New Series.—Vol. IX., No. 4—issued March, 1913.
[Price Two Shillings, net.]

Journal

OF THE

MARINE BIOLOGICAL ASSOCIATION

OF

THE UNITED KINGDOM.

THE PLYMOUTH LABORATORY.

PLYMOUTH:
PRINTED FOR THE MARINE BIOLOGICAL ASSOCIATION BY W. BRENDON & SON, LTD.,
AND
PUBLISHED BY THE ASSOCIATION AT ITS OFFICES ON THE CITADEL HILL.
SENT FREE BY POST TO ALL MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION:
ANNUAL SUBSCRIPTION FOR MEMBERSHIP, ONE GUINEA.
Agents in London:—Messrs. Dulau & Co., Ltd., 37 Soho Square, W.

On the Precipitation of Calcium Carbonate in the Sea by Marine Bacteria, and on the Action of Denitrifying Bacteria in Tropical and Temperate Seas.

By

G. Harold Drew.

With Two Figures in the Text.

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INTRODUCTION.

THE investigations in the American Tropics described in this paper were made during the summers of 1911 and 1912 under the auspices of the Carnegie Institution of Washington: the work was made possible by the invaluable help and kindness of Dr. Alfred G. Mayer, Director of the Department of Marine Biology. The investigations in Temperate waters were made from the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, and my thanks are due to the Council and Director of the Association for giving me every facility for the work.

Originally the primary object of the investigations was to make a study of the action of marine denitrifying bacteria in Tropical and Temperate seas. The discovery during the course of the experiments that these denitrifying bacteria also possess the power of precipitating Calcium carbonate from soluble Calcium salts present in sea-water has, perhaps, by its geological significance, somewhat overshadowed the interest of the primary object of the work.

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The main contentions raised in this paper are—

(1) That in the seas of the American tropics bacteria exist which are actively precipitating Calcium carbonate from the Calcium salts present in solution in sea-water. It is suggested that this bacterial action has been a very considerable factor in the formation of chalk and many other varieties of sedimentary rock, chiefly, or in part, composed of Calcium carbonate. It is also contended that the vast deposits of chalky mud now being formed to the West of the Bahamas, and in the neighbourhood of some of the Florida Keys, are being precipitated by bacterial agency, and that a similar process plays an important part in the cementation of fragments of coral and other detritus into compact coralline rock.

(2) That the destruction of Nitrates by bacterial action in the seas of the American Tropics is far in excess of that occurring in Temperate waters. Hence an explanation is afforded of the relative scarcity of plant life (and consequently of animal life) in Tropical as compared to Temperate seas, in accordance with the terms of Brandt's (2) hypothesis.

Preliminary notes on this work have already been published in the Tortugas Laboratory Reports for 1911 and 1912 (4 and 6) and in the Journal of the Marine Biological Association (5). The chronological sequence of the investigations will be followed in the account given here of the experimental work.

GENERAL CONSIDERATIONS AND PREVIOUS WORK.

It is generally conceded that the plankton of Tropical and sub-Tropical seas is far less in quantity than that found in colder waters.*

The zoo-plankton depends ultimately for its food on the phyto-plankton, hence any factor limiting the growth of the phyto-plankton, which was capable of exercising its influence in Tropical and not in Temperate or Arctic waters, might offer an explanation of this phenomenon. It has been shown by various investigators that this factor is not temperature, light, or salinity, and it has been suggested that the explanation may lie in the relative deficiency in Tropical seas of the Nitrates or nitrogenous compounds which are so essential for all plant life. A matter of common observation in support of this view is the remarkable scarcity of Algal growth in the shallow waters of Tropical shores as compared with that in Temperate regions, and the fact that in the Tropics, wherever sewage or other nitrogenous waste is poured into the sea, a free growth of Algae is found.

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* For the most recent work, and full discussion of this subject, see "The Depths of the Ocean," by Murray and Hjort (13), p. 366 *et seq.*, London, 1912.

At present no really reliable and accurate chemical method of estimating the combined Nitrogen in sea-water exists, hence the above theory cannot be directly put to the test. On the other hand, the existence of denitrifying bacteria in Temperate waters has long been known, and it would seem a fair deduction that should this bacterial destruction of Nitrates take place with greater intensity and completeness in Tropical than Temperate waters, an explanation of the relative scarcity of phyto-plankton in the former would be offered. This suggestion was first made by Brandt (2) in 1901, and is universally known as "Brandt's hypothesis." He enunciated it as follows :—

"If the denitrifying bacteria of the sea, like the closely-investigated denitrifying bacteria of the land, develop a strongly disturbing activity at higher temperatures, only a relatively small production (of phyto-plankton) would take place in the warm seas in spite of much more favourable conditions, according to the law of the minimum, owing to the great disturbance amongst the indispensable food substance; whilst, in the cold seas, more nitrogen compounds would be at the disposal of the producers owing to the retardation or suppression of the disturbing process." (*From the published English translation.*)

The presence of denitrifying bacteria has been demonstrated in Kiel Bay by Baur (1), along the Dutch coast by Gran (9), in the open waters of the North Sea and Baltic by Feitel (7) and Brandt (2 and 3), and in 1909 I identified several of the species described by Gran in samples of water obtained from the Western part of the English Channel. All these denitrifying species have a higher temperature optimum than that of their natural environment, and this is obviously a point strongly in favour of Brandt's hypothesis.

The chief difficulty in the way of putting the hypothesis directly to proof lies in the fact that at present no accurate method of determining the Nitrate contents of sea-water exists, and hence it is impossible to correlate quantitative plankton observations with direct analysis of the amount of combined Nitrogen present in sea-water in different localities. Much valuable work on this subject has been done by Raben (15), but he states that his error in control experiments averages over 30%. An exhaustive study (as yet unpublished) of all the methods of estimating combined Nitrogen in sea-water, as given by various investigators, has been made by Mr. D. J. Matthews, Hydrographer to the Marine Biological Association of the United Kingdom, and he has come to the conclusion that the limits of error in all these methods are so large as to make them quite unreliable. Since chemical methods are at present inadequate to give evidence on this hypothetical deficiency of Nitrates in warmer seas, it seemed of interest to investigate the distribution and relative activity of denitrifying bacteria in Tropical waters in comparison to those found in Temperate seas, and it was with this primary object that the present work was undertaken.

The previous researches most closely related to these investigations are those of Gran (9), who isolated a number of species of denitrifying bacteria from the inshore waters of the Dutch coast. He made use of solutions of Nitrates, Nitrites, or Ammonium salts as the sole source of Nitrogen in his culture media, which contained only a dilute solution of Calcium malate as organic nutrient material for the bacteria. He classifies the bacteria into four groups according to their reactions in pure cultures towards Nitrates or Nitrites:—

(1) Those which reduce Nitrates and Nitrites to free Nitrogen without any Ammonia formation.

(2) Those which readily reduce Nitrates to Nitrites. The Nitrite disappears slowly without perceptible formation of free Nitrogen, and some Ammonia is formed.

(3) Those which cannot reduce Nitrates to Nitrites, but which are capable of slowly removing the Nitrate without perceptible formation of free Nitrogen. Though the Nitrites are not reduced, yet they can serve as the sole source of Nitrogen for the growth of the bacteria.

(4) Those which cannot reduce, and are not capable of assimilating either Nitrates or Nitrites, but will flourish when Ammonium salts are present.

In investigations on samples of water taken in the English Channel some ten miles off Plymouth, I was able to recognize species belonging to the second group of Gran's classification, but could not detect the presence of species belonging to any of the other groups, and it would seem probable that these other groups are chiefly composed of littoral forms.

In fluid culture media inoculated with samples of sea-water and kept at a temperature of 28° C., Gran found that the formation of Nitrite was detectable in from one to two days, and that eventually all the Nitrate and Nitrite was destroyed in the majority of cases, especially if the cultures were reinoculated at intervals. In my experiments I was able to obtain similar results in cultures kept at 30° C. after eight days; in cultures kept at 15° C. the first formation of Nitrite was detectable in from five to six days, but denitrification never proceeded beyond this stage.

Baur (1) showed that the optimum temperature for growth and denitrification of the species described by him lay between 20° C. and 25° C., when the bacteria were grown in fluid culture media containing Peptone.

The most important work on the distribution of marine bacteria is that of Fischer (8) in 1886, 1889, and 1893, but he does not enter into the chemical activities of the species found, so that the observations do not throw much direct light on problems of the metabolism of the sea. The variations in the number of bacteria found in different surface samples from positions in mid-ocean are somewhat surprising and difficult to account for. Deeper samples were taken by means of a water-bottle made of brass, but in view of the now well-known bactericidal action of metals, and of copper in particular, I do not consider that any great value can be attached to these observations. With the exception of Fischer's work, little seems to have been published on the general distribution of marine bacteria.

A point that has not yet been considered is the origin of the Nitrate supply in the sea. Nitrates are absorbed by diatoms and the phytoplankton in general, and are presumably built up into complex nitrogenous compounds within the plant. If these compounds, on the death of the plant, are broken up and the Nitrogen again rendered available for use in the form of Nitrates, a series of reactions must be gone through which may well be performed by bacterial agency, and this also applies to the waste nitrogenous products of animal metabolism. In addition, it has been shown that Nitrates are actually decomposed by the denitrifying bacteria, which would thus tend to keep the Nitrate concentration down to the level necessary for their own existence, and would come into competition for this essential with other forms of plant life. If the bacteria are successful in decomposing Nitrates to the extent of entirely removing the Nitrogen from all chemical combination, as seems probable from the experiments in cultures, it follows that there must be some source of Nitrates in order that the concentration in the sea may remain constant. The existence of nitrifying bacteria, which are capable of absorbing and combining with the free Nitrogen of the air and eventually giving rise to Nitrates, has been shown by Keding (10) and Keutner (11), but these have so far only been found on the bottom close to shore, or apparently living in symbiosis with algae or plankton organisms. Similarly, Thomsen (16) has demonstrated the presence on the bottom of inshore waters of bacteria which are capable of forming Nitrites from Ammonium salts, and others which can convert Nitrites into Nitrates. It would seem possible that similar bacteria having a nitrifying action remain to be discovered in the open sea.

The precipitation of Calcium carbonate in the sea by bacterial agency is apparently a line of investigation that has not previously been suggested or followed. Both Baur (1) and Gran (9) made use of

Calcium salts in their culture solutions in order to obviate the great increase in alkalinity that resulted if Potassium or Sodium salts were used, but they have not called attention to, or apparently realized, the probable significance of this precipitation of Calcium carbonate by bacterial agency as an important factor in the formation of various sedimentary calcareous rocks in Tropical seas.

The subject of the precipitation of Calcium carbonate in sea-water has been dealt with by Murray and Irvine (14) in 1889, and again by Murray and Hjort (13) in 1912, and they ascribe the precipitation to the interaction of Ammonium carbonate, derived as an ultimate product of the decomposition of nitrogenous organic matter, with the Calcium sulphate present in sea-water, according to the equation

$$(NH_4)_2\,CO_3 + CaSO_4 = CaCO_3 + (NH_4)_2\,SO_4.$$

Expressed in the terms of the Ionic Hypothesis, this reaction can be explained by the statement that $CaCO_3$ must be precipitated when the product of the concentration of its ions $Ca^{\cdot\cdot}$ and CO_3'' exceeds a certain limit; an increase in the concentration of CO_3'' ions is produced by the advent of $(NH_4)_2CO_3$, which is partially ionized into $NH_4^{\cdot}$ and CO_3'', and hence the product of the concentrations of $Ca^{\cdot\cdot}$ and CO_3'' ions is increased, and $CaCO_3$ is thrown out of solution.

Though this reaction has been shown conclusively to occur under experimental conditions, where nitrogenous organic matter has been allowed to putrefy for some time in sea-water, yet it is obvious that its effect must be purely local, and must be confined to the immediate neighbourhood of the decaying organic body, which gives rise to the formation of $(NH_4)_2\,CO_3$.

In this paper the precipitation of $CaCO_3$ in an unorganized state alone is dealt with. The formation of the calcareous skeletons, tests, and shells of animals, and the skeletons and platelets of algae, which play an immensely important part in the constitution of marine bottom deposits, is beyond the scope of these investigations.

DESCRIPTION OF APPARATUS.

In 1911 the apparatus at my disposal was of a somewhat primitive nature, as it is difficult when on the first expedition in a new field of work to know beforehand exactly what gear will be necessary. In 1912 a more complete outfit was available, and the Carnegie Institute yacht *Anton Dohrn* was especially fitted for my requirements.

For deep-sea work the motor trawl winch was modified so as to carry fine sounding wire, and a derrick was rigged aft, projecting over the stern of the boat, over which the wire was led. The motor winch is sunk below the level of the deck, an arrangement which is to be greatly commended, as it can be covered over with hatches when not in use, and so affords great economy of deck space, and also has the advantage of bringing the weight of the winch nearer the water-line, and avoiding the unstability that may be caused when a heavy winch is fixed on deck.

The sounding wire was 2·2 mm. in diameter, and consisted of four strands of eight wires each, made of high tensile steel; the breaking strain was given at 400 lbs., but in practice I have no hesitation in saying that it far exceeded this figure. The wire was very difficult to kink, and did not show any tendency to untwist or permanently stretch under a tension of about 350 lbs.; it proved in every way satisfactory, and was supplied by Messrs. Bullivant and Co., of London.

For measuring the length of wire run out, one of the fathom measuring sheaves as made by the Telegraph Construction and Maintenance Co., of London, was used. This consisted of a sheave containing a steel wheel about 12 inches in diameter, grooved for and made especially to fit the wire; the length of wire run out is measured by the number of turns of the wheel indicated by a dial on the side of the sheave. The dial has two hands showing fathoms and hundreds of fathoms, the hands revolve backwards on winding in the wire, and so again register zero when the sounding is completed. The axle of the wheel revolves on simple bearings, so the slight inaccuracy unavoidable if ball bearings are employed is prevented.

Samples of the bottom were obtained with one of the "snapper rods," disengaging a 30-lb. iron weight on touching the bottom, also supplied by the Telegraph Construction and Maintenance Co. This consisted of two brass jaws closed by a strong spring, and kept apart by a trigger; on touching bottom the trigger was released and the jaws closed on a sample of the bottom; at the same time the 30-lb. weight, which was only held in position by the tension of its own weight, was disengaged as soon as the tension was relieved on touching bottom, and so was left behind as the wire was reeled in.

In order to tell the depth at which bottom was sounded, the wire was led through a pulley connected with a spring balance, which thus registered the tension of the wire. On touching the bottom the decrease in tension, due to the release of the weight, was shown on the dial of the balance. This arrangement was not satisfactory in rough weather, as the rolling of the yacht caused such varying tensions on the wire that it was not always possible to tell the exact depth at which the weight was disengaged.

For obtaining samples of water for bacterial analysis a special water-bottle was designed for me by Mr. D. J. Matthews. This apparatus is described in detail by Mr. Matthews in the present number of the "Journal of the Marine Biological Association of the United Kingdom," so only a brief account of it will be given here (see p. 525).

The apparatus employed by previous workers for obtaining samples of water from the deep sea for bacteriological examination has either consisted of some sort of water-bottle made of metal, or else of exhausted glass bulbs, with a neck drawn out into a capillary tube, which could be broken off at the depth from which a sample was desired. The use of exhausted glass bulbs presents considerable difficulties for depths as great as 800 fathoms: the bulbs must be strong and very thoroughly annealed, as otherwise the slight shock caused by breaking the capillary neck is liable under the great pressure to make the bulb fly into small fragments: another great disadvantage is the strong probability that the sudden reduction in pressure to which the water is exposed, as it enters the bulb, would immediately kill any bacteria in the water. The employment of a metal water-bottle seemed undesirable in view of the bactericidal action of metals: in order to settle this point some test experiments were made with various metals to see if a suitable one could be found. 100 c.c. of water from the Laboratory tanks at Plymouth, diluted 1 in 100 with sterile sea-water, was exposed for six hours to the action of about two square inches of various metals, with the following results:—

Metal.	Numbers of plates.	Number of colonies of bacteria developing from 1 c.c. after plating on Peptone Agar. Counted after 10 days.
Aluminium bronze . .	1	0
„	2	0
„	3	0
Pure copper foil . .	1	1
„	2	0
„	3	0
Brass	1	0
„	2	0
„	3	1
Pure nickel . . .	1	17
„	2	12
„	3	8
Silver (coins) . . .	1	3
„	2	2
„	3	4
Control experiment .	1	512
„	2	560
„	3	480

It is thus obvious that none of these metals are suitable for the work, and probably the only metal that could be used would be Platinum, which would be prohibitive on account of the expense.

In order to overcome these difficulties, a water-bottle on a new principle was designed for me by Mr. Matthews.

The container of the bottle consisted of a strong glass cylinder holding about 250 c.c.: this was closed at each end by thick rubber washers, through the centre of which a short piece of thin-walled rubber tubing passed, the tubing being sealed at the end within the cylinder. The washers were fixed in metal plates sliding along the guide bars of the skeleton frame in which the glass cylinder was fixed, and by sending down two messengers along the sounding wire the cylinder could first be opened at both ends and then closed at any required depth. The whole apparatus was first sterilized by steaming in a "Koch," and then the cylinder was completely filled with 95% Alcohol: the washers were kept tight on the ends of the cylinder by strong springs so that no leakage occurred. When the apparatus had been lowered to the required depth, the first messenger was sent down, this, by hitting a lever, opened the cylinder at both ends, and the alcohol, being of lower specific gravity than sea-water, diffuses out almost instantaneously, causing an upward flow of water through the cylinder. On sending down the second messenger the cylinder, with its sample of water, was tightly closed at each end by the rubber washers.

The washers, with their attached pieces of thin rubber tubing, had sufficient capability of bulging inwards to allow for the contraction of the Alcohol, due to the low temperature at any considerable depth, and to its compressibility being greater than that of sea-water, and similarly the expansion of the sample of water, as the apparatus was hauled up, was compensated for by the partial collapsing of the thin-walled rubber tubing. It is obvious that even had a slight amount of leakage occurred, a leakage inwards during the descent of the apparatus would not vitiate the results, as bacteria would promptly be killed in the 95% Alcohol, and similarly on hauling up, the leakage, if any, would be outwards, due to the expansion of the sample through the regularly increasing temperature and decreasing pressure, so that the sample would not be contaminated by any of the surface layers through which it was hauled. There was, however, no reason to suppose that any leakage occurred, and it appears that the expansibility of the rubber washers and tubing was sufficient to allow for the small changes in bulk of the fluids within the cylinder. After the first sterilization by steaming, the action of the Alcohol was relied on for sterilization between successive samples, and both experimentally and in practice this method was found to be absolutely safe, as all the marine bacteria are very readily killed by Alcohol, and they do not form resistant spores.

After the collection of a sample it was siphoned off into a sterilized glass bottle by means of a sterilized length of rubber tubing: this method was considered preferable to any arrangement of taps leading from the collecting cylinder, owing to the difficulties of cleaning and sterilization which would be involved. Part of the sample was also siphoned off into bottles, which were returned to Plymouth for analysis for salinity: these bottles had previously been thoroughly washed and rinsed with several changes of distilled water, and then dried in an oven; they were closed with rubber stoppers.

It was found in practice that this design of water-bottle worked extremely well and gave very little trouble; it is to be noted that the sample of water collected is kept only in contact with rubber and glass throughout, so that the bactericidal action of metal is avoided.

Surface samples of water were taken in wide-mouthed stoppered bottles, holding about twelve ounces; the samples were always taken from the bows of the boat when moving ahead, in order to avoid any possible contamination from the sides of the boat.

Some samples from depths up to 80 fathoms were collected off the Tortugas in 1911 in retort-shaped glass flasks of about 300 c.c. capacity, with narrow, recurved, long-drawn-out necks. These were sterilized, exhausted, and sealed; they were then lowered in an apparatus in which the extremity of the neck could be broken off at any desired depth by sending a messenger down the sounding wire, when the flasks became completely filled with water. After hauling up, a little water was shaken from the neck, and it was then sealed with the blowpipe. By this method risk of contamination from more superficial layers of water as the apparatus is drawn up is avoided, since the changes in pressure and temperature as it ascends tend to cause a continuous outflow through the narrow neck until the surface is reached.

A somewhat similar apparatus was used for obtaining deep samples from the station 70 miles west of Ushant, but the glass bulbs were smaller, and the tube leading from them was bent at right angles to itself. Considerable difficulty was caused by the breaking of the tube owing to the force of the inrushing stream of water impinging on the wall where it was bent at right angles.

If this form of apparatus is used, all sharp angles in the inlet tube should be avoided, and it should be so arranged that the inrushing stream of water spreads itself in a fan-shaped manner over the sides of the bulb, but I do not consider that any form of exhausted glass flask is suitable even for depths as small as 80 fathoms.

In Jamaica no apparatus for obtaining deep samples was available, so the primitive method of lowering a sterilized stoppered bottle with a string tied to the stopper was employed. At the required depth the stopper was pulled out until the bottle was nearly full and then allowed to fall back in place. This method can only be used for very shallow depths owing to the pressure of the water at greater depths making it impossible to withdraw the stopper.

Temperature records were obtained in the Bahamas by means of deep-sea reversing thermometers, specially made by Messrs. Negretti and Zambra, of London. They were tested up to a pressure of three tons to the square inch at the National Physical Laboratory at Teddington, and a table of temperature corrections was furnished for each instrument by the same Institution. These reversing thermometers differ from ordinary thermometers in having a constriction and S-shaped dilatation immediately above the main bulb, and in having a somewhat large secondary bulb at the upper end of the stem. The graduations are reversed, so that the lowest temperature is marked near the top of the capillary portion. On turning the thermometer upside down, the mercury thread breaks at the constriction, and fills the small bulb at the end of the capillary and also part of the capillary itself. The thermometer is read in the reversed position, and when certain corrections have been applied, the reading records the temperature at which the thermometer was reversed. The effect of the pressure of the water is avoided by having the thermometer sealed in an outer glass case. The lower end of this case is partially filled with mercury in which the bulb of the thermometer is immersed, thus allowing for rapid conduction of heat between the mercury in the thermometer bulb and the surrounding water. An auxiliary thermometer was sealed up in the same outer case as the reverser, so that the temperature at which the actual reading was taken could also be recorded. In order to calculate the correction that must be applied to the temperature registered by the reverser, three factors must be known :—

(*a*) The temperature of the thermometer at the moment of reading.

(*b*) The kind of glass of which it is made.

(*c*) The volume, expressed in degrees of the stem, of the secondary bulb and the portion of the stem below the 0° graduation.

Of these (*a*) is given by the auxiliary thermometer, and (*b*) and (*c*) were engraved on the back of the stem of each reversing thermometer.

All the thermometers were made of the glass known as Jenaer 16 III, and the apparent dilatation of mercury in this glass is $\frac{1}{6300}$. The correction to be applied to the reading of the reverser is given by the formula $\frac{(V^\circ + T)\ (T - t)}{6300}$, where T = the temperature registered by the reverser, t = the temperature shown by the auxiliary thermometer at the moment of reading, and V° = the volume, expressed in degrees of the stem, of the secondary bulb and the portion of the stem below the 0° mark of the reverser.

The thermometers were mounted in pairs in simple metal cases, and were attached just below the water-bottle. They were suspended in a vertical position by a catch forming part of the water-bottle; this was released by the first messenger, when the thermometers fall by their own weight and reverse; they were hauled up in this reversed position. This simple arrangement proved quite as satisfactory as any of the more complicated reversing frames which are generally in use.

CULTURE MEDIA AND METHODS.

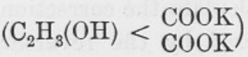
The culture media employed for isolating and counting the bacteria in plate cultures were the following:—

I. Peptone Agar.

Peptone	2·0 grammes
Potassium nitrate (KNO_3) . . .	0·5 „
Sea-water	1000·0 c.c.
Agar Agar	18·0 grammes (of fibre)

In the earlier work less Agar was used, but eventually it was found more convenient to use a stiffer jelly, and this did not appear to hinder appreciably the growth of the bacteria.

II. Potassium Malate Agar.

Potassium malate  $\left(C_2H_3(OH) < \begin{matrix}COOK\\COOK\end{matrix}\right)$	1·0 gramme
Sodium phosphate ($Na_2HPO_4\ 12H_2O$) .	0·25 „
Potassium nitrate (KNO_3)	0·5 „
Sea-water	1000·0 c.c.
Agar Agar	12·0 grammes (of fibre)

The medium was only filtered through glass wool, so that a very slight floccular precipitate of calcium phosphate was retained.

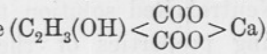
III. Peptone Gelatin.

Peptone	2·0 grammes
Potassium nitrate (KNO_3)	0·5 ,,
Sea-water	1000·0 c.c.
Gelatin	150·0 grammes

This medium was only used at the Tortugas. It was necessary to keep it cooled with ice to about 20° C., as the temperature of the Laboratory sometimes rose as high as 37° C., or even higher, and Gelatin media will not remain solid at these temperatures.

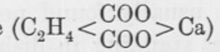
The following fluid media were used:—

I. Gran's Medium (Modified).

Potassium nitrate (KNO_3)	0·5 gramme
Sodium phosphate ($Na_2HPO_4, 12H_2O$)	0·25 ,,
Calcium malate  $(C_2H_3(OH)<\genfrac{}{}{0pt}{}{COO}{COO}>Ca)$, about .	5·0 ,,
Sea-water	1000·0 c.c.

Calcium malate is only slightly soluble in water, so can be added in excess. Gran used distilled water, and added 30 grammes of Sodium chloride per litre, but in these experiments sea-water has been used instead.

II. Calcium Succinate Medium.

Calcium succinate  $(C_2H_4<\genfrac{}{}{0pt}{}{COO}{COO}>Ca)$	2·0 grammes
Potassium nitrate (KNO_3)	0·5 ,,
Sodium phosphate ($Na_2HPO_4, 12H_2O$) . . .	0·25 ,,
Sea-water	1000·0 c.c.

This medium was boiled and filtered before sterilization, to remove the slight precipitate of Calcium phosphate. It was found that this medium with the addition of the phosphate gave a more vigorous growth than if it was omitted.

III. Calcium Acetate Medium.

Calcium acetate ($Ca(CH_3COO)_2$)	5·0 grammes
Sodium phosphate ($Na_2HPO_4, 12H_2O$) . . .	0·25 ,,
Potassium nitrate (KNO_3)	0·5 ,,
Sea-water	1000·0 c.c.

Boiled and filtered before sterilization to remove precipitate of phosphate.

IV. Peptone Calcium Acetate Medium.

Calcium acetate ($Ca(CH_3COO)_2$)	5·0 grammes
Peptone (Witte's)	0·2 ,,
Potassium nitrate (KNO_3)	0·5 ,,
Sea-water	1000·0 c.c.

The fluid media II, III, and IV were also made up with the addition of 0·2 grammes of Magnesium tartrate per 1000 c.c.

The fluid media were made up in 1500 c.c. resistance glass flasks, and 1000 c.c. of medium was used for each culture.

For other purposes a simple solution of Peptone in sea-water was employed (2 grammes to 1000 c.c.), and media were also used consisting of this Peptone solution with the addition of 0·5% of various carbohydrates, such as Cane sugar, Dextrose, Laevulose, Mannite, Lactose, etc., with sufficient Neutral Red solution to colour them, in order to test the acid-forming properties of the bacteria in the presence of Carbohydrates.

The ordinary "Koch" steam sterilizer, and an iron oven for dry-heat sterilization, were used, and gasoline cooking stoves were found to be the most satisfactory source of heat. It was found an advantage to use Petri dishes with porous earthenware covers, which enabled the water of condensation to evaporate partially; the evaporation could be checked at any time by covering the dishes with a bell-jar lined with wet filter-paper. It was usually found necessary to keep all cultures on tables with their feet standing in dishes of paraffin oil, in order to prevent the attacks of ants and other insects. In all other respects ordinary bacteriological routine was followed, and the methods need not be further particularized here.

The reduction of the Nitrate to a Nitrite in fluid culture media was tested for by the addition of 5 c.c. of 10% Sulphuric Acid and 2 c.c. of a 1% solution of Metaphenylene diamine hydrochloride to 25 c.c. of the culture. The production of a brown colouration (due to the formation of Bismarck Brown) is an indication of the presence of a Nitrite, and is an extremely delicate reaction.

The Diphenylamine and Brucine sulphate reactions were also used when testing for the presence of Nitrates.

The formation of Ammonia was tested for by the addition of 5 c.c. of 10% Potassium hydrate and 5 c.c. of Nessler's Reagent; the white precipitate formed on the addition of the Potassium hydrate does not appreciably interfere with the test, though it renders it less delicate.

Under expeditionary conditions, and in the absence of the somewhat elaborate apparatus that would be necessary in order to estimate chemically the amount of denitrification in cultures, it was only possible to compare the rate of denitrification in different cultures by noting the time taken for the first appearance of the Nitrite reaction, and the time taken for all trace of Nitrite or Nitrate to disappear. It seems that the rate of denitrification in culture media inoculated with equal volumes of samples of sea-water must be a function of the number of bacteria in the sample, the temperature at which the cultures are grown, and the specific power of denitrification of the individual species of bacteria. Considering the rapid multiplication of bacteria when the food supply is plentiful, up to a maximum determined chiefly by the accumulation of the waste products of their own metabolism, it appears that the factor of the number of bacteria in the sample may be neglected within the limits of these experiments. For example, the number of bacteria in 1000 c.c. of Gran's medium at the end of twenty-four hours would probably be much the same whether it were inoculated from a sample containing 8 or 16 bacteria per 1 c.c., similarly it was a matter of experience that the first trace of Nitrite formation was observable at about the same time, whether 5 or 10 c.c. of a given sample had been used for inoculation.

