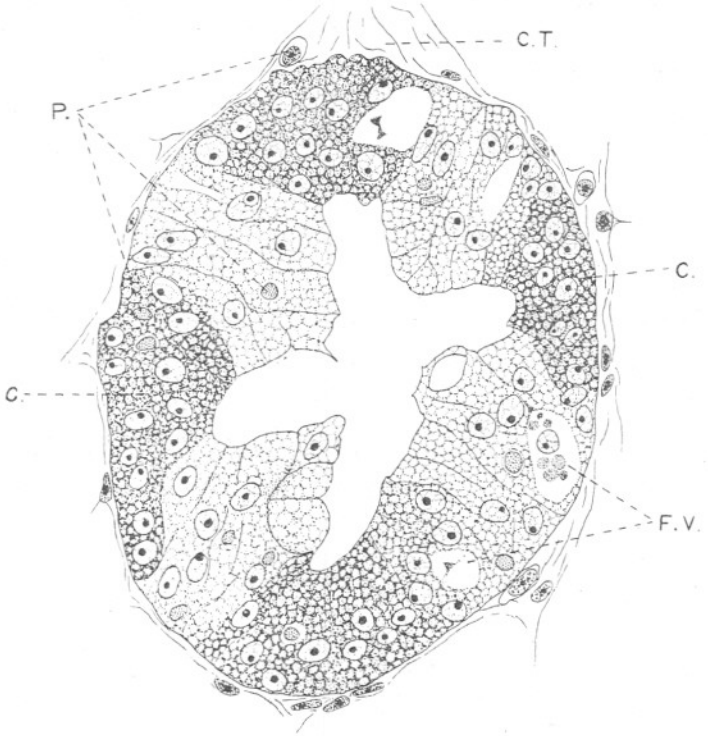

FIG. 11.—Transverse section through tubule of digestive diverticula. Iron hæmatoxylin and acid fuchsin. × 350. C., crypts of darkly staining young cells; C.T., connective tissue; F.V., food vacuoles; P., phagocytes.

Lamellibranchs, and the outline of the cells is frequently very irregular. The tubules are surrounded by a few strands of connective tissue (C.T.), but muscle is *never* present. The nuclei are very characteristic, being circular and possessing a large nucleolus. In cross section the lumen is usually tripartite or in the form of a cross, and in the crypts (C.) at the

end of the arms are low areas of darkly staining protoplasm with many nuclei, the outline of the cells being very indistinct. The remaining cells are larger, very vacuolated, and consequently more lightly staining. As I have pointed out (1926) there is every reason, " From the histological character, the distribution, and the behaviour of these small dark cells . . . for considering them young cells which, by dividing, are able to make good the loss resulting from the casting off of the old cells." Large vacuoles (F.V.), sometimes with ingested food material which stains with erythrosin, are frequently found in the older cells, while phagocytes occur everywhere. There is never any indication of secretion.

As already noted, cilia are never to be seen in sections (similar observations have been made on Ostrea by Carazzi (1896, 1897), MacMunn (1900), and Vonk (1924)), nor have I observed them in fresh material; but in many Lamellibranchs (though never possible to see more than a border cuticle in sections), it is possible to see long cilia beating in the tubules when fresh material is examined, as Potts (1923) and I (1926) have shown. As will be shown later, there is a constant stream of food particles passing into the diverticula and of rejected particles passing out, and as there is no system of circular and longitudinal muscles such as ensures a similar circulation in the Crustacea (in which the diverticula are organs of both assimilation and secretion), there is strong presumptive evidence that cilia are present in the tubules of *all* Lamellibranchs. In many cases, including Ostrea, these cilia appear to be retracted very readily, and so cannot be seen when the tissue is pressed out under a coverslip for examination.

The tubules are embedded in vesicular connective tissue, in which lie many phagocytes often containing included granules, which frequently, as MacMunn (1900) has described and figured, take the form of brown or yellow spheres, which often are blackened by osmic acid after fixation with Flemming. The nature of the pigment will be discussed in the section on Assimilation (p. 340).

(*f*) *Style-Sac.*

Except for a short diverticulum where it arises (see Fig. 3), the style-sac is united for its entire length with the mid-gut. The two cavities (Fig. 12) are separated by two typhlosoles which, however, are not so well marked as in such genera as Anodonta (Nelson, 1918) or Crepidula (Mackintosh, 1925). The epithelium of the gut is quite distinct from that of the style-sac, and will be described later. The epithelium of the style-sac (Fig. 13) is very characteristic, consisting of cells of medium height, very regularly arranged, with large oval nuclei and long, stout cilia all of the same length. The structure of the style-sac in *Crepidula fornicata* (which,

although a Gastropod, has a style and style-bearing organs of exactly the same nature as those of the Lamellibranchs) has been investigated in detail by Mackintosh (1925). He has shown that the cilia are continued into the cell where they form an "internal fibrillar apparatus," the fibres of which are greatly thickened below the nucleus, so as to form "a bundle of thick rod-like bodies." I have observed the same arrangement in Ostrea (Fig. 13, I.F.), the fibres showing very clearly after staining with iron hæmatoxylin (though whether or no they really represent fibres in the living tissue cannot be stated). Mackintosh has also demonstrated the presence of a series of "intra-epithelial" canals,

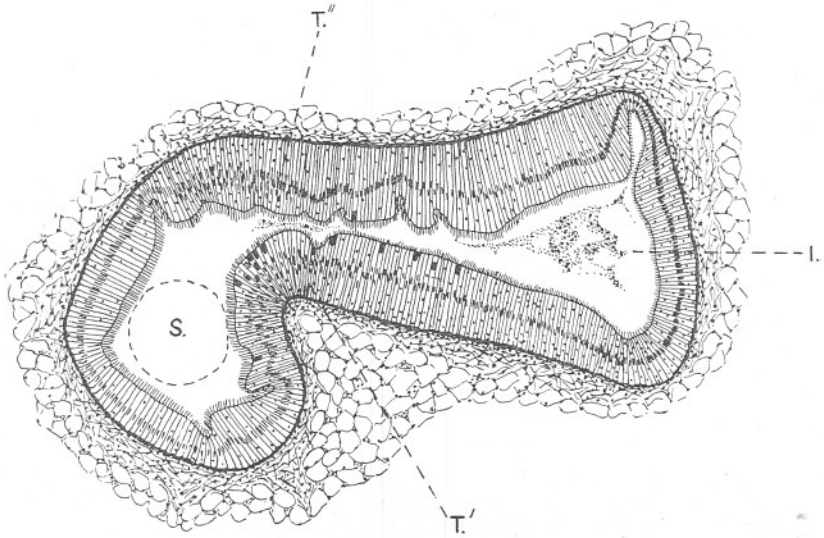

FIG. 12.—Transverse section style-sac and mid-gut. Iron hæmatoxylin and mucicarmine. × 56. I., lumen of gut; T′., larger typhlosole; T″., smaller typhlosole; S., position of style in sac.

which appear in transverse sections near the base of the cells, and are filled with a very lightly staining, stringy substance. A similar state of affairs exists in Ostrea, the canals (I.E.C.) appearing to pass through and not between the cells, though it is difficult to be certain. Mackintosh has further shown that the larger canals extend longitudinally down the style-sac, and are connected with one another by smaller canals, and this appears also to be the case in Ostrea. Judging by their staining reactions, he is of the opinion that the contents are of the nature of connective tissue fibres, and considers that the function of the whole apparatus is to lend extra strength to the epithelium, which bears a considerable strain in revolving and pushing forward the style. The epithelium of the larger typhlosole (T′. Fig. 12), which corresponds to the *minor* typhlosole

of Anodonta and Crepidula, or the *right* typhlosole of Mya as described by Edmondson (1920), consists of long, very narrow cells with cilia a little shorter than those of the groove. Mucus glands are very numerous in this region and also occur in the other typhlosole (T″.)—which is covered with long cells which gradually merge into the epithelium of the mid-gut—but never in the epithelium of the style-sac. The intra-epithelial canals also occur in the typhlosoles, but in decreasing numbers as these merge into the epithelium of the gut. Phagocytes occur everywhere, though they are not so numerous in the epithelium of the style-sac as they are in that of the typhlosoles, in which they are exceptionally numerous. The whole is surrounded by a few strands of muscle, the typhlosoles being filled in with vesicular connective tissue of the usual type.

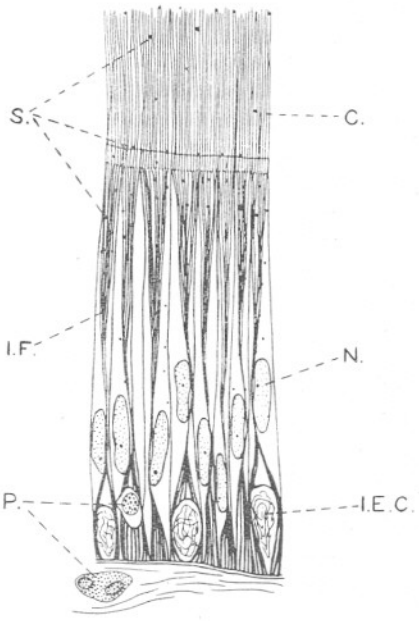

FIG. 13.—Transverse section epithelium of style-sac. Iron hæmatoxylin and acid fuchsin, secretion granules demonstrated by iron technique. × 900. C., long cilia of epithelium; I.E.C., intra-epithelial canals; I.F., internal fibrillar apparatus; N., nuclei of epithelial cells; P., phagocytes; S., droplets of secretion containing iron in solution.

It is very difficult to determine where and how the substance of the style is secreted. List (1902), Nelson (1918), Edmondson, and Mackintosh all think that it is secreted by the narrow cells of the minor typhlosole, but they have been unable to produce definite evidence. Gutheil describes and figures clear vesicular granules above the nuclei in the cells of the style-sac. In Ostrea, sections prepared for histological examination showed no sign of any secretion. It has been shown (1926) that the presence of minute droplets of secretion containing iron in solution can be demonstrated in the style-sac epithelium of *Mytilus edulis* four hours after a 0·5% solution of iron saccharate in sea-water has been injected by way of the foot. This method of demonstrating the presence of secretory cells has been employed with success for Crustacea, Insecta, and Gastropoda (for full details and literature see my papers (1924, 1926)). I have employed the same methods with Ostrea, injecting the same

solution by way of the adductor muscle, afterwards washing the animals so as to prevent any of the fluid entering the mouth. The style-sac was fixed (by the methods described in the section on assimilation) two, four, and six hours later, and sections prepared which were treated so as to demonstrate the presence of iron by the Prussian blue method, the sections being stained with alum carmine. In the style-sac of the animal which had been fixed four hours after injection, it was easily possible to distinguish fine blue granules in the cytoplasm above the nuclei and in the process of being passed into the lumen. The position of the granules is indicated in Fig. 13 (the internal fibrillæ do not appear after staining with alum carmine, but were drawn from sections stained with iron hæmatoxylin). There was *no trace of similar granules in the epithelium of the gut*, nor could I determine their presence with certainty in the narrow cells of the larger typhlosole though they are present in the cells of the other typhlosole so long as they retain the character of the style-sac epithelium. In view of the presence of these granules, it seems probable that Gutheil is correct, and that the substance of the style is secreted by the cells of the groove and not of the larger typhlosole, and that it is then revolved by the cilia of the style-sac, so that, as Edmondson has shown in his experiments on the regeneration of the style in Mya, it comes to lie against the larger typhlosole the cilia of which have a different function, as will be described in the section on ciliary currents.

The style during life lies in the groove of the style-sac, as indicated by the broken circle in Fig. 12. It is a gelatinous rod, whose structure has been described too often for further detailed description to be necessary. In the oyster the central core is very fluid and flows freely to and fro, the outer portion being firmer and consisting of co-axial layers. The style is seldom white, usually yellowish or brown, but the colour depends on the nature of the food, as in all cases where the style-sac is in communication with the mid-gut. Spirochætes of the genus Cristispira are very numerous, particularly in the outer layers, and are able to move about freely in the substance of the style.

(*g*) *Mid-Gut.*

This region (Fig. 14) is characterised in cross section by the possession of a large typhlosole with a groove down the centre. The cells of the epithelium are invariably ciliated, mucus glands are present, but not in large numbers, while there is a complete absence of muscle around the epithelium, which is bounded by a broad basement membrane. Phagocytes are very plentiful both in and around the epithelium, and in the lumen, where they are to be seen lying among the food

particles and mucus therein contained, particularly in the groove of the typhlosole.

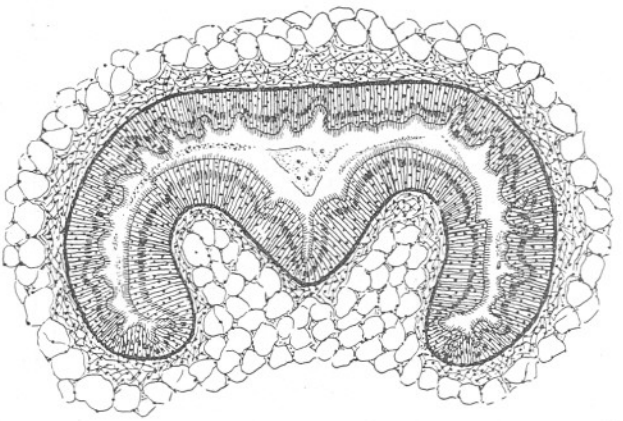

FIG. 14.—Transverse section mid-gut. Iron hæmatoxylin and acid fuchsin. Round dots in epithelium indicate phagocytes, dark masses indicate mucus glands. × 56.

(*h*) *Rectum.*

The rectum (Fig. 15) is practically circular in cross section, the lumen being larger than that of the mid-gut. The typhlosole is here thrown into more prominent folds, and the central groove is practically obliterated, owing to the coming together of the two halves of the typhlo-

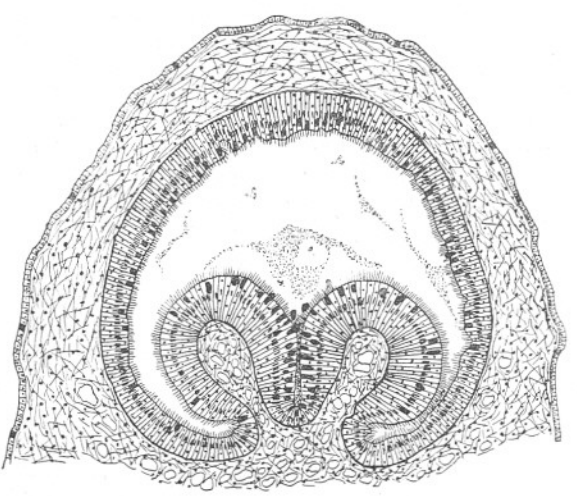

FIG. 15.—Transverse section rectum. Delafield's hæmatoxylin and erythrosin. Great numbers of mucus glands in epithelium. × 56.

sole. Mucus cells are extremely numerous, more so than in any other region of the alimentary canal (the same is true for *Mya arenaria* (Yonge (1923)). All the other cells of the epithelium are ciliated, phagocytes are very plentiful everywhere; there is no surrounding muscle, while the basement membrane is thinner than that of the mid-gut. The surrounding connective tissue is more compact than in any other region of the gut. In the lumen are found food particles, or excreta, mucus, and phagocytes.

(*i*) *The Phagocytes.*

As will have been noted from the foregoing account, one of the most striking features about the gills, palps, and entire alimentary tract is the universal presence of wandering phagocytic cells. They are always easy to distinguish because their nuclei, unlike those of the epithelial cells which are oval and lightly staining, are small, spherical and contain a great number of fine granules of chromatin, which stain darkly with hæmatoxylin. The cytoplasm of the phagocytes stains lightly with erythrosin. No less than seven different types of blood cells in the Lamellibranchs have been distinguished by de Bruyne (1896), but it is doubtful how many of these represent different stages in the same type. In this paper no attempt is made to divide the phagocytes into different types, although further work on the subject is contemplated.

The presence of these phagocytes is characteristic of the Lamellibranchs (with the possible exception of the Septibranchs), and attention has been drawn to their presence by many workers, although their great importance in the physiology of digestion in these animals has not always been recognised. Lankester (1886, 1893) seems to have been the first to note the presence of the phagocytes in the gills of green oysters; de Bruyne (1893, 1896) gave a long account of the wandering of phagocytic cells into the epithelium of the gills and mantle in a number of Lamellibranchs; Herdman and Boyce (1899) gave a full account of their activities, especially in connection with green leucocytosis in the American oyster; List (1902) noted their presence in and around the gut in the Mytilidæ; Gutheil (1912) gives a full account of their occurrence throughout the alimentary tract of Anodonta and in the connective tissue and blood-vessels, and he also observed them dividing amitotically in the region of the gut; Matthias (1914) observed the presence of great numbers of phagocytes in the ventral portion of the stomach of *Arca barbata;* Orton (1923) has noted their great numbers and widespread distribution throughout the tissues, and particularly round the gut, of *Ostrea edulis;* I have myself (1923, 1926) observed and figured them in the gut of Mya, and in connection with the digestive diverticula in the same animal and in Nucula, Cardium, and Teredo.

In the oyster they are abundant everywhere, and appear to pass freely through the tissues. Fig. 16 represents a blood-vessel from the region of the œsophagus (it is an enlarged drawing of the smaller blood-vessel shown in Fig. 9). In the lumen can be seen a mass of blood cells,

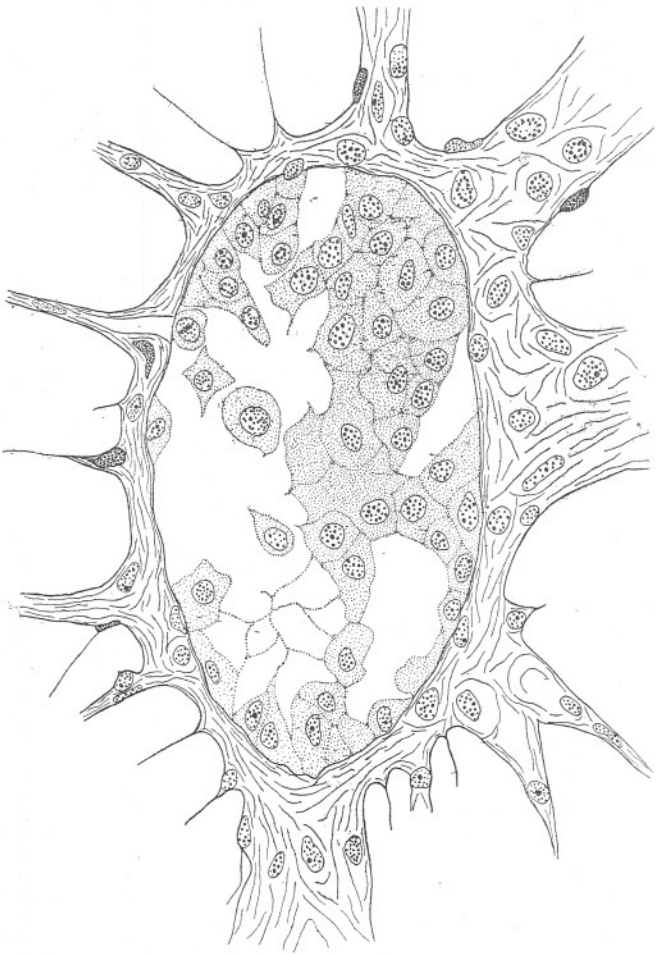

FIG. 16.—Transverse section through blood vessel near œsophagus. Many amœboid blood cells in lumen and passing through walls. Iron hæmatoxylin and acid fuchsin. × 1200.

which have probably come together as a result of fixation. Similar cells can be seen passing through the wall of the vessel, though it is impossible in this region to distinguish more than the characteristic nuclei; the nuclei of the connective tissue which forms the wall of the blood-vessel are usually smaller, spindle-shaped, and stain intensely black. There can

be no doubt that the cells are amœboid, and have the power of wandering at will through the tissues and in and out of the lumens of the gut and of the blood-vessels. An account of the very important part they play in the assimilation of food will be given in the appropriate section.

B. LARVAL OYSTERS.

The development and structure of the larvæ of *Ostrea edulis* have been described in detail by Horst (1886), while Stafford (1913) has given an

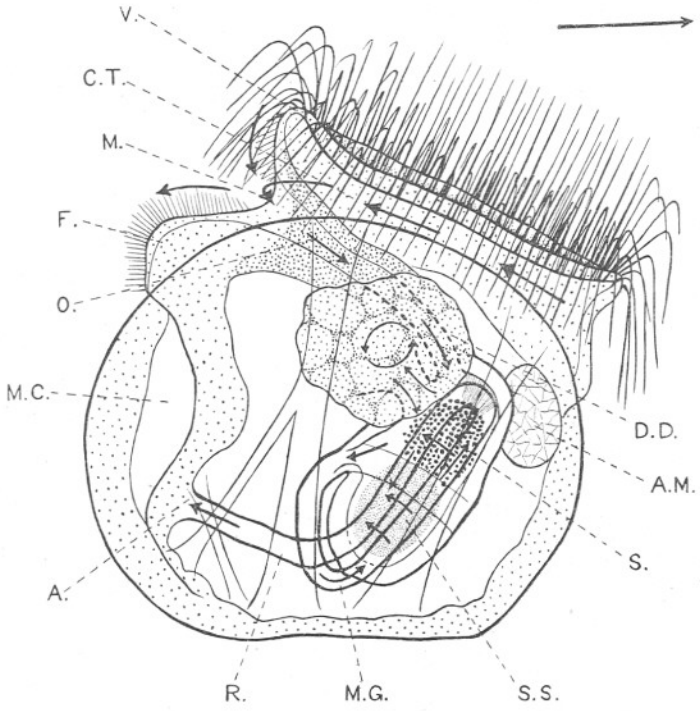

FIG. 17.—*Ostrea edulis*, veliger larva showing alimentary organs, drawn from life. × 330. A., anus; A.M., adductor muscle; C.T., ciliated tract at base of velum; D.D., digestive diverticula; F., foot; M., mouth; M.C., mantle cavity; M.G., mid-gut; O., œsophagus; R., rectum; S., stomach; S.S., style-sac with contained style; V., velum. Large arrow above figure shows direction of movement, smaller arrows in figure show direction of food currents caused by cilia.

account of the developmental stages in the American oyster, *Ostrea virginica*, with a summary of the previous work on both species. Here it is necessary only to describe the alimentary organs of the veliger larvæ of *Ostrea edulis*. Fig. 17 represents such a larva, the dimensions of whose shell were $0{\cdot}2 \times 0{\cdot}165$ mm., drawn from life so as to show the alimentary organs. The velum (V.), which is protruded—when retracted the organs

are tightly packed together and difficult to distinguish—is crowned with extremely long cilia, while there are smaller cilia round the base. The mouth (M.) lies behind the velum, between it and the rudiments of the foot (F.). It is a wide, funnel-shaped orifice which leads into an œsophagus (O.), whose thick walls are pigmented. This passes forwards and downwards in the middle line and opens into the head of the stomach (S.), an oval-shaped organ divided by an annular thickening of the wall from the style-sac (S.S.). On the posterior wall of the stomach open the two simple lobes of the digestive diverticula (D.D.), which are arranged symmetrically one on either side, their more ventral portions overlapping the œsophagus. They are darkly pigmented and even at this stage have the structure of the adult diverticula (see Fig. 42, p. 353). The style-sac contains the style which, though difficult to distinguish normally, can easily be seen if the larvæ are placed for several hours in a very dilute solution of brom thymol blue in sea-water when the substance of the style stains a light yellow, and can be seen revolving rapidly in the stomach. It may be a single oval mass (as represented in Fig. 17), or be composed of from two to four rounded masses. The mid-gut (M.G.) begins on the posterior side of the stomach at the line of its junction with the style-sac, and passes dorsally and then ventrally, describing a loop on the right side of the stomach before turning backwards as the rectum (R.), which ends in the anus (A.) on the dorsal side of the mantle cavity (M.C.). The whole of the gut is lined with large and very active cilia (cilia cannot be seen in sections of the digestive diverticula, but there is evidence that they are present in the living tissue), though in the figure the only cilia shown are the group of extremely large ones on the antero-ventral wall of the stomach. The external dimensions of the various parts of the gut are : œsophagus, 0·02 mm. ; stomach, 0·046 mm. ; mid-gut, 0·012 mm. Sections of the larvæ do not demonstrate any points in the structure of the alimentary system, which cannot be seen in an examination of the living larvæ.

C. "SPAT" OYSTERS.

The structure of the food collecting and digestive organs in recently settled or "spat" oysters, though they quickly come to resemble those of the adult, show many interesting features. Unfortunately, 1925 proved a bad year for spat at Conway, and I was unable to obtain specimens in the act of settling, and so get stages in the metamorphosis from the larval to the adult structure, a process which takes place with great rapidity. The larvæ come to lie on the left valve cementing themselves firmly to the surface by means of a secretion from the byssus gland in the temporarily developed foot. A full account of the metamorphosis of the American oyster has been given by Stafford. Fig. 18 is a

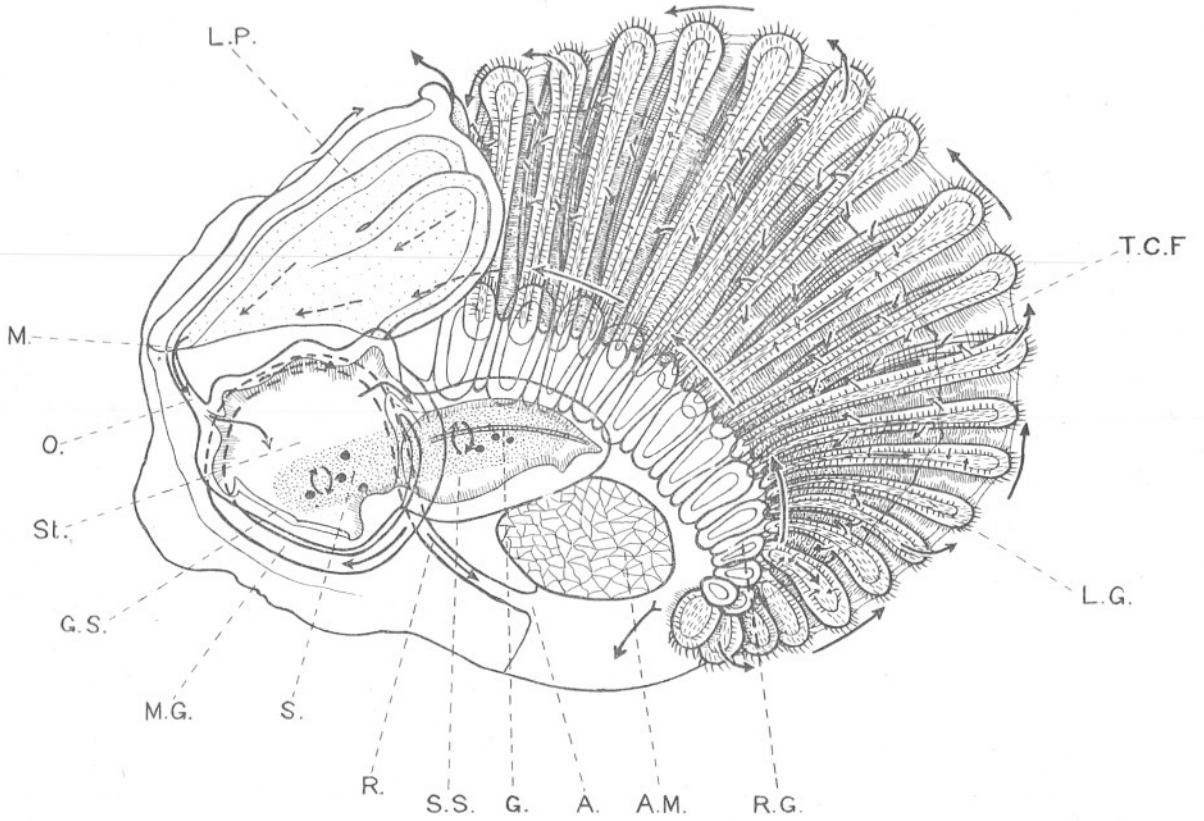

FIG. 18.—*Ostrea edulis*, "spat" shortly after settling, drawn from life after removal of shell (1·2 mm. deep), digestive diverticula not shown. × 155. A., anus; A.M., adductor muscle; G., groove down style-sac; G.S., gastric shield; L.G., left gill; L.P.; labial palps; M., mouth; M.G., mid-gut; O., œsophagus; R., rectum; R.G., right gill; S., style; S.S., style-sac; St., stomach; T.C.F., transparent connections between free ends of filaments. Arrows indicate direction of ciliary currents and movement of style.

drawing of the smallest settled oyster which I obtained, the shell (not figured) measured 1·2 mm. from the umbo to the margin and the body, after removal from the shell and consequent contraction of the mantle folds (i.e. as shown in the figure), 0·59×0·66 mm.

At this stage there is one simple gill on each side which represents the inner demibranch of the adult. There is a marked difference in the degree of development, the lower or left gill (L.G.) being much larger than the upper, right one (R.G.). Moreover, there are twenty filaments present on the left and only thirteen on the right. No firm lamella is formed, the filaments being united solely by thin strands of transparent tissue (T.C.F.) at their free extremities. The ascending and descending portions of the filaments are also unconnected. Lateral, frontal, and laterofrontal cilia are all to be distinguished on the filaments and also large cilia on the free extremities. The labial palps (L.P.) are much larger in proportion to the rest of the body than in the adult. The outer palps are completely united to form a hood which encloses the inner palps, which are united for about half their length. The mouth (M.) leads into a short œsophagus (O.), which opens into the large stomach (St.). Seen from the side this is a somewhat squarish organ with a well-developed gastric shield (G.S.) on the dorsal wall, against which bears the style (S.), which can readily be distinguished as a stout rod in which lie embedded diatoms and other particles. The wall of the stomach is covered with large cilia and so is that of the style-sac (S.S.), which forms a wide tubular diverticulum posterior to the stomach. Along one side of the sac is a narrow groove (G.). The stomach is surrounded by tubules of the digestive diverticula, though these have not been shown in Fig. 18 as they would have obscured the other organs ; they are best studied in sections. On the postero-ventral side of the stomach is the opening of the mid-gut (M.G.), still quite distinct from the style-sac. As in the adult, the gut describes a circle round the left side of the stomach before passing dorsally and backwards as the rectum (R.); the anus (A.) opens into the exhalent chamber on the dorsal side of the adductor muscle (A.M.).

Transverse sections through a slightly larger specimen—the shell was 2 mm. across—are shown in Figs. 19 and 20. The former represents a section through the middle of the stomach. Owing to the direction of the cut, the section has passed transversely through a number of the gill filaments and the disparity in numbers between the filaments of the two sides is again demonstrated. The stomach lumen is practically filled with the style, the dorsal walls—with the exception of the extreme dorsal end—being covered with the gastric shield, the remainder of the wall being thickly ciliated. On the ventral side is the opening of the mid-gut (O.M.G.); the gut has been cut twice (M.G.) in its course round

the left side of the stomach. On the left wall of the stomach opens one of the ducts (O.D.) of the digestive diverticula, tubules of which (D.D.) are present on all sides of the stomach. Unlike the adult condition, however, the ducts have the same structure as the tubules—i.e. there are no

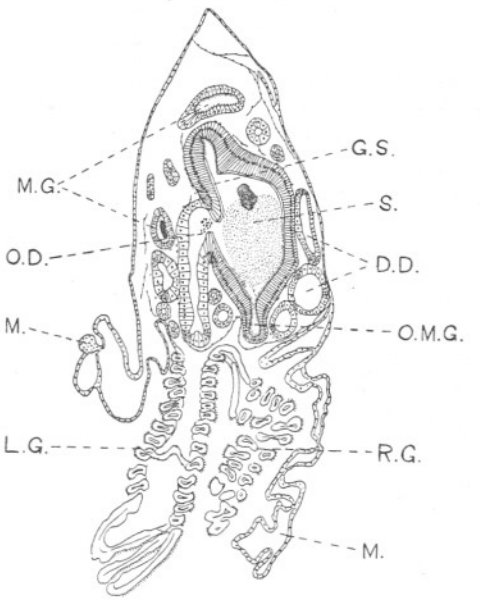

FIG. 19.—Transverse section through 2 mm. spat in region of stomach. Alum carmine. × 80. D.D., digestive diverticula; G.S., gastric shield; L.G., left gill; M., mantle; M.G., mid-gut; O.D., opening of digestive diverticula into stomach; O.M.G., opening of mid-gut into stomach; R.G., right gill; S., style.

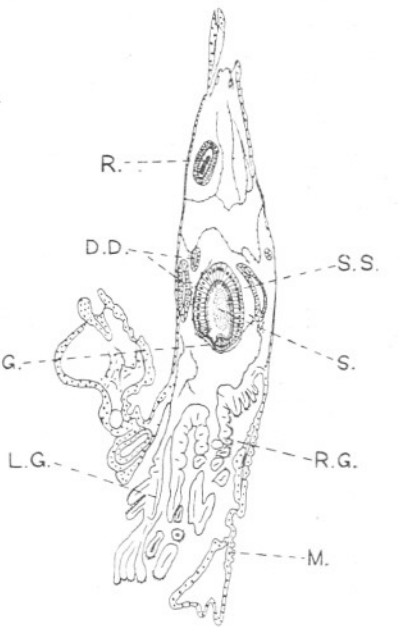

FIG. 20.—Section from same series as Fig. 19, more posterior. × 80. G., groove in style-sac; R., rectum; S.S., style-sac. Other lettering as in Fig. 19.

ducts strictly speaking. The tubules are identical in structure with those of the adult, nests of darkly staining young cells lying between the more lightly staining, vacuolated and older cells. The dark masses in the stomach, the opening of the digestive diverticula, and in the lumen of the mid-gut are iron saccharate on which the animal had been fed one day before fixation.

Fig. 20 represents a section from the same series as Fig. 19, but more posteriorly, the section passing transversely through the style-sac (S.S.), the structure of which is shown clearly. The epithelium consists of large cells, very clearly demarcated, containing large oval nuclei and covered with thick, long cilia. On the ventral side lies the groove (G.), which is formed of extremely low ciliated cells bounded on each side by a group of tall, narrow cells. It is along the line of this groove that the union of

style-sac and mid-gut must later take place, and the areas of tall cells are, no doubt, identical with the cells of the future typhlosoles. The lumen of the sac is incompletely filled by the substance of the style (S.). In view of the fact that in Ostrea the style-sac and mid-gut are separate in the larvæ and early stages of the adult, it would be interesting to know whether in species such as *Mya arenaria* in which these structures are also separated in the adult, the separation represents a persistent embryonic condition or is secondary. The structure of the style-sac in the adult Mya would suggest that there has been union between the two and secondary separation. The other points of interest in Fig. 20 are the backward prolongations of the digestive diverticula (D.D.) on either side of the style-sac, and the rectum (R.) which here contains a mass of iron in the lumen.

3. FEEDING. THE COURSE OF THE CILIARY CURRENTS.

The course of the ciliary currents was followed in the intact tissues under the binocular microscope, and in small pieces of excised tissue under the high powers of the monocular microscope.

Carmine and carborundum powder of varying grades were employed to demonstrate the direction of the currents. The literature on this branch of the subject is extensive, and reference has been made to only the most important papers.

A. ADULT.

1. In the Mantle Cavity.

(*a*) *The Gills.*

Although in the oyster the mantle folds are not united except at the point of division between the inhalent and exhalent chambers, the food current is not drawn in along the whole of the inhalent chamber since, as described and figured by Orton (1912), the mantle folds are normally opposed except for the short distance on the ventral surface between the thick lines in Fig. 1. The ingoing current is caused by the action of the lateral cilia on the gills, a fact fully established by the work of Wallengren (1905) and Orton (1912). These cilia cause a strong current of water to pass between the gill filaments from the infrabranchial chamber into the suprabranchial chamber, which is in free communication with the exhalent chamber, as shown by the dotted arrows ventral to the adductor muscle in Fig. 1.

As a result of this current, any particles in suspension in the water will be carried into the inhalent chamber. As soon as the ingoing current has passed through the comparatively narrow inhalent aperture, its

speed will be reduced and the largest particles in suspension will drop on to the mantle folds. This may be called the first selection of particles. Smaller particles remaining in suspension will be deposited on the surface of the gill which serves as a very efficient filter, the water passing between the filaments while the particles are stopped by the action of the latero-

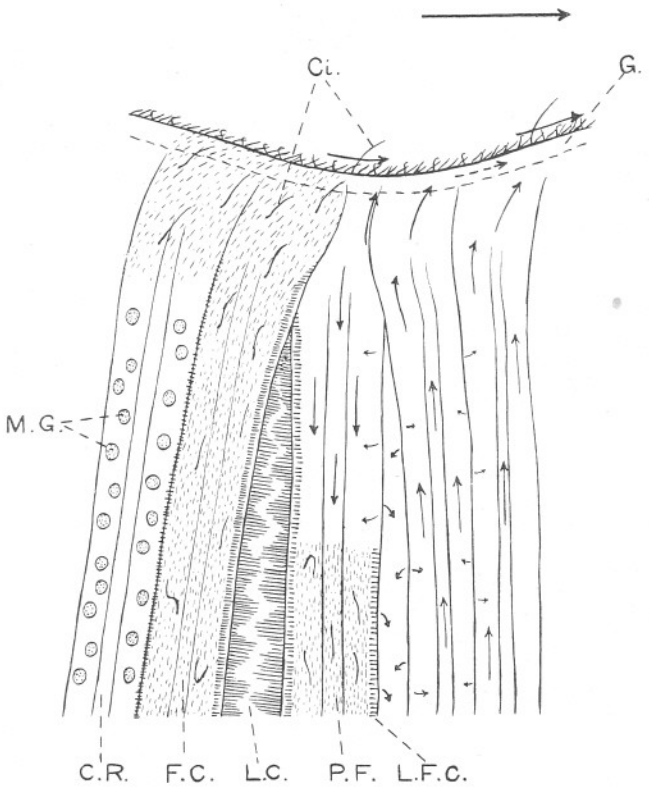

FIG. 21.—Semi-diagrammatic representation of five gill filaments and free margin of demibranch, several of the filaments being drawn apart to show cilia between. × 375. C.R., chitinous supporting rod in filament; Ci., cirri; G., ciliated groove at free margin; F.C., frontal cilia; L.C., lateral cilia; L.F.C., laterofrontal cilia; M.G., mucus glands; P.F., principal filament. Arrow above figure indicates direction of mouth, smaller arrows in figure show direction of beat of cilia.

frontal cilia (Fig. 21, L.F.C.), which lie at the edges of the filaments, so that those of adjacent filaments interlock, while at the same time they beat across the surface of the filament, and so throw particles on to the frontal surface. Fig. 21, which represents several filaments pulled apart, shows these cilia very clearly and also the lateral cilia (L.C.) beneath. On account of their consecutive beat, these cilia appear to beat up the

side of one filament and down the side of the one opposite ; but in reality the effective beat is inward into the interlamellar space which communicates with the suprabranchial chamber. The abfrontal cilia (see Fig. 6) no doubt assist in the formation of the current into the suprabranchial chamber. The frontal cilia (F.C.) are smaller than the laterofrontals, but here and there are especially large cilia, or cirri (Ci.) as they have been called by Wallengren who regards them as characteristic of the ciliated tracts along which food is carried. The frontal cilia are concerned solely with the transport of the particles which drop upon them or are thrown upon them by the laterofrontals, the contact of solid particles immediately causing the mucus glands (M.G.), with which the surface of the filaments is covered, to secrete and so entangle the particles with mucus. The beat of the frontal cilia on the principal filaments in the bottom of the grooves between the plicæ is the reverse of that on the other filaments. The former beat towards the base of the demibranch, the latter towards the free margin, as shown diagrammatically in Fig. 22. Conditions are the same on all four demibranchs. Kellogg (1915) has described a similar state of affairs in Pecten, but this difference in the beat of the cilia on two kinds of filaments is not usual in Lamellibranchs. Particles carried to the free margin are passed into the ciliated groove (G., Figs. 21 and 22), which runs along it and in which they are carried towards the palps and mouth, while particles taken to the base of the gills by the cilia of the principal filaments are also carried forward by the ciliated tracts present at the gill axes (see Figs. 1, 2, and 22).

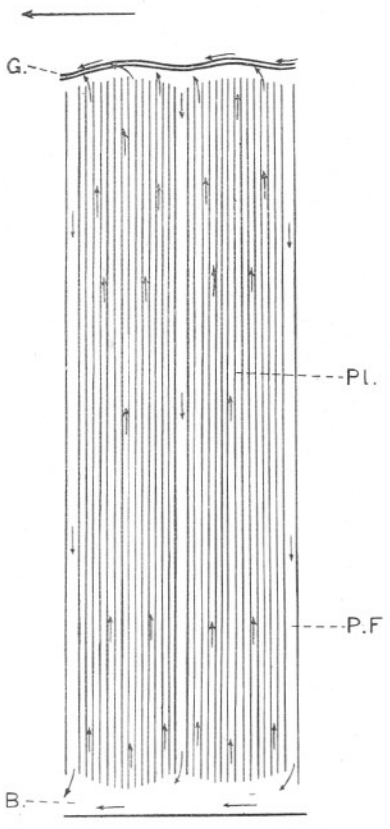

FIG. 22.—Diagrammatic figure of two plicæ and three principal filaments of gill lamella. ×10. B., base of gill, arrows show direction of current along gill axis ; G., groove at free margin ; P.F., principal filament ; Pl., plica. Arrow above figure shows direction of mouth, smaller arrows direction of currents on face of gill.

It is the opinion of Kellogg that the arrangement of the ciliary currents

on the gills is an adaptation which ensures that feeding shall take place only when very limited numbers of particles are present in the ingoing currents. These will tend to fall into the grooves and be carried by the cilia on the principal filaments to the base of the gill, and so direct to the palps. But when the water is heavily laden with silt, particles will fall on all parts of the gill, become embedded in a common mass of mucus, and *all* be dragged to the free margin of the gill under the action of the frontal cilia on the summits of the plicæ. Individual particles or thin strings of mucus in the ciliated groove are carried to the palps, as we have seen, but large particles or heavily laden mucus strings tend to fall out of the groove on to the mantle surface, from which they are expelled. Kellogg's observations have been confirmed; small particles alone are passed to the base of the gills, and from thence direct to the palps (except when they occur in such numbers that they accumulate in masses, are caught by the cilia on the plicæ and carried across to the free margin of the gill), while material in the grooves may or may not reach the palp according to its size. His deductions from these observations will be discussed in the section on the palps. There are thus *two selective mechanisms on the gills*, one on the surface of the filaments and one on the free margin, both of which act by selecting the smaller particles or masses for passage to the palps and mouth and reject the larger.

There is also a certain degree of muscular activity in the gills which has often been overlooked. Kellogg, however, with his accustomed keenness of observation, has noted (p. 674) that in Pecten "much material causes the gill grooves . . . to open wide, and then to close with so sudden a contraction that material is thrown out of them. Often this violent bending of filaments, which results in spreading open and then constricting the grooves, occurs in about a second of time. The whole demibranch, also, may present a wavy surface, and sway, fanwise, towards the mantle and inwards." Similar movements have been observed in the gills of Ostrea, the opening and sudden contraction of the grooves being of frequent occurrence. It has already been shown that horizontal muscles are present in the interfilamentar connections and also vertical muscles in the principal filaments, and it is the contraction of these muscles, presumably, which causes the movements of the gills. These types of movement, sudden contractions and bending of the filaments, result in excess of material being transferred from the grooves to the crests of the plicæ or from the surface of the gills to that of the mantle, and form yet another sorting mechanism, though of a less exact nature than the ciliary ones.

(*b*) *The Palps.*

The junction between the gills and palps is shown in Fig. 2 (p. 299), and on examination of this figure it will be seen that particles from the free margin of the gills are transferred to the middle of the inner palp face, whereas those from the gill axes (i.e. the smaller particles) pass into the groove between the palps, although, on account of the slight development of cilia in the grooves particles are passed on to the lower folds, as shown in the figure. In accordance with the terminology suggested by Kellogg, this groove will be called the lateral oral groove (L.G.), while the groove which leads from it to the mouth between the non-folded region of the palps will be known as the proximal oral groove (P.G.). There is a third, distal oral groove in Lamellibranchs in which the outer demibranchs do not extend so far forward as the inner, but this is practically absent in the oyster. Material passed on to the folded surface of the palps comes under the action of the long and very active cilia with which it is covered which, as will be described in detail shortly, conduct it either to the upper margin (U.M.) or across the palp folds in the direction of the mouth. There is a powerful backwardly directed current along the upper margin of the palps in which particles are carried back to a point marked X in Fig. 2 within a short distance of the tip, where it meets a forwardly directed current from the tip. A vortex is created in which particles are rolled into masses which, on attaining a certain size, fall off on to the mantle, directly from the left palps and by way of them in the case of the right palps.

The direction of the ciliary currents on the outer smooth surfaces of the palps is shown in Fig. 23. The cilia being shorter the currents are much weaker than those of the inner surfaces, and particles are carried diagonally backwards, those near the upper edge being transported into the upper marginal current on the inner face—and so finally rejected at X—while those in the central or lower areas are passed to the distal edge round which they are carried on to the folded surface, there to be either taken to the mouth or rejected as the case may be.

Passing now to a detailed examination of the inner, folded surface; Fig. 24 represents such a surface, the arrows showing the direction of the currents. The number of folds varies according to the age of the animal but this in no way affects their action. Each fold (as shown in the cross section in Fig. 7, p. 304) bends towards the mouth, overlapping to some extent the fold immediately proximal to it. In the middle of the exposed distal surface of each fold runs the longitudinal groove. There are no less than five distinct ciliated tracts on the exposed surface of the folds, details of which are shown in Fig. 25. Beginning with the most distal; there is a tract of cilia (*a*) which beats downward into the furrow between

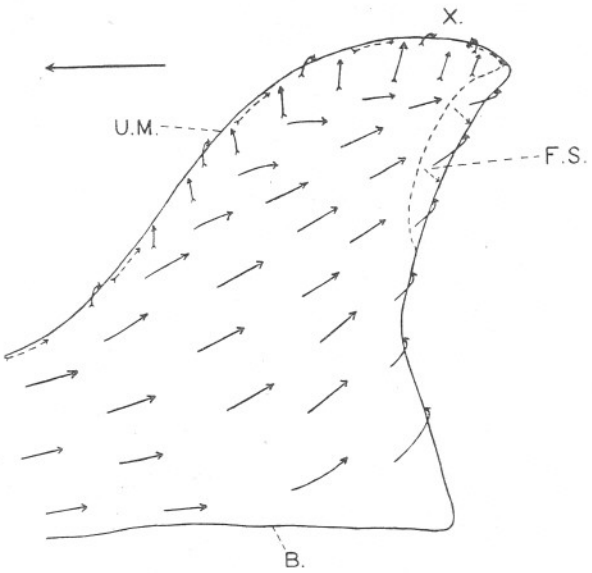

FIG. 23.—Smooth, outer surface of palp showing direction of ciliary currents. × 10. B., base of palp; F.S., folded, inner surface frequently exposed along dotted line; U.M., upper margin of palp; X., point where material rejected from palp. Large arrow shows direction of mouth.

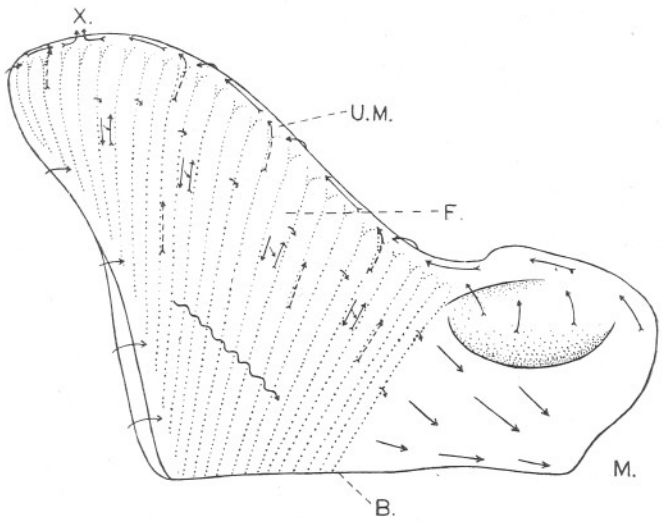

FIG. 24.—Folded, inner surface of palp showing direction of ciliary currents. × 10. B., base of palp; F., folds; M., position of mouth; U.M., upper margin; X., point where material rejected.

adjoining folds, but this region is largely covered by the adjacent overlapping fold; next there is a narrow tract (*b*) within the longitudinal groove whose cilia beat in the direction of the base of the palp; then a narrow tract (*c*) which directs particles diagonally across the palp towards the mouth; then a tract (*d*) in which particles are carried to the upper margin of the palp; and, finally, a tract of cilia (*e*) whose beat is directed at right angles to the line of the folds and towards the mouth. Moreover, in the furrows between the folds are tracts of cilia which lead, invariably, towards the upper margin (Siebert (1913) states that the cilia in the furrows in Anodonta beat in the opposite direction, but his findings have not been confirmed by other workers). The cilia on the proximal surface of the folds which are not exposed are difficult to observe, but appear in the main to beat into the furrows, and so are concerned with the rejection of particles. The path taken by particles carried on to the folded surface is the resultant of the action upon them of these different tracts of cilia, the interaction of which is very difficult to investigate. There can be no doubt, however, that the whole forms an extraordinarily efficient sorting mechanism. As the folds lie normally the effect of the five exposed ciliated tracts will be that light particles, such as carmine grains, are carried diagonally across the palp face, the individual particles being thrown lightly from fold to fold, largely by the action of the large cilia of tract (*e*), and following the somewhat serpentine course indicated by the long undulating arrow in Fig. 24. Large particles, such as carborundum, or smaller particles imbedded together in long mucus strings (which amounts to rather the same thing since the larger the particles the more mucus is secreted) tend to be drawn down within the furrows under the action of the cilia

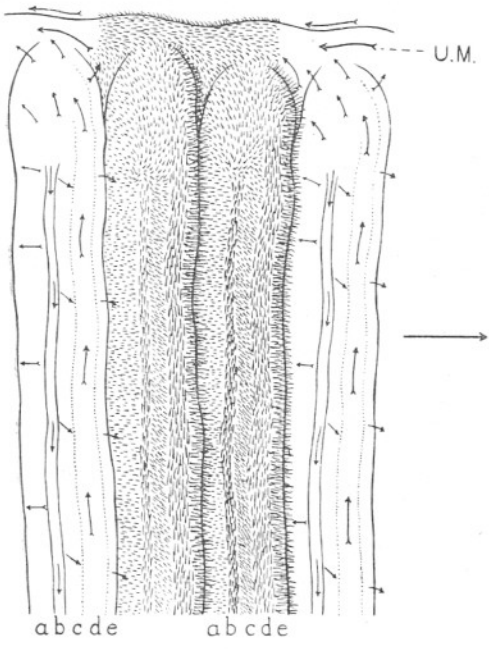

FIG. 25.—Enlarged semidiagrammatic figure of palp folds, showing direction of ciliary currnets. × 60. a, b, c, d, e, tracts of cilia on exposed surface of folds; U.M., upper margin. Large arrow shows direction of mouth.

in tract (*a*), and so, finally, expelled. So strong are the cilia in the furrows that if any portion of a mucus string comes under their influence, the whole string is carried to the upper margin. It is difficult to be certain as to the true state of affairs when the opposing palps, as always happens in life, are working in conjunction; experimentally it is only possible to examine the working of one exposed surface. Though gravity may have some influence on the selection of particles or strings of mucus lying on the under surface, that, of course, cannot influence particles attached to the upper surface, though the larger particles will tend to fall on to the under surface. Undoubtedly the mucus is of great importance; the larger the strings or masses, the more they will come into direct contact with the cilia, and the more chance that some portion will be drawn into the furrows and removed. Kellogg thinks there is a muscular retraction of the proximal edge of the folds concerned which causes particles to fall into the furrows; but I have never seen any such action in the oyster.

There is, however, an immediate muscular reaction when large particles are placed on the palp surface, the entire palp curling back in the manner shown in the left palps in Fig. 2. This is caused, no doubt, by the thick layer of longitudinal muscle which lies beneath the epithelium of the smooth surface (see Fig. 8, p. 305). As a result, the inner surface becomes convex and the folds are drawn apart, thus exposing the furrows into which the majority of the material on the palp will fall and be removed. The palps may occasionally curl inwards—by a contraction of the muscles under the epithelium of the folded surface—so that the folds are puckered and spaces left through which particles can drop into the furrows. These muscular responses are of the first importance in the functioning of the palps as was first noted by Wallengren, who originally described the different tracts of cilia on the palp folds, but who considered that, as a result of their *individual* contraction, different tracts were brought into play, and in this way the direction taken by the particles was controlled. Kellogg has described a curling over of the ventral (upper) margin of the palp in Schizotherus, whereby material is drawn off directly from the palp surface on to the outgoing marginal tract. Such a movement has not been observed in the palps of Ostrea, which are not free from one another, as in Schizotherus, but are attached for a quarter of their length. Allen (1914, 1921) follows Wallengren's account, and ascribes selection to the action of the different tracts of cilia brought into play during different states of contraction and relaxation in the folds; he claims further (apparently owing to a faulty reading of Wallengren's paper) that by this means cilia are brought into action which led particles in the *opposite* direction to that of the mouth. Grave (1916) considers that there is a reversal of the beat of certain cilia (pointing to the similar conditions

described by Parker for the sea anemone Metridium). I have never observed any sign of a reversal of cilia in the palps of any Lamellibranch, nor have Grave's opinions been supported by any more recent worker. Cobb (1918) has shown that the palps of Anodonta respond by muscular contractions to a variety of stimuli; mechanical, electrical, chemical, photic, and thermal. He also found that the detached palp reacts as effectively as one attached to the body (a fact also noted during these experiments on the palps of Ostrea), showing that "the palp contains within itself the neuromuscular organization necessary for all the responses described . . . and . . . possesses an autonomy even more complete than that of the vertebrate heart and comparable with what is shown by the tentacle of an actinian." Churchill (1924) has observed the muscular curling of the palps under normal feeding conditions in young, transparent fresh-water mussels. Nelson (1924) watched the feeding of spat oysters under similar conditions, and states that the rejection of particles is due to "reflex erection of the ridges of the palps which brings into play groups of cilia which beat away from the mouth." He does not state whether this erection is due to a general curling back of the palp surface, but describes the palps at this stage as consisting of "isolated filaments which are capable of independent movement." (This is certainly not the case in the spat of *Ostrea edulis*, where, as we have seen, the palps are more united than in the adult.) Nelson placed spat in 1/20 sat. magnesium sulphate, and states that the filaments of the palps lost the power to erect, with the result that masses of material passed over to the mouth and eventually blocked it. He concludes that feeding in the oyster is accomplished "through the delicate co-ordination of nervous, muscular, ciliary, and mucus secreting elements in which mechanical sorting of the materials plays the most important part"; an admirable summary of the state of affairs.

Herdman and Boyce (1899) have described in the oyster the presence of thin bands of muscle arising one on each side at the surface of the mantle near the anterior edge of the visceral mass and being inserted at the junction of the gills and palps, and have identified them with the protractor pedis muscle of other Lamellibranchs. They suggest that in the oyster they may function by pulling apart the inner and outer palps and gill demibranchs of each side, and so allowing food particles to reach the mouth more easily. It is difficult experimentally to prove this or to see its necessity since other Lamellibranchs function perfectly well without it, but, as they state, the opening of the shell will, by separating the points of attachment of the two muscles, cause "the opening up of the food avenues."

A considerable controversy has arisen around the question whether in the Lamellibranchs the selection of particles for swallowing is qualita-

tive or quantitative. The view that there is a definite selection of particles, having food value has been upheld chiefly by Lotsy (1893), Allen (l.c.) and Grave (1916), but the majority of workers, including List (1902), Kellogg (l.c.), Yonge (1923), Nelson (1924), and Churchill (1924) have failed to find anything other than a purely mechanical selection having as its object the reduction of the quantity of matter passed to the mouth, large particles or many small particles embedded in mucus being rejected and smaller particles or mucus masses passed on to the mouth quite irrespective of their food value. This appears to be confirmed by examinations of stomach contents by Savage (1925), and the majority of previous investigators whose work he summarises. Churchill found that when freshwater mussels were kept in suspensions of mixed organic and inorganic matter they took in a sample of everything small enough to enter the mouth. In some cases where the inorganic particles are the larger there may be—incidentally—a selection of particles having food value (as Nelson thinks is the case in spat oysters). Nothing but a purely mechanical or quantitative selection has been found in the oyster, and this has, I think, been made clear in the preceding account, but attention may again be drawn to the series of selective mechanisms which exist.*

1. The heaviest particles in the ingoing current drop on to the mantle and never reach the gills.
2. The smaller particles on the gills are carried by the cilia on the principal filaments to the base, the larger ones passing to the free margin.
3. The largest particles or mucus masses fall out of the groove on the free margin on to the mantle.
4. Muscular contractions in the gills cause material to be transferred from the grooves on to the crests of the plicæ, and from the surface of the gills to that of the mantle.
5. Material passed on to the inner face of the palps from the free margin of the gills is there most rigorously sorted, larger particles or masses being rejected and only the smallest crossing towards the mouth.
6. The smaller particles from the gill axes which pass into the lateral oral groove are not so rigorously sorted, since the folds at the base of the palps are lower and closer together and the effect of the curling back of the palp surface is much slighter.

Experiments with four grades of carborundum powder demonstrated the efficiency of these sorting mechanisms very clearly. The particles

* Lamellibranchs, such as Syndosmya, Tellina or Gari, which have long, free siphons and are classified by Hunt (1925) as deposit feeders, may exercise a certain qualitative selection by means of the inhalent siphon which is fringed with sensory tentacles. Possibly the Protobranchs may do the same, though to a less extent, by means of the extrusible appendages of the outer pair of labial palps. In both cases, however, qualitative selection, if it occurs, takes place *outside* the mantle cavity.

were in all cases dropped lightly on the middle and posterior regions of the gills, and with the following results (the coarser grades being taken first) :—

Grade 120. Particles passed to the free margin of gill ; fall on to mantle before they can reach the palps.

Grade 220. All carried to free margin ; majority drop off, a few of the thinner mucus strings reach palps, there *all* rejected.

Grade F. All carried to free margin ; comparatively little falls off, *all* rejected by palps.

Grade FF. All carried to free margin ; very little falls off, great majority rejected by palps, a very little carried to mouth.

As already stated, Kellogg is of the opinion that Lamellibranchs can only feed in waters that are comparatively clear. This has been denied by Grave (1916), Nelson (1921)—who supplies the *definite evidence* that oysters can feed in waters bearing as high as 0·4 grams dry weight of suspended matter per litre—and Churchill (1924), and, I think, with reason. Certainly the more particles carried into the mantle cavity, the more wholesale is the rejection, but, as Churchill has shown for fresh-water mussels, although the main surface of the palp is concerned with the rejection of the large masses passed on from the marginal grooves on the demibranchs, the finer matter which enters by way of the lateral oral groove will find its way to the mouth. I place a similar interpretation on my experiments, although in the oyster the selective mechanisms, both on palps and gills, are more efficient than in the majority of Lamellibranchs—a correlation, no doubt, with the sessile mode of life and consequent danger of silting up—in which the frontal cilia of all gill filaments usually beat in the same direction, and food can pass to the mouth by way of the distal and lateral oral grooves without ever coming into contact with the folds on the palps ; *Mya arenaria* is a good example of this type of Lamellibranch. Even in the oyster, however, I have observed the passage of a certain amount of material from the gills to the palps under all conditions approaching the normal (if the gills are absolutely covered with a mass of particles these are all removed, whatever their individual size, but this would never occur in nature) ; carmine grains are carried to the mouth along the lateral oral groove while the rest of the palp surface is ridding itself of carborundum. It is not impossible, however, that Lamellibranchs, and especially such highly specialised species as the oyster, feed with the *maximum of efficiency* in waters that are comparatively clear ; they can, moreover, by frequent closing of the shell valves clear the water to some extent and prevent any too great accumulation of sediment within the inhalent chamber.

It remains to describe the passage of particles from proximal folds to the mouth, the course of which is shown in Fig. 2 (p. 299). The cilia in this region are short, and matter accumulates along a line parallel to the last fold and then passes slowly in the direction of the mouth. There is never an accumulation of material about the mouth, particles which do not pass deep in the proximal oral groove being caught by ciliated tracts which lead them upwards and then either distally on to the upper margin of the inner palp face, or over on to the outer face of the inner palps. In either case they are rejected finally. Material which reaches the mouth passes slowly into the œsophagus.

(*c*) *Removal of Material from the Mantle Surface.*

Material dropped on to the surface of the mantle is carried away by the ciliary tracts shown in Fig. 1 (p. 298). There are ciliary tracts in the anterior region of the mantle cavity which carry particles back to a point about the middle of the inhalent aperture where they accumulate, since the thickened ridge which bounds the mantle is not ciliated. The masses thus formed are expelled from time to time by sudden contractions of the valves. Nelson (1921) by very ingenious experiments has shown that ejections of this nature are most numerous when the water in which oysters are living is at its maximum turbidity. Along the posterior region of the inhalent chamber, and also in the exhalent chamber, matter passes directly to the edge of the mantle, there to accumulate and be expelled in the manner described.

II. In the Gut.

Mucus laden with particles passes slowly along the œsophagus, particularly in the grooves at the extremities of the lumen, as described by Vonk (1924), into the stomach. Fig. 26 represents the stomach and œsophagus opened out for examination of the ciliary currents. As a result of the position of the cut, the ventral wall of the stomach lies on the left side of the figure. The relative positions of the various parts is seen in Figs. 3 and 4, and by reference to these and to Fig. 26 some idea of the physiology of the stomach will be gained.

Particles entering the stomach will tend to pass into the food sorting cæcum (F.C.) either directly or by way of the ciliated tract (C.T.), which leads into it from the floor of the stomach. The cæcum, as shown in Figs. 3 and 4, is a long grooved diverticulum, which extends backwards under the floor of the stomach. Fig. 27 represents it opened out, the broken lines indicating the line of the cut. The ingoing ciliated tract (C.T.) is situated on a ridge, which passes down the right side of the

cæcum to the extremity and then along the left side, terminating abruptly at a point level with the opening of the cæcum. Particles are carried very rapidly along this tract until they reach the point X, when they may be carried in either of two ways. If they are heavy (e.g. carborundum) they are rolled round and fall over into the groove on the right of the ridge, as shown by the arrows and dark mass in Fig. 27. The cilia in this groove conduct particles slowly round in the reverse direction to the cilia on the ridge and out of the cæcum into the deep ventral groove (G., Figs. 3, 4, 26, and 27), which runs across the floor of the stomach

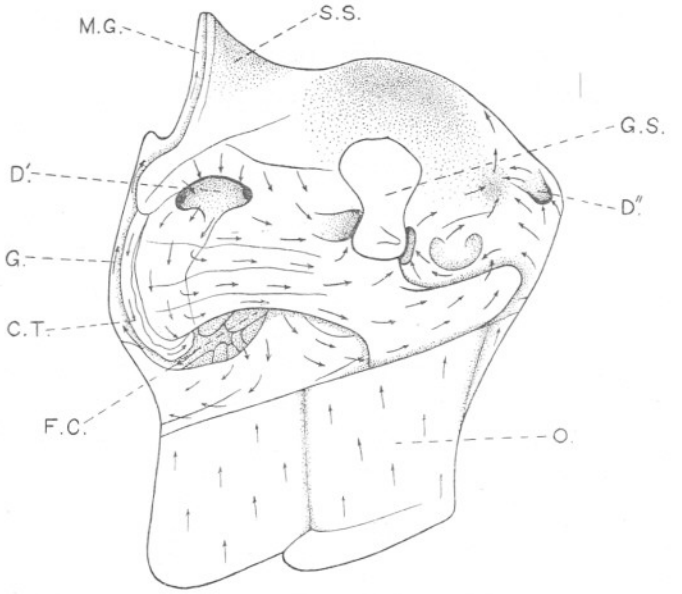

FIG. 26.—Stomach opened out to show direction of ciliary currents, cut made along right ventral surface. × 4. C.T., ciliated tract leading into food sorting cæcum; D′., larger, left duct of digestive diverticula; D″., smaller, right duct; F.C., food sorting cæcum; G., ventral groove leading from food sorting cæcum to mid-gut; G.S., gastric shield; M.G., mid-gut; O., œsophagus; S.S., style-sac.

and is continued into the mid-gut, as shown in Fig. 26. On the other hand, light particles, such as carmine grains, not embedded in great masses of mucus, pass along the ciliated tract past the point X, and are wafted out of the left side of the cæcum in the direction indicated by the dotted arrows in Fig. 27, being ultimately carried to the region of the gastric shield, as shown in Fig. 26. The cæcum constitutes yet another sorting mechanism wherein larger particles are separated from smaller ones without any apparent regard to their food value, the larger ones being removed from the stomach by way of the mid-gut, and the smaller being retained in the stomach and passed towards the head of the style.

Nelson (1918) has described a similar cæcum in Modiolus, and I have given an account of a food sorting area in the stomach of Mya (1923). In both of these cases the mechanism is more complicated than in Ostrea, presumably because in the latter the selective powers of the gills and palps are better developed, a fact testified to by the smaller size of the particles in the stomach of the oyster. The remaining cilia on the wall of the stomach either conduct particles towards the gastric shield, where they come under the action of the style, or else towards the ducts of the digestive diverticula (D′. and D″., Figs. 3, 4, and 26). These ducts are bounded on the one side by an overhanging wall over which the cilia beat *into* the opening, but on the other the opening lies flush with the epithelium of the stomach, and the cilia on this side lead particles *away* from the opening. There is thus a mechanism whereby particles enter

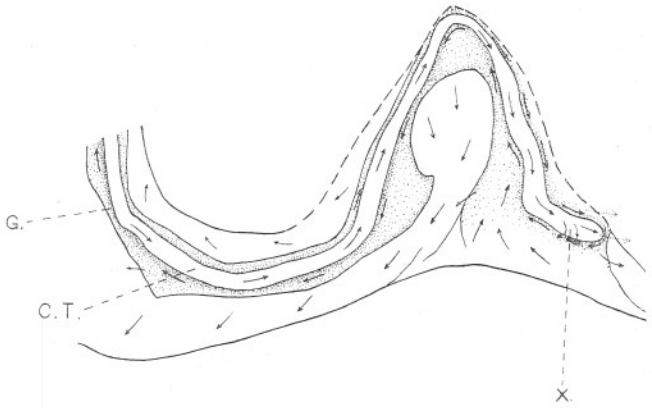

FIG. 27.—Food sorting cæcum opened up along dorsal surface, line of cut shown by broken lines. × 8. C.T., ciliated tract leading into cæcum; G., ventral groove leading out; X., point where large and small particles separated, larger passing into groove and smaller following line of dotted arrows to gastric shield region.

the ducts on the one side and leave them on the other, which is essential if a circulation is to be maintained within the diverticula.

