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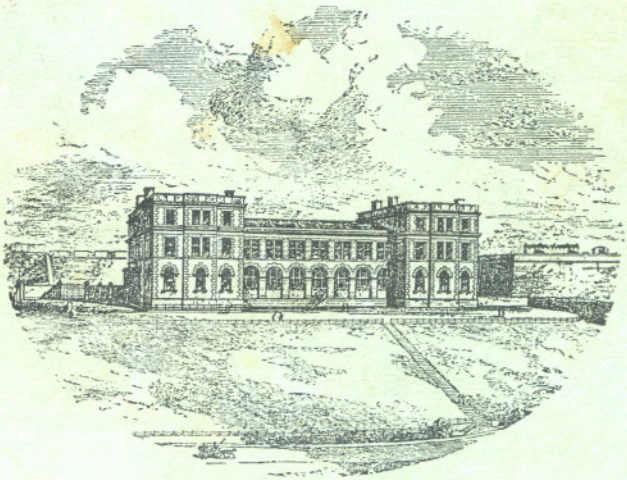

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Rays and Skates of Devon and Cornwall. II. A Study of the Fishery; with Notes on the Occurrence, Migrations and Habits of the Species.

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With 6 Figures in the Text.

Contents.

I. Growth of the Fishery.

There have been, in recent years, certain drastic changes in the sea fisheries of England and Wales. Not least among these has been the rapid rise in importance of Rays and Skates.

Prior to the beginning of the present century there was little or no demand for these Elasmobranchs in this country. Colonel Montague, writing in 1809, comments upon the immense quantities of Rays and Skates landed in Devonshire and states that they were then used chiefly for baiting crab-pots. In times of scarcity, however, some of the small ones were eaten by fishermen's families, *but were never exposed for sale.* Fifty years later this state of affairs had changed but little. Jonathan Couch, writing in 1862 (**3**, Vol. 1, p. 84), gives the following instructive account of fishing in the West of England at that time. "An adventure in the fisheries, at least in the West of England, is usually set on foot by

some practical fisherman, who provides the boat and her outfit, and who himself acts as the principal fisherman ; and who seeks his profit as owner by what is called the boat share, which commonly amounts to a fifth part of the fish sold in the market : for the remainder he has a common share with his men. But other fishes will come to the hook besides those which find a place at fashionable tables, or the public are accustomed to buy, and which, indeed, are intrinsically as valuable as any which have a ready sale. The Grey Gurnard, Scad, Comber, Power, the Wrasses, Dogfish, Rays, and Skates, are in this class, and by the fishermen they are collectively known as rabble-fish, as being rejected from the market ; and they consequently fall to the lot of the fishermen themselves, who take them for the subsistence of their families, without deducting any portion for the boat share. The Skate is the largest, and, on the whole, the most important of these rejected fishes, and the Saxon word *Skitan*, to reject, is expressive of the fact of its being so. The same word is the parent of several expressions still in common use as significant of being thrown out, aside, or rejected." Again (*loc. cit.* p. 89), in writing specifically of the Blue Skate, *Raia batis*, he says, " The Skate is never the special object of the fisherman's search and when it chances to take the hook it may give him perhaps a greater amount of trouble than the prize can repay."

During the succeeding quarter of a century the fish-eating public in this country must gradually have grown aware of the value of Rays and Skates as food-fishes, for Day (**4**, Vol. II, p. 335), writing some time between 1880 and 1884, refers to Couch's account of Rabble-fish and adds the significant statement, " Things are altered now, much of this rabble-fish going to Billingsgate and other large inland markets."

Nevertheless, Rays and Skates even then were not considered to rank as food fishes. Cunningham, in his *Natural History of the Marketable Marine Fishes of the British Islands*, published in 1896, dismisses them with only a few words in the opening general section of the book. In the main part of the work dealing with the history of particular fishes they find no place. McIntosh and Masterman also, in their *Life Histories of the British Marine Food Fishes*, published in 1897, all but ignore the Raiidæ though they, too, discourse at length on such Teleosteans as the Gobies, Rocklings, Sticklebacks, and Blennies.

Towards the opening of the twentieth century, however, a definite fishery for Rays and Skates gradually arose and by 1906, the first year for which reasonably reliable statistical returns are available, no less than 384,953 cwt. of these fishes were landed at the various ports in England and Wales, to which total Devon and Cornwall contributed 44,618 cwt.

From that time onwards until the outbreak of the Great War in 1914, the annual landings remained fairly uniform, fluctuating only between 350,000 and 400,000 cwt. The prices, however, steadily rose—apart

from two minor drops in 1909 and 1911—from an average value on landing of 11s. 2d. per cwt. in 1906 to 14s. 1d. per cwt. in 1913.

During the first five years of the post-war period (1919–1923) the landings of Rays and Skates in England and Wales steadily increased in total weight and total value. Since then, the quantity of fish landed has remained practically constant, averaging approximately 420,000 cwt. per annum (*vide* Fig. 1, p. 4).

Prices, however, gradually fell from the artificial peak produced by post-war conditions; but the average value per cwt. over any full year has never fallen below 26s. 8d. (in 1927), a figure which is almost double that of 1913. At the present time prices are steadily rising (*vide* years 1927–30 in Table I, Column 4).

TABLE I.

England and Wales.

Total Weight of Skates and Rays landed (in cwts.); Total Value (in pounds); and Average Value per cwt—1906–30 inclusive.*

Year.	Weight (in cwt.).	Value (in £).	Average price per cwt.
1906	384,953	214,556	11/2
1907	378,773	216,170	11/5
1908	381,134	225,097	11/10
1909	415,704	230,591	11/1
1910	367,678	225,127	12/3
1911	351,729	200.972	11/5
1912	368,207	235,632	12/10
1913	359,446	253,729	14/1
—	—	—	—
1919	244,656	464,998	38/–
1920	356,869	625,534	35/1
1921	375,480	684,674	36/6
1922	438,505	608,048	27/9
1923	448,436	639,098	28/6
1924	422,161	599,761	28/5
1925	399,723	577,776	28/11
1926	364,523	539,905	29/7
1927	430,508	573,644	26/8
1928	424,724	573,238	27/–
1929	446,317	614,729	27/7
1930	435,818	638,896	29/4

Within the area under survey—Devon and Cornwall—the Ray and Skate fishery is of primary importance (*vide* Fig. 2, p. 5). The various species of the genus Raia collectively constitute the heaviest landings of demersal fish. In 1929 they formed no less than 35% of the total weight and 30% of the total value of all demersal landings for the year.†

* From the Ministry of Agriculture and Fisheries Statistical Tables of Sea Fisheries.

† It is of interest also to note that, with regard to quantity of fish landed in Devon and Cornwall in 1929, Dogfish come second to Skates and Rays, with 16% of the total by weight. That is to say that slightly over 50% by weight of all demersal fish landed in Devon and Cornwall in that year was Elasmobranch.

Actually, they are of still greater importance than even these figures indicate. Forming, as they do, the only large and staple fish supply within the area throughout the year, they play a large part in attracting buyers to the district and in keeping them there, thus helping to maintain a demand for other fish as well, with higher resultant prices.

II. Fishing Methods and Gear.

Rays and Skates are demersal fishes fitted by structure and habit for life on the sea floor. They may be fished by any of the usual methods

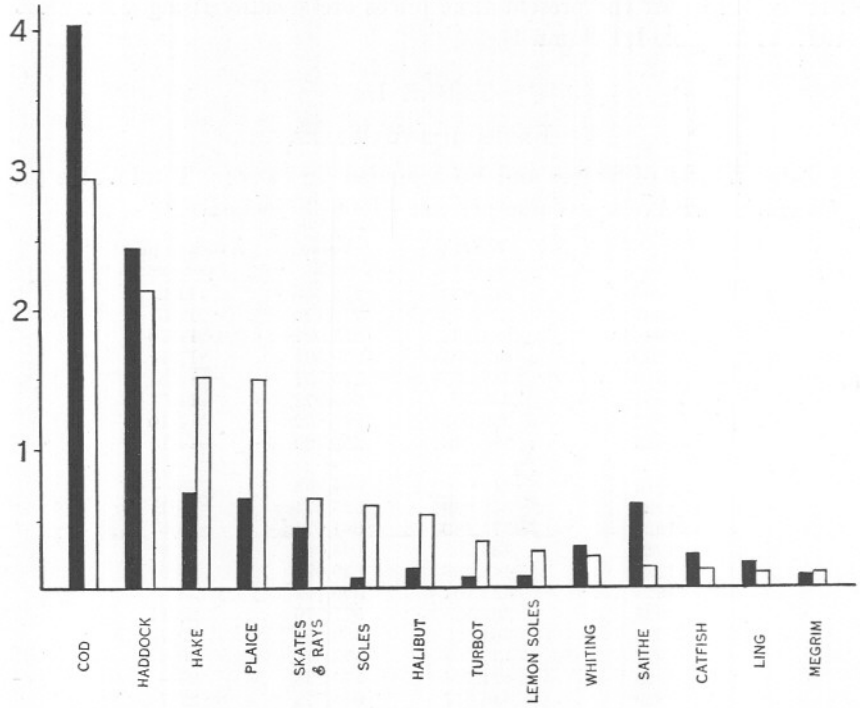

Fig. 1.—Graphical representation of the landings of the principal species of demersal fish of British taking landed in England and Wales in 1930, showing (*a*) total quantity (black columns) in millions of cwt., and (*b*) total value (white columns) in millions of pounds. *Note important position of Skates and Rays.*

employed in the capture of demersal species. In Devon and Cornwall they are caught in beam, V.D., and otter trawls, on long lines, and in set nets.

The main trawling ports are Brixham and Plymouth, from both of which fishing operations are carried out continuously over the whole year. From Brixham about 50 sailing smacks operate, all of which carry beam

trawls. Nine small steam trawlers work from Plymouth and fourteen sailing smacks, the former equipped with V.D. and otter and the latter with beam trawls. At Padstow a fairly intensive trawl fishery is usually carried on during the first three or four months of the year by East-country steamers of the drifter-trawler type. There also work from each of these

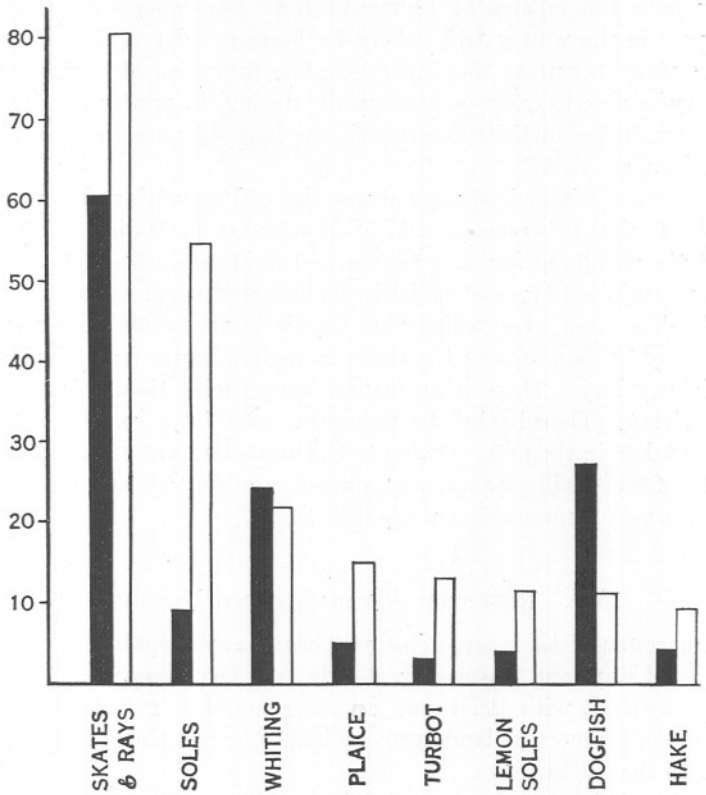

FIG. 2.—Graphical representation of the landings of the principal species of demersal fish of British taking landed in Devon and Cornwall in 1929, showing (*a*) total quantity (black columns) in thousands of cwt., and (*b*) total value (white columns) in thousands of pounds. *Note the leading position of Skates and Rays.*

ports and from the numerous other smaller harbours along the coast small motor trawlers which fish the inshore grounds.

At all seasons of the year Rays and Skates are caught on the usual trawling grounds with little variations in numbers except what can largely be attributed to weather conditions—a state of affairs which indicates a minimum of migratory movements, at any rate on a large scale. A certain amount of migration, however, does take place (*vide infra*, p. 18).

Long-lining is prosecuted mainly by Cornish fishermen with Newlyn as

the main port. A certain amount of line fishing is also carried on from various other smaller ports on the Cornish coast, e.g. Looe, Mevagissey, and St. Ives. The line fishery continues for only part of the year, roughly from April till October. This is due not to any change or movement on the part of the stock of available fish, but to the movement of the fishing fleet which congregates at Plymouth from December till February to take part in the winter drift fishery for herrings. Even though no other fishery were to attract them, the long-line fishermen of Cornwall would scarcely be able to carry on successfully during the winter months owing to the small size of their vessels and the long distance from port of the line-fishing grounds.

The set-net fishery is at present peculiar to Plymouth and of particular interest in that it is seasonal and of short duration, lasting at most from about the middle of January till the end of March. The nets are of the fixed or anchored type set in fairly shallow water and acting as " tangle nets." The area over which this fishery is prosecuted is exceedingly limited, extending around the shore in shallow water from Yealm Point to Bigbury Bay. There is an inshore spring migration of large mature *Raia clavata* (Thornbacks) to this area (*vide infra*, p. 20), which are readily taken in the nets. Owing to the limited extent of the fishing area this net fishery will support only a small number of boats, but for these it proves very remunerative while it lasts.

III. Research Apparatus and Methods.

On account of their large size and heavy cost, regular and adequate samples of Rays and Skates cannot ordinarily be delivered at the Laboratory to be dealt with at leisure. In order to obtain sufficient data large numbers of commercial landings have had to be examined in considerable detail on the fish markets.

Rays and Skates, when exposed for sale, generally are spread out in lots on the fishmarket floor, all with their ventral surfaces uppermost. In order to avoid handling the fish—too much interference with which would not be tolerated by fishermen, salesmen, or buyers—it was necessary at the outset to learn rapidly and accurately to distinguish the various species landed in this area without raising each fish to view its dorsal side. After considerable practice this was found to be possible. The diagnostic characters which have proved most useful on the fishmarket have been described in a previous paper (Steven, **8**).

The problem of obtaining measurements of the fish also presented difficulties to be overcome. Amidst the bustling activity of a busy market it is quite impossible successfully to use an ordinary measuring board for several reasons. These are :

1. This method necessitates handling and moving every fish examined.

2. Amidst the conditions prevailing an adequate number of fish could not be dealt with in the limited time available while the fish are exposed for sale.

3. Any attempt to use such a board would prove a grave hindrance to the normal activities of the workers on the market.

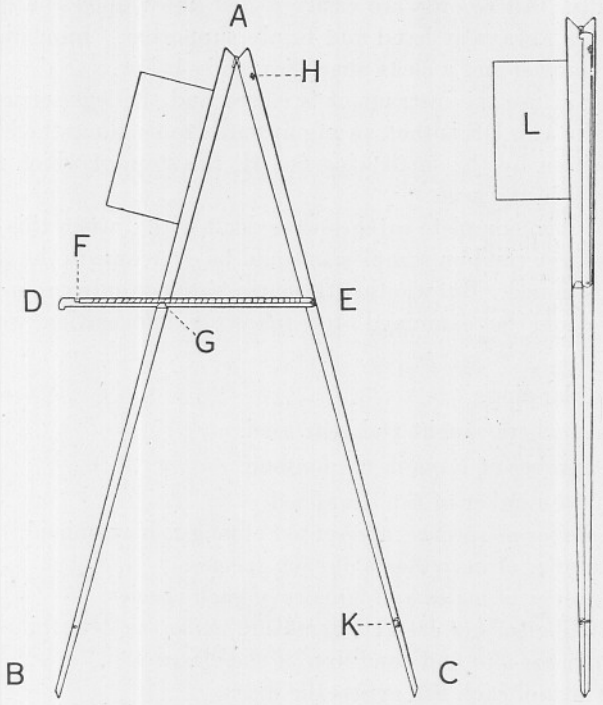

FIG. 3.—Measuring instrument used on the fishmarkets.
Left: open; Right: closed.

4. The use of an ordinary measuring board demands the services of two persons—one to carry out the measuring and another to record the data.

To overcome these difficulties an instrument was devised which renders possible rapid measurement of Rays and Skates spread out in lots, without handling or in any other way interfering with them, and at the same time enables a single unaided worker quickly and easily to record his data (Fig. 3).

The instrument is essentially a pair of large dividers 4′ 6″ in length. Twenty-two inches down from the hinge at the top an arm DE is fixed which can be moved around its point of attachment at E. When the instrument is in use the arm is dropped into a horizontal position and runs

in a small slot G (on the leg AB) open at the top. The arm DE is bevelled along its upper edge and the sloping surface graduated to indicate in centimetres the distance between the points BC of the dividers.

Attached to the leg AB, in a convenient position near the top, is a small rectangular plate L measuring 12 inches by 6 inches. On this plate are carried numerous sheets of paper held in position by two rubber bands. All records are easily jotted down upon the uppermost sheet which is always at hand and firmly supported. Immediately it is filled it is removed and a clean sheet lies ready below.

When not in use the instrument is closed and the legs secured by the catch K. The arm DE is then swung upwards to lie along the leg AC and fixed in position by the small wing-nut H, the stem of which fits into a small recess F in the arm.

When possible, complete catches were dealt with ; when this could not be done, as large random samples as could be overcome in the time available were examined. But whether the entire catch or only a representative part of it could be examined, the following information was always recorded.

1. Date of landing.
2. Locality where caught and gear used.
3. Total number of Rays in the landing.
4. (*a*) Total number of fish examined.
   (*b*) Number of species represented among fish examined.
   (*c*) Number of individuals of each species.
   (*d*) Number of males and females of each species.
   (*e*) Number of mature and immature males, as determined roughly from the size and condition of the claspers.*
   (*f*) Width of each fish across the disc.

In practice it was found necessary to use a more simplified system of notation than the usual male and female symbols (♂ and ♀) for denoting sex, and also to devise a method for recording mature and immature conditions in the males as indicated roughly by the size and condition of the claspers. The following scheme was adopted and proved highly successful.

A measurement without any accompanying symbol denotes a female fish, a horizontal stroke above the figures denotes an immature male, while mature males were indicated by a $\wedge$ over their measurements. Thus 49, $\overline{33}$, $\hat{75}$, denote a female, an immature male, and a mature male of 49, 33, and 75 cm. respectively in width across the disc.

* The females could not be so divided as there is no external morphological difference between mature and immature individuals. Since this paper was written, however, a possible method of distinguishing between them without having to open the body-cavity for examination of the gonads, has been discovered. Further work is proceeding in order to test the accuracy of the method.

A typical fishmarket sheet is shown below :

5/3/30 S.T. .....
Fishing Mount's Bay (40–50 fm.)

Landed *ca.* 1200 small, 310 large } fish.

Sample of large fish.

R. brachyura ..	$\overline{58}$	69	59	$\overline{41}$	62	$\hat{69}$	55			
	71	79	49	$\overline{64}$	$\overline{55}$	54	53			
	62	$\overline{59}$	44	$\overline{59}$	65	77	75			
R. clavata	$\overline{49}$	49	44	55	$\overline{44}$	52				
	62	41	$\hat{59}$	51	63	61	55	59		
	59	57	$\overline{42}$	$\overline{55}$	59	49	$\overline{41}$			
	48	$\overline{47}$	47	$\overline{40}$						
R. fullonica	49	40	48	49	44	42	51			
R. montagui	41	$\overline{40}$	$\overline{39}$	44	47	$\overline{41}$	39	41	$\hat{42}$	
	42	46	$\overline{40}$	$\overline{32}$	41	$\overline{39}$				
R. circularis	57	$\overline{59}$	$\overline{54}$	61	72	51	$\overline{52}$	48	46	

From such a sheet the information set out under heads 1–4 *f.* above is then directly obtainable.

IV. Species Landed and the Numerical Contribution of each to the Fishery.

In the Ministry of Agriculture and Fisheries Statistical Tables of Sea Fisheries all the species of Raia are grouped together under the inclusive heading " Skates and Rays." From these returns, therefore, no information can be extracted concerning the separate contributions of the different species to the total " Skate and Ray " landings for either the country as a whole or for any statistical region within it. By the detailed examination of large numbers of fish in the manner described above, accurate information on this point so far as the markets of Devon and Cornwall are concerned has been sought.

It has previously been recorded by Clark (**1**, p. 581) that eleven species of Raia appear more or less regularly on the fishmarkets within this area. These are shown in Table II below.

TABLE II.

SPECIES OF RAIA LANDED IN DEVON AND CORNWALL.

	Scientific name.	Usual name in Devon and Cornwall.	More generally recognised common name.
1.	*R. clavata* Linnæus.	Thornback Ray. Greeja (St. Ives). Roker (Commercial name).	Thornback Ray.
2.	*R. montagui* Fowler.	Spotted Smoothback.	Spotted Ray. Homelyn Ray.
3.	*R. brachyura* Lafont.	Blonde Ray. Smoothback Sand Ray. Calaber (St. Ives).	Blonde Ray.
4.	*R. microcellata* Montagu.	(Painted Ray).	Small-eyed Ray.
5.	*R. undulata* Lacépède.	(Marbled Ray).	Undulate Ray. Painted Ray.
6.	*R. nævus* Müller and Henle.	Cuckoo Ray. Butterfly Ray.	Cuckoo Ray.
7.	*R. circularis* Couch.	Sand Ray.	Sand Ray.
8.	*R. fullonica* Linnæus.	Owl Ray (Newlyn).	Shagreen Ray.
9.	*R. batis* Linnæus.	Common Skate. Blue Skate.	Blue Skate.
10.	*R. marginata* Lacépède.	White-bellied Skate. Mule (St. Ives). *Owl (young).	Bordered Ray (young). White-bellied skate (adult).
11.	*R. oxyrhynchus* Linnæus.	Long-nosed Skate (Bottled-nosed Skate).†	Long-nosed Skate.

The numerical compositions of the catches obtained by the two main methods of capture—long-lining and trawling—differ greatly from each

* When small, this Ray is not distinguished by the fishermen from *R. fullonica*.
† Should more correctly be applied to *R. marginata* (adult).

other and from that of the total combined landings by all types of fishing vessels.

In long-line landings, the mean numerical composition by species is as follows (Table III) :—

TABLE III.

COMPOSITION OF LINERS' LANDINGS.

R. clavata	45%
R. fullonica	22%
R. nævus	15%
R. batis	10%
R. montagui	4%
R. circularis	3%
Total supplied by six species	99%

Three species, *R. marginata*, *R. brachyura*, and *R. oxyrhynchus*, each of which occurs in small numbers, supply the remaining 1%. *R. undulata* and *R. microcellata* seldom or never occur, even as single specimens, in line catches.

The landings by steam trawlers are made up as follows (Table IV):

TABLE IV.

COMPOSITION OF LANDINGS BY STEAM TRAWLERS.

R. montagui	26%
R. brachyura	24%
R. nævus	24%
R. clavata	11%
R. fullonica	8%
R. batis	6%
Total supplied by six species	99%

The remaining 1% is made up by the five other species, all of which occur occasionally in the catches in small numbers.

Steam trawler Ray landings are generally separated for sale on the fishmarket into small and large fish, the " smalls " consisting of fish less than about 40 cm. (about 16 inches) across the disc. The compositions of the " small " and " large " fish differ fundamentally from each other. In the smalls the species occur as follows (Table V) :—

TABLE V.

COMPOSITION OF TRAWLERS' "SMALLS."

1. R. nævus	30%
2. R. montagui	29%
3. R. brachyura	22%
4. R. fullonica	8%
5. R. batis	6%
6. R. clavata	5%
Total for six species	100%

Below is shown the totally different composition of the "large" fish (Table VI) :—

TABLE VI.

COMPOSITION OF STEAM TRAWLERS' "LARGE" FISH.

1. R. clavata	33%
2. R. brachyura	30%
3. R. montagui	20%
4. R. fullonica	7%
5. R. circularis	4%
6. R. batis	4%
Total supplied by six species	98%

The remaining 2% is made up by five other species all of which occur in small numbers from time to time.

A third class of landings, from the point of view of numerical composition by species, is obtained by sailing smacks (beam trawls) and inshore motor trawlers (otter trawls). The catches by the last-named vessels are very similar to each other, both being drawn from shallower water than those of steam trawlers and liners, and are made up on an average as follows (Table VII) :—

TABLE VII.

COMPOSITION OF CATCHES BY SAILING TRAWLERS AND INSHORE MOTOR TRAWLERS.

1. R. clavata	36%
2. R. montagui	30%
3. R. nævus	23%
4. R. fullonica	8%
Total supplied by four species	97%

Two species, *R. brachyura* and *R. microcellata*, supply between them most of the remaining 3%, but all the other species present in the area occur in small numbers from time to time.

To obtain the numerical composition by species of the total landings within the area is not possible by direct methods as records of the total number of fish landed by the different types of vessel are not available. The weight of fish landed, according to the different methods of capture, is, however, obtainable from the Ministry of Fisheries official records of landings at major ports.

These reveal that approximately 37% by weight of the total trawled fish landed at the major ports in Devon and Cornwall is caught by steam trawlers and 63% by weight by wind and motor trawlers. Again, it has not been possible to obtain any accurate determination of the relation between the number of fish landed by the various methods of capture and their total weight. But from general observation of the landings it would appear that for the two classes of landings by trawlers the number/weight relations are not widely different. Calculating on this assumption, the numerical composition by species of the total *trawl* landings at the major ports of Devon and Cornwall is as follows (Table VIII) :

TABLE VIII.

Composition of Total Trawl Landings.

R. montagui	29%
R. clavata	27%
R. nævus	24%
R. brachyura	10%
R. fullonica	8%
Total produced by five species	98%

The six other species in small numbers make up the remaining 2%.

The total trawled fish forms 42% by weight of the complete total of Rays and Skates landed—by all methods of capture—and liners produce 58% of that total. Here, however, it is certain that trawled landings contain a greater mean number of fish per cwt. than do liners' landings.

To calculate the percentage numerical composition of the total landings for the area, therefore, on a basis of 42% trawl and 58% line fish will give a slightly higher value than the true one to those species which bulk largely in liners' landings. Bearing this source of error in mind, the figures obtained by calculating on this basis are nevertheless instructive. These are as shown below (Table IX) :

TABLE IX.

Composition of Total Landings at Major Ports of Devon and Cornwall.

R. clavata	37%
R. nævus	19%
R. fullonica	16%
R. montagui	15%
R. batis	7%
R. brachyura	4%
Total supplied by six species	98%

The remaining five species collectively make up the remaining 2% of the total.

The combined results for the most important species are summarised in Table X.

TABLE X.

Percentage Numerical Composition of Landings (Summary).

	Steam Trawlers.			Wind and inshore motor.	Total Trawled.	Total Landings.
	Liners.	Large.	Small.	Total.			
R. clavata	**45**	**33**	5	*11*	**36**	**27**	**37**
R. nævus	*15*	—	**30**	**24**	**23**	**24**	*19*
R. fullonica	**22**	7	8	8	8	8	*16*
R. montagui	4	**20**	**29**	**26**	**30**	**29**	*15*
R. batis	*10*	4	6	6	—	—	7
R. brachyura	—	**30**	**22**	**24**	—	*10*	4
R. circularis	3	4	—	—	—	—	—

V. Occurrence and Distribution.

Within the area under survey Clark (**1**, p. 581) gives the following general information regarding the distribution of the species.

"Numbers 1, 2, 3, 4, 6, 8, 9 (*vide* p. 10 *supra*) are of frequent occurrence in the neighbourhood, and are taken at all stages. Numbers 5, 7, 10, 11 are periodic in their appearance, but the young of 5 and 10 occur commonly on the outer grounds.

Numbers 7, 10, 11 increase in frequency with deeper water towards the western end of the Channel."

From the data set down in section IV it will be seen that *R. clavata* is the most abundant species among the total landings. It is, in fact, the most generally distributed species at all depths, and on all kinds of bottom, but

generally showing a decided preference for rough ground. Clark (**2**, p. 25) states its bathymetric distribution to be " shallow water to moderate depths," but gives no actual figures. At the western entrance to the English Channel it is abundant down to 80 fathoms, and appears not to diminish in still deeper waters, although definite data from greater depths in this particular region are not at present available. But that it is common down to over 100 fm. is shown by the results obtained during a trawling cruise in the *George Bligh* in August of last year. A series of nine trawl-hauls, each of four hours' duration, was taken roughly 80 miles N.N.W. of the " Bull " in various depths from 89 fm. down to 180 fm. As the ship was trawling specially for Hake, Rays did not figure largely in the catches, but some were present in every haul except one. Of the species taken, *R. clavata* was always most abundant down to roughly 100 fm. and was not absent in depths of from 160–180 fm. Beyond about 100 fm., however, *R. fullonica* became definitely more numerous and *R. clavata* less numerous in the catches (Table XI, p. 16).

R. brachyura is also fairly abundant in the Channel area but is practically absent from liners' catches, although numerous in trawl landings—especially those of steam trawlers. The reason for this is twofold.

(1) The main long-line fishing fleet which operates from Newlyn, fishes generally in from 60–80 fm. of water or even more. *R. brachyura*, however, according to Clark (**2**, p. 16) is confined to depths less than about 60 fm. This species has a decided preference also for sandy ground, such as is suitable for trawling, and therefore is taken by trawlers whose main fishing grounds lie in depths of under 60 fm.

(2) Liners can and do work on rough ground such as is favoured by *R. clavata* but not by *R. brachyura*, and avoid the softer trawling grounds on account of the danger to their lines. This also tends to prevent their taking *R. brachyura* in any numbers.

The periodic appearance of *R. undulata* and *R. microcellata* on the fish-market (Clark, **1**, p. 581) does not appear to be due to any periodicity in the movements or occurrence of the fish themselves. These two species are very restricted in their distribution, *R. undulata* being confined to a trawling ground 18–20 miles outside the Eddystone* and *R. microcellata* to a few sandy bays and estuaries. It is because of their very restricted distribution that those two species do not appear regularly in the landings. When the grounds on which they do occur are visited they seldom fail to appear in the catches.

R. nævus is most abundant in this area between 35–60 fm. No useful information can so far be added regarding the general distribution of the other species.

It has been found that unispecific shoals and unisexual shoals of one

* An occasional specimen may sometimes be taken off Start Point.

TABLE XI.

Serial number of haul.	Depth in Fathoms. (a) on shooting.	(b) on hauling.	(c) mean of a and b.	Rays present in Trawl. R. clavata.	R. fullonica.	R. nævus.	R. batis.	R. oxyrhynchus.
2	89	102	95½	5	—	—	—	—
1	120	89	104½	15	5	2	2	2
4	115	96	105½	45	15	3	2	1
3	102	115	108½	5	9	2	10	2
9	110	120	115	—	—	—	—	—
5	96	158	127	43	12	—	4	—
8	162	110	136	9	11	—	3	—
6	145	145	145	2	20	—	3	—
7	180	162	171	12	57	—	1	—

Extra haul off Black Rock—53° 40′ N. : 11° 20′ W.

						R. circularis.
17	137	124	130½*	7	26	4

* Trawl was fishing for part of the time in at least 150 fm.

species occur—as for instance is shown for *R. clavata* landed from the Ray net fishery at Plymouth (*vide* p. 22 *infra*). Liners' catches, too, occasionally furnish evidence of this segregation. On 23rd August, 1930, a liner which had been fishing 45 miles S.W.×W. of Carn Du Point near Mousehole, had an almost blank haul, catching only nine fish. All these nine were *R. fullonica* and *all females*.

On 3rd June, 1930, a small inshore trawler fishing near Newlyn brought in 205 Rays, 183 of which were *R. brachyura*. Of these 152 were male and 31 female.

There can be little doubt, therefore, that there do occur unispecific shoals which at times may be almost if not entirely unisexual.

Such separation of species, however, unless the shoal be very large and cover an extensive area, or if small, be alone in a fairly large area, is not noticeable as a general rule in trawl landings as there is no indication in the catch of the order in space and time in which the captured fish were taken. When emptied on deck the contents of the " cod end " are all thoroughly mixed up. Nevertheless, an almost completely unspecific and unisexual haul was made by a steam trawler in Mount's Bay in March, 1930 (*vide* p. 23 *infra*).

As a general rule, on most of the larger fishing grounds, although one species may predominate, several species are present. In those circumstances do the various species mix indiscriminately or do the members of each species tend to keep together ? An attempt to answer this question was made by taking an accurate census of every fish which came up in three hauls of a full fleet of long-lines ordinarily used by a Cornish long-liner. On the vessel in question the fleet consists of 24 baskets of line, each basket carrying roughly from 110–120 hooks about 11 feet apart.

If the various species are in separate shoals or groups there should be a tendency for the same species to appear more or less together on the same part of the line. Any such grouping will always tend to be obscured, of course, by the fact that the lines remain on the sea floor for anything up to six hours at a stretch. Therefore, even though at the outset there may be a definite distribution of the species along the line, other fish, or shoals of fish, will come along and take the hooks which have not previously been occupied. Thus the distribution of fish caught on the lines at any particular point of time unless they occupy adjacent hooks—which is unlikely —will be obscured by those which were hooked previously and by those which subsequently come along and are caught.

The possibility must also be recognised that purely as the result of chance, two or more fish of the same species may occur together here and there on the line though the population be entirely and indiscriminately mixed.

An examination of the distribution of fish along the above-mentioned

lines reveals a definite tendency for the same species to appear more or less together on the line. This grouping is too pronounced to be explained purely as the result of the laws of chance.

In Figure 4A is shown diagrammatically the catch of fish on a continuous section of line 1½ miles in length, hauled during the forenoon of 22nd July, 1931, from a fishing ground roughly 80 miles S.S.W. of Mousehole, near Newlyn, Cornwall. Each short vertical stroke denotes a hook which came up empty. A stroke produced downwards indicates a hook on which a fish of some kind was taken, while a stroke produced both downwards and upwards and ending above in a large dot denotes one on which *R. montagui* was taken. It will at once be seen that this fish shows a very definite grouping on this part of line. A similar grouping of *R. montagui* is shown in Figure 4B on a half-mile stretch of line hauled during the afternoon of 23rd July, 1931, on slightly different ground.

In Figure 5 is shown a continuous stretch of line 2½ miles long with the upwardly extended strokes denoting *R. nævus*, this being part of the same haul as the second one mentioned above. Here again a very definite grouping of the fish is seen. These results agree with other more general observations made at sea by the author when, however, a definite record of each hook and fish could not be made.

It appears, therefore, that, on the sea floor, where various species of Raia are present within a limited area at the same time, the species do not mix indiscriminately but segregate into unispecific groups or shoals.

VI. Migrations.

As yet little is known regarding the migrations and shoaling habits of the Raiidæ. What little information there is on record applies mainly to *Raia clavata*, perhaps because its movements are more marked than those of the other species or perhaps because it is the most generally distributed and most abundant Ray in inshore shallow waters and at moderate depths down to at least 80 fm. Meek (**5**, p. 41) states definitely that there is, in this species, a periodical migration inshore in summer and into deeper water for the winter. Unfortunately he gives no actual data as to depths. At the western entrance to the Channel, there is little evidence of such a wholesale inshore migration in summer at any rate within the area inside the 80 fm. line. The long-liners, which are responsible for over 50% of the total Ray landings in Devon and Cornwall, fish throughout the summer months only, mainly in depths of from 60–80 fm., and the present tendency is for the boats to go still farther offshore into ever deeper water in order to maintain the level of their catches.

Certain conditions described by Murie (**6**, p. 166), however, for the Thames Estuary are borne out by observation in this area. According to

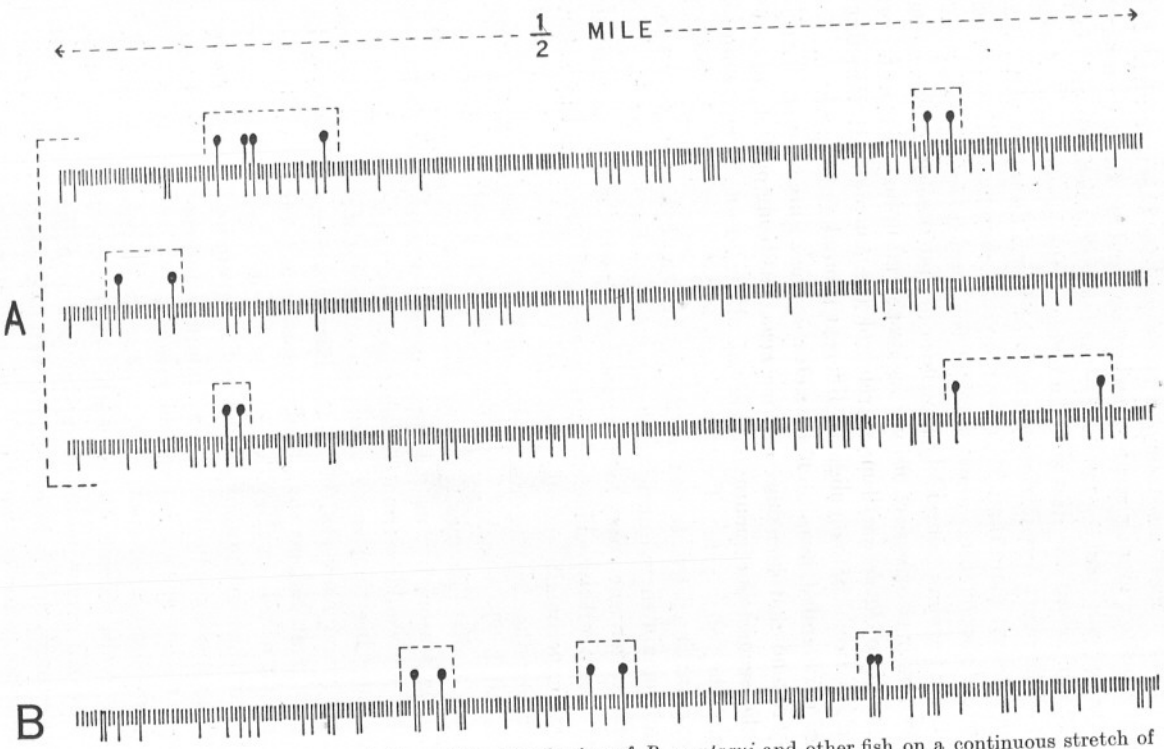

FIG. 4.—A. Diagrammatic representation of the distribution of *R. montagui* and other fish on a continuous stretch of 1½ miles of long-line—22nd July, 1931. For explanation see text, p. 18.
B. Distribution of *R. montagui* on ½ mile of long-line, 23rd July, 1931.

this author the Thornback is captured in shallow water almost at all seasons, especially during its early stages.

Examination of steam trawlers' fish landed on Plymouth market indicates clearly that this statement holds good for this area also. It is the usual custom among steam trawlers on landing Rays to separate them into " small " and " large " fish—the former consisting of fish under about 40 cm. across the disc and the latter of the larger ones. Among their small fish very few *R. clavata* are to be found at any season, giving an average of not more than 5% over the year. Among their larger fish, however, *R. clavata* forms about 33% of the total (*vide* Table X, p. 14).

The steam trawlers fish mainly in water of from 40 to 60 fm. in depth. The Ray landings of vessels fishing inside the 30-fathom line, however, consist mainly of *R. clavata*, the majority of which are of small and medium size, such as would be included in the " smalls " of steam trawlers.

Of 943 fish landed from shallow water by the research vessel *Salpa* during 1930–31 and identified and measured, 83% were *R. clavata*. Of these, 82% consisted of young individuals under 40 cm. in width across the disc.

The explanation seems to be that the young fish are hatched in shallow water and remain there during their early stages of growth, moving out into deeper water when they have reached a size of 40 cm. or over across the disc. Corroborative evidence on this point is being sought by means of marking experiments.

Migrations, either feeding or spawning, or both, do also occur, however, among the adult Thornbacks. Murie (**6**, p. 167) states that, " when long-lining in the Wallet (Thames Estuary), Rokers were few at the beginning of the fishing season, but as the sprats came about so did the Rokers multiply. They would be from 18 inches to 2 feet wide, length in proportion, and more big than small ones. As an instance of a good catch, some thirty years back (1870?) in the Barrow Deep one morning, on 28 lines, 190 great Rokers were hooked, besides several lines being lost through weight of fish on them."

It is evident that, in the above-mentioned areas of the Thames Estuary, the adult Thornbacks appear for a time in large numbers probably feeding on sprats—i.e. they show a feeding migration.

A strikingly similar inshore migration of adult Thornbacks takes place every spring in the vicinity of Plymouth. Each year, usually about the middle or end of January, while herrings are still abundant, large numbers of *R. clavata* congregate in fairly shallow water—inside the 22-fm. line—around the shore to the eastward of Plymouth Sound from Yealm Point to Bigbury Bay, and give rise to the set-net fishery already mentioned.

The landings from this net fishery present certain features of great interest. The first fish to arrive (as shown by the landings—Table XII)

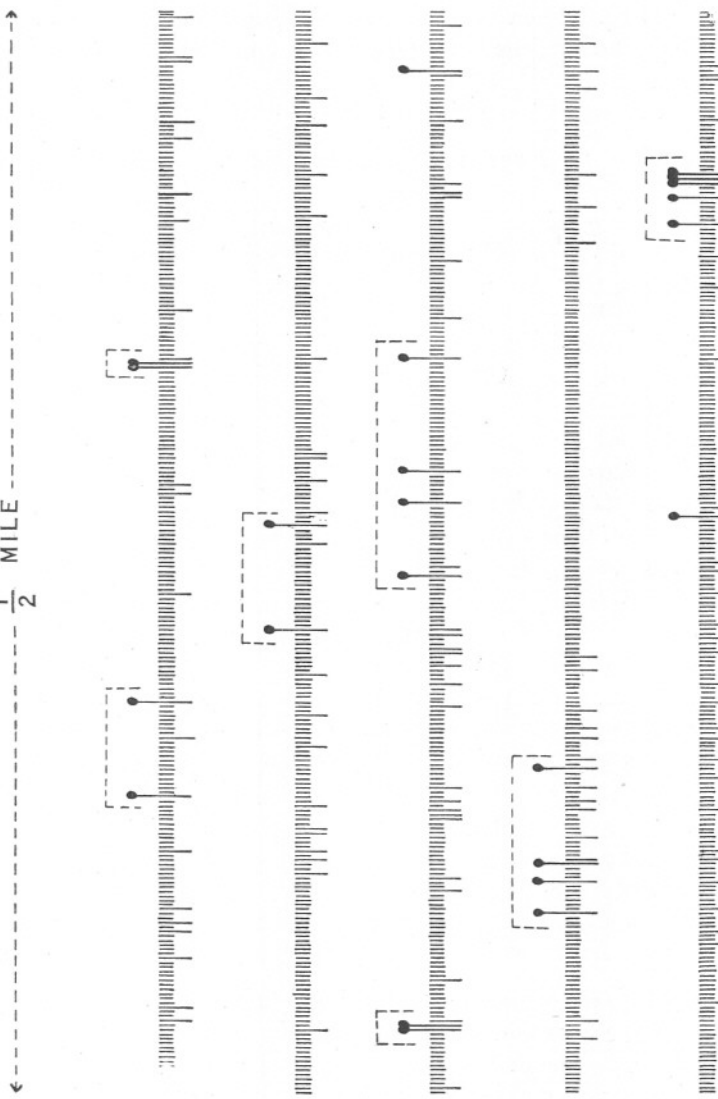

FIG. 5.—Distribution of *R. naevus* on a continuous stretch of 2½ miles of long-line—23rd July, 1931.

are almost entirely females—all fully grown gravid fish nearly, but not quite, ready to deposit their eggs. In a few weeks, adult male fish begin to appear in increasing numbers and finally landings may consist almost entirely of adult males, the females having departed and the males having taken their place. When this happens, the fishery is nearly at an end. These male fish do not remain long behind the females which have already left the area.

TABLE XII.

Landings from Ray Nets.

	1930.					
	R. clavata.		*R. brachyura.*		*R. batis.*	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
February						
20	10	192	—	—	—	—
21	10	198	—	—	2	—
24	34	199	—	—	3	3
March						
4	1	33	2	—	2	4
8	20	44				
10	2	47	6	—	—	—
11	67	37	—	—	—	—
12	28	41	—	—	—	—
15	38	—	1	—	—	—
January			1931.*			
12	2	159	1	—	—	—
13	—	121	1	1	—	—
16	2	49	—	—	—	—
19	2	198	3	—	—	—
20	5	156	1	—	—	—
22	1	75	2	—	—	—
23	17	123	2	—	—	—
February						
3	2	77	12	3		
4	—	121	2	2		

All the fish move off before " spawning " takes place. Although the females landed all contain almost ripe ova, in scarcely any of them are egg capsules to be found.

This congregation of Thornback Rays around the shore from Yealm Point to Bigbury Bay, upon which the Plymouth Ray net fishery depends, is therefore an inshore feeding migration of mature adult fish (*Vide* pp. 23 and 24).

* Owing to stormy weather the fishery this year came to a premature close.

There is evidence of similar brief inshore migrations in early spring at other points along the south Cornwall coast. In March, 1930, a steam trawler after fishing with little success for a week " off the Wolf " moved into Mount's Bay. There, in 25–30 fm. of water, on the night of Saturday–Sunday, 15th–16th March, between the hours of 7 p.m. and 2 a.m. she caught 210 large Rays, 207 of them being *R. clavata*,* of which every one, without exception, was a large female. Another trawler, fishing at the same time just outside in 45–50 fm., had the usual mixed trawl catch.

Certain fishermen say that these large Rays appear every year off Falmouth, but the trawlers cannot always get at them because they go too far in. Unfortunately, there is no inshore Ray net fishery there from which to obtain corroborative evidence. Nor is there such a fishery at any point around the coasts of Devon and Cornwall, except at Plymouth.

Evidence of a somewhat similar migratory movement on the part of *R. brachyura* is furnished by the catches of a small motor liner fishing from Newlyn. This vessel, being unable to go as far to sea as the regular fleet of larger liners working from the port, normally fishes on a small sand-bank close inshore.

On 4th June, 1930, this small liner landed 853 Rays, consisting of 824 *R. brachyura* (and 29 *R. montagui*), not a single fish being more than 50 cm. across the disc. In August of the same year, this vessel, fishing on the same bank with the same gear, was bringing in catches consisting again almost entirely of *R. brachyura*, and all 65–75 cm. in width across the disc.

As it does not seem possible for a growth of 15–25 cm. to have taken place in two months, it must be assumed that the large fish had migrated to the bank from elsewhere and that the smaller fish had moved away. Unfortunately, no observations on the landings at Newlyn were possible in the period between June, when the immature fish were being landed, and August, when the large mature fish had taken their place.

VII. Food and Feeding.

As is already well known, young Rays feed very largely upon small crustaceans, especially Amphipods and Crangonids (Clark, **1**, p. 635). In the vicinity of Plymouth the Amphipod *Ampelisca spinipes* is of primary importance, being present in large numbers on certain grounds (Steven, **6**, p. 681). As the fish increase in size they turn their attention to larger crustacea such as Upogebia, Portunus, and Corystes, and—in certain species at least—to fish.

Adult Thornbacks, however, sometimes feed entirely on fish. The

* The three others were large male *R. brachyura*. It is interesting to note that, in nearly every catch of *female R. clavata* from the Ray nets there is also nearly always present one or two *male R. brachyura*.

large Rays of this species taken by the nets already mentioned (p. 20) were in both 1930 and 1931 found to be feeding exclusively on herrings and sprats. Of several hundreds of stomachs examined, not one was found to contain anything but fish, mainly herring (sometimes as many as six in one stomach), and not more than half a dozen empty stomachs were encountered. Several large *R. brachyura* and a few large *R. batis* taken in the same locality also had their stomachs full of herrings. One of the latter, a female measuring 143 cm. across the disc, contained no less than nine large fish.

Other fish, commonly including Rays, also enter largely into the diet of adult *Raia batis*.* Of 41 stomachs of these fishes ranging from 89 cm. upwards in width of disc, examined on and between 25th and 30th July last year, 13 contained one or more Raia sp. Those specimens of which the species could be determined consisted of *R. nævus* and *R. montagui*, with one doubtful *R. clavata* among them. The full results of the examination of the stomachs are tabulated below.

FOOD OF SKATES (*R. batis*).

25th July, 1930.

Serial No. of Fish.	Width across disc (in cm.).	Food in Stomach.
1	101	*Raia nævus*
2	94	*Scyllium canicula* *Homarus vulgaris*
3	124	Empty
4	145	*Lophius piscatorius*
5	100	Raia sp.
6	108	*Acanthias vulgaris*
7	129	Empty
8	125	*Eledone cirrosa*
9	93	*Raia nævus*†
10	90	*Eledone cirrosa*
11	105	*Pleuronectes limanda*
12	98	*Lophius piscatorius.*
13	110	Empty
14	99	*Raia nævus*
15	91	Raia sp. (? clavata)
16	97	Empty
17	102	*Caranx trachurus*
18	100	Raia sp. remains

* See also Murie, **6**, p. 165.

† This fish had been caught on one of the hooks of a long-line. It was then swallowed by a Skate which was itself also caught on the same hook.

Serial No. of Fish.	Width across disc (in cm.).	Food in Stomach.
19	104	Empty
20	98	Empty
21	96	*Acanthias vulgaris*
22	95	Empty
23	109	*Cancer pagurus* *Eledone cirrosa*
7th July, 1931.		
24	116	*Acanthias vulgaris*
25	104	*R. nævus*
26	123	*R. montagui*
27	119	Empty
30th July, 1931.		
28	121	*Cancer pagurus*
29	104	*R. montagui*
30	98	*Cancer pagurus*
31	89	*Eledone cirrosa*
32	96	*Acanthias vulgaris*
33	125	Empty
34	139	Empty
35	146	Empty
36	144	*Raia nævus* *Trigla cuculus*
37	142	*Clupea pilchardus* *Cancer pagurus* *Pleuronectes microcephalus*
38	119	Empty
39	126	*R. nævus*
40	98	*R. montagui*
41	94	*Cancer pagurus* Raia sp. remains

Nine large *Raia marginata*, 90–135 cm. in width across the disc, examined at the same time, were all found to have empty stomachs. The food of six *R. oxyrhynchus*, 83–114 cm. across the disc, included *Cancer pagurus*, *Atelecyclus septemdentatus*, *Corystes cassivelaunus*, other crustacean remains, Trigla sp. and *Callionymus lyra*.

Of the foraging habits of the Raiidæ little is known. It is nevertheless certain that they depend upon " scent "—or at any rate on some sense other than sight—for the finding and recognition of their food or prey. For in long-line fishing, where the catch depends upon the fish finding and

taking the bait, there is no difference at all in the magnitude of day and night hauls. Neither the brightest day nor the darkest night appreciably affects the catches of Ray. They thus differ markedly from Turbot which, being sight feeders, are seldom caught in any number on lines during the night, but are readily taken by day (*vide* appendix, pp. 28–33 *infra*).

VIII. The Toll of the Fishery.

Further work on the Rays and Skates of the Channel Area is proceeding with a view to ascertaining, if possible, their growth-rate and of discovering some method of age determination. Only when more precise information is forthcoming on these points can any definite estimate be made of the toll of the fishery. It seems worth while, nevertheless, to mention here certain facts concerning the fishery which appear to point very definitely to a possible depletion of the stock if the present intensity of fishing continues.

The statistics at present available show an alarming decline in the total British catches of Rays and Skates from the English Channel as a whole during the last five years, the total landings from regions VII d and e (Channel) in the years 1926–1930 being 64,061 cwt., 57,344 cwt., 54,238 cwt., 50,771 cwt., and 45,037 cwt. respectively. Certain factors external to the fishery itself have, in some years at least, helped to cause this decline. But that these figures reflect a real change in the available stock of fish is indicated by events and conditions in the Cornish long-line fishery, according to the following statement by the fishermen concerning the number of hooks used and the grounds fished.

Whereas in pre-war and the immediate post-war years the Cornish long-line fleet working from Newlyn used on an average from 1000 to 1500 hooks per vessel on from $2\frac{1}{2}$ to 3 miles of lines, they now shoot from 2000 to 3000 hooks on from 5 to 7 miles of lines in order to capture approximately the same amount of fish. Moreover, instead of fishing as a rule within a radius of 50 miles from port, they are now obliged, in spite of the inadequacy of their vessels, to seek grounds up to 90 or even 100 miles distant.

As increase in the amount of gear that can be used has now attained its maximum for the type of vessel at present employed in the line fishery, and as there is a limit (now reached) to the distance from port at which they can profitably and safely work, it would appear that the landings must in future decline. It is doubtful whether the use of larger vessels capable of working more gear and of going farther away from port would arrest the possibility of this decline more than temporarily.

It seems sufficiently interesting and important finally to mention that, in line fishing, there is a complete absence of any destruction of non-

marketable small fish.* Should the question ever arise in a restricted area of devising means for the preservation of a Ray fishery, a useful preliminary step would be to consider the possibility of substituting lining for other more destructive methods of fishing.

IX. Acknowledgments.

In carrying out these investigations I have received assistance from so many persons—fishermen, salesmen, buyers, and others—that it is impossible for me here to mention them all individually. Without their aid I could not have worked. To all those, therefore, to whom I am indebted in any way for help and advice I gladly extend my thanks. I am especially grateful to Messrs. Howard and Ben Dunn for much assistance generously given throughout the whole course of the investigations.

I am also under particular obligation to Mr. E. Ford for many valuable suggestions, and for reading the manuscript before it was submitted for publication.

X. SUMMARY.

1. Until the beginning of the present century there was little demand in this country for Rays and Skates. This fishery is now of major importance both nationally and within the Devon and Cornwall area.

2. Of the eleven species of Raia present in the western area of the Channel, *R. clavata* makes the greatest numerical contribution (37%) to the total landings in Devon and Cornwall. The composition of the catches obtained by different methods of fishing varies greatly.

3. *R. clavata* is the most widely distributed species in the Channel area at all depths and on all kinds of sea bottom.

4. In a series of trawl hauls off the west coast of Ireland *R. clavata* was most numerous in the catches down to about 100 fm. From that depth down to about 170 fm. (the greatest depth fished) *R. fullonica* was most numerous.

5. Unispecific and even unisexual shoals of at least three species of Raia—*R. clavata*, *R. brachyura*, *R. fullonica*—occur.

6. When more than one species of Raia is present within the same area at the same time, the members of the different species have been found not to mix indiscriminately.

7. *R. clavata* appears to hatch out in shallow water close inshore and gradually move seawards into deeper water as it grows.

* The smallest Ray taken in the three shots recorded on pp. 30–33 (appendix) was a *R. nævus* 33 cm. (about 13 inches) in width across the disc.

8. Adult *R. clavata* show definite migratory movements, though the full extent of their wanderings is not yet known. There is an inshore migration in early spring of adult fish to a small part of the coast near Plymouth. The first fish to appear are females, males appearing later.

9. There is evidence of somewhat similar migratory movements by *R. brachyura*.

10. *R. clavata*—and possibly also *R. brachyura* and *R. batis*—at times may feed almost entirely on Herrings.

11. Large *R. batis* feed to no inconsiderable extent on other species of Raia.

12. In foraging for their food Rays and Skates depend upon some sense other than sight.

13. There has been in recent years a steady decline in the landings of Rays and Skates from the English Channel—probably due to depletion of the available stock of fish.

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XII. APPENDIX.

Below are shown in detail the complete catches of fish, and their distribution on the hooks, taken in three hauls of long-line worked from a Newlyn (Cornwall) motor liner on 22nd and 23rd July, 1931. The

interval between adjacent snoods, each of which carried a single hook, was approximately eleven feet.

The various species of fish captured are denoted by the following

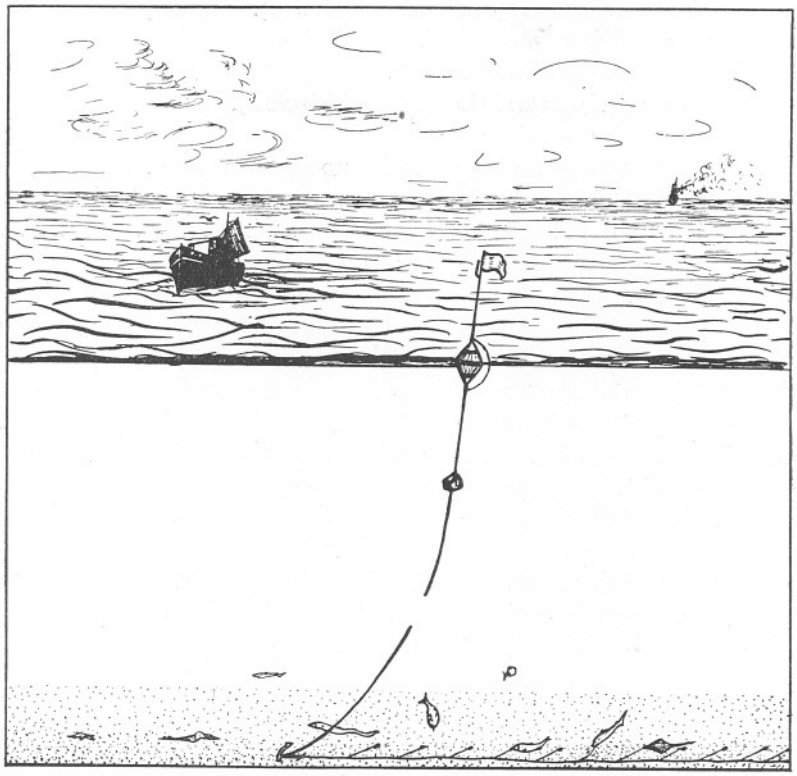

FIG. 6.—Diagram of a Cornish motor liner, and a portion of its long-line which has just been shot. The complete line carries approximately 2600 hooks and extends for a distance of between 5 and 6 miles along the sea floor. For further explanation, see text, p. 30.

symbols in which all *Rays* are indicated by letters and other species by numbers.

C = *Raia clavata* (Thornback Ray).
M = *Raia montagui* (Spotted Smoothback Ray).
N = *Raia nævus* (Cuckoo Ray).
R = *Raia circularis* (Sand Ray).
F = *Raia fullonica* (Shagreen Ray ; Owl Ray).
B = *Raia batis* (Common Skate).

1 = *Acanthias vulgaris* (Spur Dogfish).
2 = *Scyllium canicula* (Rough Dogfish).
3 = *Scyllium catulus* (Nursehound).

4 = *Rhombus maximus* (Turbot).
5 = *Conger vulgaris* (Conger Eel).
6 = *Molva vulgaris* (Ling).
7 = *Trigla gurnardus* (Grey Gurnard).
8 = *Trigla lyra* (Piper).
9 = *Gadus morrhua* (Cod).
0 = *Luidia sarsi* (Long-armed Starfish).

Every dot represents a hook on which no fish was taken.

A record of the blank hooks and fishes caught on the portion of line shown in Figure 6, p. 29, would read as follows: . . . 9 . 1 F . . . .

Here and there along the length of a line small sections frequently become tangled. Such tangled portions have not been recorded in the present census as the hooks which they carry are put entirely out of action as far as their fishing capacity is concerned.

Two of the three shots recorded below are day shots, while one—the second—is a night shot. It will be noticed that Turbot (indicated by the figure 4) are present in both the day catches but that none were taken during the night. Rays and Dogfish are abundant in all three catches.

NOTE.—The first catch here recorded was not considered satisfactory by the fishermen on account of the scarcity of *Raia clavata* (Thornback)—the most remunerative species which is mainly sought. They therefore moved from their first position before shooting the lines a second time.

First Shot. 80 miles S.S.W. of Mousehole (Cornwall). Began to shoot lines 4.30 p.m.; finished 6.5 p.m. Shot S.W. (24 baskets). Began hauling 8.30 p.m.; finished 2.15 a.m.. 22nd–23rd July, 1931.

.....2..1.....1 F .....1...M
..... M . M ..1.. F ...6...1. M ....1.....1.....4..... C ....
.....1..1.1.....1.1 0.1.....1 1 2 2.....
.....1.....1 1 1....0.1....1. N ..1.0. M .1.. M ...1....1...1 1
...1. N .0.....1.....4..... N . N . M .....1.. M ...
..... R .1..1 .. N ..... 1 ..... F .....1... N
.....N.1....1.1.....1.....1 ..1 .....1 .....
.....N .....1.1.....C1...1..1 N .....
.....1..1..1..... N .....1..... N .....
C R .1.1.. M .4 M . N .....1 1.....1 1.....1....1.....
1 1.1.....1..0... N ..1....1.....1..1.....
.....4. N .1.1....1....1 1. C ..1 1..1...1.....1 1..1.1... M .....
..... B .....1 1 6....1.. M .1..1..... N . N .... 1 .. 1 C ..1
..... M .....1.....1 F ....1.....1....1.....1....1..
.....1.....1...0.0.....1.....1.....1.....40...

. . . . . B . . 0 1 . . M . . . . . 1 . . . . . 1 0 . C 1 F . . . 1 . . . . . 1 . 1 . 1 1 . . . . 1 1 1 . . 0 . . . . . 1 . B . 1 . . N . . . . . 1 1 1 1 . . 1 . . 1 . . . N . . . . B C . . . . C . . . . . N . . . . . R . . . . 1 1 . . . . . M . . . . . 1 . 1 . C 1 1 1 . . . . N . . . . . 1 1 . . . . . C . . . . . 1 N 1 . . . . N . . . . . 1 . . . N . . C . . . . . 1 . . . . . 1 . . . . . 1 1 . . 1 . . 1 1 . . . . . N . . . . N . . . . . 1 . . 1 . . . . . 1 . . . . . F . M 1 1 . . . . . 1 . . M . C 0 . 1 . . . . . 1 . . . . C . . . 1 . . C . N . 1 C . . . . . 1 . . . 1 . . . . . C N . 0 . . . . 1 . . . F . . M 1 . 1 . . 1 . . . . 1 . 1 . N . 1 . 1 . . . . . 1 C . 1 . . M . 1 . M . 1 . . . 1 . 1 . . . . . 1 . . . . . 1 . N . . . . . N . . 1 . 1 . 1 . . . . . 1 . M . 1 . . . . . 1 . 1 . . 1 . N . . 1 . . . M . . . . R N 1 . . . . N . 1 . . . . . 1 . 2 . . . . . 2 N . . . 1 . . 1 . . . . . M . . . . . 1 . . . . . 1 . . . . . N . . . . 1 . . 1 . N . 1 N 1 . M . . . . . 1 . 1 N . 1 N . . . . N . 1 . 1 . . . . . 1 . . . . 1 . . . . . 1 . . . . . 0 1 1 1 . . . . . N . . . 1 1 . . . . . 1 . . 1 . . 1 1 C . . N . 1 . . . . 1 . 1 . . . . . 1 . . 1 . . . . 1 . . F . . . . 1 . . 1 . . 1 . . . . F . . 1 . 1 . . . . N . . . . . 1 . 1 . . N . . . . . F . . . . 1 . . . . . 1 . R 1 . . 1 . . . . . 1 . 0 . . . . . 1 4 B . . . 1 . . 2 . 1 . . . 1 . . 1 . N . . 2 . . . . . 1 1 1 . . 1 . . . . . 1 . . . . . 1 . . 1 . 1 . 1 1 M . . . . . N . . 1 . 1 . . . . . 1 . 1 . 1 6 . . . . . 1 . 1 1 . C . . C . . . . . 1 . . 1 1 . . 1 . . 1 . . 2 . . . . . 1 . N . 2 . . . B . . . . C . . 1 . N . . . . . C . 1 . 1 . . . . . 1 M . . 1 . . . . . 1 . . . . . C . . . . . . 1 . 1 . . 1 . . . . . 1 . 1 . 1 . . . . . 1 . . . . . 0 . . . 1 . . . . 1 . . . 1 . . . . C 0 6 . . . . 1 . . . . . 1 . 1 . . . 1 . . . 1 . . . . . M . 1 . . . . . N . 1 . . . . . 1 1 . . . 1 . . . . . . 1 1 . . . 1 . . . . . N . . . . 1 . . . . . 1 . . M . 1 . 1 . . N . . . . . 1 . 1 1 1 . . 1 . . . . . 1 . 1 . . . . . 1 . . . . . F . . . R 1 1 1 . M . . . . 1 . . . 1 . M . 6 . . . . 1 0 1 . . . . . 1 . 1 . . . . . R . . . . 1 . . . . 2 . . N . . . . . 1 . . . 1 . . 1 . 1 . F R 1 . . 2 . . . . . 0 . . . . B . R . . 1 . . . . . B . . . . F . R . . . . . 1 . . . . . F . . . 1 1 1 . . . . 1 1 . . . 1 1 . 1 . . . . 1 . . . . . 1 . . 1 C 1 . . . 1 . F . . R 1 . . 1 . . 1 . . M N . . . . 1 . . . . . 1 . N . . . . 1 1 1 . 1 . . . . 2 1 B . . . . . M M . . . . . C . . . . 2 . . . . 1 . . . . . 1 1 . . . . 1 . . 1 . . . . . C l . 1 . . . . 1 . . . . 1 . . . 1 1 . 1 . . . . . 1 . . . 1 . . . . . 1 . . . . . .

Second Shot. " Steamed " 8 miles S.S.W. from previous berth. Began shooting 3.30 a.m.; finished 4.50 a.m. Shot S.W. (24 baskets). Began hauling 6.15 a.m.; finished 11.10 a.m. 23rd July, 1931.

. . . . . N C . 1 . . . N . . . R . . . . . 1 6 . . . . . 1 . . . . 1 N . . . . . 6 . . 1 . . . . . N . . . . R . . . . . 1 1 . 1 . . 1 . . . . R . . . C . . . . . N . 1 . . . . . 1 1 . . . . . 1 . . . . . M . . . . . N . C . 1 . . . . C . . . . . 0 1 . . . . . M C . . . . . C N . . . . . C . . . M . . . . 1 1 . . . . . 1 . . . . . C . . . . . 1 . . . . . 1 . N . . . 1 . . . . . C . . M . 1 . M . . . . . 1 . . 6 . . . . . N 1 . 1 . 1 . . M . . . . M . . . . . 2 . . . . 1 . . . 5 . . . . . 1 . 2 . . . . . 1 . N . . . . . 1 1 . . . . 1 . . . .

1 C .1.....1... C .. R ..... F C .....N 1.11.....6.....
1.....5..... C ..... C ..... 1 .... N 1
.. C . C ..... C C 1....1.. C .1....1.1... C ...1.. C .. N .....
... C ..... C .5.. C . M . B 1..... C ..6.....6....5.6....6.6...
..6 6..5..5 5..1 C C ..1..... 6 .....1.6 C B .. C ...1..1
...6.....1 C C .6.5..... N . C 5..M .....C .1
.....1.1.....6..... R ..... C .....2..21.....
.....1..1.0..... N . N ... C 1. C ... B B 2.1..2.5 1..9 N .. N .....
..... C ....2.1.1...1 5.. N . M 1 2 1.....5 1.. C ...1 1. N ....
N C .....1 1 .... C ....1 1 .....1 0..... C .....1..... C
..... C ..... M ..0.6 M 1.....2.... C 1 .....1. 1 .....6... 1 1
.....1 N N ....6.....1 N ..2.1.1.....1 0....3.....111....1...
.....1....1.1.....0. C ..1.....B .1 1..2C0.2.1.
C ..... 2 .....1.. F 1 6...1.. C 1 .... C .2... C .... C.
B .. C .1..1....1.....1 0.....1....1.....1 C ... N 1
.....1 1..... M ..... N ..1..1 1. N ..... C ...C 1 N 1 2..C ...
C ...1.1 C .....2.1..... C ..... M ..... C ..2 C..
C .....5....0...2 C C .. B ..2....1.C...6..... N .....
1.. C ..... C .....
.....1.....6..... C .....2 F M .....6..
5 1... C .. N 6.. C ..6 C .1..... C ..... B .. N 2.. C ..
.....1 1.....1..... C 6...1 1 3.....
..... C 1..... C .... C . B ..... C ..... C .....
.....6. C ...1 F .....2 1.....1
.....1.6....0..1.....1.....1....1. C .....1..... C F ...
..1..... C .....1...6..... C ..... B ....1.... C . N 1
F....1 N C 1 1...1.0.....1.....7.1..N .....1.1.....
C .....1.. C ...2..... N .....1.. C . 3 .... C . M ....
...1 N .....0. B N ..... C 2.....6.....1
... C ..... C 1..1.....1..1.1 1... C M .... C .1. C ..... N
1 B... B . N ..... N ...1 1.....1. C .....1 1.1.1 0 1.....
..... C . C . C .....1..6... N . F ....1.....1..... M C
..... M 1 1.1.....1.....1 B . N F .6 6..1 M .1...1..... M ...
.....2..... N ...1 1.1... B .....1.....0. C 1.....
... N .1..... N ....2..... C .1..1.....1 1.. N ..... C . N

Third Shot. Same berth as No. 2. Began shooting 11.20 a.m.; finished 12.15 p.m. Shot N.E. (18 baskets only). Began hauling 2.00 p.m.; finished 6.15 p.m. 23rd July, 1931.

...1.....4....4.....4.....4.....4.....
..4.....0.....4..4.....4.....4. F
.. C .....M ..... N .....1.....N .....

.....0.....4. C .....
N N C ..... M .....0.....4.....4...0...
.....0 4.....1.....4.....
.....0.. M .1... F 1.... M .....1.....4.....1.....
... N ..... F ... N .. M .....1.... C 4.....
.....1..... M .....1.....1...
..0.....5.....2..... N N 0.....1.....
.....4.. C .....4 4 0. F ..0..... M ....4...1..
M 0..0 4..... N .1..... N ..... N .....
..... N .....1.....1..... R M .1.....
1.....1 N .....0.....1...1.....
... N ..... N ... N 8....1.....6.2..1... N .1....1.1...0.....
F .. C .....
.....2.....1.....2.....
... C ...1..... M ..... M .....1..1
....1.....0.... C . C ..... C .....1... F ....1.0.....1...C....
..1.. C .. M .....1.... C .1..... N . C .....
.....1.....1.....0..... N ..... N .. N N N .....
.....1..... C F .0.....1.....4. C .1..... C ..
.....1..... C ..... C ..... C....1.....
1. C C N .4..... C 1.. N ....1.....1...1... N .....1 C ..4..
1 1...1..1....4.. N C C C . C ..1 1... C ..0 C 1...1. C.4 N ..... C...
M ..... C 1. C . C .. C ..... C ..... N ..... F M 1.1 0 1.....
..1.1.....1.....1 C .....1.....1....1.1....0.....
.....1..1....1.....11...0.....11..1....1.....1.....
1..1. C .1.....0.1...1 1....1 N ..... M 1...1 M ... N 4
.....1.....1. C ....2.... M ..1... M .....1.....1.C...
1.....1 ..1 C 0....4 1..... M 4 M ..... C ...1 0 C.... F ....
....1 1.0.... C..1.....1.....4 1.1.....

The Bacterial Flora of the Slime and Intestinal Contents of the Haddock (*Gadus aeglefinus*).

By

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THE surface of most fish is covered with a coating of slime which increases in amount after death when the fish is held at room temperature or stowed in ice. The slime has been examined by Dr. Ingvaldsen of the Prince Rupert Station who has found that 3 c.c. of this material contain 33·348 mg. of nitrogen of which 11·9 per cent is amino-nitrogen. It is poor in carbohydrates, but along with the mineral salts contained in sea-water it appears to provide an excellent medium for bacterial growth. In the fresh fish, the slime is clear, of mucous consistency, and shows comparatively few organisms in stained films, but in fish which are not quite fresh the slime appears to be increased in amount, is opaque and increased in consistency. With increasing age the slime becomes yellowish and evil-smelling, and stained films show an enormous increase of bacteria. In the early stages of the work attempts were made to correlate the number of organisms in the slime with the age of the fish by making bacterial counts, but owing to the irregular distribution of the slime over the surface of the fish and the difficulty of making homogeneous suspensions the results were not very satisfactory, although gross changes were readily detected. A survey of the literature on the subject of fish bacteriology revealed little knowledge of the nature of the organisms usually present on the surface of freshly caught fish, and it seemed desirable to examine this question in detail as a preliminary to work on the problems of spoilage. The results which are presented in this paper are the records of a series of systematic observations upon the nature of the organisms found in the surface slime and intestinal contents of one species of freshly caught salt-water fish. The haddock was chosen as the most suitable fish for investigation because it could be obtained with regularity throughout the year. The experimental work was carried out at the Torry Research Station, Aberdeen, of the Department of Scientific and Industrial Research, and under the personal supervision of Professor J. Cruickshank, Department of Bacteriology, Marischal College, Aberdeen.

Twenty-two fish have been examined in detail for the nature of the

surface flora. Most of the fish were line caught in the inshore waters off the coast of Aberdeen, but in a few cases trawled fish from deeper waters were used. On these occasions a fish from the top of the trawl net which had presumably not come into contact with contaminated surfaces, e.g. the deck of the boat, was selected. The fish was removed from the hook or from the trawl net with the minimum of handling and transferred to a sterile tin box which was not opened until delivered in the laboratory. In all cases the time which elapsed between the catching of the fish and the arrival of the fish at the laboratory did not exceed six hours.

In a few cases samples of slime were taken by rubbing a sterile cotton-wool swab over the surface of the fish at the time of catching. The swab was replaced in its sterile glass container and conveyed to the laboratory. This method was designed to avoid contamination of the surface of the fish by the fisherman's hands. The same fisherman practised both methods and there was no appreciable difference in the results. For the majority of the isolations hormone agar made from horse heart or ox muscle was used. An agar made from fish muscle was employed on several occasions, but it was more troublesome to prepare and did not produce better or more luxuriant growth, and its use was discontinued.

On arrival at the laboratory a sample of the surface slime was taken on a sterile platinum loop and the material plated out on a series of plates. These were incubated aerobically, at first both at 22° C. and 37° C., but as growth at the higher temperature was always scarce, the lower temperature only was used in subsequent isolations. Anaerobic cultures were not made.

For the examination of the intestinal contents twelve additional fish were used. The ventral surface of the fish was sterilised by the application of a germicidal dye mixture (0·5% crystal violet and 0·5% brilliant green in 50% alcohol), or by flaming with alcohol, and an incision was made along the middle line with sterile instruments. A loop of intestine containing undigested material was cut out and placed in a sterile Petri dish ; upper or lower intestine was taken indiscriminately. The intestine was opened longitudinally with a sterile scalpel and a sample of the contents withdrawn with a platinum loop. Owing to the small number of organisms present in the contents of the intestine of fish, as compared with mammals, it was unnecessary to make dilutions of the material for primary plating and often considerable masses of the contents were spread in order to obtain good growth. Incubations were made aerobically at 22° C.

In a few cases where the intestine was empty and the mucus only was plated out, little or no growth was obtained and no isolations were made. Even where undigested food material was present, the number of organisms was always strikingly less than in mammalian intestine.

After twenty-four hours' incubation little or no growth was apparent on plates inoculated with slime or intestinal content and it was only after an interval of three days or more that colonial differences were sufficiently marked to allow of the isolation of different types. In a series of preliminary investigations it was found that air organisms and moulds were frequent contaminations on plates which were incubated for long periods, and in the subsequent work all plates were prepared and inoculated in a deep glass box, open on one side only, which was thoroughly sterilised before use. It was found generally that a platinum loopful of slime spread over three plates gave abundant growth and that as a rule well-separated colonies were obtainable from the second or third plates of the series. Subcultures of colonies were made on agar slopes from the various types and after a considerable period of incubation these were carefully examined for purity. It was frequently necessary to replate the cultures as they were often found to be contaminated with slow-growing types.

The morphological characters of the organisms were examined from agar slopes and from broth cultures and the appearances on agar and potato and in broth and gelatin were noted. Records were made of their behaviour in various carbohydrates and in litmus milk, and of their power to produce indole and acetyl-methyl-carbinol and to reduce nitrate. In the study of certain of these cultural characters, long periods (up to ten weeks) were given before the results were noted.

In addition to the information gleaned from the literature, referred to later, Bergey's *Manual of Determinative Bacteriology* (**1**) was found to be useful as a standard of reference, and the terminology employed in this paper is more or less that used by him. The identification of these organisms with types already described has been found to be difficult or impossible, partly owing to the inadequate descriptions given in the literature of many water, sea-water, and soil organisms, and partly owing to the absence of distinguishing features or reactions in many of the organisms encountered.

A notable feature of the plates made from slime was the variation in the number of colonies of chromogenic organisms. In some cases these were few or absent, in others they constituted a considerable percentage of the total number of colonies. Orange or lemon-yellow colonies were most common, but variations from a pale flesh to brown colour were seen. It was found in the course of the subsequent examination of these chromogenic organisms that little or no dependence could be placed on the intensity of the pigment as the colour varied markedly in any one type with age and with the nature of the medium. Colonies of a pure culture might show all degrees of variation in intensity of pigment on the same medium. Further, bacteria, which appeared to be in all other respects

alike, might show in culture on agar or on potato marked differences in pigment formation.

With regard to the non-chromogenic organisms the colonies were usually moist and shining, and varying most definitely in opacity, but the absence of distinctive features made it extremely difficult to recognise different types from the colonial appearances alone. As a result there was much repetition in the course of the work, and many strains of the same organism were submitted to examination. In contrast to the slime the intestinal contents showed few or no chromogenic colonies.

Surface Flora.

Of the 247 cultures isolated from haddock slime of which descriptions are appended, 140 were non-sporing bacilli corresponding in their general characters with the group Achromobacter (Bergey). He describes these as " rods, small to medium in size, occurring principally in water and soil. Form no pigment on agar or gelatin but may produce a brownish growth on potato. Cultural characters variable. Some species form acid in simple sugars, others are without action on carbohydrates. Motile or non-motile. Gram-negative." Although no accurate quantitative estimations of the relative proportions of various genera in the slime were attempted the relative numbers of Achromobacteria to other organisms in the primary cultures suggested strongly that this type preponderates in the surface slime of the haddock. Of the organisms which have been classed as Achromobacter for convenience fifteen different types may be recognised on the basis of biochemical activities, of which two are of especial interest, one, because it includes seventy per cent of the total number of cultures, and the other, because of its close similarity to an organism isolated from living halibut in the Pacific Ocean and described in detail by Harrison (**2**).

Cultures belonging to the first of these two types have been isolated from all but one of the fish examined, and were present in large numbers in practically all the samples. Ninety-seven cultures have been examined. Morphologically small non-motile coccobacilli occurring in pairs and short chains, they are distinct from any other organisms isolated. In their cultural and biochemical activities they are very similar, differing only in their actions on glucose and on nitrate. If importance is placed on these reactions they can be divided into four sub-groups or strains according as they ferment glucose or reduce nitrate.

Agglutinating sera were produced in rabbits against two cultures of this type, but although these sera were active against the homologous organisms there was no cross agglutination, and other members of the type were not affected. The absence of pigment and the general

" inertness " were in agreement with the group Achromobacter, but they have not been identified with any of the fifty-six named types of Achromobacter in Bergey's *Manual*, nor have they been described in any available literature.

Organisms of the second type have been isolated from three samples of slime only. They show marked proteolytic properties as demonstrated by their power to liquefy coagulated ox serum and differ markedly from the other types in this respect. The characters of this type are in complete agreement with those of *Achromobacter pellucidum* described by Harrison from halibut slime. This worker, however, claims to have demonstrated a filterable or " symplastic " stage of this organism, but attempts to repeat this work with the haddock cultures have so far failed.

Of the remaining thirteen types nine correspond closely to types described in Bergey's *Manual*, but are not identical with these. The other four types show sufficient resemblance to the Achromobacter to be placed in this group, but cannot be identified with any organisms described by other workers.

As regards frequency of occurrence in the cultures the next most important genus is Micrococcus, forty cultures of which have been examined. Seven types were recognised of which five were pigmented. The cocci were similar to the common air organisms and to those described by Harrison which he obtained from halibut, but differ in certain particulars. One type (19) liquefied coagulated ox serum. On two occasions cocci, showing motility of a rotatory character, have been isolated from slime. Motile cocci are seldom encountered in general bacteriological work, but Harrison and Fellers (**3**) report having isolated them repeatedly from halibut and from salmon respectively. The organisms isolated in the course of this work differ from *Rhodococcus agilis*, as described by Harrison, in the colour of the pigment produced and in fermentation reactions.

Of the remaining organisms Flavobacteria and Pseudomonas were the only types occurring in significant numbers. Of the former, twenty-seven cultures were isolated. In contrast to the Achromobacteria, in which a multiplicity of types was met with, the types of Flavobacteria were relatively constant. Only five types were found in the twenty-seven cultures. These were similar in most respects to water organisms listed by Bergey and to types isolated by Harrison. It is worthy of note that whereas the majority of Flavobacteria types are Gram-negative, four of the five types were Gram-positive. In general, the Flavobacteria were more active biochemically than the Achromobacteria, producing some change in litmus milk and in most cases liquefaction of gelatin. They were, however, less active in carbohydrate media than the Micrococci. One of the types (29) liquefied coagulated ox serum.

Organisms of the genus Pseudomonas occurred on five fish only, and

were never numerous. They were identical in all respects with the types *fluorescens*, *viscosa*, and *convexa* (Bergey).

With regard to coliform organisms it is of interest that organisms of mammalian intestinal type have not been encountered, and that only four cultures belonging to the genus Aerobacter have been met with. Two of these were *Aerobacter aerogenes* and two *Aerobacter cloacæ*. As all the fish were taken close to the three mile limit off the coast of Aberdeenshire, the absence of coliform types is noteworthy.

In the course of the work it was found that when agar plates of the primary cultures were examined in a dark room colonies might be found which were luminous. The " phosphorescence " or luminescence of fish kept under certain circumstances is a well-known phenomenon, and various types of luminescent organisms have been described from time to time in sea-water and on marine animals. Nevertheless, no mention is made of the presence of these bacteria in the literature of other workers who have made systematic observations on the bacteriology of fish. The isolation of these organisms from plates crowded with other bacteria is difficult, as the luminosity of the colonies is their only distinguishing feature, and the presence of other organisms in large numbers appears in some way to interfere with their growth or with the production of light on agar plates. Probably for the same reason it has been found that fish which have been kept for some time so that they have become brightly luminous do not readily give cultures from which luminous organisms can be isolated. In the case of fresh fish, however, there is less difficulty, and separated luminous colonies may be encountered which are readily picked off and purified. Not infrequently when the cultures of slime failed to give luminous colonies the cultures of intestinal contents of the same fish did so. These organisms show considerable variation in morphology, but in young culture they were for the most part Gram-negative bacilli or coccobacilli. Eighteen cultures have been obtained and have been studied. It is hoped to give a separate communication upon the characters of this particular group.

In view of the work of Gee (**4**) and others who have described spore-bearing organisms of the *subtilis-mesentericus* group (genus *Bacillus*), particular attention was paid throughout this work to the detection of spore-bearers. These were never isolated from slime although they were met with in the intestine.

Of minor interest because of the infrequency of occurrence, it may be mentioned that yeasts were isolated on two occasions, and that two organisms producing a bright red pigment and agreeing in general characters with the genus Serratia were obtained. One of these cannot be placed among the types described in Bergey's *Manual*.

Intestinal Flora.

Forty-six cultures have been isolated. As in the case of the slime, members of the genus Achromobacter preponderated in every sample, and in several instances were present almost in pure culture. Five different types of Achromobacter were distinguished, of which the coccobacillary type, already referred to as occurring in slime, was the only one common to both sites. The other four types were not identical with any of the Achromobacter found in the slime.

Flavobacteria were present in one sample only. These differed slightly from the Flavobacteria isolated from slime, and from an organism isolated by Harrison from halibut. *Aerobacter cloacæ* was obtained on one occasion.

In contrast to the results obtained from the examination of slime, spore-bearing organisms were present in small numbers in almost every sample of intestinal content. The characters of these have been found to agree in almost every respect with those of marine spore-bearing organisms published by Newton (**5**).

The occurrence of luminescent organisms in the intestine has already been referred to.

Reed and Spence (**6**), in the case of haddock from the mouth of the St. Croix River, Nova Scotia, in a quantitative examination of eleven samples of slime and thirty-four samples of intestinal content found Proteus types by far the most common organism (70%) in the intestinal contents, with Achromobacter 4·4%, Pseudomonas 8·7%, Flavobacter 5·6%, Aerobacter 4·6%, Bacillus 5·7%, and others 1%. In the slime the corresponding figures were Proteus 18%, Achromobacter 23%, Pseudomonas 22%, Flavobacter 8%, Bacillus 24%, Micrococci 4%. The striking feature of these results is the constant occurrence in the intestinal contents of the Proteus type which has not once been encountered in this investigation either in the slime or intestinal contents of haddocks from the North Sea. In their comparison of individual fish there was marked irregularity with regard to the occurrence of the various genera, e.g. Proteus types were frequently abundant in the slime of some fish and absent in others caught under similar circumstances. In contrast to the results recorded here, Achromobacteria were not found regularly in the intestinal contents and were absent occasionally from the slime. In the case of the intestine the same irregularity of occurrence was shown by the other groups of organisms.

It is noteworthy that Pseudomonas types were found in considerable numbers in all the samples of slime examined by these workers, and frequently in the intestinal content, whereas Harrison failed completely to isolate this organism from the slime of fresh halibut in spite of special

attempts to do so. In the present work this group was encountered in six samples of slime only. As the colonial appearances of this group are distinctive, its presence in the material examined was not likely to be overlooked. This organism has been encountered frequently in bacteriological examinations of decomposing fish (Fellers; Harrison, Perry, and Smith (**7**); and Harrison), and as it has active proteolytic properties and may be active in spoilage it is important to ascertain the source. It is a common water type, is almost constantly present in samples of ice in which fish are preserved, and is thus likely to contaminate samples of slime unless stringent precautions are taken to avoid extraneous organisms.

Reed and Spence also report the constant presence of the genus Bacillus in slime, whereas in this investigation this genus, although almost constantly present in the intestine, was never encountered in the slime. Gee also found spore-bearers in slime, the other organisms being Proteus, Pseudomonas, and Diplococcus types, but it is remarkable that he did not encounter Achromobacteria. He has also found spore-bearing organisms in the muscle of living fish, and attributes to them much of the spoilage which results on keeping, whereas other workers have found fresh muscle to be sterile.

A comparison of this work with the work of various Canadian and American investigators (Obst, **8**; Hunter, **9**; Fellers; Harrison, Perry, and Smith; and Sanborn, **10**) who have made bacteriological studies of salmon, halibut, sardines, and other fish with reference to spoilage, is difficult, as in many cases the samples were taken indiscriminately from slime, muscle, and intestine and from fish in various stages of decomposition. It is sufficient to say that most of the groups mentioned above have been met with.

CONCLUSIONS.

1. The bacterial flora of the slime and intestinal contents of North Sea haddocks has been investigated, and two hundred and ninety-three cultures in all have been examined in detail.

2. The most common organisms in both sites were Achromobacteria, and of these several types were usually present. One type in particular predominated which has not hitherto been described by other workers.

3. Organisms of the genus Micrococcus were found frequently in slime but not in the intestine.

4. Flavobacteria were present irregularly in the slime samples, but were encountered in only one sample of intestinal contents.

5. Pseudomonas types were found in the slime of a few fish, and only in small numbers. They were not met with in the intestine.

6. Spore-bearing organisms were never met with in the slime, but were present in most samples of intestinal contents in small numbers.

7. Luminescent organisms were present irregularly in the slime and in the intestinal contents.

I wish to express my deep indebtedness to Professor J. Cruickshank for his constant help and advice throughout the work.

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ACHROMOBACTER.

	1	2	3	4	5	6
Morphology	Coccobacilli, in pairs or short chains. Gram-negative.	Rods, majority small, occasional long forms. Gram-negative.	Rods, medium to long, a few swollen at one or both ends. Gram-negative.	Rods, medium size, singly and in chains, some filaments. Gram-negative.	Rods, medium thickness, long. Gram-negative.	Rods, vary in length, majority long, slender, slightly curved, others swollen and filamentous. Gram-negative.
Motility	–	–	–	–	–	+
Agar Slope	White, slightly raised, moist, opaque, smooth and shining.	Whitish, translucent to semi-opaque, slightly raised, moist and smooth.	Translucent to semi-opaque, slightly raised, smooth shining surface.	Creamy, semi-opaque, slightly raised, moist, smooth and shining.	Translucent to semi-opaque, slightly raised, smooth and shining.	Whitish, raised, opaque, tending to "flow" down slope. Later becomes transparent with buff to brown opaque particles.
	a. b. c. d.					
*Glucose	+ + – –	+	–	+	+	–
Lactose	– – – –	+ faint, late.	–	–	+	
Saccharose	– – – –	–	–	–	+	
Mannite	– – – –	+	–	–	+	
Dulcite	– – – –	–	–	–	–	
Indole	– – – –	–	–	–	–	–
*V.P.				–	–	
Nitrate	– + + –	+	–	–	+	–
Litmus Milk	No change.	Slowly becoming acid.	No change.	No change.	Litmus reduced.	Litmus reduced, milk peptonised, serum brown and slimy.
Potato	Scant and glistening.	Scant and glistening.	White, moist, glistening, limited, becoming fawn.	No growth.	Scant, whitish and glistening.	No growth.
‡Gelatin	–	–	–	–	–	+
Slime	+	+	+	+	+	+
Intestine	+	–	–	–	–	–
Related to	–	Ach. fermentationis (Chester) Bergey et al.				Ach. pellucidum, Harrison N. sp.

* Glucose + = acid
,, – = no acid
* V.P. = Voges Proskauer test

‡ Gelatin + = liquefaction
,, – = no liquefaction

	7.	8.	9.	10.	11.	12.
Morphology	Rods, medium length, rather stout, rounded ends. Gram-negative.	Rods, short and stout, rounded ends, singly, in pairs. Sometimes short chains. Gram-negative.	Rods, medium length, rather stout, rounded ends, occur singly. Gram-negative.	Rods, medium size, rounded ends. Gram-negative.	Rods, rather long and slender, occur singly. Gram-negative.	Rods, medium length, slender, rounded ends, occur singly. Gram-negative.
Motility	+	+	+	+	+	+
Agar Slope	Whitish, slightly raised, opaque, moist, surface smooth and shining.	Whitish, slightly raised, semi-opaque, moist, surface smooth and shining.	Whitish, semi-opaque, flat, moist, smooth surface, becomes transparent.	Raised, semi-opaque, moist, pink - brown tinge at foot of slope, surface smooth and shining.	Whitish, slightly raised, translucent, moist, surface smooth and shining.	Whitish, semi-opaque, tending to "flow" over slope, moist, smooth and shining.
Glucose	+	+	+	–	–	+
Lactose	–	– or faintly + late.	–			–
Saccharose	–	–	–			–
Mannite	–	– or faintly + late.	–			–
Dulcite	–	–	–			–
Indole	–	–	–	–	–	–
V.P.	–					–
Nitrate	–	–	–	+	+	–
Litmus Milk	Coagulation, marked shrinkage of clot and separation of whey, complete reduction except at surface which is pink.	Acid with or without coagulation.	Alkaline and peptonised first at surface, later throughout, litmus reduced, serum yellow.	Litmus completely reduced, peptonisation, green ring at surface.	No change.	No change.
Potato	Moist, raised, pinkish fawn becoming brown.	Pale cream, opaque, moist and shining, potato slightly discoloured.	Fawn, spreading, wet, opaque.	Sepia - brown, moist, shining and opaque, potato discoloured.	No growth.	Fawn to pinkish, opaque, slightly raised.
Gelatin	+	–	+	+	–	–
Slime	+	+	+	+	+	+
Intestine	–	–	–	–	–	–
Related to	Ach. multistriatum (Wright) Bergey et al.	Ach. multistriatum (Wright) Bergey et al.	Ach. geniculatum (Wright) Bergey et al.	Ach. geniculatum (Wright) Bergey et al.	Ach. pestifer (Frankland) Bergey et al.	Ach. venosum (Vaughan) Bergey et al.

	13.	14.	15.	16.	17.	18.
Morphology	Rods, medium length, slender, rounded ends. Gram-negative.	Rods, short and slender. Gram-negative.	Rods, small, rounded ends, occur singly. Gram-negative.	Rods, short, many degenerate and with small areas failing to stain. Gram-negative.	Rods, short and stout, occasional swollen filamentous forms, many have small areas failing to stain. Gram-negative.	Rods, short and stout, some swollen irregular forms, many show marginal staining. Gram-negative.
Motility	+	+	+	+	+	+
Agar Slope	Flat, translucent, moist, tending to spread, surface smooth and shining.	Luxuriant, whitish, translucent, flat, spreading, surface smooth and shining.	Flat, opaque, moist, smooth shining surface, white becoming pinkish.	Flat, transparent, uneven surface, growth in condensation water reddish brown.	Creamy, flat, semi-opaque, moist, surface smooth and shining.	Creamy, semi-opaque, slightly raised, moist, surface smooth and shining, becomes translucent.
Glucose	−	+	+	−	+	+
Lactose		−	+		−	−
Saccharose		+	+		−	−
Mannite		+	+		−	−
Dulcite		−	−		−	−
Indole	−	−	−	−	−	−
V.P.			−			
Nitrate	+	−	+	−	+	+
Litmus Milk	Slowly becomes alkaline.	No change.	Acid, late.	No change.	No change, organism does not grow well.	No change.
Potato	Fawn, wet, glistening, tending to spread.	Scanty, white and shining.	Opaque, slightly raised, surface smooth and shining, white becoming pinkish.	No growth.	No growth.	No growth.
Gelatin	−	+	−	−	−	+
Slime	+	+	+	−	−	−
Intestine	−	−	−	+	+	+
Related to	Ach. raveneli (Chester) Bergey et al.	Ach. litoralis Bergey et al.		Ach. guttatum (Zimmermann) Bergey et al.	Ach. pestifer (Frankland) Bergey et al.	Ach. dendriticus (Bordoni Uffreduzzi) Bergey et al.

	ACHROMOBACTER.	MICROCOCCUS.				
	19.	20.	21.	22.	23.	24.
Morphology	Rods, medium length, stout, rounded ends, occasional long filaments. Gram-negative.	Spheres, singly and in pairs. Gram-positive.	Spheres, small, in pairs. Gram-positive.	Spheres, small, many slightly oval, in pairs. Gram-positive.	Spheres, singly and in groups of four. Gram-positive.	Spheres, occur singly and in clumps. Gram-positive.
Motility	+	–	–	–	–	–
Agar Slope	Creamy, opaque, slightly raised, smooth and shining surface.	Lemon - yellow, raised, opaque, luxuriant, smooth shining surface.	Lemon - yellow, raised, opaque, moist, smooth shining surface.	Pale lemon, opaque, slightly raised, moist, becoming tough.	Lemon - yellow, raised, opaque, luxuriant, moist, smooth surface.	Apricot, slightly raised, opaque, moist, smooth shining surface, becomes tough.
Glucose	–	+ faint or –	+ faint or –	+	+	+
Lactose		–	–	late +	–	+
Saccharose		–	–	late +	+	+
Mannite		–	–	–	+	+
Dulcite		–	–	–	–	–
Indole	–	–	–	–	–	–
V.P.		–	–	–	–	–
Nitrate	–	–	+	+	+	+
Litmus Milk	No change.	Coagulation, peptonisation, litmus partially reduced, serum purplish brown.	No change.	Faintly acid late.	Alkaline.	Slowly becomes acid.
Potato	No growth.	Lemon - yellow, opaque, potato slightly discoloured.	Scanty, lemon-yellow.	Scanty, lemon-yellow.	Scanty, lemon-yellow.	Scanty, orange.
Gelatin	–	+	–	+	+	+
Slime	–	+	+	+	+	+
Intestine	+	–	–	–	–	–
Related to		Micrococcus subflavus Bumm.	Micrococcus ochraceus Rosenthal.	Micrococcus citreus Harrison, Micrococcus conglomeratus Migula.	Micrococcus varians Harrison, Micrococcus percitreus Bergey et al.	Micrococcus aurantiacus Harrison, Micrococcus perflavus Bergey et al.

	MICROCOCCUS.			FLAVOBACTER.		
	25.	26.	27.	28.	29.	30.
Morphology	Spheres, singly, in pairs and clumps. Gram-positive.	Spheres, occurring in clumps. Gram-positive.	Spheres, large, occur in pairs and clumps of four. Gram-positive.	Rods, short, average thickness, occur singly. Gram-positive in very young culture.	Rods, short and rather stout, some slightly curved, occasional long rods. Gram-positive.	Rods, varying in length, some with swollen ends. Gram-positive in very young culture.
Motility	–	–	+	–	–	–
Agar Slope	Porcelain-white, slightly raised, opaque, smooth surface, waxy-shine.	White, flat, opaque, surface smooth and shining.	Apricot, slightly raised, opaque, surface smooth, shining, becomes translucent.	Apricot, slightly raised, opaque, surface smooth and shining, becomes tough.	Apricot, slightly raised, opaque, smooth and shining surface, becomes tough.	Lemon-yellow, slightly raised, opaque, surface smooth, later wrinkled into folds.
Glucose	+	+	+ or –	–	–	+ faint or –
Lactose	+	–	–			–
Saccharose	+	+	–			–
Mannite	–	+	–			–
Dulcite	–	–	–			–
Indole	–	–	–	–	–	–
V.P.	+	–				
Nitrate	+	–	–	–	+	–
Litmus Milk	Acid with or without coagulation.	No change.	No change.	Alkaline, slow peptonisation and reduction of litmus.	Alkaline, slow peptonisation and partial reduction.	Alkaline, later peptonisation and partial reduction of litmus.
Potato	Porcelain—white, opaque, limited.	White, scanty.	No growth.	Luxuriant, apricot, opaque, moist.	Luxuriant, opaque, apricot, moist.	Yellow, opaque, raised, moist, surface smooth and shining.
Gelatin	–	+	+	+	+	+
Slime	+	+	+	+	+	+
Intestine	–	–	–	–	–	–
Related to	Micrococcus candidus Harrison.			Flavobacterium marinum Harrison, N.Sp., Flavobacterium lutescens (Lustig) Bergey et al.	Flavobacterium fucatum Harrison, N.Sp.	Flavobacterium turcosum Harrison.

	FLAVOBACTER.			BACILLUS.		
	31.	32.	33.	34.	35.	36.
Morphology	Rods, medium length, slender. Gram-positive.	Rods, medium size. Gram-negative.	Rods, short and slender, occasional long forms. Gram-positive.	Rods, average size, rounded ends, spores long, oval, narrow. Gram-positive.	Rods, long, stout with square ends, spores oval, bulging bacillus, eccentric to subterminal. Gram-positive.	Rods, long, stout, square ends, occur in chains, spores oval, subterminal bulge bacillus. Gram-positive show granules.
Motility	−	+	+	+	+	+
Agar Slope	Yellowish-green, flat, semi-opaque, moist, surface smooth and shining.	Golden, flat, translucent growth, surface smooth and shining.	Lemon-yellow, slightly raised, opaque, moist, surface smooth and shining.	Flat, translucent, wet, creamy and opaque at foot of slope, surface smooth.	Creamy, wet, spreading, raised, surface shining and smooth, later becomes uneven.	Creamy, flat, opaque, dull uneven surface.
Glucose	Faint +	+	+	+	−	+
Lactose	−	−	−	−		−
Saccharose	Faint +	+	−	+		−
Mannite	Faint +	+	−	+		−
Dulcite	−	−	−	−		−
Indole	−	−	−	−	−	−
V.P.		−				
Nitrate	−	+	+	−	+	−
Litmus Milk	Faintly acid.	Acid with or without clot, peptonisation, reduction of litmus.	Alkaline, later partial peptonisation.	Coagulation and peptonisation. Litmus reduced.	Becomes slowly alkaline and peptonised.	Peptonisation, litmus reduced, serum brownish.
Potato	Greenish-yellow, moist and shining, flat.	Pale golden, wet, spreading growth, pigment deepens.	Spreading, lemon, opaque, flat.	Brown, wet, opaque, spreading, shining, potato discoloured.	Raised, opaque, at first smooth, later wrinkling into folds, fawn becoming pinkish.	Opaque, dull uneven surface, creamy becoming fawn.
Gelatin	−	+	+	+	+	+
Slime	+	+	−	−	−	−
Intestine	−	−	+	+	+	+
Related to	Flavobacterium dormitor Harrison.	Flavobacterium diffusum Harrison, Flavobacterium rigensis Bergey et al.	Flavobacterium sulphureum Bergey et al.	Bacillus mesentericus Trevisan.	Bacillus sublanatus Wright.	Bacillus cereus Frankland.

LUMINESCENT CULTURE.

	37.
Morphology	Rods, varying in morphology, many coccal, others definitely bacillary, short and stout, some show marginal staining. Gram-negative.
Motility	+
Agar Slope	Translucent to semi - opaque, slightly raised, smooth shining surface, luminous.
Glucose	+
Lactose	−
Saccharose	−
Mannite	−
Dulcite	−
Indole	−
V.P.	+
Nitrate	+
Litmus Milk	No change.
Potato	No growth.
Gelatin	−
Slime	+
Intestine	+

Notes on the Biology of some Lamellibranchs in the Clyde Area.

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With 3 Figures in the Text.

CONTENTS.

INTRODUCTION.

FOR the past few years certain common Scottish intertidal Lamellibranchs have been studied, chiefly with a view to tracing the rate of growth and fluctuations of the various year-groups. The investigations in the Clyde Sea Area have now been extended to include certain species living below L.W.M., two of which are discussed in the present paper. The species dealt with are *Cardium edule*, *Tellina tenuis*, *Tellina fabula*, and *Abra alba*.

The measurement of length used in the various tables, both of the shell and of the annual ring, is that of the greatest antero-posterior diameter, taken to the nearest mm. above, e.g. 4·1 to 5·0 mm. being given as 5 mm. The lengths of the shells were measured on a measuring-board and those of the rings were taken by means of jointed dividers.

For sampling below L.W.M. the Robertson Bucket Dredge was used.

The dredged material was usually put through a 2-mm. sieve, with a check sample through the 1-mm. sieve.

I am indebted to Mr. R. Elmhirst, the Superintendent of the Marine Station at Millport, for his assistance in collecting most of the material on which this paper is based.

TELLINA TENUIS.

The rate of growth of this species, at and above L.W.M. in Kames Bay, has been studied for several years, but the rate of growth below L.W.M. was not satisfactorily worked out owing to scanty material.

The species occurs plentifully in Kames Bay in the intertidal region and

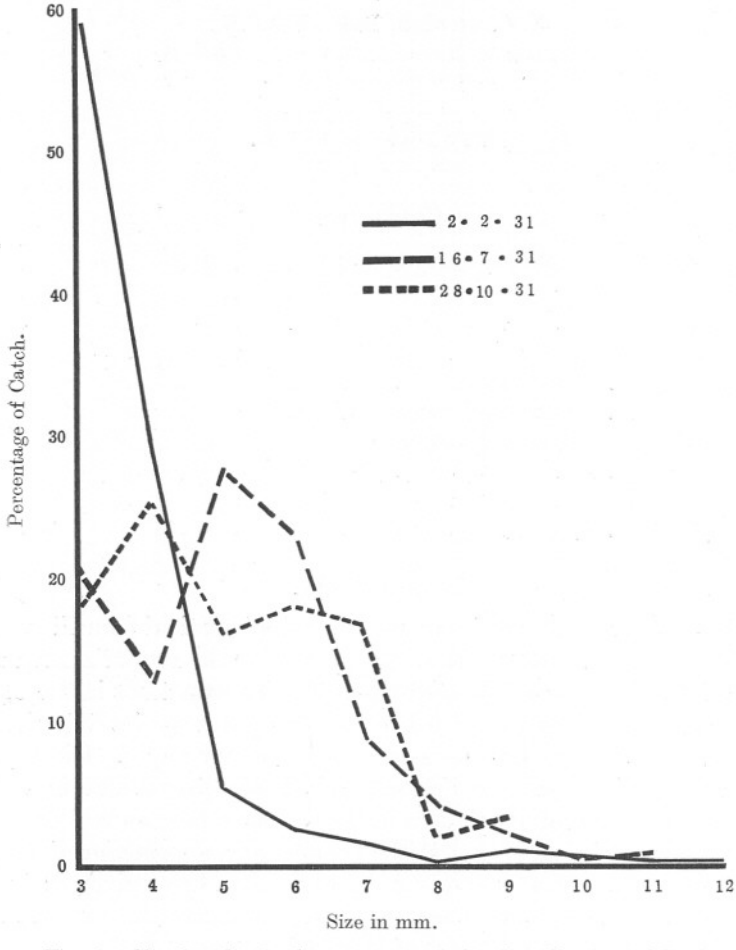

FIG. 1.—Showing the size-frequency curves for the collections of *Tellina tenuis* taken in 1½ fm. in Kames Bay, Millport, during 1931.

just below L.W.M., but does not extend seawards beyond about 2½ fathoms. When investigations were begun in 1926 a few dredgings were taken, but the material was too scanty to allow of more than the general conclusions that (1) the rate of growth in shallow water was less than at

L.W.M. and (2) spat did not survive in quantity for very long, leaving the ground comparatively sparsely populated.

Following the heavy spat-fall in the late summer of 1930 it was felt that this would prove a suitable opportunity for completing the study of the rate of growth of the species in Kames Bay over the whole of its vertical range. Investigations were confined to one station in Kames Bay (Appendix, p. 68) where both *T. tenuis* and *T. fabula* were abundant, and collections were taken three times during the year with the Robertson Bucket Dredge, a minimum of twenty hauls being taken at each visit. As already mentioned, there was abundant spat in 1930 and this is reflected in the size-frequency curve for February, 1931 (Table 3; Fig. 1), where nearly 90% of the population was under 5 mm. On this curve there was only one well-marked group, with the mode at 3 mm. On the curve for July there were two distinct modes, one at 3 mm. representing the new 1931 spat and the other, representing the older 1930 spat, at 5 mm. On the curve for October there were again two modes at 4 mm. and 6 mm. respectively, but in this case the number of specimens is so small that too much reliance cannot be placed on the figures.

The results of the dredgings taken in the autumn of 1926 are interesting and show a close parallel to those of February, 1931. Then, also, there was only one well-marked group on the ground, with a mode at 3 mm. In the middle of April, 1927, the mode was still the same, but by the middle of August it had moved to 6 mm. and the new spat gave a mode at 3 mm. In the autumn of 1927 there were two well-marked groups on the ground: the 1927 spat, mode at 3 mm., and the 1926 spat, mode at 6 mm.

The collections taken during 1928 and 1929 contained so few specimens that they need not be considered, with the exception of a collection taken in March, 1928. The size-frequency curve for that collection was the same as that of the previous autumn, showing that below L.W.M., as above, there had been no growth during the winter months.

The modes for the size-frequency curves for collections with a reasonably large number of specimens may thus be tabulated :—

TABLE 1.

	1926 *spat.*	1927 *spat.*
6.10.26	3 mm.	.
13.4.27	3 mm.	.
13.8.27	6 mm.	3 mm.
8.10.27	6 mm.	3 mm.
23.3.28	6 mm.	3 mm.
	1930 *spat.*	1931 *spat.*
2.2.31	3 mm.	.
16.7.31	5 mm.	3 mm.
28.10.31	6 mm.	4 mm.

Thus in both 1926 and 1930, following heavy spat-fall, the year-groups could be followed for a time. The 1926 spat could be traced until the spring of 1928 but seems to have died out during that year. The 1930 spat was still present as a distinct group in the autumn of 1931 but was already thinning out.

In the intervening years, with seemingly less spat, the species was comparatively scarce on the sublittoral ground, and with the few specimens taken no year-groups could be traced.

The falling-off in abundance is important and shows that the species is now best adapted for life in the intertidal area. In 1926 and 1927 so few specimens were taken that the figures are unreliable, but the 1931 collections offer suitable material. As is shown in Table 2, both *T. tenuis* and *T. fabula* decreased in abundance, especially the former.

TABLE 2.

TABLE SHOWING THE ACTUAL NUMBERS OF *T. TENUIS* AND *T. FABULA* IN 20 HAULS IN KAMES BAY DURING 1931.

	2.2.31.	16.7.31.	28.10.31.
T. tenuis	1566	301	155
T. fabula	1363	755	546

The results of the investigations, Stephen (**3**), on *T. tenuis*, both above and below L.W.M. in Kames Bay, may be thus summarised :—

The species breeds in summer. Growth is greatest in the upper parts of the beach and least below L.W.M. In 1926 and 1930 spat was unusually abundant both above and below L.W.M. Above L.W.M. the 1926 spat dominated the ground until the summer of 1930, when it largely died out and was replaced by the 1930 spat, which remains at present the dominant year-group. Below L.W.M. in both 1926 and 1930 the results were very similar in that the spat grew slowly and died off rapidly.

The size of the species at the end of each year of life in Kames Bay, judged from the modes of the various size-frequency curves, may be taken to be as follows :—

	Position on the Beach.		
	1½ fathoms.	L.W.M.	Near H.W.M.
End of first year of life	3 mm.	3 mm.	3 mm.
,, second year of life	6 mm.	7 mm.	9 mm.
,, third year of life	.	9 mm.	12 mm.
,, fourth year of life	.	11 mm.	.

TABLE 3.

TABLE SHOWING THE PERCENTAGE OF THE TOTAL CATCH AT EACH MM.-SIZE IN THE COLLECTIONS OF *T. tenuis* FROM STN. 6, KAMES BAY, 1931.

Date.	3	4	5	6	7	8	9	10	11	12	13	14	15	Total specimens.
2.2.31	58·8	29·7	5·5	2·4	1·5	0·4	0·7	0·5	0·2	0·2	0·1	.	0·1	1566
16.7.31	21·0	12·7	27·6	23·2	8·7	4·0	2·0	0·3	0·6	.	.	.	.	301
28.10.31	18·7	25·1	16·1	18·1	16·8	1·9	3·2	.	.	.	.	.	.	155

TELLINA FABULA.

This species is distributed over the shallow, sandy ground in Kames Bay from about L.W.M. springs to 10 fathoms. Its area of distribution overlaps that of *T. tenuis* in the region just below L.W.M. Prior to 1931 several attempts had been made to determine the rate of growth, but these were unsuccessful, chiefly because insufficient material was collected. The earlier work, however, was not entirely wasted since the curves contain confirmatory evidence on several points.

