REPORT FOR 1898

ON THE

LANCASHIRE SEA-FISHERIES LABORATORY

AT

UNIVERSITY COLLEGE, LIVERPOOL,

AND THE

SEA-FISH HATCHERY, AT PIEL.

DRAWN UP BY

Professor W. A. HERDMAN, D.Sc., F.R.S.,

Hon. Director of the Scientific Work;

Assisted by Mr. ANDREW SCOTT and Mr. JAMES JOHNSTONE.

WITH TWO PLATES.

LIVERPOOL:

PRINTED BY T. DOBB & Co., 229, BROWNLOW HILL.

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1899.

REPORT on the INVESTIGATIONS carried on in 1898 in connection with the LANCASHIRE SEA-FISHERIES LABORATORY at University College, Liverpool, and the SEA-FISH HATCHERY at Piel, near Barrow.

Drawn up by Professor W. A. HERDMAN, F.R.S., Honorary

With Compliments from

PROFESSOR W. A. HERDMAN, F.R.S.,
UNIVERSITY COLLEGE,
LIVERPOOL.

who will be glad to receive your publications in exchange.

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INTRODUCTION.

THIS is the first complete year of Mr. Scott's work at the Piel Hatchery and of Mr. Johnstone's work at the Liverpool Laboratory, and a glance merely at the above table of contents will show the extent and variety of the investigations that have been undertaken.

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In one of his letters to me at the beginning (June) of the research Mr. Gamble says: "We hope by the end of summer to have some sound experimental evidence of the nature of colour change in *Virbius* and possibly in *Mysis* as well. . . . Our apparatus consists partly of an arrangement for maintaining a constant stream of water through a series of observation vessels and partly of an arrangement by which air is sucked through a second set of vessels. Thus we can determine whether change of air or change of water is the most conducive to health. Then in addition to a dark box we have a series of 'light-filters' by means of which we can obtain the influence of monochromatic light on *Virbius* both in the above-mentioned vessels and under the microscope. . . . . For exact physiological work, the sea-water and gas laid on in the Laboratory at Piel are an immense advantage—to say nothing of the excellent accommodation, and from what we managed to do a few weeks ago both Keeble and I hope that before the end of September we may have settled some important questions in connection with the colour-reactions of Crustacea to various stimuli." For further particulars I refer to the authors' report given below (p 81).

This work has, at present, no obvious connection with economic fishery problems, but it is impossible to foretell, in these days of rapid advance in discovery, and in the application of science to industries, how soon the results of what now seems an investigation in pure science may turn out to have some important practical bearing. For my part I am of opinion that all knowledge of animal life in our seas is of importance, and will help us to understand the life and ways of fishes.

That a detailed knowledge of the sea and its contents (however minute) must be the basis of fishery practice and regulation is recognised in the following extract from the Memorial in favour of International Oceanographic Exploration sent last April by the Swedish Government to our Foreign Office :—"All fishing in the North Atlantic, and especially the presence of the migratory fishes, depends upon the great currents in the upper layers of the sea, and the variation of the presence in these layers of the food required by the fishes, viz., 'Plankton' or organisms of animal or vegetable origin floating in the water. A knowledge of these currents and of the quality and quantity of food they contain is necessary in order to determine the legislation required for the creation of a rational organisation of the fisheries." Then, again, the migrations of the Cod towards the Lofoten banks and fjords, and of the winter Herring into the Skagerack, seem, according to Otto Pettersson, to be regulated by the impact of cold Arctic and west Atlantic water in winter driving the fishes to those parts of the sea where the conditions are less unfavourable.

It is considerations such as these that lead naturalists to urge that fishery observations and investigations must not be restricted by any territorial or administrative boundaries, but should be extended to off-shore waters, and even the high seas, so as to follow up and unravel the factors that contribute to the distribution of our coast fisheries.

Mr. Ascroft has also stayed some time at the Piel Hatchery, and we include in this Report a short account from him of his observations on the separation of mud from sea-water by shell-fish and on the growth of mud banks upon beds of shell-fish, especially Mussels.

It may be well to note here that the well-equipped biological Laboratory with tank-house at Piel is now open, upon certain conditions, to duly qualified investigators. In addition to the Laboratory accommodation, the building provides dining-room, writing-room or library, and about eight bed-rooms for workers. No charge is made for residence, and meals are provided at a fixed and reasonable rate. The regulations for workers, as approved by the Committee, are appended to this Report.