The main agent concerned with the movement of material within the stomach, however, is the style. It was originally suggested by List that the style was probably revolved by the action of the cilia in the style-sac, but it was left to Nelson (1918), as the result of careful opening of the stomach, to observe the actual revolution of the style in the stomach of Anodonta and Modiolus. He found that the maximum number of revolutions per minute in Anodonta at 11·5° C. was 11, and in Modiolus at 25° C. was 13. In both cases the direction of the movement was clockwise when viewed from the anterior end of the animal. It has never been possible to observe the movement in the adult oyster, in which

the stomach is less exposed than in the majority of Lamellibranchs, but I have seen it in the larvæ and spat (as will be described later), while Churchill (1924) has observed it in young, transparent fresh-water mussels, and there can be no doubt, if only from the nature of the ciliation of the style-sac, that the style revolves in all Lamellibranchs. The head of the style is continually being dissolved away, and in the sticky mass become embedded particles and strings of mucus, and it may well be, as Orton (1923) has suggested, that material is in some cases drawn into the stomach as a result of the mucus strings being wound round the "shredded revolving head of the style like a capstan." As we have seen, muscle is practically absent from the gut of the oyster (in common with all Lamellibranchs except the Septibranchs), the place of peristalsis being taken by ciliary activity. One of the principal functions of the style, as Nelson (1918, 1925) has pointed out, is that of stirring and mixing particles in the stomach, an operation performed in many other animals by peristaltic contractions. Although, of necessity, the different activities of the stomach have been described separately, in life, of course, they are all proceeding simultaneously, food entering from the œsophagus, being sorted in the cæcum, being revolved in the head of the style, passing in and out of the ducts of the digestive diverticula, and being removed by way of the mid-gut all at the same time.

The disposition of the ciliary currents in the style-sac and first part of the mid-gut is shown in Fig. 28. Particles enter the mid-gut from the stomach by way of the ventral groove (G.) and pass quickly down it along the channels at the base of the typhlosoles (T′. and T″.). The cilia on the typhlosoles beat diagonally away from the stomach and into the gut. The groove of the style-sac is ridged transversely, and the beat

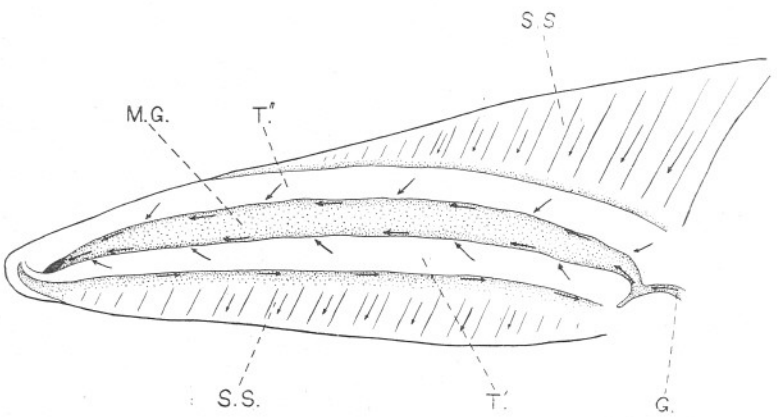

FIG. 28.—Style-sac opened along middle of surface so as to expose ciliary currents in style-sac and mid-gut. × 4. G., ventral groove in stomach; M.G., mid-gut; S.S., style-sac; T′., larger typhlosole; T″., smaller typhlosole.

of its large cilia is very difficult to determine ; particles placed upon them appear to get caught between the cilia, as they can be seen trembling with the movement of the cilia but are moved extremely slowly. Such movement as there is, however, is from right to left (looking at the style-sac from the stomach), i.e. in the direction which would revolve the style in a clockwise direction when seen from the same standpoint. There is a tract of cilia beating in the direction of the stomach on the side of the larger typhlosole (T′.), which is somewhat easier to demonstrate and is accompanied by the production of great quantities of mucus. The same disposition of cilia is found in the style-sac of Mya (Yonge (1923), and there is the same difficulty in demonstrating the direction of their beat. The style, presumably, is revolved by the first set of cilia and pushed forward by the other, and the difficulty of demonstrating the direction of beat may be due to the cilia not being adapted for the movement of small particles which rest lightly on them, but for the movement of a firm body which is pressed firmly against them. In spite of the presence of the ciliary currents leading from the style-sac into the gut over the face of the typhlosoles, a certain amount of material which has been carried down the gut gets caught up in the substance of the style, wrapped round it spirally as it revolves, and carried back to the stomach. This " retrieving function " of the style has been commented on by Nelson (1918, 1925), Allen (1921), and Orton (1923)—the latter having figured the spiral bands of mucus laden food strings wrapped round the style of the oyster—and is probably of some importance in Lamellibranchs, such as Ostrea, in which the style-sac and mid-gut are in communication.

Throughout the remainder of the gut material is passed slowly backwards under the influence of the cilia, and is finally ejected by way of the anus into the exhalent chamber (see Fig. 1), where it comes under the influence of the exhalent currents and of the cilia on the mantle surface and is removed from between the shell valves.

B. THE LARVÆ.

The arrows in Fig. 17 (p. 317) indicate the direction of the ciliary currents in the larvæ. If larvæ are placed in suspensions of carmine, indian ink or other fine particles these are thrown by the large cilia of the velum on to the ciliated tract (C.T.), which runs round the base of the velum, where they are embedded in mucus and carried back to the mouth. The velum, therefore, acts both as a swimming and as a food collecting organ. Not all the material passes into the mouth, any surplus being carried off by the cilia on the lobe which represents the rudiments of the foot (F.), so that a larva swimming through a thick suspension leaves behind it a trail of particles embedded in a long string of mucus. If

the suspension is excessively thick the larvæ become embarrassed in their movements and turn repeatedly over and over in their efforts to free the cilia from the mass of particles which they automatically collect.

Material is passed into the stomach by way of the œsophagus and is there rotated, as indicated by the arrows in the figure, by the cluster of large cilia at the anterior end and also by the action of the smaller cilia which line the wall of the stomach. At the same time, the style, in which particles become embedded, is rotating in the style-sac. The direction of rotation appears to be constant for the individual, but to vary in the different larvæ, in some clockwise, in others anti-clockwise. Nelson, on the other hand, states that in the larvæ of *Ostrea virginica* the movement is always clockwise. The speed varies greatly, as few as 36 and as many as 90 revolutions per minute having been counted. Partly as a result of this movement, particles are thrown into the cavity of the digestive diverticula in which they can be seen in active movement, as shown by the curved arrows in Fig. 17. We may therefore assume that cilia are present in the cavity of the diverticula. Particles leave the stomach by the mid-gut, and are carried rapidly through the remainder of the alimentary tract and ejected at the anus. When larvæ were kept in heavy suspensions of carmine the gut became packed with a continuous red stream, and under these conditions the action of the cilia was practically inhibited, owing to the pressure of the enclosed mass ; movement became exceedingly slow and a certain amount of peristaltic activity was observed. The gut in the larvæ is, it may be noted, unlike that of the adult, free from the surrounding tissue. The fæces are rolled into a ball by the action of the cilia in the mantle cavity, and are then expelled.

C. THE SPAT.

All mechanisms concerned with the rejection of surplus matter are well developed in the spat. Thus the cilia of the mantle are larger and more active than those in the adult, while the palps are relatively of immense size and reject the great majority of particles passed on to them from the gills, which collect them in the usual manner as indicated by the arrows in Fig. 18 (p. 319). Fig. 29 shows the palps seen from the free end after they had been dissected out of a 1 mm. spat, the two inner palps (I.P.) being enclosed by the hood formed by the outer palps (O.P.). It is extremely difficult at this early stage in their development to distinguish the direction of all the ciliary currents on these small organs. No folds are present, although there is a groove on each of the palps along which the cilia beat in the direction of the free extremities of the palps, as in the furrows of the fully developed palp, and there are ciliated tracts leading in the same direction along the outer edges of the outer palps

Other tracts lead across the palps, but, after careful examination, only one tract—on the right inner palp—was distinguished in which the cilia beat towards the base. When particles are placed upon the palps only a minute proportion succeed in passing between the inner and outer palps of each side and so reaching the mouth, the majority are passed

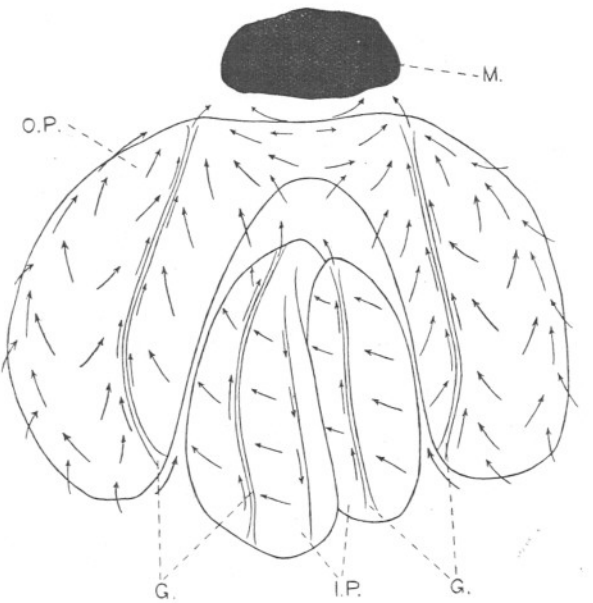

FIG. 29.—Palps from 1 mm. spat dissected out and drawn from free end, i.e. looking in direction of mouth. × 275. G., grooves on inner faces of palps; I.P., inner palps; M., mass of rejected material accumulated in depression at junction of outer palps; O.P., outer palp.

rapidly to the depression at the junction of the outer palps (see Figs. 18 and 29), where they are rolled into a large ball (M.), which finally falls on to the mantle surface. In experiments on whole spat the palps were found to be very active and to respond readily to stimulation by drawing back and upwards, probably by so doing exposing to their maximum the outgoing tracts.

Particles which succeed in reaching the mouth are passed rapidly through the œsophagus into the stomach (see Fig. 18). There they are whirled round by the cilia and in the head of the style, which in spat of this size can be seen in rapid movement through the wall of the stomach. It consists of a somewhat irregular rod which bears against the gastric shield and revolves in a clockwise direction when viewed from the anterior, the speed observed varying between 60 and 70 revolutions per minute.

Embedded in its substance, especially near the head, are particles of all sizes, the largest observed being specimens of the spherical diatom Coscinodiscus having a diameter of about 23μ. These are shown in Fig. 18. It is difficult to observe the disposition and interior of the digestive diverticula in the spat, but movements were seen within them, while peristaltic movements were well marked in the mid-gut, especially in the region nearest the stomach, particles being moved by this means and by ciliary activity through the mid-gut and rectum and expelled at the anus, which opens in the exhalent chamber.

The great development of the organs concerned with the removal of surplus matter in the spat can readily be understood, since a small sessile organism of this nature is in constant danger of being smothered by falling silt unless this can speedily be removed from within the mantle cavity. The presence of peristaltic activity in the gut of both larvæ and spat seems to indicate that the absence of peristalsis in the adult is not primitive. Peristalsis is usually well developed in the Gastropods, which may represent, in this respect at any rate, the more primitive condition.

4. ASSIMILATION.

1. Literature and Methods.

Although in animals such as the Vertebrates, digestion precedes assimilation, this is the case only to a very limited extent in the Lamellibranchs, since food is ingested directly both by the phagocytes and the tubules of the digestive diverticula and digestion then takes place *intracellularly*. The only extracellular enzymes in the gut of Lamellibranchs are those set free by the dissolution of the head of the style. In this paper, therefore, an account of the process of assimilation is given before passing to a consideration of the digestive enzymes.

I have recently had occasion (1926) to review the literature dealing with assimilation in the digestive diverticula, so that it is unnecessary to discuss the matter in detail. As a result of a study of previous work and as the outcome of my own experiments, the conclusion was reached that the digestive diverticula are organs of absorption and of intracellular digestion, since they absorb soluble matter such as iron sulphate (Carazzi (1897) on Ostrea) or iron saccharate (Yonge (1926) on Nucula, Mya, and Teredo), and ingest solid matter such as Indian ink (List (1902) on Mytilus, Potts (1923) on Teredo, Vonk (1924) on Ostrea), carmine (List on Mytilus) or blood corpuscles from dogfish (Yonge (1926) on Teredo). Sigerfoos (1908) and Potts have further shown that the Teredinidæ ingest wood fragments in digestive diverticula specialised for that purpose. Matter which may be of use to the animal such as iron (Carazzi (1897),

Yonge (1926)) or blood corpuscles (Yonge) is carried away in amœbocytes, but useless material such as Indian ink is rejected into the lumen of the diverticula shortly after ingestion and carried out of the body (List, Vonk).

Carazzi (1896, 1897) claims that iron is absorbed by the epithelial cells of the gills, palps, and œsophagus, and then carried to the digestive diverticula by way of the amœbocytes. Since, however, he kept his oysters for *four months* in a solution of iron sulphate in sea water so that they had time to become thoroughly permeated with iron, an entirely contrary interpretation may be placed on his results, namely, that the phagocytes become loaded with iron either from the digestive diverticula or by direct ingestion and then carry it to all the tissues. In the same way the Marennin from Navicula is taken in by the phagocytes and carried to all free surfaces of the oyster, so that the gills, palps, and gut are coloured green (for full details on the subject of green oysters see Lankester (1886), Herdman and Boyce (1899), Mitchell and Barney (1916), and other papers quoted by them). Gutheil (1912) found fat globules in the ciliated epithelium of the gut in Anodonta, except in the style-sac and the region of the gastric shield. He therefore concluded that the epithelium could absorb, although he carried out no controlled experiments by first starving and then feeding animals, but argued from the presence of fat in the epithelium of fresh animals. Churchill (1915, 1916) states that after keeping fresh-water mussels in very dilute solutions of soap, egg albumen, or starch stained with iodine, these substances are taken by the outer epithelial cells of the body, mantle, foot, gills, and palps, some being carried away by blood cells, which he observed on occasion between the epithelial cells. His experiments were in most cases kept up for a considerable number of days, and, though the same objection cannot be made to them as to those of Carazzi, since he plugged the mouth of many of his animals thus preventing the passage of food to the digestive diverticula, yet the presence of these substances in the epithelial cells did not necessarily mean that they had been absorbed directly by them. Canegallo (1924) kept Unio in soap solution and found that this was absorbed to a far greater extent by the epithelium of the intestine than of the gills, the fat being carried away by leucocytes. Ranson (1926) considers that molluscs can absorb organic matter in solution through any free surface as well as by the intestine. In my own work on Nucula, Cardium, Mya, and Teredo (1926) the absorption of iron saccharate was never observed except in the tubules of the digestive diverticula.

Large particles are taken in directly by phagocytes, to the universal presence of which attention has already been drawn. De Bruyne (1893, 1896) considered that they ingested damaged or degenerating epithelial

cells particularly in the gills. Gutheil (1912) has described and figured in Anodonta the passage of phagocytes laden with material from between the epithelial cells through the basement membrane and into the connective tissue and blood vessels. Cuénot (1914) has observed phagocytosis in the blood cells of Lamellibranchs, as a result of the injection of Chinese ink. Canegallo (1924), by injecting olive oil stained with Sudan III into Unio, found that this was quickly taken in by leucocytes. I have described (1923) the presence of great numbers of these phagocytes in the gut of Mya, and shown that they may contain large, hard particles such as sand grains or the tests of diatoms, often in such numbers that the gut is coloured grey. After feeding Cardium and Mya with blood corpuscles of dogfish it was found (1926) that the corpuscles were ingested by phagocytes lying between the epithelial cells in the stomach and ducts of the digestive diverticula. They were carried into the connective tissue and there digested. Reference has already been made in this paper to the presence of phagocytes in all parts, and to the fact that they often contain green or brown granules, the colour being due to a pigment investigated by MacMunn (1900) and named by him Enterochlorophyll, on account of its close relationship to chlorophyll.

Oysters after appropriate periods of starvation in water which had been passed through filter cloth were fed with suspensions in sea-water of iron saccharate, of blood corpuscles from dogfish, of a pure culture of the diatom Nitzschia, and with an emulsion of olive oil stained with Nile blue sulphate. No experiments were carried out with Indian ink, the recent and conclusive work of Vonk (1924) on Ostrea having rendered them unnecessary. Animals were removed, and the various regions of the alimentary system fixed, at varying intervals after the commencement of feeding. After feeding with iron saccharate tissues were fixed in equal parts of 5% of ammonium sulphide in 95% alcohol and Bouin's fluid, sections being later treated for ten minutes with a 10% solution in water of potassium ferrocyanide, and then for a few minutes in a very dilute solution of HCl in order to demonstrate the presence of iron by the Prussian blue reaction, the sections being stained with alum carmine. After feeding with the other substances tissues were fixed either in Flemming's strong fluid or in Bouin. If by the former method, sections were stained with a saturated solution of safranin in 70% alcohol and later differentiated in clove oil saturated with orange G, the osmicated fat by this method standing out very clearly against the red nuclei and yellow cytoplasm. After fixation in Bouin sections were stained with Delafield's hæmatoxylin and erythrosin.

Larvæ and spat were fed on carmine and iron saccharate, and fixed respectively in Bouin and in the ammonium sulphide-alcohol Bouin mixture.

II. Feeding Experiments on Adults.

(*a*) *With Iron Saccharate.*

This substance was taken in readily, a thick brown suspension in sea-water being rapidly cleared. Oysters opened within a few hours of feeding were found to have the stomach full of a brown mass of iron saccharate, a great deal of which was embedded in the head of the style. After sectioning, iron was found in the lumen of all parts of the gut up to two days after feeding (it was present in very great quantity in the rectum six hours after feeding), sometimes it was seen ingested in phagocytes lying free in the lumen and—very rarely—being carried by them in between the cells of the epithelium. *But in the epithelium of neither the gills nor the palps nor any part of the gut except the tubules of the digestive diverticula was it absorbed.* In the cells of the latter it is ingested freely, slight traces being present six hours after feeding, a maximum being reached from one to two days after feeding, very slight traces being found after three days and none after any longer period.

The typical conditions of absorption are shown in Fig. 30, which represents two cells from a digestive tubule two days after feeding with iron saccharate. The free surface of the cells is very irregular, and iron is taken into large vacuoles and accumulates in the form of irregularly round or oval masses. It is *never* absorbed in the form of fine granules or in a diffuse condition. Exactly the same conditions were found in Nucula, Mya, and Teredo, while List and Vonk found that Indian ink was taken into vacuoles in the diverticula of Mytilus and Ostrea in a similar manner. It is impossible, as Vonk has also noted, to distinguish a bounding membrane around the masses which appear to lie

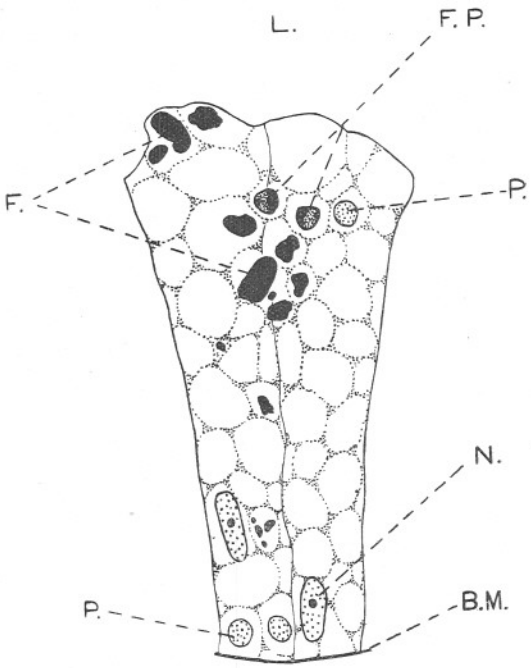

Fig. 30.—Two cells of digestive diverticula two days after feeding with iron saccharate. Iron technique. × 1350. B.M., basement membrane; F., masses of ingested iron; F.P., iron partly filling vacuoles; L., lumen; N., nucleus of cell; P., phagocytes.

free in cavities in the protoplasm, but the manner in which the iron first forms a ring (Fig. 30, F.P.) the interior of which is later filled up, seems to point to the presence of such a membrane. This manner of absorption is unlike that found in animals, such as Arthropods and Annelids, in which digestion is extracellular and only the soluble products of digestion are absorbed, and would appear to be an indication of the presence of intracellular digestion, as would also the irregular outline of the free surface of the cells.

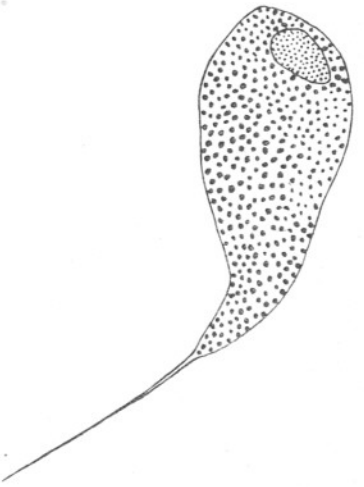

FIG. 31.—Phagocyte from connective tissue between digestive diverticula and gonad, four days after feeding with iron saccharate. Nucleus at one end, fine pseudopodium at the other, body of phagocyte full of iron. Iron technique. × 2400.

Phagocytes are almost invariably present in the cells in which iron is being ingested, they may or may not be present in the others. Usually only their nuclei (as in Fig. 30) can be distinguished, although occasionally the outline of the cells can be seen, particularly when they are full of iron which they have collected from the cells. Four days after feeding, though no trace of iron was found in the tubules, many phagocytes full of minute granules of iron were to be seen immediately round the tubules, in the connective tissue (e.g. the phagocyte in Fig. 31), and occasionally in the blood vessels and in the gonads (Carazzi considered that the final destination of the iron was the gonad). There was never any indication of rejection of iron into the lumen, in the manner described by List and Vonk after feeding with Indian ink.

(*b*) *With Blood Corpuscles.*

A quantity of fresh blood from a dogfish was added to the filtered sea-water in a large bell-jar in which a number of oysters had been starved. The corpuscles were taken in rapidly by the oysters. The stomach contents of an oyster opened three hours after the blood had been added consisted exclusively of mucus, blood corpuscles of dogfish, phagocytes of the oyster and a few ciliates and spirochætes. The style was intact. The great majority of the corpuscles were in perfect condition, the outline being smooth and the nucleus quite clear, some were lying free in the stomach, some entangled in mucus or in the substance of the head of the style, while others were in *process of being ingested by phagocytes*. This process is shown in Figs. 32 and 33; in the former a

phagocyte is beginning to engulf a corpuscle, while in the latter one has already been ingested, and is lying in a vacuole within the phagocyte which is beginning to engulf a second corpuscle. The stomach contents of an animal opened six hours after feeding were a diffuse red, probably owing to the presence of free hæmaglobin, very few corpuscles could be distingished, and there were many fewer phagocytes free in the lumen, those present being often large, and containing the remnants of many corpuscles in an advanced state of digestion. Many other phagocytes contained no ingested matter. Phagocytes with ingested corpuscles could be distinguished passing into the epithelium of the stomach. Eighteen hours after feeding there was only a slight redness in the stomach, which contained very few corpuscles, the outline of which was often serrated.

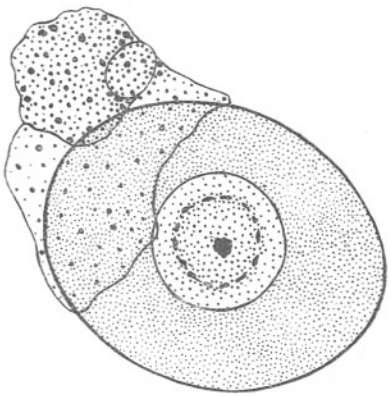

FIG. 32.—Phagocyte in stomach ingesting blood corpuscle three hours after feeding with blood from dogfish. Drawn from life. × 2400.

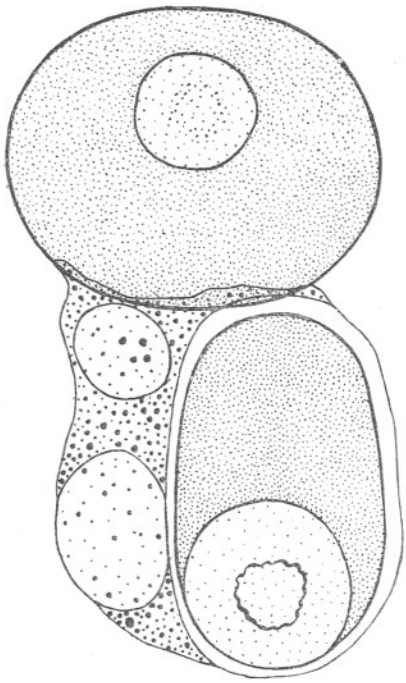

FIG. 33.—As above, phagocyte with one corpuscle ingested and beginning to ingest a second. Drawn from life. × 2400.

As a result of sectioning it was found that corpuscles are taken in between the cells of the epithelium in all regions by the phagocytes. This was most rare in the rectum, few corpuscles passing so far in the lumen, and in the digestive diverticula where, although corpuscles were occasionally found ingested in the cells, they only appeared to be digested with consequent formation of fat globules in the presence of phagocytes. Ingestion by phagocytes took place to a small extent in the gills, palps and œsophagus, to a greater extent in the mid-gut, but the principal centre of phagocytic activity was found to be the stomach and ducts of

the digestive diverticula, immense numbers of phagocytes making their appearance in the lumen and epithelium in these regions. The course of phagocytic ingestion of corpuscles was followed in detail in the stomach epithelium.

It is very difficult to remove all traces of fat from oysters, even after prolonged starvation. Feeding experiments were carried out on animals which had been starved four and eleven weeks, and in both cases, though the quantity of fat was substantially less than in fresh animals, there

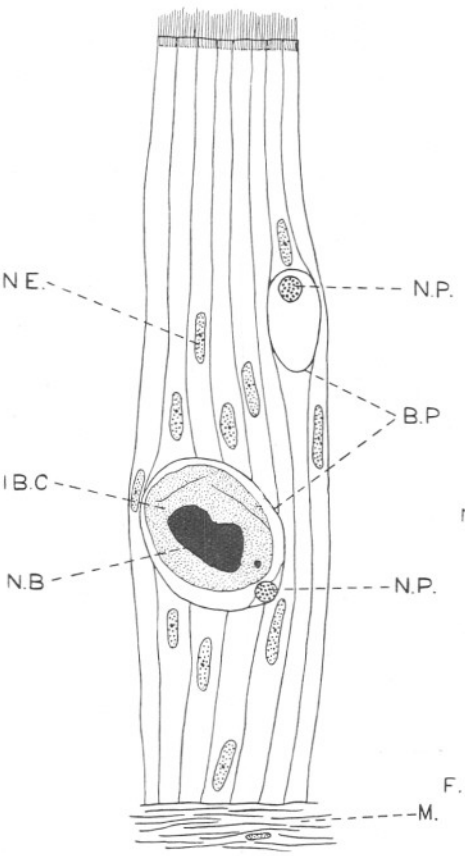

FIG. 34.—Stomach epithelium, showing ingestion of a blood corpuscle in a phagocyte three hours after feeding. Oyster starved for eleven weeks previously. Fixed strong Flemming, stained safranin and orange G. × 900. B.P., boundary of phagocyte; I.B.C., ingested blood corpuscle; M., muscle; N.B., nucleus of blood corpuscle; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

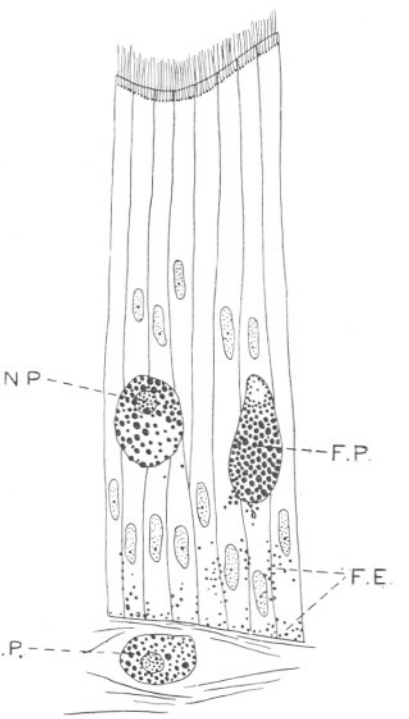

FIG. 35.—Stomach epithelium, ingestion and digestion of blood corpuscles in phagocytes six hours after feeding. Oyster starved for eleven weeks previously. Fixed Flemming, stained safranin and orange G. × 900. F.E., fat in epithelium; F.P., fat in phagocytes; N.P., nucleus of phagocyte.

was still a certain amount present, especially in the connective tissue, in the phagocytes and very occasionally at the base of the epithelial cells. Nevertheless, the difference in the fat content after feeding with blood corpuscles was quite unmistakable. Fig. 34 shows the ingestion

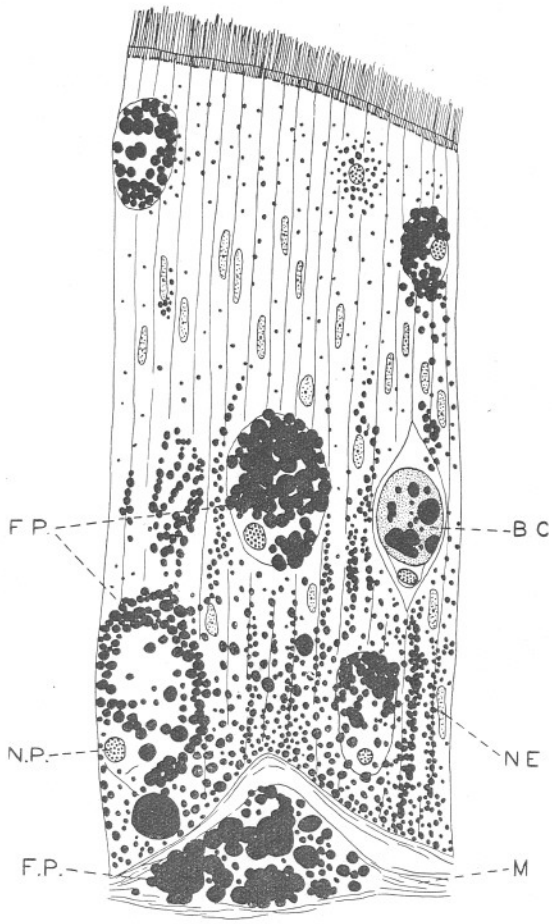

FIG. 36.—As above, twelve hours after feeding. Oyster starved for four weeks previously. Technique as before. × 900. B.C., corpuscle in early stages of digestion; F.P., fat in phagocytes; M., muscle; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

of a corpuscle three hours after the commencement of feeding. The corpuscle lies in a phagocyte in the middle of the epithelium, the outline is uneven and the nucleus is degenerating. At this period there are few phagocytes in the epithelium, and there is practically no sign of fat in either the ingested corpuscles or in the epithelial cells. The condition

six hours after feeding is shown in Fig. 35. There are now many times more phagocytes in the epithelium than at the preceding period ; the ingested corpuscles are difficult to distinguish, consisting now, as a result of the digestive action of the phagocytes, of a mass of fat globules. Two phagocytes laden with fat are shown in the epithelium in the figure and a third is seen passing through the circular muscle into the connective tissue. Fat is being passed from the phagocytes to the cells, in which it is accumulating near the base. Fig. 36 represents a portion of the epithelium twelve hours after feeding. Owing to the mass of fat globules it is difficult to distinguish the outline of the epithelial cells, of the phagocytes, and of the nuclei. Phagocytes are present in the epithelium in vast numbers ; in the figure one phagocyte contains a corpuscle (B.C.) in the early stages of digestion, the other phagocytes containing great numbers of fat globules, which in some cases probably represent the products of digestion of two or more corpuscles. Fat has passed from the phagocytes to the cells, the whole epithelium appearing black with osmicated fat. Fat is also being transported into the connective tissue, though the nuclei and outline of the phagocyte or phagocytes which carry it are obscured by fat. There is a sharp distinction between the ciliated epithelium of the stomach and the epithelium of the gastric shield area, the latter containing no fat and few phagocytes, which never contain ingested corpuscles.

One day after feeding conditions were much the same as twelve hours after, but there was a still greater accumulation of fat in the phagocytes, in the epithelium, and in the vesicular connective tissue cells in which it is deposited by the phagocytes. It is never carried by them into the blood vessels. Conditions remain substantially the same two and three days after feeding. At the end of the latter period great quantities of fat were observed at the base of the cells of the gastric shield area, phagocytes were rare, and only at the base of the cells, and it is they, presumably, which carry the fat here from the ciliated epithelium, since there is no indication that phagocytes can pass through the substance of the gastric shield. Five days after feeding, though the connective tissue contains great quantities of fat, there are only slight traces in the epithelium, while the few phagocytes which contain fat are in most cases either at the base of the epithelium or in the basement membrane. Very similar conditions prevail six days after feeding, while after eight days there is a complete absence of fat in both epithelium and phagocytes in the ciliated areas of the stomach, but in the region of the gastric shield there is still abundance of fat near the base of the cells, though phagocytes are very rarely seen. There is a reduction in the amount of fat in the vesicular connective tissue. In an oyster fixed eleven days after feeding, however, there was no trace of fat in the epithelium of the gastric shield

area, but a little, especially in the phagocytes, in the ciliated epithelium ; there was a considerable reduction in the quantity of fat in the vesicular connective tissue. Fourteen days after feeding there was a considerable quantity of fat in the ciliated epithelium, phagocytes, and connective tissue. These individual variations are due probably to variations in the number of corpuscles taken in, not all the oysters having opened their shell valves for the same period, and to the degree to which they had been deprived of fat by starvation, which depends on the amount present previous to starvation. The same degree of phagocytic activity was observed in the ducts of the digestive diverticula ; similar activity in the ducts has been described and figured (1926) for *Mya arenaria*.

(*c*) *With Olive Oil.*

An emulsion of olive oil stained red with Nile blue sulphate was injected by means of a pipette either into the mouth or mantle cavity of oysters, parts of whose shells had been drilled away so as to permit of the operation. The shell valves were then clamped, and the oysters placed in water with the drilled valve undermost, so as to prevent the light oil from floating out. The animals were examined after one day.

When opened the epithelium of the mantle, free surface of the visceral mass, gills, palps, and stomach was in many cases found to be coloured *blue* in patches, while under the microscope the gill mucus was seen to be full of phagocytes, most of them ingesting oil. Fig. 37 represents such a phagocyte. The large vacuoles (shown empty in the figure) are filled with unchanged oil, but the smaller vacuoles (black in the figure) are vividly blue, owing to the transformation of the neutral fat into fatty acids by the lipase of the phagocyte, with a consequent change in the colour of the stain. The use of Nile blue sulphate provides a very graphic demonstration of the digestion of fats. The blue colour of the epithelia was found to be due, when the tissues were cleared in glycerine, to the presence of great numbers of phagocytes, all laden with fat and fatty acids, both on the surface, in the epithelium, and in the deeper layers. The condition in the gill is shown in Fig. 38, in which

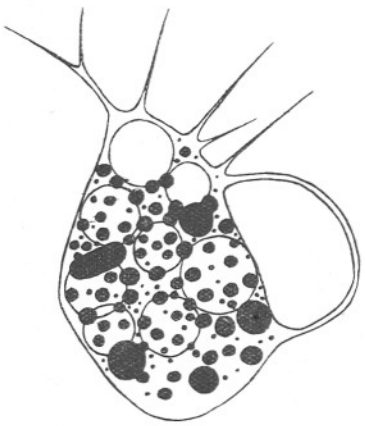

FIG. 37.—Phagocyte from gill mucus after ingestion of olive oil stained with Nile blue sulphate. Large vacuoles full of red oil, small vacuoles (shown black) containing blue stained fatty acid. Drawn from life. × 2700.

phagocytes are seen lying free on the surface of the gill, while others are passing into the tissue, and there are a line of them down the centre

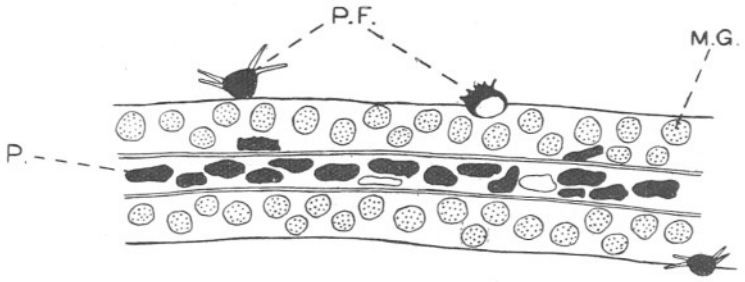

FIG. 38.—Portion of gill one day after feeding with olive oil stained Nile blue sulphate, cleared with glycerine. × 480. M.G., mucus glands; P., phagocytes with fat in centre of gill filament, others passing in; P.F., phagocytes free on surface of gill, full of fat and fatty acids.

of the filament in the blood channel, most of them containing fat. Similar conditions prevail in the mantle, as represented in Fig. 39, the centre of the mantle tentacle being deep blue with darker spots denoting

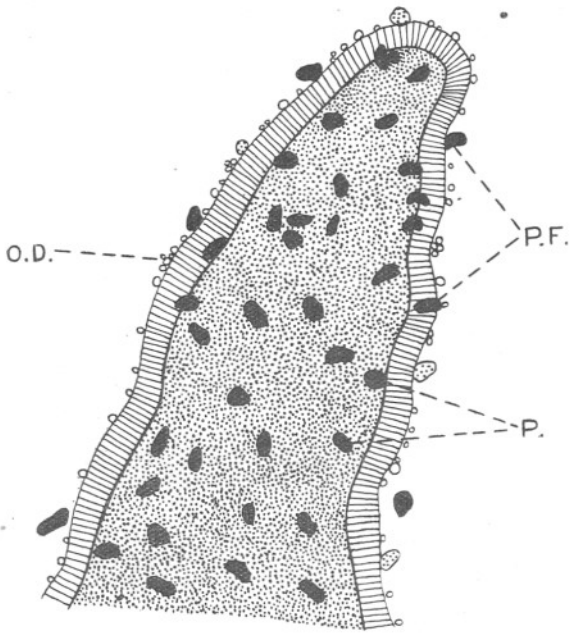

FIG. 39.—Tentacle from edge of mantle one day after feeding with olive oil stained Nile blue sulphate, cleared in glycerine. × 240. O.D., droplets of oil on surface of epithelium; P., phagocytes coloured blue owing to fatty acids, lying deep in tissues; P.F., phagocytes containing fat and fatty acids free on surface and passing through epithelium.

the presence of phagocytes near the surface. Other phagocytes are passing through the epithelium on the surface of which are more phagocytes and droplets of oil.

In the lumen of the stomach there were immense numbers of phagocytes, most of them with ingested oil. In certain cases they collected in great numbers round large droplets of oil, which had turned blue under the influence of their enzymes. *All oil droplets lying free in the stomach and not surrounded by phagocytes retained the red colour*—evidence of the absence of lipase in the stomach. Nelson (1918) also noted the absence of extracellular lipase in the stomach of other Lamellibranchs. Portions of the epithelium cleared in glycerine showed that phagocytes laden with oil were passing through it in large numbers.

(*d*) *With Nitzschia.*

Oysters which had been starved for three months were fed with a pure culture of Nitzschia, a quantity of which was added daily to the filtered sea-water. The oysters were observed to open their valves more widely than usual. One oyster was opened after seven days. In the stomach were many phagocytes ingesting Nitzschia, such as the one shown in Fig. 40, which has ingested three diatoms, and there were also very many free diatoms, while at the head of the style was a brown mass consisting exclusively of entangled diatoms. There were fewer phagocytes in the stomach than after feeding with blood corpuscles or oil. Many of the phagocytes contained green or yellow globules, the result probably of the ingestion of the brown chromatophores of the diatoms. Digestive diverticula pressed out and examined under a coverslip were largely colourless, except for the presence in some tubules of light green or brown vacuoles, which were not seen in the diverticula of starved animals. Substantially the same conditions were found after two weeks of feeding with Nitzschia.

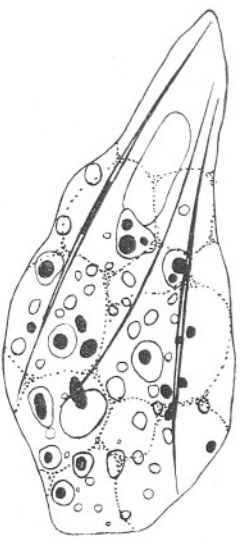

FIG. 40.—Phagocyte from stomach ingesting three Nitzschia. Drawn from life. × 2400.

Sections of the stomach and mid-gut, fixed in Flemming, showed many fat globules in the epithelium and great numbers of phagocytes. It was difficult to see ingested diatoms in the phagocytes, but in Fig. 41 is shown a portion of the edge of the stomach epithelium, in which lie two phagocytes, each containing an ingested diatom. As a result of the digestion

of the diatoms, there is a quantity of fat in the phagocytes, and some has been passed into the cells of the epithelium. Conditions are thus essentially the same as after feeding with blood corpuscles. It was never possible to detect ingested diatoms in the digestive diverticula. Vonk

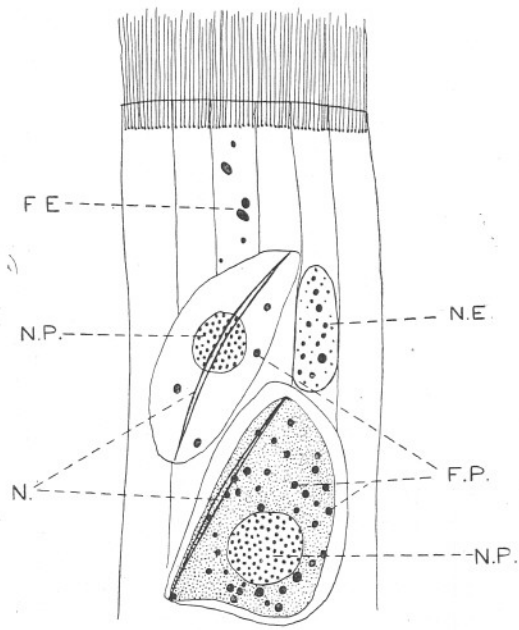

Fig. 41.—Edge of stomach epithelium, two phagocytes ingesting Nitzschia. × 2700. F.E., fat in epithelial cells; F.P., fat in phagocytes; N., Nitzschia; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

after feeding starved oysters with plankton, never observed the presence of whole diatoms in the cells of the "liver," only numerous green inclusions of very irregular form, though occasionally green algæ appeared to be taken in entire.

III. Feeding Experiments on Larvæ and Spat.

Larvæ placed in a suspension of iron saccharate in sea-water took it in in large amounts. A study of sections shows that it was assimilated *exclusively* in the cells of the digestive diverticula. Fig. 42 represents a transverse section through one of the two simple diverticula twenty-one hours after feeding with iron saccharate. This has been absorbed in large quantities and lies in discrete round masses in vacuoles in most of the

cells. It is never in the form of fine granules or diffuse. It is being passed from the cells to phagocytes, two of which are seen in the connective tissue around the diverticula, both of them so packed with iron that only the nucleus, and that with difficulty, can be distinguished.

In the spat, iron was taken in exclusively by the cells of the digestive diverticula in the same manner as in the larvæ and adult, although iron was found in the lumen of all parts of the gut (see Figs. 19 and 20).

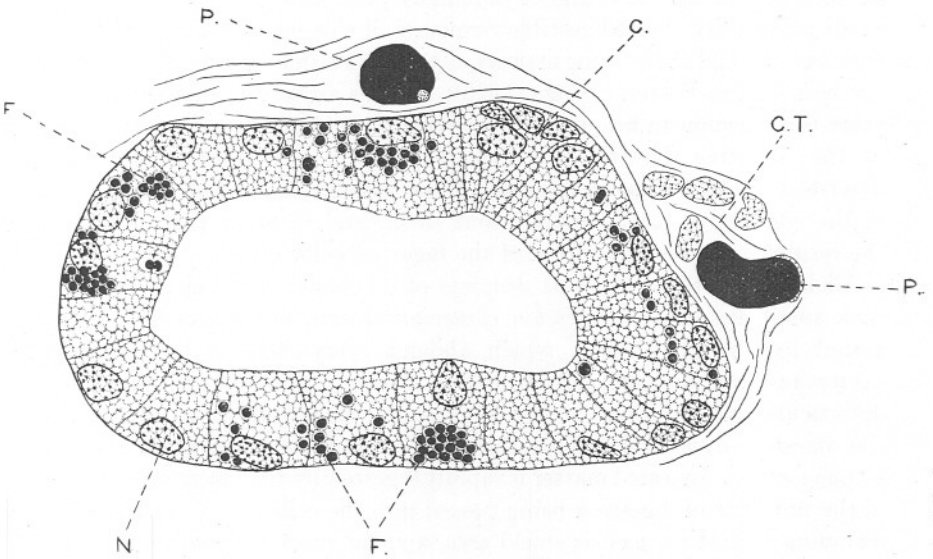

FIG. 42.—Transverse section through digestive diverticulum of one side in larval oyster. Fed on iron saccharate twenty-one hours before fixing. Iron technique. × 1800. C., crypt of young cells; C.T., connective tissue; F., iron lying in round vacuoles in cells; N., nucleus of cell of diverticulum; P., phagocytes laden with iron.

Carmine was also taken in by the spat in such quantity that in sections stained with Delafield's hæmatoxylin the lumen of the gut appeared as a uniform red. Carmine is ingested by the cells of the digestive diverticula in precisely the same manner as the iron saccharate, and in no other region of the gut.

IV. Discussion of Results.

Soluble matter, such as iron saccharate, is absorbed exclusively in the cells of the digestive diverticula in larva, spat, and adult, being invariably taken into large vacuoles and carried away by leucocytes. Presumably, therefore, the products of extracellular digestion in the stomach due to the action of the digestive enzymes from the style are here absorbed. Fine particles, such as carmine grains or Indian ink (List and Vonk), are ingested by the cells of the digestive diverticula, being also taken into

large vacuoles and being expelled later if of no food value. It may be assumed that the contents of the green or brown vacuoles, seen in the digestive diverticula of freshly fed animals (see Fig. 11), consist of finely divided vegetable matter which has been ingested by the cells and is in process of being digested intracellularly within the vacuoles. The presence of bright yellow or brown concretions, which alone remain in the diverticula after prolonged starvation, and which are also found in the lumen of the ducts and of the hinder portions of the gut represents in all probability the indigestible remnants of this intracellular digestion which is expelled in the same manner as the Indian ink. These concretions are best seen in Pecten (Yonge (1926)). It has already been shown that there is a mechanism for ensuring a circulation of particles in the tubules of the digestive diverticula. The presence of the enterochlorophyll described by MacMunn in the cells of the tubules and in the leucocytes in the connective tissue round about them, and in other parts will be the result of the decomposition of the ingested chlorophyll.

All larger particles, such as droplets of oil, blood corpuscles, or even such small diatoms as *Nitzschia closterium* forma *minutissima*, are ingested by the phagocytes, which abound everywhere in the mantle cavity and gut, but particularly in the stomach, ducts of the digestive diverticula and mid-gut. They very rarely pass into the tubules of the digestive diverticula, those that enter the ducts being there seized by phagocytes. Ingested matter is rapidly digested by the phagocytes, part of the products of digestion being passed into the cells of the epithelium, including that of the gastric shield area, and the remainder carried to the vesicular connective tissue cells, or Langer's vesicles, and there stored. *No evidence of any absorption in the epithelium of the gut or of any free surface in the mantle cavity, other than by the agency of phagocytes, was found*, and previous accounts of direct absorption by the ciliated epithelium on further investigation will probably be found to be the result of the action of phagocytes and the transference of material from them to the cells.

5. THE DIGESTIVE ENZYMES.

Digestive enzymes are present in the style and in the tissue of the digestive diverticula, the former are released into the stomach when the style dissolves and the latter remain in the tissues, where they act intracellularly. It is clear from the results of the experiments described in the last section that the phagocytes must also possess powerful digestive enzymes of various kinds. The enzymes were obtained by grinding up the styles or the excised tissue of the digestive diverticula with sand, and then extracting for two or three days with distilled water (the extracts being as efficacious as those prepared in sea-water and being easier to

deal with), toluol being used as antiseptic. Except when otherwise stated, extracts of the style were always of a strength of 1% and extracts of the digestive diverticula 10%, and incubation took place at about 30° C. Rigorous controls consisting of boiled extracts were invariably set up. All experiments were confined to adult oysters.

1. The Style.

The presence of digestive enzymes in the style was first discovered by Coupin (1900), who found an amylase and a weak invertase in the style of Cardium; this has been confirmed, amongst others, by Mitra (1901), who found amylase and glycogenase in Anodonta, Van Rynberk (1908), who found amylase and invertase in Mytilus, Nelson (1918), who found similar enzymes in Anodonta, and Yonge (1923), who found amylase and glycogenase in the style of Mya, and showed that the amylase had all the properties of a typical enzyme. More recently Berkeley (1923) has found an oxidase in the styles of several Lamellibranchs. Barrois (1889) gives a detailed chemical analysis of the styles of Cardium made for him by Lambling, who found that they consisted of 87·11% water, 12·03% solid organic matter, and 0·86% solid inorganic matter. The mass of the organic matter consisted of a globulin, though traces of a mucin or chondrin like substance were found. List also found the latter in the styles of Mytilus. Mitra (1901), in ignorance of the work of Barrois, made a thorough examination of the styles of Anodonta, with almost identical results. Mackintosh (1925) finds that the style of Crepidula consists largely of globulin with some mucus. Mitra thought that the style represented a mass of enzyme, but Nelson (1918) advances the more probable view that the enzymes are *adsorbed* on the surface of globules of albuminoid substance.

In view of the complete agreement of previous workers, I have not carried out a chemical examination of the style of Ostrea, there being no reason to doubt that it differs in any important degree from those which have been analysed.

(*a*) *Specificity.*

In common with previous workers, I have failed to find any trace of proteoclastic or lipoclastic enzymes in the style, experiments with calcified milk, methyl acetate, and phenol red milk, all giving negative results. No action was found on the glucosides, amygdalin and salicin, on pectin or on lactose, maltose, raffinose, cellulose, or sucrose (in spite of the contrary assertions of Coupin and Mitra with regard to the latter). Starch and glycogen, as in Mya, were the only substances acted on by the enzymes of the style, both being rapidly converted into reducing sugars, as shown

by their action on Fehling's and Benedict's reagents. The properties of the amylase have been studied in detail.

(*b*) *Influence of Temperature on Amylase.*

The experiments shown in Table I were carried out to determine the optimum temperature for the working of the style amylase. The enzyme was destroyed after incubation, the contents of each tube filtered and made up to the original volume.

TABLE I.

OPTIMUM TEMPERATURE OF STYLE AMYLASE.

10 c.c. of extract with 10 c.c. of 1% starch solution in each experiment.

1. Extract of 1·14 gms. of style made in 70 c.c. toluol water. Experiments incubated for 4 hours.

Temperature.	Titrated with 10 c.c. Benedict.
16° C.	9·95 c.c. needed.
25° C.	9·7 ,,
30° C.	9·2 ,,
35° C.	9·05 ,,
40° C.	8·9 ,,
48° C.	8·9 ,,
55° C.	10·0 ,,

2. Extract of 0·93 gms. of style made in 50 c.c. toluol water. Incubated for 3 hours.

Temperature.	Titrated with 10 c.c. Benedict.
40° C.	15·05 c.c. needed.
43° C.	14·35 ,,
46° C.	14·7 ,,
49° C.	15·45 ,,
52° C.	16·25 ,,

These two experiments demonstrate that the optimum temperature lies at, or a little below, 43° C. The pH was 5·9 in both experiments.

Experiments were carried out to determine the temperature of destruction. The heating of 5 c.c. of enzyme extract for fifteen minutes at eight temperatures between 100° C. and 56° C. resulted in complete destruction of the enzyme, as shown by subsequent incubation for twenty hours at 30° C. with 1% starch solution. Heating at 55° C. and all lower temperatures resulted in some of the enzyme remaining active.

It appears that the enzyme is destroyed at 56° C. There was practically no action on starch at 0° C., the enzyme being inactivated, not destroyed. It is interesting to compare the above optimum temperature and temperature of destruction with those found for the style enzyme of Mya (1923), which were 32° C. and 51° C. respectively. The difference between the optima is very striking, and may reflect differences in the habitat of the two animals ; it is known that the oyster will breed and flourish in the Norwegian pools, where the temperature may rise as high as 90° F. (32° C.), but in neither animal does the optimum temperature represent

anything approaching the temperature at which digestion must normally proceed.

(*c*) *Influence of pH.*

Table II shows the results of an experiment to determine the optimum pH for the working of the amylase.

TABLE II.

OPTIMUM pH OF STYLE AMYLASE.

Extract of 1·36 gms. of style made in 90 c.c. toluol water. 10 c.c. of extract and 10 c.c. of 1% starch solution in each experiment with acid or alkali, volume made up to 25 c.c. with water. All incubated for 6 hours at 32° C.; pH determined by Clark and Lubs' indicators.

	·01N HCl.	·01N NaOH.	Initial pH.	Titrated with 10 c.c. Benedict.
A.	3·0 c.c.	—	3·4	No reduction.
B.	0·5 c.c.	—	4·9	55·4 c.c. needed.
C.	0·3 c.c.	—	5·2	13·5 ,,
D.	—	—	5·9	9·1 ,,
E.	—	0·1 c.c.	6·2	9·7 ,,
F.	—	0·2 c.c.	6·6	10·15 ,,
G.	—	0·5 c.c.	7·3	11·6 ,,
H.	—	0·7 c.c.	7·6	13·05 ,,
I.	—	1·0 c.c.	9·3	21·0 ,,

The optimum is very sharply defined, and lies at about 5·9, i.e. at the pH produced by the dissolution of the style in water, on either side of this point, and particularly on the acid side, the efficiency of the enzyme being rapidly reduced.

(*d*) *Influence of Salts.*

In view of the fact that if the pancreatic amylase is dialysed it loses its power to act upon starch, as shown by Bierry, Giaja, and Henri (1906), and that the amylase from the liver is inactivated in the same manner (Starkenstein (1910, 1910a)), action being restored in the former case by the addition of the electro-negative chloride or bromide ions, and in the latter by the addition of sodium chloride, experiments were carried out to determine whether the amylase of the style is similarly dependent for its efficacy on the presence of electrolytes.

An extract of 0·75 gms. of style was made in 40 c.c. of toluol water. After three days the enzyme was precipitated by the addition of 200 c.c. of absolute alcohol, the precipitate being filtered off, thoroughly washed

with absolute alcohol, dried, and then dissolved in 40 c.c. of glass-distilled water. The experiments in Table III were then carried out, the sea-water used in experiments B and C being made acid until the pH approximated to the optimum.

Table III.

Action of Purified Enzyme with and without Salts from Sea-water.

10 c.c. extract with 10 c.c 1% starch solution in each experiment. Incubated for 5 hours at 32° C., enzyme destroyed and titrated.

	Added.	pH.	Titrated with 10 c.c. Benedict.*
A.	20 c.c. distilled water	5·8	175 c.c. needed
B.	20 c.c. 100% sea-water	5·7	28·05 ,,
C.	20 c.c. 200% sea-water	5·5	25·6 ,,
D.	20 c.c. dis. water and 1 drop sea-water	5·8	72·75 ,,

In the absence of the salts present in sea-water the enzyme is almost inactivated (A), action is restored to some extent by the addition of a trace of sea-water (D), and fully restored in a medium of 50% (B) or 100% (C) sea-water, action being slightly less in the former.

A series of dialysis experiments were then carried out, details of which are given in Tables IV, V, and VI.

Table IV.

Action of Dialysed Extract.

0·75 gms. style extracted in 80 c.c. toluol water for 3 days, divided into two parts, A and B, each of 40 c.c. These dialysed in separate parchments for 3 days, surrounding fluid being changed daily, contents of each finally made up to 40 c.c. 10 c.c. extract with 10 c.c. 1% starch solution in each experiment. Incubated for 5 hours at 32° C., enzyme destroyed and titrated.

		Added.	pH.	Titrated with 10 c.c. Benedict.*
A.	1.	10 c.c. distilled water	5·8	275 c.c. needed.
	2.	10 c.c. acidified sea-water	5·8	24·05 ,,
	3.	10 c.c. surrounding fluid	5·8	230 ,,
	4.	10 c.c. 1%NaCl	5·8	28·75 ,,
B.	1.	10 c.c. distilled water	5·8	290 ,,
	2.	10 c.c. 1%Na_2SO_4	5·8	290 ,,
	3.	10 c.c. 1%NaBr	5·8	50 ,,
	4.	10 c.c. 1%KCl	5·8	40·1 ,,

* Or suitable aliquot part.

TABLE V.

ACTION OF DIALYSED EXTRACT.

0·45 gms. style extracted in 40 c.c. toluol water, dialysed 3 days, water changed 4 times. Experiments conducted as in Table IV.

	Added.	pH.	Titrated with 10 c.c. Benedict.*
C.	1. 10 c.c. distilled water	6·4	241·5 c.c. needed.
	2. 10 c.c. 1%Na_2CO_3	9·8	186·5 ,,
	3. 10 c.c. 1%KI	6·6	96·5 ,,
	4. 10 c.c. 1%$CaCl_2$	5·7	23·0 ,,

TABLE VI.

ACTION OF DIALYSED EXTRACT.

0·64 gms. style extracted in 60 c.c. toluol water, dialysed 4 days, water changed 4 times. Experiments conducted as in Table IV.

	Added.	pH.	Titrated with 10 c.c. Benedict.*
D.	1. 10 c.c. distilled water	6·6	270 c.c. needed.
	2. 10 c.c. 1%MgCl	5·8	46·5 ,,
	3. 10 c.c. 1%NaF	6·0	270 ,,
	4. 10 c.c. 1%$NaNO_3$	6·2	123·5 ,,
	5. 10 c.c. 1%K_2SO_4	6·1	300 ,,
	6. 10 c.c. 1%BaCl	5·8	40 ,,

An examination of the results of these four sets of experiments shows that the dialysed extract is almost without action on starch, but that action is restored to a slight degree on the addition of 10 c.c. of the water into which the salts had passed from out of the parchment (A3), while action was completely restored as before on the addition of sea-water (A2). Action was also restored in the presence of the chlorides of sodium (A4), potassium (B4), calcium (C4), magnesium (D2), and barium (D6), and in that of sodium bromide (B3). To a less extent it was restored in the presence of the iodide of potassium (C3), the nitrate of sodium (D4) and the carbonate of sodium (C2), in spite of the high pH in the last case. There was no increase in activity in the presence of the sulphates of sodium (B2) or potassium (D5), or in that of sodium fluoride (D3). The amylase of the style appears, therefore, to need for its action the presence of electro-negative ions—preferably those of chlorine or bromine—the identity of the electro-positive ion being immaterial. Conditions are the same as in the case of the amylase of the pancreas or of the liver in Vertebrates. Since the extracts of the style made up in distilled water have the same efficacy as those prepared in sea-water it appears that these ions are present in sufficient quantity in the substance of the style.

* Or suitable aliquot part.

II. The Digestive Diverticula.

The presence of digestive enzymes in extracts of the digestive diverticula has been shown by Fredericq (1878), who found protease in Mya and Mytilus; Mitra (1901), who found amylase and invertase in Anodonta; Van Rynberk (1908), who found amylase in Mytilus; Dakin (1909), who found amylase, protease and lipase in Pecten; Heymann (1914), who found protease, lipase and a variety of sucroclastic enzymes in Ostrea; and Yonge (1923), who found in Mya sucroclastic enzymes which acted on starch, glycogen, sucrose, maltose, and lactose, also a protease acting in acid media and a lipase. Most of these workers considered that the

Table VII.

Action of 10% Extract of Digestive Diverticula on Carbohydrates, etc.

10 c.c. extract with 10 c.c. of substrate, controls of boiled extract, incubated at 30° C.

No.	Substrate.	Time.	Experiment. Titrated with 10 c.c. Benedict.	Control.
1.	1% starch	2 hrs.	4·3 c.c.	9·0 c.c.
2.	0·5 % glycogen	1 day	5·9 c.c.	9·0 c.c.
3.	5% sucrose	,,	4·8 c.c.	9·0 c.c.
4.	1% raffinose	3 days	6·8 c.c.	9·0 c.c.
5.	1% inulin	,,	8·2 c.c.	8·2 c.c.
6.	1% salicin	,,	4·2 c.c.	8·3 c.c.
7.	1% amygdalin	,,	4·2 c.c.*	8·1 c.c.
		1 c.c. boiled with 5 c.c. Barfoed's sol. 10 min.		
8.	2% maltose	1 day	Reduction	No reduction.
9.	2% lactose	,,	Reduction	No reduction.

* Smell of CN.

digestive diverticula were secretory, and that these enzymes were discharged into the stomach. As shown in detail in a previous paper (1926) there is no evidence, histological or physiological, of any secretion in the diverticula which are organs of absorption and of intracellular digestion, the digestive enzymes acting on material ingested.

(*a*) *Sucroclastic Enzymes.*

Specificity.—Owing to the presence of reducing sugars in the extract of the digestive diverticula, it is necessary to estimate the sugar in both experiments and controls. In Table VII are shown the results of experiments on the simpler carbohydrates and glucosides. Starch, glycogen,

sucrose, raffinose (to a slight degree), maltose, and lactose were all digested by the enzymes in the extract and also the two glucosides, salicin and amygdalin. Inulin was not digested. A series of longer experiments was set up to determine whether cellulose or pentosans are digested. Both of these are of great importance since cellulose must bulk large in the food

TABLE VIII.

ACTION OF 10% EXTRACT OF DIVERTICULA ON CELLULOSE, PENTOSANS, AND INULIN.

No.	Experiment.	Temp.	Time.	Titrated with 10 c.c. Benedict.
1.	A. 20 c.c. with 0·5 gm. sawdust.	32° C.	3 wks.	A. 3·2 c.c.
	B. Ditto boiled.	,,	,,	B. 5·2 c.c.
	C. 20 c.c. extract alone.	,,	,,	C. 3·2 c.c.
2.	A. 20 c.c. with 0·5 gm. sawdust.	,,	2 wks.	A. 2·55 c.c.
	B. Ditto boiled.	,,	,,	B. 3·2 c.c.
	C. 20 c.c. extract alone.	,,	,,	C. 2·55 c.c.
3.	A. 10 c.c. with 10 c.c. 1% pectin.	,,	15 days.	A. 5·0 c.c.
	B. Ditto boiled.	,,	,,	B. 7·0 c.c.
	C. 10 c.c. with 10 c.c. water.	,,	,,	C. 5·0 c.c.
4.	A. 20 c.c. with 10 c.c. 1% pectin.	,,	16 days.	A. 4·45 c.c.
	B. Ditto boiled.	,,	,,	B. 7·30 c.c.
	C. 20 c.c. with 10 c.c. water.	,,	,,	C. 4·5 c.c.
5.	A. 20 c.c. with 10 c.c. 5% gum arabic.	,,	2 wks.	A. 2·4 c.c.
	B. Ditto boiled.	,,	,,	B. 2·92 c.c.
	C. 20 c.c. with 10 c.c. water.	,,	,,	C. 2·4 c.c.
6.	A. 20 c.c. with 10 c.c. 5% gum arabic.	,,	3 wks.	A. 2·2 c.c.
	B. Ditto boiled.	,,	,,	B. 4·5 c.c.
	C. 20 c.c. with 10 c.c. water.	,,	,,	C. 2·6 c.c.
7.	A. 10 c.c. with 10 c.c. 2% inulin	,,	2 wks.	A. 5·85 c.c.
	B. Ditto boiled.	,,	,,	B. 9·8 c.c.
	C. 10 c.c. with 10 c.c. water.	,,	,,	C. 6·0 c.c.
8.	A. 20 c.c. with Ulva 3″ × 1″	,,	3 wks.	A. Ulva unchanged
	B. Ditto boiled.	,,	,,	B. Ulva unchanged.

of the oyster, while Petersen (1911), in his work on the food of oysters in the Limfjord, maintained that detritus was the principal source of food, and Boysen Jensen (1914) has shown that the main constituent of this detritus consists of pentosans. No experiments were made by him on the digestion of pentosans, although Heymann (1914), by somewhat questionable methods, found that pectin was digested by extracts of the

"liver" of the oyster. The same author also maintaining that inulin is digested, a longer experiment was set up in order to confirm the results of the experiment in Table VII. In view of the autolysis which proceeds if tissue extracts are left for any long period, resulting in the formation of additional quantities of reducing sugars, two controls were necessary for these experiments, one with boiled extract and substrate and the other with unboiled extract without substrate. Sawdust was used for the experiments on cellulose, owing to the presence in it of hemicelluloses, which do not occur in filter paper. Table VIII gives the result of these experiments.

None of these substances were digested, the only increase in reducing sugar being due to autolysis. Cellulose in the form of sawdust or as the green alga, Ulva, was not digested even after three weeks incubation. It would have been surprising if it had since the power of digesting cellulose by means of an enzyme is rare, being confined, in the Mollusca, to certain of the herbivorous Pulmonates and Tectibranchs, which secrete an extracellular cellulase and, in the Lamellibranchs, to the specialised Teredinidæ, which digest wood intracellularly (for résumé of work on this subject see Yonge (1925a)). After incubations of two and three weeks there was no indication of the digestion of the pentosans, pectin and gum arabic. It is impossible to confirm the findings of Heymann, nor is confidence in his work strengthened by the negative results of the experiments here performed on inulin. The implications of these results on the theories of Petersen and Boysen Jensen will be discussed later.

Influence of Temperature on Amylase.—Similar experiments to those carried out on the style amylase were done on the amylase from the digestive diverticula. The experiments in Table IX were performed to determine the optimum temperature.

Table IX.

Optimum Temperature of Amylase.

10 c.c. 10% extract with 10 c.c. 1% starch solution, digests in left column incubated 2 hours, in right column 3 hours.

Temperature.	Titrated with 10 c.c. Benedict.	Temperature.	10 c.c. Benedict.
18·5° C.	7·75 c.c. needed.	40° C.	5·2 c.c. needed.
28° C.	6·8 ,,	43° C.	5·0 ,,
35° C.	6·4 ,,	46° C.	5·0 ,,
40° C.	6·3 ,,	49° C.	5·2 ,,
45° C.	6·25 ,,	52° C.	5·4 ,,
50° C.	6·8 ,,		
55° C.	8·0 ,,		
60° C.	9·45 ,,		

These experiments show that, at pH 5·5, the optimum temperature is 44·5° C., i.e. slightly higher than that of the style amylase, where the pH, however, was 5·9.

Experiments to determine the temperature of destruction showed that the heating of 5 c.c. of enzyme extract for fifteen minutes at four temperatures between 100° C. and 67° C. resulted in complete destruction of the enzyme as shown by subsequent incubation for twenty hours at 30° C. with 1% starch. The enzyme remained active after heating at 64° C. and all lower temperatures. The temperature of destruction therefore, at pH 5·5, lies between 64° C. and 67° C. This is considerably higher than that of the style amylase (56° C.); there, however, the pH was 5·9, and as Compton (1921 and previous papers therein quoted) has shown that temperature optima of enzyme actions are dependent, amongst other things, on pH, there is not rigorous proof that the two enzymes are distinct in their properties. The action of the amylase from the diverticula is practically inhibited at 0° C.

Influence of pH.—The experiment in Table X was carried out to determine the optimum pH for the action of the amylase.

TABLE X.

OPTIMUM pH OF AMYLASE FROM DIVERTICULA.

10 c.c. 10% extract and 10 c.c. 1% starch solution with acid or alkali, volume made up to 25 c.c. with water. Incubated for 2 hours at 30° C.