Rate of Growth (Table 4; Fig. 2).

Material was collected from one station in Kames Bay, Millport, on clean sand at a depth of $1\frac{1}{2}$ fathom at low tide in February, June, and October, 1931.

On the size-frequency curve for February there is one well-marked mode at 4 mm., representing the 1930 spat, and there is a hint of a mode between 9 mm. and 11 mm., probably the 1928 spat. It may be assumed that in this, as in other species, there is no growth during the winter months and that the mode at 4 mm. represents the modal size of the 1930 spat at the end of the previous autumn.

On the curve for July there are two well-marked modes, one at 4 mm., representing the new 1931 spat, and another at 7 mm., representing the 1930 spat. The species, therefore, appears to breed in early summer, probably about the same time as *T. tenuis*.

On the October curve there are again two well-marked modes, one at 5 mm., representing the 1931 spat, the other at 8 mm., representing the 1930 spat.

From the figures for 1931 the rate of growth of the species in Kames Bay may be taken as follows :—

At end of First Autumn.	At end of Second Autumn.	At end of Third Autumn.
4–5 mm.	8 mm.	probably 10 mm.

In the autumn of 1930 the spat of that year had the mode at 4 mm., while in the autumn of 1931 the mode was at 5 mm. The conditions of growth would therefore seem to have been more favourable in 1931, although the result may have been due to less competition for food, since the 1930 spat was very much more numerous than that of 1931.

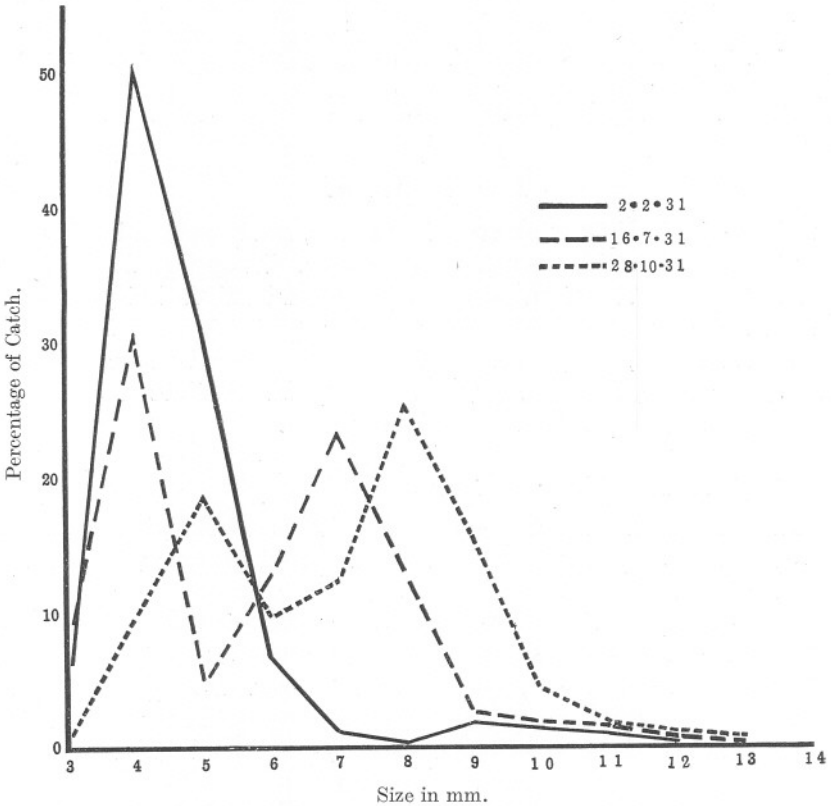

FIG. 2.—Showing the size-frequency curves for the collections of *Tellina fabula* taken in 1½ fm. in Kames Bay, Millport, during 1931.

The figures for the previous collections of *T. fabula* in Kames Bay confirm those given above and show that they represent the usual conditions in the bay. For example, during 1928 the modes of the 1927 spat were as follows :—

23.3.28	5 mm.
25.4.28	5 mm.
6.6.28	6 mm.
29.9.28	7–8 mm.

The autumn mode for the 1928 spat was at 4 mm.

T. fabula would seem to die out about the end of its third year of life, probably like *T. tenuis*, after the summer spatting. Some few specimens may be older, but there is no evidence on the curves of the survival of more than a very few individuals after that age.

The autumn of both 1926 and 1930 was a period of exceptional survival of *T. tenuis* spat. Full figures are not available for *T. fabula* over the whole period, but a comparison of the figures for 1926, 1928, and 1930 (Table 4), would suggest that these years were marked also by an abundant survival of the spat of this species in Kames Bay.

TABLE 4.

Table Showing the Size-Frequencies of *T. fabula* in Kames Bay.

A, during 1926 and 1928 when only a few dredgings were taken at one time. B, during 1931 when a minimum of 20 hauls was taken at each time.

	3	4	5	6	7	8	9	10	11	12	13	14	No. of specimens.
						A.							
6.10.26	24·7	52·5	13·7	2·0	2·0	.	2·0	.	2·0	.	.	1·0	101
23.3.28	8·3	18·2	31·4	19·0	7·5	7·5	2·5	1·7	2·5	0·8	0·8	.	121
25.4.28	10·9	21·7	22·2	20·7	8·3	5·2	3·1	3·1	3·1	1·0	0·5	.	193
6.6.28	2·6	6·6	26·0	26·9	14·3	10·9	7·4	3·0	0·9	1·3	.	.	230
29.9.28	11·5	15·4	7·7	11·5	23·1	19·2	11·5	.	.	.	.	.	26
						B.							
2.2.31	6·1	50·7	31·1	6·6	1·2	0·3	1·6	1·2	0·9	0·2	.	.	1363
16.7.31	8·7	30·8	5·0	13·0	23·2	13·0	2·8	1·4	1·4	0·5	0·1	.	755
28.10.31	0·9	9·9	18·9	9·9	12·4	25·2	15·7	4·2	1·5	1·1	0·2	.	546

Abra alba.

The rate of growth of this species was studied in Loch Striven, chiefly at Station 11 (Appendix, p. 68), samples being taken in spring, summer and autumn. These collections were supplemented by two others taken at Stations 10 and 11 (Appendix, p. 68) in Loch Striven in September, 1930, by Mr. Moore, which he kindly handed to me for examination.

Abra alba usually has well-marked rings on the shell, which may safely be taken as annual or winter rings (Ford, **2**, p. 540). Before the age of the shells can be read it must first be proved whether the first well-marked ring is the first or second winter wing. In the Clyde area in *C. edule*, for example, the first winter ring is very faint and the first conspicuous ring is really the second winter ring. In *A. alba*, however, the first distinct ring is the first winter ring, the evidence for which statement is as follows, Table 5.

TABLE 5.

TABLE SHOWING THE NUMBERS, AVERAGE SIZE, AND RANGE OF ALL SPECIMENS WITHOUT A WINTER RING AT EACH DATE OF COLLECTION IN LOCH STRIVEN DURING 1931.

Date of collection.	Number of specimens.	Range in size.	Average size.
13.2.31	1	10 mm.	10 mm.
19.6.31	.	.	.
20.8.31	64	3–11 mm.	5·6 mm.
29.10.31	42	6–11 mm.	8·1 mm.

In the February collection there was only one specimen, the smallest of the collection, which had no winter ring within the margin of the shell, the winter ring at 10 mm. being in the course of formation. In the June collection all specimens had rings and those with one ring ranged from 9 to 13 mm. In the August collection there were numerous small specimens without rings, ranging from 3 to 11 mm. and with an average size of 5·6 mm. By the end of October all specimens without a winter ring ranged from 6 to 11 mm. with an average size of 8·1 mm.

The species therefore breeds in summer, probably about the end of June and July. The first distinct ring is the first winter ring. Growth is rapid and, since the shell is thin, there is not the same limitation to growth as might be expected in a species which secretes a heavy shell. Differences in conditions from year to year will therefore affect the rate of growth considerably; for example, the first winter rings of the 1928 spat average considerably less than those of the 1929 spat (Table 6).

With regard to the other year-groups in the collections, the average sizes of the winter rings and of total lengths have been calculated for each collection (Table 6).

TABLE 6.

TABLE SHOWING THE AVERAGE SIZES IN MM. OF THE VARIOUS WINTER RINGS AND TOTAL OF *A. ALBA* IN EACH COLLECTION FROM LOCH STRIVEN IN 1931.

	0 Ring.	1 Ring.		2 Rings.			3 Rings.			
		Winter ring, 1930–		Winter ring, 1929–	Winter ring, 1930–		Winter ring, 1928–	Winter ring, 1929–	Winter ring, 1930–	
Date.	Length.	31.	Length.	30.	31.	Length.	29.	30.	31.	Length.
23.2.31	10·0	10·4	15·0	9·2	18·0	19·6	.	.	.	.
19.6.31	.	6·7	10·8	10·3	14·6	16·3	8·4	16·2	18·3	19·2
20.8.31	5·6	7·0	11·5	11·0	15·6	17·4	7·0	16·1	18·6	19·6
29.10.31	8·1	7·8	14·8	10·8	15·9	18·1	9·0	16·7	18·7	20·0

In February the collection was a very small one and does not seem to have been quite representative of the station since no 1928 spat was found, and the means are all high in comparison with the figures for the rest of the collections. Disregarding these February figures, the average size in round figures at the end of each year would seem to have been approximately as follows (Table 7).

TABLE 7.

TABLE SHOWING THE APPROXIMATE SIZE OF THE VARIOUS SPATS IN LOCH STRIVEN AT THE END OF EACH YEAR OF GROWTH.

Spat of	At end of First year.	At end of Second Year.	At end of Third Year.	At end of Fourth Year.
1931	8 mm.	.	.	.
1930	8 mm.	15 mm.	.	.
1929	10·7 mm.	15·4 mm.	18 mm.	.
1928	8 mm.	16·3 mm.	18·5 mm.	19·6 mm.

The renewal of the spat in different years varies greatly (Table 8). During 1931 the survivors of the 1929 spat were the dominant year-group on the ground. The year 1929 seems, therefore, to have been a very favourable one for *A. alba*, for not only did the spat grow more than usual, judging from the size of the first winter ring (Table 7), but it survived in unusually large numbers (Table 8).

TABLE 8.

TABLE SHOWING THE NUMBERS OF EACH YEAR'S SPAT SURVIVING IN THE COLLECTIONS TAKEN IN LOCH STRIVEN DURING 1931.

Date.	1928 Spat.	1929 Spat.	1930 Spat.	1931 Spat.
23.2.31	5	17	1	.
19.6.31	12	73	24	.
20.8.31	16	43	13	64
29.10.31	3	16	10	42
Total	36	149	48	106

The two collections taken by Mr. Moore in 1930 also show the same peculiarities, namely, the large size of the winter ring in the 1929 spat (Table 9), and the preponderance of that year-group in the collections (Table 10).

The preponderance of certain year-groups in the Abra population in Plymouth waters has been indicated by Ford (**2**, p. 544). The 1922 brood

was one such group, and judging by its size at the end of successive years (Ford, **2**, p. 543) its growth was much slower than that of Abra from Loch Striven.

TABLE 9.

TABLE SHOWING THE MEAN SIZES OF THE VARIOUS WINTER RINGS IN *A. ALBA* TAKEN AT STNS. 10 AND 11 ON 1.9.30.

Station.	1930 Spat.	1929 Spat.		1928 Spat.		
	Length.	Winter ring, 1929–30.	Length.	Winter ring, 1928–29.	Winter ring, 1929–30.	Length.
10	11·0 mm.	12·7 mm.	16·1 mm.	8·7 mm.	15·7 mm.	19·0 mm.
11	?	10·7 mm.	15·5 mm.	7·2 mm.	17.2 mm.	19·3 mm.

TABLE 10.

TABLE SHOWING THE NUMBERS OF SURVIVORS OF THE SPAT OF VARIOUS YEARS IN THE COLLECTIONS FROM STNS. 10 AND 11 ON 1.9.30.

Station.	1928 Spat.	1929 Spat.	1930 Spat.
10	3	99	1
11	17	60	?

As in the case of the material collected in 1931 so in this 1930 material the spat of 1929 formed by far the largest part of the collections, and similarly its first (1929–30) winter ring was much larger than that of the 1928 spat.

It has already been shown how in the Clyde area both *C. edule* and *T. tenuis* grow fastest at some particular level. In this case it is not easy to draw conclusions, since too much stress must not be laid on the averages of the 1928 spat for Station 10 where only three specimens were taken.

Judging from the averages of the 1929 spat, *A. alba* seems to grow at a distinctly greater rate in the shallower water, especially in the first year when growth is greatest.

There is a fair range of size in both the winter ring and in the length of the 1929 spat, and the question arises whether there is any correlation between the two, e.g. is the rate of growth in the second year of life of spat which has grown poorly in the first year less than that of spat which has grown well? In Table 11 the range and average size of the first winter ring for the 1929 spat are tabulated against each mm. in length of shell at the end of 1930. While some individuals which have grown slowly in their first year overtake by the end of the second year individuals which have grown well in their first year, on the average those individuals which have grown well in their first year tend to be larger at the end of the second year. It is noticeable, also, how growth in the second year at

Station 11 (mid-loch) was greater than at Station 10 (loch head), thus reversing the positions during the first year.

TABLE 11.

TABLE SHOWING THE RANGE AND AVERAGE SIZE OF THE 1929–30 WINTER RING IN THE 1929 SPAT FOR INDIVIDUALS AT EACH MM. OF LENGTH IN THE END OF 1930 AT STNS. 10 AND 11.

	Station 10.		Station 11.	
Length in mm. at the end of 1930.	Average size 1929–30 winter ring.	Range in size of winter ring.	Average size 1929–30 winter ring.	Range in size of winter ring.
19 mm.	14·4	14–15	.	.
18 ,,	13·7	13–15	13·0	12–15
17 ,,	13·8	11–15	12·1	11–14
16 ,,	12·6	11–14	10·7	8–12
15 ,,	12·4	11–13	11·2	8–13
14 ,,	10·8	9–12	8·2	8–12
13 ,,	11·0	10–12	8·0	7–9
12 ,,	9·3	9–10	.	.

The years 1926 and 1930 were years of specially abundant spat-fall in the Cumbrae area for *C. edule* (1926 only), *T. tenuis*, and *T. fabula*, but the conditions which favoured them do not seem to favour *A. alba*. There are no comparable figures for 1926, but 1929, not 1930, was a brood year for *A. alba*. If it is a general rule that the peak years for these species do not coincide it would be a matter of some economic importance for plaice grounds where *A. alba*, *T. fabula*, and *Nucula nitida* are the dominant forms. Such variations in the quantity of fish food in certain Danish areas brought about by the variations in the density of the constituent species have been traced by Blegvad (**1**).

Loch Fyne. CARDIUM EDULE.

During the summer of 1931, and again in the autumn of that year, several of the sand-flats on Loch Fyne were visited. The most interesting of these was at the head of the Loch, a fairly extensive stretch of coarse sand over which a heavy flow of fresh water finds its way. The Cardium zone extends over the lower half of the beach and for some little distance, to about 1 fathom, beyond L.W.M. Compared with other cockle grounds visited, this one is displaced to seaward, the brackish conditions probably being responsible.

In August, 1931, a $\frac{1}{4}$ sq. m. cut was sieved and a large sample picked up at random by hand from each of two stations, one at L.W.M. and the other at a point 100 yards up the beach. Again in October similar collections were

made, only on that date the tide was not quite so good and the October stations are therefore some little distance landward of the August ones. In addition some dredging was done, in about 1 fathom, to secure some of the large cockles which could be seen from the boat, and to determine the seaward limit of distribution.

When the intertidal collections were examined they were seen to be remarkable in that, with the exception of two old specimens from L.W.M., all belonged to the 1929 or 1930 spat, the former predominating. The few cockles secured from below L.W.M. were all very much older. That is to say above L.W.M. all the cockles had been killed off after the 1928 spat-fall but before that of 1929. Almost certainly this is to be accounted for by the specially hard winter of 1928–29. During February the frost was very severe. According to statements from local fishermen the area at the head of the loch was then frozen up to an unusual degree. Other species seem to have suffered as well. On such a ground one would expect to find *Macoma baltica* in quantity, especially in the upper levels, yet only a few old, many-ringed, and much eroded, specimens were found near L.W.M. It would appear, therefore, that only the Cardium population below L.W.M. and the old deeply buried Macoma escaped destruction by the frost.

There are several interesting points about this new cockle population. In the first place the continued absence at either station of any specimens older than the 1929 spat, such as occur below L.W.M., shows that there has been no migration. A similar conclusion was reached from a study of the sizes of the various annual rings for the cockle population on the Hunterston Sands.

The falling off in the rate of growth from the seaward to the landward area of the cockle ground, so well shown on the Hunterston beds, is very evident here also (Table 12).

TABLE 12.

TABLE SHOWING THE NUMBERS OF EACH YEAR'S SPAT, THE AVERAGE SIZE OF THE WINTER RINGS, AND LENGTH OF THE COCKLES IN THE QUANTITATIVE SAMPLES FROM THE HEAD OF LOCH FYNE.

Date of collection.	Station.	Spat of	Number of specimens.	Average size 1930–31 winter ring.	Average size.
13.8.31	L.W.M. ¼ sq. m.×2	1929	21	22·6 mm.	34·8 mm.
		1930	6	.	18·9 mm.
,,	100 yards up from L.W.M. ¼ sq. m.	1929	10	16·3 mm.	25·0 mm.
		1930	5	.	11·6 mm.
28.10.31	L.W.M. ¼ sq. m.×2	1929	14	20·2 mm.	30·7 mm.
		1930	5	.	20·1 mm.
		1931	12	.	6·0 mm.
,,	100 yards up from L.W.M. ¼ sq. m.	1929	31	19·0 mm.	26·0 mm.
		1930	3	.	15·7 mm.
		1931	0	.	.

The shells from this beach are much eroded, especially those from the upper parts. The specimens collected in August were not much damaged, but those taken in October in the upper levels were so badly eroded as to be almost, or entirely, perforated in the upper half of the shell.

The range in size of the 1929 spat taken in October is 12 or 13 mm., and it is of interest to trace the relationship between the length of the winter ring on any shell and the length of the shell itself. In Table 13 the average size of the winter rings of all specimens at each mm. is given, and at both stations it is evident that there is a definite relationship. In general, those specimens which have grown best in their first year of life are the largest at the end of their second, and *vice versa*. There are, of course, individual cases where the leeway is made up.

TABLE 13.

TABLE SHOWING A COMPARISON OF THE AVERAGE SIZES OF THE WINTER RINGS FOR INDIVIDUALS AT EACH MM. SIZE IN OCTOBER, 1931. HAND SAMPLES.

L.W.M. 13.8.31

Number of specimens.	Length in mm.	Average size of 1930–31 winter ring.
1	36	27
1	35	25
5	33	24·2
5	32	23·8
27	31	22·9
23	30	21·7
17	29	21·2
5	28	20·0
6	27	21·0
3	26	20·0
1	25	18·0
1	24	18·0

L.W.M. 28.10.31

Number of specimens.	Length in mm.	Average size of 1930–31 winter ring.
1	35	24
3	34	21
8	33	22·1
16	32	21·2
31	31	20·0
32	30	19·9
15	29	20·2
10	28	18·9
6	27	18·8
5	26	19·2

100 yards up from L.W.M. Station 13.8.31

Number of specimens.	Length.	Average size of 1930–31 winter ring.
1	33	20
1	31	25
6	30	23·2
12	29	22·1
16	28	21·2
21	27	20·2
12	26	19·2
8	25	17·9
7	24	17·0
2	23	14·5
1	21	11·0

100 yards up from L.W.M. Station 28.10.31

Number of specimens.	Length.	Average size of 1930–31 winter ring.
4	30	22·5
5	29	21·8
26	28	20·3
39	27	20·0
40	26	19·0
34	25	17·6
26	24	16·8
15	23	16·8
8	22	17·5
1	21	15
1	20	13
2	19	14·5
3	17	13·7

There is also the comparison between the weight of the shell and its length at the two positions on the beach to be considered (Table 14). This relationship has only been worked out for the August collections, the extent of the erosion in the October material making comparison impossible. For the August samples the graph of $\frac{\text{weight}}{\text{length}}$ (Fig. 3) is not a straight line, possibly in part due to the small number of specimens, but, taking the best line amongst the points, it is clear that at L.W.M. the shells are distinctly heavier, length for length, than those further up the beach.

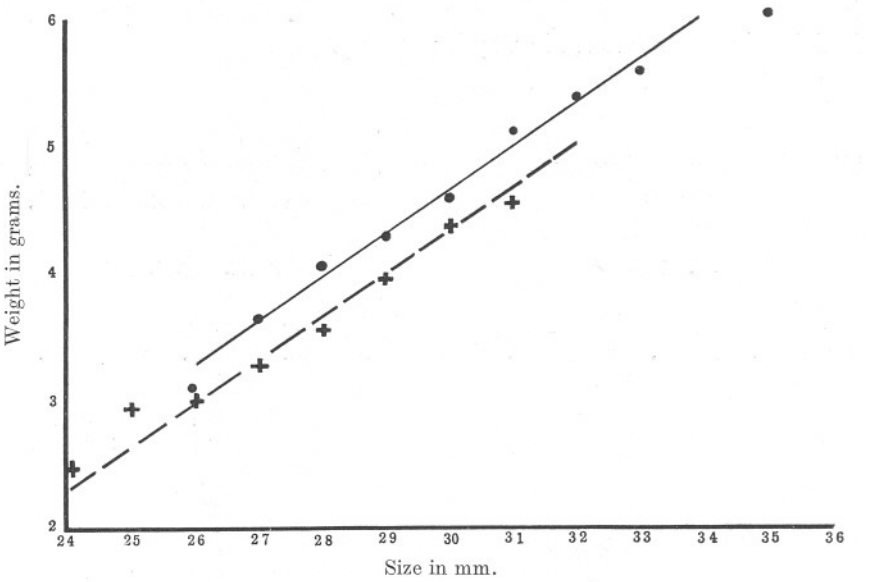

FIG. 3.—Showing the relation length to weight in *Cardium edule* shells from Loch Fyne Head. Dots represent the figures for the Station at L.W.M.; Crosses represent the figures for the Station one hundred yards up the beach.

Thus *Cardium edule* not only grows faster at the seaward part of its range, but lays down a heavier shell, i.e. has more available lime. The other common shore form, *Tellina tenuis*, on the other hand, grows best at the landward part of its range provided the beach remains uniform in nature, e.g. Kames Bay or St. Andrews Sands. Where the Tellina ground overlaps the Cardium ground *T. tenuis* gets smaller before dying out, seemingly not thriving well on the type of ground occupied by Cardium. This difference between the two species can be accounted for by the difference in feeding habits. Cardium burrows in the sand with its short siphons projecting a little way above the surface and draws in a strong current of water from which it collects its food. The further up the beach, therefore,

the longer it is exposed during ebb tide and the less time it has for feeding. Tellina, on the other hand, feeds on detritus. It has long siphons which are kept waving about, the inhalent one searching the bottom and picking up particles which can be seen travelling down the siphon. On a short even beach, therefore, even the higher levels for a short time provide a period of rich feeding for an animal of this kind.

TABLE 14.

TABLE SHOWING THE AVERAGE WEIGHT FOR EACH MM. LENGTH IN THE SHELLS OF CARDIUM FROM LOCH FYNE.

	L.W.M.		100 yards up from L.W.M.		
Length.	Number of specimens.	Average weight of shell.	Length.	Number of specimens.	Average weight of shell.
35 mm.	1	6·03	31 mm.	1	4·55
34 ,,	.	.	30 ,,	5	4·36
33 ,,	3	5·57	29 ,,	11	3·95
32 ,,	5	5·37	28 ,,	17	3·55
31 ,,	20	5·10	27 ,,	18	3·28
30 ,,	14	4·57	26 ,,	12	3·10
29 ,,	14	4·27	25 ,,	7	2·84
28 ,,	5	4·03	24 ,,	4	2·44
27 ,,	5	3·63			
26 ,,	3	3·07			

TABLE 15.

TABLE SHOWING THE AGE COMPOSITION OF THE COLLECTIONS OF *CARDIUM EDULE* FROM $1\frac{1}{2}$ SQ. M. AT STNS. 21 AND 21A IN DECEMBER, 1931.

Station.	1924.	1925.	1926.	1927.	1928.	1929.	1930.	1931.
21	.	4	39	4	3	3	2	35
21a	2	.	40	.	.	2	2	8

Hunterston Sands.

The rate of growth of the cockle and the differences in size at various levels on these sands were investigated during 1929–30, so during 1931 only a few samples were taken to confirm certain points, chiefly the age-composition of the Cardium population.

The 1926 spat was for several years the dominant year-group in the collections of Cardium and Tellina from Hunterston and Kames Bay respectively, but during 1930 the 1926 Tellina group largely died out and was replaced by the new 1930 spat. To test if parallel events had taken place on the Cardium grounds certain of the stations on Hunterston were re-examined in December, 1931 (Appendix, p. 68). In this case, however, there had been no replacement, the 1926 spat still predominating (Table 15), most of the other year-groups being represented by only one or two specimens.

The growth of the cockle was previously shown to be greatest at the seaward part of its range, and this is confirmed by a comparison of the mean sizes of the various winter rings of the 1926 spat at the two stations (Table 16). The divergence of the means at the two stations increased with each year.

TABLE 16.

TABLE SHOWING THE AVERAGE SIZE OF THE ANNUAL RINGS ON THE 1926 SPAT IN DECEMBER, 1931.

Station.	Winter ring 1927–28.	Winter ring 1928–29.	Winter ring 1929–30.	Winter ring 1930–31.	Length.
21	21·7	30·1	34·3	36·8	39·2
21a	17·7	23·2	25·6	27·6	28·8

RENEWAL OF A LAMELLIBRANCH POPULATION.

With regard to the renewal of a Lamellibranch population on any ground an interesting question arises in the light of the foregoing observations.

It has already been pointed out how, in Kames Bay in September, 1926, the population of *T. tenuis* consisted almost entirely of spat, and how in the autumn of 1930 spat again predominated after the adults had largely disappeared.

The same would seem to have been true of Cardium on the Hunterston Sands in 1926—much spat with few adults, and in subsequent years little spat. Further, in Loch Fyne, the ground was repopulated by Cardium in 1929, a year which, judging from the conditions at Hunterston, did not seem to be an outstanding brood year for Cardium.

The question therefore is: must the adult population almost die out before the ground can be repopulated? if so, is there sufficient spat produced every year to repopulate the ground? or is there a cycle with years of special spat production?

It would seem, from the results just quoted, that the clearing of the ground of adults, for example, as a result of high mortality after spawning or after unusual frost, is the important factor.

There is doubtless a high survival of spat in certain years which affects the rate of repopulation. At Loch Fyne Head, for example, it is not possible to say what difference in density of population there might have been had the frost come in another year. Simply from a study of the age-composition of a collection, without reference to the actual density of population, a poor spat on fallow ground would give the impression of a year of high spat survival.

SUMMARY.

The paper deals with four species from the Clyde Area, namely : *Tellina tenuis*, *Tellina fabula*, *Abra alba*, and *Cardium edule*.

T. TENUIS.

1. The growth at one station below L.W.M. in Kames Bay is considered. This is slower than in any other part of its vertical range.

2. Heavy spat-falls were recorded in 1926 and in 1930, but, in both cases, the spat grew slowly and died off quickly at the stations below L.W.M.

3. The general rate of growth for the species from its upper to its lower limit of distribution in Kames Bay is tabulated.

T. FABULA.

1. An investigation was made at one station in $1\frac{1}{2}$ fathom in Kames Bay during 1931.

2. A length of about 4 mm. is attained at the end of the first year ; 8 mm. at the end of the second year ; and 10 mm. at the end of its third year.

3. The years 1926 and 1930 seem to have been years of exceptional spat-survival for this species as well as for *T. tenuis*.

CARDIUM EDULE.

1. The species was studied in Loch Fyne and on the Hunterston Sands.

2. On the shore at the head of Loch Fyne all Cardium above L.W.M. were killed off by the severe winter of 1928–29. Most of the present Cardium population is derived from the 1929 spat.

3. The rate of growth is greater at L.W.M. than further up the beach.

4. Specimens which have grown slowly in their first year tend to lag behind those which have grown well.

5. The graph $\frac{\text{weight of shell}}{\text{length of shell}}$ is given. Shells from higher levels are, length for length, distinctly lighter than those from lower levels.

6. Two of the stations on the Hunterston Sands were re-examined in the autumn of 1931 and the 1926 spat still found to be the dominant year-group.

ABRA ALBA.

1. Collections from two stations, both in Loch Striven, are discussed.

2. The 1929 spat was the dominant year-group and seems to have grown specially well in its first year.

3. The rate of growth is fast, especially in the first year, and is subject to considerable variation from year to year.

4. The average age for the animal in this area would seem to be about 3 or 4 years.

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APPENDIX.

Positions of the stations at which the various species were collected.

ABRA ALBA.

Stn. 10. Loch Striven Head. Mud.
Stn. 11. Loch Striven. Mid-loch off Inverchoalain. Mud.

TELLINA TENUIS.

Kames Bay. Millport. Sand. $1\frac{1}{2}$ fms.

TELLINA FABULA.

Kames Bay. Millport. Sand. $1\frac{1}{2}$ fms.

CARDIUM EDULE.

Loch Fyne. Intertidal stretch at the head of the loch.
Hunterston Sands.
Stn. 21. South-west end of the sands about half-tide, in the middle of the cockle bed.
Stn. 21a. About 100 yards up the beach from Stn. 21.

Observations on the Fauna and Constituents of an Estuarine Mud in a Polluted Area.

By

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With 2 Figures in the Text.

CONTENTS.

INTRODUCTION.

THE estuarine mud dealt with in this paper lies on what is known as Dingle Beach, an area (the property of the Mersey Docks and Harbour Board) on the north bank of the Mersey Estuary between Liverpool and Garston. It lies in a region of much sewage pollution, as the river Mersey is used as an outlet for untreated sewage from the very densely populated districts on both of its banks ; one large and one small sewer discharge on Dingle Beach itself. The river water in this area is of a fairly low salinity (samples taken at Low Water average 13°/$_{oo}$ and at High Water 20°/$_{oo}$), has a pH of about 7·9 and contains a certain amount of chemical pollution from the tanneries, chemical works and other industrial factories on its banks. In addition, the water contains large quantities of silt in suspension which prevents, to a large extent, the penetration of light and would tend to stop up the pores of certain types of animals (Sponges, Ascidians, etc.) and to eliminate Algæ.

Dingle Beach (Fig. 1) is $\frac{3}{4}$ mile in length and about 300 yards of shore are exposed at low water. Low-water mark of ordinary tides is about

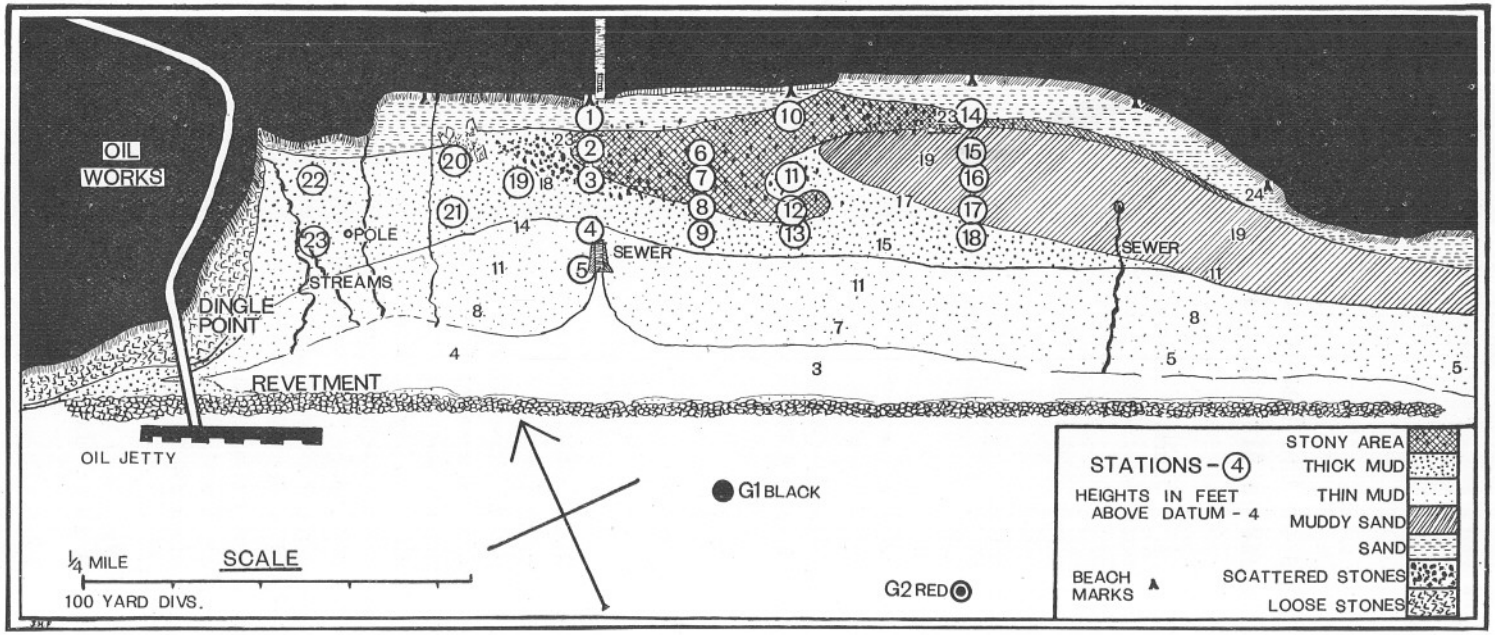

FIG. 1.—A map of Dingle Beach showing the different types of ground, the stations, tidal levels, etc. (The heights in feet above datum and general features taken from The Mersey Docks and Harbour Board chart of the River Mersey (1926). By permission.)

4 feet above datum and high-water mark about 26 feet. The foreshore can be readily divided into several fairly distinct areas :—

I. A stony area near the centre of the beach with limits of 16 and 23 feet above datum.
II. An area of very thick and apparently stable mud on the west and south of the beach, not extending below half-tide level or above 23 feet.
III. An area of thin and unstable mud extending over the lower half of the shore, never extending above half-tide level.
IV. An area of muddy sand of a very similar tidal level to that of the thick mud area but at the eastern end.
V. A sandy area, the whole length of the beach above 23 feet.

These areas and their approximate boundaries and tidal levels are shown in Figure 1.

An attempt has been made to treat these areas from three standpoints :

1. An ecological survey of the fauna present.
2. An analysis of the constituents of the mud, sand, gravel and stones in the soil, to find the exact structure of the medium in which the fauna lives.
3. A quantitative analysis of the Molluscan fauna from a series of stations.

1. Description of the Areas and an Ecological Survey of the Fauna.

I. *The Stony Area.* (Limits of tidal level :—16–23 feet above datum.)

This area consists of shingle lying on a clay bed which is about 7–12 cm. below the surface. The larger stones are toward the surface, smaller ones below, grading into sand before reaching the clay, while the spaces between the larger stones near the surface are filled by a wet mud (Fig. 2). On the border of this area, most particularly to the west side (Fig. 1), are a few large scattered stones, surrounding each of which is usually to be found a small pool the size depending on the size of the stone concerned.

The stony area contains the most varied fauna of any part of the beach. The following species are to be found :—

Clitellio arenarius O.F.M. In great abundance at the surface of the mud filling the spaces between the stones.

Nereis diversicolor O.F.M. In the gravel and coarse sand below the stones, fairly abundant (about 20 per sq. metre).

Mya arenaria L. Extremely abundant but small. It is very significant that (*a*) although Mya is so very prolific in this area it is almost absent on

other parts of the beach, and that (*b*) in places where the underlying clay bed lies deepest the Mya are largest. Specimens rarely reach 3·5 cm. in length, those apparently showing two summers' growth average 1·7 cm. in February. In one small area where there is a sudden drop in the clay bed (<30 cm.) Mya reach 5·0 cm. (averaging 3·2 cm.). No large shells have been found cast up. The spat falls in late May (2–3 mm.).

Macoma balthica (L). Extremely abundant in the mud at the surface, but average only 1–1·5 cm. in length. Both white and pink varieties are present in almost equal numbers and there does not seem to be any correlation between colour and either size or sex. The spat falls in late May and early June, a time similar to that given by Stephen (**5**) for Macoma in the Firth of Forth. This year, however (1932), small but definite quantities of spat (3 mm.) were found in February.

Cardium edule L. Very small and infrequent, rarely reaching 1 cm. in length even when showing 3 rings.

Mytilus edulis L. Occasional specimens showing up to 3 rings and measuring only 1·5 cm. in length may be found attached to small stones; they are, as a rule, partly buried in the mud.

Hydrobia ulvæ (Pennant). Scattered over the surface but in no great quantity.

Littorina littorea (L). Abundant. They are to be found crawling over the surface of the area and settling on the larger drier stones. As many as 40 may be collected from a stone of barely a cubic foot. Specimens appear to be smaller towards the N.W. end of the area.

Littorina littoralis (L). In a similar habitat to *L. littorea* but occurs only infrequently. Dead shells are common.

Carcinus mœnas (Pennant). Specimens up to 2 cm. across the carapace are to be found occasionally amongst the stones.

Gammarus locusta (L). In a similar habitat to Carcinus.

Corophium volutator (Pallas). Infrequent, buried in the mud.

Balanus balanoides (L). Present on some of the larger stones.

Dead shells of *Scrobicularia plana* (Da Costa) exist. They are mostly broken and all have apparently been dead a long time.

A diagrammatic section through the stony area showing the distribution of fauna and constituents will be found in Figure 2, page 83.

II. *The Area of Thick Mud.* (14–23 feet above datum.)

The layer of stiff mud covering this area varies from about 30–60 cm. in depth and is jet-black just below the surface. The mud, although becoming distinctly wetter at the lower extremities of the area, is nevertheless quite stable as it is covered with a thick layer of diatoms, giving the mud a chocolate colour especially in spring and summer. Bright green patches occur in places marking the position of enormous numbers

of *Euglena limosa* Gard. Mr. Ghazzawi (**3**) has identified the following diatoms from this area :—

Colletonema neglectum Thwaites ; *C. subcohærens* Thwaites ; *Melosira nummuloides* Kütz ; *Rhabdonema minutum* Kütz ; *Achnanthes subsessilis* Kütz ; *Shizonema smithii* Ag ; *Licmophora lyngbgei* (Kütz) ; *Amphora cymbifirca* Gregory ; *Synedra affinis* Kütz ; *Pleurosigma quadratum* Smith ; *P. fasciola* (Kütz) ; *Orthosira marina* Smith (not living) ; *Navicula bahusiensis* Grun ; *Nitzchia tænia* Smith ; *N. sigma* Smith.

The following species are to be found :—

Clitellio arenarius O.F.M. In great abundance at the surface.

Nereis diversicolor O.F.M. Very abundant at a depth from 10–20 cm. Occasionally on the surface. In and near the pools are to be found *N. diversicolor* of a vivid green colour resembling that of *Eulalia viridis*.

Mya arenaria L. Comparatively infrequent and small.

Macoma balthica (L). In immense numbers in the upper three or four centimetres of mud, and of a size similar to those in the stony area.

Cardium edule L. Specimens occur occasionally but are always small.

Littorina littorea (L). Plentiful toward high-water mark crawling on the surface of the mud, apparently browsing on diatoms. Skeletons of diatoms are numerous in the fæces.

Littorina littoralis (L). In a similar habitat to *L. littorea* but rare.

Hydrobia ulvæ (Pennant). Scattered fairly thickly over parts of the area.

Corophium volutator (Pallas). Only occasional specimens.

III. *The Area of Thin Mud.* (Below 14 feet above datum.)

The mud covering the area below half-tide level is of a very thin and wet nature, on which it is impossible to walk. It is possible to investigate parts of the area by using the brickwork of the sewer as a foothold. The mud is very unstable and apparently contains little macroscopic life. A few *Nereis diversicolor* occur in the thicker parts, and where occasional large stones project out of the mud, straggling specimens of *Balanus balanoides* find attachment.

IV. *The Area of Muddy Sand.* (11–23 feet above datum.)

In this area a layer of fairly clean sand lies on the surface, but removal of the upper centimetre reveals a muddy sand black in colour. The surface of the area is dotted with small holes which indicate the presence of *Corophium volutator*. Patches of diatoms and of *Euglena limosa* occur

in places but are not so abundant nor as luxurious as those on the thick mud area. The following species occur :—

Corophium volutator (Pallas). In very great abundance living just below the surface.

Nereis diversicolor O.F.M. In no great abundance.

Mya arenaria L. Infrequent and small.

Macoma balthica (L). Present, but in no great numbers.

Littorina littorea (L). Common on the borders of the stony area and comparatively big.

Littorina littoralis (L). Occasionally only.

Dead shells of *Scrobicularia plana* occur also in this area.

V. *The Sandy Area near H.W. Mark.* (Above 23 feet above datum.)

This area, at and near high-water mark, consists of coarse, clean sand. The constituent particles are mostly between 350μ and 500μ. The fauna is poor and consists of a few Littorina and Carcinus. No additional fauna is found by digging.

Stranded on the beach chiefly in summer may be found *Aurelia aurita* (L) and *Pleurobrachia pileus* (O.F.M.), while in the small pools *Crangon vulgaris* L. is abundant. *Liparis montagui* (Donovan) and *Gobius minutus* Pallas are occasional.

2. An Analysis of the Constituents of the Grounds.

I. *The Stony Area.*

Mention has already been made (p. 71) of the structure of the stony area and the general arrangement of the constituents. (See also Fig. 2.) The exact analysis of the constituents was made in the following manner. The frame used in the quantitative analysis of the Mollusca (p. 79) was used to mark out a definite area (1/16 sq. metre) and the whole of the material in that area down to the clay bed placed in a canvas bag, the collector using his own judgment as to whether an individual stone on the border line would be taken or disregarded.

This material, on reaching the laboratory, was washed through a series of sieves with round holes of the following diameters—5 mm., 4·5 mm., 4 mm., 3·5 mm., 3 mm., 2·5 mm., 2 mm., 1·5 mm., 1 mm. and 0·75 mm. Most of the material passing the last sieve was retained in a large vessel, but the silt was lost. The various grades were all dried separately and that retained on the first sieve graded by means of sieve plates with holes of 50, 25 and 10 mm. diameter ; that passing the last sieve was sifted through gauges with square holes of 500, 350 and 150μ.

Allen (1) grades material as follows :—

I. *Stones.* All inorganic material which will not pass through a 15-mm. sieve.
II. *Coarse Gravel.* Material left on a 5-mm. sieve.
III. *Medium Gravel.* Material left on a 2·5-mm. sieve.
IV. *Fine Gravel.* Material left on a 1·5-mm. sieve.
V. *Coarse Sand.* Material left on a 1-mm. sieve.
VI. *Medium Sand.* Material left on a 0·5-mm. sieve.
VII. *Fine Sand.* Passing 0·5-mm. sieve but which settles in one minute after stirring.
VIII. *Silt.* Remains in suspension at the end of one minute.

These terms, with the exception of the first, will be used here ; a 10-mm. sieve however is used as the defining mechanism for stones. Although Allen expresses the results as percentages of the total weight it was thought to be of more value to express results as volumes, this not being dependent upon the specific gravities of the various materials and as it is volume that is the biological factor concerned.

The volumes were obtained by the Archimedes principle, dry material being put into the water and all the air bubbles shaken out. Samples from two stations of the stony area are given (Table I), and as the amount of material used in each case is the same, the figures are comparable without being reduced to percentages.

To complete the total volume of about 4500 c.c. ($25^2 \times 7 = 4375$) there is required (1) water, (2) silt, and (3) living material. In this last case it will readily be seen that 295 molluscs found in this space will occupy an appreciable volume. ($295 = 1/16 \times 4720$: Stn. 7, Table VIII.)

A comparison of the two samples in Table I shows that although they compare very favourably, Station 6 (nearer high-water mark—Fig. 1) is of a slightly finer texture than Station 7 even although Station 6 contains stones above 5 cm. diameter. There is a certain amount of selection in taking a sample as the collector naturally tends to avoid including any large stone.

Mud from between the stones was analysed by means of a Nobel's Elutriator. The apparatus had four separators of internal diameters at the widest parts of 6 cm., 9·5 cm., 13·5 cm. and 17·5 cm. approximately, and was run with a constant head of water of 106 cm. (34 cm. stock and 72 cm. siphon) and the outlet pipe drawn out to form a resistance. With the tap full open the elutriator discharged at approximately 600 c.c. per minute, the rate of flow at the widest parts of the separators was therefore 212·4 mm., 84·6 mm., 41·9 mm. and 25·0 mm. per minute.

TABLE I.

Results of Analysis (Stony Area).

Material.	Particles left on a Sieve of	Approx. volumes in c.c. Station 7.	Totals.	Station 6.	Totals.
Stones	50 mm.	—		275	
,,	25 mm.	550		320	
,,	10 mm.	200	750	90	685
Coarse Gravel	5 mm.	60	60	49	49
Medium Gravel	4·5 mm.	4·0		6·5	
,, ,,	4·0 mm.	6·0		9·0	
,, ,,	3·5 mm.	6·0		8·0	
,, ,,	3·0 mm.	7·0		11·0	
,, ,,	2·5 mm.	6·5	29·5	12·0	46·5
Fine Gravel	2·0 mm.	3·5		7·0	
,, ,,	1·5 mm.	7·0	10·5	13·0	20·0
Coarse Sand	1·0 mm.	6·5	6·5	10·0	10·0
Medium Sand	0·75 mm.	12·0		13·0	
,, ,,	0·5 mm.	30·0	42·0	42·0	55·0
Fine Sand	350μ	75·0		138·0	
,, ,,	150μ	250·0		240	
,, ,,	Passing 150μ	35·0	360·0	30	408·0
Total Volume			1258·5		1273·5

TABLE II.

Wet weight of sample 15·50 *grams.*

Material.	% of wet wt.	% of dry wt.
Mud (wet)	100	—
,, (dry)	73·36	100
Water	26·64	
1st separator	50·71	69·11
2nd ,,	0·44	0·60
3rd ,,	3·83	5·22
4th ,,	1·66	2·32
Silt	8·42	11·48
Total	65·06	88·73
Error*	8·30	11·27

* The error represents a total error made by a combination of errors in :—

(1) Sampling (two samples not of identical consistency).
(2) Experimental error (including loss of material in transfer).
(3) A loss of silt from the elutriator.
(4) A loss of soluble salts.

Material falling at a rate greater than the flow would be retained in its respective separator. Silt passes over. Measurements of the materials thus separated were found to be :—

(1) greater than 100μ
(2) 60–100μ
(3) 20–60μ
(4) less than 20μ

Two identical (as far as possible) portions of mud obtained by weighing to 0·01 gm. were treated, one being dried in a low-temperature oven and the other elutriated. The apparatus was started slowly and gradually increased to full speed. Almost all the silt came over together and was collected in jars and filtered. The sediments were dried and weighed.

Mud from amongst the stones gave the analysis given in Table II by this method.

It is really this mud rather than the stones and gravel that forms the medium in which the Macoma live. (See Fig. 2.)

II. *The Area of Thick Mud.*

Double samples of mud from this area were taken in identical glass tubes (two halves of a combustion tube) fitted with rubber bungs. The two mud samples were taken as close to each other as possible so that they might be considered of identical consistency. Analyses were made of the upper layer and of a layer below 3 cm. depth. The samples were treated in the same way as the mud from the stony area (see above). The following results given in Table III were obtained.

TABLE III.

Material.	Depth 0–2·5 cm. *Wet wt. of sample* 19·04 *gm.* % of wet wt.	% of dry wt.	Depth 3–5·5 cm. *Wet wt. of sample* 18·07 *gm.* % of wet wt.	% of dry wt.
Mud (wet)	100	—	100	—
,, (dry)	65·32	100	60·87	100
Water	34·68	—	39·13	—
1st separator	38·92	58·76	18·16	29·82
2nd ,,	12·52	18·90	10·54	17·32
3rd ,,	2·81	4·35	4·46	7·23
4th ,,	5·70	8·61	2·67	4·39
Silt	0·86	1·32	8·38	13·77
Total	60·81	91·94	44·21	72·53
Error	4·51	8·06	16·66	27·47

The large error in the second sample is due to three small stones present in the first tube giving the mud a greater dry weight. It will be seen that

the lower layer contains more water and much more silt than the upper, a result to be expected from the appearance of the sample in the tube. The samples are taken from Station 11.

III. *The Area of Thin Mud.*

The results of an analysis of a sample from Station 4 at the border of the thin and thick muds is given in Table IV, and one from the really thin mud (Station 5) is given in Table V.

TABLE IV.

Material.	Depth 0–2·5 cm. *Wet wt. of sample* 20·32 *gm.* % of wet wt.	% of dry wt.	Depth 3–5·5 cm. *Wet wt. of sample* 14·28 *gm.* % of wet wt.	% of dry wt.
Mud (wet)	100	—	100	—
,, (dry)	54·24	100	58·64	100
Water	45·76	—	41·36	
1st separator	24·14	44·50	26·93	45·76
2nd ,,	6·43	11·84	10·58	18·05
3rd ,,	4·44	8·19	5·03	8·58
4th ,,	4·89	9·01	6·77	11·55
Silt	6·69	12·34	3·25	5·54
Total	46·59	85·88	52·56	89·48
Error	7·65	14·12	6·08	10·52

TABLE V.

Wet weight of sample 9·73 *grams.*

Material.	% of wet wt.	% of dry wt.
Mud (wet)	100	—
,, (dry)	27·97	100
Water	72·03	—
1st separator	nil	nil
2nd ,,	0·42	1·51
3rd ,,	2·56	9·13
4th ,,	5·34	19·09
Silt	19·64	70·23
Total	27·96	99·96
Error	0·01	0·04

IV. *The Area of Muddy Sand.*

This sand, from a glance at the sample in the tube, appears to be coarser and much freer from silt than the other areas. Table VI shows the results of an analysis from Station 16.

TABLE VI.

Wet weight of sample 21·59 *grams.*

Material.	% of wet wt.	% of dry wt.
Mud (wet)	100	—
,, (dry)	66·45	100
Water	33·55	—
1st separator	47·30	71·19
2nd ,,	9·09	12·99
3rd ,,	1·04	1·57
4th ,,	6·34	9·55
Silt	negligible	negligible
Total	63·77	95·30
Error	2·68	4·70

V. *The High-Water Zone.*

The analysis of the clean sand at high-water mark (Station 1) gives a result to be expected. (Table VII.) Sieving a sample of sand shows the particles to be mostly between 350 μ and 500 μ in size.

TABLE VII.

Wet weight of sample 15·23 *grams.*

Material.	% of wet wt.	% of dry wt.
Sand (wet)	100	—
,, (dry)	81·90	100
Water	18·10	—
1st separator	79·09	96·56
2nd ,,	0·31	0·38
3rd ,,	—	—
4th ,,	—	—
Silt	—	—
Total	79·40	96·94
Error	2·50	3·06

3. A Quantitative Analysis of the Molluscan Fauna.

The method of estimating the numbers of molluscs by marking out a definite area and sieving the contents was adopted here. To mark the area, a square wooden frame was used with internal measurements of 25 cm. and 7·5 cm. depth. The sieve was a wire framework with square holes 2·5 mm. across. In the case of the stony area where it was impossible

TABLE VIII.

Comparison of the Molluscan Fauna in Different Grounds at Similar Tidal Levels.

NUMBERS OF MOLLUSCS PER SQUARE METRE OF SURFACE.

L.la = *Littorina littorea*. L.ls = *Littorina littoralis*.
M.a = *Mya arenaria*. M.b = *Macoma balthica*.
C.e = *Cardium edule*. M.e = *Mytilus edulis*.

25 feet			*Stn.* 1. *Sand.* nil.			
23 feet					*Stn.* 10. *Gravel with mud.* L.la 272 L.ls 0 M.a 32 M.b 560 C.e 0 M.e 0	*Stn.* 14. *Gravel.* L.la 16 L.ls 0 M.a 0 M.b 208 C.e 0 M.e 0
20 feet	*Stn.* 20. *Mud (with stones).* L.la 0 L.ls 0 M.a 128 M.b 960 C.e 0 M.e 0		*Stn.* 2. *Stony.* L.la 112 L.ls 0 M.a 48 M.b 576 C.e 0 M.e 0	*Stn.* 6. *Stony.* L.la 256 L.ls 0 M.a 2016 M.b 1488 C.e 0 M.e 64		*Stn.* 15. *Muddy Sand.* L.la 0 L.ls 0 M.a 0 M.b 848 C.e 16 M.e 0
18 feet	Average of *Stn.* 19 *and* 22. *Thick Mud.* L.la 0 L.ls 0 M.a 72 M.b 5928 C.e 0 M.e 0		*Stn.* 3. *Mud with Stones.* L.la 96 L.ls 0 M.a 64 M.b 1120 C.e 0 M.e 32	*Stn.* 7. *Stony.* L.la 0 L.ls 0 M.a 544 M.b 4176 C.e 0 M.e 0	*Stn.* 11. *Thick Mud.* L.la 48 L.ls 16 M.a 336 M.b 2192 C.e 0 M.e 0	*Stn.* 16. *Muddy Sand.* L.la 0 L.ls 0 M.a 32 M.b 1827 C.e 0 M.e 0
16 feet	*Stn.* 21. *Thick Mud.* L.la 0 L.ls 0 M.a 64 M.b 5264 C.e 16 M.e 0			*Stn.* 8. *Thick Mud with a few stones.* L.la 516 L.ls 16 M.a 434 M.b 2044 C.e 0 M.e 0	*Stn.* 12. *Stony.* L.la 288 L.ls 48 M.a 3872 M.b 4736 C.e 16 M.e 16	*Stn.* 17. *Muddy Sand.* L.la 0 L.ls 0 M.a 0 M.b 416 C.e 0 M.e 0
15 feet	*Stn.* 23. *Thick Mud with fresh water.* L.la 0 L.ls 0 M.a 0 M.b 112 C.e 0 M.e 0			*Stn.* 9. *Thick Mud.* L.la 0 L.ls 0 M.a 80 M.b 3744 C.e 16 M.e 0	*Stn.* 13. *Thick Mud with a few stones.* L.la 64 L.ls 16 M.a 512 M.b 3904 C.e 32 M.e 0	*Stn.* 18. *Thick Mud.* L.la 0 L.ls 0 M.a 16 M.b 2678 C.e 0 M.e 0
14 feet			*Stn.* 4. *Border of Thick and Thin Muds.* L.la 0 L.ls 0 M.a 16 M.b 372 C.e 48 M.e 0			
11 feet			*Stn.* 5. *Thin Mud near Sewer.* nil.			

to insert the frame, as in the mud or sand, it was held as firmly as possible until the stones on the border-line had been picked out by hand. Sieving was a laborious task as it was impracticable to reach the river and the pools on the shore were never more than a couple of inches in depth.

The molluscs were then picked out of the sieve and counted, only living ones being taken ; dead and broken shells were ignored.

The stations from which the counts were made are shown in Figure 1. They have been so arranged that correlation can be made between stations in the same area but of different tidal levels, and between stations of the same tidal level but in different areas. Each count is multiplied by 16 to give the number which would be taken from a square metre of similar ground. The results are given in Table VIII and are arranged so that these correlations can be easily made out. The table is discussed in the following section.

In addition to those molluscs mentioned in Table VIII, *Hydrobia ulvæ* (which passed through the sieve) was found to be most abundant at Stations 19 and 21. One living specimen of *Scrobicularia plana* was found near Station 20.

4. Correlation of the Fauna with Type of Ground and Tidal Level.

The correlation of the fauna with both the type of ground and tidal level is expressed in Tables VIII and IX. Table VIII shows the relationship of the various stations and the actual fauna at each, with both tidal level and type of ground, while Table IX shows the comparison of average numbers of molluscs in each area with the results of the analysis of the ground constituents.

Although the absence of macrofauna at Station 1 is almost certainly due to tidal level, that at Station 5 must be due to the thin mud, a medium of waterlogged silt which would choke the breathing apparatus of most animals.

Mya arenaria seems to be associated with stones and is found in numbers only in the stony area and stations near the border (Stns. 9, 11, 13, 19, 20). The mean figure given for the stony area (Table IX) is 502 per square metre, but is increased to 1716 per sq. metre if the high-water Stations, 2, 10 and 14, are omitted. This latter figure gives a more accurate idea of their abundance. Their abundance increases from high-water mark towards a lower tidal level (*cf.* Stn. 10 with 32 and Stn. 12 with 3872 per sq. metre) and shows a marked decrease in different areas at the same tidal level. *Cf.* Station 12 (stony area), 3872 : Station 17 (muddy sand), 0 :

Station 21 (thick mud), 64. Their small size (p. 72) may be attributed to any or all of the following factors :—

1. Fairly high tidal level. There are no stony areas near low-water mark. Size as well as numbers increase in the area towards low-tidal levels.
2. Shallowness of available ground. Larger specimens are found in small areas where the clay bed lies deeper.
3. Shortage of food through overcrowding.
4. Low salinity (13°/$_{oo}$).
5. Presence of a thick mud through which their siphons have to pass (Fig. 2).

TABLE IX.

Limits of Tidal Level. L.W. (ordinary tide)=4 feet. H.W. (ordinary tide)=26 feet.	Area of Thin Mud. 4–14 ft.	Area of Muddy Sand. 11–23 ft.	Area of Thick Mud. 14–23 ft.	Stony Area. 16–23 ft.	Sandy Area at H.W. above 23 ft.
Data from Stn.	5	16	11	Average of 6 and 7	1
Water % wet weight	72·03	33·55	34·68	26·64	18·10
Volume in c.c. per 625 sq. cm. surface area, and 7 cm. depth.					
Stones	nil	nil	nil	718	nil
Gravel	negligible			108	negligible
Sand	see below			71	see below
Analysis of substance by elutriation (in the case of the stony area, of the mud from between the stones). Results expressed as % dry weight.					
Grade 1 above 100μ	nil	71·19	58·76	69·11	96·56
Grade 2 60–100μ	1·51	12·99	18·90	0·60	0·38
Grade 3 20–60μ	9·13	1·57	4·35	5·22	nil
Grade 4 below 20μ	19·09	9·55	8·61	2·32	—
Silt	70·23	nil	1·32	11·48	—
Mean No. per sq. metre of :					
Mya arenaria	0	11	200	502	0
Macoma balthica	0	1030	3071	1443	0
Cardium edule	0	5	6·4	2	0
Mytilus edulis	0	0	0	14	0
Littorina littorea	0	0	11·2	162	0
Littorina littoralis	0	0	3·2	8	0

It is significant that spat of Mya (< 3 mm.) may be found in quantity on all areas of the beach in late May, but it disappears in June on all but favourable grounds.

Macoma balthica is abundant wherever there is thick mud but becomes less so as this changes either to sand or to thin mud [*cf.* Stn. 9 (thick mud), 3744 : Stn. 4 (border of the thin mud), 372 : Stn. 17 (border of muddy sand), 416]. The mud between the stones (Fig. 2) may be quite as good a

medium for Macoma as the thick mud, for it is possible that a square metre containing stones with 1443 specimens might contain as many Macoma per actual unit of mud as the latter (homogeneous mud) with 3071 individuals. Numbers increase towards low-water mark (*cf.* Stns. 20 and 21). The restriction in sandy areas may be due to a large extent to the absence of sufficient food material on these areas as the diatoms and Euglena are mostly confined to the mud. It should be noted, however, that even in sandy areas (Stns. 15, 16 and 17) the numbers of Macoma are large compared with 150–200 per sq. metre

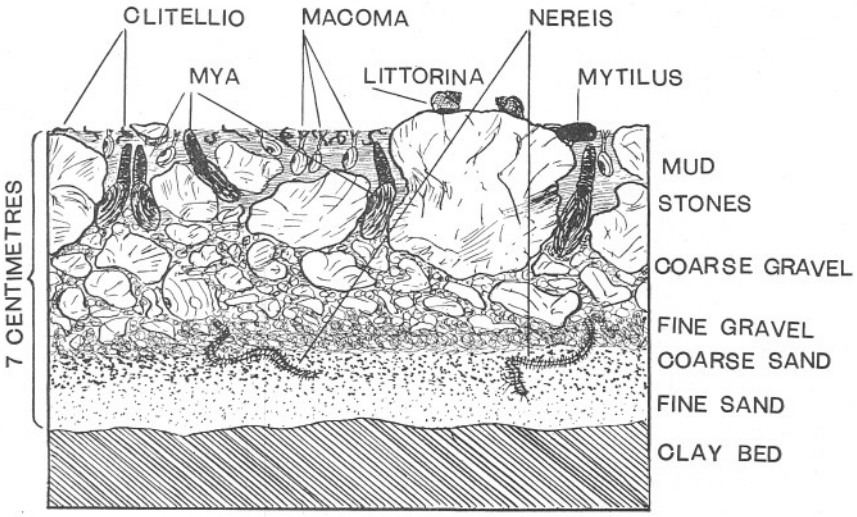

FIG. 2.—A diagrammatic section through a typical part of the stony area to show the relationships of the various constituents and the fauna.

given as a maximum for the same species in a typical area by Stephen (**5**) or with 20 given by Petersen (**4**) as typical of a Macoma community.

Cardium edule, generally considered as a co-partner with *Macoma balthica* on a muddy shore (Stephen, **6**; Petersen, **4**), is here very poorly represented. Stephen (**6**) notes that Cardium is usually found nearer low water than is Macoma, a statement that is to an extent borne out by examples from Dingle in that Cardium is most common at Stations 9, 12, 13 and 21. Specimens, however, are small (p. 72) and are obviously in an unsuitable habitat. They are in fairly equal numbers in the muddy sand and thick mud.

Mytilus edulis is much dwarfed and only found in the stony area (Table IX). The mud is probably the cause of its small size.

Littorina littorea is abundant in those areas where it may browse on diatoms in the absence of larger algæ, and at the same time benefit by

the holdfast provided by the stones. *Nereis diversicolor* and *Clitellio arenarius* are in their natural habitat. Corophium thrives well in the muddy sand, only occasional though not noticeably stunted ones occur in other areas. The other species with one exception do not require special mention. *Scrobicularia plana* must at one time have been common on Dingle Beach but has apparently almost completely died out; shells, although abundant, seem to be all old and worn and only one living specimen has been found (p. 81).

Increase in the population of the neighbourhood during late years has given rise to a big increase in sewage and hence to corresponding increase in silt deposition (**2**). It is reasonable to suppose that the bulk of the silt has been laid down quite recently (the opinion of those residing in the area tends to support the hypothesis) and in such a way the dying out of Scrobicularia might possibly be explained, though too much stress should not be laid on this point as there is the possibility that Scrobicularia is still present, deep enough in the mud to have, as yet, escaped detection.

The importance of sewage in the river must be emphasised, as sewage products not only provide abundant inorganic salts permitting a luxurious growth of diatoms which, with bacteria, form directly or indirectly the food of almost every living thing on the beach, but that it is also the probable cause of the deposition of silt which has made the foreshore the type that it is (**2**).

Dingle Beach, from the above characters, can be described as a Macoma community but differing from the typical community, "d," as described by Petersen (**4**) in the excessive numbers of molluscs present, and in the absence of forms such as Arenicola. Petersen's community, however, was below low-water mark, although that alone would not account for the difference. Although both Stephen (**5**) and Petersen (**4**) describe their grounds as "muddy" it seems probable that in consistency theirs will more closely resemble the muddy sand of Dingle than the other areas. No analysis is given by either.

Acknowledgments.

I am indebted to Prof. Orton for his continued aid and advice in the carrying out of this survey; to Mr. Ghazzawi for the identification of the diatoms, to the Dept. of Oceanography for the use of the sieves and to the Mersey Docks and Harbour Board for permission to work on the beach, and for permission to reproduce in Figure 1 the heights in feet above datum, etc., given in their chart of the River Mersey (1926).

SUMMARY.

1. An ecological survey of the fauna of Dingle Beach, Mersey Estuary, has been made.

2. A detailed analysis of the constituents of the mud, sand and gravel is given.

3. A quantitative analysis of the Molluscan fauna from a series of stations has been made.

4. Type of ground and fauna at different tidal levels are correlated. *Mya arenaria* is only found in abundance where there are stones. *Macoma balthica* is abundant wherever there is thick mud.

5. Dingle Beach is a type of Macoma community but differing markedly from the typical community described by Petersen as " d."

6. The importance of sewage in producing silt and the part played by sewage in the food chain are discussed.

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The Feeding Habits of the Galatheidea.

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With 7 Figures in the Text.

CONTENTS.

INTRODUCTION.

ALTHOUGH the Galatheidea are conspicuous members of the Anomura and occur commonly both on the shore and in deeper water, no account has yet been given of the different methods of feeding which are found within the group.

While working at the Marine Biological Laboratory at Plymouth some time ago I became interested, as previous workers have been, in the curiously modified third maxillipeds of *Porcellana longicornis*, and decided to examine the mode of feeding in this and related forms.

My best thanks are due to Dr. E. J. Allen, F.R.S., of the Marine Biological Association, and to Professor J. H. Orton, of Liverpool University, for every help and encouragement, and also to Professor J. H. Ashworth, F.R.S., of Edinburgh University, for valuable criticism of the manuscript.

Previous Work.

Dalyell (1853) describes *Porcellana longicornis* as the "fanning or ventilating crab," and mentions the alternating see-sawing action of the third maxillipeds fringed with long hairs; he associates the movements however with respiration. Gosse (1854) points out the use of the maxillipeds in feeding, comparing them with the legs of a barnacle. The account, though brief, is accurate. Zimmermann (1913) states that sweeping hairs are present on the terminal segments of the third maxillipeds in some of the Galatheidea. Potts (1915) compares the third maxillipeds of *P. longicornis* with those of Hapalocarcinus, which is said to feed in a similar way. He states that the stomach contains small unrecognisable fragments, a small proportion of planktonic organisms and occasionally pieces of algæ. Borradaile (1921) states that *P. longicornis* gathers suspended food by means of the long fringes on the third maxillipeds. He suggests that larger pieces may also be seized by the chelæ, but did not observe the process. Hunt (1925) classifies *P. longicornis* as a suspension feeder, and *Galathea nexa* as carnivorous. Orton (1927) observes that *P. longicornis* uses the third maxillipeds alternately, "like whips or lacrosse racquets," for catching suspended particles.

No account has, up to the present, been given of the feeding habits of any species of Galathea.

Bionomics.

The British Galatheidea occur both on the shore and in deeper water. *Galathea nexa*, *G. intermedia*, and *G. dispersa* occur only in deep water. *G. strigosa* can be found during very low tides in certain districts, but it is more commonly taken in deeper water. *G. squamifera* is widely distributed between tidemarks, usually below stones and in crevices.

Porcellana longicornis occurs in deep water in the Cellaria beds and in crevices of Lepralia, under stones and in the roots of Laminaria. It is also found commonly between tidemarks along with *P. platycheles*, which clings to the under surfaces of stones and hides in crevices in the rocks, relying on its protective coloration to escape detection. Zimmermann (1913) states that *P. longicornis* is found only in places scoured free from mud by the tide, while *P. platycheles* inhabits muddy areas. This is not universally the case; in the Plymouth district, both at Rum Bay and on Looe Island, the two species may be found under the same stones, although in the crevices, where a thick deposit of mud accumulates, only *P. platycheles* is present.

A considerable difference exists in the degree of activity of the Galatheidæ and the Porcellanidæ. The Galatheas have retained greater freedom of movement, using their legs for leisurely progression and being

also able to dart rapidly backwards through the water by means of violent flappings of their well-developed tails. The Porcellanas, on the other hand, have become more and more crab-like in appearance and adapted to a sedentary life in crevices and under stones. *P. longicornis* can still creep rapidly over the substratum and sometimes attempts to swim in a feeble manner by flapping its tail. *P. platycheles*, however, has become still more sedentary, scarcely moving, and relying on protective shape and coloration for safety.

Galathea dispersa has been chosen as a typical example of the Galatheidæ from which the other forms do not differ appreciably while *Porcellana longicornis* has been taken as typical of the Porcellanidæ.

STRUCTURE OF THE MOUTH-PARTS.

Galathea dispersa.

Third maxilliped.

The endopodite of the third maxilliped of *Galathea dispersa* is long and mobile (Fig. 1, a). The basal segment is not expanded to form a branchial plate and never fits tightly over the mouth ; it is, instead, long and narrow with a longitudinal crest on the dorsal side bearing a row of strong teeth. The second segment is also elongated and bears on its inner edge two rows of stout setæ. The third segment is short and broad, and carries a brush of setæ pointing dorsally and towards the middle line ; these form an ill-defined cleaning tuft which is used for freeing the antennæ and antennules from particles of dirt. The fourth segment also carries a number of serrated setæ pointing towards the middle line. The terminal segment is long in proportion to its breadth. On its distal end it bears a number of stout setæ, serrated along one edge with close-set teeth, admirably fitted for scraping small particles off the substratum. Covering these over so that they cannot be seen in a ventral view is a tuft of simple, curved setæ which follow the others in the food-collecting movements and sweep up all particles loosened by the serrated setæ. The segment is also provided on its median side with a short row of strong spines with blunt lateral projections.

In *Galathea squamifera* (Fig. 1, b) the arrangement is essentially the same, but the first segment is shorter and broader and the second, third, and fourth are fringed on their inner side with a row of long bipinnate hairs, foreshadowing the condition found in Porcellana. This intermediate condition of the maxillipeds is of considerable interest when it is realised that *G. squamifera* is intermediate in habit between *G. intermedia* and the Porcellanas, occurring with the latter below stones while *G. dispersa* lives openly on the sea floor.

Second maxilliped.

The second maxilliped is also elongated. The penultimate segment is provided with a median tuft of hairs, and the terminal segment is covered with long stout bristles, curved at the points, and provided with a double row of fine teeth.

FIG. 1.—(*a*) Ventral view of the left third maxilliped of *Galathea dispersa* ×6.
(*b*) Ventral view of the left third maxilliped of *Galathea squamifera* ×6.

First maxilliped.

The first maxilliped is a thin plate imperfectly chitinised. The basal endite of the protopodite is thickened dorso-ventrally and provided with stout curved setæ directed dorsally into the mouth opening. The distal endite is flattened dorso-ventrally and provided with similar setæ, which,

however, are directed at right angles to the others and overlap the mandibles.

Maxilla.

The maxilla is also plate-like and weakly chitinised. The two basal endites are provided with a row of stout setæ, curved dorsally towards the mouth, and are also fringed both dorsal and ventral to the seta-row with fine hairs. The two distal endites are similarly provided with hairs and setæ, but these are directed towards the middle line and cover over the mandibles.

Maxillule.

The small maxillule is weakly chitinised and the basal endite is provided with a fringe of close-set hairs covering over a row of curved setæ which are directed dorsally towards the mouth. The distal endite has similar hairs covering a row of short thick spines, which at the anterior angle give place to longer and slenderer setæ.

Mandible.

The mandible is strongly calcified and provided with a sharp incisor process separated from a smooth molar process by a deep groove. The

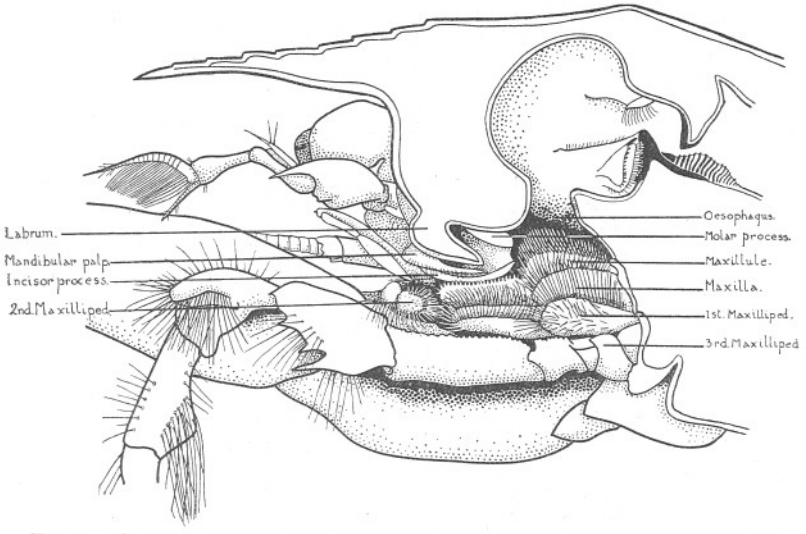

Fig. 2.—A median longitudinal section through *Galathea dispersa* to show the arrangement of the mouth-parts ×5.

palp is well developed and composed of three segments; the terminal segment is edged by a number of short spines.

In a median longitudinal section through the head (Fig. 2) the mouth

is seen lying posterior to the mandibles. The labrum is median in position, and occupies, with the terminal segment of the mandibular palp, the groove between the incisor and molar processes. The mouth opening is bounded laterally by the dorsally-directed setose fringes of the proximal endites of the three pairs of inner mouth-parts, while the mandibles are covered over ventrally by the distal endites and their setæ, which are directed almost at right angles to those of the proximal endites.

Porcellana longicornis.

Third maxilliped.

The third maxilliped (Fig. 3) of Porcellana differs greatly from that of Galathea, and in certain respects approaches more nearly to the typical

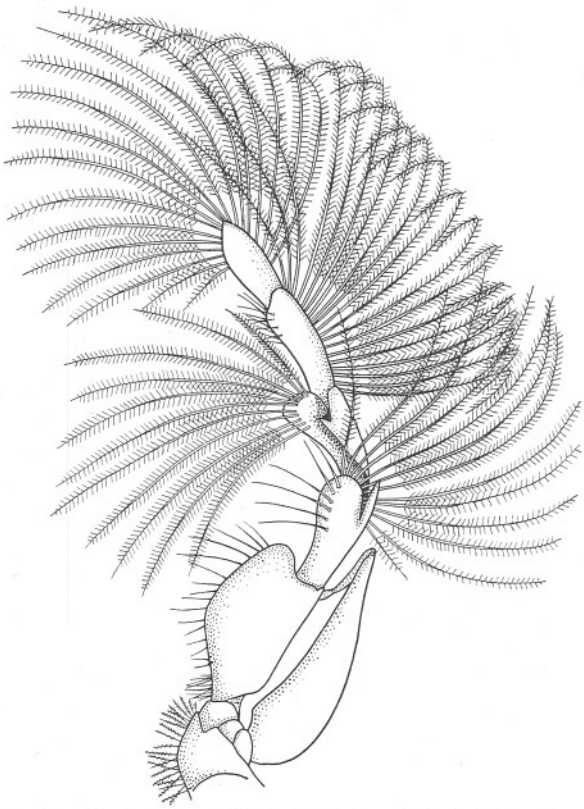

Fig. 3.—Ventral view of the left third maxilliped of *Porcellana longicornis* ×10.

Brachyuran type. The whole appendage is expanded laterally, but the three basal segments in particular have flattened expansions on their ventral surfaces. When the appendage is flexed between segments two and

three, the distal part lies parallel and median to the basal part, and the fringing hairs of the last three segments, and in places the edges of the segments themselves, lie behind these lateral expansions so that when the two appendages are approximated in the middle line and pressed against the ventral surface, a serviceable opercular plate is formed. This plate plays no part in the feeding of the animal, as it does in Carcinus (Borradaile, 1922), but is formed as a protection to the mouth-parts at any sign of danger.

The first segment of the endopodite carries a few simple hairs. The second segment is fringed on its lateral margin by a number of very long pinnate setæ. The third segment bears a row of similar setæ on its median edge, while the fourth bears them on its lateral edge, and the fifth segment carries them on the median, terminal, and lateral margins. The result of this arrangement of setæ is that a spoon-shaped scoop is formed, extending over a relatively large area. The exopodite extends as far forward as the middle of the second segment.

Second maxilliped.

The second maxilliped is similar to that of Galathea, but the terminal tuft is longer and thicker and the setæ are pinnate, instead of being toothed.

First maxilliped.

The first maxilliped is less strongly chitinised than that of *Galathea dispersa*. The basal endite of the protopodite is fringed with long, weak setæ, curved dorsally towards the mouth and overlapped ventrally by a row of fine hairs. The distal endite is covered with fine setæ which are directed towards the mid-ventral line. The endopodite is composed of two segments ; the more distal is bare, but the other is fringed with delicate setæ.

Maxilla.

The maxilla is also weakly chitinised. The proximal endite bears a number of stout setæ curved towards the mouth and covered over ventrally with long hairs pointing in the same direction. Each of the three distal endites is fringed with a brush of soft hairs.

Maxillule.

The proximal endite of the maxillule bears a dense fringe of fine setæ which are curved towards the dorsal surface and point into the mouth. These are covered ventrally by a row of hairs. The distal endite is fringed with shorter and stouter setæ directed towards the middle line.

Mandible.

The mandible does not differ except in size and the arrangement of the teeth of the incisor process from that of *Galathea dispersa*.

FEEDING HABITS.

Galathea dispersa.

The food taken by the Galatheidæ is of two sorts; large pieces of animal and vegetable material, or organic debris and micro-organisms from the deposits of the sea bottom.

An examination of the stomach contents of members of the Galatheidæ shows that the deposit-feeding method is the more usual. The species examined were *G. squamifera*, *G. strigosa*, *G. dispersa*, and *Munida rondeletii*. Always the bulk of the stomach contents was found to consist of unidentifiable detritus, fine sand, small pieces of red and green algæ, a few diatoms and unicellular algæ, parts of small crustacea, eggs, and small gastropods. In addition pieces of muscle and larger pieces of algæ were found in small quantities, showing that the animals had also been feeding on larger material. *G. dispersa* can be taken as a typical example.

Deposit feeding.

When Galathea is feeding on finely divided material the third maxillipeds are used for collecting food, and act as brooms which sweep over the substratum, into grooves and hollows, over the animal's own legs or those of its companions, and collect the diatoms, small animals, algæ, and detritus which may be there. The maxillipeds are extremely mobile at all joints, and capable of being extended both anteriorly and laterally for a considerable distance. When in use for collecting material lying on the substratum in front of the animal, they are stretched out to their fullest extent, and their tips are then pressed against the substratum, often so tightly that the terminal setæ are bent almost at right angles to the segment, as they are drawn back towards the mouth (Fig. 4). The maxillipeds are most frequently used together, but they can also work alternately. If only a small quantity of material has been collected it is lifted from the substratum in the terminal tufts of the appendages and brushed out by the second maxillipeds. If a large amount of material has been gathered, it is lifted between the terminal segments of the third maxillipeds. The substratum below the ventral surface of the animal can also be swept by the maxillipeds, which are bent then ventrally from the base and posteriorly from the second joint of the endopodite so that the opposite side of the setal tuft is used to sweep over the substratum in an anterior direction.

When the brushing movement of each maxilliped is completed, it is folded upon itself so that the third segment of the endopodite is at right angles to the other parts of the limb, and the terminal segments are lying in a plane parallel to the basal segments, but nearer to the midventral line. In this position it is easier for the relatively short second maxillipeds to reach all the setæ.

The second maxillipeds always work alternately. The terminal segments of their endopodites are stretched anteriorly, and their terminal

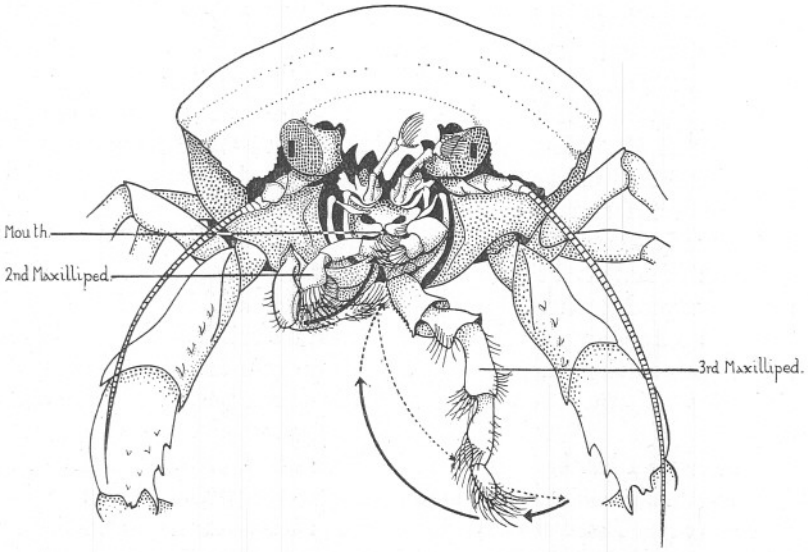

FIG. 4.—An anterior view of *Galathea dispersa* in the act of feeding on finely divided material. The left third maxilliped is fully extended and is collecting particles off the substratum; the right is flexed and the bristle bundles are about to be cleaned out by the second maxilliped. The left second maxilliped has completed the cleaning movement and is carrying the food between the inner mouth-parts to the mouth, ×4. The continuous arrows show the path of the second maxillipeds and of the third when on the substratum; the dotted arrows show the path of the latter through the water.

setæ inserted into the tufts on the third maxillipeds. They are then drawn back towards the mouth and their tips twisted ventral to, and between, their own bases where they are combed out by the inner mouth-parts (Fig. 4). Some kind of sorting mechanism is formed by these appendages, so that suitable particles are allowed to pass to the mouth while unsuitable material is thrust into the outgoing respiratory current made by the flagella of the exopodites of the two pairs of maxillipeds. Since the inner mouth-parts all lie on top of each other, and are partially obscured by the maxillipeds, it is not possible to observe the process of sorting.

Feeding on large pieces of food.

The power to detect food at a distance is not well shown in Galathea, under aquarium conditions at any rate, for pieces of fish or mussel which are out of reach of the chelæ are disregarded. When, however, food is presented to a Galathea at close range, several methods may be employed to convey it to the mouth. If the piece is large it may be seized in one chela while small pieces are pulled off by the other and passed to the maxillipeds. If the piece is small it may be passed direct to the maxillipeds, one or both of which may receive it. If very small it may be brushed out from between the pincers by the terminal brush of one maxilliped and passed direct to the mouth. If larger it may be grasped by the cleaning tufts of both the third maxillipeds and passed on to the second maxillipeds. Usually the cleaning tufts of the third maxillipeds retain a loose hold of the food, while the terminal segments are folded towards each other in the middle line and form a floor to the feeding chamber, ready to exert pressure on the food when required. The second maxillipeds do not pass the food further towards the mouth, but rotate it until a ragged corner is presented to the mandibles. The second maxillipeds take up a position with the terminal bristle bundles inserted into the food, so that they can push it away from the mouth and thus work in opposition to the third maxillipeds which press it closer to the mandibles.

When the piece has been arranged by the second maxillipeds the mandibles prepare for action. The incisor processes separate, the palps and labrum are raised, and the food is pushed in between the mandibles by the pressure of the third maxillipeds. The mandibles then close and the palps and labrum are slightly lowered. The action of the incisor processes is not usually so much a tearing action, as described for other forms, as an actual cutting movement, which takes place in three phases ; the edges close on the food ; pressure is exerted (shown by a pause) ; the mandibles overlap suddenly as they cut through. If the food is tough it may be pulled away by the second maxillipeds while the mandibles grip it, so that tearing does take place under certain circumstances. The subsequent events depend on the nature of the food. Soft material like the body of the polychæte Pomatoceros is bitten through several times without any tearing action, the whole piece being pushed further in between each bite and rapidly swallowed. Tougher material like the anterior end of the worm, which contains an internal supporting structure, is treated differently. Between each bite, which is assisted by the tearing action of the second maxillipeds, the palps and labrum descend, pushing the material up into the œsophagus and cleaning out the groove behind the cutting edge of the mandible. The piece is presented again to the mandibles,

either in the same position as before or after rearrangement. When the operculum of the worm is presented to the mandibles it is rotated many times and attempted from many angles before it can be cut through by the mandibles, although it is finally divided into four or five pieces and swallowed.

The inner mouth-parts appear to assist in retaining the food in position in front of the mandibles, but make no obvious or well-defined movements.

Porcellana longicornis.

The Porcellanidæ have abandoned the deposit feeding habits of the Galatheidæ, and also to a great extent the method of feeding on large particles, although the mandibles show no corresponding weakening of structure. Only once was a *Porcellana longicornis* observed to pass an object of any size to the mouth-parts. A small mollusc became attached to a chela and was removed by the third maxillipeds, presented to the inner mouth-parts and rejected at once in the respiratory current. Whether this was an act of feeding or merely a cleaning reaction is not certain, but appears more likely to have been the latter. On the other hand, Dalyell reports that Porcellana occasionally eats mussel in captivity, and Potts found relatively large pieces of algæ in the stomach. This observation was not confirmed either on material from deep water at Plymouth or from shore specimens from the Firth of Forth. In both cases Hunt's statement that the food of these animals consists of detritus and micro-organisms, closely comparable to that of filter-feeding ciliary feeders, was confirmed. No difference was found between the food or the method of obtaining it in *P. longicornis* and *P. platycheles*, so that the former, which is more easy to observe, has been taken as the type.

The Feeding Mechanism.

The respiratory current plays an important part in the feeding of *P. longicornis*. In contrast to Galathea where the flagella of the exopodites of both sides beat at the same time, in Porcellana they function alternately, so that water is drawn across the front of the carapace and can easily be tested by the antennules. Up to the present it has not been found possible to determine the nature of the stimulus which starts the feeding movements. The animals rarely fed in the morning unless after several days' starvation, but in the afternoon the addition of fine plankton or ground-up algæ to the water was often sufficient to start the process. This curious observation is substantiated by Dalyell's statement that the " ventilating movements " were always more active in the afternoon.

The direction of the respiratory current is shown in Figure 5, when the flagella of the right side alone are beating. As the abdomen of the crab is

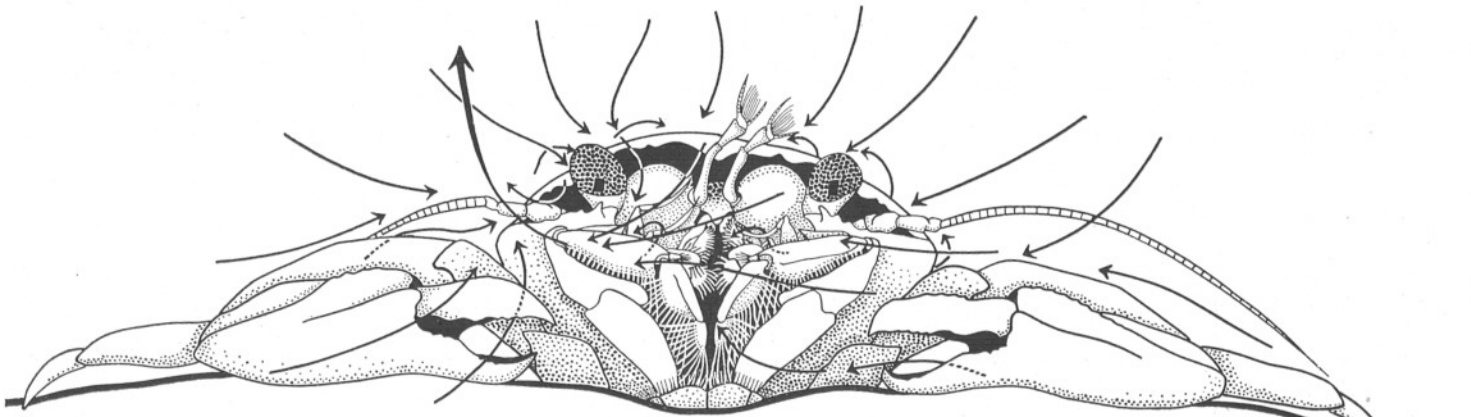

FIG. 5.—An anterior view of *Porcellana longicornis* to show the direction of the respiratory current. The flagella of the right side only are beating, ×6.

held pressed against the substratum no water is drawn over the ventral surface, but a strong current passes anteriorly over the dorsal surface. Water from the animal's left passes behind the chela of that side under the eye and antennules and across the maxillipeds, where it is joined by the water bailed out of the left branchial chamber by the action of the scaphognathite. Water from the anterior direction and from the dorsal surface of the animal on that side passes under the antennules, which are turned towards the left and flick and jerk continually. Water from the right side passes round the antennule and eye as on the other side, but before it reaches the flagella it is turned aside in an antero-lateral direction by the stream of water leaving the right flagella and derived partly from the left side of the body and partly from the right branchial cavity. Water from

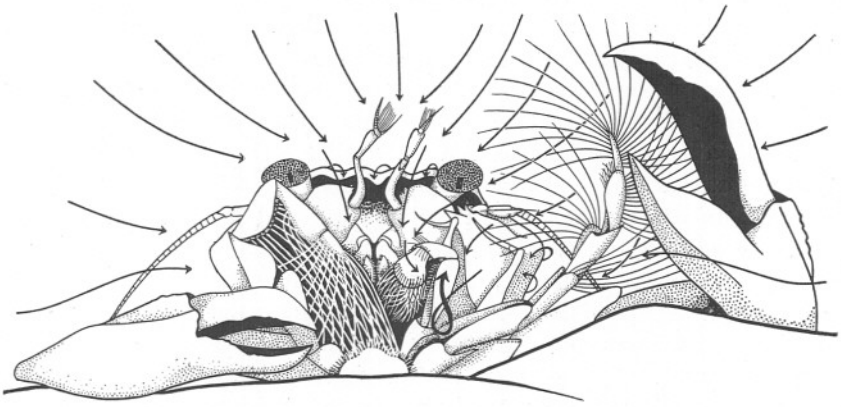

FIG. 6.—An anterior view of *Porcellana longicornis* while feeding, to show the direction of the water currents drawing food in suspension towards the animal, rapidly on the left side, more slowly on its right where the maxilliped is flexed, ×5.

the left branchial cavity joins the main stream of water from that side. Water passing forward over the dorsal surface travels in an anterior direction to the edge of the carapace, where it passes round the eyes and antennæ to join the main streams from the sides.

After a longer or shorter period the flagella of the other side begin to beat spasmodically, and after a few seconds a complete reversal of the current takes place, the left flagella alone beating.

By this means the water round the animal is kept in constant motion, particles in suspension are drawn into contact with the antennules, and the animal is made aware of the presence of suitable food in the neighbourhood.

As soon, however, as feeding commences the beating of the flagella ceases altogether, and another water current, formed by the movements of the third maxillipeds, is set up (Fig. 6). When a feeding animal is

looked at from in front, particles in suspension in the water can be seen moving towards the mouth symmetrically from all directions except from below. Water is also drawn anteriorly over the dorsal surface, and the whole mass is turned away from the mouth in an antero-lateral direction. The current is not of equal intensity on both sides of the body at the same time, owing to the alternate movements of the maxillipeds. The flexing of the maxilliped of one side causes the traction to cease, although the momentum of the water mass continues the movement while the maxilliped is unfolding again, but at a slower rate.

The capture of food is brought about entirely by the movements of the third maxillipeds. Their bases are attached on either side of the middle line and are free to move laterally through about 60° and dorso-ventrally through about 30°. When feeding commences the maxillipeds are lowered to their greatest extent (Fig. 7, a) and are then swung laterally to the limits of their movement without being unbent. When, however, they reach that point the terminal joints are unflexed, and the setæ edging them spread into position (Fig. 7, b). These hairs appear to be under muscular control. This can best be seen in a Porcellana when, with one maxilliped half-unbent, the feeding act has been suspended by some movement which disturbs the crab. When the limb is flexed the hairs on any segment lie parallel and close together, but when it is extended the hairs diverge to form an extensive net. Under the circumstances a gap is often apparent in the row of hairs when those towards the distal end have taken up their spread position, while those towards the proximal end are still parallel. Often it is possible to see one or more hairs leave the parallel series and move across the gap into position beside the others. As the feeding recommences the rest of the hairs spread out also. When the maxilliped is fully extended the hairs stand out for a considerable distance, so that a large spoon-shaped net is produced whose walls are formed by the hairs and their lateral branches. This net projects a considerable distance beyond the front of the carapace and covers a large area. As the maxilliped is flexed the basal segments move towards the middle line through an angle of about 30°. As the hairs come together a considerable volume of water passes between them and many of the particles in suspension are filtered out. The maxillipeds usually move alternately, one being fully flexed while the other is fully extended, but often over long periods of time only one will work, the other remaining in the flexed position.

The movements of the second maxillipeds are closely correlated with those of the third. As soon as one of these appendages is flexed then the basal segment of the second maxilliped on that side is moved through a small angle away from the middle line, and the distal segments are rotated anteriorally and laterally so that the terminal brush of setæ can be inserted into the bases of the series of hairs on the third maxilliped

(Fig. 7, c). The distal segments are then rotated back again into a position with the tips tucked under and into the mouth, while the third maxilliped is turned laterally and again unflexed so that the whole length of the hairs

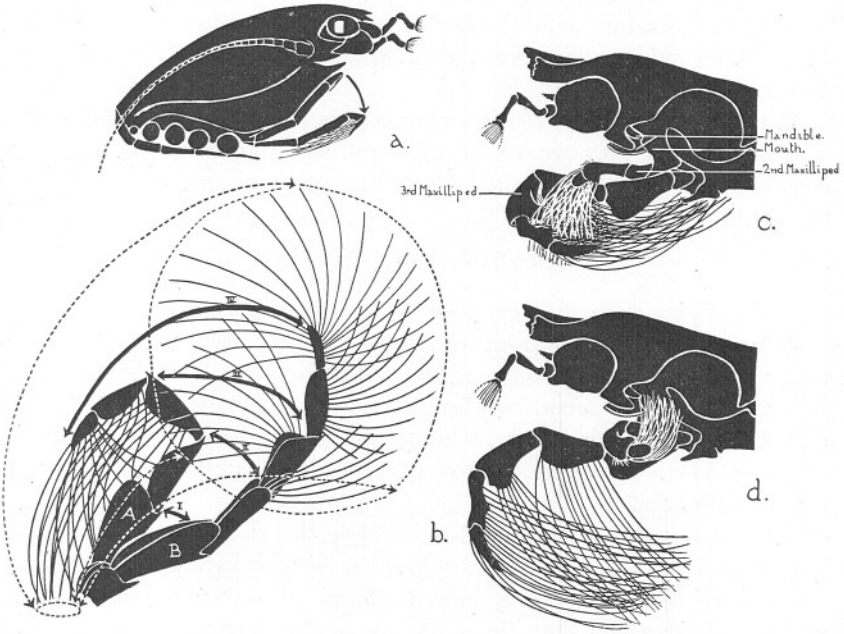

FIG. 7.—(*a*) A lateral view of *Porcellana longicornis* to show the lowering of the third maxillipeds at the commencement of feeding, ×4.

(*b*) A ventral view of a third maxilliped in two positions, A when flexed at the beginning and end of each feeding movement, B when fully extended. The continuous arrows indicate the paths taken by the segments of the appendage as it alternates between positions A and B. The movements indicated by arrows I and II are completed simultaneously and are then followed by III and IV in quick succession, when the limb is being unflexed. When the limb is being flexed the movements follow each other evenly in the reverse sequence. The dotted arrows indicate the movements of the hairs of the filter-net, ×7.

(*c*) A median section through *P. longicornis* to show the relative positions of the second and third maxillipeds at the end of each feeding movement. The third maxilliped is fully flexed and the second fully extended, the hairs on the two interlocking, ×6.

(*d*) A median section through *P. longicornis* to show the relative positions of the maxillipeds at the end of the combing movement of the second maxilliped. The third maxilliped is spreading out again and the second is fully flexed presenting the food collected from the hairs of the third maxilliped to the inner appendages and the mouth, ×6.

on it is drawn through the terminal brush of the second maxilliped (Fig. 7, d). If a great many particles have been caught one brushing may not be enough, and the regular cycle of movements is interrupted while the hairs are combed out several times in succession. The second

maxilliped is then tucked in between the inner mouth-parts and withdrawn at the same time as its neighbour from the other side approaches. The result is that each maxilliped is combed out by the other one as well as by the inner mouth-parts. That this is of considerable importance in feeding is shown by the fact that when one casting net only is being used both the second maxillipeds are working and the food is pushed from one to the other.

The exact function of the inner mouth-parts is again obscured by the maxillipeds, but they appear to select and sort the food as in Galathea, rejected material being shaken off and carried away in the respiratory stream.

CLEANING MOVEMENTS.

In the Galatheidea the fifth pair of legs are closely concerned with the cleaning of the carapace both inside and outside. Although reduced in many ways they are specially adapted for this purpose by their length, being able to reach dorsally as far as the eyes and ventrally as far as the maxillipeds, by their great mobility and by the presence of a small grasping chela. In addition a tuft of sickle hairs is present as described by Zimmermann.

In the Galatheidæ the fifth leg is often thrust inside the branchial cavity and under the posterior margin of the carapace to remove small particles which have lodged there. The appendage is then stretched forward on the ventral surface to the third maxillipeds, whose terminal tufts sweep it clean. It is also used for cleaning the ventral surface of the telson, the dorsal surface of the carapace, and the posterior appendages. The anterior appendages are cleaned on the ventral surface by the terminal brush of the maxillipeds which stretch far out in order to reach them, on the dorsal surface by the fifth leg. The antennules are cleaned by being bent sharply down and pulled several times through the cleaning tufts of the third maxillipeds. These are brought together parallel to each other in the middle line with the last three segments bent downwards, and the setæ of the cleaning tufts locked together round the antennule. They are then moved away from the ventral surface, while the antennule passes back into position. The antennæ are cleaned in a similar manner, but all the hairs of the third maxillipeds take part, the antenna being drawn along the length of the appendages. After each cleaning movement is completed the third maxillipeds are carefully brushed out by the second maxillipeds and any small food particles are sorted out and swallowed, the rest being rejected.

In the Porcellanidæ the cleaning process is in essentials the same as in the Galatheidæ, except that the fifth legs are used for cleaning both the dorsal and the ventral surface of the appendages and then presented

direct to the second maxillipeds for cleaning, since the setæ of the third maxillipeds are unsuited for this purpose.

Although the bulk of the material collected from the surface of the animal is unsuitable for food, and is at once rejected, a certain amount of edible material is swallowed, so that the cleaning movements serve the double purpose of removing dirt from the carapace and supplying the animal with food.

DISCUSSION.

Little is known about the feeding habits of other members of the Galatheidea, and since the structure of the third maxillipeds is not of importance in classification, few drawings sufficiently detailed to deduce from them the method of feeding can be found in the literature on Crustacea. Laurie (1926), however, gives a drawing of the maxilliped of *Pterolythes alobatus*, which is almost identical with that of *Porcellana platycheles*, and leaves no doubt that the method of capturing food is the same.

In other groups of the Anomura almost as little is known of the feeding habits.

In *Eupagurus bernhardus* the third maxillipeds are used in a similar way to those of Galathea (Orton, 1927). They are long and do not form an opercular plate, but sweep up particles from the bottom by means of a terminal brush of setæ. In addition the small chela is used to scrape up deposited material and pass it to the maxillipeds. Large pieces of food are also passed to the maxillipeds by the chela. The mouth-parts are poorly adapted for dividing large masses of food and small pieces are cut off with difficulty so that the bulk disappears very slowly.

Uca leptodactyla feeds upon minute organisms and organic debris which it scrapes off the sand particles in front of its burrow when the tide goes out (Matthews, 1930). The sand grains are picked up one at a time by the small chela and presented to the mouth-parts. The first and second maxillipeds are provided on their median edges with long bristles, many with spatulate ends, and it is by means of these that the sand grains are scoured.

Birgus latro has become adapted to living on land and feeds on coconuts (Darwin, 1860). The husk is peeled away and one of the eye-holes battered in with the large claw. The last pair of walking legs are provided with small narrow pincers, and these are then used to extract the food.

Upogebia pugettensis feeds entirely on suspended matter owing to its specialised habitat (MacGinitie, 1930). The animals live in burrows with constricted openings through which they cannot pass and subsist entirely

on plankton, large masses of food being rejected. The water current containing the suspended matter is drawn in by the action of the swimmerets and filtered on the hairs edging the first trunk limbs. The third maxillipeds are here modified as combs to clean the filtering hairs. They work alternately and are in turn cleaned out by the second maxillipeds.

Although a more detailed comparison cannot be carried out at present, it has been shown that in members of three out of the four subdivisions of the Anomura the typical carnivorous habit of the Decapoda has been given up or reduced in importance, and secondary methods of feeding adopted, first on bottom deposits and by still greater modification on plankton, and it is probable that there is a general tendency throughout the group to feed on fine particles of food.

In the majority of the Brachyura the third maxillipeds have lost their length and mobility, and form the relatively rigid opercular plate, but an examination of the gut contents of slow-moving crabs such as Macropodia, Inachus, and Hyas, shows that in some deposit-feeding plays a considerable part. In order to check the results obtained from examining the gut contents, several specimens of Inachus, Macropodia, *Galathea squamifera*, and *G. dispersa* were placed together in a dish containing sand, detritus, diatoms, and general vegetable matter from the sides of an aquarium tank. At the end of three hours the gut contents were examined. Inachus and both species of Galathea showed similar gut contents, very fine sand, short lengths of red and green algæ, detritus, diatoms, and unicellular plants, resembling closely the stomach contents found under natural conditions. When observed alive, Inachus was seen to pick up minute particles in the chelæ and hold them in front of the mouth where the maxillipeds brushed them from between the pincers and were in turn brushed out by the second maxillipeds which fold below the mandibles into the mouth. Macropodia had eaten nothing. This agreed with the observations on the natural food of the animal. The stomach contents show that it is a carnivorous selector, feeding almost exclusively on small crustacea, the stomachs of many examined being filled with the remains of copepods and ostracods. This type of food was not supplied to the animals under observation, so that they did not feed.

Hyas in captivity will eat mussel readily, and can be seen picking up small pieces of food with the chelæ. An examination of the gut contents suggests that the animal is only to a small extent a deposit-feeder, eating small Polychætes, pieces of Ophiuroids, and algæ mixed with a certain small amount of sand and organic debris.

One member of the Brachyura is of particular interest, Hapalocarcinus, the gall-forming crab, which spends its life shut up in a chamber in the coral into which only matter suspended in the water can enter. The

mouth-parts and probable method of feeding have been described by Potts (1915) from preserved material. The third maxillipeds are closely fringed with hairs and serve to strain plankton from the water entering the gall. Hapalocarcinus differs however from Porcellana in the great reduction of the inner mouth-parts.

The well-developed mouth-parts of the majority of the Decapoda show that they were originally carnivorous, feeding on large food masses. Departures from the normal methods of obtaining food are entirely secondary, and have been developed independently in various groups, often in connection with peculiar habitats.

SUMMARY.

The Galatheidea are divided into two families, the Galatheidæ and the Porcellanidæ, which differ from each other in their feeding habits as well as their structure.

Galathea dispersa has been taken as typical of the Galatheidæ. An examination of the gut contents shows that the food in the stomach consists of finely divided particles mixed with sand and detritus. In addition larger pieces of animal and vegetable matter are occasionally found. Observations of the animals in captivity show that they feed by two methods; either large pieces of food are seized by the chelæ and maxillipeds and passed to the mandibles or, as is more usual, the third maxillipeds are used to collect finely divided material from the substratum. The setæ on the terminal segments of the maxillipeds form a dense tuft which sweeps over the substratum, loosening and collecting small particles. The terminal tufts are cleaned out by the setæ of the second maxillipeds, and the food passed to the mouth.

Porcellana longicornis has been taken as typical of the Porcellanidæ. The stomach contents are much more finely divided than those of Galathea, and are closely comparable to those of the filter-feeding polychætes and molluscs. The third maxillipeds are fringed with long bipinnate hairs which stand out to form a large spoon-shaped scoop. The appendages are swung sideways alternately, unfolding and spreading the setæ. They are then flexed again as they move back towards the middle line, entangling particles in suspension in the water in the setæ which are in turn brushed out by the second maxillipeds and the food passed to the mouth.

It is evident that the Galatheidea have largely abandoned the predatory habits of the rest of the Decapoda, a change which has also occurred in members of other groups of the Anomura such as the Hermit Crabs and the Mud-shrimp, Upogebia.

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The Larval Stages of *Simnia patula.*

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With 1 Text Figure and Plates 1–2.

Simnia patula (Pennant)=Ovula is common feeding on *Alcyonium digitatum* and on *Eunicella verrucosa*, trawled in Plymouth waters. Mr. R. A. Todd records in the 1904 Plymouth Fauna List (Plymouth Marine Invertebrate Fauna, *Journal of the Marine Biological Association*, VII, 1904), which is quoted in the new fauna list (1931, Marine Biological Association), that "spawn probably belonging to this species has been found in April, June–July." This spawn is also well known to other members of the staff of the Laboratory and it is often found with the Simnia itself. It is now quite certain that it is the spawn of this mollusc, for it has been deposited in a plunger-jar on the glass and on Alcyonium (Jan. 19/20, 1932), and hatched out Feb. 21/22, 1932; it has been hatched out in the Laboratory, the larvæ distinguished in the plankton and reared until the crawling stage in a plunger-jar; and young stages which bridge the gulf from larva to adult have also been found. The adults will live for months in a plunger-jar feeding voraciously on Alcyonium. The life-history is described here for the first time. It is very interesting because it is quite unlike that of *Trivia europea* recently described (Lebour, 1931b), although the two are placed in the same family. Trivia bites holes in compound ascidians and lays its eggs in vase-shaped capsules embedded up to the neck in the ascidian, and these hatch out into larvæ having accessory shells rather like those of Lamellaria but with distinct differences. Simnia lays its eggs in a single layer of capsules, spreading over the Alcyonium for an inch or more in an irregular roundish mass (Text-figure 1).

Each capsule measures about 3·5 mm. across and contains numerous eggs, the capsules sticking together in a tough, slightly yellowish, but nearly colourless, layer. Each capsule is roundish but inclined to be polygonal from the pressure of its neighbours. There is no accessory shell, and the first whorl of the embryonic shell before it leaves the egg is fully formed and of a dark brownish horn colour.

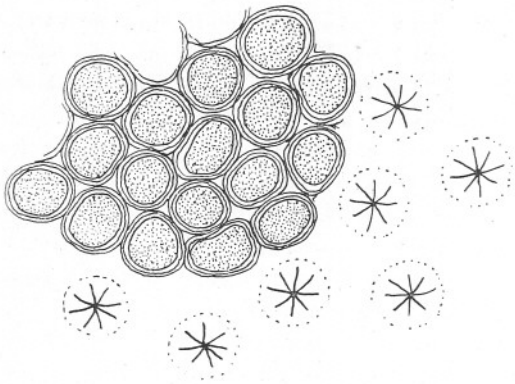
It was interesting to find that when these embryos were fixed in

Bouin-Duboscq a film separated off from the shell, presumably the periostracum, forming a covering of exactly the shape of the accessory shell in Trivia (Plate 1, Fig. 3).

Very young larvæ occur in the plankton at a similar stage to those hatched in the Laboratory and from there onwards all free-swimming stages can be found. The later stages were kept until they lost the velum. A few later crawling stages were found in shelly gravel and on Alcyonium.

It is usually in the summer that the larvæ occur, and they may be very abundant, usually from outside the Breakwater but occasionally from inside.

In July, 1931, some spawn was placed in a plunger-jar and hatched out. The capsules were in several patches on the Alcyonium and probably



TEXT-FIG. 1.—Part of an egg mass of *Simnia patula* on Alcyonium.

were laid by different individuals. Some were newly laid, the eggs being unsegmented and of a pale yellowish-orange colour. These measured about 0·13 mm. across. Others were hatching out and the larvæ inside the capsules were already veligers with a one-whorled shell. The newly hatched larva measures about 0·14 mm. or more across the widest part of the shell, the velum being bilobed with rounded lobes and measuring about 0·2 mm. across. The shell is very opaque, dark brown with a granular irregular pattern all over it (Plate 1, Fig. 8). At the extreme outer lip it begins to be reticulated, and here there is a slight tooth-like outgrowth which grows into the characteristic tooth of the outer lip separating the two sinuses supporting the velum. The velum is quite colourless with long cilia bordering the top margin and an under-layer of smaller cilia below the groove which leads to the mouth from all round the velar edge. Eyes and short tentacles are prominent, and two large otocysts, also a broad foot with a thin horn-coloured operculum (Plate 1, Figs. 1–7). It is not possible to see the internal organs owing to the thickness and

opacity of the shell. Many hundred eggs occur in each capsule, all of which apparently develop. As the larva grows the shell continues to be reticulated with a coarse network, the pattern being all over except on the first whorl which is always differentiated by its granular surface. The animal protruding from the shell is a pale yellowish white, in the early stages with dark pigment round the mouth and on the sole of the foot which disappears as the animal grows. The velum is always colourless with no pigment at all in any stage, and soon becomes four-lobed, the lobes growing very long in the later stages. When the shell measures 0·34 mm. across at its widest part the velum is nearly a millimetre across, each lobe being nearly 0·50 mm. long. When the shell is about 0·60 mm. long, its largest measurement being now from the apex of the spine to the shell siphon, the velum measures 3 mm. across, the lobes being long and narrow. At a length of 0·75 mm. the shell has three whorls and a distinct shell siphon; the eyes are conspicuous, the tentacles long and ciliated at the tips; the foot projects beyond the shell and is more or less pointed at the hind part, rounded in front and ciliated all over. The head projects beyond the velum and a siphon is beginning to form. These late stages can either swim or crawl (Plate 1, Figs. 9–16). Placed in the plunger-jar they grew to about 0·80 mm. in length and then began to lose the velum. At 0·96 mm. the velum had quite gone and the mantle was beginning to spread over the shell. The siphon was long (Plate 2, Figs. 1–5). The tooth on the outer lip which had grown almost square and much inflexed now disappears; the operculum is lost; the reticulation of the shell are replaced by straight ribs; there are now four whorls. The embryonic shell grows no further. From now onwards radiating ribs are added and it is gradually grown over and finally disappears. A stage showing the embryonic shell with the adult shell growing from it in radiating ribs was found on an Alcyonium. This measured 1·12 mm. in length from the tip of the spire to the shell siphon (Plate 2, Fig. 6). A later stage from shelly gravel, probably from an Alcyonium, showed the adult shell gradually spreading over the embryonic shell. This measured 1·84 mm. in length (Plate 2, Figs. 7–8). The shell structure showed layers added on to one another with faint ribs very much like the structure of the adult. A still later stage from Alcyonium measuring 4·80 mm. in length showed the complete absorbtion of the embryonic shell (Plate 2, Figs. 9–10). In this the animal and shell were like the adult, the mantle covering a large portion of the shell and having brown hair-like stripes separated by wide intervals.