Consequently it would appear that for purposes of comparison, and within the limits of the experiments described, if the temperature be the same for the cultures compared, the rate of denitrification is a measure of the specific denitrifying power of the particular species of bacteria.

In the work on the bacterial precipitation of Calcium carbonate, the precipitate, which was often so fine as to tend to remain in suspension, was usually obtained by centrifugalization. It was either preserved in small bottles with some of the culture fluid, or else washed first with distilled water and then with absolute alcohol, and finally allowed to dry. These precipitates were sent to Dr. F. Wright of the Carnegie Geophysical Laboratory at Washington, who with great kindness reported on their mineralogical properties.

THE INVESTIGATION OF SAMPLES OF SEA-WATER TAKEN OFF PORT ROYAL, JAMAICA.

The work at Port Royal was done in May, 1911, but was of a very preliminary nature. It was necessary to depend on a sailing-boat for obtaining the samples, but owing to the remarkable regularity with which an on-shore wind springs up every morning but little difficulty was experienced from this cause. No apparatus for obtaining deep samples was available, but samples were taken from a depth of six fathoms by means of a bottle from which the stopper was pulled by a line, and then allowed to fall back into place. A measurement of the rate of denitrification in fluid culture media inoculated with samples of sea-water was made, but isolation of the bacteria on solid media was not attempted.

The following method was employed:—

Samples of sea-water were collected in sterilized stoppered bottles from the surface, and from depths of 3 and 6 fathoms, from positions about five miles from shore, where, from a consideration of the wind and tide, the water was probably under truly oceanic conditions and unaffected by the neighbouring land.

10 c.c. of these samples were added to 1000 c.c. of Gran's medium. The cultures were kept in a moderate light, and the temperature varied from 25° to 31·5° C. The average temperature during the growth of each culture was noted.

In a typical culture made from surface water, and for which the average temperature was 29° C., the first indication of the formation of a Nitrite, as given by the Metaphenylene diamine reaction appeared after 27 hours; after 38 hours the brown colour produced in this reaction was very intense, the culture became cloudy, and on testing with Nessler's Reagent slight Ammonia formation was apparent. After 48 hours the culture became very cloudy and a scum of bacterial growth developed: the Nitrite and Ammonia reactions remained unaltered. After 63 hours the Nitrite reaction was somewhat less marked, the Ammonia reaction was unaltered, and bubbles of gas began to appear. After 72 hours many bubbles of gas were being produced, and the Nitrite and Ammonia reactions were very slight. After 86 hours the bubbling had ceased, and no Nitrite or Ammonia was present in the culture. Testing the culture for Nitrates by the Brucine and Diphenylamine reactions then showed that no Nitrate was left in the solution.

In the absence of a gas analysis apparatus the nature of the gas evolved could not be determined, but considering that it was non-inflammable, did not turn lime water milky, and that the Nitrate originally present had been destroyed, it seems strongly probable that this gas was pure Nitrogen. Thus in 86 hours 0·5 gramme of Potassium nitrate had been decomposed by bacterial growth. If a further 0·5 gramme of Potassium nitrate were then added, it was rapidly decomposed, and this could be repeated many times until the other constituents of the culture medium were used up, or the accumulation of the products of metabolism had become toxic to the bacteria.

It was found that the rate of denitrification varied with the temperature, and that in cultures kept at a temperature of between 10° and 12° C. no growth or denitrification occurred. The denitrification was always more rapid in cultures from water taken from a depth of 3 or 6 fathoms than from the surface. It was also more rapid with samples taken from the thick muddy water of a mangrove swamp, where organic matter was plentiful.

The bacteria present in the cultures were very minute, actively motile bacilli with rounded ends.

An abstract of the behaviour of a few of the cultures is given below.

1. Sample collected 5 miles S. of Port Royal, wind S.E., force 4, tide rising. Sample taken from surface. 1000 c.c. of Gran's medium was inoculated with 10 c.c. of sample.

After 20 hours a slight cloud developed in the culture, and faint Nitrite reaction was given.

After 36 hours a dense cloud developed in the culture, and strong Nitrite reaction was given.

After 60 hours a dense cloud and scum developed in the culture, and strong Nitrite and faint Ammonia reaction was given.

After 70 hours a dense cloud, scum, and bubbles developed in the culture, and faint Nitrite and faint Ammonia reaction was given.

After 84 hours culture was less cloudy, with much scum, no Nitrite or Nitrate reaction, very faint Ammonia.

The average temperature at which the culture was grown was 30° C.

2. Sample collected from same spot under similar conditions, from a depth of 3 fathoms. 1000 c.c. Gran's medium was inoculated with 10 c.c. of sample.

After 20 hours a slight cloud developed in the culture and faint Nitrite reaction was given.

After 27 hours a denser cloud developed in the culture and strong Nitrite and faint Ammonia reaction was given.

After 38 hours a dense cloud and scum developed in the culture and strong Nitrite and faint Ammonia reaction was given.

After 48 hours a dense cloud and scum developed in the culture and moderate Nitrite and faint Ammonia reaction was given.

After 63 hours a moderate cloud, thick scum, and bubbles developed in the culture and faint Ammonia reaction was given.

After 72 hours a slight cloud and thick scum, no Nitrite or Nitrate, and very faint Ammonia reaction.

The average temperature at which the culture was grown was 29° C.

3. Sample collected from a spot 6 miles S. of Port Royal, wind E.S.E., force 4, high tide (slack). Taken from surface. 1000 c.c. Gran's medium was inoculated with 10 c.c. of the sample.

After 20 hours a slight cloud developed in the culture; no Nitrite reaction was given.

After 27 hours a slight cloud developed in the culture; faint Nitrite reaction was given.

After 38 hours a dense cloud developed in the culture; strong Nitrite and faint Ammonia reaction was given.

After 48 hours a dense cloud and scum developed in the culture; strong Nitrite and faint Ammonia reaction was given.

After 63 hours a dense cloud and scum developed in the culture; moderate Nitrite and faint Ammonia reaction was given.

After 72 hours a moderate cloud, scum, and bubbles developed in the culture; very slight Nitrite and faint Ammonia reaction was given.

After 86 hours a moderate cloud and scum, no Nitrite or Nitrate and very faint Ammonia reaction.

The average temperature at which the culture was grown was 29° C.

4. Sample taken from surface water of the large mangrove swamp lying N.W. of Port Henderson. 1000 c.c. of Gran's medium inoculated with 10 c.c. of sample.

After 20 hours no cloud or Nitrite reaction.

After 24 hours slight cloud and slight Nitrite reaction.

After 40 hours strong cloud and scum, strong Nitrite and slight Ammonia reaction.

After 75 hours cloud, scum, and bubbles, no Nitrite or Nitrate and slight Ammonia reaction.

The average temperature at which the culture was kept was 30° C.

5. Subculture from Culture (1). 1000 c.c. Gran's medium inoculated with 5 c.c. of culture (1) and kept at a temperature of 10° C. to 12 C. by means of ice.

After 100 hours the culture was quite clear, and gave no Nitrite reaction. It was then removed from the ice and kept at the room temperature, which averaged 30° C.

After 107 hours a dense cloud developed in the culture, and strong Nitrite and faint Ammonia reactions were given.

After 120 hours a dense cloud, scum, and bubbles developed in the culture, and moderate Nitrite and faint Ammonia reactions were given.

After 131 hours a faint cloud, scum, and bubbles developed in the culture, and very faint Nitrite and faint Ammonia reactions were given.

After 146 hours a faint cloud and scum, no Nitrite or Nitrate, and very slight Ammonia reactions were given.

Twenty cultures from samples of water taken well out to sea from Port Royal were made, and the process of denitrification followed through with each. All gave very similar and consistent results, but the rate of denitrification decreased rapidly with the temperature at which the cultures were grown: thus at an average temperature of 27° C. the first trace of the Nitrite reaction appeared after about 40 hours, and denitrification was complete after about 100 hours.

The results of precisely similar experiments that I made with samples of water taken from the English Channel near Plymouth in the autumn of 1909, showed that there the process of denitrification was very much slower, and was never complete at the room temperature (17° C.). The first trace of the formation of a Nitrite in cultures in the modified Gran's medium, as detected by the Metaphenylene diamine reaction, occurred about the fifth day, and a large proportion of the Nitrite and Nitrate always remained, even in the oldest cultures. In similar cultures incubated at 30° C. denitrification was complete by the eighth day at earliest, but uniformly consistent results were not obtained, as in some of the cultures complete denitrification never occurred, even after several months.

It would thus appear that even under similar temperature conditions, the marine bacteria in the seas off Jamaica are much more active in causing denitrification than those found in the English Channel, and since the rate of denitrification is a function of the temperature, it follows all the more that the destruction of Nitrates by bacterial agency in the seas round Jamaica must be far in excess of that occurring in the cooler waters of the English Channel.

THE INVESTIGATION OF SAMPLES OF SEA-WATER TAKEN AROUND THE DRY TORTUGAS.

The Dry Tortugas consist of a group of eight small Keys, the largest of which, Loggerhead Key, is only about $\frac{3}{4}$ mile long by $\frac{1}{8}$th wide. They are situated about 150 miles from the mainland of Florida, and form the extreme western end of the chain of the Florida Keys. The 100-fathom line lies some 30 miles to the S. and S.W. of the

Islands and then trends round in a N.W. direction; beyond the 100-fathom line the depth increases with moderate rapidity until depths of from 1000 to 1400 fathoms are reached. To the E., N.E., and N., as far as the coast of Florida, the water is shallow, the soundings showing from 20 to 30 fathoms in most places. Beyond the 100-fathom line to the southward the influence of the Gulf Stream begins to make itself felt, though the region of maximum current velocity here lies nearer the coast of Cuba. The Tortugas Keys are of purely coral formation; they consist entirely of broken shell and coral sand, and no soil is present; the greatest elevations are the hurricane ridges, which are not more than 15 feet above sea-level, and during a hurricane the islands are sometimes completely submerged. There is no vegetation on the smaller Keys, but Loggerhead Key, on which the Carnegie Laboratory is situated, is partially covered with a growth of bushes and coarse grass. There is no fresh-water supply on the islands.

From these considerations it is obvious that the risk of contamination of samples of sea-water, taken a few miles from the Keys, through land bacteria is very small, and that such samples may be taken as being truly oceanic.

The motor-yacht *Anton Dohrn,* and smaller motor-boats, made the collection of samples an easy matter, and the well-equipped Laboratory made possible fuller investigations than those attempted in Jamaica.

A number of cultures were made in Gran's medium under conditions exactly comparable to those made at Port Royal, and the rate at which the process of denitrification proceeded was observed. The results agreed almost exactly with those obtained at Port Royal, so need not be described in detail. It thus seems that the denitrifying power of the bacteria in the seas around the Tortugas is the same as that of those around Jamaica.

Cultures were also made on various solid media, and pure cultures of the various species of bacteria were isolated by plating in Petri dishes with Peptone Agar. Samples of surface water taken from various positions round Tortugas as far as possible removed from influence of the land, and collected on sunny days, gave an average count of 14 colonies per 1 c.c. of sample. Counts of several plates from the same locality, and from different localities, showed a somewhat remarkable agreement as to the number of colonies present, the highest count ever obtained being 20 and the lowest 8 per 1 c.c. Allowing for experimental error, this shows great uniformity in the distribution of bacteria in the sea round Tortugas.

The colonies appeared to be of two kinds when grown on Peptone Agar, one much more plentiful than the other. Subcultures made from these colonies in Gran's medium showed that the bacteria forming the most common type of colony produced an active denitrification, while the others grew very slowly in this medium and produced no denitrification.

The characteristics of the denitrifying form are as follows:—

The bacterium is a very minute, actively motile short rod, with rounded ends, readily giving rise to involution forms in old cultures.

On the Potassium malate, or Peptone Agar media, colonies are visible as minute white specks after 6 to 8 hours, when the room temperature averages 29·5° C. After about 18 hours the colonies are well developed; they are white in colour, circular, but with a finely irregular outline, and have a granular appearance. Superficial colonies are much elevated at first, but as growth proceeds spread rapidly over the surface of the Agar. Deep colonies remain small, circular, and discrete.

Growth is somewhat more rapid on Peptone Agar than on the Potassium malate Agar, and the older colonies develop a brownish tinge in the centre when growing on the former medium. On Gelatin Peptone ($5°/_{oo}$ Peptone in sea-water and kept at between 20° and 25° C. to ensure the medium remaining solid) growth was very slow: in stab cultures growth proceeded slowly from the surface downwards, leaving a funnel-shaped depression of liquefied Gelatin.

Acid formation, as shown by the Neutral Red reaction, occurs in Dextrose, Laevulose, Mannite, and Cane Sugar, but not in Lactose media.

Growth is inhibited at a temperature of 10° C., but takes place slowly at 15° C.

Growth is much retarded by exposure to bright sunlight, but the bacteria are not killed by a ten hours' exposure.

The bacteria are facultative anaërobes, but growth under anaërobic conditions is very slow.

In Gran's medium growth is rapid, but no growth occurs if the Potassium nitrate be omitted, or if the Calcium malate be replaced by Calcium carbonate. Growth in a pure solution of Peptone in sea-water is slight, but becomes abundant if Potassium nitrate be added, when denitrification quickly ensues. The most rapid early growth was produced in sea-water containing $2°/_{oo}$ Peptone, $1°/_{oo}$ Potassium malate, and $0{\cdot}5°/_{oo}$ Potassium nitrate, and in this clear medium a slight floccular precipitate, presumably of Calcium salts derived from the sea-water, was soon formed. Growth was also rapid at first in a solution of $5°/_{oo}$ Potassium malate and 0·5°/$_{oo}$ Potassium nitrate in sea-water, but in this medium growth apparently ceased after a few days and denitrification was never complete; a slight precipitation occurred, and the solution was found to have very definitely increased in alkalinity.

This bacterium does not appear to have been previously described, and I propose for it the name of "Bacterium calcis," owing to its power of precipitating Calcium carbonate from solutions of Calcium salts. This point will be dealt with later in the paper.

The characteristics of the scarcer non-denitrifying form of bacterium found on the Agar plates are as follows:—

The morphological characters are exactly similar to those of *B. calcis.*

Growth on the Potassium malate Agar medium is very slow and indefinite. On Peptone Agar growth is somewhat slower than in the case of the *B. calcis.* On the surface, circular cream-coloured colonies are formed, having a brownish centre, the edges are smooth and regular, and the colony remains discrete and does not tend to spread. The deep colonies are smaller and usually ovoid in shape, and of a somewhat darker colour than those on the surface.

No growth was obtained on Gelatin media.

Acid formation, as shown by the Neutral Red reaction, occurs in Dextrose and Laevulose, but not in Cane Sugar, Lactose, or Mannite media.

Growth takes place slowly at 10° C. No visible growth occurred at 0° C., but cultures were not killed by twenty-four hours' exposure to this temperature.

Growth is retarded by light, and cultures are killed by four hours' exposure to bright sunlight.

The bacterium is a strict aërobe.

Free growth takes place in Gran's medium, but develops much slower than in the case of the denitrifying form: no growth occurs if the Potassium nitrate be omitted entirely, but takes place freely if a mere trace in excess of that normally present in the sea-water be added, though no denitrification results. Attempts were made to discover whether this bacterium had any nitrifying or denitrifying action in various culture media, but uniformly negative results were obtained. Nitrites were neither oxidized to Nitrates, nor reduced to Ammonia or free Nitrogen, and Ammonia salts were unaffected. No growth was obtained in any culture medium that did not contain at least a trace of Nitrates, so it was not practicable to ascertain whether the bacterium had a nitrifying action without the necessary facilities for quantitative work.

On one occasion samples were obtained from various depths up to

90 fathoms at a point near the Gulf Stream region, 25 miles south of Tortugas. Exhausted glass flasks, with capillary necks which could be broken off at the required depth, were used for the purpose.

These samples were plated in the Peptone Agar medium and counted with the following average results :—

Depth in fathoms.	Denitrifying forms. (Bacterium calcis.)	Non-denitrifying forms.	Number of Colonies developing from 1 c.c. of sample.
0	9	2	11
10	25	4	29
40	2	2	4
60	5	3	8
90	5	6	11

It is probable that these figures are not very reliable, especially for the greater depths, since it is possible that many of the bacteria were killed by the sudden reduction of pressure to which they were exposed as the water entered the exhausted bulb.

THE INVESTIGATION OF SAMPLES TAKEN FROM A POINT 70 MILES WEST OF USHANT ISLAND, FRANCE.

This spot was chosen as it is sufficiently far out in the Atlantic to be largely out of the influence of the English Channel water. The object was to investigate truly oceanic bacteria, and previous work in 1909 had shown that the bacterial flora of the Channel water was relatively very complicated, probably owing to the presence of littoral forms. The Marine Biological Association of the United Kingdom very kindly sent their s.s. *Oithona* from Plymouth for this work, and gave me every facility both on board and in their Laboratory. As in Tortugas, the deep samples were collected in exhausted glass flasks, and accordingly, as previously explained, the results obtained from the deep samples cannot be considered to possess any very great degree of accuracy.

Attempts were made to plate the samples in Peptone Agar on board the boat, but the result was not satisfactory, as owing to the motion of the boat the jelly set in irregular waves and lumps. Consequently the samples were kept on ice, and cultures were made from them at Plymouth 24 hours after collection. It is clear that if in the future attempts are made to make plate cultures on board a small boat in rough weather, a very delicately swung table will be necessary, or else the roll-tube culture method must be employed.

Three plates on Peptone Agar were made from each sample, 1 c.c. of the sample being used for each plate. The plates were kept at the room temperature, averaging about 20° C., and the colonies were well developed after 48 hours: they appeared to be all of one kind. A count gave the following results:—

Depth in fathoms.	Number of colonies developing from 1 c.c. of sample.
0	7
10	9
20	6
30	5
50	6
70	30
80	20

The increase in the number of colonies at 70 and 80 fathoms is somewhat remarkable, but no conclusions in this respect can be drawn from one series of observations.

The characteristics of this bacterium are as follows:—

Morphologically it resembles the *B. calcis* already described.

On Peptone Agar after about 36 hours at 20° C., the colonies are white in colour, circular, with a finely serrated outline and a coarsely granular appearance. Superficial colonies grow very rapidly, and may spread as a whitish semi-transparent growth of irregular shape over the surface of the Agar. The deep colonies remain small, globular, and discrete. In old Agar cultures a brownish tinge is developed, and the colour may diffuse through the substance of the Agar. On Gelatin Peptone growth was rapid: in stab cultures growth proceeded from the surface downwards, leaving a funnel-shaped depression of liquefied Gelatin, and eventually all the Gelatin became liquefied.

Acid formation, as shown by the Neutral Red reaction, took place in Dextrose, Mannite, and Laevulose, but not in Cane Sugar or Lactose media.

1000 c.c. of Gran's medium, inoculated on board with 10 c.c. of a surface sample immediately after collection, and kept at an average temperature of about 20° C., showed the first trace of Nitrite formation after 70 hours. After 84 hours a very strong Nitrate reaction was obtained, and a slight Ammonia reaction was given with Nessler's reagent. The process of denitrification, even after the lapse of weeks, did not extend beyond this, and no bubbles of gas were formed. Other experiments made with subcultures from Agar and Gelatin media gave similar results, so that it appears that this bacterium cannot entirely break down Nitrates at a temperature of 20° C. The optimum temperature for denitrification produced by this bacterium appears to be about 20° C., as the process was less rapid at average temperatures of 17° C. and 25° C. At a temperature of 32° C. rapid growth took place, but no denitrification resulted.

It should be noted that these temperature observations were only made with subcultures from colonies on Peptone Agar and Peptone Gelatin media, and it is possible that the power of denitrification becomes diminished after cultivation on such media. Further and more accurate temperature experiments are required in which the culture medium is directly inoculated with freshly collected samples of water.

This bacterium appears to be closely related to the *Bacterium calcis*, its chief points of difference being—

1. Lesser denitrifying power and lower temperature optimum for denitrification.
2. More rapid growth on Gelatin media.
3. Absence of acid formation in media containing Cane Sugar.

INVESTIGATION OF SAMPLES OF WATER FROM THE MARQUESAS KEYS AND THE EXPERIMENTAL PRECIPITATION OF CALCIUM CARBONATE BY BACTERIAL AGENCY.

The Marquesas Keys constitute a coral atoll which forms part of the long chain of Keys separating the Gulf of Mexico from the Straits of Florida. Within the atoll the water is very shallow, and the bottom consists of a fine chalky mud many feet deep. Samples of the water from within the atoll were sent to me at Plymouth by post, and examined fourteen days after collection.

On plating on Peptone Agar, an average of 800 colonies per 1 c.c. of the sample were obtained. These colonies appeared to be all of one species, and in appearance and all cultural characteristics were identical with the *Bacterium calcis* previously described as occurring around the Tortugas.

A suspension of these bacteria from a culture on Peptone Agar was made in sterile sea-water, and a similar suspension containing roughly the same number of bacteria was made from a third subculture on Peptone Agar of the bacteria obtained from the station 70 miles west of Ushant. 1 c.c. of each of these suspensions was then added to 1000 c.c. of the modified Gran's medium: some of these cultures were kept at an average temperature of 20° C. and others at 32° C., with the following results:—

At 20° C. cultures from Marquesas showed trace of Nitrite after 45 hours.
" " " " gave strong Nitrite reaction after 53 hours.
" " " 70 miles W. of Ushant showed trace of Nitrite after 140 hours.
" " " 70 miles W. of Ushant showed strong Nitrite reaction after 162 hours.

In both cases a slight amount of Ammonia was recognizable by Nessler's reagent when the Nitrite reaction was strong, but decomposition of the Nitrite did not proceed further, even after 14 days.

At 32° C. cultures from the Marquesas showed trace of Nitrite after 18 hours.
" " " " gave strong Nitrite reaction after 22 hours.
" " " 70 miles W. of Ushant never gave Nitrite or Ammonia reaction.

The culture from the Marquesas showed a slight amount of Ammonia formation, but the decomposition of the Nitrite did not proceed further.

From these experiments it appears that the bacteria from subcultures from the Marquesas have a much greater denitrifying power than those from subcultures from a point 70 miles west of Ushant, and that as the bacteria from the Marquesas appear to be of the same species as those investigated at the Dry Tortugas, their power of causing complete denitrification in Gran's medium has been lost by successive cultivations on Peptone Agar.

The presence of the thick layers of fine chalky mud within the Marquesas Keys, and elsewhere in many places near the Florida coast, led to a consideration of the possibility of its precipitation by bacterial agency.

Since these bacteria grow freely in Gran's medium, the Calcium salt of a simple organic acid is a sufficient source of organic food for them, and it seems probable that they would thrive in sea-water containing the products of decomposing vegetable matter, provided that the Nitrate supply, and conditions of light and temperature, were suitable. Such conditions should be especially well fulfilled by the drainage into the sea of a well-wooded country with a calcareous subsoil, and the soluble organic calcium salts would be precipitated as Calcium carbonate by the action of the bacteria. In addition, the elimination of the acid radical from the Nitrate in the process of denitrification, by whatever stages it may occur, must leave the alkaline base free to destroy the normal equilibrium of the salts in sea-water, and by increasing the alkalinity, would also result in the precipitation of Calcium carbonate.

To test this theory cultures were made in a medium having the following composition:—

Calcium succinate . . .	2·5 grammes
Potassium nitrate . . .	0·5 ,,
Sea-water	1000·0 c.c.

Calcium succinate is soluble in these proportions, and the medium is quite clear. Free growth was manifested by the cloudiness of the medium 48 hours after inoculation, and Nitrite formation was apparent.

After 96 hours the medium appeared quite milky, and this milkiness was due to the presence of exceedingly fine particles of a substance which was soluble in dilute Hydrochloric Acid with evolution of gas, and was presumably Calcium carbonate. In some cultures these particles settled as a definite sediment, but in others the particles were so minute that they showed little tendency to settle, and could only be separated by centrifugalization. The conditions determining the size of the particles formed could not be ascertained, as the size varied in cultures which were apparently made and grown under identical conditions.

The addition to cultures in which the particles of Calcium carbonate were so small as to remain in suspension of any foreign substance, such as finely powdered Calcium sulphate, or of larger particles of sand, resulted in the aggregation around them of the particles of Calcium carbonate, forming a concretion around a central nucleus. These concretions were hard and of almost crystalline appearance under the microscope, and were soluble in dilute Hydrochloric Acid with evolution of bubbles of a gas which, when the operation was performed on a microscopic slide, could be completely absorbed by running in a solution of Sodium hydrate under the cover-slip. Once this process of concretion had been initiated, it appears to progress independently of the presence of particles which act as nuclei, and a large concretion may often be found having a number of smaller concretions around it, or continued into a chain of small spheres, the whole presenting somewhat the arrangement shown by freely budding yeast cells. The deposition of this form of Calcium carbonate also takes place on the sides of the flask, and more especially over any area where the glass is scratched or roughened.

From these results it would seem strongly probable that the layers of fine unorganized chalky mud found in the Marquesas Keys are being precipitated by the action of the *Bacterium calcis*, and it would seem a reasonable suggestion that similar bacterial action may have played an important part in the formation of chalk and other limestone formations in geologic times. The formation of semi-crystalline concretions round a central nucleus at first seemed to suggest an explanation of the formation of oölite grains, but a mineralogical examination, very kindly made by Dr. Fred. E. Wright, showed that the concretions did not possess that laminated structure characteristic of oölite grains, and that their crystalline structure was nearer that of Calcite than Aragonite.

SOME CONSIDERATIONS ON THE PHYSIOGRAPHY OF THE TONGUE OF THE OCEAN AND ANDROS ISLAND, BAHAMAS, B.W.I.

The position of the Tongue of the Ocean is shown in the map on page 507, which includes the greater part of the Bahama group, and shows its position relative to Florida and Cuba. The Tongue consists of a long and narrow stretch of deep water, running in a N.N.W.–S.S.E. direction, and except at its northern end it is completely surrounded by shallows or by land. On the west, for about three-quarters of its length, it is bounded by the coast of Andros Island; south of Andros it is separated from the Santaren Channel by some 60 miles of shallow water lying over the Great Bahama Bank. To the south it is separated from the Old Bahama Channel by over 50 miles of shallows, averaging not more than 3 fathoms in depth. To the east it is separated from the deep water of Exuma Sound by from 20 to 40 miles of shallow water of from 2 to 3 fathoms in depth, and by the chain of islands and cays extending in a N.N.W. direction from Great Exuma Island. The mouth of the Tongue of the Ocean lies between New Providence Island on the east and the northern extremity of Andros Island on the west; it is here some 25 miles wide, and it maintains this width for the greater part of its length as it stretches south. Between the southern extremity of Andros and Green Cay it narrows to under 20 miles, but south of this point it expands eastwards into an almost circular terminal basin of about 35 miles diameter. The total length is about 120 miles.

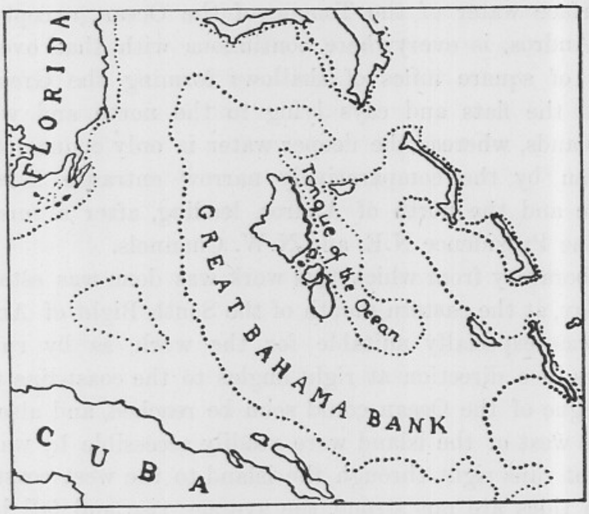


FIG. 1.—Map showing position of the Tongue of the Ocean. The dotted line shows the 100-fathom line; in most parts this almost coincides with the 5-fathom line.

To the north, the Tongue is connected by a stretch of deep water extending in a N.E. direction, with the Providence N.E. Channel, and the Providence N.W. Channel, leading respectively into the Atlantic and the Straits of Florida, and thus the Tongue of the Ocean is brought into direct connection with the two main divisions of the Gulf Stream.

The soundings in this area show a slight but regular upward gradient from a depth of 1084 fathoms at the mouth, to 740 fathoms in the southern terminal basin. Along the margins the gradient is extremely steep, and along the coast of Andros our observations showed that it was almost perpendicular at a distance of from a quarter to half a mile from the shore, but unfortunately no complete observations have been made from which this gradient could be calculated. When attempting to make soundings in this area, the sinker (a 30-lb. conical iron weight) in every case was caught upon what was probably some projection on a submarine cliff, and it was impossible to free it; after several such attempts and loss of sinkers the soundings were abandoned. The entrance to the Tongue by the Providence N.E.

Channel is of steadily increasing depth, up to 2200 fathoms at its junction with the still deeper water of the Atlantic; the Providence N.W. Channel becomes shallower at its junction with the Straits of Florida, and between Great Isaac and the western end of Bahama Island is only between 200 and 300 fathoms in depth.