	HCl.	NaOH.	Initial pH.	Titrated with 10 c.c. Benedict.
A.	5 c.c. ·1N	—	2·4	10·8 c.c. needed.
B.	3 c.c. ·1N	—	3·2	9·6 ,,
C.	1 c.c. ·1N	—	3·6	8·4 ,,
D.	3 c.c. ·01N	—	4·0	7·45 ,,
E.	2 c.c. ·01N	—	4·6	6·65 ,,
F.	1 c.c. ·01N	—	5·0	6·1 ,,
G.	—	—	5·5	5·8 ,,
H.	—	1 c.c. ·01N	5·8	6·0 ,,
I.	—	2 c.c. ·01N	6·2	6·03 ,,
J.	—	3 c.c. ·01N	6·6	6·1 ,,
K.	—	5 c.c. ·01N	7·0	6·45 ,,
L.	—	0·8 c.c. ·1N	7·8	7·3 ,,
M.	—	1 c.c. ·1N	8·6	8·0 ,,
N.	—	2 c.c. ·1N	9·6	9·4 ,,

The optimum lies at about pH 5·5, i.e. somewhat lower than that of the style (5·9). The optimum is not so sharply defined as in the case of

the style enzyme, the efficacy of the enzyme not decreasing so rapidly on either side of that point.

Influence of Salts.—A number of experiments were conducted to determine the action of salts on the activity of the amylase, the results being given in Tables XI and XII.

TABLE XI.

Action of Purified Enzyme with and without Salts from Sea-water.

15 gms. diverticula extracted in 60 c.c. toluol water, enzyme precipitated with alcohol and purified as in expts. on style. Ppt. dissolved in 40 c.c. glass distilled water. 10 c.c. extract with 10 c.c. 1% starch in each experiment, incubated for 5 hours at 32° C.

	Added.	pH.	Titrated with 10 c.c. Benedict.
A.	20 c.c. distilled water.	6·4	29·0 c.c. needed.
B.	20 c.c. 100% sea-water.*	5·8	17·4 ,,
C.	20 c.c. 200% sea-water.*	5·6	17·3 ,,
D.	20 c.c. dis. water and 1 drop sea-water.	6·4	24·4 ,,

Table XII.

Action of Dialysed Extract.

50 c.c. 10% extract dialysed for 3 days, water changed 4 times. Experiments conducted as in Table XI.

	Added.	pH.	Titrated with 10 c.c. Benedict.
A.	10 c.c. distilled water.	6·6	38·8 c.c. needed.
B.	10 c.c. 1% NaCl.	5·8	23·25 ,,
C.	10 c.c. 1% NaBr.	9·6	28·2 ,,
D.	10 c.c. 1% $CaCl_2$.	5·8	22·65 ,,
E.	10 c.c. 1% KI.	6·0	31·75 ,,

Here again the enzyme requires for its working the presence of the salts in sea-water, only a trace of which has a considerable effect (Table XI, D). The action of the enzyme is definitely inhibited after dialysis. It is more difficult to purify the enzyme from the tissue extract than from the style and hence the greater activity of the dialysed enzyme in this case. Action was restored in the presence of the chlorides of sodium and calcium (Table XII, B and D) and of the bromide of sodium (C)—the reduced action in the last case being due to the high pH, owing to the presence

* Acidified.

of impurities in the salt—and to a slighter extent in the presence of potassium iodide (E).

(*b*) *Lipoclastic Enzymes.*

The results of a series of experiments on the lipase in the digestive diverticula are shown in Table XIII.

Table XIII.

Action of 10% Extract of Digestive Diverticula on Fats and Esters.

Controls of boiled extract, incubation at 32° C.

No.	Extract.	Substrate.	Time.	Acidity in terms of ·1N NaOH. Experiment.	Control.
1.	15 c.c.	30 drops olive oil emulsion	7 days	3·2 c.c.	2·5 c.c.
2.	25 c.c.	30 drops ,, ,,	14 ,,	2·5 c.c.	2·1 c.c.
3.	20 c.c.	30 drops ,, ,,	21 ,,	1·05 c.c.	0·85 c.c.
4.	10 c.c.	10 c.c. 5% methyl acetate	7 ,,	9·0 c.c.	2·5 c.c.
5.	10 c.c.	10 c.c. 10 % ,, ,,	14 ,,	17·35 c.c.	5·15 c.c.
6.	5 c.c.	5 c.c. boiled milk with 3 c.c. 2 % Na_2CO_3 and phenol red.	17 hrs.	Yellow.	Remains Red.
7.	5 c.c.	ditto with 1 c.c. Na_2CO_3	19 ,,	Yellow.	Remains Red.

The action on olive oil is very slight even after three weeks incubation; but there is considerable action on methyl acetate, and experiments with phenol red milk were all positive. Since fat is taken in freely by the phagocytes and there digested, very little appearing in the digestive diverticula, there is probably no necessity for the presence of a powerful lipase in the latter. Indeed, the slight lipolytic action of the extract may be due, in part at any rate, to the phagocytes in the tissue extracted and *not* to enzymes from the actual absorptive tubules.

(*c*) *Proteoclastic Enzymes.*

It is very difficult to test for the presence of proteoclastic enzymes in extracts of the diverticula on account of the weakness of their action. No digestive activity on coagulated egg albumen or congo red fibrin could be demonstrated. Satisfactory results were obtained by the method of Dernby (quoted by Bodansky and Rose). A 10% solution of gelatin was prepared and a series of experiments performed with extracts of the diverticula at different hydrogen ion concentrations. At stated intervals

5 c.c. of the digests were removed and the process of digestion determined by placing them in ice for fifteen minutes, and then observing the degree of liquefaction. The gelatin is liquefied as digestion proceeds, and fails to solidify to a greater or less extent dependent upon the degree to which digestion has proceeded. Dernby used the following scale of numbers to denote the approximate degree of digestion :—

0 = Completely solid.
1 = Solid, but small pieces may be torn off by strong shaking.
2 = Solid, but the surface moves somewhat when tubes are shaken.
3 = Soft.
4 = Half liquid.
5 = Almost liquid.
6 = Entirely liquid.

Table XIV shows the result of an experiment of this nature which confirmed the results of a previous experiment. There appear to be two optima, one at a pH of about 3·7 and the other at and above pH 9·0. There is *no* action at the normal pH of the tissue extract, while optimum conditions prevail at a degree of alkalinity which cannot be present

TABLE XIV.

DIGESTION OF GELATIN BY EXTRACT OF THE DIVERTICULA.

10 c.c. 15% extract with HCl or NaOH with 15 c.c. 10% gelatin with water = 30 c.c.

No.	HCl.	NaOH.	Initial pH.	Degree of liquefaction after incubation at 32° C. for :— 2 days.	3 days.	4 days.	5 days.
1	2·5 c.c. N	—	2·2	1–2	1–2	2	3
2	1·0 c.c. N	—	3·7	5	6	6	6
3	5·0 c.c. ·1N	—	4·2	2	3	4	6
4	2·5 c.c. ·1N	—	4·8	1–2	2	2	2
5	5·0 c.c. ·01N	—	5·4	1	1	1	1
6	—	—	5·8	0	0	0	0
7	—	0·7 c.c. ·1N	6·0	0	0	0	0
8	—	1·0 c.c. ·1N	6·4	0	0	0	0
9	—	2·0 c.c. ·1N	7·3	0–1	1	1–2	3
10	—	3·0 c.c. ·1N	8·6	2	2	3	6
11	—	4·0 c.c. ·1N	9·2	2–3	3	6	6
12	—	5·0 c.c. ·1N	9·8	3	3	6	6
13	—	1·0 c.c. N	10·2	3	3–4	6	6
14	—	2·0 c.c. N	10·8	6	6	6	6
15	—	3·0 c.c. N	11·3	6	6	6	6

normally in the tissues. It may be that we are dealing here with two enzymes, as Bodansky and Rose (1922) believe to be the explanation of similar double optima obtained in experiments of the same nature on certain Coelenterates. In the case of the oyster, one enzyme may come from the digestive diverticula and the other from the phagocytes ; Heymann, indeed, states that there are three proteases in the oyster : a trypsin, a " liver " pepsin, and a blood pepsin. He knew nothing, however, of the digestive powers of the phagocytes. It is useless to call one pepsin and the other trypsin, as it is possible at the optimum of either for the extract to act on peptone with the formation of amino acids, as proved by positive results of tests for tryptophane eighteen hours after the commencement of experiments. On the other hand, *after three weeks digestion with gelatin there is no indication of the presence of amino acids* in any medium. The extract coagulates calcified milk in four or five hours.

The important point in connection with this work is not whether there are two enzymes or one, but the *weakness* of the proteoclastic enzyme. Although albumenoses are split up with formation of amino acids, gelatin is not, while albumen and fibrin are not attacked. It takes two days for the digestion of gelatin to proceed to the stage it attained in one or two hours in the experiments of Bodansky and Rose on the Coelenterates, Physalia and Stomolophus. These animals are by nature carnivorous, whereas the oyster is not, and it is an interesting fact that in the Ascidian, *Ciona intestinalis*, which also feeds by ciliary currents and has a similar diet to the oyster, it was found (1925) that there is a similar weakness of proteoclastic enzymes, though in this case digestion is exclusively extracellular. Apparently there is a correlation between the nature of the food and the variety and strength of the digestive enzymes in animals. In Ciona, as in the Lamellibranchs, the sucroclastic enzymes are very powerful. In the omnivorous Crustacea both sucroclastic and proteoclastic enzymes are highly developed (Yonge (1924)).

III. Stomach Contents.

Digests with the stomach fluid show the presence of enzymes, presumably derived from the style, capable of quickly digesting starch and glycogen. Slight traces of reducing sugars were found after three days incubation with sucrose, maltose and amygdalin, but none after five days incubation with lactose. This action is far slighter than that of the extract of the digestive diverticula and can safely be attributed to the phagocytes, great numbers of which are always present in the stomach. To the same origin, no doubt, can be attributed the traces of lipase and protease. Phenol red milk made alkaline with 2 c.c. of 2% Na_2CO_3 is

turned yellow in two hours by the action of stomach fluids and calcified milk is coagulated in the same time. These experiments were repeated with filtered and unfiltered fluid, since in the former the phagocytes would be absent. The experiments were otherwise identical in all respects and controls were set up. In the test for lipases the phenol red milk was turned yellow in two hours by the unfiltered fluid and in twelve hours by the filtered fluid; in the test for protease the calcified milk was coagulated in twelve hours by the unfiltered and in forty hours by the filtered fluid, the control also coagulating after forty hours in the latter case. As we have seen there is *no action on olive oil* by enzymes free in the stomach, only by phagocytes. There is thus no evidence that the digestive diverticula secrete enzymes into the stomach, since the only enzymes of any power proceed from the style, the traces of other enzymes having their origin in the phagocytes. The lack of powerful digestive enzymes in the digestive tract is confirmed by the presence—noted by many workers—of living and apparently unprotected organisms, both plant and animal, in the mid-gut, rectum and fæces of the oyster and other Lamellibranchs. No naked organism, unless protected chemically like intestinal parasites, could survive the action of powerful enzymes.

IV. Gill Mucus.

Gorka (1916), in a paper which I have been unable to see, but which is quoted by Vonk, states that he found enzymes in the gill mucus of Anodonta and Unio capable of digesting polysaccharides, glucosides and fat, and in the mucus of the palps he also found a protease. A series of experiments on the gill mucus of Ostrea were carried out, the mucus being obtained by covering the gills with fine carborundum and collecting the mucus laden strings and extracting them in toluol water, later filtering off the carborundum. After four days incubation no trace of action on any carbohydrate or glucoside was found, nor did digests with phenol red milk or olive oil give positive results, although there was a slight increase in acidity after two weeks incubation with methyl acetate. Traces of tryptophane were found after two weeks incubation with peptone, while calcified milk was coagulated after three days. In both cases controls gave negative results. There appear therefore to be traces of lipase and protease in the mucus, but if that is examined under the microscope many phagocytes are seen which wander freely on the surface of the gill, as already described in the section on feeding with olive oil. There seems no doubt that the slight development of enzymatic action in these experiments, and probably in those of Gorka, is due to enzymes from these phagocytes.

v. Oxidases.

It is most convenient here to refer to the presence of oxidases in the tissues. Berkeley (1923) has shown that extracts of the styles of *Saxidomus giganteus*, *Paphia staminea*, and *Mya arenaria* have a marked oxidising action on guaiacum, paraphenylendiamine, and pyrogallol in the absence of H_2O_2, and in its presence after boiling, and further that the oxidation of guaiacum takes place as rapidly in the absence of air. He suggests, in connection with his theory that the style is concerned with anærobic respiration (to which reference will be made later), that the substance is a complex of an oxidising agent and an enzyme which can convey oxygen to the tissues. He also found a slight action on guaiacum by extracts of the palps and traces by those of the mantle and " digestive gland," but none by those of the gills, gonad, or siphons.

In the oyster, extracts were made of the mantle, gills, palps, digestive diverticula, style, gonad, and muscle. Catalase was tested for by adding 2 c.c. of H_2O_2 to 5 c.c. of the extracts. There was a great evolution of oxygen, showing the presence of catalase, with the gonad and digestive diverticula, a medium evolution with the palps, gills, and muscle, but none with the style and mantle. Peroxidases were tested for with tincture of guaiacum, hydroquinone, and pyrogallol, in every case 5 c.c. of extract being used, and to it added 2 c.c. of H_2O_2 and twelve drops of freshly prepared guaiacum, 2% hydroquinone or 1% pyrogallol. With guaiacum oxidation was very slow, and after a day slight traces of activity were found only with the style, palps, and gills. After five hours hydroquinone was turned a decided green-brown colour with the style extract, a light yellowish brown with the gills, palps, mantle, and digestive diverticula, and pale yellow with the muscle and gonad. Pyrogallol after five minutes was turned dark red-brown with the style extract, a medium brown with the digestive diverticula and muscle and a light yellow with the other tissues. The action of minced tissue was also tested with the indophenol reagent in the absence of H_2O_2, the results, which are striking, being given below :—

1. Style—deep purple almost immediately.
2. Gill—deep purple in a few minutes.
3. Mantle—purple in four to five minutes.
4. Palps—light purple in ten minutes.
5. Dig. diverticula—light purple in fifteen minutes.
6. Gonad—light purple in fifteen minutes.
7. Muscle—light purple in twenty minutes.

The different reagents all give different results with the various tissue extracts, except in the case of the style which in *all cases* gives the most

decided reaction, and clearly contains, since it can act in the absence of H_2O_2, a complete oxidase system. It is strange that action on guaiacum should be so much less with the styles of Ostrea than with those of Saxidomus in Berkeley's experiments. Time has not permitted further work on this subject, but the presence of this enzyme in the style may be of great importance in the metabolism of the Lamellibranchs.

6. HYDROGEN ION CONCENTRATION IN THE GUT AND PERMANENCE OF THE STYLE.

1. Hydrogen Ion Concentration.

Table XV shows the pH of the fluid in the mantle cavity and in all regions of the gut, and of the substance of the digestive diverticula and

Table XV

pH in Fresh Oysters.

No.	Mantle cavity.	Œsophagus.	Stomach.	Style.	Dig. div.	Mid-gut.	Rectum.
1.	7·0	6·0	5·5	5·2	5·8	5·6	6·0
2.	7·1	5·9	5·4	5·2	5·8	5·5	6·0
3.	6·8	5·8	5·6	5·2	5·7	5·6	5·9
4.	7·2	5·9	5·5	5·2	5·9	5·8	5·9
5.	6·8	5·8	5·6	5·2	5·8	5·8	5·8
6.	6·8	5·6	5·6	5·2	5·8	5·8	5·8
7.	6·8	5·9	5·5	5·2	5·7	5·8	6·0
8.	7·0	6·0	5·4	5·2	5·8	5·8	6·3
9.	6·8	5·8	5·4	5·2	5·6	5·9	5·8
10.	7·1	5·6	5·4	5·2	5·7	6·0	5·8
11.	7·0	5·8	5·6	5·2	5·8	5·7	5·9
12.	6·9	5·8	5·4	5·2	5·8	5·8	6·0
Range	6·8–7·2	5·6–6·0	5·4–5·6	5·2	5·6–5·9	5·5–6·0	5·8–6·3
Mid-point of range	7·0	5·8	5·5	5·2	5·75	5·75	6·05
Average	6·94	5·83	5·5	5·2	5·77	5·76	5·93

style. Clarke and Lubs' indicators were used for the estimations, drops of fluid, or fragments of tissue being mixed with the indicators on a white plate and the colours compared with those of the same indicators added to drops of standard buffer solutions ; the usual corrections for salt error were made. The pH in twelve healthy animals all with firm, well-developed styles was determined, the range, mid-point of range and

average of each set of values being given. The results agree with those recorded (Yonge (1925b)) for Pecten, Mya, and Ensis, the style, which had in all cases a pH of 5·2, being the most acid substance in the gut, while of the fluids that of the stomach with an average pH of 5·5 is the most acid, followed by that of the mid-gut, œsophagus, rectum, and mantle cavity in the order named. The tissue of the digestive diverticula had an average pH of 5·77. Similar results were obtained with oysters starved for twelve weeks, as shown in Table XVI, the figures

TABLE XVI.

pH IN STARVED OYSTERS.

No.	Mantle cavity.	Œso-phagus.	Stomach.	Style.	Dig. div.	Mid-gut.	Rectum.
1.	7·0	5·7	5·5	5·4	5·8	5·8	6·0
2.	7·0	5·8	5·6	5·4	5·7	5·9	6·1
3.	6·8	5·8	5·65	5·4	5·8	5·8	5·9
4.	7·0	5·7	5·7	5·4	5·7	5·8	5·8
5.	7·0	5·8	5·6	5·4	5·9	5·8	6·0
6.	7·0	6·0	5·9	—	5·9	5·9	6·0
Average	6·97	5·8	5·66	5·4	5·8	5·83	5·95

being slightly higher, i.e. conditions less acid, in all cases. The style was present in five out of the six oysters. The digestive diverticula were in all cases very pale, but the pH remained practically the same as in the fresh oysters.

It was shown in the paper cited above that the origin of the acidity of the gut lay in the style and *not*, as previously thought, in a secretion from the "liver." This provides, incidently, yet further evidence that the digestive diverticula do not secrete. By removing the style from Mya or inducing it to disappear from Mytilus by keeping animals out of water for four days or by placing them in boiled or deoxygenated water for six days, it was found that the pH of the stomach rose even though that of the mantle cavity fell on account of the accumulation of CO_2. Similar results were obtained with Crepidula kept out of water for two days, and with Tapes whose shell valves had been clamped together for seven days.

The last method has been found the most satisfactory, and in Table XVII are shown the results of a series of experiments with oysters which had been clamped for one, two, three, four, five, and six days. Twelve animals were used for each experiment, each, after clamping, being replaced in the tanks and so kept at normal temperature. In all case

the style was absent, and, as a result of the accumulation of CO_2, the average pH in the mantle cavity fell from the normal average value of 6·94 to 6·7, 6·56, 6·53, 6·51, 6·44, and, finally, 6·41 respectively after from one to six days clamping, while during the same periods the pH of the stomach rose from a normal of 5·5 to 5·67, 5·7, 5·84, 5·9, 6·02, and 6·14. Thus while the pH in the mantle cavity dropped by 0·53, the pH in the stomach

TABLE XVII.

pH IN CLAMPED OYSTERS.

No.	1	2	3	4	5	6	7	8	9	10	11	12	Average
Clamped 1 day.													
pH in mantle cavity .	6·7	6·6	6·6	6·8	6·8	6·8	6·7	6·6	6·6	6·7	6·7	6·8	6·7
pH in stomach .	5·7	5·7	5·6	5·7	5·6	5·7	5·6	5·7	5·8	5·6	5·7	5·7	5·67
Clamped 2 days.													
pH in mantle cavity .	6·6	6·7	6·6	6·6	6·5	6·6	6·6	6·4	6·4	6·6	6·6	6·5	6·56
pH in stomach .	5·6	5·7	5·8	5·7	5·6	5·7	5·8	5·6	5·8	5·7	5·7	5·6	5·7
Clamped 3 days.													
pH in mantle cavity .	6·6	6·4	6·5	6·6	6·5	6·4	6·6	6·5	6·6	6·7	6·5	6·5	6·53
pH in stomach .	6·0	5·8	5·9	5·8	5·8	5·8	6·0	5·8	5·6	5·9	5·9	5·8	5·84
Clamped 4 days.													
pH in mantle cavity .	6·6	6·4	6·6	6·5	6·4	6·6	6·5	6·6	6·6	6·5	6·4	6·4	6·51
pH in stomach .	6·1	5·8	5·9	5·8	5·7	6·0	5·8	6·0	5·9	6·0	5·9	5·9	5·9
Clamped 5 days.													
pH in mantle cavity .	6·5	6·3	6·6	6·5	6·3	6·6	6·3	6·4	6·4	6·5	6·4	6·5	6·44
pH in stomach .	6·1	5·8	6·0	5·9	6·0	6·1	6·0	6·0	6·0	6·0	6·1	6·2	6·02
Clamped 6 days.													
pH in mantle cavity .	6·5	6·4	6·6	6·5	6·7	6·3	6·3	6·4	6·4	6·1	6·5	6·2	6·41
pH in stomach .	6·3	6·2	6·4	6·1	6·2	6·2	6·1	6·1	6·0	6·0	6·1	6·0	6·14

rose, on account of the absence of the style, due to decrease in the rate of secretion, by 0·64, so that it came near to that of the mantle cavity. This experiment, together with those cited above, leaves no doubt that the acidity of the gut is due to the dissolution in it of the style. It is important to note that the pH thus produced in the stomach approximates to the optimum pH for the working of the amylase of the style (5·9).

II. PERMANENCE OF THE STYLE.

The view was advanced (1925b) and has recently been reasserted (1926a) that the style is dissolved by the fluid in the stomach, and is only maintained as a result of a balance between the rate of secretion and the rate of dissolution. The view that its presence is correlated with the presence of food has been disproved by the work of Orton (1923), Martin (1923), Berkeley (1923), and Yonge (1925b), all of whom showed that Lamellibranchs retain the style after long periods of starvation, provided

they are kept perfectly healthy, whereas in the presence of abundant food the style may be absent in unhealthy animals. As shown in Table XVI, five of the six oysters starved for twelve weeks retained the style.

The style is dissolved and reformed at very different rates in different animals. After artificial extraction of the style of Mya, Edmondson (1920) found that it took seventy-four days completely to regenerate (though this may have been due in part to the injury caused by the operation), while in *Ostrea virginica* Nelson (1925) states that it is alternately formed and dissolved in a rhythmical fashion. The style is large and firm at flood tide when the animals are feeding actively, but at late ebb tide when " most of the sand has been sorted out and removed from the stomach and digestion is well under way the style may be reduced to a soft amorphous mass of jelly." A similar rhythm is shown in the production of other forms of digestive secretion, such as that of the salivary glands of Gastropods (Hirsch (1914), Krijgsman (1925)).

In *Ostrea edulis* the style is usually present as shown in Table XVIII. Out of fifteen healthy animals examined, all of which had been in the

TABLE XVIII.

CONDITION OF STYLE AND DIGESTIVE DIVERTICULA IN FRESH OYSTERS.

No.	Condition of Style.	Condition of Dig. Div.
1.	Large, firm.	Pale.
2.	,, ,,	Dark.
3.	Absent.	,,
4.	Large, firm.	,,
5.	Medium, soft.	,,
6.	Large, firm.	,,
7.	,, ,,	,,
8.	,, ,,	Pale.
9.	,, ,,	Dark.
10.	,, ,,	,,
11.	Absent.	,,
12.	Large, firm.	,,
13.	,, ,,	Pale.
14.	,, ,,	Dark.
15.	,, ,,	,,

tanks for a week so as to provide time for recovery from the effects of the journey from the beds, in only two cases was the style absent, and this was not correlated with the colour of the digestive diverticula, paleness of which Orton (1923) thought might be connected with absence of the

style. All the animals appeared in good condition; in those obviously in bad condition, with flabby watery tissues, the style is frequently absent or much reduced.

The style invariably disappears when oysters are kept out of water for any length of time. Table XIX shows the results of a series of experiments to test the speed at which the style was dissolved, thirty-six healthy oysters being kept out of water and opened six at a time at one-hour

TABLE XIX.

CONDITIONS OF STYLE AFTER REMOVAL OF OYSTERS FROM WATER.

	Oysters out of water for :—		
	1 hour.	2 hours.	3 hours.
No.	Condition of style.	Condition of style.	Condition of style.
1.	Soft, $\frac{1}{2}$ size.	Firm, $\frac{3}{4}$ size.	Absent.
2.	Absent.	Soft, $\frac{1}{2}$ size.	,,
3.	Practically intact.	Absent.	Soft, $\frac{1}{4}$ size.
4.	Intact.	Soft, $\frac{1}{4}$ size.	Absent.
5.	,,	Practically intact.	,,
6.	Firm, $\frac{3}{4}$ size.	,, ,,	Practically intact.

	4 hours.	5 hours.	6 hours.
No.	Condition of Style.	Condition of Style.	Condition of Style.
1.	Absent.	Absent.	Absent.
2.	,,	,,	,,
3.	,,	,,	,,
4.	,,	,,	,,
5.	,,	,,	,,
6.	,,	,,	,,

intervals. After one hour in only one case was the style absent, and the same conditions were found after two hours, although the styles were much reduced. After three hours only two animals possessed styles, while after four, five, and six hours in no case was a style present.

It was found in the previous work on the subject (1925b) that styles were dissolved rapidly in alkaline or slightly acid media, but increasingly slowly as the pH was reduced until at a certain critical pH—probably corresponding to the isoelectric point of the globulin of the style—it ceased to be dissolved. This critical pH varied for the styles of different animals, being 4·4 for Ensis, 4·2 for Mya, and 3·6 for Pecten, Mytilus, and Crepidula. It was suggested that the differences might be due to the fact that in the former cases the style is lodged in a separate cæcum, and is a much firmer and more resistant body than in the other three in which it lies in free communication with the gut.

In Table XX are shown a similar series of experiments carried out on the styles of Ostrea, large, firm styles being placed in tubes containing 10 c.c. of standard buffer solutions, a little toluol being added to prevent decomposition. The styles were dissolved rapidly in pH between 10

TABLE XX.

DISSOLUTION OF STYLE IN DIFFERENT pH.

pH	Length of style.	Time to dissolve.	pH.	Length of style.	Time to dissolve.
10·0	2·5 cm.	56 min.	2·6	2·6 cm.	90 min.
9·0	2·5 cm.	70 min.	2·3	3·0 cm.	22 hrs.
8·0	2·5 cm.	70 min.	1·9	2·5 cm.	15 days.
7·0	2·6 cm.	61 min.	1·65	2·4 cm.	13 days.
6·0	2·4 cm.	75 min.	1·42	2·8 cm.	13 days.
5·0	2·5 cm.	87 min.	1·25	2·4 cm.	9 days.
4·0	2·3 cm.	90 min.	1·14	3·1 cm.	7 days.
3·0	2·8 cm.	88 min.	1·04	2·6 cm.	7 days.

and 2·6, more slowly at pH 2·3 and extremely slowly—it took fifteen days for a style 2·5 cm. long to be dissolved—in pH 1·9. Below this point dissolution was also very slow, though gradually increasing in speed down to pH 1·04, in which a style of 2·6 cm. took seven days to dissolve.

Unlike the other styles that of Ostrea is dissolved in all media, although the difference between the fifteen days needed for the process at pH 1·9 and the fifty-six minutes needed at pH 10 is very striking. Repeated experiments have confirmed these figures. The isoelectric point, 1·9, is much lower than the lowest, 3·6, recorded for the other molluscs examined, and as the style in Ostrea is exceptionally unstable, this gives additional evidence of the connection between the isoelectric point and the site of formation, and consequent firmness, of the style.

It is clear from the above experiments that the style must speedily be dissolved by the fluid in the stomach. It has been shown definitely that the presence of food is *not* necessary for the formation of the style, while Berkeley's theory that the style is a reserve of oxygen which is used in anærobic respiration cannot be substantiated in view of the fact that there is no correlation between the size of the style in different species and the nature of the habitat; a criticism which has also been made by Nelson (1925). Moreover, in such animals as Siliqua, Schizothærus, Macoma (Edmondson (1920)), and Mya (Edmondson, Yonge (1923)), in which the style lies in a *separate cæcum* and so is protected from the action of the fluid in the gut, the style never dissolves even after death from starvation or from lack of oxygen. The style is continually being

dissolved, and is only maintained by the continual secretion of new substance in the style-sac ; any lowering of the vital or metabolic activities of the animals is at once reflected in a reduction or stoppage in the rate of secretion, but the rate of dissolution remains constant and the style disappears. This is a purely physical reaction which cannot take place if the style is protected in a cæcum. The gradual reduction in secretion is indicated by the gradual rise in pH in the clamped animals. Absence of a style is, therefore, an indication of lowered metabolism, and it is noteworthy that Allen (1921) has found that the style is formed less readily in autumn and winter than in summer, and has proved by experiment that this is the direct result of the difference of temperature. Edmondson also states that the style of Mya is reformed more rapidly after excision in summer than in winter. Allen further notes that there is no rhythmical loss and renewal of the style in fresh-water Lamellibranchs as there is in marine, tidal species ; the result, clearly, of the equable conditions under which the former species live. Spärck (1925) considers " that absence of the crystalline style must be interpreted as an indication of something not quite normal, as regards the state of metabolism or nutrition." Adverse conditions of any kind will cause a lowering of metabolism, and this, in Lamellibranchs such as the oyster, will result in the partial or total dissolution of the style, the state of which presents a valuable index of the condition of the animal.

7. RESERVE FOOD MATERIALS.

It is fitting that some reference should be made to the nature and distribution of the reserve food materials. So much work has already been carried out on this subject (the most recent investigation is that by Russell (1923), which contains a summary and bibliography of previous work) that further research was considered unnecessary. As already noted, fat is stored in the oyster particularly in the vesicular connective tissue cells, or Langer's vesicles, and is also present in the epithelium of the gut and of the digestive diverticula. Traces were found in the connective tissue after three months starvation. Material fixed in Carnoy's fluid and treated with iodine shows the presence of masses of glycogen in the vesicular connective tissue, but never in the epithelium of the digestive diverticula or of the gut. These results are in complete accordance with those of previous workers both on the oyster and on other Lamellibranchs.

Quantitative estimations of the fat and glycogen in oysters made by the Government Chemist (Russell) and by previous workers show that the latter is much the more abundant (ranging between 21·34 and 40·04%, according to the analyses of the Government Chemist), while " fattening."

of oysters is to be attributed, as pointed out by Mitchell (1916a), " to the accumulation of glycogen, which must be regarded as the chief storage substance for oysters." The same author (1916) has further found that oysters kept in a weak solution of glucose show an increase in the amount of glycogen in the tissues. There is a seasonal variation in the quantity of glycogen, which in the oyster, according to the estimations of the Government Chemist, is constantly high from July to January, the total carbohydrate and glycogen approximating closely, showing that practically all the carbohydrate is in the form of glycogen. It is to be assumed, as Russell points out, that " during this period the oyster accumulates reserve food substance in the form of glycogen." From February to April there is a fall in the glycogen content, although the total amount of carbohydrate remains constant, the former being presumably " broken down into an assimilative form which is then, in May and June (when the total carbohydrates fall), utilised in the formation of the sexual products " (Russell). There can be no doubt as to the primary importance of glycogen in the physiology of the oyster and all Lamellibranchs in which it seems to play the same part as does fat in the vertebrates. This throwing of the balance of metabolism on to the carbohydrate side is in close accordance with the results recorded on the nature of the digestive processes.

8. GENERAL DISCUSSION.

In the oyster the organs of feeding and digestion are specialised for dealing with small particles *exclusively*. The elaborate ciliary mechanisms in the mantle cavity with the accompanying secretion of mucus ensure the capture of fine particles in suspension, of which the selective mechanisms reject the larger particles or mucus laden masses and allow only the smaller ones to pass to the mouth. There is a reduction in the individual size and general bulk of the particles swallowed, but no indication of any selection of particles having definite food value. In the gut, cilia and mucus glands are also universally distributed, ciliary activity, either directly or by the agency of the style, having taken the place of the muscular peristalysis necessary for the passage of large particles through the gut. The style is clearly correlated, here as elsewhere, with the presence of cilia, mucus glands, and a finely divided, and principally vegetable food.

The purely mechanical process of feeding is confirmed by the results of investigations into the stomach contents of oysters and other Lamellibranchs. Thus Savage (1925) states that " the oyster appears to ingest anything suitable that it can capture, and no evidence was found to show that selection takes place." The work of Savage and previous

investigators (quoted in detail by him) shows that in the stomach are found samples of all matter in suspension in the water in which the oysters live, and it is not surprising, therefore, to find that in the Limfjord, where there is a great development of Zostera and of detritus formed by its decomposition, the stomach contents of oysters should consist largely of detritus. This has led the Danish workers, notably Petersen (1911), Boysen Jensen (1914), Blegvad (1914), and Spärck (1925), to maintain that oysters are by nature detritus eaters. Recent American workers such as Nelson (1921), Churchill (1920), and Martin (1923) all consider that animate matter, and particularly diatoms, is of primary importance in the food of the oyster. Savage found that at Orford inanimate material provided the bulk of the stomach contents, animate matter never exceeding 10%. Hunt (1925), in his account of the stomach contents of Lamellibranchs, states that they consist of a mixture of micro-organisms and detritus. He is at variance with Blegvad in the latter's classification of Lamellibranchs as detritus feeders, adding, very aptly, that " When sand-grains are numerous in a stomach the proportion of detritus is correspondingly great, and the organisms present are largely bottom-living forms, but there is no reason to suppose that this preponderance of detritus signifies its value as food any more than the abundance of sand suggests the nutritive value of silica." Reviewing these results it is seen that the majority of workers have accepted the presence of material in the stomach of oysters or other Lamellibranchs as proof that it has been *deliberately swallowed and can be digested*. The Danish workers, in particular, do not appear to have studied either the mechanism of feeding or of digestion in Lamellibranchs.

Digestion is largely intracellular either in the tubules of the digestive diverticula or in the phagocytes. This is clearly correlated with the finely divided nature of the food, which is again sorted in the food cæcum in the stomach only the most minute particles entering the ducts and tubules of the digestive diverticula, the ramifications of the latter providing the large ingesting surface typical of the gut of animals which digest intracellularly. The larger particles in the gut are taken in directly by phagocytes. The only extracellular enzymes are those of the style which act exclusively on carbohydrates. This feeble development of extracellular digestion and particularly the complete absence of extracellular protease and lipase accounts for the passage of living organisms undamaged through the gut, their presence in the rectum and fæces having been noted by many authors, including Blegvad (1914), Coker, etc. (1921), Allen (1921), and Churchill and Lewis (1924). The two first of these, however, have drawn from the presence in the fæces of living diatoms, green algæ and other plankton organisms the *quite erroneous conclusion* that these are either useless or of secondary importance as

food. Attention to this error has also been drawn by Nelson (1925). Sherwood (statement in Savage's paper) and Nelson (1921) have both noted the presence of living oyster larvæ in the fæces of the adults.

An examination of the enzymes shows that oysters are unable to digest everything which enters the stomach, whereas the contrary has been too often assumed. Thus there is no indication of digestive action on cellulose or pentosans by the enzymes of either the style or the digestive diverticula. Boysen Jensen (1914) found that pentosans were the only non-nitrogenous substances present in estimable quantities in the detritus of the Limfjord, but the only evidence he could produce as to digestion was that pentosans are digested by herbivorous mammals and that cellulose is digested by Helix, finally stating that, " We may then perhaps conclude that also bivalves are able to digest pentosan, and that the considerable amount of pentosan present in the sea bottom—besides other possible substances (hemicelluloses generally) plays an important part as non-nitrogenous nourishment for a great portion of the bottom fauna." The known facts of the comparative physiology of digestion indicate that conditions in Mammals and Gastropods have *no bearing whatever* on conditions in Lamellibranchs, in which the digestive processes are particularly characteristic, and of which the only members capable of digesting cellulose (and there is no evidence as yet that they can digest pentosans) are the highly specialised wood-boring Teredinidæ.

Like all Lamellibranchs, oysters are particularly adapted for the digestion of carbohydrates. The only extracellular enzymes are those which digest starch and glycogen, while extracts of the digestive diverticula reveal the presence of powerful sucroclastic enzymes capable of digesting a variety of carbohydrates. On the other hand, lipoclastic and proteoclastic enzymes are very weak, and fats and proteins are probably digested largely in the phagocytes. In close connection with this concentration on the digestion of carbohydrates is the storage of great quantities of glycogen which represent the principal reserve food material. There is a close parallel to these conditions in Ciona (Yonge (1925)), in which digestion is also concentrated on carbohydrates, and there are large reserves of glycogen particularly in large cells in the epithelium of the mid-gut. It is clearly this dependence on carbohydrates which has enabled the Teredinidæ to live on a diet consisting almost exclusively of the carbohydrates in wood.

It follows that the food of the oyster must consist of small organisms rich in carbohydrates, i.e. of microscopic plant life. The following table taken from the paper of Brandt (1900) shows the relative amounts of protein, chitin, fat, and carbohydrates in the ash-free dried substance of diatoms, peridinians, and copepods.

	Diatoms %	Peridinians %	Copepods %
Protein	28·7	13·7	65·1
Chitin	—	—	5·1
Fat	8·0	1·37	7·7
Carbohydrate	63·2	84·9	22·1

There is a much greater proportion of carbohydrates in the two former. Russell considers that growth is due to an increase in protein and " fattening " to an accumulation of carbohydrates ; and the connection between " fattening " and the presence of large numbers of diatoms in the food has been noted by many workers, including Nelson (1921) and Savage. No doubt, in the spring, the abundance of algal spores provides ideal food, with their high carbohydrate content and delicate structure which renders them easy to assimilate. Martin (1923) has drawn attention to the importance of nannoplankton, especially small flagellates and peridinians, in the food of the oyster, and the structure and physiology of the digestive system supports this, since it is only organisms of this size which are ingested entire in the digestive diverticula. Only fragments of diatoms seem to be so ingested—whole diatoms are digested by the phagocytes—and it is only " detritus " of *this* nature, i.e. fragments of vegetable matter containing assimilative carbohydrates, which can be of use to the oyster.

It is clear from the results of this research that ideal conditions for " fattening," and incidently reproduction, in the oyster are found in the presence of abundant supplies of diatoms, peridinians, algal spores, and other microscopic vegetable matter. It is the quantity of *carbohydrate* which is important, the protein matter necessary for growth is probably always present in excess of the demands and powers of digestion of the oysters. Such conditions are provided artificially in the " claires " at Marennes and other places along the French coast. Immense numbers of diatoms and other microscopic organisms accumulate in them, and the speed with which the oysters " fatten " is proof positive of the fitness of the environment.

9. SUMMARY.

1. The anatomy and histology of the food collecting and alimentary organs of the adult oyster are described.

2. The anatomy of the stomach is investigated with the aid of gelatin casts and attention drawn to the food cæcum, the ventral groove, and the two ducts of the digestive diverticula.

3. Cilia and mucus glands are universal throughout the food collecting and alimentary organs.

4. There is evidence that the gastric shield is composed of fused cilia.

5. There is no evidence of secretion in the digestive diverticula.

6. The histology of the style-sac resembles that described by Mackintosh for Crepidula. There is evidence that secretion of the style takes place in the groove.

7. Phagocytes are everywhere numerous in the blood vessels, connective tissue and epithelia, and free in the gut and mantle cavity.

8. The alimentary organs of the larva are described.

9. The anatomy and histology of these organs in the "spat" is described, the palps are relatively large and the gills asymmetrical. The style-sac is distinct from the mid-gut.

10. The course of the ciliary currents on the gills and palps is described and the importance of the various selective mechanisms emphasized. Selection appears to be purely quantitative, large particles or mucus masses being rejected and smaller ones accepted.

11. Muscular activity is of great importance in the functioning of both gills and palps. Reversal of cilia has never been seen.

12. Rejected matter is removed from the mantle cavity.

13. Material is sorted in the food cæcum in the stomach, larger particles passing into the mid-gut and smaller ones towards the gastric shield and ducts of the digestive diverticula, within the tubules of which there is a constant circulation.

14. The rotation of the style assists in the stirring of matter in the stomach.

15. In the style-sac are cilia, which rotate the style and others which push it into the stomach.

16. In the larva the velum acts as a food collecting organ; the style lies in an extension of the stomach and rotates rapidly. Material passes freely into the digestive diverticula.

17. In the spat rejective mechanisms are highly developed. The style revolves at a speed of between sixty and seventy revolutions per minute.

18. The tubules of the digestive diverticula are the only place where soluble matter is absorbed, in adult, larvæ, or spat.

19. Fine particles are ingested and digested intracellularly in the tubules of the digestive diverticula, the products of digestion carried away by amœbocytes, and useless matter rejected into the lumen.

20. Larger particles are ingested and digested by phagocytes in all parts, the products of digestion being carried to the vesicular connective tissue cells and there stored.

21. Enzymes in the style digest starch and glycogen. The amylase, at pH 5·9, has an optimum temperature of 43° C., and is destroyed at

56° C. The optimum medium is pH 5·9. It is inactivated by purification with absolute alcohol or by dialysis, but action is restored on the addition of chlorides or bromides and to a less extent iodides, nitrates, and carbonates, but not with sulphates or fluorides.

22. Sucroclastic enzymes in the digestive diverticula act on starch, glycogen, sucrose, raffinose, maltose, lactose, salicin, and amygdalin, but not on inulin, cellulose, or pentosans.

23. The amylase, at pH 5·5, has an optimum temperature of 44·5° C., and is destroyed at between 64 and 67° C. It has an optimum pH of 5·5, and is inactivated after purification or dialysis, action being restored in the presence of chlorides or bromides.

24. There is a weak lipase and protease, the latter has two optima at pH 3·7 and at or above 9·0 ; its action is very slow.

25. The only enzymes free in the stomach are those from the style.

26. There is no evidence of any enzymes free in the gill mucus.

27. There is a powerful complete oxidase system in the style, and a catalase in the digestive diverticula and gonad, and traces in the palps, gills, and muscle.

28. The style is the most acid substance in the gut and the cause of the acidity of the gut.

29. The style is dissolved rapidly in fluid of pH 2·3 and above, but very slowly below that point. It is readily dissolved and reformed in the oyster, its presence depending on the maintenance of the balance between the rate of secretion and the rate of dissolution. Its condition is a valuable indication of the state of metabolism.

30. Glycogen and fat are stored, particularly in the vesicular connective tissue cells, the former furnishing the principal reserve food material.

31. The presence of abundant supplies of microscopic plant life rich in carbohydrates provides ideal food for the oyster, and represents optimum conditions for " fattening " and reproduction.

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The Vertical Distribution of Marine Macroplankton. III. Diurnal Observations on the Pelagic Young of Teleostean Fishes in the Plymouth Area.

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With 8 Figures in the Text.

THIS paper records the results of two serial collections undertaken to obtain information on the behaviour of the post-larval stages of teleostean fish during the hours of darkness.

The net used was a stramin ring-trawl (2 metres diameter at mouth and 6 metres long). The first collections were taken on July 15th to 16th, 1924, at a period of full moon, at L4 ; on this occasion the hauls were so arranged that five series of collections were obtained between 3 p.m. on July 15th and 11 a.m. on July 16th ; in each series samples were taken from five different depths. The second batch of samples was obtained on June 17th, 18th, and 19th, between L4 and the Eddystone. Samples were taken from six different depths in each series, and the experiment was carried through two successive nights. There was no moon.

The material was treated as described in a previous paper (**6**), all the young fish being picked out and then the remaining plankton organisms counted directly or by sampling. The results shown by the plankton organisms on July 15th to 16th, 1924, have already been published (**6**). It is unfortunate that on both occasions the numbers of post-larvæ of the more important food fish captured were very low. This was perhaps to be expected in 1924, as mid-July is somewhat late in the season to find the very young stages still in the plankton. On this account the collections in 1925 were made in the middle of June, at which time of the year in 1924 the young fish were very abundant. In 1925, however, for some unknown reason the post-larvæ of many species apparently disappeared from the plankton a month earlier than in 1924 (**7**), and the results of the day and night collecting were most disappointing, the number of fish taken being considerably less even than in the series of collections taken on July 15th to 16th, 1924.

Table 1 gives the complete log taken on June 17th–19th, 1925 ; all times are Greenwich mean. (The log for July 15th to 16th, 1924, is given in a previous paper, **6**.)

TABLE 1.

Date : June 17th–18th–19th, 1925. Position : between L4 and Eddystone. Ship : s.s. *Salpa*.
Gear : 2-metre ring-trawl

	Depth.	Time net entered water.	Fishing time.	Time net left water.	Length of warp out.	Remarks.
1st Series	VI	2.33 p.m.	2.34½–2.45½ p.m.	2.49 p.m.	60 fathoms	
Brilliant sunshine ; sea	V	2.57 ,,	2.58–3.8 ,,	3.10½ ,,	45 ,,	
calm	IV	3.20 ,,	3.21–3.31 ,,	3.33 ,,	35 ,,	
	III	3.39½ ,,	3.40–3.50 ,,	3.51 ,,	20 ,,	
	II	—	4–4.10 ,,	—	10 ,,	
	Surface	—	4.18½–4.28½ ,,	—	—	
2nd Series	VI	7.25½ p.m.	7.27–7.37 p.m.	7.41 p.m.	60 fathoms	
Sunset 8.30 p.m. ;	V	7.49 ,,	7.50–8.0 ,,	8.3½ ,,	45 ,,	
cloudless sky ; wind	IV	8.12 ,,	8.13–8.23 ,,	8.25 ,,	35 ,,	
freshening from	III	8.32 ,,	8.32½–8.42½ ,,	8.44 ,,	20 ,,	
north ; sea slightly	II	—	8.53–9.4½ ,,	—	10 ,,	
loppy	Surface	—	9.13–9.23 ,,	—	—	Becoming difficult to read and see ; deck-lights on at 9.30 p.m.
3rd Series	VI	10.37 p.m.	10.39–10.49 p.m.	10.53 p.m.	60 fathoms	Net struck bottom. Dark.
No moon ; sea calm ;	V	11.10 ,,	11.11–11.21 ,,	11.23½ ,,	45 ,,	Dark.
wind freshening	IV	11.33 ,,	11.34–11.44 ,,	11.46 ,,	35 ,,	,,
from North as series	III	11.54½ ,,	11.55 p.m.–12.5 a.m.	12.6 a.m.	20 ,,	,,
progressed	II	—	12.15–12.25 ,,	—	10 ,,	When hauling 10 fathoms of wire
	Surface	—	12.36–12.46 ,,	—	—	were first run out by accident.
4th Series	VI	2.35 a.m.	2.36–2.46 a.m.	2.48½ a.m.	60 fathoms	Towing rather fast.
Sun rose 4.27 a.m. ;	V	2.57 ,,	2.58–3.8 ,,	3.10 ,,	45 ,,	
cloudless ; fresh	IV	3.18½ ,,	3.19–3.29 ,,	3.30 ,,	35 ,,	Getting light.
wind and slight lop	III	—	3.40–3.50 ,,	—	20 ,,	Deck-lights put out.
	II	—	3.59–4.10 ,,	—	10 ,,	Light enough to read.
	Surface	—	4.19–4.29 ,,	—	—	

5th Series	VI	7.22½ a.m.	7.24½–7.34 a.m.	7.36½ a.m.	60 fathoms	
Bright sunshine ; cloud-	V	7.44½ ,,	7.45½–7.55½ ,,	7.57½ ,,	45 ,,	
less sky ; fresh north	IV	8.6 ,,	8.6½–8.16½ ,,	8.17½ ,,	35 ,,	Clouding over ; thin flakes.
wind	III	8.26 ,,	8.26½–8.36½ ,,	8.37 ,,	20 ,,	Clouds dispersing.
	II	—	8.45–8.55 ,,	—	10 ,,	Sun shining all time.
	Surface	—	9.2½–9.14 ,,	—	—	
6th Series	VI	7.29 p.m.	7.30–7.40 p.m.	7.43 p.m.	60 fathoms	
Bright sunshine ; cloud-	V	7.52 ,,	7.53–8.3 ,,	8.4½ ,,	45 ,,	
less ; sea calm ; sun-	IV	8.11 ,,	8.11½–8.23 ,,	8.24 ,,	35 ,,	
set 8.35 p.m. ; fresh	III	8.30½ ,,	8.31–8.41 ,,	8.42 ,,	20 ,,	
north wind ; it had	II	—	8.49–8.59 ,,	—	10 ,,	Getting hard to see.
been very fine and	Surface	—	9.6½–9.16½ ,,	—	—	Deck-lights on.
sunny all day						
7th Series	VI	10.31 p.m.	10.33–10.43 p.m.	10.46 p.m.	60 fathoms	Dark.
No moon ; cloudless ;	V	10.55 ,,	10.55½–11.5½ ,,	11.7 ,,	45 ,,	,,
fresh north wind ;	IV	11.15 ,,	11.16–11.26 ,,	11.27 ,,	35 ,,	,,
sea loppy	III	—	11.36–11.46 ,,	—	20 ,,	,,
	II	—	11.55 p.m.–12.5 a.m.	—	10 ,,	,,
	Surface	—	12.13–12.23 ,,	—	—	,,
8th Series	VI	2.27 a.m.	2.28½–2.38½ a.m.	2.41 a.m.	60 fathoms	
Sun rose 4.23 a.m. ;	V	2.48 ,,	2.49–2.59 ,,	3.1½ ,,	45 ,,	
cloudless	IV	3.8 ,,	3.9–3.19 ,,	3.20½ ,,	35 ,,	Getting light.
	III	3.28 ,,	3.28½–3.38½ ,,	3.39 ,,	20 ,,	
	II	—	3.49–3.59	—	10 ,,	Deck-lights put out.
	Surface	—	4.6½–4.16½ ,,	—	—	
9th Series	VI	7.32½ a.m.	7.35–7.45 a.m.	7.47½ a.m.	60 fathoms	
Bright sunshine ;	V	7.55 ,,	7.56–8.6 ,,	8.7½ ,,	45 ,,	
cloudless ; sea calm	IV	8.15 ,,	8.15½–8.25½ ,,	8.27 ,,	35 ,,	
	III	8.34½ ,,	8.35–8.45 ,,	8.45½ ,,	20 ,,	
	II	—	8.53½–9.3½ ,,	—	10 ,,	
	Surface	—	9.11½–9.21½ ,,	—	—	

The Fishing Depths.

On both occasions the depth-recorder, kindly lent by Admiralty Authorities, was in use and tracings were obtained of the actual path of the net through the water for each haul; the tracings for the 1924 collections have been reproduced in the previous paper (**6**).

In 1924 the results were very satisfactory in that they showed that for most hauls the net fished steadily at the required depths, and did not follow an irregular course up and down through the water; also the net fished at approximately the same five depths in each series; it is, however, regrettable that no samples were obtained from the water layers between 34 metres and the bottom, at 51 m. In 1925 the depth records showed very unfavourable fishing results compared with the above. The first two series were satisfactory, but at midnight on the first night a misfortune occurred; in our efforts to get the net into the deeper layers it struck the bottom. Thereafter the results obtained by the recorder reflected the uneasiness felt by the collector, and show that the speed of the ship when towing had been increased slightly in order to avoid the possibility of a re-occurrence of the accident. As a result, as in 1924, there are no samples in the remaining six series from the water layers between 32 metres and the bottom. This is unfortunate, as we shall see when we come to consider the vertical distribution of the various species of young fish captured.

A fresh attempt to repeat the experiment will be made, this time, if possible, in May, to ensure a good supply of fish; efforts also will be made to obtain more samples from the deeper layers. This is necessarily somewhat risky; the use of the depth-recorder has shown that it is a common occurrence for the net to traverse a vertical distance of 10 or more metres in its path through the water, and that hence in the deeper water layers the possibilities of the net striking bottom at some particular instant during the haul are great; the large amount of sand brought up by the net on such an occasion cannot but be detrimental to the material of which the net is made.

The tracings obtained in June, 1925, are reproduced in Figs. 1 and 2. It can be seen that in the 3rd and 4th series the clock of the recording drum ran down after a very short time, so that in hauls III and IV of the 3rd series and II and III of the 4th series the maximum depth only was recorded. This was rectified when it became light enough to examine the clock, and it was found that the error was due to the slipping of the winding key which had become worn; for the remainder of the collections the clock was wound by means of a pair of pliers.

The average depths have been estimated as previously described in another paper (**6**), by taking the depth at ten equidistant points along

the curve and calculating the mean. The sudden increase in depth of the net at the end of haul II of the third series was due to the several fathoms of warp being accidentally allowed to run off the winch drum immediately before hauling in.

Table 2 gives the average depth for each haul.

TABLE 2.

AVERAGE DEPTH IN METRES FOR EACH HAUL ON JUNE 17TH–18TH–19TH, 1925.

	1st Series.	2nd Series.	3rd Series.	4th Series.	5th Series.	6th Series.	7th Series.	8th Series.	9th Series.
Surface	—	—	—	—	—	—	—	—	—
II	4·5	5·4	11·2	6*	3·2	3·2	5·2	3·6	4
III	16·2	14	23*	13*	7·5	9·3	12·2	8	11·1
IV	23·4	24·8	31*	18	9·9	14	16·6	13·9	12·7
V	29·8	31·3	29	26·8	20·8	19·4	20·7	23·7	23·3
VI	48·8	38·6	49	30	31·4	30·9	25·8	27·5	28·5

GENERAL RESULTS.

In Tables 7 and 8 (pp. 397–399) are given the actual numbers of each species taken in the collections. In most cases reference is made to these two tables when discussing the results for each species in detail.

This work must be regarded merely as a record of the behaviour of the various species of post-larval fish on the three nights in question, one night of which was at the time of full moon while the other two were moonless.

On all occasions the majority of species showed only a very slight movement at night. There were, however, one or two exceptions, notably the post-larvæ of *Sardina pilchardus*, which became much more abundant at night, curiously enough at all depths. The reasons for this increase in numbers shown by the post-larval pilchard are, at the moment, not quite clear ; further observations are needed. There was also evidence that certain species, e.g. *Callionymus lyra*, showed a behaviour on the moonlight night different from that on the dark nights with no moon. On the moonlight night Callionymus showed no signs of upward movement, whereas on the two dark nights their distribution stretched upwards to the surface.

As regards the night of July 15th to 16th, 1924, a study of Table 7 conveys the impression that at night there was only a slight filling up of the surface layers, in which the post-larvæ were comparatively scarce

* Maximum depth only.

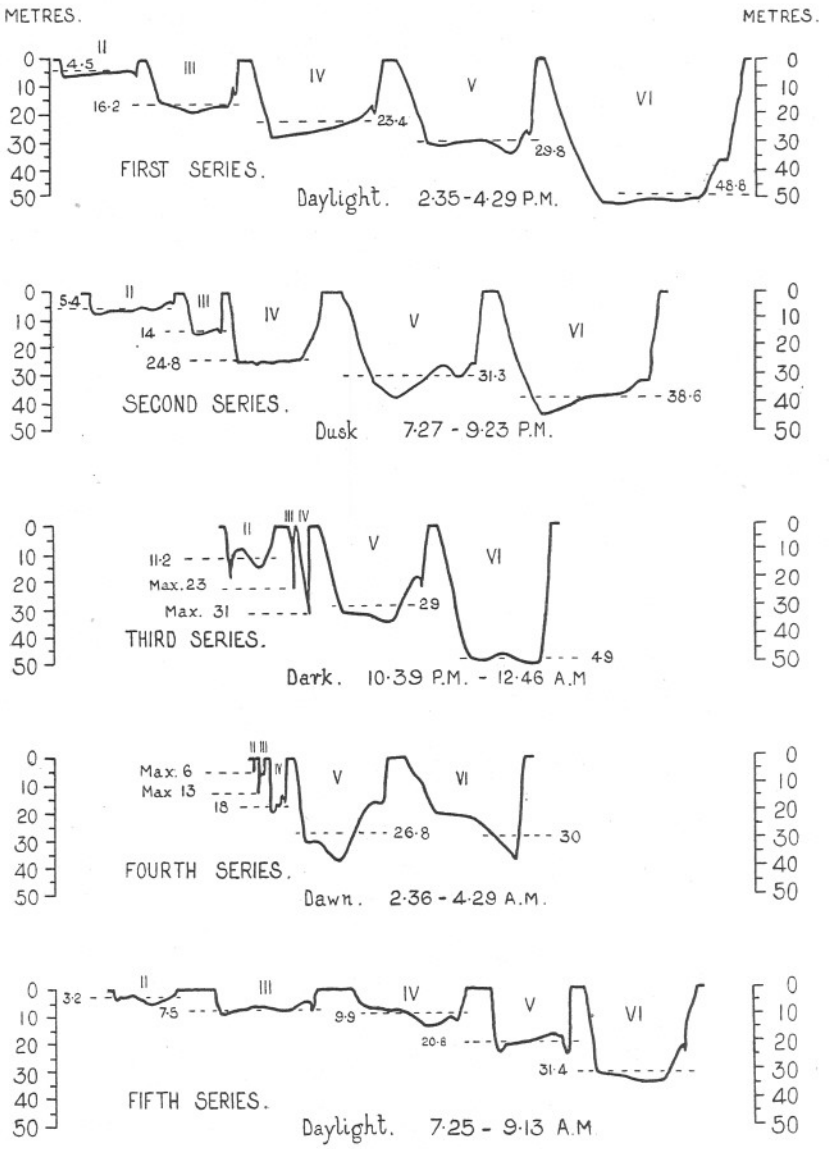

FIG. 1.—The first five series of curves given by the depth recorder indicating the path of the net through the water at five depths during the collections of June 17th–18th–19th, 1925. (The surface haul is not included.) The net enters the water on the right-hand side of each curve. The dotted lines indicate the calculated "average depths."

in the daytime, with no consequent marked diminution of numbers of fish in the deeper layers.

Table 3 gives the total numbers of young fish taken in each haul without those of *Sardina pilchardus* and *Gobius elongatus*, the post-larvæ of which species were so numerous that they would distort the true picture of events. Table 4 gives the numbers of different species of fish post-larvæ taken in each haul.

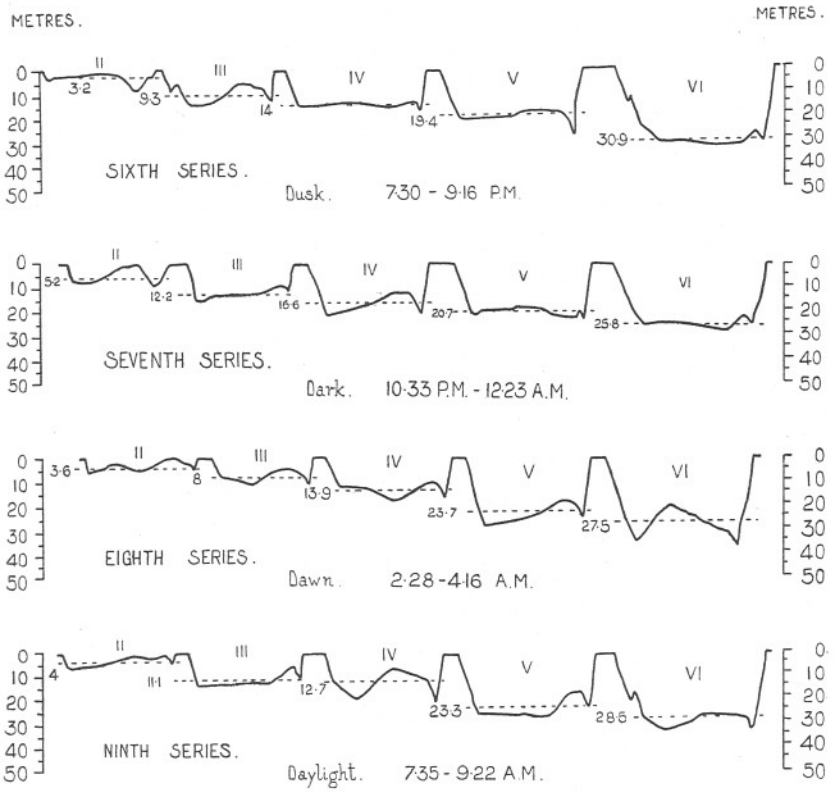

FIG. 2.—The last four series of curves given by the depth recorder indicating the path of the net through the water at five depths during the collections of June 17th–18th–19th, 1925. (The surface haul is not included.) The net enters the water on the right-hand side of each curve. The dotted lines indicate the calculated "average depths."

It will be seen from Table 4 that there were definitely more species of young fish present at the surface at night than at any other time; this agrees well with the findings of Johansen in Danish waters (**4**, p. 6). In the lower layers from about 9 metres downwards there would not appear to be much change, an obvious increase at night in the deepest layer (V), being somewhat compensated by a decrease in the layers above (IV).

TABLE 3.

TOTAL NUMBERS OF POST-LARVÆ CAUGHT IN EACH HAUL ON JULY 15TH–16TH, 1924, EXCLUSIVE OF THOSE OF SARDINA PILCHARDUS AND GOBIUS ELONGATUS.

	1st Series. 3.25 p.m. to 4.45 p.m.	2nd Series. 7.55 p.m. to 9.17 p.m.	3rd Series. 10.50 p.m. to 12.20 a.m.	4th Series. 2.50 a.m. to 4.16 a.m.	5th Series. 8.45 a.m. to 10.11 a.m.
Surface	9	8	52	14	14
II, 2–7 m.	44	46	61	43	30
III, 9–15 m.	87	48	61	121	82
IV, 16–20 m.	286	172	61	77	172
V, 30–34 m.	56	211	149	153	106
Total	482	485	384	408	404

TABLE 4.

TOTAL NUMBERS OF DIFFERENT SPECIES OF FISH POST-LARVÆ OCCURRING IN THE COLLECTIONS MADE ON JULY 15TH–16TH, 1924.

	1st Series. 3.25 p.m. to 4.45 p.m.	2nd Series. 7.55 p.m. to 9.17 p.m.	3rd Series. 10.50 p.m. to 12.20 a.m.	4th Series. 2.50 a.m. to 4.16 a.m.	5th Series. 8.45 a.m. to 10.11 a.m.
Surface	7	6	16	3	7
II, 2–7 m.	14	15	16	11	9
III, 9–15 m.	17	14	18	16	19
IV, 16–20 m.	22	22	15	16	18
V, 30–34 m.	11	15	20	17	14

TABLE 5.

TOTAL NUMBERS OF POST-LARVÆ CAUGHT IN EACH HAUL ON JUNE 17TH–18TH–19TH, 1925, EXCLUSIVE OF THOSE OF SARDINA PILCHARDUS, GOBIUS ELONGATUS, AND CALLIONYMUS LYRA.

			Dark.				Dark.		
Series	1	2	3	4	5	6	7	8	9
Surface	1	3	19	5	–	3	18	3	2
II	17	14	34	8	3	2	16	5	2
III	10	13	21	6	7	4	12	6	6
IV	22	13	17	19	5	15	25	20	13
V	70	38	15	15	5	23	64	20	26
VI	42	18		14	6	56	31	25	28

TABLE 6.

TOTAL NUMBERS OF DIFFERENT SPECIES OF FISH POST-LARVÆ OCCURRING IN THE COLLECTIONS MADE ON JUNE 17TH–18TH–19TH, 1925.

			Dark.				Dark.		
Series	1	2	3	4	5	6	7	8	9
Surface	3	4	11	5	0	2	8	1	1
II	3	9	16	5	2	4	9	6	3
III	7	9	12	6	6	5	12	7	6
IV	7	8	11	12	5	11	13	10	5
V	10	14	12	11	6	8	18	12	12
VI	11	13		13	5	12	17	12	11

In Tables 5 and 6 the results obtained on the nights, June 17th–18th–19th, 1925, are dealt with in a similar manner, except that in Table 5, which gives the total numbers of post-larvæ taken in each haul, the post-larval *Callionymus lyra* have been excluded as well as *Sardina pilchardus* and *Gobius elongatus*.

Table 5 shows that on this occasion the results were somewhat similar to those obtained on July 15th–16th, 1924 ; the numbers are very small, but they show, if anything, perhaps that the upward movement was more marked on this occasion. Table 6 shows that there was a general increase in the number of species at all depths at night except at 29 metres at 11.11 p.m. on the first night.

In the following pages the vertical distribution of each species will be discussed in detail.

CLUPEIDÆ.

SARDINA PILCHARDUS (Walb.).

If we examine the percentage distribution of *Sardina pilchardus* post-larvæ (Table 9) on July 15th–16th, 1924, we see that while tending to be most abundant between 10 and 20 metres in the daytime the young fish filled up the surface layers at dusk and midnight, and further, that at midnight more were taken actually at the surface than at any other depth. At dawn they left the surface, and the greatest number were taken at a depth of about 9 metres ; in full daylight they had resumed almost exactly the same distribution as on the previous afternoon.

In addition to this evidence of change in the vertical distribution of the post-larvæ there is a very marked increase in the numbers caught

at midnight and even more so at dawn. In fact, it would appear that we were at the time passing through a large shoal, and that at about 3 a.m. we encountered the densest region. This increase in numbers is illustrated in Fig. 3, which shows diagrammatically the actual number of individuals caught at the different depths in each series of hauls.

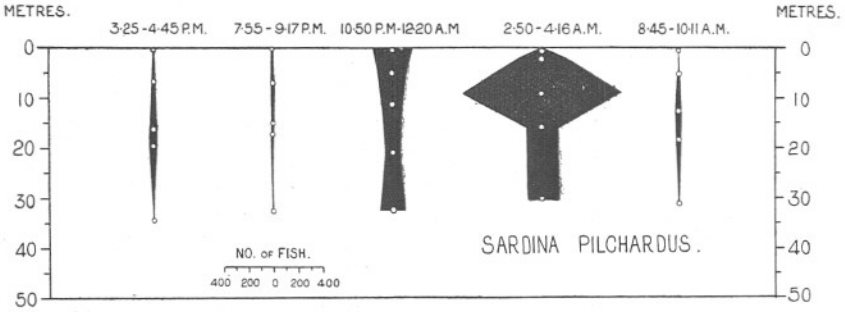

FIG. 3.—The vertical distribution of *Sardina pilchardus* post-larvæ, at the times shown, on July 15th–16th, 1924, showing the large increase in numbers at all depths at midnight and dawn. The white spots and black circles indicate the " average depths " at which hauls were taken.

In Table 10 I give the number of post-larvæ of this species taken at the different depths in June, 1925.* Here again we see a tendency to extend into the upper layer at night, and again also we note an increase in the total numbers taken at night ; although the figures are not so great as in 1924 this increase in numbers is very noticeable in the diagrammatic distribution shown in Fig. 4. On this occasion it is the night series only that show the largest catches ; at dawn, between 2 and 4 a.m., there is an

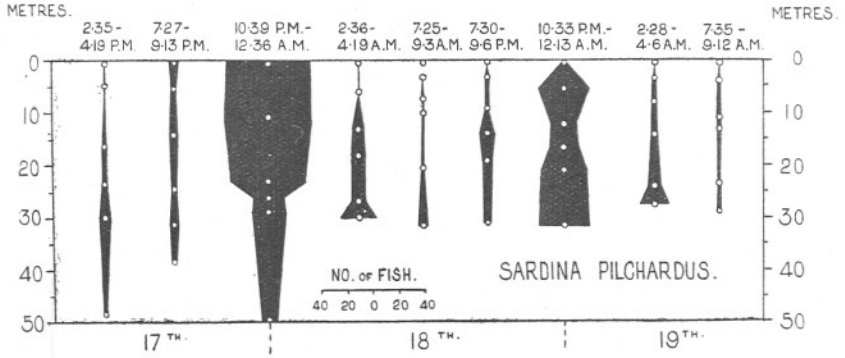

FIG. 4.—The vertical distribution of *Sardina pilchardus* post-larvæ, at the times shown, on June 17th–18th–19th, 1925, showing the large increase in numbers at all depths on the two nights. The white spots and black circles indicate the " average depths " at which hauls were taken.

* All the post-larvæ caught on this occasion were less than 20 mm. in length.

TABLE 7.