It seems that Simnia must have a long larval life, for the late larvæ lived for several weeks in the plunger jar without losing the velum. The long velar lobes apparently indicate this, for all the shore forms which have a short larval life have a bilobed and very short velum for the whole

of their free-swimming existence (*Littorina littorea*, *Lacuna vincta*, *Rissoa membranacea* and *Rissoa parva*). In shape the velum of Simnia is comparable to that of *Nassarius incrassatus* (see Lebour, 1931a) in which the larval life is very much prolonged and the crawling stage is not reached until the mollusc is of a considerable size.

These free-swimming larvæ of Simnia are important members of the outside plankton in summer, being amongst some of the largest planktonic mollusca seen, and at times they occur in great abundance with the larvæ of *Nassarius incrassatus*, these two larvæ being occasionally the only larval molluscs present in the outside waters. *Limacina retroversa* is however very often found with them.

It is interesting to find in early descriptions of planktonic larval stages that Macdonald (1859) described the larval shells of Pedicularia, and these are very similar to the Simnia shells. Dautzenberg (1899) also figures young Pedicularia showing a similar reticulated embryonic shell. Pedicularia is closely related to Simnia, and in this way seems to be nearer to it than it is to Trivia. Simroth (1895) figures two planktonic larval shells which he thinks belong to Cypræa which again closely resemble the Simnia type. Both these facts are suggestive. It is perhaps possible that Simnia and Pedicularia are more closely related to Cypræa proper than to Trivia ; and is Trivia, as Pelseneer (1926) suggests, more closely related to Lamellaria than to Cypræa ?

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PLATES 1–2. *Simnia patula*.

(Scale B is six times the scale of A.)

PLATE 1 (1–7 scale B, 9–16 scale A, Fig. 8 is still more enlarged).

FIG. 1.—Newly hatched larva from egg, 0·14 mm. across.

,, 2.—The shell of same.

,, 3.—The same treated with Bouin-Duboscq, showing pellicle round shell.

,, 4.—Larva three days old, shell 0·16 mm. across.

,, 5.—Shell of same.

,, 6.—Operculum of same.

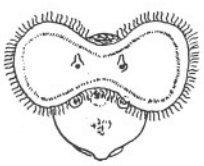
,, 7.—Larva a few days old, from plankton, shell 0·18 mm. across.

,, 8.—Sculpture of embryonic shell much enlarged.

,, 9.—Larval shell from plankton, 0·24 mm. across.

,, 10–13.—Larvæ with four-lobed velum from plankton : 10, shell 0·34 mm. across : 11, shell 0·4 mm. across : 12, shell 0·56 mm. long : 13, shell 0·75 mm. long.

,, 14–16.—Larva crawling, losing velum.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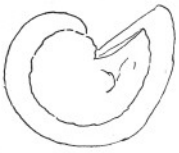
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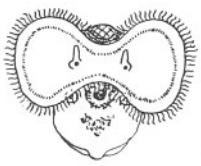
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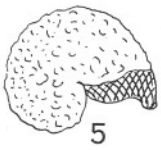
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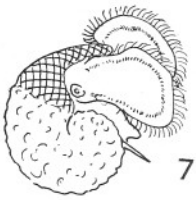
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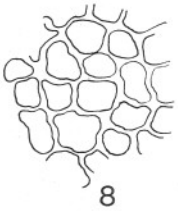
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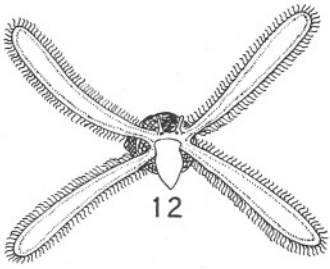
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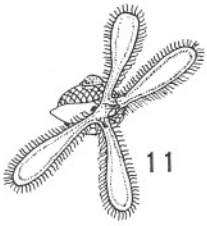
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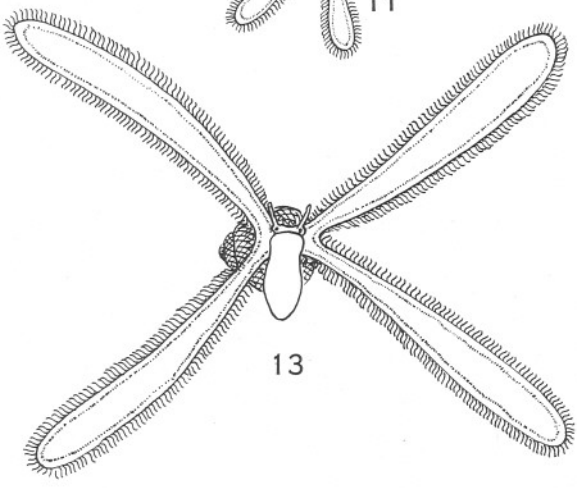
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PLATE 2 (1–9 scale A, 10 on a smaller scale).

FIGS. 1, 2.—Shell of Simnia having lost velum, showing growth at mouth of shell filling up the velar sinuses.

,, 3–5.—Simnia crawling, slightly later stages, reared from larvæ in plunger jar, 0·8 to 0·96 mm. long.

,, 6.—Later stage found feeding on Alcyonium, shell 1·12 mm. long.

,, 7–8.—Later stage, from shelly gravel, shell 1·84 mm. long.

,, 9–10.—Stage showing adult characters, shell 4·8 mm. long, from Alcyonium.

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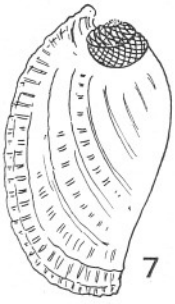

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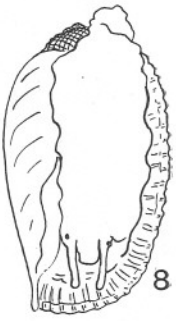

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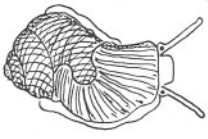

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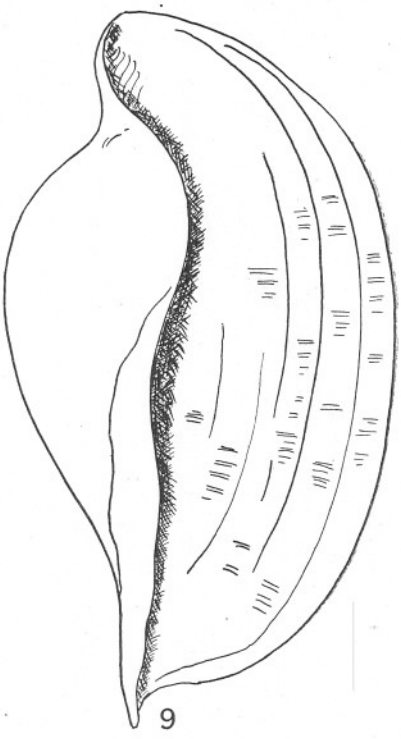

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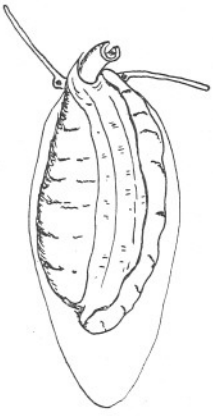

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The Eggs and Early Larvæ of Two Commensal Gastropods, *Stilifer stylifer* and *Odostomia eulimoides.*

By

Marie V. Lebour, D.Sc.

Naturalist at the Plymouth Laboratory.

With Plate 1.

Stilifer stylifer (Turton) has been recorded from Plymouth many years ago living on *Psammechinus miliaris* (=*Echinus*) and *Echinus esculentus ;* also from several other British localities (see Jeffreys, 1867, IV, p. 196). Jeffreys describes the spawn and larvæ hatched from it, but apparently these have never been figured. It had not been rediscovered at Plymouth until 1931 when in August Mr. A. D. Hobson found several specimens of *Psammechinus miliaris* from deep water beyond the Eddystone with these small commensal molluscs on them. In the first lot of about a dozen Psammechinus, three were covered with the spawn of Stilifer, one having one mollusc and two having two. In a second lot taken a few days afterwards, five were covered with spawn, one having one mollusc, two having two, and two having three. These were placed in a plunger-jar, but unfortunately most of the Stilifer left the Psammechinus and were presumably eaten by them as pieces of their shells were found. They probably did not like their surroundings. Some of the spawn was removed from the Psammechinus and placed in a fresh plunger-jar with some Nitzschia where some of the larvæ hatched out. A Psammechinus was introduced in the hope that the larvæ might settle down, but they all died after living only a few days.

The eggs were all on the dorsal surface of the Psammechinus, sometimes ten or a dozen egg-sacs on one individual. Each egg-sac is of a more or less three-cornered cushion shape, somewhat rounded, about 1·2 mm. broad and 1·1 mm. high, attached by a narrow stalk to the test of the Psammechinus (Plate 1, Fig. 1), and each sac contained about 60 to 80 eggs. Both egg-sac and eggs were colourless and very transparent. Each egg measured about 0·1 mm. long when young, and rather more as the embryo developed, and were roundish or oval in shape. Before hatching the embryonic shell is completely formed. This is a pale brownish horn colour and quite transparent. The foot and velum are well developed and otoliths and eyes are present. On hatching the shell consists of one

whorl and measures about 0·13 mm. across. It is nearly round, the velum having two rounded lobes measuring about 0·12 mm. across. A row of very long cilia edge the margin and a row of much smaller cilia below border the groove leading to the mouth which is very inconspicuous. The foot is short and round, pulled out at the posterior extremity, covered with minute cilia and bearing a very thin operculum. The eyes are very far apart (Plate 1, Fig. 2). In a day or two after hatching, the shell has grown slightly larger, the velum broader, and there is usually one tentacle present (Plate 1, Figs. 3–7). Thus the early larva is a typical veliger and like an ordinary well-developed gastropod. Unfortunately none of them lived more than a few days so that it was impossible to see any changes undergone by its settling down.

Odostomia (*Brachystomia*) *eulimoides* Hanley is common in the neighbourhood of Plymouth living on the lamellibranchs *Chlamys opercularis* and *Pecten maximus*, usually on the ears. In May and June Mr. A. D. Ritchie gave me several of these and nearly all of them were laying eggs. The eggs are usually on the ears of the shells, but sometimes on the valves. They are laid in irregular gelatinous masses, about a millimetre across, sometimes more and sometimes less, clear and colourless, each mass containing many eggs also clear and colourless (Plate 1, Figs. 8, 9). The eggs are round, measuring about 0·16 mm. across, and are peculiar because the developing embryo at first occupies only the central part, the large space between the embryo and the thick-walled envelope being occupied by a granular fluid. Batches of eggs were obtained on the following dates: 31.5.31, 1.6.31, 11.6.31, 12.6.31 (two batches), and others later on in June. In each some of the eggs were only beginning to develop and some were nearly ready to hatch. The eggs were placed in a plunger-jar with Nitzschia and some of them grew up to a certain size, but they all died before the normal dextral shell was formed. However, the larvæ were interesting whilst forming the shell. It probably only lives a short time as a veliger and is not important as a planktonic larva.

It is well known that the genus Odostomia and its allies has an embryonic shell with a sinistral twist, and that the animal is dextral even in the earliest stages. When a certain size has been attained the shell becomes dextral also, the sinistral embryonic shell remaining as the apex, usually placed almost at right angles to the rest. In *Odostomia eulimoides* this embryonic shell is almost hidden by the later whorls, but in many of its relatives the sinistral apex is clearly seen (Plate 1, Figs. 16–17).

When *Odostomia eulimoides* hatches it has an almost spherical shell of one whorl, the aperture being to the left. The anus, however, is on the right side, showing that the animal is dextral. There is a short foot drawn out posteriorly, covered with minute cilia and bearing an operculum. The velum has two oval lobes which are nearly circular and the

eyes are far apart. Two otoliths are conspicuous at the base of the foot. The intestinal mass is well differentiated, the digestive gland, stomach, œsophagus, mouth, intestine, anal gland, and anus can all be made out. The newly hatched larva measures about 0·16 mm. across the widest part of the shell and the compact velum measures about the same across. The velar lobes are edged with long cilia and below is a row of much shorter cilia, the two bordering a groove to the mouth. The anal gland is nearly black, as are also the eyes, otherwise the whole animal and shell are clear and colourless (Plate 1, Figs. 10–11). The velum very soon disappears and the animal crawls, but all the crawling stages were abnormal in the plunger-jar and soon died. The shells, however, of those which had two to two and a half whorls were interesting as they showed the transition from a sinistral to a dextral shell. The first two whorls constitute the embryonic shell ; after this a large irregular piece is added which begins on the lower surface of the outer lip. This is an outgrowth which sticks out from the lip and is not at first complete round the whole of the periphery. The more regular growth added later to this forming the dextral shell aperture results in the embryonic shell being at a right angle to the true shell and forming the curious apex characteristic of the shells of this family. A figure is given of the apex of the shell of *Eulimella acicula*, a related species, but of a different genus, showing this apex in its typical form (Plate 1, Fig. 17).

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EXPLANATION OF PLATE.

(Scale B is six times the scale of A.)

PLATE 1.

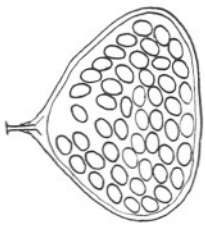
FIG. 1.—Egg-sac of *Stilifer stylifer*, 1·2 mm. across (scale A).

Scale B:
- FIG. 2.—Newly hatched larva, shell 0·13 mm. across.
- FIG. 3.—Larva a few days old, 0·16 mm. across.
- FIGS. 4–7.—Empty shells of early larvæ.

FIG. 8.—Portion of egg mass of *Odostomia eulimoides* (scale A).

Scale B:
- FIG. 9.—Egg of *Odostomia eulimoides*, 0·16 mm. across.
- FIG. 10.—Newly hatched larva.
- FIG. 11.—Larva a few days old.
- FIG. 12.—Empty shell of same.

Scale A:
- FIG. 13.—Newly hatched larva.
- FIGS. 14–15.—Later shell showing irregular growth at the margin.
- FIG. 16.—Apex of adult *Odostomia eulimoides*.
- FIG. 17.—Apex of adult *Eulimella acicula*.



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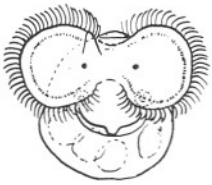

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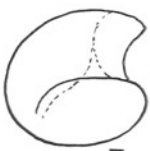

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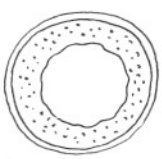

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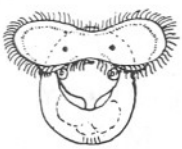

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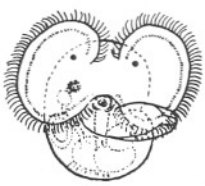

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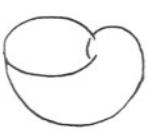

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Limacina retroversa in Plymouth Waters.

By

Marie V. Lebour, D.Sc.,

Naturalist at the Plymouth Laboratory.

With Plates 1 and 2.

Limacina retroversa (Flem.) has been known in the Channel for over twenty-seven years and was several times recorded by Gough (1907) and Bygrave (1911) in the plankton reports (see Marine Biological Association, *Plymouth Marine Fauna*, 1931, p. 265), both from inshore and from outside. In recent years the more frequent collecting in the waters round Plymouth has shown that it may be present throughout the year, although much the most abundant in summer. Not only is it found in the outside waters but it is also often present in the Sound and even at the mouths of the estuaries.

It is very important from the food point of view as it is one of the commonest planktonic molluscs and much eaten by the plankton animals. In the North Sea and in other northern waters it is a favourite food of the herring, but as the herring is not usually at Plymouth when Limacina is most abundant this is of no great consequence with regard to the Plymouth herring. Mackerel probably eat it and certainly many invertebrates, amongst which are *Clione limacina* and Sagitta, both of which have actually been seen with Limacina inside them.

Hardy (1924, 1926) showed that it forms 2·17 per cent of the total year's food of the herring in the North Sea. In other parts it may be so largely eaten by the herring as to produce the condition known as "gut pouch."

In summer enormous swarms of Limacina may be taken in one haul of the ring-trawl, or even in a coarse tow-net, outside the Breakwater. In the smaller meshed nets the young may be taken and sometimes the egg masses.

Paulsen (1910) in his plankton résumé of the International Fisheries states that nothing is known of its breeding habits, and apparently no more has been added to this statement. He gives a good account of its distribution in the international area and this is quoted by Ostenfeld in his last summary (1931).

It is really quite easy to find out something about its breeding, for the

eggs are ripe for the greater part of the year and apparently each individual may have several broods. Especially in the summer almost every adult is laden with eggs and if left overnight in a finger-bowl will almost certainly have deposited some by the next morning. A further batch of eggs is usually seen inside after one batch has been extruded. Egg-bearing individuals have been seen in almost every month (January possibly excluded), and young of all ages also both inside and outside the Sound and as far out as the most southerly stations worked. The largest numbers are always outside the Sound. Many of all ages, including adults, were recorded in the summer from near Ushant in 1929 and 1930.

The fact that it is so abundant in these waters and also that it remains throughout the year makes Paulsen's (*op. cit.*) statement that it probably does not penetrate into the North Sea through the Channel rather doubtful. It is constantly present in the plankton in the northern and central North Sea (see Hardy *op. cit.*), and the more northerly of the North Sea stations and the more easterly stations of the Channel have been little worked. It seems more probable that *Limacina retroversa* will be found to have a greater range when further investigations are made.

As nothing so far is known of the eggs and life-history of the species, the following notes are not without interest.

The eggs of *Limacina retroversa* are planktonic, floating in small gelatinous strips with the eggs scattered and widely separated (Plate 1, Fig. 1). The form is similar to that of all pteropod eggs known and very like those of *Clione limacina*, but with the eggs fewer and further apart. Each strip may measure about 2 mm. in length and 0·64 mm. in breadth, but may be larger or smaller. Sometimes they are caught in some thread-like substance. The egg measures about 0·09 mm. to 0·10 mm. in length, and 0·06 to 0·07 mm. in breadth. Both matrix and eggs are perfectly colourless and glass-like in transparency. The embryos begin to develop at once and are moving about inside the egg envelope on the first day (Plate 1, Figs. 2–4). The second day they may hatch, but are very backward in development, and, unlike Clione, have no shell at first (Plate 1, Figs. 5–6). The cilia at first are round the body, then a circular velum appears which becomes bilobed when the shell forms, which is in a few days (Plate 1, Figs. 7–17). The shell is at first round, cap-like and perfectly symmetrical, measuring about 0·05 mm. across. By the fourth day it is asymmetrical and a good deal larger, the velum bilobed, the foot a small protuberance, ciliated on its surface. The shell is now about 0·10 mm. across its widest part and the width of the velum is about the same. The alimentary canal is forming from the apical mass. These early stages were obtained from the eggs, but unfortunately it was not possible to keep them alive. The other stages were all obtained from the plankton. When the shell measures about 0·13 mm. across the larva has

the usual veliger form ; the foot pulled out to a narrow tongue behind is ciliated all over with larger cilia at the tip and bears an operculum. Otoliths, eyes and mouth are present, the velum being about 0·15 mm. across with oval lobes of the usual structure with a row of long cilia round the margin and a second row of shorter cilia beneath, the two forming a groove to the mouth (Plate 1, Fig. 18). The whole shell and animal including the velum are perfectly colourless with the exception of the digestive gland which is coloured a brownish yellow, and the anal gland near the anus which is almost black. The shell now begins to be spiral with a sinistral twist, but whilst the shell is sinistral the animal is dextral. It is not necessary to describe the anatomy of this well-known mollusc. From now onwards the veliger may be recognised by its shell which gradually acquires more whorls (Plate 1, Fig. 19 ; Plate 2, Fig 1). When the shell measures about 0·32 mm. across two small lappets are seen developing from the sides of the foot at the base. These are the " wings " which grow quickly and are soon projecting beyond the shell (Plate 2, Fig. 2). As the " wings " grow the velum dwindles. Another organ, the " balancer," appears at the same time which projects from the base near the foot and is sometimes outstretched in swimming, sometimes pressed against the body whorl of the shell. The velum is dwindling fast when the shell measures 0·37 mm. across and the wings are nearly as long as the shell (Plate 2, Fig. 3), the foot changing its shape and very soon almost disappearing. The wings grow rapidly ; the velum disappears altogether and the Limacina is like the adult (Plate 2, Figs. 4–5). The oldest shells may have four or five whorls and measure about a millimetre or more in height. The wings may be twice as long as the shell or even more. They are covered with very minute cilia and are the only organs of locomotion, the animal swimming by strong flappings of its wings. There is a small amount of rose colour in the region of the mouth, the digestive gland is brownish yellow and the anal gland black ; otherwise the whole animal and shell are absolutely colourless and transparent. In very old individuals the operculum may fall off (Plate 2, Fig. 6).

When about three-parts grown the Limacina is able to lay eggs and it probably sends out many broods during its lifetime. We do not know how long they can live, but they appear to grow very quickly and it is probable that they reach their largest size in less than a year. In spite of their many enemies, there must be an enormous number continually breeding in spring or summer, and less so at other times of year, and they must play a very important part in the economics of the sea.

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EXPLANATION OF PLATES.

(Scale B is 6 times the scale of A, and scale C is twice the scale of A.)

PLATE 1.—(Fig. 1, scale A ; Figs. 2–20, scale B.)

FIG. 1.—Eggs of *Limacina retroversa*, laid 21/22.7.30.

FIGS. 2–4.—Eggs with developing embryos, 0·09–0·10 mm. long.

FIGS. 5–6.—Early larvæ, hatched in Laboratory.

FIG. 7.—Larva with young shell, hatched in Laboratory.

FIGS. 8–13.—Empty shells of young larvæ, hatched in Laboratory.

FIGS. 14–17.—Young larvæ with shells, hatched in Laboratory.

FIG. 18.—Larva 0·13 mm. across shell, from plankton.

FIG. 19.—Larva 0·18 mm. across shell, from plankton.

FIG. 20.—Larva 0·20 mm. across shell, from plankton.

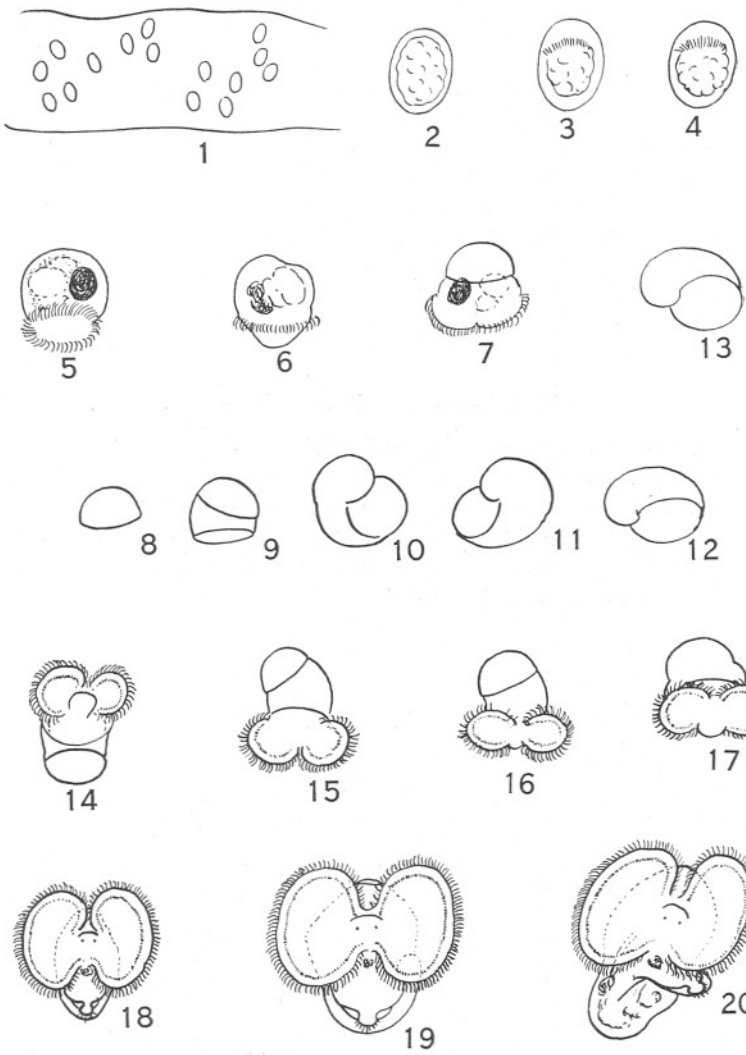
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PLATE 2.—(1–3 scale B, 4–5 scale C.)

Fig. 1.—Larva 0·25 mm. across shell, from plankton.

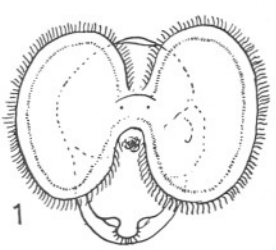
Fig. 2.—Larva 0·32 mm. across shell, from plankton.

Fig. 3.—Larva 0·37 mm. across shell, from plankton.

Fig. 4.—Limacina about three-parts grown, shell 0·72 high, from plankton.

Fig. 5.—Limacina with adult characters, 0·96 mm. high, from plankton.

Fig. 6.—Operculum of Limacina.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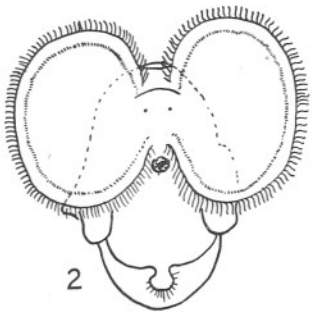
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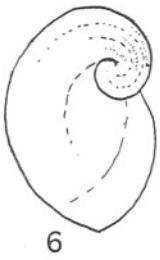
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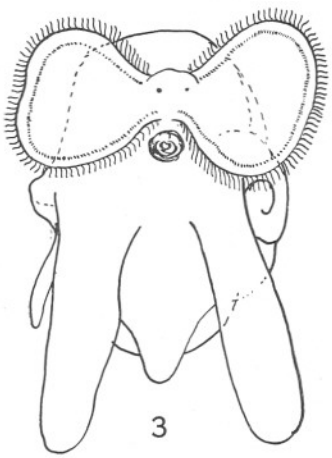
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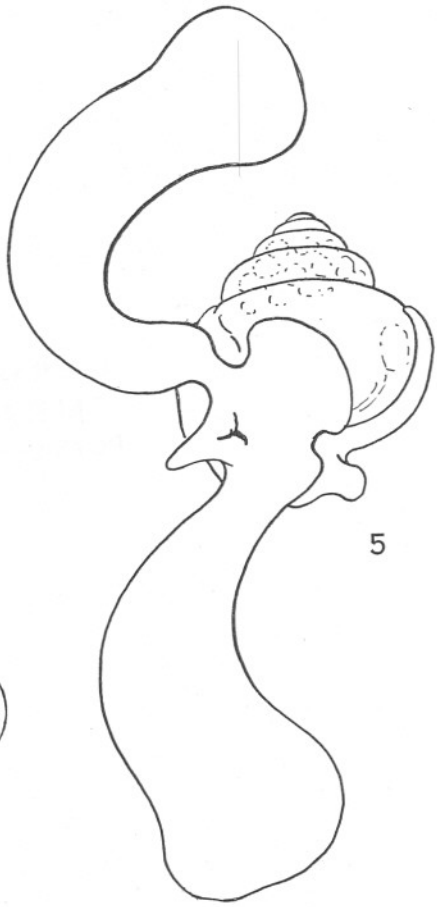
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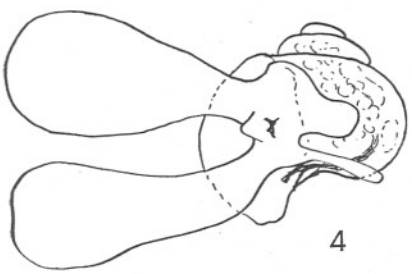
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On the Biology of Sagitta. The Breeding and Growth of *Sagitta elegans* Verrill in the Plymouth Area, 1930–31.

By

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With 2 Figures in the Text and Plate I.

PLANKTON animals are in intimate relation with the sea-water in which they live, and the physical conditions of this surrounding medium must have profound effects upon the general metabolism of such delicate organisms. One of the greatest problems in biology is an understanding of the differences that are shown in a single species according to its geographical distribution, probably caused, in marine animals, by differences in the conditions of the sea from place to place; in this respect variations in the sizes to which the animals grow are noticeable. Such differences also appear in a species in one locality when the successive broods are subject to varying conditions in the water brought about by seasonal changes. I have shown in my researches on the vertical distribution of plankton (**11**) that the copepod *Calanus finmarchicus* will behave differently at different seasons, and that this difference appears because we are dealing with separate broods which are probably physiologically distinct one from another. Similar differences are apparent also in the behaviour of other plankton animals. It has therefore proved necessary in a study of seasonal change in behaviour to know exactly how many distinct broods we are dealing with.

Since some confusion had existed concerning the species of Sagitta present in our Plymouth waters, it seemed that a close study of Sagitta broods would prove useful both in throwing light on the question of seasonal behaviour of different broods and also in clearing up the life-history of these animals, which are very common and characteristic in the plankton around our shores.

Evidence is also available that the seasonal change in size of different broods of marine animals bears some relation to the temperature of the sea-water in which they develop. If this be so it is necessary that the connexion should be more closely investigated. Research on populations of plankton animals is however much complicated by the possible introduction into the same area of animals from different water masses

which differ in physical and chemical conditions. A study of the effects of seasonal change in temperature would be very difficult in such areas because one would never be certain that the population under examination was always the indigenous one. The waters of the English Channel near Plymouth are however perhaps rather suitable for this research; continuous hydrographical observations have shown that there seem to be years in which there is little interchange of waters of *widely* differing characteristics, such as is found for instance in regions where waters of Polar and of Gulf Stream origin intermingle.

A careful examination of the Chætognath population at Plymouth has proved that there are two species which predominate in the plankton, *Sagitta elegans* Verrill* and *Sagitta setosa* J. Müller. In addition, during a year's collections three specimens of adult *Sagitta serratodentata* Krohn have been found, but this species can probably only be regarded as an occasional invader of the region under survey.†

In these pages I shall deal only with *Sagitta elegans*, describing its growth and breeding throughout a year. A similar study has been made of *S. setosa* and it is hoped to publish this in a later paper, when the two species will be compared.

Methods of Study.

Since one of the objects of this research was to gain a knowledge of the size to which Sagitta grows at various seasons of the year it was necessary that the collections should provide as many of the adult individuals as possible. For this purpose the 2-metre stramin ring-trawl is the most suitable net, and as weekly collections are made with this net to study the seasonal abundance of young fishes it was decided to make the collection serve both ends. Ideally it would have been necessary to take also concurrent hauls with a silk net to sample efficiently the young Sagitta population, but an examination of two sets of collections would have been too lengthy when it was probable that the use of the stramin net alone would produce the desired data.

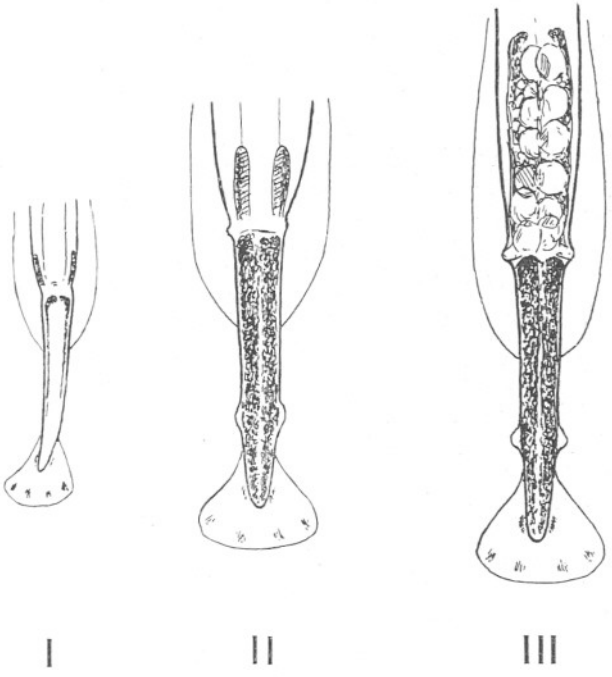
Collections were made once a week in daylight at a position 2 miles east of the Eddystone lighthouse in water about 54 metres in depth; on every occasion oblique hauls (**12**, p. 640) were taken in order to sample all water layers, and each haul was half an hour in duration. My thanks are due to Captain V. Lord and the crew of s.s. *Salpa* for their continuous care in making these weekly collections.

In each catch the total number of Sagitta (preserved in 5% formalin) was found, either by complete count, or by sample method (**10**, p. 776) if

* I am greatly indebted to Dr. H. B. Bigelow, Miss Beale, Dr. A. G. Huntsman, and Professor A. Meek for confirmation in the identification of this species.

† Two of these were taken on May 7th, 1930, 16½ and 15 mm. in length; one on September 3rd, 1930, 12 mm. in length. All three were fully mature.

the catches were large. An adequate sample was then measured in length to the nearest half millimetre below. Great care was taken that those measured were secured by fair sampling means in order that a true picture of the size distribution should be obtained on each occasion. In measuring, the tail fin was excluded, the length being that from the tip of the snout to the base of the tail. Measurements have usually been give by other workers (e.g. **2** and **4**) including the tail fin, but as this membrane is so liable to damage it was thought advisable where such



TEXT-FIG. 1.—The three stages of maturity used as criteria of development in Plate I. I, upper limit of Stage I; II, upper limit of Stage II; III, ripe adult in Stage III. Stained in alum carmine; the proportions appear narrower than natural owing to shrinkage.

large numbers were being dealt with that the more defined end of the body should be regarded as the posterior limit. Actually in the larger specimens the tail fin is just over half a millimetre in length beyond the end of the body, being proportionately slightly smaller in the younger stages. The addition of half a millimetre to my measurements will therefore give a sufficiently approximate idea of the total length of the animal.

After measuring, 30 to 60 individuals were selected to supply a good sample covering the complete range of lengths obtained. These were stained in alum carmine to emphasize the gonads and mounted in Canada

balsam. The stained specimens were examined under the microscope to find the state of maturity or otherwise of the gonads in relation to the body length.

Sagitta is a hermaphrodite animal in which the male organs mature first. The testes are situated in the posterior section of the body, or tail, and this portion becomes filled with spermatozoa and maturing spermatocytes. It is only after this stage is reached that the ovaries begin to ripen fully. The state of maturity of the animal could thus be very conveniently divided into three easily separated stages (Text-Fig. 1). Stage I included all the youngest Sagitta in which not a single sperm mother cell was visible lying loose in the tail cavity. Stage II ranged between those individuals with the first appearing spermatocytes and those in which the tail segment was packed with spermatocytes and spermatozoa, but in which the ovaries, while appearing evident, showed little sign of swelling eggs (Text-Fig. 1). Stage III contained those individuals in which the ovaries were fully ripe or ripening. The measurements obtained were the upper limit in length of those in Stage I, the upper and lower limits of those in Stage II, and the lower limit in length of Stage III. Thus often an overlapping occurred between Stages I and II and Stages II and III as was to be expected. During the process of staining and mounting some shrinkage of the animals takes place, which may amount in the longer individuals to as much as half a millimetre; no correction has been made for this shrinkage as it it so small as not materially to affect the general picture.

In Tables I to III on pages 143 to 145 are given the full number of Sagitta occurring in each collection, the results of the measurements of total length, and the data obtained from the examination of the stained specimens.

General Results.

The results of the research have been reproduced in a complete form in Plate I, in which the length distribution on each day has been reduced to a percentage basis and the data regarding state of maturity have been incorporated. This diagram, which covers the period May, 1930, to May, 1931, appears to show clearly that a brood of large individuals spawning

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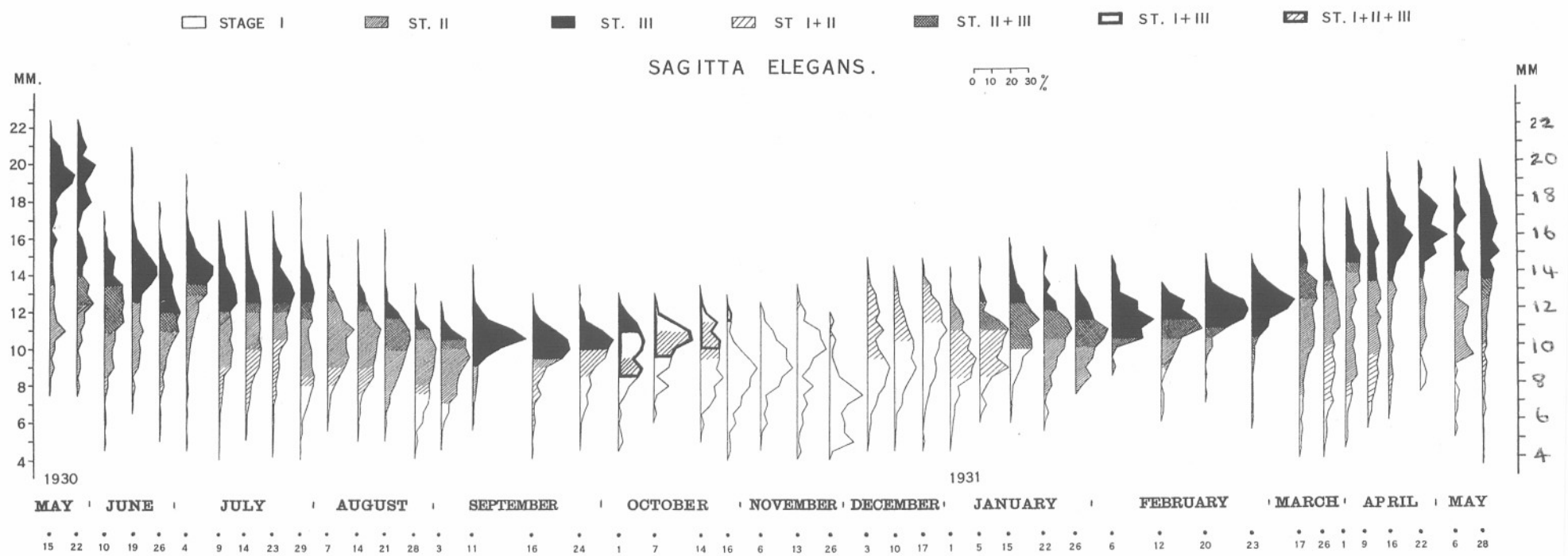
EXPLANATION OF PLATE I.

PLATE I.—The percentage size distribution of *Sagitta elegans* caught in the ring-trawl on the days given. The black, cross-hatched and shaded areas represent the various stages of development (see top of diagram for key). In this diagram the lines of demarcation between different stages must not be taken as being exact, as possible shrinkage has not been taken into account. It must also be realised that the Stage III includes both ripening and mature females, and they must not be regarded as all fully mature, though usually probably the majority are so.

PLATE I.


STAGE I
ST. II
ST. III
ST I+II
ST. II+III
ST. I+III
ST. I+II+III
SAGITTA ELEGANS.
0 10 20 30 %
MM.
MM
22
20
18
16
14
12
10
8
6
4
1930
1931
MAY JUNE JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY FEBRUARY MARCH APRIL MAY
15 22 10 19 26 4 9 14 23 29 7 14 21 28 3 11 16 24 1 7 14 16 6 13 26 3 10 17 1 5 15 22 26 6 12 20 23 17 26 1 9 16 22 6 28

in May gave rise to a brood spawning in June and July, the latest individuals of which may themselves have arisen from the early June spawners. The July spawners produced a brood which did not fully mature until September. The offspring of the September spawners developed no gonads during October and November ; in December the male organs were ripening, and in January the ovaries started to mature. A complete spawning population was thus formed in February. These adults again gave rise to a large brood spawning in April and May.

During the twelve months beginning June, 1930, and ending May, 1931, there were apparently therefore four different broods of *Sagitta elegans*, and possibly five if two broods can be accounted for in June and July during which time spawning is probably intermittent.

The diagram shows very clearly the ripening of the male products preparatory to the full maturity of the individual, and indicates also the overlapping that occurs between the different stages of development. In October there is an overlapping of the remaining adults of the September breeding population and their offspring in which no gonads are developed until the male organs start to ripen in December.

A pronounced feature is the absence of the younger stages, which is probably entirely due to the coarseness of the mesh of the stramin net used for collecting. On each occasion, however, there is an indication of the arrival of the young of new broods in the catches at the periods when probably in fact they are far more numerous than the adults. The closing up of the length-distribution curves to form compact breeding populations is also especially noticeable in September and February.

Inspection of Plate I also shows that there was a definite difference in the length attained by the adults in each successive brood. The approximate average lengths of the adults of the different broods as determined by inspection of the graphs in Plate I, without calculation, were as follows :—

May, 1930	19½–20 mm.
June, 1930	13½–14½ mm.
July, 1930	*ca.* 13 mm.
September, 1930 . . .	10–10½ mm.
February, 1931 . . .	12–12½ mm.
April–May, 1931 . . .	*ca.* 16 mm.

Coincident with this was a difference in the size at which the different broods matured, the broods with smallest adults maturing at a smaller size than those with the largest (See Plate I).

In Text-Figure 2 I have summarised the above results and included the curve for the seasonal change in the temperature of the water. In this figure the temperatures are given for the surface at the International

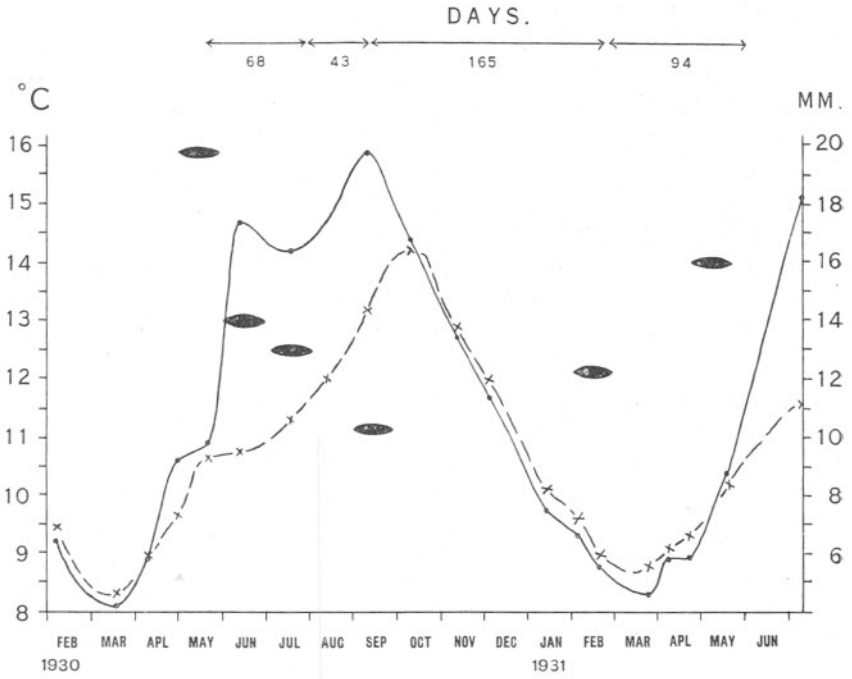
Station L5 near the Eddystone and for 25 metres at the station E1 ten miles beyond the Eddystone.* It is probable that for the months October to May this represents fairly accurately the temperature conditions at all depths in the locality where the collections of Sagitta were made; from June to September the temperature of the deeper layers was probably somewhere between that of the surface at L5 and that at 25 m. at E1, but nearer to those at E1. The black areas in Text-Figure 2 indicate the chief times of spawning as apparently shown by Plate I, and these are plotted against the average length of the spawning adults. The decrease in the length to which the Sagitta grew as the temperature rose is very evident, the smallest size occurring in September. Although the Sagitta spawning in February were born under higher temperature conditions than were those spawning in September they attained a slightly greater size; but during the period of their growth the temperature was falling and also they lived for a very much longer time than those of any other brood. It is probable that the size reached is conditioned amongst other things by the amount and type of food eaten, the temperature conditions, and the length of life, and that the latter depends on the time of the onset of maturity. At the top of Text-Figure 2 are given the periods of days elapsing between the last dates on which the different spawning populations were abundant, and this possibly gives some idea of the length of life of the Sagitta at different times of the year on the assumption that they die after spawning as seems to be indicated by Plate I.† As one would expect, the time taken to reach maturity is longer in the colder months than in the warmer; those born in February taking 94 days to full maturity in May, as against 43 days for those born at the end of July which matured in September. The length of life for the June and July brood is given as covering the whole of this period but it is probable that it should be split. The longest-lived Sagitta were undoubtedly those born in September. These did not mature until February, a period of 165 days; it must be realised, however, that for two months at any rate of this period there was no gonad development. The stimulus for gonad development was possibly removed by the high temperature in October when the water at all depths reached a temperature of over 14° C., and it is possible that somewhere in the neighbourhood of this temperature lies the limit at which successful reproduction is no longer possible. It was noticeable also that the ovaries of the September breeding population showed rather an unhealthy appearance and contained few ripe eggs,

* I am indebted to Mr. H. W. Harvey and Dr. L. H. N. Cooper for the temperature records at these stations. The salinity range was of the order of 35·00 $^{o}/_{oo} \pm$ ·20; the full data will be obtainable from the Rapport Altantique of the International Council for the Exploration of the Sea for 1930 and 1931.

† Kramp (**7**, p. 36) says: "Undoubtedly the animal dies when the eggs have been spawned."

and there was evidence that gonad development was somewhat retarded during August. During June the majority of the Sagitta would experience the temperature of the lower water layers, but as the warming of the surface waters extends to greater and greater depths during July and August many individuals must experience high temperature conditions.

After the temperature of 14° C. had been passed there was no further



TEXT-FIG. 2.—Curves of Temperature at surface at international station L5 (——) and at 25 metres at E1 (– – –), February, 1930, to June, 1931. Superimposed on these are the periods of spawning of the different broods of *S. elegans* (black areas) plotted against the approximate average length of the adults of each brood. (Left coordinate, temperature in °C.; right coordinate, length in millimetres.) Above are given the approximate periods between the times of spawning.

sign of developing gonads until the water had cooled to nearly 11° C. in December when the male organs started to develop; following on the development of the male organs the ovaries did not ripen until a temperature of about 10° C. was reached in January.

A further point of interest is that the large adults of the spring of 1931 did not grow to so great a length as did those of the spring of 1930, the average and maximum lengths being for each year 16 and 20½ mm. and 19½–20 and 22 mm. respectively. It is to be noticed that the temperature in 1930 was about half a degree lower than that in 1931; there is some evidence also that the 1930 brood was born earlier than that of

1931 and would therefore have had a longer period in which to grow. This difference in size attained in different years is a phenomenon that may throw light on the effects of temperature on growth, but it is not proposed to discuss it further until more data are available for different years.

On the Length of Mature Ovaries.

During the examination of the mounted specimens some measurements were made of the length of the mature ovaries. The ovaries were regarded as mature when they contained one or more fully distended eggs. By a number of measurements of the eggs *in situ* in the mounted material it was found that the average diameter of the largest eggs lay between ·334 and ·394 mm. This agrees fairly with figures given by Huntsman and Reid (**6**, p. 103) in which the diameters for the largest eggs lay between ·310 and ·446 mm.

There is naturally a decrease in the length of the ovaries throughout the season as the successive adults are smaller, but this decrease is not in direct proportion to that of the length. I give below figures showing the average length of ripe ovaries for adults of different sizes, and also the ratio ovary-length/body-length. These show clearly that in the smaller adults the length of the ovary is less in proportion to the total length of the body than it is in the larger adults, being about constant from 10 to 14 mm. and then increasing gradually.

Length of adults in mm.	Average length of ovary in mm.	$\frac{\text{Ovary-length}}{\text{Body-length.}}$
9	0·55	0·061
9·5	0·71	0·075
10	1·01	0·101
10·5	1·19	0·113
11	1·14	0·104
11·5	1·10	0·096
12	1·36	0·113
12·5	1·21	0·097
13	1·30	0·100
13·5	1·49	0·110
14	1·55	0·111
14·5	1·63	0·112
15	1·95	0·130
15·5	2·22	0·143
16	2·22	0·139
16·5	2·76	0·167
17	3·20	0·188
17·5	2·94	0·168
19	3·69	0·194
20	3·86	0·193

Seeing that there is no evidence of any very marked decrease in the diameter of the ripe eggs at the different times of the year it follows from the above figures that the number of offspring produced per parent must be very much greater in the early months of the year when the adults grow to a large size. In fact, in the late summer adults one rarely sees more than one or two ripe eggs present at a time.

Fully spent adults were rarely seen, which agrees with Kramp's observations (**7**, p. 36); they were most noticeable however among the remnants of the large May brood that lived over into June. It was also noticed that in many of those individuals which contained a nematode or trematode parasite the ovaries failed to develop; in obtaining the data on state of maturity for Plate I these were ignored.

As regards the actual number of Sagitta occurring in the catches it is unwise to draw conclusions from one year's results only, on account of errors due to swarming. The full data are given in Table I, page 143, and except for one catch of 7502 individuals on March 17th, 1931, there seems to be some indication that in the year under review the Sagitta were definitely most abundant during the months July and August, and the beginning of September. These would be the offspring of the June and July spawners. It has already been remarked above that the ovaries of the September breeding population appeared unhealthy, perhaps on account of the high temperature, and the figures appear to bear out this as they indicate that the brood arising from the September spawning was only a small one. There is also a tendency shown for each successive brood to decrease in numbers from their first appearance in the catches to the appearance of the succeeding brood.

Observations from other Localities.

It is hard to compare my data with those from elsewhere as there has been little work done that covers the period of a whole year. In general the change in size of adults at different times of the year has been realised, but no very definite data have been obtained. Bigelow (**1**, p. 320), for instance, says for the Gulf of Maine that "the adults average decidedly larger (up to 35 millimetres long) in March and April, when the temperature is near its lowest for the year, than in summer." The difference in the size at maturity of Sagitta from place to place has been well shown by Huntsman (**5**, p. 446), but owing to the difference in the temperatures at different depths and the intermingling of water masses in the region in which he worked it is impossible to find the exact relationship between temperature and size attained. As regards the breeding periods, Huntsman and Reid (**6**) by examination of ovaries concluded that in the Bay of Fundy, "the spawning season is a long one, extending from the end of

March or the beginning of April to September at least. September 4th would seem to be near the end of the season, as at that time a smaller proportion of individuals contained large eggs than previously." From their figures (**6**, p. 103) there was evidently no such period of the year when the nearly full-grown Sagitta were entirely without gonads as occurred off Plymouth during October and November. Bigelow (**1**, p. 315), for the Gulf of Maine, says, " On the whole, then, it is safe to say that *S. elegans* is a late spring and summer breeder in the Gulf of Maine, in so far as any considerable production is concerned, but probably it reproduces more or less throughout the entire year." Fish, for the Woods Hole region, says that in March and April, 1923, the *S. elegans* were ripe, and that the eggs were common in the plankton in April. He also says (**3**, p. 134) that " in the 16 years that *S. elegans* has been recorded, with one or possibly two exceptions, none appeared before November or remained after July." From a temperature curve given by Fish it would appear that the temperatures between July and November were falling from about 20° C. to 10° C. Kramp (**7**, p. 38), after examination of specimens taken in July and August, says, " At most stations small and full-grown specimens were found together, which indicate that the breeding period in the Greenland waters extends over a considerable space of time, though the relative numbers of individuals of the different groups at different times seem to indicate that the bulk of the specimens breed in the autumn."

From data given by Meek (**8**) for the North Sea there is evidently a change in size, during the year, but stages of maturity are not given. From his text-figures on pages 750 and 751 the largest Sagitta would appear to be as follows :—

April, 1924 . .	20 mm.	April, 1925 . .	16 mm.
July, 1924 . .	14 mm.	June, 1925 . .	21 mm.
August, 1924 . .	12 mm.	July, 1925 . .	17 mm.
September, 1924 .	13 mm.	September, 1925 .	10 mm.
October, 1924 . .	18 mm.		

According to Ritter-Záhony the distribution of *S. elegans* is (**9**, p. 17) *S. elegans elegans*, in Atlantic and apparently Pacific Ocean from 45° N. to the ice sea on the surface.* *S. elegans arctica*, Northern ice seas, circumpolar on the surface ; *S. elegans baltica*, Baltic.

The largest size given is 52 mm. by Huntsman (**5**, p. 446) for the waters of the Eastern Atlantic.

* Has been recorded as far south as the Canary Isles by L. Germain and L. Joubin (Res. Camp. Sci. Albert I, Monaco, Fasc. XLIX, 1916, p. 6).

SUMMARY.

1. A study of the adult population of *Sagitta elegans* Verrill has been made in the waters off Plymouth by weekly collections with the 2-metre stramin ring-trawl hauled obliquely in the daylight.

2. Measurements of large samples and a study of the state of development of the gonads has shown that there were apparently four, if not five, broods of *S. elegans* produced in the year.

3. These successive broods were apparently spawning in April–May, June–July, and September, 1930, and February, 1931.

4. In the offspring of the September spawning population no gonads were developed during October and November. The male organs started to ripen in December, and the ovaries in January, but the brood did not appear to be fully mature until February.

5. There was a difference in the size to which the adults of the different broods grew and at which they matured. The approximate average lengths of the adults of the spawning populations were in 1930 for May, 19½–20 mm.; June, 13½–14½ mm.; July, *ca.* 13 mm.; September, 10–10½ mm.; and in 1931, February, 12–12½ mm.; and April–May, *ca.* 16 mm.

6. Measurements of the ovaries of mature animals showed that in the smaller individuals the length of the ovary is less in proportion to the total length of the body than it is in the adults.

7. Very few fully spent individuals were to be seen; the ovaries failed to develop in many of those Sagitta which were parasitised by nematodes.

8. In the total catches the Sagitta were most abundant during July, August and the beginning of September.

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TABLE I.

Total Numbers of *Sagitta elegans* in Ring Trawl Collections.

May	15th,	1930	243	Sept.	24th,	1930	270	Jan.	26th,	1931	117
,,	22nd	,,	101	Oct.	1st	,,	102	Feb.	6th	,,	51
June	10th	,,	1324	,,	7th	,,	74	,,	12th	,,	83
,,	19th	,,	637	,,	14th	,,	303	,,	20th	,,	816
,,	26th	,,	760	,,	16th	,,	229	,,	23rd	,,	474
July	4th	,,	2218	Nov.	6th	,,	441	March	17th	,,	7502
,,	9th	,,	4377	,,	13th	,,	159	,,	26th	,,	491
,,	14th	,,	3942	,,	20th	,,	32	April	1st	,,	935
,,	23rd	,,	2142	,,	26th	,,	61	,,	9th	,,	638
,,	29th	,,	1423	Dec.	3rd	,,	730	,,	16th	,,	764
Aug.	7th	,,	7800	,,	10th	,,	360	,,	22nd	,,	76
,,	14th	,,	5210	,,	17th	,,	262	,,	30th	,,	18
,,	21st	,,	2038	,,	22nd	,,	72	May	6th	,,	91
,,	28th	,,	1018	Jan.	1st,	1931	210	,,	13th	,,	7
Sept.	3rd	,,	1222	,,	5th	,,	109	,,	28th	,,	786
,,	11th	,,	4398	,,	15th	,,	1765				
,,	16th	,,	558	,,	22nd	,,	172				

TABLE II.

Results of Examination of Stained Specimens of *S. elegans* for State of Maturity: Lengths in Millimetres.

Date. 1930	St. I. Upper limit.	St. II. Lower limit.	St. II. Upper limit.	St. III. Lower limit.
May 15th	—	8½	15	13½
,, 22nd	—	8	14	12½
June 10th	8½	7½	13½	11
,, 19th	8½	7½	12½	12½
,, 26th	8	7½	12	11
July 4th	7½	8½	13½	13
,, 9th	9	7	12	12
,, 14th	10	7	12½	12
,, 23rd	10½	7½	12½	12
,, 29th	8½	8	12½	12
Aug. 7th	9	8	13½	12½
,, 14th	9	7½	12½	12
,, 21st	7½	8½	12	10
,, 28th	8	7½	11	10½
Sept. 3rd	7	7	10½	10
,, 11th	—	7½	9	9
,, 16th	9	5½	9½	9½
,, 24th	10	8½	10	10
Oct. 1st	11	—	9½	8½
,, 7th	12	—	11	10
,, 14th	12½	9½	11½	10
,, 16th	12	—	11½	—*
Nov. 6th	12	—	—	—
,, 13th	13	—†	—	—
,, 20th	11½	—††	—	—
,, 26th	11	—**	—	—
Dec. 3rd	12½	9½	13½	—
,, 10th	13	10½	13	—
,, 17th	13	11½	13½	—
,, 22nd	11½	10	13½	—‡
1931				
Jan. 1st	11	8½	12½	—§
,, 5th	10	8½	12½	11
,, 15th	10	10	12½	10
,, 22nd	—	7½	12	10½
,, 26th	—	7½	11½	10
Feb. 6th	—	—	10½	10
,, 12th	7	9	11½	10½
,, 20th	8	8½	11	10½
,, 23rd	—	8½	10½	9½
March 17th	7	7½	14½	12½
,, 26th	10	7	13½	13
April 1st	8	7½	14½‖	14
,, 9th	9½	7	13½	13½
,, 16th	8½	7	13½	13½
,, 22nd	8½	10	12½	14
,, 30th	10	8	13½	15
May 6th	6½	9	14½	13½
,, 28th	10	8	13½	12½

* 1 only at 9½.
† 1 at 12 mm. with a few sperm morulæ.
†† 2 at 11 mm. ,, ,, ,,
** 1 at 11 mm. ,, ,, ,,
‡ One with ovary nearly ripe.
§ 1 at 13½ mm.
‖ 1 at 15½.

TABLE III.

Measurements of Body Length of *Sagitta elegans* in Millimetres.

1930	Length in mm. 4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17	17½	18	18½	19	19½	20	20½	21	21½	22	Total measured.
May 15 .	–	–	–	–	–	–	–	–	1	1	4	1	4	4	14	6	4	3	4	4	2	2	2	3	6	1	4	6	5	10	20	22	12	11	9	1	1	167
,, 22 .	–	–	–	–	–	–	–	–	1	–	1	1	–	2	1	3	3	7	4	5	2	2	4	3	2	–	1	2	6	4	3	6	8	2	4	2	1	80
June 10 .	–	–	1	–	1	1	–	2	5	5	14	12	12	16	16	29	27	28	27	27	13	13	16	5	3	–	1	–	–	–	–	–	–	–	–	–	–	274
,, 19 .	–	–	–	–	–	–	1	2	6	1	14	17	19	17	26	18	21	21	22	44	57	56	42	26	10	7	3	1	–	–	1	1	1	–	–	1	–	435
,, 26 .	–	–	–	1	1	4	1	5	14	9	18	21	30	44	60	52	64	52	45	35	41	33	18	11	5	–	–	2	–	–	–	–	–	–	–	–	–	566
July 4 .	–	–	1	–	–	1	–	4	3	4	7	10	13	20	29	34	52	53	88	85	111	113	67	38	27	8	6	1	1	–	1	–	–	–	–	–	–	777
,, 9 .	–	1	1	1	1	2	9	12	15	18	39	42	48	38	46	43	38	62	59	53	40	24	20	12	9	5	–	–	–	–	–	–	–	–	–	–	–	638
,, 14 .	–	–	–	3	2	5	13	20	23	36	56	63	66	57	62	62	61	64	57	51	32	21	15	8	6	4	1	–	–	–	–	–	–	–	–	–	–	788
,, 23 .	–	1	3	3	4	3	6	17	12	20	32	27	39	65	66	58	75	84	100	79	58	33	42	19	6	3	3	–	–	–	–	–	–	–	–	–	–	858
,, 29 .	–	1	2	8	8	19	34	48	59	64	53	51	47	38	36	54	46	55	63	54	42	17	14	7	2	4	1	–	1	–	–	–	–	–	–	–	–	828
Aug. 7 .	–	–	–	–	2	4	9	10	25	40	61	72	65	63	94	54	49	33	24	5	6	7	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–	627
,, 14 .	–	–	–	2	2	8	9	14	37	55	54	63	65	69	78	55	48	30	24	5	4	4	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	628
,, 21 .	–	–	–	1	5	4	14	17	33	48	64	70	78	91	86	64	43	10	8	3	1	–	2	1	1	–	–	–	–	–	–	–	–	–	–	–	–	644
,, 28 .	–	1	9	5	27	29	45	48	47	58	62	65	70	54	49	13	7	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	592
Sept. 3 .	–	–	1	2	7	21	22	42	41	46	60	78	65	65	29	8	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	491
,, 11 .	–	–	–	–	2	1	3	8	11	16	15	49	96	187	136	53	30	7	4	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	622
,, 16 .	–	1	–	1	4	2	3	14	7	16	22	48	59	54	35	13	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	283
,, 24 .	–	–	1	1	2	6	11	9	15	13	20	34	38	45	30	9	6	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	241
Oct. 1 .	–	–	2	1	–	1	–	3	5	10	12	8	12	13	11	9	4	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	92
,, 7 .	–	–	–	–	–	1	–	1	6	1	6	7	9	16	15	8	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	73
,, 14 .	–	–	–	1	4	1	11	18	16	27	19	22	22	24	14	21	7	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	210
,, 16 .	–	3	3	2	9	6	14	15	23	24	31	23	14	6	3	4	4	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	185
Nov. 6 .	–	–	1	2	15	8	16	11	42	49	73	61	59	36	28	10	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	418
,, 13 .	–	3	1	2	5	4	8	5	13	4	10	13	23	19	18	5	6	4	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	146
,, 20 .	–	–	–	2	3	–	3	–	1	2	4	3	1	3	5	4	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	32
,, 26 .	–	1	8	5	5	4	6	11	10	3	2	1	1	2	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	60
Dec. 3 .	–	–	2	2	4	4	10	13	21	22	27	20	13	9	19	10	14	11	10	3	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	217
,, 10 .	–	–	1	2	3	3	13	15	20	30	37	30	33	32	25	20	16	9	8	6	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	305
,, 17 .	–	–	1	–	1	2	4	8	10	18	19	17	23	24	35	27	27	23	11	11	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	263
,, 22 .	–	–	–	–	–	–	1	–	3	6	2	7	7	5	9	7	8	7	3	3	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	71

TABLE III—*continued.*

1931		Length in mm.																																					Total measured.
		4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17	17½	18	18½	19	19½	20	20½	21	21½	22	
Jan.	1	–	–	1	2	3	5	6	11	9	24	25	30	20	23	14	15	11	4	2	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	206
„	5	–	–	–	–	–	1	4	1	4	7	17	10	13	14	17	11	2	4	2	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	108
„	15	–	–	–	–	–	1	1	2	3	9	15	18	33	31	26	44	31	23	21	11	6	3	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	280
„	22	–	–	–	–	1	4	1	2	7	6	11	12	20	20	26	22	13	11	2	6	–	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	168
„	26	–	–	–	–	–	–	–	–	4	10	6	11	12	18	21	12	10	8	3	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	117
Feb.	6	–	–	–	–	–	–	–	–	–	–	1	–	1	9	8	12	7	7	2	2	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	51
„	12	–	–	–	–	–	1	1	1	–	1	3	5	7	9	18	15	9	10	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	83
„	20	–	–	–	–	–	–	–	1	–	1	–	2	9	8	25	48	53	48	24	7	3	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	231
„	23	–	–	–	–	1	1	1	2	1	1	3	4	4	11	25	28	58	69	44	24	11	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	291
Mar.	17	–	2	3	4	2	5	5	7	5	8	12	14	25	25	26	24	22	30	27	30	11	14	10	2	3	–	–	–	1	–	–	–	–	–	–	–	–	317
„	26	–	1	4	6	5	9	18	13	15	20	21	16	20	15	29	29	26	25	21	16	12	4	3	2	1	1	1	–	1	–	–	–	–	–	–	–	–	334
April	1	–	–	3	3	3	3	7	11	8	19	17	17	12	15	15	9	10	16	12	23	20	22	23	13	11	7	7	2	–	–	–	–	–	–	–	–	–	308
„	9	–	–	–	–	1	4	4	7	9	11	10	11	14	11	9	14	8	13	13	10	9	8	6	12	8	4	2	1	1	–	–	–	–	–	–	–	–	200
„	16	–	–	–	–	–	1	3	4	6	7	5	4	9	5	3	5	6	7	14	7	10	15	27	34	41	27	30	16	11	4	5	1	1	–	–	–	–	308
„	22	–	–	–	–	–	–	–	–	2	3	1	–	1	3	2	2	2	3	2	1	2	2	7	6	12	5	6	8	4	–	1	1	–	–	–	–	–	76
„	30	–	–	–	–	–	–	–	–	–	–	2	–	–	2	–	–	1	–	1	1	–	1	–	1	1	–	1	2	1	1	–	1	1	1	–	–	–	18
May	6	–	–	–	1	–	1	1	2	–	1	–	9	6	4	4	6	7	1	6	6	7	4	3	5	1	2	6	3	2	–	1	–	–	–	–	–	–	89
„	13	–	–	–	–	–	–	–	–	1	–	–	–	–	2	1	–	–	–	–	–	–	–	–	–	–	1	2	–	–	–	–	–	–	–	–	–	–	7
„	28	2	2	2	1	1	6	3	6	4	5	9	3	11	8	11	9	15	16	21	22	26	17	37	23	26	34	27	22	18	8	4	1	–	–	–	–	–	400

On the Biology of Sagitta. II. The Breeding and Growth of *Sagitta setosa* J. Müller in the Plymouth Area, 1930–31, with a Comparison with that of *S. elegans* Verrill.

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With 2 Figures in the Text and Plate II.

In a previous paper (**6**) an account was given of the breeding and growth of *Sagitta elegans* Verrill in the waters off Plymouth throughout a whole year. Similar data are now available for *Sagitta setosa* J. Müller* which also occurs commonly in the Plymouth area. The material has been treated in exactly the same way as was that of *S. elegans*. Measurements were made to the nearest half-millimetre in body-length and the Sagitta were divided into the three stages of development after examination of material stained in Alum Carmine, as described in the previous publication (p. 133 and Text-Fig. 1).

Sagitta setosa were rare in the ring-trawl catches in 1930 until September when they became fairly abundant; so that, whereas the data for *S. elegans* already published covered the period May, 1930, to May, 1931, it has been necessary to take the period September, 1930, to September, 1931, for *S. setosa*. But in order that a fair comparison may be made between the two species the observations on *S. elegans* have been continued to September, 1931, as shown in Text-Figure 1, which is supplementary to Plate I of **6**, and in Tables IV–VI.

General Results.

The results of measurements and examinations of *S. setosa* have been set out in Tables I to III, pp. 156–159. These results have been reproduced in a complete form in Plate II, in which the length distribution on each day has been reduced to a percentage basis and the data regarding state of maturity have been incorporated. The diagram which covers the period September, 1930, to September, 1931, appears to give the following information.

* I am indebted to Professor Alexander Meek for kindly confirming the identification of this species.

There was a population of adults spawning in September, 1930, which gave rise to a brood which matured and spawned during October. The offspring of this October spawning population did not fully mature until the following February; in the middle of December the male organs were ripening, and the ovaries had started to develop by the middle of January. The spawning adults in February gave rise to a brood which matured at and grew to a large size in April and May. The April and May spawners gave rise to a spawning population in June, followed by others in July and August.

The evidence obtained for *S. setosa* is not quite so clear-cut as was that for *S. elegans*. This is no doubt due to the smaller numbers of *S. setosa* available in the catches, and also to the smaller differences in the sizes attained by the adults of the different broods than in *S. elegans*. It is not, for instance, quite certain that there is a definite spawning in February; there are no indications of young coming on in the small catches which ensue. It is however noticeable that when the catches are small the smaller individuals tend to be absent, which is probably due to the selective action of the stramin net, the small Sagitta whose proportion is very low only appearing in large catches. There is however evidence that the *S. setosa* in March and April are maturing at a considerably larger size than they were in January and February, and this is taken as an indication of a different population. Throughout the summer also the catches were often not rich enough to show the full story; there is evidence, however, from the size variation that there are at least two distinct broods between June and September. But the observations with adequate numbers are not quite closely enough spaced to make it clear what has actually happened between June 9th, 1931, and August 14th. One would imagine that there might have been time for the production of two broods. On the other hand the gradual increase in the size of the adults from an average of $11\frac{1}{2}$ mm. at the beginning of July to $13\frac{1}{2}$ mm. on August 5th points to the fact that here we have perhaps one brood which has probably arisen from a spawning in June and has continued to grow in size after attaining maturity. There is indirect evidence available that this may be so, in that the larger Sagitta on August 14th have a high proportion parasitized by Nematodes. In Table III, in the last column, are

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EXPLANATION OF PLATE II.

PLATE II.—The percentage size distribution of *Sagitta setosa* caught in the ring-trawl on the days given. The black, cross-hatched and shaded areas represent the various stages of development (see top of diagram for key). In this diagram the lines of demarcation between different stages must not be taken as being exact, as possible shrinkage has not been taken into account. It must also be realised that the Stage III includes both ripening and mature females, and they must not be regarded as all fully mature, though, usually, probably the majority are so.

given the number of *S. setosa* seen to contain Nematode parasites among those that have been measured. The comparatively high number in August is obvious, and it seems possible that this may indicate that the large adults on the dates given have lived on from July, since one would expect parasites to be more prevalent in a population of old individuals. The beginning of a new brood on August 14th that grows to a spawning population in September is indicated, but spawning also appears to be taking place throughout August.

On the above assumptions then it would appear that from the period September, 1930, to August, 1931, inclusive, there were at least six breeding populations of *S. setosa*, i.e. in September, October, February, April–May, June and July–August.

Plate II also shows that there was a definite difference in the lengths attained by the adults in each successive brood. The approximate average lengths of the adults of the different broods as determined by inspection of the graphs in Plate II without calculation were as follows :—

September, 1930	11½–12½ mm.
October, 1930	9½–10 mm.
February, 1931	11½–12 mm.
April–May, 1931	14 –14½ mm.
June–1st half July, 1931 . . .	11½ mm.
2nd half July–1st half August, 1931 .	12½–13½ mm.
2nd half August–September, 1931 .	12 –11 mm.

Observations on *S. elegans* from June to September, 1931.

As stated above, the observations on *S. elegans* were continued until September, 1931, and the results obtained are given in Tables IV, V, and VI, and represented graphically in Text-Figure 1. The broods in the summer of 1931 followed the same general trend as did those for 1930. The approximate average lengths for the adults were in June *ca.* 12½–13½ mm., July 12–12½ mm., and end of August and beginning of September 11 mm. It was noticeable that in 1931 the brood developing during August reached maturity slightly earlier than did the corresponding brood in 1930. There are slight differences in the sizes attained by the adults at corresponding times in the two years ; Text-Figure 2 shows that there are also slight temperature differences between the two years. Until similar data are obtained over a period of a number of years and in different localities, it is however premature to attempt to correlate slight variations with environmental conditions.

Comparison between *S. elegans* and *S. setosa*.

Comparing the results for the two species of Sagitta shows that there is a similarity in the behaviour of the two species in that neither of them breed during the months of November, December, and early January.

There were, however, certain definite differences.

1. *S. setosa* produced a brood maturing in October, while in *S. elegans* the last pronounced spawning took place in September.

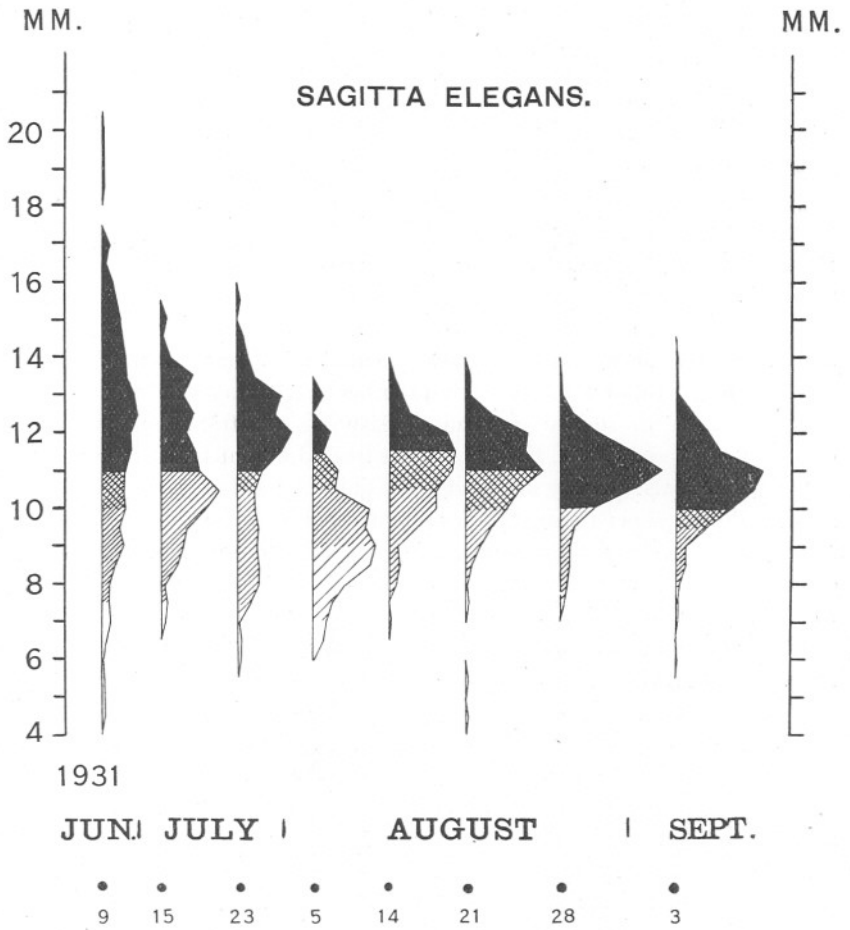
2. Of the broods that lived through the winter without spawning that of *S. elegans* started to mature the earlier; the male organs maturing earlier in December, and the female gonads earlier in January, than in *S. setosa*.

3. There was a distinct difference between the sizes to which the adults of the different species grew at different times of the year. In *S. elegans* the average size of adults between September, 1930, and September, 1931, varied from 10–10½ mm. in September, 1930, to *ca.* 16 mm. in April and May, 1931, and 11 mm. at the end of August and beginning of September, 1931. For *S. setosa* the average lengths for the adults at the corresponding times were 11½–12½ mm., 14–14½ mm., and 11–12 mm. respectively. The range of size variation over a comparable period is thus greater for *S. elegans* than for *S. setosa*. The brood of *S. setosa*, however, which matured in October, 1930, had the low average size for the adults of 9½–10 mm., but there was no mature brood of *S. elegans* during that month to compare it with.

The summarised results are set out in Text-Figure 2 together with the temperature conditions prevailing at the time.

In addition to the above differences between the species a comparison of their actual abundance in the catches is of interest. These figures are given in Tables I and V, and in Table I of **6**. Up to September, 1930, *S. elegans* definitely predominated over *S. setosa*. From that date until August, 1931, the two species are of about the same abundance, but towards the end of August and beginning of September in 1931, *S. setosa* became the predominating species, when the very large catches of 19,725 and 31,666 were recorded on September 3rd and 10th respectively.

The occurrence of two very large individuals among the 31,666 caught on September 10th is of interest. During the sorting of the material almost all the individuals over 13 mm. in length were picked out: the measurements of these including some ranging down to 11 mm. are given in the last line in Table III. There were in all 126 specimens, but as these only amounted to 0·4% of the whole catch their presence has only been



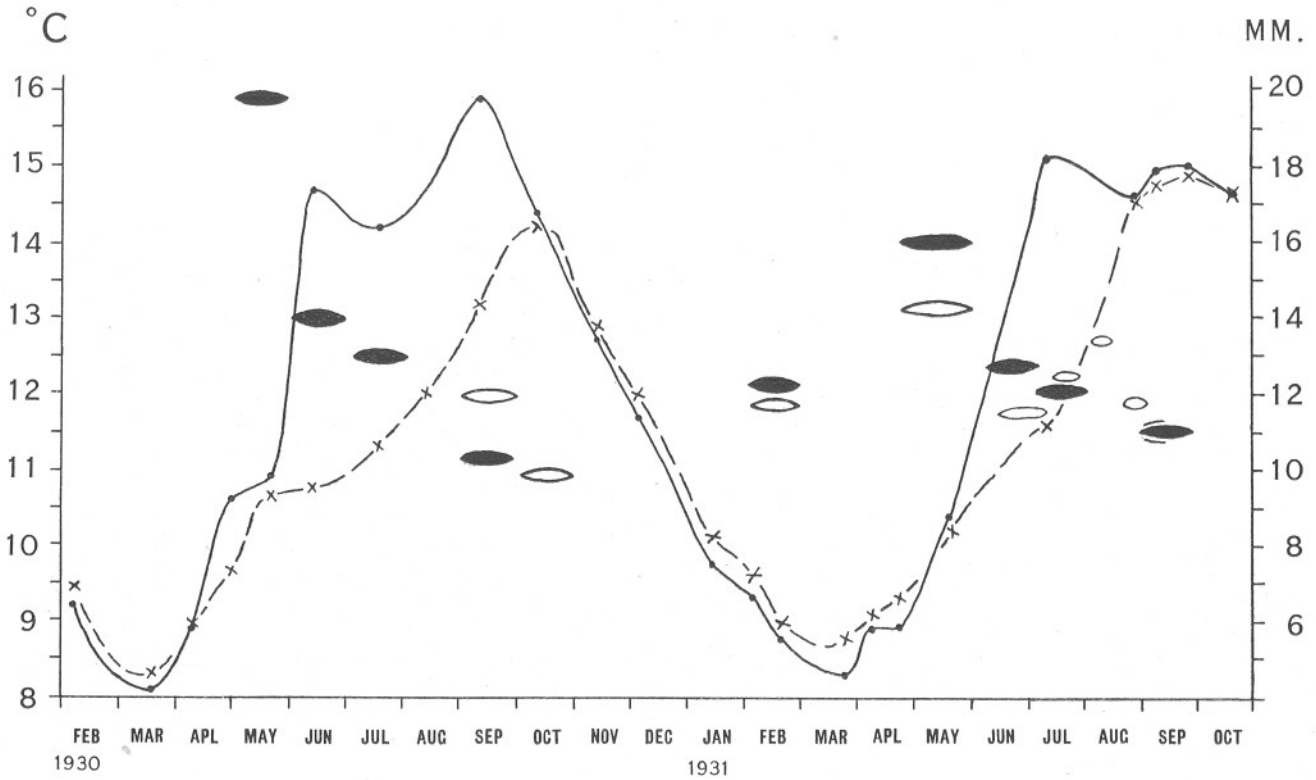
TEXT-FIG. 1.—The percentage size distribution of *Sagitta elegans* caught in the ring-trawl on the days given, June to September, 1931. (For further description see explanation of Plate II.) This figure is supplementary to Plate I of **6**.

indicated in Plate II by a dotted line. Two individuals measuring respectively 19½ mm. and 22½ mm. in length were the largest taken in the whole year and they can probably be regarded as abnormal; both had the appearance of being spent.

The significance of the differences between the two species is not clear and cannot be discussed until more data are obtained and the exact geographical range for each is mapped out together with information of the environmental conditions they can withstand in different localities.

In marine animals the existence of so-called "geographical races" has been known for many years, and attention has especially been turned to their study in such fishes of commercial value as the herring. In marine invertebrates differences in the form of the individuals of a single species from place to place are continually being reported and have been indicated from time to time for most groups of plankton animals. But it is desirable that these observations should be continued over periods of time in different localities as has here been done for Sagitta. While much attention has been given to so-called "temporal variation" in plankton crustacea of fresh waters, little work has been done in this line on marine plankton animals. To my knowledge the only detailed observations covering a long period are those of Adler and Jespersen (**1**) on the size attained by certain species of Copepods, while I myself have carried out similar observations on the adults of *Calanus finmarchicus* during a period April to September inclusive (**5**). In certain localities where hydrographic conditions are complicated it will be difficult to sift "geographic races" from seasonal broods. But a number of observations in many localities may help to throw light on the effects of the environmental conditions on the growth and form of marine animals. A recent paper by Steuer (**7**) brings together a certain amount of information on geographical races of copepods brought about apparently by hydrographic conditions, individuals of a species tending to grow to larger sizes in cold southern waters as compared with the warmer regions, and also when living in the deeper colder layers as opposed to surface-living forms. Slight structural modifications are also indicated. A perusal of his paper emphasizes the need for more detailed seasonal observations in all localities.

It seems unnecessary to say that a species cannot be regarded as a series of standard organisms, but that their reactions to their environment must depend to a great extent upon the conditions under which they live and grow. The formation of geographical and seasonal broods must therefore be taken into consideration in a study of the animals' behaviour and has an obvious bearing on such studies as that of vertical distribution (see Russell, **5**).



TEXT-FIG. 2.—Curves of Temperature at surface at international station L5 (——) and at 25 metres at E1 (– – –). Superimposed on these are the periods of spawning of the different broods of *S. elegans* (black areas) and *S. setosa* (white areas) plotted against the average length of the adults of each brood. (Left coordinate, temperature in °C.; right coordinate, length in millimetres.)

On the Ovary Length of *S. setosa*.

The results of a number of ovary measurements have shown that on an average the lengths of the ovaries of *S. setosa* are shorter in proportion to the body-length than are those of *S. elegans*. The following figures give the results of averaging a number of measurements, but it must be realised that there is considerable variation in ovary length among individuals.

Length of adults (mm.).	Average length of ovary (mm.).	Ovary-length / Body-length.
11	0·96	0·088
11·5	0·96	0·084
12	0·99	0·083
12·5	1·10	0·088
13	1·143	0·088
13·5	1·19	0·088
14	1·32	0·094
14·5	1·63	0·112
15	1·60	0·107
15·5	1·82	0·117

If these figures are compared with those given in **6**, p. 138, for *S. elegans*, it will be seen that the ovaries of *S. elegans* are always proportionally longer than those of *S. setosa* except for those of 14½ mm. length which are equal.

These figures also indicate that as with *S. elegans* there is a tendency for the larger individuals to have relatively larger ovaries in *S. setosa*. From about 10 to 14 mm. the proportion ovary-length/body-length is about constant, but above this length there was a sudden increase. These latter large individuals will have been the adults of the April–May population.

In comparison with the eggs of *S. elegans* those of *S. setosa* as measured *in situ* in stained and mounted specimens are much smaller, being about ·13 to ·19 mm. in diameter.

Previous Observations on *Sagitta setosa*.

Few records are to be found on the occurrence of *S. setosa*. Ritter-Záhony (**3**) gives its distribution as only English Channel, North Sea, Skagerak and Kattegat. He notes that there are three types which differ in fin proportions, especially in the length of the front fin. The specimens that I have examined at Plymouth agree very closely in fin-outline and proportions with the Helgoland type shown in Fig. 1, A, on p. 8 of **4**. Meek (**2**), however, has recorded *S. setosa* in the Northumbrian

plankton agreeing with the Kattegat type in appearance. Ritter-Záhony (**4**, p. 9) gives the largest observed size as 14 mm. total length. Apart from the two large individuals of 19½ mm. and 22½ mm. on September 10th, 1931, my records in Table III show that 16 to 17 mm. seems to be about the limit of size in this area (to which ½ mm. should be added to allow for the tail fin).

SUMMARY.

1. A study of the adult population of *Sagitta setosa* J. Müller has been made in the waters off Plymouth by weekly collections with the 2-metre stramin ring-trawl hauled obliquely in the daylight.

2. Measurements of samples and a study of the state of development of the gonads has shown that there were apparently at least six broods of *S. setosa* during the period September, 1930, to August, 1931, inclusive.

3. The successive broods were apparently spawning in September and October, 1930, and February, April–May, June, and July–August, 1931.

4. In the offspring of the October spawning population no gonads were developed during November. The male organs started to ripen in the middle of December, and the ovaries in the middle of January.

5. There was a difference in the size to which the adults of the different broods grew. The approximate average lengths of the adult of the spawning populations were in 1930 for September, 11–12½ mm.; and October, 9½–10 mm.; and in 1931, February, 11½–12 mm.; April–May, 14–14½ mm.; June–1st half July, 11½ mm.; 2nd half July–1st half August, 12½–13½ mm.; and 2nd half August–September, 12–11 mm.

6. A comparison is made between these data for *S. setosa* and the data previously published (**6**) for *S. elegans*.

7. The need for similar observations in these and other plankton animals in different localities is stressed.

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TABLE I.

TOTAL NUMBERS OF *SAGITTA SETOSA* IN RING-TRAWL COLLECTIONS.

Sept.	11th, 1930	760	Jan.	15th, 1931	339	June	9th, 1931	530
,,	16th ,,	46	,,	22nd ,,	270	,,	16th ,,	18
,,	24th ,,	87	,,	26th ,,	101	July	8th ,,	1
Oct.	1st ,,	45	Feb.	6th ,,	2	,,	15th ,,	106
,,	7th ,,	369	,,	12th ,,	17	,,	23rd ,,	120
,,	14th ,,	683	,,	20th ,,	239	,,	30th ,,	10
,,	16th ,,	258	,,	23rd ,,	134	Aug.	5th ,,	7
Nov.	6th ,,	300	March	17th ,,	221	,,	14th ,,	325
,,	13th ,,	120	,,	26th ,,	333	,,	21st ,,	2545
,,	20th ,,	73	April	1st ,,	1048	,,	28th ,,	7893
,,	26th ,,	84	,,	9th ,,	1872	Sept.	3rd ,,	19,725
Dec.	3rd ,,	1080	,,	16th ,,	385	,,	10th ,,	31,666
,,	10th ,,	162	,,	22nd ,,	27			
,,	17th ,,	376	,,	30th ,,	18			
,,	22nd ,,	78	May	6th ,,	42			
Jan.	1st, 1931	249	,,	13th ,,	8			
,,	5th ,,	59	,,	28th ,,	175			

TABLE II.

RESULTS OF EXAMINATION OF STAINED SPECIMENS OF *S. setosa* FOR STATE OF MATURITY: LENGTHS IN MILLIMETRES.

Date. 1930	St. I. Upper limit.	St. II. Lower limit.	St. II. Upper limit.	St. III. Lower limit.
Sept. 11th	—	—	—	9½
,, 16th	6½	7	9	8½
,, 24th	6	6	9½	10
Oct. 1st	6½	—	9	9
,, 7th	8½*	7½	9†	9
,, 14th	8½	—	8½	8½
,, 16th	8½	7	8½	8½
Nov. 6th	11	—	9½	9½
,, 13th	9½	—	9	9
,, 20th	9½	—	—	—
,, 26th	10	—	—††	—
Dec. 3rd	10	—	—	—**
,, 10th	11½	—	—	—
,, 17th	11	10	11½	—
,, 22nd	11½	9½	—	—§
Jan. 1st, 1931	10½	9½	—	—‖
,, 5th	10	8½	10	—
,, 15th	10½	10	12	—
,, 22nd	11	10½	11½	11
,, 26th	11	9	11	10½
Feb. 12th	9	9	10	9½
,, 20th	8½	7½	11½‡	10

Date. 1931	St. I. Upper limit.	St. II. Lower limit.	St. II. Upper limit.	St. III. Lower limit.
Feb. 23rd	9	7	11	10
March 17th	9	9	12½	11½
,, 26th	—	9½	13	11½
April 1st	10½	7½	13	12
,, 9th	6	—	13	11½
,, 16th	—	9	13½	12
,, 22nd	—	—	11	12
,, 30th	—	—	—	12½
May 6th	—	—	—‡‡	11
,, 13th	—	—	—	12
,, 28th	—	6½	12	9½
June 9th	—	6½	9	9½
July 15th	—	—	—	10
,, 23rd	—	8½	9½	10
,, 30th	—	—	—	10
Aug. 5th	—	—	—	10
,, 14th	5½	6	10	9½
,, 21st	6½	7	10	9
,, 28th	9	8	11	10½
Sept. 3rd	6½	7½	11	10½
,, 10th	9	8½	10	10

* 1 at 12 mm.
† 1 at 13 mm.
§ 1 at 10½ mm.
†† 1 at 9½ mm.

** 1 at 11 mm.
‖ 2 at 10½ and 9½ mm.
‡ 2 at 13½ and 12½ mm.
‡‡ 2 at 12½ and 11 mm.

TABLE III.

Measurements of Body-Length of *Sagitta setosa* in Millimetres.

1930		Length in mm.																								Total measured.	Number with Nematodes.
		4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17		
Sept.	11	–	–	–	–	–	–	–	–	–	–	1	1	2	3	7	25	54	51	14	10	2	–	1	–	–	–	–	171	2
„	16	–	–	–	–	1	2	1	1	4	2	5	1	1	2	6	8	6	6	1	–	–	–	–	–	–	–	–	47	1
„	24	–	2	–	3	7	16	12	14	8	6	2	1	3	1	1	1	–	–	–	1	–	–	–	–	–	–	–	77	–
Oct.	1	–	–	–	–	–	1	4	1	1	1	4	6	6	7	3	4	5	1	1	1	–	–	–	–	–	–	–	46	–
„	7	–	–	–	–	4	2	2	10	13	16	20	49	51	39	19	24	24	11	6	2	–	–	–	–	–	–	–	292	1
„	14	–	–	1	3	4	11	12	21	23	24	37	47	38	19	13	7	6	2	–	–	–	–	–	–	–	–	–	268	2
„	16	1	4	7	11	11	16	19	22	17	21	30	36	24	17	5	6	3	–	–	–	–	–	–	–	–	–	–	250	2
Nov.	6	–	–	1	5	12	17	31	54	45	44	35	24	15	15	3	–	–	–	–	–	–	–	–	–	–	–	–	301	–
„	13	1	3	5	11	8	14	11	16	20	8	12	3	3	1	1	–	–	–	–	–	–	–	–	–	–	–	–	117	–
„	20	–	–	3	3	12	2	11	9	16	10	4	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	73	–
„	2	1	2	4	5	14	11	13	6	11	6	5	1	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	82	–
Dec.	3	–	–	1	7	7	13	18	25	25	21	20	19	9	4	–	1	–	–	–	–	–	–	–	–	–	–	–	170	–
„	10	–	2	2	2	7	15	26	20	22	9	21	17	4	3	1	1	–	–	–	–	–	–	–	–	–	–	–	152	–
„	17	–	–	–	2	5	5	9	17	28	18	26	26	20	7	2	3	–	1	–	–	–	–	–	–	–	–	–	169	–
„	22	–	–	1	3	2	–	3	6	8	11	9	13	5	3	7	2	1	–	–	–	–	–	–	–	–	–	–	74	–
1931																														
Jan.	1	–	1	1	5	7	28	20	32	35	36	28	17	13	6	4	1	–	–	–	–	–	–	–	–	–	–	–	234	–
„	5	–	–	–	–	2	4	–	3	4	13	10	9	6	5	–	–	–	–	–	–	–	–	–	–	–	–	–	56	–
„	15	–	–	–	–	1	4	3	9	11	13	16	30	24	28	20	17	5	4	–	–	–	–	–	–	–	–	–	185	1
„	22	–	–	2	1	–	10	11	11	15	30	30	19	36	34	24	12	11	–	–	–	–	–	–	–	–	–	–	246	–
„	26	–	–	–	–	–	1	4	6	4	8	12	16	11	19	12	5	3	–	–	–	–	–	–	–	–	–	–	101	1
Feb.	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	2	–
„	12	–	–	–	–	–	–	1	–	1	–	–	3	2	3	2	1	3	1	–	–	–	–	–	–	–	–	–	17	–
„	20	–	–	–	–	–	2	2	1	5	7	8	8	7	18	16	27	18	19	4	2	2	1	–	–	–	–	–	147	–
„	23	–	–	–	–	–	–	2	3	3	2	7	7	9	4	16	14	21	11	7	1	–	–	–	–	–	–	–	107	–
March	17	–	–	–	–	–	–	–	–	–	–	–	1	3	1	4	3	5	9	13	8	3	1	–	–	–	–	–	51	–
„	26	–	–	–	–	–	–	–	–	–	–	1	2	4	3	9	15	25	36	55	42	18	5	1	–	–	–	–	216	1
April	1	–	–	–	–	–	–	–	–	1	1	1	6	6	6	19	23	23	15	33	19	10	3	–	–	–	–	–	166	1
„	9	–	–	–	–	–	–	–	–	–	–	–	2	–	3	1	8	17	35	32	41	15	7	1	–	–	–	–	162	2
„	16	–	–	–	–	–	–	–	–	–	–	–	1	2	3	4	8	9	12	32	31	29	17	8	3	–	–	–	159	2
„	22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	3	6	8	3	5	–	–	–	–	27	–
„	30	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	2	4	5	–	1	–	–	18	1
May	6	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	2	1	4	4	10	10	5	1	4	–	–	42	1
„	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	3	1	2	–	–	–	–	8	–
„	28	–	–	–	2	2	1	4	7	6	15	13	7	14	6	8	4	5	5	2	6	6	21	19	13	3	5	1	175	3

June	9	–	–	2	–	–	3	5	3	3	12	16	23	33	27	42	53	31	30	11	8	13	9	7	11	1	–	–	343	–
„	16	–	–	–	1	–	–	1	–	–	–	1	1	1	3	3	–	2	2	–	–	1	–	1	–	–	1	–	18	–
July	15	–	–	–	–	–	–	–	–	–	–	–	3	4	16	19	27	17	12	4	1	2	1	–	–	–	–	–	106	–
„	23	–	–	–	–	–	–	–	–	1	1	1	3	3	6	10	15	26	24	23	4	2	1	–	–	–	–	–	120	2
„	30	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	1	2	5	–	–	–	–	–	–	–	–	10	–
Aug.	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	2	1	1	–	–	2	–	–	–	–	–	7	1
„	14	–	1	–	–	1	8	8	4	8	4	6	6	10	14	13	11	20	39	48	51	21	6	6	–	1	–	–	286	20
„	21	–	1	5	2	8	9	16	14	21	23	28	28	44	37	39	34	29	31	29	22	10	–	1	–	–	–	–	431	14
„	28	–	–	–	–	–	–	–	2	2	10	10	9	23	32	41	44	46	39	23	14	8	1	2	–	–	–	–	306	6
Sept.	3	–	–	1	2	1	4	6	5	6	9	13	27	20	27	31	40	33	19	15	3	2	1	–	–	–	–	–	265	1
„	10	–	–	–	–	–	1	–	3	8	9	13	24	34	63	64	62	24	16	2	1	–	–	–	–	–	–	–	324	–
„	10*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*4*	*5*	*15*	*21*	*16*	*17*	*18*	*12*	*13*	*2*	*1*	–	–	*126*	8

* 0·4% of whole catch: also 1 of 19½ mm. and 1 of 22½ mm.

TABLE IV.

Measurements of Body-Length of *S. elegans* in Millimetres.

	Length in mm.																															Total measured.
1930	4	4½	5	5½	6	6½	7	7½	8	8½	9	9½	10	10½	11	11½	12	12½	13	13½	14	14½	15	15½	16	16½	17	17½	18	18½	19	19½	20	
June. 9	–	1	2	1	–	4	9	6	9	15	25	21	27	26	27	36	34	44	39	31	29	26	23	17	12	4	7	–	–	2	2	1	1	481
„ 16	–	–	–	1	1	1	2	–	1	3	1	1	1	–	–	–	–	1	–	–	–	–	2	–	–	–	–	1	–	–	–	–	–	16
July 8	–	–	–	–	1	–	1	2	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
„ 15	–	–	–	–	–	–	2	3	2	9	13	15	25	33	22	20	15	19	13	18	5	2	3	–	–	–	–	–	–	–	–	–	–	219
„ 23	–	–	–	–	1	3	1	10	18	18	16	18	15	15	21	36	45	32	36	15	9	6	–	1	–	–	–	–	–	–	–	–	–	316
„ 30	–	–	–	–	–	4	1	2	2	3	3	2	–	–	1	1	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	22
Aug. 5	–	–	–	–	–	2	3	4	7	13	14	12	13	5	6	2	4	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	87
„ 14	–	–	–	–	–	–	1	–	6	9	7	25	42	41	55	57	50	18	11	5	–	–	–	–	1	–	–	–	–	–	–	–	–	328
„ 21	–	1	–	1	–	–	–	2	1	3	9	16	26	33	49	38	39	16	3	3	–	–	–	–	–	–	–	–	–	–	–	–	–	240
„ 28	–	–	–	–	–	–	–	3	6	7	8	12	28	63	84	62	30	10	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	316
Sept. 3	–	–	–	–	1	–	2	1	3	9	8	27	50	70	77	40	28	15	3	2	2	–	–	–	–	–	–	–	–	–	–	–	–	338
„ 10	–	–	–	–	–	–	–	–	–	2	–	1	2	4	7	4	4	1	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	28

TABLE V.

TOTAL NUMBERS OF *S. ELEGANS* IN RING-TRAWL COLLECTIONS.

June	9th, 1931	434	Aug.	5th, 1931	88
,,	16th ,,	16	,,	14th ,,	824
July	8th ,,	5	,,	21st ,,	1058
,,	15th ,,	219	,,	28th ,,	2570
,,	23rd ,,	544	Sept.	3rd ,,	5152
,,	30th ,,	22	,,	10th ,,	256

TABLE VI.

RESULTS OF EXAMINATION OF STAINED SPECIMENS OF *S. ELEGANS* FOR STATE OF MATURITY: LENGTHS IN MILLIMETRES.

Date.	St. I. Upper limit.	St. II. Lower limit.	St. II. Upper limit.	St. III. Lower limit.
June 9th	—	7½	11	10
,, 16th	7	8	9½	?
July 15th	—	7½	11	11
,, 23rd	7½	7	11	10½
,, 30th	7	7½	11½	11½
Aug. 5th	9	7	11½	10½
Aug. 14th	8	7	11½	10½
,, 21st	—	7½	11	10
,, 28th	—	7	10	10
Sept. 3rd	6½	7½	10	9½
,, 10th	—	8½	11	10½

The Determination of Nitrate in the Sea by Means of Reduced Strychnine.

By

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Assistant Chemist at the Plymouth Laboratory.

With 1 Figure in the Text.

THOMPSON and Johnson (**7**) have criticised the method of analysis for nitrate in sea-water by means of reduced strychnine (**2**, **3**, **4**). They state : " Plans had been made to use Harvey's (1928)* method for the nitrate ions but considerable difficulty was encountered in preparing the reagent. Furthermore, any oxidising material present in the water would rapidly react with the reagent. Thus iodate ions appeared to give a reaction similar to the nitrate ions. Other investigators on the Pacific Coast have experienced considerable difficulty with this particular nitrate reaction and in general it has been discarded as being unreliable from a quantitative standpoint."

In view of the precautions necessary, it seems desirable to stress that the method has given a good general picture of seasonal changes in nitrate in the sea, that the reagent presents no undue difficulty of preparation and that it has been used with success by a number of independent workers in Europe.

Following in detail the method of preparation described in (**4**), little difficulty has been experienced by the writer in obtaining good batches of reagent from the majority of the strychnine reductions. It was sometimes necessary to continue the reduction for 36 hours (3 days of 12 hours each) to obtain a product of suitable strength. The two most probable sources of contamination are an impure atmosphere such as that of a large industrial town, and impure reagents, particularly sulphuric acid containing nitric acid. Wattenberg (private communication through Mr. Harvey) avoids any risk of atmospheric contamination by passing a current of an inert gas through the stoppered reduction flask. This variation has not been tried at Plymouth.

Impure sulphuric acid is the commonest source of trouble. A few preliminary experiments have been made to devise a method for removing

* Reference **5** in appended bibliography.

nitric acid from sulphuric acid. Most of the impurity in a grossly contaminated acid may be removed by heating for one or two hours at 300° C. with a few crystals of ammonium sulphate. The method, which is dependent on the breakdown of ammonium nitrate at a high temperature to nitrous oxide and water, has not yet been conclusively shown to remove the last trace of nitric acid. It may be worth following up by anyone

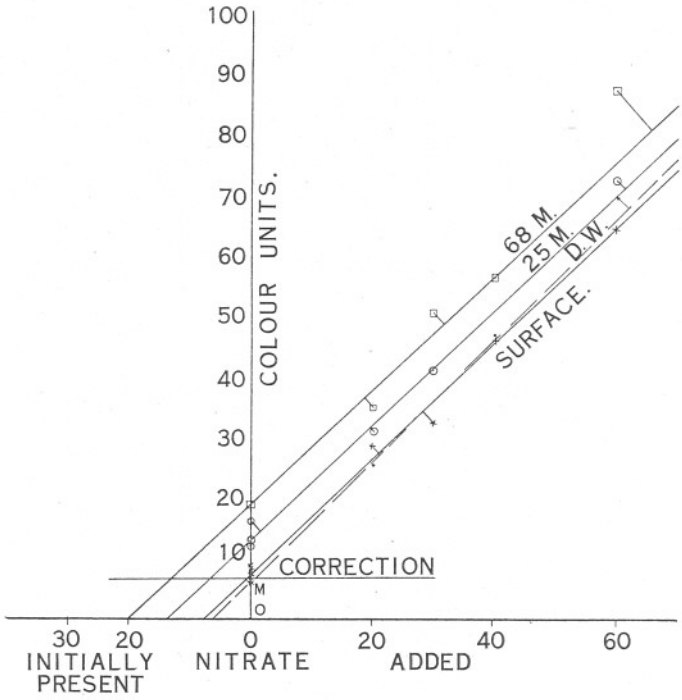

FIG. 1.—Graphical Method of Evaluating Nitrate from Colour Measurements.

Samples collected and preserved at E1, July 10, 1931, analysed July 20–21 with reagent six weeks old. Curves for water from surface, 25 m. and 68 m. (bottom) and for distilled water redistilled from baryta (D.W.) as marked. The horizontal line shows the blank correction for colour given by distilled water alone.

unable to obtain commercially a nitrogen-free sulphuric acid. The colour given with distilled water by a satisfactory reagent did not exceed that given by 5–8 mg. of nitrate-nitrogen per cubic metre.*

In order to carry out the determination of nitrate, 6 c.c. of the reagent were added to 5 c.c. portions of sea-water or of distilled water to some of

* Atkins (*Nature*, 1932, **129**, p. 98) has recently achieved the same object by cautiously adding hydrogen sulphide or ammonium sulphide in such quantity that a trace of nitric acid remains, sufficient to give the faintest perceptible colour with a diphenyl-benzidine reagent.

which 10, 20, 30, 40, or 60 mg. per cubic metre of nitrate-nitrogen had been added. The resultant colour in each case was compared 18 hours later in a Duboscq colorimeter with a standard solution of safranine. This dyestuff matches solutions containing up to 50 mg./m.^{3} of nitrate-nitrogen exactly and the difference in shade for higher concentrations is scarcely significant. Its solutions at the required dilution obey Beer's Law. A 0·0008% solution of safranine (from Grübler, dyeing strength not stated) has been arbitrarily taken as containing 100 colour units, and with a good batch of nitrate reagent is equivalent to from 90 to 130 mg. of nitrate-nitrogen per cubic metre. When examining solutions weaker than this the safranine solution has been proportionally diluted. This is a simple quantitative method of evaluating the sensitivity of a batch of reagent and would enable a comparison to be made between the products of different laboratories. The safranine does not fade appreciably in the colorimeter during several hours' continuous working with a "daylight" lamp and its tint is not altered by traces of hydrochloric acid fumes.

When the colour given by various samples had been found in terms of the standard colour unit, the actual nitrate content was evaluated by a graphical method (Fig. 1). Since the colour developed by a reagent less than six weeks old with distilled water is less than that with sea-water the blank correction in such a case may be too small.

In the Figure the results for distilled water redistilled from baryta and E1 surface water are nearly co-linear, showing that the surface water was completely exhausted of nitrate. The length OM represents colour due to reagent alone. The 25-metre and 68-metre E1 samples with added nitrate gave greater depths of colour which gave linear curves on the graph parallel with the first two. In these cases a certain amount of nitrate was initially present in the water.

The method of calculation is shown by the following fictitious example :

Suppose that the reagent gives 7 colour units with distilled water and 54 units with an unknown sample, and that the graph shows that 100 colour units are given by sea-water containing 90 mg. of nitrogen per cubic metre present as nitrate+nitrite without allowing for the blank correction.

Since 100 colour units =90 mg./m.^{3} N.

Then 54 ,, ,, $=\frac{54\times 90}{100}=48{\cdot}6$ mg./m.^{3} N.....(1)

And 7 ,, ,, $=\frac{7\times 90}{100}=6{\cdot}3$ mg./m.^{3} N.....(2)

Subtracting the blank correction (2) from (1), the true amount of nitrate (+nitrite) present is 48·6—6·3=42·3 mg./m.^{3} N.

This method of evaluating the results is in practice very simple.

Interference by Organic Material.

Presence of organic material, particularly phytoplankton during the spring or autumn outbursts, may completely invalidate the method unless first removed (**4**). Samples preserved with mercuric chloride brought in from the International Station, E1, on March 23, 1931, gave an absurd set of results. Centrifuging the samples in an electric centrifuge for fifteen minutes was found to remove the source of trouble, so that when various known amounts of nitrate were added to three of the samples the usual linear relation between colour and nitrate concentration was obtained.

When much plankton is present in the water, it would seem essential to centrifuge the samples or to remove living and dead organic matter in some other way before making the determination. This is of much importance since nitrate figures are of most interest when the plankton outbreak is occurring.

Thus, during twelve months' work the method, given due care, has been found well able to give concordant results, such as those shown in Figure 1. An occasional high value, due apparently to contamination, had to be discarded.

Influence of Iodate.

The depth of colour developed by different amounts of iodate, calculated as mg. iodine per cubic metre, is shown in Table I as if it were due to nitrate expressed as mg. nitrogen per cubic metre. It will be seen that the ratios

TABLE I.

Colour Development Due to Iodate.

Iodate expressed as iodine mg./m.^{3}	Increase in colour due to iodate expressed in terms of nitrate-nitrogen mg./m.^{3}
42,400	71·4
21,200	28·6
4,240	3·4
4,240	2·9
2,120	1·5
2,120	1·5
1,060	1·2
1,060	3·0

are very large and that the colour due to 4,000 mg. of iodine per cubic metre gives a depth of colour little greater than the experimental error inherent in the method. Such a relatively enormous amount of iodine

is known not normally to be present in sea-water (**6**) and therefore the possibility that iodate interferes may be dismissed.

Iron has already been investigated (**2**) and found to be without appreciable effect in the concentrations in which it is present in the sea except possibly in inshore waters. The zero values found in summer also support the contention that other oxidising agents are not likely to interfere.

Effect of Nitrite.

The reagent is recognised as determining both nitrate and nitrite, but it had not been finally shown whether equivalent amounts of each give rise to the same intensity of colour. If the relation is definitely known and the nitrite has been determined with the Griess-Ilosvay reagent, the true amount of nitrate present may be found by difference. It seems that solutions containing added nitrite give more erratic results than those containing added nitrate. Two possible reasons may be adduced for this. The free nitrous acid produced in presence of sulphuric acid is certainly more unstable than nitric acid and may tend spontaneously to decompose. Furthermore, nitrous acid may be more liable to react with organic matter.

One set of determinations made on water, collected on September 25, 1931, at E1, 5 metres depth, which was not centrifuged gave an excellent linear relation which showed nitrite exactly equivalent to nitrate. In four other sets on samples collected on July 10, September 8 and November 30, 1931, from E1 surface or 25 metres, less satisfactory curves were obtained since the experimental points did not show good alignment and the ratio $\frac{\text{colour due to nitrite}}{\text{colour due to nitrate}}$ varied from 0·54 to 0·80. Some of these samples were centrifuged (including that which gave the figure 0·54), some not. Ideally, therefore, the ratio approaches unity, but under standard working conditions it appears often to be about 0·7 or 0·8.

As a corollary, when nitrite is very high in the sea (Atkins, **1**, found up to 38·9 mg. of nitrite-nitrogen per cubic metre at E1 on August 29, 1928) difficulty may be anticipated in getting concordant results with the reagent.

Acknowledgements are due to Mr. H. W. Harvey of this Laboratory for valuable criticism and advice, and to Dr. E. J. Allen for his general interest.

SUMMARY.

The recent criticism of Thompson and Johnson (**7**) of the method of determining nitrate in the sea by means of reduced strychnine is held to be unsound.

Preparation of the reagent given due care and pure materials has offered little difficulty.

A possible method for the preparation of nitrate-free sulphuric acid is outlined.

The effect of iodate in concentrations likely to be met in the sea is negligible.

Interference by organic material has been avoided by first centrifuging all samples obtained during periods of plankton activity.

The relative intensity of colour given by nitrate and by nitrite nitrogen is discussed.

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Nitrate in Sea-water and its Estimation by means of Diphenylbenzidine.

By

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With 4 Figures in the Text.

Introduction.

Several methods have been used for the estimation of nitrate. First there stands the method employed by Raben and his co-workers and carried out in connection with Brandt's researches. This depends upon the reduction of the nitrate to ammonia, which is distilled off and nesslerised. These researches have been summarised by Brandt (1927). In the course of time a number of minor modifications tending to increase accuracy were made in the method, notably that of Buch (1923).

Gad-Andresen (1923) carried the reduction of ammonia a step further by means of alkaline sodium hypobromite, with liberation of free nitrogen, the volume of which was then measured. This method appears to give accurate results but is somewhat tedious. It was examined also by Orr (1926).