The surface water of the Tongue of the Ocean, except along the coast of Andros, is everywhere continuous with that overlying the thousands of square miles of shallows forming the Great Bahama Bank, and the flats and cays lying to the north and west of the Exuma Islands, whereas the deeper water is only connected with the outer ocean by the comparatively narrow entrance between New Providence and the north of Andros, leading, after a turn of about 90°, into the Providence N.E. and N.W. Channels.

The Laboratory from which this work was done was established at Golding Cay, at the eastern mouth of the South Bight of Andros; this position was especially suitable for the work, as by running out a few miles in a direction at right angles to the coast-line the middle of the Tongue of the Ocean could soon be reached, and also the mud-flats to the west of the island were readily accessible by water, as the South Bight runs right through the island to the west coast. In this region the tides are not strong, the average rise and fall being from 2 to 3 feet. Much difficulty was experienced in getting any definite information as to the set of currents in the Tongue; our local pilot stated that a current would set in a southerly direction for weeks at a time, and then without any apparent reason or change of wind would reverse and set in a northerly direction for several weeks, but such information derived from the negro natives cannot be relied on. When taking observations on May 8th, May 11th, and May 23rd, we experienced a distinct southerly drift on each occasion, but the amount of this drift was not determined, and in addition the drift caused by the wind was an unknown factor. On May 8th the wind was S.S.E., of about force 1 at 8.30 a.m., freshening to about force 3 at 10.30 a.m.; a rough estimate from the landfall on returning gave the drift of the boat as about 2 miles south during the four hours occupied in working the station; the boat had a large awning and exposed a considerable area to the wind, and had drifted this distance against the wind, so it would seem that on that occasion there must have been a strong current setting south.

Andros Island consists of a limestone formation, the exact nature of which has been dealt with by Wayland Vaughan (17). The greater part of the island is very flat, and is only elevated a few feet above sea-level: a few irregular undulations, never more than 100 feet high, are found especially along the east coast. There is evidence to show that formerly the level of the land was much higher than at present, and signs of rapid erosion of the rock are everywhere obvious. One of the most remarkable features is the absence of soil even in the well-wooded parts of the island, the trees and bushes growing directly out of crevices and holes in the rock, and giving rise to practically no leaf mould. In the numerous "pot holes" which occur all over the island, a small deposit of black leaf mould can be found, and these "pot holes" are the favourite places for the cultivation of sugar-canes and bananas. The erosive action of water on the rock is especially noticeable where the slow drainage from an inland swamp can be traced in its course to the sea: in such a locality the hard rock is eroded, honeycombed, and undermined to a most remarkable degree, even though the amount of drainage, except after the heaviest rains, can scarcely be more than a slow trickle. Erosion of the rock along the sea-coast, where it is exposed to the action of the sea-spray, is also very marked. From the occurrence of this erosion it is obvious that all the water draining from the land into the sea must contain a high proportion of Calcium salts in solution.

Towards the west of the island the land is remarkably flat, and near the coast consists of white chalky mud, which has partially dried, and in places has formed a harder crust on the surface. These half-dried mud-flats slope almost imperceptibly into the sea, and are continuous with the submarine flats which extend some sixty miles off the west coast with an average depth of from two to three fathoms. The mud forming these submerged flats is very soft, and near the coast it was easily possible to push a twelve-foot sponge pole down to its full length into it without touching any harder material: the surface layer of the mud for a depth of about six inches is of a creamy white colour, but below that it is of a greyish tinge and has a slight odour of sulphuretted hydrogen. Unfortunately there was no opportunity of obtaining information as to the real thickness of this layer of mud, nor of investigating more than the surface layers at any distance from the coast.

Microscopical examination showed that this mud was almost entirely composed of minute unorganized particles of Calcium carbonate. Near the shore a good deal of organic matter, chiefly in the form of decaying mangrove roots, was present. Further out little organic matter was noticeable, but it was not possible to examine the deeper layers of the mud in these situations: the only organic matter that was seen consisted of the rootlets of a species of Zostera, which was found in occasional patches some miles off the coast.

BACTERIAL INVESTIGATIONS IN THE DEEP WATER IN THE TONGUE OF THE OCEAN.

Continued bad weather during the whole of our stay at Andros greatly added to the difficulties of these investigations, and on this account it was only found possible to work three stations. The last two were worked under the most disadvantageous conditions, the quick roll of the boat making the filling of the water-bottle with Alcohol, and the syphoning off of the sample under sterile conditions, a matter of the greatest difficulty.

The first station worked was situated six miles due east of Golding Cay, the second 14 miles E. of Golding Cay, and the third 10 miles E.N.E. ¼ E. of Golding Cay. The three stations were thus situated at the angles of a triangle which was nearly equilateral, the base being a little longer than the sides, and running due east and west.

At the first station, worked on May 8th, bottom was sounded at 822 fathoms. The sea was calm at first, with a S.S.E. swell, but became choppy later. The wind was S.S.E., force 0 to 1 at 8.30 a.m., freshening to about force 3 at 10.30 a.m. The sample of the bottom obtained by the snapper rod was of a very stiff clay-like consistency, greyish white in colour, and was composed of very minute unorganized particles of Calcium carbonate, containing a few pteropod and globigerina shells. The following temperatures, to which the necessary corrections have been applied, were recorded:—

Depth in fathoms.	Temperature in degrees Centigrade.	
Surface	26·90	
10	25·90	
50	25·14	
100	22·00	
200	17·13	
400	9·03 8·93	average = 8·98
600	4.78 4·62	average = 4·70
Bottom (822 fths.)	4·00 3·94	average = 3·97

These samples, without previous dilution, were plated in Peptone Agar, 1 c.c. of the sample being used for each plate. The Agar was cooled to just under 40° C. before plating. It is very necessary that this temperature should not be exceeded, as many marine bacteria are very sensitive to heat: the use of Agar at as high a temperature as 45° C. will cause the death of a large proportion of the bacteria, though in the process of plating they can only be exposed to this temperature for a very short time. The cultures were kept in the dark at the room temperature (averaging about 28° C.), and at the end of 24 hours a free growth of colonies was apparent. At the end of 48 hours the plates were counted with the following results:—

Depth.	Number of colonies developing from 1 c.c. of sample.	
Bottom (822 fathoms)	0	
„ „	3	
„ „	1	
600 fathoms	17	
„ „	14	
400 fathoms	15	
„ „	16	
200 fathoms	1760	Very much overcrowded, indications of presence of many more colonies which have not developed owing to overcrowding.
„ „	1500	
100 fathoms	uncountable owing to overcrowding.	
„ „	„ „ „ „	
50 fathoms	uncountable „ „ „	
„ „	„ „ „ „	
10 fathoms	uncountable „ „ „	
„ „	„ „ „ „	
Surface	uncountable „ „ „	
„	„ „ „ „	

From these counts it is apparent that the number of bacteria falls off at some point between 200 and 400 fathoms.

The second station was worked on May 11th, at a point 14 miles due east of Golding Cay. The sea was calm at first, and the wind E.N.E., force 1, but later in the day a heavy swell set in and the wind freshened to about force 4; eventually the weather became so bad that it was impossible to work, and the station had to be abandoned before it was completed. Bottom was sounded at 890 fathoms, but there was some stray on the wire, so that the true depth was probably about 825 fathoms, as shown by the chart. The bottom consisted of fine white calcareous ooze; no remains of pteropods were seen, but some globigerina shells were present. The following temperatures were recorded :—

Depth in fathoms.	Temperature in degrees Centigrade.
Surface	26·30
10	26·40
50	24·89
100	22·63
200	17·42
300	14·37 14·27 average = 14·32
400	9·86 9·73 average = 9·79
Bottom	4·17 4·13 average = 4·15

Samples down to a depth of 200 fathoms were diluted 1 in 100 with sterilized sea-water before plating. The following results were obtained after 48 hours' growth :—

Depth in fathoms.	Number of colonies developing from 1 c.c. of sample.
Surface	16,200
10	13,100
50	14,000
100	14,000
200	15,000
300	14
400	12

The figures given represent the mean of the number of colonies developing in the two plates that were made from each sample. It is here apparent that the number of bacteria per 1 c.c. falls off very rapidly between 200 and 300 fathoms.

The third station was worked on May 23rd at a point 10 miles

E.N.E. ¼ E. of Golding Cay. The wind was east, of about force 4: as it had been blowing for the previous ten days without intermission, the sea was so rough that it was only possible to work when steaming slowly ahead into the wind. This resulted in the production of a great deal of stray on the sounding wire, so that the number of fathoms of wire run out is greater than the actual depth at which the samples were taken; these differences will be large for the more superficial samples, but small at greater depths, as the wire strays in a curve whose gradient becomes very steep a little below the surface, under these conditions.

The following temperatures were recorded:—

Length of wire run out in fathoms.	Temperature in degrees Centigrade.	
Surface	27·10	
20	26·50	
100	22·80	
160	18·83	
250	14·97 15·00	average = 14·98
350	10·85 10·84	average = 10·85

At this point the station had to be abandoned owing to the bad weather. The samples down to 160 fathoms were diluted 1 in 100 with sterilized sea-water before plating in Peptone Agar; the remaining two were plated undiluted. At the end of 48 hours the following counts were made:—

Length of wire run out in fathoms.	Number of colonies developing from 1 c.c. of sample.
Surface	15,000
20	15,500
100	13,700
160	13,300
250	14,300
350	16

These numbers represent the mean of the number of colonies in the two plates made from each sample.

The colonies developing in all the cultures were only of two kinds, the *Bacterium calcis*, and the non-denitrifying species already described. The non-denitrifying species formed a relatively small proportion of the total, and they were not found at all in cultures made from samples taken below 250 fathoms. As they appear to be comparatively chemically inactive, and as nothing is at present known concerning the part played by them in the metabolism of the sea, they will not be further considered here.

A consideration of these results obtained in the Tongue of the Ocean shows that the waters down to a depth of somewhere about 300 fathoms in April, 1912, contained an enormously larger number of bacteria than the water in the neighbourhood of Tortugas in June, 1911. The number of bacteria falls off from about 14,000 to about 12 per 1 c.c. between depths of 250 and 350 fathoms; the temperature at 250 fathoms was about 15° C., and at 350 fathoms about 11° C., and it was shown in June, 1911, at Tortugas that *B. calcis* will grow slowly at 15° C., but that growth is totally inhibited at 10° C. It would thus seem that the observed distribution of the bacteria agrees fairly with what might be expected from the temperature conditions.

As regards these observations as to the occurrence of bacteria in small numbers at depths below 350 fathoms, the possibility of experimental error must be considered: a leakage into the water-bottle of 0·25 c.c. as it was being hauled up through the last 300 fathoms would account for the number found, and there are also many possible sources of error in the process of siphoning off the sample, and making the cultures, where a permanent Laboratory is not available. It is possible that the water below 350 fathoms was really sterile, though if so the constancy of the results obtained is curious, if it is to be ascribed to experimental error. In any case, the small number of bacteria found at depths below 350 fathoms can play no part in the metabolism of the sea, since it has been shown that at the temperatures obtaining at these depths *B. calcis* is incapable of growth.

The much greater abundance of bacteria in the surface waters of the Tongue of the Ocean than in the waters round Tortugas may perhaps be accounted for by the fact that in the former locality by far the greater part of the surface water must flow over the immense chalky mud flats and shallows which bound it in most directions, and, as will presently be shown, these mud flats are phenomenally rich in bacteria, and are probably still being deposited by bacterial agency.

HYDROGRAPHIC OBSERVATIONS IN THE TONGUE OF THE OCEAN.

The samples of water taken for hydrographic observations were analysed by Mr. D. J. Matthews at Plymouth. With great kindness he calculated the results, and from his notes the following observations and conclusions are drawn.

The samples were analysed for salinity in comparison with the standard sea-water supplied by the Central Laboratory of the Conseil International pour l'Exploration de la Mer, and hence the results are strictly comparable with all other analyses published under the auspices of this International Council.

At the first station, 6 miles east of Golding Cay, the following results were obtained:—

Depth in fathoms.	Temperature.	Cl‰.	S‰.	σ_0.	σ_t
0	26·90	20·06	36·24	29·12	23·70
10	25·90	20·46	36·96	29·71	24·57
50	25·14	20·43	36·91	29·66	24·76
100	22·00	20·28	36·64	29·45	25·48
200	17·13	20·08	36·27	29·15	26·48
400	8·98	19·58	35·37	28·43	27·43
600	4·70	19·49	35·21	28·30	27·90
822	3·97	19·367	34·98	28·11	27·79

Note.—The original surface sample was lost owing to breakage of the bottle in transit to England. The analysis was made on a sample taken three days later at the same spot.

In this table Cl ‰ means the weight of Chlorine in grammes found in 1000 grammes of sea-water. S‰ means the salinity, or the total weight of salt in grammes found in 1000 grammes of sea-water. σ_0 represents the specific gravity of the sample at 0° C., and σ_t represents the specific gravity of the sample at the temperature "t" at which it was collected, with no correction for pressure.

At the second station, 13½ miles east of Golding Cay, the following results were obtained:—

Depth in fathoms.	Temperature.	Cl‰.	S‰.	σ_0.	σ_t
0	26·30	20·25	36·58	29·40	24·15
10	26·40	20·33	36·73	29·52	24·23
50	24·89	20·395	36·84	29·61	24·78
100	22·63	20·36	36·78	29·56	25·42
200	17.42	20•12	36·35	29·21	26·47
300	14·32	19·81	35·79	28·76	26·75
400	9·79	19·56	35·34	28·40	27·27
890	4·15	(No sample; bottle did not work.)			

Owing to the uncertainty of the depths at the third station, due to the bad weather and consequent stray on the wire, it was decided not to include these observations in a consideration of the hydrographic conditions, and to make what deductions were possible from the results obtained at the two stations given.

It is interesting to note that at the *Challenger* Station, No. 27, in 22° 49′ N., 65° 19′ W., March 28th, 1873, where the depth was 2960 fathoms, the actual temperature reading at 200 fathoms was 17·22° C., and that this agrees more closely with the temperatures in the Tongue of the Ocean than that taken from the *Challenger* smoothed curve, which was 18·17° C.

From this diagram it can be seen that the surface salinity increases from west to east very rapidly, 0·34‰ in 7½ miles, but the surface temperature is fairly uniform, between 26° C. and 27° C.

At both stations the salinity increases downwards to a maximum probably lying between 10 fathoms and 50 fathoms, but more rapidly at Station I, so that from 10 fathoms to 50 fathoms the salinity decreases from west to east.

Below 100 fathoms the conditions are closely similar at both stations, as far as the observations go; the salinity decreases fairly rapidly to 400 fathoms and then more slowly to the bottom.

The temperatures decrease rapidly and uniformly from the surface to about 500 fathoms, then more slowly to the bottom.

There is practically no thermocline (German *sprungschicht*) at any depth, and the salinity shows only a poorly marked discontinuity layer, confined to the upper stratum. This absence of a thermocline is remarkable.

In general, below about 250 fathoms the temperatures and salinities agree with the nearest stations of the *Michael Sars* in the open Atlantic;

above this depth they are higher, and differ from them and the open ocean, north of the belt of calms, in the absence of a temperature thermocline and in the maximum salinity being found below the surface. The latter points either to a considerable local supply of fresh water, or to a current of lower salinity from either the Florida stream or the region of equatorial calms. Unfortunately we have no reliable salinity observations for the two latter.

The following section shows the vertical distribution of layers of different salinities and temperatures at the two stations in diagrammatic form :—

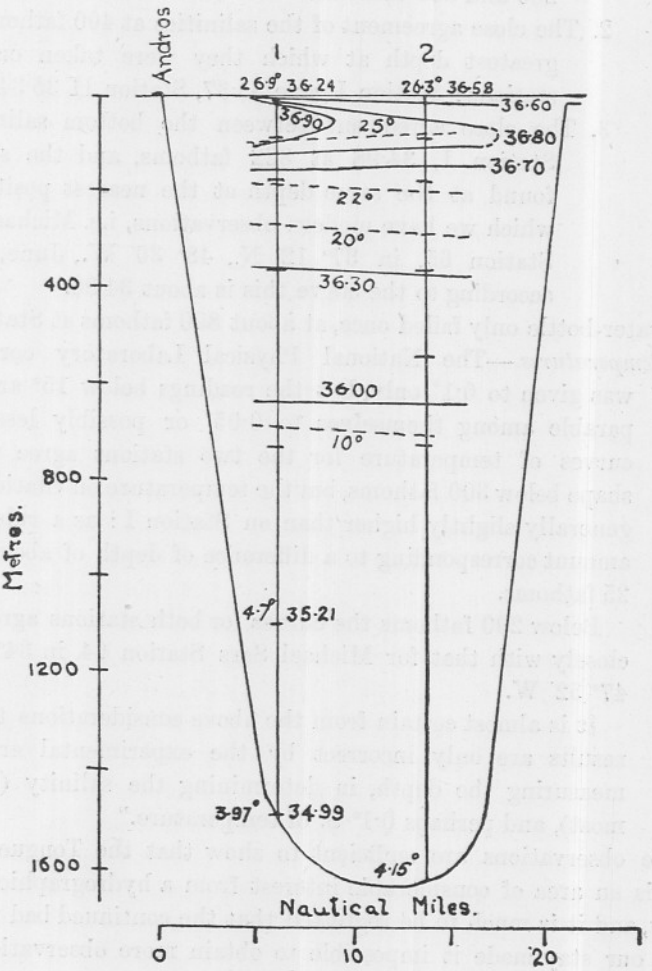


FIG. 2.—Section across the Tongue of the Ocean from Golding Cay, Andros Island eastwards. Broken lines = isotherms, continuous lines = isohalines.

With regard to the accuracy of the work, Mr. Matthews makes the following remarks:—

" *The accuracy of the observations.*

(a) *Salinity.*—The method of taking the samples from the water-bottle was rather inconvenient, as a siphon was used; the samples were also very small, but well preserved. The water-bottle itself might have leaked or closed at the wrong depth, as was the case with earlier models. That this was not so is shown by:

1. The sharp fall in the number of bacteria at between 200 and 300 fathoms.
2. The close agreement of the salinities at 400 fathoms, the greatest depth at which they were taken on both stations: Station I gave 35·37, Station II 35·34.
3. The close agreement between the bottom salinity at Station I, 34·98 at 822 fathoms, and the salinity found at the same depth at the nearest position at which we have modern observations, i.e. Michael Sars, Station 65, in 37° 12′ N., 48° 30′ W., June, 1910: according to the curve this is about 34·96.

The water-bottle only failed once, at about 890 fathoms at Station II.

(b) *Temperatures.*—The National Physical Laboratory correction was given to 0·1° only, but the readings below 15° are comparable among themselves to 0·05° or possibly less. The curves of temperature for the two stations agree well in shape below 300 fathoms, but the temperature on Station II is generally slightly higher than on Station I: as a rule by an amount corresponding to a difference of depth of about 20 to 25 fathoms.

Below 200 fathoms the curves for both stations agree very closely with that for Michael Sars Station 64, in 34° 44′ N., 47° 52′ W.

It is almost certain from the above considerations that the results are only incorrect by the experimental errors in measuring the depth, in determining the salinity (0·02 at most), and perhaps 0·1° C. of temperature."

These observations are sufficient to show that the Tongue of the Ocean is an area of considerable interest from a hydrographical point of view, and it is much to be regretted that the continued bad weather during our stay made it impossible to obtain more observations and samples.

BACTERIAL INVESTIGATION OF THE CHALKY MUD-FLATS WHICH ARE BEING DEPOSITED TO THE WEST OF ANDROS ISLAND.

Samples of the mud were taken from the western entrance of South Bight, and from points two and three miles out from the shore: practically identical results were obtained from all these localities. The sample at the mouth of the Bight was taken in about 4 feet of water, that two miles out in 7 feet, and that three miles out in 8 feet. The samples were necessarily taken from the surface of the mud.

For bacterial examination, one part of this mud was shaken up with three parts of sterilized sea-water; this was allowed to settle for 15 minutes, and then the clearer surface layer was diluted 1 in 1,000,000 with sterilized sea-water. The diluted fluid was plated in Peptone Agar, 1 c.c. being used for each plate. The count of a number of plates after 48 hours gave 40 colonies as an average, and thus the surface mud itself must contain about $40 \times 4 \times 1{,}000{,}000 = 160{,}000{,}000$ bacteria per 1 c.c. The actual number in the mud may exceed this figure, since a large proportion of the bacteria would possibly settle with the larger particles after the first dilution.

The bacteria found in these cultures were nearly all the *B. calcis*, only occasionally were a few colonies of the non-denitrifying species seen.

A sample of the water taken from the surface at a spot three miles out from the western entrance of South Bight gave a count of 35,000 colonies per 1 c.c., the great majority of these being *B. calcis.*

Subcultures of *B. calcis* were made in Gran's medium, and in the Calcium succinate, Calcium acetate, and Peptone Calcium acetate media, whose composition has already been given. Denitrification in all these media was rapid and eventually complete, and was accompanied by the precipitation of Calcium carbonate. In the last three media, which contained no solid matter and were quite clear and transparent before inoculation, this precipitation was manifested after twelve hours by the formation of a thick white cloud in the fluid, readily distinguishable from the cloudiness produced merely by bacterial growth. The development of this precipitate continued rapidly during the first forty-eight hours, but in many cases it was composed of such fine particles that they showed little tendency to settle to the bottom of the flask; in other cases larger particles were formed, and a sediment similar in appearance to the chalky mud of the mud-flats was produced. The exact conditions determining the size of the particles precipitated could not be ascertained, as the size varied largely in cultures made at the same time, in the same media, and kept apparently under the same conditions. The addition of Magnesium tartrate in small quantities (0·2 grammes per 1000 c.c.) to the culture media seemed to induce the precipitation of larger particles, but it did not appreciably affect the rate of growth of the bacteria.

In some of the older cultures that had been kept for a week or more, the sides of the flasks were coated with a thin layer consisting of extremely minute rhombohedral crystals of Calcium carbonate. Occasionally these crystals formed around small bubbles that had remained near the surface of the fluid, the weight of the crystals eventually caused the bubbles to sink, and then the contained gas became dissolved; in this way a number of small hollow spheres were formed, their walls consisting of minute crystals of Calcium carbonate. The formation of these curious bodies occurred especially readily in the Calcium succinate medium to which 0·2 grammes of Magnesium tartrate per litre had been added. The deposition of Calcium carbonate in a distinctly crystalline form was only noted in old cultures, and then it was in an amount relatively extremely small when compared to the precipitate of apparently amorphous Calcium carbonate.

Specimens of the precipitates from some of the culture media were sent to Dr. Fred. E. Wright, of the Geophysical Laboratory of the Carnegie Institution, who with great kindness examined them, and described them as follows:—

"Preparation I. Precipitate from medium composed of—

Calcium acetate	5·0 grammes
Potassium nitrate	0·5 ,,
Peptone (Witte's)	0·2 ,,
Sea-water	1000·0 c.c.

Filtered and sterilized.

"Contains two substances: (1) Fine grains of a strongly birefracting, apparently uniaxial, optically negative substance, and with refractive index about 1·66. This is probably calcite. The grains are isolated, and no evidence of spherulitic crystallization was observed. On treatment with very dilute Hydrochloric Acid, a noticeable evolution of Carbon dioxide took place. (2) Scattered through the preparation are fine needles of a weakly birefracting substance of about 1·525 refractive index; extinction angle large. These needles are evidently selenite (hydrated Calcium sulphate).

"Preparation II. Precipitate from medium composed of—

Calcium succinate	2·0 grammes
Magnesium tartrate	0·2 "
Potassium nitrate	0·5 "
Sea-water	1000·0 c.c.

"This preparation consists largely of a cryptocrystalline aggregate of a weakly birefracting substance, whose refractive index is about 1·52 to 1·53. This substance proved too fine for further determination. Scattered through this substance are rounded and irregular patches of a second cryptocrystalline substance of strong birefringence, which gives off CO_2 when treated with dilute hydrochloric acid, and is probably calcite.

"Preparation III. Precipitate from a medium composed of—

Calcium acetate	5·0 grammes
Sodium phosphate (Na_2HPO_4, $12H_2O$)	0·25 "
Potassium nitrate	0·5 "
Sea-water	1000·0 c.c.

"This preparation is again very fine grained, and consists (1) in large measure of minute grains of a substance which agrees with calcite in its optical properties in so far as they could be determined. On immersion in dilute HCl a distinct evolution of CO_2 gas was observed. (2) Of a substance whose grains are somewhat coarser than the calcite grains, their birefringence being medium to weak; refractive index about 1·525; biaxial and apparently optically positive; probably selenite, but not crystallized in the usual manner."

The small quantity of hydrated Calcium sulphate present in these precipitates is undoubtedly derived from that in solution in the sea-water with which the media were made up, but the reason of its precipitation is difficult to explain since no such precipitation occurred in culture media kept uninoculated under similar conditions as control experiments. It would therefore appear that this deposition of Calcium sulphate, along with the Calcium carbonate, must in some indirect way be the result of bacterial action, and it would seem a possible suggestion that the odour of sulphuretted hydrogen noticeable in the deeper layers of the mud-flats might be due to the reduction of the Calcium sulphate to a sulphide and subsequent decomposition of the sulphide by bacterial action.

These observations have shown that on the chalky mud-flats of the Great Bahama Bank the *B. calcis* is found in enormous numbers, and also that this bacterium is capable of precipitating Calcium carbonate from fluid media containing soluble Calcium salts. It would seem a fair deduction that these mud-flats have been precipitated by the action of the *B. calcis* on the soluble Calcium salts carried into the sea by drainage from the land, where extensive and rapid weathering of the limestone rock is in progress.

CONCLUSION.

The observations so far available are too few, and the area they cover too small, to attempt to make any broad generalization at present. However, it can be stated with a fair degree of certainty that the very extensive chalky mud-flats forming the Great Bahama Bank, and those which are found in places in the neighbourhood of the Florida Keys, are now being precipitated by the action of the *Bacterium calcis* on the Calcium salts present in solution in sea-water. From this the suggestion is obvious that the *Bacterium calcis,* or other bacteria having a similar action, may have been an important factor in the formation of various chalk strata, in addition to the part played by the shells of foraminifera and other organisms in the formation of these rocks. Dr. T. Wayland Vaughan has also suggested that the Miami oölite, and other oölitic rocks, may owe their origin to the occurrence of some diagenetic change in the precipitate of very finely divided particles of Calcium carbonate, produced in this way by bacterial action. If this view as to the formation of chalk and oölite rocks is correct, it would seem probable that these strata must have been deposited in comparatively shallow seas, whose temperature approximated to that of tropical seas at the present time.

It has also been shown that bacterial denitrification is far more rapid and complete in the tropical seas round Jamaica, the Dry Tortugas, and the Bahamas, than in the temperate waters of the Bay of Biscay and the English Channel, and hence an explanation is provided of the relative scarcity of plankton and algal growth in the former localities, in accordance with the terms of Brandt's (2) hypothesis.

The distribution of the bacteria, both as to numbers and species, has been shown to vary at different localities and at different depths, but there are at present too few observations to enable any conclusions or generalizations to be drawn.

As it at present stands, the investigation can at most be considered to offer a mere indication of the part played by bacterial growth in the metabolism of the sea. To obtain a real insight into the question, it would be necessary to make more extensive bacterial and chemical observations in Tropical, Temperate, and Arctic waters, to study the bacteriology of other areas where Calcium carbonate is being precipitated from the sea, and to make further investigations in the Laboratory into the chemistry of the reactions that can be brought about by various species of marine bacteria.

REFERENCES.

1. Baur, E. Ueber zwei denitrifizierende Bakterien aus der Ostsee. Wiss. Meeresunters. Kiel. VI. 1901.
2. Brandt, K. On the Production and the Conditions of Production in the Sea. Conseil Internat. Rapp. et Proc. Verb. III. 1905.
3. Brandt, K. Ueber die Bedeutung der Stickstoffverbindungen für die Produktion im Meere. Botan. Centralblatt. XVI. 1904.
4. Drew, G. Harold. Report of Preliminary Investigations on the Marine Denitrifying Bacteria. Year Book No. 10 of the Carnegie Institution of Washington. 1911.
5. Drew, G. Harold. The Action of some Denitrifying Bacteria in Tropical and Temperate Seas, and the Bacterial Precipitation of Calcium carbonate in the Sea. Jour. of the Marine Biolog. Assoc. of the U.K. Vol. IX., No. 2. 1911.
6. Drew, G. Harold. Report of Investigations on Marine Bacteria carried on at Andros Island, Bahamas, B.W.I., in April, 1912. Year Book No. 11 of the Carnegie Institution of Washington. 1912.
7. Feitel, R. Beiträge zur Kenntnis denitrifizierender Meeresbakterien. Wiss. Meeresunters. Kiel. VII. 1903.
8. Fischer, B. Die Bakterien des Meeres. German Plankton Expedition, 1894.
9. Gran, H. H. Studien über Meeresbakterien I. Bergens Museums Aarbog No. 10. 1901.
10. Keding, M. Weitere Untersuchungen über stickstoffbindende Bakterien. Wiss. Meeresunters. Kiel. IX. 1906.
11. Keutner, J. Über das Vorkommen und Verbreitung Stickstoffbindender Bakterien im Meere. Wiss. Meeresunters. Kiel. VIII. 1905.
12. Matthews, D. J. A Deep-sea Bacteriological Water-bottle. Journ. Mar. Biol. Assoc. of the U.K., Vol. IX, No. 4. 1913.
13. Murray, Sir John, and Hjort, J. The Depths of the Ocean. London, 1912.

14. MURRAY, J., and IRVINE, R. On Coral Reefs, and other Carbonate of Lime Formations in Modern Seas. Proc. Royal Soc. of Edinburgh. Vol. XVII. 1889.