YOUNG FISH CAUGHT, JULY 15TH–16TH, 1924. FULL MOON.

	Time.	*Depth in metres.*	Sardina pilchardus.	Gadus merlangus.	G. minutus.	G. luscus.	Onos sp.	Raniceps raninus.	Capros aper.	Arnoglossus sp.	Rhombus maximus.	Scophthalmus norvegicus.	Pleuronectes microcephalus.	Solea variegata.	Ammodytes lanceolatus.	Caranx trachurus.	Callionymus sp.	Labrus bergylta.	L. mixtus.	Ctenolabrus rupestris.	Crenilabrus melops.	Centrolabrus exoletus.	Trachinus vipera.	Scomber scomber.	Gobius elongatus.	Lebetus scorpioides.	Blennius pholis.	B. ocellaris.	B. gattorugine.	Trigla sp.	Lepadogaster gouani.	L. bimaculatus.	Liparis montagui.	Lophius piscatorius.	*Total Young Fish.*
DAYLIGHT	4.34 p.m.	*S.*	11	–	–	–	–	–	–	–	1	–	–	–	2	2	–	–	1	–	–	3	1	–	–	–	–	–	–	–	–	–	**21**
July 15th	4.18 „	*6·6*	33	–	–	–	1	–	–	–	2	1	–	–	5	3	–	1	7	1	–	16	9	–	–	–	1	3	–	–	1	–	–	**86**
	4.1 „	*13·6*	70	–	–	–	–	–	1	–	1	10	–	2	22	12	14	–	2	3	–	10	2	68	–	–	1	4	2	–	4	–	1	**225**
	3.44 „	*19·5*	70	–	–	–	1	1	–	1	–	37	7	4	36	8	62	–	4	2	1	1	–	3	106	1	1	–	5	6	–	4	–	1	**362**
	3.25 „	*34·5*	8	–	–	–	–	–	–	–	–	1	1	3	4	–	42	–	–	–	1	–	–	–	62	1	–	–	1	–	–	2	–	–	**126**
DUSK	9.7 p.m.	*S.*	15	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	1	–	–	1	15	–	–	–	1	–	–	–	–	–	**38**
	8.49 „	*7*	34	1	–	–	–	–	–	–	–	8	–	5	1	3	7	–	–	–	11	3	1	2	89	–	–	–	4	1	–	1	–	–	**171**
	8.32 „	*15·1*	19	–	–	–	–	–	–	–	–	3	–	3	4	–	12	–	–	2	4	1	1	11	154	–	1	–	1	5	–	–	–	–	**221**
	8.15 „	*17·1*	30	–	–	–	–	1	1	1	–	25	1	16	15	1	47	1	2	7	9	8	3	21	171	–	–	–	3	7	–	–	1	2	**373**
	7.56 „	*32·3*	9	–	1	–	2	–	–	–	–	13	8	12	57	–	101	–	–	4	2	1	–	2	118	–	–	–	–	5	–	3	–	–	**338**
DARK	12.9 a.m.	*S.*	332	–	–	–	1	–	–	11	1	1	2	–	5	4	3	–	–	4	–	2	–	4	23	1	–	1	12	–	–	–	–	–	**407**
	11.52 p.m.	*5·8*	215	–	–	–	1	–	–	2	–	2	1	–	5	10	1	–	–	4	1	10	–	10	52	1	–	–	11	2	–	–	–	–	**328**
	11.34 „	*11·2*	171	–	–	–	–	–	1	1	–	5	5	1	24	5	6	–	–	1	1	1	–	4	87	–	–	1	2	2	–	1	–	–	**319**
	11.16 „	*20·7*	111	–	–	–	–	–	–	1	–	4	3	1	18	3	12	–	–	1	–	5	–	4	95	–	–	–	5	3	–	–	–	1	**267**
	10.52 „	*32·2*	193	–	1	–	–	–	–	2	–	9	5	4	24	4	71	–	–	3	1	4	2	2	210	2	–	–	5	5	–	4	1	–	**552**
DAWN	4.6 a.m.	*S.*	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	–	–	–	2	–	–	–	–	–	**22**
	3.51 „	*1·9*	384	–	–	–	–	–	–	4	–	2	–	–	–	7	–	–	1	17	–	1	–	8	26	–	1	–	–	2	–	–	–	–	**453**
	3.34 „	*9*	1276	–	–	–	–	–	–	9	–	2	1	–	17	30	2	–	1	8	4	17	1	14	237	–	–	–	13	2	–	–	–	–	**1634**
	3.14 „	*16*	260	–	–	–	–	–	–	3	–	4	1	2	26	5	8	–	1	–	–	7	2	10	213	–	–	1	2	5	–	–	–	–	**550**
	2.52 „	*30·1*	272	–	–	1	–	–	–	2	–	12	12	6	49	6	44	–	–	1	–	3	–	1	187	1	–	–	4	7	–	4	–	–	**612**
DAYLIGHT	10 a.m.	*S.*	4	–	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	1	1	–	–	1	2	–	–	–	6	–	–	–	–	–	**18**
July 16th	9.43 „	*5·3*	20	–	–	–	–	–	–	–	–	–	–	–	5	–	2	–	–	2	7	1	–	9	10	–	–	–	4	–	–	–	–	–	**60**
	9.26 „	*12·5*	45	–	–	–	–	–	–	5	–	2	–	3	15	5	19	–	4	2	10	5	3	2	103	–	1	1	–	2	1	–	–	2	**230**
	9.8 „	*18·3*	41	–	–	–	1	–	–	3	–	14	3	12	47	–	59	–	–	3	10	5	–	3	236	2	–	–	4	4	–	1	–	1	**449**
	8.46 „	*31·1*	8	–	–	–	–	–	–	1	–	8	3	2	24	–	50	–	–	3	2	4	–	–	105	–	–	–	2	2	–	5	–	–	**219**

TABLE 8.

YOUNG FISH CAUGHT, JUNE 17TH–18TH–19TH, 1926. NO MOON.

	Time.	*Depth in metres.*	Sardina pilchardus.	Gadus merlangus.	G. minutus.	G. luscus.	Onos sp.	Merluccius merluccius.	Arnoglossus sp.	Rhombus sp.	Scophthalmus norvegicus.	Zeugopterus punctatus.	Z. unimaculatus.	Pleuronectes limanda.	P. microcephalus.	Solea vulgaris.	S. variegata.	Ammodytes tobianus.	A. lanceolatus.	Callionymus sp.	Labrus bergylta.	L. mixtus.	Crenilabrus melops.	Ctenolabrus rupestris.	Centrolabrus exoletus.	Scomber scomber.	Gobius elongatus.	Lebetus scorpioides.	Blennius pholis.	B. gattorugine.	Trigla sp.	Agonus cataphractus.	Lepadogaster bimaculatus.	*Total Young Fish.*
DAYLIGHT	4.19 p.m.	*S.*	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	**3**
June 17th	4.0 „	*4·5*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	16	–	–	1	–	–	–	–	**18**
	3.40 „	*16·2*	4	3	–	–	–	–	–	–	–	–	–	–	–	–	1	–	6	–	–	–	–	1	5	1	–	–	–	–	–	–	**21**
	3.21 „	*23·4*	4	7	–	–	–	–	–	–	4	–	–	–	–	–	7	–	40	–	–	–	–	–	3	–	–	–	–	–	–	–	**66**
	2.58 „	*29·8*	10	44	–	–	–	–	–	–	3	–	–	–	2	–	5	–	45	–	–	–	1	–	7	–	–	–	–	3	–	–	**125**
	2.35 „	*48·8*	3	33	–	1	–	–	–	–	1	–	–	1	–	–	1	–	30	1	–	–	–	–	1	–	–	–	–	–	–	2	**75**
DUSK	9.13 p.m.	*S.*	8	2	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	**12**
	8.53 „	*5·4*	4	5	–	–	–	–	1	–	1	–	–	–	2	–	–	–	10	–	–	–	–	–	3	–	–	1	–	1	–	–	**28**
	8.32 „	*14*	7	2	–	–	–	–	–	–	1	–	–	–	2	–	–	–	39	–	–	–	–	1	6	1	–	–	–	–	–	–	**60**
	8.13 „	*24·8*	5	4	1	–	1	–	–	–	–	–	–	2	–	–	–	–	85	–	–	–	–	–	4	–	–	–	–	1	–	–	**103**
	7.50 „	*31·3*	7	10	6	–	1	–	–	–	4	–	–	1	1	–	8	–	352	–	1	–	–	–	4	4	–	1	–	–	–	–	**401**
	7.27 „	*38·6*	4	5	2		1	–	–	–	–	–	–	1	2	–	1	–	248	–	–	–	–	–	3	4	–	1	–	1	–	–	**274**
DARK	12.36 a.m.	*S.*	66	1	–	–	–	–	1	–	1	1	–	–	–	–	–	–	38	–	–	–	–	–	2	21	–	1	1	1	–	–	**134**
	12.15 „	*11·2*	67	1	2	–	–	–	–	–	5	–	–	2	3	–	5	–	47	1	–	–	1	–	4	29	–	–	1	2	–	1	**177**
	11.55 p.m.	*23*	58	–	1	–	–	–	–	–	1	–	–	2	4	1	–	–	52	–	–	–	–	–	5	17	–	–	1	2	–	–	**148**
	11.34 „	*26**	24	1	1	–	–	–	–	–	2	1	–	–	5	–	4	1	59	–	–	–	–	–	2	7	–	–	–	–	–	–	**107**
	11.11 „	*29*	29	2	3	–	–	–	–	–	–	1	–	–	1	–	2	1	145	–	–	–	–	–	1	22	–	–	–	1	–	1	**209**
	†10.39 „	*49*	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	**–**

Dawn	4.19 a.m.	*S.*	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	1	–	–	–	7
	3.59 „	*6*	1	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	1	–	–	10
	3.40 „	*13*	9	1	–	–	1	–	–	–	–	–	–	–	–	1	–	–	–	5	–	–	–	–	–	3	–	–	–	–	–	–	–	20
	3.19 „	*18*	11	5	–	–	1	–	–	1	–	–	–	1	–	1	–	–	–	17	–	–	–	1	–	6	9	–	2	1	–	–	–	56
	2.58 „	*26·8*	12	3	–	–	–	–	–	–	2	–	–	1	1	–	–	–	1	71	–	–	–	–	–	4	8	–	–	–	2	–	1	106
	2.36 „	*30*	28	–	1	–	–	–	–	–	1	1	–	1	–	–	3	–	2	74	–	–	–	–	1	–	2	4	–	–	1	1	–	100
Daylight	9.3 a.m.	*S.*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8.45 „	*3·2*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	2	–	–	–	–	–	–	–	3
June 18th	8.27 „	*7·5*	4	1	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	2	–	–	–	1	–	4	–	–	–	–	–	–	–	13
	8.6 „	*9·9*	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	1	–	4	1	–	–	–	–	–	–	9
	7.46 „	*20·8*	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26	–	–	–	1	–	–	1	–	–	3	1	–	–	34
	7.25 „	*31·4*	8	–	–	–	–	–	–	–	2	–	–	2	–	–	2	–	–	18	–	–	–	–	–	–	–	–	–	–	–	–	–	32
Dusk	9.6 p.m.	*S.*	–	2	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
	8.49 „	*3·2*	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	2	1	–	–	–	–	–	–	8
	8.31 „	*9·3*	3	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	2	–	–	1	–	–	–	–	–	–	8
	8.11 „	*14*	12	2	–	–	–	–	–	–	–	–	1	–	3	–	–	–	–	8	–	–	–	1	1	5	5	1	1	–	–	–	–	40
	7.53 „	*19·4*	8	5	–	–	–	–	1	–	4	–	–	1	–	–	2	–	–	9	–	–	–	–	–	10	–	–	–	–	–	–	–	40
	7.30 „	*30·9*	7	9	–	–	–	–	1	–	7	–	–	17	3	–	6	–	–	31	–	–	–	1	–	9	2	–	–	–	3	–	–	96
Dark	12.13 a.m.	*S.*	3	2	–	–	–	–	–	–	2	–	–	–	–	–	3	–	–	12	–	–	–	–	–	6	2	–	–	–	5	–	–	35
	11.55 p.m.	*5·2*	39	1	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	41	–	–	–	–	–	8	12	–	–	2	3	–	–	108
	11.36 „	*12·2*	21	–	1	–	–	–	1	–	2	–	–	–	1	–	1	–	–	79	–	–	–	–	–	3	7	–	1	1	1	–	–	119
	11.16 „	*16·6*	23	1	1	–	–	–	1	–	7	–	–	–	3	–	5	–	2	96	–	–	1	–	–	2	19	–	–	–	2	–	–	163
	10.55 „	*20·7*	36	3	4	–	1	–	1	–	12	–	1	1	6	–	22	–	–	147	–	1	1	–	–	6	8	1	1	–	3	–	–	255
	10.33 „	*25·8*	39	4	1	1	–	–	–	–	3	–	–	–	1	–	6	–	2	182	3	–	–	2	1	4	9	–	–	1	–	1	1	261
Dawn	4.6 a.m.	*S.*	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
	3.49 „	*3·6*	2	1	–	–	2	–	–	–	–	–	–	–	–	–	–	–	1	2	–	–	–	–	–	1	–	–	–	–	–	–	–	9
	3.28 „	*8*	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	1	–	–	–	–	3	2	–	1	1	–	–	–	17
	3.9 „	*13·9*	6	10	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	6	1	–	–	–	–	3	4	–	2	1	1	–	–	36
	2.49 „	*23·7*	10	4	–	–	–	1	–	–	2	–	–	–	–	–	7	–	–	59	–	–	1	1	–	3	2	–	–	2	5	–	–	97
	2.28 „	*27·5*	25	1	1	–	–	–	–	1	4	–	–	–	–	–	3	–	1	75	1	–	–	–	–	2	10	–	–	–	–	–	1	125
Daylight	9.12 a.m.	*S.*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2
June 19th	8.54 „	*4*	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	3
	8.35 „	*11·1*	5	3	–	–	–	–	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	–	12
	8.16 „	*12·7*	4	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	–	–	–	–	–	3	1	–	–	–	–	–	–	29
	7.56 „	*23·3*	1	2	–	–	–	–	–	–	8	–	–	2	2	–	5	–	1	21	–	–	–	–	–	2	3	–	–	2	2	–	–	51
	7.35 „	*28·5*	4	13	–	–	1	–	–	–	7	–	–	1	3	–	1	–	–	9	–	–	–	–	–	10	2	–	–	–	2	–	–	53

* Maximum depth, 31 m. : 26 m. given as possible average.

† Net struck bottom: only a few fish found amongst bottom material.

indication of increase in numbers in the bottom haul both on the 18th and the 19th ; it is unfortunate that there are no deeper hauls to show whether they were even more abundant below. It is interesting to note

TABLE 9.

POST-LARVÆ OF SARDINA PILCHARDUS CAUGHT IN THE RING-TRAWL ON JULY 15TH–16TH, 1924.

		Depth in metres.	5–10 mm.	11–15 mm.	16–24 mm.	Total number of fish.	Percentage distribution.
1st Series	Surface	—	11	—	—	**11**	*5·7*
3.25-	II	6·6	33	—	—	**33**	*17·2*
4.45 p.m.	III	13·6	60	10	—	**70**	*36·5*
Daylight	IV	19·5	61	7	2	**70**	*36·5*
	V	34·5	8	—	—	**8**	*4·1*
2nd Series	Surface	—	5	10	—	**15**	*14·1*
7.55-	II	7	23	11	—	**34**	*31·7*
9.17 p.m.	III	15·1	11	7	1	**19**	*17·8*
Dusk	V	17·1	16	12	2	**30**	*28·1*
	V	32·3	4	2	3	**9**	*8·3*
3rd Series	Surface	—	160	156	16	**332**	*32·5*
10.50 p.m.	II	5·8	141	66	8	**215**	*21·1*
-12.20 a.m.	III	11·2	124	31	16	**171**	*16·7*
Dark	IV	20·7	67	34	10	**111**	*10·8*
	V	32·2	142	41	10	**193**	*19*
4th Series	Surface	—	16	—	—	**16**	*0·7*
2.50-	II	1·9	326	57	1	**384**	*17·4*
4.16 a.m.	III	9	982	258	36	**1276**	*57·7*
Dawn	IV	16	187	61	12	**260**	*11·8*
	V	30·1	173	91	8	**272**	*12·3*
5th Series	Surface	—	3	1	—	**4**	*3·3*
8.45-	II	5·3	17	3	—	**20**	*17·0*
10.11 a.m.	III	12·5	37	5	3	**45**	*38·2*
Daylight	IV	18·3	29	8	4	**41**	*34·8*
	V	31·1	5	2	1	**8**	*6·7*

that the catch actually at the surface at midnight on 18th–19th was very small. ‡ This was also shown by certain other plankton organisms.

Now we have said that in 1924 there was a possibility that we might have run into a shoal of young pilchard at night ; this might also be put

forward as an explanation for the 1925 results. It would be a curious coincidence, however, if this has occurred on three occasions, and especially on two successive nights as it did in 1925. A further possibility would be that in the light the greatest numbers descend to below 30 metres or just above the bottom. There is no sign that such was the case in the first two series on June 17th, 1925, when we were fortunate enough to fish the net well down in the deeper layers. The question remains, did they escape the net on all occasions except in the dark ? I do not think that young pilchard, all less than 20 mm. in length, would be able to avoid so large a net as the ring-trawl, but on this question we need more observations. The only explanation that remains is the possibility of swarm formation by the small fish. If this were so one would expect that the swarm formation occurred in the daytime, when the small numbers caught could be accounted for by the fact that no swarms were encountered by the net.

It is obvious that it is of no avail to try and bring forward explanations of these curious results until we have far more observations.

TABLE 10.

POST-LARVÆ OF SARDINA PILCHARDUS ON JUNE 17TH–18TH–19TH, 1925.

			Dark.				Dark.		
Series	1	2	3	4	5	6	7	8	9
Surface	1	8	66	1	—	—	3	—	—
II	—	4	67	1	—	4	39	2	1
III	4	7	58	9	4	3	21	5	5
IV	4	5	24	11	1	12	23	6	4
V	10	7	29	12	2	8	36	10	1
VI	3	4	12	28	8	7	39	25	4

GADIDÆ.

GADUS MERLANGUS L.

In the collections in 1924, only one post-larval whiting occurred : this was taken at 7 metres at dusk.

In June, 1925, they were more numerous, but the results (see Table 8, p. 398) do not point to any definite migration at night ; however, while none were taken right on the surface in the daytime, one or two were taken there by the dusk, midnight, and dawn hauls. Nearly all the specimens were between 7 and 20 mm. in length. The following table

shows the numbers of larger specimens taken, ranging between 22 and 50 mm. in length.

			Dark.				Dark.		
Series	1	2	3	4	5	6	7	8	9
Surface	–	–	–	1	–	–	–	1	–
II	–	–	1	3	–	–	–	1	–
III	–	–	–	1	–	–	–	–	–
IV	–	–	–	–	–	–	–	6	–
V	1	1	–	1	–	–	–	2	–
VI	2	–	–	–	–	–	–	–	–

It will be seen that the larger number of young whiting of this size were taken at dawn on both days, when they occurred at most depths, including the surface. At each of the depths IV and V in series 8 one small *Cyanea capillata* was captured.

Gadus minutus (O. F. Müll.).

Post-larvæ of *Gadus minutus* were very scarce on both occasions; however the results would point to quite a definite upward movement on the part of some at night. On July 15th–16th, 1924, two specimens only were captured, 14 and 12½ mm. in length. The first of these was taken at 32 metres at 7.56 p.m., and the other at the same depth at 10.52 p.m.; none occurred in any of the daylight or dawn hauls.

On June 17th, 18th, and 19th, 1925, specimens, 6½–12 mm. in length, were taken in the hauls shown in the following table.

Series	1	2	3	4	5	6	7	8	9
Surface	–	–	–	–	–	–	–	–	–
II	–	–	2	–	–	–	–	–	–
III	–	–	1	–	–	–	1	–	–
IV	–	1	1	–	–	–	1	–	–
V	–	6	3	–	–	–	4	–	–
VI	–	2	–	1	–	–	1	1	–

Here we see definite indications that the post-larvæ were extending their distribution upwards at dusk and midnight on the first night, and at midnight on the second night, reaching a level at a depth of 11 and 12 metres respectively, at which depth it is very unusual to meet with them in the daytime (**7**, p. 119).

BOTHIDÆ.

ARNOGLOSSUS SP.

On July 15th–16th, 1924, at 3.44 p.m. one post-larva only of Arnoglossus, probably *A. laterna* (Will.), was taken at 19·5 m. ; at 8.15 p.m. one at 17·1 m. ; at midnight eleven were caught at the surface, and one or two at every other depth sampled ; at 4·6 a.m. none were taken quite at the surface, but four at 1·9 m., nine at 9 m., and three and two at 16 and 30 m. respectively. In daylight the next day none were caught at the surface or at 5 metres, but five, three, and one were taken at 12·5, 18·3, and 31·1 m. respectively. Thus it would appear that this species showed quite a definite migration towards the surface at midnight, together with a total increase in numbers.

In 1925, on June 17th, between 2.30 and 4.20 p.m., no Arnoglossus post-larvæ were taken ; at 8.53 p.m. one only was taken at a depth of 5·4 metres, and at midnight one at the surface ; in the next two series, dawn and daylight, on the 18th, none were caught, but at dusk between 7.30 and 9.6 p.m. one was taken at each of the following depths : surface, 9·3 m., 19·4 m., and 30·9 m. ; between 10.30 p.m. and 12 midnight one occurred at 12·2 m., one at 16·6, and one at 20·7 m. In the two following series, at dawn and daylight on the 19th, again no specimens were captured. Thus on this occasion also there occurred an increase in numbers in the darker hours, possibly indicating an upward movement.

SCOPHTHALMUS NORVEGICUS (Günther).

The numbers of *S. norvegicus* post-larvæ, all between 4 and 10½ mm. in length, taken on July 15th–16th, 1924, are given below.

	1st Series. 3.25 p.m. to 4.45 p.m.	2nd Series. 7.55 p.m. to 9.17 p.m.	3rd Series. 10.50 p.m. to 12.20 a.m.	4th Series. 2.50 a.m. to 4.16 a.m.	5th Series. 8.45 a.m. to 10.11 a.m.
Surface	—	5	1	—	—
II, 2–7 m.	1	8	2	2	—
III, 9–15 m.	10	3	5	2	2
IV, 16–20 m.	37	25	4	4	14
V, 30–34 m.	1	13	9	12	8

These figures show that while in the daytime these post-larvæ appeared to avoid the water layers between the surface and a depth of about 13 metres, at night they extended their distribution upwards, so that a few occurred at the surface itself ; the greater number, however, still kept to the deeper layers.

From June 17th to 19th in 1925 we again see a similar behaviour, although in this case the numbers of individuals captured are small (Table 8, p. 398); on the second night the majority occurred, as in 1924, below a depth of 10 metres, and on the first night the largest catch was from 11 m., but the numbers taken on this night were very small.

PLEURONECTIDÆ.

Pleuronectes microcephalus (Don.).

On July 15th–16th, 1924, post-larvæ of *P. microcephalus*, 6–12 mm. in length,* occurred in daylight, at dusk, at dawn, and again in daylight, only below 15 metres, except for one at 9 metres at 3.34 a.m. In the dark, however, between 10.52 p.m. and 12.9 a.m. they were distributed from the surface downwards (see Table 7, p. 397).

In June, 1925, there was evidence that they extended upwards at dusk and remained so at midnight, on both nights on which the collections were taken, but they never actually reached the surface. They were all between $5\frac{1}{2}$ and $12\frac{1}{2}$ m. long. In the daytime none were caught above 20 metres; there is again evidence of a slight increase in numbers at night, possibly indicating that in the daytime they were restricted to some deep-lying zone not sampled by the net (Table 8, p. 398).

SOLEIDÆ.

Solea variegata (Don.).

Post-larvæ, 4 to $8\frac{1}{2}$ mm. in length, of *S. variegata*, were only taken throughout the period of collecting on July 15th–16th, 1924, below 10 metres, except at 8.49 p.m., when five were caught at a depth of 7 metres; this time, however, the majority still occurred below 10 metres (Table 7, p. 397). There was therefore little indication of change in vertical distribution throughout the night.

Again on June 17th to 19th, 1925, they mostly occurred below 10 metres. There was a slight indication of extension upwards of their distibution at midnight on the 18th, when three were caught at the surface, at this time, though, they were again more abundant in the deeper layers (see Table 8, p. 398).

CARANGIDÆ.

Caranx trachurus (L.).

Post-larvæ of *Caranx trachurus*, between 4 and 8 mm. in length, were taken on July 15th–16th, 1924; while the largest catch was made at

* At 11.16 p.m. at 20·7 metres one metamorphosing specimen $16\frac{1}{2}$ mm. in length was captured, and at 31 m. at 8.46 a.m. a late post-larva of $14\frac{1}{2}$ mm.

13·6 metres at 4 p.m. on July 15th, at 11.52 p.m. most were caught at 5·8 m., and at 3.34 a.m. on July 16th the majority were taken at 9 m. (Table 7, p. 397). In June, 1925, none were taken.

AMMODYTIDÆ.

Ammodytes lanceolatus (Lesauv.).

Post-larvæ of *Ammodytes lanceolatus*, mostly between 5 and 20 mm. in length, showed on the night of July 15th, 1924, no very marked increase in numbers in the surface layers. At this time there was a full moon. From Fig. 5 it would appear that they moved slightly downwards at dusk, and that at midnight they were evenly distributed between the depths of 10 and 30 metres, avoiding the layers from the surface down to 5 and 6 metres, although there were relatively more at this level than in the daytime. In the daytime they were most abundant below a depth of about 15 metres.

The following table gives the sizes and total numbers of specimens taken in each haul of the five series :—

	Depth in metres.	Length in millimetres. 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total number of fish.
1st Series	*S.*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6·6	–	–	–	–	–	–	1	–	–	–	–	–	1	–	1	–	–	–	–	–	3
	13·6	1	–	1	–	3	1	5	2	5	1	1	1	–	1	–	–	–	–	–	–	22
	19·5	–	4	1	–	4	3	5	7	7	–	3	–	1	1	–	–	–	–	–	–	36
	34·5	–	1	–	–	–	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	4
2nd Series	*S.*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	7	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
	15·1	2	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	4
	17·1	1	1	3	1	2	3	1	1	1	1	–	–	–	–	–	–	–	–	–	–	15
	32·3	1	1	2	3	6	11	6	9	5	2	5	3	–	–	1	–	–	–	1	1	57
3rd Series	*S.*	–	–	1	–	–	–	–	1	1	1	1	–	–	–	–	–	–	–	–	–	5
	5·8	–	–	1	–	1	–	2	–	–	–	–	–	–	–	–	–	–	1	–	–	5
	11·2	1	3	1	1	4	3	4	2	3	1	–	–	–	1	–	–	–	–	–	–	24
	20·7	–	–	–	1	1	2	4	3	3	1	1	1	–	1	–	–	–	–	–	–	18
	32·2	1	–	–	2	–	2	4	3	4	3	–	3	1	1	–	–	–	–	–	–	24
4th Series	*S.*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	1·9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	9	1	5	4	1	1	2	1	1	–	1	–	–	–	–	–	–	–	–	–	–	17
	16	3	6	1	3	4	3	2	1	–	2	–	–	–	–	–	1	–	–	–	–	26
	30·1	4	6	–	1	4	5	7	9	3	5	3	1	–	–	–	1	–	–	–	–	49
5th Series	*S.*	–	–	–	–	–	1	1	–	–	–	1	–	–	–	–	–	–	–	–	–	3
	5·3	1	–	–	–	–	–	–	–	1	1	1	1	–	–	–	–	–	–	–	–	5
	12·5	–	2	2	2	1	1	3	–	2	–	1	–	1	–	–	–	–	–	–	–	15
	18·3	1	7	2	3	5	5	6	6	5	3	1	2	–	1	–	–	–	–	–	–	47
	31·1	–	–	2	3	4	2	2	7	3	1	–	–	–	–	–	–	–	–	–	–	24

In the collections on June 17th–19th, 1925, very few post-larval stages of this species occurred. The numbers were too small to show any indication of movement (Table 8, p. 398).

Johansen (**4**, p. 11) gives records of this species for April in Danish waters, which show a huge increase in the numbers taken at night over those caught in the daytime. It is noteworthy, however, that these stages were from 13 to 91 mm. in length, very much larger than the specimens I have taken, and it is more than probable that at this length the young sand-eels are congregated together in shoals and have already

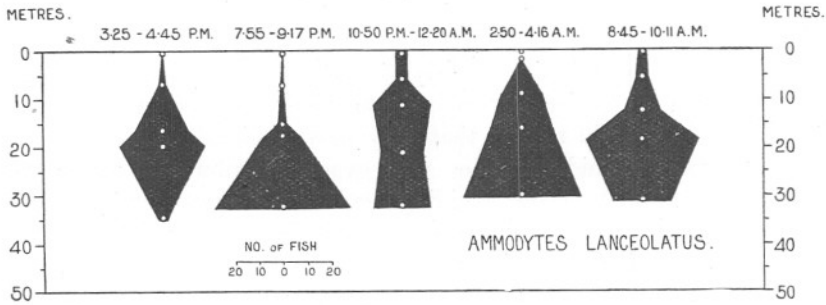

FIG. 5.—The vertical distribution of *Ammodytes lanceolatus* post-larvæ, at the times shown on July 15th–16th, 1924. The white spots and black circles indicate the "average depths" at which hauls were taken.

adopted their typical habit of burrowing in the sand, in which case they may well have been either in the deeper layers or buried in the bottom in the daytime. Further, the extraordinary rapidity with which these fish can move, a whole swarm dispersing with lightning speed, would enable them to avoid the net in daylight. At this stage, in our waters, they are mostly quite close inshore, when on clear days one can see the large swarms swimming about over sandy bottoms near the rocks; a sudden movement on the part of the observer or the casting of a stone into the water will disperse the shoal immediately.

CALLIONYMIDÆ.

CALLIONYMUS SP.

The specimens captured on these occasions were mostly the post-larvæ of *Callionymus lyra* (L.). A few *C. maculatus* (Rafin.) were, however, present. Of the large number taken on July 15th–16th, 1924, only twenty-five specimens of *C. maculatus* were seen; these were specimens in a sufficiently advanced stage of development to be distinguishable from *C. lyra* by the pigmentation of the ventral fin (Fage **3**, p. 136, and Mielck **5**, p. 232). It is possible that a few earlier stages may also have been present. In June, 1925, probably the post-larvæ of *C. maculatus* were even more scarce, e.g. among the 248 specimens taken at 7.27 p.m. on June 17th from 38·6 metres none were seen.

On July 15th–16th, 1924, at the period of full moon, the post-larvæ of *Callionymus* sp. showed no signs of making any upward movement during the night. An examination of Table 11 and also of Fig. 6, which gives diagrammatically the distribution at different times, shows that there was even an indication that the young Callionymus went deeper

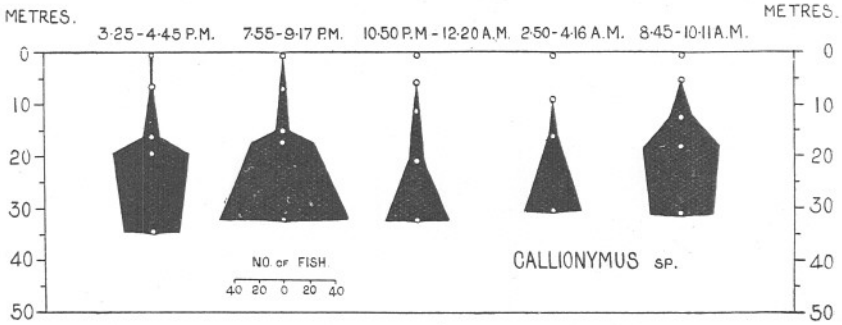

FIG. 6.—The vertical distribution of *Callionymus* sp. post-larvæ, at the times shown on July 15th–16th, 1924. The white spots and black circles indicate the "average depths" at which hauls were taken.

during the night and early hours of the morning than in the daytime. In the afternoon and at dusk on July 15th and in full daylight on the morning of July 16th there was a sudden increase in the numbers of these post-larvæ below 15 metres, and above this level very few were taken; at midnight and dawn, however, they were relatively scarce even down to a depth of 20 metres.

TABLE 11.

NUMBERS OF POST-LARVÆ OF CALLIONYMUS SP. IN EACH HAUL ON JULY 15TH–16TH, 1924.

	1st Series. 3.25 p.m. to 4.45 p.m.	2nd Series. 7.55 p.m. to 9.17 p.m.	3rd Series. 10.50 p.m. to 12.20 a.m.	4th Series. 2.50 a.m. to 4.16 a.m.	5th Series. 8.45 a.m. to 10.11 a.m.
Surface	2	—	3	—	—
II, 2–7 m.	3	7	1	—	2
III, 9–15 m.	14	12	6	2	19
IV, 16–20 m.	62	47	12	8	59
V, 30–34 m.	42	101	71	44	50

In the two daylight series (1 and 5) and the dusk series (2) all the post-larvæ were between 3·5 and 7·5 mm. in length. At midnight, however, at 20·7 metres, three specimens, 8 mm. long and two of 8·5 and 9·5 respectively, were caught; while in the same series at 32·2 metres there were four 8 mm. long and two bottom-stages of 12·5 and 14 mm. At dawn at 30 metres two 9·5 mm. and one 10 mm. long were caught; these

were very early bottom stages, one of which was a *C. maculatus*. This is an indication of an upward movement from the deeper layers of the older stages.

On the two nights in June, 1925, when there was no moon, there was apparently definite evidence of a vertical movement upwards at night (see Table 12 and Fig. 7). In the daylight hardly a single specimen was

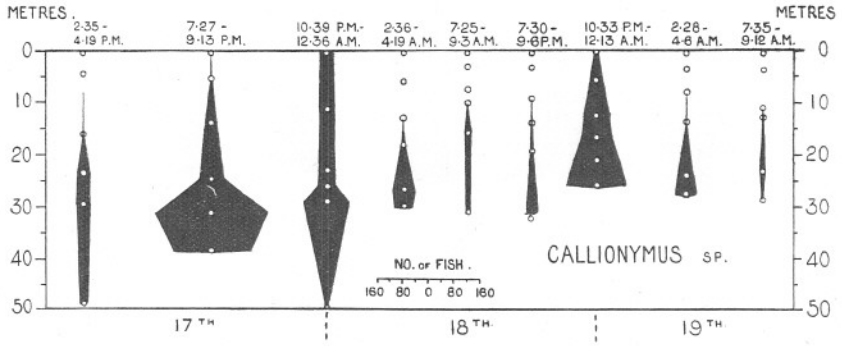

FIG. 7.—The vertical distribution of *Callionymus* sp. post-larvæ, at the times shown, on June 17th–18th–19th, 1925, showing the increase in numbers at all depths on the two nights. The white spots and black circles indicate the "average depths" at which hauls were taken.

taken in any of the hauls above 15 metres, but at night post-larvæ were caught at the surface itself, although they were apparently still far more numerous in the deeper layers.

TABLE 12.

NUMBERS OF POST-LARVÆ OF CALLIONYMUS SP. IN EACH HAUL ON JUNE 17TH–18TH–19TH, 1925.

			Dark.				Dark.		
Series	1	2	3	4	5	6	7	8	9
Surface	1	1	38	1	—	—	12	—	—
II	1	10	47	1	—	1	41	2	—
III	6	39	52	5	2	—	79	4	—
IV	40	85	59	17	2	8	96	6	11
V	45	352	145	71	26	9	147	59	21
VI	30	248	5	54	18	31	182	75	9

Again, as we noticed to be the case with the pilchard post-larvæ, we find a curious increase in the total number of young Callionymus caught at night in June, 1925. To show that this is not due to a vertical movement of fish of the small bottom-living stages I append the following

figures, giving the actual sizes of the specimens taken in two of the hauls : at 29·8 metres in Series 1 and 31·3 metres in Series 2. It will be seen that in neither case can any of these be said to be true bottom living forms, but that they are all true planktonic post-larvæ. Ehrenbaum (**2**, p. 106) says that *Callionymus lyra* takes to the bottom at a length over 10 mm., and Mielck (**5**, p. 235) gives about 11–12 mm. for the corresponding stage of development of *C. maculatus* (?).

	Length in millimetres.												
	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10
1st Series, 29·8 m.	–	3	10	9	7	4	3	2	3	3	1	–	–
2nd ,, 31·3 m.	3	15	51	29	58	42	58	37	36	13	8	–	2

The possible explanations for this increase in numbers will be the same as those already stated under the description of the vertical distribution of *Sardina pilchardus* post-larvæ (p. 401). Perhaps, judging from the actual figures which show such a large increase in the deeper layers at night, it is most probable that in mid-June, a time at which the light is at its strongest and its penetration into the water probably deepest, the Callionymus larvæ were, in the daytime, congregated either in the water layer a few metres above the bottom or actually resting on the bottom itself. The figures for daytime catches shown in Tables 6 and 8, pp. 155 and 157, of a previous paper (**7**) would indicate that on June 4th and July 1st these post-larvæ occurred higher in the water. Tables 3 and 4, pp. 152 and 153, of the same paper show, however, that in mid-June, 1924, on the 17th and 25th days of the month, in the daytime they were also well above the bottom ; on both these dates the weather was dull. In June, 1925, the amount of sunshine recorded in the South-west of England was exceptional, and may well have been the cause of the fish seeking the deepest layers, if such should turn out to be a correct interpretation of the figures shown by my collections. I give below the figures showing the average daily sunlight for the weeks at this time of year in 1924 and 1925.

	Average number of hours per day.	
	1924	1925
22nd week of the year	5·2	12·1
23rd ,, ,, ,,	2·2	12·6
24th ,, ,, ,,	6·5	10·6
25th ,, ,, ,,	6·7	9·7
26th ,, ,, ,,	4·4	7·9

Atkins, in a paper on Phosphate Content in this volume of this Journal (**1**), gives on p. 457 a graph that illustrates this exceptional amount of sunshine in June, 1925.

LABRIDÆ.

Post-larval wrasses were scarce on June 17th–19th, 1925; but on July 15th–16th, 1924, young *Ctenolabrus rupestris*, *Crenilabrus melops*, and *Centrolabrus exoletus* were present throughout the series. It has been shown (**7**, p. 131) that these forms are somewhat irregular in their vertical distribution, and may be taken right at the surface in daylight; little information can be obtained from the figures (Table 7, p. 397), except that at any rate they did not definitely migrate to the surface at night in which case they would have been taken in greatest numbers at that region.

SCOMBRIDÆ.

Scomber scomber (L.).

The post-larvæ of the mackerel have been shown to occur as much in the upper layers as deeper down in the daytime, even being taken right on the surface (**7**, p. 133).

On July 15th–16th, 1924, they were most abundant at 5 or 6 metres in the two daylight hauls and at midnight; at dusk and dawn, however, they would seem to have been deeper in the water, between 9 and 17 metres. This may, of course, be due to uneven horizontal distribution, because while at dusk on June 18th, 1925, they were mostly at 20 to 30 metres, at dusk on the 17th there was no indication of this.

GOBIIDÆ.

Gobius elongatus Canest.

Post-larvæ of *Gobius elongatus* on July 15th, 1924, showed a distinct extension of their distribution into the upper and surface layers at night. An examination of Table 13 and of Fig. 8, however, will show that the *bulk* of the young fish were still well below the actual surface.

Table 13.

Numbers of Gobius elongatus post-larvæ in each Haul on July 15th–16th, 1924.

	1st Series. 3.25 p.m. to 4.45 p.m.	2nd Series. 7.55 p.m. to 9.17 p.m.	3rd Series. 10.50 p.m. to 12.20 a.m.	4th Series. 2.50 a.m. to 4.16 a.m.	5th Series. 8.45 a.m. to 10.11 a.m.
Surface	1	15	23	4	2
II, 2–7 m.	9	89	52	26	10
III, 9–15 m.	68	154	87	237	103
IV, 16–20 m.	106	171	95	213	236
V, 30–34 m.	62	118	210	187	105

It is curious that in the 3rd Series (Dark) the largest catch was made by the bottom haul, as with Callionymus (p. 407), indicating a possible descent of some individuals (see Fig. 5); no conclusion, however, can be drawn from a single observation, unevenness in horizontal distribution always having to be borne in mind.

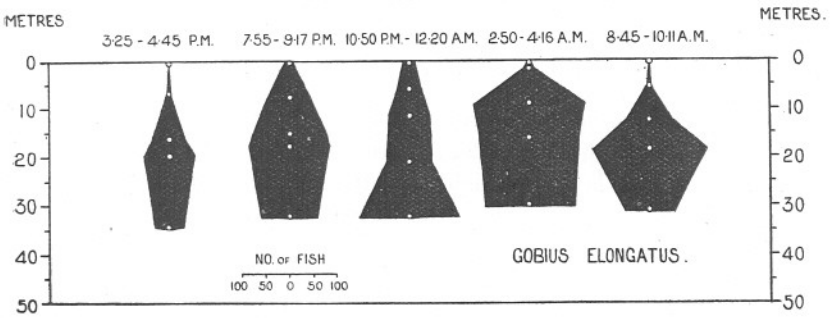

FIG. 8.—The vertical distribution of *Gobius elongatus* post-larvæ, at the times shown, on July 15th–16th, 1924. The white spots and black circles indicate the "average depths" at which hauls were taken.

In June, 1925, post-larval Gobies were extremely scarce in the daytime; at night, however, there was a marked increase in numbers at all depths, and they appeared to have been fairly evenly distributed from the surface downwards (Table 8, p. 398).

LEBETUS SCORPIOIDES (Coll.).

Post-larvæ of *Lebetus scorpioides* were extremely rare on both the occasions on which serial collections were made.

On July 15th, 1924, in Series 1 two only were taken, one from a depth of 19·5 m. and the other from 34·5 m. In Series 2 no specimens were captured. In Series 3 one was taken at the surface, one at 5·8 m., and two at 32·2 m. In Series 4 only one was caught, and that from 30·1 m.; and in Series 5 two were taken, both from 18·3 m. In June, 1925, none were taken, except at 8·11 p.m. and 10·55 p.m. on the second night, June 18th, when they were caught at 14 and 20·7 metres respectively (Table 8, p. 398).

Johansen, in April, 1925, only took this species in the upper water layers, in Danish waters, between the hours of 12 midnight and 2 a.m. (**4**, p. 10).

BLENNIIDÆ.

Blennius pholis (L.) and Blennius ocellaris (L.).

Post-larvæ of *Blennius pholis* and *Blennius ocellaris* were too scarce on both occasions to show any evidence of their behaviour (Tables 7 and 8, p. 397).

Blennius gattorugine (L.).

Blennius gattorugine post-larvæ did not avoid the upper layers in the daytime on July 15th–16th, 1924; there is an indication of an accumulation in the surface layers at night, but the numbers are too small to be conclusive. In June, 1925, they were very scarce.

TRIGLIDÆ.

Trigla sp.

In July, 1924, post-larvæ of *Trigla* sp., which were living in the daytime below a depth of about 7 metres, showed a slight extension upwards of their vertical distribution at dusk, dark, and dawn, but on no occasion was any specimen caught at the surface itself (Table 7, p. 397). On the two nights in June, 1925, however, when there was no moon, although the numbers taken were very small, there is an indication of a more marked upward movement (Table 8, p. 398).

GOBIESOCIDÆ.

Lepadogaster bimaculatus (Penn.).

In July, 1924, post-larvæ of *L. bimaculatus* showed no signs of any diurnal change in their vertical distribution, being taken mostly below 20 metres; one was taken at 7 m. at dusk. In June, 1925, only seven specimens were caught in the whole series of collections; of these two were taken in daylight, three in the dark, and two at dawn.

CYCLOPTERIDÆ.

Liparis montagui (Donov.)

Only two post-larvæ of *L. montagui* occurred in the July, 1924, collections; one was taken at 8.15 p.m. from 17 metres and one at 10·52 p.m. from 32 m. There were none caught in June, 1925.

AGONIDÆ.

Agonus cataphractus.

In all the collections one post-larvæ of this species only occurred; this was from 25 metres at 10.33 p.m. on June 18th, 1925.

LOPHIIDÆ.

Lophius piscatorius (L.).

Post-larval *L. piscatorius* only occurred in June, 1924, when only seven specimens were taken altogether; there was no evidence of upward movement at night (Table 7, p. 397).

SUMMARY.

1. Results are given of collections made with the stramin ring-trawl to study the vertical distribution of post-larval teleostean fishes throughout the period from daylight, through the hours of darkness, to daylight on July 15th–16th, 1924, and June 17th, 18th, and 19th, 1925. At the time of collecting in July, 1924, it was full moon; in June, 1925, there was no moon.

2. In no case, except perhaps that of *Sardina pilchardus* post-larvæ, was there evidence of marked upward migration at night; the general impression for most species was that while the type of distribution shown in daylight was somewhat modified at night, only very few individuals moved up to the surface layers. There was, however, a notable increase in numbers of certain species at all depths at night, whether this was an indication of a movement up from layers very close to the bottom is not known; it will need many more observations before the exact significance of this can be fully understood.

3. The numbers of post-larvæ taken were in the case of most species unfortunately very small on all occasions; it is not possible, therefore, to come to very definite conclusions.

4. A possible indication of the effect of moonlight was shown by the post-larval *Callionymus lyra*, for while in July, 1924, at the time of full moon they showed no upward movement at night, in June, 1925, with no moon a certain proportion reached the surface layers.

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The Vertical Distribution of Marine Macroplankton IV. The Apparent Importance of Light Intensity as a Controlling Factor in the Behaviour of Certain Species in the Plymouth Area.

By

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With 7 Figures in the Text.

INTRODUCTION.

If, on a day in the summer, we take a series of collections of plankton from different depths, in full daylight, in water about 50 metres deep a few miles beyond the Plymouth Breakwater, we find that the plankton exhibits an ordered vertical distribution. In the upper 6 or 7 metres plankton animals are scarce, and then suddenly an increase in their abundance takes place, which exists nearly to the bottom. Analysis of the catches, however, shows that the various species that compose the plankton do not all appear to adopt the same type of vertical distribution; in fact, the total plankton distribution is the sum of a number of different types of distribution. If we look at a table recording the numbers of different species caught at, say, six depths, we find that as we go deeper new species appear in the catches that were not represented in the collections from the layers above.

If we repeat the above experiment on different days, we find that the type of vertical distribution shown by each species is fairly consistently the same from day to day with relation to that of the other species, though the actual depth units may vary.

Fig. 1 gives the vertical distribution shown by about ten different species on three separate days. The diagrams are based on results obtained by collections with the stramin ring-trawl, the fishing depths of which have been obtained by a graphic depth-recorder. In each case six different depths were sampled. The figure shows the percentage vertical distribution for each species, i.e. each catch is expressed as a percentage of the total number of that species caught at all six depths. Table 1 gives the actual numbers and percentages for the three different days.

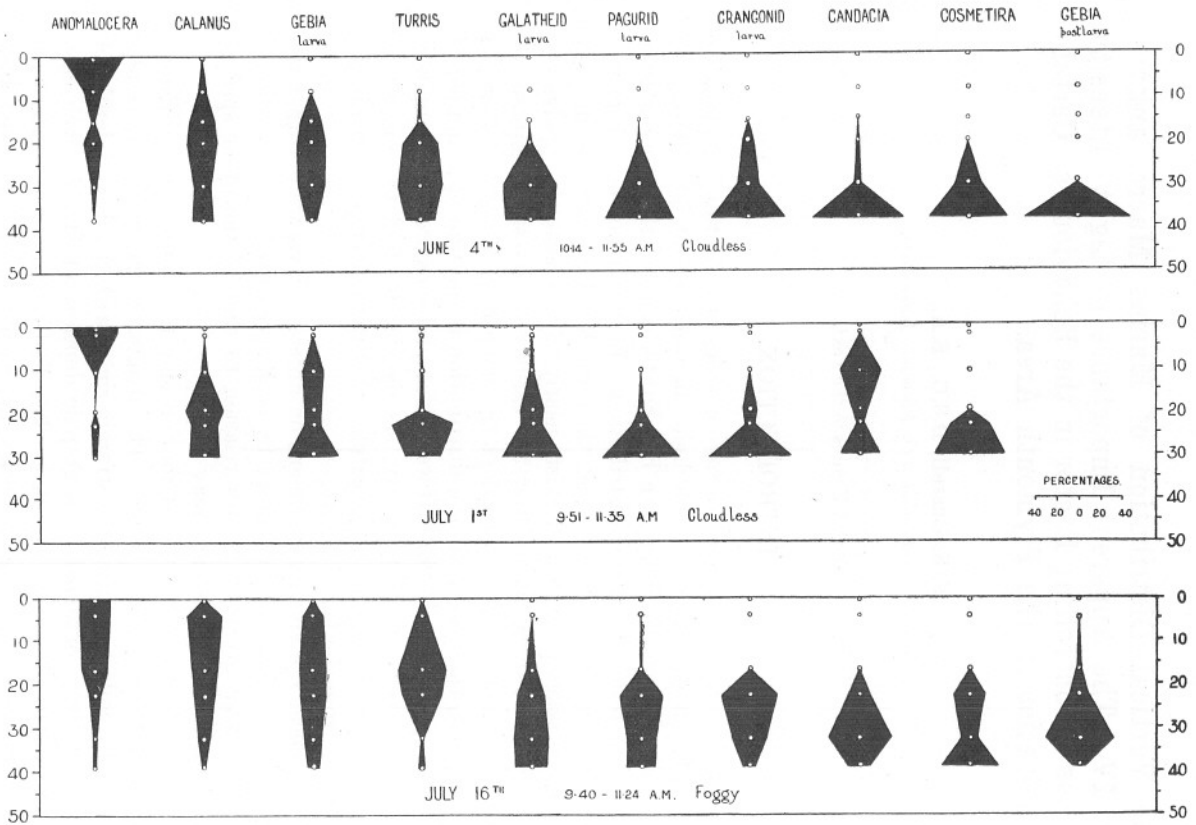

FIG. 1.—The percentage vertical distribution of the above-named species on June 4th, July 1st, and July 16th, respectively, in water more than 50 metres deep as shown by collections with the stramin ring-trawl. The depths are in metres: the white spots and black rings indicate the average depths at which hauls were taken.

TABLE 1.

JUNE 4TH, 1925. 10·14–11·55 A.M. BRIGHT SUNSHINE.

	Anomalocera patersoni.	Calanus finmarchicus.	Gebia larvæ.	Turris pileata.	Galatheid larvæ.	Pagurid larvæ.	Crangonid larvæ.	Candacia armata.	Cosmetira pilosella.	Gebia post-larvæ.
Surface	37	50	—	—	—	—	—	—	—	—
	55·3%	*1·3%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0'0%*	*0·0%*	*0·0%*
8 m.	12	320	20	—	10	10	—	10	—	—
	18%	*8·2%*	*1·4%*	*0·0%*	*0·1%*	*0·3%*	*0·0%*	*2·7%*	*0·0%*	*0·0%*
15 m.	3	960	260	16	60	—	—	—	—	—
	4·4%	*24·7%*	*19·3%*	*3·7%*	*0·9%*	*0·0%*	*0·0%*	*0.0%*	*0·0%*	*0·0%*
20 m.	12	1060	620	136	200	70	5	20	—	—
	18%	*27.3%*	*45·9%*	*31·1%*	*3·2%*	*2·7%*	*11·7%*	*5·4%*	*0·0%*	*0·0%*
30 m.	3	650	330	170	3120	810	8	30	322	—
	4·4%	*16·7%*	*24·5%*	*39·1%*	*49·9%*	*31·2%*	*18·6%*	*8·1%*	*25·6%*	*0·0%*
38 m.	—	850	120	114	2870	1710	30	310	938	2
	0·0%	*21·8%*	*8·8%*	*26·2%*	*45·9%*	*65·8%*	*69·8%*	*83·8%*	*74·4%*	*100%*

JULY 1ST, 1925. 9·51–11·35 A.M. BRIGHT SUNSHINE.

	Anomalocera patersoni.	Calanus finmarchicus.	Gebia larvæ.	Turris pileata.	Galatheid larvæ.	Pagurid larvæ.	Crangonid larvæ.	Candacia armata.	Cosmetira pilosella.	Gebia post-larvæ.
Surface	15	7	1	—	—	—	—	—	—	—
	41·7%	*0·1%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	
2 m.	14	8	5	—	3	—	—	1	—	—
	38·9%	*0·2%*	*0·1%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·7%*	*0.0%*	
11 m.	2	380	740	17	160	—	—	60	—	—
	5·5%	*9·4%*	*19·1%*	*2·3%*	*3·7%*	*0·0%*	*0·0%*	*42·6%*	*0·0%*	
19·8 m.	—	1510	620	50	960	10	40	20	—	—
	0·0%	*37·2%*	*16%*	*7·0%*	*22·2%*	*7·1%*	*14·3%*	*14·2%*	*0·0%*	
23·1 m.	3	960	590	410	830	30	30	10	19	—
	8.3%	*23·7%*	*15·3%*	*57·5%*	*19·2%*	*21·5%*	*10·7%*	*7·1%*	*36·6%*	
30·2 m.	2	1190	1910	237	2370	100	210	50	33	—
	5·5%	*29·4%*	*49·4%*	*33·2%*	*54·9%*	*71·5%*	*75·0%*	*35·5%*	*63·4%*	

[1] 1

TABLE I.—*continued.*

JULY 16TH, 1925. 9·40–11·24 A.M. FOGGY.

	Anomalocera patersoni.	Calanus finmarchicus.	Gebia larvæ.	Turris pileata.	Galatheid larvæ.	Pagurid larvæ.	Crangonid larvæ.	Candacia armata.	Cosmetira pilosella.	Gebia post-larvæ.
Surface	52	157	25	—	—	—	—	—	—	—
	29·6%	*0·9%*	*0·3%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*
4 m.	46	5450	1530	10	—	—	—	—	—	—
	26·1%	*33·4%*	*21·2%*	*11·5%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*
16·5 m.	44	4140	1870	38	320	20	—	—	1	1
	25·1%	*25·4%*	*25·9%*	*43·7%*	*9·7%*	*3·3%*	*0·0%*	*0·0%*	*0·2%*	*6·2%*
22·2 m.	20	3420	1760	30	880	240	80	20	173	3
	11·4%	*21·1%*	*24·4%*	*34·5%*	*26·6%*	*40·7%*	*53·3%*	*20%*	*30·4%*	*18·8%*
32·3 m.	7	2070	1100	3	1130	170	50	60	94	10
	3·9%	*12·7%*	*15·3%*	*3·4%*	*34·2%*	*28·9%*	*33·3%*	*60%*	*16·5%*	*62·5%*
38·8 m.	7	1040	920	6	980	160	20	20	301	2
	3·9%	*6·4%*	*12·8%*	*6·9%*	*29·6%*	*27·2%*	*13·3%*	*20%*	*52·9%*	*12·5%*

It will be seen from this table and from Fig. 1 that these species exhibit fairly constant types of vertical distribution relative to one another. That there are discrepancies is natural, owing to the unevenness in horizontal distribution that may bring in errors large enough to distort the true picture of the vertical distribution. The presence of *Candacia armata* high in the water on July 1st is unusual: it can be seen from the table, however, that the numbers were rather small to be significant, these being obtained by examining a sample one-tenth of the whole catch. It may well have been that below 30 metres they were far more numerous. On the same day between 12·25 and 2·6 p.m. the numbers were for this species:

Surface	3·5 m.	8·8 m.	21·3 m.	27·1 m.	36·7 m.
2	—	—	—	20	120

These figures show an increase below 30 metres.

The order in which the species have been placed in the figure and table is based on an examination of seventeen separate stations of a similar nature, the general impression thus given being that they show a gradual descent in the region of their maximum abundance. These are, of course, only ten of the thirty or forty different species that

occur in the collections. The actual results for all the stations will be given in a future paper.

It will be noticed also in Fig. 1 that many species are considerably higher in the water on July 16th than on June 4th. The significance of this will be discussed later.

Comparison between the Vertical Distribution of certain Species in relation to Light.

Let us for the moment put aside all outside factors that may affect the vertical distribution of any one species, except the factor, *light intensity*. Of all the changing factors that make up the environment of the organism in offshore waters in this region this shows the greatest range of variation. Now let us suppose that an animal has a so-called *optimum* intensity of illumination, that is, that if given a range of in-

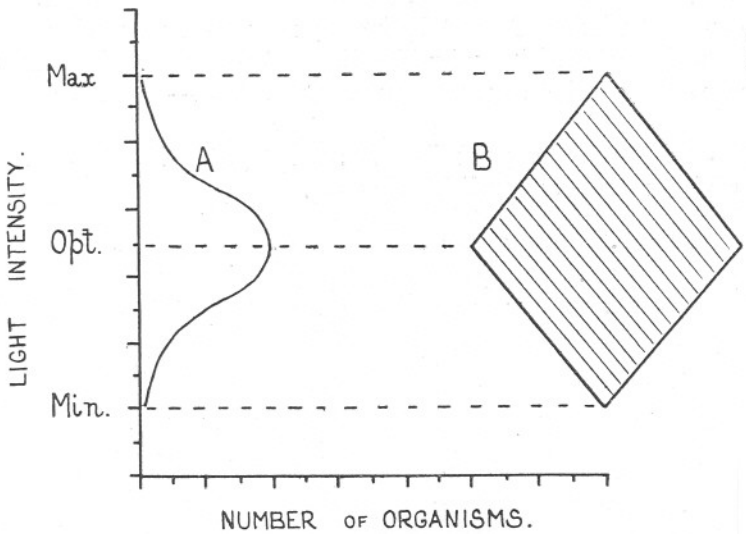

Fig. 2.—A, Hypothetical distribution curve. B, Distribution figure that would be obtained if hauls were taken with a net at the points of maximum, optimum, and minimum abundance.

tensities to choose from it would select this optimum ; by what mechanism this is brought about does not concern us at the present. Let us assume also that besides having an optimum intensity it has also a range of illumination outside which for some unknown reason it does not elect to pass : it will then have a *maximum* intensity of illumination and a *minimum*. Let us also assume that the maximum and minimum occur equidistant in light intensity units from the optimum. We should then imagine its distribution to be that of the order shown by the curve A in

Fig. 2. (A similar curve for vertical distribution has been suggested by Rose, **12**, p. 529.) If the light intensity units be also regarded as depth units, and if collections are made by the net in the region of the maximum, optimum and minimum light intensities, we shall obtain a symmetrical distribution figure* as that marked B (Fig. 2).

Now we know that the intensity of light in the sea does not decrease in direct proportion with the depth, but that it decreases in geometrical progression. That is, for every metre of depth the same fraction of the light present a metre above is absorbed. If, for instance, we have at the surface 100 units of light, and if at a depth of 1 metre there are 50 units, then at a depth of 2 metres there will be $\frac{50}{2}$ units, at 3 metres $\frac{25}{2}$, and at 4 metres $\frac{12{\cdot}5}{2}$, and so on; this is, of course, for *pure* water.

In Fig. 3, curve A represents the actual illumination in the sea, twenty miles off the coast of Plymouth, at different depths in October. This curve was drawn from figures obtained by means of a photo-electric cell, with maximum sensitivity for blue light (Poole and Atkins, **10**), the actual readings being as follows (from **10**, p. 192) :—

October 1st, 1925. E1 (ten miles S.W. of Eddystone Lighthouse). Sea surface : glassy, very slight oily swell.

Time.	Light.	Depth.	% light.
2.11 p.m.	Weak Sun	Air	100†
1.58 ,,	,, ,,	1·5	71·2
1.46 ,,	,, ,,	6·1	41·2
1.18 ,,	,, ,,	8·9	28·3
12.53 ,,	,, ,,	12·2	18·6
12.40 ,,	Dull	18·3	7·92
12.31 ,,	,,	24·4	2·93
12.19 ,,	,,	34·8	0·54
1.35	Weak Sun		

Suppose that the optimum intensity for a certain animal be 35 units and that it has a range of 65 units : then, if the optimum lies midway between the maximum and minimum, the maximum will have a value of 67·5 and the minimum 2·5 units. From the curve, in Fig. 3, we can see that the maximum intensity will be found at a depth of about 2 metres, just over 5 metres above the depth at which the optimum occurs, i.e,

* In all figures illustrating vertical distribution straight lines, rather than arbitrary probability curves, have been drawn between the points, the depths at which hauls are made not being sufficiently close at times to allow of any nearer approximation.

† Percentage of light transmitted by actual surface was 95%.

7.5 m. The minimum intensity, however, will be very much further than the maximum from the optimum in depth units, at about 26·5 metres, i.e. 19 metres below the optimum. If then we take collections from 2 metres, 7·5 m. and 26·5 m., i.e. in the regions of maximum, optimum,

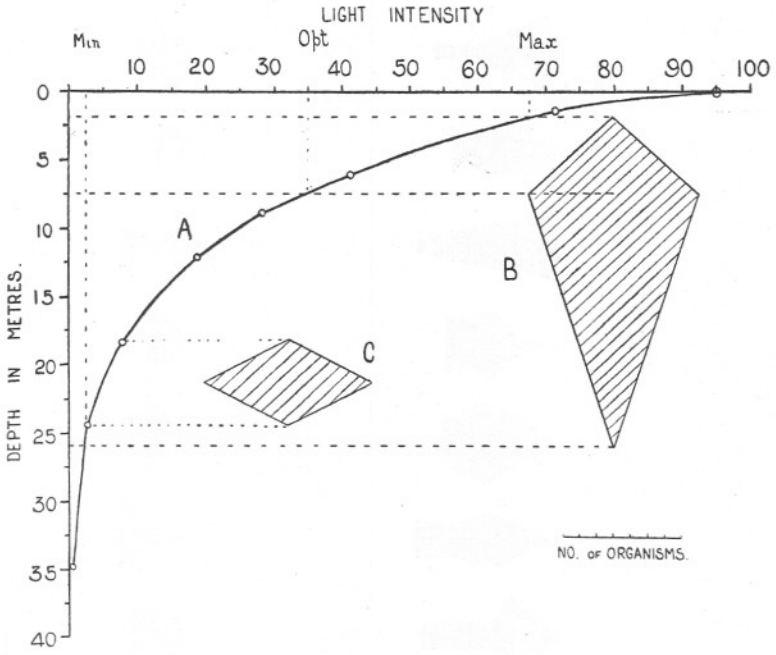

FIG. 3.—A, Curve of percentage light intensity at the international Station E1 on Oct. 1st, 1925, from figures obtained by Poole and Atkins, **10.** B, Vertical distribution diagram of an organism having a high optimum light intensity and living through a wide range of intensities. C, Vertical distribution diagram of an organism having a low optimum light intensity and living through a narrow range of intensities.

and minimum intensities, we shall get a distribution figure of the type shown, B. At whatever depth the optimum occurs if the ratio $\frac{\text{range}}{\text{optimum}} = \frac{67{\cdot}5}{35}$ we shall get approximately the same type of distribution. The smaller the value $\frac{\text{range}}{\text{optimum}}$ becomes, however, the more nearly symmetrical will the distribution figure be. This is exemplified by C, which shows the distribution of an organism whose optimum is 5 light intensity units and range 5 units, i.e. 2·5 to 7·5, so that the ratio $\frac{\text{range}}{\text{optimum}} = \frac{5}{5} = 1$.

Fig. 4 gives some of the types of distribution shown by the copepod, *Calanus finmarchicus*, and the medusa, *Cosmetira pilosella*, at different

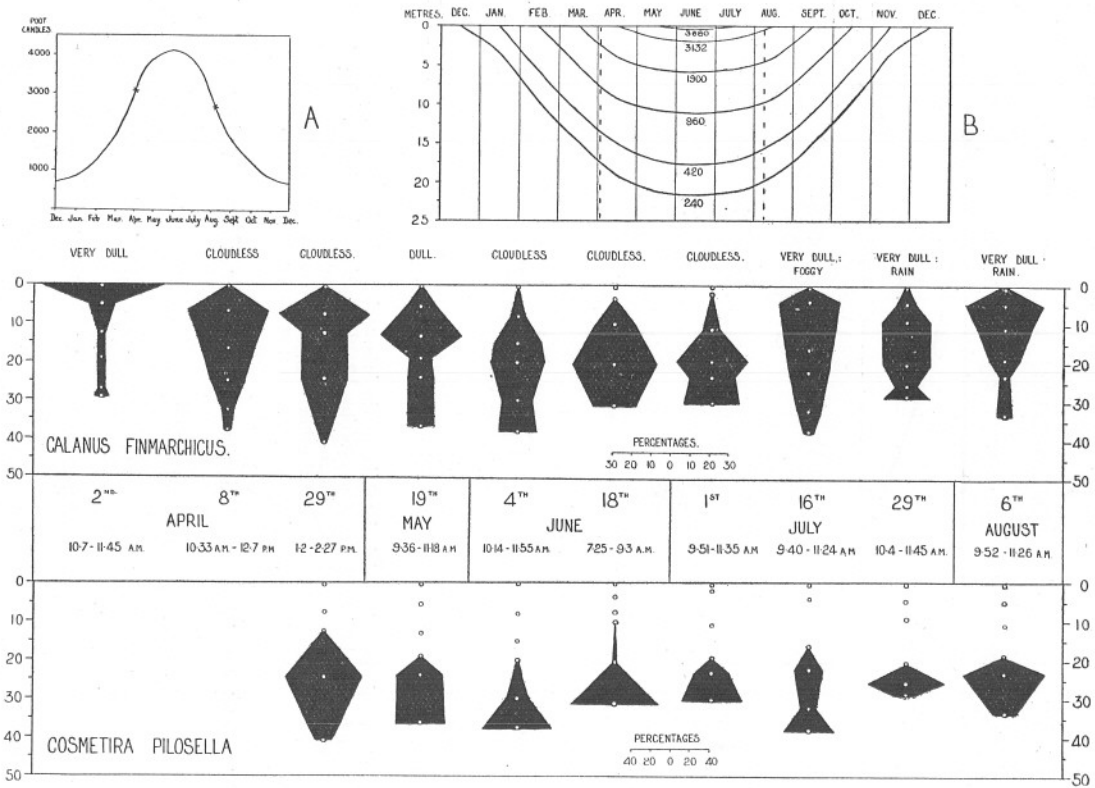

FIG. 4.—A, Curve of seasonal change in light intensity (skylight) in air (from Dictionary of Applied Physics). B, Seasonal iso-intensity curves of light plotted against depth : the intensities are in foot candles. The lower portion of the figure shows the vertical distribution of *Calanus finmarchicus* and *Cosmetira pilosella* on different days in water over 50 metres in depth. The white spots and black circles indicate the average depths at which hauls were made.

dates between April and August, in daylight, drawn from results obtained by taking plankton samples at six different depths. Calanus is a very suitable example to take, because owing to the large mesh of the ring-trawl all the younger stages filter through and only fully grown specimens are retained. This is important, as it has been shown by Farran (**3**) and others that the younger individuals live higher in the water than the older, in which case the distribution figure obtained would embody two or three separate types of distribution running gradually one into the other, if the collections contained mixtures of small and large stages.

The actual numbers of each species taken on the different occasions are given in Table 2. The full details of conditions, times, and depths are to be found in a previous publication (**14**, pp. 149–151). If we examine in Fig. 4 the vertical distribution of Calanus on different days we notice that on April 8th, July 16th, and August 6th the types of distribution shown approximate very closely to the hypothetical distribution marked B* in Fig. 3. There are also indications of this type of distribution shown on some of the other days, but it must be remembered that errors due to irregular horizontal distribution are liable to distort any picture of the true vertical distribution. On the three days mentioned above it is very evident that samples were taken at different depths right through the vertical range of distribution of Calanus, and that one was somewhere near the region of maximum abundance. If the theory outlined above holds for *Calanus finmarchicus*, it would appear that the full-grown animal prefers a comparatively high light intensity, and can also exist in a considerable range of intensities.