Harvey (1926) criticised these methods on the ground of their tediousness and of their being vitiated by a systematic error, which lowered their value, if it did not render them useless, in comparing the nitrate content of water which had been almost depleted of this constituent. The drastic method of reduction employed gives rise to ammonia from amino acids and possibly from proteid matter suspended in the water. Harvey applied to the analysis of nitrate in sea-water a colorimetric method introduced by Denigès as a test for bromine and subsequently used by him in 1911 as a test for nitrites and nitrates in water. This depends upon the oxidation of a reduced strychnine compound in sulphuric acid solution. The correct reduction product is not very easy to prepare and any method involving the use of strong sulphuric acid has certain obvious drawbacks and possible sources of error. Nevertheless, in Harvey's hands, this reagent has given good results whereby the changes in the English Channel have been studied throughout the year (1926, 1928). This method is well adapted for use in ships and rich results have been obtained

by recent expeditions using it. At a meeting in Copenhagen in 1928 Brandt, Buch and Giral summarised the methods of estimating nitrogenous compounds in sea-water, and Harvey, Sund and Wattenberg gave their experiences using the reduced strychnine method. Gran (1930) published some of his results obtained in the North Sea with Klem and Braarud. Bigelow and Leslie also used the method in Monterey Bay in 1928. They report a difficulty in securing a suitable reagent. Moberg (1928, 1929) also used this method in the Pacific and introduced slight modifications. Ibañez (1929), too, made analyses by this method with water from off the Spanish coast. Wattenberg with Böhnecke and Hentschel obtained most interesting results in the open sea between Iceland and Greenland. Furthermore, Ruud (1930) investigated the nitrate content in the Antarctic, in the Weddell Sea, using reduced strychnine and the colorimeter devised by Sund. Kreps and Verjbinskaya (1930) also used the method in studying the Barents Sea in the Arctic. In addition to all these, it was used with conspicuous success by Helge Thomsen (1931) who worked with Sund's colorimeter and determined nitrates in the Atlantic, Mediterranean, Indian Ocean, Pacific Ocean as well as the seas around South China, the Celebes Sea, Sulu Sea and the Caribbean Sea. Thompson and Johnson (1930) comment on the difficulty in preparing the reagent and point out that any other oxidising material present in the water would also react with it; in particular they mention iodate as giving a similar reaction. An accompanying paper in this Journal by Cooper considers the validity of the conclusions which led Thompson and Johnson to reject the method as being unreliable from the quantitative standpoint. It may however be pointed out that it seems to have been used quite successfully by a number of other workers who obtained results at least sufficiently accurate for their purpose. Bini (1929) apparently intended to use this method for examining the water of the Red Sea, but decided that it was not a convenient one to use on board ship; accordingly his column for nitrate is entirely filled with a succession of blanks. This does not however hinder him from putting forward the old theory that denitrifying bacteria are responsible for the low values of nitrate in the Red Sea. The theory that the absence of nitrate in tropical waters, or its presence in small quantities only, is due to the action of denitrifying bacteria appears to the writer to be at variance with the available evidence. From the action of denitrifying bacteria, in cultures rich in organic substances, the deduction has been made that these bacteria will act similarly in a dilute inorganic solution, such as sea-water, which is normally supersaturated with oxygen and provides no source of energy for this process. It is hard to see why bacteria should expend energy in taking oxygen from nitrate when there is already an ample supply of the gas dissolved in the water around them. Further-

more, the consumption of nitrate nitrogen by fixed algæ and by diatoms can readily be demonstrated so that its absence in tropical waters can be looked on as due to its complete absorption by algæ in the well-illuminated upper regions. Again, the results of Thomsen, for the Mediterranean, show that the waters of this sea may be divided into seven regions. Working in the month of May he found an appreciable amount of nitrate in the surface waters of the third and fifth areas and little or none in any of the others except at one station in the first area. Phosphate on the other hand was found to be absent from the surface waters and down to very considerable depths, such as to 150 metres or more, in all or nearly all the stations examined. It is obvious therefore that the presence of nitrate, in the stations at which it was found, can be accounted for by the fact that, though nitrate and phosphate had both been brought up by vertical mixing in these regions, yet the phosphate supply had become exhausted; consequently plant growth could proceed no further and nitrate only remained in the surface water. As in the English Channel, it appears that the phosphate is the first to be exhausted and that as the season goes on nitrate becomes completely used up. This is due to the slow regeneration of phosphate which enables the nitrate to be taken up in the course of time.

Colorimetric methods have long been in use for the examination of the nitrate content of fresh water, notably the phenol (Sprengel, 1864) and cresol (Lindo, 1888) disulphonic acid and the carbazol (Hooker, 1888) methods. These are vitiated by presence of chloride, so obviously cannot be used in sea-water.

Another method based on the use of diphenylamine in sulphuric or acetic acid has been much used since introduced by Kopp (1872) and a large number of papers have been devoted to its examination.

The diphenylamine method was critically examined by Tillmans (1910) and he and Sutthoff (1911) applied it to the estimation of nitrates and nitrites in water. By the addition of sodium chloride to the reagent the degree of accuracy was increased, and they claim that results are uniformly obtainable to an accuracy of 0·1 mg. nitric acid per litre. They state that nitrites, if present, react similarly to nitrates and combined determinations may be made. In sea-water it is desirable to make determinations not to 0·1 but to 0·001 milligrams of nitrate nitrogen per litre. As far as the writer is aware the diphenylamine method has been applied to the analysis of the nitrate content of sea-water only by Tschigirine and Daniltchenko (1930) in the Black Sea, for the peculiar conditions of which they obtained interesting results. The diphenylamine test is not of course a specific test for nitrates, like the phenoldisulphonic reaction. Ekkert (1925) examined a number of substances which might possibly give the reaction and found that it was given by nitrate, nitrite, chlorate,

perchlorate, bromate, iodate, chromate, bichromate, molybdate, vanadate, ferricyanide and by hydrogen peroxide. None of these substances are, however, likely to interfere in the estimation of nitrate and nitrite in sea-water, with the possible exception of iodate, which will be discussed later.

The Diphenylbenzidine Test.

In spite of the large number of papers devoted to its study since introduced by Kopp (1872) it was felt by Letts and Rea (1914) that the diphenylamine reaction was not entirely reliable. The probable course of the reaction has been studied by Kehrmann and Micewitz (1912) who pointed out that the diphenylamine was first of all reduced to tetraphenyl-hydrazine and that this became rearranged to give diphenylbenzidine. Further, the removal of hydrogen and the presence of sulphuric acid resulted in giving an imonium compound.

1. $2(C_6H_5{\cdot}NH{\cdot}C_6H_5)-2H=(C_6H_5)_2{\cdot}N{\cdot}N{\cdot}(C_6H_5)_2$
2. $(C_6H_5)_2{\cdot}N{\cdot}N{\cdot}(C_6H_5)_2=C_6H_5{\cdot}NH{\cdot}C_6H_4{\cdot}C_6H_4{\cdot}NH{\cdot}C_6H_5$
3. $C_6H_5{\cdot}NH{\cdot}C_6H_4{\cdot}C_6H_4{\cdot}NH{\cdot}C_6H_5-2H+H_2SO_4=$
$C_6H_5{\cdot}N{\cdot}C_6H_4{\cdot}C_6H_4{\cdot}NH(C_6H_5){\cdot}O{\cdot}SO_3H$

Letts and Rea thought that the chances of this rather complex reaction going astray might be considerably lessened by starting with diphenyl-benzidine instead of with diphenylamine. Wieland (1913) had prepared diphenylbenzidine, by oxidation of diphenylamine, in the course of his researches upon the production of the blue colour. Letts and Rea started with carefully purified diphenylbenzidine and found that the reaction depended on the temperature, the time and the proportions of the reagents. Their table gives results for nitrate nitrogen in water varying from 14·00 to 0·10 parts per 100,000. The stronger solutions were diluted for estimation and comparisons were made in small crucibles. A colorimeter was not used. Nitrites, where present, were removed by means of potassium permanganate. Smith (1917) considered the diphenylben-zidine reaction to be about twice as sensitive as the diphenylamine reaction, in addition to being of greater reliability.

Preliminary tests on sea-water showed that the diphenylbenzidine reaction appeared to work well. Using sea-water and a dilute solution of the reagent in pure sulphuric acid it was found that, when the sea-water was diluted with an equal volume of distilled water, the ratio of the nitrate content of the diluted to the undiluted sea-water was 1 : 1·70 after 24 hours and 1 : 1·91 after 46 hours. Comparisons were carried out in a Kober colorimeter. Tests were made on a number of samples of sea-water which had been stored in the laboratory for some months. The results are shown in Table 1. The figures obtained by the use of diphenylbenzidine, shown

in the column marked A, indicate that the method is likely to give consistent results with sea-water. Indeed, the accord shown with the values obtained by the reduced strychnine method is remarkable. The actual amount of nitrate present in the C and A series cannot have been the same,

TABLE 1.

COMPARISONS OF NITRATE FOUND IN SEA-WATER AT INTERNATIONAL HYDROGRAPHIC STATION E1 ON VARIOUS DATES, TAKING THE 70 M. VALUES AS 100 PER CENT FOR EACH WATER COLUMN.

The values under C are those kindly supplied by L. H. N. Cooper, from his own analyses (see accompanying paper) by the reduced strychnine method, carried out on preserved samples. Under A are shown those obtained using the diphenylbenzidine method; the samples were stored in semi-darkness, without preservative, till mid-October, 1931.

	April 7.		April 22.		May 18.	
m	C	A	C	A	C	A
0	—	—	100	107	25	122
5	106	98	80	80	100	108
10	85	88	80	77	100	102
15	—	—	95	97	100	(59)
25	—	—	105	95	137	103
50	—	—	88	98	150	102
70	100	100	100	100	100	100

so it seems that regeneration of nitrate must have proceeded in the A series in a manner roughly proportional to the amount of nitrate originally present. This is very remarkable, in view of the results of Issatchenko (1926), for he failed to find nitrate producing bacteria in the water column except near the bottom. It appears, however, that in our samples a certain measure of nitrate regeneration has proceeded throughout the column, since the absolute amounts found under the heading A are greater than those under C, as shown by later work. This point must be left over for future investigation. It remains to consider the conditions necessary to obtain the best results when the reaction is carried out in sea-water.

PROPORTIONS OF WATER AND REAGENT.

Letts and Rea recommend that the reaction should be carried out by taking 0·5 ml. of solution and adding to it 1·2 ml. of pure concentrated sulphuric acid. Heat is developed, but before adding diphenylbenzidine the mixture is allowed to cool down to room temperature. When cool

0·3 ml. of a solution of diphenylbenzidine in concentrated sulphuric acid is added and the colour produced is examined after a few minutes and again after an hour or more. To study this point a series of quartz test tubes were taken and to them 1·00 ml. of a solution containing 0·001 mg. of nitrate nitrogen was added. Water, which had been previously distilled, was redistilled from barium hydroxide through a glass condenser and accurately-measured small volumes were added to dilute the 1 ml. already in the tube. Pure sulphuric acid containing diphenylbenzidine was then run in to make a total volume of 11·0 ml. in each case. Figure 1

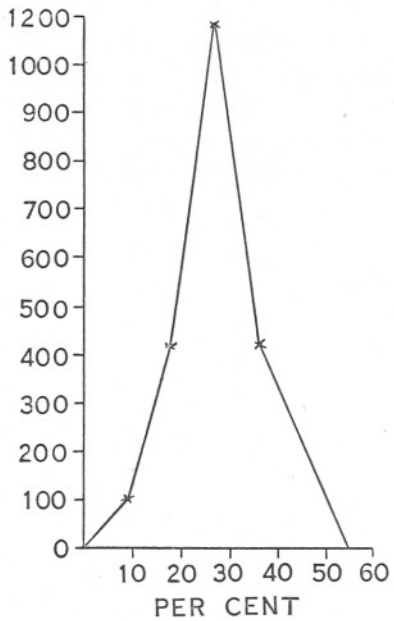

FIG. 1.—The abscissæ denote percentages of water, by volume, in the sulphuric acid mixtures. The ordinates are arbitrary colour units. The nitrate concentration was constant throughout.

shows the intensity of colour in relation to percentage of water by volume. The maximum was found with the proportion of 3 of water to 8 of pure acid, by volume, which is equivalent to 27·3 per cent. Such a mixture gives the greatest evolution of heat. This is close to the value 25% recommended by Letts and Rea. It may be noted how sharply production of colour falls off once the maximum has been passed. This appears to be due to the dissociation of the sulphuric acid imonium compound shown in the equation already given. It was thought at first that, instead of using a colorimeter, it might be possible to titrate back this oxidised

substance using a reducing reagent such as a sulphide or titanous chloride. This however was found to be impossible owing to the large effect exerted on the colour by dilution with water alone. The proportions recommended by Letts and Rea were adopted for use in sea-water, the actual volumes being five times as great.

The Concentration of the Diphenylbenzidine Sulphuric Acid Reagent.

Table 2 shows the concentration of diphenylbenzidine used by various workers.

TABLE 2.

Effect of Concentration of Diphenylbenzidine upon the Intensity of Colour Produced in the Reaction.

With 0·001 mg. nitrate nitrogen per tube, 2·5 ml. water + 6·0 ml. acid + 1·5 ml. acid reagent.

Grams per 100 ml. of H_2SO_4.		Time since mixing. 1·5 hrs.	25 hrs.	9 days.
0·004	Atkins	100	100	Blue, paler on top.
0·020	Letts and Rea	200	254	Blue at bottom, yellow halfway down with brownish precipitate.
0·100	Snell	205	74*	Light green, no precipitate.
1·000	Yoe	—	—	—

It is obvious that the concentration of 20 mg. per 100 ml. of acid is sufficient for dilute solutions to attain the maximum speed of reaction without undue loss of stability.

According to Kolthoff and Sarver (1930) the green colour produced with Snell's proportions is due to the combination of the blue salt with the excess of diphenylbenzidine.

Relation between Colour Intensity and Nitrate Concentration.

A series of tubes was made up containing distilled water, acid and acid reagent in the proportions shown in Table 2. The series consisted of tubes containing 0, 10, 20 and 40 mg. per cubic metre of added nitrate nitrogen. The depth of colour produced after 21 hours is shown in Figure 2. The ordinates are colour units, taking that produced by the 20 mg. tube as 100. It will be noticed that a certain amount of colour is given by distilled water, owing to the traces of nitrate in it and to the nitrate present

* Colour distinctly green.

in sulphuric acid. By producing the curve backwards, so as to cut the concentration axis, it may be seen that the distilled water and acid between them produced a concentration of 27 mg. of nitrate nitrogen per m^3.

In Figure 3 the same process has been repeated, using sea-water which had been stored in a large carboy for some months, so that all sediment had settled out of it. This was found to contain only a minute trace of nitrite, less than 1 mg. per m^3 of nitrite nitrogen. The colour produced by the sea-water with 40 mg. has been taken as giving 100 colour units. It may

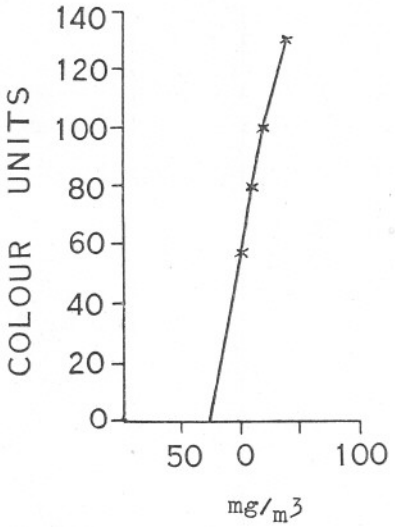

FIG. 2.—The concentration of added nitrate nitrogen in the distilled water is shown by the abscissæ, read from 0 towards the right. The concentration resulting from that originally in the water, as increased by the sulphuric acid, may be read off towards the left.

be seen that the more concentrated solutions depart from the linear relationship, though it was found that on allowing to stand for 44 hours the curve became straighter at the greater concentrations, though still not a straight line ; for obvious reasons it seemed desirable to limit the time of standing for comparing the tubes to about 20 hours. It may be seen from Figure 3 that the sea-water tubes contain 75 mg. per m^3, not correcting for the amount of nitrate in the acid. Separate determinations in which acid was substituted for distilled water or sea-water showed that the concentration in the sulphuric acid was about 7 mg. of nitrate nitrogen per m^3. When correction for this has been made according to the method

previously described (Atkins, 1930), the sea-water was found to contain 72·5 mg. per m^3. It is not of course claimed that the decimal place is of any real significance.

The Degree of Accuracy Obtainable.

Much time was expended in trying to get results which were of a reasonably high degree of accuracy, and the variations between tubes prepared

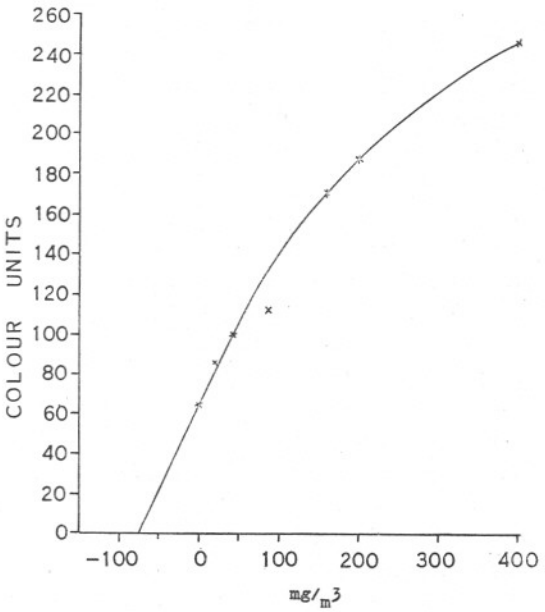

Fig. 3.—The concentration of added nitrate nitrogen in a sample of sea-water is shown by the abscissæ, read from 0 towards the right. The concentration resulting from that originally in the water, as increased by the sulphuric acid, may be read off towards the left.

in an apparently identical manner were found, at the outset, to be very baffling.

First of all, one must use pure sulphuric acid, such as the nitrogen-free product supplied by the British Drug Houses Ltd. This acid was only produced by the firm after it had been reported to them that the acid supplied as pure sulphuric acid really contained considerable quantities of nitric acid. No better success had attended the use of foreign and other British acids (Harvey, 1926). Even the " Arsenic Test " sulphuric acid (B.D.H.) was liable to contain appreciable amounts of nitrate nitrogen.

The nitrogen-free acid at present being supplied is exceptionally good and gives no colour after standing with the reagent for a day, or gives only very slight colour. The latter indeed is to be preferred, since the absence of all colour leaves one in doubt as to whether the acid may not have a slight reducing action.

It has been found by the writer (1932) that even sulphuric acid, which gives a marked reaction for nitrate, can be entirely freed by the cautious addition of ammonium sulphide or preferably of hydrogen sulphide. This leaves only a minute amount of sulphur, too small to produce a turbidity, and the quantity used should be adjusted so that the reagent still produces a very faint colour with the acid.

A number of samples of distilled water were compared and it was found that in general our laboratory supply could be used. The Plymouth town supply is a very pure upland water and the laboratory where analyses were carried out is free from the fumes usually present in a chemical laboratory. Moreover, owing to the prevailing winds blowing in from over the sea, it is only rarely that the atmosphere is contaminated by nitrogen acid fumes, though this happens occasionally when the wind is from the east. Freshly prepared laboratory-supply distilled water from a hard glass bottle, used for years only for distilled water, was compared with the same water specially redistilled from a glass vessel containing barium hydroxide. The distillation was carried out in a room from which all nitric acid bottles had been removed some months previously, no flames were burning in the room and distillation was effected by means of an electric hot plate, while a sea breeze was blowing through the room. When these samples of distilled water were treated as usual and the resulting 10 ml. mixtures were compared in quartz tubes after standing for one day, it was just possible to distinguish them. The laboratory distilled water gave a tint to which it was impossible to ascribe a definite colour, but in the case of the water distilled from the barium hydroxide and from the hot plate no suspicion of a tint could be seen. It was found, however, that on standing in a laboratory, in the absence of bottles of nitric acid, but in which flames were occasionally burning, both the distilled water and the sulphuric acid were liable to absorb a trace of nitric or nitrous acid.

Great care must be taken in cleaning all the vessels used, with pure strong sulphuric acid ; white glass, especially optical glass used in colorimeters, is liable to give off very considerable amounts of nitrate.

Though the colour production is most intense when the acid reagent is added directly to the sea-water, so that the mixture becomes very hot, yet it is impossible to control the temperature with any success owing to differences in the rate of adding the acid and differences in the thicknesses of the walls of the test tubes used. Accordingly Letts and Rea recommend

that the water and sulphuric acid should first of all be mixed and allowed to stand till room temperature has been reached, and after that the reagent should be added in another quantity of acid. This subsequent addition results in only a very slight evolution of heat. The mixing is carried out as follows: The sea-water is first of all added to the carefully cleaned quartz tubes (glass may also be used), then the pure sulphuric acid is run in, attention being paid to the times of delivery being closely the same in these additions, so that the loss of acid on the sides of the burette, owing to the viscosity of the sulphuric acid, may be the same in each case.

It is necessary when dissolving diphenylbenzidine in sulphuric acid to stir the mixture extremely thoroughly. Unless this is done quite appreciable differences in concentration may exist when the solution is poured into the burette. It is necessary to mix very carefully, with a rod, the contents of the tubes after the addition of the acid to the reagent. Even after what had appeared to be an adequate amount of stirring, it was found on occasion that the colour developed unevenly.

As previously remarked the presence of flames in the room is to be avoided and all risk of contamination by floating dust particles should be reduced to a minimum. Apparently minute particles of dust may either contain nitrates or conversely may reduce sulphuric acid so that nitrate present in the mixture is lost.

When using sea-water it is essential, unless the bottles have stood for a considerable time, to centrifuge so that all plankton organisms may be thrown down. This modification, introduced into Harvey's method by Cooper, is a very necessary one in plankton-rich water.

Having taken all precautions mentioned and having executed each operation with the greatest care, erratic results are still obtained occasionally. Thus, when a series of tubes had been examined in the colorimeter after 20 hours, and had been kept for 24 hours more, it was found that one tube which formerly gave a value rather lower than the average had increased to a more normal value. In that case apparently the reaction had been delayed, either by this tube having been slightly cooler than the others at the start, or by inadequate mixing. On the other hand, one tube which gave quite a normal value after 20 hours gave a decidedly high result after the further period of standing. This can only be explained by some chance contamination.

In Table 3 are shown the results of a series in which all precautions were taken. It may be seen that the divergence from a mean value is about 5% for the shorter period of standing. On the amount of nitrate nitrogen present, however, this only amounts to 10^{-8} of a gram. Errors in measuring out three small volumes are of course included in this figure.

TABLE 3.

To Show Degree of Accord Obtainable.

Six tubes taken, each with 2·50 ml. of centrifuged sea-water containing 72 mg. per m^3 of nitrate nitrogen, 6·00 ml. of pure sulphuric acid added and when cold 1·50 ml. of acid reagent. The cups were each reversed in the colorimeter and the mean values of right and left are given. The amount being analysed is 0·000,18 mg., so 5 per cent error is 0·000,01 approx.

Tube.	Time since mixing. 18 hrs.	42 hrs.
A . . .	100·0	100·0
B . . .	102·0	97·0
C . . .	97·0	91·0
D . . .	102·3	104·2
E . . .	99·6	103·1
F . . .	105·3	109·9
Mean . .	101·2	101·0

Effect of Sodium Chloride on the Reaction.

Tillmans showed that the addition of sodium chloride to the diphenylamine reaction led to an increase in the intensity of colour. It must obviously have the same effect on the diphenylbenzidine reaction.

Table 4 shows the results obtained with a series of tubes. It appears, therefore, that one could make up a set of standards containing known amounts of nitrate nitrogen dissolved in a solution made to be 0·4% with sodium chloride. On account however of a possible reducing action of traces of organic matter in the sea-water, it is preferable to use sea-water to which known amounts of nitrate have been added.

TABLE 4.

Effect of Sodium Chloride upon the Intensity of Colour Produced in the Reaction.

Each tube had 0·001 mg. of nitrate nitrogen in 2·5 ml. of water or salt solution+6·0 ml. of H_2SO_4 and when cold 1·5 ml. of diphenylbenzidine sulphuric acid reagent. The tube without chloride is taken as 100 in each case ; obviously the colour in the 69-hr. tube was the greater, but variation with time is not being considered here.

NaCl per cent in water.	Time since mixing, in hours. 0·5	21	69
0·0	Deepest colour*	100	100
0·2	Less colour	113	131
0·4	,, again	121	133
1·0	,, again	121	131
2·0	,, again	121	129
3·0	Least save control	121	129
3·0†	Very slight blue	7·6	6·2

Effect of Iron Salts upon the Reaction.

With regard to the action of iron preliminary experiments were carried out at the very start of this investigation using 1 ml. of solution to 10 of the acid reagent. The heat developed on mixing them was of course considerable. Ferric chloride solutions of the concentration $m/10^2$ and $m/10^4$ were used. Neither gave any more colour than that obtained with distilled water and acid alone. It is probable, however, that the ferric

TABLE 5.

Comparison of Effect of Nitrate, Nitrite and Ferric Iron upon the Intensity of Colour Produced.

Mixtures 0·001 mg. nitrate nitrogen, 0·001 mg. nitrite nitrogen and M/10,000 Fe (ic) chloride, 1·0 ml., viz. 0·0056 mg. Fe in 2·5 ml. water. Examined after 20 hrs.

(*a*)	Nitrate, allowed to cool after acid, then acid reagent	100
(*b*)	Nitrite ,, ,, ,, ,,	67·3
(*c*)	,, ,, ,, ,, ,,	71·2
(*d*)	,, cooled‡ under tap, then acid reagent	71·2
(*e*)	,, ,, ,, ,,	69·9
(*f*)	Iron, allowed to cool, then acid reagent	26·6
(*g*)	,, ,, ,, ,,	27·0
(*h*)	Acid blank, no heat developed	4·4

chloride was reduced by the strong hot sulphuric acid, so the experiments were repeated in the cold using the normal proportions and the results are shown in Table 5. From these it may be seen that ferric iron is about 21 times less effective, weight for weight, than nitrate nitrogen. Since it has been shown by Harvey (1925) that no ferric iron is present in sea-water

* This was the last tube to be mixed, and so had stood a lesser time than the others.
† Control with no added nitrate.
‡ The cooling was done after acid had been added to the other tubes also.

and that only small amounts of iron in any condition are normally found, it appears obvious that no error from the presence of iron can be expected in coastal waters. It is, however, just possible that in the great ocean depths there may be small amounts of interference due to the possible presence of iron in a ferric state and in concentration much greater than normally found in shallower seas. Ferrous salts gave no colour with the reagent.

The Reaction with Nitrite.

According to Tillmans and Sutthoff (1911) the action of nitrate and nitrite is similar, so that combined determinations may be made using diphenylamine. The same should be true starting with diphenylbenzidine, so that the use of permanganate by Letts and Rea to remove nitrite, as such, seems unnecessary, and risky too, since permanganate itself gives the reaction. It is known however that free nitrous acid is decomposed on heating, and much heat is developed when the sulphuric acid is added. Table 5 shows that in the reaction, as ordinarily carried out, about 30 per cent of the nitrous acid is destroyed. From Table 6 it may be

TABLE 6.

Comparison of Effect of Nitrate and Nitrate, as in Table 5.

		20 hrs.	44 hrs.
(*a*)	Nitrate, allowed to cool, then acid reagent	100	100
(*b*)	,, ,, ,,	101	—
(*c*)	,, ,, ,,	108	—
(*d*)	Nitrite, cooled at once under tap, then acid reagent	106	106
(*e*)	Nitrite, allowed to cool, then acid reagent	65	61
(*f*)	,, stood in boiling* water a few minutes, then acid reagent	66	61

seen that if the tube be cooled at once this loss of nitrous acid may be prevented or much reduced. Even with some additional heating the destruction does not amount to the theoretical value according to the equation :—

$$3HNO_2 = HNO_3 + 2NO + H_2O.$$

As however the amount of nitrite in sea-water is normally small the loss is not of much practical importance, but with high nitrite—as much as 38 mg. nitrite nitrogen per m^3 has been found (Atkins, 1930)—the tubes should be cooled immediately.

* The beaker was removed from the flame, to avoid contamination from that source. The temperature was therefore rapidly falling.

The Possible Interference of Iodide, Iodate, Arsenite and Arsenate.

Sea-water is known to contain iodide, which with strong sulphuric acid liberates hydriodic acid. It appeared possible that this might act as a reducing agent and so lessen the production of colour due to nitrate. It has been shown by Reith (1930) that there is in sea-water about 43 mg. per m^3 of total inorganic iodine, corresponding to a normality of $0{\cdot}34 \times 10^{-6}$. Potassium iodide was accordingly made up in $m/10^3$ concentration and diluted in successive steps of ten times down to $m/10^6$. These four dilutions were prepared with laboratory distilled water and it was found that the most concentrated solution gave a bright yellow due to the liberation of iodine. The other three gave scarcely any more blue than the blank. The fact that they did give a very faint blue in excess of that given by the blank is probably due to the presence of a trace of iodate. Had the iodide, or rather the free hydriodic acid, exerted any reducing action, the tubes should of course have been perfectly colourless. This possible reducing action was further tested by mixing equal volumes of these iodide solutions, from $m/10^4$ to $m/10^6$, with a solution containing 0·001 mg. of nitrate nitrogen per ml. A highly concordant series was obtained in which no difference could be seen between the tubes containing nitrate and those with nitrate and added iodide.

As already mentioned, Thompson and Johnson pointed out that iodate also gave the reaction with the reduced strychnine reagent. Cooper, in an accompanying paper, has shown that this reaction, though given, is very much less sensitive, so that 4000 mg. per m^3 of iodine as iodate produce only as much colour as 3 mg. of nitrate nitrogen. That is to say that iodate may be entirely neglected, even if present, in making an estimation by this method. As regards the presence of iodate, Winkler (1916) found a sample of sea-water from the Adriatic to contain 38 mg. per m^3 total iodine, of which the iodide ion accounted only for 8 mg. and iodate the remainder, there being no organically bound iodine. Correcting to normal salinity the total iodine came to 51 mg.

It was found that with the diphenylbenzidine reagent nitrate and iodate behaved identically, using equivalent proportions, namely, $m/10^3$, $m/10^5$ and $m/10^6$. Consequently it seems that iodate present in sea-water will be recorded as nitrate. Nevertheless, when a comparison was made between the two methods of analysis, Cooper obtained, for samples from Station E1, taken on October 20th, 27 mg. nitrate nitrogen per m^3 for the surface water and 29 mg. as an average for 0, 5, 25, 50 and 70 m., whereas the diphenylbenzidine method gave 28 mg. for the surface and 31·5 mg. from samples of the whole water column mixed together. The

agreement is very close, and it appears to rule out the presence of iodate in this sample of sea-water in any significant quantity.

To reduce iodate to iodide Winkler added 5 ml. N/100 As_2O_3 solution and 10 ml. strong hydrochloric acid to each litre of sea-water and allowed to stand for half an hour. To effect the same object Reith used, for each 500 ml. of filtered sea-water, 100 mg. of sodium hydrogen sulphite and 5 ml. iodide free 4N hydrochloric acid and allowed a few minutes only for the reaction.

Before studying the effect of these reagents upon the diphenylbenzidine reaction with iodate, it is advisable to consider their behaviour separately. Arsenic is known to exist in sea-water, as much as 25 mg. per m^3, reckoned as As_2O_3, having been found by the Government Chemist (Orton, 1924) in water from the English Channel in November. It has further been shown by Atkins and Wilson (1927) that most, if not all, of this exists in the form of arsenite.

Ekkert's list of substances giving the diphenylamine (and consequently diphenylbenzidine) reaction does not mention arsenate. On testing solutions of arsenic acid the blue colour was always produced. By varying the concentration, however, it was proved that the colour was not due to arsenic acid but to some impurity, bearing no constant ratio to arsenic from sample to sample. It is obvious, therefore, that the arsenate, if any, present in sea-water, is without effect upon the estimation of nitrate.

To test the effect of arsenious acid six tubes were prepared. A, B and C contained each 1·0 ml. of $m/10^3$ KNO_3, and D, E, F a corresponding amount of KIO_3. A and D received each 2·0 ml. of water, and C and F the same volume of a solution of As_2O_3 containing 70 mg. per litre. B and E received each 1·0 ml. of water and 1·0 ml. of arsenious solution. After standing with acid for about $2\frac{1}{2}$ hours the reagent was added. The nitrate tubes were absolutely unaffected by the arsenious acid. The iodate tubes showed slight reduction, amounting to 7 per cent for F. Obviously a greater concentration of arsenious oxide is required, and the amount present in the sea would thus appear to be quite without effect upon the nitrate reaction.

Tubes were similarly treated, adding to each, instead of arsenious acid, solid sodium hydrogen sulphite the size of a pin's head. The nitrate remained unaffected, but the iodate was almost completely destroyed, the colour produced being only about one-third again as intense as the blank with distilled water.

On testing with sea-water, however, it was found that the addition of a larger amount of solid sodium hydrogen sulphite did bring about an appreciable reduction of nitrate, though the addition to each tube of traces of the solid was without effect. By " traces " is meant an amount

which was just visible on the end of a knife ; this is, however, a very large excess as regards the minute amount of nitrate.

The reaction was then tried on two samples of sea-water, one stored for ten months, the other freshly drawn. To the usual 2·5 ml. was added 1·0 ml. of $m/10^5$ sodium hydrogen sulphite and 1·0 ml. of $m/10^3$ solution. On adding the usual reagents it was found that no reduction had been effected by the $m/10^5$ solution, but that the $m/10^3$ solution had reduced the nitrate—or nitrate plus iodate—to about one-third.

Iodate alone was then tried with $m/10^5$ solution. Since the total iodine present is probably not far from $0{\cdot}34\times10^{-6}$ normal, using 2·5 ml. of iodate and 1·0 ml. of sulphite, solutions should leave the latter in excess. On proceeding with the reaction it was found that 25 per cent of the iodate had been destroyed. Obviously sufficient time had not elapsed for the reaction to become completed ; only the usual cooling time had been allowed. It would appear advisable, therefore, to treat the water with the reducing reagent and to acidify slightly, so that Reith's method for removal of iodate may be allowed to proceed to completion before the full amount of sulphuric acid is added. The fact that no reduction was effected in the two samples of sea-water by adding the $m/10^5$ solution of reducing agent, whereas this reduced a pure iodate solution, appears to indicate that in these samples iodate was not present in any appreciable quantity ; this seems the more likely since the reagent is more active in reducing nitrate in sea-water than in fresh, owing doubtless to the chloride, which interferes as in the permanganate and other oxidations.

There is, however, another reason to account for the small effect produced by the iodate known to be present in sea-water. The following reactions must be considered :—

(*a*) $3I_2+6KOH=5KI+KIO_3+3H_2O$.
(*b*) $HIO_3+5HI=3I_2+3H_2O$.

Thus it may be seen from (*a*) that iodine set free in an alkaline solution, such as sea-water, reverts to iodate to the extent of one-sixth. Further oxidation may of course increase the amount, but the ratio iodite—iodate in sea-water does not appear to have been adequately studied. On acidifying sea-water, however, as is done in the diphenylbenzidine reaction, the iodate present is destroyed by the iodide ; only the excess of the iodate, if there is any, will be left to simulate nitrate. This was tested as follows : To each of two tubes 1·0 ml. of $m/10^4$ potassium iodate was added ; then the first received 1·0 ml. of water and the second 1·0 ml. of $m/10^3$ potassium iodide, the molecular ratio being accordingly 1 : 10, a good excess over the required 1 : 5. On completing the test as usual the first tube gave the usual deep blue, the second only a light colour due to the pink of the iodine in strong acid, the blue of the blank,

and perhaps slightly more. The colours could not be matched. The iodate has been destroyed by the excess iodide.

Two tubes were then prepared similarly save that $m/10^5$ iodate was used and $m/10^4$ iodide. At this dilution the reaction between iodide and iodate does not appear to proceed, the colorimeter readings being almost identical.

With sea-water, however, the reaction does appear to take place (cp. the difference also in the sodium hydrogen sulphite reaction in sea-water), for when two tubes containing 1·0 ml. of sea-water received respectively 1·0 ml. of water and of $m/10^4$ potassium iodide, the blue colour given by the untreated sea-water was the more intense, in the approximate ratio 10 : 9. Since it contained 72·5 mg./m^3 nitrate nitrogen, including iodate, the corrected value becomes 67·5 mg/m^3. It should be noted that the excess of potassium iodide used here was very great, 1·0 ml. of $m/10^4$, whereas the total iodine is only about 0·34 $m/10^6$. A considerable excess appears to be needed, as even in sea-water 1 ml. of $m/10^4$ iodide did not completely destroy 1 ml. of $m/10^5$ iodate.

It remains to consider the possible effect of the iodine liberated according to equation (*b*) upon the course of the diphenylbenzidine reaction. Tubes were made up containing 1·0 ml. of $m/10^5$ potassium nitrate with 1·0 ml. of water, 1·0 ml. of $n/10^5$ iodine with water as before and 1·0 ml. of each solution. On completing the reaction as usual the nitrate solutions gave an intense blue, the iodine solutions gave a light blue, with a faint pink shade, and the mixture gave an approximately additive result. The iodine therefore does not seriously interfere with the reaction even when present in a far greater amount than in sea-water, viz. $n/10^5$ as against less than $n/10^6$. The use of the potassium iodide method of destroying iodate seems to be quite legitimate.

Effect of Miscellaneous Reagents on the Reaction.

When at the start trouble was experienced with the presence of traces of nitric in the sulphuric acid an attempt was made to use phosphoric acid instead of sulphuric acid. Glacial acetic acid has been used in the diphenylamine reaction, but the reaction does not then seem to be as sensitive as when sulphuric acid is used. It was found that syrupy phosphoric acid gave no trace of blue colour with the diphenylbenzidine, but on the addition of a trace of nitrate an intense blue was produced. It can be used in testing fresh water, though no experiments have been carried out to see whether it is quite as delicate as when using sulphuric acid. With sea-water, however, a precipitate is given with phosphoric acid. An attempt was made to remove the calcium and magnesium in the sea-water by adding phosphate, and the supernatant liquid was then used for the test; it was found, however, that a precipitate was still produced, due apparently to the sulphate and possibly chloride. It was

unnecessary to seek farther because sulphuric acid sufficiently pure was then obtained. It was found, moreover, if a light blue colour was produced when the reagent was mixed with sulphuric acid, that this could be completely destroyed by gently warming the mixture. Acid grossly contaminated with nitric acid however, though the colour became slightly paler on heating, still gave an intense blue.

As previously mentioned the blue colour is given with ferric salts. An attempt was made to avoid this by the use of small quantities of citric acid; this however only delayed the onset of the blue, and the citric acid was soon decomposed by the strong sulphuric acid. It was found that the blue colour produced in the reaction could be decolorised by the addition of ferrous sulphate, which however leaves a white turbidity. Ammonium sulphide vapour though effective in preventing the formation of the blue compound, if added before the reagent, does not decolorise it when added subsequently. It was also found that potassium permanganate, potassium chromate, bichromate and ferricyanide gave the reaction, but not potassium ferrocyanide, which however does not destroy the blue colour once it has been formed, and may give a white turbidity. It is, of course, obvious that all the reagents which give the blue colour with the diphenylamine reagent will also give it with diphenylbenzidine.

Stability towards Light.

The red colour given by the reduced strychnine reaction is unstable towards light, even towards the diffuse daylight of the laboratory, and special precautions have to be taken to protect it. It was found, on the contrary, that the blue colour produced in the diphenylbenzidine reaction is extremely stable, as may be seen by inspecting Table 7; indeed the

TABLE 7.

Stability towards Light and Exposure to Air.

Three tubes were prepared and examined after three hours. They were then treated as follows:—

After 3 hrs.			4 days.	11 days.
(*a*)	100	Rubber cap and kept in dark	100	100
(*b*)	106	Do. but in south window*	103	109
(*c*)	102	Covered with beaker, kept in dark	99	107
		As before, but covered and uncovered.		
(*d*)	100	Kept in diffuse light, under beaker	100	100
(*e*)	101	,, ,, ,, uncovered	108	107

* This position was slightly warmer than in the dark, in a north room.

solution exposed to light gave a slightly more intense colour than that kept in the dark. This is beyond the range of experimental error and may be attributed to the fact that the room was somewhat warmer. Apparently the uncovered tubes absorb traces of nitrate or nitrite from the air, and though they also absorb a little water, so that the upper surface is of reduced intensity, or almost colourless, yet the absorption of nitrogen acids preponderates.

Rate of the Reaction and Preparation of Permanent Colour Standards.

Difficulty was experienced in studying the rate of the reaction owing to the fact that no substance could be found which readily matched its colour. It was however shown by comparing the intensity produced by solutions of different concentrations that even after 66 hours the reaction had not entirely ceased at room temperature. The following were examined and found not to give a match to the blue produced in the diphenylbenzidine reaction: Methyl blue, methylene blue, alizarine blue; Victoria blue, British Drug Houses, also the following five, Nile blue sulphate, water blue, aniline blue water soluble, cotton blue and thymol blue alkaline range; toluidin blue and readily soluble Berlin blue 1 A, both Grübler; xylenol blue, Cooper Laboratory; cyanin, Metz; xylene cyanole, FF, Sandoz; also ammonia copper sulphate and the blue colour produced in the Denigès phosphate reaction. Of the oxidation reduction indicators indigo tetrasulphonate and indigo monosulphonate, Lamotte, were also tried, and the latter gave the best match of all tried, provided that comparisons were made in the diffused daylight of a greyish sky. In sunlight or with a blue sky, however, the match was not so good. As a test of the identity of the tint of the indigo monosulphonate and the blue produced in the reaction, comparisons were made in yellow, green and blue light. The values found were closely the same. It was also found that a better agreement between consecutive readings could be obtained by using a Schott and Gen green filter, VG2, 1 mm., owing to the great sensitiveness of the eye to green light and variations in green tints. The only drawback to using indigo monosulphonate appears to be the fact that it is an oxidation-reduction indicator and liable to undergo alteration on standing. The monosulphate, however, while freely soluble in warm water, deposits when the water cools and so gives a colour which is not sufficiently intense for the stronger solutions which it may be desired to examine. It was found that an alcoholic solution was satisfactory, taking equal volumes of absolute alcohol and water, and diluting the solution then with absolute alcohol, till it was approximately the colour of that produced by 0·001 mg. of nitrate nitrogen with diphenylbenzidine. This solution was then boiled, in order that any reducing substances

present might exert their full effect upon the colour intensity ; no change was however appreciable. On standing in a south window for four days the intensity fell off to an obvious extent, but the solution stored in the dark appeared to have remained unaltered. The solution is undoubtedly sufficiently stable for use as a standard provided it is checked against nitrate solution made up in sea-water. Figure 4 shows the course of the diphenylbenzidine reaction with a sea-water containing 72 mg. nitrate nitrogen per litre and allowed to stand at room temperature. It was compared with a saturated aqueous solution of indigo monosulphonate, and

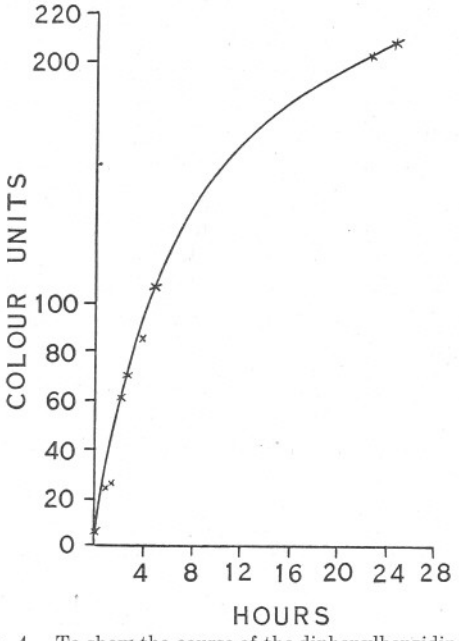

FIG. 4.—To show the course of the diphenylbenzidine reaction with sea-water containing nitrate, at about 12° C.

allowed to stand in diffused light. The measurements were not continued after the second day, because even in a diffused light this weak aqueous solution had deteriorated somewhat. It appears obvious, however, that the increase in colour which may be obtained by allowing the reaction to stand over for another day is not compensated by the additional consumption of time or by the risk of contamination previously mentioned. Riehm (1930) recommends the preparation of colour standards by diluting the blue given by known amounts of nitrate, by the addition of sulphuric acid of the same concentration as used in the reaction, viz. the final concentration. The diluted scale is said to be stable for 48 hours at least.

SUMMARY.

1. The diphenylbenzidine reaction of Letts and Rea has been examined and is recommended for use in sea-water ; 2·5 ml. of sea-water is mixed with 6·0 ml. of the purest strong sulphuric acid and then allowed to cool. Subsequently 1·5 ml. of a sulphuric acid solution of diphenylbenzidine is added, the concentration being 20 mg. per 100 ml. of acid. The colour should be compared, after 20–24 hours, with a standard solution made up by adding a definite quantity of nitrate to the sea-water. A blank correction should be used. Diphenylbenzidine used must be recrystallised from boiling toluene.

2. If the sulphuric acid used is found to give a blue colour with diphenylbenzidine, this may be removed, if not too intense, by warming the acid for a few minutes. With a more intense blue the nitric acid present may be eliminated by previous cautious treatment with hydrogen sulphide. Phosphoric acid may be used instead of sulphuric acid with fresh water, but not with sea-water.

3. A series of tests on the same sample of sea-water may be found to agree to within 5 per cent. With the amount used the agreement found was to less than 0·000,01 mg. The intensity of the colour produced in the reaction when nitrate is dissolved in distilled water is increased by the addition of sodium chloride. When examined after 20 hours it is found that a 0·4 per cent solution of sodium chloride produces as much effect as a 3 per cent solution.

5. Nitrites are decomposed by the sulphuric acid in the reaction so that the full effect of nitrites present is not exerted. By cooling at once after the addition of the acid the loss is however not material, especially as the amount of nitrite in sea-water is usually far less than that of nitrate.

6. Ferric iron is found to give the reaction, but only to the extent of 1/20 of that produced by nitrate nitrogen, weight for weight, and causes no error in sea-water.

7. Iodate and nitrate produce the blue colour in amounts which are proportionate to their molecular concentration. Iodide does not appear to interfere nor do arsenite or arsenate. Iodate, if present, should be removed with sodium hydrogen sulphite in very dilute solution or preferably by dilute sodium iodide, since the trace of iodine liberated does not cause appreciable interference. The iodide already in sea-water along with iodate destroys a portion of the latter in any case.

8. The colour produced in the diphenylbenzidine reaction is stable towards light and may be matched in diffused daylight from a grey sky

with indigo monosulphonate. Comparison is carried out, for preference using a green colour filter; the Schott and Gen filter VG2 has been found suitable.

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The Copper Content of Sea-water.

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Introduction.

The existence of copper in plants and animals has long been known. For an account of early analyses in seaweeds and marine animals reference may be made to Quinton (1912). In recent years the study of the distribution of copper has become of increasing importance in connection with researches on its nutritional value. Analyses have been given of the copper content of many vegetables and fruits (Remington and Shiver, 1930) and of the oyster in relation to the cure of nutritional anæmia (Levine, Remington and Culp, 1931). Orton (1924) collected a number of results bearing on the copper content of oysters. Some of these values are extraordinarily high and the oysters have a greenish appearance from the excess copper, which however may be removed after relaying on clean grounds. There is, however, always a small residual amount of copper, the function of which is unknown. The source is probably particulate inorganic matter in the abnormal cases and the food in those with a normal copper content. This would point to the existence of copper in diatoms and algal spermatozoa, such as those of the Fucaceæ. Copper too is a constituent of the respiratory pigment in Crustacea. Its source is again to be sought in the phytoplankton. One would therefore expect to find a seasonal change in the copper left free in sea-water. Except in the case of the respiratory pigment it was not however known whether the copper was present as an essential or accidental constituent of the cell. Sommer (1931), however, also Lipman and Mackinney (1931), have recently shown that copper is essential for the growth of higher plants, such as barley and flax. Orr (1929) has pointed out our lack of knowledge as to how the concentration of copper in pasture soils varies. It is not known whether some soils contain too little copper.

The first comprehensive survey of the distribution of copper appears to be that of Dieulafait (1879). He proved its presence in both igneous and sedimentary rocks. All but one out of a large number examined gave a detectable amount of copper on 100 g. sample ; the amount varied from one to fifty times. His spectroscopic method enabled him to identify in an absolute manner as little as 10^{-6} g. of copper. He refers to the early

(1850) detection of copper, in the ash of Fucus, by Malaguti, Durocher, and Sarzeau, and to the similar work by Forchhammer (1864).

As a consequence of the universal presence of copper in rocks, Dieulafait pointed out its existence in ancient and modern seas. After many failures he succeeded in demonstrating its presence in brine, from salt marshes, of density 1·36. The method is based upon precipitation with sulphuretted hydrogen and redissolving in nitric acid. Seeing that his brine was concentrated so that 1 litre represented 200 to 250 l. of natural sea-water, his results give 10–12·5 mg. of copper per cubic metre. These he considers minimum values, obtained by finding the least volume of brine in which he could qualitatively detect copper.

When the copper content of sea-water was first sought for by the writer in 1928 the only analysis known was one by the Government Chemist, London (Orton, 1924), which gave approximately 0·2 parts per million or 200 mg./m^3. This value related to a sample of water, from 70 metres depth, taken by me near the Wolf Light off the Lizard Hd., English Channel, on November 12, 1921.

Methods of Analysis for Copper.

The above sample was analysed for copper by concentrating 800 ml. of filtered water to 150 ml. The tint of 50 ml. was then compensated, after the addition of three drops of concentrated hydrochloric acid, by the addition of very dilute potassium bichromate. It was then saturated with sulphuretted hydrogen and compared with similar tubes containing known amounts of copper.

When a number of samples have to be examined the avoidance of filtration and evaporation is however desirable, so the potassium ethyl xanthate method of Scott and Derby (see Scott, 1922) was tried. It was found that as little as 0·01 mg. copper per 100 ml., namely, 100 mg./m.^{3} gave a noticeable brown tint. This seemed an appropriate degree of delicacy and during 1928 a number of samples were thus examined. Values from about 40–110 mg./m^3 were obtained, but the tints were so faint that complete reliance could not be placed on the results. The surface values were as a rule lower than the deeper, and beyond 50 mg. the difference from the blank became practically indistinguishable. Moreover, with this reagent, there is always a very faint tint in absence of copper—when made up in glass distilled water.

Later on Callan and Henderson's (1929) colorimetric method was tried, using sodium diethyl-dithio-carbamate

$$\begin{array}{l} \diagup N(C_2H_5)_2 \\ C{=}S \\ \diagdown SNa \end{array}$$

This is a readily soluble white crystalline substance, used in 0·1 per cent slightly alkaline aqueous solution; it is perfectly colourless and 10 ml. added to 100 ml. of glass distilled water gives no tint, nor is any tint given with our Laboratory supply from a copper still with block tin spiral condenser. With this reagent 0·01 mg. copper per 100 ml. gives a far more intense colour than it does with potassium ethyl xanthate. The sensitivity is about twice as great, and the fact that the reagent is colourless is an added advantage. It was examined in 12 cm., 100 ml. Nessler tubes, in 15 cm., 100 ml. Hehner tubes, and in 30·5 cm., 200 ml. Hehner tubes. With the 15 cm. tubes it was possible to distinguish 0.002 mg. in 100 ml., viz. 20 mg./m^3 of copper from the blank, but not in the Nessler tubes. In the long Hehner tubes 0·001 mg. in 100 ml., viz. 10 mg./m^3 could be distinguished at the 200 mark as giving a distinct yellowish tint, and a very slight tint at 150 mark; at 100 mark it was indistinguishable from the blank. One would therefore expect this reagent, used with long tubes, to be a possible one for use with sea-water if it contains 50–200 mg./m^3. No colour is however given, even with winter samples, when the copper content should be at a maximum, under conditions that would definitely detect 20 mg./m^3. There is therefore a disagreement between the Government Chemist's result and my own xanthate and carbamate values. The latter are, however, in good agreement with the following xanthate analysis. A carboy of sea-water, from Station E1, surface, taken on March 23, 1931, was concentrated to one-fifth of its volume and, after making allowance for a blank correction, was found to contain 50 mg./m^3 of copper, corresponding to 10 mg. for original sea-water.

Again, use was made of the standard method of electro-deposition. One litre of the same carboy of water was raised nearly to boiling and was electrolysed for one hour at 2·0 volts, the copper being deposited upon platinum gauze. Electrolysis of the hot solution was continued for a further two hours with fresh gauze. Electrolysis with a third piece of gauze was continued for an hour.

The copper deposited was dissolved in copper-free water acidified with the purest sulphuric acid. After neutralisation the volume was made up to 10 ml. in each case and the copper was estimated colorimetrically. The first and second periods of electrolysis deposited, respectively, 0·0040 and 0·0057 mg. copper, the third gave none, total 0·0097 mg. from one litre or 9·7 mg./m^3. The current must be continued till the liquid has been lifted away from below the electrodes to avoid loss by redissolving of the copper. The method of electro-deposition is very simple and the colour intensity is so intense that accuracy is greater than in the volume concentration method.

It remains to explain the discrepancy between the earlier results and the

concordant results just mentioned. My attention was drawn by L. H. N. Cooper to some bolts on the inside of the standard water-bottle. The gun-metal bolts were corroded and covered with verdigris. Consequently all samples are liable to contain, and according to the analysis given do contain, traces of added copper. The sea-water used in the later analyses was from a glass carboy filled, from the surface, with a wooden bucket. The water-bottle is carefully washed with fresh water after each cruise, but the sample I took for the Government Chemist was taken on the fourth day of a cruise, after we had taken shelter in Falmouth Harbour for two days, which gave opportunity for the accumulation of an additional amount of verdigris.

It remains to consider the presence of iron in sea-water as a source of error in direct examination. According to Ansbacher, Remington and Culp (1931) with the xanthate method, 0·050 mg. of iron gives the same colour as 0·0123 of copper. On testing the carbamate method, however, the writer found that it required a concentration of 1·32 mg. per litre of ferric iron to give as much colour as 0·01 mg. per litre of copper. The interference is therefore negligible. It is, of course, absent altogether when the copper is deposited electrically.

SUMMARY.

1. On comparing the potassium ethyl xanthate and the sodium diethyl-dithio-carbamate methods for estimating copper the latter was found to be preferable because (*a*) It is at least twice as delicate, (*b*) The reagent is absolutely colourless, (*c*) In very dilute solution ferric iron causes little or no disturbance.

2. Estimation of copper may be carried out by electro-deposition from one litre of sea-water at about 90–100° C. for three hours at 2·0 volts with subsequent re-solution and colorimetric estimation using the carbamate method.

3. In agreement with the spectroscopic determinations of Dieulafait in 1879, it was found that sea-water contains about 10 mg. per cubic metre of copper.

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On the Use of Sodium Bicarbonate and Calcium in the Rectification of Sea-Water in Aquaria.

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THE use of sodium bicarbonate for the maintenance of the proper hydrogen ion concentration and bicarbonate content in marine aquaria using a closed circulation, was recommended by Breder and Howley (**1**). It was pointed out by them that this substance was more suitable for such purposes than quicklime which is used in the Plymouth Aquarium, because the latter " disproportionately increases the calcium content." Atkins (**2**) disagrees with this opinion chiefly on the grounds that sulphates from metabolized food do not tend to increase the acidity of the water.* Atkins quotes Smith (**3**) as stating that the urinary SO_4 comes from ingested sea-water, and neglects entirely the fact that Smith had reference to the bulk of the urinary salts. As Smith remarks on page 494, a fraction of the urinary SO_4 is of metabolic origin, and this fraction is of course the only SO_4 which is significant in the problem discussed by Breder and Howley.

The maintenance of a proper hydrogen ion concentration in sea-water depends as much upon the bound CO_2 ($BHCO_3$) as upon the free CO_2, and it was shown by Breder and Howley that the bound CO_2, which practically represents the entire buffering capacity of sea-water, tends to be depleted in aquaria water in the course of time. This depletion is due primarily to the oxidation of the protein fed as food to the fishes, and the subsequent excretion by the fishes of neutral sulphates, phosphates, etc. The base with which these acids are neutralised (within the body of the fish) is ultimately drawn from the bicarbonate of sea-water, thus increasing the acidity of the latter (at a given CO_2 tension) by lowering the $BHCO_3$. At the same time the fishes are deprived of a salt ($BHCO_3$) which is essential to the maintenance of their own alkaline reserve. The metabolism of one pound of meat produces a quantity of sulphuric and phosphoric

* Or decrease the alkalinity as Atkins prefers to express it.

acids roughly equivalent to 2·5 grams of $NaHCO_3$, or the quantity contained in 12 litres of sea-water. The restoration of the alkaline reserve ($BHCO_3$) by the addition of $NaHCO_3$ (or Na_2CO_3) is more rational than by the addition of lime, since the resulting increase in Na is insignificant in comparison with the quantity originally present, whereas this cannot be said of Ca, of which sea-water contains only a small amount. The original bicarbonate would be entirely replaced in about $2\frac{1}{2}$ years in the New York Aquarium system. Using $NaHCO_3$ to restore the alkali reserve, the total Na would be increased only about 0·5 per cent during this time, whereas if lime is used the total Ca would be increased by more than 10 per cent or 20 times as much in proportion to the natural molar content.

While the practical danger of over-dosage may be not very immediate, for those concerned with the management of public aquaria, it is comforting to have such possibilities as remote as possible. A comparison of the two papers in question clearly shows that the attempt at the New York institution has been aimed at keeping the chemical nature of the stored sea-water more nearly constant than at Plymouth, both by mechanical and chemical means. The degree of accuracy desirable is doubtless of a controversial nature, but in the absence of exact knowledge of the optimum conditions for each species involved, it was deemed best to limit the deviations from natural sea-water as much as possible.

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On the Effect of Long Continued Additions of Lime to Aquarium Sea-water.

By

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THE contention of Breder and Howley (1931), and Breder and Smith (1932) that liming may lead to an increase in calcium in aquarium water is correct. In January, 1932, when the salinity of the Plymouth tank water was 38·0‰ the calcium content was about 0·62 g. per litre compared with about 0·39 g. found in the sea-water off Plymouth by the permanganate method. Thus compared with the normal calcium content of water of 38‰ salinity a 46% excess was present. One liming did not increase the calcium content appreciably, which suggests that the water is at the present time (Jan., 1932) saturated with respect to calcium. Other indirect evidence lends some support to this statement.

Excess base determined by Wattenberg's method (1930) (which only determines base in combination with carbonic or other volatile acid) was 4·2 milliequivalents per litre compared with 2·35–2·4 milliequivalents in the water of the English Channel, whilst the pH was 7·9 compared with 8·0–8·3. The fish are therefore likely to have no difficulty in maintaining their internal alkaline reserve.

Any considerable increase in sulphate should be remedied by precipitation of calcium sulphate since it seems probable that sea-water is nearly saturated with respect to this salt.

Although there is certainly a definite increase in calcium due to regular liming of the aquarium water, the excellent condition of the fish and delicate invertebrates such as the echinoderms shows that it is of little consequence. Since the most important factors appear to be control of pH and adequate aeration there appears to be no sufficient reason to change the current Plymouth practice, which has been carried out without any untoward effects for eight years.

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[It is obvious that Messrs. Breder and Howley are correct in their suggestion that the addition of lime to sea-water, in an aquarium, may raise its calcium content. This has been established by the analyses carried out by L. H. N. Cooper at my request. The increase in calcium, however, appears to be without any observed injurious effect. W. R. G. A.]

The Development of *Nereis pelagica* Linnæus.

By

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With 12 Figures in the Text.

CONTENTS.

1. Introduction.

Although a great deal of work has been done on the development of various species of the Nereids, and the general outlines of the development of a Nereid worm are well known, there still remain several species about which we have comparatively little information. Such a species is *Nereis pelagica* Linn. which appears to have been reared previously by only one worker, Herpin (1926), who, however, did not get it to grow past a comparatively early stage, and whose experience differed considerably from mine. These differences will be pointed out at the end of the paper.

The work of which this is a record was done five years ago. Publication has been delayed by various causes, one being the hope that it might be possible to repeat the rearing and so add various details to the description. An opportunity for repetition not having presented itself the account is given as it stands.

2. Method.

The larvæ were reared in a plunger-jar filled with outside sea-water which had been filtered through fine bolting silk. By the time the young worms were ready to feed the jar contained a good growth of diatoms. Larvæ were examined in cavity slides without pressure and while free to

move about. A drop of saliva—as mentioned by Herpin—added to the sea-water in the cavity slide proved very effective in slowing down the movements of the active creatures without in any way killing or distorting them. In such a solution they survived for hours and after having been washed free from it lived for days in finger bowls. Such larvæ, however, were never returned to the plunger-jar. This saliva method has not proved successful in the case of any other Polychæte larvæ which I have tried. The drawings were all made from living specimens—frequently while confined in saliva—with the aid of a squared net micrometer, drawing in the first place on to squared paper. These drawings were then checked, as far as possible, from specimens fixed in Bouin and mounted in Canada Balsam. Bristles were examined in Farrant's Medium.

3. General Account of the Development.

On the afternoon of 4th February, 1927, Mr. William Searle collected on the shore at Rum Bay three heteronereids of *Nereis pelagica*, two males and one female. These were placed in a dish in the Laboratory. During the late afternoon one of the males showed considerable activity in swimming round and round the dish and was still doing so at 10 p.m. The other two worms rested quietly on the bottom. The following morning both males were swimming rapidly, the female resting, apparently exhausted, on the bottom. She had spawned and the water was milky with countless eggs. Some of these were put into finger bowls containing fresh outside sea-water. In order to make sure that the eggs were fertilized the males were cut open and a little of the sperm which gushed out through the cuts was added to them. Some of the eggs were then transferred to a plunger-jar. The female was also cut open and a few remaining eggs shaken out into clean sea-water and sperm added in the usual way when making an artificial fertilization.

The eggs contained a large number of oily-looking globules which in some cases were observed to run together to form fewer larger globules. Each egg was about 180μ in diameter and lay in a spherical cavity about 225μ in diameter enclosed by a very thick (about 150μ) gelatinous and very transparent envelope, the inner boundary of which was quite distinct but the outer difficult to see. As Herpin (1926) has pointed out this jelly is not adhesive as is the case with *Perinereis cultrifera* and *P. marioni*, in which species it sticks the eggs to the side of the bowls and possibly to rocks. In *N. pelagica* it is almost certain that the eggs are pelagic.

The eggs which had been naturally spawned segmented during the morning, but those which were artificially fertilized did not show the first cleavage until five hours afterwards. Late on February 6th a good proportion of the embryos began to rotate, pressing themselves against the

inner surface of the gelatinous envelope. This they continued to do during the following day. On February 8th, three and a half days after fertilization, the majority hatched out, a number having done so some hours previously. They swam actively and often a thin membrane, apparently the last remains of the egg envelope, clung to them for a time before being finally thrown off. The fate of the gelatinous main mass of the envelope was unfortunately not made out.

A lateral view of a recently hatched larva is shown in Figure 1. In essentials it corresponded exactly to the free swimming type of Nereis larva known in such detail from the work of E. B. Wilson (1892) and others. Three pairs of parapodia were present, each parapodium having noto- and neuropodial chæta-sacs which contained chætæ whose tips

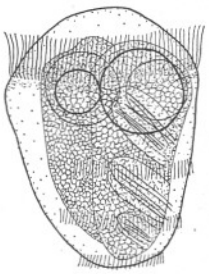

FIG. 1.—View of right side of recently hatched larva 3½ days old. ×156. Actual length approx. 215μ.

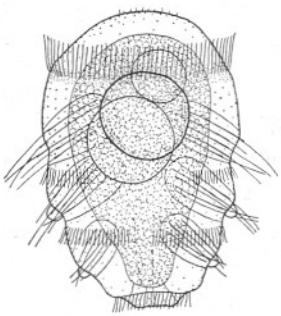

FIG. 2.—Dorsal view of larva about 4½ days old. ×156. Actual length approx. 250μ.

already protruded to the exterior. The prototroch was a complete ring of cilia, arranged in a single row. Underlying it was an irregular band of pale pink pigment. Three paratrochs were present, the first two with dorsal gaps, the anterior possessing the largest of these. The third paratroch was almost a complete ring, but a slight dorsal gap may have been present. As will be apparent later it was not a true telotroch. It was suspected that the single row of fine cilia forming each of these paratrochs was itself broken up into short rows of cilia placed end to end but with slight gaps between the ends. Four large and conspicuous oil globules of differing size were situated anteriorly. Four was the usual number of these globules, but the number as well as the size varied. The internal structure was not clear, the region of the gut being granular. A day later (Fig. 2) the chætæ protruded for a considerable distance and in the second and third pairs of parapodia a lobe was growing out between the noto- and neuropodial sacs. A few short cilia, possibly sensory, had appeared at the anterior extremity. The dorsal gap in the second para-

troch was narrower than previously. The third paratroch had a ventral gap. Another day later and the condition shown in Figure 3 was reached. The chætæ were really long and distinctly articulated. Two pairs of pinkish eye-spots had appeared and anterior to them a row of cilia on each side formed an akrotroch. The dorsal gap in the second paratroch

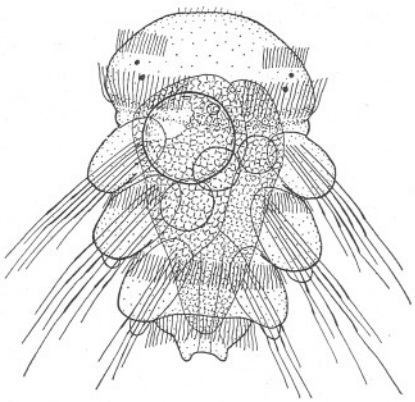

FIG. 3.—Dorsal view of larva about 5 days old. ×156. Actual length approx. 286μ.

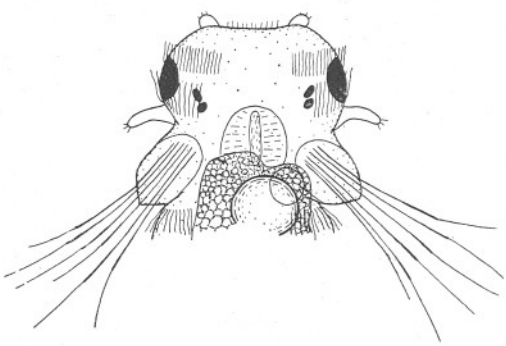

FIG. 4.—Dorsal view of head of larva about 7 days old. ×156.

had closed up, but it now had a ventral one as had also the first and third. The dorsal and ventral portions of the prototroch had each disappeared for a short distance, thereby dividing that organ into lateral, albeit still lengthy, portions. The pink pigment in the prototrochal region still formed a conspicuous speckled band. Anal cirri were appearing and the mouth could be distinguished (about one o'clock of the large oil-globule in Figure 3). The following day the first signs of the tentacles were seen

at the anterior end of the prostomium and the first pair of tentacular cirri were visible as small ventro-lateral buds posterior to the prototroch. A further day's growth and they reached the comparative size shown in Figure 4. The eyes were larger and were brown in colour while a pair of large brown pigment patches anterior and lateral to them were a conspicuous feature of the head. These patches varied greatly among different individuals, some were without them altogether, others had a patch on one side only. According to Herpin they are developed out of the pink pigment of the prototrochal region which no longer formed a band below

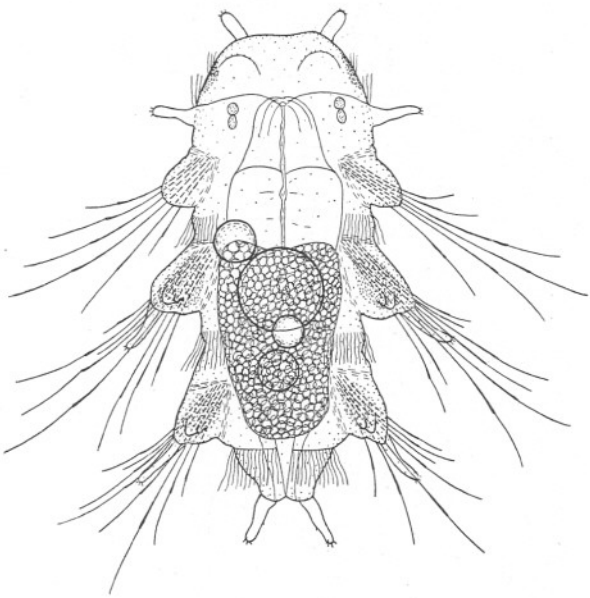

FIG. 5.—Ventral view of larva about 9 days old. ×156. Actual length (exclusive of tentacles and caudal cirri) approx. 380μ.

that organ at this stage. The dorsal gap in the prototroch had widened since the last stage, but the larvæ still swam actively although at times they crawled. For the next few days they could either swim or crawl, but as the cilia gradually disappeared crawling superseded swimming until by the time the stage shown in Figure 6 was reached they were almost entirely crawlers, although they still swam occasionally for short distances along the bottom. When swimming the chætæ were laid along and pressed close against the body with their tips directed backwards.

We must now return to consider the stage shown in ventral view in Figure 5. This larva, about nine days after fertilisation, and two days older than the one whose head is shown in Figure 4, is specially interesting.

The prototroch had disappeared except for a few cilia on either side of the head, and the akrotroch was likewise confined to two short lateral tracts. The first paratroch showed little or no change, but the second had reacquired the wide ventral gap shown in the figure. The third paratroch had a slight ventral gap but was usually complete dorsally. In the specimen here illustrated the brown pigment on the sides of the head was granular. The eyes could be seen through the transparent head. The tentacles and tentacular cirri were lengthening and the palps had

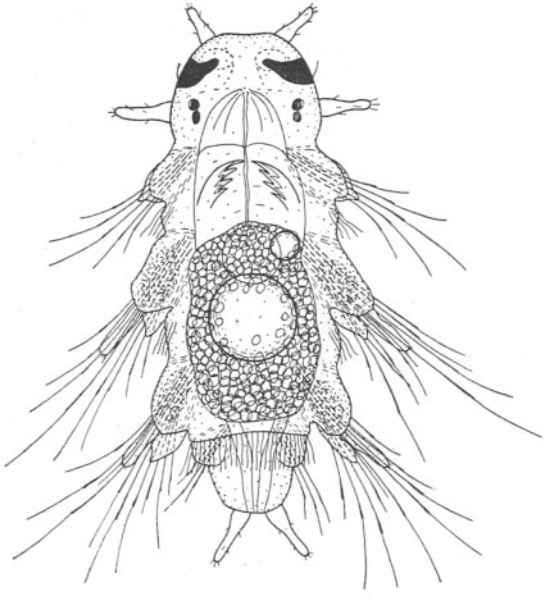

FIG. 6.—Dorsal view of larva 18 days old. ×156. Actual length (exclusive of tentacles and caudal cirri) approx. 400μ.

appeared on the under surface of the head anterior to the line of the prototroch. The first pair of parapodia still consisted of noto- and neuropodial sacs only, but the second and third pairs in addition to the now long middle lobe showed the rudiments of the ventral lobe and of the ventral cirrus (see also Fig. 7). The dorsal cirrus did not appear until considerably later. In the middle of each parapodium of the last two pairs there was a mass of granular tissue constituting, apparently, a gland. This mass became very conspicuous and the granules highly refringent when the living creature was put into a drop of saliva. The anal cirri were fairly long. The buccal and pharyngeal regions were marked out, but the middle portion of the gut was still very granular and with it were

closely associated the large oil globules which had shifted backwards from the anterior position they occupied during the earlier stages.

A day or two after the last stage the jaws became visible and chætæ of a fourth pair of parapodia appeared posterior to the third paratroch. Growth for the next few days was rather slow and at eighteen days old (Fig. 6) the larva was only a little further advanced than it was at nine days (Fig. 5). The jaws and fourth pair of parapodia were conspicuous, the neuropodium of the second and third pairs of parapodia was larger, and a central lobe was growing out between the bristle bundles of the first

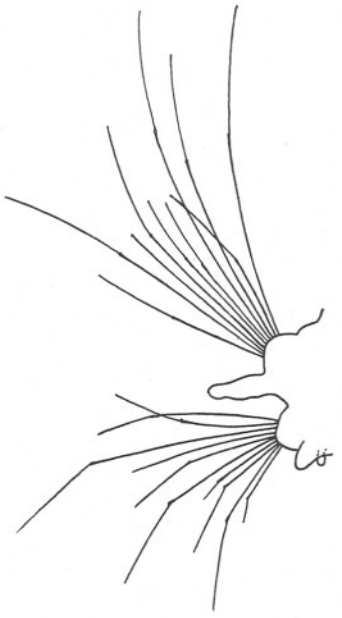

FIG. 7.—Outline of second parapodium of right side, viewed from in front, of a larva about 10 days old. ×270.

pair. The ciliation was still more reduced, the prototroch had quite gone, the akrotroch almost so, while the first and second paratrochs were only short lateral rows. Only the third paratroch had undergone little or no change. Growth continued slowly. The palps became fairly well developed and mobile. The buds of a second pair of tentacular cirri arose ventral to the first pair. The middle lobe of the first pair of parapodia lengthened and the bristles of this pair began to fall out. The bristles with long terminal appendices of the other parapodia were being replaced by bristles with shorter terminal appendices. Dorsal cirri appeared on the second, third, and fourth notopodia, although in some cases on the second they lagged behind those developed on the posterior

two pairs. Thus was reached the condition shown in Figure 8 of a larva thirty-four days old. It will be noticed that the oil globules were much smaller than formerly and the middle tissues of the gut less granular. The introvert was protrusible and the creature was feeding, the stomach containing a brownish mass of diatomaceous material. The cilia had finally all disappeared. Figure 9 is of a still more advanced stage, but the

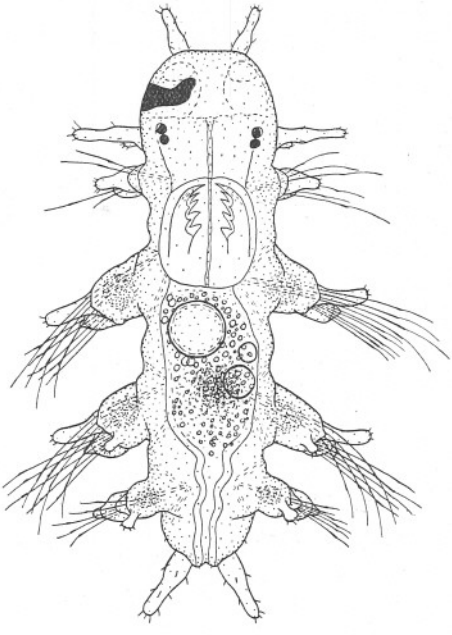

FIG. 8.—Dorsal view of a larva 34 days old. ×156. Actual length (exclusive of tentacles and caudal cirri) approx. 430μ.

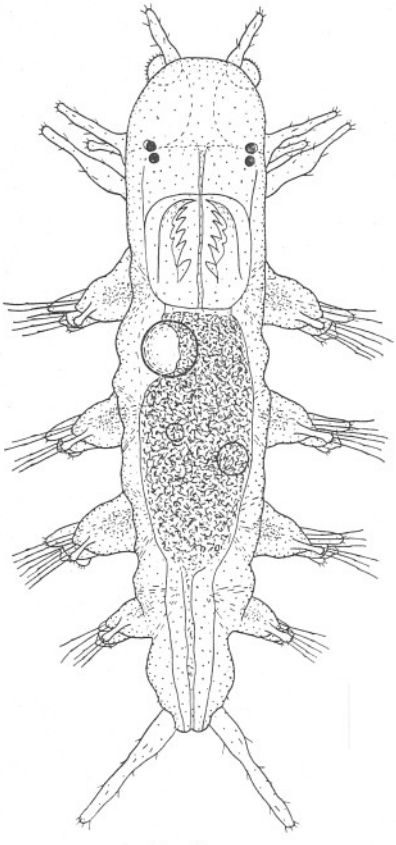

FIG. 9.—Dorsal view of a young worm 33 days old. ×156. Actual length (exclusive of tentacles and caudal cirri) approx. 560μ.

specimen was actually a day younger. Great variation existed as to the state of development of different individuals at this time, some having grown much faster than others. There was also variation in the stages at which different organs appeared; thus in some the first pair of parapodia lost their bristles before the fifth pair of parapodia were visible, in others afterwards. The worm shown in Figure 9 had lost the bristles of the first pair and the middle lobe on each side formed a long tentacular cirrus and was directed forwards just behind the first two pairs. The fifth larval pair

of parapodia was well developed, making in all four adult pairs. The last of the bristles with long appendices had fallen out. The stomach was full of diatomaceous material, although oil globules were still present.

Individuals continued to grow at very different rates, and some were definitely monstrous with malformed heads, etc. Of those which grew the most rapidly one worm had seven adult pairs of parapodia and the eighth pair forming forty days after fertilization. Seven days later two specimens were seen with eight pairs and the ninth and tenth forming. The brown pigment patches on the head had disappeared and the prostomium was acquiring the adult shape. On the same day as the last was seen a specimen with ten pairs of adult parapodia and the eleventh and twelfth pairs forming. In this specimen the buds of the fourth pair of tentacular cirri could be distinguished ventral to the third pair, the latter being those which had originally developed as lobes between the bristle bundles of the first pair of larval parapodia and which by now were much the longest of the tentacular cirri. Figure 10 shows a young worm sixty-five days old. It had twenty-one pairs of parapodia with the twenty-second, twenty-third, and twenty-fourth in process of formation. It is interesting to note that in these the dorsal cirrus is almost the first part to appear, in the anterior two or three pairs it was almost the last. An outline of the tenth parapodium of a worm at this stage is given in Figure 12. All the adult lobes were present, but it had not quite reached the adult shape. The general appearance of the worm was very like that of the adult except that from above the segments looked squarer. Paragnaths were appearing but were very difficult to see. These young worms were living in tubes which they had formed and fastened to the bottom of the plunger-jar. The walls of the tube were of a parchment con-

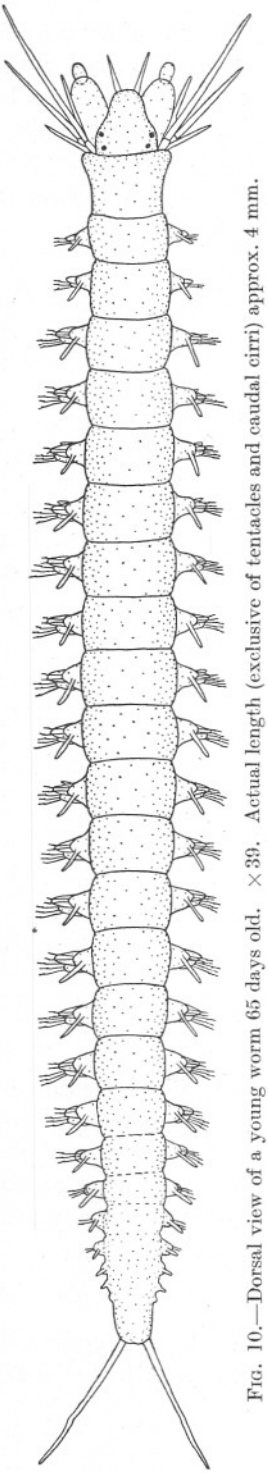

FIG. 10.—Dorsal view of a young worm 65 days old. ×39. Actual length (exclusive of tentacles and caudal cirri) approx. 4 mm.

sistency. They were feeding on diatoms. By the end of July, or about five and a half months after fertilisation, some of the worms had reached an average length of one centimeter and had forty to fifty chætigerous segments. The paragnaths showed the typical adult pattern and were easily visible. The following February, one year after fertilisation, the largest of the surviving worms was one and a half

FIG. 11.—Bristles of a larva about 18 days old. ×756. (*a*) simple capillary, (*b*) heterogomph falciger, (*c*) homogomph falciger, (*d*) homogomph spiniger.

centimeters (measured when fixed) and had approximately sixty chætigerous segments. During the whole year of rearing the water in the plunger-jar was not changed nor was fresh water added to make up for that lost by evaporation. Some of the worms lived for some weeks longer without any great changes and eventually died.

4. THE SUCCESSION OF THE BRISTLES.

While reading the following account the Table showing the approximate number of the bristles during the different stages should be consulted.

In the early stage with three chætigerous segments (Fig. 5) the bristles were mainly homogomph spinigers of the type shown in Figure 11, d. The appendices of these bristles varied in length from approximately 70μ to 35μ, the long ones predominating, and they were spined. Herpin's figures (1926, Fig. 3, b, c) do not show these spines. The first parapodium had about seven spinigers in its dorsal bundle and about five in its ventral, while the second and third parapodia each carried about ten dorsally and five to seven ventrally. In addition to these all the neuropodia bore about two homogomph falcigers (Fig. 11, c) with spined appendices of approximately 20μ long. The shorter appendices of the spinigers approached in structure to these falcigers and it is doubtful if there was any great significant difference between them. The dorsal and ventral bundles of the second and third parapodia each contained one simple capillary bristle slightly spined at its distal extremity (Fig. 11, a). The noto- and neuropodia of these segments each had an acicule ; no acicules were ever present in the first pair of larval parapodia.

By the time that four chætigerous segments were present (Fig. 6) certain changes had taken place. Homogomph spinigers still predominated, but the dorsal bundle of the first parapodium had now only about four ; the second and third, five or six, and of course there was none in the fourth. Moreover, the bristles with the longest appendices had fallen out and the lengths of these now varied from 55μ to 30μ, and there was a larger proportion of the shorter ones. The dorsal bundle of the fourth parapodium had only one or two (according to stage of development) homogomph falcigers (Fig. 11, c). The ventral bundles of the first three parapodia had lost all their homogomph spinigers except one or two. The first had in addition two homogomph falcigers while the second and third carried three to five of these (Fig. 11, c) as well as two to three heterogomph falcigers (Fig. 11, b) which were not represented in earlier stages. They still retained their single capillary bristle. The ventral bundle of the fourth parapodium had about two homogomph and one heterogomph falcigers.

TABLE SHOWING SUCCESSION OF BRISTLES (NUMBERS APPROXIMATE).

		Larval Parapodium Number	1	2	3	4	5
Approx. stage of Fig. 5.	Notopodium	homogomph spinigers	7	10	10		
		capillary bristle	–	1	1		
	Neuropodium	homogomph spinigers	5	6	6		
		,, falcigers	2	2	2		
		capillary bristle	–	1	1		

		Larval Parapodium Number	1	2	3	4	5
	Notopodium	homogomph spinigers	4	6	6	–	
Approx.		,, falcigers	–	–	–	1	
stage		capillary bristle	–	1	1	–	
of	Neuropodium	homogomph spinigers	2	1	2	–	
Fig. 6.		,, falcigers	2	5	5	2	
		heterogomph falcigers	–	2	2	1	
		capillary bristle	–	1	1	–	
Approx.	Notopodium	homogomph spinigers	2	3	3	–	
stage		,, falcigers	–	–	–	2	
of		capillary bristle	–	–	1	–	
Fig. 8.	Neuropodium	homogomph falcigers	2	5	4	3	
		heterogomph falcigers	–	3	3	2	
Approx.	Notopodium	homogomph falcigers	–	2	2	2	2
stage	Neuropodium	homogomph falcigers	–	4	4	4	1
of		heterogomph falcigers	–	3	3	3	2
Fig. 9.							

At the transition stage (Fig. 8) the number of homogomph spinigers was still further reduced. The dorsal bundle of the first parapodium (which was rapidly becoming a tentacular cirrus) retained only two or three of the shorter variety, while the ventral bundle had two or three homogomph falcigers. All these were in process of falling out. The second and third parapodia had dorsally three or four homogomph spinigers with appendices of the medium and short lengths, again showing a reduction in number on the previous stage. The capillary bristles were falling out, likewise those of the neuropodium. The dorsal bundle of the fourth parapodium still had two homogomph falcigers. The ventral bundles of the second and third parapodia had lost all their homogomph spinigers but had about five homogomph falcigers and two to four heterogomph falcigers. The ventral bundle of the fourth parapodium had three or four homogomph falcigers and two or three heterogomph falcigers.

Finally, at the stage shown in Figure 9, the first pair of parapodia had lost all their bristles and become tentacular cirri, while the second and third larval parapodia (first and second adult) had shed the last of the homogomph spinigers. Their dorsal bundles, as well as those of the third and fourth adult parapodia, each carried two homogomph falcigers of the type shown in Figure 11, c. The ventral bundles of the first three pairs of parapodia possessed three or four falcigers of the same type and had in addition two or three heterogomph falcigers of the type shown in Figure

11, b. The neuropodia of the fifth pair each carried one homogomph and two heterogomph falcigers.

I have not followed in detail the subsequent history of the bristles, but a few words on their condition at the stage of Figure 10 will not be out of place. The first two parapodia, which were smaller than the remainder, had lost their dorsal bristles entirely. The remaining parapodia, except those developing at the posterior end, were all very similar to the tenth, which is illustrated in Figure 12. The bristles on the whole corresponded to those found in the adult except that dorsally there were two homogomph falcigers similar to, but of a more robust type than that shown in Figure 11, c. The adult possesses homogomph spinigers in this position and actually some of the more anterior parapodia at this stage had such

FIG. 12.—Outline of tenth left parapodium of a young worm 66 days old. ×270.

spinigers, suggesting that the falcigers are replaced from in front backwards. The ventral bundle resembled that of the adult; above three homogomph spinigers, below them a heterogomph falciger, then a heterogomph spiniger and finally lowest of all three heterogomph falcigers. All these bristles resembled the adult patterns.

At the age of one year approximately the first twenty parapodia (except the first and second) on either side carried dorsally about three homogomph spinigers of adult type, while the remaining posterior notopodia had only one bristle each, and that a stout homogomph falciger, of the kind found in the same situation in the adult. The ventral bristles were similar to those of full-grown specimens.

5. COMPARISON WITH HERPIN'S LARVÆ.

Herpin (1926) has also reared larvæ of *N. pelagica* from the egg, but his specimens behaved somewhat differently from mine. They hatched out at a later stage, one that appears to have been nearly identical with that

shown in Figure 3. They were then seven days old. While in the egg capsule they had one ciliated girdle, the prototroch, which disappeared before they were actually liberated. Thus on hatching they were incapable of swimming and crawled at once. My larvæ on the other hand were provided with several ciliated girdles, or parts of girdles, and swam strongly for the first few days, only gradually taking to crawling as the cilia were slowly lost. They could indeed still swim a little when eighteen days old (Fig. 6). Herpin's larvæ developed more slowly than mine ; the last stage he figures was twenty-eight days old and was, if anything, a little less advanced than my larvæ at eighteen days. He declares that at eighty-three days his larvæ were scarcely more developed than this. In the absence of more abundant data it does not seem justifiable to speculate as to the cause of these interesting differences.

6. SUMMARY.

(1) Larvæ of *Nereis pelagica* Linnæus were reared from the egg, and the young worms, which developed from them, to the age of one year.

(2) The larvæ from an early stage possessed three chætigerous segments. At first after hatching they swam strongly by means of a prototroch, an akrotroch and three paratrochs, but as the cilia gradually disappeared they crawled more and more, finally abandoning swimming altogether.

(3) The head developed a pair of tentacles anteriorly and a pair of palps ventrally. A pair of tentacular cirri arose posterior to the prototroch and a little later a second pair ventral to them.

(4) The first pair of parapodia lost their bristles, and a lobe which had grown out between the noto- and neuropodial chæta-sacs became a third and posterior pair of tentacular cirri. Much later a fourth pair developed ventral to them.

(5) About the time when the larvæ ceased to swim (18 days old) a fourth pair of larval parapodia (third adult pair) appeared, to be followed by a fifth, sixth, etc. At sixty-five days old worms had about twenty-one pairs of parapodia, at one year they had sixty and were then about 1·5 cm. long.

(6) During larval development a constant succession of bristles was seen. In the earliest stages the bristles were mainly homogomph spinigers with long appendices ; later these fell out and were replaced by homogomph and heterogomph falcigers with shorter appendices. Later still these were replaced by bristles of the adult type.

(7) It is pointed out that these larvæ differed from those which Herpin reared in that they hatched earlier and instead of crawling as soon as liberated they swam for several days by means of their ciliated girdles.

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A Note on *Balanophyllia regia*, the only Eupsammiid Coral in the British Fauna.

By

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With 2 Figures in the Text.

THE Eupsammiidæ are one of the most interesting families of the Madreporaria. They have an exceptionally wide range of distribution, being found alike in temperate and tropical seas. In the latter they were probably originally confined to deep water, where the majority of them still occur, but they have extended their vertical range (Yonge, 1930) and various species are now found on many of the coral reefs in the Indo-Pacific region. Thus *Dendrophyllia ramea* occurs in moderately deep water in the Mediterranean and has also been found in the English Channel off Roscoff (Lacaze-Duthiers, 1897), while other species of this genus are common near the surface on many of the Pacific coral reefs, the bright orange-coloured polyps of *Dendrophyllia manni* being, for example, very conspicuous on the fringing reefs at Kaneohe Bay on the Island of Oahu, Hawaii (Edmondson, 1929 ; Yonge, 1930).

It is characteristic of the Eupsammiidæ that they never possess zooxanthellæ, even when in association with true reef-building corals (Yonge and Nicholls, 1931 a) which always contain them. This fact was of great assistance in the course of work during the Great Barrier Reef Expedition on the significance of the relationship between corals and zooxanthellæ by providing a natural control to experiments on reef-building corals (Yonge and Nicholls, 1931 b ; Yonge, Yonge and Nicholls, 1932). The yellowish-green corpuscles which always occur in great numbers in the tissues of both Dendrophyllia and Balanophyllia are not algæ, as Boschma (1924) has suggested, but probably wandering cells containing masses of excrement.

Only one species of the Eupsammiidæ is contained in the British Fauna. This is *Balanophyllia regia*, a solitary cup coral, which was discovered by Gosse (1860) on the perpendicular sides of a rock pool at Ilfracombe on the north coast of Devonshire in 1852. Gosse called this " the scarlet and gold star-coral," and the specific name he gave it refers to " the royal

colours in which the animal is arrayed." This species was also found a little later by Charles Kingsley at Lundy Island. At Plymouth, as recorded in the Fauna List (1931), it was originally found by Mr. William Searle, the Laboratory collector, in May, 1906, on the vertical sides of a small cave at Sandway Cellar, Sandway Point, Cawsand. Specimens have frequently been obtained by him from the same locality since then, and a few were also found on the Renny Rocks in February, 1929.

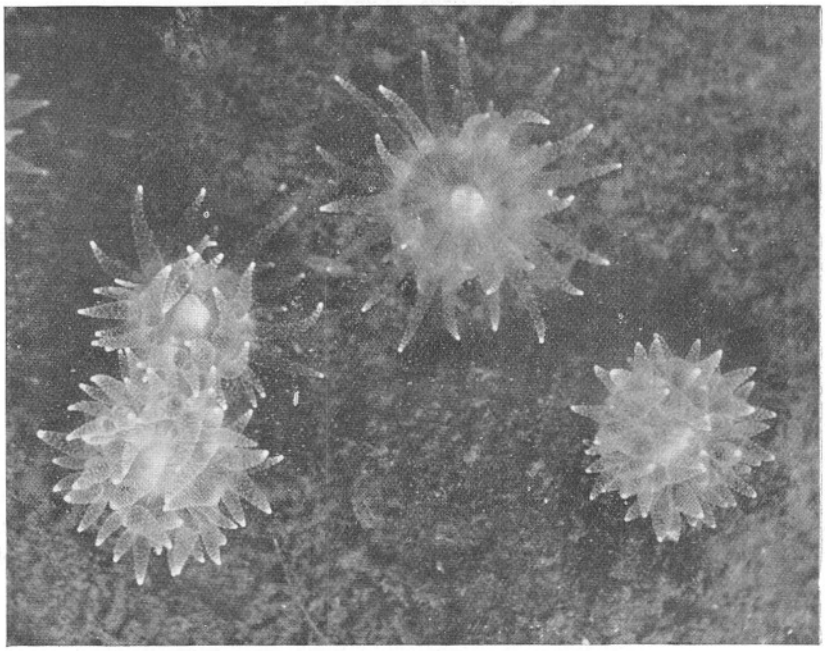

Photo. D. P. Wilson.

FIG. 1.—Group of four *Balanophyllia regia* in various stages of expansion. The fully expanded polyp shows the large mouth and oral disc and the tentacles with their slightly bulbous tips and opaque white spots. × about 2.

Lacaze-Duthiers (1897), to whom we owe most of our knowledge on this species, found it both at Roscoff in the English Channel and at Banyuls in the Mediterranean. Like Dendrophyllia, the genus occurs also in the Pacific. *Balanophyllia bairdiana* was dredged by the Great Barrier Reef Expedition from a depth of 16 fathoms and was used, along with Dendrophyllia, as a check on experiments with reef-building corals. Boschma (1924) records the presence of a species of Balanophyllia on the lower surface of large colonies of reef-building corals in the Java Sea, while van der Horst (1922) records sixteen species taken by the Siboga Expedition at depths ranging from 9 to 580 metres.

Nine specimens of *Balanophyllia regia* were obtained for me from Cawsand by Mr. William Searle about the middle of March, 1930, so that I might compare their feeding reactions with those of Dendrophyllia. The observations made, which have been published elsewhere (Yonge, 1930), showed that, like Dendrophyllia, Balanophyllia has a relatively large mouth and oral cone, with two rows of tentacles which varied between 27 and 36 in number and have considerable powers of extension (see Figure 1). Large pieces of meat were readily seized by them and immediately swallowed. The ciliary currents were much weaker than those of reef-building corals (many of which live in regions where much silt is present) and carry material away from the disc and tentacles and are never concerned with food-collection as they are in certain reef-building corals. As noted by Lacaze-Duthiers, the tentacles when fully expanded are not conical and obtusely pointed as stated by Gosse, but long and slender with terminal knobs like Caryophyllia.

Balanophyllia regia lives for indefinite periods in captivity. The specimens collected in 1930 are still alive in the Laboratory after two years. On April 28th, 1930, four planulæ were found in the bottom of the bowl containing the corals. These were light orange in colour and pear-shaped with the mouth at the narrow end, and about 1 mm. long and 0·75 mm. wide at the broad end. They were about half the size and paler in colour than the planulæ of *Dendrophyllia manni* which I obtained in quantity at Honolulu, and which have been described by Edmondson (1929). The planulæ were transferred to a separate bowl and kept under observation. They settled at once to the bottom. The planulæ of *Dendrophyllia manni* swim near the surface for several days before sinking to the bottom. Since the planulæ of Balanophyllia were not actually observed at the time of extrusion by the adult it is impossible to say whether they have the same habits, but as the adults were kept under circulation it seems possible that the planulæ would have been carried away had they swum near the surface. This matter needs further investigation.

Although Edmondson reports that the planulæ of Dendrophyllia may take as long as thirty days to settle and metamorphose, one of the planulæ from Balanophyllia settled and fixed itself to the glass the day after it was obtained. The other three, though they remained alive for several days, failed to settle.

The young Balanophyllia was kept under observation and is still alive in the Laboratory after two years. Although the development of *Balanophyllia regia* has been described and figured by Lacaze-Duthiers, he does not record the actual time taken, and a short summary of my own observations may be of some value in extending and confirming his work.

The newly settled coral was round and very flat with a diameter of 1·2 mm. After one day the twelve mesenteries were plainly seen and also

the twelve tentacles which consisted of minute stumps. The diameter at the base was now about 2 mm. owing to the rapid spreading out of a thin, colourless layer around the periphery. When meat juice was given the mouth opened widely and the whole polyp expanded, the fragments being drawn in by the cilia lining the stomodæum without any assistance from the rudimentary tentacles. After two days the tentacles were appreciably larger and with greater powers of contraction away from the

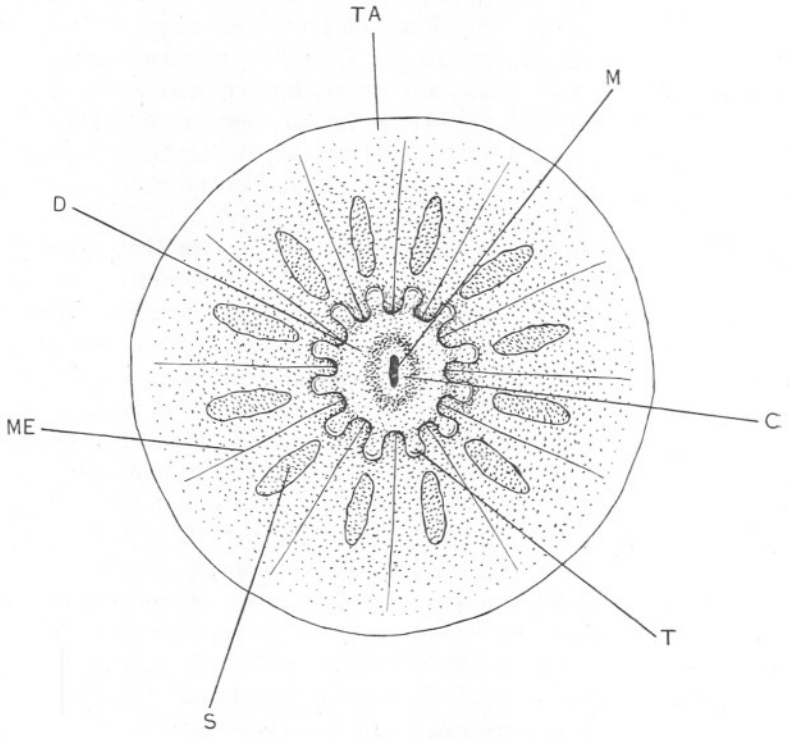

FIG. 2.—Appearance of young polyp, 26 days after fixation. × 34. C., oral cone; D., disc; M., mouth; ME., mesentery; S., septum; T., tentacle; TA., transparent area round periphery of base.

mouth, a relatively large disc being thus exposed. After this development took the form of an increase in the length and power of the tentacles, the raising of the height of the polyp and the appearance of the twelve septa above the basal plate and between the mesenteries. At the end of sixteen days a piece of meat about one quarter the size of the polyp was swallowed with ease, the oral cone extending greatly and the column reaching a height equal to the diameter of the base which still remained about 2 mm. The tentacles were a little larger but still of little use. After twenty-six days the coral had the appearance shown in Figure 2. Meat was readily

swallowed when placed on the disc, and the elongating tentacles were now of definite assistance. The diameter of the base was 2·5 mm. and the twelve septa were very clearly seen.

At the end of 38 days the animal was in all respects a fully formed polyp. The tentacles could expand to over 1 mm. and were very transparent with the characteristic opaque spots upon them. They could seize and hold meat tenaciously. The diameter of the base was about 4 mm. and the outer ends of the septa were bifurcate. The coral was not examined again until it was nearly six months old, when it was found to have increased its diameter to 5 mm. and its tentacles from twelve to twenty-four. These now consisted of six large ones separated by groups of three smaller ones. Apart from the fact that it has increased its diameter to 6 mm. and that its tentacles can now extend to a length of 5 mm. and are all approximately of the same size, the coral remains in the same condition at the present time (Feb. 26th, 1932). It is possible that growth would have been quicker in the sea than in the circulating water in the Laboratory where food is not so abundant.

This note has been written to draw attention to a very interesting member of the British fauna and to the especial interest of the Eupsammiid corals, and also to emphasize the fact that there is at Plymouth not only an imperforate coral, *Caryophyllia Smithii*, but also a perforate coral, *Balanophyllia regia*, some knowledge of the breeding period of which has been obtained. In conclusion, I wish to thank Mr. D. P. Wilson for the beautiful photograph reproduced in Figure 1.

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Note on an Unusual Specimen of *Asterias rubens* L.

By

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With 1 Figure in the Text.

A VERY small, globular Echinoderm was brought to the Dove Marine Laboratory in September, 1931. Its locality of capture is not known, but it is thought to have been brought in by a child who had collected it on the local rocks. The first impression was that of one of the flatter Echinoids. Examination revealed only characteristic Asteroid features. Figures 1, A and B, are photo-micrographs of the living specimen in sea-water, taken on September 16th with a 4-in. objective. The following is a description made on the same date.

Test, globular, similar in shape to *Echinus miliaris*. Diameter, 9·8 mm., height 7·2 mm.

Oral surface. Mouth central, turning downwards—in the centre of a 5-radiate depression ; the radii with no spines, but bearing a double row of large tube-feet in the grooves, each terminating in a well-defined sucking disc. Large plates overhung the mouth in each inter-radial area ; these could be opened or closed to a considerable extent and were well furnished with spines carrying one to three straight pedicellariæ. No indications of teeth or of any structure resembling an Echinoid peristome.

The oral surface passed insensibly into the ab-oral. Closer to the oral than to the ab-oral surface a narrow zone of small, irregular rectangular plates encircled the "test." These were richly provided with sessile straight pedicellariæ, but had no tube-feet.

Ab-oral surface. This was composed of irregularly-shaped plates with no signs of radial symmetry, no apical system, and no division into ambulacral or adambulacral areas. There were no spines or tubercles. An extensive system of pointed, extensible, delicate papulæ gave a characteristic appearance when fully extended. Most of the plates, especially the larger ones, had one or more paxillæ similar to those of *Asterias rubens*. Scattered pedicellariæ of both crossed and straight types were present.

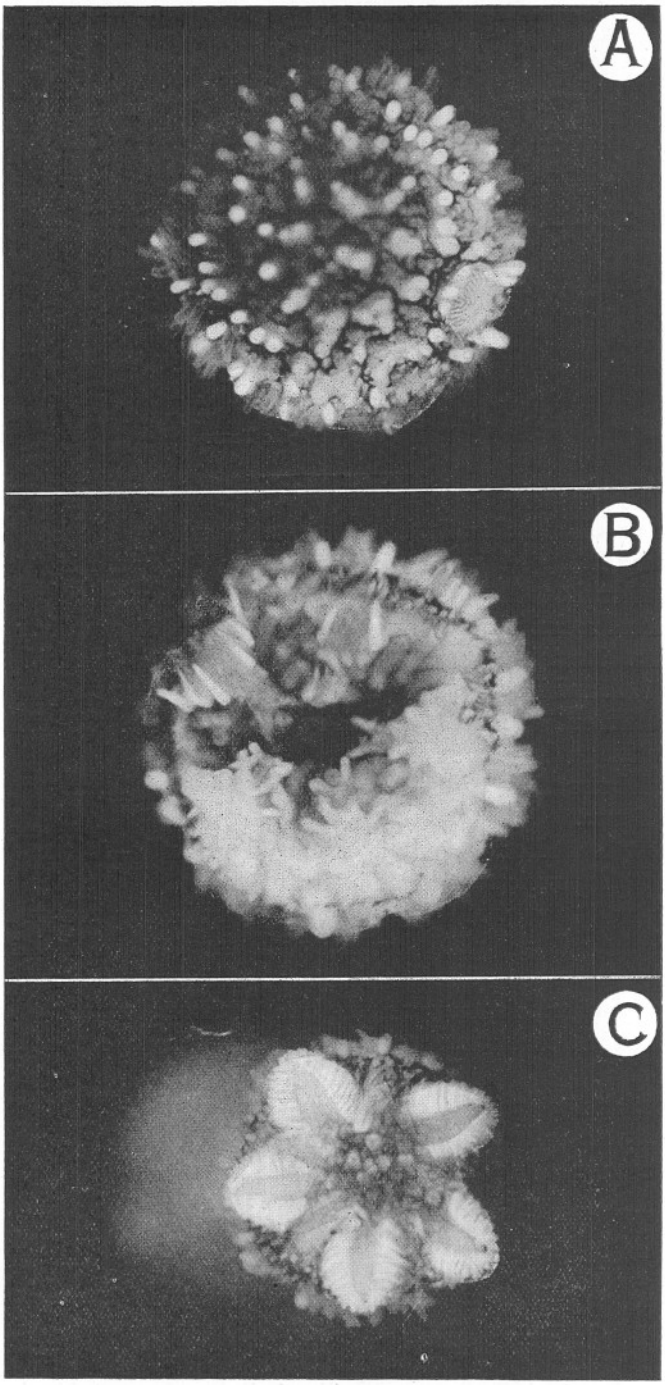

FIG. 1.—An unusual specimen of *Asterias rubens*. Photo-micrographs of the living animal in sea-water.

A.	Sept. 16th, 1931.	Ab-oral surface.	× 5.
B.	do.	Oral surface.	× 5.
C.	Dec. 1st, 1931.	Oral surface.	× 2.

A large and conspicuous madreporite was present, situated inter-radially, and a minute functional anus in the centre of this surface.

The *colour* of the plates was white with a tinge of pink; the margins of the plates outlined in brown. The madreporite was a bright rosy pink.

A large, eversible stomach was observed to be extruded for the capture of food.

The animal was clearly an Asteroid, in spite of its unusual form. It continued to live healthily in captivity. By September 30th the radial grooves had become extended outwards and dorsally (in an ab-oral direction). On October 11th these extensions measured 3–4 mm. in length from the inner point of each inter-radial plate; ambulacral plates and spines were now clearly visible and a few very fine tube-feet had appeared in the extensions of the grooves. At the apex of the oral surface of each arm the red sensory spot was first noticed on this date. A number of attempts were made to obtain photographs of these stages, but none turned out sufficiently good to reproduce, owing to the extreme activity of the animal and to its habit of recurving the arms back close to the "test," so that focussing was impossible. The arms continued to grow and the animal to increase in girth without losing its characteristic spherical shape. Figure 1, C, was taken on December 1st, 1931. On this date, 10 weeks later than that when Figures 1, A and B, were taken, the "test" had a diameter of 18 mm.; and a height of 15 mm.; the arms had grown to 7–9 mm. measured as before. On December 26th the animal crawled over its partition into the next tank where it was captured and eaten by a *Solaster papposus* during my absence.

There appears to be no way of deciding whether this specimen represents an interesting example of regeneration or an abnormal development. It would be of interest to speculate on the verdict of a systematist confronted with the specimen when it was first photographed but presented to him as a fossil.

Specific Differences in the Gonadial Spicules of *Echinus esculentus* (Linnæus) and *Psammechinus miliaris* (Gmelin).

By

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With 4 Figures in the Text.

THE observations here recorded were made as a result of a suggestion by Professor Orton. While examining material of *Psammechinus miliaris* he noted the presence of C-shaped spicules in the gonad. He kindly passed the material on to me, suggesting that an examination of other common Echinoids might prove interesting.

In searching through the relevant literature no record was found of the occurrence of gonadial spicules in *E. esculentus* or *P. miliaris*, two of our commonest Echinoids, whose specific characters are carefully described by Mortensen (**5**). Such spicules are, however, recorded by Mortensen (**1**) and Stewart (**2** and **3**) in several forms, including Temnopleurus, Stephanocidaris and Salmacis by the former and Echinostrephus, Dorocidaris and *Echinus sphæra* by the latter.

Living *P. miliaris* were obtained from Plymouth, Whitstable and Millport, spirit specimens from Plymouth, Port Erin and the River Mersey Bar Lightship ; living *E. esculentus* from Millport and Port Erin and preserved material from Plymouth and Port Erin. A small portion of the genital gland was removed and cleared in glycerine. The arrangement and shape of the spicules is thus rendered distinct. Approximately 100 specimens of *P. miliaris* and 36 of *E. esculentus* have been examined in this way. In both forms the spicules are situated all over the surface of the gonad (♂ and ♀) with their tips penetrating the gland like a series of either " staples " or " calipers "(Fig. 1). In *P. miliaris* the majority are C-shaped with a distinct bulging in the middle of the arc on its inner and outer margins (Fig. 2), whereas the majority in *E. esculentus* are C-shaped but without the central bulging (Fig. 3). Although the general form of the spicules in *E. esculentus* and *P. miliaris* is that of an arc, several variations may occur. In both forms the degree of curvature varies considerably. A slight bulging in the middle of the arc on the outer

margin is exhibited by certain spicules of *E. esculentus*, while others, although few in number (approximately 12 in a 1000 spicules examined), present a bulging in the middle of the arc on the inner margin also ; thus they resemble the knobbed spicules of *P. miliaris*. In the latter animal some spicules (18 in 200 examined) are smooth crescents like those of

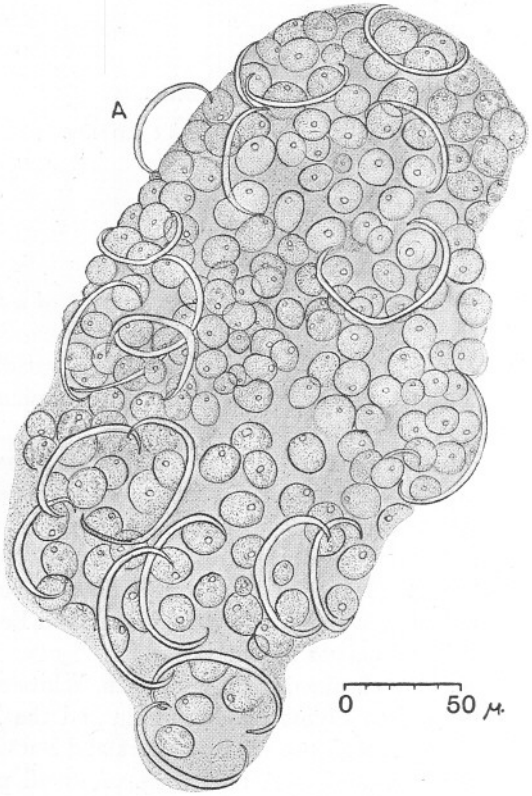

FIG. 1.—A portion of the gonad (♀) of *Echinus esculentus* cleared in glycerine to show arrangement of spicules. (Spicule A has been added here in profile from another view.)

E. esculentus and others, particularly the " caliper " type, lack the inner bulging. Triradiate forms with the three rays of almost equal length may also occur in *P. miliaris* (approximately 15 in a 1000 spicules examined) and one has been seen in *E. esculentus*. In *P. miliaris* an unusual enlargement of the central knob on its outer margin was however observed in certain spicules of the normal type from all the localities mentioned, although particularly in animals from the River Mersey, These are apparently stages in the development of triradiate spicules. All degrees

in the development of this enlargement were noted. In some it was slight, in others it was a definite ray, but only half the usual length, while a few spicules were triradiate with the three rays of equal length. S-shaped spicules may be present in both species, and forms with bifurcated tips were present in the Whitstable specimens of *P. miliaris*. Although these variations do occur and both species possess similarly shaped spicules, but in very different proportions, it may be said that approximately 99% of those in *E. esculentus* are smooth crescents whereas approximately 85% in *P. miliaris* exhibit a bulging in the middle of the arc in its inner and outer margins.

Size offers a further distinction between the gonadial spicules of these two species. In *P. miliaris* the mean value is 27μ and in *E. esculentus* 50μ, the measurements given being the outside measurement across the long axis of the arc of 1204 spicules taken from 54 *P. miliaris* (2 Plymouth, 12 Whitstable, 20 Port Erin, and 20 River Mersey specimens); and 1058 spicules from 15 individuals of *E. esculentus* (2 from Plymouth and 13 from Port Erin). (See Fig. 4.) In *E. esculentus* the range in size of the spicules is also greater than in *P. miliaris*. The size range appears to vary but little with the size of the individual. (See Table I.) In addition to the samples given in the table, about 20 specimens of *E. esculentus* from Port Erin showed gonadial spicules with an average length of approximately 50μ.