During the course of his work in hatching Cod, Plaice, and other fish at Piel last spring, Mr. Scott made a number of coloured drawings of the various embryonic and larval stages of our common fishes. We hope that these may be published on a future occasion, when the series is more complete.

Having succeeded so far, and shown that the work can be conducted with the sea-water pumped up at Piel, Mr. Scott is naturally anxious to be supplied with a pond in which to keep spawning fish, and with a proper outfit of hatching boxes, so as to be able to carry on operations on a much larger scale. As Capt. Dannevig in Norway and the Scottish Fishery Board (lately at Dunbar, and for the future at Aberdeen) have adopted a special form of hatching apparatus, in which the little cubical boxes containing the developing eggs are rocked up and down constantly in the water of a larger tank, with, so far as we can ascertain, very good results, it certainly seems desirable that we should try some of these "Dannevig rocking boxes" at Piel, for comparison with the simple tanks with constant flow of water, which we used last year. Five sets of rocking boxes have, therefore, been ordered from Norway, where they are made under the direction of Capt. Dannevig, and are expected to arrive at Piel in a few days. They will be set up along the east wall of the tank-house, and the plain tanks, which will also be in use this coming season, are now being moved into the adjoining portion of the verandah, which is being enclosed for the purpose so as to form an extension of the tank-house.*

The provision of a satisfactory spawning pond, in which the parent fish can be kept in considerable numbers until they produce their eggs, is a more difficult matter. The Scientific Sub-Committee have had the matter under consideration at several meetings, and I have gone carefully into the question of alternative sites, on the ground, with the Chairman, the Superintendent, Mr. Scott, and the Engineer to the Railway Co. (our landlords at Piel). The difficulty is to get a site which is sufficiently near to the hatchery, sufficiently protected from heavy seas, which can be excavated to a sufficient depth, and which will enable the pond to be constructed at a reasonable cost. A tidal pond, such as that now being constructed at Bay of Nigg, Aberdeen, by the Fishery Board for Scotland, has certain obvious advantages, the chief of which is that no pumping is required, but it is open to the objection that it must of necessity (at Piel) be on the shore, and, therefore, exposed to the seas.

We are trusting for one year more to such supplies of spawn as can be obtained by the steamer or from the trawlers, but if these supplies are as poor as they were last year, we shall require in early summer, at the latest,

* Since the above was written the hatching boxes have arrived, and the necessary changes in the accommodation have been carried out.

to decide upon the position and nature of the spawning pond, in order that it may be completed in time for the following season.

Mr. Johnstone's work at Liverpool has been partly the investigation in the Laboratory of any specimens that were sent to us, partly assisting me in the work, and partly (with Mr. Tom Mercer) looking after the Fisheries Museum, and the Circulating Fisheries Exhibition now at Preston. A good deal of Mr. Johnstone's time in the Laboratory has been taken up with the examination of Mussels and other shell-fish, at all times of the year, and from various parts of the district. The evidence as to the spawning habits of the Mussel obtained from the microscopic characters of the reproductive organs is rather puzzling, but it now seems most probable that the Mussel commences to produce mature reproductive elements in the middle of winter,* and continues to emit eggs and sperms in small quantities for the first six months of the year (probably in increasing amount after April), during which time large eggs are always to be found in the ovary; and then, in the middle of summer, produces rapidly a much greater number of ova, so as to clear out the contents of the ovary, which suddenly (about end of July) undergoes a great change, easily recognisable under the microscope as the "spent" condition. It then, after a brief interval, proceeds to develop fresh ovarian tubules, and load up rapidly with young ova, which develop during the autumn and early winter in time to be mature at the end of the year. The full details of these changes in condition will be found in Mr. John-

* We have obtained Mussels with completely formed active spermatozoa in the middle of December. Mr. Ascroft has found the free-swimming larva at Lytham in April and May, and the first "strikes" of young Mussels are frequently found here upon Algæ in June.

stone's section of the Report at p. 36, and the various stages are illustrated by two plates. I have also started Mr. Johnstone on an investigation of the structure of our edible Cockle, and hope in next year's Report to publish his full detailed account of that animal.