To turn now to the vertical distribution of *Cosmetira pilosella*, we see from Table 2 that this species was never taken in any numbers above a depth of 20 metres in daylight. Thus it seems evident that the medusa in question shows a preference for a low light intensity : further, in Fig. 4 it can be seen that on April 29th, July 29th, and August 6th, the distribution figures were curiously symmetrical compared with those of Calanus. In fact, they approximate very nearly to the type shown as C in Fig. 3. (See also Fig. 6, p. 432, July 17th, 2.35–4.19 p.m., and 7.27–9.13 p.m., July 18th, 2.36–4.19 a.m., and July 19th, 2.28–4.6 a.m., and 7.35–9.12 a.m.)

* It will be remembered that in this hypothetical distribution, B, the optimum zone was imagined as lying midway between the maximum and minimum in light intensity units. It is quite to be expected that in nature such would not be the case, but that there would be a skew either towards the high or low intensity. From the actual distributions shown in Fig. 4 it should be possible to test such a point, if the light intensities in the region of maximum abundance, and near the upper and lower limits of the vertical distribution be known.

TABLE 2.

CALANUS FINMARCHICUS

	April 2nd.	April 8th.	April 29th.*	May 19th.	June 4th.	June 18th.	July 1st.	July 16th.	July 29th.	August 8th.
Surface	868	20	16	6	50	—	7	157	546	1005
	65·3%	*0·8%*	*0·5%*	*0·3%*	*1·3%*	*0·0%*	*0·1%*	*0·9%*	*2·5%*	*3·8%*
II	183	1100	1541	381	320	7	8	5450	2260	10,520
	13·8%	*41·9%*	*47·3%*	*17·9%*	*8·2%*	*0·5%*	*0·2%*	*33·4%*	*10·4%*	*40·5%*
III	59	740	785	890	960	175	380	4140	5570	7000
	4·5%	*28·2%*	*24·1%*	*41·8%*	*24·7%*	*12·4%*	*9·4%*	*25·4%*	*25·3%*	*27·2%*
IV	59	488	820	250	1060	285	1510	3420	5470	4100
	4·5%	*18·6%*	*25·2%*	*11·8%*	*27·3%*	*20·3%*	*37·2%*	*21·1%*	*24·8%*	*15·9%*
V	52	160	100	300	650	620	960	2070	2670	1370
	4·0%	*6·1%*	*3·1%*	*14·1%*	*16·7%*	*44·0%*	*23·7%*	*12·7%*	*12·2%*	*5·3%*
VI	105	120	—	300	850	320	1190	1040	5480	1800
	7·9%	*4·6%*		*14·1%*	*21·8%*	*22·7%*	*29·4%*	*6·4%*	*24·8%*	*6·9%*

COSMETIRA PILOSELLA.

	April 2nd.	April 8th	April 29th*.	May 19th.	June 4th.	June 18th.	July 1st.	July 16th.	July 29th.	August 8th.
Surface	—	—	—	—	—	—	—	—	—	—
			0·0%	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*
II	—	—	—	—	—	—	—	—	—	—
			0·0%	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·0%*
III	—	—	11	—	—	—	—	1	—	—
			1·9%	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*0·2%*	*0·0%*	*0·0%*
IV	—	—	447	—	—	—	—	173	51	9
			79·3%	*0·0%*	*0·0%*	*0·0%*	*0·0%*	*30·4%*	*0·8%*	*1·4%*
V	—	—	106	281	322	1	19	19	4650	511
			18·8%	*47·1%*	*25·6%*	*5·2%*	*36·6%*	*16·5%*	*79·1%*	*83·3%*
VI	—	—	—	316	938	18	33	301	1180	94
				52·9%	*74·4%*	*94·8%*	*63·4%*	*52·9%*	*20·1%*	*15·3%*

Again applying the above argument it would appear that *Cosmetira pilosella* shows a preference for low light intensities, and is adapted only for a relatively small range of intensity.

Fig. 5 gives the vertical distribution of two species of copepods, *Centropages typicus* and *Temora longicornis*. It can be seen that while Temora apparently preferred a low intensity of light Centropages lived under conditions of comparatively high light intensity, and was adapted to a considerable range of intensities, the distribution figure following very closely that of B in Fig. 3 (p. 421).

* Only five depths sampled.

The actual numbers of each species taken were :—

July 16th, 1925. Closing metre net.

	Surface.	2·7 m.	6·5 m.	25·8 m.	26·8 m.	41·7 m.
Centropages	5,860	11,560	21,680	6,980	6,080	1,840
	10·9 %	*21·4 %*	*40·1 %*	*13 %*	*11·2 %*	*3·4 %*
Temora	40	40	—	5,820	11,320	29,820
	0·08 %	*0·08 %*	*0·0 %*	*12·4 %*	*24·1 %*	*63·5%*

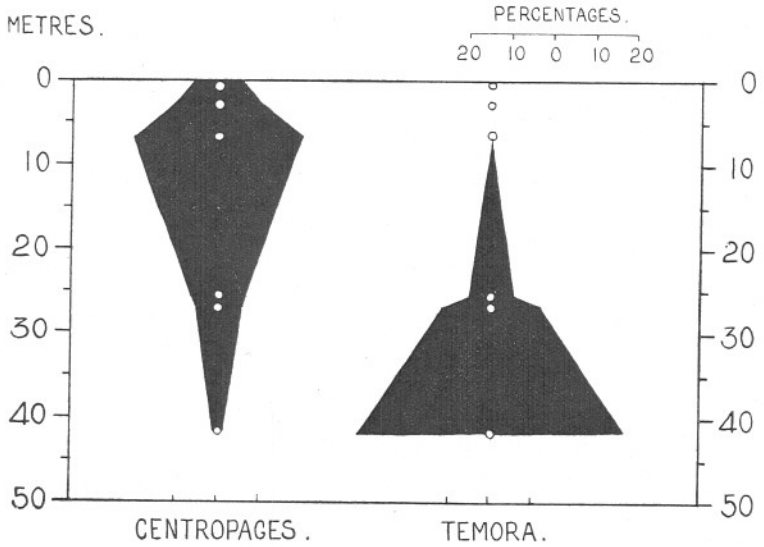

FIG. 5.—The percentage vertical distribution of *Centropages typicus* and *Temora longicornis* on July 16th, 1925, in water over 50 metres deep, as shown by collections with a closing metre net. The white spots and black circles indicate the average depths at which hauls were taken.

SEASONAL VARIATION IN LIGHT INTENSITY CORRELATED WITH OBSERVATIONS ON SEASONAL CHANGES IN VERTICAL DISTRIBUTION OF CERTAIN ORGANISMS.

In Fig. 4 (p. 422), A is a curve representing the approximate change in the average midday illumination in air for each month in the year, expressed in foot candles. This curve is copied from Fig. 57 on p. 448 of the *Dictionary of Applied Physics*, Vol. IV, which is based on 9½ months' observations taken at the National Physical Laboratory from March to December, 1914. The description says that "direct sunlight was always shielded from the test cards," so that the figures may be taken as representing skylight only.

I have, however, treated this light as direct sunlight, and estimated the light intensity at different depths in the sea in each month in the year. Since the light has been regarded as direct sunlight it has been necessary to take into consideration the altitude of the sun at different times of year, and, hence, the amount of light lost in the sea by reflection from the surface and the angle of refraction.

I am indebted to Dr. H. H. Poole for the following approximate figures from which the results have been worked :—

TABLE 3.

Date.	Altitude of sun at noon.	Percentage of total illumination transmitted by water surface.	Secant of angle of refraction.
Dec. 21st	16°	32%	1·45
Nov. 21st and Jan. 21st	20°	42%	1·42
Oct. 21st and Feb. 21st	29°	60%	1·33
Sept. 21st and Mar. 21st	40°	76%	1·22
Aug. 21st and April 21st	52°	87%	1·13
July 21st and May 21st	60°	92%	1·08
June 21st	63°	93%	1·06

The intensity of illumination was estimated for each month at five different depths, viz. 5, 10, 15, 25, and 35 metres, the formula employed being $I = I_0 e^{-\mu x}$, where μ is the coefficient of absorption and x the length of the path of the rays through the water, this being the depth multiplied by the secant of the angle of refraction, which can be seen from the above table to vary for each month. The coefficients of absorption, μ, were those calculated by Poole and Atkins on the occasion on which the results embodied in the curve A of Fig. 3 were obtained, they varied for different depths, being 0·110 from 0–10 metres, 0·117 from 10–20 metres, and 0·133 from 20–30 metres ; this last value was used in this case also for estimating the illumination at 35 m.

The following table gives the figures in foot candles for each month (1 foot candle = 10·764 metre candles).

	Air.	I_0*	I_5	I_{10}	I_{15}	I_{25}	I_{35}
Dec. 21st	750	240	108·12	48·71	18·84	1·93	0·28
Nov. 21st and Jan. 21st	1000	420	192·35	88·06	34·75	3·74	0·57
Oct. 21st and Feb. 21st	1600	960	461·96	222·28	93·02	11·53	1·96
Sept. 21st and Mar. 21st	2500	1900	971·18	496·59	223·30	32·91	6·49
Aug. 21st and April 21st	3600	3132	1682·3	903·44	431·13	73·10	16·27
July 21st and May 21st	4000	3680	2031·9	1121·7	552·98	101·46	24·14
June 21st	4100	3813	2128·1	1188·3	593·34	112·33	27·44

* This is the illumination immediately beneath the surface, obtained by multiplying the air illumination by the percentages given in Table 3.

From the above figures curves were drawn for each month, and from these Fig. 4 B was constructed. This diagram shows the *iso-intensity* lines plotted against depth throughout the season. The curves naturally have no direct significance, being based on purely arbitrary figures, but they are inserted here to illustrate the principle which would appear to underlie the behaviour of certain plankton animals in the daytime throughout the seasons. In practice, of course, these curves would not be smooth, but would be extremely wavy according to variations in weather conditions, daily changes in illumination being enormous from one moment to another under certain conditions, such variations being as much as 80% in a few minutes (**2**, p. 449).

From this diagram (B) we see then that if an animal is to be adapted to a certain light intensity we should expect it to show variations in depth throughout the year, and that it should be at its deepest at mid-day on a sunny day in the middle of June.

Fig. 4 shows the daylight distributions of *Calanus finmarchicus* on different days between April 2nd and August 6th, 1925. It can be clearly seen that there is a gradual descent of the region of maximum abundance from the beginning of April to June 18th, when the sun is near its maximum altitude and intensity, and that after that a gradual ascent is shown. There are evident discrepancies, but on examination of the weather conditions existing at the time the collections were made these become explained. For instance, we should not expect so marked a change in the distribution between April 2nd, when the Calanus were crowding at the surface, and April 8th, when the depth of maximum abundance was around 7 metres ; but this would appear to be explained by the fact that on April 2nd the weather was very dull and overcast, and that on April 8th there was bright sunshine. Again the rise shown for July 16th to August 6th appears to be unexpectedly high, but here it is evidently due to the fact that on these occasions the weather was very dull and foggy, with mist and rain.

It can be seen from the figure that the region of maximum abundance has sunk by June 18th from very near the surface to about 20 metres ; probably it would have been even deeper if the collections on June 18th had been taken at midday when the light is at its strongest : they were, however, taken between 7·25 and 9·3 in the morning. Now a glance at diagram B shows that the intensity of 3132 foot candles occurring at the surface in April has sunk only to about 2 metres in mid-June. This is because the air illuminations on which the figures are based are skylight and not direct sunlight. If the figures had been for direct sunlight the curve A would have been very much steeper, and consequently the iso-intensity lines in B would have gone deeper.

As an illustration I compare the intensities I have calculated for

September 21st with those obtained by Poole and Atkins on October 1st, 1925. It will be seen that they agree very nearly. Both are expressed in metre candles.

Theoretical Intensities, Sept. 21st.		Actual Intensities, Oct. 1st, 1925.	
Air	26,896	Air	22,400*
Just below surface	20,446	1·5 m.	15,000
5 m.	10.449	6·1 m.	9,280
10 m.	5,343	8·9 m.	5,770
15 m.	2,402	12·2	3,970
25 m.	354	18·3 m.	1,450
35 m.	70	24·4 m.	470
		34·8 m.	132 } †
			94 }

The weather conditions noted for October 1st, 1925, were " Dull " and " Weak Sun." On September 3rd, however, in bright sun the air illumination was three times as great, being 68,700 metre candles (**10**, p. 192).

If now we examine the seasonal changes in the vertical distribution of *Cosmetira pilosella*, as shown in Fig. 4, it would appear that there is not so marked a change ; it is, however, difficult to tell for certain, as it is evident that on May 19th, June 4th and 18th, and July 1st the whole vertical range of distribution was not sampled. However, in Fig. 6 (p. 432), we see that in the afternoon of June 17th, between 2.35 and 4.19 p.m., the whole range was apparently sampled and the zone of maximum abundance lay around 30 metres ; if the distribution on this date be compared with that for July 29th and August 6th it is obvious that the zone of maximum abundance has moved up from about 30 m. to about 25 m., a movement of only about 5 metres, while *Calanus finmarchicus* showed between July 18th and August 6th a vertical rise of nearly 20 metres of the zone of maximum abundance. If this signifies that Cosmetira has followed a certain low light intensity in its seasonal changes in depth, the small variation in its depth is contrary to expectation ; because, in theory, the the iso-intensity curves for low intensities should move through a slightly greater vertical range between April and August than those for high intensities. It may, of course, be that proximity to the bottom comes in as an interfering factor, in this case it being at just over 50 metres. On the other hand, it may be an indication of the actual changes that do occur in the light intensity in the sea in these areas.

* This value varied from 16,000 to 24,500 m.c. during the series, and the values quoted here are not corrected for this, but are the observed values. The corrected values are recorded as percentages in an unnumbered table on p. 420, plotted in Fig. 3.

† Surface = 24,500 and 17,400 m.c. respectively.

In the course of my investigations I have always used a small silk tow-net attached to the warp just at its junction with the bridles of the ring-trawl. The collections obtained by this net have furnished abundant evidence that the deeper layers are very much richer in plant life than the water layers nearer the surface ; this is true both of diatoms and Phæocystis, the species of diatoms, of course, varying with the seasons. This plant-life presumably has sunk down from the upper layers in which active assimilation and reproduction occurs, and must be constantly accumulating in the deeper layers (*vide* also Gran **5**, p. 123).

Owing to the scattering of light by these countless small diatoms in suspension in sea-water the *apparent* coefficient of absorption of the sea-water will tend to be raised, and hence it is possible that the difference in intensity in the deeper layers between the period before the diatoms are abundant and May or June may not be relatively so great as the changes that occur in the upper layers during the same season. To illustrate this point I took collections on April 9th and April 13th this year (1926) in the same region about ten miles from land. Before and after collecting I used a Secchi's disc, 20 cm. in diameter, to ascertain the transparency of the water.

On April 9th it was cloudless, with slight haze, but the breeze was fresh so that the surface of the sea was considerably ruffled ; on April 13th it was cloudless, the atmosphere was clear and at the same time the sea surface was very calm with an almost glassy smoothness. It was therefore to be expected that the light penetration of April 13th would be greater than that on April 9th, the Secchi disc, however, disappeared from sight at the following depths on the two days :—

April 9th, Eddystone 1 mi. W.	11.27 a.m.	10 metres.
	1.25 p.m.	12 ,,
April 13th ,, ,,	11 a.m.	12 ,,
	12.55 p.m.	10 ,,

From these figures it would appear that there was possibly a great difference in the transparency at a depth of about 10–12 m. on the two days. Collections with the ring-trawl showed that, while there was a considerable amount of animal life at the surface and at about 3 metres on April 9th, it was markedly scarce in these layers on April 13th. This would perhaps indicate a higher intensity in the surface layers on the latter date than on the former ; yet, how was it that the Secchi disc disappeared at approximately the same depth on the two days ? The answer is probably to be found in the results shown by the small tow-net, which being of " medium " mesh (50 strands to the inch) would only show signs of diatoms in the catch if they were extremely abundant in the sea-water.

Now the collections with this net on the two days showed the following results :—

April 9th.		April 13th.	
Surface	No green tinge.	Surface	No green tinge.
3 m.	,, ,,	3 m.	Faint green tinge.
7 m.	,, ,,	13 m.	Deep green tinge and thick catch of Phæocystis and Biddulphia, etc.
15·5 m.	,, ,,	27·6 m.	Ditto.
22·4 m.	,, ,,	35·4 m.	Ditto.
32·4 m.	,, ,,	41 m.	Ditto.

The thick growth of plant-life, which started evidently somewhere between the surface and 13 metres, may have been the cause of the disappearance from view of the Secchi disc at about 10 metres on April 13th at which depth the transparency of the water would be greatly reduced. In which case the indications from the ring-trawl catches are that the upper layers had a stronger illumination on April 13th than on April 9th, while the evidence produced by the Secchi disc would indicate that on the two dates there may not have been so great a difference in the illumination at about 10–12 metres.

A further example of the apparent large change in depth of an animal living in the upper layers throughout the season, in daylight, compared with the slight change of one living at deeper depths and lower intensities, is furnished in a previous paper (**14**). Here, on p. 118 is given the vertical distribution of post-larval Gadoid fishes between April 2nd and June 17th : it can be seen that the post-larvæ of *Gadus merlangus*, the whiting, show a marked change as the season advances, deserting the surface and upper layers as the light intensity grows. The post-larvæ of the Poor Cod, *Gadus minutus*, however, which apparently always lived at a low light intensity, below 20 metres, showed no marked seasonal change in their distribution in daylight.

Diurnal Variation in Light Intensity and Diurnal changes in the Vertical Distribution of certain Plankton Animals.

Apart from seasonal change in light intensity there is also a regular diurnal fluctuation, i.e. in the passage of time from midday, through the hours of darkness, to midday. Corresponding with these changes in intensity we very often find alterations in the vertical distribution of plankton animals.

Of the actual changes that do occur in the vertical distribution of plankton animals throughout the twenty-four hours in the sea little is

at present known. The majority of records have been based on the comparison of collections from surface layers only, or other depths; such collections, while indicating that the surface layers become filled up by certain animals at night, or that some forms are taken at smaller depths at night than in the daytime, give no definite information about their actual vertical distribution at different times.

In 1924 I carried out a series of collections throughout the twenty-four hours, on a moonlight night in July; the results showed that while some species actually crowded into the surface layers at night, depleting the lower water layers, others, apparently, merely extended their daytime distribution into the upper layers, so that they became evenly distributed from the surface downwards (**13**, pp. 783 and 785). Again other forms showed apparently no change in their vertical distribution throughout the hours of darkness, while many animals, living on or near the bottom in the daytime, moved up through a vertical distance of 10 or 20 metres at night (**13**, pp. 787 and 789). It would appear from the diagrams referred to in this previous paper (**13**) that those forms that were evenly distributed had lost even their minimum intensity, and were wandering anywhere through the water layers. There is an indication that they have picked up their optimum intensity at dawn and massed around it, moving down with it as the day advances. A somewhat similar suggestion* has already been put forward by Michael to explain the diurnal changes in the vertical distribution of *Sagitta bipunctata* in the San Diego region (**9**). It seems probable, however, that other factors may underlie the nocturnal habits of certain animals, as the apparent indication of no change in the vertical distribution of certain species at night cannot be explained on this hypothesis, neither can the massing of certain species right at the surface between sunset and sunrise.

In 1925 I repeated this experiment in mid-June; at this time there was no moon. The collecting on this occasion was carried through two successive nights, so that the second night acted more or less as a control on the first. Most species showed identical types of behaviour on the two nights indicating that the methods of collecting were probably sound. It was noticeable that certain animals showed a more marked movement upwards on this moonless night than on the moonlight night in July, 1924. The full results of these collections will be given in a future paper.

In Fig. 6 I have given the vertical distribution of three species at the different times in June, 1925. These are for the copepod *Calanus finmarchicus* and the medusæ, *Turris pileata* and *Cosmetira pilosella*. The actual numbers of each species taken in each haul are given in Table 4.

* He suggests that, while being attracted upwards by bright twilight conditions at dusk and dawn, at night, the twilight stimulus being removed, they return to deeper layers where they find optimum conditions of temperature, salinity, etc.

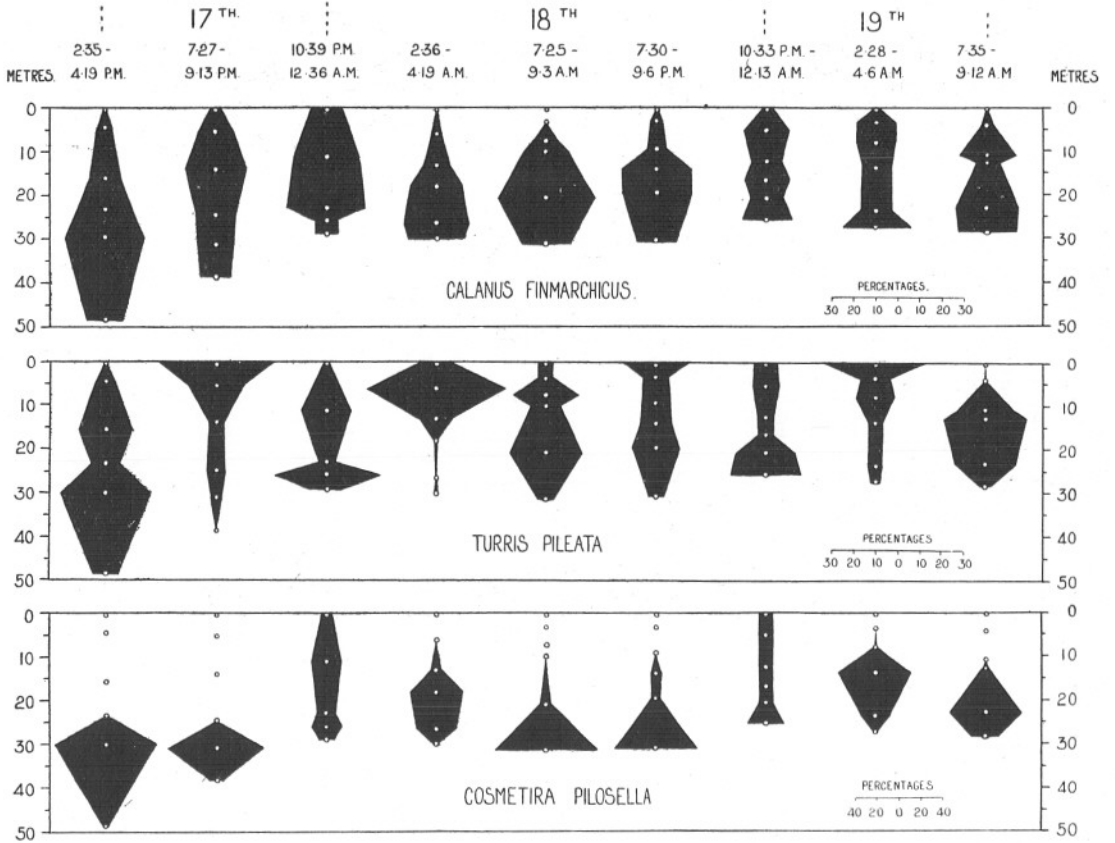

FIG. 6.—The percentage vertical distribution of *Calanus finmarchicus*, *Turris pileata*, and *Cosmetira pilosella*, at the times shown, on June 17–18–19th, 1925. The white spots and black circles indicate the average depths at which hauls were taken.

TABLE 4.

CALANUS FINMARCHICUS.

	Daylight. 2.33 to 4.10 p.m.	Dusk. 7.25 to 9.4 p.m.	Dark. 10.37 p.m. to 12.46 a.m.	Dawn. 2.35 to 4.29 a.m.	Daylight. 7.22 to 9.14 a.m.	Dusk. 7.29 to 9.16 p.m.	Dark. 10.31 p.m. to 12.23 a.m.	Dawn. 2.27 to 4.16 a.m	Daylight. 7.32 to 9.21 a.m.
Surface	40	200	1330	21	—	44	400	45	12
	0·7%	*5·5%*	*13·6%*	*1·1%*	*0·0%*	*1·2%*	*4·5%*	*1·8%*	*0·2%*
II	410	600	2850	110	7	220	1840	465	314
	6·9%	*16·7%*	*29·0%*	*5·5%*	*0·5%*	*6·3%*	*21·3%*	*18·8%*	*6·2%*
III	870	1010	3520	235	175	330	1230	460	1260
	14·7%	*27·6%*	*36·0%*	*11·8%*	*12·4%*	*9·4%*	*14·2%*	*18·6%*	*25·9%*
IV	1490	690	1030	480	285	1160	1810	340	570
	25·2%	*18·9%*	*10·5%*	*24·2%*	*20·3%*	*33·1%*	*21·0%*	*13·8%*	*11·8%*
V	2140	640	1050	610	620	1110	1330	360	1410
	36·2%	*17·5%*	*10·7%*	*30·7%*	*44%*	*31·6%*	*15·4%*	*14·6%*	*28·9%*
VI	970	510	*	530	320	640	2000	800	1310
	16·2%	*13·5%*		*26·6%*	*22·7%*	*18·2%*	*23·2%*	*32·4%*	*26·9%*

TURRIS PILEATA.

Surface	—	117	1	28	4	97	21	134	1
	0·0%	*53·4%*	*2·9%*	*15·0%*	*7·8%*	*30·5%*	*11·1%*	*48·1%*	*0·4%*
II	6	55	8	121	4	38	23	39	4
	9·7%	*25·2%*	*23·7%*	*64·8 %*	*7·8%*	*12·1%*	*12·0%*	*14·0%*	*1·7%*
III	15	16	3	28	15	41	16	50	58
	24·6%	*7·2%*	*8·4%*	*15%*	*29·5%*	*12·9%*	*8·3%*	*18·0%*	*25·8%*
IV	8	18	17	3	7	48	16	19	86
	13·2%	*8·2%*	*50%*	*1·6%*	*13·8%*	*15·1%*	*8·3%*	*6·8%*	*38·3%*
V	25	12	5	2	17	70	53	21	63
	41·1%	*5·3%*	*14·9%*	*1·0%*	*33·4%*	*22·1%*	*27·8%*	*7·5%*	*28·0%*
VI	7	2	*	5	4	24	62	16	13
	11·5%	*0·9%*		*2·6%*	*7·8%*	*7·4%*	*32·5%*	*5·7%*	*5·7%*

COSMETIRA PILOSELLA.

Surface	—	—	20	—	—	—	38	—	—
	0·0%	*0·0%*	*10·9%*	*0·0%*	*0·0%*	*0·0%*	*11·7%*	*0·0%*	*0·0%*
II	—	—	53	—	—	1	42	—	—
	0·0%	*0·0%*	*28·7%*	*0·0%*	*0·0%*	*0·3%*	*13%*	*0·0%*	*0·0%*
III	2	—	33	19	—	8	41	4	—
	0·2%	*0·0%*	*17·8%*	*10·2%*	*0·0%*	*2·6%*	*12·6%*	*4·2%*	*0·0%*
IV	7	1	52	91	—	36	44	65	11
	0·6%	*0·3%*	*28·1%*	*48·7%*	*0·0%*	*11·7%*	*13·5%*	*67·8%*	*8·6%*
V	1217	251	27	66	1	30	47	25	85
	93·0%	*87·2%*	*14·6%*	*35·3%*	*5·2%*	*9·8%*	*14·5%*	*26·1%*	*66·4%*
VI	79	36	*	11	18	233	113	2	32
	6·0%	*12·5%*		*5·9%*	*94·8%*	*75·7%*	*34·8%*	*2·0%*	*25·0%*

* Net struck bottom.

the details of time, depths, etc., are given in another publication in this volume of the Journal (**15**, p. 388). It can be seen from Fig. 6 that the behaviour of *Calanus finmarchicus* differed in June, 1925, from that shown for July, 1924, see Fig. 6 on p. 791 of the previous publication (**13**). The figure shows that there was apparently merely an extension of the distribution into the surface layers on June 17th at "dusk" and "dark,"* and similarly at "dark" and "dawn" on the 18th and 19th, whereas in July, 1925, there was a massing at the surface at "dusk," an even distribution from surface downwards at "dark," and an apparent accumulation round an optimum intensity at about 10 metres at "dawn." The differences between the two observations may possibly be correlated with differing intensities occurring at different times of the year.

Turris pileata, which, in 1924 (**13**, p. 783), showed a massing at the surface at midnight, in 1925, as shown by Fig. 5, tended to mass at the surface at dusk and dawn apparently seeking the deeper layers again at night. It should here be noted that the day distributions at 2.35 to 4.19 p.m. on June 17th and 7.25 to 9.3 a.m. on June 18th are probably misleading; they can be seen from Table 4 to be based on too low figures. It is probable, however, that that shown for 7.35 to 9.12 a.m. on June 19th represents more nearly the true daylight distribution.

The results obtained for *Cosmetira pilosella* are very clearly defined: the figure speaks for itself, and shows that about midnight these medusæ were evenly distributed from the surface downwards, and that at dawn they appeared to be massed around an optimum intensity, which they followed downwards as the daylight increased in strength.

The behaviour of these three species of plankton animals has been inserted here to illustrate how the diurnal changes in light intensity probably play an important part in controlling the behaviour of some plankton animals from day to day. It is, however, evident that a very much larger number of observations of this type must be made at all times of the year, before we can hope to arrive at the true significance of these diurnal changes in vertical distribution.

DISCUSSION.

Let us now consider the various external factors that may be of importance in controlling the behaviour of these plankton animals in this region.

Rose, in an admirable paper on the biology of plankton (**12**), comes to the following general conclusion. The majority of pelagic animals are adapted to an optimum intensity of light. Each species, and even each

* There is an indication that there may have been a depletion of layers below about 25 metres, but unfortunately the catch from the deeper layers was lost through the net striking the bottom.

individual, may have its own characteristic optimum intensity. Each animal, further, is affected by numerous physico-chemical factors, either external or internal in origin. The optimum zone of distribution changes with the age of the animal and its physico-chemical state at any moment, and rises or falls according to a number of external or internal factors which may interfere, such factors being temperature, hydrogen ion concentration, salinity, etc. Rose gives his factors the following order of importance :—

1. Light, which, under average conditions, has clearly a predominating influence.

2. Temperature, which becomes very important and can even overwhelm the effect of light when it passes 20°.

3. Other factors of the medium (concentration, aeration, etc.).

It is then very evident that my observations in the field tend to confirm Rose's ideas.

In considering the vertical distribution of plankton animals there are two factors that must always be borne in mind.

1. *The geographical locality.* Factors may exert a powerful influence in one latitude, that in another region may have little effect.

Rose experimented in the laboratory with copepods both from Roscoff in the English Channel and Banyuls-sur Mer on the Mediterranean coast. He noticed that the temperature zone between about 20° and 25° was of considerable importance for many of the species examined, in that, between these points there lay critical temperatures at which phototropism was reversed and at which different species of copepods, which were uniformly distributed in jars containing water of lower temperatures, crowded to the bottom of the vessel in which they were living (**12**, pp. 485 and 512). Further, this held good for copepods both from Roscoff and Banyuls-sur-Mer. In the Channel the highest temperature reached by the upper 5 or 6 metres in the summer is just over 16° ; it would seem unlikely, then, that high temperature would play an important part in the behaviour of certain plankton animals in this region ; on the other hand, the surface waters of the Mediterranean may reach so high a temperature as 27° in the summer, in that region therefore high temperature might well be considered a factor of great importance in the behaviour of plankton animals in the sea. M. Rose has also expressed a similar opinion to me by letter.

2. *The type of environment.* Just as variations in certain factors may be of a sufficient range to become important in controlling the behaviour of certain animals in one geographical region, but not in another, as, for instance, temperature, so in the same manner a factor may or may

not have importance in different types of environment in the same locality. In confined spaces such as ponds and rock pools the changes that occur in the water are extreme compared to those in the open sea. For instance, Atkins (**1**, p. 768) found that the seasonal changes of pH and temperature in the following two environments were :—

	pH	pH Range.	Temperature.	Temperature Range.
Rock Pool	8·57–8·01	0·56	21·4°–8·2° C.	13·2° C.
E1, 20 miles S.W. of Plymouth Breakwater	8·27–8·14	0·13	16·2°–9·9° C.	6·3° C.

In shallow water round the shore the variations in such factors are also more extreme than in the open sea.

My observations were made in the neighbourhood of the Eddystone Lighthouse, where the conditions do not differ essentially from those existing at the International Station, E1 ; if anything the ranges of the various factors are very slightly greater in this region. It has been noted that the pH variation is 8·27–8·14, such changes are so slight as to be of doubtful importance in affecting the behaviour of such plankton animals as are discussed in this paper ; the salinity range is in the neighbourhood of 35·40–35·13°/$_{oo}$ (**1**, p. 768). It remains then to discuss the possible effect of temperature change throughout the season ; this in the surface layers is quite considerable, amounting to 6° or 7°.

In Fig. 7 is given the change in temperature that took place twenty miles from the coast throughout the year 1925, the same year in which the seasonal observations of plankton animals given in this paper were made. The figure has been based on the following temperature observations (Table 5) for which I am indebted to Mr. H. W. Harvey :—

TABLE 5.

Station E1. Lat. 50°·02′ N. G.L. 4°·22′ W. Depth, 74 m.

Depth in metres.	Jan. 19th.	Feb. 17th.	March 14th.	April 22nd.	May 13th.	June 3rd.	July 8th.	Aug. 5th.	Aug. 31st.	Oct. 1st.	Nov. 11th.	Dec. 11th.
0	10·8°	9·8°	9·3°	9·8°	10·7°	12·6°	16·0°	15·4°	16·30°	14·9°	11·8°	10·8°
5	10·79°	10·01°	9·24°	9·69°	10·5°	11·95°	15·75°	15·22°	16·30°	14·50°	—	—
10	10·79°	—	9·19°	9·47°	10·3°	10·92°	14·3°	15·10°	15·75°	14·00°	12·8°	10·97°
15	10·79°	10·01°	9·19°	9·44°	9·99°	—	12·03°	14·90°	14·93°	13·83°	—	—
20	—	—	—	—	9·96°	10·55°	11·85°	13·15°	13·40°	13·70°	12·85°	10·97°
25	10·79°	10·01°	9·18°	9·44°	—	—	—	12·02°	13·32°	—	—	—
30	—	—	—	—	9·96°	10·33°	11·80°	12·02°	13·15°	13·60°	12·83°	10·97°
40	—	—	—	—	—	—	—	—	—	13·58°	—	—
50	10·79°	10·01°	9·16°	9·44°	9·96°	10·00°	11·80°	12·01°	12·80°	13·59°	13·00°	10·97°
ca. 70	10·79°	10·01°	9·16°	9·44°	9·95°	10·00°	11·80°	12·00°	12·80°	13·57°	13·05°	10·97°

It can be seen, both from Fig. 7 and the table, that the surface temperature was rising steadily from the middle of March until August 31st, when it reached a maximum of 16·30° C. If temperature were the factor that drove certain plankton animals from the surface in summer it would be expected that the forms living in the upper layers would be at their maximum depth towards the end of August. Actually results showed

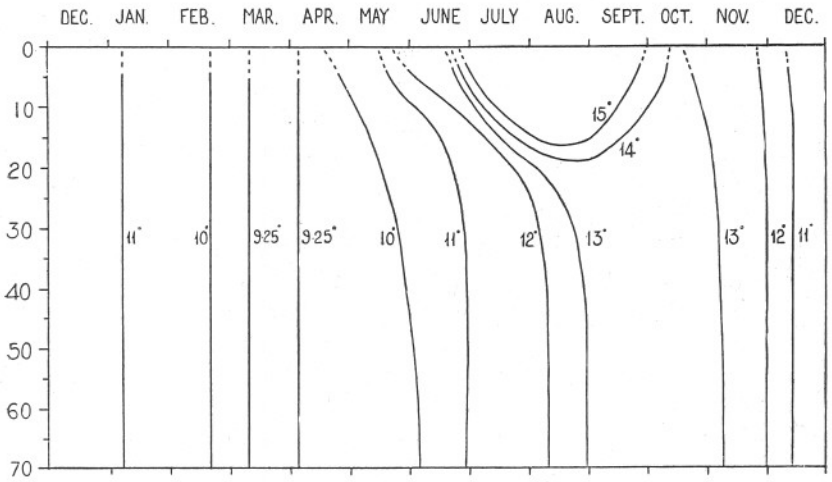

FIG. 7.—Diagram showing the seasonal changes of temperature that occurred at all depths at the international station, E1, in the year 1925. The depths are in metres, and degrees are Centigrade.

that they were deepest in mid-June when the light intensity was presumably at its highest, and that in July and August, when the temperature was still rising, they were higher in the water.

These observations point to the apparent paramount importance of light intensity—and by that we must understand certain compositions of light, owing to the selective absorption of sea-water—in controlling the behaviour of certain plankton animals.

That the slight changes occurring in other factors have no effect is by no means expected. It is necessary first to obtain actual readings of light intensity at different depths throughout the season correlated with plankton collections made at the same time as the light measurement. It should be possible then to find if the optimum intensity chosen by an animal at any one time remains constant throughout the season, or whether other factors may intervene to change it. The necessity for laboratory experiments on the effects of different factors on animals kept under varying light intensities is evident. Experiments employing light of the various compositions likely to be met with at different depths in the sea would be of great interest.

In this respect it is interesting to speculate on the possible importance played by the longer ultra-violet rays. It may well be that if these rays are present above a certain concentration they may exert a harmful or even lethal effect on certain organisms. The harmful effects of short ultra-violet rays in too great quantities on the higher animals is well known, and one would naturally suppose that plankton animals, which for the most part are transparent and have no direct pigmentary protection from the penetration of light rays, are especially susceptible. Huntsman (**7**) has demonstrated the destructive action of sunlight on *Calanus finmarchicus*, *Meganyctiphanes norvegica*, *Thysanoëssa inermis*, and other plankton species, and suggests that "the bathymetric distribution of such forms will be determined by the amount of sunlight in any region, and the distance to which it penetrates the water." Fox (**5**) also found that ultra-violet rays were the most active in causing Echinoderm larvæ and Paramoecium to seek the bottom of a jar lighted from the side.

Knudsen (**8**) found, by a spectro-photographic method, that the absorption in sea-water of the violet end of the spectrum was even more marked than that of the red rays, and that the green penetrated farthest. Grein (**6**), however, demonstrated the presence of ultra-violet rays (300–400 $\mu\mu$) at great depths, up to 1000 metres and more. It is a significant fact that Knudsen worked in water 9 m. deep in the Nyborg Fjord, while Grein made his observations in depths of over 1500 m. in the Mediterranean. The effect of fine particles in suspension on scattering light of small wave lengths is great and it is probably in this fact that the explanation lies as to the apparent disagreement of Grein's and Knudsen's results. In shallow waters and waters near to the coast the amount of matter in suspension is very great as compared with ocean water far from land. Close to land then the effect will be to increase the *apparent* coefficient of absorption of the shorter wave-length rays. In the case of Knudsen's observations the optimum wave-length for penetration was found to be in the green portion of the spectrum, 510 $\mu\mu$. From this point in the spectrum the coefficient of absorption increased towards the red end, due to the *actual* absorption of these rays by the sea-water; a similar and more marked increase was also shown towards the violet end, presumably in this case this was to a large extent *apparent* absorption, due to the scattering of the short wave-length rays by particles in suspension. It seems then quite permissible to suppose that as one moves away from the coast and its turbid waters to the clearer and purer water of the open sea there will be a gradual increase in penetration along the spectrum from the region of the green towards the violet and ultra-violet, until very nearly the maximum amount of ultra-violet light is able to penetrate. In pure water, free from particles in suspension,

there is hardly any absorption of the green end of the yellow light, 558 $\mu\mu$; the absorption of ultra-violet is, however, much greater. Raman (**11**, p. 53) quotes figures found by Count Aufsess, showing that for pure water selective absorption in the visual region ceased for wave-lengths less than 558 $\mu\mu$, the coefficients of absorption for the two wave lengths, 522 $\mu\mu$ and 494 $\mu\mu$, being 0·00002. Shelford and Gail (**16**, p. 157), in a table showing the transmission of light of different wave-lengths by pure water, give the maximum penetration as being in the region of 537 $\mu\mu$, and they give for ultra-violet light (300 $\mu\mu$) a penetration slightly greater than that for orange, 600·5 $\mu\mu$. It seems then probable that in the region in which I have carried out my plankton observations, ten miles from the coast, that the presence of a certain amount of ultra-violet light is to be expected in the upper water layers.

I should like to take this opportunity of thanking Dr. E. J. Allen F.R.S., and the Staff of this laboratory for much assistance; I am also especially indebted to Dr. H. H. Poole, Dr. W. R. G. Atkins, F.R.S., Mr. H. W. Harvey, and Mr. C. F. A. Pantin, for helpful criticism and advice.

SUMMARY.

1. Results of collections with the ring-trawl to obtain evidence on the vertical distribution of plankton animals in daylight at different times of the year and at night are given.

2. The vertical distribution of *Calanus finmarchicus* and *Cosmetira pilosella* is discussed in relation to the distribution of light intensity in the sea.

3. Results show that light intensity is apparently the external factor of greatest importance in determining the vertical distribution of these plankton animals in this region.

4. Many more observations at sea correlated with simultaneous records of light intensity at different depths are required, together with laboratory experiments on the effects of various factors on the behaviour of plankton animals kept under different conditions of light intensity.

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The Precipitation of Calcium and Magnesium from Sea Water.*

By

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With 2 Figures in the Text.

Apparently calcium carbonate is precipitated in certain parts of the ocean by processes which are inorganic in so far as that the calcium does not first form a true constituent of organisms (Clarke, 1920, p. 128). The conditions governing solubility of calcium in sea-water have been reviewed by Johnston and Williamson (1916) with the conclusion that surface layers of the ocean are approximately saturated, and that slight natural changes, particularly in carbon dioxide tension, might suffice to cause precipitation.

The molar concentration of magnesium in sea-water is about five times that of calcium. In marine sediments magnesium carbonate is a constituent which varies in proportion according to the organic remains producing them (Clarke and Wheeler, 1922). It is a frequent constituent of shells, but much less abundant than calcium. The mode of formation of magnesium-containing deposits is often obscure.

Magnesium hydroxide is precipitated from sea-water by addition of small amounts of alkali. In fact, both magnesium and calcium are in a delicate equilibrium where slight changes in alkalinity and carbon dioxide tension may cause precipitation. The delicacy of these equilibria and the nearness of their points of maximum sensitivity to natural conditions make calcium and magnesium particularly subject to changes induced by organisms. Likewise they are two of the most important elements biologically, both in amount and specific effect. It is therefore significant to examine the solubility conditions for both elements together, and to determine the conditions for their precipitation.

* I wish to express to Dr. Allen my appreciation for the extension of laboratory facilities and material and helpful personal interest, and to Dr. Atkins and Mr. Harvey for many suggestions and practical help.

The solubility product constants for the two carbonates are, at 16 degrees—

$$(1)\ K_{MgCO_3} = 1{\cdot}4 \times 10^{-4} \quad \text{(Johnston, 1915).}$$
$$(2)\ K_{CaCO_3} = 0{\cdot}98 \times 10^{-8} \quad \text{,,} \quad \text{,,}$$

The solubility product constant for calcite is practically equal to that for calcium carbonate (Johnston and Williamson, 1916).

In sea-water at pH 8 the excess base, which is practically a measure of $[HCO_3^-]$ is about $2{\cdot}5 \times 10^{-3}$ N. From the equation

$$(3)\quad [CO_3^=] = \frac{k_2\,[HCO_3^=]}{[H^+]} \quad \text{by substitution}$$
$$[CO_3^=] = 1{\cdot}35 \times 10^{-4}$$

From (2) $[Ca^{++}]\,[Co_3^=] = 0{\cdot}98 \times 10^{-8}$
and, from (2) and (3)

$[Ca^{++}] = 0{\cdot}73 \times 10^{-3}$ representing the solubility limit for $CaCO_3$ at pH 8 in sea-water.

By actual determination

$$[Ca] = 1 \times 10^{-2}$$

Therefore, sea-water at pH 8 is super saturated with $CaCO_3$.

The solubility product constants for magnesium hydroxide and calcium hydroxide are

$$(4)\ K_{Mg(OH)_2} = 1{\cdot}2 \times 10^{-11} \quad \text{(Johnston, 1915).}$$
$$K_{Ca(OH)_2} = 4{\cdot}1 \times 10^{-6} \quad \text{,,} \quad \text{,,}$$

In sea-water

$$[Mg] = 0{\cdot}05.$$

Substituting in (4)

$$[OH^-] = \sqrt{\frac{1{\cdot}2 \times 10^{-11}}{5 \times 10^{-2}}} = 1{\cdot}6 \times 10^{-5}$$

pH 9·2 is attained by photosynthesis experimentally and probably naturally (Atkins, 1922), and *Ulva* can apparently produce a pH close to 10. It therefore appears that magnesium hydroxide might be precipitated under natural conditions. In these calculations no allowance is made for activity factors or for the influence of neutral salts.

Addition of alkali to pH 10 is known to produce a precipitate in sea-water. Qualitative examination of such a precipitate showed CO_2, Ca, and Mg.

After preliminary tests, the two series reported in Tables 1 and 2 were made in which graded amounts of NaOH and Na_2CO_3, respectively, were added to samples of sea-water. After at least 24 hours shaking, the mixtures were filtered and a sample of filtrate analysed for Ca by McCrudden's (1909–10) method with $KMnO_4$. The residue in the flasks was

TABLE 1.

ADDITION OF NaOH TO 200 ML. SEA-WATER.

Number	NaOH added gram molecules per liter.	pH	In precipitate. Ca. gram molecules per liter.	Mg. gram molecules per liter.	% total Ca.	% total Mg.
11	0·00209	9·3	0·0002	0	10	0
12	0·00625	9·5	0·0003	0·00015	14	1·5
13	0·0174	10·0	0·00039	0·00161	19	16
14	0·0523	10·5	0·00039		19	
15	0·156	11·5	0·00083		38	
7b	0·278	11·6	0·00125	0·00857	57	86
Sea-water			0·00212	0·01		

TABLE 2.

ADDITION OF Na_2CO_3 TO 200 ML. SEA-WATER.

Number.	$\frac{Na_2CO_3}{2}$ added gram molecules per liter.	pH	In precipitate. Ca. gram molecules per liter.	Mg. gram molecules per liter.	% total Ca.	% total Mg.
18	0·00285	9·1	0	0·0006	0	0·8
19	0·00855	8·5	0·000875	0·00005	3·8	0·5
20	0·0259	9·3	0·0017	0·00005	81	0·5
21	0·0740	10·0	0·00917	0·0004	95	4·0
22	0·134	10·6	0·00183	0·0042	86	40
17b	0·438	11·0	0·00193		91	
Sea-water			0·00212	0·010		

washed into the filter with a small amount of 70% alcohol, and the filter washed once with alcohol. The residue was dissolved in standardized H_2SO_4 and titrated with NaOH. The difference between H_2SO_4 and NaOH was equivalent to ([Mg]+[Ca]) in the precipitate. [Ca] being determined in the filtrate, [Mg]=([Mg]+[Ca])−[Ca].

This procedure for determination was worked out with suggestions from Mr. H. W. Harvey. Qualitative tests on the amount of 70% alcohol used for washing showed only traces of Ca and Mg. Willstätter's method was not noted until later. It is similar, but uses alcohol and acetone titrations to effect a separation.

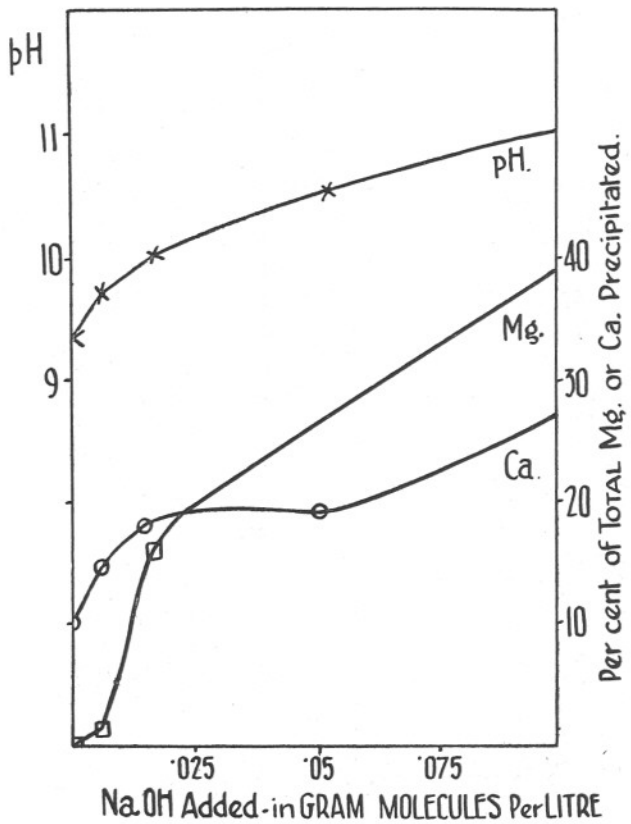

Fig. 1. Titration of sea-water with NaOH (part of curve from points given in Table 1).

Colorimetric pH determinations and Mg precipitated are consistent with many potentiometric titrations made in 1924.

Fig. 1 represents the lower part of the curve drawn from points given in Table 1, showing the relation of per cent Ca and Mg precipitated and pH against NaOH added. Fig. 2 shows data from Table 2 for Ca and Mg precipitation and pH against Na_2CO_3. It is conspicuous that either with Na_2CO_3 or NaOH, Ca exceeds Mg in the precipitate. Na_2CO_3 precipitates much more Ca, and relatively little Mg up to pH 10. A

small amount of Mg is precipitated by Na_2CO_3. These facts agree with the much greater solubility product of Mg CO_3. NaOH precipitates increasingly less Ca above pH 10, conforming with the greater solubility of $Ca(OH)_2$ than of $CaCO_3$.

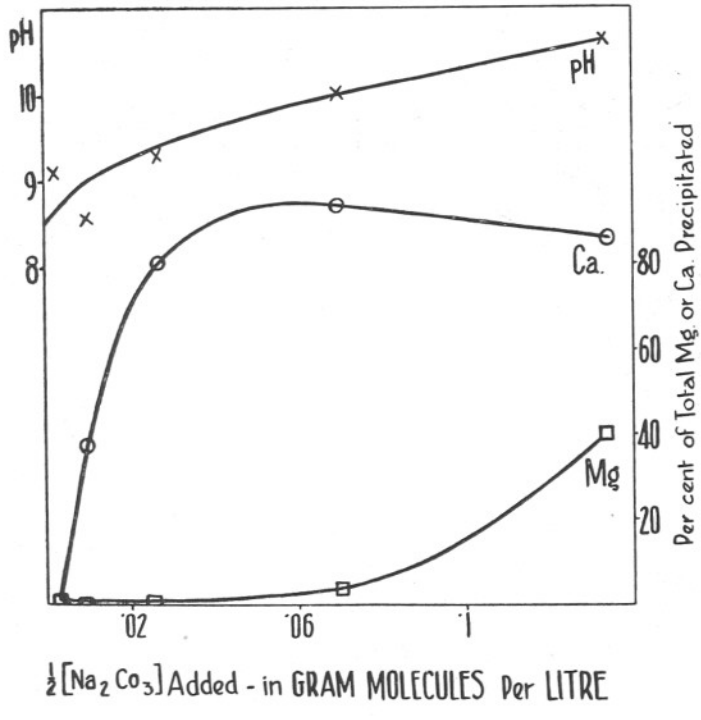

FIG. 2. Titration of sea-water with Na_2CO_3.

Unfortunately, methodical uncertainty is greatest in the regions of low alkalinity and principal biological importance. The investigations show that Ca and some Mg may be precipitated under possible conditions of natural sea-water alkalinity, although it is another question as to how frequently this alkalinity is attained. The same conditions governing precipitation outside of the organism may explain the excess of Ca over Mg in organic "formed" precipitates, as alkalinity necessary for magnesium precipitation is much more difficult for the organism to attain, especially within its tissues.

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The Phosphate Content of Sea Water in relation to the Growth of the Algal Plankton. Part III.

By

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With 5 Figures in the Text.

THE first two papers of this series, which appeared under slightly different titles (1923, 1925), showed that there is a seasonal change in the phosphate content of the surface water of the English Channel, thus confirming the work of Matthews, who found that in spring and summer almost all the phosphate was used up, presumably by plants. It was further shown that such changes were experienced by the deeper water also, so that when the surface water was at its minimum phosphate value the bottom water, 70 metres, at Station E1 was reduced to 11 mg. per cubic metre, less than a third of its winter and maximum value.

A comparison of the years 1923 and 1924 made it clear that the changes were qualitatively identical, and quantitatively very nearly so, differing only slightly in the maximum and minimum values, though more noticeably in the dates at which these values were reached.

Since all the phosphate is used up when and where the illumination is sufficient it appears to be the factor limiting further growth, so knowing approximately its concentration in the phytoplankton it is possible to make an estimate of the wet weight of vegetable matter produced. This is obviously a minimum value, for in its simplest form it neglects, in this estimate of the annual crop, the possibility of a portion of the phosphate being used twice over, or more often, through decay, or the consumption of the plant cells by animals—the latter process being followed in time by excretion or decay.

Again, since the deeper the water the less is the light intensity, it seemed probable that water from the region of total darkness might be far richer in phosphate than that near the bottom in the comparatively shallow English Channel. This supposition was confirmed to some extent by the results recorded in Tables 11, 12 and 13 of Part II.

Furthermore, the bright light experienced even in winter in latitudes nearer the equator gives reason to suppose that under such conditions

the seasonal changes in phosphate concentration will be less marked than they are here. Data confirming this view are given in Table 14 of Part II. There is another reason why the surface waters in warm latitudes should remain poor in phosphate, namely, the stability of the water owing to the marked temperature gradient, so that warm water is normally present at the surface. In our northerly latitudes, as the water surface cools in autumn, a stage is reached when the bottom water is the warmer. A condition of instability is thus produced and a very complete vertical mixing ensues, save in special cases where differences in salinity are sufficient to maintain the higher density at the bottom.

In the present paper data on the seasonal changes for 1925 are presented, and their onsets compared with those of the two preceding years. Owing to the assistance of scientific colleagues and others who so kindly provided the author with water samples, records of temperature and of position, it has also been possible to include analyses of water from a wide area of the Atlantic Ocean and the North Sea, including specimens from great depths, unobtainable hitherto, also some from the Pacific Ocean. These afford information concerning the seasonal changes, or their absence at different latitudes, and are of particular interest in permitting a conclusive test to be made as to the richness of deep ocean water.

It may be added that the depth series results for August 8th, 1924, in the Faroe-Shetland Channel, given in Part II, Table 12, has now been definitely proved to have been vitiated by the phosphate yielded by the white glass bottles; this was suspected at the time, and the bottles when tested later by storage gave up phosphate to distilled water. Fortunately, however, another series from near the same position became available this year, and the analyses are shown in Table 9. The green glass bottles in general use yield no phosphate.

Seasonal Changes in the Phosphate Content at L Stations, 1925.

Table 1 shows the changes in phosphate undergone by the surface water from L1, in the Sound in front of the Laboratory, past L5 (the Eddystone), right out to the International Hydrographic Station E1, twenty-two miles from L1. Similar data for 1923 and 1924 have been presented in Parts I, Table 5, and II, Table 1. This year, however, a series of bottom samples, from L4, three miles clear of the headlands, was also examined. The low summer values, denoting complete exhaustion of phosphate supplies, are clearly shown, the figures for August 31st being specially noteworthy, for it is seen that in spite of a considerable regeneration of phosphate early in the month, it has been very completely used up right down to the bottom; this change has taken place

to an even greater extent than at the same depth at E1, for L4 being nearer the shore there is rather more vertical mixing of the water. This series is naturally somewhat irregular, owing to the presence of phosphate carried out by the river water. The irregularity is, however, far less than might be expected.

TABLE 1.

SURFACE VALUES FOR PHOSPHATE AS P_2O_5 IN MG. PER M^3 FROM L1 TO E1, 1925, AND L4, 40 M.

Station	Jan. 19th	Feb. 17th	Mar. 14th	April 22nd	May 13th	June 3rd	July 8th	Aug. 5th	Aug. 31st	Oct. 1st	Nov. 11th	Dec. 11th	Jan. 12th
L1	—	25	26	—	5	—	3	6	—	—	—	28	48
L2	39	41	28	2	0	—	2	6	—	28	—	27	43
L3	28	—	27	—	0	4	—	6	—	22	23	27	40
L4	28	33	38	3	1	2	3	7	1	15	18	25	40
L4 (40)	32	29	46	26	5	19	6	23	2	20	24	24	—
L5	28	29	29	2	0	4	2	8	1	6	24	24	40
L6	30	31	14	—	0	4	3	2	—	7	26	19	40
E1	31	33	23	1	1	3	0	5	0	8	25	12	—*
Analysed	Jan. 21st	Feb. 20th	Mar. 17th	April 23rd	May 16th	June 4th	July 10th	Aug. 6th	Sept. 5th	Oct. 2nd	Nov. 13th	Dec. 17th	Jan. 12th

SEASONAL CHANGES IN THE PHOSPHATE CONTENT AT STATION E1.

In Table 2 the data given in Parts I, Table 7, and II, Table 3, are continued. The results for the three years are shown in Fig. 1 for the surface water and in Fig. 2 for the bottom. The high values for 15 m. on March 14 and 10 m. on June 3 are evidently due to the inclusion of a particle rich in phosphate or to chance pollution of the water by fish, etc. Such instances have been noted previously. Special care was taken this year always to examine the samples when quite fresh, so that the high values shown for August 5 are undoubtedly due either to regeneration of phosphate at, or to the movement of water, richer in phosphate, to, E1. This confirms the apparent regeneration shown in August and September of the 1923 curve, though in these cases a delay of about ten days between the drawing of the sample and its analysis introduced some measure of doubt. Regeneration, a process which we must imagine to be continually active, becomes noticeable only when the phosphate set free remains in a region poorly illuminated. Thus as may be seen in Table 2 and Fig. 3 there was a noticeable renewal of phosphate in the bottom water between May 13 and June 3. During this time, be it noted, the bottom water remained at 10·0°, whereas the

* May safely be taken as 40.

surface water rose from 10·7° to 12·6°; but by July 8 the bottom had risen to 11·8°, denoting a considerable mixing with the warmer surface water, which by this time had risen to 16·0°. Consequent upon the mixing the phosphate content fell to slightly under half its June value. Between

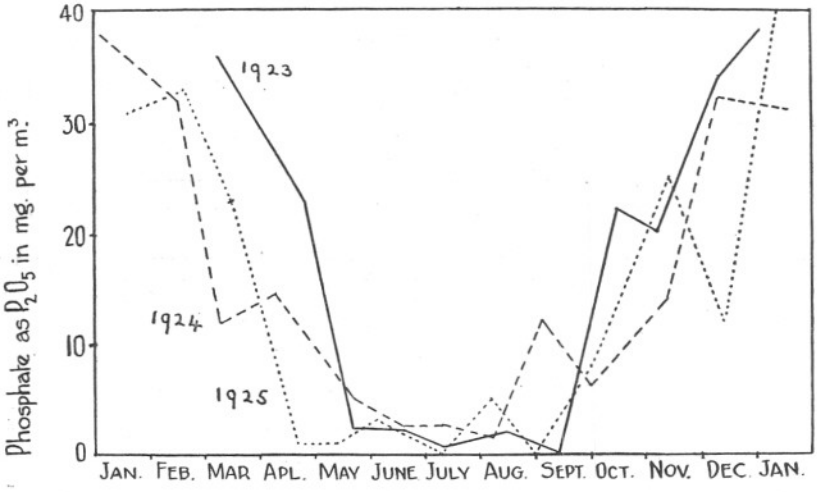

FIG. 1.—Seasonal changes in phosphate concentration at Station E1, surface.

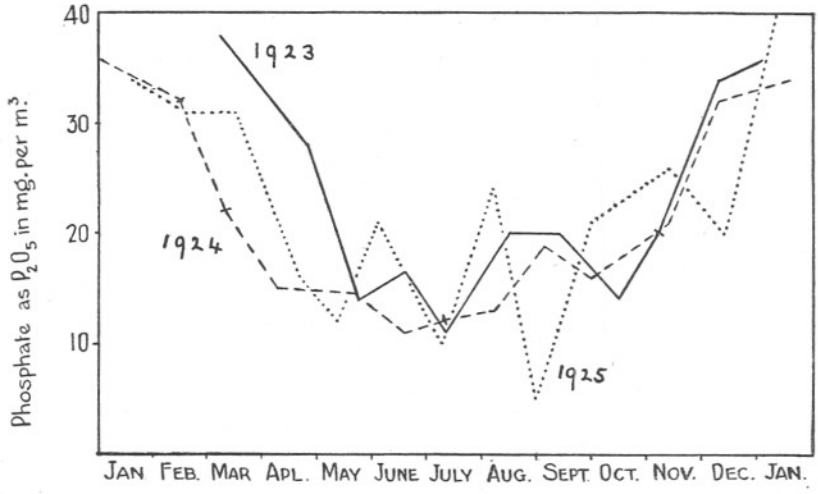

FIG. 2.—Seasonal changes in phosphate concentration at Station E1, bottom, 70 metres.

July 8 and August 5 the bottom only rose 0·2°, and again the bottom water became enriched with phosphate. The August 31 series shows a gain of 0·8° for the bottom, and a fall in phosphate to about one-fourth of its previous value. This stripping of the water, so that from 20–70 m.

it contained only 6 mg. per m^3, has been more complete this year, 1925, than in either of the two previous years. Unfortunately it was not possible to obtain samples early in September to see whether the minimum had been reached, but October and November analyses showed the normal autumnal preponderance of regeneration.

For 1923 the average consumption at Station E1, surface to bottom, 70 metres, was 29·6 mg. of P_2O_5 per m^3, leaving a balance of 7·4 out of the original 37 mg., which, however, was a March value. For 1924

TABLE 2.

VALUES FOR PHOSPHATE AS P_2O_5 IN MG. PER M^3 AT STATION E1, SURFACE TO BOTTOM, DURING 1925, ALSO SURFACE AND BOTTOM TEMPERATURES, THEIR DIFFERENCES AND THE DIFFERENCES BETWEEN SUCCESSIVE OBSERVATIONS OF BOTTOM TEMPERATURE.

Depth	Jan. 19th	Feb. 17th	Mar. 14th	April 22nd	May 13th	June 3rd	July 8th	Aug. 5th	Aug. 31st	Oct. 1st	Nov. 11th	Dec. 11th	Jan. 12th
0	31	33	23	1	1	3	0	5	0	8	25	12	40*
5	—	31	25	—	4	6	3	7·5	2	11	—	—	—
10	—	—	28	3	5	104	5	7	2	11·5	26	19	—
15	—	—	38	—	5	7	6	9	4 5	14	—	—	—
20	—	—	—	—	7	12	5	16	6	17	—	19	—
25	31	—	27	5	7	—	5	23	—	19	—	—	—
30	—	—	—	—	11	17	7	24	6·5	19	26	19	—
40	—	—	—	—	12	—	7	—	6·5	24	—	—	—
50	32	—	30	13	—	21	9	—	—	20	—	—	—
60	—	—	—	—	—	—	10	—	—	21	—	—	—
70	34	31	31	16	12	21	10	24	5	21	26	20	—
Analysed	Jan. 21st	Feb. 20th	Mar. 17th	April 23rd	May 16th	June 4th	July 10th	Aug. 6th	Sept. 5th	Oct. 2nd	Nov. 13th	Dec. 17th	Jan. 12th
0	10·8°	9·8°	9·3°	9·8°	10·7°	12·6°	16·0°	15·4°	16·3°	14·9°	11·8°	10·8°	9·9°*
70	10·8°	10·0°	9·2°	9·5°	10·0°	10·0°	11·8°	12·0°	12·8°	13·6°	13·0°	11·0°	—
Δt	0·0°	–0·2°	0·1°	0·3°	0·7°	2·6°	4·2°	3·4°	3·5°	1·3°	–1·2°	–0·2°	—
Δ′t	—	–0·8	–0·8	+0·3	0·5	0·0	1·8	0·2	0·8	0·8	–0·6	–2·0	—

the corresponding figures were: used up, 28·3 mg.; balance unconsumed, 8·7 mg.; winter concentration having again been 37 mg. For 1925 the maximum was probably missed; it occurred about January 2 in 1924, but no samples were available till January 19th this year. However, December 9th, 1924, gave 32 mg. and January 19th 31·9 mg.; accordingly taking 32 as the maximum, and the minimum value of August 31, namely, 5·1 mg., the amount used up was 26·9 mg. per m^3. These, however, are gross values. They take no account of the amounts

* In reality these are values for L6, but as L3–L6 gave same value for phosphate water was uniform and values may be taken for E1. Weather too bad to do depth series, water probably uniform to bottom.

set free by regeneration and again utilized. Were this known accurately the total crop could be found with a correspondingly increased exactness. An attempt may be made to do this by finding out, for each year, the total consumption of phosphate, including what is ascertained to have been available owing to regeneration or replenishment by water which may have moved into the area studied.

Data showing the monthly loss, viz. utilization, of phosphate and the gain, viz. regeneration, for the whole water column, surface to bottom at Station E1 are shown in Table 3 and in Fig. 4 for the three years.

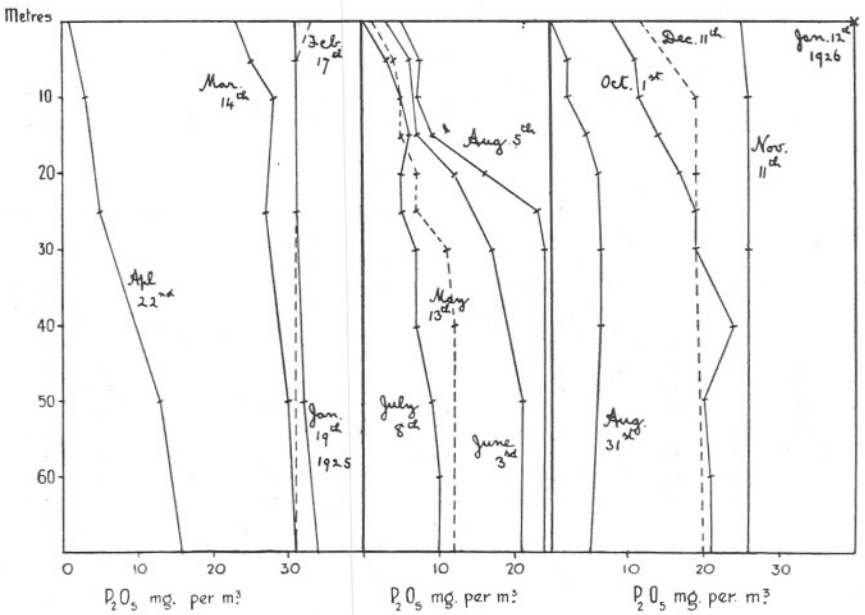

FIG. 3.—Variation of phosphate concentration with depth at Station E1 during 1925. For the sake of clearness the curves for the months have been plotted in three sections.