TABLE I.

	No. of individuals.	Locality.	Range in diameter of test.	No.* of spicules measured.	Range in size of spicules.	Average size of spicules.
P. miliaris	20	Port Erin	0·6–1·2 cm.	402	14–40μ	25·9μ
,,	20	R. Mersey	1·6–2·5 ,,	400	16–58μ	30·0μ
,,	5	Plymouth	1·7–5·4 ,,	424	16–48μ	23·9μ
,,	7	Millport	2·7–3·8 ,,	200	17–59μ	28·6μ
,,	12	Whitstable	3·0–4·6 ,,	302	16–53μ	27·8μ
E. esculentus	3	Port Erin	1·4–2 ,,	100	32–76μ	43·7μ
,,	5	Millport	7·3–9·0 ,,	200	32–74μ	48·7μ
,,	13	Port Erin	7–10 ,,	858	22–80μ	47·3μ
,,	2	Plymouth	larger than 10 cm.	200	28–78μ	49·1μ

* (Only a proportion of the spicules were used for the graphs in Fig. 4.)

The average size of the spicules in *P. miliaris* taken from the five localities given above would indicate that size is doubtfully correlated with the size of the individual. A definite statement cannot be made concerning this relationship until specimens of all sizes have been examined from each locality. It may be that a particular locality has an approximately constant average size of spicule for all its individuals, large and small; or it is possible that the average size of the

FIG. 2.—Spicules from the gonad of *Psammechinus miliaris*.

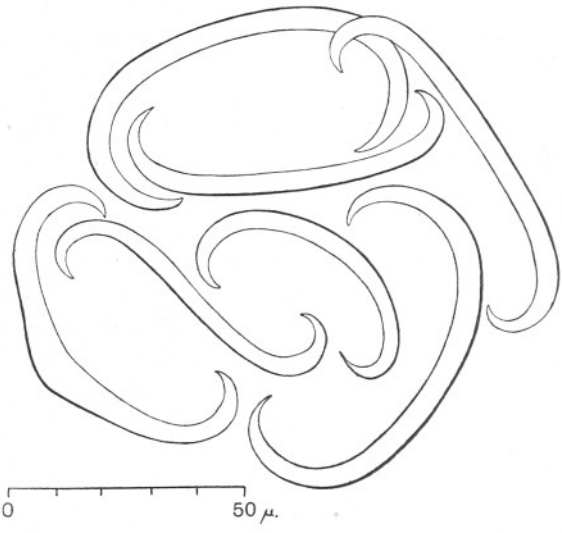

FIG. 3.—Spicules from the gonad of *Echinus esculentus*.

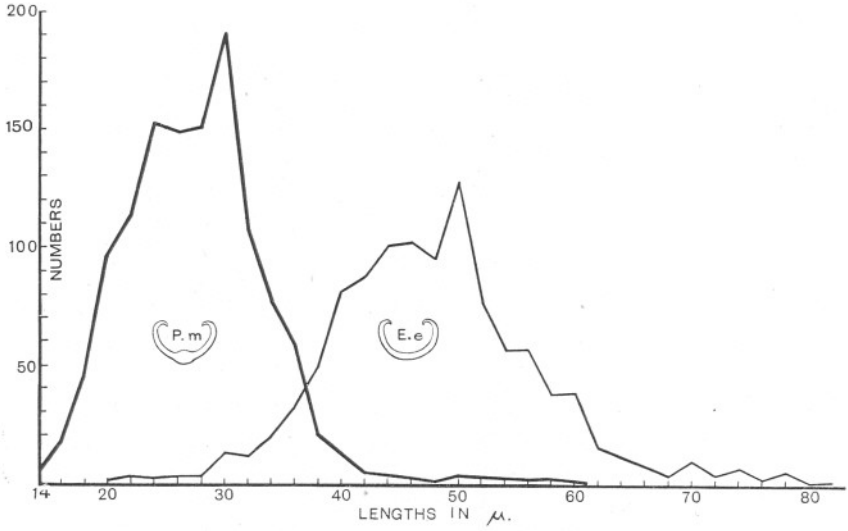

FIG. 4.—Graphs showing the variations in the lengths of 1204 unselected gonadial spicules of *P. miliaris* (thick lined P.m) and 1058 unselected gonadial spicules of *E. esculentus* (thin lined E.e) measured to the nearest 2μ and plotted on the same base.

spicules may be correlated with the size of the individual in a particular locality. In the case of *E. esculentus* the larger specimens examined have spicules with a somewhat greater average length than the smaller ones, nevertheless the size range differs only slightly in the two sets of individuals.

In the spent gonads of both types studied the spicules are very numerous and more obvious than in the ripe gland. In this connexion it is interesting to quote a sentence from Stewart (**4**) in his paper on Dorocidaris in which he says, " I find great variation in the number of spicules although their general form is constant in similar specimens, those having comparatively small genital tubules having spicules most abundant, whereas where the tubules are of large size (female ?) the spicules are often very scanty and small." If the number of gonadial spicules is constant, it would follow that they would be more difficult to find in the large ripe organ than in the compact spent gland.

The knobbed spicules of *P. miliaris* resemble the gonadial spicules of *Echinus sphæra* figured by Stewart (**2**). Since *Echinus sphæra* (Müller) is now recognised as synonymous with *E. esculentus* (Linnæus) (Mortensen, **5**), it appears as though the gonadial spicules of *E. sphæra*, drawn by Stewart, are not those of *E. sphæra* (Müller) synonymous with *E. esculentus* (Linnæus).

The walls of the genital tubules near their origin from the genital rachis are densely packed with the typical C-shaped spicules in both *P. miliaris* and *E. esculentus*.

Spicules appear to be absent from the gonads of *Echinocardium cordatum* since a dozen freshly preserved specimens from Port Erin showed no trace of these structures.

While considering the spicules of the common Echinoids it may be of interest to note the occurrence of C-shaped spicules, similar to those in the gonad, in the walls of the axial sinus and alimentary canal and in the epithelium lining the test of *E. esculentus* and *P. miliaris* although they are very scarce in the axial sinus of *P. miliaris*. No record of their occurrence has been met with in the literature consulted. Similar spicules occur in the tube feet of *E. esculentus* as recorded by Chadwick (**6**). Apart from the perforated plates of the terminal disc, which are similar to those of *E. esculentus*, spicules appear to be absent from the tube feet of *P. miliaris*. A careful examination of 50 tube feet from several fresh specimens of this species did not reveal any of these structures.

The foregoing observations indicate that the distribution of the C-shaped spicules in both species is the same as that of the coelomic epithelium. Chadwick (**6**), however, clearly states that the spicules of the tube feet of *E. esculentus* lie in the connective tissue.

The abundance of spicules varies in different parts of the individual.

They are most plentiful in the walls of the gonad and alimentary canal and less abundant in the epithelium lining the test and covering the axial sinus.

If, as it may be surmised, the function of the spicules is to support the structures with which they are associated, then their distribution is as one might expect. As the gonads develop and increase in size and weight, a considerable pressure will be set up against the epithelium enveloping the gland. The latter must of necessity stretch. As it does so its spicules become scattered, but will still serve to support the distended wall until the gonad is ripe. After the escape of the germ cells the epithelium will contract and so bring the spicules together in dense groups such as are characteristic of the spent gland. The spicules in the walls of the alimentary canal may likewise give support while permitting of a considerable amount of distention. The epithelium lining the test does not perform any particular supporting function and one would expect the spicules therein to be relatively sparse, as in fact they are normally.

All preparations were cleared in glycerine and the appended figures were drawn from such preparations.

These researches were carried out while holding State and Liverpool Education Committee Research scholarships.

My thanks are due to Professor Orton and also to Dr. Mortensen for his courtesy in supplying references to literature, and his assistance in the identification of the small *P. miliaris* from Port Erin.

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The Fæcal Pellets of the Trochidæ.

By

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With 12 Figures in the Text.

Fæcal Pellets of the following species are described :—

Gibbula cineraria (Linn.).
G. umbilicalis (Da Costa).
G. tumida (Montagu).
G. magus (Linn.).
Cantharus (*Jujubinus*) *clelandi* (Wood).
Calliostoma zizyphinum (Linn.).

Of the various molluscan fæcal pellets so far described, none have shown a very high degree either of internal differentiation, or of external sculpturing. In the latter respect the most complicated are perhaps those of the Nuculidæ (Moore, **1**) and the Pectinidæ (Moore, **2**). In neither of these groups is there any trace of internal localisation of different types of material, but in the Mytilidæ (Moore, **2**) there is, in some species, a sorting of the finer material to the lateral regions of the fæcal ribbon, and of the courser material to the centre. There is not however any clear-cut line of demarcation between the two regions.

In the present group there is, in all the species described except *Calliostoma zizyphinum*, a localisation of the constituent materials according to their grade into certain definite regions of the pellet; and there is further, in all except Calliostoma, a very complex system of surface sculpturing.

The pellets of *Gibbula umbilicalis* and *G. cineraria* may frequently be seen on the shore, where their peculiar shape makes them easily recognisable. Moorhouse (**3**), speaking of *Trochus niloticus* from Low Isles, on the Great Barrier Reef, says: "Feeding appears to proceed at every opportunity, so that the amount of fæcal matter deposited is very great. This fæcal track has often been the means of tracing an animal that was

otherwise hidden." He does not however give any description of the fæces.

The methods used for collecting, and preparing sections of the pellets, have already been given in a previous paper (Moore, **2**). There is one potential source of error which calls for special comment. When a pellet contains an area in which are deposited any large mineral particles occurring in the food material, a section in which such large particles occur is very liable to be damaged in cutting. At the same time, a section of the same pellet through an area where there do not happen to be any large particles, is more likely to remain intact. And, since it is generally the case that only a certain proportion of the sections cut are undamaged, an examination of the most perfect sections is apt to give the erroneous impression that there is no localisation of the different types of material. This can only be avoided by correlation of the results from sectioning, with an examination of dissected specimens; and in this particular group the pellets can quite easily be dissected with a needle.

Unless otherwise stated, the localities given for the various species refer to specimens collected between tide marks and, except where stated, the pellets from a considerable number of specimens have been examined. I am indebted to Dr. E. J. Allen for the material from Plymouth; and to Miss M. W. Parke for the material from Loch Hine, in Ireland; also to the Editor of *Nature* for permission to reproduce Figure 10.

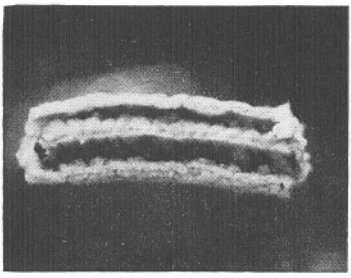
Gibbula cineraria (Linn.). [=*Trochus cinerarius* of Forbes & Hanley.] Localities: Plymouth; Loch Hine, Ireland; Port Erin; littoral, and from 5 to 20 fathoms.

G. umbilicalis (Da Costa). [=*Trochus umbilicatus* of Forbes & Hanley.] Localities: Plymouth; Port Erin.

The pellets of these two species are so alike that it has not been possible to differentiate between them. They are shed in the form of a rod, which breaks into lengths of three to five times the diameter. They are usually brown and sandy in appearance, although both these characters are variable according to the nature of the food eaten: when the animal has been feeding mainly on algæ, more or less translucent pellets may be found.

In the case of *G. cineraria*, an animal with a shell 1·5 cm. in diameter, forms pellets with an average diameter of 0·6 mm.

The pellets are roughly circular in section, but on the ventral side there are two deep V-shaped longtitudinal grooves, with an upstanding ridge between them. This ridge is supported on either side by a series of rounded buttresses, which constitute the internal walls of the ventral grooves, and are separated from one another by deep, narrow clefts. These buttresses can be seen in Figures 1 and 3. The crest of the mid-ventral



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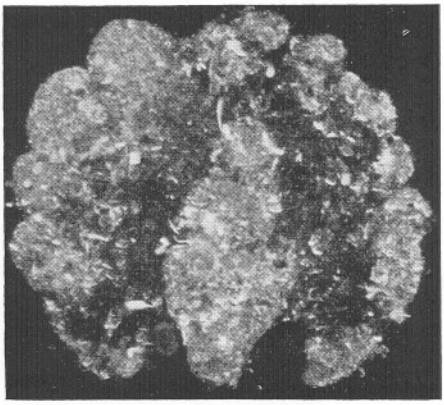

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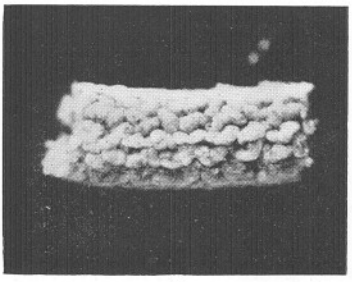

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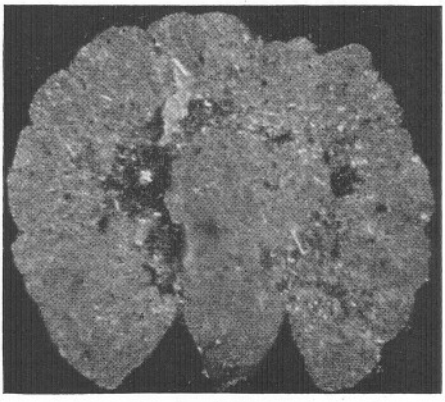

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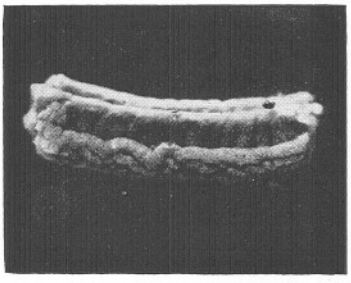


3

6

Gibbula umbilicaris.

FIG. 1.—Ventral view.
,, 2.—Dorsal view,
,, 3.—Ventro-lateral view.
,, 4.—
,, 5.— } Transverse sections.
,, 6.—

ridge may be either rounded or flattened, and in some cases it appears to be of a gelatinous consistency, and can be dissected away unbroken from the rest of the pellet with a needle.

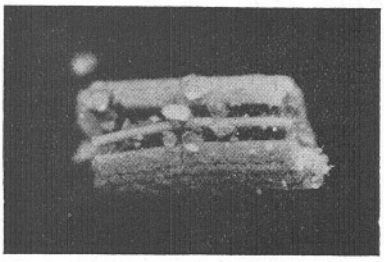
The ventro-lateral lips bounding the ventral grooves are usually smooth, but the rest of the dorsal and lateral regions of the surface of the pellet are cut by deep grooves into about ten rounded longitudinal ridges, and these ridges are thrown into tightly packed lateral undulations. In the pellets of specimens collected from the littoral zone of the shore these undulations are usually of a rather irregular nature, and the loops of neighbouring ridges do not lie opposite one another on successive ridges. In specimens of *G. cineraria* taken at a depth of from 5 to 20 fathoms, off Port Erin, the ridges on the pellets tended to be more compact, and their undulations more regular, although never attaining the regularity of certain allied species. This tendency towards the formation of more regular pellets by specimens from deep water, as compared with those occurring on the shore, is found also in *G. magus*, and it would be interesting to know whether a similar phenomenon is found in other animals also, and if so, what is its significance.

In an experiment, *G. cineraria* was fed on a pure algal culture, containing no gritty matter, but the pellets retained their typical form, although of a very loose consistency. Under natural conditions, extraneous particles tend to adhere to the pellets, so that the fine details of their sculpture may be obscured.

In transverse section the pellet shows two distinct types of material, in regions more or less sharply marked off from one another. In one of these there are only fine particles, and these are fairly firmly bound together—presumably by some material like mucus—so that if the pellet is crushed, the regions composed of this material tend to remain intact. The mid-ventral ridge, and a central region attached to, and forming a base to this ridge, are formed of this fine material, as is the whole of the region comprising and underlying the dorsal and lateral ridges. These two regions meet near the bottoms of the ventral grooves.

Between these two areas of fine material there is a region of variable extent, in which are found any coarse particles such as shell fragments, or large sand grains, which the pellet contains. Although this region is not clearly defined in all pellets, it is typically present, and may include large empty cavities between the individual particles.

Owing to the difficulty of cutting such sandy material, it is not possible to show a single section which will illustrate all these points, but a general idea of the structure of the pellet may be obtained from a comparison of those shown in Figures 4–6, with the photographs of entire pellets in Figures 1–3.



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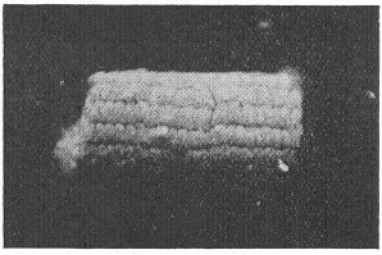

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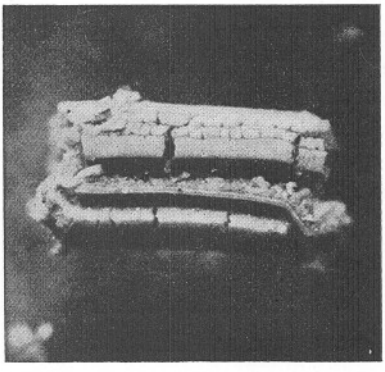

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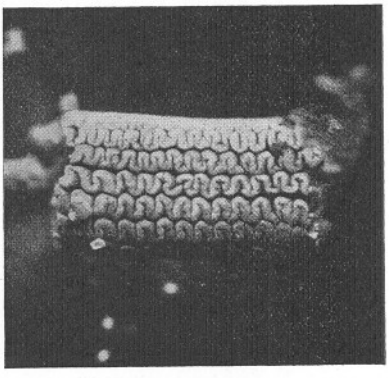

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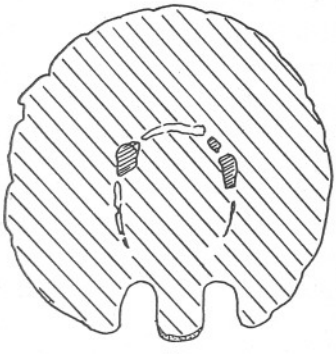

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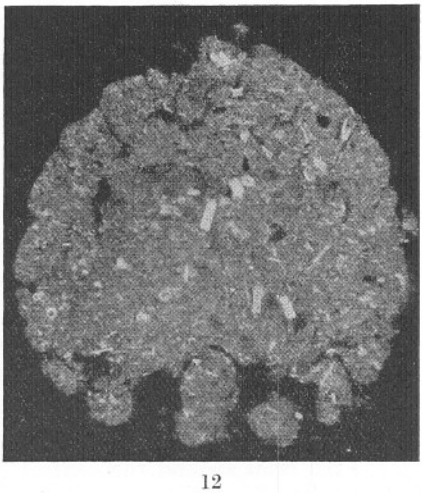

12

FIG. 7.—*Cantharus clelandi*, ventral view.
,, 8.— ,, ,, dorsal view.
,, 9.—*Gibbula magus*, ventral view.
,, 10.— ,, ,, dorsal view.
,, 11.—} ,, ,, Transverse sections.
,, 12.—}

Gibbula tumida (Montagu). [=*Trochus tumidus* of Forbes & Hanley.] Locality : Port Erin, 5 to 20 fathoms.

The pellets of this species are of a similar type to those described above, but with the sculpturing of a more regular pattern. The ventral grooves and ridge are similar, with the exception of the buttresses, which are much narrower in this species, and may even be absent altogether. The mid-ventral ridge also may be narrower in this species. The dorsal and lateral system of ridges is of the same type as in the previous species, except that here there are more numerous ridges, and the undulations in them are considerably more regular. The ridges, being more numerous, are also thinner, and the undulations are of a finer pattern than those of *G. cineraria*. They tend also to lie opposite one another on successive ridges, and may even be almost as regularly arranged as they are in *G. magus*. The grooves which separate these ridges are deeply cut, but their distinctness is obscured by the small size of the pellets, and the relative coarseness of the sand of which they are composed. In transverse section the pellets show the same type of localisation of the coarse and fine grade materials as do those of *G. cineraria*.

Gibbula magus (Linn.). [=*Trochus magus* of Forbes & Hanley.] Localities: Port Erin, littoral (one specimen only), and from 5 to 20 fathoms.

The pellets of this species are of the same type as those of the preceding, but of an even more regular pattern. The ventral grooves occupy a relatively smaller area of the surface of the pellet, and the buttresses of the mid-ventral ridge are inconspicuous from the surface, although clearly visible if the pellet is dissected. The gelatinous tip to the mid-ventral ridge is usually prominent, and can be clearly seen in Figure 9. The dorsal and lateral system of ridges are generally deeply cut and clearly defined ; the ridges are more numerous than in *G. cineraria*, and their undulations are usually very regular, as seen in the example shown in Figure 10. There may sometimes, however, be a system of secondary undulations superimposed on the first, and giving rise to a more complex pattern. It is noteworthy that the pellets of a single specimen which was found on the shore between tide marks were of a very much less regular type than is usual in those from deeper water.

In transverse section the localisation of material seen in the preceding species is much less noticeable, so that the region of coarse grade material may appear to be altogether absent, as in the section shown in Figure 12 ; but dissection of the pellet generally shows a certain amount of coarse grade material around the central core. The rest of the pellet consists of fine material, and frequently contains numerous diatom tests.

From an animal with a shell 3·0 cm. in diameter, the pellets average 1·4 mm. in diameter.

Cantharus (*Jujubinus*) *clelandi* (Wood). [=*Trochus millegranus* of Forbes & Hanley.] Localities: Port Erin, littoral (one specimen only), and from 5 to 20 fathoms.

The pellets are very similar to those of *Gibbula magus*, except that the sculpturing of the dorsal and lateral surfaces is not so deeply cut. This may be associated, as in *G. tumida*, with the small size of the animal and its pellets, and the relative coarseness of the sand of which the latter are composed. (Figs. 7 and 8.)

The undulations of the ridges, as in *G. magus*, are very regularly disposed, and the ventral grooves are restricted, as in that species, to a relatively small area of the ventral surface. The mid-ventral ridge is thin, and its buttresses are reduced or absent. As in *G. magus*, there is not much coarse material in the pellet, so that a transverse section shows little localisation of material, but coarse particles may be found in the usual region if the pellet is dissected.

From an animal with a shell 1·0 cm. in diameter, the pellets average 0·4 mm. in diameter.

Calliostoma zizyphinum (Linn.). [=*Trochus zizyphinus* of Forbes & Hanley.] Localities: Plymouth; Port Erin, littoral, and from 5 to 20 fathoms.

The pellets, in marked contradistinction to those so far described, are in the form of rods, circular in section, but devoid of any surface sculpturing, and with no localisation of the materials inside. They are of a rather loose, sandy consistency, and the surface of pellets of animals collected in deep water is usually rougher than that of specimens from the littoral zone. The pellets of var. *lyonsii* do not differ from those of the typical form from the same ground. In general the pellets do not contain any material as coarse as the larger particles found in Gibbula and Cantharus, but it is an interesting fact that the pellets of many individuals collected on the shore are composed almost entirely of sponge spicules.

From an animal with a shell 2·0 cm. in diameter, the pellets average 0·75 mm. in diameter.

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The Shell Gravel Deposits, and the Infauna of the Eddystone Grounds.

By

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With 4 Figures in the Text.

THE brief survey of the Eddystone shell gravel and of its infauna, the results of which are described in this paper, was begun in January, 1931, and extended over a period of twelve months. The original object of the survey was to make comparison of the present fauna with that found by Allen (1) on this ground during the course of a series of dredgings taken over a wide area in the Plymouth and adjacent waters, and to note the extent of the change, if any, in the faunistic character of the ground. It soon became evident, however, that if the work were to be made quantitative, it would not be feasible to effect comparison, since use would have to be made of instruments other than those adopted by Allen.

The present scope of the work includes the results of an examination of the substratum in which the members of the infauna live, its constitution, development, and conservation, a short faunistic survey of the ground, and a consideration of a few factors of importance in the bionomics of a well-defined infauna community.

Much assistance has been given by Dr. Allen, and by the members of the staff of the Plymouth Laboratory, to all of whom I am deeply indebted. To Captain Lord of the s.s. *Salpa*, whose advice and help at sea have been of the greatest value, and to all the members of the crew, I express my grateful thanks.

METHODS OF COLLECTION.

Positions of stations, a list of which is given in Table I of the Appendix (p. 272), have been determined by means of a single bearing on the Eddystone Lighthouse, and the distance as given by the Barr and Stroud Rangefinder, Type F.T. 32, with 80 cm. base. Positions can be determined quickly and accurately with an error of about 1·5% at 3500 yards —the greatest distance of working from the Stone. It was originally

intended to sample the fauna over the area quantitatively by means of a $\frac{1}{10}m^2$ Petersen grab ; on most occasions, however, the instrument failed to bring up any gravel owing to the insertion of large pieces of shell between the teeth. On May 20th, 1931, twelve dips resulted in only two good hauls. Without doubt the instrument works well on the finer deposits, but in order to maintain a uniform method throughout it has been found necessary to make use of the Conical Dredge fitted with a canvas bag, and with the exception of a few hauls taken with a fine-meshed Naturalist's Dredge in order to define the limits of the shell gravel on the reef border, all sampling has been done with the Conical Dredge.* This type of instrument, as used in quantitative work, has been somewhat adversely criticised, and with some justification. It is worth while considering the limits within which reliable work can be accomplished with the dredge.

In the first place, the instrument is not effective in taking a sample of the epifauna of the ground. Members of the epifauna which attach themselves to shell fragments and to stones are of course captured by any instrument capable of digging into the bottom deposit, but the epifauna also includes predatory species, active enough to get out of the way of the dredge. Not a single actively moving crustacean, mollusc, or echinoderm has been taken during the course of the work. Specimens of *Ebalia tuberosa*, *E. tumefacta*, *Portunus pusillus*, *Conilera cylindracea*, and *Amphioxus lanceolatus*, all of which—and particularly the latter—are capable of fairly rapid movement, have been caught, but these are really members of the infauna, and are scooped up with the gravel.

With regard to the infauna, there is no indication in the scanty comparative records of any serious discrepancy in the conical dredge haul as compared with that of the grab. Ford (**8**) concludes, after comparing the two, " that the conical dredge is capable of taking a good sample under favourable circumstances, and will give a good idea of the general community formation." Only two grab hauls are available for comparison as a result of the present survey. Hauls 21 and 23 (Appendix, pp. 277–8) show the numbers of the various species from 1 litre of the grab samples, and may be compared with Hauls 22 and 29 respectively, taken in the same vicinity, and representing the fauna of 1 litre of gravel from the conical dredge. The numbers of individuals of the Mollusca, Echinodermata, Crustacea, Polychæta, and Nemertini, and the number of species taken in the two hauls of each type, are given for comparison below.

* Diameter of the mouth of the dredge = 1 ft. 6 in.
Total length = 2 ft. 10 in.
Length of canvas bag = 2 ft. 1 in.
Diameter of hinder end of the dredge = 6½ in.

NUMBER OF INDIVIDUALS FROM 2 LITRES OF GRAVEL FROM CONICAL DREDGE AND GRAB HAULS.

	Conical Dredge.	Grab.
Mollusca	16·0	15·5
Echinodermata	18·0	51·0
Crustacea	3·0	1·0
Polychæta	8·0	9·5
Nemertini	2·0	1·5
Number of species taken	18	21

Except in the numbers of *Echinocyamus pusillus*, there is no suggestion of greater efficiency on the part of the grab. The most obvious and most serious objection to the use of the conical dredge in quantitative work lies in the fact that it must be hauled a considerable distance before it can be filled; at least two adverse factors are thus involved.

1. Movement over the bottom may not be smooth, an excess of the surface layer being obtained.
2. The sample collected is a general one from a large area, the grab sample being a particular one from a small area.

Before considering these two points, it would be well to indicate the approximate distance which the dredge has been made to travel during a haul of 4–5 minutes' duration. Unfortunately, during the greater part of the work the distance was not noted, but towards the end of the survey the positions of the ship at the time of shooting of the dredge, and at the time when the dredge had been pulled to a position vertically below the stern, were taken. The observations made on December 11th are given in Table 1, below.

TABLE 1.

	Position at beginning of haul. Eddystone bearing.	Position at end of haul. Eddystone bearing.	Distance travelled by the dredge.
1.	E. 1000 yards.	E.¼N. 1075 yards.	90 yards.
2.	E. 1725 ,,	E.¼N. 1660 ,,	105 ,,
3.	E. 2350 ,,	E. 2500 ,,	150 ,,
4.	N.E. 2600 ,,	N.E.¼E. 2720 ,,	185 ,,
5.	N.E. 3000 ,,	N.E. 3150 ,,	150 ,,
6.	N.W. 1670 ,,	N.W. 1690 ,,	20 ,,
7.	N.W.¼W. 2000 ,,	N.W. 2000 ,,	100 ,,

It is not claimed that the figures given in the last column are strictly accurate; the distances travelled by the dredge, as calculated from a consideration of the initial and final positions, are only approximate, since in the first place bearings have only been taken to the nearest $\frac{1}{4}$ point of the compass, and secondly, no allowance has been made for the error of the rangefinder. It is sufficient, however, to show that the maximum distance of hauling is about 200 yards, this maximum being attained when the ship slips away on a rapidly moving tidal stream. Hauling over this distance eliminates any possibility of detecting "patchiness" of fauna, but since the object of the survey has been rather to indicate the general nature of the fauna within arbitarily selected areas, the only danger is that in hauling over this distance more than one of such areas may have been sampled. In comparing the fauna from the different parts of the ground, difference in texture of the soil has been made the factor for division into areas, and areas differing in Representative Number* by 1·0 have been selected. In order to get from a gravel of Representative Number (R.N.) x, to one of x+1, or x—1, it is necessary to travel at least 500 yards, as reference to Figure 1 will show. Within 200 yards, gravels of R.N. difference 0·3—0·4 may be sampled. There is, then, a possibility of overlapping from one area into the next, and in comparing the faunas from gravels of different texture, the R.N. must be understood to be liable to an error of the order of $\pm 0{\cdot}2$.

The probability of collecting an excess of the surface layer of the deposit, by using the conical dredge, involves

(*a*) The collection of a number of surface-living animals out of proportion to the number in the deeper layers of the soil.

(*b*) The collection of an insufficient quantity of the sub-surface gravel deposit.

The first point was considered when it was shown that there is reason to believe that the dredge is probably as efficient as any other instrument at present used in quantitative work, for obtaining a fair sample of the infauna. For the collection of a gravel sample, the grab is definitely inferior, the small amount of soil taken being exposed to the wash of the water during hauling, with the loss of a good deal of the finer particles. The middle portion of the conical dredge sample is not so exposed, and the finer grades are retained.

The routine adopted during work at sea has been as follows. When in the neighbourhood of a station selected prior to the cruise, the ship has been brought on to the required bearing relative to the Stone, and manœuvred into position, instructions as to the distance from the Eddystone, as given by the rangefinder, being given from time to time. When the

* For the explanation of the term Representative Number, and for the method of its derivation, see p. 247.

ship had been brought into position, the dredge was dropped over the stern on the port side, and the boat was allowed to slip away with the tide, or by giving a turn or two to the engines until 60 fathoms of warp had been paid out, when the warp was made fast. After an interval of about two minutes, hauling was commenced, and continued slowly and steadily until the dredge had just left the bottom, the final bearing and distance then being taken.

As soon as the dredge was on deck, its contents were tipped into a bath, and two bottles (of about $2\frac{1}{2}$ litres capacity) were filled with the middle portion of the gravel, and taken back to the Laboratory ; the rest of the gravel was searched on deck in order to obtain a qualitative estimate of the fauna. From the one bottle, about $\frac{1}{2}$ litre of gravel was removed, and sieved according to Allen's method (**1**), whereby particles are retained on a series of sieves with circular perforations of 15·0 mm., 5·0 mm., 2·5 mm., 1·5 mm., 1·0 mm., and 0·5 mm. diameter, the material passing through the finest sieve being divided into two portions, one of which settles within 1 minute after stirring up with water, the other—remaining in suspension —being filtered off, dried, and weighed. After drying and weighing the remaining grades, the percentage composition of the sample was determined.* Texture has been expressed by assigning to each gravel a Representative Number, as used by Borley (**4**). The method of deriving the R.N. is illustrated by the analysis of a sample taken at a distance of 1725 yards W. of the Eddystone, and given below. The percentage of each grade is multiplied by the diameter of the smallest particles in that grade (i.e. the diameter of the perforations of the sieve on which the particles composing the grade are retained). Fine sand is given a diameter of 0·1 mm., and the silt is neglected. The sum of the various products is divided by 100, the value so obtained being the R.N. of the sample.

For quantitative estimation of the fauna, 1 litre of gravel was taken from

	Sieves on which particles are retained.	A. Grade.	B. Percentage Composition.	A × B.
Very Coarse Gravel	15·0 mm. sieve	15·0	0·16	2·40
Coarse Gravel	5·0 mm. ,,	5·0	9·12	45·60
Medium Gravel	2·5 mm. ,,	2·5	37·84	94·60
Fine Gravel	1·5 mm. ,,	1·5	26·02	39·03
Coarse Sand	1·0 mm. ,,	1·0	11·35	11·35
Medium Sand	0·5 mm. ,,	0·5	8·13	4·06
Fine Sand	Passes through 0·5 mm. sieve	0·1	5·70	0·57
Silt	do. but in suspension after 1 minute	0·0	1·67	0·00
			99·99	197·61

Representative Number $\frac{A \times B}{100} = \frac{197{\cdot}61}{100}$
$= 1{\cdot}98$

* Large, living bivalves and echinoderms were removed from the sample previous to sieving.

the second bottle and sieved, all living animals retained on the 15, 5, 2·5, and 1·5 mm. sieves being picked out by searching the gravel, a little at a time, under water in shallow enamel dishes. The time and labour required for searching increases with decreasing size of particle, and the limit for all practical purposes may be set at the 1·5 mm. grade. The importance of examining the gravel of this grade with some thoroughness may be gathered from the fact that the mollusc *Astarte triangularis*, which from the point of view of numbers is the most important mollusc in the shell gravel, has been found in grade 1·5 only. Small polychætes, crustacea, and nemertini were obtained by repeated shaking of the sample with water, and straining the liquid through cheese-cloth. In addition, measurements of all undamaged dead mollusc valves from grades 15, 5, 2·5, and 1·5 of the dried gravel, as used for determining the R.N., were made, and will be referred to later.

The Nature of the Bottom.

Dredging in the immediate neighbourhood of the Eddystone reveals the fact that there is a considerable area where there is little or no veneer of shell covering the bare rock. The limits of this type of ground, from which the dredge comes up empty, or with a fauna obviously associated with a solid substratum, are indicated in the chart (Fig. 1). The greater area of the bottom surrounding the Eddystone reef is covered with shell gravel, extending over an area bounded by the circumference of a circle of 3000–4000 yards radius, with the exposed reef as its centre.

Three well-marked submarine ridges run out to the N.E., S.E., and N.W. of the exposed reef, upon which the present light and the stump of the old Smeaton Tower stand. The sides of the reef slope down rapidly into about 30 fathoms of water, and Worth (in **1**) notes that the summit and a portion of the sloping sides are gneissic in character, whilst triassic fragments preponderate at points more distant from the main reef.

Examination of the bottom samples taken during the course of the present work made it clear that gneiss is the main inorganic constituent of the samples taken in the immediate neighbourhood of the reef, but that sandstones and pebbles are found to the exclusion of gneiss at points no more distant from the reef than 1800 yards. No attempt has been made to separate the gneissic and triassic rocks, all of which have been included in the general term of "matter of inorganic origin." Table 2 gives the amount of such material in grammes per 1000 gm. of a sample, as selected from grades 15, 5, 2·5, and 1·5. The greatest quantity of inorganic matter is found,

(1) In samples taken near the exposed reef—at a distance of 1000 to 1500 yards.

(2) In samples taken at distances of 2000 yards or more from the reef.

To the E. and N.E. of the Stone (in Table 2, as Eddystone bearing W. and S.W.), however, there is a progressive decrease up to distances of 2500 and 3500 yards, respectively, a circumstance to which reference will be made later. The first series of gravels have as their main inorganic constituent, gneiss, the second, sandstones and pebbles. Between the two series is an area, the gravels of which contain very little inorganic matter.

Unbroken and broken mollusc valves constitute the greater part of the larger fragments of the shell gravels, whilst in the lower grades, echinoid spines, polyzoan "stalks" and a few unbroken and broken mollusc valves and *Echinocyamus pusillus* tests are found together with sand. Altogether some 30 species of lamellibranchs are represented. Undamaged valves, echinoid tests and gastropods, have been picked out from grades 15, 5, 2·5, and 1·5, and their numbers as they occur in a series of samples are given in Table II of the Appendix.

TABLE 2.

NUMBER OF GRAMMES OF MATTER OF INORGANIC ORIGIN AND OF UNDAMAGED SHELL PER 1000 GM. OF GRAVEL.*

	Position of station. Eddystone bearing.	Gm. of inorganic matter.	Gm. of undamaged shell.
N.E.	N.E.¼N. 1850 yards.	3·62	51·50
	N.E. 2600 ,,	305·77	4·38
	N.E. 3000 ,,	520·03	4·13
N.W.	N. by W. 1090 ,,	244·33	11·06
	W.N.W. 1075 ,,	123·30	4·51
	N.W. 1670 ,,	89·30	29·71
	N.W.¼W. 2000 ,,	87·99	24·56
W.	W. 1075 ,,	62·43	16·69
	W. 1100 ,,	21·87	9·07
	W. 2525 ,,	0·79	5·85
S.W.	S.W. 1700 ,,	348·70	11·30
	S.W. 2600 ,,	57·18	11·97
	S.W. 3500 ,,	23·37	6·63
S.E.	S.S.E. 1010 ,,	112·02	12·02
	S.E. by S.¼S. 1760 ,,	12·00	25·02
	S.S.E. 2175 ,,	203·41	4·60
E.	E. 1725 ,,	30·00	25·28
	E. 2350 ,,	513·22	6·64

* Broken shell, polyzoan and echinoid remains, and fine sand, account for the greater part of nearly all the gravels.

Excluding the gastropods, of which it is difficult to make an exact census, it is notable that the molluscs *Glycymeris glycymeris*, *Astarte triangularis*, *Gafrarium minimum*, *Chione ovata*, *Chione fasciata*, and the echinoid *Echinocyamus pusillus*, comprise at least half the total number of undamaged specimens, and are frequently found in such numbers as to constitute 80–90% of the whole. All the species, the shells of which have been taken in any number in the gravel, have also been taken alive either in the conical or in the naturalist's dredge. Table 2 will show that, generally speaking, the greatest aggregations of undamaged shell occur in the gravels where the inorganic matter is in the least quantity; the figures in the table represent the number of gm. of undamaged shell per 1000 gm. of gravel.

Three well-defined gravel areas are thus to be found.

(1) *The Inner Shell Gravel*, in which gneiss is the chief component of the inorganic material, and in which the concentration of undamaged shell is low.

(2) *The Middle Shell Gravel*, where very little matter of inorganic origin is to be found, and where the concentration of shell, broken and unbroken, is high.

(3) *The Outer Shell Gravel*, where pebbles and sandstones are found in quantity, and where undamaged shells are not found in large numbers.

The approximate limits of these areas are indicated in the chart (Fig. 1).

The Representative Number of a sample has been shown to be a measure of the degree of coarseness of a deposit, a quality determined to a very great extent by the degree of scour to which the bottom is exposed. Worth in (**1**), in considering samples taken in the neighbourhood of the Eddystone, notes that "with one exception the fine textures occur at some considerable distance from the reef, while the coarse textures are clustered around the reef or around the Hand Deeps."* During the course of the year a sufficient number of samples have been taken to show that essentially this is the case, as will be seen by reference to Figure 1, where the R.N.s of samples taken at various positions around the Eddystone reef are shown.

The Representative Numbers of samples taken along lines radiating from the Eddystone are given in Table 3. The figures without brackets are the R.N.s as calculated from the amounts of all types of material remaining on the various sieves. A decrease in value of the R.N. is found as the samples are taken at progressively increasing distances from the Light; in one or two instances, however—in the Outer Shell Gravel area—

* The Hand Deeps are about 4¼ miles N.W. of the Eddystone.

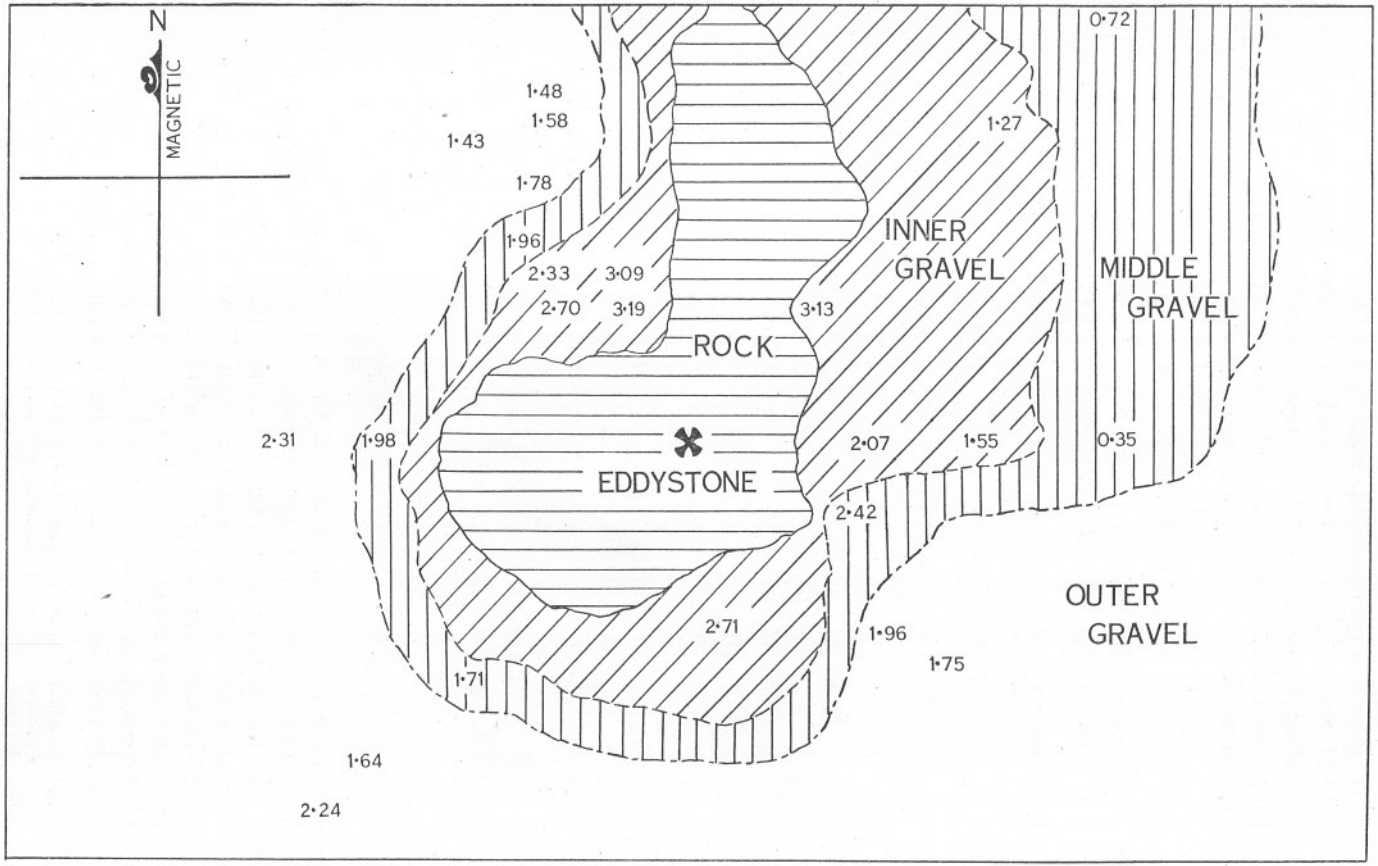

FIG. 1.—Chart showing the Representative Numbers—indicated thus, 2·42—of the gravel samples taken round the Eddystone reef, and the approximate limits of the bare rock, and of the Inner, Middle and Outer shell gravel areas. Scale: 1 cm.=440 yards,

Area without gravel covering, shown thus
Inner shell gravel area, shown thus //////
Middle shell gravel area, shown thus
Outer shell gravel area unshaded

the R.N. is higher at a point with the same bearing on, but further from the Stone. If now the R.N. be recalculated for the sample, after all the inorganic matter has been removed, the new values (Table 3, bracketed figures) for "shell alone,"* show a steady decrease in value the greater the distance from the Stone. The inference is, that the shell is showing a

TABLE 3.

REPRESENTATIVE NUMBERS OF GRAVELS AS CALCULATED FOR THE WHOLE SAMPLE AND FOR THE SHELL CONTENT ONLY.

(Middle Gravel stations are shown in italics, the R.N. for the whole sample, and for the shell content only, differing by less than 0·10.)

	Position of station. Eddystone bearing.	R.N. whole sample.	R.N. shell only.
N.E.	*N.E.¼N. 1850 yards.*	*1·71*	(*1·70*)
	N.E. 2600 ,,	1·64	(0·91)
	N.E. 3000 ,,	2·24	(0·74)
N.W.	N. by W. 1090 ,,	2·71	(1·89)
	W.N.W. 1075 ,,	*2·42*	(*2·35*)
	N.W. 1670 ,,	1·96	(1·78)
	N.W.¼W. 2000 ,,	1·75	(1·65)
W.	W. 1075 ,,	2·07	(1·93)
	W. 1700 ,,	1·55	(1·42)
	W. 2525 ,,	*0·35*	(*0·35*)
S.W.	S.W. 1100 ,,	3·14	(1·80)
	S.W. 2600 ,,	1·27	(1·17)
	S.W. 3500 ,,	*0·72*	(*0·66*)
S.E.	S.S.E. 1010 ,,	3·09	(2·91)
	S.E. by S.¼S. 1760 ,,	*1·99*	(*1·97*)
	S.S.E. 2175 ,,	1·48	(1·24)
E.	*E. 1725* ,,	*1·98*	(*1·92*)
	E. 2350 ,,	2·31	(1·24)

progressive segregation according to size, the larger particles being the more abundant near the reef, the smaller particles more so away from the reef, but that the effect is masked, to the W. and S.W., by the intrusion of inorganic matter from outside grounds, or from the outer portions of the reef. Table 3 indicates clearly stations of the Middle Gravel area (figures in italics), for since there is but little matter of inorganic origin within this area, the Representative Numbers, as calculated for the whole sample and for the shell content alone, differ but little in value. It will be seen

* Inorganic matter has been removed from grades 15, 5, 2·5 and 1·5; "shell alone," therefore, refers to a gravel from which the greater part of the inorganic matter has been removed. See also page 257.

that in the western half of the area round the Eddystone—with Eddystone bearing N.E.$\frac{1}{4}$N., E., and S.S.E.—the Middle Gravel stations are to be found about 1800 yards distant from the main reef. To the E. and N.E., however, the Middle Gravel area is much more extensive, and is expressed most typically at stations from 3000 to 3500 yards distant (Fig. 1).

Evidence as to the extent of movement of particles over the sea floor is of a very contradictory nature. Authorities differ in opinion as to the depth at which wave action ceases to affect the equilibrium of particles lying on the sea bed, but the general opinion seems to indicate that wave action may be felt down to a depth of at least 600 feet in the open ocean (**11**, p. 80). The passage of an oscillatory wave causes particles to move in an orbit perpendicular and opposite to the direction of propagation of the wave, in such a way that the particle moves back to its original position after the passage of the wave. Particles of small size and of low density will move before particles of larger diameter and of greater density, Formulæ have been developed for determining the force required to move particles of known average diameter and density, and are quoted by Borley (**4**), Owen (**13**) and others. It is sufficient, however, to realise that storm waves may lift particles from the sea floor, subsequently to be transported by currents and other agencies, with the result that there is a tendency to segregation of particles of comparable density according to size.

The direction and strength of the currents and eddies around the Eddystone reef must be variable in the extreme, and be dependent on the varying forces of wind and tide, so that it is useless to speculate on the probable resultant direction of the submarine disturbances conditioned by these forces, but it seems probable, in view of the fact that segregation takes place along lines radiating from the exposed reef, that once a particle has been raised from the floor by the passage of a wave, gravity will be the controlling factor in effecting its movement. Johnson (**11**, p. 208), speaking of the removal of graded cliff material from beaches, says, " the seaward inclination of the beach greatly facilitates the removal of débris into deep water ; for . . . if oscillatory waves produce equal impulses alternately landward and seaward, débris on an inclined plane must travel down the slope, whereas on a horizontal bottom it might remain in one place indefinitely."

Immediately surrounding the exposed reef there is little or no covering of shell over the bare rock. In the comparatively shallow water of this region, even slight wave action will be felt, and the steep incline will assist in the passage of particles to a less disturbed and more level bottom. More distant from the Stone, the incline and depth of the bottom are sufficient to allow large pieces of shell and smaller pieces of gneiss to remain in an equilibrium position, and increasing depth associated with a

less marked gradient will permit of the maintenance of equilibrium of smaller shells and rock fragments.

In order to show more precisely this segregation of particles according to size, the sizes of the dead valves of the commoner species of molluscs as they occur in the various gravel samples have been noted, and for this purpose measurements of all undamaged valves from grades 15, 5, 2·5 and 1·5 have been made. The average lengths of the valves of the molluscs *Glycymeris glycymeris*, *Chione ovata*, *Chione fasciata*, and *Gafrarium minimum*, and of the tests of the echinoid *Echinocyamus pusillus*, taken from the various samples are given in Table 4.

TABLE 4.

	Eddystone Bearing.		
	E. 1725 yds.	E. 2350 yds.	
Glycymeris glycymeris	3·91 mm. (172)	3·00 mm. (53)	
Gafrarium minimum	3·54 mm. (71)	2·92 mm. (58)	
Chione ovata	3·07 mm. (71)	2·57 mm. (55)	
Chione fasciata	3·89 mm. (39)	3·27 mm. (33)	
Echinocyamus pusillus	3·72 mm. (27)	2·70 mm. (14)	
	N.E.¼N. 1850 yds.	N.E. 2600 yds.	N.E. 3000 yds.
Chione ovata	2·63 mm. (175)	*3·26 mm. (67)	*3·32 mm. (39)
	N. by W. 1090 yds.	N.W. 1670 yds.	N.W.¼W. 2000 yds.
Glycymeris glycymeris	3·58 mm. (59)	3·43 mm. (217)	3·09 mm. (201)
Gafrarium minimum	3·89 mm. (65)	3·50 mm. (190)	3·13 mm. (139)
Chione ovata	3·03 mm. (21)	*3·26 mm. (122)	2·95 mm. (159)
Chione fasciata	4·63 mm. (27)	4·12 mm. (73)	3·65 mm. (93)
Echinocyamus pusillus	3·60 mm. (33)	3·32 mm. (78)	3·07 mm. (66)
	W. 1075 yds.	W. 2525 yds.	
Gafrarium minimum	3·31 mm. (77)	2·35 mm. (28)	
Chione ovata	2·89 mm. (59)	2·44 mm. (44)	
	S.W. 1100 yds.	S.W. 3500 yds.	
Echinocyamus pusillus	3·84 mm. (18)	2·86 mm. (36)	
	S.S.E. 1010 yds.	S.E. by S.¼S. 1760 yds.	
Echinocyamus pusillus	3·60 mm. (40)	3·42 mm. (90)	

Measurements have been made along the anterior-posterior axes of the mollusc valves, and along the oro-anal line of Echinocyamus, in each case to the nearest ¼ mm. Where the range of size is great, e.g. in *Chione fasciata*, where all lengths from 1·5 to 20 mm. are to be found, only those between 1·5 and 8·5 mm. have been selected. Obviously, the same range must be adopted throughout if a true comparison is to be made. Inclusion of large valves which are probably not moved over the bottom to any extent, only obscures any variation in average size of the smaller valves, due to selective transport and segregation. The maximum limiting size of 8·5 mm. has been chosen, because a natural break in the frequency distribution of all the valves—with the exception of *Echino-*

cyamus pusillus which does not attain this length—occurs near this point ; the lower limit of 1·5 mm. is the limit below which the possibility of picking out and counting becomes impracticable. Average length values have only been obtained where a sufficient number of valves have been measured ; the numbers are given in brackets in Table 4. With the exception of those values of average length marked with an asterisk, there is a distinct fall in average size of the valves and tests at stations taken at intervals from the reef, seawards. A possible reason for the aberrant values for *Chione ovata*, at stations 2600 and 3000 yards S.W. of the Eddystone, is discussed later, in the consideration of the shell gravel community.

Evolution and Conservation of the Shell Gravel Bottom.

It has been shown that the number of species contributing to the Eddystone shell gravel is not great, and that all are animals which are found alive in this particular type of deposit ; the question arises as to whether this particular shell gravel area is formed from the calcareous and siliceous remains of animals which have actually lived their lives in the neighbourhood of the reef, and which have in the course of time accumulated to form the extensive deposits, or whether there has been intrusion of inorganic matter and shell, from the sea bed outside the area surrounding the sloping sides of the reef. The fact that the valves of the Abra (Syndosmya) group, and of other molluscs which live in deposits other than gravel, are found only on the fringe of the muddy gravel, and then only to the extent of less than 1% of the total undamaged shell, precludes the possibility of extensive intrusion of outside forms into the gravel area. Some migration of sandstones and of pebbles probably occurs, but only into the Outer Shell Gravel deposits ; on the other hand it is not known to what extent the intruded material is derived from the outer edges of the reef.

The Outer Shell Gravel deposits are found (within the area investigated) to the S.E., S.W., W., and N.W. of the Stone, intrusion being most marked to the S.W. and W., and less so to the N.W. as is shown by the differences between the R.N.s calculated for the whole sample, and for the shell content only, of the deposits of these areas (Table 5).

TABLE 5.

Position of station. Eddystone bearing.	R.N. whole sample.	R.N. shell only.	R.N. whole sample—shell only.
N.E. 3000 yards.	2·24	0·74	1·50
N.E. 2600 ,,	1·64	0·91	0·73
E. 2350 ,,	2·31	1·24	1·07
S.S.E. 2175 ,,	1·48	1·24	0·24

It must be concluded that the outside material does not move up into the deposits found within a distance of at least 1700–1800 yards from the

reef, a possibility much to be expected since such a movement would involve passage from a region of comparative stability, to one of more unstable equilibrium. The Inner and Middle Shell Gravel areas are thus composed of mollusc valves, polyzoan "stalks" and the like, animals which have lived on the rocky, or on the gravel ground, and dying there, have left their remains, which have become sorted out roughly according to size, during the course of which, attrition followed by removal to deeper water has taken place. The outward movement is slow but continuous, and is compensated by the continual addition of the hard skeletons of the recently dead animals.

Areas where movement and segregation are well marked have gravels with a percentage composition of the following type.

	Grade.	Eddystone bearing. S.S.E. 1010 yards.	N.E.¼N. 1850 yards.	W. 2525 yards.
Very Coarse Gravel	15·0	5·12	1·14	0·00
Coarse Gravel	5·0	18·04	4·53	0·05
Medium Gravel	2·5	36·86	21·97	0·32
Fine Gravel	1·5	26·32	30·98	1·83
Coarse Sand	1·0	8·81	20·87	6·39
Medium Sand	0·5	3·42	16·54	39·50
Fine Sand	0·1	1·20	3·51	51·58
Silt	0·0	0·23	0·46	0·33
Representative Number		3·09	1·71	0·35

This type of deposit is characteristic of the Inner and Middle areas, inorganic matter when present, being gneissic in origin. There is a grade in which the maximum percentage of material occurs, the various amounts decreasing regularly above and below this. Such gravels will be referred to as being of the A type.

Gravels such as are found in the Outer Shell Gravel area, are of the B type, showing two maxima, one in the higher, the other in the lower grades. Their constitution is typified by the following three samples.

	Grade.	Eddystone bearing. E. 2350 yds.	N.E. 3000 yds.	N.E. 2600 yds.
Very Coarse Gravel	15·0	0·30	1·56	1·16
Coarse Gravel	5·0	22·64	23·35	20·18
Medium Gravel	2·5	29·91	20·93	13·80
Fine Gravel	1·5	15·83	13·48	8·58
Coarse Sand	1·0	8·28	6·05	6·25
Medium Sand	0·5	10·81	5·14	8·63
Fine Sand	0·1	11·01	27·36	38·15
Silt	0·0	1·21	2·13	3·25
Representative Number		2·31	2·24	1·64

Evidently the conditions under which these deposits are found, are of a much quieter nature than those giving rise to the first type, for there is present a much greater percentage of medium and of fine sand, and of silt. The large percentages of the higher grades result from the intrusion of stones from the outer grounds, for if these are removed from the grades 15, 5, 2·5, and 1·5, and the percentages recalculated, the results are as follows :—

	Grade.	Eddystone bearing.		
		E. 2350 yds.	N.E. 3000 yds.	N.E. 2600 yds.
Very Coarse Gravel	15·0	0·61	0·61	1·67
Coarse Gravel	5·0	3·70	4·43	3·50
Medium Gravel	2·5	16·73	4·75	6·75
Fine Gravel	1·5	16·51	4·69	6·94
Coarse Sand	1·0	16·51	12·74	9·00
Medium Sand	0·5	21·58	10·81	12·45
Fine Sand	0·1	21·94	57·57	55·00
Silt	0·0	2·42	4·49	4·68
Representative Number		1·24	0·74	0·91

The " shell alone," from the B type gravel, is more nearly like the A type in composition, and will be referred to as the A, or shell component of the B type.

It has not been found practicable to pick out the matter of inorganic origin from grades lower than 1·5, so that the bracketed figures in Table 3 (p. 252), and the percentages for " shell alone " given above, do not really show the distribution of the shell in the different grades. Since, however, the greatest bulk of the inorganic matter is in the higher grades, much of the original bias is removed, although the true percentage composition of shell alone would show slightly smaller values in the grades 1·0, 0·5, and 0·1, with correspondingly larger values in grades 15, 5, 2·5, and 1·5. For present purposes, however, the modified R.N. will be taken as indicating the shell component of the B type gravel.

The differences between the A and B gravels are illustrated graphically in Figures 2, 3, and 4, where the divisions of the abscissæ represent the grades 5·0, 2·5, 1·5, 1·0, 0·5, and 0·1 (grade 15, of little importance, being omitted for the sake of simplicity), and the ordinates, the percentage composition of each grade.

Figure 2 shows the single maximum of the A type gravel, which tends to be displaced to the right with decreasing submarine disturbance, and coarseness of texture. The B type (Fig. 3) has two maxima, the one on the right corresponding to the single maximum of the A type gravel, and resulting from the heaping up material segregated by tidal scour and other hydro-

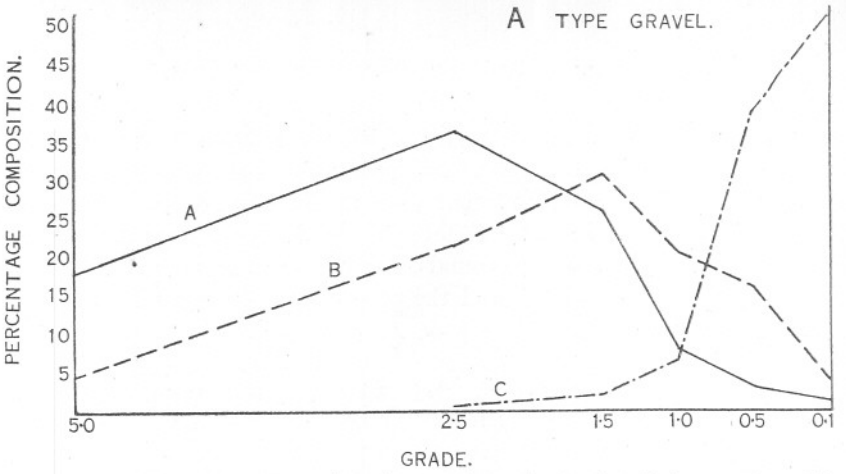

FIG. 2.—Percentage composition of the A type of gravel. A. Sample from station with Eddystone bearing S.S.E. 1010 yards. B. N.E.¼N. 1850 yds. C. W. 2525 yds.

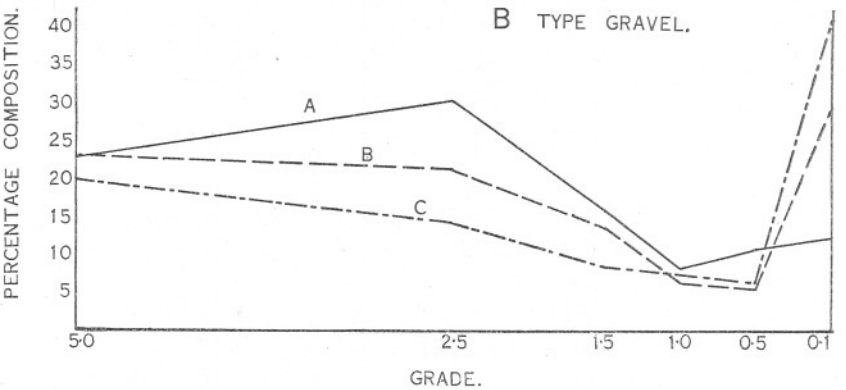

FIG. 3.—Percentage composition of the B type of gravel. A. Sample from station with Eddystone bearing E. 2350 yards. B. N.E. 3000 yds. C. N.E. 2600 yds.

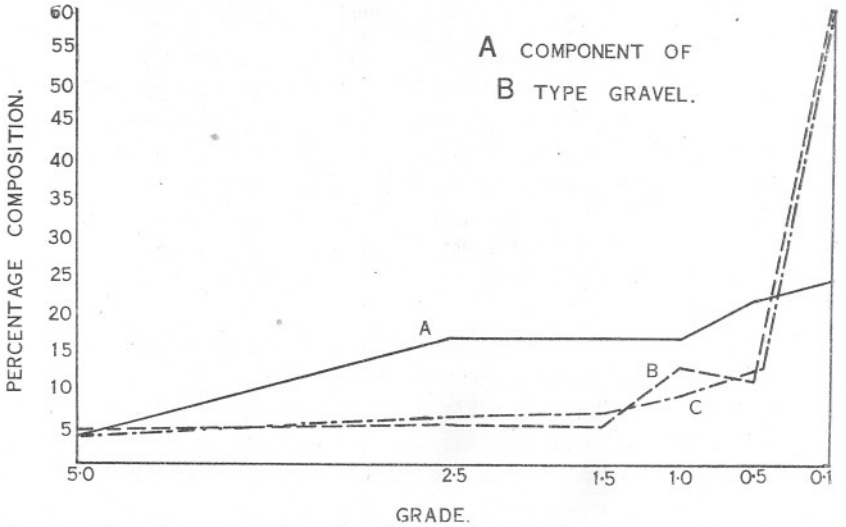

FIG. 4.—Percentage composition of the A component (shell component) of the B type of gravel. A, B and C represent the same three stations as in Fig. 3.

dynamical forces, while the maximum on the left results from the introduction of outside material. The distribution of the grades of the shell component of the B type is illustrated in Figure 4, where it will be seen that the condition approaches that found in the finer of the A type gravels (Eddystone, 2525 yards W. in Fig. 2). The similarity between the shell components of the gravels at stations 2600 and 3000 yards S.W. of the Stone is at once apparent, and it appears that at a distance, at the most of 2600 yards in this quarter, the true Eddystone shell ceases to show any segregation, the increase in coarseness being due entirely to intrusion from outside. Why large fragments should be moved towards the Stone, when evidence is such as to suppose that any resultant dynamic force is in a direction away from the reef, is not clear, but it is probable that heavy swells coming in from the S.W. move the large fragments in a N.E.'ly direction, to positions where they are in equilibrium, whilst gravity combined with less severe wave action would account for the segregation of the lighter shell particles in a direction seawards from the reef.

The prevailing S.W.'ly swells, moving material in a N.E.'ly direction, are instrumental in causing, by intrusion of outside material, a reduction in area of the gravels of true Eddystone origin—gravels of the Inner and Middle areas—on the western side, whereas to the east, the tendency is towards the extension of the reef material and of shell; there being no counterbalancing intrusion from the east, the Inner and Middle gravels are found to occupy, on this side of the Eddystone, a comparatively large area. The general characteristics of the gravels may be summarised briefly, thus :—

A Type Gravel.

Eddystone gneiss, the main constituent of inorganic origin. The percentage composition shows a single maximum. No intrusion of outside material, exhibited.

B Type Gravel.

Sandstones and pebbles, the main constituents of inorganic origin. The percentage composition shows two distinct maxima. Area of considerable intrusion.

INNER SHELL GRAVEL.
Gneiss in relatively large quantity.
R.N. 2·0–3·0.

MIDDLE SHELL GRAVEL.
Gneiss not present in any quantity.
R.N. 1·0–2·0.

OUTER SHELL GRAVEL.
Represented only in areas of little disturbance.
R.N. relatively high.

The Fauna of the Eddystone Grounds.

(a) *The Fauna of the Rocky Bottom.*

At the beginning of the year, a short preliminary survey of the North and North-West quarters of the Eddystone ground was made, using a

Naturalist's Dredge fitted with a fine-meshed net. On this side of the Stone, a considerable area of the side of the reef is devoid of a gravel covering, and has associated with it a rich attached fauna, together with an abundance of predatory species. The approximate limits of this ground are indicated in the chart (Fig. 1). It is not proposed to consider this epifaunistic community in any detail ; a list of the animals taken is given in Table III of the Appendix (p. 274). An interesting addition to the Plymouth fauna was a sponge, subsequently found by Mr. H. J. N. Borley to be a species new to science, and named by him *Pseudaxinella alleni*. A description of the sponge is to be found in the Journ. Mar. Biol. Assoc., N.S., Vol. XVII, No. 3, 1931. The sponge *Eurypon clavatum* (Bowerbank), for the identification of which I am again indebted to Mr. Borley, is also a new record for the Plymouth area.

(b) *The Infauna of the Shell Gravel.*

In all, 36 hauls, the positions of which are given in Table I of the Appendix (p. 272), have been taken on the gravel—9 with a Naturalist's Dredge fitted with a fine meshed net, 2 with the Petersen $\frac{1}{10}$ m.^{2} grab, and 25 with the conical dredge provided with a canvas bag. Of the conical dredge hauls, 22 were examined in a quantitative manner, 1 litre of the contents of the bag being used for this purpose. For purposes of identification, the authorities quoted in the *Plymouth Marine Fauna*, 2nd Edition, 1931, have been consulted. Quantitative result of the hauls are listed at the end of the Appendix (pp. 274–8), and Table IV of the Appendix indicates the range of the various species over the ground, as derived from the results both of the qualitative and quantitative hauls. Grouping is made on the basis of texture of the bottom deposit ; 4 series, from deposits of R.N. greater than 3·0, between 2·0 and 3·0, between 1·0 and 2·0, and less than 1·0, being enumerated.

Excluding the incrusting polyzoa, of which only a few have been found —on stones and shells in the finer gravels—and members of the microfauna which appears on examination to be rich in small crustacea, a total of 112 species have been taken. Of these, however, 45 have been obtained once only, and only 14 species are found in any numbers—*Amphioxus lanceolatus* (Pallas), *Glycymeris glycymeris* (L.), *Astarte triangularis* (Montagu), *Gafrarium minimum* (Montagu), *Chione fasciata* (da Costa), *Chione ovata* (Pennant), *Ampelisca spinipes* Bœck, *Conilera cylindracea* (Montagu), *Echinocyamus pusillus* (O. F. Müller), *Polygordius lacteus* Schneider, *Prœgeria remota* Southern, *Glycera lapidum* Quatrefages, *Mystides limbata* de St. Joseph, and *Lumbriconereis impatiens* Claparède. Nowhere, however, on the gravel, are the numbers of animals comparable to those found on the more silty soils.

Amphioxus lanceolatus (Pallas). Is fairly well distributed over the whole of the shell gravel. It is impossible to state with any degree of certainty the relative abundance of the animal on the clean and finer gravels. The method used for quantitative sampling, whilst excellent for determining the numbers of molluscs and of polychætes, is of little use when applied to such an actively moving form as the lancelet. In the first place, the animal is probably able, in some degree, to evade the dredge, and secondly, whilst a bottle is being filled with a sample to be examined quantitatively, any Amphioxus which may be taken will almost certainly wriggle away and bury themselves in the larger pile of gravel which is to be searched qualitatively. The indications are, however, that Amphioxus is commoner in the coarser, than in the finer deposits. Whilst not attempting to attach any real significance to the numbers taken in each haul of the conical dredge, it is interesting to note the relative frequency of occurrence of Amphioxus in the various grades of soil, as shown below.

R.N. of gravel.	>3·0	3·0–2·0	2·0–1·0	<1·0
Average number of Amphioxus per haul	11·5	8·0	5·5	0
Number of hauls	4	6	13	2*

With the exception of one of the hauls marked with an asterisk, in which very little gravel was brought up, the gravel samples, although not of equal, were of comparable volume.

Amphioxus appears to be most abundant in the gravel N.N.W. of the Eddystone—here, the scour along the bottom maintains a coarse deposit for some 2000 yards in a seaward direction—but the lancelet is likely to be found in any haul taken over a type A gravel. It will be necessary to work systematically over the B type of gravel deposit before attempting to make any estimate of the frequency of occurrence on this type of soil.

Glycymeris glycymeris (Pallas). Although this mollusc has not been found in any great numbers, the number of valves in the gravel suggest that it is one of the most important of the molluscs living on the shell gravel. The records given by Allen (**1**), Ford (**8**), and Steven (**22**), indicate its restriction to gravel grounds, and although the numbers taken are not sufficient to justify any assertion of its distribution within the gravel area, it appears that the most consistent appearance of the mollusc is in the clean deposits.

Astarte triangularis (Montagu). Numerically, this is quite the most important mollusc found during the course of the work. In many hauls it has outnumbered the rest of the molluscs put together, although its very small size—2·0–2·5 mm.—renders it very inconspicuous. Jeffreys (**10**) says that it is "local but gregarious on all our coasts from the northern extremity of Shetland to the Channel Isles, in sand, at depths of from 3–60 fathoms; it is remarkably abundant at Lewis in the Outer

Hebrides, and at Guernsey." More recent records are not numerous ; Colgan (**5**) records it as being common off Clare Island in 5, 10, 15, and 19 fathoms, but does not state the nature of the subsoil.

Gafrarium minimum (Montagu). Of a wide distribution, this species appears to occur mainly on gravel bottoms, but is not restricted to them. Whilst many whole valves were taken during the dredgings, it is remarkable how small a percentage contained living animals ; although such is true of all the molluscs found on the ground, the proportion of dead to living forms is particularly large in this species and in *Chione ovata*. At 8 stations at which the numbers were noted, of 95 Gafrarium examined, only 15 were alive.

Chione fasciata (da Costa). Ford (**8**) makes use of this mollusc (as *Venus fasciata*) in characterising the shell-gravel community, and contrasts the latter with the communities of the muddier deposits, where *Venus gallina* is the characteristic mollusc. It is only necessary to mention here that *Chione fasciata* has been found in fair numbers over the whole of the ground surveyed, and that the valves contribute in large numbers to the make-up of the deposit.

Chione ovata (Pennant). As with Gafrarium, the total number of living animals is small in comparison with the number of whole valves in any sample ; for the same 8 stations, of 104 whole valves, only 9 were found to contain living animals. *C. ovata* is not characteristic of shell gravel, but is found on a variety of bottoms. In this respect it is interesting that in the two hauls (46 and 47), taken to the S.W. of the Eddystone, where the gravels are of the B type, and contain a large proportion of silt, the valves of this mollusc are more common than are those of any other, and it is probable that the increase in average size at stations taken progressively seawards from the reef, contrary to the general direction of gradation, is due to the fact that conditions are more favourable to growth in the muddier soils, with the result that the average size is greater at death, water movement not being sufficient to transport and segregate the valves as in other quarters of the ground. If too, as we have seen, the centre of distribution of this species and of *Gafrarium minimum* is in a muddier soil, whilst the other common molluscs on the ground are characteristic of, and find their optimum conditions in, the shell gravel, it is not surprising that the proportion of dead to living should be higher in these species than in any of the others.

The occurrence of the polychæte *Prœgeria remota* in large numbers is worthy of notice. The genus and species were constituted by Southern, and the genotype is described by him (**19**). With regard to its habit, Southern says, " it is a small species, living on a bottom of sand and shells, or gravel, and would escape capture by the dredge unless special precautions were taken, . . . the Clew Bay specimens were obtained by

carefully washing fine gravel." There are not enough records for one to be sure whether or not this species is restricted to bottoms of coarse texture.

Ampelisca spinipes Boeck, *A. brevicornis* (A. Costa), and *A. tenuicornis* Lilljeborg, are all found living in the shell gravel, the former in deposits of coarse texture (R.N.>1·0), whilst the latter do not appear to trespass into deposits of R.N.>2·0. Between the two values of R.N. 2·0 and R.N. 1·0, there is a certain amount of overlapping of the two groups; Steven (**22**), working over the "corner" grounds, has noted a similar distinction.

Of the forms which are not true gravel dwellers, the two molluscs *Abra prismatica* (Montagu), and *Abra alba* (Wood), and the polychæte *Owenia fusiformis* Delle Chiaje, have been taken in the finer deposits only. The living Abras were found in the gravel 2525 yards E. of the Eddystone, at which point the maximum concentration of dead valves of these species is found.

The Eddystone Shell-Gravel Community.

The method adopted by Petersen (**17**) of naming the different animal communities by means of short terms, derived by abbreviation of the generic or specific names of the characteristic species of the communities, has the great advantage of simplicity, rendering unnecessary the listing of all the animals taken, some of which occur in small numbers only. Species which are dominant, both by number and by weight, are selected for this purpose. Seasonal animals are of little use in characterising the community, and finally, it is of practical importance to adopt for this purpose such forms as can easily be preserved and identified; and for this reason, molluscs and echinoderms are usually chosen.

Petersen laid particular stress on depth as the primary factor in determining the distribution of the nine communities found on the level sea bottom of the Danish waters—"animal communities of any water, will always be found to group themselves according to depth" (**16**, p. 7). The importance of the bottom deposit in this respect has however been recognised for some time by naturalists. Allen (**1**) has mapped out the distribution of the marine fauna over the different types of bottom, near the 30 fathom line, in the Plymouth and neighbouring waters. More recently, Ford (**8**), using Petersen's method of definition of the communities, has concluded that in the Plymouth area, "at least two distinct main series of level bottom animals exist alongside one another, . . . the one expressing itself in several recognisable forms in deposits in which fine grades predominate, the other being restricted to coarser soil, with its typical form restricted to clean shell gravel." Both are Venus–Spatangid associations, the former being an *Echinocardium cordatum–Venus gallina* (EcVg) association, the other a *Spatangus purpureus–Venus fasciata* (SpVf) association. The SpVf community, so characteristic of the Eddy-

stone shell gravel, has received surprisingly little attention from marine ecologists, largely, no doubt, because most quantitative work has been done in areas where conditions are not conducive to the formation of gravel deposits. The SpVf community may be regarded as a component of Petersen's deep Venus community (v), which is apparently, as Ford (**8**) has pointed out, a composite association ; indeed, without any doubt, Petersen never sampled a true shell gravel deposit at any time in the Danish waters. Stephen's (**21**) *Echinocardium cordatum–Tellina fabula* (EF) community, and Spärck's (**20**) *Mactra elliptica* community, have something in common with the SpVf association, but are evidently of a composite nature.

Of the characteristic molluscs of the Eddystone shell gravel, we can speak with some degree of certainty. Variations in numbers from year to year, of the various forms, might lead successive observers to name different characteristic forms, according to the relative numbers of the different species present at the particular periods of observation, but taken over a period of years, one should be able to distinguish between those forms which are most truly characteristic, and those which are not. Such a record exists (if the hypothesis that there is little or no intrusion from outside grounds into the coarser deposits, be accepted) in the graveyard of the species—the shell deposit. Considered from the point of view of the living fauna, and with reference to their occurrence on other grounds, we should be prepared to name as characteristic the following bivalves : *Glycymeris glycymeris*, *Chione fasciata*, with a more reserved opinion regarding *Astarte triangularis*, *Cardium scabrum*, and *Cardium ovale*. The first three species occur in relatively large numbers over the whole of the ground, and all contribute heavily to the total of valves in the deposit. From 1800 gm. of gravel—100 gm. from each of 18 stations—*Astarte triangularis* is the best represented, with 760 dead valves, *Glycymeris glycymeris*, with 600, and *Chione fasciata*, with 400. The Cardium are much less numerous—from the same 18 stations, *Cardium scabrum* showed 66, and *Cardium ovale*, 190 dead valves. Of the non-characteristic forms, *Gafrarium minimum*, *Chione ovata*, and *Echinocyamus pusillus* are by far the most common, with totals of 560, 530, and 330 respectively. The numbers of valves in the gravel do not of course give an absolute measure of the relative abundance of the various species in the gravel over a period of years, since the shells of some will be able to withstand attrition for a longer period than will others, but the overwhelming numbers of valves in the gravel of those species found alive in any numbers, enables us to point out more easily the characteristic species.

Of the Archiannelids and Polychætes, there is no such past record, but it is evident that with the exception of *Polygordius lacteus*, and possibly *Prægeria remota*, none are entirely confined to the shell gravel, some indeed,

such as *Nephthys hombergi* and *Glycera lapidum*, have a wide distribution, both geographical and with relation to the bottom deposit.

The distribution of the species over the whole area is such as to suggest that any one of the three lamellibranchs, *Glycymeris glycymeris*, *Chione fasciata*, and *Astarte triangularis*, associated with *Spatangus purpureus*—which although few in numbers, is characteristic of the ground—is sufficient to characterise the community. The polychætes are less important in this respect, since they are for the most part ubiquitous species, but the presence of *Owenia fusiformis*, together with the substitution of *Ampelisca brevicornis* and *A. tenuicornis* in the finer gravels, for *Ampelisca spinipes* of the coarser deposits, indicates the possible necessity of creating sub-communities of the SpVf association, although at this stage such a division would be unjustified. It is hoped in the near future to work along lines radiating from the Eddystone into the transition areas, in order to determine more precisely the limits of distribution of these and other species.

Environmental Conditions in the Shell Gravel.

Within a very small area where conditions of light, temperature, and salinity are almost constant, the most important limiting environmental conditions are related to

(1) The nature of the bottom, its texture and stability.
(2) The availability of the food supply.

The texture and stability of the gravel bottom has been considered in some detail, and it has been shown that the gravel, lying as it does as a covering over the solid sea floor, does not provide the stable base required by animals which attach themselves at an early stage to the substratum, and remain attached for the period of their adult lives. The abundance of hydroids, sponges, and cirripedes, on the sides of the Eddystone reef, show that it is not the inability of the larvæ to attach themselves, owing to the turbulent water conditions, that limits the distribution of the adults to quieter regions, but rather that the excessive movement of the bottom during stormy periods causes irreparable damage to the adults. The attached epifauna of the gravel is accordingly very scanty, and is practically absent from the Inner Gravel region. More removed from the main centre of disturbance, *Sarcodictyon catenata*, *Alcyonium digitatum* and a few hydroid zoophytes such as *Sertularella gayi* and *Nemertesia antennina* are to be found, but not in any quantity.

The extent of water movement also determines the amount of detritus and fine matter a gravel can hold. Without referring to the burrowing ability of the animals composing the infauna, it is of interest to note the feeding habits of the leading species ; the commonest species are listed in Table 6 below, according to their mode of feeding.

TABLE 6.

Suspension Feeders.	Detritus Feeders.	Carnivores.	Of doubtful feeding habit.
Ampelisca, 3 spp.[1, 2]	*Spatangus purpureus*[2]	*Conilera cylindracea*[1]	
Glycymeris glycymeris[1, 2]	*Echinocyamus pusillus*[2]	*Nephthys hombergi*[1, 2]	*Astarte triangularis*
Gafrarium minimum[2]	*Echinocardium flavescens*[2]	*Glycera lapidum*[1, 2]	*Polygordius lacteus*
Chione fasciata[1, 2]	*Tellina crassa*[1, 2]	*Onuphis conchylega*[2]	*Mystides limbata*
Amphioxus lanceolatus[1]	*Tellina pygmæa*[1, 2]	Hyalinœcia spp.[2]	*Prægeria remota*
		Lumbriconereis impatiens[1, 2]	

Those animals, the stomachs of which have been examined during the course of the work, are marked thus, [1], otherwise the authority is that of Hunt (**9**), indicated thus, [2]. It is interesting to find that the typical gravel forms are suspension feeders, whilst the common detritus feeders and carnivores are species which, with the exception of *Spatangus purpureus*, are represented on other grounds, an observation much to be expected, since one would expect to find suspension feeders more typically on a ground where the bottom is constantly stirred up, than in a deposit where the food supply lies mainly in the microfauna associated with the finer grades of the deposit.

Mortality among the Bivalves, and the Depredations of *Natica alderi*.

Mortality among the shell gravel molluscs, if we are to judge by the frequency of occurrence of the dead valves in the deposit, is heaviest, as might be expected, in the younger stages. Obviously, it would be useless to plot the frequency of the different sizes, and point to the maxima as representing periods of greatest mortality, for it has been shown that in the coarser deposits small valves are washed away to regions of less disturbance. Even so, the maximum is below a size of 4·5 mm. at all positions on the ground. Death is due to a variety of causes, either to starvation, or to being eaten by fish or other carnivores, or perhaps in the younger stages to mechanical injury caused by the shifting bottom. The only cause of which there is any measure, is that due to the gastropod *Natica alderi*. Here, as Davis (**6**) has shown, there is a means of evaluation. Natica bores through one of the valves of a lamellibranch, and after feeding on the soft tissues of the animal, leaves the two valves, which in the course of time separate and are added to the deposit. By counting all the bored valves of a species and pairing off with an equal number of unbored valves, the number of bivalves killed by Natica is obtained. Half the remaining number of single valves represents the number which have met their death in other ways, probably by starvation, since those eaten by fish are crushed and cannot subsequently be identified.

Table 7 gives the numbers of 9 species of lamellibranchs (taken from 26 stations) bored by Natica, and of those which have met their death in other ways. Of the 9 species, the ratio of

$$\frac{\text{molluscs killed by Natica}}{\text{molluscs killed by agencies other than Natica or fish}}$$

is greatest with the little *Astarte triangularis*, and least with *Cardium ovale*. No particular preference of the gastropod for the larger forms is to be seen, and there is no constant size preference within a single species. The mortality is much lower than on the Spisula beds in the North Sea, where Davis (**6**) found on one occasion a mortality as great as 88%, but the conditions on the Eddystone grounds are very different. The concentration of lamellibranchs is very low, and the distribution is probably not so patchy as on the Spisula beds ; consequently, any invasion of Natica would be less disastrous in its results. The depredations are, however, sufficiently far-reaching to be of great importance in the economy of the community.

TABLE 7.

	Size.	Total Bivalves Bored by Natica.	Total Bivalves Unbored.	Percentage Bored.	
Glycymeris glycymeris	>15·0 mm.	0	5	0·0	
	5·0–15·0 mm.	25	156	13·8	21·5
	2·5– 5·0 mm.	171	545	23·9	
	1·5– 2·5 mm.	33	130	20·3	
Astarte triangularis	1·5– 2·5 mm.	248	267	48·2	48·2
Gafrarium minimum	5·0–15·0 mm.	30	120	20·0	
	2·5– 5·0 mm.	81	529	13·3	14·9
	1·5– 2·5 mm.	28	143	16·4	
Chione ovata	5·0–15·0 mm.	21	72	22·6	
	2·5– 5·0 mm.	122	392	23·7	20·9
	1·5– 2·5 mm.	34	205	14·2	
Chione fasciata	>15·0 mm.	0	2	0·0	
	5·0–15·0 mm.	63	62	50·4	32·8
	2·5– 5·0 mm.	143	301	32·2	
	1·5– 2·5 mm.	21	99	17·5	
Cardium ovale	5·0–15·0 mm.	2	2	50·0	
	2·5– 5·0 mm.	20	250	7·4	7·2
	1·5– 2·5 mm.	3	69	4·2	
Cardium scabrum	5·0–15·0 mm.	7	29	19·4	
	2·5– 5·0 mm.	20	73	21·5	20·9
	1·5– 2·5 mm.	5	19	20·8	
Pseudamussium similis	5·0–15·0 mm.	24	37	39·3	
	2·5– 5·0 mm.	88	286	23·5	25·6
	1·5– 2·5 mm.	0	3	0·0	
Nucula spp.	5·0–15·0 mm.	5	22	18·5	
	2·5– 5·0 mm.	15	70	17·6	16·8
	1·5– 2·5 mm.	0	7	0·0	

SUMMARY.

1. The limitations of the Conical Dredge as used in obtaining a sample for quantitative estimation of the fauna of a ground are discussed, and are found to be related to

(*a*) The great distance over which the instrument must be hauled, with consequent inability to detect patchiness of fauna.
(*b*) The inability of the dredge to capture members of the epifauna.

2. The nature of the shell gravel, its position relative to the Eddystone reef, and the various factors conditioning the degree of coarseness of the deposit and the segregation of its elements are considered. The main points of note are,

(*a*) There are three well-defined areas, within which the gravels have their own particular characteristics.

(1) The *Inner Shell Gravel* area and the *Middle Gravel* area, composed of material of inorganic and organic origin, the former being gneissic in character, and the latter being formed of the remains of animals which normally live on this particular type of deposit.

(2) The *Outer Shell Gravel* area, a mixture of matter of local and of outside origin. The area is more extensive on the western than on the eastern side of the reef.

(*b*) Segregation of particles according to size occurs, and is illustrated by the movement of the valves of a number of species of molluscs. Wave action and the action of gravity are considered to be the chief factors in inducing movement of particles, which movement occurs in directions radiating from the reef outwards.

3. The epifauna of the rocky bottom is referred to briefly.

4. The infauna of the shell gravel has been examined quantitatively and qualitatively, and the range of the various species over the ground noted.

5. The nature of the shell-gravel community and its relation to some of the important environmental factors, are discussed.

6. Mortality among the lamellibranchs, and the depredations of the gastropod *Natica alderi* are considered ; figures relating to the numbers of lamellibranchs killed by *N. alderi* are given.

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APPENDIX.

Table I. List of hauls taken during the year 1931, with bearings on, and distance from the Eddystone, of the selected stations. Representative Numbers of gravel samples are given.

Table II. The numbers of the various undamaged shell components of 100 gm. of each of 18 gravel samples are tabulated, and show that

(1) Of the lamellibranchs, *Glycymeris glycymeris*, *Astarte triangularis*, *Gafrarium minimum*, *Chione ovata*, and *Chione fasciata*, are by far the most important as regards numbers.

(2) The samples from the Middle Gravel area contain the greatest number of undamaged valves. The positions of the stations from which the samples referred to in the table are drawn, are given below. Bearings of the Eddystone are given.

Outer Gravel area stations.		Middle Gravel area stations.		Inner Gravel area stations.	
43.	E. 2350 yards.	42.	E. 1725 yards.	28.	S.S.E. 1010 yards.
27.	S.S.E. 2175 ,,	30.	S.E. by S.¼S. 1760 ,,	38.	S.W. 1100 ,,
44.	N.W. 1670 ,,	35.	S.W. 3500 ,,	36.	S.W. 2600 ,,
45.	N.W.¼W. 2000 ,,	34.	W. 2525 ,,	33.	W. 1075 ,,
46.	N.E. 2600 ,,	39.	W.N.W. 1075 ,,	37.	W. 1700 ,,
47.	N.E. 3000 ,,	41.	N.E.¼N. 1850 ,,	40.	N. by W. 1090 ,,

TABLE III. A list of the species taken from the rocky bottom N.–N.W. of the Eddystone, showing the abundance and variety of the attached forms, in marked contrast to the condition on the gravel bottom where the attached epifauna is scarcely represented.

TABLE IV. The range of the species found living in gravel is shown (Nemertini and Polyzoa excluded). Probably the only significant limitations of distribution are,

(1) *Ampelisca spinipes* to gravels of coarse texture, as contrasted with *A. tenuicornis* and *A. brevicornis* in the finer deposits.

(2) *Owenia fusiformis* which, although present in the finer deposits, is absent from the more typical shell-gravel grounds.

The list of quantitative hauls includes 22 hauls from which 1 litre of gravel from the conical dredge has been searched quantitatively, and 2 grab hauls, from which also 1 litre of gravel has been taken.

TABLE I.

List of Hauls.

Date. 1931.	No. of Haul.	Bearing on, and distance from, the Eddystone Light.	Instrument of capture. C.D. = Conical Dredge. D. = Naturalist's Dredge. G = $\frac{1}{10}$ m² grab.	Nature of ground. R. = Rock. G. = Gravel. Rgh. = Rough.	Bottom sample examined for texture. (S.) R.N. given.
Jan. 21st	1	S.E.¼S. 1225 yards.	D.	G.	
	2	S.E.¼S. 1625 ,,	D.	G.	
	3	S.E.¾S. 2000 ,,	D.	G.	
	4	S. 1175 ,,	D.	R.	
	5	S. by W. 1300 ,,	D.	R.	
	6	S. 2100 ,,	D.	R.	
Feb. 2nd	7	S.½E. 2420 ,,	D.	R.	
	8	S.S.W. 1300 ,,	D.	R.	
	9	S.S.W. 1300 ,,	D.	R.	
	10	S.W. 1100 ,,	D.	R.	
	11	S.E. by S. 1700 ,,	D.	G.	
	12	S.E.¼S. 1800 ,,	D.	G.	
	13	S. 680 ,,	D.	R.	
	14	S. 590 ,,	D.	R.	
Feb. 16th	15	S.S.E. 2600 ,,	D.	G.	
	16	S.S.E. 1965 ,,	D.	G.	
	17	S.S.E. 1760 ,,	D.	G.	
	18	S.S.E. 1325 ,,	D.	G.	
May 20th	19	S.S.E. 820 ,,	C.D.	G.	S. 3·19
	19(a	S.S.E. 640 ,,	C.D.	R.	
	20	S.E.¾S. 2150 ,,	C.D.	G.	S. 1·43
	21	S.E. by S. 1320 ,,	G.	G.	
	22	S.E. by S. 1720 ,,	C.D.	G.	S. 1·78
	23	S.E.½S. 1920 ,,	G.	G.	
May 1st	24	S.E.½S. 1260 ,,	C.D.	G.	S. 2·33
	25	S.E.¼S. 1030 ,,	C.D.	G.	S. 2·70
	26	S.E.½S. 1500 ,,	C.D.	G.	S. 1·96
July 23rd	27	S.S.E. 2175 ,,	C.D.	G.	S. 1·48
	27(a)	S.S.E. 2025 ,,	C.D.	Rgh.	
	28	S.S.E. 1010 ,,	C.D.	G.	S. 3·09
Sept. 3rd	29	S.S.E. 2000 ,,	C.D.	G.	S. 1·58
Sept. 10th	30	S.E. by S.¼S. 1760 ,,	C.D.	G.	S. 1·99
Oct. 1st	33	W. 1075 ,,	C.D.	G.	S. 2·07
	34	W. 2525 ,,	C.D.	G.	S. 0·35
Oct. 15th	35	S.W. 3500 ,,	C.D.	G.	S. 0·72
	36	S.W. 2600 ,,	C.D.	G.	S. 1·27
	37	W. 1700 ,,	C.D.	G.	S. 1·55
Oct. 29th	38	S.W. 1100 ,,	C.D.	G.	S. 3·14
	38(a)	S.W. 850 ,,	C.D.	R.	
	39	W.N.W. 1075 ,,	C.D.	G.	S. 2·42
	40	N. by W. 1090 ,,	C.D.	G.	S. 2·71
	40(a)	N.E. by N.½N. 1175 ,,	C.D.	R.	
Dec. 9th	41	N.E.¼N. 1850 ,,	C.D.	G.	S. 1·71
	41(a)	N.E.¼N. 1090 ,,	C.D.	R.	
	41(b)	N.E. 2800 ,,	C.D.	Rgh.	
Dec. 11th	42	E. 1725 ,,	C.D.	G.	S. 1·98
	42(a)	E. 1000 ,,	C.D.	R.	
	42(b)	E. 1525 ,,	C.D.	R.	
	43	E. 2350 ,,	C.D.	G.	S. 2·31
	44	N.W. 1670 ,,	C.D.	G.	S. 1·96
	45	N.W.¼W. 2000 ,,	C.D.	G.	S. 1·75
	46	N.E. 2600 ,,	C.D.	G.	S. 1·64
	47	N.E. 3000 ,,	C.D.	G.	S. 2·24

TABLE II.

NUMBERS OF MOLLUSC VALVES AND ECHINOCYAMUS TESTS (Per 100 gm. of gravel).

GRAVEL NUMBER (See Table I for positions).

	42	43	28	30	27	38	36	35	33	37	34	40	39	44	45	41	46	47
Nucula spp.	1		1	1	2	3	5	2	2	2	1	3	2	5	1	10	1	
Anomia spp.		1				4	17	5	20	6	3	4	3	4	6	26	1	1
Monia sp.																		
Arca tetragona	1			1		1						1		5	8	13		
Arca lactea	13	12	4	15	1	4	6	5	5	7		7	2	20	18	56		
Glycymeris glycymeris	75	21	21	75	8	8	16	17	6	13	4	12	1	87	93	152		
Modiolus phaseolinus						4	4	1	5	6	2	3	4	5	3	11	1	
Pecten spp.	1	5	2	2			5	5	2	1	1	2	1	2	6	10	10	4
Pseudamussium similis	3	4	3	12	10		23	22	1	1	9	2	1	13	17	67	1	
Lima subauriculata	1	1					3	1	1		3	1	2			1		
Astarte triangularis	15	3	20	65	9	10	25	45	17	33	21	9	26	68	97	296		1
Cyprina islandica		2														3		
Myrtea spinifera																	1	
Kellia spp.	2	3					2	1				1	1	2	2			
Tellina crassa																		
Tellina donacina											2							
Tellina pygmæa	1						2	2		1	13		1		4	7		
Abra spp.	1						2		1		2					3		
Gari tellinella							2				1				1			
Mactra elliptica					1		1	2			6		2		1	3	1	1
Dosinia spp.								1										
Gafrarium minimum	33	30	20	52	9	8	32	25	18	15	9	15	9	84	68	135	2	1
Venus casina				1					1		1							
Chione ovata	24	33	6	42	26	4	60	52	17	8	15	8	9	37	52	118	13	9
Chione fasciata	40	19	6	53	8	4	15	15	3	7	4	5	5	49	67	97	1	3
Paphia rhomboides	2	2		1	1		4		1	1		1		4	4	3	2	1
Cardium scabrum	4	2	1	4	3	1	7	9	2	1	3			5	10	14		
Cardium ovale			11	1		4	28	14	20	10	7	12	7	10	11	52		
Hiatella arctica	3					5	13	2	9	6	1	5	4	2	3	25		
Gastropoda	85	22	63	38	4	75	78	11	150	89	1	70	38	100	93	142	5	2
Echinocyamus pusillus	16	11	12	27	3	3	11	11	12	7	2	10	9	40	41	113	1	
Others		4					1			1	2		1	4		7	1	1
Total (excluding gastropods)	236	153	107	352	81	62	284	237	143	126	112	101	90	446	513	1222	36	22

TABLE III.

List of Species taken on the Sides of the Eddystone Reef, to the N.W. of the Stone.

PORIFERA.

Sycon coronatum
Tethya aurantium
Cliona celata
Polymastia mammillaris
Pseudaxinella alleni
Vibulinus stuposus
Myxilla incrustans
Raspailia hispida
Raspailia ramosa
Desmacidon fruticosa
Eurypon clavatum

HYDROZOA.

Clytea johnstoni
Lafœa dumosa
Lafœa fruticosa
Diphasia attenuata
Diphasia pinaster
Diphasia pinnata
Diphasia tamarisca
Sertularella gayi
Sertularella polyzonias
Abietinaria abietina
Sertularia cupressina
Hydrallmania falcata
Thujaria articulata
Kirchenpaueria pinnata
Plumularia setacea
Plumularia catharina
Nemertesia antennina
Thecocarpus myriophyllium
Aglaophenia tubulifera

ANTHOZOA.

Alcyonium digitatum
Eunicella verrucosa
Epizoanthus sp.
Gephyropsis dohrni
Caryophyllia smithi

POLYCHÆTA.

Lepidonotus squamatus
Lagisca extenuata
Scalisetosus assimilis
Typosyllis armillaris
Eunice harassi
Sabella pavonina
Pomatoceros triqueter
Serpula vermicularis

POLYZOA.

Scrupocellaria scruposa
Bicellaria ciliata
Bugula flabellata
Membranipora rosselli
Cellaria fistulosa
Cribrilina figularis
Microporella ciliata
Microporella violacea
Lepralia foliacea
Lepralia pertusa
Chorizopora brongniarti
Smittia trispinosa
Schizoporella linearis
Cellepora pumicosa
Cellepora ramulosa
Crisea cornuta
Crisea denticulata
Crisea eburnea
Diastopora patina
Stomatopora johnstoni
Lichenopora hispida
Domopora truncata
Idmonea serpens

CRUSTACEA.

Scalpellum scalpellum
Verruca strœmia
Balanus spongicola
Balanus crenatus
Pyrgoma anglicum
Leucothoë spinicarpa
Galathea strigosa
Porcellana longicornis
Pilumnus hirtellus
Pinnotheres pisum
Pinnotheres ? veterum
Eurynome aspera

MOLLUSCA.

Lepidopleurus asellus
Anomia ephippium
Monia patelliformis
Arca tetragona
Modiolus barbatus
Modiolus phaseolinus
Kellia suborbicularis
Hiatella arctica
Emarginula fissura
Calliostoma papillosum
Simnia patula
Erato lævis

ECHINODERMATA.

Antedon bifida
Luidia sarsi
Luidia ciliaris
Porania pulvillus
Marthasterias glacialis

Ophiothrix fragilis
Ophiactis balli
Psammechinus miliaris
Echinus esculentus
Holothuria forskali
Cucumaria lactea

TUNICATA.
Polycarpa pomaria
Polycarpa fibrosa
Ascidia mentula
Phallusia mammillata
Trididemnum tenerum

TABLE IV.

List of Species Found on, or in, the Shell Gravel, Polyzoa and Nemertini Excluded.

× denotes presence in one or more samples. * denotes forms taken once only.

	Representative Number of gravel.			
	>3·0	3·0–2·0	2·0–1·0	<1·0
HYDROZOA				
Sertularella gayi		×	×	
Abietinaria abietina			×	
*Plumularia setacea			×	
Nemertesia antennina			×	
ANTHOZOA.				
Sarcodictyon catenata		×	×	
*Alcyonium digitatum			×	
Epizoanthus incrustatus			×	×
Ilyanthus mitchelli		×	×	
*Caryophyllia smithi			×	
POLYCHÆTA.				
Polygordius lacteus	×	×	×	×
*Eunoë nodosa		×		
*Lagisca extenuata			×	
*Sthenelais boa			×	
Mystides limbata	×	×	×	
Syllis spp.	×	×	×	
Nephthys hombergi		×	×	×
Prægeria remota		×	×	×
Glycera lapidum	×	×	×	×
Glycera gigantea		×	×	
Glycera sp.	×	×	×	×
*Eunice harassi		×		
Onuphis conchylega	×		×	
Hyalinœcia tubicola	×		×	
Hyalinœcia bilineata	×	×	×	
Lumbriconereis impatiens	×	×	×	
*Aricia sp.			×	
*Polydora sp.				×
Owenia fusiformis				×
*Petta pusilla			×	
Lanice conchylega			×	×
Terebellides strœmi				×
*Serpula vermicularis			×	
*Hydroides norvegica			×	
*Pomatoceros triqueter			×	
Maldanids	×	×	×	×
GEPHYREA.				
*Phascolosoma vulgare	×			
CRUSTACEA.				
*Pyrgoma anglicum			×	
*Sacculina sp.			×	
*Nannastacus sp.				×
Conilera cylindracea		×	×	
*Lyssianassidæ			×	
Ampelisca brevicornis			×	×
Ampelisca tenuicornis				×
Ampelisca spinipes	×	×	×	
Urothoë marina			×	×
*Leucothoë spinicarpa			×	
*Ceradocus semiserratus		×		
Mœra othonis		×	×	
Lembos longipes	×			×
*Leptocheirus hirsutimanus				×
*Megamphopus cornutus	×			
*Phtisica marina			×	
*Crangon allmani			×	
Galathea dispersa		×	×	
*Galathea strigosa			×	

	Representative Number of gravel.			
	>3·0	3·0–2·0	2·0–1·0	<1·0
Porcellana longicornis			×	
Upogebia deltaura		×	×	
*Eupagurus cuanensis			×	
*Anapagurus lævis			×	
Portunus pusillus			×	
Portunus depurator		×	×	
*Atelecyclus septemdentatus			×	
Ebalia tuberosa			×	
Ebalia tumefacta		×	×	
MOLLUSCA.				
Nucula nucleus		×	×	×
Nucula radiata		×	×	×
Glycymeris glycymeris	×	×	×	
Modiolus phaseolinus			×	×
Chlamys varia		×	×	
*Chlamys tigerina			×	
Pseudamussium similis			×	
*Lima subauriculata		×		
Astarte sulcata		×	×	
Astarte triangularis	×	×	×	
*Kellia suborbicularis	×			
Tellina crassa	×	×	×	
Tellina donacina		×	×	×
Tellina pygmæa			×	×
*Abra prismatica				×
*Abra alba				×
Gari tellinella	×		×	
*Gari costulata	×			
Gafrarium minimum	×	×	×	
Venus casina	×	×	×	×
Chione ovata	×	×	×	×
Chione fasciata	×	×	×	
Paphia rhomboides	×	×	×	
Cardium scabrum	×	×	×	
Cardium ovale	×	×	×	
*Lævicardium crassum			×	
Psammsolen candidus		×		
*Emarginula fissura			×	
*Mangelia linearis			×	
*Calliostoma papillosum			×	
Trivia europœa		×		
Natica alderi			×	×
*Turritella communis		×		
*Scaphander lignarius			×	
*Doto fragilis			×	
ECHINODERMATA.				
*Astropecten irregularis			×	
*Luidia ciliaris			×	
Ophiothrix fragilis		×	×	
Ophiura texturata		×	×	
Opiura affinis		×	×	
Echinocyamus pusillus	×	×	×	×
Spatangus purpureus	×	×	×	×
Echinocardium flavescens			×	
Cucumaria hyndmani	×	×	×	
TUNICATA.				
*Eugyra arenosa			×	
*Polycarpa fibrosa				× 1
CEPHALOCHORDA.				
Amphioxus lanceolatus	×	×	×	×

STATIONS NORTH-EAST OF EDDYSTONE.

No. 38. 1100 yards N.E. Oct. 29th, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	4
Astarte triangularis	16
Tellina crassa	1
Gafrarium minimum	1
Chione ovata	1
Echinocyamus pusillus	7
Amphipod (indet.)	1
Polygordius sp.	1
Syllid sp.	1
Mystides limbata	1
Glycera lapidum	2
Amphioxus lanceolatus	1

No. 36. 2600 yards N.E. Oct. 15th, 1931. Per 1000 c.c. of gravel.

Nucula radiata	3
Glycymeris glycymeris	1
Astarte triangularis	2
Tellina crassa	1
Tellina pygmæa	1
Venus casina	1
Chione fasciata	1
Echinocyamus pusillus	2
Ampelisca brevicornis	
Polygordius sp.	2
Lanice conchylega	1
Terebellid tube	1

No. 35. 3500 yards N.E. Oct. 15th, 1931. Per 1000 c.c. of gravel.

Nucula radiata	1
Modiolus phaseolinus	1
Tellina pygmæa	1
Chione ovata	1
Ampelisca brevicornis	1
Urothoë marina	2
Glycera lapidum	1
Lanice conchylega	1
Terebellides stroemi	1
Owenia tubes	5

STATIONS EAST OF EDDYSTONE.

No. 33. 1075 yards E. Oct. 1st, 1931. Per 1000 c.c. of gravel.

Lima subauriculata	1
Astarte triangularis	7
Tellina pygmæa	2
Chione fasciata	3
Paphia rhomboides	1
Echinocyamus pusillus	8
Polygordius sp.	1
Syllis sp.	1
Glycera lapidum	3
Mystides limbata	1
Sarcodictyon catenata	2 pieces

No. 37. 1700 yards E. Oct. 15th, 1931. Per 1000 c.c. of gravel.

Nucula radiata	1
Glycymeris glycymeris	1
Astarte triangularis	3
Gafrarium minimum	1
Chione fasciata	1
Echinocyamus pusillus	6
Urothoe marina	1
Syllis sp.	1
Glycera lapidum	6
Mystides limbata	1
Hyalinœcia sp.	1
Aricia sp.	2
Amphioxus lanceolatus	3

No. 34. 2525 yards E. Oct. 1st, 1931 Per 1000 c.c. of gravel.

Astarte triangularis	2
Tellina pygmæa	3
Abra abra	1
Venus casina	1
Natica alderi	1
Echinocyamus pusillus	7
Ampelisca brevicornis	2
Ampelisca tenuicornis	1
Polygordius sp.	5
Glycera lapidum	1
Prægeria remota	5
Terebellid sp.	1
Owenia fusiformis	1
Maldanids	2
Owenia tubes	2
Amphioxus lanceolatus	

STATIONS IN THE SOUTH-EAST QUARTER OF THE EDDYSTONE.

No. 39. 1075 yards E.S.E. Oct. 29th, 1931. Per 1000 c.c. of gravel.

Echinocyamus pusillus	1
Ampelisca spinipes	1
Polygordius sp.	1
Glycera lapidum	3
Hyalinœcia sp.	1
Eunoë nodosa	1
Amphioxus lanceolatus	2

No. 40. 1090 yards S. by E. Oct. 29th, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	3
Chione fasciata	1
Echinocyamus pusillus	10
Nephthys sp. (juv.)	1
Maldanid	1

No. 44. 1670 yards S.E. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	1
Astarte triangularis	4
Chione ovata	1
Anapagurus lævis	1
Galathea sp. (juv.)	1
Polygordius sp.	1
Syllids	2
Glycera lapidum	2
Prægeria remota	1
Maldanid	1
Nemertini	1
Micrura sp.	2

No. 45. 2000 yards S.E.¼S. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	7
Tellina pygmæa	1
Gafrarium minimum	1
Chione ovata	1
Chione fasciata	2
Echinocyamus pusillus	14
Ampelisca spinipes	1
Ebalia sp. (juv.)	1
Syllids	2
Phyllodocids	1
Glycera lapidum	2
Lumbriconereis impatiens	1
Amphioxus lanceolatus	

STATIONS SOUTH-WEST OF EDDYSTONE

No. 41. 1850 yards S.W ¼S. Dec. 9th, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	11
Nucula sp.	1
Gafrarium minimum	2
Chione ovata	2
Chione fasciata	2
Cardium ovale	2
Echinocyamus pusillus	3
Ophiura ? affinis (juv.)	2
Polygordius sp.	5
Syllis vittata	1
Syllids	2
Phyllodocids (juv.)	2
Lumbriconereis sp.	1
Glycera lapidum	5
Prægeria remota	15
Polychæta indet.	1
Amphioxus lanceolatus	2

No. 46. 2600 yards S W. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Nucula sp.	1
Echinocyamus pusillus	4
Spirontocaris cranchi	1
Polygordius sp.	1
Glycera sp.	1
Glycera lapidum	?
Phyllodocids	1
Owenia tubes	3

No. 47. 3000 yards S.W. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Paphia rhomboides	1
Echinocyamus pusillus	5
Conilera cylindracea	1
Cheirocratus sundevalli	1
Polygordius sp.	1
Polynoid	1
Lumbriconereis impatiens	5
Glycera lapidum	1
Nephthys sp.	1
Hyalinœcia bilineata	1
Arenicola sp.	1
Sabellids (juv.)	3
Pomatoceros triqueter tubes	many
Maldanids	2
Owenia tubes	2
Nemertesia antennina	1 colony

STATIONS WEST OF EDDYSTONE.

No. 42 1725 yards W. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Nucula sp.	2
Glycymeris glycymeris	2
Astarte triangularis	7
Tellina pygmæa	1
Gafrarium minimum	2
Chione ovata	1
Echinocyamus pusillus	17
Ophiuroid sp.	1
Ebalia sp. (juv.)	1
Megamphopus cornatus	2
Lembos longipes	1
Polygordius sp.	3
Syllis sp.	2
Glycera sp.	1
Glycera lapidum	2
Lumbriconereis impatiens	2
Lumbriconereis sp.	1
Mystides limbata	1
Sabellid sp. (juv.)	1
Maldanids	6
Micrura sp.	2
Micrura sp. (juv.)	many

No. 43. 2350 yards W. Dec. 11th, 1931. Per 1000 c.c. of gravel.

Chione fasciata	1
Gnathia maxillaris	1
Megamphopus cornutus	1
Polynoid	1
Syllids	2
Phyllodocids (juv.)	3
Glycera lapidum	4
Lumbriconereis impatiens	1
Mystides limbata	1
Ephesia gracilis	1
Maldanids	4
Terebellid tubes	2
Owenia tubes	1
Lepralia foliacea	pieces
Epizoanthus incrustatus	4 colonies

STATIONS NORTH-WEST OF EDDYSTONE

$\frac{1}{10}$m² Grab samples

No. 21. 1320 yards N.W. by N. May 20th, 1931. Per 1000 c.c. of gravel.

Nucula radiata	0·5
Glycymeris glycymeris	2·0
Astarte triangularis	0·5
Astarte sulcata	0·5
Gafrarium minimum	2·0
Venus casina	1·0
Chione ovata	0·5
Chione fasciata	1·5
Ophiuroid (juv.)	0·5
Echinocyamus pusillus	17·5
Mæra othonis	0·5
Ceradocus semiserratus	0·5
Syllis cornuta	0·5
Eusyllis sp.	0·5
Glycera lapidum	0·5
Prægeria remota	3·0
Nemertini	1·5
Sarcodictyon catenata	1 colony

No. 23. 1920 yards N.W.¼N. May 20th, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	1
Astarte sulcata	1
Tellina pygmæa	2
Pseudamussium similis	1
Chione ovata	2
Echinocyamus pusillus	33
Prægeria remota	2
Syllids	1
Onuphis conchylega	2
Onuphis tube	1

STATIONS IN THE NORTH-WEST QUARTER OF EDDYSTONE.