15. RABEN, E. Über quantitative Bestimmung von Stickstoffverbindungen im Meerwasser. Wiss. Meeresunters. Kiel. VIII. 1905.

16. THOMSEN, P. Über das Vorkommen von Nitrobakterien in Meere. Wiss. Meeresunters. Kiel. XI. 1910.

17. VAUGHAN, T. WAYLAND. Report in Year Book No. 12 of the Carnegie Institution of Washington. 1913.

A Deep-sea Bacteriological Water-bottle.

By

Donald J. Matthews.

With Four Figures in the Text.

In the past, deep-sea water samples for bacteriological purposes have been taken either by means of water-bottles provided with taps, and so made that the sample came in contact with metal, or in evacuated sterilized glass vessels drawn out to a fine point and sealed in the flame. The water-bottle is difficult to sterilize on account of the taps, and the results obtained are vitiated by the bactericidal action of metals.* The sealed glass tube is free from these defects, but at even moderate depths it is liable to collapse on the sudden change of pressure when the end of the capillary portion is broken off.

The water-bottle here described has none of these disadvantages, and has been used by Mr. G. H. Drew down to depths of 800 fathoms with complete success. It was designed and made at short notice, and time did not allow of more than one hurried trial before packing for shipment. Experience has shown since that many small alterations might be made which would render it more convenient, though not more accurate, in use; but as no opportunity of making and testing an improved model is likely to present itself in the near future, it is here shown in its original form.

The body of the water-bottle consists of a brass cylinder *a* of about 250 c.c. capacity, lined with a glass tube *b*. It moves freely by means of the guides *c* on the side frames *d*, which are made of brass rod and connected by circular plates *h* and *i*. Above and below the central cylinder are movable plates *e* and *f*, with recesses in which fit rubber washers, shown by shading. The washer plates and cylinder are pressed downwards by spiral springs *m* and *n* working against the fixed plate *g*, and can be held up against this pressure by two rods, only one of which, *l*, is shown in the section. The shorter rod, *l*, is fixed to the upper washer plate by a thread and lock nut which allow of a small vertical adjustment; it can be held up against the spring *m* by a bent lever bracketed on to the top plate (Fig. 3). The upper end of the

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* G. H. Drew on "The Precipitation of Calcium Carbonate in the Sea," etc. This Journal, Vol. IX, p. 479.

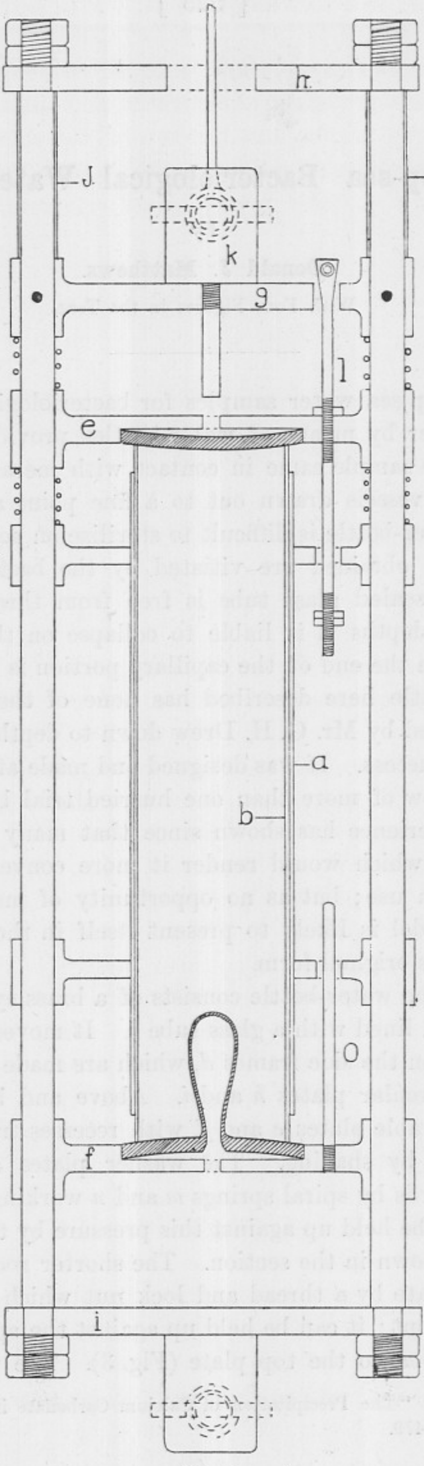

FIG. 1.

lever projects through the top plate; a broad messenger dropped down the wire drives this projection downwards and disengages the lower end of the lever from the hole in the top of the short rod *l*, allowing the upper washer plate to fall. Another longer rod, not shown, is similarly fixed to the lower washer plate; it passes freely through holes in the various plates and guides and engages with a second bent lever. The position of these two levers with respect to one another and the side frames is shown in Fig. 2. They project through the top plate at unequal distances from the centre, the inner one engaging with the long rod fastened to the lower washer plate. To prepare the water-bottle for use the lower washer plate is lifted slightly, so that it presses against the glass lining; alcohol (95 %) is then poured in, and the cylinder further raised until it is closed by the upper washer; the inner lever is now made to engage with the longer rod, and the outer one with the short rod *l*; this closes the bottle tightly. It is now lowered to the required depth, and the first messenger (the inner, smaller one in Fig. 4) is dropped down the line. This disengages the lever holding up the longer rod, but is not wide enough to touch the other. The lower washer plate falls to the bottom of the frame, and the cylinder also falls, but not so far, until it is stopped by the lock nuts on the lower end of the rod *l*. It is now open widely at each end,

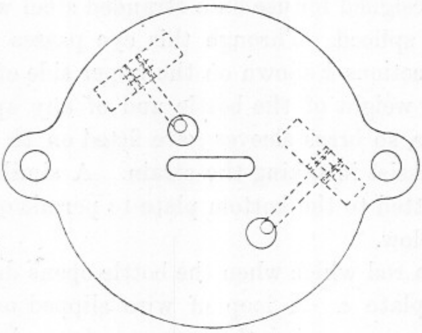

FIG. 2.

FIG. 3.

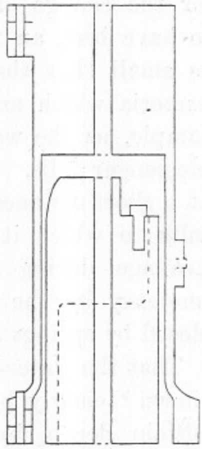

FIG. 4.

and the alcohol is displaced by the water. The second messenger, which is hollowed out below so as to pass over the first, is allowed to fall down the wire, and its broad base strikes the lever holding up the shorter rod. This allows the upper washer plate to fall on to the top of the cylinder, and the spiral springs keep the bottle tightly closed. It is now hauled up and a sample removed by a sterilized rubber tube.

The bottle is designed for use on a stranded steel wire, in the end of which an eye is spliced. Through this eye passes a screw threaded through two projections *k* shown on the upper side of the fixed plate *g*. The whole of the weight of the bottle and of any apparatus below it falls on this plate, so brass sleeves *j* are fitted on to the frames below the top plate to assist in taking the strain. A similar pair of cheeks with a screw is fitted to the bottom plate to permit of other apparatus being attached below.

At *o* is shown a rod which when the bottle opens drops till it is flush with the lower plate *c*. A loop of wire slipped over this makes it possible to release a reversing thermometer frame hanging below the bottle, or a messenger to actuate other apparatus.

The great defects to which water-bottles are liable are leakage and closing at the wrong moment. During descent leakage inwards might easily take place as the alcohol contracts on account of falling temperature and rising pressure. To counteract this, the lower washer has been made with a large dilatation ending blindly inwards but open to the sea at the other end; this would stretch slightly and compensate for the change of volume to a certain extent. It seems, however, to have been an unnecessary precaution. Leakage inward would be so small that the alcohol would remain strong enough to kill any bacteria which might enter, and could not affect the salinity of the sample, as the water-bottle is thoroughly washed out when the first messenger falls. Indeed, the escape of the alcohol is so rapid that at a short distance below the surface the sudden precipitation of the salts to which it gives rise has the appearance of an explosion. Leakage during hauling up would be outwards, and a pumping tendency by the rubber washers is not likely, as the water-bottle is closed by springs and not by weights.

That the water-bottle neither leaks nor closes at the wrong time is shown * clearly by the sharp fall in the number of bacteria below a certain depth, by the close agreement between the salinity at 400 fathoms at neighbouring stations, and by the agreement between the salinities at the greatest depth at which it has been used and those found by the *Michael Sars* at the same depth during her cruise in the

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* Drew, loc. cit.

North Atlantic in 1910. There has been no reason to doubt any of the results obtained with it, and on the single occasion when it failed to close it appears to have been lying on the bottom. It is true that at one station the results are decidedly difficult to explain, but in this case the weather made it necessary to keep the boat moving ahead while the water-bottle was out, and the depth actually reached is problematical; the stray was so great that at the time it was estimated that it might be only half that shown by the amount of wire out.

Various improvements might be made which would add considerably to the convenience of the water-bottle. In particular, the releasing rods should be arranged centrally, by means of elbows in the case of the longer one. As at present made, the supporting rods are placed asymmetrically with regard to the springs, and there is consequently a twisting moment which tends to jamb the guides on the frames; this can be prevented at present only by a very careful adjustment of the strength of the various springs.

The larger messenger is also somewhat unsatisfactory; in spite of numerous holes bored in its lower half to allow the water to escape, it takes about half an hour to fall through 800 fathoms, and at the same time it oscillates from side to side so violently that the wire quickly wears out the central hole to a funnel shape.

The Echinoderms collected by the "Huxley" from the North Side of the Bay of Biscay in August, 1906.

By

W. De Morgan.

My thanks are due to Dr. Allen for permitting me to examine this collection.

In all 292 specimens were obtained, but of these 16 were young immature Asteroidea and Echinoidea. It is impossible to identify these with any degree of certainty, and I have not hazarded an opinion about them.

The remainder belong to 17 genera and 24 species. The Ophiuroidea were particularly numerous, 190 specimens belonging to that class.

The classification followed is that of Professor MacBride in the Cambridge Natural History.

ASTEROIDEA.

Fam. ASTERINIDÆ.

Palmipes placenta (Pennant).

Station V. One specimen. 109 fathoms.
Bottom coarse sand and shells.

Fam. PENTAGONASTERIDÆ.

Dorigona subspinosa (Perrier).

Pentagonaster subspinosus. E. Perrier. *Bull. Mus. Comp. Zoology,* Vol. IX, No. 1, p. 21. 1881.

Pentagonaster subspinosus. E. Perrier. *Nouv. Arch. du Mus. d'Histoire Naturelle,* t. VI, p. 234. Pl. VI, Fig. 1. 1884.

Nymphaster protentus. Sladen. *Challenger* Reports, Vol. XXX, p. 203. Pl. L, Figs. 3 and 4. Pl. LIII, Figs. 9 and 10. 1889.

Nymphaster subspinosus. Bell. *Catalogue of British Echinoderms in British Museum,* p. 75. 1892.

Nymphaster subspinosus. Norman in Bourne. *Journal Marine Biological Association,* Vol. I, p. 327. 1890.

Dorigona subspinosa. E. Perrier. *Travailleur et Talisman.* Echinoderms, p. 375. 1896.

STATION XIII. One specimen. 412 fathoms.

A fine specimen. R = 102 mm., r = 25 mm. Breadth at base of arm 16 mm. Very few pedicellariæ. Colour in alcohol, light biscuit-brown.

FAM. ASTERIIDÆ.

Asterias rubens, Linnæus.

STATION II. One specimen. 75 fathoms.

OPHIUROIDEA.

FAM. OPHIOLEPIDIDÆ.

Ophiura ciliaris, Linn.

STATION V.	109 fathoms.	60 specimens.
STATION IX.	240 fathoms.	6 specimens.
STATION XII.	246 fathoms.	3 specimens.

The specimens dredged from Stations IX and XII are small, the largest having a disc only 5 mm. in diameter. They are remarkable for the thick cushion-like appearance of the discs, which were very convex above, and 2½ to 3 mm. thick. This may be due to their removal from a considerable depth.

Ophiura albida, Forbes.

STATION I.	75 fathoms.	1 specimen.
STATION II.	75 fathoms.	1 specimen.
STATION V.	109 fathoms.	1 specimen.

Ophiura affinis, Lütken, 1859.

Ophiura Grubei. Heller. *SB. AK. Wien*, XLVI, p. 431. Pl. II, Figs. 13–16. 1863.

Ophiura Normani. Hodge. *Trans. Tynes. Nat. Field Club*, V, p. 296. Pl. XVI. 1863.

Ophioglypha affinis. Lyman. *Ill. Cat. Mus. C.Z.*, I, p. 52. 1865.

Ophioglypha affinis. Lyman. *Chall. Rep. Oph.*, p. 77. 1882.

Ophiura affinis. Bell. *Brit. Mus. Cat.*, p. 111. 1892.

Station I.	75 fathoms.	1 specimen.
Station II.	75 fathoms.	15 specimens.
Station V.	109 fathoms.	14 specimens.
Station XI.	146 fathoms.	4 specimens.
Station XII.	246 fathoms.	2 specimens.

The diameters of the discs measured dry were as follows :—

Station I.	1 (6 mm.).
Station II.	3 (2 mm.), 1 (2·5), 1 (3), 2 (5), 2 (5·5), 4 (6), 1 (6·5) mm.
Station V.	1 (2), 1 (4), 2 (6·5), 4 (7), 3 (7·5), 2 (8), 1 (8·5), 1 (9) mm.
Station XI.	2 (3), 2 (4·5) mm.
Station XII.	1 (5), 1 (6) mm.

One specimen is recorded from Plymouth (*Journal M.B.A.*, Vol. V, N.S., 1897–9). This was from Bolt Head shell gravel ground. It agrees in all respects with the *Huxley* specimens. Diameter of disc, 4·5 mm.

Four specimens were also obtained from a collection made in the English Channel by Mr. Crawshay, at Station V, 20 miles S. 19° W. of the Eddystone (*Jour. Mar. Bio. Assoc.*, IX, p. 336). The discs of these measure 7, 6, 6, and 3·5 mm. in diameter, and in other respects they are similar to the *Huxley* specimens.

In all the above specimens the number of lateral spines is constant, namely three. The uppermost spine is the longest, and reaches half-way up the next dorsal plate of the ray. This exactly agrees with Lütken's description. Heller also states that there are three lateral spines.

Hodge (**5**), however, states that there are five lateral spines, and Jeffrey Bell (**1**) " about five."

Jeffrey Bell (**1**) describes as a " very small species."

Hodge (**5**) says that the disc " in well-grown individuals measures about ¼ inch."

Lütken says that it is 5 to 6 mm. in diameter.

The *Huxley* specimens show that it grows to a much larger size. The largest dried specimen from Station V, 109 fathoms, was 9 mm. diameter.

In descriptions of *Ophiura affinis* emphasis is laid on the regular rosulation of the primary plates. Judging from the *Huxley* specimens this is true only of the smaller and presumably younger individuals. In these there is a distinct rosette, consisting of a central and five surrounding plates, separated by smaller scales. As the animals increase in size the arrangement of the plates is not so regular, and they increase in number. Generally, however, indications of the primary arrangement may be traced.

The arm combs have a variable number of spines. In some specimens there were twelve on each side. On each side of the notch there are a variable number of small spinules, sometimes six on each side; and in the centre of the notch a small cluster. There may be a variable number of spines on the first upper arm-plate. Occasionally there is a complete row on each side of the arm-plate, which in continuation with those on the sides and centre of the notch form a complete curve of spinules. The arrangement is very variable, but the notch and arm-plate never appear to be quite destitute of spines.

In the *Huxley* collection, and other spirit-preserved specimens examined, the arms are banded at regular intervals with a darker shade.

Ophioconis Forbesi (Heller).

Station V. 109 fathoms. 1 specimen.

The disc is rather distorted, but the diameter would be about 6 mm.

Ophioconis Forbesi is described as *Pectinura Forbesi* by Heller in *Untersuchungen über die Litoralfauna des adriatischen Meeres. Kaiserlischen Akad. der Wissenschaften.* 1862. Vol. XLVI, p. 423. Pl. II, Figs. 5–8.

See also Lütken, *Additamenta ad historiam Ophiuridarum*, Part III, p. 98. 1869.

AMPHIURIDÆ.

Amphiura elegans (Leach).

Station I. 75 fathoms. 5 specimens.
Station V. 109 fathoms. 6 specimens.

Ophiactis Balli (Thompson).

Station I. 75 fathoms. 1 specimen.
Station VII. 444 fathoms. 4 specimens.
Station XIII. 412 fathoms. 6 specimens.

Ophiactis abyssicola (Sars).

Amphiura abyssicola. Sars. *Norg. Ech.* (1361), p. 18. Pl. II, Figs. 7–12.

Ophiocnida abyssicola. Lyman. *Ill. Cat. Mus. C.Z.*, I. 1865, p. 12.

Ophiactis abyssicola. Bell. *Brit. Mus. Cat. Echinoderms*, p. 123.

Station VII. 444 fathoms. 5 specimens.

Bell describes it as a rather small species. The two largest *Huxley* dried specimens have a disc diameter about 5 mm.

It is at once distinguishable from *Ophiactis Balli* by the unequal thickened disc scales and spines, and the large radial plates.

Ophiacantha abyssicola, G. O. Sars.

Station XII. 246 fathoms. 17 specimens.
Station XIII. 412 fathoms. 3 specimens.

All are young animals. The largest has a disc 5·5 mm. diameter, and arm 20 mm. long, as nearly as could be measured on a dry specimen. Bell (**1**) describes *abyssicola* as " a small species."

Diameter of disc 9 mm., and R said to =10 r. *O. abyssicola* has been obtained by the *Travailleur et Talisman* Expedition (**6**), p. 288, between Lat. N. 35° 42′, Long. W. 8° 40′, and Lat. N. 44° 5′, Long. W. 9° 25′ 40″ in depths from 112 to 1226 metres, and by the *Caudan* Expedition between Lat. 45° 57′, Long. 6° 41′, and Lat. 46° 40′, Long. 6° 58′ in 400 to 1700 metres. Koehler remarks (**6**) that all these specimens are identical with those from the coasts of Norway.

Bell (**1**) makes the translucency of the arm spines the key of the species, and this characteristic and the moniliform appearance of the arms is very marked in the *Huxley* specimens.

Grieg (**3**) remarks that *O. abyssicola* seems to be subject to sundry small variations as regards the spines on the disc, the aculeation of the branchial spikes, and their number, which is variable, and should not, in his opinion, be regarded as specifically diagnostic.

The spinulation of these young specimens is worthy of note. Both the dorsal and ventral surfaces of the disc are covered with thin subcircular imbricated scales. All the dorsal scales bear a knob or granule crowned with two to six very fine thorns. These thorny knobs are not present on all the ventral scales. As the disc increases in size the thorns grow longer, but the basal knob or granule remains about the same size. As the disc increases in size the thorns appear to coalesce into a single longer aculeated spine. This spine is much constricted at the proximal end, and easily breaks off from the knob. In the adult probably all the longer spines ultimately break off, leaving the disc covered with knobs or granules, as figured by Grieg. I have observed similar changes in *Ophiactis Balli*, and they are probably common to other Ophiurids.

Ophiacantha bidentata, Retzius.

Asterias bidentata. Retzius, 1805.

Ophiura Retzii. Nilsson, 1817.

Ophiocoma arctica. *M. Troj. Syst. Ast.*, 1842.

Ophiocoma echinulata. Forbes, 1852.

Ophiacantha Grœnlandica. *M. Tr. Arch. f. Nat.*, 1844.

Ophiacantha spinulosa. Sars, *Norg. Ech.*, 1861.

Ophiacantha spinulosa. Lyman, *Ill. Cat. Mus. C.Z.*, I., 1865, p. 93 and figure.

Ophiacantha bidentata. Jeffrey Bell, *Cat. Brit. Mus.*, p. 127.

STATION VII.	444 fathoms.	9 specimens.
STATION X.	146 fathoms.	10 specimens.

O. bidentata was taken by the *Talisman et Travailleur* Expedition between Lat. 19° 19′, Long. 20° 20′, and Lat. 39° 33′, Long. 12° 11′ 30″ in depths from 1965 to 2590 metres. All the specimens were small, the largest not more than 9 mm. diameter.

It was found by the *Caudan* Expedition in Lat. 45° 47′, Long. 6° 15′ in 17 metres.

The largest of the *Huxley* specimens has a disc diameter of only 5·5 mm., and the others are considerably smaller. Bell (**1**) gives the disc diameter as 13 mm. The *Huxley* specimens are therefore probably all young and immature. Descriptions of the species vary in certain points.

Bell (**1**) describes the mouth papillæ as "not numerous," but inconstant in number, and to some extent in position, as is the mouth shield in form. The disc covered with very short spines.

Lyman (**8**) gives *O. spinulosa* as the type of the genus, as its special mark "Disc evenly covered with short spines." The mouth papillæ, however, he describes as "standing well apart; six or seven to each angle of the mouth—two outermost on each side small, flattened, somewhat rounded, blunt; innermost one sharp, conical, rounded, resembling the lowest tooth, which also might be well enough considered as a mouth papilla. Teeth seven." The *Huxley* specimens agree in all these points, except that there are only four teeth. This might be accounted for by their age.

Lütken (**7**) describes and figures *O. bidentata.* The *Huxley* specimens agree with the figure, except in the magnified appearance of the spines. In the *Huxley* specimens the dorsal and ventral faces of the disc are covered with short, smooth, cylindrical spines, in some cases bifurcated. They stand on a slightly thorny stump or grain.

The plane of junction between the stump and the cylindrical spine is constricted, so that the spine might easily be brushed off the grain, as in the case of *O. abyssicola*. There is no indication of scales on the disc as in *O. abyssicola*, and the spines are equally thick on the ventral and dorsal surfaces.

Considering the immaturity of the specimens, and the variability of spinulation in young ophiurids, it would be rash to finally diagnose these as *O. bidentata*, but their general appearance makes it probable that they belong to that species.

OPHIOCOMIDÆ.

Ophiocoma nigra, O. F. Müller.

Station VII.	$\frac{\cdot}{444}$ fathoms.	18 specimens.

ECHINOIDEA.

Cidaridæ.

Cidaris papillata (Leske), 1778.

Station XI.	146 fathoms.	17 specimens.
Station XII.	246 fathoms.	14 specimens.
Station XIII.	412 fathoms.	19 specimens.

ECHINOTHURIIDÆ.

Phormosoma luculentum, Agassiz.

Station XII.	246 fathoms.	1 specimen.
Station XIII.	412 fathoms.	3 specimens.

This species is described and figured by A. Agassiz in his report on the *Echinoidea*. The voyage of H.M.S. *Challenger*, Vol. III, p. 97, Pls. IX, X, XA, Figs. 3–7; Pl. XXXIX, Fig. 8; Pl. XL, Figs. 31–36; Pl. XLIV, Figs. 25–27.

It was obtained by the *Caudan* Expedition (**6a**), and in the N. Atlantic by the *Hirondelle*.

ECHINIDÆ.

Echinus norvegicus, Düb. o. Kor, 1844.

Station V.	109 fathoms.	1 specimen.
Station VII.	$\frac{\cdot}{444}$ fathoms.	1 specimen.
Station XII.	246 fathoms.	3 specimens.

These are young animals. The measurements of the largest taken at Station XII are: Diameter of test, 13·5 mm.; height of test, 7·5 mm.; length of longest spine, 13 mm.

Although collected three years ago, preserved in spirit, and dried, the red patches on the test are quite clear in the larger specimens.

CLYPEASTROIDEA.

Fibularidæ.

Echinocyamus pusillus, O. F. Müller.

STATION XII. 246 fathoms. 2 specimens.

SPATANGOIDEA.

Spatangidæ.

Spatangus purpureus (O. F. Müller).

STATION II. 2 specimens.
STATION XII. 246 fathoms. 2 specimens.

Spatangus Raschi (Loven).

STATION IX. 240 fathoms. 30 specimens.

Echinocardium pennatifidum, Norman.

STATION XI. 146 fathoms. 4 specimens.

HOLOTHUROIDEA.

ASPIDOCHIROTA.

FAM. HOLOTHURIIDÆ.

Stichopus tremulus. Gunnerus (Östergren).
Stichopus Richardi. Hérouard.
Stichopus Richardi. R. Perrier.
Holothuria tremula. Gunnerus. 1767. Düben and Koren, Sars, Théel, Jeffrey Bell.
Holothuria elegans. F. O. Müller, Jäger, Brandt.

STATION IX. 240 fathoms. 7 specimens.
STATION XII. 246 fathoms. 1 specimen.

Up to 1896 this species was regarded as a true Holothurian, possessing only one bundle of genital tubes. In 1896, Östergren (**9**) demonstrated that it has two bundles of genital tubes, and consequently, as a true Stichopus, should be called *Stichopus tremulus.*

R. Perrier, in his examination of the Holothurians of the *Talisman* *et Travailleur* Expedition, also recognized the two bundles of genital tubes (**10**), p. 485, and included his specimens in the genus Stichopus.

Hérouard (**4**), p. 8, described what he thought to be a new species under the name of *Stichopus Richardi.* R. Perrier (**11**) called attention to the resemblance between this new species and *Holothuria tremula*, and also how it differed in the arrangement of the ambulacra, and in possessing two bundles of genital tubes. Perrier then read Östergren's work, and concluded that *Stichopus Richardi* and *Stichopus tremulus* are identical.

He then made certain additions to Östergren's description, and gives figures of the sclerites. Hérouard (**4**), Pl. VIII, gives drawings of the sclerites of *Stichopus Richardi* which agree with Perrier's. He, however, does not figure the "spire" (or "tige," as Perrier has it) of the disc, which is very characteristic.

The *Huxley* specimens agree with Perrier's description of *Stichopus tremulus* in external form, in the shape of the sclerites, and in possessing two bundles of genital tubes, one on each side of the dorsal mesentery. Both Théel (**12**) and Jeffrey Bell (**1**) make the absence of C-shaped deposits characteristic of *Holothuria.* Perrier describes arciform spicules in the ambulacral papillæ, and in the ventral feet of *Stichopus tremulus*, and such arciform spicules appear in the *Huxley* specimens, some being sufficiently curved to warrant the description C spicules.

These specimens have been some years in spirit, but in places a faint rosy tinge is still discernible.

Stichopus regalis (Cuvier).

Holothuria columnæ. Cuvier. 1817.
Holothuria triquetra. Della Chiaje. 1828.

Station XI. 146 fathoms. 1 specimen.

Stichopus regalis is easily recognized by the flattened ventral surface, and the well-marked division between that and the slightly convex dorsal surface. The discs of the tables also are very characteristic. They have a fairly unbroken margin, and want the sharp marginal teeth found in *Stichopus tremulus.* They are perforated by numerous holes, Théel (**12**).

Perrier's (**11**) description is very similar. He states that in young specimens the four rods are longer in proportion to the diameter of the basal disc, and are more convergent at the top. Also that in young specimens, the four rods are smooth, and end in a point, while "chez les adults elles sont hérisées de dents à leur extremité et aussi sur une certaine longueur au-dessous de cette extremité."

In the *Huxley* specimen some of the discs have 54 perforations, the average being about 44.

This specimen, which has been three years in spirit, still gives a very fair notion of the colour as described by Perrier.

CRINOIDEA.

Comatulidæ.

Antedon bifida (Pennant = rosacea).

Station V. 109 fathoms. 2 specimens.

Antedon flava, Koehler.

Station XIII. 412 fathoms. 2 specimens.

I am indebted to Dr. Koehler, of Lyons, for identifying this species.

Two specimens were obtained by the *Caudan* Expedition from a depth of 1410 metres, and it is described and figured (**6a**), p. 9, Figs. 20 and 21.

Actinometra pulchella. Pourtalès.

Antedon alata. Pourtalès, *Bull. Mus. C.Z.*, 1878, Vol. V, No. 9, 215.

Antedon pulchella. Pourtalès, *ib.*, 216.

Actinometra pulchella. 1881. P. H. Carpenter, *ib.*, Vol. IX, 4, p. 10.

Actinometra pulchella. 1888. P. H. Carpenter, *Challenger* Reports, Vol. XXVI, p. 304, Pl. IV., LII.

Actinometra pulchella. 1896. Koehler, *Campagne du Caudan*, p. 100.

Station VII. $\frac{}{444}$ fathoms. 2 imperfect specimens.

Station XIII. 412 fathoms. 15 specimens, of which two are good, the others badly mutilated.

This species was obtained by the *Blake* [Carpenter (**2**)], at a great number of stations, but at depths nowhere over 300 fathoms and rarely exceeding 200 fathoms. The *Porcupine* specimens were dredged at 477 fathoms and those of the *Dacia* at 533 fathoms. The *Caudan* specimens were from depths varying from 400 to 1710 metres.

Except two species of Rhizocrinus, this is the only Crinoid common to the European and Caribbean seas, and the only European species of *Actinometra*.

LIST OF SPECIES AND STATIONS AT WHICH THEY OCCURRED.