The years are, in broad outline, very similar, thus in each the mid-winter value is a maximum and the July a minimum at 7·4, 8·7 and 6·9 mg. per m³, though the last value is exceeded in 1925 by the late August value, 5·1 mg., the absolute minimum for the water column for the three years. Inspection of Fig. 4, however, shows clearly that striking differences exist. Thus in 1923, April, and to a greater degree May, witnessed a marked increase in the phytoplankton ; there was further increase in July and scarcely any in September. In 1924 development had commenced in February, and the March increase slightly exceeded that of April, 1923 ; April, May and June show a progressive wane in the increase ; thereafter down-grade changes predominate, save for a slight

production at the end of September. The 1925 season was late at the outset, even March showing less development than February, 1924. The end of March and the first half of April witnessed, however, a remarkable outburst in the phytoplankton. Table 2 and Figs. 1 and 3 show that almost all phosphate in the well-illuminated regions has been used up, even at 25 metres only 5 mg. per m^3 remained. As might be expected there was no further increase in May, and in June there was a marked regeneration. Thus it is seen that the vernal development of phytoplankton was a maximum in May, 1923, March, 1924, and April,

TABLE 3.

PHOSPHATE CONTENT OF THE WATER COLUMN, SURFACE TO BOTTOM, 70 METRES, AT STATION E1, WITH DIFFERENCES BETWEEN THE CONSECUTIVE OBSERVATIONS.

	1923.		1924.		1925.	
	P_2O_5 in mg. m^3	Δ	P_2O_5 in mg. m^3	Δ	P_2O_5 in mg. m^3	Δ
Jan.	—	—	37·0	+3·0	31·9	−0·1
Feb.	—	—	32·0	−5·0	31·1	−0·8
Mar.	37·0	—	22·1	−9·9	28·3	−2·8
April	27·7	−9·3	15·0	−7 1	8·5	—19·8
May	11·6	—16·1	12·1	—2·9	9·2	+0·7
June	13·2	+1·6	9·1	−3·0	15·8	+6·6
July	*7·4*	−5·8	*8 7*	−0·4	6·9	−8 9
Aug.	16·1	+8·7	10·1	+1·4	19 2	+12·3
Sept.	13·9	−2·2	16·1	+6·0	*5·1**	−14·1
Oct.	16·0	+2·1	14·9	−1·2	18·2	+13·1
Nov.	20·0	+4·0	20·1	+5·2	25·9	+7·7
Dec.	34·0	+14·0	32·0	+11·9	18·6	−7·3
Jan.†	—	—	—	—	40	+21·4
Total consumption	—	33·4	—	29·5	—	53·8
Max. minus minimum	—	29·6	—	28·3	—	26·9†
Total regeneration	—	33·4‡	—	24·5	—	61·7
Gain in free phosphate A	—	−3·0	—	−5·0	—	+8§
Gain in free phosphate B	—	−0·0	—	−5·0	—	+7·9

1925. Furthermore, late in June and early in July, 1925, the May and June regeneration, coupled with the vertical mixing (which is indicated by the rise in temperature in the bottom water recorded in Table 2), resulted in an outburst of phytoplankton similar to that of 1923, but greater. The remainder of July and early August were periods in which down-grade changes were well marked. But by late August vertical mixing, as again indicated by a rise in bottom temperature, afforded sufficient phosphate for a development of phytoplankton, which was

* Aug. 31st. Minimum values are in italics.
† Jan. 12th, 1926.
‡ Including value for Jan. 2, 1924.
§ Taking Dec., 1924, value as maximum.

about three-quarters of the April outburst, almost as great as that of May, 1923 (the maximum for that year), and greater than in any single month of 1924. The lower part of Table 3 shows that the total consump-

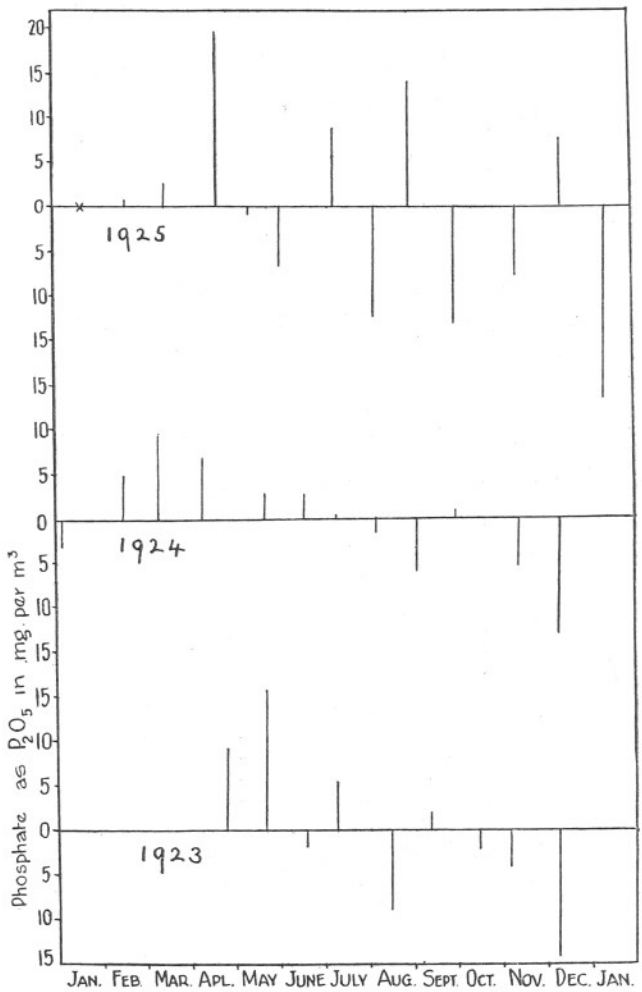

FIG. 4.—The production of phytoplankton may be deduced from the uprights above the zero line; in reality these lines show the difference in the phosphate content of the water between two successive observations. Increase in phosphate, being equivalent to destruction of phytoplankton is represented below the zero line.

tion proved for 1925 is considerably greater than for the other two years; but it must be borne in mind that this may only be apparent, due to the alternation of the processes of consumption and regeneration. It is none the less of biological importance. The results for "Gain in free

phosphate" have been arrived at in two ways: (A) by subtracting the initial and final values, and (B) by subtracting the total consumption from the total regeneration. There is a difference of 3 mg. between the two for 1923, for 1924 they agree, and for 1925 the difference is 0·1 mg. The concordance must be considered satisfactory. The "A" values are obviously the most correct; it is to be noted that they indicate a slight retention of phosphate during 1923 and 1924, with an exactly equal total liberation by January, 1926. From the March, 1923, value and the latter, however, 3 mg. more phosphate is shown as free at the end of the three years than at the beginning. The January, 1923, results were not obtained. The foregoing figures make it quite clear that the phosphate cycle is essentially a closed one. There is no evidence that

TABLE 4.

DATES WHEN CERTAIN PHOSPHATE CONCENTRATIONS WERE REACHED AT E1, SURFACE. TAKING THE 1923 DATES AS ARBITRARY STANDARDS THE ADVANCES OR RETARDATIONS OF 1924 AND 1925 ARE RECORDED.

P_2O_5 mg.	1923	1924	1925	Ahead in days, 1924	Ahead in days, 1925
30	Mar. 30th	Feb. 18th	Feb. 25th	41	33
20	April 28th	Feb. 28th	Mar. 20th	59	39
10	May 10th	April 28th	April 6th	12	34
2·5	May 21st	June 18th	April 18th	−28	33
10	Sept. 28th	Sept. 2nd	Oct. 6th	+26	—
		Oct. 20th		−22	−8
20	Oct. 11th	Nov. 22nd	Nov. 1st	−15	+6
	Nov. 7th		Dec. 21st	—	−44
30	Dec. 1st	Dec. 6th	Jan. 2nd	−5	−32

water movement affected this area materially during the period under consideration.

The differences between the corresponding seasons in the various years have already been discussed. A closer comparison may be made by a study of Tables 4 and 5, which show the dates at which certain phosphate concentrations were reached, as obtained by reading off from Figs. 1 and 2.

It is of interest to consider why the vernal outburst of phytoplankton, as indicated by phosphate consumption should have been earliest in 1924, latest in 1923, with 1925 occupying an intermediate position. In Fig. 5 are shown the mean monthly sunshine records for England, S.W. These

are plotted for the 15th of each month, save for November and December, 1925, when the values taken were from the Plymouth records only, averaged over the periods between consecutive phosphate determinations. Inspection reveals the fact that the vernal outbursts occur in the three years in the same order as do the amounts of spring sunshine. Later on in the year the same exact correlation cannot be traced, for the phosphate is all used up near the surface. During the summer, as already mentioned, the important point is how extensively the phosphate from the depths is brought into the upper layers. In the autumn as the light becomes reduced and regeneration preponderates, the quantity of light will again determine how far the phytoplankton die out. There is, however, another factor, the smoothness of the sea, for in rough water much

TABLE 5.

DATES WHEN CERTAIN PHOSPHATE CONCENTRATIONS WERE REACHED AT E1, BOTTOM. TAKING THE 1923 DATES AS ARBITRARY STANDARDS THE ADVANCES OR RETARDATIONS OF 1924 AND 1925 ARE RECORDED.

P_2O_5 mg.	1923	1924	1925	Ahead in days, 1924	Ahead in days, 1925
30	April 14th	Feb. 20th	Mar. 17th	54	28
20	May 9th	Mar. 17th	April 11th	53	28
11	July 9th	June 18th	July 6th	21	3
			June 1st	—	—
20	Nov. 6th	Nov. 4th	July 29th	—	—
			Sept. 29th (final)	2	38
30	Dec. 1st	Dec. 4th	Dec. 28th	−3	−27

of the light is scattered and reflected at the surface, and the quantity entering is correspondingly reduced. The year 1924 had no November outburst of phytoplankton (see Fig. 4), and Fig. 5 shows that its sunshine average was low. However, 1923 and 1925 were very similar in sunshine, yet only 1925 gives evidence of a remarkable development of diatoms as mentioned in the author's paper on silicate in sea-water. Fig. 4 is constructed from the average phosphate content of the whole water column, and shows no phytoplankton development in the autumn. Fig. 1, however, which concerns surface data only, does show a decided fall in phosphate in November, and so indicates plant growth, though regeneration of phosphate in the deeper water masks the result to some extent. But if the 46th to 49th weeks of each of the two years, 1923 and 1925, are considered, namely, approximately the periods between

the observations (November 7th and December 10th and November 11th and December 11th respectively), it is seen that 1923 averaged 3·1 hours and 1925 only 3·0, so it is evident that the amount of sunshine alone will not explain the difference, nor will the total daylight, for the dates are almost identical. It may be noted that the vertical circulation must have been more pronounced in 1925 than in 1923 over the November

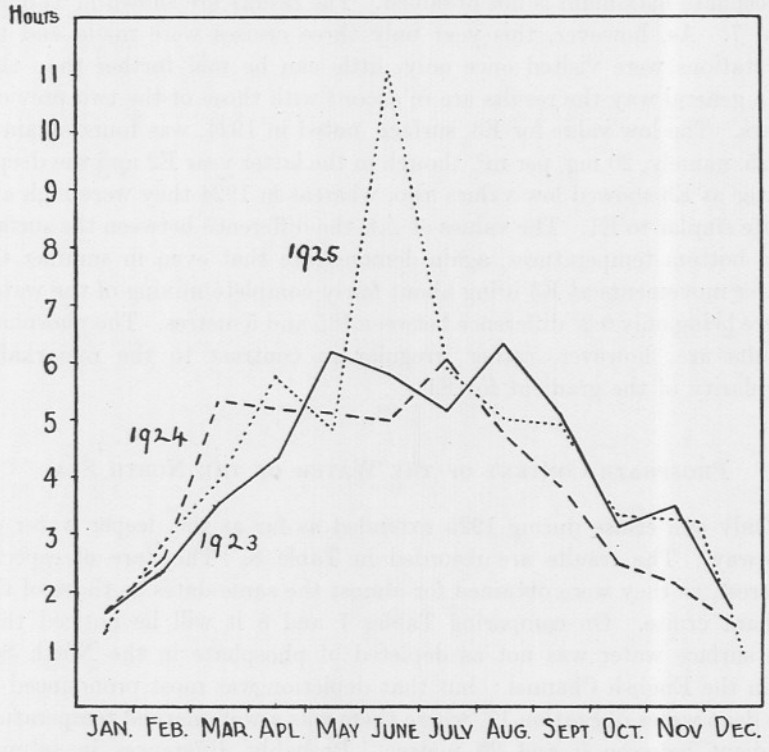

FIG. 5.—Mean monthly sunshine records for England, S.W. (including Wales), plotted for 15th of each month. For the sake of clearness the curves for the months have been plotted in three sections.

to December interval, for in November, 1923, the temperature difference between surface and 25 m. was only −0·17°, and 25 m. was the same as the bottom. It is possible that movement may have aided the mixing. In December the surface was 0·30° cooler than 25 m., and the latter 0·05° cooler than the bottom. In 1925, however, there was −1° difference between surface and 25 m., and the bottom was 0·2° warmer than the latter in November, whereas in December the reversed gradient from surface to bottom was only 0·2°.

Seasonal Changes in the Phosphate Content of the Water of the English Channel at the E and N Stations.

These stations, with the exception of E1 which has already been discussed, are only visited five times a year at the most, and the programme does not, unfortunately, include a visit at midwinter, so the phosphate maximum is not obtained. The results are shown in Tables 6 and 7. As, however, this year only three cruises were made and the N stations were visited once only, little can be said further than that in a general way the results are in accord with those of the two previous years. The low value for E3, surface, noted in 1924, was found again in 1925, namely, 20 mg. per m^3, though in the latter year E2 and the deeper water at E3 showed low values also, whereas in 1924 they were high and quite similar to E1. The values of $\triangle t$, the difference between the surface and bottom temperatures, again demonstrate that even in summer the water movements at E3 bring about fairly complete mixing of the water, there being only 0·2° difference between 105 and 5 metres. The phosphate results are, however, rather irregular in contrast to the remarkable regularity of the gradient for E1.

Phosphate Content of the Water of the North Sea.

Only one cruise during 1925 extended as far as the deeper water off Norway. The results are recorded in Table 8. They are of especial interest, as they were obtained for almost the same dates as those of the Ushant cruise. On comparing Tables 7 and 8 it will be noticed that the surface water was not as depleted of phosphate in the North Sea as in the English Channel; but that depletion was most pronounced in the deep water of Station 17, where there was a well-marked temperature gradient between 0 and 20 metres. Probably differences in salinity account for the inverted gradient in temperature in the deeper water at this station. The richness of the bottom water in phosphate at all the stations is very remarkable, Stations 2–16 average 44 mg. per m^3, with, except No. 16, bottom at 60–80 metres. Station 17 has 49 mg. at 350 m. Evidently the season is later in the North Sea than in the Channel, but this explains only the lesser depletion of the surface, not the richness of the bottom. In Part II, Tables 10 and 11, it may be seen that though one value for May, 1924, was up to 41 mg., none of the winter values, or the spring deep water values, were as high as those found in 1925. A somewhat richer than usual algal plankton might, therefore, have been expected that year, or at least a more marked outburst at one time.

Table 6.

Phosphate as P_2O_5 in mg. per m^3 on cruise of February 17th to 18th, 1925. Surface and Bottom Temperatures are shown below. Analysed February 20th.

Depth.	E 1.	Mid.‡	E 2.	E 3*.	N 1.	Mid.	N 2.
0	33	19	16	20	20	21	26
5	31	—	19	—	26	—	26
15	—	—	21	—	25	—	31
50	—	—	—	—	—	—	31
70	31	—	—	—	—	—	—
90	*	—	21	—	—	—	30
100	*	*	*	22	24	*	*
0	9·8°	10·8°	10·3°	10·5°	10·4°	10·4°	10·1°
Bottom	10·0°	—	10·7°	10·8°	10·6°	—	10·3°

Table 7.

Phosphate as P_2O_5 in mg. per m^3 and Temperature on Cruises of May 13th to 14th and July 8th to 9th, 1925.

Depth.	E1.		E2.		E3.		E1.		E2.		E3.	
	t°.	P_2O_5.	t°.	P_2O_5.	t°.	P_2O_5.	t°.	P_2O_5.	t°.	P_2O_5.	t°.	P_2O_5.
0	10·7	1	11·8	0	10·8	0	16·0	0	15·6	0	12·6	2
5	10·50	4	10·70	10	10·80	21	15·75	3	14·80	3	12·10	20
10	10·30	5	10·40	13	10·68	25	14·30	5	13·90	5	12·10	17
15	10·00	5	—	11	—	24	12·03	6	12·35	12	12·08	25
20	9·97	7	10·32	11	10·46	24	11·85	5	—	5	12·08	17
25	—	7	10·32	7·5	—	16	—	5	12·00	12	—	18
30	9·97	11	10·32	9	10·45	18	11·80	7	—	—	12·02	20
40	—	12	10·32	15	—	17	—	7	—	12	—	31
50	9·97	—	10·32	12	10·45	22	11·80	9	11·90	—	11·98	24
70	9·96	12	10·32	12	10·45	—	11·80	10	11·88	13	11·92	23
80	*	*	—	—	—	23	*	*	—	—	—	—
90	*	*	10·32	10	—	—	*	*	11·88	10	—	—
105	*	*	*	*	10·20	16	*	*	*	*	11·90	25
Δt	0·7	—	1·5	—	0·6	—	4·2	—	3·7	—	0·7	—

Intermediate stations on course to E3, surface :—

May, 18 miles on from E1, P_2O_5=0. July, P_2O_5=1.
„ 26 „ „ E2, P_2O_5=0. „ P_2O_5=0.

* Not as far as E 3, position 48°56′N, 4°54′W.
‡ Midway between adjacent stations.

Whether the source of the higher phosphate content of the North Sea in May, 1925, was regeneration during the winter or an influx of richer water cannot as yet be decided till the hydrographic results deduced from salinity changes become available; the phosphate content of the water in the Faroe-Iceland and Faroe-Shetland Channels in July, 1925, was, however, such as to afford no argument against the possibility of a movement of water rich in phosphate into the North Sea. These results are shown in Table 9. It may be added that reference to Part II, Table 12, makes it clear that in 1924 the Shetland results would have been definitely against such a view, for the values were from 8–25 mg. per m^3, save for one high value, 79 mg. in warm water south of the Wyville Thomson ridge at 800 m. As previously mentioned the August values recorded in that table are vitiated by phosphate from the glass. For some unex-

TABLE 8.

PHOSPHATE IN DEEP WATER OFF NORWAY AND IN NORTH SEA IN GENERAL, MAY 14TH TO 16TH, 1925. ANALYSED MAY 25TH. POSITION: 57°57′N., 6°45′E. FOR STA. 17.

	Sta. 17		Sta. 16		Sta. 12	Sta. 10	Sta. 8		Sta. 6	Sta. 4	Sta. 2	
Depth in metres	P_2O_5 mg. per m^3.	t°.	P_2O_5.	t°.	P_2O_5.	P_2O_5.	P_2O_5.	t°.	P_2O_5.	P_2O_5.	P_2O_5.	t°.
0	6	9·08	10	8·94	13	13	11	8·74	14	13	15	8·34
20	10	5·98	—	7·60	—	—	—	8·45	—	—	—	7·45
40	31	5·20	—	5·57	—	—	—	6·74	—	—	—	6·34
60	35	5·83	40	5·98	37	43	49*	6·30	48	44†	45*	6·33
100	40	6·63	—	6·95	*	*	*	*	*	*	*	*
150	41	7·10	—	7·04	—	—	—	—	—	—	—	—
200	41	6·56	—	7·04	—	—	—	—	—	—	—	—
250	44	6·75	—	6·88	—	—	—	—	—	—	—	—
300	46	6·48	—	6·79	—	—	—	—	—	—	—	—
350	49	6·08	*	*	—	—	—	—	—	—	—	—

plained reason the July, 1925, Shetland values are far higher than those of March to May, 1924. Such variations may afford the key to the periodic changes in the productiveness of the North Sea fishing grounds. The results recorded in Table 9 illustrate well the storage of phosphate in the deeper waters.

PHOSPHATE CONTENT OF THE WATER OF THE OPEN ATLANTIC.

In Table 10 are shown a few values for the open water south of Ireland and in the Bay of Biscay. The inshore water surface values are high in winter, and the May results show how the lines of equal phosphate content move upwards as the shore is approached. Thus at 60 m. near

* Depth, 70 m. † Depth, 80 m.

TABLE 9.

PHOSPHATE CONTENT AND TEMPERATURE IN (A) THE FAROE-ICELAND CHANNEL, 4/7/'25, AT 62°53'N., 9°05'W.; IN (B) THE FAROE-SHETLAND CHANNEL, 6/7/'25, AT 61°27'N. AND 4°23'W.; ALSO (C) AT 61°02'N., 3°22'W. ANALYSED 22 AND 23/7/'25.

	A.		B.		C.	
Depth.	P_2O_5	t°	P_2O_5	t°	P_2O_5	t°
0	70*	10·23	30	10·95	23§	12·90
10	30	10·20	30	10·12	15	11·89
20	30	9·90	27	9·61	21	10·92
30	30	9·43	23	9·38	—	10·42
40	41	8·64	43	8·38	33†	10·15
60	38	8·32	53	7·83	—	9·66
80	53	8·08	70	7·67	40	9·44
100	60†	8·03	57	7·52	43	9·30
200	57	7·74	57	6·96	—	8·42
300	57	7·51	—	5·33	54	7·30
400	57	2·03	—	2·49	—	6·09
500	59	3·38	43	0·99	54	+2·22
600	★	★	—¶	+0·17	57	−0·48
700	★	★	—¶	−0·36	—	−0·60
800	★	★	—¶	−0·47	59	−0·70
900	★	★	43‡	−0·50	★	★
1000	★	★	58	−0·56	★	★

TABLE 10.

PHOSPHATE AND TEMPERATURE AT VARIOUS POSITIONS AND DEPTHS ON THE ATLANTIC SLOPE.

Date.	Depth in m.	P_2O_5 in mg. per m^3	t°.	Position. N.	Position. W.	Notes.
3.2.'25	0	34	10·7	51°20′	9°38′	Near Fastnet.
5.2.'25	0	44	9·7	51°25′	8°10′	Both analysed, 19.2.
14.5.'25	0	15	11·1	50°34′	11°17′	Analysed, 5.6.
,,	402	32	10·2	,,	,,	Depth, 1038 m.
,,	1006	60	8·8	,,	,,	Diatoms (Rhizosolenia) very abundant in tow-net.
12.5.'25.	0	14	10·6	49°20′	8°00′	
,,	60	35	9·9	,,	,,	
,,	134	35	9·9	,,	,,	
8.6.'25.	366	32	—	45°50′	9°00′	Analysed, 13.7.
,,	824	54	—	—	—	In Bay of Biscay.

* A considerable amount of suspended matter in water sample.
† Result checked against fresh standard.
‡ Noted at time of analysis as obviously a much weaker tint than 1,000 m. value.
§ Noted as definately stronger than 10 m. value.
¶ Three bottles broken.

the coast 35 mg. was found, and 32 further out at 402 m. and the same at 366 m. in the Bay. The bottom values for the deep water are almost identical in Tables 9 and 10, 58 and 60 mg. respectively at 1000 m.

Table 11 contains similar results for the very deep water further south, between the latitudes of Lisbon and the Canary Isles. Again the accumulation of phosphate in the depths is very marked, but the most striking result is the complete removal of phosphate from the upper layers down to 50 m., with as little as 5 mg. at 75 m. This is due apparently to the fact that photosynthesis is active to greater depths owing to the more

TABLE 11.

PHOSPHATE CONTENT AND TEMPERATURE AT 37°44′N. AND 13°21′W., ON OCTOBER 12, 1925, SAVE THAT AT 3000 M., TAKEN ON OCTOBER 16, AT 29°59′N., 15°03′W. ANALYSED OCTOBER 30 TO NOVEMBER 2.

m.	P_2O_5 in mg. per m^3.	t°
0	0	21·10
40	—	21·00
50	0	20·01
75	5	17·31
100	8	15·10
150	10	15·06
200	22	13·86
300	44	12·25
500	50	10·94
1000	74	9·55
2000	78	4·81
3000	88	3·10

intense illumination in southerly latitudes. The behaviour of nitrates in the same water has been mentioned in this connection previously (1925). The temperature gradient shows complete mixing of the water down to 40 m., and nearly complete down to 50 m. Again 75 m. is a region of steep gradient and uniformity then exists between 100 and 150 m., to be followed by a continual, though irregular decrease of temperature to great depths.

It is of interest to consider the extent of the possible vertical circulation of the water at an open station such as this. Obviously irregularities of the bottom are without effect, and the factors concerned are, firstly, density changes due to cooling, and, secondly, wave motion. It is generally admitted that thermal conduction is negligible in water masses in com-

parison with convection effects, so cooling due to the cold bottom water may be left out of account. Consequently the temperature of the lower portion of the isothermal water column cannot fall below that of the surface. The July isothermal lines show a temperature of about 22° at this station, the corresponding January value being 12·5°. This may not be quite the minimum judging by experience further north, but it is probably not far from 12°. It seems legitimate, therefore, to conclude that vertical circulation due to cooling will be such that it extends downwards to the depth where 12° is found, namely, at slightly over 300—say, 350 metres. Above this the temperature will be uniformly that of the surface, neglecting a slight lag. This is what is found in the English Channel in winter right down to the bottom, 70–80 m. Below 350 m. we may expect the change to be negligible. It results that the phosphate enrichment of the surface water will be that produced by the mixing, together with that due to regeneration from the organisms which liberate phosphate in the first 350 metres, but the larger animals sink when they die and so carry down their phosphate to greater depths. Taking 46 mg. per m^3 as the value for 350 m. and averaging the values of the column up to the surface the result is 20 mg. per m^3. This is, of course, a minor limit as regeneration which must take place cannot be calculated. As an upper limit we may hazard 46 mg.—for the depletion at the surface is due to removal of phosphate by algæ under the action of light. It seems, however, very unlikely that the major limit is even approached at this latitude, where illumination is never very greatly reduced. Indeed, the minor limit of 20 mg. neglects the fact that near the surface, even in winter, photosynthetic processes probably preponderate in these latitudes. According as we proceed northwards the surface cooling becomes greater and the deep water temperatures are also lower, so the vertical circulation will proceed to progressively greater depths. Conversely near the equator* the temperature changes throughout the year are small, so what vertical circulation there is must be due in the main to wave motion—which cannot be effective to any very great depth—and not to density changes. Thus no considerable seasonal change in phosphate content is to be expected. Another important factor in increasing the yearly phosphate turnover in northerly latitudes is the great reduction in the light, which is, of course, the fundamental cause of the cycle wherever found. The further north we go the greater is the reduction in both sunlight and daylight, till the region of complete darkness is reached. In that it must be imagined that there is great destruction of plant life during the winter, so that the onset of sunlight in the spring finds the water rich in phosphate.

* There is, however, a region of upwelling on a vast scale near the equator, as cold bottom water replaces that blown polewards by the trade winds.

Information about the phosphate cycle in the open ocean is provided by the data of Table 12, which shows how completely the southern waters are deprived of phosphate in summer, whereas even in December and in March the amount present at the surface is small. By a fortunate chance the samples on the northerly route were taken near the dates of the southerly in August. Even allowing for the fact that they were 8 m. samples, not surface, it is clear that in them phosphate was far from being exhausted.

Table 13 records the results of analyses made of water from the Pacific, but owing to prolonged storage, from five months to a year, many of the

TABLE 12.

PHOSPHATE VALUES AND TEMPERATURES IN THE ATLANTIC, LIVERPOOL TO PARA, SURFACE SAMPLES TAKEN BY S.S. *Hildebrand*. ALSO FROM SOUTHAMPTON TO QUEBEC, FROM ENGINE ROOM INTAKE, 8 M. BELOW SURFACE BY C.P.R. STEAMER; LATTER TAKEN 26–27/8/'25. ANALYSED 12/10/'25.

18.3–1.4.'25. Analysed 26.5. P_2O_5 mg./m³.	t°.	13.8–26.8.'25. Anal. 5.9. P_2O_5 mg./m³.	t°.	17.12–30.12.'25. Anal. 8.1.'26 P_2O_5 mg./m³.	t°.	Lat. N.	Long. W.	Approximate latitude.
6	26	0	28	34	28	0°20′S.	47°	Off Para.
—	27	—		20	28	1°	46°	—
—	27	—		6	27	2°	44°	—
6	26	0	27	6	26	9°	39°	Mouth of Orinoco.
—	25	2	24	7	25	19°	30°	Between Jamaica and Cuba.
—	18	1	22	5	21	28°	21°	N. Florida and Canary Is.
9	14	2	20	7	17	38°	10°	Azores and S. Portugal.
19	11	2	16	7	12	48°	7°	Ushant or Brest.
—	—	23	13	—	—	53°	40°	Galway or S. Labrador.
—	—	22	11	—	—	52°	50°	Mth. of Shannon or S. Labrador.
—	—	25	10	—	—	52°	55°	Queenstown or Str. of BelleIsle.

results must be greatly vitiated by regeneration. On the whole, however, the water round the Galapagos and Marquesas Islands seems richer than that in the open ocean. The most interesting values are the first two, surface and 366 m., which, with 47 and 167 mg. per m³ are exceptionally high and probably indicate an upwelling of deep water. The 366 m. temperature is far lower than in the Atlantic, as shown in Table 11, where this value is not reached till below 1000 m. It is of interest to recall J. Schmidt's (1925) comparison of the water on opposite sides of the Panama isthmus. He found surface water on both sides nearly saturated with oxygen, but whilst at 50 m. the Atlantic is still nearly saturated the Pacific had only 0·25 as much as at the surface, and at 400–500 m. the Pacific water was practically devoid of free oxygen,

whereas the Atlantic had 40–50 per cent of that at the surface. Thus it looks as if the deeper water on the Pacific side was rich in decomposing organic matter which absorbed oxygen and liberated phosphate.

TABLE 13.

PHOSPHATE VALUES AND TEMPERATURES IN THE PACIFIC, SAMPLES TAKEN BY THE *St. George*. NOT ANALYSED TILL 13TH TO 18TH OF JULY, 1925.

Date.	P_2O_5 mg. per m^3.	t°.	Position.		
15.7.'24	47	26·1	2°6′N.	81°38′W.	Off S. America between Panama and Galapagos Is.
15.7	167	9·1	,,	,,	Do., 366 m.
17.7	13	25·1	2°9′N.	84°43′W.	Between Panama and Galapagos Is.
19.7	12	25·0	2°43′N.	88°31′	Between Panama and Galapagos Is.
21.7	34	24·5	1°55′N.	86°39′	Between Panama and Galapagos Is.*
23.7	18	24·0	1°34′N.	89°00′	N. of Galapagos Is.
24.7	68	21·7	—	—	Midway between Bindloe and James Is., Galapagos.
9.8	53	24·2	2°10′N.	90°17′	N. of Galapagos Is.
11.8	35	26·5	4°43′N.	87°44′	N. of Galapagos Is.
15.8	39	27·0	5°53′N.	83°34′	Latitude of Cocos Is. and to E. of it.†
17.8	16	27·5	6°17′N.	81°38′	S. of Panama towards Galapagos Is.
2.12	7	25·2	3°43′N.	94°10′	N.W. of Galapagos Is.
6.1	17	23·3	1°23′N.	104°30′	Open Pacific, on course Galapagos→ Marquesas.
9.12	21	21·4	0°08′N.	109°05′	Open Pacific, on course Galapagos→ Marquesas.
12.12	14	22·4	2°20′S.	114°7′	Open Pacific, on course Galapagos→ Marquesas.
15.12	5	24·2	5°12′S.	120°8′	Open Pacific, on course Galapagos→ Marquesas.
18.12	26	25·6	6°48′S.	126°11′	Open Pacific, on course Galapagos→ Marquesas.
21.12	27	26·0	8°21′S.	131°58′	Open Pacific, on course Galapagos→ Marquesas.
24.12	15	26·8	9°35′S.	136°30′	E. of Marquesas on course from Galapagos.
9.1.'25	5	27·2	9°50′S.	139°10′	Midway between Tata Hiva and Tahuata, Marquesas.
15.1	38	26·3	9°10′S.	139°50′	About 10 miles S.W. of Wa Huka Is.‡
30.1	19	26·9	10°10′S.	138°50′	Motane Is., Marquesas due N., 10 miles off.
5.2	34	27·0	11°1′S.	139°17′	S. of Marquesas Is.
6.2	10	28·0	12°37′S.	139°48′	S. of Marquesas Is.
7.2	25	27·4	13°55′S.	141°4′	Near Napuku Atoll, S.W. from Marquesas.
9.2	6	27·7	13°55′S.	141°4′	S.W. of Marquesas.
10.2	35	27·8	15°44′S.	144°23′	Near Tairo Atoll, Low Archipelago, S.W from Marquesas.

It may be added that the isothermal maps of the Atlantic show a cold area, under 21° on the N.W. coast of Africa at about 25°–32°N. latitude, surrounded by a wide belt of warmer water, This would appear

* Current sets off continent here, E. to W.
† Current E. to W. in Pacific round Marquesas.
‡ Current sets towards continent, W to E.

to indicate a region of upwelling, which would probably be rich in phosphate at moderate depths and rich in plankton near the surface.

In conclusion the writer wishes to acknowledge his obligation, and to express his thanks to Mr. H. W. Harvey and the Captain and crew of the *Salpa* for assistance in obtaining water samples and temperatures, also to the Scotch and Irish Fishery Departments for samples obtained from Aberdeen and off the Irish coast, to the Air Ministry for the samples obtained through the courtesy of the officers of the s.s. *Hildebrand*, to Dr. C. Crossley for the Pacific samples, to Dr. L. T. Hogben for those on the Canadian route, also to Mr. C. F. Hickling for samples off the Irish coast in winter.

SUMMARY.

1. A comparison of the years 1923, 1924 and 1925 as regards the phosphate content of the water at Station E1 in the English Channel has shown that the vernal diminution was earliest in the year 1924, and latest in 1923, the extreme difference being approximately two months. These differences stand in direct relation to the spring sunshine, that of 1924 for the daily average of the 9th to 12th weeks inclusive being 2·3 hours in excess of the normal, 1923 being 0·1 hr. in deficit and 1925 being 0·6 hr. in excess. Since the phosphate diminution is proportional to the increase in phytoplankton the year 1924 must have been exceptionally early in this respect.

2. The year 1925 was in general similar to the other two in having a summer phosphate minimum and a winter maximum; but it was noticeable for marked periods of regeneration of phosphate, followed by utilization, within the main cycle.

3. The maximum value at E1 was 40 mg. per m^3 of phosphate as P_2O_5, the minimum 5·1 mg. for the whole water column, 0–70 metres.

4. Much additional evidence has been found to show that the deep water of the ocean is a reservoir of phosphate, containing 50–80 mg. per m^3, or more.

5. The water of the North Sea was markedly richer in phosphate in the spring of 1925 than in that of 1924, as was also the water around the Faroe-Shetland Channel in July, 1925, as compared with the previous July. A causal connection may be sought.

6. In tropical waters the intense light normally results in the utilization of all phosphate down to at least 50 metres, and the winter cooling never suffices to effect mixing with the deeper water. At about 38°N. latitude the water may be expected to be isothermal in winter to a depth of 350–400 metres, and further north the isothermal column has its base deeper down; accordingly the seasonal phosphate cycle must become more pronounced.

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On *Lumbricillus Scoticus* n. sp.

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With 3 Figures in the Text.

A SMALL orange-coloured Enchytraeid Oligochaete has been known for some years to occur fairly abundantly in certain sheltered parts of the shore on the Cumbraes. It is also generally present, though scarce, on exposed parts of the shore. Hitherto it has been confounded with other species of Lumbricillus, but is now described as a new species.

LUMBRICILLUS SCOTICUS SP. NOV.

The worms are during life of an orange colour. They are relatively short and stout, measuring when preserved 7–9 mm. in length, and having sometimes a width of as much as 1 mm. at the clitellum. The number of segments is fairly constant—30 in 3 specimens, 31 in a fourth.

The *prostomium* is short, bluntly triangular, broader at the base than long.

The *setæ* have the lumbricilline curve. In the ventral bundles there are from 10 to 14 setæ in front of the clitellum, from 8 to 13 in the segments behind this. The lateral bundles contain 6 to 8 setæ in front (once 9), 7 to 9 (very frequently 8) behind, decreasing to 6 at the posterior end.

The *clitellum* includes, besides segments xii and xiii, a little of xi and perhaps also of xiv.

There are numerous *gland cells* in the integument, which stain deeply with hæmatoxylin.

The *cœlomic corpuscles* are numerous, narrowly or broadly spindle-shaped or occasionally oval in form, 36μ or less in length, and nucleated.

The *septal glands* are three pairs, in segments iv–vi.

There is a pair of small *post-pharyngeal bulbs*, but no salivary glands.

The *dorsal vessel* begins at septum 11/12.

The anteseptal portion of the *nephridia* is small, about one quarter the length of the post-septal, and the lumen shows no windings as it passes through this part of the organ. The post-septal portion is somewhat pear-shaped, with the narrower end behind. The duct is rather shorter than the post-septal, and comes off from the hinder end of the latter, curving ventralwards towards the surface.

The *cerebral ganglion* (Fig. 1) is one and a half times as long as broad ; its sides are almost parallel, converging slightly backwards ; there is a small indentation behind, and a larger one in front.

The *testes* are " divided," each being composed of a number of pear-shaped sacs, attached by the narrow end to septum 10/11, extending forwards and backwards in segments x and xi, and reaching as far in front as segment ix.

The *male funnels* are three or four times as long as broad, with a thin everted somewhat flangelike lip. The *vas deferens* is narrow, consists of a few coils, and is confined to segment xii.

The *penial body* is round, 0·2 mm. in diameter, compact, of the lumbricilline type. In it can be distinguished :—

(*a*) The *vas deferens*, which passes through in a dorso-ventral direction ; it immediately becomes wider on entering the penial body, both the size of the lumen and the thickness of the wall increasing ; it joins the next structure shortly before reaching the ventral surface at the male pore.

(*b*) An irregular tubular cavity, triradiate as seen in sections, which ends blindly above at the dorsal surface of the penial body, and joins the vas deferens lower down, as just stated ; this cavity thus constitutes a diverticulum of the male duct.

(*c*) A mass of cells, the bulkiest constituent of the penial body ; these cells seem to belong to and to spread out from the wall of the diverticulum just described.

(*d*) A pear-shaped aggregate of cells, perhaps glandular, on the anterior side of the mass of the penial body ; the swollen ental portion of the aggregate is solid, the ectal narrower portion or stalk is hollow and tubular, and joins the lumen of the male duct below the union of the diverticulum and vas deferens, a little before the combined tube reaches the surface.

(*e*) A muscular capsule surrounds the greater part of the mass, but is somewhat deficient above, and also on the anterior side, where the pear-shaped gland is situated. In this position there is, in front of the gland, a strong muscular bundle attached below to the body-wall just in front of the penial body, and passing upwards to be attached again to the parietes above.

(*f*) Lastly, within the penial body there is, in addition to the cells which constitute the greater part of the mass, a little muscular tissue between the several components, and a few loose cells. In the ventral portion of the penial body, just within the body-wall, there is a certain amount of vacant space, left unoccupied by any of the above structures.

The *spermathecæ* are irregularly swollen tubes ; though the ental end of each is fused with the wall of the œsophagus, there is no patent communication between the cavity of the spermatheca and the œsophageal lumen. There is no great distinction externally between ampulla and duct ; but the ectal portion of the apparatus—about two-fifths of its

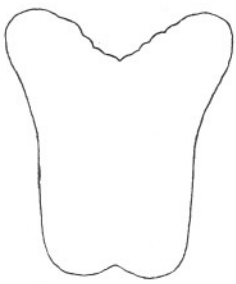

FIG. 1.—*Lumbricillus scoticus ;* cerebral ganglion.

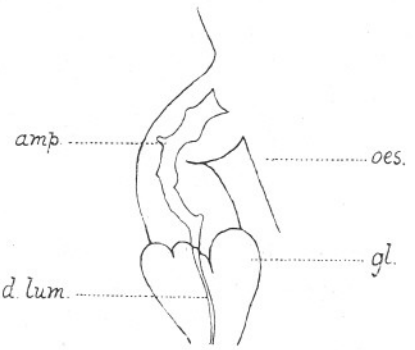

FIG. 2.—*Lumbricillus scoticus ;* spermatheca. *Amp.*, ampulla ; *d. lum.*, lumen of the duct; *gl.*, mass of gland cells causing a swelling round the ectal end (duct) of the apparatus ; *œs.*, œsophagus. The figure has been drawn from an organ isolated by dilaceration ; in sections the cavity of the ampulla appears wider than here shown.

length—is broader, owing to a development of gland cells, and may be called the duct (Fig. 2) ; the lumen is here much narrower.

These gland cells are the lining epithelium of the tube ; they have become much elongated outwards, so that the longitudinal muscular fibres of the wall, which in the upper part of the apparatus are as usual outside the epithelium, here pass amongst the epithelial cells, which extend outwards between and beyond them ; the nuclei of the cells are mostly external to the muscular layer. The region of these elongated cells is sharply delimited above (*v.* Fig. 2) ; the swelling which they occasion is lobulated on its surface, as is well seen in tangential sections of this portion.

A *ventral* (" *copulatory* ") *gland*, lobulated, and of considerable size, is present in segment xiv ; it leaves uncovered a little of the dorsal surface of the nerve cord. In one specimen a small lobe occurs in segment xv,

but this is only a part of the large gland in xiv which has spread backwards into the next segment. In a second specimen the gland is confined to xiv.

Remarks.

In the large number of setæ per bundle, and in its short and stout habit, the present worm resembles *L. minutus* (Müll.). It differs, however, in the fact that the nephridial ducts come off from the hinder end, not from the middle of the post-septal portion ; and in having a single mass of gland cells round the spermathecal duct, while Michaelsen's diagnosis (in the " Tierreich ")* of *L. minutus* would indicate that this worm has *two* series of glands in relation with the duct.

It seems to us impossible, therefore, especially in view of the very scanty details we possess concerning *L. minutus*, to unite the two ; though, notwithstanding, we find it difficult to get rid of a suspicion that they may after all really be identical.

Habitat.

L. scoticus inhabits the mid-tidal zone, being associated with and generally creeping on *Ascophyllum nodosum*, but is also occasionally found on the surface of stones on which that weed grows. Preference is shown for parts of the Ascophyllum which are decaying or soft, particularly the upper and spent tips of the weed. Examination of gut contents reveals the presence of bits of epidermis and cells from the degenerating spent tips of the Ascophyllum.

Breeding.

The cocoons (Fig. 3) of *L. scoticus* are found abundantly in spring on the bases of Ascophyllum plants and occasionally on other parts of the plants, chiefly in the shelter of air vesicles which have been opened.

They may occur singly or in bunches of six or seven, which are usually at various stages of development.

The cocoons are made of a clear straw-coloured horny material, are low and roundish, closely attached to the weed, and measure 1·6–2·2 mm. long, 0·9–1·2 mm. broad by about 0·45 mm. high. Each cocoon contains 11–14 eggs about 0·3 by 0·23 mm., and of a pale orange colour. The upper part is marked with striations (omitted in figure). A dimpled thickening occurs at each end.

Cocoons are found from January to September, being most plentiful in April and May. They may be got occasionally, as may the worms,

* *Michaelsen, W.* Das Tierreich X, Oligochæta, 1900, p. 82.

among seaweeds lying about h.w.m. This appears to be due to their being washed up with their food plant. This occurrence of the worms at the drift line suggests that they can withstand a certain exposure to fresh water. Experimentally about 3 hours in fresh water causes œde-

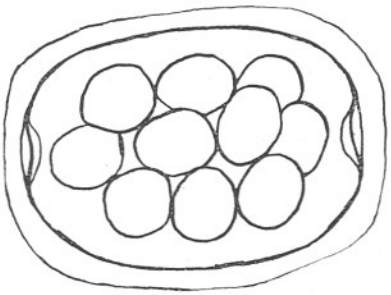

FIG. 3.—*Lumbricillus scoticus ;* cocoon.

matous swelling, followed by recovery if replaced in sea-water ; on the other hand, they live quite happily in water of as low salinity as 20% of sea-water.

GROWTH.

The young emerge by a slit which appears along the margin of the cocoon. They may be almost 2 mm. long when extended, and have usually only twenty segments. Their setæ are arranged in groups of four with a few groups of five anteriorly and three posteriorly. Immature specimens of all sizes may be obtained in the spring by washing a handful of Ascophyllum in water, preferably half and half sea-water and fresh water.

Young worms of 5 or 6 mm. length, with about twenty-seven segments, about one month old, show the clitellum formed, and the presence of yolk in segments xii and xiii. They are fully grown and mature at two months.

The Moulting Stages of the Pea-Crab (*Pinnotheres pisum*).

By

D. Atkins.

With Plates I–V and 4 Figures in the Text.

Pinnotheres pisum is a small crab with a carapace between about 2·1 and 18 mm. wide. The females are commonly found parasitic in the mussel, *Mytilus edulis*, though they have been recorded from other bivalves. The males are free-swimming and are comparatively rarely found in mussels. These small crabs are never abundant, and not more than one female has ever been found in a mussel.

Sexual Dimorphism.

It is well known that there is a marked sexual dimorphism in *P. pisum*. This is, no doubt, due to the difference in the mode of life of the two sexes, the male being active and free swimming, while the female is parasitic. Contrary to what usually occurs in the Brachyura, the adult male is much smaller than the adult female. The male has a carapace varying in width between 3·6 and 7·7 mm., while that of the female may reach a size of 18 mm.

Description of the Male.

Normally the males, young and adult, are of one form (Plate I, Figs. 1 and 2). A few abnormal crabs, however, have been found, and it is hoped to refer to them in a later paper.

The carapace of the male *P. pisum* is almost circular; very strong and hard and for the most part glabrous. It is very light grey or fawn in colour, with a conspicuous pattern of pale yellow areas outlined with darker yellow or yellowish orange. There is a slight variation of the pattern in different males; in the larger crabs the yellow areas increase in size and fuse to cover the greater part of the dorsal surface of the carapace. Lines and areas of colour also occur on the chelipeds and legs, while on the ventral surface are a few, more or less symmetrically placed,

pale yellow spots. There are frequently numerous black, with an occasional red, chromatophores scattered over the body.

The chelipeds (Plate IV, Fig. 15) are hairy ; the palms broad and rather swollen. There are two rows of setæ beneath the chela ; one reaches from the base of the palm to the tip of the finger, while that which is visible on the inner surface extends only slightly beyond the base of the immovable finger. These two rows are widely apart at the base of the palm, but converge distally. In the longer row the setæ, which point towards the tip of the finger, are stout and curved. A large tooth is present near the base of the dactylus, and fits a slight notch in the propodial finger. This notch has a small tooth at either end. Both biting surfaces bear stiff setæ, and towards the tips of the fingers small, closely set, spines. In some males the small teeth on the propodial finger are absent, as well as the curved spines from both fingers.

The walking-legs (Plate IV, Figs. 17, 19, 21) are strong, somewhat flattened, and exceedingly hairy, the long hairs being plumose. The second and third legs are especially hairy, the three distal segments bearing two thick fringes of very long hairs, one attached on the lower margin and one near the upper margin on the posterior surface. The extreme hairiness of the legs, as well as their flattened form, assists in maintaining a free swimming existence. The second and third legs are subequal in length, the second being slightly the longer. The first leg is the next in length, and the fourth leg the shortest. The short, curved dactyli end in short, horny tips.

The abdomen is narrow and tapering. Two small, transversely ridged, nodules of chitin are present on the fifth thoracic somite, and these fit into two pockets on the sixth segment of the abdomen. By this arrangement the abdomen is securely fastened to the thorax.

The copulatory organs of the male (Text Fig. 1) are large ; the first appendage is blade-like and hairy, with the tube, or rather the closed groove, running along its inner side. Numerous rosette glands are present round the lower portion of the groove. The second appendage is rod-like with a swollen base. The distal portion is normally carried within the groove of the first appendage. This stylet, unlike the first, is almost hairless, and is without glands.

Growth Stages of the Female.

The female occurs under two forms : the young female is almost indistinguishable from the male, while the next and subsequent stages are entirely different in appearance, and are what may be considered typically female in form. This change in form occurring between Stage I and II would appear to be related to the change in the mode of life, though

it would seem that it can only take place after copulation. One exception has been found among the material so far examined; a Stage I female more nearly resembling the female form than the male though with empty spermathecæ.

After the typically female form is assumed in Stage II, the further growth stages are mainly a development of those structures connected with reproduction, together with a general increase in size. Somewhat similar growth stages have been described for the female *Hapalocarcinus* by Potts (**6**).

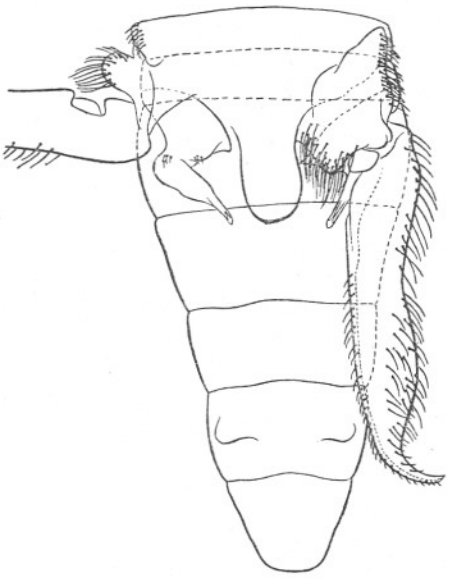

FIG. 1. Male abdomen and copulatory appendages. The first appendage of the right side is turned aside to expose the second appendage. Magnification, ca. 16⅔.

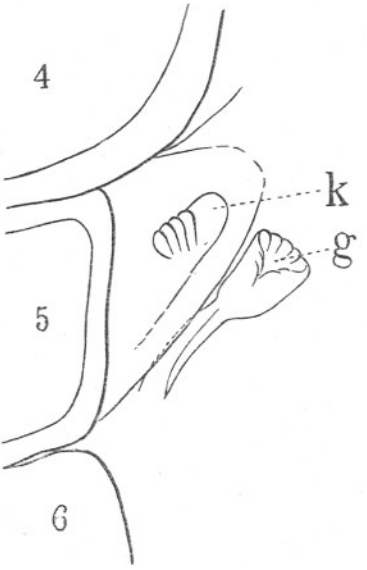

FIG. 2. Genital aperture and chitinous knob of right side of a Stage I female. g = genital aperture, k = chitinous knob; 4, 5, 6 = 4th, 5th, 6th thoracic somites. Magnification, ca. 71.

The female crab becomes parasitic in the mussel, *Mytilus edulis*. The parasitic life has exerted a considerable influence on the structure of the female, which is modified to a certain extent. It is large and extremely passive; the carapace with the rest of the exoskeleton being no longer needed for protection is soft and membraneous; the eyes are very minute, and in the fully adult crab invisible from the dorsal surface.

As noted by Orton (**4**) the female only reaches its adult form after passing through a number of growth stages. The greater part of the work recorded in this paper has been to determine these stages. The majority of them have been verified by a series of moults.

Stage I (Plate II, Figs. 3, 4). The young Stage I female, which has been found with a carapace varying in width between 2·1 and 4·9 mm., can only be distinguished from the male by the genital openings and the abdominal appendages. Indeed, the resemblance is so close that Orton (**4**) records that, on obtaining a female form moulted from a supposed male crab, he thought that he had a case of protandry until careful examination of the moult revealed the presence of the full number of abdominal appendages characteristic of the female. There is an exceedingly slight difference in the shape of the abdomen, which is a very little broader, and does not taper quite so much as in the male, while the sides of the segments are slightly convex. The locking apparatus is as well developed as in the male, and is very close to the oviducal apertures, which are on the sixth thoracic somite (Text Fig. 2). All four pairs of pleopods characteristic of the female are present, though not fully developed. The first two pairs (Plate V, Figs. 23, 24) are distinctly biramous, though there is not such a difference in length between the exopodite and endopodite of the first pair as there is in the adult. The third and fourth pairs are uniramous as in later stages. There are, at this stage, very few hairs on these appendages.

The ovary exists as paired narrow tracts of oocytes anteriorly ; these join in the thorax, then divide again to extend into the abdomen. The ovary at this stage is not visible externally.

Similar male-like females have been recorded by Rathbun (**7**) as occurring in the American species, *P. maculatus*, *P. margarita*, *P. taylori*, and *P. concharum*. These hairy, male-like females are probably at first free swimming, but after a time enter a mussel where copulation takes place.

Females of this stage have been found with spermathecæ full of sperm, others with spermathecæ empty (Plate II, Fig. 4), while again some have been found with one spermatheca full and one empty (Text Fig. 3). In this stage the oviducts are narrow and their external apertures very small, so that the accurate adjustment of the tips of the long first copulatory appendages of the male during copulation must be a process of considerable difficulty, as evidenced by the occurrence of Stage I females in which insemination is not complete, one spermatheca being empty. One of the crabs found in this condition was a tiny one with a carapace measuring only 2·1 mm. across (Text Fig. 3). The difficulty of the process must be increased by the great discrepancy in size which often exists between individuals of a pair.

It would appear, therefore, that copulation takes place during this stage ; that *P. pisum* is peculiar in copulating precociously at an extremely early age. The majority of the larger females examined have been found to have their spermathecæ full of mature sperm, but occasionally an

adult occurs with the spermathecæ almost if not quite empty. It is extremely probable that sperm from the first copulation is sufficient to fertilize several batches of eggs. The occurrence of an occasional adult female with empty spermathecæ, would seem to point to the possibility that copulation may take place more than once. Males have been found all the year round within the same host as females of all stages, including those in berry, though it has been found that " a newly moulted female (adult) appears to have no charm for a male " (**4**).

There would appear to be no relation in age and size between the male and female of a pair ; there is often a great difference, for young males

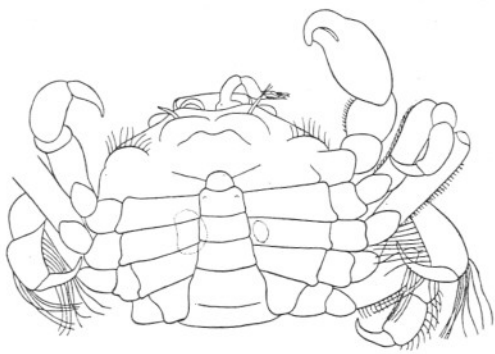

FIG. 3. Stage I female (carapace 2·1 mm. in width), with one spermatheca full and one empty. Stained with alum carmine and cleared in oil of winter green.

have been found with large adult females while the opposite may occur, though, of course, cases occur where there is little difference in size between individuals of a pair.

Orton (**4**) notes that : " It would appear that copulation normally takes place inside the host, and that the males visit mussels in their search for females, since unwary male crabs have been found with their legs or bodies trapped by the mussel closing its shell before the crab could get inside. These crabs survive the rough treatment by reason of their extraordinarily strong carapaces, and creep inside the mussel later when it must perforce relax and open its shell in order to breathe. The male-like female has a similarly hard carapace which prevents the animal being crushed to death if unluckily trapped by the mussel destined to become a host. Individual crabs have been found to be lacking a leg which might very well have been lost in this dangerous operation."

The change from the male-like female to the next stage is very striking. It undoubtedly depends upon and follows copulation, in this, offering a striking difference to Cancer (**5**), in which " ecdysis will not take place in

the female so long as there is a supply of spermatozoa in the spermatheca."

Stage II (Plate II, Figs. 5, 6). Females of this stage have been found with a carapace varying in width between 3·3 and 5·8 mm. The carapace is more or less circular ; thin and membraneous. It is translucent whitish or yellowish, without a colour pattern, although there are usually a few pale yellow spots on the ventral and dorsal surface. The front is as advanced, and the eyes are as well developed, as in the male and male-like female.

In this and the following stages the chelipeds (Plate IV, Fig. 16) are slender, the palms being reduced in width, and there is only one row of setæ on the lower edge of the chela. The walking-legs (Plate IV, Figs. 18, 20, 22) are more slender than those of the preceding stage, not so flattened and with very few hairs, though the degree of hairiness varies somewhat. The relative length of the legs is the same as in the male. The second and third legs bear only a scanty fringe of short hairs, attached near the upper margin on the posterior surface of the last three segments, which represents the much thicker and longer fringe present in the male.

The abdomen has increased in width, and is now more than half the width of the sternum. Anteriorly it extends beyond the chelæ sterna, but is very little further forward than in Stage I. The locking apparatus has disappeared. The pleopods are further developed and more hairy (Plate V, Figs. 25, 26).

There is a certain amount of variation between individuals of the same stage, which may be due to differences in general conditions. Three specimens (one crab and two moults) have been taken with the abdomen rather narrower than that of the specimen figured, but with no other difference.

All specimens so far obtained, belonging to this stage, have been found to have the spermathecæ densely packed with sperm.

Stage III. In this stage the abdomen has increased still further in width, and reaches further forward. The pleopods (Plate V, Fig. 27, 28) are rather more hairy than in the preceding stage.

Two variations of this stage occur :—

(*a*) The abdomen has increased greatly in width ; at its middle it overlaps the sternum, but anteriorly extends very little if any further forward than in Stage II. Two crabs, with carapace 5·0 mm. and 4·75 mm. wide respectively, having these characters have been obtained moulted from crabs of the previous stage. The one with carapace 5·0 mm. across is figured in Plate II, Figs. 7, 8.

(*b*) The abdomen is only slightly wider than in Stage II, but reaches further forward. The two specimens which have these characteristics

are both 5·0 mm. wide. The field note on the specimen figured in Plate II, Figs. 9, 10, is "trace of gonad seen through carapace." The second one showed no sign of gonad externally. It is thought that these two crabs should be placed in Stage III, but they have not been verified by moults.

Stage IV (Plate III, Figs. 11, 12). Females of this stage have a carapace varying in width between 6·5 and 16 mm. The carapace is rather wider than long; smooth, shining, and rather stiffer than in the preceding stages. Some specimens have yellow spots on the ventral surface and legs, others are without them. The spots appear to consist of three or four cells in a stellate arrangement. There may also be scattered black and red chromatophores on the dorsal and ventral surfaces, but in none of the stages after Stage I is there any indication of a colour pattern such as Bell (**1**) both describes and figures for the adult female of *P. pisum*.

The front is less advanced than in Stage III, and though the eyes are very small they are still visible from the dorsal surface.

The abdomen in this stage reaches just posterior to the propodites of the outer maxillipeds, when the latter are covering the mouth. The abdomen is broad and overlaps on to the coxopodites of the legs. It is deeply hollowed, as is also the thorax, though to a less extent. The abdominal appendages are well developed and hairy (Plate V, Figs. 29, 30). The exopodite is rudimentary in the first pleopod, but very long and blade-like in the second. Hairs are present along the edge of the abdomen and sternum, and there is also a slight growth of hairs stretching in a semicircle across the thorax between the chelipeds, following the outline of the terminal segment of the abdomen. Scattered rosette glands occur on the pleopods and on the inner surface of the abdomen.

The degree of development of the ovary, of course, varies in different crabs. In specimens in which there are a considerable number of yolk-laden eggs, the ovary shows a deep red through the carapace, and where it has attained its full development it occupies the greater part of the body space, and almost the entire dorsal surface of the carapace appears of a deep red colour. The ovary extends nearly to the tip of the abdomen.

Stage V (Plate III, Figs. 13, 14). Females of this stage have been found between 9 mm. and 18 mm. wide. The carapace is wider than long, and often rather quadrilateral in shape. The front is very narrow, about one-fifth the width of the carapace, and is hardly visible from the dorsal surface. The eyes are feebly developed, being very minute and quite invisible from above.

The abdomen is rather larger than in the preceding stage. Laterally it overlaps on to the basipodites of the legs, while anteriorly it completely covers the mouth parts, reaching just posterior to the eyes. When the crab is feeding the last segment of the abdomen is bent rather sharply

inwards, so that the mouth is uncovered. The abdomen is deeply hollowed, as is also the thorax. Longer and more numerous hairs are found at this stage, on the edge of the abdomen and of the sternum, between the bases of the chelipeds and on the pleopods.

There is not a very great difference between Stage IV and Stage V; some crabs have been found with the abdomen reaching as far forward as in Stage IV, but as wide as in Stage V and vice versa. The variation in the degree of concavity of the abdomen may account for this difference, or it is possible that reproduction may take place in Stage IV, and that growth may go on after reproduction has commenced.

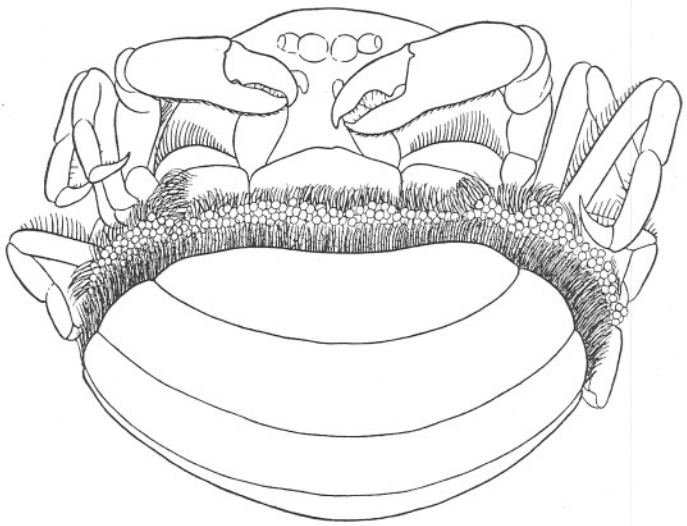

FIG. 4. Ovigerous female. Magnification, ca. $4\frac{1}{3}$.

Ovigerous Female (Text Fig. 4). The smallest berried female found had a carapace 7·5 mm. wide. The very numerous eggs are carried in the cavity formed by the hollowed thorax and deeply hollowed abdomen. The space between the side of the abdomen and the thorax besides being very small, is well guarded by long fringing hairs. The long blade-like exopodite of the second pair of pleopods, fringed with long and numerous hairs, fits along the inside of the gap as far forward as the fifth segment of the abdomen, and gives a double protection to the eggs.

In a good many instances the size of the pea-crab and its host was noted, and the figures are given in the accompanying table. It will be seen that there is a rough relationship in size between the female crab and its host, the larger crabs being found in the larger mussels. Hornell and Southwell (**3**) have noted this for *P. placunæ* found in the window-

pane oyster (*Placuna placenta*) from the coast of Okhamandal in Kattiawar. They say : " Immature shells, as is natural, less frequently revealed the presence of commensal pea-crabs ; when they did occur the crabs were more or less immature. It would seem that the crabs grow towards maturity concurrently with their hosts."

Dr. Orton tells me that judging from the size of the mussels from which Pinnotheres have been taken, it is probable that the female crab attains sexual maturity easily in its first year. Additional evidence in favour of this is the scarcity of the early stages which would seem to point to the probability that the female passes through the various growth stages very rapidly. Of the first three stages, Stage I is perhaps the least scarce. It may be that a pause occurs here before a male enters the mussel and copulation takes place. During this time growth and moulting probably go on, but without a change of form, females of this stage having been found varying in size between 2·1 and 4·9 mm.

TABLE OF MEASUREMENTS OF FEMALE *Pinnotheres pisum* AND HOST (*Mytilus edulis*).

Stage.	Pea-crab mm. in width.	Mussel mm. in length.	Stage.	Pea-crab mm. in width.	Mussel mm. in length.
Stage I (abnormal)	4·0	41	Berried	10·0	58
I	4·9	62	,,	10·0	65
III	5·0	60	,,	10·0	67
IV	6·5	80	,,	10·0	68
IV	6·5	82	,,	10·0	68
IV	7·5	56	,,	10·0	68
IV	7·5	58	,,	10·0	70
IV	7·5	68	Stage V	10·5	68
Berried	7·5	90	V	10·5	73
,,	8·0	53	IV	10·5	84
,,	8·0	58	Berried	11·0	75
,,	8·5	65	,,	11·0	76
,,	9·0	60	Stage IV	11·5	79
,,	9·0	65	V	12·0	80
,,	9·0	69	IV	12·0	79
,,	9·5	73	V	15·0	104
Stage V	9·5	72	V	15·0	90

The work recorded in this paper was undertaken at the suggestion of Dr. J. H. Orton, to whom I am indebted for advice, help, and information. I should like to express my thanks to him for sending me some of his

material as well as arranging for a further plentiful supply. The material came through the Marine Biological Association, Plymouth; a great deal of it from the Tollesbury and Mersea Native Oyster Fishery, Essex, through the kindness of Mr. French, and some from the Yealm Oyster Fisheries, Devon.

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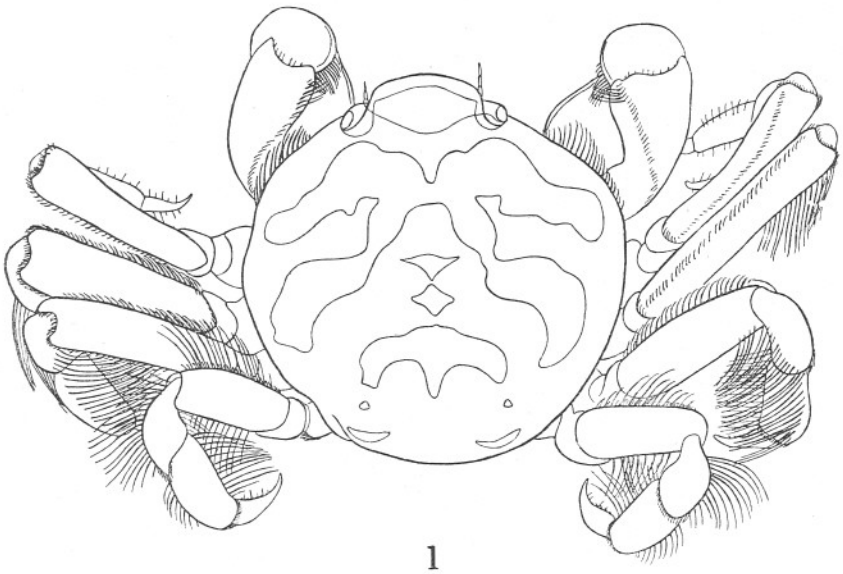
EXPLANATION OF PLATES I TO V.

The outlines of the drawings were made with camera lucida.

PLATE I.

Pinnotheres pisum, male × ca. 8½.

Fig. 1. Dorsal view. The colour pattern is indicated.
Fig. 2. Ventral view.



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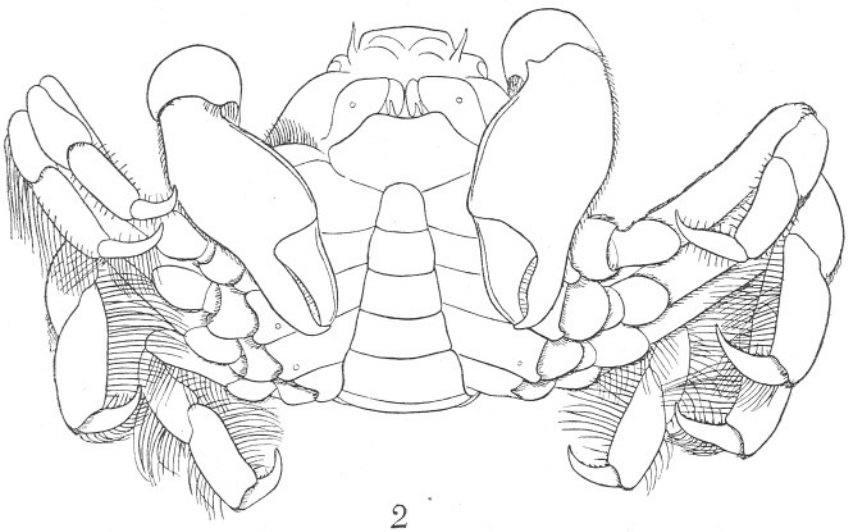

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DEL. D. A.