No. 19. 820 yards N.N.W. May 20th, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	3
Astarte triangularis	1
Gari tellinella	1
Gari costulata	1
Gafrarium minimum	5
Venus casina	1
Chione ovata	1
Cardium ovale	1
Paphia rhomboides	1
Echinocyamus pusillus	19
Ampelisca spinipes	1
Polygordius sp.	fragments
Lumbriconereis impatiens	3
Onuphis conchylega	1
Glycera sp.	1
Micrura sp.	1
Phascolosoma vulgare with Loxosoma phascolosomatum	

No. 28. 1010 yards N.N.W. July 23rd, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	2
Astarte triangularis	8
Gafrarium minimum	1
Chione fasciata	1
Echinocyamus pusillus	5
Spatangus purpureus	1
Cucumaria sp. (juv.)	1
Glycera sp.	1
Hyalinœcia sp.	1

No. 22. 1720 yards N.W. by N. May 20th, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	1
Astarte triangularis	2
Astarte sulcata (juv,)	2
Gafrarium minimum	1
Chione ovata	1
Cardium scabrum	1
Echinocyamus pusillus	10
Ophiura sp. (juv.)	2
Ampelisca spinipes	1
Lyssianassa sp.	1
Syllis cornuta	1
Goniada sp.	1
Micrura sp.	.

STATIONS IN THE NORTH-WEST QUARTER OF EDDYSTONE.

No. 30. 1760 yards N.W. by N.¼N. Sept. 10th, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	1
Astarte triangularis	8
Gafrarium minimum	1
Gari tellinella	1
Echinocyamus pusillus	1
Polygordius sp.	several portions
Glycera lapidum	2
Glycera sp.	1
Syllid	1
Sarcodictyon catenata	1 piece

No. 29. 2000 yards N.N.W. Sept. 3rd, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	4
Chione fasciata	3
Lævicardium crassum	1
Echinocyamus pusillus	5
Echinocardium flavescens	1
Ampelisca spinipes	1
Polygordius sp.	fragments
Glycera lapidum	3

No. 20. 2150 yards N.W.¼N. May 20th, 1931. Per 1000 c.c. of gravel.

Astarte triangularis	1
Astarte sulcata (juv.)	2
Tellina donacina	1
Gafrarium minimum	4
Venus casina	1
Chione ovata	1
Chione fasciata	1
Cardium ovale	2
Echinocyamus pusillus	4
Ophiuroid sp. (juv.)	1
Mæra sp.	1
Urothoë sp.	1
Ampelisca spinipes	1
Glycera lapidum	1
Onuphis conchylega	1
Hyalinœcia bilineata	1
Nemertesia antennina with Doris spawn	

No. 27. 2175 yards N.N.W. July 23rd, 1931. Per 1000 c.c. of gravel.

Glycymeris glycymeris	1
Pseudamussium similis	1
Astarte triangularis	2
Gafrarium minimum	1
Chione ovata	1
Venus casina	1
Natica alderi	1
Echinocyamus pusillus	3
Ophiothrix fragilis (juv.)	2
Conilera cylindracea	1
Ampelisca spinipes	1
Eupagurus cuanensis	1
Portunus pusillus	1
Prægeria remota	1
Onuphis conchylega	2
Glycera lapidum	2
Lumbriconereis impatiens	1
Syllid	1
Maldanid	1
Hyalinœcia tubes	4
Onuphis tubes	2
Nemertini	3

A Quantitative Study of the Fauna of the Sandy Beach at Port Erin.

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With 8 Figures in the Text.

Contents

Introductory.

In previous papers from this Station, one of us (Bruce, **2** and **3**) has attempted to define certain of the physical and chemical influences which affect the animal and plant life of the sandy beach. The present paper embodies the results of an intensive faunistic survey of the sandy beach at Port Erin, Isle of Man, carried out in September, 1931, and seeks to correlate the observed distribution of the macro-fauna with the range of physical conditions already established and amplified in the course of the present work.

Such a study enables a comparison to be made with the results of similarly conceived surveys on Scottish shores recently carried out by Stephen (**7**, **8**) and Elmhirst (**5**). Marked differences of faunal distribution are evident, as between the various localities. Many of the Scottish beaches examined by Stephen were comparatively sheltered, and harboured a very large molluscan population. The beach at Port Erin is subject to much disturbance by westerly storms, and is, on the whole, of coarser grade—in consonance with these conditions, molluscs are practically absent, while polychætes and crustaceans compare favourably in density of population, but not in number of species, with most of the Scottish stations examined.

Description of the Area.

Port Erin Bay, near the south-west extremity of the Isle of Man, is more or less rectangular in outline, about half a mile wide, and open to the west. It is sheltered, on the north, by the bold cliffs and slopes of Bradda Head, which rise steeply to a height of 350 feet. On the east is the lower ground on which the village of Port Erin is built, and this terminates on the shore in steep bluffs or " brooghs " of glacial drift. From High-Water Mark at the foot of the brooghs, a wide stretch of smooth sand extends seawards for at least 500 feet at Low-Water Springs, and this constitutes the immediate area (Fig. 1, p. 283) of our survey. On the south of the bay are low cliffs, composed, like Bradda Head, of " Barrule Slates " of supposedly Cambrian age.

Although the bay is open to the west, some measure of protection is afforded on that side by a ruined breakwater, but this is covered at High-Water Neaps, and in any case extends only across the southern half of the opening. East of the breakwater, and at the south end of the sandy beach, a short half-tide pier, and a length of quay-wall, both directed to the north-east, give rise to a small protected area of beach with the usual harbour characteristics of finer grade, rich accumulation of detritus, and blackening below the surface. Apart from this area, the sand of the beach is clean, and of moderately fine grade. In general, there is a definite increase in the proportion of coarse material, up to 2 mm. diameter, towards H.W.M., and more particularly towards the north end of the beach, where denudation has almost entirely removed the finer materials, leaving an area of coarse gravel with pebbles (Fig. 3, p. 290). The observed distribution of the beach-deposits is due, in part to the flood-current which enters the bay from the north-west, and in part to the harbour-works, which arrest the materials carried by the clockwise eddy in the bay. The slope of the beach, in the southern and middle portion, is fairly uniform, about 1 in 40, but in the northern part (lines D and E on *Plan*,

Fig. 1) the section is markedly concave, flattened below Mean Sea Level, and steep above it. There are, of course, local depressions and banks which modify the general section, and the whole contour is liable to alteration as a result of winter storms.

The beach is traversed by several streamlets of fresh water, as shown in Figure 1, but the extent and influence of this factor is discussed under "salinity," p. 284, and on p. 292.

Stations.

For the purpose of sampling the fauna, and determining the physical character of the ground, in as representative a manner as possible, five lines, or "transects," were set out, more or less radial to the curve of the beach, and upon each line five observation-stations were plotted, at successive tidal levels, from Low-Water Equinoctial Springs to High-Water Neaps (Fig. 1). In addition to these twenty-four stations (one being common to two lines), two others, designated "I.H." and "O.H." on the plan, are representative of the conditions in the inner and outer harbour respectively, while a third, "R," is in an area partly sheltered by outcropping rocks at the extreme north end of the sandy beach.

The positions of all stations were determined by direct measurement or by prismatic compass-bearings, and accurately laid down on the 25-in. Ordnance Sheet, Isle of Man, XV, 12. Accurate levels were taken at all stations, and referred to the nearest Ordnance Bench Mark (Table 1). The interpolation of tidal contours (Fig. 1) between the stations was facilitated, in some cases, by actually plotting the position of the water's edge.

TABLE 1.

Levels at Stations on Port Erin Beach.

(Heights in feet above Ordnance Datum.*)

Station	A	B	C	D	E
1.	−9·00		−9·00	−9·00	−9·00
2.	−4·78	−6·61	−6·63	−7·50	−7·10
3.	−0·04	−1·92	−0·82	−2·64	−2·98
4.	2·96	3·47	2·70	3·05	1·82
5.	6·60	6·54	7·08	7·06	4·87

I.H. 2·06; O.H. −5·80; R. −2·36

Collection of Samples.

The area covered by each sample—a quarter of a square metre—is that commonly accepted in ecological work, and used by Stephen and Elmhirst (*loc. cit.*) in their surveys of Scottish beaches.

A wire frame, 50×50 cm., was used to define the area, and the sand, dug out as quickly as possible to within a short distance of the frame,

* Ordnance Datum in the Isle of Man is Mean Sea Level at Douglas.

and to a depth of about 20 cm., was transferred to a large galvanised bath-tub. At almost every station, two such samples were dug, and in a few important areas, three. The contents of the bath-tub were sieved through a 2-mm. sieve with circular holes, which was plunged up and down in the sea, the material retained by the sieve being set aside for later examination.

Various contingencies, such as flooding, or collapse of the hole, combined to render precision impossible, but after a little experience, allowance could be made for these, so that a fair uniformity of sampling was attained.

In addition to the large sample, a small tin, holding about 1 litre, was filled and closed with an airtight lid, for chemical tests and mechanical analysis of the finer material. This sample was taken at about the mid-depth of the hole.

METHODS AND RESULTS.

A. Chemical and Physical.

The chemical tests were carried out as soon as possible—sometimes within an hour, always within two hours—after the collection of the sample.

Hydrogen-ion concentration. The sand had usually settled sufficiently in its tightly sealed tin to permit of the necessary 10 c.c. being pipetted off. Where this was not possible owing to the dryness of the sample, the sand was transferred to a Buchner funnel, and sufficient water removed by slight suction. It was shown that, provided the suction was slight and not long-continued, no appreciable change in pH resulted from this treatment, owing doubtless to the high excess-base content and buffer-capacity of the interstitial water. Cresol red and m-Cresol purple were found to cover the range of pH observed ; comparison was made with a series of Clark and Lubs standard buffer-mixtures, in a suitably screened rack, and a correction for salt-error, varying with the salinity of the sample, was applied to the reading, the result being stated to the nearest 0·05 pH (Table 2).

TABLE 2.

pH of the Interstitial Water on the Sandy Beach at Port Erin—September.

(Values are corrected for salt-error.)

Station	A	B	C	D	E
1.	7·85		—	7·60	7·80
2.	7·95	7·95	7·95	7·95	7·95
3.	7·85	7·95	7·85	7·80	7·90
4.	7·95	7·95	7·90	7·85	7·80
5.	—	7·95	(dry)	(dry)	(dry)

I.H. 8·20 ; O.H. 8·25 ; R. 7·80

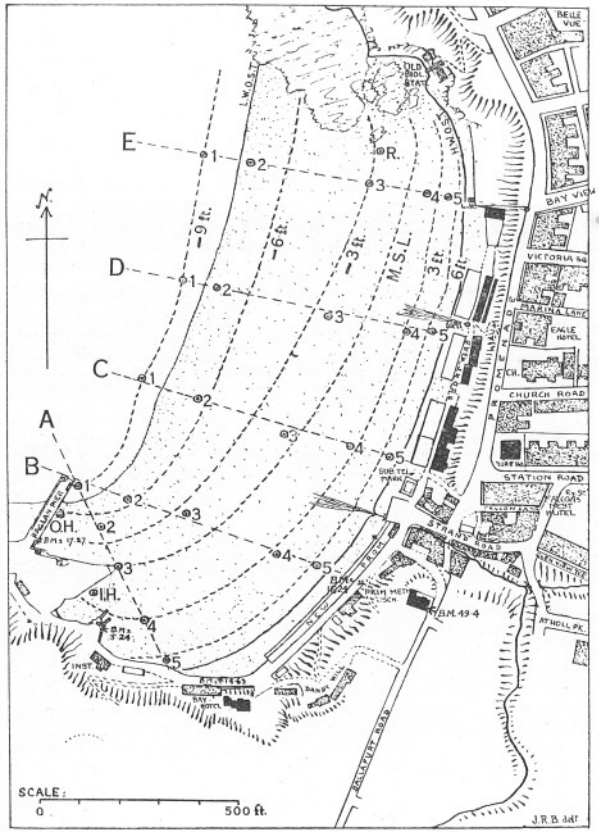

FIG. 1.—Plan of Port Erin Beach, showing Tidal Contours and Sampling Stations.

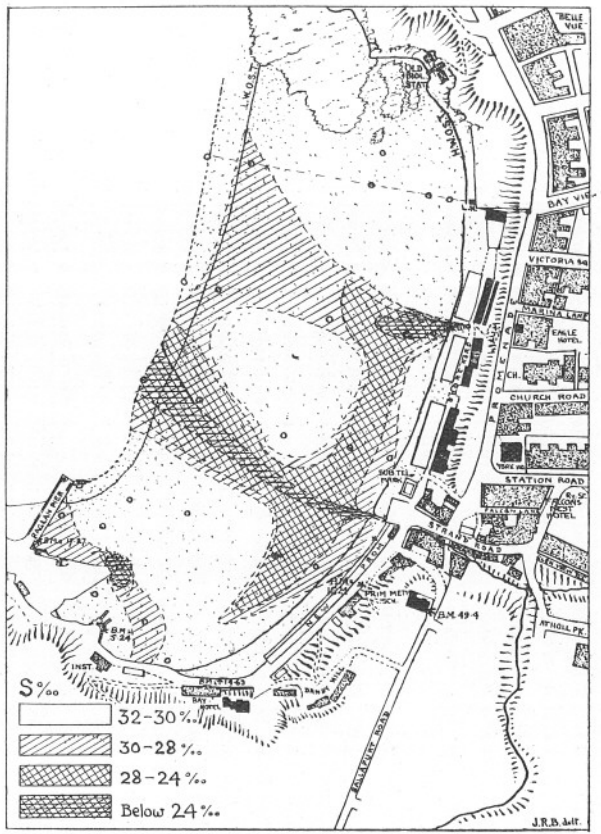

FIG. 2.—Isohalines of the Interstitial Water of the upper 20 cm. of the Beach.

It will be seen that the variations of pH, as between the different stations, are quite small, and that there does not appear to be any significant relation with situation or other factors, except at Stations I.H. and O.H., where high values of pH are associated with fine grade, and the presence of sulphides, as attested by the blackness of the deposit. This association has been noted previously, at these Stations (Bruce, **3**).

Salinity. The specific gravity of the water drained or filtered from the sand-sample, was determined by means of a small hydrometer, reading to 0·5 (water=1000). In view of the considerable sampling error, due to contamination of the sample with either sea-water or fresh drainage-water from the surface of the beach, it was felt to be unjustifiable to aim at a higher degree of accuracy than this. Using the hydrometer-reading, and the temperature of the water in the hydrometer-jar, the corresponding salinity-value (grammes total salts per 1000 grammes sea-water) was read from " Knudsen's Tables." The salinity of the sea, during the period (14th–23rd September, 1931) in which the beach observations were made, varied between 33·0°/$_{oo}$ and 33·4°/$_{oo}$, with a mean semi-diurnal value of 33·16°/$_{oo}$ (Table 3). The results are of much interest, especially when, as in Figure 2, they are expressed in the form of salinity-contours, or " isohalines." In this figure, the data of Table 3 are coupled with observations on the ground as to the actual direction of the streams, etc., and the result conveys a fairly complete picture of the salinity conditions in the interstitial water of the upper 20 cm. of the beach. The influence of the fresh-water streams, spreading fanwise towards L.W.M., was, of course, to be

TABLE 3.

SALINITY OF THE INTERSTITIAL WATER ON THE SANDY BEACH AT PORT ERIN—SEPTEMBER.

(S=grammes total salts per 1000 grammes sea-water.)

Station	A	B	C	D	E
1.	31·7		23·2	30·8	30·8
2.	31·5	31·0	25·8	29·1	28·4
3.	23·2	31·0	30·4	29·6	30·9
4.	28·0	24·0	26·7	2·7	30·6
5.	30·6	29·3	(dry)	(dry)	(dry)

I.H. 30·8; O.H. 27·9; R. 32·2

expected, but the southerly extension of the low salinity areas above half-tide level, and the generally low salinity in the harbour area, introduce important factors into those areas of the beach, which are discussed, in their biological significance, on p. 292.

Organic Matter. An attempt was made to determine the organic-matter content of the sands by various methods, including, of course, the standard procedure of "loss on ignition." Unsatisfactory results were obtained, due, in large measure, to the high calcium carbonate content of the material. Other methods, involving wet oxidation, were also tried, but found to be vitiated by the chloride which is always present in beach material. Under the circumstances, discussion of this important factor is deferred until a reliable method, now being sought, is available.

Mechanical Analysis. The material available from each Station for mechanical analysis consisted of

(*a*) the large amount of stones and gravel retained by the 2-mm. sieve, after the animals had been removed in the course of the biological examination of the material, and

(*b*) the much smaller amount, about 1 kg., of unsieved and unsorted material, forming the residue from which the interstitial water, for chemical and other tests, had been filtered.

The proportions of coarse material in the several grades, 2–4 mm., 4–6 mm., and above 6 mm., were determined by sieving the entire mass of the larger sample (*a*) through a series of round-holed sieves, which were rotated in water, the amount retained by each being weighed wet, after draining and shaking out as much water as possible. By actual determination, the percentages of the several grades so obtained differed by less than one per cent from the values obtained by the usual but laborious procedure of weighing the original material dry, sieving in water, and again drying and weighing each fraction. The percentage of all material above 2 mm. in the entire mass of sand and stones, at each station, was calculated on the assumed weight of the sample dug as 72·5 kg.—that being the weight of a check sample taken under typical conditions. The mechanical analysis of the finer grades, up to 2 mm., was carried out on the smaller sample (*b*). This involved both sieving and elutriation. About 25 g. of the undried material was sieved, in water, through the following series of round-holed sieves—2 mm., 1 mm., 0·5 mm. Material retained by the 2-mm. sieve was rejected, as already included in the coarse-grade fractions above. The fractions retained by the 1-mm. and 0·5-mm. sieves were transferred to tared filter-papers, dried, and weighed. The fraction passing 0·5 mm. was transferred, in the wet state, to the separating vessel of a single tube elutriator, which was worked with fresh water, at 14°–15° C.

At first, a current of 6·7 mm./sec. was applied; this carried over all material up to 0·1 mm. diam., but the amount of this grade was negligibly small in every case, and it was disregarded in the subsequent calculations.

The rate of flow was then increased to 25 mm./sec., when the grade 0·1–0·25 mm. was carried over. This was collected, allowed to settle, decanted off, transferred to a filter-paper, dried and weighed, in the usual manner, the same process being applied to the residue in the elutriator, constituting the grade 0·25–0·5 mm. From the data so obtained, the percentage of each fraction in the total sand-grade (passing 2 mm.) was calculated.

TABLE 4.

MECHANICAL ANALYSES OF SANDS FROM PORT ERIN BEACH.

Station.	Above 2 mm. >6 mm. %	Above 2 mm. >4 mm. %	Above 2 mm. >2 mm. %	Proportion of coarse material (all above 2 mm.) to *total* coarse and fine. %	Below 2 mm. 2·0–0·5 mm. %	Below 2 mm. 0·5–0·25 mm. %	Below 2 mm. 0·25–0·1 mm. %
A1, B1	54·2	22·5	23·3	0·08	0·27	7·04	92·80
A2	80·2	10·9	9·1	1·10	0·56	3·36	96·29
A3	72·7	9·6	17·8	0·03	0·08	6·96	93·30
A4	95·0	1·7	3·3	0·04	0·11	3·75	96·30
A5	62·6	11·1	26·4	0·05	0·01	8·77	91·40
B2	76·9	10·7	12·5	1·13	1·82	6·38	92·01
B3	87·9	6·6	5·5	2·06	1·59	10·70	87·84
B4	86·0	7·8	6·2	1·25	0·68	24·05	75·25
B5	82·8	7·7	9·6	1·10	0·55	30·95	68·60
C1	71·8	18·9	9·4	1·05	2·11	1·44	96·68
C2	73·7	11·5	14·8	4·70	5·18	37·50	57·30
C3	73·8	12·3	13·8	7·50	11·26	43·50	45·25
C4	73·8	11·8	14·6	11·20	7·73	45·80	46·40
C5	89·6	5·3	5·3	40·00	11·79	55·60	32·60
D1	54·7	25·1	20·3	1·29	0·36	2·60	97·21
D2	67·3	17·2	15·6	1·25	0·93	2·46	96·80
D3	51·1	25·5	23·4	12·30	6·83	27·60	65·70
D4	47·1	21·8	31·1	23·70	22·54	34·80	42·60
D5	52·1	22·2	25·7	43·70	40·22	44·50	15·40
E1	46·9	17·3	35·8	0·11	0·62	6·16	93·40
E2	37·2	29·1	33·7	2·70	4·57	3·83	91·79
E3	56·8	19·7	23·5	5·07	2·67	10·61	86·90
E4	37·6	20·3	42·2	62·70	37·84	33·95	28·15
E5	40·8	26·0	33·2	50·00	40·70	32·55	26·70
I.H.	44·7	19·4	36·0	0·20	0·54	1·70	97·89
O.H.	52·3	21·7	26·0	0·50	0·62	1·52	98·18
R.	41·8	26·6	31·7	1·56	2·72	5·82	91·70

In this way, the grade-composition of the two classes of material, above and below 2 mm. diam., are separately expressed, but related by an overall percentage (Table 4). A similar convention has been adopted, in the case of deep-water deposits, by Davis (**4**), who regards the materials above and below a certain limiting grade, which he places at 1·5 mm. diam., as of differing biological significance. We believe the separation-

limit at 2 mm., as adopted in this paper, to be preferable on biological grounds, at least so far as the animals of the intertidal area are concerned. In addition, it permits of an uninterrupted sequence of comparison between the deposits of the intertidal beach and the true " soils " of the adjacent coasts, of which mechanical and other analyses, when available, are expressed on the 2-mm. basis, as accepted in agricultural practice. In Figure 3, an attempt is made to illustrate diagrammatically the grade-distribution on the beach, along the several transects. In the diagram, some slight liberties have been taken with the actual figures, in order to avoid confusion. The grades above 2-mm. diam. are treated as a whole, and amounts of these grades below 3% are disregarded, as adventitious.

B. Biological.

A preliminary investigation of the fauna of the sandy portion of Port Erin Bay was made by one of us (M. E. P.) in April, 1929, and this was continued and extended, in September, 1931, along quantitative lines. The nomenclature adopted corresponds, in general, with that of the new Plymouth Fauna List (**6**). The actual counts are those of the number of organisms per quarter square-metre, but in the Table of Occurrence the mean population per square-metre is given, together with the number of samples upon which the estimate is based. The results are quantitatively valid only for the season studied, and an extension of the survey to other times in the year would probably reveal some seasonal variation in frequency and distribution.

The results for all species found are given in the following Table, while in Figures 4–8 an attempt is made to indicate diagrammatically the relative frequency of a few dominant species.

Notes on the Species.

Harmothoë lunulata (Delle Chiaje). A single specimen, at L.W. Springs, at Station A1, B1. Probably a migrant from its normal habitat, under stones and among Laminaria, on the seaward side of the pier.

Phyllodoce lineata (Claparède). Occurred in two samplings, at C1 and D1, in clean sand of fine grade, at extreme L.W.M.

Nereis diversicolor, O. F. Müller. Comparatively rare, found only at the Inner Harbour station.

Nephthys cæca (O. F. Müller). (Fig. 4, p. 290.) A dominant type, all along the bay, except at the extreme south. Always appears to prefer clean sand, and to avoid the black sand, with its characteristic features of low oxygen-content, and presence of sulphides. Grade also appears to be an important factor in determining the distribution of this species, since it tends to extend to a higher tidal level where the sand is fine.

Table of Occurrence of Species on the Sandy Beach at Port Erin, September, 1931.

(Mean number of specimens per square metre recorded at each Station.)

Station	A1, B1	A2	A3	A4	A5	†A1, B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5	E1	E2	E3	E4	E5	R	O.H.	I.H.
No. of samples taken	2	2	3	2	2	2	2	3	2	2	2	2	3	1	2	2	2	2	1	1	2	2	2	1	1	2	2	2
Harmothoë lunulata	2	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Phyllodoce lineata	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..
Nereis diversicolor	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
Nephthys cæca (Fig. 4)	2	2	..	..	..	2	20	16	2	..	8	8	1·3	..	..	12	8	2	..	..	14	2	4	..	..	16	..	2
Nerine cirratulus (Fig. 5)	..	..	1·3	2	..	..	..	1·3	10	..	..	..	1·3	4	..	..	..	..	..	..	2	..	6	4	..	..	..	..
Magelona papillicornis	..	..	..	..	..	..	..	..	2	..	2	2	..	..	..	8	12	..	..	..	16	..	..	..	..	..	..	..
Capitellids (young)	72	..	1·3	..	..	72	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	..
Arenicola marina (Fig. 6)	16	12	12	16	..	16	2	5·2	8	..	2	2	3	..	..	4	4	12	..	..	4	12	4	..	..	6	10	10
Lanice conchilega	..	..	..	..	..	..	6	..	..	..	2	..	..	..	..	2	2	..	..	..	..	..	..	..	..	..	..	..
Eurydice pulchra (Fig. 7)	..	..	..	..	..	..	..	..	64	..	..	2	20	32	2	..	2	..	..	4	..	..	..	4	4	..	..	..
Idotea sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*	..	..	..	..	..	*	..	..	..
Bathyporeia sp.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..	..	..	..	..	..	..	..
Urothoë marina (Fig. 8)	..	..	..	..	..	..	..	28	12	..	..	..	5	..	..	..	..	2	..	..	2	..	4	..	..	2	..	..
Gammarus sp.	..	..	..	..	..	..	..	1·3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..	..	..
Carcinus mænas	..	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..
Echinocardium cordatum	..	..	..	..	..	..	2	..	..	..	2	..	..	..	..	2	2	..	..	..	..	..	..	..	..	..	..	..
Ammodytes tobianus	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..	2	..	..	..	4	2	..	..	..	..	..	..

† The data from Station A1, B1 are re-inserted here to avoid apparent discontinuity of distribution. * Present, but no count made.

Nerine cirratulus (Delle Chiaje). (Fig. 5, p. 293.) A distinctly intertidal species, having its maximum density above Mean Sea Level. Grade appears to have little influence on its distribution.

Magelona papillicornis, Fr. Müller. Individuals are most numerous near L.W.M., and decrease in numbers towards the south end of the beach. Low salinity may be an inimical factor, although at this low level on the beach its influence must be too transient to be of great importance.

Capitella sp. Young specimens of a Capitellid, probably *C. capitata*, were obtained in large numbers, just within the Raglan Pier at L.W.M. A few were also seen at A3 and in the Outer Harbour. The sand was black or grey at all these stations.

Arenicola marina, L. (Fig. 6, p. 293). A dominant species. Very numerous at all but the highest level on line A—this may be associated with the harbour conditions of fine grade and blackness of the sand beneath the surface. *Arenicola marina* is usually abundant in this type of habitat, and has been shown to be capable of withstanding low oxygen tension (Borden, **1**).

Lanice conchilega (Pallas). In fine, clean sand, at and a little above L.W.M.

Eurydice pulchra, Leach (Fig. 7, p. 294). In large numbers, high up on the beach, near and above M.S.L., and in greatest abundance on lines B and C. It was not recorded at all in the samplings taken in April, 1929. This is paralleled by observations at Millport (Elmhirst, **5**), where the species is found, in winter, in shallow water below L.W. Springs, while in summer it is found further inshore.

Idotea sp. Young specimens, probably of *I. neglecta*, G. O. Sars, were found in considerable numbers in the sievings from D4 and E5, which were very stony, as well as in finer sand.

Bathyporeia sp. A single specimen, from D5, in a coarse stony sample.

Urothoë marina (Bate), (Fig. 8, p. 294). In fairly large numbers, particularly along line B, which also showed the greatest population of *Eurydice pulchra*.

Gammarus sp. Occasional specimens, probably *G. campylops*, Leach, from B3 and E5.

Carcinus mœnas (Pennant). Only occasional specimens in the samples, although a relatively common shore form.

Echinocardium cordatum (Pennant). Only at extreme L.W.M., towards the southern end of the beach, but not actually within the harbour. It occurs where the sand is clean.

Ammodytes tobianus L. Occasional specimens, at L.W.M., towards the northern end of the beach, on lines C, D, and E.

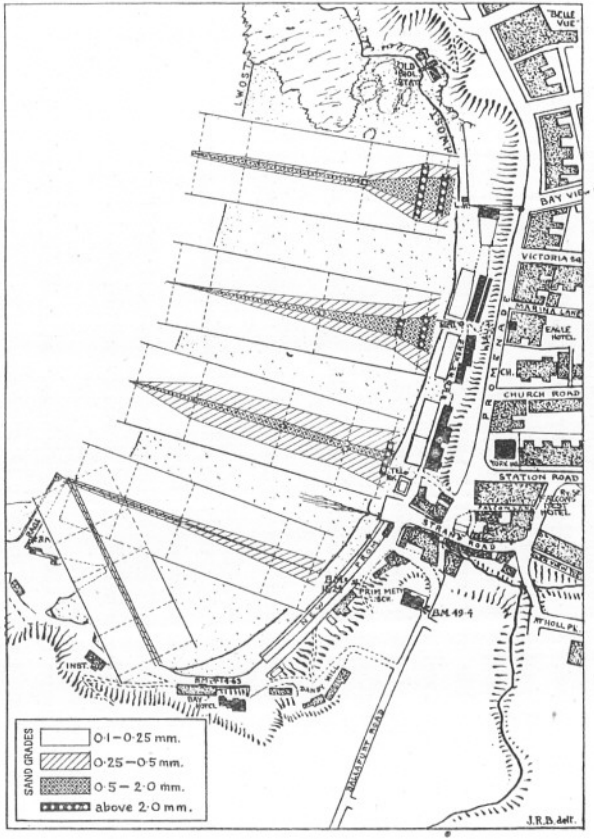

FIG. 3.—Composition of the Sand-grade, and Relative Amount of Stones and Gravel, in the upper 20 cm. of Port Erin Beach. (The proportion of the successive fractions of the sand-grade (up to 2 mm.) is denoted by the relative width of the shaded bands. The lengths of the superposed black bars are proportional to the percentage of coarse material (above 2 mm.) in the entire mass.)

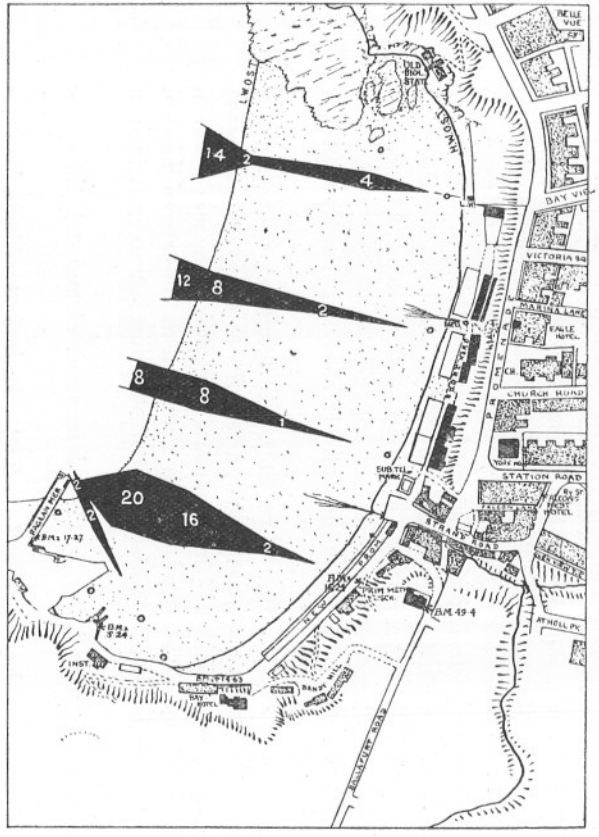

FIG. 4.—Distribution and Relative Frequency of *Nephthys cæca* (O. F. Müller) on Port Erin Beach. The width of the bars is proportional to the density of population at each Station.

GENERAL REMARKS.

It is clear that certain factors exert a paramount influence upon the observed faunal distribution in the area under survey—such are grade, tidal level, salinity, and exposure—and it is intended to restrict the discussion to these and their mutual relations.

Grade.

Regarded as an environmental factor, the grade-composition of a beach-sand is of a very complex character. Not only may a sand consist of materials of widely varying grade, and of varying proportions of those grades, but the influence which it exerts upon its animal population may depend upon totally distinct mechanisms at different points in its range of variation. A case in point is that of a polychæte, which swallows the sand in which it lives, in order to extract nourishment from the associated organic matter. The amount and nature of the sand which the worm can swallow is clearly a factor which powerfully influences its distribution. There is a limiting size of particle, depending upon the capacity of the œsophagus, above which the material will be rejected as unsuitable. This larger material may, however, exert an influence in a different direction, since, if large enough, it will contribute greater stability to the deposit, or even afford beneficial shelter. Yet again, the presence of a large proportion of pebbles may render it difficult for the worm to burrow, although the grade of the deposit between the pebbles may be far below the ingestion limit.

Tidal level.

The effect of tidal level on the beach fauna is obviously one of great importance, since definite zones of distribution, more or less restricted to certain levels of the beach, are characteristic of a wide variety of species. The effect of such directly acting factors as exposure to air, light and heat, food supply, salinity changes, etc., can in most cases be studied experimentally, but in defining environmental conditions it is necessary to know not only the magnitude of the factors, but the period and duration of their incidence. For this reason it is of great importance to determine and record the exact level (in relation to mean sea level or other datum) of the point of observation. Where, as in the present survey, the levels have been accurately determined, the symmetry of the tide curve assumed (an assumption permissible under open coastal conditions), and the mean amplitude of the tide known, an exposure factor may be calculated for each station (Bruce, **2**). If the distribution of definite species of animals on the shore is to be compared for various localities, sufficient data, as indicated above, must be given for the calculation of an exposure factor.

At Port Erin, the tidal curve is practically a symmetrical one, and the range has a mean value of 15–16 feet.

At Port Erin two main zones may be recognised :—(1) a lower zone, extending from L.W.M. to M.S.L., in which *Arenicola marina* and *Nephthys cæca* are dominant, with *Magellona papillicornis*, *Echinocardium cordatum* and *Ammodytes tobianus* in smaller numbers, and (2) an upper zone, from M.S.L. to H.W.M., where the dominant species are *Eurydice pulchra*, *Urothoë marina*, and *Nerine cirratulus*.

Salinity.

The influence of salinity upon the distribution of animals inhabiting the open waters of the sea is known to be great. While probably no less important in the inter-tidal region, the direct results of salinity changes are less evident, because obscured by the interaction of tidal and other factors. If, for example, the system of isohalines represented in Figure 2 were invariant, we should expect to find definite areas and zones of distribution of special forms, more or less corresponding with the salinity contours. On the contrary, no very close relationship can be traced. It must be remembered, however, that a salinity of $24^{\circ}/_{\circ\circ}$ near the top of the beach will have a far greater influence, owing to the longer period of its action, between consecutive high waters, than the same salinity at L.W.M., where the condition will only obtain for a short time. The low value, for instance, at C1, was recorded at extreme low water of equinoctial springs, and might only be realised at that station for a few minutes on three or four days in the year. Under such circumstances, it could have no abiding influence upon distribution in general. Tidal level apart, salinity is subject to other and non-calculable influences—the fluctuations of rainfall affect the volume of the streams and the washing-out of salts from the beach, while wind and other factors bring about complete changes in the course of the streams across the beach even in a single season. No very close correlation, therefore, must be looked for, between the distribution of the fauna and the salinity of the beach, especially in the case of those forms in which a life-duration of several years tends to nullify short period influences.

Exposure.

Exposure is apparently the major factor in which the rich sandy beaches of the Clyde Area differ from that at Port Erin. No living molluscs were taken in any of the samples during this survey, although *Ensis siliqua* and *Lutraria lutraria* occur, and the former was actually being speared by fishermen, at D1 and D2, at a depth below that of our sampling.

Cardium edule and *Tellina tenuis* are recorded as only occasionally

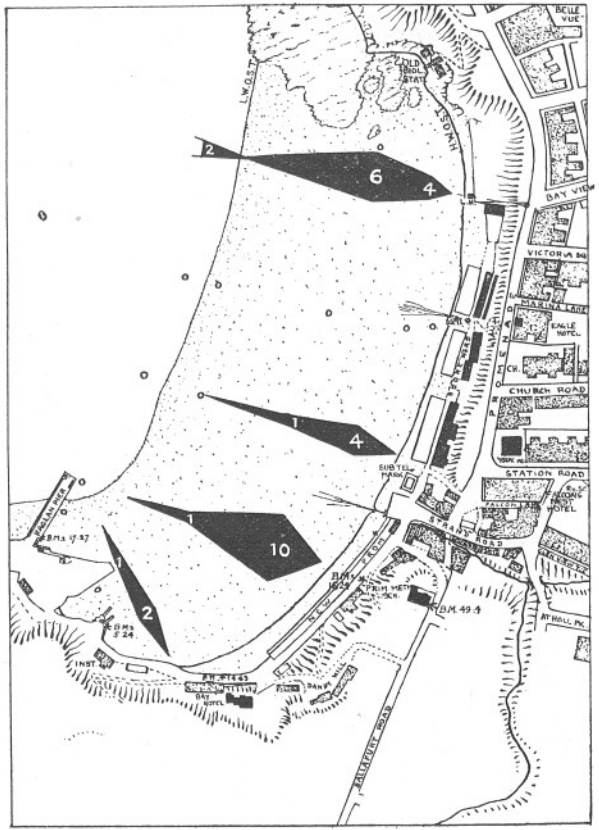

FIG. 5.—Distribution and Relative Frequency of *Nerine cirratulus* (Delle Chiaje) on Port Erin Beach.

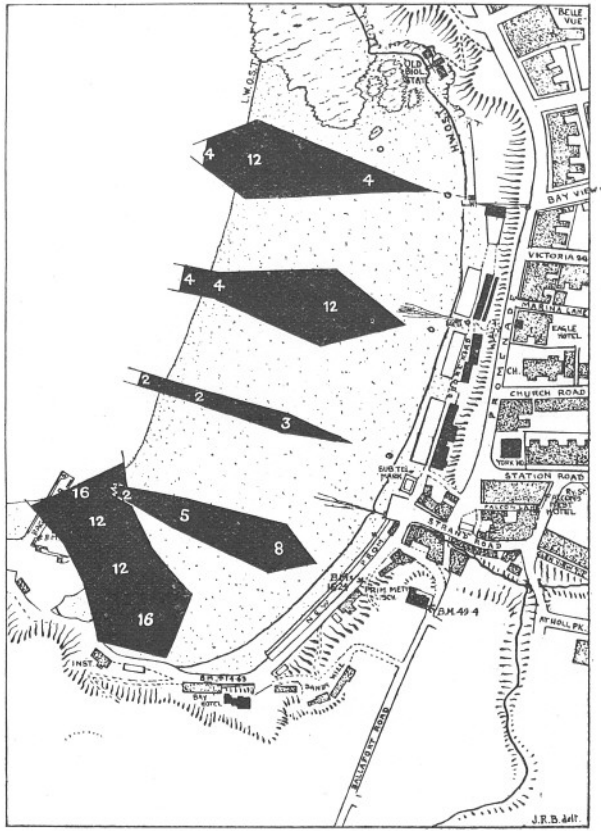

FIG. 6.—Distribution and Relative Frequency of *Arenicola marina* L. on Port Erin Beach.

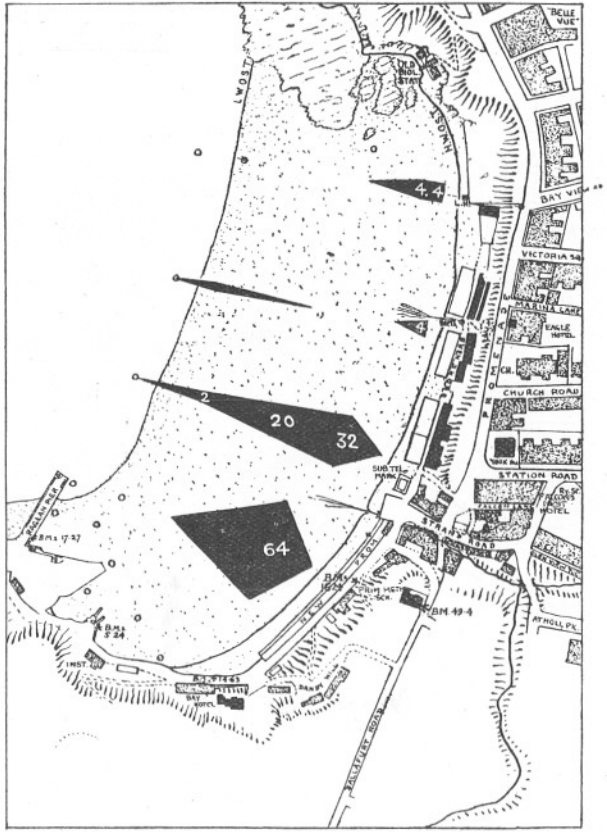

FIG. 7.—Distribution and Relative Frequency of *Eurydice pulchra* Leach on Port Erin Beach.

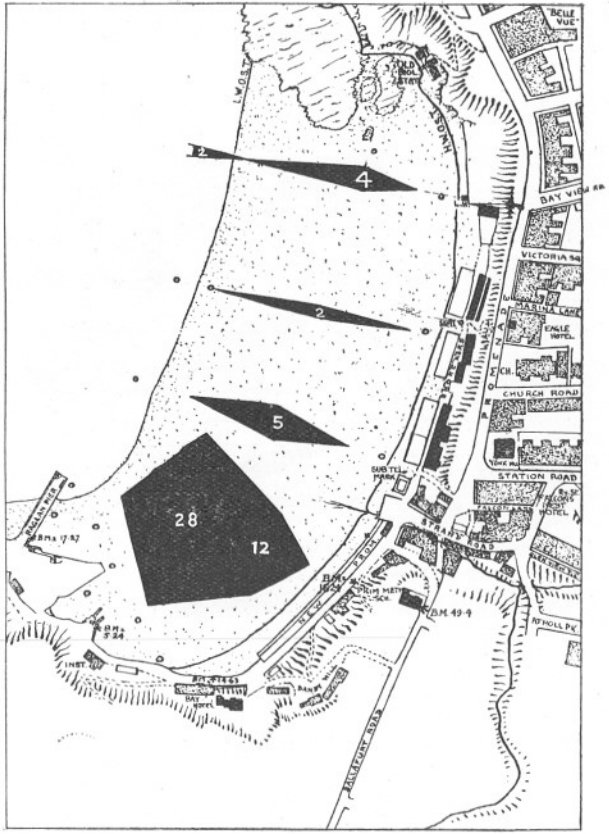

FIG. 8.—Distribution and Relative Frequency of *Urothoë marina* (Bate) on Port Erin Beach.

present in the bay, below L.W.M., but it is of interest to record that numbers of fine large *Cardium edule* and *Paphia pullastra* are to be found in the gravel at the bottom of the sea-water baths in Port Erin Bay—in this situation they obtain much more shelter than on the open beach (Walton, **9**).

Shelter from wave-shock is secured in the lee of boulders, rocks, and artificial harbour works. Almost invariably, there are concomitant factors of fine grade, high detritus-content, oxygen shortage, and blackness of the sand due to ferrous sulphide.

The authors desire to record their appreciation of much help afforded by their colleague Miss M. Parke, B.Sc., both during the shore-work and the subsequent compilation of this report.

SUMMARY.

A survey of the sandy beach at Port Erin, Isle of Man, was made in September, 1931. Observations were made, at a number of stations, involving simultaneous records of the macro-fauna and its density, and certain physical and chemical factors of the sand and interstitial water, as well as tidal level on the beach. The results enable certain general conclusions to be drawn as to the causes of the observed distribution of species, and of the differences between the faunas of this and some Scottish beaches. Plans are given, indicating tidal contours, salinity, and grade composition, *in situ* on the beach, together with frequency diagrams for five dominant species.

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The Salinity of the Water Retained in the Muddy Foreshore of an Estuary.

By

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From the Laboratory of the Marine Biological Association, Middlesbrough.

It has been shown by Reid (1930) that the salinity of the water retained in a sandy foreshore at low tide may be considerably higher than that of a stream of brackish water flowing over it. A few similar observations have been made on the salinity of the water held in the muddy foreshore of the estuary of the River Tees.

The mud banks exposed at low tide are very soft and glutinous, and contain a high percentage of organic matter derived largely from sewage and industrial wastes with which the estuary is polluted.

At low tide, a small hole, some 6 inches deep, was dug in the mud, about 3 feet from the water's edge, and the water which slowly percolated into it was removed in a pipette and filtered. A sample of estuary water was taken at the same time a few feet offshore and the salinity of both samples was determined. The results are shown in the following table, where the average salinity at high water near the estuary bottom at the stations sampled is also given :—

TABLE I.

Distance from sea in miles.	Salinity of water in mud at low tide %	Salinity of water offshore at low tide %	Average salinity of water at high tide %
6	28·4	12·6	30·0
6	22·3	14·2	30·0
10	11·5	0	25·0
$11\frac{1}{4}$	5·0	0	21·0

It seems probable that non-burrowing animals living in an estuary are subjected to greater variations and to lower minimum values of salinity than are burrowing forms at the same distance from the sea. It has been observed that, both in the clean, sandy estuary of the Tay, and in the polluted, muddy estuary of the Tees, burrowing marine animals are, on the whole, relatively more abundant in the central part of the estuary than

non-burrowers. In Table II the number of species of burrowing animals found in each of four sections of the Tees estuary, expressed as a percentage of the total number of burrowing forms present at the estuary mouth, is compared with the corresponding percentage for non-burrowing animals. Similar figures are given for the estuary of the Tay. Owing to the greater length of the Tay estuary, the distances of the lettered sections from the sea are different in the two cases, but the average salinity in any section over all states of the tide is approximately the same in the two estuaries :—

TABLE II.

Section of estuary.	Distance from sea in miles. Tees.	Tay.	Average salinity over all states of the tide. %	Tees Estuary. Percentage of the number of species present at the estuary mouth. Burrowing animals.	Non-burrowing animals.	Tay Estuary. Percentage of the number of species present at the estuary mouth. Burrowing animals.	Non-burrowing animals.
G	9 to 10½	15 to 17½	13	13	1	24	13
H	7½ to 9	12½ to 15	16	22	2	33	15
I	6 to 7½	10 to 12½	20	22	8	52	30
J	4½ to 6	7½ to 10	25	70	28	52*	57

CONCLUSIONS.

It has been found that the water retained in the muddy foreshore of an estuary at low tide is more saline than the estuary water itself at the same distance from the sea. It is suggested that this retention of salt by the bottom and shore deposits may be a factor favouring the growth of burrowing animals in the central part of an estuary.

The investigation described in this paper was carried out as part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and is published by permission of the Department.

REFERENCE.

Reid, D. M. 1930. Salinity Interchange between Sea-Water in Sand and Overflowing Fresh-water at Low Tide. Journ. Mar. Biol. Assoc., N.S., Vol. XVI, No. 2, pp. 609–614.

* This section of the Tay estuary has a steep rocky foreshore, hence comparatively few burrowing animals were found between tidemarks.

Salinity Interchange between Salt Water in Sand and Overflowing Fresh Water at Low Tide. II.

By

D. M. Reid,

Department of Biology, Harrow School.

With 4 Figures in the Text.

THE following is a continuation of the work described in a previous paper (Reid, 1930). The intention was to amplify that work by the investigation of the conditions of salinity interchange on sandy shores of less ideal type. It was considered likely that various factors might affect the condition of the water held by the sand. For instance, the rate of flow of the fresh water and its depth; irregularity in the slope of the beach; the depth of the sand and the nature of the floor on which it rests. The rate of flow of the water is not a simple factor however. It is correlated with the slope of the beach in that faster streams are usually on steeper beaches. The effect of this dual factor will be discussed later.

In the previous paper the depth of the sand appeared to be considerable, at least it was sufficiently deep not to interfere with the sampling. In the present case some of the beaches were composed of a very shallow layer of sand resting on a bed of shingle or solid rock. In one case there was a good deal of shingle mixed with the sand. With so many factors to deal with it is somewhat difficult to assign to each its particular effect.

METHODS.

Sampling was carried out as already described in the previous paper. The samples were titrated with $AgNO_3$ standardised against Normal Sea-water. No attempt was made to estimate the pH.

WHITESAND BAY (ST. DAVID'S), PEMBROKE.

The sets of readings taken in this bay are very much more numerous than in the other instances—the object being to study at each point the changes in salinity that take place at successive intervals of time after the sand has been uncovered by the tide and to study the variation with tidal conditions.

To avoid inaccuracies in sampling, at each station from H.W.M. a number of samples were collected in a line across the stream parallel to H.W.M., and these samples were combined for analysis.

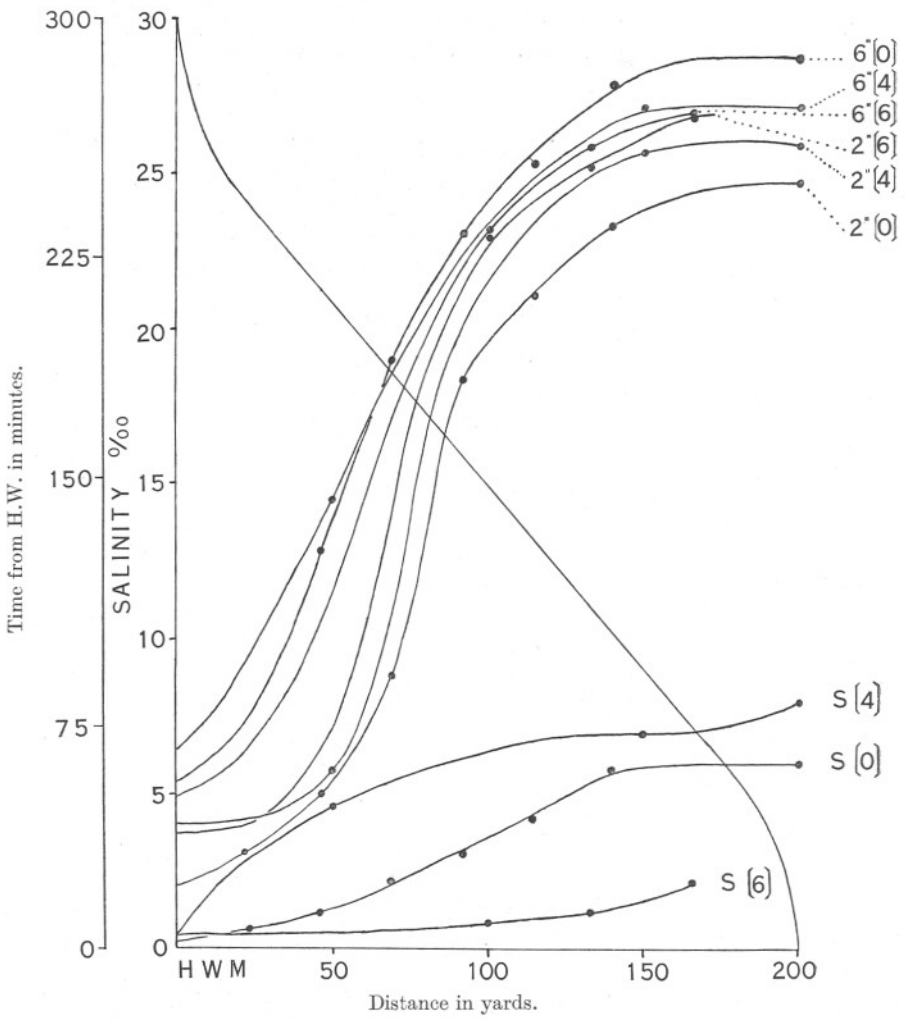

Fig. 1.—Whitesand Bay (St. David's). S(0)=S‰ of surface water just after the tide had uncovered that point. 2″(6), etc.=S‰ at that depth 6 hrs. after the tide had uncovered that point.

Besides this, an investigation of the variation of salinity of the water in the sand with the state of the tide was made by spacing out the intervals of sampling so that they fell, as nearly as could be arranged for the work, on the dates of Spring, Neap, and Intermediate Tides. The points on the

various curves (Fig. 1) represent the results of analyses of all samples taken on various days (under the particular conditions of time and depth stated on the curve), and the fact that they lie on a smooth curve, although readings were taken on different days when different tidal conditions obtained, show that if the distances be measured from H.W.M. of the day in question the tidal conditions do not affect the salinity interchange.

As in the case quoted in the previous paper, the sand in this bay is fairly homogeneous down to a depth of at least 12 inches when pebbles

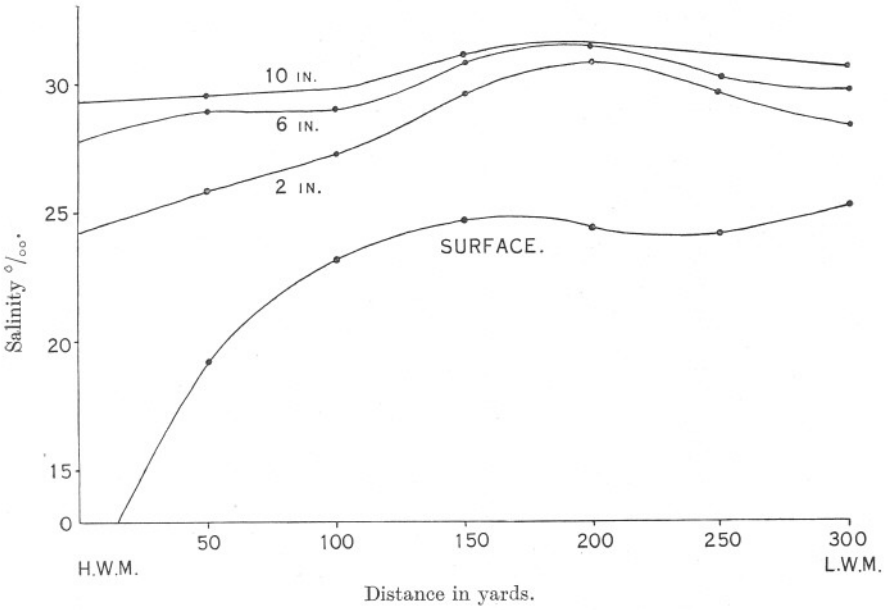

FIG. 2.—Bude River.

begin to be sufficiently numerous to make sampling uncertain. These pebbles, however, are far enough below the surface to have little, if any, effect on the problem.

The Whitesand Bay conditions closely resemble those previously described with the important exception that here the slope, though regular, is steeper. As a consequence of this the overflowing water travels at a greater pace and there is a mechanical draining out of salt water from the sand which will account for the absence of that flattening out of the curves which is so marked in the previous case. Despite this absence of a flattened area there is a marked tendency of the part of the curves to maintain a kind of equilibrium at the place where they are crossed by the simple

harmonic curve of the rate of tidal rise and fall. The harmonic curve was drawn in accordance with the formula

$h=\frac{1}{2}$ r Cos θ where h=height of tide above mean tide level.
r=range of tide.

$$\theta \text{ is an angle} = \frac{\text{Interval from H. or L.W.}}{\text{Duration of rise or fall.}} \times 180°.$$

Whitesand Bay (St. David's).

TABLE OF SALINITY READINGS.

Dates 26 and 30/8/31 and 7/9/31. Weather settled and calm.
Tidal Data. Depth of Flowing Water 1–6 in.
Maximum Tidal Range 22·7 ft. Speed of Flowing Water 3–4 ft. p. sec.

	Distances from H.W.M. (yd.).													
Salinities °/₀₀	0	23	46	50	69	92	100	115	133	140	150	166	200	240
Tide just off.														
Surface	0·2	0·6	1·1		2·1	3·0		4·1		5·8				5·4
2 in.	2·0	2·8	5·0		8·8	18·4		21·1		23·4				24·7
6 in.	5·4		12·8		19·0	23·1		25·3		28·0				28·0
Tide 4 hr. off.														
Surface	0·4			4·6							6·9		8·0	
2 in.	4·0			5·7							25·7			
6 in.	6·4			14·4							27·2		27·1	
Tide 6 hr. off.														
Surface	0·4						0·8		1·1			2·1		
2 in.	3·7						23·0		25·2			26·8		
6 in.	4·9						23·1		25·9			26·8		
Tide 11 hr. off.														
Surface	0·2													
2 in.	0·4													
6 in.	2·3													

Bude River, Cornwall.

This river differs from the others in that it flows over the sand in a well-defined channel for a large part of its course. It is comparatively deep (12 inches) and has a speed of about 3 ft. per sec.

It is, however, the bed on which the sand rests which is of greatest interest. The sand layer is fairly shallow, being little more than 10 inches deep at the points where the samples were taken. Below the sand there is a rock floor formed by the trough of the syncline whose sides form the lateral boundaries of the bay and whose axis lies at right angles to the tide marks. The general slope of the beach is fairly regular and so would tend to make the conditions fairly normal were it not for the fact that the stream does not flow directly from H.W. to L.W.M. in a straight line but cuts across the beach diagonally until, at about 180 yd., it comes close to the uprising side of the anticline. At this point, then, there is what amounts to a rock wall having against it a rampart of sand which, in turn, acts as a

salt reservoir for the sand below the stream and thus accounts for the sudden increase in salinity at this point (see Fig. 3).

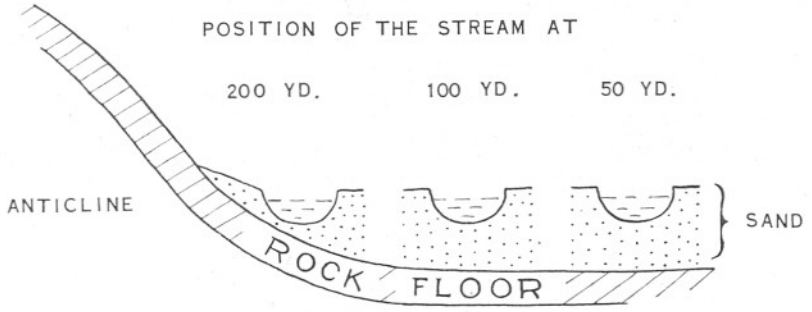

FIG. 3.—Diagrammatic section through part of Bude Bay.

BUDE RIVER (BUDE BAY).

TABLE OF READINGS.

Date 11/9/29. Weather, Fine. Wind, O.

Tidal Data.

Ht. of High Tide 11/9/29 21·2 ft.

Max. ht. Spring Tide 23·9 ft.

Ht. of Low Tide 11/9/29 3·1 ft.

Average Tidal Range 18·5 ft.

Time 3–4 p.m.

Temp. Air 20° C., Water 19° C.

Depth of Flowing Water 3–8 in.

Speed of Flowing Water 2–3 ft. p. sec.

Station.	S% of Surface Water.	S% at 2 inches.	S% at 6 inches.	S% at 10 inches in the Sand.
H.W.M.	8·3	24·3	27·8	29·3
50 yd.	10·2	25·9	28·9	29·5
100 yd.	23·2	27·3	29·0	
150 yd.	24·7	29·6	30·8	31·1
200 yd.	24·4	30·8	31·4	
250 yd.	24·2	29·6	30·2	
300 yd. below H.W.M.	25·3	28·4	29·8	30·6

Beyond the 200 yd. point (see Fig. 2) the drop in salinity seems to be accounted for by the fact that the channel becomes less well defined and steeper, so that more rapid interchange takes place between the water in the sand and the overflowing water.

It seems probable that the even concentration gradient with depth is due to the well-defined channel and the rock floor acting as an impervious layer. Thus the salt water withdrawn will tend to be replaced more rapidly and evenly by the salt water from the surrounding sand than it would be were the sand of greater depth or not resting on an impervious bed.

Red River (St. Ives Bay), Cornwall.

At first sight the investigations in this locality appear to show conditions so variable as to be of little interest or value. The slope of the beach and the depth, speed, and course of the overflowing fresh water vary irregularly from station to station. The maximum depth to which the sampler could be driven through the sand was 12 inches. At that depth it came into contact with a layer of shingle which underlies the sand and crops out

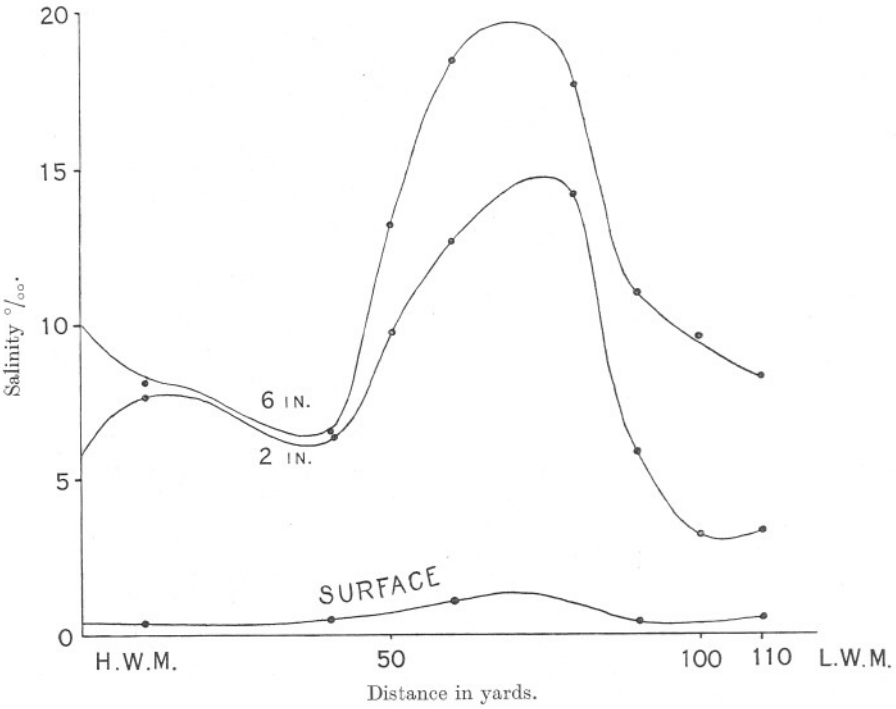

Fig. 4.—Red River (St. Ives Bay).

near H.W.M. The overflowing water at no time shows any real increase in salinity (see Fig. 4). Where the sand and pebbles are fairly evenly mixed (i.e. for the first 40 yd. from H.W.M.) a rapid fall in the salinity of the water in the sand is indicated and is probably due to its quick removal by the overflowing water as soon as the tide has receded. In the next part, from 50–80 yd., the great increase in the salinity of the water in the sand is due to a very disturbing condition but one that is probably common to a good many beaches. This is a well-defined layer of pebbles below the sand. Such a layer of pebbles will provide an under-

ground channel for the fresh water and prevent it from coming into contact with the overlying sand and its contained water so that practically no salinity interchange takes place.

Beyond the 80 yd. point the rapid fall in the salinity of the water in the sand is explained by the emission of the water from the underground channel; in fact, the conditions are similar to those found in the first 40 yd.

In general it may be stated that, since the stream flows through this open shingle-sand mixture it very soon removes the bulk of the available salt after the tide has receded and thereafter there is no more salt to remove.

Red River (St. Ives Bay).

TABLE OF READINGS.

Date 9/9/30. Weather, Fine. Wind, O.

Tidal Data.

Ht. of High Tide 9/9/30 15·6 ft. Time 3–4 p.m.
Max. ht. Spring Tide 24·1 ft. Depth of Flowing Water 1–6 in.
Ht. of Low Tide 9/9/30 5·4 ft. Speed of Flowing Water 3–4 ft. p. sec.
Average Tidal Range 12·6 ft.

Stations.	S°/₀₀ of Surface Water.	S°/₀₀ at 2 inches.	S°/₀₀ at 6 inches.
H.W.M.	0·46	5·78	10·0
10 yd. below H.W.	0·34	7·9	8·02
40 yd. below H.W.	0·46	6·24	6·31
50 yd. below H.W.		9·73	13·1
60 yd. below H.W.	1·03	12·6	18·4
80 yd. below H.W.		14·1	17·5
90 yd. below H.W.	0·34	5·84	10·9
100 yd. below H.W.	0·25	3·15	9·56
110 yd. below H.W.	0·57	3·32	8·24

CONCLUSIONS.

The conclusions which can be drawn from the present work do not in any way alter the views expressed in the previous paper on this subject, *viz.*, that marine burrowing animals living in estuaries are in no way affected by the presence of overflowing fresh water at low tide provided they can burrow down to a depth of about 12 inches. Only the animals which live on the surface have to be capable of adapting themselves to varying conditions, as shown by Pantin (1931).

The effect of the rate of flow of the surface water is not quite clear. In general, the faster the water runs the more quickly it appears to leach out the salt from the sand. However, the slope-factor also interferes because faster flowing streams will occur on steeper beaches, while the

greater direct drainage through the sand in the steeper beaches will remove some of the salt water and allow its place to be taken by the overflowing fresh water.

The Whitesand Bay example shows very clearly that tidal effects are such that a very short time under the tide will bring the water in the sand back to a state of high salinity. It therefore appears to be more difficult to reduce the salinity of the water in the sand than to increase it.

I have great pleasure in recording my thanks to Mr. W. H. Barrett of Harrow School for the help he has given me in this work.

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Some New Eye-Colour Changes in *Gammarus chevreuxi* Sexton. Part II.

By

E. W. Sexton, A. R. Clark, and G. M. Spooner.

CONTENTS.

I. INTRODUCTION.

In a previous paper (**9**) attention was drawn to the occurrence of five different "Mutant Stocks" of 'Red-eye' deviations from the normal black eye. Various differences in the characteristics of these stocks were indicated. For instance, Stock I Red-eye is a stable Bright Red behaving as a simple mendelian recessive, whilst in Stock V all kinds of shades of eye-colour occur with various sorts of changes during life.

It has since become clear, from the study of the inheritance of the eye-colours concerned, that we have to deal in each case with the effects of a

single gene* change. Reciprocal crosses have shown that a separate gene is involved at least in Stocks I, II, IV, and V.

In the present paper various hitherto unpublished data are brought together so that all relevant facts may be available for making as full comparison as possible of the five different recessive types to which reference has been made, and one other which has appeared since. Two further additions, the ' Flesh ' and the ' Beet ' Red-eyes, are being treated in a separate publication (p. 337).

The recessives here considered all show some deviation from the normal Black-eye in the production of pigment in the retinal cells. Though they are characterised as exhibiting redness in the eye to a more or less marked degree, the main characteristic which they share in common is *some kind of inhibition or retardation in the production of the black pigment* (*melanin*).† In practice it happens that, since a deposit of melanin below normal density allows the red pigment that is present to show up, the degree of redness is a useful criterion of the state of melanin deposition. Various stages between complete absence and all but full concentration of melanin are represented by a gradation of shades from bright red to dark chocolate. It is useful, however, to focus attention on the state of the melanin in preference to the directly observable resultant effect of the two pigments, because the formation of red pigment is not always maintained at a constant level, and, particularly in some strains, may be subject to wide fluctuation of its own. The red pigment is at least liable to vary in respect to *quantity*, if not also in ways directly affecting the *quality*.

The state of pigmentation of the ommatidia is not only quite readily estimated from the shade of colour observable during life, but it may be known from more direct information given after preservation of the specimen in alcohol. The red pigment having dissolved away, whatever melanin was present is left.

The circumstances of the first occurrence of these recessives are stated here, but discussion of them is deferred until the whole question of the origin of mutant types in *Gammarus chevreuxi* is considered on another occasion.

The genetic details of the Stocks here described are tabulated in the form of pedigree charts, which are too bulky for publication, but which

* The expression ' gene ' is used in this paper for no further purpose than implying that the instances under consideration are directly comparable with others which, in the current language of the geneticist, are described in terms of genes. For instance, each Red-eye stock is comparable to familiar examples where a single gene difference is said to be involved.

† The black pigment is denoted throughout as melanin, but it is to be emphasised that this pigment in Amphipods has not been subjected to investigation. While the work on the body pigments of other Arthropods, particularly that of J. Verne on Decapoda (Arch. de Morph. Gen. & Exp. 1923, XVI, p. 1, etc.), would seem to justify the assumption that the pigment is a member of the class of melanins, there is as yet no proof, and very little is known of its specific chemical properties.

are being preserved so as to be available for reference at the Marine Biological Laboratory, Plymouth.

The following abbreviations for eye-colours are used :—

B	. .	Black
RB	. .	Reddish Black
PB	. .	Purplish Black
DR	. .	Dark Red
DP	. .	Dark Purple
P	. .	Purple
RP	. .	Reddish Purple
DIR	. .	Dark Intermediate Red
IR	. .	Intermediate Red.

These eye-colours (except DP and DIR) are figured in **9**, Plate VIII.

II. STOCK I RED-EYE.

1. Main Stock.

The designation 'Stock I Red-eye' is given to the original red-eye 'mutant,' which appeared in 1912 and which provided the chief material for the first Mendelian study in Gammarus (Sexton and Wing, **1**; Allen and Sexton, **2**). It has been more thoroughly investigated than any other of the eye-colour recessives, for in addition to the inbred stock which was kept by us for over 15 years, the strain is being maintained in the Zoology Department at Oxford, where it has formed the subject of important investigations by E. B. Ford and J. S. Huxley (**3**, **6**, **7**, **8**).

At normal laboratory temperatures (i.e. ranging round 14° C., and seldom exceeding 17° C.) the recessives in the main inbred stock are sharply distinguished from the normal dominants, since the eye in them remains bright red throughout life. In appearance this red is typically of a quality (Normal Red, **9**, Plate VIII) characteristic of the state when red pigment alone is present in the retinal cells. The production of dark pigment is in fact so completely retarded that either none is ever formed at all or else a small quantity is deposited in the older, central, ommatidia during the later stages of life. In ageing individuals the central darkening may be quite noticeable, especially as it is generally accompanied by a fading of the red.

The evidence derived from the appearance of the eye in living animals has been supplemented by the examination of a number of reds preserved in spirit. The following generalisation on melanic pigment formation is therefore possible. In early stages the eyes are always pure 'Normal' red (**9**, Plate VIII), that is, all the ommatidia have a full quota of red pigment without a trace of melanin. This condition is typically main-

tained throughout the span of an average adult life, but in long-lived individuals there has been found a certain amount of melanin deposition spreading from the central ommatidia. In more exceptional cases this melanin formation may start earlier, even, in one instance, when the individual was still immature. That some variation, however, of this sort should occur is hardly surprising, for one important environmental condition known to influence melanin deposition, namely, temperature, is not kept at a constant level, and its fluctuations may of themselves be sufficient to account for it, even if no other influences, whether environmental or hereditary, are present. But that the latter are not to be excluded is clear from the work of Ford and Huxley (**6**) who have discovered three distinct modifying factors which actually do affect the extent of pigment deposition in Stock I reds.

At higher temperatures the production of melanin is much accelerated. We have only recently had the chance to observe this ourselves, but reference may be made to the account given by Ford and Huxley (**6**) to whom we owe the discovery of the influence of temperature on the phenotypical expression of red-eye recessives and the interpretation of its theoretical significance. There is every reason to suppose that the reds in the strain with which these authors deal behave in essentially the same way as those in our Main Stock, from which they were originally derived. For the present, attention is drawn to the gradual darkening, due to the deposition of melanin, which occurs during life at higher temperatures (well summarised in **7**, Fig. 3, p. 71). This darkening is evidently comparable with that which takes place rapidly in the normal black-eyed form during the few days preceding extrusion. A state of equilibrium is eventually reached at early maturity. The darkening starts in the centre of the eye, and, until equilibrium is reached, is here all the time relatively more advanced. The reason for this is that the older ommatidia are situated in the centre, and each ommatidium undergoes a similar process of change from red to dark chocolate.

The effect of the presence of the recessive gene concerned (r_1r_1), as the above authors point out, thus appears in the light of a retardation in the normal process of deposition of melanin and therefore receives expression in physiological or dynamic terms. The fact, however, of the occurrence of a final stable state which is typically lighter than the normal black, shows that other differences are correlated with the mere change in rate, and calls for more detailed interpretation.

2. Crosses involving Stock I Red-eye.

Various crosses involving Mutant Stock I have been made at one time or another. These include matings with specimens both from other

laboratory Stocks and from the wild. Of more particular interest are the results obtained from crossing Stock I 'reds' and other mutant types. These results are given under the accounts of the mutant type involved and need not be considered here.

In some cases the progeny of the crosses have not been carried beyond the F_2 or F_3, but in others they were in-bred for several generations, so giving rise to independent strains.

As far as the effects of the Stock I Red-eye gene are concerned, in none of the strains so derived is any deviation from the expected simple mendelian behaviour shown. There remains the question of the phenotypic character of the Stock I recessives.

It is noteworthy that, whereas in the Main Stock the recessives are of a remarkably uniform type and subject to little variation, when however all the r_1r_1 types from other strains are taken into consideration, a considerable range of variation is to be found. Illustrations may be given of those strains in which occur some of the more striking deviations from the "normal" Red-eye.

(1) A conspicuous example is provided by the descendants of a cross between a Stock I "red" and a heterozygous "black" from Mutant Stock III (p. 313). The r_1r_1 types, of which a number have been reared, are characteristically distinct from the typical "normal" reds Main Stock I. At laboratory temperatures they develop considerable darkening in the centre of the eye. The process starts not long after extrusion, but progresses slowly. Even in long-lived individuals the eye never passes beyond the state of dark red centre with light red periphery. (The effect of higher temperatures has not so far been tested.) Practically all individuals behave in the same way, and so bear an absolute, and not merely average, distinction from those of the Main Stock. The distinction is particularly definite in immature stages.

The genetic relations of this strain of reds, the numbers of which have become much reduced through cannibalism, are now being examined. Evidently some modifying factor, or set of factors, has been introduced in the cross. But whatever the actual genetic interpretation, the empirical result affords an example of how the effect of a particular gene may be modified when introduced into a new strain.

In certain other strains there seems to be a greater tendency for darkening to develop than in the Main Stock, but not to the extent seen in the Stock III Cross.

It may be added that in this strain a tendency of the red pigment to diminish somewhat in intensity has been noticed.

(2) Another strain which has been carried on for several generations was derived from a cross between the recessives of Stock V and Stock I ("HC," see p. 321). The Stock I "reds" which have segregated out have

been of the " normal red " type with no unusual tendency to darken, but most show deficiency in red pigment. Some adults indeed have so little red pigment as to be almost colourless. This is noteworthy, because the strain is composed entirely of Stock V recessives (see p. 321), among which loss of red pigment during immature stages is characteristic (p. 331).

(3) A certain range of variation within a single strain (" CHC " 788) is shown in the F_2 and F_3 of a mating between one of the above-mentioned HC Stock (this specimen did not carry the Stock I gene) and a Main Stock I " red." Among a number of surviving Stock I recessives hardly any two are alike. There is not only variation in intensity of red pigment, but in the extent to which darkening, of which almost all specimens have some, occurs in the centre of the eye. At extrusion several were noted as being pale,—orange instead of bright red ; but this condition does not seem to have had any influence on the pigmentation in later life.

By comparison with other stocks which have been investigated more extensively, it is very probable that the variation in the intensity of the red pigment, as seen here, in (2), and in other strains, is partly non-hereditary. Yet, as a difference is shown between some strains, there is evidently a hereditary distinction somewhere. What, indeed, can be said to be inherited is a greater or less susceptibility to " environmental " influence. (For a fuller consideration of the inheritance of red pigment deficiency in another Stock, see p. 331).

(4) The issue from a cross between a Stock I " red " (from the Oxford Main Stock) and a ' Flesh ' red (see p. 347) provides a further example. These animals were reared in the incubator at an average temperature of from 21° C. to 23° C. In the F_2, there was variation in the degree of redness among specimens proved recessive for r_1. Some came almost to lack pigment altogether, having a somewhat lilac colour, with the centre a darker purple. Since the ' Flesh ' strain at these temperatures is characterised by tendency to great reduction of red pigment, no doubt here again some modifying factor introduced in the cross was at work.

From these illustrations it is seen that in some strains the character of the recessives is more or less uniform, in others liable to fluctuation, particularly in respect to the red pigment. Instances where there is comparative uniformity are provided (1) by the Main Stock (with which may be classed strains derived from certain crosses), in which full red pigmentation is maintained and only little, if any, darkening occurs ; and (2) by the strain derived from the cross with Stock III, in which occurs conspicuous central darkening starting early in life, and a certain tendency for the intensity of the red to diminish. The latter case is no doubt susceptible of interpretation in terms of the action of modifying factors of the sort, described by Ford and Huxley (**6**, **7**), which influence the rate of darkening

of r_1r_1 types at higher temperatures. But in this case the influence is greater than that of any of the modifying factors hitherto described.

III. STOCK II RED-EYE.

An account of Stock II and strains derived from it is under preparation (a preliminary note is contained in **4** and a short reference to the Stock in **9**, p. 191). An in-bred "Main Stock" and strains derived from cross matings with Stock I have been investigated.

The recessives are characterised by a red-eye resembling that of Stock I. Their simple Mendelian behaviour has been fully verified, as has the fact that the hereditary factor involved (which is designated as gene r_2) is distinct from, and its action quite independent of, that of Stock I (r_1).

Matings between reds of the two Stocks give *all blacks*, with a 9 : 7 F_2 ratio of black to red.

The actual figures are :—	585 : 451
expected :	583 : 453

The phenotypical expression of r_2r_2 types of the Main Stock II, at laboratory temperatures, is identical with the r_1r_1 types of Main Stock I, giving typical 'Normal Red' eyes. The formation of the black melanin is completely inhibited in all but a few exceptional examples of adults, in which a faint deposit of melanin could be seen in the centre of the eye after preservation. It is generally characteristic of Main Stock II r_2r_2 types that at laboratory temperatures no melanin is deposited. And indeed just as complete an inhibition of melanin was seen in the earlier families which were kept at an average temperature of between 20° and 21° C. The effect of a temperature of higher than 21° C. is not known.

IV. ACCOUNT OF STOCK III.

A number of wild pairs were brought into the laboratory in Sept., 1922. Some were placed in an incubator at 21° C., and some kept under laboratory conditions. Of the former, four pairs gave an F_2, two containing red-eyed specimens and giving rise to Stocks II and IV respectively; while of the latter, 16 pairs gave offspring, and in five cases there was some sort of deviation from the normal black eye-colour.

In one case Bright Reds appeared in the F_1 generation. Out of 5 survivors from a family of 89, 2 were found to have bright red eyes. All of these died without issue. The same ♀ gave a second family of 357 with another ♂, but all the survivors were black, and so were all the F_2 obtained from at least 4 pairs.

In three cases a slight reddening was noticed in blacks of the F_1 generation, but in neither case was the family carried beyond a scanty F_2.

In the fifth case, which came to be known as Stock III, the F_1 were all black but some F_2 changed to Reddish Black. In the F_3 and F_4 three different gradations of red were found at birth, which were classified as Reddish Black, Dark Red, and Light Red. The main stock soon died out through cannibalism, but the strain has persisted in a cross (with Stock I) which is still under investigation.

The red colour that appeared here is peculiar to this Stock. It is not the vermilion "Normal" Red of Stocks I and II, but a tone between that colour and the "New" Red of Stock V with a concentration of slightly darker colour in the centres of the eyes.

Details of the appearance of redness in the eye are as follows. Out of a large F_1 family, 39 became mature and all were black. There was only one F_2 family of any size, in which 8 out of 26 survivors to maturity were found to be Reddish Black.* (It does not look as if these reddish specimens were distributed uniformly among the broods.)

From this F_2 family, 7 matings gave offspring, which are tabulated as follows :—

Parents.	Young at birth.	Survivors.
Black × Black	4 B + ? 2	—
Black × Black	1 B	—
Black × Black	15 B	—
Black × Black	9 B	—
Reddish Black × Reddish Black	7 B	—
Black × Reddish Black	58 B (of which a number were slightly reddish)	3 B 3 RB
Reddish Black × Reddish Black	32 B (of which a number were slightly reddish) 1 DR 1 Red	1 Mosaic eye 3 irregular RB

It is this last family that is of main interest. The first brood contained 5 B and 1 with the left eye "Mosaic," i.e. an eye with some of the ommatidia jet-black and some bright red (in this instance the 3 anterior were red and the 3 others black); the second brood were 3 Reddish Blacks, all with irregular-shaped eyes and 1 Dark Red; in the third brood 1 Light Red appeared with 6 Blacks; the fourth consisted of 5 B and 6 RB; the fifth of 4 B and 2 RB. There was no F_4 from any of these families.

Of the remainder of the small F_2 families (which altogether gave only 11 Black survivors), only one, the eye-colours of which are not known, produced young, giving an F_3 family of 21 B and 1 RB with mosaic left eye. Seven of the Blacks survived, one changing to RB, its left eye

* Three of these Reddish Blacks had irregularly shaped eyes, and one had a Mosaic Right Eye with jet-black ommatidia in the centre and some bright red round the margin.

becoming 'mosaic' (Fig. 2 on Plate III of this Journal, Vol. XVIII). The matings obtained in this F_2 family gave the following results :—

Parents.	Young at birth.	Survivors.
Black × Black	93 B (one or two slightly reddish)	6 B, 1 RB
Black × Black	235 B (a few slightly reddish)	31 B, 6 RB
	8 DR	5 DR un., 2 DR→IR
	2 Light Red	2 unchanged
Above Black ♀ mated in bowl	6 B (one slightly reddish)	1 B
	3 DR	1 DR→RB
	1 Light Red	

Very few F_5 and F_6 broods were obtained.

One Light Red was crossed with a Stock I Red, and another with a Stock II Red, the young in each case being black. Other crosses were also made.

Altogether 4 'mosaic' eyes appeared, and one or two others in crosses.

On analogy with Stock V we may ascribe the appearance of reddish eyes to the influence of a gene r_3, the independence of which from r_1 and r_2 has been shown.

V. ACCOUNT OF STOCK IV.

Stock IV, a small stock which did not survive very long, was descended from one of the pairs brought in from the wild in September, 1922, and kept at a constant temperature of 21° C. (Stock II arose at the same time). The only F_1 pair produced a large family of 19 broods, and among this F_2 a certain number of red-eyed specimens were found. The young were not examined at birth for eye-colour, but, judging from the survivors, it is probable that the reds were occurring in a 1 to 3 ratio. The number of matings among the F_2 blacks producing families containing reds is such as would be expected if a proportion of one homozygous to two heterozygous occurred among the blacks. The families from these matings in which reds occurred gave a total of 157 Black and 41 Red, thus approximating to the expected 3 to 1 ratio.

Though a number of F_3 families were obtained, the stock dwindled rapidly in the 4th and 5th generations, and then died out. Altogether no more than 1463 specimens were concerned in the Main Stock, but a considerably greater number resulted from various crosses in which specimens of the Main Stock were used.

The results of various matings fall in line with the hypothesis that, as in the case of previous red-eye types (Stocks I and II), the red eye-colour was behaving as a simple mendelian recessive character, involving

a single gene, which may be named r_4. The recessive, r_4r_4 individuals have red eyes.

(a) "*reds.*" A feature concerned with the "reds"* of this stock was a certain variability in the shade of red. About half were born with the eye a very dark red, and the rest either bright red or slightly darker than bright red. There was always a clear distinction between the 'Dark Reds' (DR) and the rest, which are classed as 'Light Reds' (LR). The survivors of the former were found to lighten to 'Light Red' as they grew up. The darker shade of red colour was due to the presence of some dark pigment mixed with the red. Such a shade is represented at a stage in the darkening of the normal black eye in the embryo, and the gradual darkening in the later stages of Stock I "reds" when kept at a high temperature (**6**).

The form taken by the "reds," both in the main stock and those that appear in crosses—a total of 834—is summarised below.

1. MAIN STOCK

Total extruded.	Survivors. Died before maturity.	Reached maturity.
Dark Red 244	11 DR→LR	13 DR→LR
Light Red 296	7 unchanged LR	31 LR

2. STRAINS FROM CROSSES

Dark Red 139	16 { 7 unchanged; 9 DR→LR }	18 { 4 DR unchanged; 14 DR→LR }
Light Red 160	7 unchanged LR	22 unchanged LR

There is thus a strong tendency to redden ; it is the rule in Dark Reds, and among Light Reds also there is a higher proportion of true bright reds at maturity than in the early stages. There is no instance of darkening.

Whatever genetic relation, if any, there exists between the Dark and Light Reds, is at present obscure.

In the *main stock*, among the few matings that were made, Light × Light gave all Light, and Dark × Dark gave Dark and Light. The figures of matings between reds are as follows :—

Light × *Light*.	Main Stock.
10 Lights	(258)
5 Lights	(258)
6 Lights	(514a)
2 Lights	(473)
5 Lights	(106 × 111)
65 Lights	(106b)

* The term '"red"' is applied to recessive individuals (*rr*). In the same way '"black"' refers to individuals with the dominant gene R. 'Black' and 'Red' refer to special types of eye-colour in the same way as 'Reddish Black,' 'Dark Red,' etc. In other words, 'Black' refers to a phenotype, while '"black"' refers to a genotype.

Dark × *Dark*. Main Stock.

17 Darks .. 42 Lights (216)
36 Darks .. 19 Lights (153b)
11 Darks .. 6 Lights (1658)

Dark × *? Dark*.*

142 Darks .. 80 Lights (115d)

But 4 Light × Light matings that were made in the *crosses* each gave both Darks and Lights.

Light × *Light*. Cross with Stock I Albino.

4 Darks .. 5 Lights (3786b3)
10 Darks .. 16 Lights (3786b2)
7 Darks .. 1 Light (3786b)
20 Darks .. 22 Lights (3954b)

Dark × *Dark*. Crosses.

3 Darks (2053)

A further point is that the distribution of Darks and Lights, when they occur together among different broods of a family, certainly does not appear to be as regular as would be expected on the assumption that a genetic relation existed between them.

(b) "*blacks*." In the great majority of cases pure and impure alike have unchanged black eyes. Some, however, show a tendency to redden, gradually changing to a Reddish Black shade (**9**, Plate VIII). The figures, for the main stock, are as follows :—

Total extruded.	Survivors. Died before maturity.		Reached maturity.	
Black 916	74	68 Black unchanged 6 B→RB	147	143 B. unchanged 4: 3B→RB 1→DR→RB

Out of 221 survivors, 10 showed signs of reddening. This is known to occur occasionally among heterozygotes of Stock I and II Red-eye, but here it seems that the individual need not necessarily be heterozygous.

The evidence for this :—Out of the very few matings involving this type, in two cases a B→RB mated with a specimen carrying r_4 has given all black young.

(i) Brood 365 B→RB ♀ × Red ♂ 375, gave family of 16 B (2 to maturity, unchanged). (ii) Cross 307, B→RB ♀ × B ♂ 584 (impure for IV Red) gave a family of 90 Black.

* In this mating the ♀, TH 115d, was hatched Dark Red and lightened to Red. The ♂, from an earlier brood of the same family, was not examined until mature, and was then recorded as Light Red. There seems little doubt but that it was of the same constitution as the female.

(c) *Contrast between "blacks" and "reds."* While the majority of the "blacks" are of the typical normal appearance, a few, as noted above, developed slight reddening. Coming to the "reds," we have seen that all are not uniformly similar. Two distinct classes exist—those born Dark Red and those Light Red throughout life. Though in the Main Stock all the Dark Reds lightened as they grew up, a few in strains derived from crosses remained Dark Red. Those born Light Red varied among themselves to a certain extent, some resembling bright Normal Red, and others almost as dark as Intermediate Red (**9**, Plate VIII). A slight, but definite, variability is thus shown by each class, especially the second. Yet if ample allowance is made for all variation among the "reds," in no case does the eye colouration fall outside the extreme limits, Dark Red unchanged to Normal Red unchanged. There is therefore always a clear distinction between the red-eye recessives and even the most reddish of the dominant "blacks."

(d) *Further consideration of Stock IV recessives.* We have hitherto followed the methodological practice of referring to the recessives as being 'red-eyed' in contrast to 'black-eyed.' The contrast, however, as in the case of other 'red-eye" recessives, is more justifiably stated in some such terms as 'eye greatly lacking in black pigment' as distinct from 'eye with full quantity of black pigment.' For the sake of comparison with other Stocks we may attempt to summarise what is known of the presence or absence of the melanic pigment in the reds.

It is safe to assume that all eyes classed as 'Dark Red' owed their dark appearance to the presence of a certain amount of the melanic pigment. Unfortunately there is only a single preserved specimen to which reference can be made—a ♀ which was born Dark Red and changed to Light Red (TH 115d). This specimen, as expected, showed a faint dark deposit. It cannot be said whether Light Red eyes all contain a dilute deposit of melanin, but, while a number of them probably contain a little, the brighter of them do not differ in appearance from Stock I reds and evidently contain none. At any rate it can be said that those which were Dark Red at extrusion and subsequently lightened did not maintain the melanin production exhibited at first. It may be noted that in these examples the relative concentration of melanin, rising to maximum round about the period of extrusion and then declining, follows a strikingly different course from that to be seen under any conditions in darkening Stock I recessives.

The earlier families of the Stock were kept at an average temperature of between 20° C. and 21° C., but no difference was observed between any of the "reds" of these families and those reared at laboratory temperatures. The effect of higher temperatures is not known.

The concentration of the red pigment showed no fluctuation in any

r_4r_4 types from the Main Stock (and in only one instance in strains derived from crosses—broods TH3786 and TH4244—in which the red decreased, giving a purplish appearance to the eye). A full colouring of red was characteristically maintained.

VI. STOCK V RED-EYE.

Since the account of this stock given previously (**9**) a great deal of further information has been accumulated. It is now seen that a much simplified description is possible. The origin of the Stock and character of the early broods which were kept in the heat at temperatures between 20·8° C. and 28° C. can be summed up shortly. The F_2 from a certain wild pair, brought into the laboratory in February, 1928, and kept at a temperature gradually increasing from 20·8° C., gave individuals with various shades of red eyes, some of which darkened and some of which lightened during life. In subsequent families there appeared all shades intermediate between Black and Red, involving changes in either direction and of various extents, as well as temporary or permanent change to some shade of purple. (The purple colour we consider to be the result of a decrease in the red pigment without a corresponding increase in black.) On the whole, black was dominant to any kind of red, and reds mated together nearly always gave reds of some sort, but even here there were exceptions (H558, H748, Family H577). The discovery of genetic ratios among the families presented many difficulties.

After a time the numbers fell off rapidly and it was thought advisable to remove the Stock to cooler laboratory conditions. Not long after this was done the previous account was given.

1. Later Families of the Main Stock kept at Laboratory Temperature.

Since removal from the incubator one very noticeable character of all the families generally has been a distinction between individuals that may be called "black" and "red" respectively, the ratios conforming with either all "blacks," or all "reds," or "black" to "red" in a proportion of 3 to 1, or "black" to "red" in equal numbers. In fact, "reds" behave as simple recessives to "blacks." Matings give, in all cases, expected results. That is, "reds" mated together give all "reds"—quite a large all "red" branch of the Stock is being maintained (descendants of F_6 broods in **9**, Chart I); "black" mated with "red" gives either all "black," or "blacks" and "reds" in approximately equal numbers; and "blacks" mated together give either all "blacks," or a 3 to 1 ratio. In all cases where a black is known from its parentage to be impure, it has given results according to expectation.

The eye-colour of " reds " at birth is frequently Reddish Black, sometimes Dark Red, frequently Intermediate Red, and quite frequently Bright Red. There is in most cases at least a temporary change to some shade of purple. The Reddish Blacks generally lighten during life, but some remain a Purple Black (=an RB in which red pigment has disappeared); while Bright Reds, though a few remain unchanged, generally darken to some extent. There is often a considerable fluctuation in colour shade.

" Blacks " usually have black eyes unchanged throughout life, but sometimes they gradually redden to a Reddish Black, and occasionally are decidedly reddish when young or when just extruded. The behaviour of the 112 " black " individuals which survived to maturity is shown below.

***B** unchanged	91	
B→**RB**	16	
B→**RB**→PB	1	(984)
RB→B→**B**	3	(1053 and 882)
RB→B→**RB**	1	(1053)

In addition, among 101 specimens which survived but died before maturity, there was 1 case of a B→RB and 4 of RB→B.

It may be mentioned here that there is good evidence that the blacks which show a certain redness at some stage or another are not necessarily heterozygous. In the above list one of the B→RB specimens behaved genetically as a pure black. Other instances will be noted later, and if, as is highly probable, a certain section of Stock V (**9**, Chart 2) is a pure ' dominant ' strain, 4 other examples would be provided, including the above B→RB→Purple Black. Of 30 " blacks," from various families, known to be impure, 27 were Black unchanged, and 3 changed to RB. There is no indication here that heterozygotes have any greater tendency to redden than the homozygous blacks.

Generally speaking, individuals fall readily into one or other of the two classes—" blacks " or " reds." But doubtful cases occur now and then. It is easy to see how ambiguity may arise. It is always possible, for instance, that a " red " born black may sometimes occur (but, for that main section of Stock V with which we are dealing, it would clearly be at most only a very occasional occurrence and does not invalidate the reckoning of all specimens born black, which do not survive, as " blacks "). Reddish Blacks which do not survive are, however, often doubtful. Usually those that survive turn out to be " reds," but certain instances have been given above in which they darkened and proved themselves " blacks." When such borderline cases occur, the general character of the brood is often a sure enough guide of the category to which the

* State at maturity in thick type.

individual belongs. There is one other exceptional case to be mentioned, namely, an Int. Red which darkened to almost black. This proved to be a "red."

Summarising, it may be said that the families of Stock V kept at laboratory temperatures show clearly enough the presence of a recessive gene with which is associated instability in pigment deposition in the eye. Later it has to be considered how this gene was behaving when the Stock was kept in the incubator, whether any modifying genes are influencing its expression, or whether the effect of any other gene at all can be discovered within the stock.

2. Results from a Cross between Stock V and Stock I (HC Strain).

Some useful information has been obtained from a strain derived from a cross between reds of Stock V and reds of Stock I. Several hundred specimens are involved.

The bulk of this strain is descended from a single pair of Stock V "reds" (one of which was impure for Stock I red), and consequently consists entirely of specimens recessive for the Stock V factor.* Here then is a chance of seeing how far Stock V "reds" may vary in eye-colour. It should be mentioned that the original Stock V individuals involved in the cross came from the F_3, that is, the next generation after that in which Stock V "reds" first appeared, and the early families, including the first generation after the above-mentioned pair, were reared in the heat.

The specimens of this recessive stock show a tendency towards the darker shades of red and purple. One striking character is the frequent occurrence of black, or almost black, eyes, particularly at birth. In these cases there is always a falling off in black pigmentation, the colour generally becoming Purple or Reddish Purple. The darkest individuals were some born Black and not lightening further than Purple Black. There are also one or two cases of RB→B.

It can summarily be said that at birth individuals could be nearly Black, Dilute Black, Reddish Black, Dark Red, Dark Intermediate Red, Intermediate Red, Bright Red, or Mixed Red.† RB and IR are most frequent. Survivors show a great variety of changes, e.g. Black→various

* Among these will, of course, be specimens which carry Stock I Red-eye and some recessive for that factor. The latter are double recessives for both Stock I and Stock V Red-eye, and in appearance are indistinguishable from Normal Stock I "reds." The double recessives are not included in this account, but reference has been made to them above (p. 311).

† Certain individuals at extrusion appeared intermediate between 'New Red' and 'Normal Red,' and to these the term 'Mixed Red' was applied. It is probable that they were extreme examples of Stock V recessives with an unusually large supply of red pigment.

forms of purple or RB; RB→B,→PB, unchanged, → lighter reds or purples; DR→PB, →other purples, →B, unchanged, or →lighter red; IR→RB, or purples. On the whole there is considerable uniformity in the eye-colour of members of the same brood, both at birth and subsequently, but the broods of one family may differ from each other considerably, as do also successive generations.

In this cross, therefore, the "reds" are on the average darker than in the red strain of the main stock described above and show a wider range of variation. It shows particularly the sort of difficulty to be expected in distinguishing "reds" from "blacks" when they occur in the same family.

3. Results of Matings of various Stock V specimens with Outside Blacks.

Six "blacks" and six "reds"—superfluous or cannibal ♂♂—were mated with outside wild black ♀♀. From these matings quite large F_2 generations were obtained.

Members of each F_1 generation, as is to be expected, are all Blacks, but in some cases there is reddening later in life. Those reaching maturity that are known to be impure included

37 Black unchanged.
7 Black reddening to RB.
Another case of Black reddening to RB proved to be pure.

The F_2 generations fall into three categories. (1) All families (i.e. offspring from each F_1 pair) are entirely "black"; (2) one family out of every few contains some "reds"; (3) all families contain some "reds." These types of F_2 occurred according to expectations. That is to say, all families contained "reds" when the original Stock V parent was a "red," and the other two types of F_2 occur when it was a "black." In other words, the results come out as expected if it is supposed that some "blacks" are pure and others impure, while the "reds" are all recessive. In one case all F_2 families would be black, in the second one out of every four F_1 matings should give "reds" in a 3 to 1 ratio, and in the third case all F_2 families should be 3 to 1 ratios of "black" to "red."

One of the Stock V specimens involved was a Black which became Reddish Purple after maturity (OH745). The results of the cross showed that this specimen was a pure recessive. The other five recessives were: RB→RP (723); RB→P→IR→R (786); RB→IR→R (713); RB→DR→IR→R→RP (725); RB→P→RP (748). Among the six "blacks" one B unchanged (715), and a B→RB (774) proved pure; and two B unchanged (717 & 746) impure, the other two (739 & 761) being still doubtful.

The F_2 families in which reds appear clearly approximate to 3 to 1 ratios, but the actual proportions are confused by the frequent occurrence of Reddish Blacks. Though some,* and probably most, of these are to be reckoned as recessives, yet in some cases they are almost certainly to be included with the blacks. The families are useful in showing the type of 3 to 1 ratio family to be expected in the Main Stock.

These crosses, then, confirm the supposition that the deficiency of melanin which occurs in Stock V, can be referred primarily to the effects of a single recessive gene.

A special point in connection with one of these crosses remains to be mentioned. The original Stock V parent concerned was the B→RB→RP (OH 745) ♂ referred to above, which proved to be a pure recessive, reds appearing in all of five F_2 families. Now in one of these families there appeared individuals with approximately ' Normal Red ' eyes, resembling those of Stock I in lacking dark pigment entirely. They were regularly distributed among broods of the family, which was constituted as follows :—

85 Black ; 5 RB ; 25 DR and IR ; 33 Normal Reds.

The Normal Reds occurred in a proportion of 1 to 3 of all other colours, among which there was a typical 3 to 1 ratio of Stock V " reds " ; and it looks very much as if a new genotype had been introduced.

In the descendants of this family, Normal Reds have segregated out in mendelian proportions, independently of Stock V " reds," and back crosses with " reds " from the Main Stock V have proved that a separate gene must indeed be involved. This gene is designated r_6. A branch of the strain which is free from the Stock V recessive gene has been set apart as Stock VI.

The appearance of the r_6r_6 types in one out of 5 F_2 families indicates that one of the original parents (Stock V ♂ and Outside ♀) was heterozygous for r_6. Suspicion is at once thrown on the ♀, which would therefore be regarded as either having mutated, or as having been heterozygous in the wild.

4. The Early Families of Stock V Kept in the Heat.

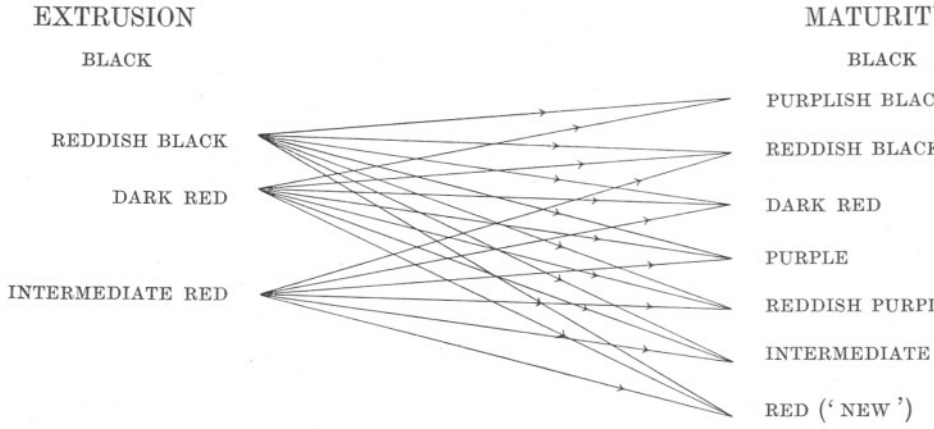
(a) *The recessive ' red-eye ' gene.* Among the later families of the stock the effects of a certain red-eye-colour gene have been discovered (see Section 1). It has now to be considered whether this gene can be traced back within the early families of the Stock kept in the incubator, and how far this assumption of its presence helps in elucidating the inheritance—or absence of it—of Stock V eye-colours. Different crosses with outside blacks (Section 3) have shown that various red-eyed individuals

* Tested by mating back to Stock V recessives.

actually are recessives, all apparently for a single gene. On the face of it, then, it is likely that one principal recessive gene has been handed down through the stock and lies at the bottom of the eye-colour instability. Considerable variation has so far been found among the "reds" (i.e. recessives) even when it is possible to distinguish fairly definitely between them and the "blacks." But in these earlier families no clear distinction can be drawn, which means that still greater variation occurs here. The results of the cross with Stock I (Section 2) have also given a warning. So we must be prepared for recessives born black, and perhaps also for a variation towards redness in the heterozygotes; in short, for an 'overlapping' between the redder "blacks" and the darker "reds."

To cut the story short, it is in fact found possible to trace the career of the recessive gene through most of the families of the Stock. There is some uncertainty in several branches owing to the small number of survivors or smallness of the families, but reasonable certainty has been obtained sufficiently often to have shown up any discrepancy in this method of interpretation. If it be assumed that the same genotypes occur in the earlier families, the following are the conclusions reached on the phenotypic character of recessives and dominants.

(b) "*reds*." The colour shown by specimens known to be recessive for the greater part varies within the limits of the range of colour change represented by the following scheme.



Among 142 individuals every one of these changes has occurred, nearly all of them several times. The course of change is not always uniform and direct, but subject to fluctuation. For example, a specimen starts, say, at RB and at maturity is RP. Before this it may have passed through an IR stage, or a PB stage, or both. Redness may fall off and later increase again. Also a gradual change after maturity may occur. But the above

scheme covers all changes that have been found to occur over *any* chosen period.

There remain certainly 3, probably 12, cases where "reds" have assumed a darker colour than any included in the above scheme. The three include a B→RB→RP (OH745) (see Section 3), which is known to be recessive from the result it gave when bred from, a Black→Red, and a Black→PB, both the latter belonging to a brood (748) in a pure recessive branch.

Now, in the later families of the Stock there has been no case of "reds" ever having a colour even approaching Black; on the other hand, in the cross with Stock I (Section 2) some broods were black at birth. It is therefore valuable to have corroborative evidence on whether there is a right to expect black "reds" in these early families of the Main Stock. There is some evidence that this is so—and that the occurrence might even be a common one.

The evidence is as follows:—(1) Family 628, in the 5th generation of a pure "red" branch, was composed of 4 Blacks and 15 Reddish Blacks (**9**, Chart 4). Of the two surviving Blacks one changed to Purple Black, and the other went all the way to Bright Red. (2) Family 658a, which contains the Black→RP specimen mentioned above and in Section 3, might be considered a 3 to 1 ratio, yet the eye-colours at birth were 113 Black and 8 Reddish Black, in which case a number of "reds" besides the particular specimen were born black. (3) There is a certain branch which almost certainly should rank as pure recessive. It arose from an F_3 pair, one DR unchanged, the other B→RB. Their family contained, at birth, 16 B, 43 RB, and 31 IR, most irregularly distributed among the broods (**9**, p. 216). Of the blacks, the 2 survivors reddened to IR. The next generation contained mostly RB's (at birth) and 1 Black; but the only third generation family, derived from 2 RB→Purplish, was composed of 21 B and 5 RB. (4) It is probable that family 563b, containing 18 B (2 unchanged) and 16 RB (2→PB), is all recessive. If this is so (and considerable difficulties in the interpretation of related families arise, if it is not), then an instance would be provided in which "reds" may even be Black unchanged!

It is noteworthy that in these small apparently recessive families which have given blacks (at birth), the family as a whole is on the dark side, viz:—

1 B	.. ..	13 RB (448–3)
21 B	.. ..	6 RB (577)
18 B	.. ..	16 RB (563b)
4 B	.. ..	15 RB (628)

(c) "*blacks*." The form taken by the "blacks" is generally unchanged Black. The 10 specimens known to be pure are all Black unchanged, as are also 29 out of 41 impure. Among the impure are also the following:—

4 B→RB
1 B→RP
6 RB unchanged
1 IR→RB.

So it appears that a certain range must be allowed for variation in the heterozygous "Blacks." This range overlaps that of the darker forms of the "reds." An unchanging Reddish Black, for instance, may equally well be a "black" or a "red," and does in fact occur for both in the same brood of one of the F_2 families. Fortunately abnormal specimens like the IR→RB do not seem to occur often.

(d) *Character of the original F_2.* The original F_2 family (**9**, Chart I) in which red-eyed individuals first appeared is found to contain the following recessives :—

IR→DR	IR unchanged
DR→RB	DR→RB
RB→Red	RB→IR
RB unchanged	

and the following heterozygous "blacks" :—

RB unchanged	RB unchanged
RB unchanged	B→RB
B unchanged	B unchanged
B unchanged	

while one B unchanged is known to be pure.

It is not possible to discover the character of this F_2 family. The 5th, 6th, and 7th broods seem to be producing "blacks" and "reds" in a 3 to 1 ratio, but it is not easy to see where many recessives might be found among the first 4 broods, of which unfortunately few survived.

(e) *Conclusion.* The effects of a recessive gene can be traced back into the early families of Stock V. The higher temperature seems to have had the effect of widening the range of phenotypic expression, thus very largely obscuring the difference between recessives and dominants.

5. Variations in Stock V Recessives.

It has been seen that r_5r_5 types of the Main Stock V show considerable variation in eye-colour shade and in the course of change during life. The explanation of the wide range of observed colour shades in terms of pigmentation is that both the red and the black pigment vary, and do so independently of each other (**9**, pp. 204–206).

The question next arises as to whether there are hereditary relations underlying any of these variations. In considering this point it is convenient, and indeed necessary, to treat the two pigments separately. With each pigment the general behaviour has first to be examined.

Black Pigment. At extrusion there is always at least some melanin present, even when the eye is bright red. Confining attention to the Main Stock at laboratory temperatures, we find that the amount present may

vary from very slight (New Red) to considerable (Reddish Black). This implies that there has occurred in the few days preceding extrusion a process of melanin deposition similar to that which occurs at this time in the normal black eye, but retarded to a greater or lesser extent. During life, the intensity of melanin is usually either maintained at a constant level, not changing from its condition at extrusion, or else shows a gradual decline. The course followed is generally uniform, involving, so far as can be seen, no larger fluctuations than would be expected to result from variations in growth rate, etc. It is to be noted that the intensity does not *increase* to any great extent beyond the condition reached at extrusion. If, indeed, the earliest growth stages are excepted, it can be said that no general increase in intensity occurs. All the more conspicuous examples of darkening after extrusion are confined to cases of darkening in early life. It thus appears that at a certain time, which may coincide with extrusion or come not very long after, the concentration of melanin reaches a point after which it increases no further, but remains at equilibrium or gradually falls away.

To this extent, then, is it possible to generalise on the behaviour of the melanic pigment in Stock V recessives. A slow accumulation of melanin continues until a certain critical stage, after which deposition suffers a check. From this point deposition may be sufficient to maintain the same relative concentration, but more often is so retarded that the intensity declines steadily. The amount of melanin accumulated before the critical stage may vary considerably, and the time of occurrence of the critical stage varies over a period between extrusion and some time during early life. The extent of subsequent fall of intensity may vary from nothing to that seen in eyes which are Reddish Black at birth and New Red at maturity.

A striking characteristic is that in all eyes,* whatever the shade and whatever the course of change in pigmentation, the melanin is uniformly distributed through all the ommatidia. This state of affairs contrasts strongly with that seen in a darkening Stock I "red," in which the melanin concentration decreases from the centre to the periphery (see p. 310). The latter case is susceptible of the simple explanation that each ommatidium undergoes the same cycle of change, and the older ommatidia in the centre will therefore be at a more advanced stage of darkening. It follows that the slower or the more limited the course of darkening, the less contrast will be shown between the centre of the eye and the periphery and the more uniform the distribution of pigment. Accordingly among Stock V recessives, in which darkening is either negligible or restricted to

* With possible exceptions among early growth stages. This statement is based mainly on the examination of preserved material, which consists almost entirely of later immature stages and adults. It may be expected that specimens which do not reach the maximum darkening till, say, the 2nd moult should show a darker centre.

a period of short duration in early growth stages, there is no opportunity for the centre to become appreciably darker than the more peripheral parts. Here expectations are fulfilled. If, however, all ommatidia follow the same course of change in eyes in which the concentration steadily falls, it is to be expected that the central ommatidia would be at a more advanced stage of lightening than those situated nearer the periphery, so that the eye would show a pale centre and a darker outer border. Such a condition is never found. The newer ommatidia on the periphery acquire the concentration of melanin that exists in more central ommatidia but do not surpass it, even though the older have previously passed through a considerably darker stage. It is therefore clear that the process which leads to decrease in melanin concentration prevents the ommatidia from following their individual course of change. It is a process which affects the whole eye, and not the ommatidia as discrete units.

We are therefore brought to the recognition of two distinct phases in the deposition of melanin. Phase 1, evident in earlier stages, results in accumulation of melanin, and is comparable to the darkening process occurring in the normal black eye and in Stock I "reds" at higher temperatures. Phase 2, coming into effect at, or not long after, extrusion, is marked by a check in the further accumulation of melanin, affecting the eye as a whole, and results in uniformity of pigment distribution over the whole eye, which may remain in a state of equilibrium or gradually grow paler. There are reasons for supposing that at the onset of phase 2 a physiological change in the process of melanin formation is involved. This is considered at a later stage.