STATION No.	I	II	V	VI	VII	IX	X	XI	XII	XIII
Latitude, N.	48° 25′	48° 24′	47° 48′	47° 46′	47° 36′	48° 7′	48° 7′	48° 10′	48° 7½′	48° 7½′
Longitude, W.	6° 28′	6° 28′	7° 46′	7° 46′	7° 31′	8° 13′	8° 13′	8° 11′	8° 13′	8° 13′
Depth in fathoms	75	75	109	—	444	240	146	146	246	412
Bottom Deposit	—	—	Coarse sand and shells.	—	—	Fine sand.	Fine sand.	Fine sand.	Fine sand.	Sand, mud, and hard ground.
Palmipes placenta	...	...	1	...	...	...	...	...	...	...
Dorigona subspinosa	...	...	...	...	...	...	...	...	...	1
Asterias rubens	...	1	...	...	...	...	...	...	...	...
Ophiura ciliaris	...	...	60	...	...	6	...	...	3	...
Ophiura albida	1	1	1	...	...	...	...	...	...	...
Ophiura affinis	1	15	14	...	...	...	...	4	2	...
Ophioconis Forbesi	...	...	1	...	...	...	...	...	...	...
Amphiura elegans	5	...	6	...	...	...	...	...	...	...
Ophiactis Balli	1	...	...	...	4	...	...	...	...	6
Ophiactis abyssicola	...	...	...	...	5	...	...	...	...	...
Ophiacantha abyssicola	...	...	...	...	...	...	...	...	17	3
Ophiacantha bidentata	...	...	...	...	9	...	10	...	...	...
Ophiocoma nigra	...	...	...	...	18	...	...	...	...	...
Cidaris papillata	...	...	...	...	...	...	...	17	14	19
Phormosoma luculentum	...	...	...	...	...	...	...	...	1	3
Echinus norvegicus	...	...	1	..	1	...	...	...	3	...
Echinocyamus pusillus	...	...	...	...	...	...	...	...	2	...
Spatangus purpureus	...	2	...	...	...	...	...	...	2	...
Spatangus Raschi	...	...	...	...	...	30	...	...	...	...
Echinocardium pennatifidum	...	...	...	...	...	...	...	4	...	...
Stichopus regalis	...	...	...	...	...	...	...	1	...	...
Stichopus tremula	...	...	...	...	...	7	...	...	1	...
Antedon bifida	...	...	2	...	...	...	...	...	...	...
Antedon flava	...	...	...	...	...	...	...	...	...	2
Actinometra pulchella	...	...	...	...	2	...	...	...	...	15
Young forms "incertæ sedis":										
Asteroidea	...	...	...	...	4	...	...	...	1	4
Echinoidea	...	...	...	...	...	...	...	...	7	...

BIBLIOGRAPHY.

1. Bell, F. Jeffrey. Catalogue of the Echinoderms in the British Museum. (Nat. Hist.)
2. Carpenter, P. H. Proceedings of the Royal Society of Edinburgh. 1883, 4.
3. Grieg, James A. The Norwegian North Atlantic Expedition. 1876–8. Ophiuroidea.
4. Hérouard. Les resultats des campagnes scientifiques. Monaco, t. XXI.
5. Hodge. Transactions of the Tyneside Naturalists Field Club, V, 1863.
6. Koehler, R. Expéditions scientifiques du *Travailleur* et du *Talisman* pendant 1880–83. Ophiures.

6a. Koehler, R. Campagne du *Caudan*. 1896.

7. Lütken, Chr. Fr. Additamenta ad historiam Ophiuridarum. Part III.
8. Lyman, Theodore. Ill. Cat. of Museum Comp. Zoology at Harvard. No. 1. Ophiuridae.
9. Östergren, Hjalmar. Ueber die Holothurien Norwegens. Bergens Museums Aarbog. 1896. No. 12.
10. Perrier, R. Expéditions scientifiques du *Travailleur* et du *Talisman*. 1880–3. Holothuries.
11. Perrier, R. Sur les Holothuries draguées par le *Travailleur* et *Talisman*. C. R. de l'Acad. des Sciences, t. CXXVI, p. 1664.
12. Théel. Report on the Holothuria of *Challenger* Expedition.

Description of a New Species of Brackish-water *Gammarus* (*G. chevreuxi*, n. sp.).

By

E. W. Sexton.

With Five Figures in the Text.

THE *Gammarus* here described is found in Chelson Meadow, a large tract of reclaimed land lying near the mouth of the Plym, and protected from the tidal water by an embankment. The land is drained by ditches, which empty into the Plym at low water by means of sluice-gates. The sea-water enters in volume only at the highest tides over the top of the sluice-gates, but there is a constant slight infiltration of salt water through the embankment.

I have named the species in honour of M. Edouard Chevreux, who has always assisted me most generously with his knowledge of the group as well as with specimens from his collection.

Gammarus chevreuxi resembles *G. locusta* more nearly than any of the other species of the genus, but there are certain constant characters, e.g. in the antennae, the fourth sideplates, the third uropods, etc., which seem to me of sufficient specific value to justify its establishment as a separate species.

DESCRIPTION.

Gammarus chevreuxi is a small species, the largest male measuring 13 mm. in length, and the largest female 9 mm.

The animals are delicate in appearance, and so transparent that their internal organs show plainly through the cuticle.

The *cuticle* in both sexes has a remarkable sensory armature, that of the pleon in particular. All the pleon-segments are covered with microscopic spinules, longest and densest dorsally. Segments 1 to 4 are the most spinose; the spinules of segments 5 and 6 are shorter and finer. The cuticle of the peraeon is not produced in spinules, but has a surface like a fine file, the head and anterior segments being the smoothest. All over the body are scattered microscopic sensory cleft-tipped hairs, each hair set in a little pocket in the skin, some single, some in rows of four to six. Each peraeon-segment carries one of these rows in the median line; pleon-segments 4 to 6 each have at least three of these rows in line with the usual spine-clusters characteristic of the genus; and the telson has two rows, one on each side.

Sideplates rather small; the fourth (Fig. 1) forms one of the distinguishing specific characters. The posterior expansion is produced downwards, but not nearly as much as in *G. locusta;* it rounds into the inferior margin, and has two setae inset.

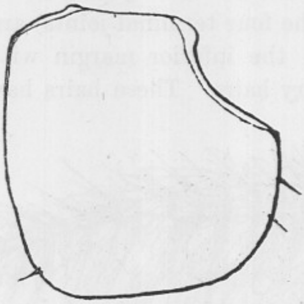

FIG. 1.—Sideplate 4. ♂ *Gammarus chevreuxi*, n. sp. ×27.

Pleon. Hind margin of segments 2 and 3 straight, with a few setules inset, postero-lateral corners produced to a short acute angle. Segments 4 to 6 rounded dorsally, 5 and 6 very short; the dorsal spine-clusters of these segments have each two divergent spines; the lateral clusters usually with two or three spines and a few hairs in each cluster.

Head. Lateral lobes not much produced, truncate, upper angle obtuse, rounded below; sinus rather deep; post-antennal angle produced, subacute.

Eyes reniform; pigment usually black coated with white, but occasionally a few individuals are found in a brood with red pigment instead of black.

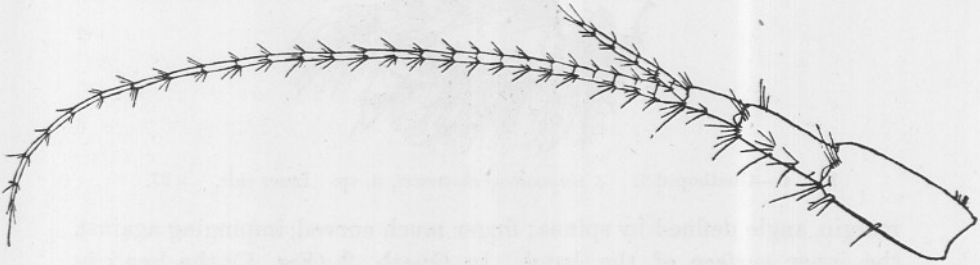

FIG. 2.—Antenna 1. ♂ *Gammarus chevreuxi*, n. sp. Inner side. ×27.

Antenna 1 (Fig. 2). The first joint of the peduncle is about equal in length to the second and third taken together. The number of joints in the flagella varies with age and sex; one of the largest males had thirty-five in the primary flagellum and seven in the accessory.

A large female had twenty in the one and four in the other, the flagella being shorter and more setose than in the male.

Antenna 2 (Fig. 3) of the male forms one of the distinguishing characters of this species, easily separating it from the other species of *Gammarus.* The fourth and fifth joints of the peduncle and the flagellum (excepting the four terminal joints) are clothed on the inner surface as well as on the inferior margin with dense tufts of long exceedingly fine sensory hairs. These hairs have very delicate coiled tips and are found only in the male, on the second antennae, the gnathopods, the first peraeopods, the third uropods, and the telson. The flagellum in the male is sixteen-jointed. The female has only a few clusters of long outstanding straight setae on the fourth and fifth joints of the peduncle, and short setae on the flagellum.

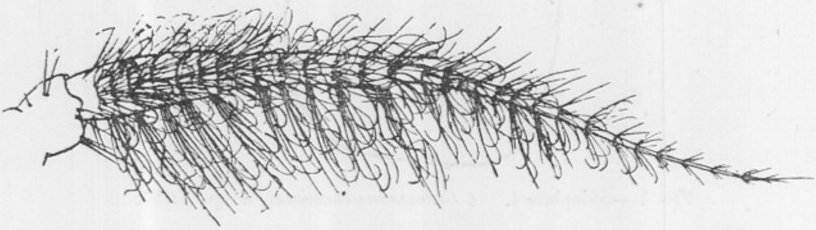

FIG. 3.—Antenna 2. ♂ *Gammarus chevreuxi*, n. sp. Inner side. × 27.

Gnathopods 1 and 2 rather small, not much difference in their size. In the male, Gnath. 1 (Fig. 4) has the sixth joint pyriform, palm oblique, indented, with one stout truncate spine midway on the palmar margin, angle defined by spines; finger much curved, impinging against the inner surface of the hand. In Gnath. 2 (Fig. 5) the hand is broader, palm slightly oblique. In both gnathopods the hand, especially on the inner side, is provided with numbers of the coiled sensory hairs, the fifth joint also carrying a few.

FIG. 4.—Gnathopod 1. ♂ *Gammarus chevreuxi*, n. sp. Inner side. × 27.

In the female the fifth and sixth joints of Gnath. 1 are practically subequal in length, but the fifth is much wider distally than the sixth;

palm slightly oblique, palmar margin crenulate and beset with small sensory spines, palmar angle with one long and two short spines on the outer side, and one long and one short on the inner. In Gnath. 2 the fifth and sixth joints are equal in length and of equal width throughout, both provided posteriorly with clusters of straight setae, palm transverse, with the margin rounded, and spines as in Gnath. 1.

Peraeopod 1 in the male has the posterior margins of the fourth, fifth, and sixth joints beset with clusters of the sensory coiled hairs.

Peraeopod 3 elongate, the hind expansion of the basal joint lightly crenulate, hind lobe free. Peraeopods 4 and 5 not much longer than pp. 3; the basal joint expanded above, gradually narrowing to the distal angle, where one or two strong spines are inset. In the female these joints, especially that of the fifth, differ in shape from those of the male; they are expanded above, but about two-thirds down they suddenly narrow; several long plumose hairs are inset on the inner side. All the hinder peraeopods are furnished with spines, most numerous on the fifth.

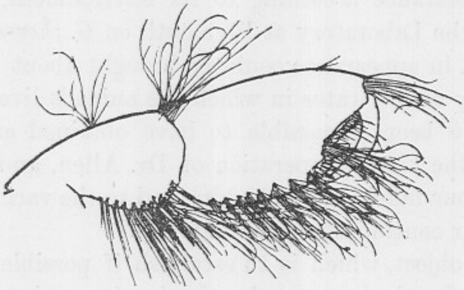

FIG. 5.—Gnathopod 2. ♂ *Gammarus chevreuxi*, n. sp. Outer side. × 27.

The *incubatory lamellae* occur on segments 2 to 5.

The *branchial vesicles* are on segments 2 to 7; they are borne on stalks, are ovate in form, and densely fringed with tangled hairs round the tip and along the posterior margin, especially in the male.

Uropods. The first pair extend beyond the second. In the third the inner ramus is not much more than a half the length of the outer, both thickly beset in the male with tufts of the characteristic sensory hairs, intermixed with long straight setae. This pair of uropods is shorter in the female, and is furnished with long spines and straight and feathered setae.

The *telson* has an apical group of three spines; of the two lateral groups the upper one carries two spines and one or two setae, and the other one spine and one or two setae.

Notes on the Life History of *Gammarus chevreuxi*

By

E. W. Sexton and Annie Matthews, M.Sc.

INTRODUCTION.

The present paper is the outcome of an investigation made on a species of *Gammarus* inhabiting both fresh and brackish water (**4**, p. 657). As this species (*G. zaddachi*) seemed to show a marked variation in appearance according to its environment, we instituted experiments in the Laboratory at Plymouth on *G. chevreuxi* to see if a similar variation in appearance could be brought about by varying the degree of salinity of the water in which the animals lived.

It would have been impossible to have obtained any measure of success without the kind co-operation of Dr. Allen, and we wish here to acknowledge our indebtedness to him, and to the various members of the staff for their continual and willing help.

Our ultimate object, which is to establish if possible a variation in the appearance of a given species by altering its environment, has had to stand over for a time, until other more pressing problems were settled. The first of these was how to ensure a constant supply of healthy animals, able to live and breed under Laboratory conditions, and the next was to find if it were possible to bring them to live in fresh water and in sea-water. In both of these experiments we have succeeded far better than we anticipated. We have now (January, 1913) the young of the *fifth* generation, bred from the animals captured in June; and, after some three months' gradual altering of the original water, we have some individuals living and breeding in absolutely fresh water, while others of the same stock are living and breeding in pure sea-water. Other problems in the life history have still to be worked out, this paper being intended only as a summary of the work as far as we have gone. Some of our conclusions will no doubt need revision later.

It must be remembered that the results here given are all obtained under artificial conditions. If it were simply a question of multiplying generations the conditions could have been made more like the natural surroundings, with stones, and plenty of mud and dirt provided for the shelter the animals seek when they moult, etc. Our method of work, however, necessitated daily observations being taken without unnecessary disturbance, and the bowls had therefore to be kept in the light, free from dirt and dust, and with only just sufficient ulva for food and not enough to provide a hiding-place.

We chose for our experiments the brackish-water *Gammarus chevreuxi*, as being easily obtainable in the neighbourhood. It occurs in the ditches draining Chelson Meadow (see previous paper).

GENERAL METHODS.

We brought our first specimens to the Laboratory on June 4 of last year (1912), together with some of the water, ulva, and the isopod *Sphaeroma serratum*, from the same ditch.

The next day two mated pairs were taken, and daily observations commenced. We kept these in jars partly covered to exclude dust, but a sufficient supply of air was ensured by a continuous bubbling of air through the water. We have found since that this is not essential, provided enough air can be supplied in some other way, such, for instance, as exposing a large surface of water to the air. In this way several broods have done well in large crystallizing dishes, about twelve inches across and two-thirds full of water, dust being excluded as in the jars. We consider, however, that we get better results, with the older animals at any rate, when the water is aerated.

With regard to the young, experience has shown us that the best method of observing them through their various stages to sexual maturity, and of obtaining their moults as they are cast, is to place each individual in a separate finger-bowl, half full of water, and almost wholly covered with a glass plate. The water is aerated once a day with a pipette, and a little ulva is given for food.

All the dishes and jars have been kept in ordinary diffused daylight, not protected in any way.

The temperature in the Laboratory in summer probably does not differ much from that in the ditches, but in winter it is considerably higher. It varies through a number of degrees at different times of the day and on different days. The animals, however, appear but little affected by this variation. The only point we have noticed is that the rate of development and the time of reaching sexual maturity are retarded by a lower temperature. In the Laboratory from October to December the temperature of the water in the moult-bowls varied between 10°–16° C.; in another room not artificially heated the range was from 4°–10° C. during the same period. In order to test the difference, if any, in the rate of moulting, a brood was divided, half being kept in the Laboratory and half in the other room, and it was found that some of the young took twice as long over their first moult in the colder place—in fact, some of those in the Laboratory had accomplished a second moult before all the young in the other room had finished their first.

The main food is ulva, supplemented by enteromorpha, and the rotting leaves of oak, beech, and sycamore. Certain kinds of animal food are eagerly taken, such as *Sphaeroma serratum* from the ditches in all stages of putrefaction, but this is a diet we give sparingly for fear of fouling the water.

Occasionally healthy males will attack and devour weak or sickly females, but they have not been seen eating dead ones. The animals will tear up their cast skins or "moults," and if these are not removed within, say, twenty-four hours, they will disappear completely. The inference is that they are eaten, and we feel sure this inference is correct, although we have no direct proof. It is certainly the case in other genera—two *Jassa*, for instance, were actually watched in the Laboratory devouring their skins immediately after ecdysis; and again, we have never found any torn pieces of a moult that has disappeared in the dirt pipetted daily out of the bowls.

Both male and female feed during the carrying period of the mating—not only cropping the ulva while resting on it, but holding pieces with their gnathopods while swimming. The young feed while in the incubatory pouch; their intestines are full when they emerge.

REPRODUCTION.

The conclusions arrived at by Holmes (**3**) and Embody (**2**) on the mating of Amphipods are, as we understand, as follows:—(1) that neither sight nor smell is concerned in the mating of a pair; (2) that they meet accidentally; and (3) that the female is wholly passive throughout, and indeed that the male recognizes her as a female by this passivity. Our results, as far as we have gone, indicate on the contrary that mating is not one-sided but mutual, and that a female when in the right physiological condition will at times seek the male, and, on the other hand, when not in this condition will actively resist him. The meeting of the sexes in the first place seems accidental, but the one certainly appreciates the presence of the other by the touching of the antennae. The antennae are provided with highly developed sensory organs, in which the olfactory sense may or may not be located, but we feel certain that in our species the animals always employ the antennae in meeting others, and in recognizing their own species.

Sexual maturity in both sexes is attained when the animals are about half grown. The time they take in reaching maturity varies greatly, but the cause of such variation is not yet known. The young produced in summer appear to become mature in much less time than those hatched later, but this cannot be laid down as a rule, as there is evidently some other potent factor at work besides temperature. The first brood of Pair I was extruded on June 19th and reached maturity on July 25th, i.e. in thirty-six days. Sometimes, however, a later brood will reach maturity before an earlier; for instance, Brood II of Pair II, extruded July 4th, did not reach maturity till September 4th, a period of sixty-two days, while Brood IV of the same pair, extruded July 29th, became mature on September 9th, only forty-two days.

The male takes the female when their respective gonads are becoming mature and carries her sometimes for days before mating takes place. This occurs once a fortnight with the female, so that, in ideal conditions, twenty-six broods could be produced annually. This would imply that breeding continues throughout the year, and such we believe to be the case, at any rate in a mild climate like Plymouth. It has gone on uninterruptedly in our jars during the seven months we have been working, from early in June to mid-January, and breeding pairs are still found in the ditches in Chelson Meadow.

The male, on the other hand, seems to have no regular mating period, but can fertilize a succession of females with an occasional period of rest. One young male, which became sexually mature on September 28th at the age of fifty-two days, by October 1st had fertilized the three females in the same jar. An interesting point to note is that the last female had only two eggs, and these were thrown off the next day, which may have been due to the fact that the eggs were not properly fertilized, and also suggests that the number of eggs extruded *may* bear some relation to the condition of the male. Again, in Brood I of Pair I a male and a female became mature in thirty-six days; four days later four other females were fertilized by this male and another, and again the last females mated had a small number of eggs, five and seven respectively, while the first female had eighteen. How many times in succession a male can mate we do not yet know, because we have never had a succession of females in the right condition. If a male is placed in a dish with several females whose eggs are at different stages of development, he chooses one with advanced eggs, mates with that one first, and then with the others in succession. If, however, he is placed with only one female, and that one with very young eggs, he does not attempt to take her until the eggs are five or six days old, and neither would the female permit herself to be carried by him till then. We have a few records of a male carrying a female for this length of time, eight or nine days before the young are extruded from the pouch, but he will only do it when there is no riper female present. The relative size of the male and female in mating does not seem of much importance; at times a big male will take a very small female, and vice versa.

We have watched the whole process of the moulting of the female, followed by fertilization and oviposition, and find that it is practically the same in our species as in *Gammarus pulex*, as described by Della Valle (1).

In the case watched, the last young one of the previous brood was extruded at 2 p.m. (December 14th, 1912), while the male was holding the female. They swam about together afterwards, stopping occasionally to seize and eat pieces of ulva.

A periodic convulsive movement on the part of the female, increasing in frequency and violence, led up to the casting of the skin at 7 p.m. the following day. Meanwhile the male employed not only the second gnathopods, but the first and second peraeopods as well in holding the female. At times the male arched himself, bringing the urosome up to rest against the fifth peraeon-segment of the female, and then suddenly straightened out, rasping the uropods along the female's cuticle. During the whole time the male kept the lower antennae bent over the head of the female, so touching both pairs of her antennae. About two hours before the moult the female commenced a series of violent rapid jerks, bringing the head and urosome together, and then straightening suddenly, at intervals stiffening the body in the form of a comma, with the head bent downwards, the body moving with a sort of convulsive tremor. Finally the male ceased swimming, and they both lay quiet, while the female, helped by the male, freed the head from the old cuticle with the gnathopods. This process and the ensuing rest occupied not more than five minutes. Then the male suddenly arched the body as before, and in straightening again pushed off the posterior portion of the moult with his uropods, assisted by the upward heaving of the female's body. He did not relinquish his hold for a moment. After moulting the female lay absolutely still, without even a movement of the pleopods, while the male kept up a steady current with his. In a few minutes they commenced to swim as before.

About an hour and a quarter after the moulting the male began to turn the female round, and after several efforts he succeeded in getting into the position described by Della Valle. At 8.30 p.m. the first ejection of sperm into the pouch was effected in four or five spasmodic movements. After lying quiet in this position for a minute or so, the male resumed the dorsal clasp, but five minutes later the whole process was repeated. Once more after an interval the male resumed the original dorsal position and swam again. After another five minutes—at 8.40 p.m.—the female suddenly struggled free, and did not allow the male to carry her again, darting away if he approached. Just at the moment they separated the first egg was seen emerging from the aperture of the oviduct. The last egg passed from the ovaries exactly twenty-four minutes later, and the newly extruded mass hung in the pouch in spheroidal form, the eggs held together by a glutinous secretion. The number of eggs was forty.

In another case watched, a distinct oviduct was apparent during the passage of each egg from the ovary to the exterior. This was clearly distinguishable as a thin light line only during the actual extrusion of the egg. The eggs laid first were pushed forward in the pouch by the later ones, and it may be mentioned here that the front ones were seen to hatch first.

The female *can* moult alone after extruding a brood from the pouch, so the presence of a male is not imperative for the moult, but our experience agrees with Embody's, that eggs are never deposited unless a male is present. In the absence of a male the eggs develop enormously in the ovaries, and are separated by distinct spaces, as happens during the normal development in the pouch, but what ultimately becomes of them has not yet been traced.

We have tried several experiments to determine the extreme interval that can elapse between any given moult and the subsequent oviposition, and we find that on the third day oviposition is still possible. If a male is kept away for four days or more after the female's moult, no mating occurs until the fourteenth day, i.e. until the next period begins, and such mating is preceded by a second moult.

It is evident that in the female moulting is directly connected with mating. The animal of course increases in size also, but as the moults occur at fortnightly intervals this increase is too gradual to be noticed at the time. With the male, on the contrary, moulting appears to be purely a growth process. The moults are at long intervals, and the increase in size very noticeable. The oldest male we have, extruded June 19th, became sexually mature July 25th, and has only been observed to moult three times since, on Aug. 1, Sept. 4, and Nov. 5.

The number of eggs in the broods we have counted varies between five and forty-four, about thirty being a fair average. The number seems to increase with age, as one would expect, e.g. one female increased the number gradually from eighteen to forty-four. The eggs when laid have the characteristic blackish-green colour of the ovary; about a week later they turn brown, then yellow, the body of the embryo being now distinctly visible. They are hatched about the twelfth to the fourteenth day, and the young are extruded from the pouch the day after. Discomfort will hasten the extrusion, e.g. irritation with a brush, lack of water, etc.

Our results show that the age of sexual maturity is from thirty-six days onward, but we are inclined to think the earlier age the more normal. Counting the time from oviposition, only fifty days, therefore, elapse between one generation and the next, and this period is probably less in the heat of summer. We have now (Jan. 2, 1913) after seven months, the young of the fifth generation.

THE YOUNG.

The young remain one day, or at the most two days, in the incubatory pouch after hatching. The four pairs of incubatory lamellae with their interlacing hairs form a continuous pouch except for a small aperture in front and one behind, where the lamellae are separated at the top by the width of the female's body. Through these apertures the young emerge, but the female can close the entrances at will by bending down her projecting mouth-parts, and by lateral compression with her gnathopods and peraeopods. At times some of the young come half-way out, others again creep right out at the back and crawl along her ventral surface between the bases of her hinder peraeopods, then suddenly double back and re-enter the pouch. But if once they get outside the peraeopods, the swirl set up by beating pleopods prevents their ever re-entering. They are exceedingly active when inside, changing places continually; when they are once out they swim vigorously, and if they meet a piece of ulva or anything that can serve as shelter they climb in and hide themselves.

We have noticed an occasional female with red eyes instead of black, among the adults. A small proportion of the newly hatched young also have red eyes, the rest of their colouring being normal.

We have been puzzled by a great difference in size among the individuals in any given brood. Sometimes this is apparent when they are first extruded, at other times some gain enormously in size while growing as compared with the others, and finally some members of a brood become sexually mature much earlier than others. It may be simply that the weaklings lag behind, but as the male and female differ so much in size at sexual maturity it may perhaps be a sexual distinction. The results of our experiments may clear this point later.

We have isolated 130 newly hatched young from different broods, and are collecting and numbering the moults as they occur. We hope when they attain sexual maturity to be able to trace back in the moults the changes undergone, and the sexual distinctions if any. Certainly the last moult before maturity shows sexual differentiation, for the female already has the incubatory lamellae present, though only partially developed, and the male has a few of the coiled sensory hairs on the antennae, telson, etc.

The time taken in the moulting of the young appears to be much shorter than with the adult. For a few days previous they are less active than usual, and a flocculent sediment is noticed in the bottom of the bowl. This, we think, is the secretion, probably lubricative, of which a copious flow precedes ecdysis, oozing from between the terga, and from all the joints of the antennae, the peraeopods, etc. Della Valle (**1**, p. 111) refers to this in describing the moulting of the female. Several young have been watched moulting; they used their anterior appendages in loosening the old cuticle of the head, but the whole process, pulling off the head covering and slipping out of the posterior portion of the cuticle, only occupied three or four seconds.

The first moult seems to be the most critical; in one brood fifteen out of thirty-two died during the first moult. Afterwards the mortality is comparatively small. Where the period between moults has been longer than normal, the individual is undersized and evidently weakly, in many cases not surviving the next ecdysis. Below are tabulated side by side the rate at which two broods moulted, kept under exactly the same conditions in the cold room. The right-hand table refers to a brood seven days younger than the other, and it will be seen that the rate of development was quicker in the younger brood. Number *d* in Brood I was much larger than the others when hatched, number *k* much smaller. This one did not grow perceptibly after its second moult, and died seventeen days later in the effort to moult again. Number *e* did not grow much after the second moult, and died just after its fourth moult. Number *i* took the longest time yet recorded for a third moult—twenty-six days; it is an exceedingly small specimen, and had great trouble in getting rid of the old cuticle.

BROOD I.

EXTRUDED 5.11.1912.

	PERIOD IN DAYS BETWEEN MOULTS.					
	Moult 1.	Moult 2.	Moult 3.	Moult 4.	Moult 5.	Moult 6.
a	8	10	14	22	13	18
b	8	11	17	12	15	18
c	9	10	15	11	14	16
d	9	13	15	12	16	17
e	9	13	18	12 died directly after moult.		
f	10	10	14	8	11	14
g	10	10	15	14	14	
h	10	10	17	10	14	16
i	11	10	26	10 died in moulting.		
j	11	11	14	16	11	15
k	11	11	17 died in moulting.			
l	11	11 dead two days later.				
m	11	11 dead two days later.				
n	12	12 dead two days later.				
o	13	9	13	27 died in moulting.		
p	13	12	13	9	13	20
q	13	14 died in moulting.				

BROOD II.

EXTRUDED 12.11.1912.

	PERIOD IN DAYS BETWEEN MOULTS.					
	Moult 1.	Moult 2.	Moult 3.	Moult 4.	Moult 5.	Moult 6.
a	7	9	11	8	10	13
b	7	9	12	23	15	23
c	7	9	13	12	12	13
d	8	8	11	10	13 died in moulting.	
e	8	9	11	9	10 died directly after moult.	
f	8	9	11	15	16	17
g	8	10	11	22	11 died in moulting.	
h	8	10	12	12	12	16
i	8	14	10 dead nine days later. Very small.			
	8	14	11	11	8 died in moulting.	
	8	14	15	15 died in moulting.		
l	8	16 died during moult.				
m	9	11	11	9	11	11
n	9	11	12	9	17	19
o	9	13	8	9	9	11
p	9	13	12	11	11 very small one.	
q	10	13	9	24	14	
r	11	15	15 died during moult.			
s	13	13	9	10	13 died in moulting.	

Another brood kept in the Laboratory gives a record of four moults in twenty-eight days. As contrasted with the two broods tabulated above, the period between the moults was much less, due no doubt to the higher temperature. Another instance is the "control brood" for Brood II, which was kept in the warm Laboratory; they were one day ahead of Brood II in the first moult and seven days ahead at the second moult.