PLATE II.

Pinnotheres pisum, female × ca. 5.

The crabs figured in this plate and in Plate III were first drawn in outline, then soaked in acid alcohol, stained with alum carmine and cleared in oil of winter green.

FIG. 3. Stage I, dorsal view. The colour pattern is indicated.

FIG. 4. Stage I, ventral view. The empty spermathecæ are shown by dotted lines.

FIG. 5. Stage II, dorsal view.

FIG. 6. Stage II, ventral view. The full spermathecæ and the pleopods are shown by dotted lines.

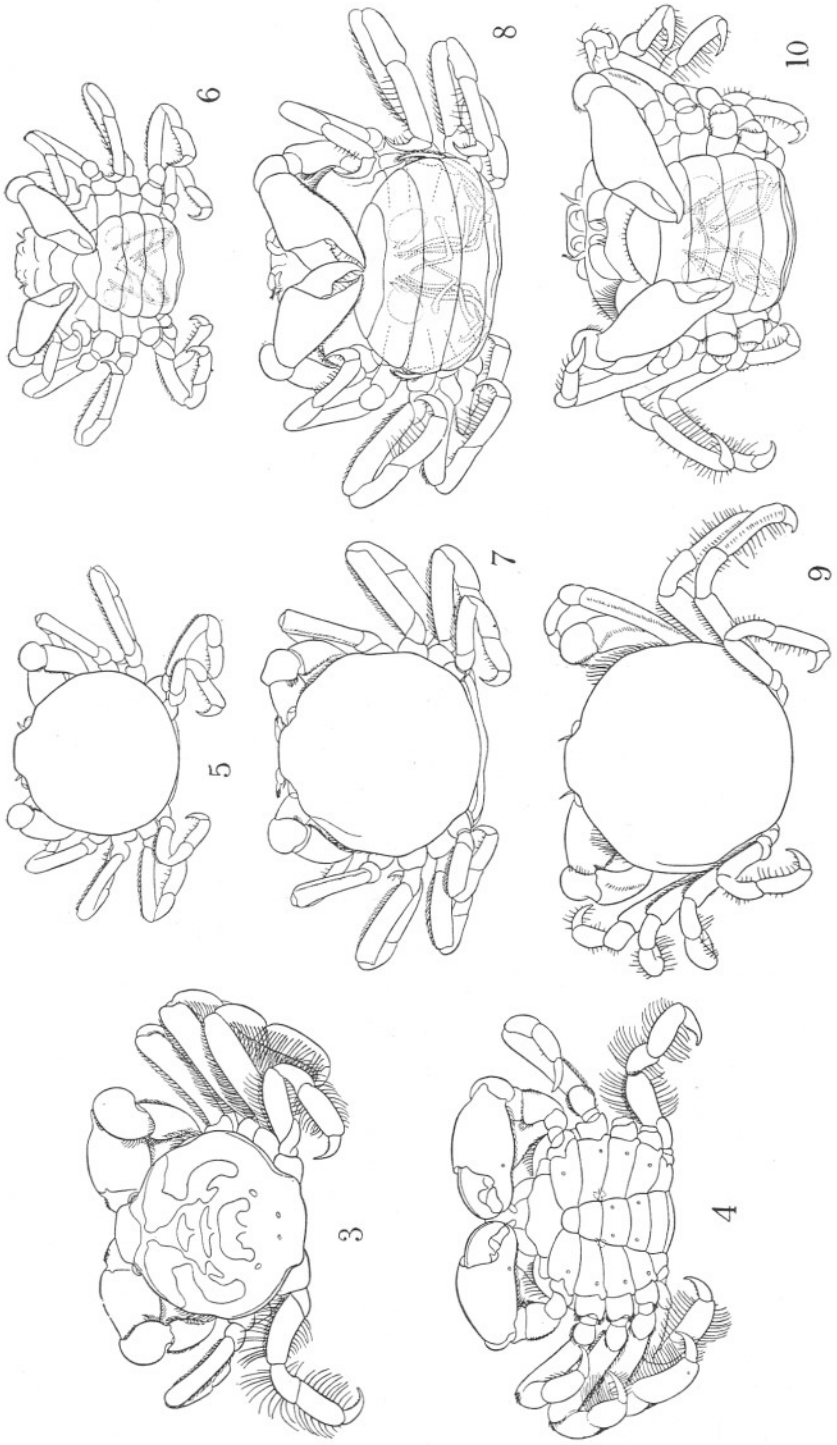
FIG. 7. Stage III (a), dorsal view.

FIG. 8. Stage III (a), ventral view.

FIG. 9. Stage III (b), dorsal view.

FIG. 10. Stage III (b), ventral view.

PLATE II.



DEL. D. A.

PLATE III.

Pinnotheres pisum, female, × ca. 5.

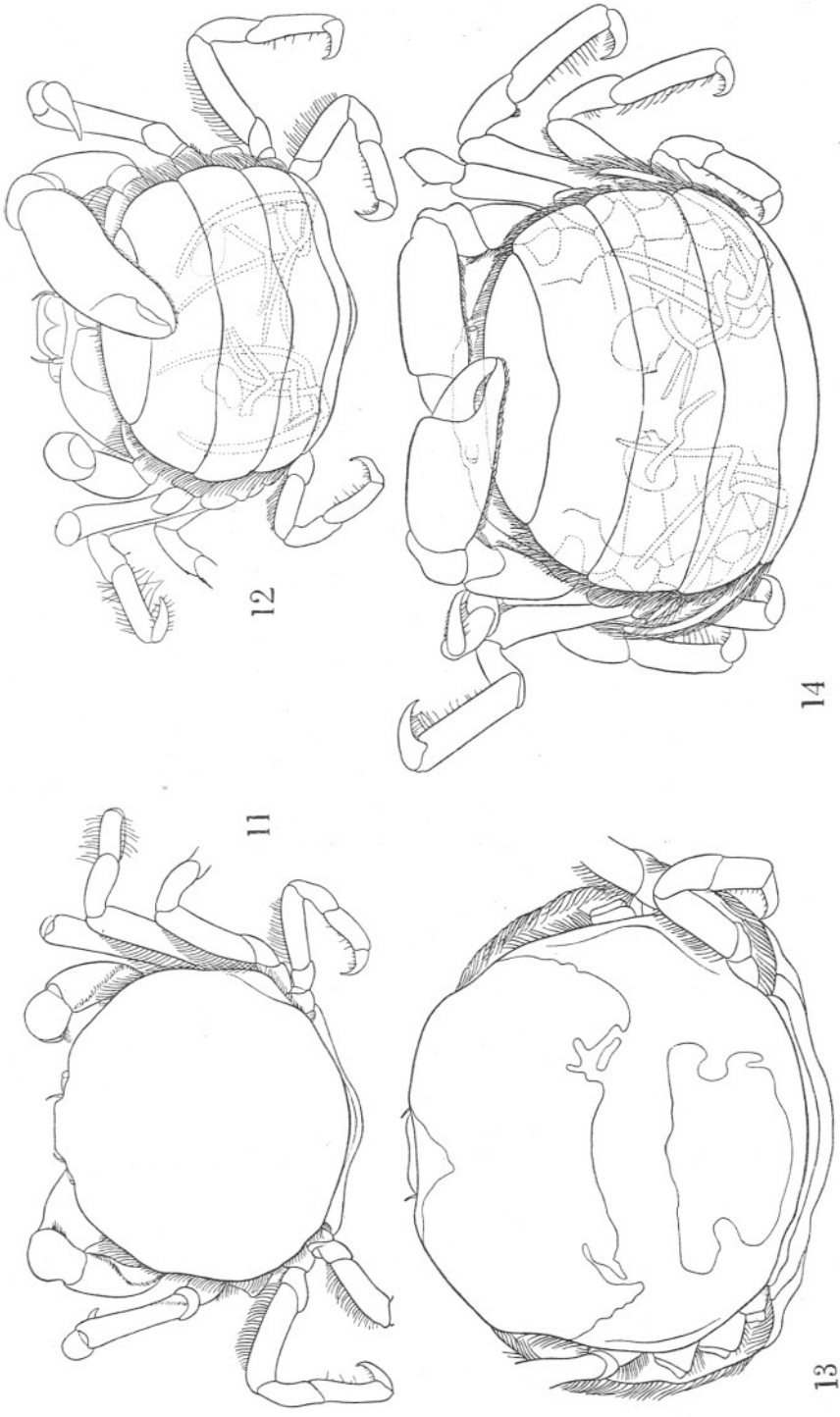
FIG. 11. Stage IV, dorsal view.

FIG. 12. Stage IV, ventral view. The spermathecæ and the pleopods are shown by dotted lines.

FIG. 13. Stage V, dorsal view. The gonad is shown in outline. The abdomen is seen extending beyond the bases of the legs.

FIG. 14. Stage V, ventral view.

PLATE III.

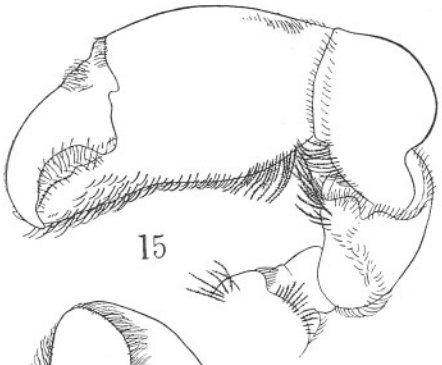


DEL. D. A.

PLATE IV.

Peræopods of right side of male and Stage II female, dorsal view, × ca. 14.

FIG. 15. Cheliped of male.
FIG. 16. Cheliped of Stage II female.
FIG. 17. First walking leg of male.
FIG. 18. First walking leg of Stage II female.
FIG. 19. Third walking leg of male. The second walking leg is very similar, but slightly longer.
FIG. 20. Second walking leg of Stage II female. The third walking leg is very similar, but slightly shorter.
FIG. 21. Fourth walking leg of male.
FIG. 22. Fourth walking-leg of Stage II female.



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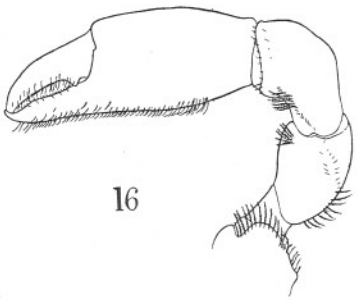

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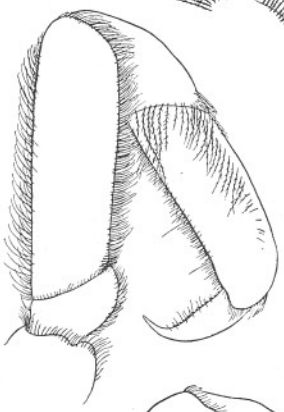

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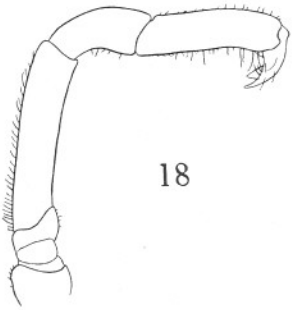

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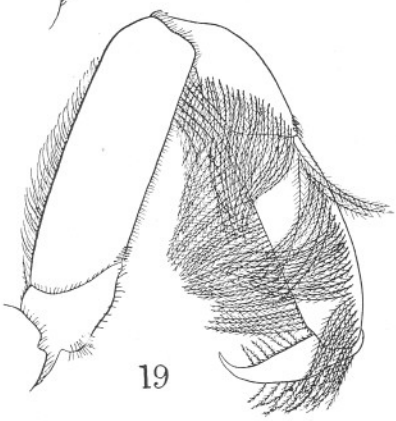

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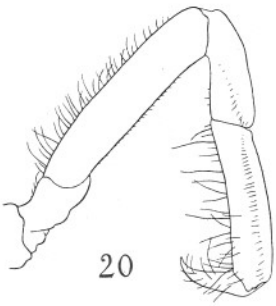

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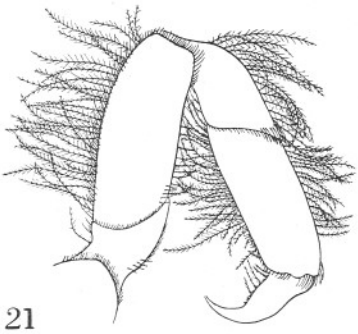

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DEL. D. A.

PLATE V.

First and second right pleopods of female.

FIG. 23. Stage I female. First pleopod—drawn from a moult—× ca. 57¼.
FIG. 24. Stage I female. Second pleopod ,, ,, ,, × ca. 57¼.
FIG. 25. Stage II female. First pleopod, × ca. 17½.
FIG. 26. Stage II female. Second pleopod, × ca. 17½.
FIG. 27. Stage III female. First pleopod, × ca. 17½.
FIG. 28. Stage III female. Second pleopod, × ca. 17½.
FIG. 29. Stage IV female. First pleopod, × ca. 12½.
FIG. 30. Stage IV female. Second pleopod, × ca. 12½.

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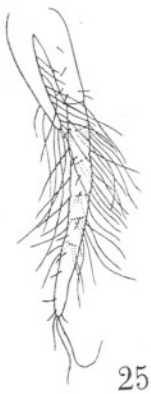

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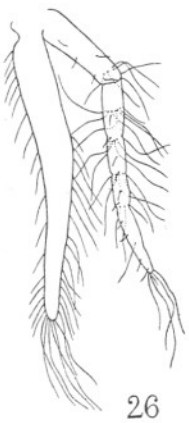

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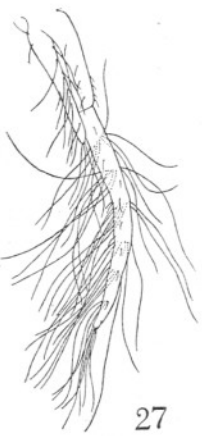

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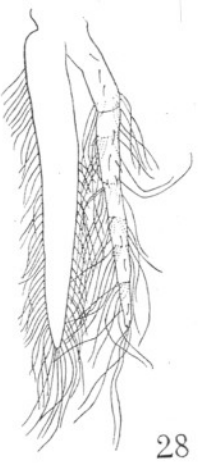

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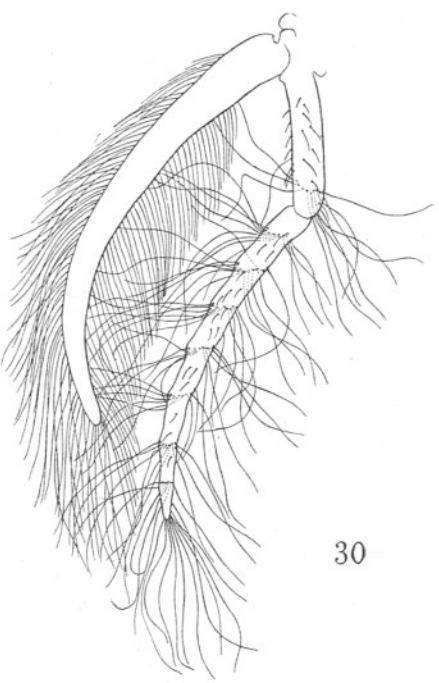

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A New Type of Luminescence in Fishes. II.

By

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Department of Oceanography, Liverpool University.

With 2 Figures in the Text.

THE experiments described in the present paper were carried out during the winter months of 1925–6 on the steam trawler *Tamura*, by the courtesy of Messrs. Neale and West, Ltd., of Cardiff. I am especially indebted to Captain Drennan, not only for the facilities for work which he provided, but for his continual encouragement in the difficult conditions under which most of the work was done. I am also greatly indebted to Professor E. Newton Harvey, whose correspondence has suggested the lines of the work, and to Professor Ramsden, of the Biochemical Department of Liverpool University, for criticising the results.

THE SECRETION.

The gland and the structure of the luminiferous epithelium of *Malacocephalus lævis* were described in a previous paper (1925). To that description it is necessary to add a note concerning the nerve supply of the gland. Careful examination has led to the belief that there is no special nerve passing to the gland, especially from the rectum. There is a network of nerve cells in the connective tissue capsule of the gland, from which fibres pass up into the folds of tissue which project into the lumen ; this network seems to be supplied by nerves arising from the dermis below the gland. The actual secreting activities of the gland are largely controlled, in my opinion, by other agencies, such as the blood supply.

As described in the earlier paper, the secretion arises in the cells lining the folds of tissue. It appears as fine granules lying in the cytoplasm between the nucleus and the external surface of the cell, and the appearance of a cell partially charged with granules is strikingly similar to that of the cells of the salivary glands figured by Bowen (1925). In fact, it is often possible to make out darkly staining masses in the neighbourhood of the nucleus, which may be indications of a network of Golgi bodies. In any case, secretion in the two cases seems to be closely similar.

Förster (1912), describing the formation of the granules in the secreting cells of Pholas, shows that they arise between the meshes of a network in the cytoplasm. The granules of Pholas, like those of *M. lævis*, have a clear central part which does not stain deeply with iron-hæmatoxylin, and an outer layer which stains very deeply.

The granules appear first near the nucleus, but multiply very rapidly in number, and soon form a dense mass which bulges out into the lumen of the gland. This process is figured somewhat diagrammatically in Fig. 1. There are comparatively few intermediate stages, so that the formation of the granules would seem to be very rapid. There is no indication that a spent cell can ever regenerate a fresh crop of granules.

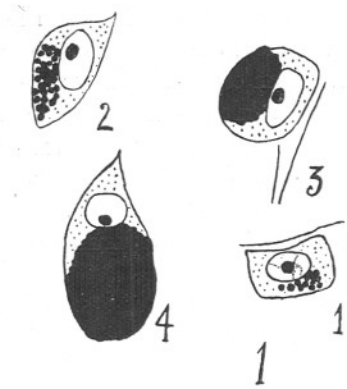

FIG. 1.—Four stages in the appearance of the luminiferous granules in the cells of the ventral gland of *M. lævis*. In 3 and 4 they form a dense mass.

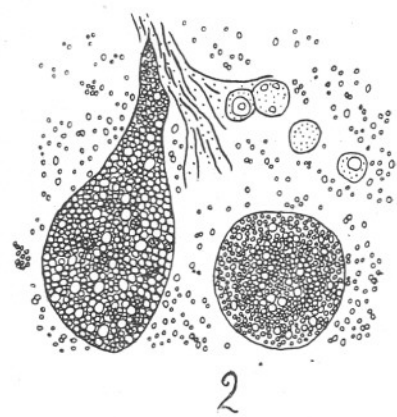

FIG 2.—The appearance of a fresh suspension of the secret on, fixed in formalin. Two globes are seen with fragments of connective tissue and some broken-down epithelial cells. Very numerous granules are also seen.

Towards the exit of the gland the tissues are seen to be breaking down, and the mass contains great numbers of secreting cells, with large nuclei, none of which bear any granules. In such glands the portion of the gland which is still actively secreting is at the periphery, as described previously.

This may be compared with the account of the luminous organs on the lower jaw of Monocentris given by Okada (1926). He finds that the epithelium of the secreting tubules is completely destroyed in the process of secretion, but that it is replaced by the constant proliferation of the cells at the base of the tubules. In this fish, also, the secretion is granular, but Okada was not able to trace its formation in the secreting cells. Since in both Pholas and *M. lævis* the appearance of the secretion is

sudden, it is possible that the rare intermediate stages may have been overlooked.

When a drop of fresh secretion, squeezed from a specimen of *M. lævis*, is examined under the microscope, it is seen to consist mainly of a dense mass of globular bodies (henceforward to be referred to as globes) measuring between 30 and 40μ, but rather variable in size. These are identical with the clumps of secretion seen in sections, but in the fresh condition one notices what was then missed, namely, that the clumps of granules, when they burst from the mother cell, are still enclosed in a cytoplasmic sheath. The secretion, as obtained in this way, also contains fragments of the other tissues of the gland, such as strips of connective tissue, and many spent secreting cells. The appearance of a suspension of the gland, as fixed in formalin, is indicated in Fig. 2.

The globes are seen to be rapidly breaking up, and the process is much more rapid if a drop of secretion is placed in contact with a drop of sea-water. Each globe is usually somewhat pear-shaped, and rupture seems to occur most commonly at the stalk. The enclosed granules escape, and increase in size to a marked extent when set free. They now appear as highly refractive oval bodies, each between 1 and 2μ in length, which show a distinct greenish fluorescence. They seem to have a fluid interior bounded by a membrane. In my view, these granules are the actual source of luminescence.

The plasmatic envelope is seen especially distinctly if the globe happens to adhere to the slide; the rolling of the ship, which causes the whole body of fluid in the drop to move from side to side, soon draws the sheath out into a stalk, at the end of which the clump of granules is suspended.

If a suspension of the secretion in a drop of sea-water is examined under the microscope in the dark, groups and chains of brilliant green globes can be seen, but by working in suitable conditions of light and darkness it is found that not all the globes seen under ordinary illumination, are luminescent. Only those which are actually breaking up, and where the escaping granules are still in a mass, are visible as points of light. In an older solution there is no longer any luminous object upon which one can focus, the reason being apparently that each individual granule does not give out sufficient light to be seen under the microscope, a fact which is also true of luminous bacteria. In an older solution, in which all the globes have broken up, the granules are scattered throughout the fluid.

The globes all break up and allow the granules to escape in the natural course of events, but the process is greatly hastened by shaking, which causes mechanical rupture of the envelope. To the naked eye the change, on shaking, is one from a liquid containing very numerous points of bluish light, to one exhibiting a uniformity diffuse bright green lumines-

cence. It is at this point that the light is most brilliant. Breaking up of the globes, however, probably takes place in natural conditions by the mechanical violence of ejection of the secretion.

The Effect of Fresh or Distilled Water.

Fresh or distilled water at once puts out the light of the secretion when this is poured into it. Examination under the microscope shows that the plasmatic envelopes of the globes undergo instant cytolysis, while the granules remain in masses; but as far as observation allows, the granules do not swell to any marked extent, nor appear any different from granules luminescent in sea-water.

If sea-water is added to such a fresh-water suspension of the secretion of *M. lævis* within a few seconds, the light returns, to some extent, but after a couple of minutes an irreversible change seems to have taken place, and no addition of sea-water or hypertonic salt solution will restore the light.

Experiments with Pure Solutions.

(a) *Sea-water.* Three test tubes were set up (i) containing 5 c.c. of sea-water and 5 c.c. of fresh water; (ii) containing 2 c.c. of sea-water and 8 c.c. of fresh water; (iii) containing 1 c.c. of sea-water and 9 c.c. of fresh water. To all three were added roughly equal quantities of secretion. In (i) feeble luminescence took place, but in (ii) and (iii) it was extremely feeble, and extinct in three hours.

This suggested that the first effect necessary for luminescence is a solution of suitable osmotic pressure. It also explained how, if one used a fish which had not been washed with fresh water or wiped, a faint luminescence took place on squeezing the secretion into fresh water.

On the other hand, a hypertonic solution of sea-water, made by adding strong salt solution, soon extinguished the light, and the change was irreversible.

(b) *Salt Solutions.* It was found that luminescence occurred in distilled water solutions of pure NaCl, KCl, and K_2SO_4, isotonic with sea-water, without any notable difference from the luminescence in sea-water. For the remaining experiments, therefore, distilled water was not used, but the ship's tap water (pH 7·4).

The following substances were used: KCl, NaCl, $MgSO_4.7H_2O$, KNO_3, $CuSO_4$, together with cane sugar, alcohol, and glycerine.

Strong solutions of these substances in distilled water were taken out, and from these, by dilution with tap-water, solutions of known strength could be prepared.

In each case test tubes were set up in a series, such that the solutions contained in them were of increasing strength. To these tubes were then added roughly equal amounts of secretion, and the tube noted which gave the brightest and most sustained luminescence. Owing to the easily understood difficulty of placing quantitatively equal amounts of secretion in each tube, no attempt was made to get anything but an approximate optimum strength in each case.

The results were as follows: copper sulphate, alcohol, and glycerine would not support the luminescence in any concentration, the first probably because of a directly poisonous effect, and the last two, probably because their great permeability renders their presence in water ineffectual in causing osmotic pressure.

The remaining substances will support luminescence as readily as a corresponding amount of sea-water, with a reservation on account of hydrogen ion concentration, as will be explained in the sequel. The optimum strengths, in each case, are given below in the first column; in the second column are given the strengths which De Vries, in his classical experiments with plant cells, found to produce equal effects i.e. to be isotonic with one another.

Cane Sugar.	30 per cent.	27–28 per cent.
$MgSO_4.7H_2O$	5 ,, ,,	26–28 ,, ,,
KNO_3	6 ,, ,,	6–7 ,, ,,
NaCl	2 ,, ,,	3–4 ,, ,,
KCl	2–4 ,, ,,	4–5 ,, ,,

There is thus a noteworthy correspondence between the two sets of figures, with the exception of $MgSO_4.7H_2O$, and the solutions are roughly isotonic. But magnesium has a peculiar effect on living matter; Bayliss (1920) quotes several authors who have demonstrated its anæsthetic action, and its use as an anæsthetic in marine biology is well known. Its abnormally low value in the present case may be due to a peculiar effect on the granule membrane. A discussion of all these effects will follow later. As a matter of fact, there is a slight difference in behaviour between e.g. NaCl and KCl, in duration of luminescence, and possibly these ions differ in their effects on the granules, as Gray (1923) shows that they differ in their action on the cilia of the gills of *Mytilus*.

It is obvious, therefore, that we are here dealing with an osmotic effect. In suitable conditions osmotically, the luminous material will diffuse out of the granules and luminescence will occur. With solutions of greater or lesser strength luminescence becomes increasingly feeble and short lived.

In all these cases, as long as there is any luminescence taking place, a restoration of the suitable conditions will restore the light to a con-

siderable extent, but once luminescence has vanished, it cannot be restored.

Effect of Hydrogen Ion Concentration.

None of the solutions used in the preceding experiments were as brilliant as a corresponding fresh sea-water suspension of the secretion. Now the sea-water was found to have a pH in the neighbourhood of 8·4, whereas the artificial solutions were between 7·4 and 7·6. This suggested that, as in so many other cases, acidity inhibits and alkalinity favours oxidation in the present case, since luminescence is undoubtedly an oxidation. To test this, a 2 per cent solution of pure NaCl in tap water, which was found to support luminescence best, was used. To test tubes of this liquid were added one drop, two drops, and so on of arbitrary solutions of HCl and NaOH. The pH was found by the colorimetric method, using solutions which had been treated identically with the above.

At pH 9·6 luminescence was brighter than in a corresponding measure of sea-water, and much more sustained. Above this however, luminescence became increasingly feeble, and would probably cease to take place much above ten.

On the other hand, between six and seven luminescence became more and more feeble and short lived, until at 5·4 extinction was almost instantaneous, the secretion being precipitated as a greenish yellow powder. When a portion of this precipitate was neutralised with sodium bicarbonate after half an hour, a feeble light returned, but twelve hours later this process failed to recover the light.

In a similar way, acidifying, after an interval, would not restore the light of a solution which had been extinguished with alkali.

These results with acid solutions suggest that the dimming of a glowing suspension of *M. lævis* secretion is due, in the first place, not so much to an exhaustion of the luminous material, as to an increasing acidity due to the products of its own activity. The change in hydrogen ion concentration of about 150 c.c. of sea-water, to which the secretion had been added, was observed at four-hour intervals by the colorimetric method. The results were :—

After	0	hours	pH was	8·4*
,,	4	,,	,,	,, 7·8
,,	8	,,	,,	,, 7·4
,,	12	,,	,,	,, 7·2
,,	16	,,	,,	,, 7·2
,,	20	,,	,,	,, 7·2

* Values uncorrected for salt error. Correction to be subtracted is 0·2 units.

Unfortunately, a control experiment with sea-water alone was not done at the same time. Owing to the respiration of the organisms contained in it, fresh sea-water naturally tends to become acid when stood in darkness, and this may have accounted for some part of the rapid decrease in alkalinity found in this experiment.

This change, involving an increase in acidity, was correlated with the brilliance of luminescence. There is always a rapid falling away from the first intense brilliance, though luminescence will continue feebly for as much as six days. Moreover, after eight hours bacteria commence putrefaction of the liquid. A stale suspension swarms with various kinds of bacteria and infusoria.

There is thus reason to believe that, in the very artificial conditions of experiment, the loss in brightness during luminescence is due to the increased acidity (or diminished alkalinity, rather) of the medium.

The acidity may be caused partly by free CO_2, for on filtering a suspension of the secretion into a fresh vessel, there is an unmistakable increase in brightness, which in this case might be due to a temporary decrease in CO_2 and increase in oxygen during filtration. The effect is temporary only. A filtered solution is extinguished earlier than a non-filtered one, as will be explained below.

The Effect of Calcium Ions.

It seemed worth while to investigate the effect of calcium ions on the luminescence. It seems that their presence or absence has no effect in this case. Luminescence was bright and sustained in a liquid composed of 7 c.c. of pure KCl solution isotonic with sea-water, and 3 c.c. of a saturated solution of potassium oxalate. Indeed, there is no reason to doubt that luminescence would have occurred in the pure solution of potassium oxalate.

The Effect of Cytolytic Agents.

The granules seem highly resistant to cytolytic agents. As mentioned earlier, fresh or distilled water produces no obvious effect, though it destroys at once the plasmatic envelope of the globe. Neither saponin nor sodium glycocholate seemed to produce the slightest effect, either in sea-water or in fresh water. In the former luminescence was as brilliant and as sustained as in ordinary sea-water ; in the latter there was instant extinction, as in the absence of the reagent.

Luminescence was not extinguished on allowing a suspension of the secretion to stand for eight hours over chloroform and carbon tetrachloride, nor was it extinguished by toluene. All these reagents, however, precipitated the luminous material on shaking up, the first two giving a

thick white sediment, the last named giving a solid plug of white material. This is, no doubt, the luminiferous substance, which is probably protein in nature, and has been coagulated at the surface of the droplets into which the oils break up on shaking. Even so, however, a faint light persists, in the case of carbon tetrachloride, after shaking.

Oxygen.

Abundant oxygen is necessary for luminescence. A drop of pure secretion, placed between glass slides, at once becomes dark except at the edge of the drop, which now appears as a bright blue ribbon. As mentioned in the previous paper, a strong suspension of the secretion soon ceases to be luminescent except at the surface. If a test tube full of such a suspension is warmed by the hand, one can see the light creeping down the sides of the tube as convection currents are set up. Also, if one blows small bubbles of air through the dark part of the liquid from a pipette, each droplet gives rise to a dazzling light.

But this phenomenon of " ringing " may appear in an old (acid) solution, and here again the explanation may be that, owing to the acidity of the liquid, conditions suitable for luminescence have again become confined to the surface. Yet oxidation is not notably hastened by the shape of the containing vessel. Luminescence seems to last as long in a flat, shallow jar, as in a tall narrow test tube. This strongly suggests that the dimming of the luminescence is rather due to an inhibition by the products of its own activity, than to a lack of oxygen or an excess of CO_2.

Effect of Light.

A suspension of the secretion of *M. lævis* was divided into two portions. One was poured into a test tube and hung up in the rigging in bright sunlight. The other was kept below, in darkness. After three hours the exposed tube was brought below and the two solutions were compared. The tube exposed to light was glowing extremely feebly, while the control was in full brilliance. That the effect was not produced by the increased oxidation due to the fresh breeze, was shown when the control was hung in a similar position all night without affecting its brilliance.

Effect of Temperature.

A jar containing a fresh suspension of the secretion of *M. lævis* in sea-water was placed in the ice box for several days. On being examined it was found to be very faintly luminescent with a pale blue light. On allowing this liquid to attain air temperature, luminescence reappeared

as normal. This is the only method so far found for preserving the secretion for any length of time ; even here, however, there is slow oxidation, for a suspension which has been preserved in this way does not remain luminescent very long.

At body temperature (approximately 37–38° C.) extinction is considerably hastened, a test tube being extinct in ten hours at this temperature, where the control was still bright after two days.

When the temperature rises to 47° C., the light disappears very suddenly, though dimming begins slightly before this. If the tube is plunged into cold water at once, some of the light returns; but destruction of the mechanism of luminescence is very rapid at that temperature, and only a few seconds serves to cause an irreversible change.

At 55° C. the change is completely irreversible. Luminescence seems, therefore, to follow the usual rule, in that it is hastened up to a certain point, after which the hastening effect is first balanced, and then masked, by destructive effects. There were obvious experimental difficulties in the way of a more accurate determination of the effect of temperature on luminescence. But Harvey (1922) showed that in Photoblepharon and Anomalops, whose light is due to symbiotic bacteria, the light dims at 40° C. and 38° C. respectively ; and is extinguished at 41–42° C., and 43° to 44° C., respectively. On the other hand, Pholas luciferase is not destroyed until 60° C. is reached ; in the present case, therefore, dimming and extinction may be associated with a destruction of the properties of the membrane, rather than with any direct effect on the luminiferous materials.

The Effect of Filtration.

The more thoroughly the suspension is filtered off, the feebler and shorter lived is the luminescence of the filtrate. The filter papers used would not remove all the granules, even when four layers were tried. The filtrate, in this case, was only luminescent for three hours. The temporary effect of filtration in increasing the brightness of luminescence has been referred to.

The filtrate from a suspension of *M. lævis* secretion gives negative results in all experiments tried. It will not give the luciferin-luciferase reaction with a luciferin solution which has also been filtered. Reducing agents will not restore the light (Na_2SO_3, $NaNO_2$), though they cause a temporary increase of brightness in a feebly luminescent suspension. Here the effect is probably caused by the alkalinity of these substances.

CONCLUSIONS.

Quite apart from any chemical considerations the form in which the luminiferous substances are present in the secretion must be examined. Apparently these are present in the granules, whose origin can be seen under the microscope, and these are set free from their investing sheath of cytoplasm either naturally or by violence. The cytoplasmic sheath plays no important part in luminescence.

In suitable conditions osmotically the luminiferous substances diffuse out of the granules, and in a medium of the right alkalinity, oxidation with luminescence occurs at the surface of the granule membrane. There is very little luminescence free in the medium, since a well-filtered suspension will give a filtrate in which luminescence is very transient; hence the amount of fuel-material free in the fluid at any moment must be very small. This explains the great difficulty experienced in attempting to demonstrate the luciferin-luciferase reaction.

There is an optimum temperature, which is probably low, and the process occurs best in darkness. In natural conditions all these requirements are met, and the great number of granules give an enormous surface area for luminescence, to sustain which there is a continual supply of fresh material diffusing out. This type of luminescence should therefore be a very efficient source of light for whatever purpose it is designed.

In this connection a note by Osorio (1912) is of great interest. He says that Portuguese fishermen use the secretion to smear over pieces of dog-fish for use as bait; they believe that the fish are attracted to the hooks by the light. Osorio considered the light due to bacteria.

In the artificial conditions of experiment the granules never become exhausted, since conditions automatically become unsuitable long before exhaustion occurs. It is therefore impossible to say whether the granules are permanent bodies, or whether they finally dissolve away. They appear quite unchanged after twenty-five hours, during which luminescence has passed its most brilliant phase.

Moreover, the luminiferous materials themselves may be unstable, for on drying the secretion, even at low temperatures over calcium chloride, the power of luminescence is lost, and cannot be regained by moistening with either fresh or salt water. But the act of drying would probably suffice to destroy the permeability of the granules.

DISCUSSION.

Each granule of the secretion of *M. lævis* is bounded by a membrane whose permeability depends upon the environment, but which normally keeps the light-producing substances within the granule. It is rendered impermeable by fresh or distilled water, and the change of permeability is irreversible. Hypertonic solutions also cause an irreversible change. The granules differ in this respect from *Amœba* (Pantin, 1923) and from the cilia on the gills of *Mytilus* (Gray, 1922), where the change caused by these reagents is reversible, and where complete recovery takes place on restoring suitable conditions. Pantin points out that cytolysis did not take place for some hours when an *Amœba* was placed in triple-strength sea-water. An irreversible change had taken place in the granules of *M. lævis* in considerably less time than this, but much stronger solutions were used (four or five times the optimum in some cases).

In a similar way the effect of acid may be simply an inhibition of oxidation, as acid inhibits the oxidation of pyrogallic acid, or it may inhibit luminescence by rendering impermeable the granule membrane. Pantin (*loc. cit.*) shows that increasing the acidity of the medium inhibits contractility in the species of *Amœba* which he studied, but that the process is completely reversible on transferring to a normal medium. Gray (*loc. cit.*) finds that a very low pH(3·4) is required to cause complete stoppage of ciliary activity in the gills of *Mytilus*, and that complete recovery takes place when the medium is made alkaline. The effect of great alkalinity was not studied by Pantin on account of a change which took place in the composition of the medium in such conditions. Probably, its effect on the granule membrane in the present case is to destroy its permeability. It will be noted that the granules themselves are very resistant to cytolysis.

Pantin suggests that, in the case of *Amœba*, "the rise in hydrogen ion concentration may increase the gelation of the protoplasm"; and J. Graham Edwards (1924) shows that the effect of acid on fresh-water amœbæ is to make the surface of the *Amœba* gelatinous and adhesive, and that the change is irreversible if the action is too prolonged. But Gray considers it "impossible to accept the suggestion that the normal activity of the ciliated cells is upset by acids owing to a disturbance of the cell surface." "Evidence is advanced which suggests that the presence of acid prevents the conversion of chemical energy into kinetic energy."

This effect of acid on the cilia of *Mytilus*, and the explanation suggested by Gray, might be applied in some measure to the present case. Luminescence must be regarded as a conversion of chemical energy into light

energy, and the rate of conversion might likewise depend upon the removal of acid, that is, upon the alkalinity of the medium. In vitro a luminescent suspension rapidly increases the acidity of the medium, with a correspondingly rapid fading in the brilliance of the light. In this case, however, the presumed irreversible effect of acid on the granule membrane must also play its part.

The essential difference between the behaviour of a cell and that of these granules seems to lie in the fact that the cell shows great power of recovery, the granules little or none. Usually, once the light has dimmed or vanished there is no means of restoring its former brilliance. I consider that this is due to the readiness with which irreversible changes take place in the granule membrane.

This difference compares in an interesting way with the behaviour of non-nucleated fragments of various Protozoa and algæ, as quoted by Wilson (1925). These fragments likewise lack the power of recovery and regeneration, though destructive metabolism may still continue ; whereas nucleated fragments can regenerate the missing structures and recover their former condition. It suggests that, in both cases, a lack of the power to recover is associated with the absence of nuclear material.

SUMMARY.

Experiments are described which indicate that there is a physiological problem quite apart from the chemical problem of luminescence in the secretion of *Malacocephalus lævis* (Lowe). The luminiferous substances are present in granules, which behave as though each were bounded by a membrane whose permeabilities resemble those of a typical cell, but differ from a cell in that they have little or no power of recovery from adverse conditions.

For optimal luminescence they require (i) a medium of a certain osmotic pressure, (ii) a certain range of alkalinity, (iii) a certain range of temperature, and (iv) abundant oxygen. Sea-water is not necessary for luminescence.

If they are exposed to extremes of acidity or alkalinity, or of hypotonic or hypertonic solutions, irreversible changes rapidly set in in the membrane of the granule, whereby the power of luminescence is lost.

In artificial conditions the rapid fading of the light from the initial brilliance is probably due to an increasing acidity caused by the accumulation of the products of oxidation.

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Abnormal Vertebræ in Herrings.

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With 3 Figures in the Text.

An important part of recent investigations on herrings at Plymouth has been the collection of statistical data on the number of vertebræ, and when this report was written a total of 6869 fishes had already been examined with regard to this character. In the case of each of 105 fishes, however, the true number of vertebræ could not be stated with confidence, owing to marked irregularities in one or more vertebræ.* These abnormal specimens are of more than passing interest. The abnormalities themselves are such that it would be well worth while to make a careful morphological study of "fresh" specimens to determine the extent to which nerves and muscles are affected. The determination of the distribution of abnormalities along the vertebral column suggests a possible explanation of the way in which they may have arisen. Moreover, there remains the question as to how the abnormal vertebræ should be counted when endeavouring to ascertain the true total number of vertebræ for statistical or genetic purposes. In the paragraphs which follow, the 105 cases of abnormality are described and their significance both in numbers and position discussed, but it should be remembered that all observations and measurements were made on dried skeletons, which had been prepared by boiling and cleaning.

The Characters of the Abnormalities.

In each of the skeletons under review, abnormality is localised in one or more distinct vertebral elements, which may be classified according to their features. The summary in Table 1 shows that a total of 142 such elements were found in the 105 skeletons obtained, but that generally a single skeleton had only one abnormal element.

* *Orton* (**3**) has given a detailed description of similar cases of irregularity obtained during his investigations on herring at Plymouth in 1914.

TABLE 1.

No. of abnormal elements per skeleton	1	2	3	5	7	Totals.
Number of skeletons	82	17	3	2	1	105 skeletons.
Total of abnormal elements	82	34	9	10	7	142 abnormal elements.

In the following description of the arbitrary classes into which the abnormal elements have been grouped, sufficient data have been included to indicate the relative frequency of occurrence of the several classes.

Class 1.

A typical vertebral element of this class has the appearance of an incipient " double " vertebra. It bears an extra pair of hæmal and neural spines arising from the median portion of the centrum, where there is a more or less distinct vertical ridge. There is, however, no suture or epiphysial growth such as occurs between two normal vertebræ. In abnormal elements from the anterior region of the body there is also an associated doubling of the epineural, pleural, and epipleural bones. In length these " double " vertebræ vary from 1 to $1\frac{1}{2}$ times that of normal adjacent vertebræ. In the cases included in this class a gradation can be noted, from those in which only an extra neural arch and an extra hæmal arch occur with no distinct ridge to the centrum, to others which have almost the appearance of two normal, though undersized, vertebræ (see Fig. 1A).

In 57 of the total of 105 skeletons the abnormal elements are exclusively of this class, and in 52 instances a single skeleton exhibits a single abnormal element. In a further 6 skeletons an abnormality of this class is accompanied by an irregularity of another class. Of the total of 142 abnormal elements examined, 71 are included under Class 1.

Class 2.

Abnormal elements of this class are analogous in structure to those of Class 1, but are triplicate in formation. The length of the compound element is less than twice that of normal vertebræ. In 14 skeletons these triplicate vertebræ are exclusively present, while in 3 other cases they occur with other abnormal elements.

Class 3.

Compound vertebræ of apparent " quadruple " formation occur in 3 skeletons, but in no case does the length of the element exceed twice that of normal vertebræ (see Fig. 1C).

Class 4.

In 21 skeletons interesting bilateral asymmetry occurs. One side of an ordinary-sized vertebra has the structure described for either Class 1 or Class 2, while the other appears normal. Occasionally one side is of Class 1 and the other of Class 2.

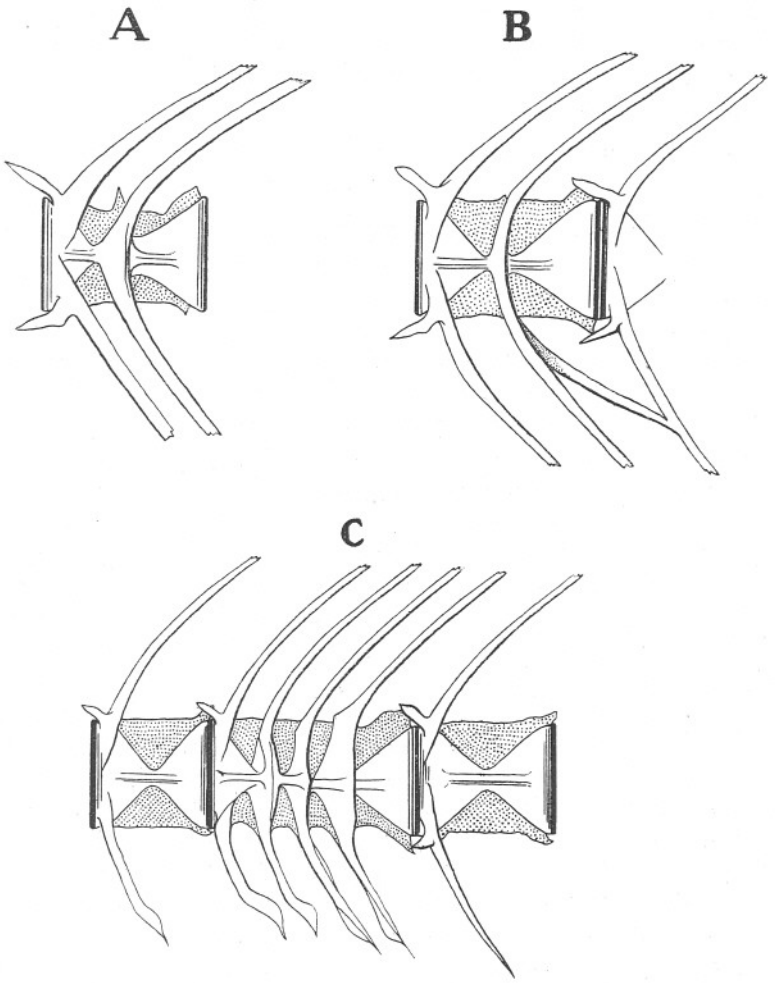

FIG. 1.—A. Typical example of abnormal vertebra of Class 1.
33rd vertebra of a herring 29 cm. in length.

B. Abnormalities of Classes 1 and 5 occurring together.
21st vertebra of a herring 22 cm. in length.

C. Abnormality of Class 3.
Fusion of vertebræ 25–28 incl.; in a herring 28 cm. long.
Vertebræ 24 and 29 are normal.

Class 5.

This class was created to include a number of minor abnormalities which were considered to be of no immediate importance.

(*a*) One of the neural or hæmal spines of a single vertebra or of one of the components of a " double " vertebra, becomes attached obliquely to the neural or hæmal spine of the opposite side of the preceding vertebra or vertebral component. The fellows of these spines remain single and unattached (see Fig. 1B).

(*b*) A slight twisting of a part of the vertebral column involving several adjacent vertebræ.

(*c*) Minor irregularities of certain vertebræ which may be due to mechanical injury at some earlier stage in the life-history.

In most instances these minor irregularities occur in association with those of the earlier classes, but in any case there is no visible differentiation of the centrum suggesting a compound formation.

In a short essay on the " numerical signification of fused vertebræ," Schmidt (**4**) discussed the question as to how certain peculiar vertebral elements in reared trout should be counted in his investigations on the inheritance of the number of vertebræ. In each of 8 samples of the offspring from diallel crossings, he found a number of individuals (average ca. 10%) in which the vertebra fifth from the end of the tail showed a peculiarity impossible to ignore and which placed him in doubt as to whether he was dealing with a single or a double vertebra. He was able to show, however, that the average number of vertebræ for the " abnormal " specimens in the 8 samples closely approached that for the " normal " specimens if a numerical value of $1\frac{1}{2}$ was assigned to the irregular vertebra. On this evidence Schmidt advanced the theory that " vertebrate animals can realise fractional parts of vertebræ, but it is evident that such individuals are numerically inferior to what we above called normal individuals. In reality the former are just as normal as the latter. In both cases it is the individual's genetic structure in connection with its environment in the sensitive period, which is deciding the total realised ; but it seems as if whole numbers in such organs as vertebræ are more easily realised than fractional parts."

We have carried out some counting trials with our abnormal herrings and the results have proved instructive. It will be seen from Table 2 that if we count the irregular vertebræ as single units the average number of vertebræ for the 95 specimens examined is 54·40, the variates ranging from 48 to 57. These values are much lower than those obtained from

"normal" specimens. If, however, we assume that the "abnormal" vertebræ each represent several whole vertebræ and assign to them an equivalent numerical value, then the average closely approaches that for the "normals."

For the third counting trial all specimens which showed more than one vertebral irregularity were excluded in order that the material upon which the count was to be made should be such that Schmidt's theory and method might reasonably be applied to it. But it is clear from

TABLE 2.

Total No. of Vertebræ.	No. of "Normal" Skeletons.	No. of "Abnormal" Skeletons. Abnormal element counted as: One Vertebra.	x Whole Vertebræ.
48	—	1	—
49	—	—	—
50	—	1	—
51	—	2	—
52	—	3	—
53	3	5	2·5*
54	70	26	3
55	1921	46	24
56	4157	10	47·5*
57	595	1	16
58	18	—	2
Totals	6764	95	95
Arith. mean.	55·79	54·40	55·82
	Difference from 55·79	−1·39	+·03

Table 3 that the adoption of the numerical value of $1\frac{1}{2}$ for a double vertebra produces a less satisfactory "fit" to the normal average than that resulting from the use of the value of 2.

The fact that in the reared trout abnormality was localised in a particular vertebra in the tail region is worthy of notice. If for the moment it be conceded that fractional parts of vertebræ can actually be realised, the

* In some skeletons it is not possible to decide upon the exact number of vertebrae. In the case of a skeleton whose whole total number appears to be either 53 or 54, the entry of $\frac{1}{2}$ is made to each of the variants 53 and 54.

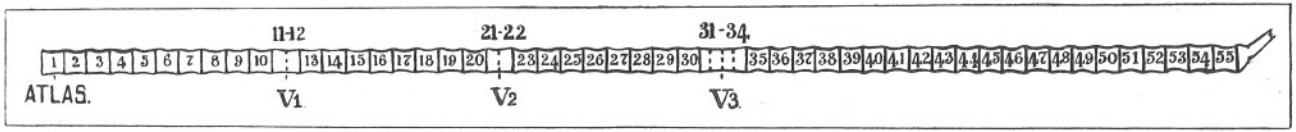

FIG. 2.—Diagram to illustrate method of defining the serial position of abnormal vertebral elements.

V_1. Abnormality of Class 1 (fusion of vertebræ 11 and 12).
V_2. Abnormality of Class 1 (fusion of vertebræ 21 and 22).
V_3. Abnormality of Class 3 (fusion of vertebræ 31–34 inclusive).

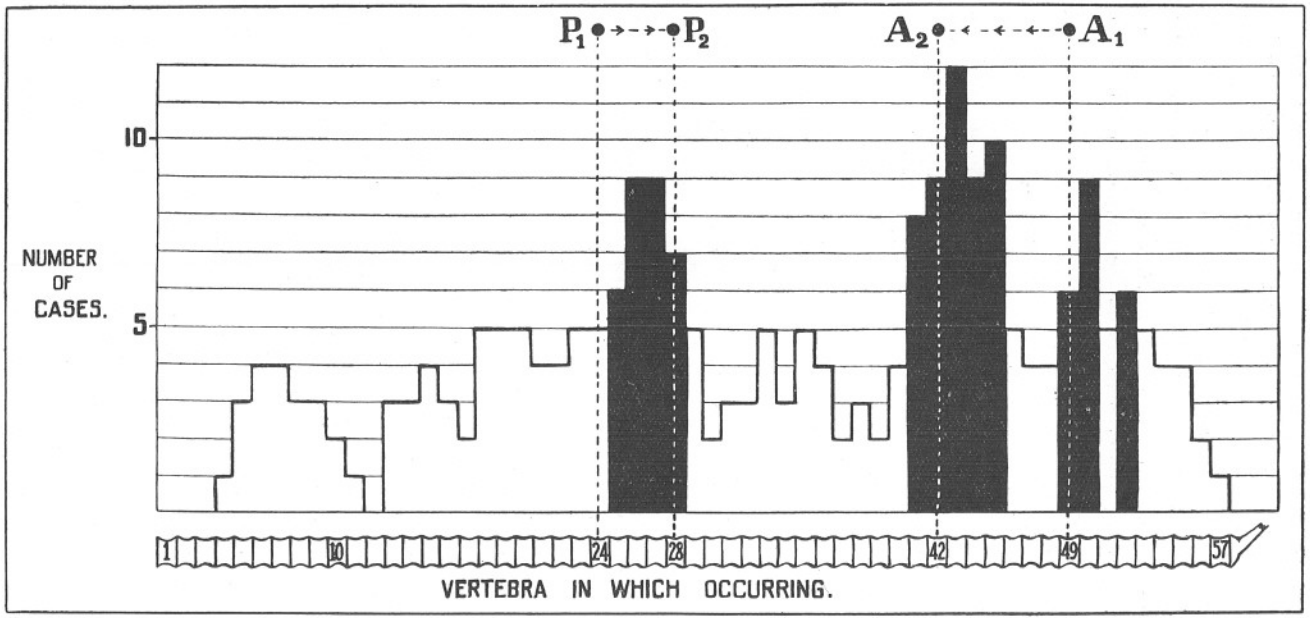

FIG. 3.—Distribution of abnormality along vertebral column. Diagrammatic representation of data summarised in Table 4 (p. 516). Vertebræ involved on more than five occasions are blocked in black.

A_1. Initial position of anus in post-larvæ. } *vide* Lebour (2)
A_2. Final „ „ in young fish. }

P_1. Initial position of pelvics in post-larvæ. } *vide* Lebour (2)
P_2. Final „ „ in young fish. }

localisation of the abnormality suggests that one particular vertebra is alone able to express itself in a broken number. In the herring, cases of irregularity have been observed in almost every vertebra throughout the vertebral column, but there is a distinct indication that some parts

TABLE 3.

Total No. of Vertebræ.	No. of Skeletons. "Double" element counted as: 1 Vertebra.	1½ Vertebræ.	2 Vertebræ.
52	1	—	—
52·5	—	1	—
53	2	—	1
53·5	—	2	—
54	15	—	2
54·5	—	15	—
55	32	—	15
55·5	—	32	—
56	7	—	32
56·5	—	7	—
57	—	—	7
57·5	—	—	—
Total	57	57	57
Arith. mean.	54·74	55·24	55·74
Difference from "normal" mean (55·79)	−1·05	−·55	−·05

are more susceptible than others. Table 4 and Fig. 3 express the relative frequency of abnormality for each vertebra along the vertebral column. To obtain these data, the total number of vertebræ in each skeleton was ascertained after making the appropriate numerical allowance for the number of whole vertebræ apparently involved in each abnormality, and the serial position of each abnormally presented vertebra was noted. The method of recording will be clear after reference to the hypothetical case shown in Fig. 2. It will be observed in Figure 3 that while vertebræ, Nos. 1, 2, 3, and 12 are the only ones in which abnormality has as yet not been seen, twelve of the vertebral series have each been involved on more than five occasions. The distribution of these twelve vertebræ is of especial interest: four of them are consecutive, forming the group, vert. 25–28,

inclusive, and the remaining eight occur in the region, vert. 41–52. Now in Fig. 3 we have indicated the initial and final positions of the anus (A_1 and A_2) and of the pelvic fins (P_1 and P_2) with respect to the vertebræ during the early life of the herring, as determined by Lebour (**2**, p. 451). These positions fall within the range of the twelve vertebræ just enumerated. In view of the limited number of skeletons examined it would be obviously unwise to attach definite significance to this coincidence in position, but we may at least suspect that there is some connection between the formation of abnormal vertebral elements and the differential growth of the post-larva. It would appear from Lebour's results, however, that the number of myotomes and hence, presumably, the number

TABLE 4.

Number of cases of Abnormality.	Serial numbers of Vertebrae involved.		Totals exclusive of 24–28, and 43–49.	Totals 24–28, and 43–49 only.
0	*1, 2. 3, 12.*		4	
1	*4, 11, 57.*		3	
2	*10, 17, 30, 37, 39, 56.*		6	
3	*5, 8, 9, 13, 14, 16, 31, 32, 34, 38.*		10	
4	*6, 7, 15, 21, 22, 36, 40, 54, 55.*	*47, 48.*	9	2
5	*18, 19, 20, 23, 29, 33, 35, 51, 53.*	*24, 46.*	9	2
6	*52.*	*25, 49.*	1	2
7		*28.*		1
8	*41.*		1	1
9	*50.*	*26, 27, 42, 44.*	1	4
10		*45.*		1
12		*43.*		1

of vertebræ, is fixed before the "migration" of the anus and pelvics begins; so that our abnormal vertebral elements would thus have to be considered as the result of partial coalescence of several whole vertebræ—a proposition already regarded as likely from other considerations.

It is not suggested that the movements of the anus and pelvics are alone concerned in the formation of abnormalities, for while it is true that irregularities most frequently occur in the region of the anal and pelvic movement, cases occur elsewhere in numbers. Lebour states that the dorsal fin which lies originally over the region, vert. 33–42/43 comes to lie over vert. 26–34/35. The total range of this movement fills the gap between P_1 and P_2, and A_1 and A_2. Then posteriorally the anal fin moves with the anus. Anteriorally the pectorals find attachment, and in Fig. 3 there is a marked peak at vert. 6–7. The suggestion is rather that the whole effect of differential growth should be taken into account.

Finally, it is interesting to note that Gemmill (**1**, p. 52 footnote) refers to a statement from Hubrecht (*Klassen u Ordn.* 6, *Abt.* 1–3, p. 60) that in shark-like fishes, pathological coalescence of vertebræ is commonest at the places of connection of the paired fins with the vertebral column.

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A General Survey of Larval Euphausiids, with a Scheme for their Identification.

By

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With 1 Figure in the Text.

WHILST working at the life histories of the euphausiids occurring in the English Channel, and also having had opportunities of examining the larvæ of several other species belonging to different genera from various localities, the writer noted certain features in the larvæ which usually enabled the genus to be distinguished easily. The chief specimens were obtained from plankton collected by Mr. F. S. Russell within a radius of twelve miles from Alexandria, Egypt, whilst working for the Egyptian Fisheries Department. The larval euphausiid material from this collection is rich, and an account of it will soon be published. In the meantime the following general notes are offered, as at present little is known of the systematics of euphausiid larvæ. As several workers are occupied with this group, so important from the point of view of the Fisheries, it seemed advisable to bring all that is known together. In so doing it has been found possible to form a working scheme, from which, being given a series of larvæ of any species, one is enabled to determine the genus. For this scheme the order of development of the pleopods has been taken as the main factor, as it is found that the pleopods develop differently in several of the most important genera.

There are eleven genera of euphausiids recognised, i.e. Bentheuphausia, Pseudeuphausia, Euphausia, Thysanopoda, Nyctiphanes, Meganyctiphanes, Nematoscelis, Thysanoessa, Nematobrachion, Tessarabrachion, and Stylocheiron.

Nothing is so far known of the larval stages in Bentheuphausia, Nematobrachion, and Tessarabrachion, and of Pseudeuphausia only enough to show that the young larva somewhat resembles that of Nyctiphanes. These four are, until more is known, left out of the following scheme. There remain seven genera, viz. Euphausia, Thysanopoda, Nyctiphanes, Meganyctiphanes, Nematoscelis, Thysanoessa, and Stylocheiron, the life histories of which are more or less known and these form the basis of the present paper.

Whilst working out the Channel species the complete series of larval stages was found and described (Lebour, 1924, 1925, 1926a) in *Nyctiphanes Couchii*, *Meganyctiphanes norvegica*, and *Thysanoessa inermis*. Miss Jorgensen (1925), from the North Sea material, has described several stages of *Thysanoessa longicaudata* and a few specimens of the same species were described from the Atlantic (Lebour 1926a), whilst Mr. R. Macdonald from Millport is preparing a description of the larval stages of *Thysanoessa Raschii*. From the Alexandria material an almost complete set of larval forms of *Stylocheiron Suhmii* was obtained and several stages of *S. abbreviatum*, a description of which is now published (Lebour, 1926b), and besides these there are in the Alexandria material almost complete series of the larval forms of *Thysanopoda æqualis*, *Nematoscelis microps*, and *Euphausia Krohnii*, with a few specimens of another larva, almost certainly an Euphausia. With these, together with the few published descriptions of larvæ by Sars, Hansen, Tattersall, and others, we can draw up a fairly comprehensive review of larval forms which considerably adds to our knowledge of the group.

The general order of development in the Euphausiidæ is the following : first the egg, which may be carried by the female (known in the genera Nyctiphanes, Stylocheiron, and Nematoscelis) or may be shed into the sea at an early stage of development (known in Meganyctiphanes and Thysanoessa). For several genera, however, the mode of egg deposition is not known, although it is probable that Euphausia and Thysanopoda shed them into the sea, and that Tessarabrachion and Nematobrachion carry them. In Nyctiphanes the eggs are carried until an advanced stage (Lebour, 1924), the nauplius only occurring inside the egg sac, and the young being shed into the water as a pseudometanauplius covered by a skin which is immediately sloughed on emergence and the metanauplius freed. Nauplii and metanauplii of Nematoscelis and Stylocheiron have not yet been seen. It is suggested by Calman (1909) that the young may emerge at an advanced stage in Stylocheiron, the eggs being so large. In the Alexandria material there are three Calyptopis stages, almost certainly belonging to *Stylocheiron Suhmii*. The smallest and youngest of these which is a first Calyptopis is quite small, measuring 1·28 mm. in length, and might easily have come from one of the large eggs.

The typical procedure in an euphausiid is for a nauplius to hatch out of the egg into the water and this first nauplius almost immediately to slough its skin and become a second nauplius, this changing into a metanauplius. These are well described by Metschnikoff (1871) and Sars (1898). Typical forms are *Meganyctiphanes norvegica* and *Thysanoessa inermis* (Lebour, 1924, 1926a ; Brook and Hoyle, 1888 ; Elmhirst, 1924). No others are as yet completely known. In the Alexandria material metanauplii of *Euphausia Krohnii* were found. Thus the only euphausiid

nauplii certainly known are those of *Nyctiphanes Couchii* (passed within the egg sac), *Meganyctiphanes norvegica*, and *Thysanoessa inermis*. Metanauplii have been described of *Nyctiphanes australis* (Sars, 1885), *N. Couchii* (Lebour, 1924), *Meganyctiphanes norvegica* (Sars, 1898 ; Lebour, 1924 ; Elmhirst, 1924), and *Thysanoessa inermis* (Lebour, 1926a). All of these are much alike, but differ in the armature of the carapace.

From the metanauplius comes the first Calyptopis, giving rise to the second and this to the third. All three stages are known in *Nyctiphanes australis* (Sars, 1885), *N. Couchii* (Lebour, 1924), *Meganyctiphanes norvegica* (Sars, 1898 ; Lebour, 1924 ; Elmhirst, 1924), *Thysanoessa inermis* (Lebour, 1924), *Thysanoessa longicaudata* (Jorgensen, 1925), *Euphausia Krohnii* (Sars, 1885, as *E. pellucida*, and also in the Alexandria material), the second and third Calyptopis of *Thysanopoda tricuspidata*, in which peculiar larval eyes are shown, are described by Sars (1885) and the three Calyptopis stages of what is almost certainly *Stylocheiron Suhmii* have been found in the Alexandria material. All the Calyptopis stages have the same general appearance with the eyes covered by the carapace, and the abdomen having no segments in the first, five in the second, and six in the last stage. There is no evidence to show that more than two naupliar and three Calyptopis stages exist in any species.

The last Calyptopis changes to the first Furcilia stage with the eyes uncovered by the carapace. In most of the species this has no pleopods, and it is probable that this stage without pleopods occurs in all. Such a Furcilia is known in *Nyctiphanes Couchii*, *Meganyctiphanes norvegica*, *Thysanoessa inermis*, *T. longicaudata*, *Nematoscelis microps*, *Thysanopoda tricuspidata*, and *Stylocheiron Suhmii*. In the following Furcilia stages the pleopods develop successively until all are setose and biramous, when they are capable of being used as swimming organs and the Furcilia then changes to a Cyrtopia, in which the antennæ are no longer used for swimming, the same being differentiated into scale and flagellum.

Generally speaking when once the Cyrtopia stage is reached it is fairly easy to place the specimen, at any rate in its genus, therefore a knowledge of the Furcilia stages is the more important for elucidating the life histories. It is proposed to deal more elaborately with those Furcilia stages in which the pleopods are developing, and on these base the scheme which will help to place the specimen in the genus to which it belongs.

It is found that the pleopods develop in different ways in several different genera of which the first three or four are the same and the last two or three are the same, but in between there are different orders of development, some of which appear to be characteristic of certain genera. If therefore a fairly complete series of larval stages is obtainable of any form we can place it within a group, and if this does not indicate

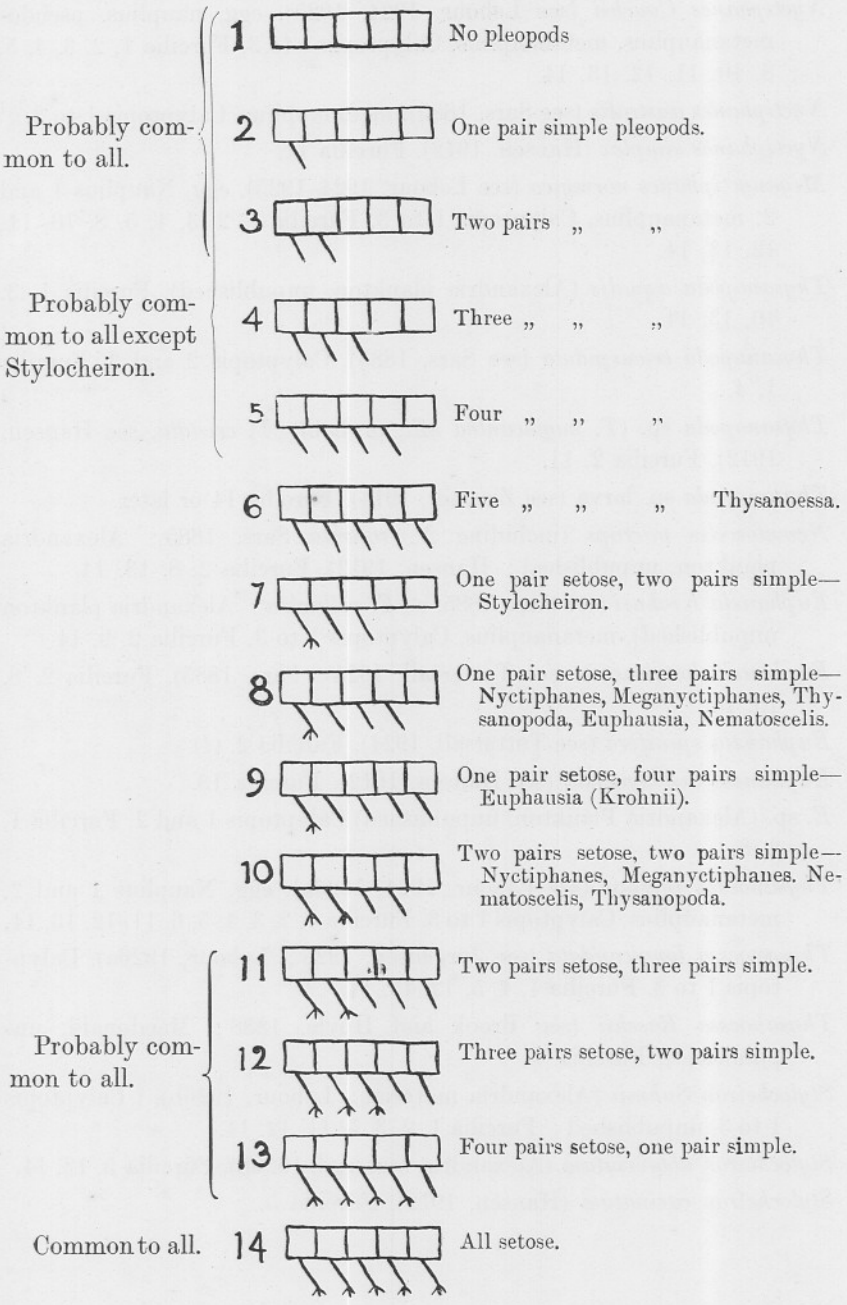
the genus a few other known characters will probably identify it. Unfortunately a complete series of Furcilia stages is rarely obtainable, but enough has been elucidated within the last few years to bring a large amount of material together, and a survey of these brings much evidence to bear in favour of their grouping the genera for the purpose of larval identification. It is not suggested that this grouping should necessarily indicate natural phylogeny, for we find in some cases, *e.g.* Nyctiphanes and Nematoscelis, that genera develop alike which are usually placed in different families. At present the facts are indicated as a key to facilitate the identification of genera. The reason is probably to be found in the fact that more hurried growth is necessary for some than for others. Thus we find that one group develops the pleopods as slowly as possible so that all five are simple buds before any become setose, *e.g.* Thysanoessa. Here probably the swimming powers are delayed as none of the pleopods can as yet be used. Closely similar is *Euphausia Krohnii*, in which the first pleopod is setose, whilst four are simple, and then the group which is most commonly found, where the first is setose with three simple and the last is not yet formed. To this group belong the majority, including Nyctiphanes, Meganyctiphanes, Thysanopoda, Nematoscelis, and Euphausia, the last being slightly different, as, at any rate in the *Krohnii* group and possibly in all, there are, as shown above, four simple buds behind the first setose pleopod. In all the other genera cited the second pleopod becomes setose before the last bud appears. Finally, we have Stylocheiron in which the first pleopod is setose with only two simple buds behind. We have thus a gradual quickening of the development culminating in Stylocheiron.