The above consideration is based on the character of the recessives in the Main Stock kept at laboratory temperatures. It should however be mentioned that the behaviour of r_5r_5 types in other strains, and in the earlier families of the Main Stock kept in the incubator, falls into the same general category. In the case of recessives reared at incubator temperatures, it is noticeable that at extrusion the average level of melanin concentration is decidedly higher than in those hatched in the laboratory. This accords well with expectations; for if the early phase of melanin deposition is comparable with that occurring in the normal eye and in reds of Stock I, then it should be accelerated with increase in temperature. During the second phase, however, the higher temperature does not appear to have brought about darkening; and further tests substantiate the supposition that during this phase heat has no effect on the rate of melanin deposition. In the strain derived from a cross with Stock I (see p. 321), the eye-colour sometimes reaches Black before phase 2 sets in.

To summarise the effect of the recessive gene r_5 on the formation of melanic pigment would involve repetition of the second paragraph of this section. More briefly stated, the influence of the gene is seen in (1),

the retardation of melanin accumulation in phase 1, and (2), the inhibition of phase 1 at an early stage by the introduction of whatever influences underly the occurrence of phase 2.

The main variations that are found in r_5r_5 types are in respect to the following :—(a) degree of retardation of melanin deposition in phase 1, as evidenced by eye-colour at extrusion ; (b) time of onset of phase 2, indicated by the extent of darkening during the first week or two of life ; (c) in the extent of decrease in concentration of melanin during phase 2. The presence of modifying hereditary factors may be sought in reference to these sources of variation. In respect to (a), eye-colours recorded at extrusion constitute the available data. These can be investigated for indications of hereditary relations. As regards (b), the information available is not adequate ; but what is known of (c) affords a possible basis for further study.

(a) That eye-colour at extrusion is at least partly dependent on non-inherited influences is evident from the striking dissimilarity that often exists between broods of the same family. Some examples are given in Table I.

(In these examples eye-colours are grouped as follows :—Red, IR (Intermediate Red), DIR (Dark Intermediate Red), DR (Dark Red), RB (Reddish Black), and B (Black). Since all eyes at extrusion contain red pigment, the darkness of the red is a fair indication of the concentration of melanin present.)

In all instances there is a statistically significant irregular distribution among the broods of the eye-colour types contained in the family. This means that the occurrence of different eye-colour types cannot be explained wholly in terms of genetic segregation.

These instances illustrate a general tendency among recessives both of the Main Stock and of other strains. It is clear that differences in eye-colour at birth cannot be simply related to genotypic differences until at least some correction can be introduced which eliminates distinctions between different broods of the same pair.

There is also a noticeable tendency for members of the same brood to exhibit identical characters, while a similar uniformity does not exist among the whole family.

As regards the relation between parents and offspring of successive generations, the results are again negative. No correlation is apparent.

When these facts are taken together, it must be concluded that underlying hereditary factors could only be detected by statistical treatment requiring prohibitively extensive numerical data.

(c) During the second phase, the concentration of melanin may remain uniform or gradually decrease. The information on this matter is however of a much more limited kind than that treated in (a). In the first place,

TABLE I.

Families Reared at Laboratory Temperatures.

No. 838 b—i.

Brood			
No. 1.		4 DR	5 IR
2.			9 IR
3.	9 RB	.	9 IR
4.		7 DR	13 IR
5.	15 RB	1 DR	
6.			3 IR
7.	7 RB	6 DR	

No. 830 a—ii.

Brood			
No. 1.		9 IR	
2.		18 IR	
3.	8 RB	18 IR	
4.	2 RB	19 IR	
5.	5 RB	7 IR	
6.	11 RB	16 IR	
7.	16 RB	4 IR	

No. 1004—ii.

Brood			
No. 1.			4 IR
2.	8 RB		
3.		9 DIR	
4.		14 DIR	
5.			10 IR
6.	8 RB		
7.			12 IR
8.	5 RB		3 IR
9.	4 RB		

No. 831 c 2.

No. 1.		12 IR	
2.		22 IR	
3.		12 IR	8 Red
4.	20 RB	3 IR	

Families Reared in Incubator.

No. 515 a.

Brood			
No. 1.		3 IR	
2.		2 IR	
3.	5 DR	2 IR	
4.		15 IR	
5.	10 DR	2 IR	
6.	9 DR	2 IR	
7.	5 DR	2 IR	
8.	11 DR	1 IR	
9.	12 DR	8 IR	
10.	25 DR		
11.	8 DR	5 IR	

No. 314 b.

Brood			
No. 1.			15 IR
2.		15 RB	2 IR
3.	9 Black		
4.		12 RB	10 IR
5.	7 Black	16 RB	4 IR

No. 330 a & b.
(a 1 : 1 ratio of R_5r_5 to r_5r_5.)

Brood				
No. 1.	6 Black	.	.	4 IR
2.	7 B	.	.	5 IR
3.	8 B	.	.	14 IR
4.	13 B	.	5 RB	
5.	11 B	.	6 RB	. 1 IR
6.	6 B	.	6 RB	. 5 IR
7.	9 B	.	4 RB	. 10 IR
8.	10 B	.	11 RB	. 1 IR
9.	8 B	.	5 RB	
10.	7 B	.	10 RB	
11.	18 B	.	3 RB	

attention is necessarily confined to individuals which survive for some length of time after extrusion. The numbers in each family are thus greatly reduced. Secondly, the amount of the red pigment varies considerably. This means that the resultant eye-colour gives only an indication of the concentration of melanin. Owing to the fluctuation of the red pigment, it is only possible to derive an approximation of the course of change in the melanin, which at the most is not subject to very wide variation.

All that can be said is that while on the one hand there is a noticeable tendency for members of the same family to behave in the same way; on the other hand no relation can be discovered between successive generations, and there are indications of other non-hereditary fluctuations.

The conclusion on the matter of variations in melanin pigmentation of Stock V recessives may be stated thus: while no hereditary relations among them have been discovered, the variations are at least partly dependent on non-hereditary influences.

Red Pigment. While fluctuation in the concentration of red pigment occurs very frequently among Stock V recessives—and indeed is responsible for the more striking changes in eye-colour—there is no reason to suppose that the gene r_5 has necessarily any direct effect upon it. It is only because the r_5r_5 types are deficient in melanin that in them alone can changes in the red pigmentation be observed. It is not therefore possible to go beyond the statement that, in Main Stock V and in strains derived from it, fluctuation in the red pigment is prevalent. This characteristic in itself contrasts Main Stock V with, for example, Main Stock I, in which the r_1r_1 types exhibit an ample and unvarying concentration of red pigment.

An opportunity for observing the variation that occurs with respect to the red pigment is afforded particularly by branches of Stock V composed entirely of r_5r_5 individuals. The strain HC derived from a cross with Stock I is also useful in this respect (see p. 321). Confining attention, however, to the Main Stock, we may briefly refer to such definite statements as can be made at the present moment.

It may be recalled that eyes frequently acquire a purple shade of greater or lesser degree. (The shades are classed as Purplish Black, Purple, and Reddish Purple.) The cause of this is a loss of red pigment. In Purplish Black and Purple eyes it may be almost or entirely absent. The dull purple colour, given by a dilute deposit of melanin, becomes brighter and more reddish as the concentration of red pigment increases. The appearance, then, of a purple shade may be used as an indication of decrease in red pigment.

At extrusion, no instances are known in which red pigment is sufficiently lacking to give a purple shade. For the course of change after extrusion, generalisation is difficult. Some examples retain a more or less ample concentration throughout life; in others, however, a decrease becomes evident some time during the earlier immature stages. The course of change which is more usual than any other is a loss of red pigment during immature stages followed by a revival in its production coinciding approximately with the onset of maturity. Examples exhibiting this type of change are sufficiently numerous to deserve special comment, and invite further investigation. It sometimes happens that the revival in the production of pigment is delayed, or is only slight, or does not occur at all. Also the amount present after the revival may fluctuate.

It is exceptional for those examples which appear to have retained their

red pigment all the time up to maturity to lose it afterwards (at any rate, before signs of ageing set in). Therefore two main classes of individuals may be distinguished. One typically retains pigment through life ; the other shows loss during immature stages, and usually a revival about the time of onset of maturity. The genetic relationship between these two classes may be tested.

The information available suffers from certain limitations, such as its concern with survivors only, and the comparatively infrequent examination of specimens. It cannot on this account be expected to give more than indications.

There is an unmistakable tendency to resemblance between members of the same family, even though many broods be involved. Thus many families are constituted predominantly of one or the other of the two types of individuals. This is a point in favour of the presence of underlying hereditary factors. On the other hand there is no obvious relation between parents and offspring of successive generations. The question of hereditary relations therefore remains open. In any case at least minor non-hereditary fluctuations have to be admitted.

Examination of Stock V recessives in other strains does not give any additional help. In some families both ' purpling ' and ' non-purpling ' individuals occur, apparently unequally distributed among the broods, but the numbers are not sufficient to warrant any definite statement on this point.

The above consideration of the variations among Stock V recessives justifies the following conclusions. Differences in eye-pigmentation in part at least cannot be explained in terms of hereditary differences. The clearest piece of evidence for this is the frequent significant disparity between broods of the same family. That is, the variations are at least partly of an ' environmental ' nature. At the same time no modifying hereditary factors have revealed their presence. If any are at all directly concerned, then they exert their influence—on the average character of the family—in such a way as to be detected only by very extensive numerical data.

Elucidation of the inheritance of Stock V eye-colours cannot proceed further until more is known of the environmental factors which influence the particular form of unstable eye-colour associated with individuals recessive for the gene r_5. Consideration has to be given not only to obvious external factors such as temperature, conditions of the water in the culture bowl, state of food, and so on ; but also to ' internal ' factors of the kind that have been demonstrated by Ford and Huxley (**7**, pp. 71–76) who, in emphasising the dynamic aspect of eye-pigmentation, show how variations in rate of pigment production, growth of ommatidia, and general body-

growth, in relation to one another, may be sufficient to give marked variation in eye-colour. Also something may depend on the cytoplasmic legacy with which the individual started off, thus introducing as a possible effective factor the physiological condition of the mother at the time the eggs are formed.

VII. STOCK VI RED-EYE.

The origin of a new type of red-eye among the F_2 from a mating between a Stock V recessive ♂ and Outside ♀ has been described on p. 323.

The eye-colour of Stock VI " reds " is of a Normal Red, very like that of Stock I. At laboratory temperatures no melanin develops in the eye at least before maturity (older specimens have not yet been investigated). As in the " reds " of all strains that are connected with Mutant Stock V, there is a tendency for the amount of red pigment to diminish.

Stock VI Red-eye behaves as a simple mendelian recessive character, and its appearance is ascribed to the presence of a gene r_6. So far r_6 has proved distinct from r_1 as well as from r_5. Investigation is being continued.

VIII. SUMMARY OF " RED-EYE " RECESSIVE TYPES.

A description has been given of 6 Stocks (Mutant Stocks I to VI), each of which contains red-eyed individuals of some form or another.

It is found that in each case the occurrence of redness is associated with a single recessive gene which influences the process of melanic pigment production. A separate gene is involved in at least Stocks I, II, IV, and V. The genes are denoted respectively r_1, r_2, r_4, and r_5, replacing the ' normal ' genes R_1, R_2, R_4, and R_5. Stock III evidently contains a recessive gene (r_3), which is definitely distinct from r_1 and r_2, and probably also, judging from its phenotypic expression, from r_4, r_5, and r_6. The gene r_6 is distinct from at least r_1 and r_5.

Each Main Stock consists of the inbred strain derived from the mating from which the recessive form originally arose. Investigation of the recessive types has been focussed on the respective Main Stocks, but at the same time it has been extended to strains derived from cross-matings or matings with animals from the wild. Each recessive type, in fact, has been studied in one or more inbred strains. Since differences are sometimes noticed in the phenotypic expression of one recessive type in different strains, a distinction between strains is maintained in discussion as well as in practice.

The effect of these genes is seen in some form of retardation or inhibition of the melanic pigment in the retinal cells. That they may also have some direct effect on the production of red pigment is not impossible,

but evidence on this point with respect to observed variations is so far to the contrary. It has been found that the quantity of red pigment may greatly decrease or otherwise fluctuate ; but these fluctuations are characteristic of certain strains and it seems that they can be observed in any of the *rr* types contained in those strains. So far no special investigation has been carried out with the view to determining what hereditary differences may underly the variation of the red.

r_1

The recessives (r_1r_1) of Main Stock I, at laboratory temperatures, have bright red eyes—with few exceptions melanin production is completely inhibited until old age. At 23° C., however, melanin appears a few days after extrusion, and gradually accumulates, until well before maturity the colour of the central ommatidia has reached a reddish brown or even black (see **6**, Fig. 1a). Strains darkening at different rates have been separated and the influence of genetic modifying factors has been discovered (**6**, p. 115). It is characterististic of the Main Stock that the red pigment retains a full concentration through life.

In some strains derived from cross-matings melanin deposition takes place more readily. A conspicuous instance of this is afforded by a strain derived from a cross with Stock III, in which the r_1r_1 types characteristically showed darkening already in immature stages. In certain strains a marked deficiency in red pigment occurs.

The effect of the gene r_1 is greatly to retard the normal process of accumulation of melanin in the eye. The extent of its action is influenced by temperature, as well as by the presence of modifying genes.

r_2

The r_2r_2 types of Main Stock II resemble, at laboratory temperatures, the r_1r_1 types of Main Stock I, failing to develop melanin to the same degree. The effect of higher temperature is, however, not known. A full quantity of red pigment is maintained in recessives of the Main Stock. So far as is known, r_2 exerts the same effect as r_1.

$?\ r_3$

On analogy with Stock V, the occurrence of red-eyed forms in Stock III is ascribed to the action of a gene r_3. Different grades of eye redness occur. The action of the gene r_3 is probably to be compared with that of r_5.

r_4

The r_4r_4 types were either Dark Red at extrusion and subsequently lightened to a Light Red, or they were Light Red through life. Among certain strains derived from crosses, there occurred a few specimens which remained Dark Red. The brightest of the Light Reds evidently contained no melanin, but probably some of those born Light Red and certainly all the Dark Reds carried a deposit.

The effect of the gene r_4 is rather different from that of r_1. There may

be a slow accumulation of melanin before extrusion, but afterwards production is inhibited, if not altogether, at least to such an extent that it does not keep pace with growth-rate. There is a suggestion of two distinct phases, as in the case of $r_5 r_5$ types.

r_5

The recessives of Stock V ($r_5 r_5$ types) show partial inhibition of melanin formation, the extent varying considerably among individuals. The colour shades and the changes which occur during life owe their variety to the fact that both the black and the red pigment are subject to independent variation. The red pigment fluctuates in all strains investigated.

Concerning the course of melanin production in recessives, the following generalisation seems justifiable. The pigment slowly accumulates (phase 1) until a point is reached beyond which no further relative increase takes place, but either a state of equilibrium is maintained, or the intensity gradually declines (phase 2). The intensity attained at the critical point, which occurs at some time in early immature stages, varies considerably. During phase 2 it is characteristic that the melanin is distributed uniformly over the eye, and there is evidence that temperature has no accelerating influence.

The effect of the gene r_5 may summarily be stated as follows. The normal process of melanin accumulation is retarded to a varying degree, and at a certain point is checked by the introduction of whatever conditions underlie the equilibrium state characteristic of phase 2.

No other genes, either modifying the effect of r_5, or underlying the fluctuations in the red pigment, have been detected. Significant differences in the composition of broods of the same family, and other indications, show that the variations among $r_5 r_5$ types are at least in part of a non-hereditary nature.

r_6

The $r_6 r_6$ types have a bright red eye. The gene r_6 appears to inhibit melanin production as effectively as r_1. So far it has been proved distinct from r_1 and r_5.

Reference may be made to two further recessive types which are dealt with in a separate paper (p. 337 of this Journal, Vol. XVIII). These are 'Flesh' Red-eye and 'Beet' Red-eye, associated with the genes *f* and *t* respectively (Stock VII). The effect of *f* is very similar to that of r_1, but has been proved distinct; while *t* has a different effect from any previously encountered, and may be reasonably regarded as definitely distinct from any recessive genes hitherto described.

Our best thanks are due to Mr. E. B. Ford for reading over the preliminary manuscripts of this paper, and for the help of his opinion on

several points; and once again we have to express our indebtedness to Dr. E. J. Allen for much valuable advice and assistance.

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An Experiment on Breeding Wild Pairs of *Gammarus chevreuxi* at a High Temperature, with an account of Two New Recessive Types of Red Eye.

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With 1 Figure in the Text.

1. Results of Rearing the F_2 from 13 Wild Pairs.

On each of two former occasions when specimens of *Gammarus chevreuxi* Sexton have been brought from the wild into an incubator and kept at 21° C. or more, red-eyed recessive types have been reared among the F_2 progeny (**4**, pp. 190 and 194). In November, 1930, an opportunity occurred for making further tests on this point. Twenty-three wild pairs, taken in Chelson Meadows on November 19, were placed on the following day in the incubator, the temperature of which at first averaged between 21° and 22° C., but was raised after a few days to an average of between 22° and 24° C. The young which were extruded from eggs laid in the wild were discarded. Those of subsequent broods were reared with a view to obtaining as many F_2 families in each stock as would give a reasonable opportunity for segregating recessive characters to appear. The F_2 young were examined for recognisable variations that might indicate the presence of a recessive character.

Of the original pairs, 8 gave no young; 2 a small F_1 but no F_2, while 13 gave a smaller or larger number of F_2 families. The results obtained from the rearing of the 13 stocks are summarised in Table I.

The eyes of the original pairs and all the F_1 were of the normal type; that is, black with white accessory pigment.

It may be noted, however, that neither the black nor the white pigment was always present in a full concentration. Among those brought in from the wild, many showed a slight deficiency of black, appearing very dark purplish or slightly reddish rather than jet-black. The deficiency shown in a tendency to reddishness was accentuated in many of the F_1 families. In most of the stocks definitely Reddish Black (**4**, Plate VIII) individuals were noted. Reddishness was most conspicuous in stocks *II*, *XIV*, *XVIII*, and *XX*. On the other hand a more typical concentration of black seemed characteristic of *IX*, *XVI*, *XXII*, and a cross between *XXI* and *XXIII*.

Some of the original wild specimens also showed a noticeably thin reticulation of white pigment. A greater or lesser deficiency in this pigment was found subsequently in all stocks, particularly in *XIV*, *XVIII*, *XXII*, and *XXIV*. The deficiency, which involved thinning of the reticulation or irregular clumping of the pigment, developed gradually during life.

Among the F_2, as the table shows, 10 stocks produced nothing but blacks of the normal type, but 3 stocks gave, in some of their families, certain conspicuous variations in eye pigmentation, which were found to behave as mendelian recessive characters. These recessive types included the following.

1. *No-White*, appearing in stock *XVI*, has the white accessory pigment of the eye completely lacking. It resembles (and indeed proved to be

TABLE I.

RESULTS OF REARING THE F_2 FROM 13 PAIRS INTRODUCED FROM THE WILD INTO INCUBATOR (NOV. 1930).

No. Stock.	1. Character of F_2.	2. OCCURRENCE OF RECESSIVES. Number of families in which recessives appeared.	Family examined in which recessive was first found.	Ratio Recessive : Normal.	3. Total number of F_2.	4. Number of adequate F_2 families, or approximate equivalent.
I	All Black Normal				108	4
II	All Black Normal				255	13
VI	All Black Normal				259	4 or 5
IX	All Black Normal				212	6 or 7
XI	All Black Normal				56	2 or 3
*XIV**	"*Flesh*" *Red-eye* appears in some families	7 out of 35	2nd	approx. 1 to 3 ratio	2,917	c. 40
	"*Beet*" *Red-eye* appears in some families	6 out of 35	4th	approx. 1 to 3 ratio		
XV	"*Flesh*" *Red-eye* appears in some families	2 out of 3	1st	apparent 1 to 3 ratio	54	3
XVI	Black "*No-white*" eye appears in some families	3 out of 7	3rd	apparent 1 ro 3 ratio	234	6 or 7
XVIII	All Black Normal				116	6
XX	All Black Normal				158	10
XXII	All Black Normal				178	9
XXIII	All Black Normal				28	1
XXIV	All Black Normal				152	6 or 7

TOTAL NUMBER OF PAIRS 13

EFFECTIVE NUMBER OF PAIRS (i.e. equivalent value of pairs giving a fully adequate F_2, calculated from probabilities given by figures in column 4) . (approx.) 10

Hence 4 recessive genes occurred in an equivalent of 10 pairs (= *circ.* 20 individuals).

N.B. Only those Stocks which gave an F_2 are included. The young were not generally examined immediately after extrusion.

genetically identical with) the already familiar No-White recessive form which occurred in the earliest of the Mutant Stocks (1).

2. *Flesh Red-eye* appeared in two separate stocks, *XIV* and *XV*. The eye is typically of a pale red colour. The production of black pigment is postponed and greatly retarded, so that even in old specimens there is no more than a central darkening. (For further description see p. 345.)

* This stock (*XIV*), containing two recessive forms, is being maintained as Mutant Stock VII.

3. *Beet Red-eye* appeared in stock *XIV* and segregated independently of Flesh Red-eye. The eye has a red colour at extrusion, but there is an appreciable amount of dark pigment present, which gives it the appearance of New, Intermediate, or Dark Red (**4**, Plate VIII). During the earlier growth stages the eye darkens rapidly to a Reddish Black, or even Black, the final state varying among individuals. Beet Red is quite unlike any other recessive form hitherto discovered (see p. 350).

There is no reason for supposing that the occurrence of these recessive forms is in any way connected with the tendency to reddishness or deficiency in white pigment noted above. Not only did the latter conditions exist in stocks which produced no recessives, but in those stocks in which the recessives occurred, pure and impure dominant were affected alike.

The manner of occurrence of the recessive forms is susceptible of the simple explanation that one of the original parents of the stock had been heterozygous for the recessive factor involved. Half the F_1 would therefore be heterozygous, and on the average 1 in 4 F_2 families would contain recessives in a 1 to 3 ratio. That this condition actually held is strongly supported by evidence from data dealt with in subsequent sections of this paper.

This implies that, among the 26 original parents, there existed 4 recessive genes in a heterozygous state, the gene for Flesh occurring twice. The question whether any of the original parents may have mutated or whether the genes were brought in from the wild, is now receiving special investigation, and cannot be discussed at this stage.

Since the probability of detecting the recessive genes carried among the animals which constituted the original pairs is dependent on the number of F_2 families obtained from an in-bred F_1, there is in practice always a greater or lesser chance that any present will escape notice. In this case, the presence of 4 genes was detected among the 26 animals ; but for an estimate of the *total* number of such genes as were present, this constitutes a minimum figure. Calculation, based on the number of F_2 families in each case, shows that among the 13 stocks there was an approximately 77% chance of recessives appearing. If 77% probability gives 4, 100% probability should give 5—hence for an estimate of the number of recessive genes carried among the animals introduced into the incubator, the experiment shows 5 in 26. This can also be expressed as 4 in approximately 20. While the figures are far too small to indicate the true proportion, whether it be of the number of genes in the wild population, or of the mutation rate, it is convenient for purposes of comparison with similar experiments to state the results in this way. The estimated 10 pairs may be described as the " effective " number of pairs, this being an estimate of the number of pairs that would, in this case, give 4 recessive characters if a full chance were allowed for all recessives to appear.

2. Occurrence of Recessive Forms.

"*No-White.*"

No-Whites occurred in three out of seven F_2 families in stock *XVI*, constituted as follows :

	F_1 *pair.*	F_2 *family.*
1	8 Black Normal	
2	45 B. Normal	
3	21 B. Normal	4 B. No-White
4	4 B. Normal	
5	44 B. Normal	
6	52 B. Normal	9 B. No-White
9	14 B. Normal	7 B. No-White

In addition, there were 18 B. Normal and 6 B. No-Whites in a brood bowl which contained, among others, ♀ and ♂ of Pair 6.

Though, on the average, only 1 in 4 adequate families would be expected to contain No-Whites, the above results are not incompatible with the supposition that No-White appears as a consequence of the heterozygosity of one of the original pair *XVI*.

It so happens that the original ♂ *XVI* was involved in two other matings. An F_2 generation was obtained from each, and in each case No-Whites appeared.

(1) Mating with ♀ *VII*.

F_1 survivors : ♂, 2 ♀♀.

F_2	25 Black Normal	5 B. No-White.

(2) Mating with ♀ *XV*.

The F_1 were divided among five bowls, several reaching maturity in each. The F_2 young were periodically removed, the total proportions in each bowl being constituted as follows :

Bowl 1	44 Normal	1 No-White
2	19 Normal	6 No-White
3	72 Normal	12 No-White
4	43 Normal	—
5	147 Normal	8 No-White
Total	325	27

Random mating would be expected to give a 15 to 1 proportion, with a wide deviation for samples of a few of each sex.

These results are sufficient to warrant the conclusion that ♂ *XVI* was heterozygous for No-White.

From matings between these No-Whites and those of Mutant Stock I (i.e. the Stock containing the original Red-eye recessives, Allen and Sexton, **1** : for No-White see p. 326), No-Whites only were obtained. It is therefore concluded that the same gene (*w*) is involved. Since No-Whites have occasionally been found in the wild (this is stated on Mrs. Sexton's

authority), this recessive gene is doubtlessly distributed among the wild population. There is no need to go further afield for an adequate explanation of the appearance of the recessive No-Whites.

"Flesh Red-eye."

Animals with pure red eyes, varying from "Normal Red" (**4**, Plate VIII) to almost colourless, occurred in several of the various F_2 families of stock *XIV*, as well as among the few of stock *XV*.

In stock *XV* the F_2 families were as follows :

Pair 1	8 Black	3 Flesh Red
♀4 ♂4	6 Black	
♀4 ♂3	13 Black	
Pairs 6 (2 ♀♀, 3 ♂♂)	20 Black	3 Flesh Red

In stock *XIV* Flesh Reds were given by the following matings :

Pair 6	59	Black	24	Flesh Red
Pair 16	39	,,	20	,,
♀18× ♂6	47	,,	15	,,
Pair 22	83	,, †	29	,,
♀28×♂21	54	,, †	23	,,
♀49b×♂14	53	,,	24	,,
♀10× ♂42	25	,, †	9	,,
♀10× ♂46	36	,, †	12	,,
Pair 33	29	,, †	6	,,
♀33×♂36	11	,, †	6	,,
♀4× ♂2	15	,,	5	,,
♀9× ♂2	27	,,	10	,,
♀12 ♂14	17	,, †	5	,,
♀12 ♂21	8	,, †	2	,,
♀30×♂36	15	,,	6	,,
♀13b ♂16	7	,, †	6	,,
♀13b ♂22	16	,,	4	,,
♀31× ♂31	25	,,	10	,,
♀31× ♂32	10	,,	4	,,
♀54×♂31	13	,,	2	,,
♀ 7*	5	,, †	2	,,
♀10*	4	,, †	3	,,
♀16*	4	,,	1	,,
♀22*	6	,,	1	,,
	608		229	

N.B. The broods were not necessarily examined at extrusion. The recessives, however, gave not the least indication of being less viable than the dominants.

* Mated in brood bowl. † Including Beet Reds (see below).

The total ratio in these families is 608 to 229, or 2·66 to 1. The divergence from the expected 628 to 209 proportion (for which the larger families are responsible) is less than twice the standard deviation and is not to be regarded as significant.

A considerable number of the F_1 of stock *XIV* were mated among each other, the matings being controlled so that the genetic potentialities of as many individuals as possible might be revealed (see section 3). Among 72 (36 ♀♀ and 36 ♂♂) which gave any F_2, 28 (16 ♀♀ and 12 ♂♂) were involved in matings giving "Flesh Reds," while 28 (11 ♀♀ and 17 ♂♂) gave adequate families containing no Flesh when mated with individuals that were known to carry it. There were, in addition, 4 pairs of unknown constitution which gave adequate families containing no Flesh.

This result is in agreement with the expected genetic interpretation that approximately half the F_1 were heterozygous, and half homozygous dominant. Stated in genetic terms, therefore, 28 were shown to be heterozygous; while the number of homozygous is 30—including 28, proved directly, and a further 2, representing the probable excess of homozygous over heterozygous in the 4 pairs mentioned above. So, if the recessive gene concerned is denoted as *f*,

30 represents the number of *FF* types
28 ,, ,, *Ff* ,, .

Ample evidence that the Flesh Reds in stock *XIV* were genetically identical with those in stock *XV* was obtained by different crosses between the two stocks.

The ♀ of original pair *XV* was also mated with the ♂ of *XVI* (see p. 340). The F_2 from this mating has already been described. Though ample opportunity was given for recessive characters to show (cf. appearance of No-White), no Flesh Reds appeared. Hence it may be concluded that ♀ *XV* did not carry Flesh Red. The onus therefore falls on the ♂.

The above results fall in line with the supposition that one member of each of the original pairs *XIV* and *XV* was heterozygous for a recessive gene *f* (in *XV* it must have been the ♂). The *ff* types have a red eye, characteristically of a pale normal shade. (For further details of the character of *ff* types see p. 345.) The expected equal proportions of homozygous and heterozygous in the F_1 is well seen in stock *XIV*.

It may be further noted that the recessive Flesh Reds gave no signs of being weaker, less productive, less vigorous, or in any way less viable than the normal dominants. This is all the more noteworthy as the whole stock was conspicuously healthy.

The 80 F_2 Flesh which reached maturity included 35 ♀♀ and 45 ♂♂.

"*Beet Red-eye.*"

Individuals with darkening red eyes occurred among the young in some of the F_2 families of stock *XIV*. It was soon found that these

showed a characteristic course of colour change, falling into a definite category to which the term " Beet Red-eye " was applied (see p. 350). While it was always possible to distinguish Beets from Flesh Reds, Beets could only be unquestionably separated from Blacks in the earliest stages.

It was also soon evident that the Beets were segregating in the manner of a recessive character, the segregation being independent of that of the Flesh Reds. Some matings gave Flesh only, others Beet only, and others gave both. The composition of the families in which Beets appeared was as follows :—

a.	♀10 × ♂42	20	Black	5	Beet Red (f)
	♀10 × ♂46	28	,,	8	,, (f)
	♀10 × *	2	,,	2	,, (f)
	♀13a × ♂36	18	,,	6	,,
	♀13b × ♂22	14	,,	2	,, (f)
	♀13b × ♂43	37	,,	9	,,
	Pair 14	0	,,	2	,,
	♀19 × ♂14	33	,,	3	,,
	♀19 × ♂20	10	,,	4	,,
	Pair 22	9	,,	3	,, (f)
	♀28 × ♂21	44	,,	10	,, (f)
	Pair 33	20	,,	9	,, (f)
	♀33 × ♂36	7	,,	4	,, (f)
	♀35 × ♂22	6	,,	2	,,
	♀43 × ♂22	1	,,	2	,,
	♀43 × ♂42	31	,,	7	,,
	♀43 × ♂43	9	,,	3	,,
	♀43 × ♂52	15	,,	1	,,
	♀49a × ♂14	27	,,	4	,,
	♀12 × ♂21	6	,,	2	,, (f)
		337		88	
b.	♀10 × ♂18	48	Black	17	Beet Red
	♀4 × ♂17	5	,,	1	,,
	♀7 *	3	,,	2	,, (f)
	♀12 *	3	,,	2	,,
	Pair 14	2	,,	2	,,
	♀15 *	2	,,	2	,,
	♀31 *	6	,,	1	,,
		69		27	

a. Broods examined within 2 days of extrusion.

b. Broods examined a little time after extrusion, but Beets still absolutely distinguishable from the Blacks.

Some other families and broods in which the Beets could not be separated with certainty from the Blacks, owing to age, are not included.

(f) These families also contained Flesh Reds.

* Mated in brood bowl.

The figures show an excess of Blacks over Beets on the expected 3 to 1 ratio, which is very clearly not due to the postponement of examination of the broods. If attention is confined to those examined early (a), the excess is twice the standard deviation and may indicate a significant divergence from the simple mendelian proportions. The excess is greater still, being nearly $2\frac{1}{2}$ times the standard deviation, if only the larger families (from both a and b) of over 15 are taken into account, the proportion then being 325 to 81. The figures are too small to justify any more definite pronouncements, but at least provide grounds for more detailed investigation.

Beets were subsequently found to breed true, thus confirming their genetic character. It may be supposed that a recessive gene *t* is involved.

The tests for the genetic constitution of the stock *XIV* F_1, as described for Flesh Red, can be applied at the same time to Beet Red (see p. 342). If *t* is taken to denote the recessive gene involved, the results give

31 *TT* (12 ♀♀, 17 ♂♂, 2 sex unknown).
30 *Tt* (16 ♀♀, 13 ♂♂, 1 ,, ,,).

This conforms with expectations. Presumably, therefore, either the ♂ or the ♀ of pair *XIV* was heterozygous for *t*.

Among the 80 F_2 Beets which survived to maturity, 42 were ♀♀ and 38 ♂♂.

3. Stock *XIV*—Distribution of Heterozygotes among the F_1 Generation.

From the results given by various matings among them, it was possible to deduce the genetic constitution of a considerable number of the F_1

TABLE II.

	♀	♂	sex unknown	Total.
FF	11	17	2*	30
Ff	16	12		28
Total	27	29	2	58
TT	12	17	1† 1*	31
Tt	16	13	1†	30
Total	28	30	3	61

FFTT			*FFTt*		
♀ 4	♂ 11	Total 15	♀ 5	♂ 4	Total 9
FfTT			*FfTt*		
♀ 6	♂ 4		♀ 9	♂ 7	
sex unknown 1		Total 11	sex unknown 1		Total 17

* To allow for certain pairs which gave no recessives; a correction which places the chances of detecting homozygotes and heterozygotes on equality.

† In this case shown by the constitution of the young.

family of *XIV*. It is assumed that two recessive genes, *f* and *t*, are involved. Reference has already been made to the results, which are summarised in Table II.

It is seen that homozygous dominants and heterozygotes of both types occurred in approximately equal numbers, and that the two recessive genes are at least largely independent of each other. The figures involved, however, are not sufficient to show minor deviations from the normal, as, for instance, partial linkage between *F* and *T*.

The distribution of the 72 F_1 adults investigated among the different broods of the F_1 family is as follows :—

Brood 1	♂ *FfTT*	♂ *FfTt*	2 ♂♂ unknown	
	♀ *FFTT*	♀ *FF–*	2 ♀♀ unknown	
,, 2	♂ *FFTT*	♂ *FFTT*	♂ *FFTT*	♂ *FFTT*
	♂ *FFTT*	♂ *Ff–*	♂ *FFTT*	♀ *FfTT*
	♀ *FfTt*	♀ *FfTt*	♀ *Ff–*	♀ unknown
,, 3	♂ *FFTt*	♂ *FF-*	♂ *FfTt*	♂ *FfTt*
	♀ *FFTt*	♀ *FfTT*	♀ *–Tt*	♀ *–TT*
,, 4	♂ *FFTT*	♂ *FFTt*	♂ *FFTt*	♂ *FfTt*
	♂ *FfTt*	♂ *–TT*	♂ *–Tt*	♂ *–Tt*
	♀ *FFTt*	♀ *FFTt*	♀ *FfTt*	♀ *FfTt*
	♀ *FfTt*	♀ *FfTT*	♀ *–TT*	♀ *FFTT*
,, 5	♂ *FFTT*	♂ *FFTT*	♂ *FfTT*	
	♀ *FfTT*	♀ *FfTt*	♀ *FfTt*	
,, 6	♂ *FFTT*	♂ *FFTT*	♂ *FfTt*	♂ *FfTT*
	♀ *FfTt*	♀ *FF–*	♀ *–Tt*	
,, 7	♂ *FF–*	♂ *FfTt*	♂ and ♀ unknown	
	♀ *FFTT*	♀ *FfTT*	♀ *FFTt*	
Broods 8 and after		♂ *FFTt*	♂ *FfTt*	♂ *FfTt*
	♂ *–TT*	♀ *FFTt*	♀ *FfTt*	♀ unknown
	♀ *FFTT*			

From the above it may be seen that the genotypes are not irregularly distributed. It may also be pointed out that heterozygotes of both kinds appeared in the first brood that the *XIV* pair produced in the laboratory.

4. Character of "Flesh" and "Beet" Recessives.

We may now consider more fully the appearance of Flesh and Beet recessives, and express more precisely the effect of the genes *f* and *t*.

This account may be compared with that of other recessive " red-eye " characters surveyed elsewhere (Sexton, Clark, and Spooner, p. 333 of this Journal, Vol. XVIII).

ff types.

The earliest examples of this class had eyes of a " Normal Red " shade (**4**, Plate VIII), but much paler than the typical " Normal " Red of Mutant Stock I. The appearance suggested a pinkish flesh colour, and the name " Flesh Red " was given to the recessive type. Among those reared in the incubator this pale red was the most usual colour, but the intensity of the red was subject to variation, and eye-colours ranging from typical Normal Red to colourless were obtained. At the same time, as the animals grow older, the central ommatidia begin to show darkening, owing to the production of melanic pigment.* In examples of more advanced age a thin deposit of melanin, concentrated in the centre, had formed over the whole eye.

When reared at laboratory temperatures (*circ.* 15° C.), the *ff* types did not show the same tendency to lose red pigment. The eyes were of a typical Normal Red or only slightly lighter. Though examples with very pale, and even colourless, eyes occurred in certain families, the contrast between the eye-colours in the laboratory and those in the incubator was most striking. A further difference lay in that in the laboratory darkening occurred either not at all or only to a very slight degree.

Though the above variations in the red pigment call for special consideration, for our present purpose of defining the character of *ff* types the red pigment has only secondary relevance. The essential difference between the *ff* recessives and the normal black-eyed form is that the eyes of the former are very greatly or entirely deficient in melanin. In the absence of melanin the existing red pigment becomes visible. This point is discussed in connection with other " red-eye " types (p. 308 of this Journal, Vol. XVIII). All these are more accurately termed " melanin-deficiency " types ; for it is only in respect to melanin formation that we are certain that the presence of the recessive gene is felt. Admittedly the production of red pigment, though involving a very different kind of chemical process, may yet be affected more or less directly ; but (a) there is no means of detecting any difference, as the red pigment is invisible in the black eyes of the dominants, and (b), even if there were a difference, it has still to be shown that it is not a secondary phenomenon dependent on the actual presence of black pigment and not on the genes affecting it. While, in the light of known instances of one gene affecting two widely different visible characteristics, it is not impossible that the black and red pigment should be affected at the same time, this should

* See footnote, p. 308.

not be assumed until there is definite reason for doing so. As yet no such reasons are forthcoming from possible sources of positive evidence. For instance, (i) no other body characters are known to be visibly influenced by the melanin deficiency genes, and there is no greater probability of the red pigment being affected than other characters. (ii) Evidence that the genes affect the red pigment would be obtained if there were differences in the state of the red pigment between two recessives, and if the difference were proved constantly to be associated with these recessive types. A notable difference exists, for example, between the r_1r_1 types and the *ff* types of their respective Main Stocks, at temperatures of 22° to 24° C.; a full concentration of red pigment being shown by the former and a marked deficiency by the latter. But, as is shown in the results of a cross-mating described below, the difference is not maintained when the two genotypes segregate in the same family.

In discussion of the gene *f*, it is seen from the above considerations that in the present state of knowledge we have to regard its sphere of action as limited to the process of melanin production.

The effect of *f* is very similar in kind to that of the original Red-eye gene, r_1. The process which normally takes place in the 2 or 3 days preceding extrusion, resulting in rapid accumulation of melanin in the eye, is very greatly, if not completely, retarded. At laboratory temperatures the younger mature specimens have usually not yet even started to produce melanin, and at most show only a very slight deposit in the central ommatidia. All older specimens have produced at least a little, but the darkening does not go far. In the incubator, the same effect is seen as in the case of the r_1r_1 types, namely, that the production of melanin is accelerated. Darkening may start in later immature stages, and most mature specimens have an appreciable central dark patch. It was, however, noticeable that the *ff* types darkened distinctly less readily than r_1r_1 types from the Oxford Stock which were being kept at the same time. This difference is borne out in the results of the cross-mating described below (see Fig. 1).

A mating was made between a Flesh Red ♂ and a ♀ of the above-mentioned Stock I r_1r_1 types (which was also a No-White). The F_1 were all Black, and an approximate 9 : 7 ratio of Blacks to Reds was obtained from matings among them.

	Obtained	Expected.
Black	89 (28 No-Whites)	86 (21 No-Whites)
Red	63 (14 No-Whites)	66 (17 No-Whites)

The F_2 " reds," among which would be expected recessives for each type (r_1r_1F and ffR_1), as well as double recessives, were reared to maturity and

TABLE III.

SUMMARY OF "REDS" IN F_2 OF CROSS BETWEEN "FLESH" RED AND "STOCK I RED."

	At Birth.	Reached Maturity.	Survived for Mating.	Proved Steriłe.	Proved Flesh (*ff*).	Proved Stock I Red (*rrF*).	No Result.
Normal White	49	33 { 21♂ 12♀	26 { 19♂ 7♀	4 { 2♂ 2♀	13 { 8♂ 5♀	8 { 8♂ –	1 { 1♂ –
No-White	14	4 { 3♂ 1♀	3 { 3♂ –	—	2 { 2♂ –	1 { 1♂ –	
Total	63	37 { 24♂ 13♀	29 { 22♂ 7♀	4 { 2♂ 2♀	**15** { **10**♂ **5**♀	**9** { **9**♂ –	1 { 1♂ –
Preserved			26 { 21♂ 5♀	2 { 2♂ –	14 { 9♂ 5♀	9 { 9♂ –	1 { 1♂ –

Genetic constitution		
	1 *ffRR*	3 *rrFF*
	2 *ffRr*	6 *rrFf*
	1 *ffrr*	–
	11 *ff* ?	9
	—	
	15	

as many as possible were tested by back-matings with the Flesh Stock, etc., for the purpose of ascertaining whether they were " Flesh " (ffR_1 or ffr_1r_1) or Stock I Red only (r_1r_1F). The results are given in Table III.

It may be noted at this point that the majority of the reds, though most

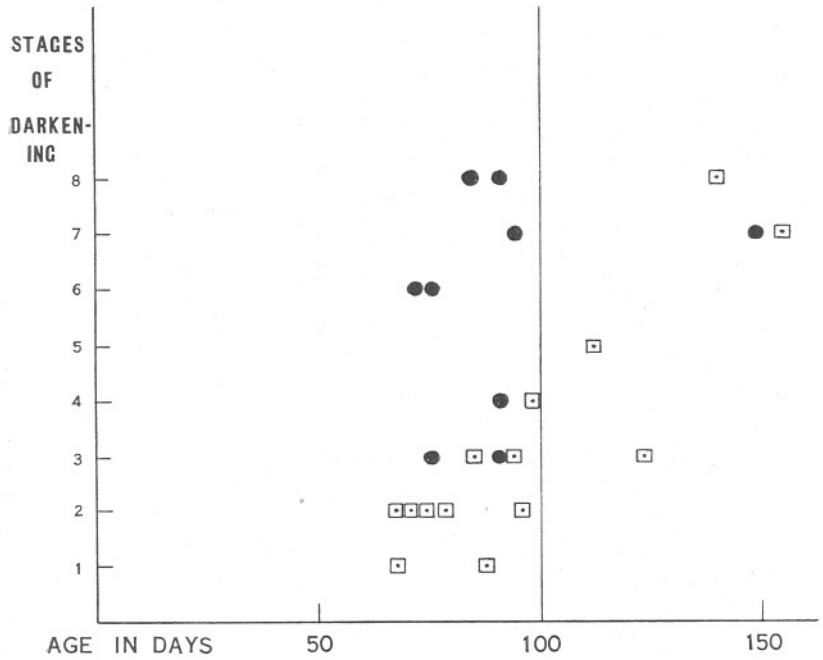

FIG. 1.—Showing that f inhibits melanin to an appreciably greater extent than r_1.
● = r_1r_1F types (maximum age value).
⊡ = ff types (minimum age value).

N.B. Since for several specimens the data of extrusion was known only within limits, the age-value cannot always be expressed exactly. However, in all cases a *maximum* has been given to r_1r_1F types, and a *minimum* to ff types. This has a considerable effect in reducing the difference between the two types, but even so the average darker state of the r_1r_1F types is unmistakable.

had plenty of red colouring when young, became deficient in red pigment as they grew older. In some cases it was almost entirely lost. There was also considerable variation in the rate of darkening. While it was always possible from later immature stages onwards to predict with some certainty that particular individuals would prove rrF and others ff, in earlier stages, and often later on, it was quite doubtful as to which type the specimen belonged.

After a sufficient family had been procured to prove genetic constitution, the F_2 " reds " were immediately preserved in spirit. When time had elapsed for the red pigment to dissolve away, examination was made of the amount of melanin that was left deposited in the eye. The 25 specimens preserved were separated into 8 classes according to the amount of melanin present. (These bear no relation to the 14 stages between red and black, used by Ford and Huxley.)

In the case of 23 it was known whether the specimen was an *ff* or an r_1r_1F type. The stage of darkening attained in these is plotted (Fig. 1) against age when preserved, the two genetic types being distinguished. The amount of pigment acquired in any given time is seen to vary considerably—no doubt partly owing to the segregation of modifying factors ; but it is quite evident that on the average the r_1r_1 types darken quicker than the *ff* types. This is particularly noticeable between the ages of 70 to 100 days. Since all other factors that might affect melanin production are, at least on the average, the same for both classes, it may be concluded that the gene *f* has a greater inhibitory influence (at these temperatures) than r_1.

With reference to the state of the red pigment among these " reds," it has been mentioned that the majority developed a marked deficiency. This variation is characteristic of the Flesh Stock, but in Stock I " reds " the pigment maintains a full concentration at incubator as well as at laboratory temperatures. Nevertheless the variation in the F_2 of the cross applied to r_1r_1F, as well as to *ff*, types. One particularly striking instance was provided by a ♂ which had a typical dark centre, but had the red colour almost completely lacking. The resulting effect was a lilac shade, very dark in the centre and fading out towards the periphery.

The question of hereditary factors underlying the variation in the red pigment requires special investigation. It has been pointed out elsewhere how in some in-bred strains a uniform concentration is maintained, while in others instability occurs through the strain, whatever recessive " melanin-deficiency " types may be contained in it.

Beet Reds.

At extrusion Beet Reds have red eyes, but, as there is always a certain amount of melanin intermixed with the red pigment, the colour is never of a pure " Normal Red." It varies, in fact, from " New Red " to " Dark Red " (**4**, Plate VIII). Subsequently, during the earlier growth stages, the eye darkens fairly rapidly, until it reaches a point at which the intensity of melanin remains more or less stable. The eye colour at this stage varies considerably among individuals. While typically approximating to a Reddish Black shade (**4**, Plate VIII), it is sometimes decidedly more reddish, and sometimes indistinguishable from Black.

Evidently the gene *t* retards the process of melanin deposition to degree comparable with a rapid-darkening r_1r_1 type (see Ford and Huxley, **2**). The resemblance, however, between the two cases cannot be carried further. In the first place, there is no striking dissimilarity between those reared in the incubator and others reared in the laboratory. No exact data are so far available, but the indication is strong that the difference in temperature has little influence on pigment deposition relative to body growth. Secondly, examination of specimens preserved in alcohol shows that at each stage during the process of darkening the melanin is uniformly distributed over the whole eye, and not concentrated in the older, central ommatidia, as in darkening r_1r_1 types. At each moult the new ommatidia show the same concentration of melanin as those in the more central part of the eye. While in the r_1r_1 types each ommatidium undergoes a similar course of pigment change, in the Beets the ommatidia are affected as a group, irrespective of their age. It appears, therefore, that the gene *t* affects some stage in the processes underlying melanin production other than that affected by r_1 and *f*.

Comparison of the Effects of Different Genes on Melanin Deposition.

An increasing number of genes are being found to influence the course of melanin deposition in the eye of *Gammarus chevreuxi*. A close resemblance is seen between the effects of some, a striking difference between others. Thus the effects of r_1 and r_2, so far as is known, are indistinguishable. Both retard very considerably the rate of melanin deposition. The gene *f*, as has been seen above, acts in essentially the same way, but has not quite such a powerful retarding influence. On the other hand, there exist between the effects of r_1 and r_5, or r_1 and *t*, differences which seem to be differences of kind rather than of degree. This suggests that different stages in the chemical processes underlying melanin production are affected, and calls for more exact comparison of the types concerned.

In their detailed investigation of r_1r_1 types, Ford and Huxley (**2** and **3**) gave prominence to the fact that the essential action of the gene r_1 is a retardation of the normally rapid process of melanin deposition in the individual ommatidia. At the same time they noted that the process was in the average case not brought to completion—a state of equilibrium was attained before the concentration of melanin was still decidedly below maximum. Except in the most rapidly darkening forms, this equilibrium phase was reached at temperatures of 20°–23° C. approximately at the time of onset of maturity. With this state of affairs the condition in r_5r_5 types may be contrasted.

An account of the influence of the gene r_5 has been given elsewhere

(see p. 328 of this Journal, Vol. XVIII). Two phases in the course of melanin production are distinguished. It is now suggested that the second phase—in which (i) no marked increase in darkening takes place,* (2) temperature differences have apparently no effect, and (3) all the ommatidia attain to an equal concentration of melanin—corresponds to the equilibrium phase among darkening r_1r_1 types. The *main* result of the presence of the gene r_5 is therefore that the stable phase is brought on very much sooner, in fact, some time during the earliest growth stages. The first phase, during which melanin is being accumulated, is of very much shorter duration in r_5r_5 types. It takes place, for the most part, before extrusion, sometimes almost as rapidly as in normal eyes. On the other hand, in r_1r_1 types, the melanin does not begin to appear until after extrusion, and at room temperature may not appear at all unless the animal lives to a considerable age.

If this comparison is justified, then (i) during the first phase in r_5r_5 types darkening should be more advanced in the centre of the eye, and should be accelerated by heat; and (2) during the second phase of r_1r_1 types, increase of temperature should have no effect, and the pigment should become uniformly concentrated over the whole eye. These points are susceptible of verification.

During the second phase, some influence is apparently at work which prevents the concentration of melanin in any part of the eye from passing a certain limit. Individual ommatidia, it seems, can darken to a certain point, but no further. Hence a uniform concentration is attained over the whole eye. A possible explanation for this phenomenon is that a precursor of the melanin cannot be formed at a sufficiently rapid rate.

While r_2, r_6, and f fall into the same category as r_1; r_4, and apparently r_3, have an effect of the kind exhibited by r_5. The r_4r_4 types, as is apparent from those born with a Dark Red eye, evidently enter on the second phase at about the period of extrusion, if not before. They differ from the r_5r_5 in showing a less variable rate of darkening in phase 1, and in a more complete inhibition of melanin production in phase 2.

The effect of the gene t, however, is different from any of the above in certain essentials. Among the tt there is uniform distribution of pigment over the whole eye during the time darkening is in progress. Since the new ommatidia at each growth-stage acquire their pigment rapidly, it follows that the rate of melanin deposition is not greatly retarded. This indicates the presence of a limiting factor of the kind seen in the second phase of, e.g., r_5r_5 types. This factor at first imposes severe restrictions, but as growth proceeds its influence becomes progressively less. The process of darkening seen during immature stages is thus the

* The relative concentration of melanin may gradually become less during this phase, especially if the phase starts early in life.

result, not of the retardation of the normal darkening process within the individual ommatidia (as in the r_1r_1), but of gradual increase of the maximum concentration attainable in the eye as a whole, owing to the gradual removal of this limiting factor.

There are, to sum up, indications that genes may influence the course of melanin production in the following ways : (1) in retarding the normal process of melanin deposition in the individual ommatidia ; (2) in imposing a limit on the concentration of melanin attainable in any part of the eye ; and (3) in shifting this limit. Following up these differences would seem to promise a fruitful line of study, which, if brought in connection with the chemistry of melanin formation, should go far towards stating the actions of the different genes in terms of reference to particular chemical processes.

My grateful acknowledgements are due to Mrs. E. W. Sexton, who has freely acquainted me with the details of previous investigations, and other useful information arising from her intimate knowledge of Gammarus. I am indebted to Miss A. R. Clark for supervising the stock during the summer of 1931 and for the help of her experience in such matters as distinguishing shades of eye-colour. I have finally to thank Dr. E. J. Allen for his kind interest and valued advice.

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Degeneration and Loss of the Eye in the Amphipod *Gammarus chevreuxi* Sexton. Part I.

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With 7 Figures in the Text and Plates III, IV, 5, and 6.

CONTENTS.

This paper is divided into two parts. The first, written in 1921, when holding the Ray Lankester Investigatorship, contains a description of an irregular coloured-eye strain which developed in the original stock of *Gammarus chevreuxi*. This Stock, now known as Stock I, was brought into the Laboratory from the wild in June, 1912.

In the second part, to appear later, a résumé will be given of the occurrence of eye-irregularity in the family Gammaridæ, as far as it can be traced up to the present time.

PART I.

1. IRREGULARITY IN THE COLOURED-EYE.

The eyes in the wild specimens of *Gammarus chevreuxi* are always black, reniform in shape, and convex. They are composed of a number of ommatidia arranged in rows, the number increasing at each growth-stage from about 10 in the newly-hatched to 70 or 80 in the full-grown. The structure is similar to that of the Gammarus eye described by

Parker (**1**, pp. 66–73). Briefly, each ommatidium consists of a two-celled cone, and 5 pigmented retinular cells, four large and one small, arranged around a central axis, the rhabdome. The space between the ommatidia is filled with the " accessory pigment-cells," rather large cells containing an opaque white pigment. Viewed from the surface, the white pigment gives the effect of a network over the eye, with the ommatidia showing as black spots in the meshes.

Several mutations have arisen. The first one (*Red-eye*), affecting the retinal pigment, was the appearance in the retinal cells of red pigment instead of black. The red proved to be a simple mendelian recessive (**2**, p. 22, Pl. I, Fig. 3).

In another mutation (*No-white*), affecting the accessory pigment, the superficial white pigment was absent. This, too, proved another mendelian recessive (**3**, pp. 326–341, Pl. VII, Fig. 5).

A third mutation (*Albino*) affected the structure of the eye, and was the most striking of all, involving the loss of the coloured retinal cells, and the breaking up of the ommateum. The whole eye presented a degenerate appearance, only the cones of a few ommatidia were left, scattered irregularly in a mass of the accessory pigment. The shape of the white mass and the number of cones varied not only in each individual, but very often in the eyes of the same individual (**2**, Pl. I, Figs. 9 and 10 ; **3**, p. 274).

This mutation also was shown to be heritable, the perfect coloured form being dominant over the imperfect albino form. The albino condition was always linked with the imperfect shape and structure.

Besides these variants from the normal, another has arisen which seems heritable in some degree, but which, so far, cannot be interpreted in any simple mendelian way. " *Spotted*," the name given to this variant, refers to the presence of spots, patches, or streaks of the white accessory pigment found, apart from the eye itself, on the cephalon, less frequently on the first peræon-segment, sometimes but rarely on the second peræon-segment. The spots, while differing considerably in size and shape, are usually situated in certain definite positions, either along the lateral line often deep in the tissues, or on the dorsum and superficial (**4**, pp. 352–366). They may remain in the same position through life, or they may change in place, as well as size and shape, at each moult (see Text-fig. 7, p. 365).

The interesting point about this variant is that it seems to be connected with any marked departure from the normal ; for example, in the Albinos, where the eye is degenerate and reduced, the spots are large and of very frequent occurrence, whilst in the perfect-eyed normals spots are not often developed, and when present are small. They occur also with the No-white mutation, and it is specially noteworthy that in the so-called " One-sided No-whites " (animals which have one eye normal, i.e. with

the retinal colour and the superficial white pigment, and one eye No-white, i.e. with the retinal colour but without the white pigment) there are, almost without exception, large spots and patches of white on the same side as the No-white eye. Even when a One-sided No-white animal is spotted on both sides, the spots are much larger on the No-white side.

Amongst the mutants the Albino-eye was the only one in which the structure was affected, the degree of degeneracy extending as far as the loss of the retinular cells. Attempts made from time to time to produce an imperfect coloured eye (i.e. an eye in which the factors controlling the organisation of the ommateum could be affected without inhibiting the production of the retinular cells) met with little success until a mating (CN.1*b*) was made in which " Spotted " was combined with the three recessive types, Red, No-white, and Albino.

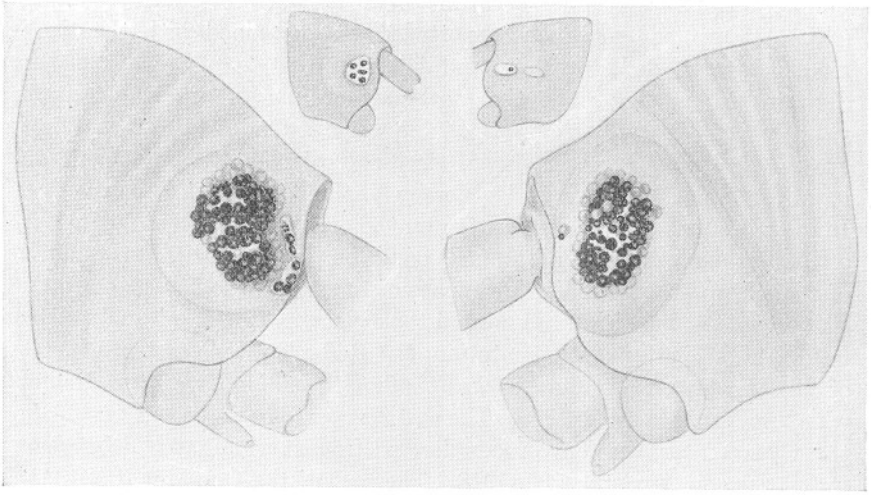
From this mating came the most extraordinary range of variation yet recorded for any marine form. The range extended from the perfect eye—perfect in structure, shape, size, and pigmentation, through an infinite number of stages of degeneracy to the complete loss of the eye. In view of all that has been written on the origin of the blind fauna it is a significant fact that blind animals could be produced within the limits of a single species in such a short space of time and in so few generations. The first departure from the normal took place in 1912, and was, as has been said, a change in pigmentation, the Red-eye ; the most important of all, affecting the structure, was that of the Albino-eye—the first of which appeared in June, 1915. The mating now to be described was made three years later, the four broods derived from it were hatched between October 1 and November 25, 1918. The first of the One-eyes (CN.183*h*) appeared in the F_3 on February 19, 1920 ; the first of the No-eyes (7 in CN.228 brood) on May 11, 1920, so that within 8 years of the bringing in of the first pair from the wild, the No-eyed form had arisen.

There were other irregularities in this stock besides the irregularity of the eyes described below, e.g. (*a*) the shape of the head was frequently abnormal, almost always so in those animals with one or both eyes missing. As will be seen in the figures (Plates IV, 5 and 6) where this malformation occurred the shape of the brain was altered, and the front margin of the head looked as if a slice had been cut off, sometimes so far back as to expose the bases of the Second Antennæ, always hidden in the normal (*cf.* Text-fig. 7).

(*b*) Another malformation found in a good number of specimens was caused by the loss of the First Antennæ, and the consequent sinkage of the anterior portion of the head. The shape of the eye was usually, though not always, affected (Plate III, Fig. 1). This peculiarity, sporadic through the stock, was probably pathological in origin. It did not appear to be heritable. No-antenna pairs gave normal-antenna offspring, and,

though some of these lost their first antennæ later, yet there is no case recorded through the generations, of a single animal hatched without antennæ. The figure given on Plate 6, No. 22 of Brood CN.349, appears at first glance to contradict this statement. The young one at birth had no first antenna on the right side, but that this must have been due to an accident while hatching was shown on examining it after its moult, when the antenna could be distinctly seen regenerating.

(*c*) And finally, there was a marked irregularity in the reproductive organs. Many cases of sterility were noted, in particular a No-antenna



TEXT-FIG. 1.—CN.228*f* ♂. Upper figure shows the right and left sides of the head at birth, May 11, 1920. A month later the Right eye had 2 ommatidia separate, the Left eye had increased in size, but was much smaller than the right, triangular in shape with the spot almost coalesced. The lower figure was taken just after maturity had been reached—July 27, 1920. ×45.

Black ♂ M. III (CN.126), and many intersexes of differing degrees of intersexuality developed (**5**, pp. 510 and 544; also details in the List, appended).

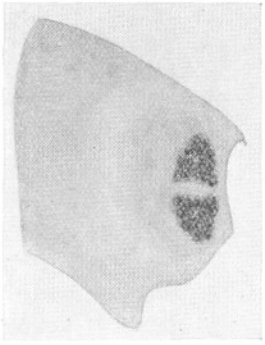
The figures given in the List are correct for the amount of irregularity at birth, but it is not possible to estimate the amount which could develop later, or to obtain any adequate data for investigation of the inheritance. The chief difficulty is that the mortality amongst the abnormals is very much higher than in the normals, so much so indeed that hardly any of the extreme types survived the first moult, and of these only one or two lived to mate. One fact comes out very clearly in this work and that is, that the farther removed an animal is from the normal, the lower is its viability. Another difficulty consequent on this is that while we know

that irregularity frequently develops in animals born normal, we have no means of judging to what extent, owing to the small proportion of survivors.

1. Types of Irregular Coloured-eyes.

Some of the different types of eyes are shown in the figures and may be briefly defined as follows :—

(*a*) *Reduction in size*, (see Brood 349, Plates IV, 5, and 6) of very usual occurrence in this strain, varies from 1 to 2 ommatidia less than normal, to a mere speck of colour (Plate 6, Fig. 20). These reduced eyes are often wedge-shaped on hatching (Text-fig. 1) and may remain so more or less



Text-fig. 2. Divided eye. Black No-white ♂.
July 7, 1920. ×45.

through life (cf. right eye of CN.210*c* on p. 365) or they may alter completely as in this specimen.

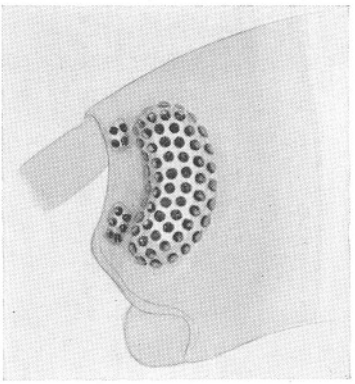
(*b*) *Increase in size* is also frequent. In some cases the eyes are larger than normal at birth (Plate 5, Fig. 15), in others the size increases disproportionately at each moult ; in others again it is due to the loss of the first antennæ, and the consequent alteration in the shape of the head (see Plate III, Fig. 1).

(*c*) *Divided eye* (Text-fig. 2) represents a Black No-white ♂ from the same No-white strain as the ♂ CN.1*b* used in this mating. The division may be transverse, as in the figure ; or longitudinal ; or the ommateum may be so divided as to look like two separate eyes (Plate 5, Fig. 16) ; or the two parts may be one behind the other as in the CN.210*n* described below. Another instance of this last type is CN.293—a Black that became Intersex and developed great irregularity, especially in the left eye, which grew to more than twice the size of the right. The right eye was of normal size, deeply indented on both margins with a small

" cluster " separate ; the left looked like two large eyes, joined together, with the ommatidia of different sizes.

(*d*) *Lobed eyes*, much less frequent than the divided, though evidently not far removed structurally. In one instance, CN.289*f*, both eyes were lobed on the front margin ; in another, CN.285*c*, the right eye had the hind margin cut into two equal lobes, while in a third, HN.69*c*, the front margin of the right eye was lobed.

(*e*) *Ommatidia separate* from the ommateum, either singly or in clusters.



TEXT-FIG. 3.—CN.157(2). Intersex 5 months old. July 7, 1920. ×45.

The clusters may be of any size, and may lie in any position near the eye (Text-fig. 3). This is of frequent occurrence.

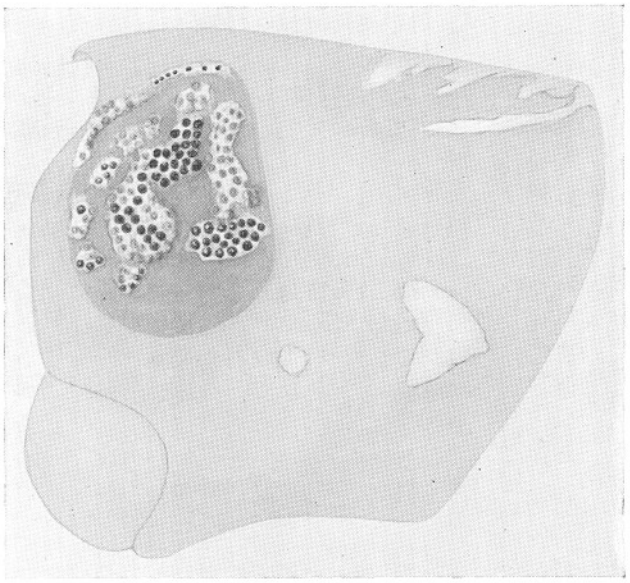
(*f*) *Scattering of the ommateum* (Text-fig. 4) shows the type (*e*) carried to the extreme, with the whole eye broken up.

(*g*) *Mosaic eye*, a term applied to an eye in which part of the retinal cells are of one colour, and part of another. There may be black, red, and colourless ommatidia in the same eye (See Plate IV for black and red ; Text-figs. 4 and 5 for black and colourless ; **2**, Plate I, Fig. 7, for red and colourless). The Figure 2 on Plate III is taken from another Stock, TB Stock III described on p. 315 of this Journal (Vol. XVIII).

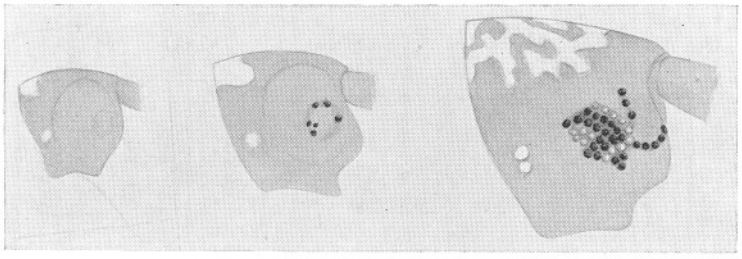
2. Loss of One or Both Eyes.

In all, 40 animals were hatched with eyes missing, 22 with one eye, and 18 with both eyes. In the F_3 generation in which they first appeared, the One-eyes numbered 12, 5 with the right eye, and 7 with the left missing, all with large spots, and 8 with dorsal patches also. Of those with No-right eye, four (CN.183*h*, 199*d*, 249*c*, and 220*b*) had an irregular eye on the

left side, while in one, CN.199*e*, the eye was reduced to one speck of reddish colour. Two of the No-left eyes also (CN.209*d*, and 228*n*) had only a speck of reddish colour on the right side, in CN.210*c*, *f*, and *n*



TEXT-FIG. 4.—CN.379*b*. ♂ hatched January 31, 1921. Died February 15, 1922, and figured. At birth, the left eye was very small, round, with 5 microscopic ommatidia, pigment dilute ; large triangular white patch behind. ×45.



TEXT-FIG. 5.—CN.220*b*. Head, right side. ×45.

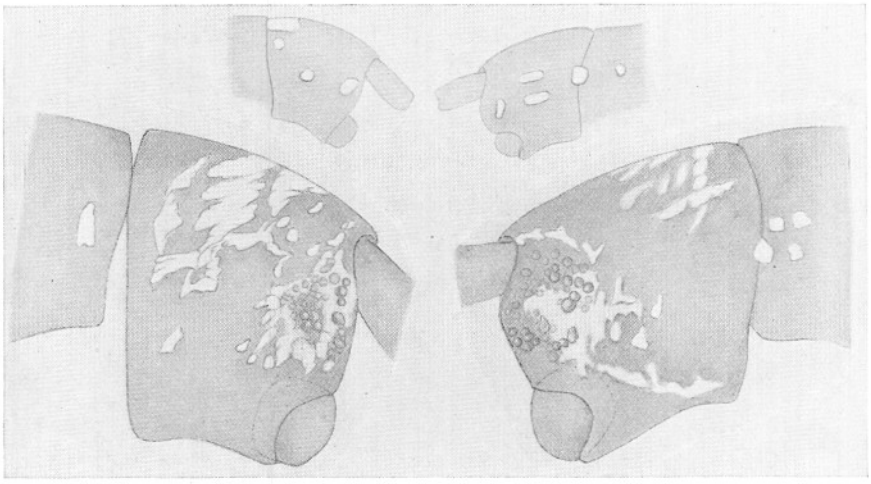
(p. 366), the right eye was irregular, in CN.228*h* it was almost normal, and in CN.269*e* quite normal.

Only four came to maturity ; one CN.210*c* (Text-fig. 7) remained unchanged, no second eye being produced ; in the other three, CN.210*f*, 210*n*, and 220*b*, a very irregular one gradually developed. An illustration

is given of CN.220*b* in Text-fig. 5, showing three stages of its growth, at birth, May 11, 1920 ; at the age of a month, on June 8, when the developing right eye was first noted ; and again on July 27, just before maturity was reached. Later on, September 8 and October 13, it was seen that the eye had greatly increased in size, and scattered, with many colourless ommatidia joined to the two curving lines of black ones. The dorsal patch then covered most of the head. The left eye at the same stages was very small at first, consisting of only 2 reddish black ommatidia ; on June 8, it was round and flat with 8 ommatidia ; on July 27 triangular, and almost No-white.

There were 10 One-eyes in the F_4 generation, all from one family ; CN.339*g* and *o* had No-right eye, and a microscopic left eye. The other 8 were in Brood CN.349, and are figured in Plate IV, Figs. 5 and 10 ; Plate 5, Figs. 15 and 18 ; and Plate 6, Figs, 19, 20, 23, and 24.

The No-eyes in the F_3 generation numbered 11, 6 from one family, CN.228*e*, *g*, *j*, *m*, *p*, and *r* ; 261*b* and *h* ; 257*c* and *d* ; and 269*f*, all spotted except one. Only 2 lived to maturity, both females,



TEXT-FIG. 6.—CN.228*m* ♀. Two stages shown. At birth May 11, 1920, at maturity August 6, 1920. ×45.

CN.228*m* and *p*, and both developed in the same way. Two stages of CN.228*m* are shown in Text-fig. 6, at birth and at maturity. In July a few colourless cones were seen, which increased in number after the next moult. It died on August 6, 1920.

In the F_4 generation 7 No-eyes were found in the same family : 3 in CN.339 (*c*, *d*, and *k*) and 4 in CN.349 (see Plate 6, Figs. 21, 22, 25, and 26).

II. DETAILS OF THE MATING (CN.1*b*) WHICH PRODUCED THE IRREGULAR COLOURED-EYES.

The male used in this mating was a Black No-white, heterozygous for red, from pure No-white unspotted stock; the female was an Albino dorsally spotted, descended from dorsally spotted ancestry for four generations. It was derived from the C.17*b* family of Albinos previously referred to (**4**, p. 350), in which one Black perfect-eyed young appeared amongst its 248 Albino imperfect-eyed offspring—the only instance ever seen of a coloured-eye occurring amongst the Albinos in the hundreds of families kept under observation.

The pair CN.1*b* had 4 broods, comprising 107 young, of which 75 were normals, 31 spotted, and 1 was a Black No-white. The appearance of a recessive in the F_1 generation was the first departure from the normal in this family.

In the F_2 generation, the first irregular coloured-eyes appeared. There were only a few, 8 out of the 1,391 young hatched, in which a definite irregularity was found, all Black eyes, all affected on one side only, 4 with the right eye and 4 with the left eye irregular; six were spotted, five on the same side as the irregularity, and one with the left eye affected had the spots on the right side. Two others, No-whites, 1 Black and 1 Red (in XXII), had a slight irregularity in the left eyes.

Compared with the remarkable developments in the succeeding generation, the irregularity in the F_2 was trifling, being shown chiefly in the uneven and indented margin of the ommateum. Only in one instance was it marked; in that animal (169*d*) the right eye was much smaller than the left, irregular in outline, and in the arrangement of the ommatidia, with the pigment very dilute, reddish, and faint; the left eye was of normal size and intensely black.

Besides these 8 definitely irregular-eyed animals, a few others were not quite normal: 5 in IX as follows: 2 Albino No-whites, spotted, one with very large spots on both sides of the cephalon, and on the first peræon-segment, the other with a small spot on the right side; 2 others, Black ♂ and Black No-white ♀ with the first antennæ missing, eyes normal; and 1 Black No-white which developed irregularity. In XIX there were 3, as follows: 1 Black One-sided No-white, spotted, with the left eye No-white and large patches and spots on each side; 1 Black No-white with the right eye much smaller than the left; and 1 Albino, spotted dorsally, with the left eye transversely divided into two pieces. In XXII 1 Albino, spotted, had the right eye almost in two pieces.

It is noteworthy that the irregularities were given by only three or four of the animals breeding. In Brood IX the 4 irregular-eyed, and the 5 others just mentioned were from one male mated with three females of its

own brood. In Brood XIX, the 4 irregular-eyed were from one female, as well as the divided-eye Albino, and the Black No-white with different-sized eyes.

All of these died without offspring, most of them before reaching maturity.

In the F_3 generation, 1,879 young were hatched. The development of the irregularity in the eyes increased enormously (see List, p. 383). It is safe to say that all the families were affected, the individual members differing only in degree. Certain families showed it in every brood, others in some of the broods, often the later ones. In a few families only, all the offspring were normal-eyed at birth, but in these also irregularity developed, as shown in the few that survived.

It was amongst the offspring of Family 2 of the Brood IX referred to above (in which 4 irregular-eyed animals appeared in the F_2) that irregularity developed to the greatest extent in the F_3 generation.

The young produced by its inter-matings numbered 237; only 130 were normal-eyed at birth (43 spotted), all the other 107 being abnormal. Of the 107, 86 had irregular eyes as follows: 82 Blacks, 31 with both eyes irregular, 27 spotted, 4 unspotted; 32 with the right eye irregular, 23 spotted, 9 unspotted; and 19 with the left eye irregular, 11 spotted, 8 unspotted; and 4 Black No-whites, 3 with the right eye affected, and 1 with the left.

The remaining 21 are the most remarkable animals that have yet appeared in this species. They all had *one or both eyes missing*, frequently accompanied by malformation of the brain and of the head, particularly of the front margin, which in some cases was so retracted as to expose the proximal joints of the second antennæ, normally covered by the lateral angles (see Text-fig. 7, and Brood 349). All but 2 of the animals were spotted. Those with both eyes missing numbered 10. Of the One-eyed, 5 had the right eye missing, 6 the left; all were spotted, most with the one eye irregular. All died without offspring, except one, CN.210*n*, a One-eye which gradually developed the second eye.

The 210 brood, in which this animal appeared, is described in detail to show the range of variation, and the changes undergone in growth.

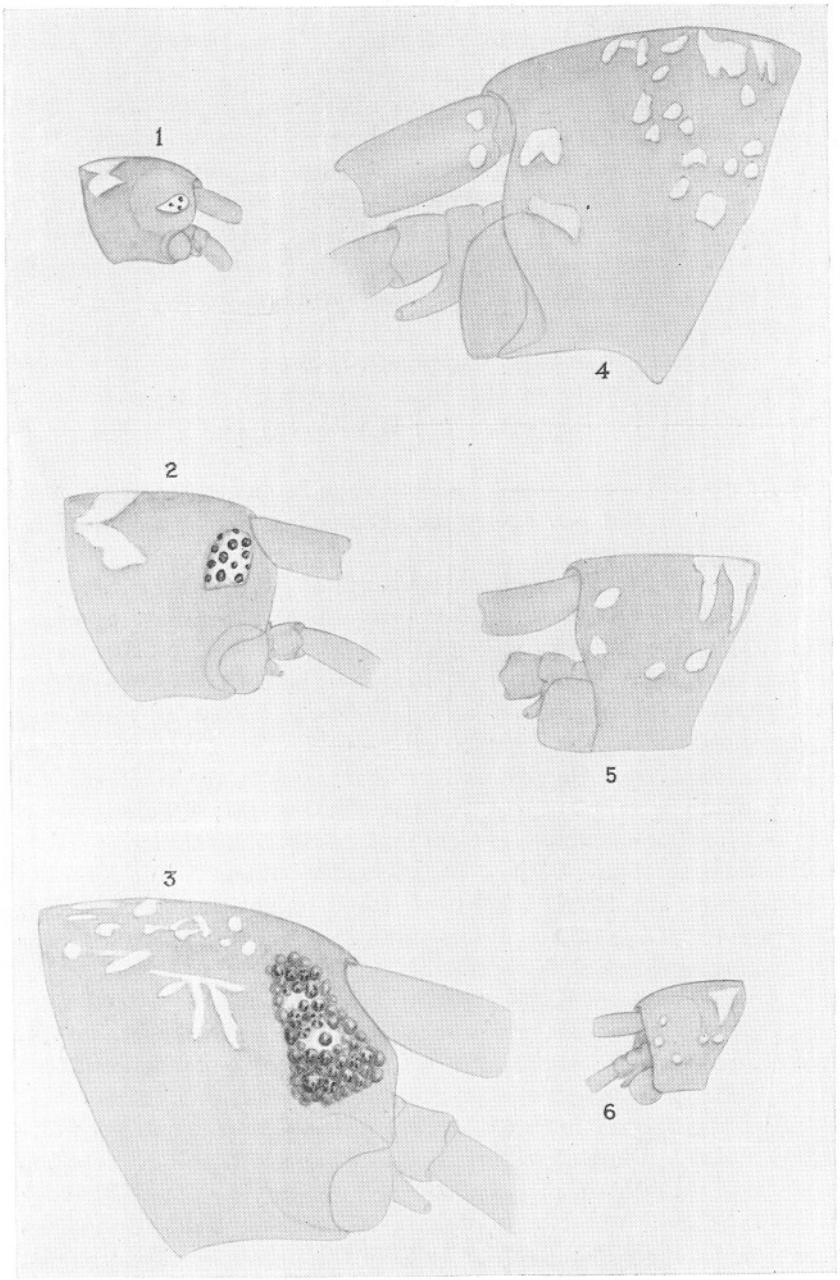
It consisted of 17 Black-eyed, extruded on April 18 and 19, 1920:—

(*a*) 1, normal eyes unspotted; and 1, normal but reticulation uneven:

(*b*) 1, with right eye normal; left eye irregular, wedge-shaped, drawn out behind; spot above position 4.*

(*bb*) 1, normal eyes; very deep spot pos. 4, and dorsal streak far back. Its growth was unusually slow, 4 months being taken instead of 6 weeks to reach maturity, a ♀. A male was added and they mated; ♀ eaten.

* See **4**, p. 353, for a diagram of the usual positions of spots. Position 4 is on the head, in the mid-lateral line, just over the anterior end of the stomach.



TEXT-FIG. 7.—CN.210*c* One-eye. 1 Right side of head, 6 Left side, on hatching April 18, 1920; 2 and 5 on June 21; and 3 and 4 at maturity September 10, 1920. ×45.

(*c*) 1, with the *left eye missing ;* (Text-fig. 7) head greatly malformed on the left side, the basal joints of the second antenna being exposed ; 3 white spots along the front margin on the left side and 2 in pos. 4 ; very large dorsal patch extending down both sides of the head ; right eye wedge-shaped, and drawn out behind, very small with only 3 ommatidia ; small spot low down on first peræon-segment. It was examined at intervals. No eye was developed on the left side through life ; the white superficial spot nearest the " eye-position " remained unchanged till after maturity was reached, when it divided into two pieces. The dorsal patch also broke up into streaks and spots and spread all over the top of the head down to the eye level.

The right eye grew very large, triangular in form, with very little white reticulation. The shape of the head was normal on the right side, very abnormal still on the left, but with the post-antennal angle produced.

This animal was a ♀ ; a male was added on September 7, mating took place, the ♀ moulted and laid eggs on September 10 and was killed by the male the same day.

(*d*) 1, normal eyes, a ♀, with a large white streak right side, pos. 4, and uneven reticulation in the left eye. Slight irregularity appeared later in the left eye. An Albino No-white ♂ was added on August 19, and eggs were laid August 20. Two broods were hatched (CN.315 and 330), 20 in all, of which 7 were irregular-eyed. The ♀ was eaten by a ♂ after moulting on October 4.

(*dd*) 1, normal eyes ; large dorsal streak far back on the head.

(*e*) 1, right eye irregular in shape, with 5 ommatidia ; 2 white spots at the base of the maxillipeds. Left eye nearly normal, large, about 8 ommatidia, spot, pos. 4. This one, a ♀, mated with an Albino No-white ♂, and had one brood (CN.338) of 10, 4 of them irregular-eyed. As it grew older it developed great irregularity in its eyes, in the shape, and in the size and arrangement of the ommatidia.

(*f*) *Left eye missing ;* large patch, pos. 4, and 2 streaks extending to dorsal. Right eye small, irregular, with 4 ommatidia ; spot, pos. 4. This animal was examined at intervals, and on July 5 it was noted that a small almost microscopic eye was growing on the left side. It consisted of 1 black speck, and 3 minute colourless ommatidia. The patch of white had increased and formed large masses of white streaks all over the top of the head ; the head margin was very irregular, and cut so far back that the eye-speck was close to position 4, in fact the width of the head was not much more than half the width of the right side. The irregular right eye had become triangular in shape. The animal, a ♂, moulted with difficulty on August 21, a very thin moult, and died on August 30.

(*g*) 1, normal eyes, with a large streak left side, pos. 4, and a deep spot

at the same point, right side ; a spot in the lateral position, right side, and 1 below and behind the eye.

(*gg*) 1, normal eyes, spotted left side. This one reached maturity, a ♂ ; it was very weakly, and died on July 29.

(*h*) 1, with the right eye small, irregular, drawn out behind wedge-shape with only 4 ommatidia ; a spot each side in pos. 4.

(*j*) 1, with very irregular right eye, microscopic, consisting of only 1 ommatidium surrounded by white, with a reddish spot behind ; and a very large drawn-out patch of white at pos. 4. Left eye normal, a small spot pos. 4.

It was examined on May 1 ; the right eye now had 2 or 3 black ommatidia, no white reticulation. Examined again on July 1 after a moult ; right eye very irregular, about 4 ommatidia, long, practically No-white, colour dilute, some ommatidia purplish, others colourless. Left eye normal in shape, and number, size, colour, and arrangement of the ommatidia. Spots unchanged. It was slow in reaching maturity, and died on August 13, a ♂.

(*l*) 1, with right eye irregular ; reticulation of left eye uneven ; unspotted.

This one, a ♀, mated first with an Albino No-white ♂, and had 2 broods (CN.337 and 348) consisting of 19 young, 4 irregular-eyed ; then with a ♂ (CN.259) of the same family and had one brood of 8 (CN.370), 6 of them irregular-eyed. It died January 20, 1921.

(*m*) 1, normal eyes, right with uneven reticulation, and small spot. Reached maturity, a ♀, and was tried with two males ; no mating ; died on October 6, 1920.

(*n*) 1, *with left eye missing ;* large white spot, pos. 4, with a smaller one in front of it ; right eye exceedingly irregular with very uneven reticulation, and small spot just behind the eye.

The animal was examined at intervals. On June 9, the beginning of an eye on the left side was noted, 3 tiny black specks on the upper edge of the ommateum, and 7 or 8 black ommatidia around the margin ; the spots had broken up into 3 large round masses, and a great number of red globules were seen in the cephalon around the œsophagus and the anterior end of the alimentary canal. Right eye much larger, still very irregular in shape, spot coalesced with the eye.

Examined on October 13 ; the left eye now very large with about 40 ommatidia, exceedingly irregular in shape, almost as if composed of two eyes, a small round regular eye of 16 ommatidia with a large crescent-shaped one fitting closely around it posteriorly. It was tried with several males, including the CN.220*b* (which, hatched a One-eye, also developed the second eye), but no mating took place. With Albino No-white males it mated twice, and had 2 broods, with one a brood of 8 (CN.321), 5

irregular-eyed ; with the other, a brood of 12 (CN.379), all irregular-eyed (one of these is figured in Text-fig. 4). It was eaten by the ♂ after moulting on February 11, 1921.

(*o*) 1, with right eye irregular, 4 ommatidia ; left eye normal, unspotted.

In the F_4 generation 1,001 young were produced of which 410 came from this Family 2, IX. The total number of irregular-eyed out of the 410 was 120 : 76 Black, of which 29 were irregular in both eyes, 23 spotted, 6 unspotted : 20 had right eye irregular, 12 spotted, 8 unspotted : and 27 had left eye irregular, 17 spotted and 10 unspotted : and 1 Red unspotted had left eye irregular.

The remaining 43 are of great interest.

41 (CN.339 and 349) came from a mating of a Black No-white ♂, CN.257, with a Black normal-eyed ♀, CN.249 (see List, p. 389), 2 regular-eyed animals from the Family 2, IX, in which the greatest amount of irregularity occurred. (The pair from which the female was derived gave 20 normals to 32 irregulars, 9 No-eyes and 4 One-eyes.) All the young from the mating were irregular-eyed, with no two eyes alike, 7 were No-eyes, 4 of them spotted, 10 were One-eyed, 7 with the right eye missing, and 3 with the left. Brood 349 is figured on Plates IV, 5, and 6.

The other 2 (CN.336) came from a mating of irregular-eyed Black ♂ and ♀ from brood CN.259. The male's eyes were both irregular, the right very large and almost square in outline, the left narrow, very uneven reticulation, ommatidia not in rows, and the colour dilute. The ♀ had the left eye large and square, and the right practically normal. It became an Intersex, but whether intersexuality developed after the brood was hatched or before, it is impossible to say ; the previous moults were examined but only tiny fragments were left, nothing to show the condition of the animal at the time.

The eyes in these 2 young were very extraordinary at birth :—

(*a*) is the only one yet seen which had eyes of different colour when hatched. On the right side was an elongate patch of white exactly like an ordinary Albino in appearance, but the young one was too delicate to keep out of water long enough to make certain if any cones were present. The left eye was Black, very small and flat with a marked difference in the size of the ommatidia, 4 larger and 3 microscopic. Eight weeks later this eye had the shape of an Albino, large and scattered with no definite outline to the ommateum, broken reticulation, numerous black ommatidia, many separate above. In the right eye black ommatidia had appeared above the white patch, in a small No-white cluster, with 2 apart. The animal was a ♀, exceedingly small and delicate. No male could be found with a sufficiently good record to place with it, and it died at 6 months unmated.

(*b*) This specimen looked as if it also had an Albino-eye on the right side, broken into three patches, but under a high-power an almost imperceptible speck of colour could be seen in each patch ; a large mass of white behind at pos. 4. Left eye Black, small (6 ommatidia), and flat, with a white spot behind and a very long and very large dorsal streak extending down the side of the head. Eight weeks later a minute Black eye, almost No-white, had developed in front of the patches, right side. The head was so much malformed on that side as to form a deep depression in which the eye lay. Left eye large, and roughly triangular with the wide end below. It died just before reaching maturity.

SUMMARY.

A description has been given of a mating which produced a great range of eye-irregularity in the offspring. Not only were the size, shape, and pigmentation affected, but the eye-structure itself, many cases occurring in which one eye or even both eyes were missing at birth.

This experiment adds another proof to the statement that the farther removed from the normal an animal is, the lower its viability. The mortality was much higher amongst the abnormals, and breeding experiments with the survivors were exceedingly difficult on account of the trouble experienced not only in rearing them to maturity, but in finding suitable mates. The cannibalism of the males of this stock made it almost impossible to use them, and small, not too vigorous, males had to be sought in the Albino No-white strain.

In three of the One-eyes, i.e. those in which one eye was present at birth, and no trace of a second could be seen (even with a high power), a very imperfect second eye developed later, after several moults had taken place. An example, CN.220*b*, is illustrated in Text-fig. 5 (List, p. 384).

In one case and one only of the One-eyed animals which survived several moults, the missing eye was never developed. The specimen CN.210*c* is figured in Text-fig. 7.

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EXPLANATION OF THE PLATES.

PLATE III.

FIG. 1.—Red ♀, R.E. 106, showing the "No-antenna" condition and the irregularity of the eye. ♀ just mature, laid eggs, and died April 11, 1920. ×58.

FIG. 2.—TB.814*c*. ♂ from Stock III, p. 315 of this Journal, showing the "mosaic" eye. 8 months old, August 10, 1925. ×58.

PLATE IV.

Brood 349, see p. 368 for ancestry. Hatched October 26, 1920. ×58.

FIG. 1.—Right eye tiny, with 2 round black ommatidia and 1 long red one. Left eye large. Died trying to moult on November 5.

FIG. 2.—R. eye microscopic, 1 black ommatidium surrounded by a thin line of white, 2 minute reddish ones behind. L. eye small, 2 long black omm., very large dorsal patch. Died November 5.

FIG. 3.—R. eye large, irregular, with a reddish cone at the top and one at lower margin; no white pigment. L. eye minute, only 2 tiny B. omm. each surrounded by a clear space. Died in moulting November 5.

FIG. 4.—Both eyes exceedingly small, 3 omm. in clear space, with dark reddish patch below, adjoining eye: no white in eye, but a small, very deep white spot on edge of brain lobe. L. eye round, with 4 microscopic cones. This one lived to February 10, 1921, when both eyes were about the same size, the left one lobed, the right square. It moulted on November 10, but hardly grew at all, measuring only 1·75 mm. at death. A normal animal would have been mature at that age.

FIG. 5.—*No eye* right side, head and lobe malformed. Left eye exceedingly small, 1 B. omm. and 1 faint reddish spot behind. Died in trying to moult November 5.

FIG. 6.—Head malformed, both eyes very small and irregular, the left with 1 reddish omm. and 2 black. This one was spotted on the first peræon-segment. It moulted on November 10, died January 20, 1921.

FIG. 7.—Eyes very small. Right with 1 B. omm. and 4 microscopic colourless ones, white retic. L. eye with 2 large B. omm. and 2 small above, reddish patch behind eye, deep; spotted.

FIG. 8.—BN. Looks like 2 eyes on RS. L. eye as if 2 eyes were joined together, one behind reddish. Died in moulting November 5.

FIG. 9.—BN. Large R. eye, 7 omm. L. eye also large but very irregular, looks like 1 round B. spot surrounded by a clear reddish space, with 1 B. omm. separate above. Died trying to moult November 5.

FIG. 10.—*No eye* R. side. L. eye BN., small, round, with light reddish patch behind, as large as eye. Died November 5 trying to moult.

PLATE 5.

From a Painting. ×58.

Normal Black eye of newly hatched young, for comparison.

FIG. 11.—R. eye microscopic, 1 B. omm. surrounded by white with tiny deep B. spot in front and 1 behind, deeper than eye. A huge white spot in lateral position, much larger than the eye. L. eye very large. Lived till May 9, 1921, and was then only 2·5 mm. in length. (Only 10 joints. in flagellum, Ant, 1 ; 3 in access. flag., and 7 in flagellum, Ant. 2.)

FIG. 12.—Both eyes large, but irregular in shape and number of omm. Much spotted, with large dorsal patch. Died in moulting November 5.

FIG. 13.—R. eye minute, with a little white streak at lower edge of lobe. Head malformed, and lobe deeply dented, touching the margin. Tiny eye L.S. with white retic. and only 3 micro. omm. Dead on November 8.

FIG. 14.—Both eyes exceedingly small, with reddish omm. and hardly any white. L. eye oval, with 2 minute reddish lenses.

FIG. 15.—*No eye* L.S. Lobe malformed. R. eye very large, drawn out, ommatidia of different sizes.

FIG. 16.—2 distinct eyes on Right side. 1 with fair-sized B. omm. in eye-position and 2 smaller omm., white pigment, and spots ; 1 lateral with 2 omm. L. eye large. Died in moulting November 6.

FIG. 17.—Both eyes very small. R. with only 2 omm., 1 large and irregular and 1 small. Practically No-white. Died after moulting November 5.

FIG. 18.—*No eye* L.S. Huge triangular patch pos. 4 ; dorsal patch. Head on R. side greatly malformed and eye reduced to 2 B. specks. Died in moulting November 8.

PLATE 6.

From a Painting. ×58.

FIG. 19.—*No eye R,S.* Head greatly malformed. L. eye far back in lobe, in lateral position, may be due to malformation, eye very minute. Died after moult November 8.

FIG. 20.—*No eye L.S.* Lobe and head malformed. R. eye reduced to 1 tiny B. speck, slightly drawn out backwards. Moulted November 15 and died November 27.

FIG. 21.—*No eyes.* Head and lobes malformed. Much spotted. Moulted November 10, died November 27.

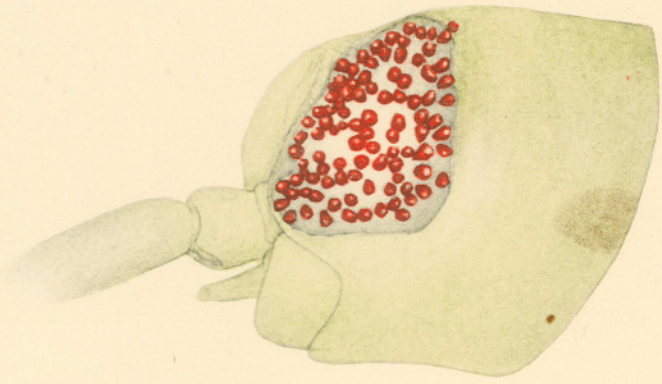
FIG. 22.—*No eyes.* Head greatly malformed. R. ant. missing at birth, probably through an accident. Moulted and died November 10. The antenna was regenerating. Figure taken from moult.

FIG. 23.—*No eye R.S.* Head malformed. L. eye microscopic, 2 omm. Moulted November 15 and died November 18.

FIG. 24.—*No eye R.S.* On L.S. a tiny speck of B. in middle of lobe, lateral position, represents eye. Died November 15.

FIG. 25.—*No eyes.* Head malformed, more so on L.S. Patches of white very deep. Moulted November 10, died December 13.

FIG. 26.—*No eyes.* Unspotted. Moulted November 11, died November 15.



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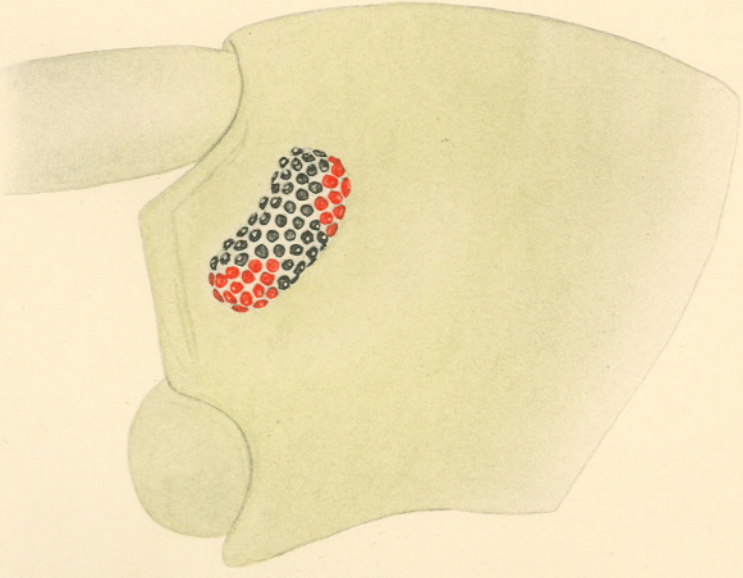

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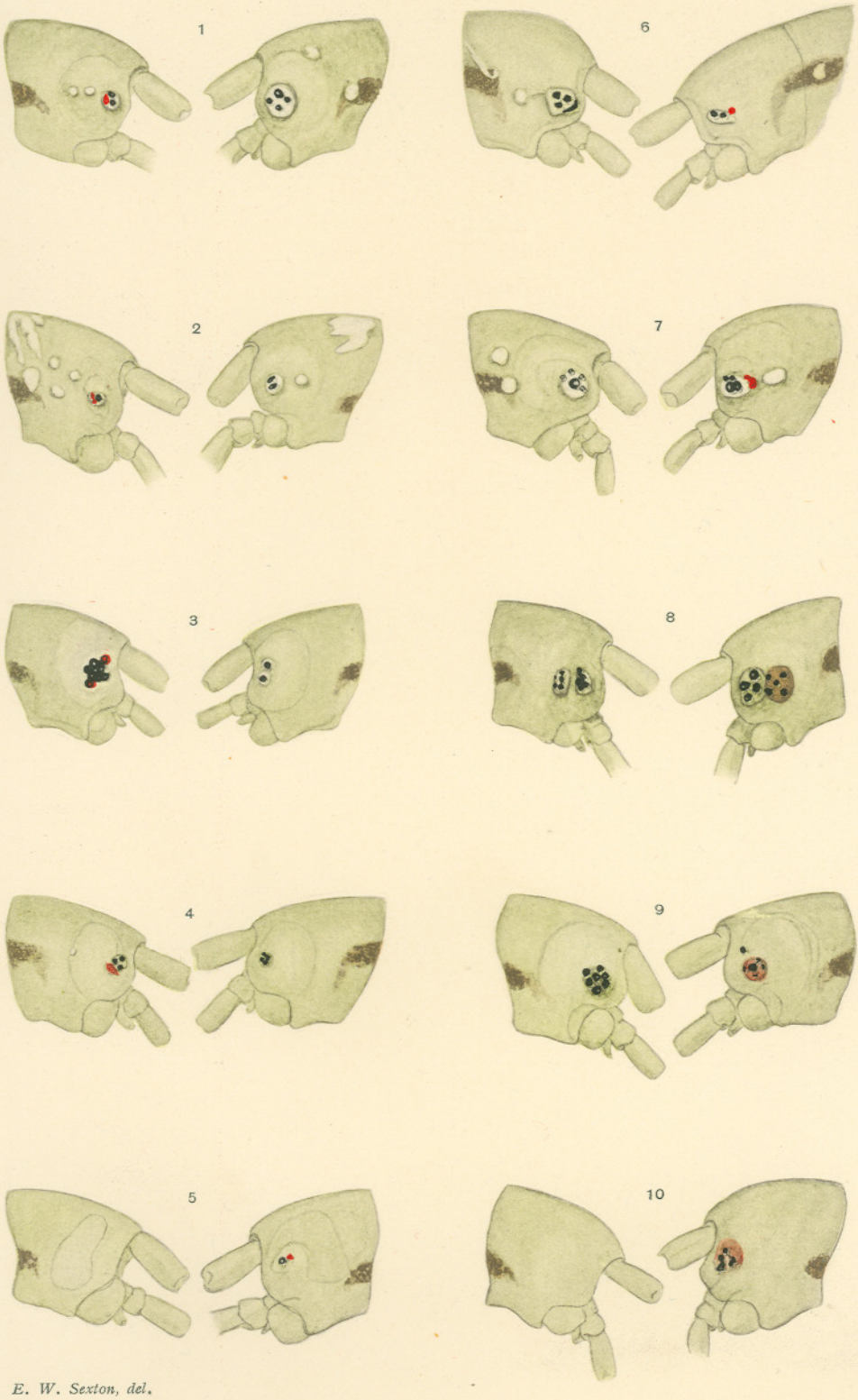

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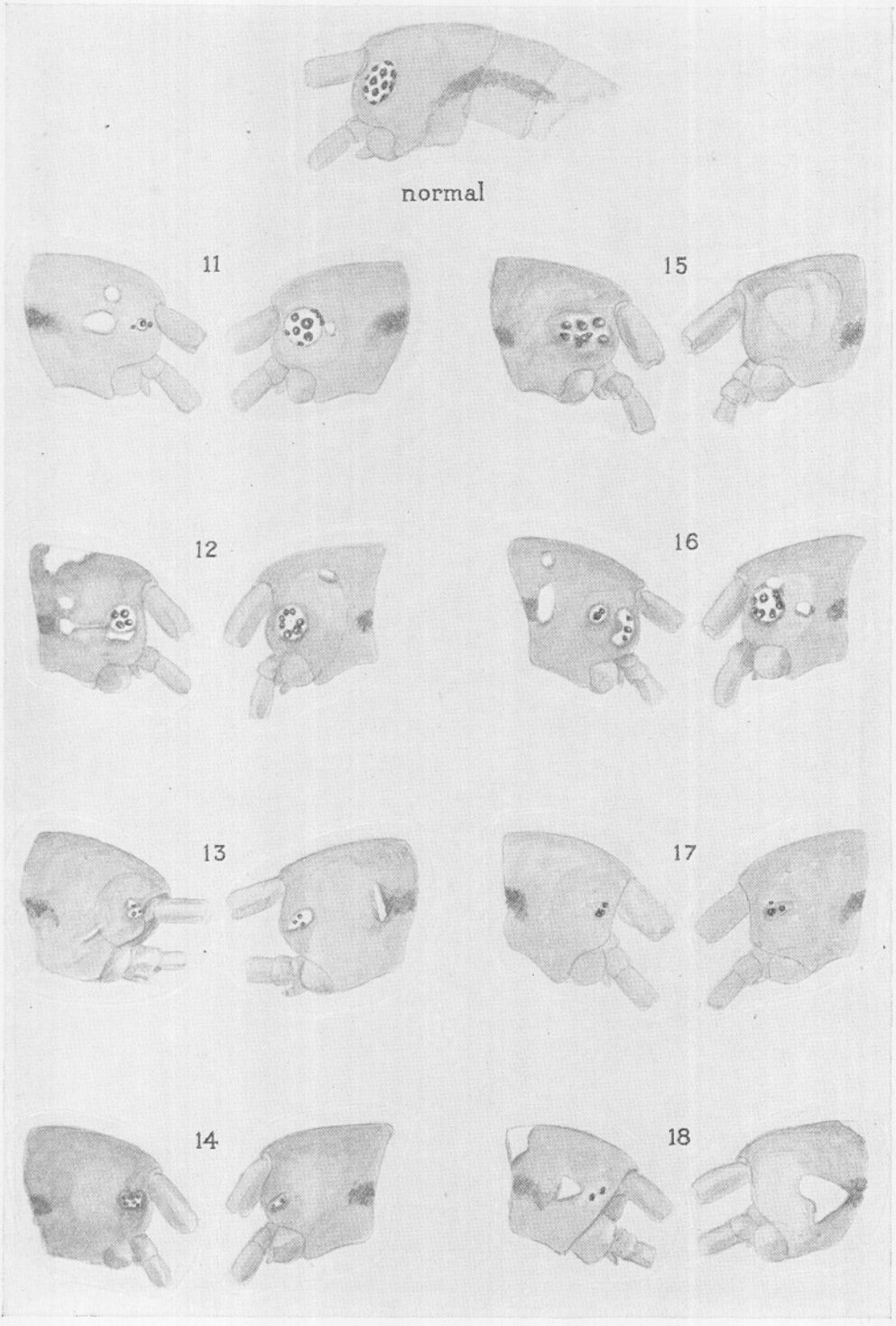
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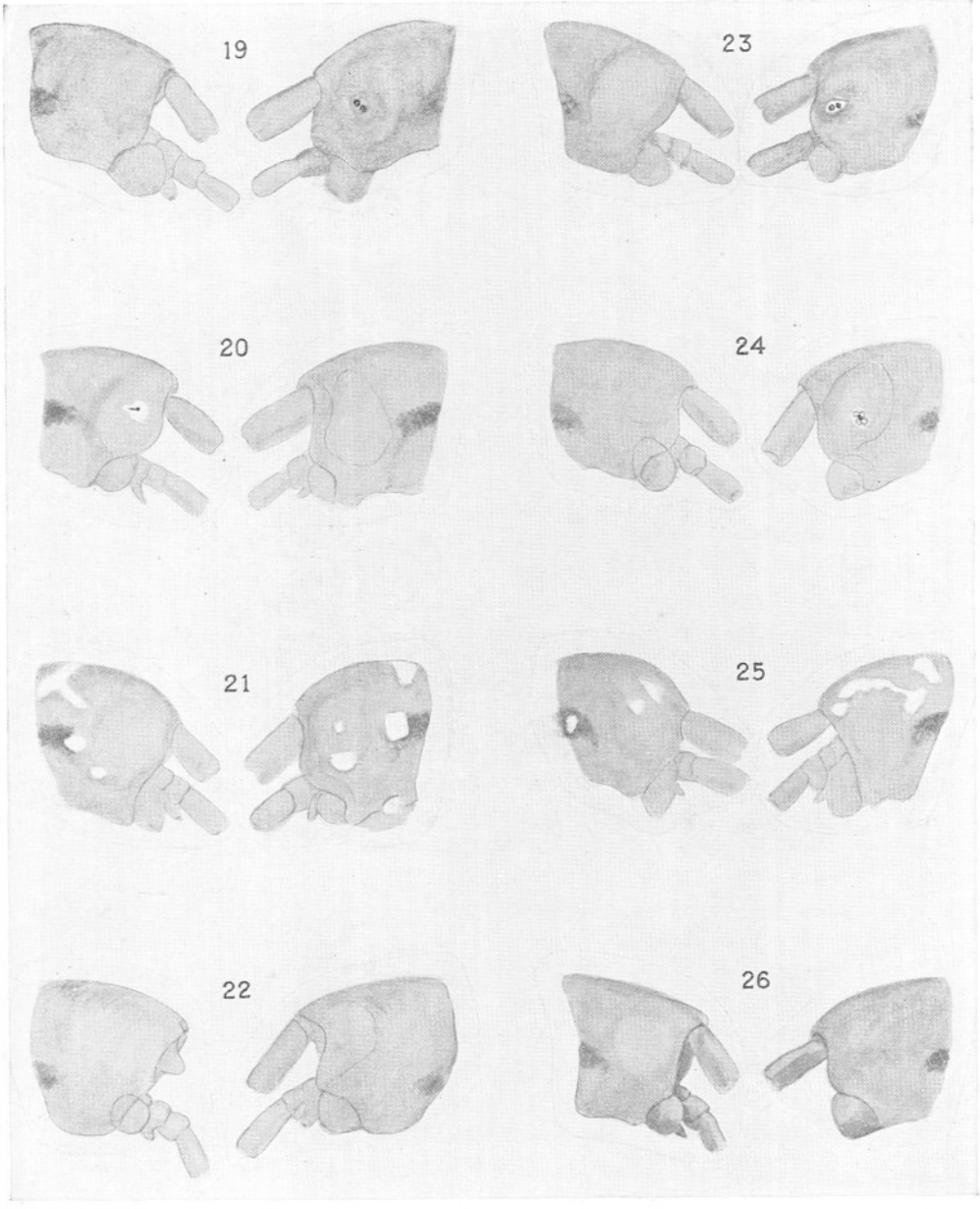
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III. LIST SHOWING THE OCCURRENCE OF THE IRREGULAR-EYE CHARACTER IN THE OFFSPRING FROM THE MATING CN.1*b*.

The abbreviations used are as follows:—B, Black; BN, Black No-white; R, Red; RN, Red No-white; A, Albino; AN, Albino No-white; R, right when used with "eye"; RS, right side; L, left; and LS, left side.

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
F_1 Generation.												
IX	24	Normals	19	5	–							
XIV	38	,,	25	13	–							
XIX	32	,,	19	12	1							Recessive appearing in the F_1 generation.
XXII	13	,,	12	1	–							
F_2 Generation.												
IX.												
Family 1.	B ♂ 1 with B ♀ 1*a*.											
46	7	Normals	1	–	1	1	–	–	2	1	1	
55	11	Normals	3	2	2	1	–	1	1	1	–	
	2	*Irregulars*	–	*2*	–	–	–	–	–	–	–	*1 with irregular R eye; 1 with L eye.*
65	18	Normals	4	3	4	1	1	–	–	2	*3	*1 of the AN, very spotted.
Family 2.	Same ♂ with B ♀ 1*b*.											
103	22	Normals	9	2	3				3	1	*4	*1 of the AN with a spot.
112	27	,,	10	5	4				2	4	2	
Family 3.	Same ♂ with B ♀ 1*c*.											
119	6	Normals	2	2	–				–	–	2	
126	31	,,	14	7	6				2	2	–	1 B ♂ No antenna=M III in records, p. 358.
129	28	,,	13	2	9				2	–	2	
131	10	,,	4	2	1				1	1	1	
140	22	,,	9	1	5				3	3	1	
145	18	,,	6	4	4				1	3	–	
156	21	Normals	11	3	3				3	1	2	1 BN ♀ No antenna.
	2	*Irregulars*	–	*2*	–				–	–	–	*Both with irregular L eye.*
158	25	Normals	12	5	3				3	2	–	
166	32	,,	9	6	6				5	5	1	1 BN developed irregularity.
175	28	,,	14	3	5				2	2	2	
Family 4.	B ♂ 2 with B ♀ 2.											
45	6	Normals	2	–	2				–	–	2	
53	15	,,	6	1	4				4	–	–	
63	20	,,	13	1	1				3	1	1	
73	29	,,	12	–	11				3	1	2	
79	27	,,	15	1	4				2	3	2	
84	34	,,	13	–	7				9	3	2	
93	28	,,	17	1	1				5	1	3	
100	28	,,	11	1	8				3	1	4	
109	18	,,	12	–	3				3	–	–	
117	9	,,	4	–	2				1	2	–	
Family 5.	B ♂ 4 with B ♀ 4.											
58	10	Normals	5	1	1	1	–	–	1	–	1	
68	19	,,	9	1	1	4	–	–	4	–	–	
76	21	,,	9	–	2	4	–	1	4	–	1	
85	8	,,	1	–	1	3	–	1	2	–	–	
Family 6.	Matings in brood-bowl.											
35	1	Normal	–	–	1				–	–	–	
38	4	Normals	2	–	1				–	1	–	
39	4	,,	4	–	–				–	–	–	
40	5	,,	1	1	–				2	1	–	

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
XIV.												
44	6	Normals	1	3	1				–	1	–	
47	4	,,	1	–	–				3	–	–	
51	6	,,	3	–	2				–	1	–	
XIX.												
Family 1.		B ♂ 1 with B ♀ 1*a*.										
59	5	Normals	4	–	1				–	–	–	
70	1	Normal	–	–	–				1	–	–	
Family 2.		Same ♂ with B ♀ 1*b*.										
80	14	Normals	10	–	3				1	–	–	
Family 3.		Same ♂ with B ♀ 1*c*.										
102	4	Normals	3	–	1				–	–	–	
111	37	,,	12	5	4				6	8	2	
116	12	,,	8	1	1				–	–	2	
124	25	,,	8	3	8				3	2	1	
133	14	Normals	6	3	2				–	1	2	
	2	*Irregulars*	*1*	*1*	–				–	–	–	*Both with R eye irregular*
134	5	Normals	1	3	–				–	1	–	
	2	*Irregulars*	*1*	*1*	–				–	–	–	*1 with R eye irregular; and 1 spotted with L eye.*
135	30	Normals	12	3	5				6	*2	2	*1 Spotted Albino with L eye divided into 2 pieces.
Family 4.		♂ 2 with ♀ 1*c*.										
169	11	Normals	1	–	1	1	–	2	1	3	2	
	1	*Irregular*	–	–	*1*	–	–	–	–	–	–	*This BN had R eye much smaller than L.*
Family 5.		♂ 2 with ♀ 5.										
122	21	Normals	10	3	4	–	–	–	1		–	
Family 6.		B ♂ 2 with B ♀ 2.										
61	5	Normals	2	1	1	–	–	–	–	–	1	
69	13	,,	5	–	2	4	–	–	1	–	1	
78	18	,,	5	–	4	3	–	3	1	1	1	
83	13	,,	3	–	1	3	–	2	4	–	–	
Family 7.		B ♂ 3 with B ♀ 3.										
67	4	Normals	1	–	1				2	–	–	
77	11	,,	9	–	–				2	–	–	
82	20	,,	11	–	2				3	1	3	
90	22	,,	8	1	7				1	2	3	
95	21	,,	9	1	5				2	2	2	
105	17	,,	9	1	–				5	1	1	
114	25	,,	12	–	5				6	1	1	
Family 8.		B ♂ 5 with B ♀ 5.										
87	7	Normals	2	2	2				–	–	1	
92	8	,,	2	*2	2				1	1	–	*1 was a One-sided No-white LS.
99	9	,,	2	–	4				1	1	1	
108	11	,,	4	–	4				1	2	–	
Family 9.		B ♂ 6 with B ♀ 6.										
81	5	Normals	1	3	–				1	–	–	
89	9	,,	3	2	–				3	–	1	
96	16	,,	8	2	2				2	1	1	
106	17	,,	7	2	4				1	2	1	
Family 10.		B ♂ 6 with ♀ 1*c*.										
88	12	Normals	7	2	1				1	–	1	
Family 11.		Matings in brood-bowl.										
52 ♀ 1	4	Normals	*4	–	–							*The one survivor was an Intersex (**5**, p. 516).
71 ♀ 5	5	,,	3	–	1				–	–	1	
75 ♀ 4	9	,,	4	–	1				2	1	1	
XXII												
Family 1.		B ♂ with B ♀ 1.										
66	17	Normals	3	–	2	3	–	1	1	5	2	
Family 2.		Same ♂ with B ♀ 2.										
94	22	Normals	8	1	5	1	–	1	2	2	2	
101	29	,,	5	2	6	6	–	1	3	3	3	
110	24	,,	9	1	2	3	1	2	2	2	2	

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Family 3.		Same ♂ with B ♀ 3.										
118	13	Normals	2	2	2	1	1	–	2	2	1	
	1	*Irregular*	–	–	*1*	–	–	–	–	–	–	*L eye irregular.*
125	21	Normals	7	1	2	5	2	–	1	2	1	
128	9	,,	5	–	–	–	–	1	1	–	2	
138	8	,,	2	2	3	–	–	–	–	1	–	The 3 BN are reddish black.
141	18	,,	6	3	2	3	1	–	1	2	–	
149	4	,,	1	–	–	1	–	–	1	–	1	
155	15	,,	6	–	4	1	–	1	1	2	–	
162	17	,,	5	2	3	3	–	3	1	–	–	
168	25	,,	9	1	3	5	1	2	1	–	3	
178	15	,,	3	1	2	1	1	–	3	3	1	
186	22	,,	4	1	4	5	–	1	2	3	2	1 of the Spotted Albino with R eye almost in 2 pieces.
194	17	,,	6	3	5	–	1	–	1	1	–	The Spotted Albino with R eye rather irregular.
224	12	,,	6	–	3	1	–	1	–	1	–	
258	15	Normals	7	1	2	1	1	2	–	–	1	
	1	*Irregular*	–	–	–	–	–	*1*	–	–	–	*L eye irregular.*
Family 4.		Same ♂ with B ♀ 4.										
56	9	Normals	6	1	1	–	–	–	1	–	–	

F_3 Generation.