Our travelling "Fisheries Exhibition" is working its way through the more important towns of the county. At the beginning of March, 1898, after a very successful period at Liverpool, when it was repeatedly visited by many interested in the subject, the exhibition was transferred to the Royal Museum, Salford, where it remained till the end of October. In the meantime, a circular was issued from the County Offices, Preston, stating that this Fisheries Exhibition might be obtained on loan, on certain terms to meet the expenses of packing, unpacking, re-arranging, and carriage. Applications were received from the Museums of Preston, Warrington, Bolton, and St. Helens; and early in November the exhibition was transferred to the Harris Free Museum at Preston, where it will remain for some months. The transference of the cases and collections (which have to be very carefully packed and unpacked) from one institution to another occupies Mr. Johnstone, with the assistance of T. Mercer and a joiner, for about a fortnight, and it occasionally happens that there are specimens which become accidentally broken or damaged, or for some other reason require to be replaced from the Laboratory, and re-labelling is sometimes necessary. All this, and the correspondence connected therewith, has taken up a not inconsiderable part of our Liverpool Assistants' time during the past year; but I think we are all agreed that it is well worth doing. The collection, it will be remembered, was originally formed for the Fisheries Exhibition at the Imperial Institute in 1897, and as it mainly illustrates our local fisheries and local investigations it is important that it should be shown *locally*, and to the people who are interested in these fisheries and who contribute to the expense of regulating them. The educational value of the exhibition must also be considerable, and may perhaps be gauged by the keen interest shown by visitors.

I can speak to that from my own experience when the collections were at Liverpool; and I may also quote a few sentences from letters sent to me by Mr. B. H. Mullen, M.A., the Curator of the Museum at Salford when the Exhibition was in that town, as follows:—

"The Fisheries Exhibition is a very great attraction here, and must be doing a lot of good. During the past three days we had almost 1,000 more visitors than during the same period in 1897. I place the greater part of this to your most interesting exhibition." . . .

"You will notice that in those four months (June, July, August, September) last year over 95,000 persons visited our museum." . . .

"I estimate the number of persons who visited the Museum while your exhibit was here to be 119,852—say, 120,000."

Mr. Mullen prepared, partly from the catalogue given as an appendix in our last Report, a "Popular Guide," which was largely sold to visitors to the Salford Museum at the price of one halfpenny. All this can scarcely fail to do much good in interesting the public in the importance of Fishery questions, and in disseminating correct and useful information as to the work of the Lancashire Sea-Fisheries Committee.

It is proposed that the Exhibition should be sent in April, 1899, to Warrington, and after that to Bolton, and then St. Helens, and any others of our neighbouring towns that can provide suitable accommodation, in the order of their formal application.* The permanent home of the Exhibition, when not on loan, is in the Fisheries Museum at University College, Liverpool.

It may be mentioned, in this connection, that last May Professor Boyce and I exhibited at the Royal Society the practical results of our investigations on Oysters and Disease which are discussed further on in this Report.

The educational aspect of such matters naturally leads me to a question of Technical Instruction, which has arisen almost simultaneously on the Scientific Sub-Committee and at University College, Liverpool, viz.:—The formation of a "School of Fisheries Science," or a curriculum of instruction in the sciences which underlie Fisheries knowledge and investigations. In November the Scientific Sub-Committee drew up the following report:—"Having regard to the increasing development of knowledge on Fishery questions, the Sub-Committee has considered a scheme for the establishment of a School of Fishery Science, prepared by Professor Herdman, and is now in communication with the Technical Instruction Committee of the County Council, with the view to this, or some suitable scheme for enabling students to obtain advanced instruction in Fishery Science, being adopted. It has also been considered desirable, if arrangements can be made, to allow the Sea-Fishery Bailiffs to avail themselves of opportunities for instruction in more advanced knowledge than they possess of sea-fish, their habits and food, in order that they may be able to instruct the fishermen in matters of which, at present, there is probably considerable ignorance, and upon which accurate knowledge would be advantageous." This report was approved by the General Committee at the meeting in November.

* Applications should be sent to Prof. Herdman, University College, Liverpool.

The matter has also been fully considered by a committee at University College, which has drawn up a detailed curriculum of instruction, and the scheme now only awaits a conference between the University and the County authorities before becoming a working department in a most important branch of technical knowledge. With our extensive scientific Laboratories and the Fisheries department and Museum at University College, with our sea-fish hatchery and experimental tanks at Piel, and with the steamer and system of bailiffs under Mr. Dawson, we possess already organised and co-ordinated in Lancashire such a mechanism for instruction in Fishery knowledge from the scientific, from the industrial, and from the administrative sides, as probably does not yet exist elsewhere in the country.