EXPERIMENTS IN VARYING THE SALINITY.

Great variations in the salinity of the water can be endured by this species, but too sudden a change tries them severely. We have made some experiments with large adults. In one instance a male and a female were taken from the brackish water and put into fresh water on July 24th; two days later the female died, the male survived till the seventh day. Again, on July 24th, a male carrying a female was put into sea-water; they both moulted, and five days later were separate, but no eggs were produced. On August 3rd the male was again carrying the female, and on the 7th she moulted again, and again they were separate and no eggs present. On August 21st they were again paired, and on the 24th another moult was found, and the female was dead. The male died on the 26th, so he had lived thirty-three days and she thirty-one. Evidently the sexual impulse was still there, but the power to produce was affected. In a third case, two half-grown females were put straight into sea-water. One had very young eggs, which were soon thrown off; the other had two partly hatched eggs, which hatched and were extruded four days later. The two females are still alive, a month later, and their ovaries are much enlarged. The extruded young are swimming about in the sea-water.

Similar experiments just commenced with newly hatched broods promise some interesting results. They show that the first moult is critical; if that be survived they continue to flourish, but the period between moults is much longer than normal. One brood of seven *G. chevreuxi* were put into fresh water; they took from eighteen to twenty-one days to reach the first moult, and all died, either in moulting or directly after. Some nine *G. pulex* put straight into brackish water all accomplished their first moult safely, but took thirteen to fifteen days instead of seven to reach it; nine others of the same brood put into water one-third brackish to two-thirds fresh took nine days to this moult.

Finding the sudden change too drastic, a number varying in size were put on September 9th into two bell-jars. Small quantities of fresh water have been added to the one from time to time at a few days' interval, so that now the water is fresh. Although the original large adults have disappeared, we still have breeding pairs mixed with their own progeny in this jar. To the individuals in the second bell-jar small quantities of sea-water have similarly been added from time to time, and the water is now full-strength sea-water. The large adults died off in this case also, but their numerous progeny are flourishing and breeding.

The papers referred to above are :—

1. 1889. Della Valle, A. "Deposizione, fecondazione, e segmentazione delle uova del *Gammarus pulex*." Atti. Soc. Nat. Modena. Ser. III, Vol. VIII.

2. 1912. Embody, G. C. "A Preliminary Study of the Distribution, Food, and Reproductive Capacity of some Fresh-water Amphipods." Internat. Revue, Leipzig, 1912.

3. 1903. Holmes, S. J. "Sex Recognition among Amphipods." Biol. Bull., V.

4. 1912. Sexton, E. W. "Some Brackish-water Amphipoda, etc." Proc. Zool. Soc., September, 1912.

Notes on the Development of *Mytilus edulis* and *Alcyonium digitatum* in the Plymouth Laboratory.

By

Annie Matthews, M.Sc.

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1. Mytilus edulis.

No very definite statement has yet been made as to the time when *Mytilus edulis* spawns at Plymouth. However, in 1911 records of spawning in the Laboratory tanks were made in January, February, and March, and in 1912 two specimens removed from the tanks spawned in early May.

Between May 10th and 21st, 1912, 100 mussels from Plymouth Pier were kept in a Laboratory tank, but as they did not spawn they were then opened, and many found to be either spent or only partly ripe. Examination of samples brought in between May and August seemed to indicate that the spawning season was finished, and occurred therefore in the early spring.

Various attempts were made at artificial fertilization from apparently ripe members of the selected hundred, but in one case only was fertilization successful—May 21st. A piece of ripe ovary and of ripe testis were shaken about in separate finger-bowls containing "outside" water, and thus ripe eggs and spermatozoa were freed in the respective bowls. At 12 noon a few drops of water containing spermatozoa were added to the finger-bowl containing ova, and at 4 p.m. that day many of the ova were developing—some showing Polar Bodies, others the early segmentation stages. Next morning the ciliate trochospheres were swimming at the top of the water in the finger-bowl, and these were removed with a pipette to two small "Breffits"* containing outside water, to which a few drops of a Nitzschia culture were added. Development gradually proceeded, the velum at this time being of very large relative size, and as Wilson states ("5th Annual Report Fishery Board for Scotland, 1886-87"), the shell muscles and alimentary canal are now elaborated, the valves of the shell being finely pitted and almost semicircular in shape.

However, while Wilson states that his larvae (now twelve days old)

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* Wide-mouthed jars of green glass of about 2 litres capacity.

never progressed beyond this stage, the specimens reared in Plymouth continued to grow and develop, and are still doing so, although very much below normal size, judging by specimens of this year's mussels brought in from outside. The valves gradually assumed a shape rather like a minute edible cockle, and about July 22nd, a purple colour—the beginning of the prismatic shell substance—appeared at the edge of the valves, behind the velum, and extending to the posterior edge of the shell. The smallest individual in which the prismatic shell substance was visible measured ·21 mm. long × ·19 mm. high. The larvae still swam with the velum and the foot was growing rapidly in size. The eye spot was present and five gill filaments, and the valves measured ·31 mm. long × ·24 mm. high. From now onward the larvae grew at varying rates, some fixing by a byssus, while many others remained unattached and much smaller, creeping about the jar. The purple colour gradually extended over the valves, fading at its edges into brown. On August 6th the foot had become very long, thin, and active, the gill cilia were long and powerful, and the velum was decreasing slowly in size. Later the foot was frequently used as a creeping organ, and on August 15th I drew one specimen that could both swim with the velum and creep with the foot; it measured ·29 mm. high × ·32 mm. long, and had six gill filaments. A similar specimen measured ·32 mm. high × ·38 mm. long, so that the young mussel ceases to swim at a much later stage than the individual Wilson saw (see Wilson, loc. cit.).

From time to time more Nitzschia was added to the jars as food, and the outside water renewed, in which they lived.

Other specimens drawn on the same day had lost the large velum and the power of swimming, and were crawling with the long tongue-like foot about the glass jars. Measured specimens were:—

·35 mm. high × ·413 mm. long, with 5 gill filaments.
·385 „ × ·46 „ „ 8 „
·41 „ × ·574 „ „ 10 „

In the last individual the shell resembled a minute adult shell, being much thicker and of elongated oval shape, and dark blue in colour.

On August 27th most of the young mussels were crawling about the jar, many near the water surface, the largest measuring ·74 mm. high × 1·16 mm. long, with 15–16 gill filaments. The eye spot was still visible through the shell. During September they attached themselves to the glass at various levels—some at the water surface—by a delicate byssus, all but one being fixed by October 4th. When removed forcibly they soon refixed. About October 15th the jars were aerated by a slow, fine air jet to keep the food in circulation, and this is still kept up, the mussels slowly increasing in size.

The largest individual in the jars at present measures 2·2 mm. long ×1·5 mm. high.

Note.—On August 7th, 1912, several swimming larvae, including one mussel that could both swim and crawl, were placed in a Breffit with a little of the water in which they were brought in. The jar was then filled with outside water, and the mussel, three Anomia sp. larvae, and several gastropod larvae developed and grew in this jar. No food has been added. The mussel is much larger than those wholly reared in the Laboratory, and measures 4 mm. long × 2·0 mm. high. The Anomia sp. measures 10·25 mm. across the widest diameter of the shell.

2. **Alcyonium digitatum.**

Male and female colonies of *Alcyonium digitatum* were placed in a tank on January 26th, 1912, and from January 27th to February 3rd, eggs in various stages of segmentation were pipetted out of the tank where they were floating, into Breffits containing Berkefeld or outside water. Hence the early critical stages of maturation and fertilization took place in the tank water. On January 28th many advanced morulæ passed into a curious irregular stage, which in turn became a round ciliate planula. This elongated gradually to an oval swimming planula, and as development proceeded the shape became pear-like, the larva swimming with the broad anterior pole forward, and simultaneously rotating on its axis. The characteristic reddish-brown colour of the egg gradually became pale cream as the larvae absorbed the yolk, and planarian-like contractile movements were observed when they were irritated by light, pipettes, etc.

Later they floated towards the base of the Breffits or near the surface film, with the long axis vertical and the anterior pole upwards, and on February 6th, some larvae had fixed on the glass at the surface film. Shrinkage now took place along the long axis, so that the oval larva became short and dome-shaped, and through the glass Breffits the eight mesenteries were visible. Some larvae settled on glass rods, glass cover-slips, and pieces of paraffin which were introduced into the Breffits at this time. By invagination of the ectoderm at the free pole of the larva, the mouth and stomodeum now arose,—fifth day of fixation, and as the yolk was absorbed it became paler and more transparent. Later eight simple tentacles appeared round the mouth, so that the larva now resembled a small anemone. By February 17th the tentacles were well developed and bore 2–3 lateral branches, and they waved gracefully about in the water if undisturbed, but retracted completely if shaken or disturbed. Forty days later the solitary polyp had grown very considerably, but no lateral buds had arisen. The base of the polyp now was approximately 1 mm. in diameter. The only food added to the water was a little Nitzschia from time to time, but they were never seen taking it in.

They lived healthily until April 3rd,—two months approximately, but were then preserved, as flagellates had attacked them.

This work is being continued and amplified, and it is hoped that a complete account of the development of *A. digitatum* will soon be forthcoming.

Notes on the Structure and Mode of Action of the "Oval" in the Pollack (*Gadus pollachius*) and Mullet (*Mugil chelo*).

By

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With Seven Figures in the Text.

DURING the summer of 1911 I conducted at the Plymouth Biological Station a series of experiments on the living active gas-gland associated with the bladder of certain marine fish, the results of which are recorded in a paper published in the *Anatomischer Anzeiger* for 1911 (Bd. XL, p. 225). Whilst so employed I incidentally made some observations on the structure and mode of action of the "oval" in fishes, and since my conclusions differ in several particulars from those of Nusbaum and Reis (*Bull. Acad. d. Sciences Cracovie*, 1905, p. 778; *Anatomischer Anzeiger*, Bd. XXXI, 1907, p. 169), I think it as well to put them on record. Most of my observations were made on the Pollack. If a weight be attached to this fish so as to cause the gas-gland (oxygen gland) to become active and to pump oxygen into the bladder, it will be found that the oval strongly contracts, so as to prevent the additional gas forced into the bladder from escaping into the blood. The oval, it may be mentioned, is a large oval area usually situated in the dorsal posterior wall of the bladder. It differs from the rest of the bladder wall in that it alone is permeable to the contained gases, and, like the ductus pneumaticus in Physostomi, permits their escape when the conditions require it. In the Pollack the oval is normally widely open and is invisible to the naked eye, but on the gas-gland being caused to become active in an unusual degree, the oval becomes strongly contracted and is then a very conspicuous structure inside the bladder. This contraction of the oval is of course effected by muscles, and the result of it is to cause the thin-walled permeable area to become more or less completely shut off from the general bladder cavity, the walls of which, as just mentioned, are impermeable.

According to the observations of Nusbaum and Reis on the ovals of Perca, Lucioperca, and Ophidium, the oval has the following structure:—The ordinary wall of the bladder is composed of three layers: an inner elastic and muscular layer covered internally by the squamous epithelium lining the bladder cavity, a middle conjunctive and vascular layer, and an outer fibrous layer. At the periphery or edge of the oval there is developed a special band of circular smooth muscle fibres, which by contraction can lessen and obliterate altogether the area of the oval exposed to the gases in the general bladder cavity. Over this area, limited externally by the circular band just mentioned, the inner layer is quite absent, only the squamous epithelium being present, and this latter in consequence abuts directly on the middle layer, in which, in the oval area, the capillary system is much developed.* In the region of the oval, therefore, the gases contained in the bladder can come into very close contact (only separated by the squamous epithelium) with the numerous capillaries of the oval contained in the middle layer. Attached to the edge of the oval, immediately external to the circular muscle band, are numerous radial muscle fibres (belonging to the inner layer surrounding the oval), the function of which is to act in opposition to the circular band and enlarge the oval area. The foregoing statements and the mode of action of the oval, according to Reis and Nusbaum, are illustrated by Figures 1–4 (devised from the statements and diagrams of these authors). It will be seen that, according to these authors, the closure of the oval, in the fishes studied by them, is effected by the simple contraction of the circular muscle band (the radial muscles slackening), the squamous epithelium being thereby raised from contact with the blood-vessels and separated from them by the muscles. I presume that these statements of Nusbaum and Reis are based upon the study of actual sections of closed and open ovals; otherwise I should doubt their accuracy, because this mode of action of the oval is quite unlike that of the oval in the Pollack and the Mullet, because I find it difficult to believe that the squamous epithelium ever becomes separated from the capillary plexus in the manner asserted, and finally because, if Tracy (*Anat. Anzeiger*, 1911) is correct in his interesting view that the oval is homologous with the posterior chamber of the Carp bladder and the distal part of the ductus pneumaticus of Physostomi (Fig. 7), these statements are improbable *a priori*. It is evident that if the edge of the oval is homologous with the circular edge of the septum separating the anterior and posterior chambers of the Carp or Siphonostoma bladder, then it might naturally be anticipated that the

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* The so-called "wundernetz"—a bad term, since this special capillary development has nothing to do with the rete mirabile duplex situated on the artery and vein supplying the gas-gland (*vide* my *Anat. Anzeiger* paper already mentioned and *Proc. Zool. Soc., Lond.*, 1911, p. 183).

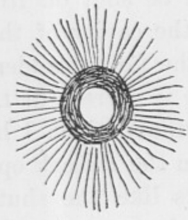

Fig. 1 Open Oval (Surface View). Fig. 2. Semi-closed Oval.

Fig. 3. Open Oval (in section).

Fig. 4. Closed Oval (in section). The squamous epithelium is separated from the capillaries.

Diagrams adapted from the description & diagrams of Nusbaum & Reis.

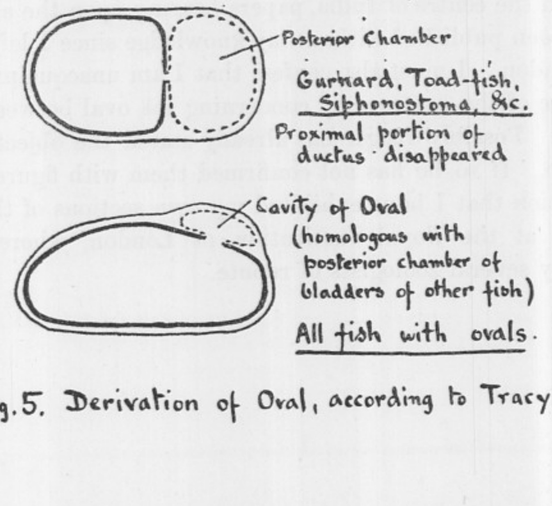

Fig. 5. Derivation of Oval, according to Tracy.

oval area would be shut off from the general bladder cavity in the same way that the cavity of the posterior chamber is shut off from the anterior in the Carp bladder. My investigations prove that this last anticipation is correct in the case of the ovals of the two fish (Pollack and Mullet) which I have examined. As shown by Figs. 5 and 6, the oval in the Pollack opens and closes by means of a circular fold which works like the shutter of an iris diaphragm. The impermeable inner layer of the bladder wall is shown by a thick line, and, as the figures indicate, this ceases at the edge of the open oval, the oval area merely being covered by the thin layer of squamous epithelium. As the figures also indicate, the circular fold is formed by the actual rotation of the tissue round the edge, hence the more closely shut the oval the deeper is its cavity. This deep cavity of the closed or nearly closed oval is very obvious in the actual bladders of the Pollack, Mullet, and other fish, but, according to the statements of Nusbaum and Reis, it does not exist in the species they examined. I am, of course, quite ready to admit that all ovals may not work on the same principle—in *Dactylopterus volitans*, e.g., I find that the oval-like structure has in section an appearance different in several particulars from that of the normal oval; at the same time, I shall feel more satisfied that the mode of action of the oval in Perca, Lucioperca, and Ophidium is different from that of the oval of Gadus, Mugil, and other fish, if Nusbaum and Reis would supply us with figures constructed from observations of actual sections of the open and closed oval instead of mere diagrams which, to say the least, look very hypothetical. The figure of the open oval of Lucioperca published by Reis (Kraków, *Rozpr. Akad.*, 1906, pp. 639–670) is of little use as evidence in the present connection.

I must point out, in conclusion, that, literature being not easily accessible in the centre of India, papers bearing upon the above subject may have been published without my knowledge since I left University College, London. I must also confess that I am unacquainted with the exact nature of the controversy concerning the oval between Nusbaum and Jaeger. Possibly Jaeger has already stated the objections I have urged above. If so, he has not confirmed them with figures. Finally, I may mention that I have exhibited my own sections of the open and closed oval at the Royal Institution of London, where they were examined by several zoologists of repute.

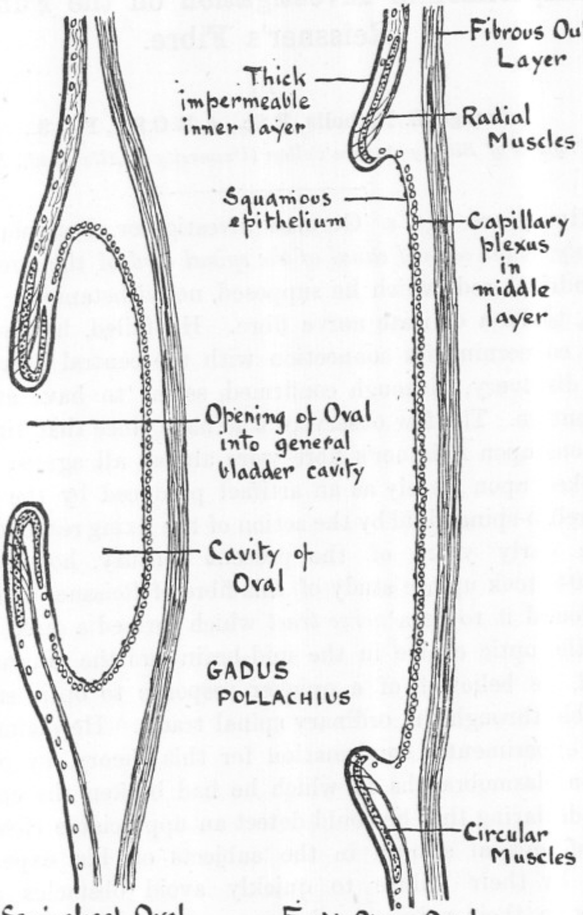

Fig. 6. Semi-closed Oval. Fig. 7. Open Oval.
Diagrammatic figures constructed from sections.

An Experimental Investigation on the Function of Reissner's Fibre.

By

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SOME fifty years ago a German investigator, Reissner, discovered *lying freely in the central canal of the spinal cord* of the lamprey a very fine cylindrical rod, which he supposed, notwithstanding its unusual situation, to be a delicate nerve fibre. He failed, however, to learn anything concerning its connection with the central nervous system, and his discovery, although confirmed, seems to have attracted but little attention. The few observers who have since that time recorded observations upon Reissner's fibre were almost all agreed that it was to be looked upon merely as an artifact produced by the coagulation of the cerebro-spinal fluid by the action of the fixing reagents employed.

In the early years of the present century, however, Sargent (1900–1904) took up the study of this fibre of Reissner and announced that he found it to be a *nerve tract* which formed a direct connection between the optic centre in the mid-brain and the musculature, and permitted, he believed, of a quicker response to optic stimuli than was possible through the ordinary spinal tracts. He claimed to have obtained experimental confirmation for this theory, by observations made upon elasmobranchs, in which he had broken the continuity of the fibre, declaring that he could detect an appreciable slowing in the passage of optical stimuli in the subjects of his experiments as exhibited by their failure to quickly avoid obstacles placed unexpectedly in their path.

My own observations upon Reissner's fibre and related structures in the central nervous system, which were begun in 1907 and have continued until the present time, while establishing beyond question the fact that the fibre is really a preformed structure,* have at the same time shown conclusively that it is not a nerve fibre or a nerve tract.

I have been able to demonstrate that the fibre takes its origin from an extraordinary epithelial organ which lies beneath the posterior

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* Edinger, as recently as 1908, had affirmed that it was merely an artifact.

commissure. This structure, for which the name sub-commissural organ has been proposed (and which, as I shall hope to show in a paper now nearly ready for publication, develops from an anlage in the brain, which is serially homologous with the anlage of the lateral and pineal eyes), *must be looked upon as an intra-cerebral sense organ.* In early development a paired structure, it takes up, in many forms, a median dorsal position, and in almost all vertebrates becomes in the adult a most conspicuous structure in the mid-brain.

It is from the internal (ventricular) aspect of the cells of this organ that Reissner's fibre arises as a large number of cilia-like fibrillae, which converge beneath and behind the posterior commissure into a rod-like structure which may, at its anterior end, be either paired at first or single and median. In either case it shortly becomes a single median thread and stretches backward as such to the extreme hind end of the central nervous system. Beneath the rhombo-mesencephalic fold it frequently comes to lie in a well-marked dorsal median groove (the "isthmic canal"), which deepens with age, and which may be paired if the paired character of the fibre is maintained so far caudally.

Through the central canal of the spinal cord Reissner's fibre may be traced backwards lying centrally and apparently supported at frequent intervals by cilia from the ependymal cells.

At the actual extremity of the spinal cord (filum terminale) the central canal widens out into a sub-spherical space which was named by Retzius* the sinus (ventriculus) terminalis. This chamber is not, however, wholly enclosed within the nervous system, for, posteriorly, the ependymal epithelium—which alone constitutes this part of the filum terminale—fails entirely, and there is left a wide opening which I have called the "terminal neural pore." The wall of the sinus terminalis is thus completed posteriorly only by the connective tissue sheath of the spinal cord. Into this meningeal wall Reissner's fibre, flaring out into a trumpet-like end, passes and is inserted.

If in freshly killed material Reissner's fibre be cut, it recoils spirally in both directions from the point of section, forming dense tangled knots such as would be formed in a thin elastic thread which, held firmly at one end, *was twisted from the free end continually in one direction.* In this reaction, as also in its straining reactions, in its origin in the brain, and in its ending in the meninges, Reissner's fibre is altogether unlike any known nerve.

Since then it is not a nerve, Sargent's "Optic Reflex Theory" can no

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* Retzius knew of its occurrence only in Amphioxus and Cyclostomes. It is to be found even better developed in Elasmobranchs and Teleosts.

longer be maintained, and the question of its function (and that of the related sub-commissural organ) is reopened.

The supposed inaccessibility of the fibre had led Sargent to operate upon it in the region of the hind-brain, and his experiments were therefore open to the serious objection that they involved great risk of grave damage to the brain itself. My discovery, however, that the fibre is, in the lower vertebrates at any rate, comparatively readily accessible in the region of the tail (where it actually passes out of the central canal of the sheltering spinal cord through the terminal pore), suggested the practicability of experimental work upon Reissner's fibre without danger of damage to the central nervous system.

The experiments, which consisted simply in breaking the fibre by a slight incision at the end of the filum terminale, were carried out in the Laboratory of the Marine Biological Station at Plymouth, in the summers of 1910 and 1911, and I desire here to acknowledge my obligations to the British Association for the Advancement of Science, and the Senate of London University for the use of their Tables, and also to the Royal Society for a Grant which enabled me to carry out the more extensive series of experiments in 1911. My thanks are also due to Dr. Allen and the other members of the staff for the courteous way in which they met my wishes and facilitated the carrying out of the work.

In all, the experiment was carried out upon some seventy specimens (dogfish and rays), and a short account of the earlier experiments has already been published (*Anat. Anz.*, Bd. XL, pp. 409–432). In compliance with the conditions under which the licence to conduct the experiments was granted the specimens were anæsthetized—a most unnecessary precaution (in view of the trivial character of the operation, which rarely drew a drop of blood), and one which, in the case of some of the subjects of experiment, proved a much more serious matter than the operation itself.

Upon these anæsthetized specimens the necessary prick was quickly inflicted and the specimens returned to the tank. Subsequent observation (extending in different cases over a period of less than an hour to as much as three weeks or more) showed that apart from a slightly different action in swimming, which I found almost impossible to analyse and describe, the only discoverable effect of the operation was that many of the specimens when at rest adopted a pose which was markedly unlike that of the normal animal. In the normal specimen at rest the under surface of the head and the lower lobe of the caudal fin touch lightly upon the supporting surface, and the entire long axis of the body extends in a straight line. In these subjects of the experiments (in which subsequent microscopic examination of the material showed that the fibre had been broken) the animal was found to retire to the darkest part of the tank and there to remain sluggishly with head and tail sharply uplifted, and often with the body sharply bent or in a sinuous curve. In the rays, the whole body was often strongly arched transversely as well. The reaction lasted for a longer or shorter period, and was usually very pronounced.

Subsequently, in the aquarium, specimens were found showing this reaction, and the examination by sections of their central nervous system showed that in each case Reissner's fibre had been broken in life, presumably by some recent accident.

Thus the principal result of the breaking of Reissner's fibre in the living animal appears to be that the animal adopts, while at rest, an unnatural pose, and probably also swims with a slightly different action. This lends support to the suggestion put forward by Dendy (*Nature*, December, 1909), that the apparatus forms part of a mechanism for automatically regulating flexure of the body.

A List of Blood Parasites of Sea Fish taken at Plymouth.

By

Herbert Henry, M.D.

The following is the result of an investigation with regard to the occurrence of haemoprotozoa in sea fish taken in the neighbourhood of Plymouth:—

A. *In May*, 1911.

	Number examined.	Number with haemogregarines.	Number with trypanosomes.	Number with new parasites.
Solea vulgaris . .	4	4(1)	—	—
Callionymus lyra . .	15	5(2)	1(3)	—
Cottus bubalis . .	10	2(4)	—	—
Blennius pholis . .	6	6(5)	—	—
,, gattorugine .	1	—	—	—
Gobius paganellus . .	9	2(6)	—	—
Agonus cataphractus .	5	1(7)	1(8)	—

B. *In August–September*, 1912.

Pleuronectes platessa .	13	2(9)	—	—
Callionymus lyra . .	4	—	—	—
Blennius pholis . .	2	2(5)	—	—
,, gattorugine .	2	2(5)	—	—
Gobius ruthensparri .	5	—	—	—
,, minutus . .	4	—	—	—
,, paganellus . .	31	3(6)	—	—
Motella mustela . .	9	—	—	1(10)
Cottus bubalis . .	13	2(4)	—	—
Rhina squatina . .	5	—	—	—
Solea lutea . . .	6	1(11)	—	—
,, vulgaris . .	13	12(1)	—	—
Gasterosteus spinachia .	8	—	—	—
Scomber scomber . .	36	—	—	2(12
Agonus cataphractus .	1	—	—	—
Siphonostoma typhle . }	12	—	—	—
Syngnathus acus . . }				

LIST OF PARASITES WITH REFERENCES.

(The numbers are the index figures in the above table.)

1. Haemogregarina simondi . . . . Laveran et Mesnil, "Deux Hémogrégarines nouvelles des Poissons," Compt. rend. Acad. d. Sc., Paris, 1901, tome cxxxiii., p. 572.
2. Haemogregarina quadrigemina . . Brumpt et Lebailly (see ref. 3).
3. Trypanosoma callionymi . . . . Brumpt et Lebailly, "Description de quelques nouvelles espèces de Trypanosomes et d'Hémogrégarines de Téléostéens marins," Compt. rend. Acad. d. Sc., Paris, 1904, tome cxxxix., p. 613.
4. Haemogregarina cotti . . . . . . Brumpt et Lebailly (see ref. 3).
5. Haemogregarina bigemina . . . . Laveran et Mesnil, 1901 (see ref. 1).
6. Haemogregarina polypartita . . . Neumann, "Studien über protozoische Parasiten im Blut von Meeresfischen," Ztschr. f. Hyg. u. Infectionskrankheiten, Leipzig, 1909, Bd. xiv., Heft 1, S. 1.
7. A Haemogregarine (to be described).
8. A Trypanosome (to be described).
9. Haemogregarina platessae . . . . . Lebailly, "Sur quelques Hémoflagellés des Téléostéens marins," Compt. rend. Acad. d. Sc., Paris, 1904, tome cxxxix., p. 576.
10. A new parasite (to be described).
11. Haemogregarina clavata . . . . . Neumann (see ref. 6).
12. A new parasite (to be described).

All fish were carefully examined for ecto-parasites, as possible carriers of infection, with the following results:—

1. On two specimens of Solea vulgaris, there was found a leech Trachelobdella lubrica? (HARDING: "A Revision of British Leeches," Parasitology, Cambridge, vol. iii., No. 2, pp. 136–9).
2. On five specimens of Solea vulgaris, there was found a trematode, Phyllonella soleae (BRONN: Das Thierreich, Leipzig, 1879–93. Abth. I A, Bd. iv., S. 363, S. 527).
3. On two specimens of Scomber scomber, a parasitic copepod, Caligus scombri (BASSET, SMITH: Ann. and Mag. Nat. Hist., 1898, vol. ii., p. 83, pl. 14, fig. 2. THOMAS SCOTT: "Notes on some Parasites of Fishes," Nineteenth Annual Report of Fishery Board of Scotland, 1901).

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY.

Experimental Metaplasia. I. The formation of columnar ciliated epithelium from fibroblasts in Pecten. By G. Harold Drew.

(*Journal of Experimental Zoology*, Vol. X, 1911, pp. 349-379.)