The possible (or known) stages in development are given in the diagram and numbered 1 to 14 (Text Fig. 1). Each division represents an abdominal segment, the simple pleopods being indicated by one stroke, the setose pleopods by a fringed stroke. The figures do not necessarily refer to actual Furcilia stages. Thus 6 in the diagram is the sixth Furcilia of Thysanoessa, but 7 in the diagram is the fourth Furcilia of Stylocheiron and 8 in the diagram is the sixth Furcilia of Nyctiphanes.

The following list enumerates all known larval stages, numbered according to the diagram, in all the species where they are known, as far as the Furcilia numbered 14. There may, however, be several Furcilia stages with all the pleopods setose. In this list there are many gaps, but it will be seen that closely related genera and species belonging to the various genera agree as to the development, and that so far no larval stages are known which disagree.

FIG. 1.

DIAGRAM TO ILLUSTRATE THE DEVELOPMENT OF PLEOPODS IN EUPHAUSIID FURCILIA STAGES.



Early Larval and Furcilia Stages known in the Various Species.

Nyctiphanes Couchii (see Lebour, 1924, 1925), egg, nauplius, pseudo-metanauplius, metanauplius, Calyptopis 1 to 3, Furcilia 1, 2, 3, 4, 5, 8, 10, 11, 12, 13, 14.

Nyctiphanes australis (see Sars, 1885), metanauplius, Calyptopis 1 to 3.

Nyctiphanes simplex (Hansen, 1912), Furcilia 11.

Meganyctiphanes norvegica (see Lebour, 1924–1925), egg, Nauplius 1 and 2, metanauplius, Calyptopis 1 to 3, Furcilia 1, 2, 3, 4, 5, 8, 10, 11, 12, 13, 14.

Thysanopoda æqualis (Alexandria plankton, unpublished), Furcilia 1, 3, 10, 13, 14.

Thysanopoda tricuspidata (see Sars, 1885), Calyptopis 2 and 3, Furcilia 1, 4.

Thysanopoda sp. (*T. monacantha* aff., probably *T. cristata*, see Hansen, 1912), Furcilia 2, 11.

Thysanopoda sp. larva (see Zimmer, 1914), Furcilia 14 or later.

Nematoscelis microps (including *N. rostrata* Sars, 1885; Alexandria plankton, unpublished; Hansen, 1912), Furcilia 2, 8, 13, 14.

Euphausia Krohnii (see Sars, 1885, as *E. pellucida*; Alexandria plankton unpublished), metanauplius, Calyptopis 1 to 3, Furcilia 2, 9, 14.

Euphausia longirostris (see Tattersall, 1924; Sars, 1885), Furcilia 2, 8, 13, 14.

Euphausia spinifera (see Tattersall, 1924), Furcilia 2. (?)

Euphausia distinguenda (see Hansen, 1912), Furcilia 13.

E. sp. (Alexandria Plankton, unpublished) Calyptopis 1 and 2, Furcilia 1, 2, 8.

Thysanoessa inermis (see Lebour, 1924, 1926a), egg, Nauplius 1 and 2, metanauplius, Calyptopis 1 to 3, Furcilia 1, 2, 3, 4, 5, 6, 11, 12, 13, 14.

Thysanoessa longicaudata (see Jorgensen, 1925; Lebour, 1926a), Calyptopis 1 to 3, Furcilia 1, 4, 5, 12, 13, 14.

Thysanoessa Raschii (see Brook and Hoyle, 1888; Macdonald, unpublished), Furcilia 6.

Stylocheiron Suhmii (Alexandria material; Lebour, 1926b), ? Calyptopis 1 to 3, unpublished; Furcilia 1, 2, 3, 5, 11, 12, 14.

Stylocheiron abbreviatum (Alexandria material, 1926b), Furcilia 5, 12, 14.

Stylocheiron carinatum (Hansen, 1912), Furcilia 5.

The larvæ described by Colosi (1922) as *Nematoscelis microps* seem to belong either to Nyctiphanes or Meganyctiphanes.

It is thus seen that the evidence is in favour of a certain order of development of the pleopods in the various genera, and so far there is none against this. So that on finding a larva with one pair of pleopods setose and two pairs simple one would immediately place it in the genus Stylocheiron, and on finding one with five pairs of simple pleopods it would be placed in the genus Thysanoessa. Fortunately these distinctive stages nearly always seem to occur as though certain stages were dominant. Such dominant stages are Furcilia 5 (presumably the fourth Furcilia stage) in Stylocheiron, and Furcilia 6 (presumably the sixth Furcilia stage) of Thysanoessa ; also Furcilia 9 (presumably the seventh Furcilia stage) of *Euphausia Krohnii*.

In the Nyctiphanes group of this scheme, which includes Nyctiphanes, Meganyctiphanes, Thysanopoda, and Nematoscelis, the first two, with the Thysanopoda sp. of Zimmer (1914), are recognisable by their truncated rostra. Other Thysanopoda and Nematoscelis may be very much alike, but distinguishable in many of the Furcilia stages by the growth of the second thoracic limb, which is very long in Nematoscelis. Euphausia apparently has Furcilia 8 in common with the Nyctiphanes group, but differs in *E. Krohnii* (and possibly in other species) by going through the Furcilia 9 stage, which does not occur in the Nyctiphanes group.

There is distinct evidence of the jumping of stages in some species. If kept in aquaria both Nyctiphanes and Meganyctiphanes may jump stages. Mr. Elmhirst and Mr. Macdonald from Millport tell me that Meganyctiphanes may jump several stages, but they always jump into a stage known for that genus. Thus Nyctiphanes might jump from a stage with three simple pleopods to one with two setose and two simple, but it would not jump to five simple pleopods, which is characteristic of Thysanoessa, neither would it jump to one pair setose and four simple, which is characteristic of *Euphausia Krohnii*. Mr. Elmhirst has kindly provided me with some notes on Meganyctiphanes (reared in aquaria) in which one jumped from one to three pairs of simple pleopods and one which jumped from three pairs of simple pleopods to three pairs setose and two pairs simple, that is from Furcilia 4 to 9, thus missing out eight altogether. Thus we may expect the development of any species to proceed in a particular way even though stages may be jumped, and it is quite possible that in some of those given in the lists the jumping of stages is the usual thing.

Taking all these facts into consideration we may now suggest a key for the elucidation of larval euphausiids, so as to place them in their respective genera. Only eight genera are taken, as no larval forms of the other three are known. Pseudeuphausia is included, as it can be

recognised in the larval stage by its rostrum (Hansen, 1912), but the development of its pleopods is unknown.

The genera may be grouped thus :—

Group I. Second pleopod becomes setose before the appearance of the fifth—Nyctiphanes, Meganyctiphanes, Thysanopoda, Nematoscelis.

Group II. A stage present with all five pleopods simple—Thysanoessa.

Group III. A stage present with first pleopod setose and four simple—Euphausia (Krohnii) (the stage preceding this having one pair setose and three simple).

Group IV. A stage present with the first pleopod setose and two simple Stylocheiron.

Key.

I. Rostrum truncated.

1. Rostrum deeply emarginate anteriorly—**Pseudeuphausia.**
2. Rostrum almost straight anteriorly.

(*a*) Abdominal segments without lateral processes.

α. Carapace very broad—**Meganyctiphanes.**

ß. Carapace not very broad—**Nyctiphanes.**

(*b*) Abdominal segments with lateral processes—**Thysanopoda** (in part. See *T. sp.* Zimmer, 1914).

II. Rostrum pointed or rounded.

1. First pleopod becomes setose before the fourth appears—**Stylocheiron.**
2. Fourth pleopod appears before any are setose.

(*a*) Fifth pleopod appears before any are setose—**Thysanoessa.**

(*b*) First pleopod becomes setose before the fifth appears.

α. Fifth pleopod appears before the second is setose—**Euphausia** (*E. Krohnii*).

ß. Second pleopod becomes setose before the fifth appears.

(i) Second thoracic leg becomes long at an early stage—**Nematoscelis.**

(ii) All thoracic legs short—**Thysanopoda** (in part, *e.g. T. æqualis*).

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A New Method for Quantitative Sampling of the Sea-bottom.

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With 2 Figures in the Text.

THE apparatus described in this paper has been specially designed for the taking of samples in cases where it is important that the finer constituents of the sample should be adequately retained. In commencing a study of the micro-fauna and micro-flora of the sea-bottom near Plymouth the writer found that, in samples taken by the " Petersen " (2) 1/10 sq. metre bottom-sampler, or by the conical dredge, the silt and finest material was liable to be washed away to a variable and non-measurable extent while hauling the apparatus. Quantitative observations on very small organisms were judged impossible by these methods and attention was directed towards devising an apparatus which would retain the finer portions of the deposits.

Sounding-tubes of the " Naumann " and " Lundqvist " types, described by Lundqvist (1), though very efficient in sampling soft bottoms of clay or mud, cannot be used for sampling sands and gravels, which, besides being too resistant to the boring of the tube, are not sufficiently cohesive to be retained as a sample by this method.

A method has now been devised which, in brief essentials, is as follows. A metal chamber, hermetically sealed by a glass diaphragm, is lowered to the bottom, on reaching which the diaphragm is automatically broken. The pressure of the overlying water-column forces into the chamber a sample of the bottom, which is prevented by a " trap " device from escaping when the apparatus is raised. For the original suggestion of utilising the pressure of the overlying water-column for the separation of a sample from the bottom, as well as for much help and advice in the experimental design of the apparatus, the writer is indebted to Dr. G. P. Bidder.

A photograph of the apparatus, which may be referred to as a " Vacuum Grab," is shown in Text Fig. 2. It is best described in detail by reference

to the drawing (Text Fig. 1). It is made throughout of brass. The cylindrical " pressure-chamber," PC, is provided above with a lug, L, by which it can be shackled to a sounding-wire, and is closed below by a massive screw-stopper, S, the joint being made hermetically tight by a rubber washer, W. This stopper, which carries the remainder of the apparatus, is bored through its centre, the upper half of the boring being of less diameter than the lower. A shoulder is so formed with a downwardly-directed, horizontal face, SF. This face is accurately turned and smoothly finished. To the upper bore is permanently fitted the " trap-tube," TT, which projects into the " pressure-chamber " and is turned through a right-angle at its upper end. The lower, larger bore is threaded and into it is screwed the sleeve, Sl, which carries the sliding " sampling-tube," ST. A series of ports, P, is cut in the sides of the sleeve, towards its upper end, in order to provide escapement for water entering the mouth of the " sampling-tube " during descent. The " sampling-tube " slides easily within the sleeve, the extent of its movment being limited by two screws, Sc, which are fitted, one on each side into the " sampling-tube " and slide in vertical slots, VS, in the sleeve. When the " sampling-tube " is in position at the upper limit of its sliding range, its sides completely close the ports in the sleeve. The lower end of the " sampling-tube " is expanded into a bell-shaped mouth, while at its upper end is the bayonet-striker, BS, a narrow, bayonet-shaped piece, projecting inwards and upwards to a striking-point which lies in the centre of the bore. It will be seen that the only entrance to the " pressure-chamber " is by way of the " sampling-tube " and " trap-tube." Between these two the glass diaphragm, GD, is inserted, being sealed by a stiff vaseline preparation to the shoulder-face, SF.

In practice, the sleeve, with " sampling-tube," is detached and the glass disc inserted. The sleeve is then screwed in, hand-tight, and the instrument sent to the bottom at the end of a sounding-wire. When the lower end of the " sampling-tube " reaches the bottom the " sampling-tube " is arrested, but, owing to the sliding sleeve, the remainder of the apparatus continues to sink until the glass disc touches and is broken by the bayonet-striker. At the moment of breakage the pressure inside the chamber is equal to atmospheric pressure, whilst that outside is equal to atmospheric pressure plus the pressure of the overlying water-column, which is approximately equivalent to an additional atmosphere's pressure for every 10 metres of depth. On breakage of the glass, therefore, a pressure equal to that of the water-column overlying the sea-bottom operates to force a sample from the area marked out by the lower edge of the " sampling-tube " up and through the trap-tube into the " pressure-chamber," where it falls to the bottom and cannot escape.

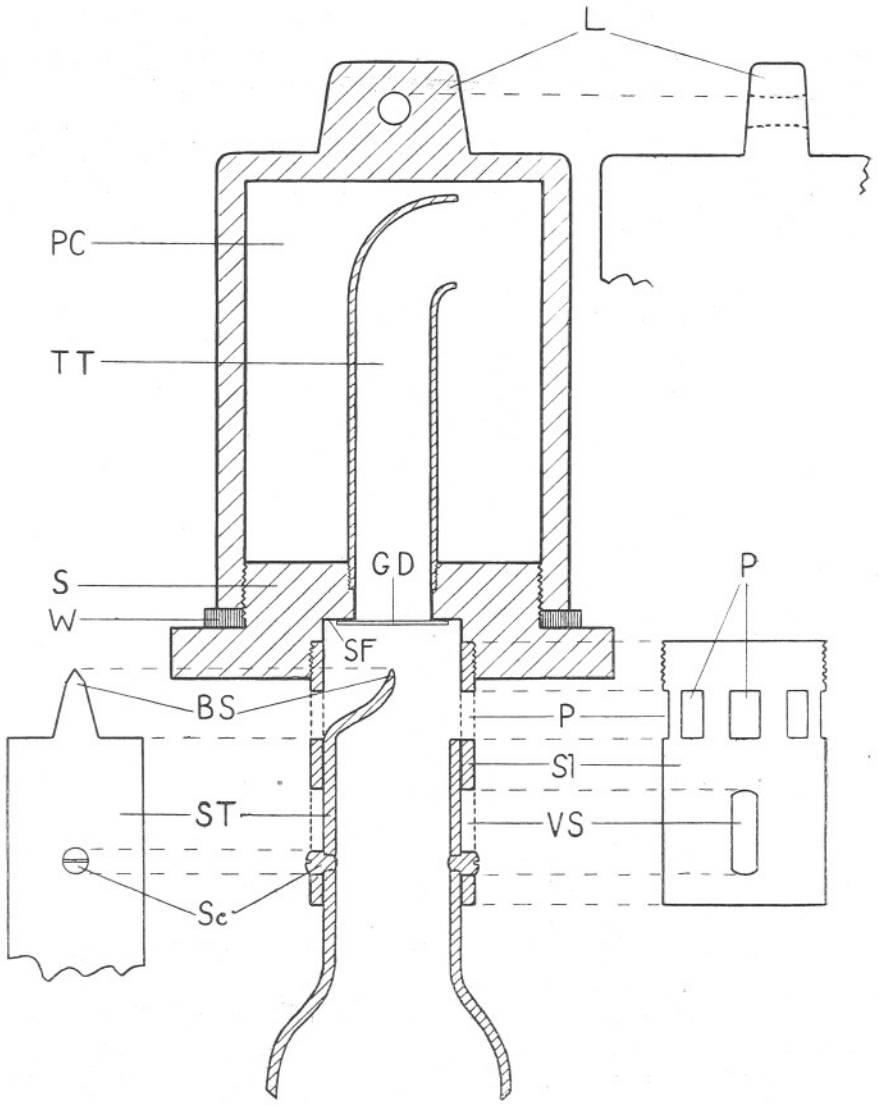

FIG. 1.—Section of "Vacuum Grab," side view, with projections illustrating lug, sleeve, and "sampling-tube." $\times \frac{2}{3}$

BS = "bayonet-striker."
GD = glass diaphragm.
L = lug for attachment to sounding-wire.
P = port in sleeve.
PC = "pressure-chamber."
S = screw-stopper.
Sc = screw in "sampling-tube": travels in slot, VS.
Sf = shoulder-face to which glass diaphragm is sealed.
Sl = sleeve.
ST = "sampling-tube."
TT = "trap-tube."
VS = vertical slot in sleeve.

Samples taken in this way, whose volume is too small to rise above the level of the upper opening of the "trap-tube," are subject to no loss of their finer constituent particles. With larger samples, however, there is a slight loss due to removal of fine particles in suspension in such part of the water-content as lies above this level. This loss, however, can be corrected for, because it varies in constant proportion to the

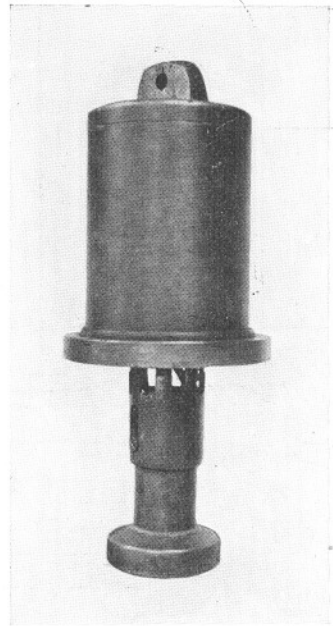

FIG. 2.—Photograph of "Vacuum Grab."

volume of the sample, and, therefore, to the depth at which the sample has been taken.

The samples taken by the above method enable a quantitative gravimetric and volumetric analysis of the constituents. To obtain values, however, relating to the area of bottom sampled it is necessary to know the relation between surface area and volume in samples of respectively different constitution and taken at different depths. To this end a series of experiments was conducted in the laboratory and at sea, in which samples were taken of artificial bottoms of known constitution, and in which the size of the sample was varied by varying either the pressure or the depth. In the laboratory the different pressures necessary for sampling were obtained by exhausting the "pressure-chamber" to a varying extent. In the experiments at sea the depth was varied. The materials used in constructing the artificial bottoms varied from fine sand

to shell-gravel in which the largest particles did not, on the average, exceed 1 cm. in diameter. The bottoms were constructed by arranging successive layers of material, each 1 cm. deep, in a straight-sided bucket. In each layer small, coloured beads of different shape and colours were so arranged that when a sample had been taken it was possible, by counting the numbers of each kind of bead in the sample, to reconstruct the area of each layer sampled. It was also possible to estimate the volume by this method, and the degree of correspondence between this estimated volume and the measured volume afforded a useful test of the accuracy of the method. The result of these experiments has been to show that, as expected on theoretical grounds, the area sampled does not vary materially either with different types of soil or with different pressures. The shape of the sample is approximately a cylinder of the same area of cross-section as that of the lower end of the "sampling-tube," the lower portion of the cylinder being rounded off or cup-shaped. With increasing pressure the instrument samples from a greater depth in the bottom-material, but not from a wider area: it digs, or rather sucks, a deeper though not a wider hole. These experiments have also shown that, as was suspected, there is a wafting away of a certain amount of surface material owing to disturbance of the immediately overlying water by the descending "sampling-tube." The ports cut in the sides of the sleeve, which are open during descent, but are closed automatically by the sliding mechanism on reaching the bottom, minimise this disturbance and limit the wafting away to a peripheral area. The experiments have demonstrated the extent of the area affected, so that the surface area truly sampled is known with sufficient accuracy. The details of these experiments, which are not yet completed, will be given in a later paper dealing with the samples obtained.

The above-described model of a "Vacuum Grab" has been specially designed for working under the submarine conditions which exist in the neighbourhood of Plymouth. It has been worked satisfactorily at all depths tried, which range from 10 metres to 70 metres. For working in depths up to 50 metres, a thickness of 1·5 millimetres for the glass diaphragm was found sufficient: from 50 to 70 metres, a thickness of 2·0 millimetres proved adequate. The "Vacuum Grab" is particularly suitable for work on bottoms of sand and fine gravel, samples of which as previously pointed out, cannot be obtained with the ordinary sounding-tube. For sampling muds and oozes, however, whose resistance to the sounding-tube is small, and whose cohesive properties are great, the sounding-tube would be more satisfactory than the present apparatus, for it possesses the great advantage of taking a stratified sample. For this reason the "Vacuum Grab" is not likely to compete with the sounding-tube as a bottom-sampler in the deep sea, where oozes and

clays constitute the bottom; but in shallow and moderately shallow waters, where it is required to work over a greater range of types of bottom, it is, so far as the writer is aware, the only means yet described of obtaining quantitative samples with respect to the finer constituents.

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Marine Biological Association of the United Kingdom.

Report of the Council, 1925.

The Council and Officers.

Four ordinary meetings of the Council were held during the year at which the average attendance was twelve. All the meetings were held in the rooms of the Royal Society, and the Council wishes to express to the President and Council of the Society the thanks of the Association for the facilities provided.

The Plymouth Laboratory was visited and inspected by a Committee of six members of the Council in March. The Building Committee has also had several meetings in connection with the building of the new laboratories.

The Plymouth Laboratory.

The engines and pumps circulating sea-water through the Aquarium and Laboratory tanks have been in constant service, no extensive repairs or alterations having been called for during the year. An attempt to improve the water circulating through the tanks, by constantly adjusting the acidity by the addition of lime, has met with considerable success, and many invertebrate animals, which were formerly difficult to keep alive, can now be maintained in a healthy condition for a long time. Weekly consignments of fishes and invertebrates have been sent to the Aquarium of the Zoological Society in London.

The new building, which is being erected by voluntary subscriptions without financial aid from the Government, was commenced early in the summer and has been completed, with the exception of the fittings. The building, which is in direct communication with the main Laboratory,

is situated to the north of that building, between it and the Citadel wall. It consists of two stories, each with a floor space of 60 ft. by 24 ft., nearly all of which is available for laboratory use. The actual accommodation for work of the Laboratory as a whole has therefore been nearly doubled.

A stone building containing three rooms, with about one-sixth of an acre of land, situated on the shore at Fisher's Nose, to the south-east of the Laboratory, has been rented from the War Office, and is now in use as a store especially for nets and gear belonging to the boats. The land has been found particularly useful for the treatment of large nets, such as those supplied to the *Discovery* Expedition, with copper soap which is being used as a preservative.

The sheds at Pier Cellars in Cawsand Bay, which have been rented at a nominal sum, also from the War Office, have been of service in connection with Dr. W. R. G. Atkins' investigation of the penetration of light into the sea.

The Boats.

The steam drifter-trawler *Salpa* has worked continuously throughout the year. Lloyd's half-time machinery survey and annual boiler survey were carried out during the year and the reconditioning necessary proved to be slight, showing that the vessel had been well looked after and small defects made good as they arose. The survey was completed at a minimum of cost and at a minimum of loss of sea-going time.

The motor boat *Gammarus* has been busily engaged in daily collecting in and about the Sound, and the sailing boat has been available to relieve her at short notice when necessary.

The Staff.

Dr. C. M. Yonge has been appointed a temporary Assistant Naturalist for two years, to carry out a special investigation on the feeding and the physiology of digestion in the oyster.

No changes have taken place in the permanent scientific staff during the year.

The Development Commissioners having provided for the training in marine biology and fishery science at the Laboratory of two Student Probationers, Mr. H. O. Bull, of Derby, and Mr. R. Palmer, of University College, Reading, have been selected, and will commence work early in 1926.

Occupation of Tables.

The following investigators have occupied tables at the Plymouth Laboratory during the year :—

Dr. I. Amemiya, Tokyo (Ecological Studies of Ostrea).
Prof. P. R. Awati, Bombay (General Zoology).
N. J. Berrill, Bristol (The Effect of Environment on Development in Tunicates).
J. Bertram, Berkhamsted (General Zoology).
Dr. G. P. Bidder, Cambridge (The Currents of Grantia).
H. O. Bull, Derby (Larval Fishes).
Dr. H. Graham Cannon, London (Crustacean Embryology. Feeding Habits of Crustacea).
Prof. R. Chambers, New York (Action of Electrolytes upon the Protoplasm of Amœba).
L. R. Crawshay, Plymouth (Sponges).
W. C. De Morgan, Plymouth (Protozoa)
W. E. Drake, Bristol (Algæ).
Miss G. H. Faulkner, Aberdeen (Filograna).
M. H. Finkelstein, Edinburgh (Electrolyte Composition of Blood of Aplysia).
Prof. Ruggles Gates, London (Algæ).
Dr. T. Gislèn, Upsala (Animal Communities and Echinoderm Biology).
Miss I. Gordon, London (Development of Echinoderms).
J. Gray, Cambridge (Ciliary Movement in Ctenophores and Pectens).
Dr. H. P. Hacker, London (Distribution of Mosquito Larvæ in relation to the chemical constitution of waters).
L. A. Harvey, London (Cytology of the Development of Ciona).
C. C. Hentschel, London (Gregarines and Parasitic Ciliates).
H. R. Hewer, London (Colour Change in Fishes).
C. F. Hickling, Cambridge (Hake and their Food).
A. D. Hobson, London (Physiology of Aplysia. Artificial Parthenogenesis).
Mrs. A. D. Hobson, London (Shore Fauna. Artificial Parthenogenesis).
Dr. L. T. Hogben, Edinburgh, Ray Lankester Investigator (Action of Electrolytes on Invertebrate Contractile Tissues. Circulatory System of Crustacea).
Dr. I. Irving, California (Secretion of Calcium carbonate).
Miss W. L. Johnson, Birmingham (Larval Ascidians).
S. Jones, Cardiff (Effect of Electrolytes on Rhythmic Pulsation of Medusæ).
A. G. Lowndes, Marlborough (General Zoology).
Miss C. Lucas, London (Protozoa).
Miss S. M. Manton, Cambridge (Development of Hemimysis Lamornæ).
Prof. W. Mielck, Heligoland (General Zoology).
Miss O. S. Mundy, Plymouth (Vertebrate Embryology).
R. Palmer, Reading (Cytology of Gammarus).
F. T. K. Pentelow, Cambridge (Myxosporidia).
Dr. H. H. Poole, Dublin (Penetration of Light in Sea-water).
F. A. Potts, Cambridge (Excretion in *Capitella capitata*).
F. Raw, Bournville (Appendages of Crustacea and Polychæta).
M. L. Richardson, Edinburgh (Circulatory System of Crustacea).

T. Howard Rogers, Birmingham (Structure of Radula of Buccinum).
A. E. A. Salem, Cambridge (General Zoology).
Dr. L. G. Saunders, Cambridge (General Zoology).
Dr. E. A. Spaul, London (Cytological Studies of Crustacea).
Dr. Edgar Stedman, Edinburgh (Action of parasympathetic drugs on Dogfish heart).
Dr. and Mrs. E. Stedman, Edinburgh (Respiratory Pigments of Crustacea. Physical Chemistry of Hæmocyanin).
C. C. Stockman, Cambridge (Respiration of Marine Animals).
Miss M. Vincent, Cambridge (Parasitic Protozoa).
G. P. Wells, Cambridge (Growth of Echinoderms).
Prof. W. N. F. Woodland, London (Cestodes from Fishes).
Dr. C. M. Yonge, Edinburgh (Physiology of Digestion in Mollusca).

The usual Easter Vacation Course in Marine Zoology was conducted by Dr. J. H. Orton, and was attended by thirty-seven students from Oxford, Cambridge, London, Birmingham, and Edinburgh. A second Course conducted by Dr. Orton was held during the Summer Vacation, attended by eight students.

An Advanced Course in Comparative Physiology and Experimental Biology, conducted by Mr. C. F. A. Pantin, was also held during the Summer Vacation and was attended by eleven students.

Dr. E. W. Shann brought a class of ten boys from Oundle School, Mr. A. G. Lowndes a class of six from Marlborough College and Monkton Combe School, and Rev. L. D. Sayers brought two boys from Eton College during the Easter Vacation.

General Work at the Plymouth Laboratory.

Dr. Orton has continued his general studies in Marine Bionomics, and has completed for publication the report on the Survey of the Fal Oyster Beds in a form suitable for use by oyster cultivators, oyster dredgermen and others interested in oyster culture.

Owing to the cost of printing, funds could only be found to print a Summary of the Report, which was distributed free to dredgermen and others in the Fal and Truro districts.

As a result of the Survey the Fal and Truro oyster committees agreed with the oyster dredgermen, with the approval of the Ministry of Agriculture and Fisheries, to restrict the hours of fishing and raise the ring from $2\frac{1}{2}$ inches to $2\frac{5}{8}$ inches ; but failure to take advantage of the recommendations as to laying cultch has undoubtedly resulted in the loss of an opportunity, in the favourable weather of 1925, to restore the fishery from a declining to a recovering condition.

Dr. Orton has himself investigated some of the research problems recommended in the report mentioned above, and found them of much scientific and economic interest. In comparing the breeding potential-

ities of oysters growing shell fast with those growing shell slowly (dumps), it was found that the former show (1) periodicity in spawning correlated irregularly with full moon ; (2) almost as intense breeding in September as in July, correlated with good growth of shell ; (3) absence of storage of reserve products (as glycogen) during growth and breeding.

The probability that rhythmical (and probably lunar) breeding is regular—that is, occurs in monthly cycles—under favourable conditions is being further investigated. The intense breeding at the end of the summer in September is a fact of economic importance, and in favourable weather may be taken advantage of, as has been shown by experiments, to increase the season's catch of oysters by 33 or even 50 per cent.

Dumpy oysters, it was found, do not breed so regularly as fast-growing oysters, but are nevertheless good spawners and valuable for purposes of reproduction. Since these oysters tend to accumulate on the beds and occur in a large proportion below the legal size, they are a natural safeguard against the annihilation of the beds. They form an ever-present nucleus of a spawning stock ready to take advantage of exceptionally favourable seasons for spatfall to improve the condition of the fishery. Dumpy oysters should therefore be preserved carefully in the present reduced condition of the beds.

Dumpy oysters, unlike those with fast-growing shells, retain a large amount of their reserve material (chiefly glycogen) even when they spawn in summer ; hence there can be little doubt that the problem of fattening is closely concerned with that of growth, as well as breeding and feeding. The problem of fattening is being investigated on the broad lines indicated. Winter-fattening, although largely due to a separate storage of reserve products, is also due in part to the proliferation of the sex-organ.

Further attempts have been made with cage experiments to procure a 100 per cent change of sex in oysters, and to fix the age of change of sex of Crepidula isolated in the sea. Owing to the difficulties in carrying out these experiments complete success has not yet been attained. Experiments have also been made on the rate of change of sex of the oyster, and also data have been accumulated from more than 500 cases of sex-change with a view to *establishing* the normal conditions.

The behaviour of Portuguese and native oysters in certain concentrations of T.N.T. (Trinitotroluene) has been investigated by Dr. Orton in experiments carried out in the Plymouth Laboratory tanks, and an account of the results forwarded to the Fisheries Branch of the Ministry of Agriculture and Fisheries.

Mr. Ford has worked chiefly on herrings and the herring fisheries. During the winter season of 1924–25 twenty-four samples each of about

one hundred fish from commercial landings at Plymouth were examined. Since then samples from landings at Milford Haven, Padstow, Mevagissey, Brixham, and Brighton have been obtained, as well as further samples from the Plymouth area, and collections are still being made. Statistical data are accumulating on length, age and growth, sexual condition and number of vertebræ. Several important facts can already be stated, as they have become manifest during the actual collection of the material. Spawning fish have been present in the Plymouth catches as early as September 30th (1925), and as late as March 2nd (1924). Throughout the Plymouth winter season of 1924–25, herrings whose scales exhibited five summer-growth zones numbered, on average, over 50 per cent per sample. Fishes of the same sample may differ considerably in their growth history as indicated by their scales, both in the length at which the first winter ring was formed, and in the interval growth between successive rings. The average number of vertebræ per sample does not remain constant, and differences having mathematical significance have been obtained. It is hoped, therefore, that a complete analysis of the samples may yield information as to the origin of the different shoals.

During the autumn Mr. Ford obtained some interesting results by projecting a bright beam of light downwards on to the surface of the water inside the Breakwater at night. A trawler deck light was suspended over the side of the motor boat *Gammarus*, and fed with acetylene. A surprising quantity of animal life was collected in a short while, some organisms being definitely attracted, while others could be seen making their way across the illuminated area, but may not have been definitely interested in the light. Young sprats and pilchards up to "britt" size were captured, and it will therefore be of interest to learn whether young herrings can be attracted by light in a similar way. Adult pollack were taken in quantity by hook and line used in the illuminated area. Absence of wind is necessary for the satisfactory collection of small organisms under these conditions, for a puff of wind will at once sweep away an assemblage of them which has gathered below the light.

Dr. M. Lebour has continued her investigations on the Euphausiidæ of the Plymouth district, and, helped by material procured by Mr. C. F. Hickling from the Atlantic, has completed the life history of both *Nyctiphanes Couchii* and *Meganyctiphanes norvegica*, the accounts being published in the Association's Journal in October, 1925. She has submitted the specimens of Thysanoessa to Dr. Hansen of Copenhagen, who pronounces them all to be *T. inermis*. An account of the life history of this species (containing both the forms *inermis* and *neglecta*) is now ready for the press, thus completeing the Euphausiid fauna of the English Channel. She has also examined plankton hauls regularly and continued the investigation of the food of adult herrings.

Dr. Lebour's book, entitled *The Dinoflagellates of Northern Seas*, was published by the Association in October, 1925, and is on sale at the price of 12s. 6d. A small paper has also been published by Dr. Lebour on the eggs and first larval stage of *Typton spongicola*, which were hatched in a plunger jar. The first larval stage of the crab *Bathynectes longipes* was also hatched in a plunger jar.

A further paper by the same naturalist on the young of *Stylocheiron Suhmii* and *S. abbreviatum*, from plankton collected by Mr. F. S. Russell in the neighbourhood of Alexandria, Egypt, is being published in the Proceedings of the Zoological Society.

The usual hydrographic stations have been worked in the *Salpa* by Mr. H. W. Harvey. The data are sent to the International Council for publication and to the French Fishery Department. The monthly record since 1921 of conditions 10 miles south-west of the Eddystone rocks has thrown light on the part played by meteorological conditions, particularly by evaporation and by currents on the temperature of the sea, which shows marked variations from year to year. It is of interest that during the last four herring seasons at Plymouth the highest weight of herrings per landing has occurred each year during that month when the sea temperature has approached most nearly to 10°–11°C.

A method for estimating *nitrates* in sea-water has been worked out by Mr. Harvey, who finds that diatoms, etc., had utilised all, or practically all, the nitrate in the water 10 miles south-west of the Eddystone by the beginning of August, after which a regeneration of nitrate occurred, commencing in the deeper water. Bacterial action, causing the formation of nitrates from added ammonium salts, was found to take place in samples of the deeper water in August, and in surface water to which a little detritus had been added, but not in surface water alone.

A number of samples of surface water from the Atlantic, distant from land, were found to be almost denuded of nitrate, while the deep water was particularly rich. In deep water off the west coast of Ireland and in the Faeroe-Shetland Channel in early summer, where mixing of the surface with deeper water is probable, the surface layers were relatively rich in nitrate, and, in the former position, were notably rich in plankton.

Mr. F. S. Russell has again been investigating the vertical distribution of the pelagic stages of young fishes. From April to August he worked a number of stations, fishing at five or six depths at each, with the ring trawl; in every case the Admiralty depth recorder was used. Indications shown last year that certain species may have a specific vertical distribution in the daytime would seem to be confirmed by this year's work. Attached to the ring trawl was a small silk tow-net, so that it is hoped to compare the vertical distribution of the copepods that compose the food of young fishes with that of the fishes taken from the same body

of water. Mr. Russell has sampled or counted all the plankton organisms taken in these hauls with the ring trawl and hopes shortly to publish a paper on the vertical distribution of macro-plankton in daylight. From June 16th to June 19th he again carried out a series of hauls in order to observe diurnal variations in vertical distribution. On this occasion he succeeded in carrying his observations through *two* nights, so that the second night may act to a certain extent as a "control" on the first. At this time there was no moon, and it is interesting to find that certain animals rose higher in the water at night than they did on the previous occasion in July, 1924, when there was a full moon.

Mr. O. D. Hunt is continuing an investigation of the micro-fauna and micro-flora of the bottom deposits. No attempt is at present being made to deal with the bacteria and smaller protozoa, but attention is being confined to larger forms, ranging in size from the smallest diatoms to the largest copepods and foraminifera.

The difficulty experienced at first from the fact that the finer particles and smaller organisms were in part washed away during the process of obtaining bottom samples, which interfered with any quantitative study, has at length been overcome by the construction of a new bottom-sampling mechanism by means of which samples can be obtained with little such loss. This apparatus, a description of which will be published at an early date in the Journal, depends for its working upon the increase of pressure which occurs as the depth of water increases.

Stations have been chosen for investigation on a line extending from Whitsand Bay (4–5 fathoms) to the Eddystone (30 fathoms), comprising a wide rage of types of deposit, from fine mud to shell gravel. Comparisons between the fauna and flora of these different types and the seasonal variations of each are being worked out. The quantitative data so far available are insufficient to allow of any generalisations, except that certain kinds of organisms, such as foraminifera, bottom-living diatoms, ostracods and bottom-living copepods, small gastropods, free-living nematodes, etc., are outstanding in abundance and occur in such numbers that a quantitative study of their distribution and its seasonal variations is obviously of importance. Numbers of the young stages of the larger benthic animals are met with, and it is hoped that the investigations will throw some light on the distribution of these also.

Mrs. Sexton's experiments on heredity in the eye colour of *Gammarus chevreuxi* have been continued and considerable progress has been made. Several new mutations affecting the body as well as the eye colour have appeared since those mentioned last year. The stock in which these changes have occurred has become much more healthy and the broods larger than they were when the mutation first appeared. This is a great help in studying the different problems presented by the experiments,

and it is hoped that it will soon be possible to publish some account of the work. Miss A. R. Clark has again given valuable help in this research.

A thorough study of the feeding processes and the physiology of digestion in the oyster is being carried out by Dr. C. M. Yonge. The anatomy and histology of the alimentary tract and of the mantle, gills, and palps have been investigated, and the direction of the ciliary currents has been worked out. The selective mechanisms on the mantle, gills, and on the palps especially, is very highly developed and only very fine particles find their way into the stomach. The sorting mechanism in the stomach is not so well developed as in Lamellibranchs which take in sand grains and other large particles. The feeding processes of the larvæ and spat were studied by Dr. Yonge during a visit to the Fisheries Experimental Station at Conway; the crystalline style was observed in rapid revolution in both larvæ and spat. Oysters have been experimentally fed with iron solution, fat stained with nile blue sulphate, and with blood corpuscles from dogfish. Fine particles and soluble substances are absorbed by the cells of the digestive gland, and large particles are ingested by phagocytes which are everywhere present in the wall of the gut and pass freely into the lumen. The stomach is the most acid region of the gut owing to the dissolution within it of the head of the style. The digestive gland contains enzymes which can digest a wide range of carbohydrates, but there is no evidence that cellulose, pectin, or pentosans can be digested; there is also a weak protease and a weak lipase. The style contains amylase (which is inactivated in the absence of certain salts), glycogenase, and an oxidase.

Department of General Physiology.

During the year Dr. Atkins has continued his work on the phosphate content of sea-water, and the seasonal changes were traced through a third year. At Station E 1 the total consumption of phosphate was somewhat greater than in the previous years, the minimum being late in August. Further determinations have been made upon the phosphate content of water from the depths, down to 3100 metres (1800 fathoms), and the result already reported, that the deep water acts as a reservoir of phosphate, has been substantiated. The samples necessary for such work were obtained through the courtesy of Dr. Stanley Kemp, of the R.R.S. *Discovery*, also of the Scottish and Irish Fishery Boards. Work on the seasonal variations on the silica content of sea-water has also been continued. In conjunction with Mr. Pantin a new buffer mixture for the alkaline range was prepared and standardised by the hydrogen electrode.

Dr. H. H. Poole continued his work, in conjunction with Dr. Atkins, on the photo-electric measurement of the penetration of light into sea-water. This was carried out at the Cawsand Bay (Pier Cellars) Station and on the *Salpa*, on which it proved feasible to work as long as it was mechanically possible to lower the gear, the apparatus being lashed down. The apparatus was also used in comparing the light intensity inside and outside the wood at Cawsand. In the sea both intensity and wave-length alter; in the wood variation in intensity is the main factor concerned.

Experiments on the preservation of hemp, cotton, and silk nets by the use of a mixed copper soap dissolved in petrol or in benzol were continued. All tow-nets now issued are so treated, and the large nets for the R.R.S. *Discovery* were also treated. Mr. H. W. Harvey's suggestion that resin should be added to improve the adherence of the soap mixture to the fibre has proved very valuable, but even without resin a piece of hemp net has remained sound after fifteen months in water. This piece was treated in September, 1924, and again in July, 1925. Single threads cannot be broken by hand.

The action of ions on amœboid movement has been studied by Mr. C. F. A. Pantin by an accurate quantitative method. In general the results most nearly resemble those obtained from *rhythmically* contractile mechanisms (e.g. heart, cilia, etc.). There are, however, significant differences which may shed considerable light on the general nature of the action of ions on the cell-membrane. The conclusions are extensive and cannot be briefly summarised: they will appear shortly in two papers in the *British Journal of Experimental Biology*.

Experiments were conducted by Mr. Pantin, in collaboration with Prof. R. Chambers, Cornell University, U.S.A., to determine the action of ions on the nuclear membrane. On micro-dissecting out the nucleus of a marine amœba into solutions of different ions, it appears that solutions of potassium chloride with a small amount of calcium are most effective in maintaining the nuclear membrane normal. Definite conclusions cannot be reached without further experiment by the same method. It is hoped to do this next summer.

A simple accurate method of determining the dissociation curve of hæmocyanin has been worked out by Mr. Pantin in collaboration with Prof. L. T. Hogben. It is suitable for class work. The dissociation curve of hæmocyanin, and the effects of temperature, hydrogen ion concentration and salts, resemble those of hæmoglobin. A continuation of the work by Dr. Hogben will appear shortly.

Mr. Pantin has also studied the quantitative estimation of copper by the Guaiacum method. The reaction is enormously affected by the hydrogen ion, and also modified by salts. Provided the solution to be

tested is accurately buffered, and due allowance is made for the salt content, this method is suitable for the micro-estimation of copper in *artificial* sea-water : 10^{-9} parts of copper can be detected under optimal conditions.

Published Memoirs.

The following papers, the outcome of work done at the Laboratory, have been published elsewhere than in the Journal of the Association :—

ALLEN, E. J. *Life in the Sea.* Phases of Modern Science, pp. 124–127. Royal Society, 1925.

AMEMIYA, I. *Hermaphroditism in the Portuguese Oyster.* "Nature," Vol. CXVI, 1925, p. 608.

ATKINS, W. R. G. *The Ocean regarded as a Pasture.* Marine Observer, Vol. II, 1925, pp. 162–164.

ATKINS, W. R. G. *The Preservation of Fishing-nets, Mosquito-nets, and Tent Fabrics.* "Nature," Vol. CXV, 1925, pp. 761–762.

ATKINS, W. R. G., AND HARVEY, H. W. *The Variation with Depth of Certain Salts utilised in Plant Growth in the Sea.* "Nature," Vol. CXVI, 1925, p. 784.

CROFTS, D. R. *The Comparative Morphology of the Caecal Gland (Rectal Gland) of Selachian Fishes, with some reference to the Morphology and Physiology of the similar Intestinal Appendage throughout Ichthyopsida and Sauropsida.* Proc. Zool. Soc., 1925, pp. 101–188.

DUNKERLY, J. S. *The Development and Relationships of the Myxosporidia.* Quart. Journ. Micr. Sci., Vol. LXIX, 1925, pp. 185–216.

ELLIS, A. E. *Land Mollusca on the Mewstone.* Journ. Conch., Vol. XVII, 1924, pp. 187–188.

GURNEY, R. *The Larval Development of some British Prawns (Palæmonidæ), II. Leander longirostris and Leander squilla.* Proc. Zool. Soc., 1924, pp. 961–982.

HARINGTON, C. R. *Report on Examination of Raft and Test Pieces at Plymouth, November,* 1923. *Remarks on Dr. Harington's Report, dated November,* 1923, *relating to his recent visit to Plymouth.* Fifth (Interim) Report of the Committee of the Institute of Civil Engineers, 1925, pp. 13–17.

HARVEY, H. W. *Hydrography of the English Channel.* Conseil Perm. Int. Explor. Mer. Rapp. et Proc. Verb., Vol. XXXVII, 1925, pp. 59–89.

HENTSCHEL, C. C. *Notes on Hophtophrya (Anoplophyra) brasili (Léger and Duboscq), an Intestinal Ciliate of the Polychæte Worm Cirratulus.* Parasitology, Vol. XVII, 1925, pp. 217–220.

HOGBEN, L. T. *Studies on the Comparative Physiology of Contractile Tissues. I. The Action of Electrolytes on Invertebrate Muscle.* Quart. Journ. Exp. Physiol., Vol. XV, 1925, pp. 263–312.

LEBOUR, M. V. *The Dinoflagellates of Northern Seas.* Plymouth, 1925. Price, 12s. 6d.

LEBOUR, M. V. *The Food Chains in the Sea.* Trans. Plymo. Inst., Vol. XVII, 1922–1924 (1925), pp. 29–35.

MACDONALD, A. D. *Action of Adrenalin on the Perfused Fish Heart.* Quart. Journ. Exp. Physiol., Vol. XV, 1925, pp. 69–80.

NEWTH, H. G. *The Early Development of Astropecten irregularis, with Remarks on Duplicity in Echinoderm Larvæ.* Quart. Journ. Micr. Sci., Vol. LXIX, 1925, pp. 519–554.

ORTON, J. H. *Summary of a Report on a Survey of the Oyster Beds in the Fal Estuary, in November,* 1924, *with Notes on the Biology of the Oyster.* Falmouth, 1925.

ORTON, J. H. *On the Efficiency of the Petersen Grab.* "Nature," Vol. CXV, 1925, p. 156.

ORTON, J. H. *Possible Effects on Marine Organisms of Oils Discharged at Sea.* "Nature," Vol. CXV, 1925, pp. 910–911.

ORTON, J. H. *The Conditions for Calcareous Metabolism in Oysters and other Marine Animals.* "Nature," Vol. CXVI, 1925, p. 13.

ORTON, J. H. *The Production of Oysters (O. edulis) on English Beds in Relation to New Observations on Breeding Phenomena.* "Nature," Vol. CXVI, 1925, pp. 673–674.

PALMER, R. *The Chromosome Complex of Gammarus chevreuxi Sexton.* "Nature," Vol. CXVI, 1925, p. 785.

PANTIN, C. F. A., AND ROGERS, T. H. *An Amphoteric Substance in the Radula of the Whelk (Buccinum undatum).* "Nature," Vol. CXV, 1925, pp. 639–640.

POOLE, H. H. *On the Photo-electric Measurement of Submarine Illumination.* Scient. Proc. R. Dublin Soc., Vol. XVIII, 1925, pp. 99–115.

RUSSELL, F. S. *Depth-recording with Plankton-nets.* "Nature," Vol. CXV, 1925, pp. 603–604.

STEDMAN, ELLEN, AND STEDMAN, EDGAR. *Hæmocyanin. Part I. The Dissociation Curves of the Oxyhæmocyanin in the Blood of some Decapod Crustacea.* Biochem. Journ., Vol. XIX, 1925, pp. 544–551.

The Library.

The thanks of the Association are again due to numerous Foreign Government Departments, and to Universities and other Institutions at home and abroad for copies of books and current numbers of periodicals presented to the Library. Thanks are due also to those authors who have sent reprints of their papers to the Library.

Finance.

The special thanks of the Association are due to the various donors to the Building Fund, who have made possible the erection of the new laboratories. For this purpose the sum of £3,610 17s. 6d. has been subscribed. Thanks are also due for the grants for the maintenance of the Laboratory which have been so generously made by the Fishmongers' Company (£600 and £150 advance for next year), the Ray Lankester Trustees (£20), the British Association (£20), and the Universities of Oxford, Cambridge, London, Bristol, Birmingham, and Leeds.

Vice-Presidents, Officers, and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1925–26 :—

President.

Sir E. Ray Lankester, K.C.B., LL.D., F.R.S.

Vice-Presidents.

The Duke of Bedford, K.G.
The Earl of Stradbroke, C.V.O., C.B.
The Earl of Balfour, K.G., F.R.S.
Viscount Astor.
Lord Montagu of Beaulieu.
Lord St. Levan, C.V.O., C.B.
The Right Hon. Sir Arthur Griffith-Boscawen.
The Right Hon. Sir Austen Chamberlain, K.G., M.P.
Sir W. B. Hardy, F.R.S.
The Right Hon. Sir Arthur Steel-Maitland, Bart., M.P.
George Evans, Esq.
Sir Nicholas Waterhouse, K.B.E.
Prof. W. C. McIntosh, F.R.S.
G. A. Boulenger, Esq., F.R.S.
J. O. Borley, Esq., O.B.E.

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H. H. Dale, Esq., C.B.E., M.D., F.R.S.
Prof. J. C. Drummond, D.Sc.
G. P. Farran, Esq.
Prof. J. Stanley Gardiner, F.R.S.
Prof. W. Garstang, D.Sc.
Prof. E. S. Goodrich, F.R.S.
Prof. S. J. Hickson, D.Sc., F.R.S.
Prof. A. V. Hill, Sc.D., F.R.S.
H. G. Maurice, Esq., C.B.
C. Tate Regan, Esq., F.R.S.
Prof. D'Arcy Thompson, C.B., F.R.S.
Prof. D. M. S. Watson, F.R.S.

Chairman of Council.

Sir Arthur E. Shipley, G.B.E., D.Sc., F.R.S.

Hon. Treasurer.

George Evans, Esq., 1, Wood Street, London, E.C. 2.

Hon. Secretary.

E. J. Allen, Esq., D.Sc., F.R.S., The Laboratory, Citadel Hill, Plymouth.

The following Governors are also members of Council :—

G. P. Bidder, Esq., Sc.D.
E. T. Browne, Esq.
Lothian D. Nicholson, Esq. (Prime Warden of the Fishmongers' Company).
W. T. Brand, Esq. (Fishmongers' Company).
George Evans, Esq. (Fishmongers' Company).
His Honour Judge Chapman (Fishmongers' Company).
Lothian D. Nicholson, Esq. (Fishmongers' Company).
Major Nigel O. Walker, O.B.E. (Fishmongers' Company).
Prof. G. C. Bourne, D.Sc., F.R.S. (Oxford University).
Sir Arthur E. Shipley, G.B.E., D.Sc., F.R.S. (Cambridge University).
P. Chalmers Mitchell, Esq., C.B.E., D.Sc., F.R.S. (British Association).
Prof. E. W. MacBride, D.Sc., F.R.S. (Zoological Society).
Sir Sidney Harmer, K.B.E., F.R.S. (Royal Society).

List of Annual Subscriptions

Paid during the Year, 1st April, 1925, to 31st March, 1926.

	£	s.	d.
Dr. W. M. Aders	1	1	0
E. J. Allen, Esq., D.SC., F.R.S.	1	1	0
G. L. Alward, Esq.	1	1	0
Dr. Ikusaku Amemiya	1	1	0
Prof. J. H. Ashworth, D.SC., F.R.S.	1	1	0
G. T. Atkinson, Esq. (1913–1917)	5	5	0
H. F. Barnes, Esq.	1	1	0
Prof. W. Bateson, F.R.S. (the late)	1	1	0
Lieut.-Col. T. T. Behrens	1	1	0
Mrs. M. G. Bidder	1	1	0
E. J. Bles, Esq., D.SC. (the late)	1	1	0
L. A. Borradaile, Esq., SC.D.	1	1	0
E. G. Boulenger, Esq.	1	1	0
Colonel Sir Henry Bowles, Bart. (1924 and 1925)	2	2	0
Dr. A. Bowman	1	1	0
Sir J. Rose Bradford, K.C.M.G., M.D., D.SC., F.R.S.	1	1	0
Brighton Public Library	1	1	0
L. R. Brightwell, Esq.	2	2	0
H. H. Brindley, Esq.	1	1	0
H. O. Bull, Esq.	1	1	0
R. H. Burne, Esq., M.A. (1925 and 1926)	2	2	0
S. F. Bush, Esq.	1	1	0
Raymond R. Butler, Esq.	1	1	0
L. W. Byrne, Esq.	1	1	0
Dr. W. T. Calman, F.R.S.	1	1	0
H. Graham Cannon, Esq., D.SC.	1	1	0
G. S. Carter, Esq.	1	1	0
Prof. C. Chilton (1925 and 1926)	2	2	0
Dr. James Clark	1	1	0
J. F. Coonan, Esq.	1	1	0
L. R. Crawshay, Esq., M.A.	1	1	0
Carried forward	40	19	0

	£	s.	d.
Brought forward	40	19	0
Prof. Otto V. Darbishire	1	1	0
Dr. W. Cameron Davidson	1	1	0
F. M. Davis, Esq.	1	1	0
W. C. De Morgan, Esq.	1	1	0
G. Despott, Esq.	1	1	0
Director of Agriculture and Fisheries, Travancore, S. India (1924 and 1925)	2	2	0
F. A. Dixey, Esq., F.R.S.	1	1	0
C. C. Dobell, Esq., F.R.S.	1	1	0
H. V. Dobson, Esq., J.P.	1	1	0
Prof. J. S. Dunkerly, D.SC., PH.D.	1	1	0
Howard Dunn, Esq., J.P.	1	1	0
George Evans, Esq.	1	1	0
G. P. Farran, Esq.	1	1	0
G. Herbert Fowler, Esq., PH.D.	1	1	0
Dr. E. L. Fox	1	1	0
H. Munro Fox, Esq. (1924 and 1925)	2	2	0
Miss E. A. Fraser, D.SC.	1	1	0
Prof. F. W. Gamble, D.SC., F.R.S.	1	1	0
S. G. Gibbons, Esq.	1	1	0
Prof. E. S. Goodrich, F.R.S.	1	1	0
J. R. Groome, Esq.	1	1	0
Sir Eustace Gurney	1	1	0
Dr. H. P. Hacker	1	1	0
Wilfred Hall, Esq.	1	1	0
A. C. Hardy, Esq.	1	1	0
A. E. Hefford, Esq.	1	1	0
C. C. Hentschel, Esq.	1	1	0
Prof. Sydney J. Hickson, D.SC., F.R.S. (1924 and 1925)	2	2	0
Prof. A. V. Hill, F.R.S.	1	1	0
W. T. Hillier, Esq., M.R.C.S.	1	1	0
T. V. Hodgson, Esq. (the late), 1925 and 1926	2	2	0
Capt. G. C. L. Howell	1	1	0
P. Hoyte, Esq.	1	1	0
Prof. Julian S. Huxley	1	1	0
J. J. Judge, Esq.	1	1	0
Carried forward	81	18	0

	£	s.	d.
Brought forward	81	18	0
Sir Frederick Keeble, C.B.E., SC.D., F.R.S. (1924 and 1925)	2	2	0
R. Kirkpatrick, Esq.	1	1	0
The Hon. Lionel Lindsay	1	1	0
J. J. Lister, Esq., F.R.S.	1	1	0
J. R. Lumby, Esq.	1	1	0
Stanislaus Makovski, Esq.	1	0	0
G. I. Mann, Esq.	1	1	0
D. J. Matthews, Esq.	1	1	0
J. C. Maude, Esq.	1	1	0
Capt. W. N. McClean	1	1	0
J. H. Midgley, Esq.	1	1	0
Milford Haven Trawler Owners' and Fish Salesmen's Association, Ltd. (1924 and 1925)	2	2	0
W. S. Millard, Esq.	1	1	0
P. Chalmers Mitchell, Esq., C.B.E., D.SC., F.R.S.	1	1	0
Major A. R. Moncrieff	1	1	0
C. C. Morley, Esq.	1	1	0
National Museum of Wales	1	1	0
H. G. Newth, Esq.	1	1	0
Chas. Oldham, Esq.	1	1	0
G. Ord, Esq.	1	1	0
G. W. Paget, Esq.	1	1	0
T. A. Pawlyn, Esq. (1925 and 1926)	2	2	0
Pawlyn Brothers (1925 and 1926)	2	2	0
F. T. K. Pentelow, Esq.	1	1	0
Plymouth Corporation (Education Committee)	1	1	0
Plymouth Corporation (Museum Committee)	1	1	0
Plymouth Proprietory Library	1	1	0
Port of Plymouth Incorporated Chamber of Commerce	1	1	0
Mrs. Horace Porter (1925 and 1926)	2	2	0
E. A. Robins, Esq.,	1	1	0
J. H. Robinson, Esq.	1	1	0
T. Howard Rogers, Esq.	1	1	0
Charles H. Rudge, Esq.	1	1	0
E. S. Russell, Esq., D.SC.	1	1	0
J. T. Saunders, Esq.	1	1	0
Carried forward	123	17	0

	£	s.	d.
Brought forward	123	17	0
R. E. Savage, Esq. (1924 and 1925)	2	2	0
Edgar Schuster, Esq., D.SC.	1	1	0
W. L. Sclater, Esq.	1	1	0
Major R. B. Seymour Sewell, I.M.S.	1	1	0
Miss Lilian Sheldon	1	1	0
Thos. and Wm. Smith, Ltd. (1925 and 1926)	2	2	0
States Committee for Fisheries, Guernsey	1	0	0
A. C. Stephen, Esq.	1	1	0
The Right Hon. Lord St. Levan C.B., C.V.O.	1	1	0
Ernest J. Stream, Esq., M.A. (1924–1926)	3	3	0
H. H. Sturch, Esq.	1	1	0
E. J. Tabor, Esq.	1	1	0
H. E. Tabor, Esq.	1	1	0
J. M. Tabor, Esq.	1	1	0
S. Takeda, Esq.	1	1	0
Prof. W. M. Tattersall	1	1	0
Harold Thompson, Esq.	1	1	0
Sir Herbert Thompson, Bart.	1	1	0
Sir John Thornycroft, F.R.S.	1	1	0
Torquay Natural History Society	1	1	0
Major Nigel O. Walker, O.B.E.	1	1	0
Lieut.-Col. H. J. Walton, I.M.S., M.D., F.R.C.S., C.M.Z.S.	1	1	0
Arthur W. Waters, Esq., F.L.S.	1	1	0
Prof. D. M. S. Watson, F.R.S. (1924 and 1925)	2	2	0
Mrs. F. J. Weldon	1	1	0
W. A. Willes, Esq.	1	1	0
Ronald Winckworth, Esq., M.A., F.R.G.S.	1	1	0
W. B. Woodrow, Esq.	1	1	0
Total	£158	9	0

List of Donations to the Building Extension Fund

For the Year 1st April, 1925, to 31st March, 1926.

	£	s.	d.
International Education Board	750	0	0
The Worshipful Company of Fishmongers (Second donation)	250	0	0
The Royal Society	100	0	0
The Institution of Civil Engineers	100	0	0
Magdalen College, Oxford	25	0	0
Dr. G. P. Bidder	500	0	0
George Evans, Esq.	21	0	0
E. J. Allen, Esq., D.SC., F.R.S.	10	10	0
W. T. Brand, Esq. (Second donation)	10	0	0
R. H. Burne, Esq., M.A.	5	5	0
Pawlyn Brothers	5	0	0
Birmingham University Zoology Club	3	3	0
Prof. W. Bateson, F.R.S. (the late)	2	2	0
Stuarts and Jacks, Ltd.	2	2	0
Prof. J. Bayley Butler	1	1	0
J. T. Cunningham, Esq.	1	1	0
T. A. Pawlyn, Esq.	1	1	0
Total	£1,787	5	0

For the Year commencing April 1st, 1926.

	£	s.	d.
Miss M. V. Lebour, D.SC. (Second donation)	5	15	0
C. F. Hickling, Esq.	2	2	0
Total	£7	17	0

Year 1924–25	1,823	12	6
Year 1925–26	1,787	5	0
Year commencing 1st April, 1926	7	17	0
Total	£3,618	14	6

THE MARINE BIOLOGICAL ASSOCIATION

Dr. *Statement of Receipts and Payments for the*

GENERAL

	£	s.	d.	£	s.	d.
To Balance from 31st March, 1925 :—						
Cash in hand	37	5	2			
Cash at Bank	490	9	5	527	14	7
,, Grants :—						
Ministry of Agriculture and Fisheries Grant from Development Fund	10,100	0	0			
Fishmongers' Company	750	0	0			
British Association	20	0	0	10,870	0	0
,, Subscriptions				158	9	0
,, Composition Fee				15	15	0
,, Donations				3	3	0
,, Sale of Specimens (*less* Purchases)				915	18	5
,, ,, Fish (*less* Expenses)				37	13	7
,, ,, Nets, Gear, and Hydrographical Apparatus				870	12	8
,, Table Rent (including Cambridge University, £52 10s. ; Trustees of the Ray Lankester Fund, £20 ; Birmingham University, £10 10s. ; London University, £50 ; Bristol University, £25)				278	14	0
,, Tank Room Receipts				369	15	8
,, Interest on Investments :—						
4% War Stock	3	2	8			
4% New Zealand Stock	13	2	10			
Deposit Account	4	2	2	20	7	8
,, Royalties on Films				1	0	4
				£14,069	3	1

The Association's Bankers hold on its behalf :—
£410 14s. 8d. 4% New Zealand Stock, 1943-63.
£78 9s. 4d. 4% War Loan, 1929-42.
£51 War Savings Certificates.

BUILDING

	£	s.	d.
To Balance at Bank 31st March, 1925	1,825	14	7
,, Donations	1,787	5	0
,, Interest on Building Fund Deposit	68	6	4
	£3,681	5	11

OYSTER NUTRITION

	£	s	d.
To Grant from Development Fund	365	0	0
,, Transfer from General Fund	1	10	3
	£366	10	3

PUBLICATION OF DR.

	£	s.	d.
To Balance at Bank 31st March, 1925	75	0	0
,, Grant from Development Fund	150	0	0
,, Sale of Book	81	19	0
	£306	19	0

OF THE UNITED KINGDOM.

Year 1st April, 1925, to 31st March, 1926. Cr.

FUND.

	£ s. d.	£ s. d.
By Salaries :—		
Director	1,012 10 0	
Physiologist	910 0 0	
Naturalists	2,985 3 5	
Hydrographer	518 6 8	5,426 0 1
,, Laboratory Wages (including National Insurance)		1,622 1 6
,, Annual Upkeep of Library		384 13 9
,, Scientific Publications :—		
Journal, Vol. XIII, No. 4, and Vol. XIV, No. 1	500 13 10	
Less Sales	77 8 6	423 5 4
,, Annual Upkeep of Laboratories and Tank Rooms :—		
Buildings and Machinery	245 4 8	
Electricity, Gas, Coal, and Water	319 0 1	
Chemicals and Apparatus	527 18 4	
Rates, Taxes, and Insurance	124 9 10	
Travelling	100 8 8	
,, Challenger Society Meetings	41 19 10	
Stationery, Postages, Telephone, Carriage, and Sundries	385 18 3	1,744 19 8
,, Annual Maintenance and Hire of Boats :—		
Wages (including Diet Allowance, National Insurance, and Casual Labour)	1,513 14 6	
Coal and Water	585 4 3	
Maintenance and Repairs, with Nets, Gear, and Apparatus	1,293 6 4	
Boat Hire and Collecting Expeditions	9 15 4	
Insurance	340 7 7	3,742 8 0
,, Interest on Bank Loans		12 6 7
,, Transfer to Oyster Nutrition Research Grant		1 10 3
,, Balance :—		
Cash in hand	87 15 8	
Cash at Bank	624 3 1	711 18 9
		£14,069 3 11

FUND.

	£ s. d.
By Expenditure on Building and Equipment	2,342 9 3
,, Balance, Cash at Bank	1,338 16 8
Note.—Further liabilities on the Building Fund to the amount of £1,500 have been incurred.	
	£3,681 5 11

RESEARCH GRANT.

	£ s. d.
By Salary and Expenses	366 10 3
	£366 10 3

M. V. LEBOUR'S BOOK.

	£ s. d.
By Printing and Publication	268 8 5
,, Balance, Cash at Bank	38 10 7
	£306 19 0

Examined and found correct,

(Signed) N. E. Waterhouse.
L. D. Nicholson.
P. Chalmers Mitchell.
J. O. Borley.

3 *Frederick's Place,*
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27th April, 1926.

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OBJECTS

OF THE

Marine Biological Association

OF THE UNITED KINGDOM.

THE ASSOCIATION was founded at a Meeting called for the purpose in March, 1884, and held in the Rooms of the Royal Society of London.

The late Professor HUXLEY, at that time President of the Royal Society, took the chair, and amongst the speakers in support of the project were the late Duke of ARGYLL, the late Sir LYON PLAYFAIR, the late Lord AVEBURY, the late Sir JOSEPH HOOKER, the late Dr. CARPENTER, the late Dr. GÜNTHER, the late Lord DALHOUSIE, the late Professor MOSELEY, the late Mr. ROMANES, and Sir E. RAY LANKESTER.

The Association owes its existence and its present satisfactory condition to a combination of scientific naturalists, and of gentlemen who, from philanthropic or practical reasons, are specially interested in the great sea fisheries of the United Kingdom. It is universally admitted that our knowledge of the habits and conditions of life of sea fishes is very small, and insufficient to enable either the practical fisherman or the Legislature to take measures calculated to ensure to the country the greatest return from the "harvest of the sea." Naturalists are, on the other hand, anxious to push further our knowledge of marine life and its conditions. Hence the Association has erected at Plymouth a thoroughly efficient Laboratory, where naturalists may study the history of marine animals and plants in general, and where researches on food-fishes and molluscs may be carried out with the best appliances.

The Laboratory and its fittings were completed in June, 1888, at a cost of some £12,000, and from that time until 1926 a sum of over £6,500 has been spent on additional buildings. Throughout this period investigations, practical and scientific, have been constantly pursued at Plymouth. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council; in addition, naturalists from England and from abroad have come to the Laboratory, to carry on their own independent researches, and have made valuable additions to zoological and botanical science, at the expense of a small rent for the use of a working table in the Laboratory and other appliances. The number of naturalists who can be employed by the Association in special investigations on fishery questions, and definitely retained for the purpose of carrying on those researches throughout the year, must depend on the funds subscribed by private individuals and public bodies for the purpose. The first charges on the revenue of the Association are the working of the sea-water circulation in the tanks, stocking the tanks with fish and feeding the latter, the payment of servants and fishermen, the maintenance of a research steamer and other collecting boats, and the salaries of the Resident Director and Staff. At the commencement of this number will be found the names of the gentlemen on the Staff.

The purpose of the Association is to aid at the same time both science and industry. It is national in character and constitution, and its affairs are conducted by a representative Council, by an Honorary Secretary and an Honorary Treasurer, without any charge upon its funds, so that the whole of the subscriptions and donations received are devoted absolutely to the support of the Laboratory and the prosecution of researches by aid of its appliances. The reader is referred to page 4 of the Cover for information as to membership of the Association.

CONTENTS OF NEW SERIES, Vol. XIV., No. 2.

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Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, &c.; and have access to the books in the Library at Plymouth.

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