If the County Technical Instruction Committee will help in meeting the necessary expenses of selected students passing through a two or three years' curriculum, and the Laboratory expenses of showing to the bailiffs and a limited number of fishermen the methods of investigation and the microscopic, chemical, and other facts which they hear about in lectures and reports, it can scarcely be doubted that much more will be done thereby in the dissemination of real and accurate knowledge than can possibly be effected by imperfect and sporadic courses of lectures to the fishermen.

Turning now to such special work as I have been able to do myself for this Report:—

(1.) I have considered it useful in the present state of affairs to discuss somewhat fully Mr. Fryer's criticism of Sea-Fish Hatching in America, which appeared in the last Report of the Inspectors for England and Wales (1898). I have had some correspondence on the matter with the United States Fish Commission, and I may quote here the following sentences from a letter recently received from the Commissioner :—" For about ten years the Cod work has been attended with marked success, and in Massachusetts has resulted, not only in establishing the in-shore Cod fishery on grounds long exhausted, but, through favourable distribution of the fry, in extending the fishery to other waters not originally frequented by the Cod." . . . " Some investigations made a few years ago by the Commission indicated that the value of the Cod now annually taken on new grounds is at least several times greater than the entire yearly expenditures of the Commission for fish-cultural work, and is increasing each season."

(2.) The investigation into the condition of Oysters from various localities and under various circumstances, and their relation to infective diseases in man, which I have been carrying on during the last three years in conjunction with Professor Boyce and Dr. Kohn is now concluded, and our full memoir on the subject with illustrations of the detailed evidence will soon be published. I have therefore considered it right to lay before you in a special section of this report the final conclusions at which we have arrived on " The Oyster Question ; " and I have appended to it a reprint (from our paper at the Bristol meeting of the British Association in September) of Dr. Kohn's account of the presence of iron and copper in certain Oysters. I think it is clear that what the public wants at present is an assurance that the Oysters they buy and eat come from grounds that are above suspicion. There is a great opportunity for an independent authority—either " Health " or " Fisheries," " Central " or " Local "—to take up the matter, and after due investigation to license or certify certain grounds or certain Oysters upon the lines I have indicated in the conclusions on p. 66.

W. A. Herdman.

January, 1899.

Fish Hatching Work at Piel.

(Andrew Scott.)

At the beginning of December, 1897, I entered upon my duties at Piel, and at once proceeded to place everything in working order for the approaching spawning season. Various little defects in the machinery and tanks were discovered and put right. By far the most serious defect was found in the large overhead store cisterns in the tank room, where considerable leakage was taking place, making the room uncomfortable to work in. However, with some difficulty and expenditure of time, they were made fairly water-tight, and at the end of twelve months are now practically free from leaks.

The wooden hatching tanks used in the previous season were cleaned out, fresh sand put in the filtering compartments, and a constant circulation of sea-water established. All the apparatus was in satisfactory working order by the end of January.

On January 27th the steamer visited the spawning grounds in the central part of the Irish Sea, between the Isle of Man and Lancashire, where a few hauls with the fish trawl and surface nets were taken. No mature fish were obtained, but in the tow-net collections made at the "Top end of the Hole" and "On the Shoals" a few fish eggs were observed. The grounds were again visited during February, and although no spawning fish were found, a marked increase in the number of floating eggs was noticed. A quantity of living tow-net material brought into the Laboratory was found to contain three forms of fish eggs, in various stages of development—in some the larvæ were quite lively. These eggs were carefully picked out and placed in glass aquaria, with a constant circulation of sea-water. In the course of the same evening a few of the larvæ hatched out, and two forms were identified, from the arrangement of the colouring matter, as being the larvæ of Cod and Flounder. The third, a colourless larva, was not identified, and only lived a few days. The Cod and Flounder larvæ lived for twelve days. By this time the yolk sac had been completely absorbed, but the larvæ made no attempt to feed, although kept supplied with plankton taken in the tow-net.

On March 9th the steamer again visited the spawning grounds, and this time secured a number of mature Cod, Haddock, and Plaice, from which a quantity of eggs were obtained and fertilized. These, however, were probably not quite mature, and at the end of the following day they had all died and sunk to the bottom of the tanks, no development having taken place. The tow-nettings contained an increased number of developing eggs of Cod, Flounder, Plaice, &c.