THE implantation of small pieces of the ripe ovary of *Pecten maximus* or *Pecten opercularis* into the adductor muscle of another animal of the same species was found to result at first in the formation of a closed cyst within the muscle, lined with layers of fibroblasts. Complete degeneration and disintegration of the ovarian tissue within the cyst occurred in a few days, and then the cyst contained only an orange-coloured granular substance, presumably derived from the yolk, and numbers of blood corpuscles. After the lapse of from twenty-one to thirty-two days, changes occurred in the innermost layer of fibroblasts lining the cyst. They reverted to an embryonic type, and afterwards became converted into columnar ciliated epithelium, which formed a continuous layer lining the cyst. The changes resulting in this formation of ciliated epithelium from fibroblasts were followed clearly step by step, and once formed, the ciliated cells persisted unaltered for at least 120 days, which was the longest period for which the animals could be kept alive under experimental conditions.

Experiments were performed showing that this change is not produced by the implantation of any of the other tissues of Pecten, by neutral foreign bodies which would merely act as a source of mechanical irritation, by the transplantation of the ripe ovarian tissue of other Lamellibranchs, or by the transplantation of pieces of the ovary of *Pecten opercularis* into the adductor muscle of *Pecten maximus*, and vice versa.

Other experiments showed that the development of ciliated epithelium does not occur if pieces of the immature or spent ovary be implanted, and that it is prevented by treating the ripe ovary with a suspension of the sperm in sterile sea-water before implantation. Also that it does not occur if the ovary be killed by physical or chemical agents before implantation. A series of experiments were made to eliminate the possibility of the origin of the ciliated epithelium lining the cysts from the ciliated cells of the oviduct, which might be present in pieces of the ovary that were implanted, or from the layer of epithelial cells forming the outer coating of the adductor muscle, which might be carried inwards by the transplanting needle.

It thus appears that the conversion of fibroblasts into ciliated epithelium is a specific reaction following the implantation of the ripe living ovary.

These observations were the result of nearly a thousand experiments, of which the majority were performed on *Pecten opercularis.*

It appears that this conversion into ciliated epithelium of the inner layer of fibroblasts lining a cyst formed round a piece of the ovary, which has been implanted into the adductor muscle of Pecten, is a specific reaction that occurs only when the ripe living ovary of an animal of the same species is implanted. The reaction takes place long after all trace of organized structure in the implanted tissue has disappeared, and it is difficult to conceive of its being due to any other cause than the presence of some definite chemical substance within the cyst, which is characteristic of, and specific for, each species.

Examination of the contents of the cysts showed, in all cases where the development of ciliated epithelium had occurred, that an orange granular substance, and blood corpuscles in various stages of degeneration, were present. These orange granules resembled in appearance the orange-coloured yolk substance of the ripe ova, and the amount of this granular substance within the cysts seemed to be independent of the length of time during which the implanted tissue was allowed to remain in the muscle. If implantation of pieces of the ovary of approximately equal size were made, examination of the contents of a cyst after six days showed as much of this substance present as in a similar cyst after 120 days; hence it appears that this substance cannot escape through the cyst wall. When it is considered that the development of the ciliated epithelial lining only occurs as a reaction to the implantation of ripe ova, containing a plentiful supply of the orange-coloured yolk substance, there is at least a possibility that the orange substance within the cysts bears a close chemical relation to the yolk substance, and that the development of ciliated epithelium from the fibroblasts lining the cyst is a specific reaction to its presence.

Though admittedly based on no experimental evidence, it is suggested as a possible explanation of the phenomena that some substance is formed as a result of the ingestion of these orange granules by the blood corpuscles, and their subsequent degeneration within the cyst: that the granules themselves remain unchanged, and are again set free on the disintegration of the corpuscles, and that their action on the protoplasm of the corpuscles is merely catalytic. This substance, produced from the blood corpuscles, is probably a fluid, and would be slowly and continuously formed as long as blood corpuscles could pass through the walls of the cyst. The action of this substance on the fibroblasts forming the walls of the cyst is to delay their return to the spindle shape typical of the resting condition, and eventually to set up those changes in the inner layer of fibroblasts resulting in their conversion into ciliated epithelium.

G. H. D.

A Review of the British Marine Cercariae. By Marie V. Lebour, M.Sc. (*Parasitology*, Vol. IV, No. IV, January, 1912.)

THE work in this paper on *Spelotrema excellens* Nicoll was partly carried out at Plymouth in April, 1911. The first host of this worm seems most commonly to be *Littorina obtusata; L. rudis* and *Paludestrina stagnalis* are also first hosts for it. The tailed cercaria occurs in sporocysts in the digestive gland of these molluscs, and possesses a stylet in its head by which presumably it bores its way into its second host, the green crab, *Carcinus maenas.* Here it encysts and gradually enlarges, loses its stylet, and assumes the *Spelotrema* form, the walls of the cyst thickening until a certain size is reached, when the cercaria rests. The full-grown cysts are found all the year round, but no young stages in the winter. All the Plymouth crabs seem to be infected and in almost every organ, the digestive gland and muscles being the favourite parts. The final host of the worm is probably the herring gull, *Larus argentatus.*

M. V. L.

Contributions to the Knowledge of the Laminarias. (*Beiträge zur Kenntnis der Laminarien.*) By C. Killian. (*Zeitschrift für Botanik*, 1911, Heft 7.)

NOTWITHSTANDING the large number of papers on Laminaria, its development was until recently but little understood, for it was only in 1910 that Drew published an account of the first successful cultures. Thanks to the previous work of that author, the writer succeeded in getting the swarm spores of *Laminaria digitata* to germinate. First of all there developed a germinal thread consisting of a few cells; from certain of these cells secondary, one-layered laminae sprouted, which were joined to the germinal thread by colourless rootlet cells. Later these rootlet cells disappeared, and then the laminae, freed from the germinal threads, developed on their lower sides rhizoids which established a direct attachment to the rocks. In the young plants the cell divisions proceed at first regularly over the whole surface. It is only later that the intercalary growing point is formed between the rhizoids and the leaf; in addition growth tissue develops at the edge of the leaf and makes the extension of the surface in breadth possible. Gradually the young plant becomes many-layered; the outer layers consist of isodiametric, the innermost of elongated cells with rich intercellular substance; only the point remains for the time one-layered. It must, however, be noted that in the stalk the rows of newly formed central tissue remain near together; in the leaves, on the other hand, where the growth in breadth strongly predominates, the original longitudinal rows become more and more pulled asunder. A further complication is added, in that (1) between these rows of the internal tissue connections already existed which are now simply mechanically pulled apart, and (2) new connections of this kind are formed. These new connections occur through two cells, which develop at opposite points on the longitudinal rows, growing towards one another and becoming fused. From a cell resulting from such fusion a chain of cells then forms by transverse division. In still later stages the single cells of the longitudinal and transverse rows of the central tissue, whose power of growth ceases, become mechanically deformed through the rapid growth of the leaf. In this way there finally result tube-shaped cells which, according to their age and the degree of the tension to which they are subjected, differ from one another in appearance. We can thus understand the confused mass of cells of all possible shapes which is presented by a section through the central tissue of an old plant. Similar pictures such as one finds in other Laminarias suggest that in them also tissue development takes the same course.

A point in the anatomy of the haptera is worth noting, viz., that in these there are internally no elongated, tube-shaped cells. As is well known, these algae possess at first an adhesive disc from which the organs of attachment take origin, the construction and shape of the latter being modified by external conditions.

It is well known that the different representatives of the Laminariae are characterized by regularly split leaves. In order to understand the processes which bring about the formation and the repair of these slits, experiments were undertaken by the author with a view to explaining the reactions and tissue-formations which follow as the result of wounding. It was shown that *Laminaria digitata* after being wounded reacted rapidly and vigorously. The differentiation in the stem, leaf, and haptera, the age and the corresponding specialization of the tissue cause manifold differences in the regeneration, and the direction and character of the wound also exert an influence. As regards the tearing process, which may be especially well seen in *Laminaria hyperborea*, it appeared that these plants slit their leaves autonomously. This is made clear by a microscopical investigation of the leaf surface in the neighbourhood of such a tear. The epidermis at such a place bends inwards from both leaf faces more and more into the inner tissue. One cannot, be it noted, speak of any external wounding. When the epidermis has forced itself in this way into the central tissue the latter finally becomes torn and the further growth of the epidermis closes the wound. From this normal slit-formation and healing we must distinguish the purely mechanical splitting, which heals in exactly the same way as the wounds in the experiments already mentioned. In nature both kinds of slit-formation run into one another. In other Laminarias a similar state of things seems to exist.

Field observations show that Laminarias have definite requirements as to light, heat, salinity, change of water, etc., at each different age. Differences in the external form of the different parts of the thallus clearly depend upon changes in these conditions. Laboratory experiments showed that Laminarias from different localities in the neighbourhood of Plymouth possessed different degrees of elasticity, and algae from places where wave movement was slight were stretched, with one and the same load per unit area of section, to a greater extent than the more resistant individuals from very exposed localities. Plants which grew under similar conditions showed, on the contrary, an equal degree of extension. *Laminaria digitata* stretched similarly to *L. saccharina*, whilst the surf-loving species *L. hyperborea* and *bulbosa* were more resistant.

K.

Notes on the Choanoflagellate Genera *Salpingoeca* and *Polyoeca*, with Description of *Polyoeca dumosa*, sp. n. By J. S. Dunkerly, B.Sc. (*Annals Mag. Nat. Hist.*, Ser. 8, Vol. V, Feb., 1910, pp. 186–191.)

Note on our Present Knowledge of the Choanoflagellata. By J. S. Dunkerly, B.Sc. (*Journ. Quekett Micr. Club*, April, 1910, pp. 19–24.)

THE following is a summary of observations on some Flagellata at Plymouth Marine Biological Station during the month of August, 1909.

Certain late division stages of *Salpingoeca vaginicola* and the setting free of the daughter cell were observed, and a new species of *Polyoeca*, *P. dumosa*, was described from material taken from a tank in the Laboratory, the type species, *P. dichotoma* S. K. not having been recorded since 1874. The above results were described with figures in *Ann. and Mag. Nat. Hist.*, Ser. 8, Vol. V, 1910, and in addition to the above, the following species of Choanoflagellidae were found:—

Codonosiga botrytis J. Cl.
Codonocladium umbellatum Tat.
Salpingoeca vaginicola St.
" *ampulla* S. K.
" *urceolata* S. K.
" *napiformis* S. K.
Polyoeca dumosa n. sp.

Although careful search was made, none of the double-collared forms, *Diplosiga* Frenz. or *Diplosigopsis* Francé, were found, and I have been unable to find these in material from many different localities. That another interpretation than that of a double collar may be placed upon some of the published figures of these forms I have pointed out in another place (*Journ. Quekett Microsc. Club*, Ser. 2, Vol. XI, 1910).

J. S. D.

Marine Biological Association of the United Kingdom.

Report of the Council, 1911–12.

The Council and Officers.

Four meetings of the Council have been held during the year, at which the average attendance has been 13. The meetings have been held in the rooms of the Royal Society and of the Linnean Society, and the thanks of the Association are due to the Councils of these two Societies for allowing their rooms to be used. A Committee of the Council visited the Plymouth Laboratory on June 1st, and inspected both the buildings and boats belonging to the Association.

The Plymouth Laboratory.

Some expense has been incurred this year in re-pointing the upper parts of the south front of the building. This was rendered necessary owing to the exposed position of the building and the exceptionally wet and stormy weather of the past winter, in consequence of which the walls became damp and the ends of the roof joists were threatened. It is hoped that the work now done will prevent a repetition of the trouble. In other respects the buildings are in good condition, and the engines and pumps are in good working order. The Aquarium has been repainted and redecorated throughout, and the general equipment of the Laboratory has been maintained in an efficient state.

It was again necessary to hire a room at the Yacht Club during the Easter vacation in order to accommodate the students attending classes at the Laboratory.

The Boats.

The *Oithona* was refitted during the winter, and has been running again since the beginning of March.

Col. G. M. Giles was kind enough to present to the Laboratory a twenty-five-foot motor-boat, which is proving very useful for work in Plymouth Sound. The late Mr. W. I. Beaumont—whose recent death by accidental drowning whilst yachting in Loch Fyne is a cause of deep regret to the many friends who have met him at the Laboratory, which he has constantly visited during the last seventeen years—also presented the Laboratory with a small punt, which is very convenient for use with the motor-boat.

A floating cage or tank has been constructed and moored in Cawsand Bay. This has proved very useful for storing animals of various kinds before bringing them to the Laboratory. It has been found that animals, especially those captured in deeper water, live much better in the Laboratory tanks after having spent some time in this floating tank.

The Staff.

In addition to the members of the staff mentioned in last year's Report, Mr. L. R. Crawshay has been employed temporarily to complete his report on the fauna of the deep-water grounds of the English Channel which lie to the south-west of the Eddystone.

Occupation of Tables.

The following Naturalists have occupied tables at the Plymouth Laboratory during the year:—

K. H. BARNARD, B.A., Cambridge (General Zoology).
Prof. CHAS. CHILTON, D.SC., New Zealand (Amphipoda).
W. DE MORGAN, Plymouth (Experimental Zoology).
Miss D. DALE, Cambridge (assisting Mr. G. R. Mines).
Prof. A. DENDY, F.R.S., London (Sycon).
G. H. DREW, B.A., Plymouth (Tissue Transplantation).
Miss K. DUFFY, Manchester (Cycloporus).
Señor FERRER, Madrid (General Zoology).
H. M. FUCHS, Cambridge (Hybridization of Echinus).
E. S. GOODRICH, F.R.S., Oxford (Polychaeta).
J. GRAY, Cambridge (Experimental Zoology).
Dr. M. HASPAR, Marburg (Polyzoa).
H. LYSTER JAMESON, D.SC., London (Mussels).
K. R. LEWIN, B.A., Cambridge (Protozoa).
Miss JORDAN LLOYD, Cambridge (Experimental Zoology).
Mrs. MATTHEWS, Plymouth (Development of Alcyonium).
G. R. MINES, M.A., Cambridge (Physiology of Heart Muscles).
G. E. NICHOLLS, B.SC., London (Nervous System of Fishes).
C. SHEARER, M.A., Cambridge (Experimental Zoology and Dinophilus).
GEOFFREY SMITH, M.A., Oxford (Blood of Carcinus).
Prof. J. STEPHENSON, D.SC., Lahore (Polychaeta).
J. TAIT, D.SC., Edinburgh (Blood of Crustacea).
Miss A. W. THOMSON, Oxford (Fish Parasites).

The usual Easter Vacation Course in Marine Biology was this year conducted by Mr. L. R. Crawshay, and was attended by thirteen students from Oxford and Cambridge. Dr. Cresswell Shearer again brought a class of five students from Cambridge for a practical course in Experimental Embryology.

Mr. E. J. Lewis brought a class of seven boys from Oundle School for practical work during the last fortnight of April, and Messrs. J. T. Cunningham and H. B. Lacey, with four students from the South-Western Polytechnic, Chelsea, spent Whitsuntide in working at the Laboratory.

General Work at the Plymouth Laboratory.

The Director has continued his experimental investigations into the conditions of growth of marine plankton diatoms. Several of the latter can now be cultivated in almost entirely artificial solutions prepared from the purest chemical salts obtainable, and in this way an opportunity is afforded for studying directly the effect of minute changes in the composition of the medium in which these diatoms live. It is hoped that these experiments will eventually throw considerable light upon the causes which bring about the variations in the quantity of minute vegetable life which take place in the sea itself. Since it is this minute vegetable life which forms the fundamental food-supply of all marine animals, an exact knowledge of the conditions under which it can best flourish is of importance from both a theoretical and a practical point of view.

Mr. D. J. Matthews has been carrying out investigations on the chemistry of sea-water. These investigations have been directed chiefly to those points in which the Laboratory tank-water differs from the normal sea-water of the district. Determinations of the hydrogen-ion concentration by Sörensen's colorimetric method showed that the tank-water was decidedly less alkaline than the outside water, the alkalinity measured by this process being the true alkalinity as opposed to the titration alkalinity, which only measures the quantity of base combined with acids volatile when boiled with a dilute mineral acid. Analysis has shown that this deficient alkalinity is due not only to an excess of carbonic acid, but also to a far greater excess of nitrates and phosphates. A quantity of carbonate of soda added gradually to the water caused a considerable temporary improvement, and plutei were reared nearly to metamorphosis under the circulation. The method has been discontinued on the large scale on account of the continual rise in the relative amount of sodium salts which it causes, but small-scale experiments in which it is possible to measure the changes more accurately and to keep a complete record of the results are being carried out. As the nitrates, and also the large excess of ammonia are almost certainly of bacterial origin, some experiments have been made on treatment with a solution of bleaching powder. This reduced the ammonia to one-third and killed all the bacteria, but the method was abandoned as it caused temporary discomfort to the anemones and other invertebrates in the tank. Treatment by electrolysis is now being tried and has given encouraging results. The ammonia is reduced and all the bacteria killed without affecting any other living organisms present, whether fish or invertebrates, and samples of the water removed in flasks while still smelling of hypochlorous acid gave rise to an abundant growth of diatoms and green algae. There is a possible commercial application of this process to the treatment of shell-fish from sources open to contamination, and experiments on these lines are being carried out.

The dissolved oxygen has been determined from time to time, and the water has been always found saturated with this gas, even when the tank was cut off from the general circulation and run on aeration alone.

At Christmas there is always considerable difficulty in obtaining food for the fish, and advantage was taken of this period of enforced starvation to determine the rate of increase of the bacteria. A small tank containing a rather large number of pollack was cut off from the circulation and run on aeration alone. After three days, during which the fish had no food, the number of bacteria was only 3200 per cubic centimetre; five hours after feeding the number had risen to 46,000, and next day, after a second lot of food had been given, there were over 150,000 per cubic centimetre. This last number is probably much higher than the average for the whole system.

Colorimetric examination of the outside water has shown that the alkalinity during the early part of the spring of the present year (1912) was far less than during the previous late summer and autumn.

Mr. F. J. Bridgman has been occupied in an investigation of the age of plaice found in the western portion of the English Channel. The otoliths of a large number of fishes obtained from the neighbourhood of Plymouth and from the bays on the Devon coast to the east of Start Point have been examined for this purpose.

During the winter a Report on the Natural History of the American Slipper-Limpet (*Crepidula fornicata*) was prepared by Mr. J. H. Orton for the Kent and Essex Sea Fisheries Committee. In preparing this report it became evident that definite information as to the food of Crepidula would be valuable to the Kent and Essex oyster-farmers, whose oyster-beds are being overrun with this animal. A careful examination of the ingested food of the slipper-limpet was therefore undertaken, and a comparison made with the ingested food of oysters taken from the same grounds. This comparison established the curious fact that both kinds of animals were feeding on almost exactly the same food, that is, the various kinds of organisms found floating in the sea. After this conclusion had been arrived at, the way in which the slipper-limpet obtains its food was discovered. This observation confirmed the conclusion that the slipper-limpet feeds on the same food as the oyster, for it was found that it feeds in essentially the same way as the oyster, namely, by using its gill as a water-pump, and at the same time as a sieve for filtering the organisms from the water-current it produces. It was therefore made clear that the slipper-limpet is a keen competitor with the oyster for food and space.

This report was followed up by investigations leading to an explanation of the mechanism of the food-current in the slipper-limpet, the oyster, and other Gastropods and Lamellibranchs, and to slight additions to our knowledge of the mode of feeding in the Oyster, the Scallop, the Cockle, the Mussel, and some other bivalves.

Mr. Orton has also been occupied with general studies on the invertebrate fauna of the neighbourhood. A special feature of the collecting during the year has been the working of a fresh piece of rich shore collecting-ground immediately north of the Mewstone, where large gatherings of *Echinus miliaris*, *Cucumaria saxicola*, *Cucumaria normani*, and many other invertebrates have been made. Investigations are being made on the apparent pairing habits of *Echinus miliaris* and on the question of specific difference in the two species of Cucumaria mentioned above. A research on rate of growth in invertebrates has been begun by laying down marked bottles on the various grounds in Plymouth Sound. Useful material for this research was obtained last September from the floating raft moored in Cawsand Bay.

Mr. L. R. Crawshay has completed his report on the fauna of the deeper portion of the English Channel to the south-west of the Eddystone, a region which was practically unexplored previous to his investigations. This report is published in the Journal of the Association. In connection with the work, Dr. R. Hartmeyer, of Berlin, has described the ascidian fauna.

Messrs. Shearer, De Morgan, and Fuchs published a valuable paper on the hybridization of Echinoids in the Journal in October last, and they have since that time been continuing experimental work on the same subject.

Mr. G. H. Drew, who holds a Beit Memorial Fellowship for Medical Research, has worked at the Laboratory for the greater part of the year, and has continued to carry out experimental investigations on tissue growth, which bear directly on the cancer problem.

Mr. G. R. Mines, of the Cambridge Physiological Laboratory, assisted by Miss D. Dale, also of Cambridge, carried out at the Laboratory during last summer an important physiological investigation on the relations to electrolytes of the heart muscles of different species of skates and rays and of the mollusc, *Pecten maximus.* Mr. Mines's work showed what useful facilities the Laboratory can offer for physiological investigation, and it is the desire of the Council to do all in their power to encourage researches of this kind.

A fifth Blue Book has just been published by H.M. Stationery Office, containing the Fourth Report upon the work done by the Marine Biological Association in connection with the International Investigation of North Sea Fisheries. This report contains detailed memoirs on the experiments with marked plaice carried out in the North Sea, and on the transplantation of plaice from the inshore grounds to the Dogger Bank. The details of the trawling experiments made by the s.s. *Huxley* in the years 1906–9 are also recorded.

Published Memoirs.

The following papers, either wholly or in part the outcome of work done at the Laboratory, have been published elsewhere than in the official publications of the Association:—

BLOOMER, H. H. *Anatomy of British Species of Psammobia.* Proc. Malac. Soc., vol. 9, 1911, pp. 231–9.

DREW, G. H. *A Note on some attempts to cause the Formation of Cytolysins and Precipitins in certain Invertebrates.* Journal of Hygiene, vol. 11, 1911, pp. 188–92.

DREW, G. H. *A Note on the Application of Giemsa's Romanowsky Stain to the Blood and Tissues of Marine Invertebrates.* Parasitology, vol. 4, 1911, pp. 19–21.

LEBOUR, M. V. *A Review of the British Marine Cercariae.* Parasitology, vol. 4, 1912, pp. 416–56.

MACBRIDE, E. W. *Two Abnormal Plutei of Echinus, and the light which they throw on the Factors in the Normal Development of Echinus.* Quart. Journ. Micr. Sci., vol. 57, 1911, pp. 235–50.

MATTHEWS, D. J. *Scottish Hydrographical Research.* Internat. Revue, Bd. 4, 1912, pp. 493–504.

MINES, G. R. *Note on the Mechanism of Discharge of the Cuvierian Organs of Holothuria nigra.* Quart. Journ. Micr. Sci., vol. 57, 1912, pp. 301–4.

MINES, G. R. *On the Relations to Electrolytes of the Hearts of Different Species of Animals. I. Elasmobranchs and Pecten.* Journal of Physiology, vol. 43, 1912, pp. 467–506.

NICHOLLS, G. E. *An Experimental Investigation on the Function of Reissner's Fibre.* Anat. Anzeiger, Bd. 40, 1912, pp. 409–32.

ORTON, J. H. *An Account of the Natural History of the Slipper-Limpet (Crepidula fornicata), with some remarks on its occurrence on the Oyster Grounds on the Essex coast.* Printed by the Kent and Essex Sea Fisheries Committee, Jan. 1912, for distribution amongst the Committee.

Sexton, E. W. *On the Amphipod Genus Leptocheirus.* Proc. Zool. Soc., 1911, pp. 561–94.

Sexton, E. W. *A new Amphipod Species, Tryphosites alleni.* Ann. & Mag. Nat. Hist., ser. 8, vol. 7, 1911, pp. 510–13.

Shearer, C. *The Problem of Sex Determination in Dinophilus gyrociliatus.* Quart. Journ. Micr. Sci., vol. 57, 1912, pp. 329–71.

Smith, G. *Studies in the Experimental Analysis of Sex. VII. Sexual Changes in the Blood and Liver of Carcinus maenas.* Quart. Journ. Micr. Sci., vol. 57, 1911, pp. 251–65.

Taylor, T. H. *An Aid in the Study of Nematocysts.* Proc. Roy. Phys. Soc. Edin., vol. 18, 1912, pp. 235–40.

Woodland, W. N. F. *On some Experimental Tests of Recent Views concerning the Physiology of Gas Production in Teleostean Fishes.* Anat. Anzeiger, Bd. 40, 1911, pp. 225–42.

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The thanks of the Association are due for the following books and current numbers of periodicals presented to the Library during the past year:—

Académie Imp. des Sciences de St. Pétersbourg. Bulletin.
American Museum of Natural History. Annual Report.
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Australian Museum. Memoirs.
—— Records.
—— Report.
Bergens Museum. Aarsberetning.
—— Aarbok.
—— An Account of the Crustacea of Norway, etc. By G. O. Sars.
Bermuda Biological Station for Research. Contributions.
Bernice Pauahi Bishop Museum, Honolulu. Fauna Hawaiiensis.
—— Memoirs.
—— Occasional Papers.
Board of Agriculture and Fisheries. Annual Report of Proceedings under the Salmon and Fresh-water Fisheries Acts.
—— Annual Report of Proceedings under Acts relating to Sea Fisheries.
—— Monthly Return of Sea Fisheries, England and Wales.
—— Report of Proceedings of Annual Meeting.
—— Report on the Research Work of the Board in relation to the Plaice Fisheries of the North Sea.
Bristol Naturalists' Society. Proceedings.
British Association for the Advancement of Science. Report.
British Museum. Catalogue of the Fresh-water Fishes of Africa in the British Museum.
Brown University. Contributions from the Biological Laboratory.
Bulletin Scientifique de la France et de la Belgique.

Bureau of the Productive Industries, Formosa. Icones Plantarum Formosanarum, nec non et Contributiones ad Floram Formosanam.
Bureau of Science, Philippine Islands. Journal of Science.
Cairo Zoological Gardens. Report.
Carnegie Institution of Washington: Dept. of Experimental Evolution. Annual Report of the Director.
—— Dept. of Marine Biology. Annual Report of the Director.
—— Papers from the Tortugas Laboratory.
—— On Germinal Transplantation in Vertebrates. By W. E. Castle and J. C. Phillips.
—— Experiments with *Drosophila ampelophila* concerning Evolution. By F. E. Lutz.
Ceylon Marine Biological Laboratory. Reports.
Club Montanyenc, Barcelona. Butlletí.
College of Science, Tokyo. Journal.
College voor de Zeevisscherijen. Verslag van den Staat der Nederlandsche Zeevisscherijen.
Colombo Museum. Director's Report.
—— Spolia Zeylanica.
R. Comitato Talassografico Italiano. Bollettino.
—— Memoria.
Comité du Laboratoire de Carlsberg. Comptes Rendus.
Conchological Society of Great Britain and Ireland. Journal of Conchology.
Conseil perm. internat. pour l'Exploration de la Mer. Bulletin Trimestriel des Résultats acquis pendant les Croisières Périodiques.
—— Publications de Circonstance.
—— Bulletin Hydrographique.
—— Bulletin Statistique.
Cornwall Sea Fisheries Committee. Reports.
Danish Biological Station. Report.
Dept. of Agriculture, etc., Ireland. Report.
—— Scientific Investigations.
Dept. of Commerce and Labor, Bureau of Fisheries, U.S.A. Pamphlets.
—— Report of the Commissioner of Fisheries.
—— Reports in relation to the Condition of Seal Life on the Rookeries of the Pribilof Islands, and to Pelagic Sealing in Bering Sea and the North Pacific Ocean in the years 1893–5. With Atlas.
Dept. of Fisheries, New South Wales. Annual Report.
—— On the Need for more Uniformity in the Vernacular Names of Australian Edible Fishes. By D. G. Stead.
—— The Future of Commercial Marine Fishing in New South Wales. By D. G. Stead.
Dept. of Marine and Fisheries, Canada. Annual Report.
Dept. of Trade and Customs, Melbourne. *Onchocerca gibsoni:* The cause of Worm Nodules in Australian Cattle. With Notes on Worm Nests in Australian Cattle and in Camels. By J. B. Cleland and T. H. Johnston.
—— Zoological Results of the Fishing Experiments carried out by the F.I.S *Endeavour* 1909–10.
Deutschen Fischerei-Vereins. Zeitschrift für Fischerei.
Deutscher Seefischerei-Verein. Mitteilungen.

Dominion Museum. Bulletin.
Durban Museum, Natal. Report.
La Feuille des Jeunes Naturalistes.
Field Museum of Natural History. Report.
Finnländische Hydrographisch-Biologische Untersuchungen. Abhandlungen.
Fisheries Society of Japan. Journal.
The Fisherman's Nautical Almanac. By O. T. Olsen.
Fishery Board of Scotland. Annual Report.
—— Scientific Investigations.
Fiskeri-Beretning.
Government of Bombay. The Present Depletion of the Oyster Bed of Sind; its Causes and the Remedies. By J. Hornell.
Government Museum, Madras. Report.
Illinois State Laboratory of Natural History. Bulletin.
Imperial Cancer Research Fund. Report.
Indian Museum. Memoirs.
—— Records.
—— Report.
Institut Océanographique. Annales.
R. Irish Academy. Proceedings.
Kommission zur wissenschaftlichen Untersuchung der Deutschen Meere, etc. Wissenschaftliche Meeresuntersuchungen.
Kommissionen for Havundersögelser, Copenhagen. Meddelelser.
—— Skrifter.
K. Bayerischen Akademie der Wissenschaften, München. Abhandlungen.
—— Sitzungsberichte.
Kgl. Danske Videnskabernes Selskab. Oversigt.
—— Skrifter.
K. Fysiografiska Sällskapets i Lund. Handlingar.
Kgl. Norske Videnskabernes Selskab. Skrifter.
Laboratoire Biologique de St. Pétersbourg. Bulletin.
Lancashire and Western Sea Fisheries. Superintendent's Report.
Leland Stanford Junior University. Publications.
Linnean Society. Journal.
—— Transactions.
Liverpool Biological Society. Proceedings and Transactions.
Madras Fisheries Bureau. Bulletin.
Manchester Microscopical Society. Annual Report and Transactions.
Marine Biological Association of the West of Scotland. Report.
Marine Biological Laboratory, Woods Holl. Biological Bulletin.
—— Report.
Marine Biological Station, Port Erin. Report.
Marine Dept., New Zealand. Report.
Mededeelingen over Visscherij.
Meteorological Office. Monthly Pilot Charts, North Atlantic and Mediterranean.
—— Monthly Pilot Charts, Indian Ocean and Red Sea.
—— Annual Report of the Committee.
R. Microscopical Society. Journal.
Musée Océanographique de Monaco. Bulletin.