IX

Family 1
Brood 65 B ♀, with ♂ 126, Fam. 3.

Family 2.
Brood 103 Pair 1 B ♂ with B ♀.

164	5	Normals	2	2	1	
172	6	,,	5	1	–	
183	13	Normals	4	4	*5	*1 Intersex (183*m*, **5**, p. 532).
	9	*Irregulars*	–	**8*	*1*	**1 Intersex (183*c*, **5**, p. 538) both eyes irregular ; 2 others irreg. both eyes ; 5 R eye irregular.*
	1	**One-eye**	–	*1*	–	**Right eye missing.**
190	6	Normals	1	2	3	1 of the spotted had the head malformed.
	11	*Irregulars*	*5*	*6*	–	Unspotted. *1 with R eye irregular ; 4 with L eye (1 of these was No-white RS).* Spotted : *2 with both eyes irregular ; 2 with R eye ; 2 with L eye (1 of these had only 1 microscopic speck of colour).*
209	7	Normals	4	2	1	
	5	*Irregulars*	*2*	*3*	–	Unspotted : *Both eyes irregular.* Spotted : *2 with both eyes irreg. 1 with L eye.*
	1	**One-eye**	–	*1*	–	**Left eye missing,** *and R eye only a tiny speck of red.*
Brood 103 B ♂ 2 and B ♀.						
174	4	Normals	–	3	–	
Brood 103 B ♂ 2 and B ♀ 3.						
173	9	Normals	5	**4*	–	**1 developed irregularity in L eye.*
	7	*Irregulars*	*1*	*6*	–	Unspotted : *both eyes irregular.* Spotted : *3 with both eyes irregular ; 2 with R eye ; 1 with L eye.*
210	8	Normals	2	6	–	1 unspotted, and 3 spotted with very irregular white pigment ; 1 spotted developed slight irregularity LS.
	6	*Irregulars*	*2*	*4*	–	Unspotted : *R eye irregular.* Spotted : *3 with R eye ; 1 with L eye.*
	3	**One-eyes**	–	*3*	–	**Left eyes missing** ; *R eye irregular in all and much spotted.* (*One Figured* on p. 365.)
259	7	Normals	6	1	–	
	9	*Irregulars*	*3*	**6*	–	**Intersex (**5**, p. 521) with L eye irregular.* Unspotted : *2 with R eye irreg. ; 1 with L eye.* Spotted : *2 with both eyes ; 4 with L eye, including Intersex ; (one of these developed irregularity in R eye).*

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Brood 103 B ♂ 2 with BN ♀ 112*b*.												
199	8	Normals	–	5	–				1	2	–	
	11	*Irregulars*	*1*	***10**	–							*Intersex *with 2 truncate spines.* Unspotted : *1 with L eye irregular.* Spotted : *2 with both eyes (1 with a lateral coloured spot) ; 8 with R eye.*
	2	**One-eyes**	–	*2*	–							**Right eye missing** *in both ; left eye very irregular (1 had only a microscopic speck of colour).*
228	9	*Irregulars*	*2*	*7*	–							Unspotted : *1 with both eyes irreg. ; 1 with L eye.* Spotted ; *all 7 with both eyes irregular.* (Text-fig. 1.)
	8	**Eyes missing**	–	*2*	–							**6 No-eyes** (Text-fig. 6), *(1 with microscopic speck of colour in the lateral position RS) ; 2 with* **Left eye missing** *(1 had R eye normal, in the other there was only a speck of colour).*
249	8	Normals	6	1	–				–	1	–	
	1	*Irregular*	–	*1*	–				–	–	–	*Both eyes irregular.*
	1	**One-eye**	–	–	–				–	*1*	–	**Right eye missing.**
261	4	Normals	2	2	–							
	11	*Irregulars*	*5*	*6*	–							Unspotted : *4 with R eye irregular ; 1 with L eye.* Spotted : *3 with both eyes ; 2 with R eye ; and 1 with L eye.*
	2	**No-eyes**										**2 No-eyes spotted.**
Brood 112 Pair 1 B ♂ and B ♀.												
198	3	Normals	1	–	1				1	–	–	
	2	*Irregulars*	–	*1*	*1*							*L eyes irregular.*
220	3	Normals	2	–	1							
	1	*Irregular and* **One-eye**	–	*1*	–							**Right eye missing,** *L eye irregular.*
241	16	Normals	8	1	3				2	2	–	Nearly all with thin or uneven reticulation.
	3	*Irregulars*	–	*2*	*1*				–	–	–	Spotted : *1 with both eyes irregular ; 1 with R eye.* BN *with R eye irregular.*
257	17	Normals	9	1	3				3	***1**	–	*Intersex (**5**, p. 534).
	2	**No-eyes**										**2 No-eyes,** *unspotted.*
268	6	Normals	3	1	1				1	–	–	
	2	*Irregulars*	–	*1*	*1*							B spotted, *both eyes irregular :* BN *with R eye.*
Family 3.												
Brood 126 B ♂ with B ♀ 65 of Fam. 1.												
200	17	Normals	5	8	–				–	4	–	All with reticulation uneven and irregular.
	4	*Irregulars*	*1*	*1*	–				–	*2*	–	B unspotted : *L eye irregular.* Spotted : *R eye. A with L eye irregular.*
211	29	Normals	19	4	–				–	6	–	
	4	*Irregulars*	–	*4*	–				–	–	–	*1 with both eyes irregular ; 3 with L eye.*
231	17	Normals	8	6	–				–	3	–	1 of the B unspotted had part of the ommateum separate LS ; it joined together in 3 months.
	5	*Irregulars*	*1*	*1*	–				–	*3*	–	B unspotted : *R eye irregular.* Spotted : *L eye.* A, spotted : *1 with divided R eye ; 2 with minute L eye.*
248	23	Normals	9	9	–				2	3	–	All with very uneven reticulation.
	4	*Irregulars*	–	–	–				–	*4*	–	*1 with very minute irregular eyes ; 1 with R eye, and 2 with L eye microscopic.*
260	3	Normals	1	1	–				–	1	–	
	2	*Irregulars*	*1*	*1*	–				–	–	–	*R eyes irregular.*
269	24	Normals	11	***11**	–				–	2	–	*2 Intersexes.
	4	*Irregulars*	–	*1*	–				–	*3*	–	B spotted : *both eyes irregular.* A *1 with R eye and 1 with L eye very irregular.*
	1	**No-eyes**										**No-eyes.** *No cones seen, but specks of white.*
277	18	Normals	6	8	–				3	1	–	
	13	*Irregulars*	*4*	*8*	–				–	*1*	–	B unspotted : *1 with both eyes irregular ; 1 with R, and 2 with L eye.* Spotted : *4 with both eyes irregular; 1 with R, and 3 with L eye.* A spotted : *R eye irregular.*

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
286	23	Normals	12	8	–				1	2	–	
	4	*Irregulars*	–	*3*	–				–	*1*	–	B *all with L eye irregular* : A *with R eye.*
299	6	Normals	2	2	–				1	1	–	1 of the B spotted had a very malformed head ; the other had a spot joined on outer margin of eye.
	1	*Irregular*	–	*1*	–				–	–	–	*R eye irregular.*
314	15	Normals	7	*5	–				2	1	–	*Intersex.
	4	*Irregulars*	*1*	*3*	–				–	–	–	Unspotted : *L eye irregular.* Spotted : *2 with R eye ; 1 with L eye. All slightly.*
Brood 126*a* Pair 1 B ♂ and B ♀.												
182	6	Normals	2	–	1				1	–	2	
	1	*One-sided No-white*	–	–	–				–	*1*	–	A spotted, *with L eye no-white.*
Brood 126*a* Pair 2 B ♂ and B ♀.												
181	4	Normals	2	–	–				1	1	–	
Brood 156 B ♂ and 112 BN ♀ from Fam. 2.												
310	3	Normals	–	–	2				–	–	1	1 BN developed irregularity in both eyes.
Brood 166*b* B ♂ and ♀ spotted.												
254	7	Normals	2	3	–				–	2	–	Albinos with very small eyes and large spots.
262	7	Normals	4	1	–				–	2	–	
	1	*Irregular*	–	*1*	–							*L eye irregular.*
Family 4. Mating in 63 bowl.												
123	6	Normals	5	–	–				1	–	–	
Family 5. No F_3 offspring.												
Family 6. No F_3 offspring.												
XIV. No F_3 offspring.												
XIX.												
Families 1, 2, and 3. No F_3 offspring.												
Family 4.												
Brood 169 Red ♂ and B ♀.												
216	14	Normals	11	*1	2							*Intersex. Mated (**5**, p. 527).
226	21	Normals	18	1	2							
	2	*Irregulars*	–	*1*	*1*							B *with L eye slightly irregular.* BN *with R eye slightly.*
Family 5. No F_3 offspring.												
Family 6.												
Brood 69 Red ♂ 3 and Red ♀ 2.												
189	21	Normals				18	3	–				
196	28	,,				23	5	–				
208	25	,,				24	1	–				
225	17	Normals				16	1	–				
	1	*One-sided No-white*				–	*1*	–				*L side no-white.*
233	36	Normals				35	1	–				Spotted Red developed irregularity in both eyes.
	2	*Irregulars*				*1*	*1*	–				*Both with R eye irregular.*
Brood 69 Same Red ♂ 3 and B ♀.												
127	13	Normals	10	3	–							
130	15	,,	14	1	–							
132	23	,,	19	4	–							
136	20	,,	19	1	–							
139	18	,,	14	4	–							
142	21	,,	16	5	–							
151	23	Normals	16	7	–							
	1	*Irregular*	–	*1*	–							*Both eyes irregular.*
157	28	Normals	*22	6	–							*2 Intersexes, both of which developed irregularity (**5**, pp. 520 and 534).
165	21	Normals	16	5	–							
	3	*Irregulars*	3	–	–							*2 with R eye irregular ; 1 with L eye.*

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Brood 69 Same Red ♂ 3, with B ♀ 96 of Fam. 9.												
265	25	Normals	*18	*7	–							*2 Intersexes (5, pp. 526 and 530).
	1	*Irregular*	*1*	–	–							*R eye irregular.*
274	35	Normals	30	5	–							1 spotted B developed irregularity in both eyes.
	4	*Irregulars*	*3*	*1*	–							*1 unspotted with R eye irregular; the others with both eyes irregular.*
284	36	Normals	*31	5	–							*Intersex. The largest (5, p. 531) : 1 of the unspotted almost No-white.
	1	*Irregular*	*1*	–	–							*R eye irregular.*
293	24	Normals	*24	–	–							*2 Intersexes (5, pp. 519 and 542), one of which developed irregularity in both eyes. Left eye twice size of Right, as if 2 eyes were placed one behind the other. Another B developed irregularity, portion separate.
	1	*Irregular*	–	*1*	–							*R eye irregular.*
Brood 69 Red ♀ 2 in brood bowl.												
115	8	Normals	2	1	5							
Brood 69 Same Red ♀ 2 with A bino ♂												
121	9	Normals	3	2	4							
Family 7												
Brood 105 Pair 1 B ♂ and B ♀.												
163	1	Normal	1	–	–							
170	19	Normals	16	3	–							
179	23	,,	20	3	–							
187	32	,,	20	12	–							
195	37	,,	35	2	–							
204	41	,,	30	11	–							
223	20	Normals	7	13	–							Unspotted : 2 with malformed head. Spotted : 1 with patchy reticulation.
	1	*Irregular*	–	*1*	–							*L eye irregular.*
Family 8. No F_3 offspring.												
Family 9.												
Brood 96 B ♀ mated in Fam. 6.												
Brood 106. Matings in bowl.												
143	35	Normals	*18	6	–				5	6	–	*Intersex : 1 unspotted A developed irregularity.
146 ♀ 1	19	,,	10	1	–				4	4	–	
148 ♀ 2	16	,,	7	3	–				4	2	–	
Brood 106 Pair 1 B ♂ and B ♀.												
152	13	Normals	5	5	–				3	–	–	
	1	*Irregular*	*1*	–	–				–	–	–	*R eye irregular.*
159	13	Normals	9	1	–				1	2	–	
Brood 106 Pair 2 B ♂ spotted dorsally and B ♀.												
154	2	Normals	2	–	–							
171	10	,,	6	4	–							
180	10	,,	7	3	–							
188	25	,,	20	5	–							
197	27	Normals	19	8	–							
	2	*Irregulars*	–	*2*	–							*1 with R eye No-white and irregular, 1 with R eye practically No-white.*
205	34	Normals	21	13	–							
	3	*Irregulars*	*2*	*1*	–							Unspotted : *1 with both eyes irregular; 1 with L eye.* Spotted : *R eye irregular.*
218	35	Normals	26	*9	–							*Intersex (5, p. 543), developed slight irregularity.
Families 10 and 11. No F_3 offspring.												
XXII.												
Family 1. No F_3 offspring.												

Brood Number.	Number in Class.	Description	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Family 2.												
Brood 101 B ♂ 2 and Red ♀												
160	1	Normal	1	–	–							
167	15	Normals	9	6	–							Unspotted: 1 developed irregularity and lost its antennæ.
177	19	,,	8	11	–							
185	17	,,	13	4	–							
192	16	,,	12	4	–							
203	28	,,	15	13	–							
215	14	,,	12	2	–							
Brood 110 B ♂ and B ♀.												
176	1	Normal	–	–	1	–	–	–				
184	2	Normals	1	–	–	–	–	1				
191	10	Normals	3	1	3	2	–	1				
	1	*Irregular*	*1*	–	–	–	–	–				*L eye slightly irregular.*
214	9	Normals	2	5	1	1	–	–				Red one developed irregularity.
	2	*Irregulars*	–	*2*	–	–	–	–				*1 with both eyes irregular; 1 with R eye.*
245	18	Normals	7	3	2	3	–	3				
Family 3.												
Brood 125. Red ♂ and ♀.												
150	1	Normal	–	–	–	1	–	–				
Family 4. No F_3 offspring.												
Cross-matings.												
IX. Fam. 3. 166*c* *No antenna* A ♂ × XIX Fam. 9. B ♀ 159.												
238	12	Normals	11	1	–							
263	20	Normals	14	6	–							
	2	*Irregulars*	*1*	*1*	–							Unspotted: *L eye slightly irregular, almost No-white.* Spotted: *L eye irregular.*
271	15	Normals	9	6	–							
	4	*Irregulars*	*4*	–	–							*2 with both eyes irregular; 1 with R eye; and 1 with L eye.*
280	10	Normals	10	–	–							
290	24	Normals	17	7	–							
	1	*Irregular*	–	*1*	–							*R eye irregular.*
301	35	Normals	27	8	–							
327	4	,,	3	1	–							
328	8	,,	*8	–	–							*Intersex (5, p. 544).
IX. Fam. 3. 156 B ♂ × XXII. Fam. 3. B ♀ 141.												
243	4	Normals	2	–	–	–	1	–	–	1	–	
253	20	,,	4	2	1	5	1	1	1	4	1	
XIX. Fam. 6. 121 BN ♂ × XXII. Fam. 3. B ♀ 141 (sister to the above).												
255	25	Normals	10	9	–	2	2	–	–	2	–	
IX. Fam. 3. 166*d* BN ♂ *irregular eyes* × BN ♀ 113 from CN.1*c* (similar mating to CN .1*b*=same ♂ and dorsally spotted A ♀.												
267	4	Normals	–	–	4							
276	21	,,	–	–	21							
287	13	,,	–	–	13							
295	8	,,	–	–	8							
IX. Fam. 3. 166*a* *No antenna* A ♂ × AN ♀ C 132 from AN+R stock.												
239	9	Normals							2	7	–	Unspotted: 1 developed spots.
275	14	,,							8	6	–	
IX. Fam. 3. 175*d* AN ♂ + B × Red ♀ M13 from Original Red Stock.												
RF.1	6	Normals	*6	–	–							*Intersex (5, p. 533).
IX. Fam. 2. 112 B ♂ 1 × B ♀♀ 223 Fam. 7, XIX.												
347	16	Normals	12	4	–							
363	1	Normal	1	–	–							
IX. Same 112 B ♂ × B ♀ 232 spotted. Fam. 6 XIX.												
353	15	Normals	12	3	–							
358	9	Normals	9	–	–							
	3	*Irregulars*	*2*	*1*	–							Unspotted: *1 with R eye; 1 with L eye irregular.* Spotted: *1 with R eye.*
366	3	Normals	3	–	–							

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
IX. Same 112 B ♂ × Red ♀ from M13. Original stock.												
357	3	Normals	2	1	–							
	2	*Irregulars*	*1*	*1*	–							Unspotted: *R eye irregular, developed irregularity in L eye.* Spotted: *both eyes irregular.*
373	5	Normals	3	2	–							

F_4 Generation.

IX. Family 2.
Brood*164. No F_4 offspring.

Brood 172. (1) B ♂ × B ♀ 171. Fam. 9, XIX.

242	3	Normals	2	1	–							
285	23	Normals	*26	3	–							*Intersex (**5**, p. 542)
	1	*Irregular*	*1*	–	–							*R eye irregular.*

Brood 172 (2) B ♂ × B ♀ (2) 171. Fam. 9, XIX.

230	6	Normals	4	2	–							
	1	*Irregular*	*1*	–	–							*R eye irregular.*
251	1	Normal	1	–	–							
279	18	Normals	16	2	–							
288	16	Normals	14	2	–							
	1	*Irregular*	*1*	–	–							*R eye irregular.*
356	18	Normals	13	5	–							

Brood 183. No F_4 offspring.
Brood 190. No F_4 offspring.
Brood 209 B ♂ × B ♀ 206b, *irregular* with L eye much smaller; became normal and eyes same size. Fam. 6, XIX.

304	12	Normals	9	3	–							

Same 209 B ♂ × B ♀ 246 (F_3). Fam. 3. IX.

305	6	Normals	6	–	–							
	1	*Irregular*	–	*1*	–							*R eye irregular.*
323	19	Normals	*12	7	–							*2 Intersexes (**5**, p. 536).
	2	*Irregulars*	*1*	*1*	–							Unspotted: *L eye irregular.* Spotted: *R eye irregular.*

Brood 174. No F_4 offspring.
Brood 173 B ♂ × B ♀.

236	2	Normals	1	1	–							
247	10	Normals	4	6	–							
	4	*Irregulars*	–	*4	–							*Intersex, *both eyes irregular* (**5**, p. 529); *2 with both eyes irregular and 1 with R eye.*
278	4	Normals	–	4	–							
	1	*Irregular*	–	*1*	–							*L eye irregular.*
289	6	Normals	*4	2	–							*Intersex (**5**, p. 528); Spotted: 1 lost both First Antennae, and developed great irregularity in both eyes; lobed; the other developed irregularity in R eye.
	6	*Irregulars*	*1*	*5	–							*Intersex (**5**, p. 533); Unspotted: *R eye irregular, developed in L also* Spotted: *2 with both eyes, 1 with R eye, and 2 with L eye irregular (one of these became irregular in R eye and intersex).*
298	17	Normals	11	6	–							
	4	*Irregulars*	*2*	*2*	–							*All with both eyes irregular.*

Same ♂ 173 × A ♀ 169. Fam. 4. XIX.

235	3	Normals	3	–	–							
	7	*Irregulars*	*1*	*6*	–							Unspotted: *R eye irregular.* Spotted: *1 with both eyes, 1 with R, and 4 with L eye irregular (the one with R eye irregular, developed irregularity in L eye also.*
237	2	Normals	2	–	–							
	4	*Irregulars*	*3*	*1*	–							Unspotted: *2 with both eyes irregular, and 1 with R.* Spotted: *R eye irregular.*

Brood Number	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Brood 173 B ♂ dorsal spot, *developed irregular L. eye* × B ♀ 171. Fam. 9. XIX.												
246	15	Normals	11	4	–							
Brood 173*h* B ♀ *both eyes irregular* × BN ♂ *Divided-eye* (see text-fig. 2)												
296	15	Normals	10	5	–							
302	7	,,	6	1	–							
312	11	Normals	9	2	–							
	2	*Irregulars*	–	2	–							*Both eyes irregular; (one with R eye divided in 2 pieces).*
318	18	Normals	18	–	–							
	5	*Irregulars*	4	1	–							Unspotted: *all with L eye irregular.* Spotted: *both eyes irregular.*
Brood 210*d* B ♀ spotted, developed *irregular L eye* × AN ♂ II.												
315	3	Normals	3	–	–							
	4	*Irregulars*	1	3	–							Unspotted: *L eye irregular.* Spotted: *1 with both eyes; 2 with L eye irregular.*
330	10	Normals	6	4	–							
	3	*Irregulars*	–	3	–							*1 with R eye, 2 with L eye irregular.*
Brood 210*l* B ♀ *R eye irregular* × AN ♂ V.												
337	4	Normals	4	–	–							
	2	*Irregulars*	–	2	–							*1 with both eyes, 1 with R eye irregular*
348	11	Normals	7	4	–							Unspotted: 1 survivor developed irregularity in both eyes.
	2	*Irregulars*	–	2	–							*Both with L eye irregular.*
Brood 210*e* B ♀ *irregular both eyes* × AN ♂ V.												
338	6	Normals	1	2	–	2	1	–				
	4	*Irregulars*	1	2	–	1	–	–				B unspotted: *both eyes irregular.* Spotted: *1 with both eyes, 1 with R eye irregular.* Unspotted: R, *with L eye.*
Brood 210*n* **One-eye** *left eye missing, and R eye irregular* × AN + R ♂ II.												
321	3	Normals	1	2	–							
	5	*Irregulars*	3	2	–							Unspotted: *1 with R eye, 2 with L eye irregular.* Spotted: *1 with both eyes, and 1 with L eye irregular.*
Brood 210*n*, **One-eye** same B ♀ × AN + R ♂ from same stock as II and V.												
379	12	**All irregular**	3	9	–							Unspotted: *1 with both eyes, 2 with L eye irregular.* Spotted: *6 with both eyes, 2 with R, and 1 with L eye irregular.*
Brood 210*l* B ♀ as above × B ♂ 259*j*, same family, *L eye irregular at birth, R eye developed irregularity later.*												
370	2	Normals	1	1	–							
	6	*Irregulars*	3	3	–							Unspotted: *both eyes irregular* Spotted: *1 with R eye, 2 with L eye irregular.*
Brood 259*h* B ♂ and B ♀ (♂ *both eyes irregular,* ♀ *left eye very irregular, became Intersex).*												
336	2	**Irregulars** (see p. 368 for description).										
Brood 199. No F_4 offspring.												
Brood 228. No F_4 offspring.												
Brood 249 B ♀ × BN ♂ 257 of this same family.												
339	15	**All Irregular** 4 3 and 3 All eyes different.				unspotted B or BN?						
		No-eyes 3 (2 spotted).										
		One-eyes 2 Both with **Right eye missing** *and*							*Left eye microscopic.*			
349	26	**All Irregulars,** all with different eyes. Figured on Plates IV, 5 and 6.										
		No-eyes 4 (2 spotted).										
		One-eyes 8 (5 with **Right eye missing** and 3 with **Left eye missing**).										
Brood 261. No F_4 offspring.												
Brood 198. No F_4 offspring.												
Brood 220 B ♂ **One-eye,** developed the second eye (See Text-fig. 5) × AN ♀ from C. stock.												
319	19	Normals	3	–	1	2	1	2	4	1	5	=9 Coloured: 10 Albino (one ♂ lost its First Antennae).
Brood 241. No F_4 offspring.												
Brood 257. 1 BN ♂ (see Brood 249 above).												
Brood 268. No. F_4 offspring.												

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
IX. Family 3.												
Brood 200.	A ♂ and A ♀ both spotted.											
283	3	Normals							2	1	–	
292	11	,,							4	7	–	
303	16	,,							9	7	–	
311	6	,,							4	2	–	
320	8	,,							4	4	–	
345	16	Normals							9	7	–	
	3	*Irregulars*							–	*3*	–	*1 with R eye, and 2 with L eye very irregular.*
Brood 211c	B ♂ spotted dorsally, *L eye irregular* × B ♀.											
351	3	Normals	3	–	–							
Brood 211e	B ♂ spotted dorsally, *irregular L eye*					*which became normal* × B ♀ unspotted from Brood 245. Fam. 2, XXII.						
344	10	Normals	5	2	–				–	3	–	
	1	*Irregular*	*1*	–	–				–	–	–	*L eye slightly irregular.*
Brood 231	B ♂ and B ♀ both spotted.											
359	9	Normals	5	1	–	2	1	–				
	3	*Irregulars*	*1*	*2*	–	–	–	–				Unspotted: *L eye irregular.* Spotted: *1 with R eye, and 1 with L eye irregular.*
365	14	Normals	6	1	–	4	3	–				B spotted with L eye No-white.
	3	*Irregulars*	*1*	*2*	–	–	–	–				Unspotted: *L eye irregular.* Spotted: *1 with R eye, 1 with L eye irregular.*
Brood 248	B ♂ × Red ♀, 243 dorsally spotted (same family).											
355	13	Normals	7	–	–				2	4	–	
Brood 260.	No F_4 offspring.											
Brood 269	B ♀ spotted × RN ♂ from No-white Stock.											
368	22	Normals	3	–	6	4	1	8				
	1	*Irregular*	*1*	–	–	–	–	–				*R eye slightly irregular*
Brood 269b	B ♂ spotted × BN ♀ from No-white Stock.											
369	3	Normals	3	–	–							
Broods 277: 286: 299: 314: 212: 181: 182						254: 262: 310:			and 123. No F_4 offspring.			
XIX. Family 4.												
Brood 216.	No F_4 offspring.											
Brood 226.	No F_4 offspring.											
Family 6.												
Broods 189: 196: 208: and 225.	No F_4 offspring.											
Brood 233	Red ♂ × Red ♀.											
339a	3	Normals				3	–	–				
Broods 127 and 130.	No F_1 offspring.											
Brood 132	Pair 1 B ♂ and B ♀.											
207	22	Normals	12	4	–	5	1	–				
234	5	,,	1	3	–	–	1	–				
256	37	,,	16	11	–	8	2	–				
266	27	Normals	14	5	–	7	1	–				
	4	*Irregulars*	*1*	*1*	–	*2*	–	–				Unspotted: B *and* R *with L eye irregular.* Spotted: B *with R eye irregular.*
273	18	Normals	13	–	–	5	–	–				
Brood 132 B	B ♀ × B ♂ 142 (same family).											
206	3	Normals	–	1	–	–	2	–				
	4	*Irregulars*	–	*2*	–	–	*2*	–				Spotted: B *1 with both eyes irregular, 1 with L eye*; R *1 with both eyes irregular, and 1 with R eye.*
217	11	Normals	6	5	–							
	6	*Irregulars*	*3*	*2*	–	–	*1*	–				Unspotted: *2 with R eye irregular, 1 with L.* Spotted: B, *1 with R, and 1 with L*; R, *1 with R eye irregular.*
240	4	Normals	2	2	–							
	2	*Irregulars*	*1*	*1*	–							Unspotted: *R eye irregular.* Spotted: *L eye irregular.*
264	13	Normals	8	3	–	1	1	–				
272	12	Normals	7	2	–	2	1	–				
	2	*Irregulars*	*1*	–	–	–	*1*	–				Unspotted: B *with both eyes irregular.* Spotted: R *with R eye.*

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
282	16	Normals	8	4	–	4	–	–				All with very flat eyes, not convex as is normal.
	3	*Irregulars*	*1*	*2*	–	–	–	–				Unspotted : *L eye irregular*. Spotted : *2 with both eyes irregular*.
291	5	Normals	3	1	–	1	–	–				Pigment almost red.
	1	*Irregular*	–	*1*	–	–	–	–				*R eye irregular*.
Brood 136 Pair 2, B ♂ and B ♀.												
193	*1*	*Irregular*	*1*	–	–							*Both eyes irregular*.
Brood 136 Pair 3, B ♂ and B ♀.												
201	5	Normals	5	–	–							
	1	*Irregular*	*1*	–	–							*R eye irregular*.
213	6	Normals	6	–	–							
	7	*Irregulars*	*4*	*1*	–	*1*	*1*	–				Unspotted : B, *1 with both eyes irregular, 2 with R eye, and 1 with L eye ;* R, *1 with R eye*. Spotted : B *with L*, R *with R eye*.
229	10	Normals	7	2	–	1	–	–				
	15	*Irregulars*	*3*	**7*	–	–	*5*	–				*Intersex (5, p. 523). Unspotted : *1 with R, 2 with L eye*. Spotted : B, *2 with both eyes, 3 with R, 2 with L eye ;* R, *1 with both eyes irregular, 1 with R eye, and 3 with L eye*.
Brood 139. No F_4 offspring.												
Brood 142. 1 B ♂ mated with ♀ 132 (see above).												
Brood 151 B ♂ × B ♀ 105*a* (same family).												
219	12	Normals	7	5	–							
	4	*Irregulars*	*2*	*2*	–							Unspotted : *R eye irregular*. Spotted: *1 with R, 1 with L eye*.
232	37	Normals	31	6	–							
	1	*Irregular*	*1*	–	–							*R eye irregular*.
Brood 157. No F_4 offspring.												
Brood 165*d* B ♂ dorsally spotted × B ♀ spotted.												
227	2	Normals	–	1	–				1	–	–	
	1	*Irregular*	*1*	–	–				–	–	–	*R eye irregular*.
250	7	Normals	3	2	–	2	–	–				
	1	*Irregular*	–	*1*	–	–	–	–				*R eye irregular*.
Brood 165 B ♂ *R eye irregular* × Red ♀ RE 66 from Original Stock.												
244	10	Normals	3	–	–	7	–	–				
Brood 265. 1 B ♂ spotted.												
Brood 274. 1 B ♀ spotted and *developed irregularity*. 265 B ♂ × 274 B ♀.												
342	6	Normals	2	3	–	1	–	–				
	17	Normals	9	4	–	4	–	–				
Broods 284 : 293 : and 115. No F_4 offspring.												
Brood 121. 1 B ♂ with B ♀ 141 (XXII).												
Family 7. Broods 163 : 170 : 179 : 187 : 195 : and 204.						No F_4 offspring.						
Brood 223. 2 ♀♀ with ♂ 112. IX.												
Family 9. Brood 143 B ♂ × B ♀ 105. Fam. 7. XIX.												
222	10	Normals	8	2	–							
Broods 146 : 148 : and 152. No F_4 offspring.												
Brood 159 B ♂ and B ♀.												
221	10	Normals	10	–	–							
Brood 154. No F_4 offspring.												
Brood 171. 2 ♀♀ put with Brood 172. Fam. 2. IX. Broods 180 : 188 : 197 : 205 : and 218. No F_4 offspring.												
XXII. Family 2. Broods 160 : 167 : and 177. No F_4 offspring.												

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Brood 185c B ♂ and B ♀-both dorsally spotted.												
252	11	Normals	2	5	1	–	2	1				
270	5	,,	2	1	–	1	–	1				
281	10	,,	2	5	2	–	–	1				
Broods 203 : 215 : 176 : 184 : and 191. No F_4 offspring.												
Brood 214 BN ♂ and B ♀ *irregular eyes.*												
294	10	Normals	4	1	–				–	2	3	
Brood 214 same BN ♂ and B ♀ spotted.												
297	2	Normals	2	–	–							
Brood 245 B ♀ × A ♂ 253*b*. Fam. 3. IX.												
341	2	Normals	1	1	–							
	3	*Irregulars*	*1*	*2*	–							Unspotted : *R eye irregular.* Spotted: *L eyes irregular.*
Brood 245 B ♀ × B ♂ 211c. Fam. 1. IX.												
344	10	Normals	5	2	–				–	3	–	
	1	*Irregular*	*1*	–	–							*L eye very slightly irregular.*
Brood 245 R ♀ × BN ♂ *divided eye* (Text-fig. 2).												
343	21	Normals	5	3	4	4	–	5				
	2	*Irregulars*	–	*1*	–	–	–	1				*Both with L eye irregular.*
Family 3.												
Brood 150. No F_4 offspring.												
Cross matings.												
Broods 238 : 263 : and 271. No F_4 offspring.												
Brood 280 B ♂ and B ♀.												
324	3	Normals	2	1	–							
Broods 290 : 301 : 327 : 328 : and 243. No F_4 offspring.												
Brood 253 with Brood 245 (above).												
Broods 255 : 267 : 276 : 287 : and 295. No F_4 offspring.												
Brood 239c A ♂. very spotted × Red ♀ from Original Stock.												
306	5	Normals	2	–	–	3	–	–				
Broods 275 : 347 : 353 : 358 : 357 : 373 : 363 : 366. No F_4 offspring.												

F_5 Generation.

IX. Family 2.												
Broods 230 : 251 : 279 : 288 : 242 : and 285. No F_5 offspring.												
Brood 356 B ♂ and B ♀.												
419	1	Normal	1	–	–							
434	10	Normals	10	–	–							
447	8	,,	7	1	–							
Broods 304 : 305 : and 323. No F_5 offspring.												
Brood 236 B ♂ × A ♀ dorsally spotted *very irregular* from Albino Stock.												
300	11	Normals	7	4	–							
Brood 236 same B ♂ × Red ♀ 233 *irregular.* Fam. 6. XIX.												
307		**All Irregulars**	*1*	*3*	–							Unspotted : *L eye irregular.* Spotted : *2 with both eyes, and 1 with L eye irregular.*
		One-eye	–	*1*	–							**Left eye missing.**
Brood 236 same B ♂ × Red ♀ 233 (2) *irregular.* Fam. 6. XIX.												
308	12	Normals	5	7	–							
	2	*Irregulars*	*1*	*1*	–							Unspotted : *R eye irregular.* Spotted: *both eyes irregular.*
322	7	Normals	4	3	–							
	1	*Irregular*	1	–	–							*L eye irregular.*
331	27	Normals	25	2	–							
Broods 247 and 278. No F_5 offspring.												
Brood 289*f* B ♀ *eyes very irregular, and lobed. No antenna* × BN ♂ *No antenna developed*												*irregularity* from CN 224 Fam. 3. XXII.
380	6	Normals	4	2	–							
	2	*Irregulars*	–	*2*	–							*1 both eyes irregular, 1 with L eye irregular.*
394	11	Normals	10	1	–							

Brood Number.	Number in Class.	Description.	Black. Unspotted.	Black. Spotted.	Black. No-white.	Red. Unspotted.	Red. Spotted.	Red. No-white.	Albino. Unspotted.	Albino. Spotted.	Albino. No-white.	Notes.
Brood 298 B ♂ and B ♀.												
421	12	Normals	6	1	–	5	–	–				2 have slightly irregular pigment.
Broods 235 and 237. No F_2 offspring.												
Brood 246 B ♀ spotted × B ♂ 209 same stock (see						under ♂).						
Brood 296 B ♂ and B ♀.												
354	2	Normals	2	–	–							
360	6	,,	4	1	1							
367	7	,,	6	–	1							1 unspotted B with slightly irregular pigment.
Broods 302 and 312. No F_5 offspring.												
Brood 318 B ♂ and B ♀.												
372	4	Normals	–	–	2				1	1	–	
	2	*Irregulars*	2	–	–				–	–	–	*Both eyes slightly irregular.*
Broods 315 : 330 : 337 : 348 : 338 : and 321. No F_5 offspring.												
Brood 379*b* B ♂ (Text-fig. 4) × B ♀ 394 (F_3 gen.) fro						m 289 *No antenna*			mating (above).			
429	10	Normals	2	4	2				1	–	1	
Broods 370 : *336 : 339 : 349 :* and 319. No F_5 o						ffspring.						
Family 3.												
Broods 283 : 292 : 303 : 311 : 320 : 345 : 351 : 3						44 : 359 : 365 : 3			55 : 368 : and 3			69. No F_5 offspring.
XIX. Family 6.												
Broods 339*a* : 307 : 207 : 234 : 256 : 266 : and 27						3. No F_5 offsprin			g.			
Brood 206*b* B ♀ spotted, *very irregular eyes which bec*						*ame normal* × B ♂			209 Fam. 2, IX.			
304	12	Normals	9	3	–							
Brood 206*b*. Same B ♀ × B ♂ 217*b* dorsally spotted,						same family.						
313	6	Normals	–	1	–	4	1	–				
	1	*Irregular*	–	–	–	–	*1*	–				*Both eyes irregular*
317	9	Normals	8	1	–	–	–	–				
332	21	,,	14	4	–	3	–	–				
335	13	,,	9	2	–	2	–	–				
Brood 206*a* R ♀ *irregular eyes* × same B ♂ 217*b*.												
333	3	Normals	2	–	–	1	–	–				
346	11	,,	8	2	–	–	1	–				
Brood 240. No F_5 offspring.												
Brood 264 Red ♂ and B ♀.												
329	4	Normals	3	–	–	1	–	–				
Broods 272 : 282 : 291 : 193 : 201 : 213 : and 229						. No F_5 offspring			.			
Brood 219 B ♀ *clusters of ommatidia sep. in both eyes*						× B ♂ 223 spotted			(XIX).			
325	1	Normal	1	–	–							
326	4	Normals	4	–	–							
340	7	,,	5	2	–							
Brood 232 (see under ♂ 112) : 227 : and 250.						No F_5 offspring.						
Brood 244 B ♂ *No antenna* × Red ♀ from Pure Red						Stock.						
350a	6	Normals	3	–	–	3	–	–				
350b	13	,,	3	1	–	9	–	–				
Broods 342 and 352. No F_5 offspring.												
Family 9.												
Brood 222. No F_5 offspring.												
XXII. Family 2.												
Broods 252 : 270 : 281 : 294 : and 297. No F_5 off						spring.						
Brood 343 RN ♂ *spotted* and RN ♀.												
378	4	Normals	–	–	–	–	–	4				
Broods 341 : and 344. No F_5 offspring.												
Cross matings												
Broods 324 : and 306. No F_5 offspring.												

Abstracts of Memoirs

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY.

The Osmotic Properties of Medusæ.

By J. B. Bateman.

J. Exp. Biol., 9, *pp.* 124–127, 1932.

The erroneous statement made by Gortner (1929) that the water content of medusæ is 99·8 per cent, is corrected by reference to earlier works (Krukenberg, 1880 ; Mœbius, 1880, 1882 ; Vernon, 1896 ; Myers, 1919). Macallum's statement (1903) that slowness of diffusion in the medusæ is responsible for the existence of considerable osmotic differences between the animal and the surrounding sea-water, is corrected by experiments in which the vapour pressure of the jelly was compared with that of the external medium. The animals, whether alive or dead, rapidly came into osmotic equilibrium with their environment. " Bound " water determinations on slices of the jelly disc, by A. V. Hill's " direct " and " differential " methods (1930), showed nearly all of the water to be capable of acting as a solvent for added substances.

J. B. B.

On the Influence of the Extra-Cardiac Nerves upon Sino-Auricular Conduction in the Heart of Scyllium.

By J. J. Izquierdo.

Journal of Physiology, Vol. LXIX, No. 1, *pp.* 29–47, *March*, 1930.

Previous observers had reported, both for the mammals and cold-blooded animals, that during faradization of the vagus nerves blocks are produced or increased and conduction intervals are lengthened. Cases of " reversed " vagus action, in which stimulation of the nerve produced the opposite effects, were also known and explained as due to the stimulation of sympathetic fibres included in the vagus trunk.

Nevertheless, neither the " pure " sympathetic effect in the hearts in which the " reversed " effects had been described was known, nor sufficient evidence was at hand to prove that improvement and slowing of conduction are always characteristic of vagal and sympathetic stimulation.

In view of this double interest, two first series of experiments made by the author in the turtle (*Am. Journ. of Physiol.*, LXXXVIII, p. 195, 1929) and in the dog (*Am. Journ. of Physiol.*, XCI, p. 696, 1930) were completed at the Laboratories of the Marine Biological Association with a third one made on the heart of Scyllium. The results of the experiments, which were published in the *Journ. of Physiol.*, LXIX, p. 29, 1930, are summarized as follows :—

1. Faradization of the nerves coursing along the walls of the ducti Cuvieri brought about two groups of effects upon conduction between *sinus and auricle* impaired by pressure.

(*a*) *Primary effects.* After a stimulation which slows the sinus rhythm or produces a partial block, and independently of the slowing produced which by itself should improve conduction, the S-A intervals are lengthened (50–75%) and gradually return to normal after the stimulation is over.

Neither normal nor impaired conduction were influenced by the stimulation.

(*b*) *After-effects.* Stronger faradization, producing total blocks and arrest of the heart, are followed by an extremely brief secondary phase of opposite character.

2. *After atropinization*, the vagal effects are no longer observed. Instead, the S–A intervals are found appreciably shortened both during and after the end of the stimulation.

This shows that the influence of the vagus upon the basal segments of the heart of Scyllium consists in lengthening conduction, while the sympathetic influence, although less notable, has an opposite character.

J. J. I.

The Larval Stages of Caridion, with a Description of a New Species *C. steveni.*

By Marie V. Lebour, D.Sc.

Proc. Zool. Soc., London, May, 1930, *pp.* 181–194.

The larvæ which were regarded by Sars as belonging to *Pandalus borealis* and *P. bonnieri* are now found to belong to Caridion. Sars' *P. borealis* is the larva of *Caridion gordoni* and Sars' *P. bonnieri* is the larva of a new species, here called *C. steveni*. *C. gordoni* is a deeper water species and has not been found at Plymouth in the adult state, although the young stages and the larvæ occur, the larvæ fairly commonly. *C. steveni* is occasionally to be found between tide-marks and its larva occurs with that of *C. gordoni* or separately. The larvæ, post-larvæ, and young stages are described in this paper and the new species *C. steveni* described

in its adult state. The larvæ of the latter species were hatched out in the Laboratory, and the late larvæ of both species kept until they turned into post-larvæ and young.

M. V. L.

The Larvæ of the Plymouth Caridea.—I. The Larvæ of the Crangonidæ. II. The Larvæ of the Hippolytidæ.

By Marie V. Lebour, D.Sc.

Proc. Zool. Soc., London, April, 1931, *pp.* 1–9.

The larvæ of all the species of Crangon and Philoceras occurring at Plymouth are described and all but one have been hatched from the egg. *Philocheras sculptus* larva is described for the first time.

The larvæ of the two species of Hippolyte known from Plymouth are described. Both were hatched from the egg. *H. prideauxiana* larvæ are described for the first time. *H. fascigera* Gosse is shown by hatching its larvæ to be a variety of *H. varians*.

M. V. L.

Further Notes on Larval Brachyura.

By Marie V. Lebour, D.Sc.

Proc. Zool. Soc., London, April, 1931, *pp.* 93–96.

There are additions to the " Larval Stages of the Plymouth Brachyura." Amongst other things *Pisa biaculeata* has been hatched from the egg in a plunger jar, the zoea being more like Inachus and Macropodia than it is like Hyas, although there are certain important differences. A table shows the relationship with these crabs.

M. V. L.

Preliminary Studies on the Bacterial Cell-Mass (accessory cell-mass) of *Calandra oryzæ* (Linn.) : the Rice Weevil.

By K. Mansour.

Quart. Jour. Micro. Sci., 73 (3) 421–436, 2 *plates*, 4 *figs.*, 1930.

Examination of the ovaries has shown the presence of bacterial cells at the tips of the ovarioles. From these cells the bacteria pass to the germarium and infect the growing oocytes at a very early stage. During embryonic development an alimentary bacterial cell-mass is formed in all eggs. In the eggs destined to give rise to females a second bacterial

cell-mass is formed close to the genital cells. This later on gives rise to bacterial cells at the tips of the ovarioles. A study of the feeding habits of Calandra and *Hylobius abietis*, another similarly infected weevil, throws doubt on the supposed digestive rôle of this kind of bacteria.

K. M.

Tyrosinase in Crustacean Blood.

By Kathleen Godwin Pinhey.

Brit. Jour. Exp. Biology, Vol. VII, p. 19, 1930.

The blackening of crustacean blood when it is shed, or in clots at wounds, is caused by an enzyme similar to, if not identical with, the tyrosinase systems previously described in various invertebrates, fungi and in the higher plants. The components of the system are an enzyme contained in the blood corpuscles, from which it is freed on cytolysis, and its substrate tyrosine, free in the blood stream. The enzyme is by definition a tyrosinase, since it will bring about the oxidation of tyrosine with the ultimate production of melanin, deriving the oxygen necessary for the reaction from the air. The tyrosinase content of the blood is not constant, nor does it undergo a seasonal variation. The possibility of shorter cycles in tyrosinase activity has not been investigated.

The blood will accelerate the oxidation of diphenylenediamine and α napthol to the blue indophenol derivative; but as this reaction is comparatively insensitive to NaCN, it is unlikely that it is due to an indophenol oxidase.

The enzyme is inhibited by low molecular concentrations of NaCN, indicating the presence of a metallic group as an active part of the enzyme molecule. The activity of the enzyme is also depressed by H_2S, $CuSO_4$, $FeCl_3$, sodium fluoride, sodium pyrophosphate, and the alcohols. Of the latter, ethyl alcohol is effective in concentrations of 2·7 molar, while methyl has no effect in 3·5 molar concentration. This is the expected result from Warburg's hypothesis, but as tyrosine is insoluble in alcohol, and the amounts of the higher alcohols which could be introduced into the watery solution were too small to have any affect, a series could not be investigated. Thymol, phenyl urethane and urethane in certain concentrations will depress the oxidation of tyrosine by the enzyme, but higher concentrations of these reagents increase the rate of oxidation. A possible explanation of this effect is discussed.

K. G. P.

Studies on some Sporozoa in Polychæte Worms. I. Gregarines of the genus Selenidium.

By Harendranath Ray.

Parasitology (Cambridge), Vol. XXII, pp. 370–398, 1930, *with* 4 *plates and* 3 *figs. in the text.*

1. The main facts in Brasil's (1907) account of the intracellular schizogony in *Selenidium caulleryi* from *Protula tubularia* have been confirmed. Early stages in the sporogony of this species are described now for the first time.

2. Intracellular schizogony is described for the first time in *Selenidium mesnili* Brasil from *Myxicola infundibulum*, and here also the early stages in sporogony are noted for the first time.

3. The life-histories of Caullery and Mesnil's two unnamed species of *Selenidium* (now called *S. spionis* (Koll.) and *S. foliatum* n.sp.) from *Scolelepis fuliginosa* have been exhaustively studied. No schizogonic phase has been seen in either. The gametocytes, here described for the first time, are found to develop fully only after escape into the sea. The gametocytes of *S. spionis* are ovoid, $108\mu \times 60\mu$, and contain spores with four sporozoites: those of *S. foliatum* are spherical, 70μ in diameter, and their spores contain eight sporozoites.

4. Precocious association is observed in *S. foliatum*. In this species two associates are of very different sizes—the larger is $230–250\mu \times 40–50\mu$, and the smaller $40–50\mu \times 15–30\mu$—and when stained *intra vitam* with neutral red, the cytoplasm of the shorter of the two behaves differently from that of the longer and shows innumerable pink granules.

5. New or hitherto little-known species of Selenidium are recorded from *Cirratulus cirratus*, *Branchiomma vesiculosum*. *Sabella pavonina* and *Terebella lepidoria* and the morphological characters of the trophozoites are described.

6. A discussion follows of the value of the diagnostic characters of the genus Selenidium. It is suggested that this genus requires drastic revision and will probably have to be dismembered. Stress is laid, however, on the occurrence in all the gregarines examined, and at all the described stages of their development, of characteristic chromatic bodies at the anterior end, structures which have hitherto escaped the notice of most observers.

H. N. R.

Studies on some Sporozoa in Polychæte Worms. II. *Dorisiella scolelepidis* n. gen., n. sp.

By Harendranath Ray.

Parasitology (Cambridge), Vol. XXII, pp. 471–480, 1930.

The organism dealt with here is the first gut-inhabiting organism remotely resembling Coccidia that has ever been described in detail from an annelid worm. The chief characters are : chromatic granules present at the anterior end at all stages ; two types of schizogony, with merozoites arranged " en barillet " ; the macrogametocytes are migratory ; no oocyst is developed round the zygote ; two spores are formed, each containing eight sporozoites. Type-species : *Dorisiella scolelepidis*, in the epithelial cells of the gut of *Scolelepis fuliginosa* Clpde.

H. N. R.

Book Notice.

"A Handbook of the British Seaweeds."

By Lily Newton, Ph.D., F.L.S.

Pp. xii+478. *London. The Trustees of the British Museum (Natural History).* 1931. 15*s.*

Since 1871 the main means of identifying British seaweeds has been Harvey's *Phycologia Britannica*. As long ago as 1887 George Murray, in a review of Hauck's *Die Meeresalgen*, said that "a corresponding book on British algæ is more needed now than at any time since the study has been earnestly taken up." Many additions have subsequently been made to our flora, with increasing difficulties for collectors who have not access to libraries and herbaria. The present handbook thus meets a long-felt want. The blue-green, green, brown, and red marine algæ are all included and identification is aided by both generic and specific keys. There are a large number of illustrations: habit and structural drawings are given for each genus and for the most part these are excellent. The arrangement is fittingly based on Batters' *Catalogue of the British Marine Algæ* (1902), for he himself intended to write some such book but unfortunately died in 1907. His catalogue will still be of use to supplement the details of distribution in these islands which Dr. Newton gives for each species.

The aim has been to include the whole of the British seaweeds, but one notices a few species which do not appear to be mentioned. Among these are *Callymenia Larteriæ* Holmes (North Devon, Clare Island, Guernsey), *Ptilothamnion lucifugum* Cotton (Clare Island), *Ctenosiphonia hypnoides* Falkbg. (Guernsey). Perhaps the most important omission is that of *Colpomenia sinuosa* Derb. & Sol., a brown alga from the Indian Ocean, which was first noticed in 1907 and has since spread widely on the south and west coasts (Cotton, *Kew Bulletin*, 1908, 1911); it has been described as a pest in oyster-beds in France. The beginner would probably confuse it with *Leathesia difformis*.

These are, however, but minor criticisms of a valuable and well-produced book; considering the wealth of illustrations it is cheap and should do much to stimulate interest in seaweeds. There is no longer the band of amateur algologists so conspicuous in the last century and still remembered in many specific and generic names, enthusiasts such as Dr. John Cocks, who is said not to have missed a single low tide at Plymouth or Falmouth for twenty years, and observers like the Rev. J. H.

Pollexfen, who blindfolded could tell most of the Orkney algæ by their smell alone. The purely collecting phase has passed and there are not likely to be many more macroscopic additions to our flora. Recent work has however shown what an astonishing variety of life-cycles may be found in the marine algæ—red, green, and brown—and how much of their biology still remains to be elucidated. To quote Murray again : "access to the study is so specially easy in this country that it may be regarded as a national duty to keep a Greville always with us." Be this as it may, with the facilities now afforded by our several marine biological stations and by Dr. Newton's handbook marine algology should be considerably furthered.

M. A. W.

Marine Biological Association of the United Kingdom.

Report of the Council, 1931.

The Council and Officers.

Four meetings of the Council have been held in London during the year, at which the average attendance was sixteen. The thanks of the Association are due to the Royal Society, in whose rooms at Burlington House the meetings have been held. A Committee of six members of the Council visited and inspected the Plymouth Laboratory in April.

The Council received with regret the resignation of Mr. George Evans from the post of Honorary Treasurer, which took place in April. Mr. Evans had been Honorary Treasurer for sixteen years, during a period of considerable expansion in the work of the Association, and had rendered signal service by the care he had exercised over its finances.

Mr. Evans has been succeeded in the Hon. Treasurership by Mr. Nigel O. Walker, one of the Governors representing the Worshipful Company of Fishmongers.

The Council records with regret the death of Mr. Lothian D. Nicholson, who was also a Governor representing the Fishmongers' Company.

The Plymouth Laboratory.

The buildings and fittings have been maintained in a good state of repair, which included overhauling and painting the woodwork of the south, east, and west sides of the main building and relaying the worn floor in the entrance passage.

The Otto gas engine, installed in 1888, has been done away with and a motor pump and an air compressor, both working on the Corporation electric supply, have been obtained for circulating water and air through the tanks. The variable-voltage direct-current dynamo has been re-installed, driven by an electric motor.

The boiler supplying the heating system throughout the laboratories has been enlarged to cope with the new Library and the building extension in progress, and is now fired by oil fuel with automatic temperature control. Satisfactory heating and economical working are being obtained from it.

Extension of Laboratory Buildings.

The new Library, the necessity for which was pointed out in last year's Report, has been completed, in accordance with plans prepared by Mr. E. H. A. Barron, F.F.A.S., of Plymouth, the architect appointed by the

Council. The accommodation provided both for books and for readers is most convenient and suitable, and this extension has been greatly appreciated by all our workers.

The old galvanized-iron store near the main entrance gates has, through the generosity of Mr. E. T. Browne who contributed £310 for the purpose, been rebuilt in lime-stone, and the bridge connecting the south and north buildings has been faced with dressed stone. These alterations have not only increased our storage, but have very greatly improved the appearance of the approach and entrance to the Laboratory.

The old Library room has been converted into a good laboratory for three workers, and the former librarian's room is to be used for the type museum of local fauna.

The north building is being extended in an easterly direction to provide more laboratory accommodation, partly in place of that taken for the new Library and partly to increase facilities for visiting workers. This has been made possible through the generosity of the Rockefeller Foundation of New York, whose Trustees have made a grant to the Association of such sum as may be necessary (including certain equipment) up to £4642. It is hoped that the new laboratories may be completed before Easter.

The Ship and Motor-Boat.

The steam drifter *Salpa* has been kept in good order and both hull and engines are in first-class condition. She has been kept steadily at work during the year, with the exception of the necessary periods in harbour for refit.

The motor-boat *Gammarus* has had a thorough overhaul by the man who originally built her. She was found to be in excellent condition, only minor defects requiring attention.

The Staff.

At the invitation of the Oyster Growers and Dealers' Association of North America and the National Shellfish Association, Dr. C. M. Yonge spent July and August in the United States and attended the annual conference of these bodies. Under their auspices he visited the most important centres of oyster culture along the Atlantic seaboard.

The only change in the staff during the year has been the promotion of Mr. G. M. Spooner from Student-Probationer to an additional post of Assistant Naturalist, which was sanctioned by the Development Commissioners. In order to effect an economy during the present financial stringency, no appointment is being made immediately to the Student-probationership vacated by Mr. Spooner.

During the year H.M. Treasury agreed to provide the funds necessary for the Association to adhere to the Federated Superannuation System for Universities, and made the provision retrospective as from January 1st, 1927. It will be a very great advantage to our staff to be members of this superannuation system.

Occupation of Tables.

The following investigators have occupied tables at the Plymouth Laboratory during the year:

Dr. C. Amirthalingam, London (Central nervous system of Scyllium).
Miss D. Atkins, London (Pinnotheres and Loxosoma).
J. B. Bateman, London (Osmotic pressure of body fluid of marine animals).
Miss A. Bidder, Cambridge (Cephalopod larvæ. Digestive system of Loligo).
Miss M. V. Bishop, London (General Zoology).
Miss M. A. Borden, Canada and London (Respiration and the function of hæmoglobin in Planorbis and Arenicola).
H. J. H. Borley, Cambridge (Sponges).
Prof. D. W. Bronk, Philadelphia and London (Energy exchanges in crab muscle).
Prof. H. Graham Cannon, Sheffield (Crustacean blood system).
Capt. R. Charles, London (General Zoology).
J. S. Colman, Cambridge (Shore Zoology).
B. Dawes, London (Plaice cytology).
A. C. Downing, London (Energy exchanges in crab muscle).
Dr. G. H. Faulkner, London (Alcyonidium).
T. P. Feng, London (Osmotic pressure of body fluids).
E. B. Ford, Oxford (Gammarus).
D. C. Gall, London (Submarine photometer).
Miss E. Georgeson, Leeds (Budding and regeneration in Ascidians).
A. Graham, Sheffield (Digestion of Gastropods).
J. Gray, Cambridge (Animal movement).
O. I. Green, Oxford (Food of fishes).
I. T. Hamilton, Cambridge (Ecology).
Prof. C. R. Harington, London (Sea Action Committee).
J. E. Harris, Cambridge (Viscosity of protoplasm).
C. H. Hartley, Eton (General).
G. T. D. Henderson, Bristol (Distribution of fish eggs and larvæ).
Prof. A. V. Hill, London (Energy exchanges in crab muscle).
A. D. Hobson, Edinburgh (Fertilisation and parthenogenesis in invertebrates).
S. W. Hutcherson, Ramsgate (General Zoology).
Prof. J. S. Huxley, London (Gammarus).
Dr. O. G. Ibañez, Madrid (Oceanographical chemistry).
C. C. John, London (Development of Spadella).
Miss M. Johnson, Birmingham (Rate of Scaphognathite beat in Decapods).
E. I. Jones, London (Parasites in Nucula and fish).
Dr. A. B. Keys, California (Library).
J. A. Kitching, Cambridge (Echinoid eggs).
Mrs. M. K. Krainska, Warsaw (Development of Decapods)
S. H. Lele, Liverpool and Madras (Thymus in fish).

Dr. D. McClean, London (Effect of testicular extract on permeability of ova).
Prof. D. L. Mackinnon, London (Parasitic Protozoa).
Dr. F. G. Maskell, Oxford and Wellington, N.Z. (Tunicata).
H. B. Moore, Port Erin (Fæces of marine animals).
Dr. Th. Mortensen, Copenhagen (Sea stars and brittle stars).
R. Naidu, Liverpool and Madras (Plankton).
Prof. T. C. Nelson, New Brunswick, N.J. (Physiology of feeding in Lamellibranchs).
A. G. Nicholls, Australia (Ligia).
F. P. O'Neill, Newcastle (Plankton).
C. F. A. Pantin, Cambridge (Respiratory pigments of Cucumarians).
J. L. Parkinson, London (Osmotic pressure of body fluid of marine animals).
Miss M. Parke, Port Erin (Marine algæ).
L. E. R. Picken, Cambridge (Osmotic regulation in Sipunculids).
J. A. Ramsay, Cambridge (Respiration of Holothuria).
Miss N. Reed, London (Parasitic Protozoa).
A. D. Ritchie, Manchester (Muscles of Pecten and Ostrea).
The Hon. V. Rothschild, Cambridge (Microdissection).
J. T. Sanderson, Cambridge (Parasitic Protozoa on Calanus).
Dr. F. F. Schacht, London (General Zoology).
Miss F. E. Smith, Plymouth (Nemertines).
F. G. W. Smith, London (Development of Nassa).
Miss F. A. Stanbury, Plymouth (Substitution of various salts by Diatoms in artificial sea-water).
J. L. Steward, Cambridge (Parasitology).
Miss M. A. Tazelaar, London (Parasitic Protozoa).
Dr. G. Teissier, Paris (General Zoology).
E. F. Thompson, Cambridge (Inorganic constituents of invertebrate bloods).
Dr. Jan Verwey, Holland (Building, construction and apparatus of Laboratory).
Dr. Wolsky, Hungary (Gammarus).
Dr. D. Wrinch, Cambridge (Cell division).
T. Z. Young, Oxford (Nervous system of fishes and cephalopods).

The research ship *Atlantis*, which had been built in Copenhagen for the Woods Hole Oceanographic Institute, remained for ten days at Plymouth before proceeding on her first voyage across the Atlantic. The following members of her scientific staff spent some time at the Laboratory making final arrangements of apparatus for the research work which they carried out with success on their voyage : Capt. C. O. Iselin, Dr. G. L. Clarke, Dr. F. Zorell, Mr. R. B. Montgomery, and Mr. H. Bigelow.

Meetings of the Challenger Society and of the Biochemical Society were held at the Laboratory during the summer, and an afternoon was spent there by the members of the Museums Association, who were holding their Annual Conference in Plymouth.

The usual Easter Vacation Course in Marine Zoology was conducted by Mr. D. P. Wilson and Mr. G. A. Steven, and was attended by forty

students from Oxford, Cambridge, London, Edinburgh, Belfast, Liverpool, Birmingham, Sheffield, Reading, and Portsmouth.

An advanced course in Comparative Physiology and Experimental Biology was conducted by Mr. A. D. Ritchie and Mr. R. J. Pumphrey and was attended by five students.

During the Easter Vacation Mr. J. M. Branfoot brought six students from Oundle School; Mr. M. W. Barr brought one from Harrow; Dr. Shann, two from Rugby; Mr. S. R. B. Pask, seven from St. Paul's; Mr. H. Foy, three from Malvern College; Mr. A. S. Gillespie, five from Dauntsey School; and Mr. H. C. Wilson, three from Monkton Combe.

At Whitsuntide Mr. Leigh-Sharpe, of the Chelsea Polytechnic, conducted a class of thirteen students.

The Scientific Work of the Plymouth Laboratory Staff.

In the following account of the work of the Laboratory staff an attempt is made to arrange the descriptions of the different investigations that are being undertaken in such a way that their bearing may be indicated on the general plan of work which is being followed. We commence with the physical and chemical characteristics of the environment, pass on first to the vegetable plankton which forms the fundamental food supply of the sea, and then to studies on the animal plankton which is immediately sustained by this vegetable food. Then follows work on the ecology, physiology, and genetics of the invertebrate animals which form the fauna of the sea floor, feeding on the plankton or on each other, and themselves constituting the food of the bottom-living fishes. This leads finally to studies on fishes themselves and on the commercial fisheries which provide in this country an important supply of food for man.

Physics and Chemistry of the Environment.

Dr. Atkins and Dr. H. H. Poole have again collaborated on photo-electric photometry, and in continuation of their work with colour filters, rendered possible by the General Electric Company's cell, type CMV6, measurements were made at sea to determine separately the penetration of blue, green, yellow, and red light. A few determinations of the penetration of ultra-violet around 3600 Å were made. Measurements were also carried out with a combination which indicates the penetration of an approximation to white light. In previous years only the blue component could be measured and it was the advent of a cell with a greater range of colour sensitivity that permitted the measurement of white light this year. Obviously, once it was possible to measure the latter, any of the components could be measured. The number of depth-series

now carried out in the open sea has reached a total of eighty-two. Of these twenty-four were performed this year. Dr. Poole and Dr. Atkins exhibited the various types of apparatus used in the course of their work at the Scientific Exhibition of the Royal Dublin Society Bi-centenary celebrations.

Mr. D. C. Gall, of Messrs. H. Tinsley & Co., collaborated with Dr. Atkins in the production of a slightly modified form of Dr. H. H. Poole's apparatus for submarine photometry, special precautions being taken to avoid electrical leakage in damp weather. This, together with a reproduction of Dr. J. H. J. Poole's neon lamp photometer, was supplied to the U.S.A. Research Ship *Atlantis*, and Dr. G. L. Clarke and Mr. Montgomery reported that the apparatus worked satisfactorily down to nearly 200 metres, during their crossing of the Atlantic.

The continuous hydrographic records between Plymouth and Ushant, which have been carried out for some years past by Mr. H. W. Harvey, have been maintained during the year, the data having been collected by Dr. L. H. N. Cooper at the same time as water samples for his other researches described below. The records are published by the International Council for the Exploration of the Sea in the annual Rapport Atlantique and much of Mr. Harvey's time is occupied in working up these data. The meeting of the International Council at Copenhagen held in March was attended by Mr. Harvey, who also, at the invitation of the Council, spent two months in co-operation with Dr. K. Buch and Dr. H. Wattenberg on an investigation of the first and second dissociation constants of carbon dioxide in sea-water. The final results of this will be published by the Council.

During the year Dr. L. H. N. Cooper, in order to obtain comprehensive data on the problem of the variation in the amounts of nutrient salts in the sea, has made frequent determinations of temperature, phosphate, silicate, nitrate, nitrite, ammonia, pH, alkaline reserve, and oxygen in the English Channel. Salinity data will also be available to show the character of the water dealt with. Wattenberg's new method for ammonia in sea-water by direct Nesslerisation has been applied with success, although it is not as sensitive as could be wished for the problem in hand. The seasonal variations in ammonia are somewhat erratic, as might be expected of a substance which is at once an intermediate stage in the breakdown of dead protein material to nitrate and also, probably, a direct source of nitrogen for growth. As in the case of phosphate, ammonia originating from the land appears to be without appreciable effect more than a few miles out.

The behaviour of ammonia and nitrite in spring affords indirect evidence that both are readily available as sources of nitrogen for growth. Nitrite, an intermediate in the oxidation of ammonia to nitrate, is readily detect-

able in spring and autumn, up to 13 mg. of nitrogen per cubic metre. However, a very accurate series of analyses in July showed that at the surface stations, L3 to E1 inclusive, there was less than 0·02 mg. of nitrogen per cubic metre or less than 2 parts in 100,000,000,000, probably the most complete exhaustion of a minor constituent yet recorded.

The sunless and rather stormy summer had a marked effect on the hydrodynamical conditions, so that the vertical distribution of phosphate was quite different from that found during any former summer. The same unusual conditions must have influenced the other substances studied.

There is some evidence that solution of calcium from the bottom may be sufficient to affect the alkaline reserve, but since the observed variations are small—about 1 in 200—the data will have to be carefully weighed before a final conclusion can be reached. It is expected that the results of Dr. Cooper's investigations will be ready for publication in the next number of the Journal.

Plankton.

Mr. Harvey has continued work on the rate of growth of the plankton diatom *Nitzchia closterium* under conditions of constant light and temperature with varying additions of nutrient salts and of land washings. Miss F. A. Stanbury, of the Plymouth Technical College, has continued to work, under the direction of Dr. Atkins, on the growth of diatoms.

Mr. F. S. Russell has continued his researches on the biology of the plankton animal Sagitta in the Plymouth area. By means of weekly collections with the ring-trawl it is hoped that the complete life stories of both *Sagitta elegans* and *Sagitta setosa* will be known. As with Calanus, it was found that *Sagitta elegans* which were born in the cold months of late winter and early spring grew to a very large size compared with that of the successive broods which appeared during the summer. An examination of the reproductive organs has shown that during the period mid-October, November, December to mid-January the Sagitta were not breeding; in January the ovaries began to ripen and in March the offspring of those which had lived over from the previous October appeared in the ring-trawl catches. These Sagitta matured at a size of 2 or 3 mm. greater than their parents did, and it is this brood which attains the great growth in size in May; practically all of these large Sagitta had died off by the middle of June. Similar data have been obtained for *Sagitta setosa* but have not yet been worked out; there are already indications, however, that the life story of this species is different from that of *Sagitta elegans*.

In July and August Mr. Russell made observations on the vertical distribution of plankton at sea in conjunction with Dr. Atkins. By means of quick attachment and releasing gear, six nets were fished

simultaneously at different depths; the fishing depths were found by using the Admiralty depth recorder on the bottom net and by suitably weighting the remaining nets to ensure that they fished in a straight line. At the same time measurements of the air illumination were made by Dr. Atkins, and either immediately before or after fishing a depth series of light intensity readings was taken. Seven series of collections were made under conditions of different light intensity. The material has not yet been worked up.

Weekly observations on the seasonal abundance of the young fishes have been continued, and a count of all the plankton contained in the weekly ring-trawl collections has been made throughout a whole year. Mr. Russell has published in the Association's Journal a paper on the biology of Cyanea and, also, a further paper on his researches on the vertical distribution of plankton completing the observations for four series of twenty-four hours' collections. He has prepared a paper on the importance of Copepods as a factor in oceanic economy to be read at the Fifth Pacific Science Congress in Vancouver in 1932 (now postponed to 1933). This is a review of the present position of research on the biology of Copepods in the North Atlantic and neighbouring seas and embodies much work that has been done at this laboratory in past years.

Experiments have been conducted by Mr. G. M. Spooner on the reactions to light of various plankton animals. It is so far clear that with a number of different species that are attracted or repelled by light, it is the direction of the light, rather than the change of intensity from moment to moment, that is effective in determining the course of movement.

With a view to completing our knowledge of larval forms occurring in the plankton, Dr. M. V. Lebour has spent most of her time in studying larval gastropods, which have proved peculiarly interesting. The aim has been to identify the spawn and larvæ of all those which have planktonic stages and to follow the life history of each species, and she is now able to identify many mollusc larvæ in their free-swimming stages. *Nassarius reticulatus* has been reared from egg to crawling stage in a plunger-jar, the process taking two months, during which time the larvæ fed almost exclusively on diatoms. *N. incrassatus* has also been reared from the egg for part of its larval life, the later stages being taken from the plankton and the crawling stages therefrom reared to the adult. A paper on the life-histories of both species has appeared in the current number of the Association's Journal, as well as papers on the life-history of *Clione limacina* and of the cowrie, *Trivia europea*. The latter mollusc has an accessory larval shell somewhat similar to the Echinospira shell of Lamellaria. The eggs, first recognised in 1926 by Professor Pelseneer on the French coast embedded in compound ascidians, and hatched out

by him, have never until now been obtained from the Trivia themselves. In June 1930 they were laid in a plunger-jar, hatched, and kept alive for a few days. The older stages, hitherto unknown, were obtained from the plankton and kept until metamorphosis took place, being fed on compound ascidians. The free-swimming life of both the Nassarius species and of Trivia is long and all three larvæ are very important members of the plankton. Further researches have led to the elucidation of the life-history of *Simnia patula*, which is totally unlike that of Trivia ; of *Limacina retroversa*, which is found to breed from spring to autumn in great abundance, and of many others the study of which is not complete. Among the latter are species belonging to the Turridæ, *Triphora perversa*, and certain new Echinospira larvæ, all of which have handsome and elaborate veliger stages and a long larval life. A large number of species have been hatched from the egg and partially reared and many shore forms with planktonic larvæ are being studied.

In continuation of Dr. Lebour's work on larval Crustacea, a paper is now ready for the press on the life-history of *Spirontocaris cranchi*, which has not been worked out before. It has proved specially interesting on account of the supposed affinity of the species with Hippolyte. Its larvæ, however, are much more like those of Caridion and Pandalus. Work on the Alpheidæ, distinguishing the larvæ of the two species of Alpheus, is nearly finished. Two papers on the life-history of the decapods have been published in the April Proceedings of the Zoological Society (1931) : " The Larvæ of the Crangonidæ and the Larvæ of the Hippolytidæ," and " Further Notes on the Larval Brachyura." A second paper on larval Galatheidæ, finishing those of the Plymouth district, has been published in the June number of the Association's Journal.

Continuing his studies of the Polychæte larvæ of the plankton, Mr. D. P. Wilson has completed his work on the development and metamorphosis of the Mitraria larva of *Owenia fusiformis*. The larvæ reared and fixed during the summer of 1930 proved to be excellent material, better in all respects than that previously obtained. A large number of larvæ of all stages were either sectioned or prepared as whole mounts, and the study of these has completed in detail the story of the development from the trochosphere to the newly metamorphosed worm. Many drawings have been made from these sections and other illustrations prepared for publication, and the greater part of the work has been written up. It is difficult without the drawings to give much indication of the results achieved from the study of the new sections. They mainly concern the complicated way in which the head is fitted on to the trunk at metamorphosis, and the almost equally complicated and unusual way in which the segmental blocks of coelomic mesoderm surround the

intestine during the growth of the larva. Much detail has been added to that already ascertained for all the stages, and two points of special interest may be mentioned here. In the late larva the folds of the developing worm trunk are suspended by two muscle fibres in a way similar to that in which the folds of the Polygordius endolarva are suspended. The second point concerns the digestion of the prototroch after metamorphosis ; its broken-down cells are ingested intracellularly by the cells of the stomach-wall. This is the first case of intracellular digestion recorded for an Annelid and it only takes place when the creature's own tissue is digested. Diatoms, etc., its ordinary food, are digested extracellularly in the usual manner.

Invertebrates of the Sea Floor (*Ecology, Physiology, Genetics*).

In January of this year Mr. J. E. Smith commenced work on an ecological survey of the bottom fauna of the grounds in the immediate neighbourhood of the Eddystone. The area under survey has been restricted in the main to the shell gravel deposits. Bottom deposits of coarse texture are indicative of considerable water disturbance, and provide for an animal community of which *Amphioxus lanceolatus*, *Timoclea ovata*, *Glycymeris glycymeris*, *Clausinella fasciata*, *Gafrarium minimum*, *Astarte triangularis*, *Spatangus purpureus*, *Echinocyamus pusillus*, and *Glycera lapidum* are important members. The distribution of the various species within the community is being investigated. The work has been made, as far as possible, quantitative. The Petersen Grab has been found to be unreliable in its results on these grounds, owing to the fact that closure before hauling in is usually prevented by the intervention of shell between the teeth with consequent loss of material. The Conical Dredge has therefore been used practically throughout the work. The chief disadvantage of the instrument is that it must be towed for some distance in order that it may be filled. For this reason any gravel sample has to be considered as indicating an average sample over an area of as much as 100 yards radius. This is a serious disadvantage when the whole area under investigation is limited. Against this disadvantage there are numerous small advantages which the Conical Dredge has over the Grab. The quantity of gravel in the dredge is such that three separate determinations may be made from the contents of a single haul.

The major part of the contents of the dredge has been used for a qualitative investigation of the fauna, whilst for quantitative work 1000 c.c. of gravel have been examined. A third portion has been graded by sieving and the representative number of the gravel, indicative of the degree of coarseness, determined. For quantitative work, all living

molluscs and echinoderms of greater length than 1·5 mm. have been recorded. Lower grades than 1·5 mm. have been found to contain very little unbroken shell, in consequence of which it has been considered that only a very small proportion of the living fauna has been overlooked. The importance of a careful examination of the 1·5 mm. grade may be judged from the fact that practically all living *Echinocyamus pusillus* and about half the living molluscs are found in this grade.

The gravels themselves are made up, at least in the coarser grades, of a limited number of species of broken and unbroken shell, but the relative numbers of the various species represented vary considerably, as does also the amount of matter of inorganic origin. It is hoped that a comparison of the distribution of the living and dead faunas may give some indication of the movement of shell due to submarine currents and eddies. A study of the distribution of the various grades of gravel has already made possible a rough estimation of the main centres of disturbance round the Eddystone. As might be expected, these correspond with the submarine ridges which extend outwards from it.

Dr. C. M. Yonge's work on the permeability of the uncalcified chitin in Decapod Crustacea is now nearing completion. Previously it had only been known that this allows water to pass through freely under the influence of osmotic pressure. It has been found that the chitin is composed of two portions, a lamellated inner layer and a very thin, hyaline surface layer. The two have different properties. The former has an iso-electric point at pH 3·5 and the latter at pH 5·1. Of more importance, however, is the presence within the surface layer of adsorbed lipoid. This has been demonstrated both by the specific lipoid fixation and staining of Ciaccio, and by detailed experiments on the permeability of chitin. Thus fresh chitin (from which the underlying tissue has been previously removed by maceration in fresh water) is very slightly permeable to decinormal solutions of strong acids and alkalies, but similar concentrations of fatty acids or of ammonia penetrate with much greater speed. These latter substances are all lipoid solvents. Even when similar concentrations of these substances are present on both sides of the chitin there is still a flow from the side with the lipoid (in life the free surface) to the other. The fresh membrane has thus a selective and polarised permeability analogous to that of a living membrane. After treatment with lipoid solvents such as absolute alcohol, chloroform, or ether (but *not* organic solvents such as acetone or methyl acetate which are *not* lipoid solvents), the chitin becomes much more permeable. Decinormal hydrochloric acid, for instance, may then pass through the membrane at more than thirty times its previous speed. Fatty acids, on the other hand, pass through only a little faster than before (if the lipoid is only partially

removed they may even pass through *less* quickly), their speed of penetration being now dependent, apparently, entirely on their strength, i.e. their degree of dissociation. The lipoid may also be removed by exposing the membrane to normal solutions of strong acids or alkalies which hydrolyse it. The absence of the lipoid from the surface layer of chitin after these various treatments was confirmed by means of sections of portions of chitin fixed and stained by Ciaccio's method.

This surface layer of chitin, by rendering largely impermeable to dissolved substances the uncalcified chitin of the fore and hind guts, the gills and elsewhere, is clearly of the first importance in assisting in the insulation of the animal from its environment. Its origin is being investigated. Sections of moulting animals show that only the underlying, thick layer of chitin is secreted by the epithelial cells. Evidence is being accumulated which points with increasing certainty to the tegumental glands as the source of the lipoid. The function of these glands, characteristic of the Decapod Crustacea, where they occur in great numbers beneath the epithelium, has been unknown and has given rise to many theories. They have fine ducts which pass through the underlying chitin and open at the surface, and the course of these can be followed by a fine line of red when the chitin has been stained for lipoids. They apparently only secrete at the time of moulting.

This work has an added interest in view of its relations to that of Professor A. V. Hill and his collaborators on changes in the osmotic concentration of the blood of Crustacea, and other marine invertebrates, in different concentrations of sea-water.

A review of digestive processes in marine invertebrates and fishes has been prepared and published in the *Journal du Conseil*, attention being especially drawn to the evolution of extracellular from intracellular digestion, and the specialization of digestive enzymes. A knowledge of the latter is in many cases essential before the true food of an animal can be determined.

Three further papers have been published on work done during the Great Barrier Reef Expedition, on the structure, distribution and physiology of the zooxanthellæ of corals and their relationship to the animals in which they live. The corals, specialised carnivores like all coelenterates, *cannot* digest the algæ but eject them when their metabolism is lowered by any agency, starvation, excessive heat, lack of oxygen, etc. In a healthy coral the zooxanthellæ multiply until they pack the endoderm, but their numbers are clearly controlled by the quantities of excretory products, carbon dioxide, nitrogen, phosphorus and sulphur, produced by the animal, all of which can be utilised by the plants which invariably intercept them before they can be discharged into the surround-

ing water. The very great abundance of symbiotic algæ in animals which digest amongst carbohydrates only glycogen—and that very slowly—points to the possibility that such animals may obtain much of their carbohydrates from proteins by deaminisation, thereby releasing large quantities of nitrogen, phosphorus, and sulphur which in turn permit of the growth in the tissues of great numbers of zooxanthellæ. The animal is benefited by the automatic removal of its abundant excretory products. It is hoped shortly to test the truth of the assumption that such animals do obtain their carbohydrates largely from protein—a matter of fundamental importance in an adequate estimation of the food chains in the sea—by experiments at this laboratory on anemones with and without zooxanthellæ.

The work on the Mendelian inheritance of eye-colour in *Gammarus*, carried on by Mrs. Sexton, with the assistance of Miss A. R. Clark and Mr. G. M. Spooner, has been directed this year to the analysis of the unpublished data previously accumulated, and their comparison with newly acquired facts. A paper has been prepared in which much of this previously unpublished matter has been collected, dealing with the different stocks where variations in eye-pigmentation have occurred. Amongst other points discussed are, the hereditary relations of different variations in eye-colour; a comparison of the different types of eye-colour and colour-changes, so that a closer understanding may be reached on the action of the hereditary factors underlying them; and a consideration of the question of the origin of recessive characters as to how far they may be present in the wild. This question of the origin of recessive characters is now receiving special attention. An experiment, conducted by Mr. Spooner, on the rearing of the F_2 of some wild pairs introduced into a temperature of 21–25° C. gave results which called for further investigation. Four recessive eye-characters were obtained from a number of wild pairs. Taken in conjunction with some previous results obtained by Mrs. Sexton, a high proportion of either heterozygosity or mutation (or a combination of both) in adults brought in from the wild is indicated. In order to obtain more decisive information on this point, an experiment on a large scale is now being carried on.

Fish and Fisheries.

Mr. E. Ford has continued his herring researches. The commercial landings during the winter drift-net fishery of 1930–31 at Plymouth showed a predominance of fish born during the winter of 1924–25. Hence, for three successive seasons the fishery has depended in the main upon this very rich year-class 1925. Looking back over the past seven seasons,

it is seen that the three year-classes, 1920, 1923 and 1925, have contributed most to the success of the industry :—

Season.	Dominant Year-classes.	Age.
1924–25	1920	5
1925–26	1920	6
1926–27	1920 and 1923	7 and 4
1927–28	1923	5
1928–29	1925 and 1923	4 and 6
1929–30	1925	5
1930–31	1925	6

During the fishery of 1930–31, two matters arose which subsequently formed subjects for enquiry by the Devon Sea Fisheries Committee, and upon which the Laboratory supplied relevant information. In both instances, objections were raised by the drift-net fishermen to the activities of other fishermen using different fishing gear. On the one hand, it was contended that herring stocks were being harmed as the result of trawling by motor-boats in Bigbury Bay whereby large numbers of fish actually spawning were being taken, and spawn already deposited was being destroyed by over-riding with the trawl. The second complaint was directed against the putting-out of standing nets for rays during the drifting season, resulting in damage and loss by the fouling of drift-nets with buoys and lines attached to the ray-nets. Knowledge of the biological facts of the two cases proved useful in the Committee's investigations.

Two samples of spawning herrings were analysed for sex, length, age, and number of vertebræ in order to determine whether fishes of the same sex and age agreed in average number of vertebræ. The results, which have an interesting bearing on herring " race " conceptions, are being prepared for publication. Mr. Ford has also continued his studies on growth in length during the larval life and metamorphosis of fish. Reports have been published in the Journal of the Association on the facts noted for the sprat, pilchard, and freshwater eel. Important questions in ontogeny and phylogeny are considered.

Mr. G. A. Steven has continued the researches on the rays and skates of the western area of the Channel which he began last year. Attention has been devoted mainly to a study of the fishery, both by observations on the fish markets and by trips to sea on commercial vessels. At the outset it was found necessary to learn rapidly to distinguish the different species as they lay exposed for sale on the fishmarket floor. A paper on " Methods of Rapid Identification on the Fishmarket " has been prepared and

published in the Journal of the Association. The separate contributions of each of the eleven species present within the area to the total Ray and Skate landings in Devon and Cornwall have been ascertained. *Raia clavata*, the Thornback Ray, is found to be the most abundant species, forming approximately 37% numerically of the total commercial landings, followed by *R. naevus*, 19%; *R. fullonica*, 16%; *R. montagui*, 15%; other species all under 10% each. Much useful data has also been acquired upon the general distribution and migrations of certain of the species. It has been found that uni-specific and even uni-sexual shoals of fish occur. There is also evidence that, where several species are present within the same restricted area, they are not mixed indiscriminately but members of the same species tend to remain together. In order to acquire additional data on this point a record was taken of every fish which came up on more than 15 miles of long-line carrying over 8000 hooks. Marking experiments have also been commenced and 197 fishes have been marked and liberated from s.s. *Salpa* in the vicinity of Plymouth. Two of these fishes, both *R. clavata*, have been recovered after having been at liberty for six months or more after marking. One showed a growth of exactly 3 cm. across the disc in six months and the other 5·2 cm. in eight months. A paper on " Methods of Preparation of Rays and Skates for Market " has been prepared and is awaiting publication.

The investigations into the food of Shags and Cormorants, begun two years ago by Mr. V. C. Wynne-Edwards, in co-operation with the Cornish Sea Fisheries Committee, have been continued by Mr. Steven. The stomachs of 44 birds shot near Port Isaac and Newlyn have been examined. The data acquired were in complete agreement with those previously obtained and lend no support to the view that these birds prey to any appreciable extent on fishes of economic importance. As a result of these investigations, the Cornish Sea Fisheries Committee has ceased payment of 1/– for the head of every Shag and Cormorant forwarded to them and has recommended that these birds should from henceforth receive protection under the Wild Birds' Protection Act.

The experiments on the growth of plaice in the pond at Pier Cellars, undertaken for the Ministry of Agriculture and Fisheries, were completed in 1930. Mr. B. Dawes has this year published in the Journal a report on the work during the third and final year, and also a statistical study of growth and maintenance in the plaice, based on the data of the experiments carried out in 1929 and 1930 at Cawsand and Lympstone.

In continuation of his work on fish behaviour Mr. G. M. Spooner has been accumulating information on the comparative form of the brains of bony fishes, with the special aim of comparing the relative development of the different nerve-centres with the fish's sensory capacities. A paper

by Mr. Spooner on schooling in fish was published in the Journal of the Association, Vol. XVII, No. 2.

Dr. W. R. G. Atkins has continued to study the question of the preservation of fishing nets. In his opinion what is now wanted is that the methods already tested with much success, should be widely used. The durability of certain fibres in sea-water is being tested for the Empire Marketing Board and the preservative action of various tar distillates, supplied by the Chemical Research Laboratory, Teddington, is also being studied. Some years ago the use of resin as a binding agent, offering an alternative to tar, was tried with copper oleate. Copper resinate, specially prepared by Messrs. Wm. Bailey and Son, Wolverhampton, is now being tested.

River Tees Survey.

The biological and chemical survey of the estuary of the River Tees, commenced in April 1929, has been continued through the year. Dr. B. A. Southgate holds the appointment of Superintendent of the Laboratory, physiologist and chemist, with Mr. R. Bassindale as biologist. Attention has been devoted mainly to the cause of the death of salmon and sea trout smolt as they enter the deoxygenated and polluted belt of the estuary during their seaward migration in April, May, and June. Previous observations had indicated cyanide, from coke oven effluents, as the main cause of mortality, enhanced by low concentrations of dissolved oxygen, there being reason to believe that tar acids were hardly ever present in sufficient concentration to be toxic and that the low concentrations of oxygen were of themselves insufficient to account for the mortality, particularly during the earlier weeks of the smolt run when the water is colder and less deoxygenated than towards the end.

Preparations were accordingly made for intensive observations during the smolt run of 1931. Dr. Southgate had discovered that a trace of formaldehyde destroyed the toxicity of dilute solutions of cyanide, while Mr. Bassindale found that the gill colour of trout dying from cyanide poisoning was of a brighter scarlet than when the fish were dying either from tar acid poisoning or, even more so, from asphyxiation. Improvements were also made in the method of estimating minute quantities of cyanide in estuary water.

Detailed observation of the condition of the estuary water was commenced on April 15th and continued until the end of the migration on June 6th. A large proportion of the samples of estuary water collected were lethal to trout and the great majority of the samples were rendered innocuous by the addition of formaldehyde, indicating that cyanides caused the toxicity. The colour of the gills of dying smolts also indicated

cyanide poisoning, and analysis showed the presence of cyanide often in lethal concentrations. Tar acids were not found to be present in lethal concentration and it had been previously established that their toxicity was not additive to that of cyanide.

Netting for smolts was carried out at high water by day and by night near the upper limit of the polluted belt, the captured fish being marked and liberated. The estuary banks were searched daily for dead and dying smolts. Neither the numbers of fish netted nor the number found dead showed any relation between the numbers migrating and the range of the tide, but the position of dead and dying smolt varied with the movement of the polluted belt, which penetrates further up river on spring tides. The few marked smolt which were recaptured or found dead suggested that fish may remain several days near the head of the estuarine water before proceeding seaward.

At the instigation and with the assistance of Messrs. Imperial Chemical Industries, Ltd., and Messrs. Dorman, Long & Co., Ltd., an investigation was then commenced on the treatment of coke oven effluents in order to destroy their cyanide toxicity by means of a waste product produced in quantity in the district.

Throughout the year work was continued on assessing the " oxygen demand " of the sewage, trade effluents and of the mud on the river bottom, on the effect of varying salinity and poisons upon various marine animals, and on the distribution of the fauna. Mr. Harvey has visited Middlesbrough from time to time and keeps in contact with the administration and the various problems under investigation by such visits and by correspondence.

The Library.

The new Library building has already been referred to in an earlier section of this report.

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, or received in exchange for the Journal. Thanks are also due to those authors who have sent reprints of their papers, which are much appreciated.

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PLYMOUTH MARINE FAUNA. Second Edition, 1931. *Being notes of the local distribution of species occurring in the neighbourhood, compiled from the records of the Laboratory of the Marine Biological Association.* (*With one Chart.*) Plymouth. Published by the Marine Biological Association of the United Kingdom. Copies can be obtained from The Director, Marine Biological Laboratory, Citadel Hill, Plymouth, Devon. Price 2s. 6d. (3s. post free).

Finance.

The Council have again to express their thanks to the Development Commissioners for their continued support of the Plymouth Laboratory. They are grateful also for generous grants from the Fishmongers' Company (£600), the Royal Society (£50), the British Association (£50), the Physiological Society (£30), the Ray Lankester Trustees (£20), the Universities of Cambridge (£105), Oxford (£52 10s.), London (£52 10s.), Bristol (£25), Birmingham (£15 15s.), Manchester (£10 10s.), Sheffield (£10), the Imperial College of Science and Technology (£10), as well as to the subscribers to the Building Fund, especially to the Trustees of the Rockefeller Foundation, whose grant of £4,642 has already been referred to, and Mr. E. T. Browne, who has added £310 to the donation of £500 which he made in the last financial year.

Vice-Presidents, Officers and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1932–33 :—

President.

The Lord MOYNE, P.C., D.S.O.

Vice-Presidents.

The Duke of BEDFORD, K.G.
The Earl of STRADBROKE, K.C.M.G., C.B., C.V.O.
The Earl of IVEAGH, C.B., C.M.G.
Viscount ASTOR.
Lord ST. LEVAN, C.B., C.V.O.
The Right Hon. Sir AUSTEN CHAMBERLAIN, K.G., M.P.
Lord NOEL-BUXTON.
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Sir NICHOLAS WATERHOUSE, K.B.E.
G. A. BOULENGER, Esq., F.R.S.
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Prof. H. MUNRO FOX.
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ROBERT GURNEY, Esq., D.SC.
Prof. C. R. HARINGTON, F.R.S.
Prof. A. V. HILL, F.R.S.
S. KEMP, ESQ., D.SC., F.R.S.
G. C. Robson, Esq.
E. S. RUSSELL, Esq., D.SC.
J. M. TABOR, Esq.
Prof. G. I. TAYLOR, F.R.S.

Chairman of Council.

Prof. E. W. MACBRIDE, D.SC., F.R.S.

Hon. Treasurer.

NIGEL O. WALKER, Esq., 38, Regent Street, Cambridge.

Secretary.

E. J. ALLEN, Esq., D.SC., LL.D., F.R.S., The Laboratory, Citadel Hill, Plymouth.

The following Governors are also members of the Council :—

G. P. BIDDER, Esq., SC.D.
E. T. BROWNE, Esq.
GEORGE EVANS, Esq.
The Lord MOYNE, P.C., D.S.O.
H. G. MAURICE, Esq., C.B. (Ministry of Agriculture and Fisheries).
OWEN HUGH SMITH, Esq. (Prime Warden of the Fishmongers' Company).
NIGEL O. WALKER, Esq. (Fishmongers' Company).
GUY WOOD, Esq., M.B., M.R.C.P. (Fishmongers' Company).
Prof. G. C. BOURNE, D.SC., F.R.S. (Oxford University).
J. GRAY, Esq., F.R.S. (Cambridge University).
Sir P. CHALMERS MITCHELL, KT., 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).

BALANCE SHEET

	£ s. d.	£ s. d.
SUNDRY CREDITORS:		
On Open Account	332 4 10	
Wages accrued	43 17 2	
		376 2 0
BUILDING FUND:		
As at 31st March, 1931	2,190 6 11	
Add: Donations:		
Mr. E. T. Browne (for Store)	310 0 0	
Rockefeller Foundation	2,650 0 0	
Sundry	14 4 0	
Interest on Deposit	18 2 0	
	5,182 12 11	
Less: Expenditure:		
Library 1,890 7 0		
New Store 310 0 0		
Rockefeller Laboratories .. 2,650 0 0		
Sundries 46 17 4		
	4,897 4 4	
		285 8 7
(*Note.*—Further Liabilities of £1,895 have been incurred in connection with the Building Fund.)		
RESERVE FOR DEPRECIATION OF BOATS AND MACHINERY:		
As at 31st March, 1931	250 0 0	
Add: Transfer from Income and Expenditure Account	250 0 0	
		500 0 0
SURPLUS:		
As at 31st March, 1931	5,755 9 4	
Add: Composition Fees	15 15 0	
	5,771 4 4	
Less: Deficit for the year as per Income and Expenditure Account	146 2 7	
		5,625 1 9
		£6,786 12 4

TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

I report that I have examined the above Balance Sheet with the books of the Capital expenditure on erection of Buildings on Land held on lease from the War Sheet is properly drawn up so as to exhibit a true and correct view of the state of given to me and as shown by the books of the Association.

34 *and* 35 *Bedford Street,*
Plymouth. 26*th April,* 1932.

(Signed) JOS. M. TABOR, G. C. ROBSON } *Members of Council.*

31ST MARCH, 1932.

	£ s. d.	£ s. d.
BOATS AND EQUIPMENT, as per Valuation as estimated by the Director at 31st March, 1931 :		
S.S. *Salpa*	2,000 0 0	
Motor-boat	150 0 0	
Nets, Gear and General Equipment	27 0 0	
		2,177 0 0
LABORATORY APPARATUS, ENGINES AND PUMPS :		
As per Valuation as estimated by the Director at 31st March, 1931	400 0 0	
Additions during the year at cost	140 12 8	
		540 12 8
LIBRARY :		
As per Valuation as estimated by the Director at 31st March, 1931	1,800 0 0	
Additions during the year at cost	731 2 10	
	2,531 2 10	
Less : Depreciation	545 11 5	
		1,985 11 5
STOCK OF SPECIMENS, CHEMICALS AND JOURNALS at Valuation as estimated by the Director		350 0 0
SUNDRY DEBTORS		83 19 0
PREPAYMENTS		103 1 10
INVESTMENTS, at Market Value at 31st March, 1931 :		
£410 14s. 8d. New Zealand 4% 1943/63	344 15 0	
£352 2s. 3d. Local Loans 3%	232 7 10	
		577 2 10
(Market Value at Date—£577 4s. 6d.)		
CASH AT BANK AND IN HAND :		
Lloyds Bank Limited, Current Account	155 9 10	
Coutts Bank, Current Account	30 19 10	
Coutts Bank, Deposit Account	765 4 1	
Cash in Hand	17 10 10	
		969 4 7
		£6,786 12 4

Association and have obtained all the information and explanations I have required. Department is excluded. Subject to this remark I am of opinion that the Balance the Association's affairs, according to the best of my information and the explanations

(Signed) N. E. WATERHOUSE, *Auditor.*

INCOME AND EXPENDITURE ACCOUNT

	£ s. d.	£ s. d.
To Salaries, including Institutions Contribution to Superannuation		6,877 14 5
,, Laboratory Wages, including National Insurance and Institutions Contribution to Superannuation ..		2,091 14 8
,, Depreciation of Library		545 11 5
,, Scientific Publications, *Less* Sales		603 1 1
,, Upkeep of Laboratories and Tank Rooms:		
Buildings and Machinery	256 3 4	
Electricity, Gas, Coal, Oil and Water	294 8 7	
Chemicals and Apparatus	384 10 1	
Rates, Taxes and Insurance	115 3 3	
Travelling	130 15 7	
Stationery, Postage, Telephone, Carriage and Sundries	375 15 11	
Specimens	124 12 6	
		1,681 9 3
,, Maintenance and Hire of Boats:		
Wages, including Diet Allowance, National Insurance and Casual Labour	1,617 10 2	
Coal, Water, Oil, Petrol, etc.	311 8 10	
Maintenance and Repairs, with Nets, Gear and Apparatus	456 12 6	
Purchase of Material for Nets for Sale, excluding Labour	299 15 8	
Boat Hire and Collecting Expenses	12 17 1	
Insurance	271 15 11	
		2,970 0 2
,, Printing of "Plymouth Marine Fauna"	168 0 0	
Less : Grant from H.M. Treasury	163 0 0	
		5 0 0
,, Transfer to Depreciation Reserve Account ..		250 0 0
		£15,024 11 0

YEAR ENDED 31ST MARCH, 1932.

	£ s. d.	£ s. d.
By GRANTS:		
Ministry of Agriculture and Fisheries, Grant from Development Fund	11,364 9 0	
Fishmongers' Company	600 0 0	
British Association	50 0 0	
Royal Society	50 0 0	
		12,064 9 0
,, SUBSCRIPTIONS		235 3 3
,, DONATIONS		6 8
,, SALES:		
Specimens	1,083 19 8	
Fish (*less* expenses)	52 17 5	
Nets, Gear and Hydrographical Apparatus	480 7 3	
		1,617 4 4
,, TABLE RENT		446 12 6
,, TANK ROOM RECEIPTS		470 11 6
,, INTEREST ON INVESTMENTS AND BANK DEPOSITS ..		26 16 10
,, SALE OF DR. M. V. LEBOUR'S BOOK	2 6 4	
,, SALE OF "MARINE FAUNA OF PLYMOUTH"	14 18 0	
		17 4 4
BALANCE, BEING DEFICIT FOR THE YEAR		146 2 7
		£15,024 11 0

List of Annual Subscriptions

Paid during the Year, 1st April, 1931, to 31st March, 1932.

	£	s.	d.
Dr. W. M. Aders, O.B.E.	1	1	0
Hiroaki Aikawa, Esq. (1930, 1931, 1932)	3	3	0
E. J. Allen, Esq., D.SC., LL.D., F.R.S.	1	1	0
G. L. Alward, Esq.	1	1	0
C. Amirthalingam, PH.D. (1931, 1932)	2	2	0
Prof. J. H. Ashworth, D.SC., F.R.S.	1	1	0
The Rt. Hon. Lord Askwith, K.C.B., D.C.L.	1	1	0
W. H. Barrett, Esq.	1	1	0
G. R. de Beer, Esq.	1	1	0
J. Bělehrádek, Esq., M.D.	1	1	0
N. J. Berrill, Esq., PH.D. (1929, 1930, 1931)	3	3	0
Mrs. M. G. Bidder	1	1	0
Birkbeck College	1	1	0
W. Birtwistle, Esq.	1	1	0
H. H. Bloomer, Esq.	1	1	0
H. Moss Blundell, Esq. (1930, 1931)	2	2	0
Mrs. H. Moss Blundell	1	1	0
Dr. J. Borowik	1	1	0
L. A. Borradaile, Esq., SC.D.	1	1	0
C. L. Boulenger, Esq.	1	1	0
Prof. G. C. Bourne, D.SC., F.R.S. (1930, 1931)	2	2	0
A. Bowman, Esq., D.SC.	1	1	0
Prof. A. E. Boycott, F.R.S.	1	1	0
Sir J. Rose Bradford, Bart., K.C.M.G., M.D., D.SC., F.R.S.	1	1	0
J. M. Branfoot, Esq.	1	1	0
Brighton Public Library	1	1	0
R. Brown, Esq.	1	1	0
Miss E. M. Browne	1	1	0
H. O. Bull, Esq.	1	1	0
R. H. Burne, Esq., F.R.S.	1	1	0
M. Burton, Esq. (1931, 1932)	2	2	0
R. R. Butler, Esq.	1	1	0
L. W. Byrne, Esq.	1	1	0
Carried forward	43	1	0

	£	s.	d.
Brought forward	43	1	0
Prof. H. Graham Cannon, SC.D.	1	1	0
J. N. Carruthers, Esq.	1	1	0
Paymaster Captain Charles, R.N. (Retd.)	1	1	0
Dr. J. Clark	1	1	0
R. S. Clark, Esq. D.SC. (1931, 1932, 1933, 1934)	4	4	0
Coastguard and Fisheries Service, Alexandria	1	1	0
Prof. F. J. Cole, D.SC., F.R.S.	1	1	0
J. S. Colman, Esq.	1	1	0
J. F. Coonan, Esq.	1	1	0
J. Omer Cooper, Esq.	1	1	0
L. R. Crawshay, Esq.	1	1	0
Miss D. R. Crofts, D.SC.	1	1	0
Norman Cuthbertson, Esq.	1	1	0
Prof. Otto V. Darbishire	1	1	0
Dr. W. Cameron Davidson (1929, 1930, 1931)	3	3	0
F. M. Davis, Esq.	1	1	0
Ben Dawes, Esq. (1930, 1931, 1932)	3	3	0
G. Despott, Esq.	1	1	0
Director of Agriculture and Fisheries, Travancore	1	1	0
F. A. Dixey, Esq., F.R.S.	1	1	0
C. C. Dobell, Esq., F.R.S.	1	1	0
P. Eggleton, Esq., D.SC.	1	1	0
George Evans, Esq.	1	1	0
Prof. C. Lovatt Evans, F.R.S.	1	1	0
G. P. Farran, Esq.	1	1	0
R. A. Fisher, Esq., SC.D., F.R.S.	1	1	0
Fisheries Survey Committee, Capetown	1	1	0
E. Ford, Esq.	1	1	0
G. Herbert Fowler, Esq., PH.D.	1	1	0
Dr. E. L. Fox	1	1	0
Prof. H. M. Fox	1	1	0
Miss E. A. Fraser, D.SC.	1	1	0
Prof. F. E. Fritsch	1	1	0
Prof. J. Stanley Gardiner, F.R.S. (1929, 1930, 1931)	3	3	0
R. D'O Good, Esq.	1	1	0
Prof. E. S. Goodrich, D.SC., F.R.S.	1	1	0
Alastair Graham, Esq.	1	1	0
A. P. Graham, Esq.	1	1	0
Ronald Grant, Esq.	1	1	0
Carried forward	93	9	0

	£	s.	d.
Brought forward	83	9	0
Dr. A. M. H. Gray	1	1	0
J. R. Groome, Esq. (1931, 1932)	2	2	0
Wilfred Hall, Esq.	1	1	0
Ian I. Hamilton, Esq.	1	1	0
Prof. A. C. Hardy	1	1	0
Prof. C. R. Harington, F.R.S.	1	1	0
Cecil B. Harmsworth, Esq. (1928, 1929, 1930)	3	3	0
H. W. Harvey, Esq.	1	1	0
G. T. D. Henderson, Esq.	1	1	0
C. C. Hentschel, Esq.	1	1	0
C. F. Hickling, Esq.	1	1	0
Prof. Sidney J. Hickson, D.SC., F.R.S.	1	1	0
Prof. A. V. Hill, F.R.S.	1	1	0
W. T. Hillier, Esq., M.R.C.S.	1	1	0
Prof. Lancelot T. Hogben, D.SC.	1	1	0
Dr. E. G. Holmes	1	1	0
Hull University College	1	1	0
O. D. Hunt, Esq.	1	1	0
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Dr. G. Lapage	1	1	0
A. G. Lowndes, Esq.	1	1	0
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B. J. Marples, Esq.	1	1	0
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Capt. W. N. McClean	1	1	0
Milford Haven Trawler Owners and Fish Salesmen's Association, Ltd.	1	1	0
W. S. Millard, Esq.	1	1	0
Carried forward	136	10	0

	£	s.	d.
Brought forward	136	10	0
Sir P. Chalmers Mitchell, Kt., C.B.E., D.SC., F.R.S.	1	1	0
F. W. Moorhouse, Esq.	1	1	0
C. C. Morley, Esq.	1	1	0
Mount Desert Island Biological Laboratory	1	1	0
Dr. J. Mukerjii	1	1	0
National Museum of Wales, Cardiff	1	1	0
National Institute of Turkish Fisheries	1	0	0
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R. G. Neill, Esq.	1	1	0
H. G. Newth, Esq. (1930, 1931)	2	2	0
A. G. Nicholls, Esq.	1	1	0
J. A. Nicholson, Esq. (1932, 1933)	2	2	0
J. R. Norman, Esq.	1	1	0
Office Scientifique et Technique des Pêches Maritimes	1	1	0
Charles Oldham, Esq.	1	1	0
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Messrs. Peacock and Buchan	1	1	0
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Prof. E. Percival (1931, 1932)	2	2	0
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Plymouth Education Authority	1	1	0
Plymouth Public Library	1	1	0
Plymouth Proprietary Library	1	1	0
Port of Plymouth Incorporated Chamber of Commerce	1	1	0
Portsmouth Municipal College	1	1	0
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H. C. Regnart, Esq.	1	1	0
D. M. Reid, Esq.	1	1	0
E. A. Robins, Esq.	1	1	0
G. C. Robson, Esq.	1	1	0
The Hon. Victor Rothschild	1	1	0
Carried forward	181	12	0

	£	s.	d.
Brought forward	181	12	0
Chas. H. Rudge, Esq. (1932, 1933)	2	2	0
E. S. Russell, Esq., D.SC.	1	1	0
F. S. Russell, Esq., D.S.C., D.F.C.	1	1	0
Capt. The Hon. Lionel St. Aubyn, M.V.O.	1	1	0
The Rt. Hon. Lord St. Levan, C.B., C.V.O.	1	1	0
J. T. Saunders, Esq.	1	1	0
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B. Sen, Esq.	1	1	0
Miss Lilian Sheldon	1	1	0
N. Smedley, Esq.	1	1	0
B. Webster Smith, Esq.	1	1	0
F. G. W. Smith, Esq.	1	1	0
G. M. Spooner, Esq. (1931, 1932)	2	2	0
States Committee for Fisheries, Guernsey	1	1	0
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Mrs. N. S. Steven	1	1	0
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Eric J. Tabor, Esq.	1	1	0
Harold E. Tabor, Esq.	1	1	0
J. M. Tabor, Esq.	1	1	0
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Prof. G. I. Taylor, F.R.S.	1	1	0
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Sir Herbert F. Thompson, Bart. (1931, 1932)	2	2	0
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Torquay Natural History Society	1	1	0
R. C. Vernon, Esq. (1931, 1932)	2	2	0
H. M. Vickers, Esq.	1	1	3
A. Walton, Esq.	1	1	0
Sir Nicholas E. Waterhouse, K.B.E.	1	1	0
Prof. D. M. S. Watson, F.R.S.	1	1	0
E. C. Weberman, Esq.	1	1	0
Mrs. F. J. Weldon	1	1	0
Dr. K. B. Williamson	1	1	0
D. P. Wilson, Esq.	1	1	0
Carried forward	228	17	3

	£	s.	d.
Brought forward .	228	17	3
Mrs. D. P. Wilson (1931, 1932)	2	2	0
R. S. Wimpenny, Esq.	1	1	0
Ronald Winckworth, Esq., F.R.G.S.	1	1	0
V. C. Wynne Edwards, Esq.	1	1	0
C. M. Yonge, Esq., D.SC., PH.D.	1	1	0
Total .	£235	3	3

List of Donations to the General Fund

For the Year, 1st April, 1931, to 31st March, 1932.

	£	s.	d.
Sir H. Bowles, Bart.	0	6	8
Total .	£0	6	8

List of Donations towards the Building Extension Fund

Paid during the Year, 1st April, 1931, to 31st March, 1932.

	£	s.	d.
Trustees of Rockefeller Foundation	2,650	0	0
E. T. Browne, Esq. (for New Store)	310	0	0
Prof. J. Stanley Gardiner, F.R.S.	5	0	0
C. F. A. Pantin, Esq. (Second donation)	5	0	0
Prof. T. A. Stephenson, D.SC.	3	3	0
Miss D. Atkins	1	1	0
Total	£2,974	4	0

The Journal of Experimental Biology

(Late *The British Journal of Experimental Biology*)

EDITED BY
J. GRAY

ASSISTED BY

J. Barcroft, A. J. Clark, J. B. S. Haldane, J. S. Huxley, W. H. Pearsall,
S. C. Brooks, F. A. E. Crew, E. Newton Harvey, M. H. Jacobs, E. Ponder,
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THE

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