Amongst some living fish brought in by the steamer from this expedition was a mature female Flounder. These fish were placed in the tanks, and on the following day the Flounder was observed to be shedding its eggs. The fish was therefore taken out, and the eggs pressed out into a jar, and successfully fertilized with the milt of a male Flounder that had been captured, amongst other fishes, in the Barrow Channel by Mr. Wright, a few days previously. Development proceeded rapidly, and eight days later all the larvæ hatched out, there having been practically no mortality amongst the eggs. The larvæ lived without loss for fourteen days. The contents of the yolk sac had been absorbed several days previously. At this stage a marked mortality set in, and during the next few days the larvæ died off rapidly. Notwithstanding the various experiments tried to persuade them to feed, at the end of eighteen days all had died.

On March 16th the steamer arrived with a further supply of eggs from Plaice and Flounder. None of the Flounder eggs, and very few of the Plaice eggs, had been fertilized. On the 18th March a supply of Cod eggs was brought in by the steamer. Many of these were fertilized, and the development proceeded rapidly.

The spawning grounds were again visited on March 22nd, and a number of sailing trawlers were boarded when hauling in their nets. Many mature fish had been captured, and a supply of Cod, Haddock, Plaice, Flounder, and Dab eggs were obtained. The eggs of the Haddock, Plaice, and Dab did not fertilize, nor did many of the Cod and Flounder.

The surviving eggs of the Plaice fertilized on the 16th, of the Cod on the 18th, and of the Flounder and Cod on the 22nd, and 23rd March developed satisfactorily, hatching out at the end of eighteen, sixteen, eleven, and thirteen days respectively. Throughout these periods a considerable daily mortality was observed, which was, no doubt, due largely to the condition of the eggs when fertilized. In several cases the larvæ were clearly visible through the membrane of the eggs when death occurred.

Through the kind permission of the Fishery Board for Scotland, the steamer was allowed to visit the Clyde and trawl there for spawning fish. Three visits were made, but on only one of these, the first, were mature fish obtained, and the eggs successfully fertilized. The eggs were from Plaice, and the quantity on arrival at Piel measured 150 cubic centimeters. This was by far the greatest individual quantity of eggs received during the season. On the first visit the eggs of the Witch, Dab, Grey Gurnard, and Haddock were also obtained; but the Gurnard and Haddock eggs did not fertilize. The Dab and Witch eggs were fertilized, and underwent partial development, but died after three days.

Development in the Plaice eggs proceeded through its usual course with scarcely any mortality, and at the end of twelve days, the hatching of the larvæ commenced, and was completed on the following day. Shortly after hatching was completed the larvæ were carefully transferred to glass aquaria, through which a constant circulation of water was maintained, and when they were a week old various experiments were tried to persuade the larvæ to feed. To one jar material collected in the filter was added; to a second, Diatoms; to a third, Copepoda; to a fourth, plankton from the tow-nets, and to a fifth, "Mussel broth" obtained by squeezing up the living shell-fish and passing the semi-fluid mass through a fine sieve. All these experiments were of no avail, and the larvæ began to die off when they reached the age of fourteen days. During the following fourteen days there was a considerable daily mortality, and at the end of twenty-nine days all the larvæ were dead, they, apparently, having made no attempt to feed, as no trace of food could be seen in the stomach when examined under the microscope.

Other expeditions were made to our own spawning grounds in search of mature fish, but no more fish eggs could be obtained.

The method of collecting fertilized eggs by the steamer is one of some difficulty and uncertainty, owing to the stormy weather sometimes experienced during the spring of the year. It may happen that just at the very time spawning fish are on the ground it is unsafe for the steamer to venture out. The collecting of eggs from fish caught in the net of an ordinary trawler is also unsatisfactory, owing to the circumstance that when the eggs are ready for shedding the least pressure on the sides of the fish forces the eggs out. Fish caught in a trawl-net are generally mixed up with a large quantity of debris, especially after a four or five hours' drag, and the weight of this "rubbish" pressing against the ripe fish forces the eggs out before the net is emptied on deck. The majority of the eggs that are brought in to our hatchery from these expeditions, therefore, are not perfectly mature, consequently the eggs may not be fertilized at all, or if fertilized, die off before hatching out. It is from this cause that the high mortality arises.