Museo de La Plata. Catálogo de la Seccion Antropológica.
—— Revista.
Museo Nacional, Buenos Aires. Anales.
Museo Nacional de Chile. Boletin.
Museum of Comparative Zoology, Harvard College. Bulletin.
—— Memoirs.
—— Report.
Muséum National d'Histoire Naturelle, Paris. Bulletin.
The Museums Journal.
Natal Scientific Society. Transactions and Proceedings.
Natural History Society of New Brunswick. Bulletin.
Natural History Transactions of Northumberland, Durham, and Newcastle-on-Tyne.
Naturforschende Gessellschaft in Basel. Verhandlungen.
Naturhistorischen Museum, Hamburg. Mitteilungen.
Nederlandsche Dierkundige Vereeniging. Verslag.
New York Academy of Sciences. Annals.
New York Zoological Society. Bulletin.
New Zealand Institute. Transactions and Proceedings.
Norges Fiskeristyrelse. Aarsberetning vedkommende Norges Fiskerier.
Northumberland Sea Fisheries Committee. Report on Scientific Investigations.
La Nuova Notarisia.
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Oxford University Museum. Catalogue of Books added to the Radcliffe Library.
Physiographiske Forening, Christiania. Nyt Magazin for Naturvidenskaberne.
Plymouth Institution. Report and Transactions.
Quarterly Journal of Microscopical Science. (Presented by Sir E. Ray Lankester, K.C.B., F.R.S.)
Queensland Museum. Annals.
Rijksinstituut voor het Onderzoek der Zee. Helder. Jaarboek.
—— Verhandelingen.
Royal Commission on Sewage Disposal. Reports.
Royal Society of Edinburgh. Proceedings.
—— Transactions.
Royal Society of London. Philosophical Transactions.
—— Proceedings.
—— Year-Book.
Royal Society of Victoria. Proceedings.
Scottish Oceanographical Laboratory. Report of the Scientific Results of the Voyage of S.Y. *Scotia*.
Selskabet for de Norske Fiskeriers Fremme. Norsk Fiskeritidende.
Senckenbergische naturforschende Gesellschaft, Frankfurt. Bericht.

Smithsonian Institution. Opinions rendered by the International Commission on Zoological Nomenclature.
Sociedad Geográfica de Lima. Boletin.
Sociedad Scientifica de São Paulo. Revista.
Societas pro Fauna et Flora Fennica. Acta.
—— Meddelanden.
Société Belge de Géologie, etc. Bulletin.
—— Nouveaux Mémoires.
Société Centrale d'Aquiculture et de Pêche. Bulletin.
Société de Géographie de Finlande. Atlas de Finlande.
Société d'Océanographie du Golfe de Gascogne. Mission Arctique commandée par M. Charles Bénard. VI. Stations Scientifiques. Cartographie Météorologie.
—— Le Sauvetage Maritime. By E. Debrosse.
Société Imp. Russe de Pisciculture et de Pêche. Vyestnīk R'ibopom'shlennostī.
Société Zoologique de France. Bulletin.
—— Mémoires.
South African Museum. Annals.
Station Aquicole de Boulogne-sur-Mer. Annales.
Kgl. Svenska Vetenskaps-Akademien. Arkiv för Botanik.
—— Arkiv för Zoologie.
—— Handlingar.
Tôhoku Imperial University, Japan. Science Reports.
Torquay Natural History Society. Journal.
Transvaal Museum. Annals.
United States National Herbarium. Contributions.
United States National Museum. Bulletin.
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—— Report.
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University of California. Publications. Zoology, Physiology, Botany.

University of Pennsylvania. Contributions from the Botanical Laboratory.

—— Contributions from the Zoological Laboratory.

University of Toronto. Studies.

Kgl. Vetenskaps Societeten, Upsala. Nova Acta.

—— Tvahundraarsminne.

Visscherij-Station, Batavia. Mededeelingen.

Visschershaven, Ijmuiden. Jaarsverslag.

Zoologische Sammlung des Bayerischen Staates. Beiträge zur Naturgeschichte Ostasiens.

Zoological Society of Japan. Annotationes Zoologicae Japonenses.

Zoological Society of London. Proceedings.

—— Transactions.

Zoologisches Museum, Berlin. Bericht.

—— Mitteilungen.

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Alward, G. L. The Development of the British Fisheries during the Nineteenth Century, with Special Reference to the North Sea.
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Billard, A. Note sur un nouveau genre et une nouvelle espèce d'Hydroïde *Sibogella erecta*.
—— Note préliminaire sur les espèces nouvelles de Plumulariidae de l'Expédition du *Siboga*.
—— Note sur le *Plumularia catharina* Johnst.

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Cushman, J. A. A Monograph of the Foraminifera of the North Pacific Ocean. Pt. II. Textulariidae.
Dakin, W. J. Notes on a New Coccidian (*Merocystis kathae* n. gen. et sp.) occurring in the Renal Organ of the Whelk.
—— Notes on the Biology of Fish Eggs and Larvae.
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Hamburger, C., and von Buddenbrock. Nordische Ciliata mit Ausschluss der Tintinnoidea.

Harding, W. A. A Revision of the British Leeches.

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Hartmeyer, R. Die geographische verbreitung der Ascidien.

—— Revision von Heller's Ascidien aus der Adria. I. Die Arten der Gattung *Ascidia*.

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Hatschek, B. Das neue Zoologisch System.

Helland-Hansen, B. Neue Forschungen im Nordlichen Atlantischen Ozean.

Herdman, W. A. On the occurrence of *Amphidinium operculatum*, Clap. & Lach., in vast quantity, at Port Erin (Isle of Man).

Heron-Allen, E., and Earland, A. The Recent and Fossil Foraminifera of the Shore-Sands at Selsey Bill, Sussex.

Hickson, S. J. On Ceratopora, the Type of a New Family of Alcyonaria.

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Hoek, P. P. C. Les Clupéides (le Hareng excepté) et leurs migrations.

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—— Note sur la Phylogénese de l'Insecte.

—— Sur l'Ontogénèse de l'Insecte.

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—— Beiträge zur Charakterisierung des Tier- und Pflanzen-lebens in den von der *Deutschland* wahrend ihrer Fahrt nach Buenos Ayres durchfahrenen Gebieten des Atlantischen Ozeans.

Lohmann, H. Untersuchungen über das Pflanzen- und Tierleben der Hochsee im Atlantischen Ozean während der Ausreise der *Deutschland.*

M'Intosh, W. C. On the Storm of January, 1912, in the Bay of St. Andrews.

Man, J. G. de. Helminthologische Beiträge.

—— Sur deux espèces et une variété nouvelles du Genre Palaemon Fabr. provenant du Congo Belge.

Mangan, J. The Occurrence of *Necrobia* and *Dermestes* in Cotton Bales.

Masterman, A. T. Third Report on Later Stages of Pleuronectidae.

Mayer, A. G. Medusae of the World.

—— Ctenophores of the Atlantic Coast of North America.

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—— On the Relations to Electrolytes of the Hearts of Different Species of Animals. I. Elasmobranchs and Pecten.

Murie, J. "Slipper-Limpet" or "Boat-Shell" (*Crepidula fornicata*): its Introduction and Influence on Kent and Essex Oyster-Beds.

Nicholls, G. E. An Experimental Investigation on the Function of Reissner's Fibre.

Okamura, K. Some Littoral Diatoms of Japan.

Orton, J. H. An Account of the Natural History of the Slipper-Limpet (*Crepidula fornicata*), with some Remarks on its Occurrence on the Oyster-Grounds on the Essex Coast.

Paulsen, O. The Plankton on a Submarine Bank.

Pettersson, O. Studien über die Bewegungen des Tiefenwassers und ihren Einfluss auf die Wanderung der Heringe.

Popta, C. M. L. Ueber Fische von Wladiwostok und von Blagoweschtensk a. Amur, gesammelt von Herrn Dr. P. v. Wittenburg.

—— Vorlaüfige mitteilung über neue Fische von Lombok.

—— Ichthyographische Übersicht von Lombok.

—— La fonction de la Vessie aèrienne des Poissons.

Ritchie, J. New Species and Varieties of Hydroida thecata from the Andaman Islands.

—— Note on a Rare Plumularian Hydroid, *Cladocarpus formosus.*

—— Is the Hydroid, *Selaginopsis mirabilis,* a Native of British Seas?

—— Contribution to our knowledge of the Hydroid Fauna of the West of Scotland. Being an Account of Collections made by Sir John Murray, K.C.B., on S.Y. *Medusa.*

—— Two Unrecorded *Challenger* Hydroids from the Bermudas, with a note on the Synonymy of *Campanularia insignis.*

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—— On the Distribution of the Thorny Lobster (*Palinurus vulgaris*) in British Waters.

—— On an Entoproctan Polyzoon (*Barentsia benedeni*) new to the British Fauna, with Remarks on Related Species.

—— Supplementary Report on the Hydroids of the Scottish National Antarctic Expedition.

Ritchie, J., and McIntosh, D. C. On a Case of Imperfect Development in *Echinus esculentus.*

Schaxel, J. Das Verhalten des Chromatins bei der Eibildung einiger Hydrozoen.

Schaxel, J. Plasmastrukturen, Chondriosomen und Chromidien.

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—— Die Morphologie des Eiwachstums und der Follikelbildungen bei den Ascidien. Ein Beitrag zur Frage der Chromidien bei Metazoen.

—— Die Bedeutung des Chromatins nach Untersuchungen an Metazoenzellen.

Schröder, B. Adriatisches Phytoplankton.

—— Phytoplankton von Westindien.

Sexton, E. W. A New Amphipod Species, *Tryphosites alleni.*

—— On the Amphipod Genus Leptocheirus.

—— The Amphipoda collected by the *Huxley* from the North Side of the Bay of Biscay in August, 1906.

Shearer, C., De Morgan, W., and Fuchs, H. M. Preliminary Notice on the Experimental Hybridization of Echinoids.

Shipley, A. E. Strongylosis in the Sheep.

—— The Diseases of Hive-Bees.

Southwell, T. The Pearl-Inducing Worm.

Stebbing, T. R. R., and Herdman, W. A. The Terms Polyzoa and Bryozoa.

Tait, J. A Simple Method of Observing the Agglutination of the Blood Corpuscles in Gammarus.

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—— Blood Coagulation in the Amphipod, Gammarus.

Taylor, T. H. An Aid in the Study of Nematocysts.

Vayssière, A. Sur les Opisthobranches et sur les Marséniadés du golfe de Tadjourah.

Walker, A. O. On *Gammarus campylops* Leach.

—— Notes on *Jassa falcata.*

Woodcock, R. Colour Varieties of *Donax variegatus* (Gmelin) from the Channel Islands.

Woodland, W. N. F. On some Experimental Tests of Recent Views concerning the Physiology of Gas Production in Teleostean Fishes.

Donations and Receipts.

The receipts for the year include the grants from His Majesty's Treasury (£1000) and the Worshipful Company of Fishmongers (£400), Special Donations (£204), Annual Subscriptions (£149), Rent of Tables in the Laboratory (£71), Sale of Specimens (£478), Admission to Tank Room (£94).

The following is a list of the Special Donations :—

	£	s.	d.
G. P. Bidder, Esq.	100	0	0
H. Swithinbank, Esq. . . .	100	0	0
Dr. Edgar Schuster . . .	4	4	0
G. H. Fox, Esq.		10	6
	£204	14	6

Vice-Presidents, Officers, and Council.

The following is the list of gentlemen proposed by the Council for election for the year 1912–13:—

President.

Sir E. Ray Lankester, K.C.B., LL.D., F.R.S.

Vice-Presidents.

The Duke of Abercorn, K.G., C.B.
The Duke of Bedford, K.G.
The Earl of Ducie, F.R.S.
The Earl of Stradbroke, C.V.O., C.B.
Lord Avebury, F.R.S.
Lord Walsingham, F.R.S.
The Right Hon. A. J. Balfour, M.P., F.R.S.
The Right Hon. Joseph Chamberlain, M.P.
The Right Hon. Austen Chamberlain, M.P.
W. Astor, Esq., M.P.
G. A. Boulenger, Esq., F.R.S.
A. C. L. Günther, Esq., F.R.S.
A. R. Steel-Maitland, Esq., M.P.
Sir John Murray, K.C.B., F.R.S.
Rev. Canon Norman, D.C.L., F.R.S.
Edwin Waterhouse, Esq.

Members of Council.

W. T. Calman, Esq., D.Sc.
Prof. A. Dendy, D.Sc., F.R.S.
G. H. Drew, Esq.
Prof. F. W. Gamble, D.Sc., F.R.S.
Sir Eustace Gurney.
Commander M. W. Campbell Hepworth, C.B., R.N.R.
Prof. J. P. Hill, D.Sc.
E. W. L. Holt, Esq.
Prof. E. W. MacBride, D.Sc., F.R.S.
P. Chalmers Mitchell, Esq., D.Sc., F.R.S.
Edgar Schuster, Esq., D.Sc.
Geoffrey W. Smith, Esq.
Prof. D'Arcy W. Thompson, C.B.

Chairman of Council.

A. E. Shipley, Esq., D.Sc., F.R.S.

Hon. Treasurer.

J. A. Travers, Esq., Tortington, Arundel.

Hon. Secretary.

E. J. Allen, Esq., D.Sc., The Laboratory, Citadel Hill, Plymouth.

The following Governors are also members of the Council:—

G. P. Bidder, Esq., M.A.
The Earl of Portsmouth (Prime Warden of the Fishmongers' Company).
Sir Richard Martin, Bart. (Fishmongers' Company).
The Hon. Nathaniel Charles Rothschild (Fishmongers' Co.).
Prof. G. C. Bourne, D.Sc., F.R.S. (Oxford University).
A. E. Shipley, Esq., D.Sc., F.R.S. (Cambridge University).
Prof. W. A. Herdman, D.Sc., F.R.S. (British Association).

Dr. *Statement of Receipts and Payments for*

		£ s. d.	£ s. d.
To Balance from last year:—			
Cash at Bank		316 0 4	
Cash in hand		6 14 9	
		322 15 1	
Less Bank Loan		300 0 0	22 15 1
,, Current Income:—			
H.M. Treasury		1,000 0 0	
The Worshipful Company of Fishmongers, including £200 on account of 1912–13		600 0 0	
Annual Subscriptions		146 4 0	
Composition Fee		15 15 0	
Rent of Tables, including £20 from the Trustees of the Ray Lankester Fund		71 19 6	1,833 18 6
,, Extraordinary Receipts:—			
Donations, per Report			204 14 6
,, Balance:—			
Loan from Bank		400 0 0	
Overdraft at Bank		63 1 0	
		463 1 0	
Less Cash at Bank	120 10 8		
Cash in hand	0 1 2	120 11 10	342 9 2
Amount overdrawn on General Account		542 9 2	
Less Repairs and Renewals		200 0 0	
		£342 9 2	
			£2,403 17 3

Examined and found correct.

(Signed) N. E. Waterhouse.
Wyndham Birch.
L. W. Byrne.
W. T. Calman.

3 Frederick's Place,
Old Jewry, E.C.
26*th June*, 1912.

the Year ending 31*st May*, 1912. **Cr.**

		£ s. d.	£ s. d.
By Current Expenditure :—			
Salaries and Wages—			
Director		200 0 0	
Hydrographer		150 0 0	
Naturalist		250 0 0	
Additional Naturalist		133 6 8	
Assistant Naturalist		150 0 0	
Salaries and Wages, and Compensation paid		716 12 2	
		1,599 18 10	
Less Compensation recovered from Employers' Liability Assurance Corporation		40 1 8	1,559 17 2
Travelling Expenses			45 12 2
Library		122 9 3	
Less Duplicates sold		3 4 9	119 4 6
Lecture Expenses			31 11 11
Journal		62 1 9	
Less Sales		14 6 0	47 15 9
Buildings and Public Tank Room—			
Gas, Water, and Coal		118 7 11	
Stocking Tanks and Feeding		32 15 1	
Maintenance and Renewals		94 18 5	
Rent, Rates, Taxes, and Insurance		70 4 8	
		316 6 1	
Less Admission to Tank Room		94 1 6	222 4 7
Laboratory, Boats, and Sundry Expenses—			
Glass, Apparatus, and Chemicals		212 9 5	
Less Sales		35 13 1	
		176 16 4	
Purchase of Specimens		66 11 6	
Maintenance and Renewal of Boats, Nets, Gear, etc.	359 15 5		
Less Sales	21 18 11	337 16 6	
Insurance of s.y. *Oithona*		18 17 7	
Coal and Water for Steamer		106 18 3	
Stationery, Office Expenses, Printing, etc.		146 16 6	
		853 16 8	
Less Sale of Specimens		477 19 1	375 17 7
By Bank Interest			1 13 7
			£2,403 17 3

Walter Ibbotson Beaumont.

Many workers at the Plymouth Laboratory will have heard with deep regret of the death of Mr. Beaumont, who was accidentally drowned whilst yachting at Tarbert, Loch Fyne, on May 3rd, 1912. Mr. Beaumont first came to Plymouth in 1895, and from that time he spent several months of each year at the Laboratory. He had commenced the study of biology under the late Professor Milnes Marshall, in Manchester, and subsequently went to Cambridge, where he entered Emmanuel College. He was chiefly interested in faunistic work, and contributed valuable papers on nemerteans, schizopods, and nudibranchs from Port Erin, Plymouth, and Valencia. He was also a keen student of bird-life, and did much useful work in connection with bird-marking and bird migration.

George Herbert Grosvenor.

Mr. Grosvenor will always be remembered by a large number of younger English biologists from the fact that for a number of years he conducted the annual Easter Vacation Course in Marine Biology at the Plymouth Laboratory, and it was under his guidance that they obtained their first acquaintance with the wealth of living creatures that are found in the sea. He was a man of a singularly cheerful and attractive disposition, a keen observer, and an enthusiastic naturalist. His principal contribution to marine biology was the paper in which he demonstrated that the nematocysts of nudibranchs are derived from the hydroids upon which they feed. Latterly Mr. Grosvenor worked chiefly at agricultural entomology in connection with his post of lecturer in that subject at Oxford. Mr. Grosvenor was drowned whilst bathing at Polzeath, in Cornwall, on September 4th, 1912, in an attempt to help a companion who was in difficulties.

George Harold Drew.

The sudden death of Mr. Drew, at Plymouth, on January 30th, 1913, at the age of thirty, came as a great shock both to his fellow-workers at the Laboratory and to his many friends elsewhere. Since leaving Cambridge as an undergraduate he had spent most of his time in Plymouth, and the originality and successful character of his work had marked him out as one of the most promising of the younger English biologists. His experimental work on tissue growth, which he undertook in connection with the study of cancer, yielded results which are bound to have much influence on future researches on this subject. In addition to his pathological investigations, Mr. Drew made a special study of marine bacteria and of the general problems of the metabolism of the sea. In connection with this work he made expeditions to the West Indies in 1911 and 1912, and some results of his observations are published in the paper which appears in the present number of this journal, the final proofs of which he had corrected shortly before his death. He was a man of much originality of mind and independence of character, and possessed a charm of personality which made his friendship peculiarly attractive.

E. J. A.

BY-LAWS

MEMBERS.

1. The Association shall consist of Governors, Founders, Life Members, and Annual Members, from whom shall be chosen the Council, Officers, and not more than twenty Vice-Presidents.

2. A number of Associate Members, not exceeding fifty in all, may be elected from among persons connected with Marine Fisheries or interested in Marine Botany or Zoology.

COUNCIL.

3. The affairs of the Association shall be conducted and its laboratories managed by a Council consisting of the Governors, the Officers, the Prime Warden of the Fishmongers' Company for the time being, and fourteen other Members to be chosen annually who shall be eligible for re-election, except that the two senior elected Members of Council according to date of election shall retire each year, and shall not be eligible for re-election until they have been out of office one year. In case of equal seniority the order of retirement shall be determined by lot. None of the above provisions as to retirement shall apply to any one member or officer of each of the three following Government Departments, namely, the Board of Agriculture and Fisheries, the Scottish Fishery Board, and the Board of Agriculture and Technical Instruction for Ireland, whom being a member of Council the Association may desire to re-elect as the representative of his Department on the Council. A Meetings of the Council five shall be a quorum.

4. The Council shall have power to appoint from time to time from among their number such Committees as the Council may deem expedient, and may delegate to such Com-

mittees such of the powers of the Council (including power to affix the Seal to documents relating to matters referred to or dealt with by such Committees) as the Council may determine. When executive functions are exercised by such Committees three shall be a quorum. Such Committees shall periodically report their proceedings to the Council, and shall conduct their proceedings in accordance with the directions of the Council.

OFFICERS.

5. The Officers of the Association shall consist of a President, a Chairman of Council (who shall be *ex-officio* a Vice-President), an Honorary Treasurer, and an Honorary Secretary, all of whom shall be chosen annually from amongst the Members, but shall be eligible for re-election.

6. In the event of any vacancy occurring in the Council, or among the Officers or Vice-Presidents, the Council at their next or any later Meeting after such vacancy has been made known, shall elect some duly qualified person to the vacant office.

7. The President shall preside at the Meetings of the Association, and shall regulate the discussions and proceedings thereat.

8. In the absence of the President at any meeting of the Association, a Vice-President shall preside; and, in the absence of all the Vice-Presidents, a Member of the Council shall preside; and, if no Member of the Council be present at any Ordinary Meeting, the Members present shall appoint such Member as they shall think fit to be Chairman.

9. The Chairman of Council shall preside at the Meetings of the Council, and shall regulate the discussions and proceedings thereat. In his absence, the Members of the Council present shall appoint such Member of the Council as they think fit to act as his deputy.

10. In case of an equality of votes at meetings of the Association, or of the Council, the Member presiding shall have a second or casting vote.

11. It shall be the duty of the Hon. Treasurer to receive all sums of money due to the Association, and to disburse all sums payable by the Association out of the funds in his hands.

12. No payment exceeding £15 (except for rent, taxes, or wages) shall be made by the Hon. Treasurer without the consent of the Council.

13. The Accounts of the Hon. Treasurer shall be audited previous to each Annual Meeting by a Committee of two Members of the Council and two Members of the Association, to be appointed by the Council, of which Committee three shall be a quorum.

DIRECTOR.

14. A Director of the Laboratory, who shall also act as Assistant Secretary of the Association, shall be elected by the Council, and shall be paid such a salary as shall from time to time be determined.

15. It shall be the duty of the Director to maintain the laboratories, aquarium and library, and other property of the Association, in a state of efficiency; to superintend and direct the scientific work at the laboratories; to prepare and edit the publications of the Association; to keep a list of all the Members of the Association, together with their addresses; to summon Meetings of the Association and of the Council; to conduct all correspondence; to take minutes of the proceedings of the Association and the Council, and generally to act under the direction of the Council in all matters connected with the affairs of the Association.

16. The Council may employ an Assistant to the Director, who shall receive such remuneration and shall be subject to such directions as they shall from time to time determine.

ELECTION, WITHDRAWAL, REMOVAL, AND PRIVILEGES OF MEMBERS.

17. Every candidate for election as a Member or Associate Member shall be proposed in writing by a Member, and such proposal shall be forwarded to the Director, who shall lay it before the Council at the next succeeding Meeting.

18. The method of voting for the election of Members shall be by ballot, and a majority of the Council balloting shall elect.

19. The payments to be made by the Members shall be as follows:—A Governor shall pay £500, and a Founder shall pay £100. An Ordinary Member shall pay £1 1s. annually. The annual contribution may be compounded for at any time on payment of £15 15s. Any University of the United Kingdom or other Corporation or body approved by the Council, on the payment of £500 to the Association in the name of the University or other Corporation or body, and for the purpose of acquiring the right herein specified, shall, if the Council of the Association assent thereto, become a Governor of the Association and acquire the perpetual right of nominating annually one Member of the Council of the Association to serve for one year (from the Annual Meeting in one year to that in the following year), and any Member of the University, Corporation or body subscribing £100 or more to such fund of £500, shall, in virtue of such subscription, become a "Founder" of the Association.

20. The annual contribution shall become due on the 1st January in advance; but any Member elected in the months of October to December inclusive, and paying a contribution during that period, will not be called upon for a second contribution for the year following his election.

21. Every Member having paid all sums due to the Association shall be at liberty to withdraw therefrom, upon giving notice in writing to the Director.

22. Whenever written notice of a motion for removing any Member shall be delivered to the Director, signed by the President or Chairman for the time being on the part of the Council, or by five or more Members, such notice shall be sent by post to each Member seven days before the next Annual or Special Meeting of the Association, when such motion shall be taken into consideration and decided by ballot. If a majority of the Members balloting shall vote that such Member be removed, he shall be removed from the Association accordingly.

23. Whenever any Member shall be in arrear for two years in the payment of his annual contribution, notice thereof in writing shall be sent to him by the Hon. Treasurer, and in case the contribution shall remain unpaid, the Hon. Treasurer shall give notice thereof to the Council, who shall cause a similar notice to be sent to the Member with an intimation that at the expiration of three months he will be liable to have his name erased from the list of Members. In default of payment the Council may order his name to be erased accordingly.

24. Members shall have the right to be present, to state their opinions, and to vote at all Meetings of the Association; to propose candidates for admission as Members; to introduce visitors at Meetings of the Association; to have personal access and to introduce strangers to the laboratories, and to make use of the library, subject to such regulations as the Council may from time to time prescribe.

25. Members shall be eligible to any office in the Association, provided they are not more than one year in arrear in the payment of the annual contribution.

26. A Member shall not be entitled to vote on any occasion until he shall have paid his contribution for the year last past.

ASSOCIATE MEMBERS.

27. Associate Members shall not be required to pay any contribution to the funds of the Association, but are expected to communicate information to the Association on Marine Fisheries and on Marine Zoology or Botany, to supply specimens, and to advance the objects of the Association in other ways.

28. Associate Members shall not be eligible to any office in the Association, nor shall they be entitled to vote at any Meeting.

29. Associate Members shall have personal access to the laboratories and library of the Association, subject to the regulations of the Council in force for the time being.

ANNUAL MEETING.

30. The Annual Meeting of the Association shall be held in April in each year on such day and at such time as the Council shall from time to time direct.

31. The object of the Meeting shall be to receive from the Council their annual report on the affairs of the Association, and to elect the Council, Officers, and Vice-Presidents for the ensuing year.

32. The Council shall cause to be prepared a list containing the names of Members whom they shall recommend to be elected as the Council, Officers, and Vice-Presidents, for the year ensuing, and a copy of such list shall be hung up in the Office of the Association not later than the 15th March.

33. If any five or more Members shall desire to substitute for any of the names in the said list, the names or name of any other Member or Members, they shall give notice in writing to that effect, specifying the names or name proposed to be substituted; such notice to be given on or before the 31st March to the Director, who shall cause the same to be forthwith hung up in the Office of the Association.

34. If no such notice is given to the Director, the Members named in the list prepared by the Council shall be held to be elected as the Council, Officers, and Vice-Presidents for the ensuing year.

35. If any such notice is given, the election shall be by ballot at the Annual Meeting, and the President or Chairman shall appoint two or more Scrutineers from the Members present, not being Members of the Council, to superintend the ballot, and to report the result to the Meeting.

36. Any balloting list containing a greater number of names proposed for any office than the number to be elected to such office shall be rejected by the Scrutineers.

37. No ballot shall be taken unless five or more Members vote.

SPECIAL MEETINGS.

38. The Council may at any time convene a Special Meeting of the Association, and shall do so upon the requisition of any twenty or more Members presented to the Council. A notice thereof shall be sent to every Member whose last known residence was in the United Kingdom, seven clear days before such Meeting shall take place, and the general nature of any proposition to be submitted to such Meeting shall be stated in the notice.

39. No vote shall be taken at any Special Meeting, unless twenty or more Members shall be present in person or by proxy. Members absent from the Meeting may vote either by proxy, or by previously sending their votes in writing to the Director.

40. Any of the By-Laws of the Association may be repealed or altered or others adopted in lieu thereof at an Annual or Special Meeting, but no resolution for effecting any such repeal or alteration shall be proposed at any Annual Meeting unless at least fourteen days' notice in writing specifying the terms of such resolution shall have been given to the Director, or unless it has received the sanction of the Council.

June, 1912.

CONTENTS OF NEW SERIES, Vol. IX., No. 4.

NOTICE.

TERMS OF MEMBERSHIP.

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition Fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the Laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the Laboratory for research, with use of tanks, boats, &c.; and have access to the books in the Library at Plymouth.

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