An instance of this was demonstrated during the past season. A large female Plaice, fully distended with eggs, was brought in by the steamer from one of the expeditions, and was kept alive in one of the tanks. In the course of a few days however, it turned sickly, and to all appearances was in a dying condition. The eggs were therefore pressed out into a bucket containing a small quantity of sea-water, and mixed with the milt of a male Plaice that had been brought in along with the female. None of the eggs floated, although examination showed a number of them to have been fertilized, and to be undergoing development. The living ones were carefully picked out and placed in a jar by themselves. Notwithstanding a considerable daily mortality, development proceeded rapidly, and in a few days the little fishes were showing very clearly through the egg membranes, but only two hatched out. A number of the embryos reached the hatching-out point and then died. The two that did hatch out were feeble in their movements, and lay on their backs at the bottom of the jar. They were very different from the larvæ of the Clyde Plaice, and only lived a few hours.

It is clearly evident then, that fish eggs for hatcheries must be under the most natural condition obtainable. The only way to secure this is to collect the fish towards the end of the previous year, or very early in the spawning season, and keep them alive in a suitable pond, allowing them to shed the eggs by their own efforts.

That the water is satisfactory for the purpose is proved by the result of the past year's hatching work. After passing through the filter it was perfectly pure and transparent, the specific gravity varying from 1·0025 to 1·0026, and the temperature from 4° C. to 4·8° C.

The mortality of the larvæ kept after hatching is very serious. Further experiments will be tried, as it may be that we have merely not yet hit upon the proper food. It may, however, be found best to set the young fish free before they reach the critical stage in their development.

Observations on the Occurrence and Habits of Leptocephalus.

(Andrew Scott.)

In recent years much light has been thrown upon this peculiar group of fishes (the Leptocephalidæ), which were at one time considered to be fully developed animals and classed by naturalists as such, under the generic name of *Leptocephalus.* Thanks to the observations made at Roscoff, in France, in 1886, the form then known only as *Leptocephalus morrisii*, was actually observed to change into the Conger eel *(Conger vulgaris)*, and later, in 1891-95, two Italian investigators, Grassi and Calandruccio, carried on careful experiments on the *Leptocephalus brevirostris* taken in the Straits of Messina. They found that this so-called species went through a transformation, changing into the common Eel, *Anguilla vulgaris.** So that there can no longer be any doubt now that the *Leptocephali* of the older naturalists are only the larval stages of Eels.

* Proceedings Royal Society, London, vol. LX., No. 363. Dec., 1896.

That there is still a considerable amount of definite information wanted regarding the movements and true habitat of these larvæ in our own seas, may be gathered from the fact that the records of their capture along the British coasts are few and far between.

In our surface tow-net gatherings, taken along the Lancashire coast during the past year or two, we have occasionally found a little flat, transparent fish, which has been entered on our lists as "*Leptocephalus* sp." In January, 1896, three were taken in the estuary of the Wyre, by tow-net worked from the steamer. In April of the same year, one was taken by tow-net worked off Lytham Pier. These were forwarded to us by Mr. Ascroft. In January, 1897, one was taken in the Mersey, off New Brighton, and in the year finished (1898), a number of individuals have passed through my hands, as follows :—

On April 26th, Mr. J. Wright, Chief Fishery Officer at Piel brought into the Laboratory a tow-net collection he had made in the vicinity of the north end of Roa Island, and amongst the material was a living *Leptocephalus*. This specimen was kept alive for a few days, but eventually died. Shortly afterwards, May 18th, while I was collecting young flat-fishes in the shore pools and gutters at low water in the same neighbourhood, three *Leptocephali* were captured. From that date onwards, to the end of June, when the weather and tide permitted, careful examination of the shores was made, with the result that eighteen specimens of this hitherto rare fish were obtained; others were seen but not captured.

The method adopted for the capture of these and other young fish, and which proved very successful, was the following one :—Advantage was taken of the fact that, during the ebb of the tide here, there is a rapid fall of water, which has scooped out regular well-defined gullies in the more level stretches of the shore, and although the adjoining parts of the shore may be almost dry, there is still a considerable current sweeping down these gullies. By closing up the smaller gullies, and digging gutters between them and the larger ones, we were able to divert almost the whole of the retiring water on certain parts of the shore into one or two main gullies, which we afterwards partially closed up with stones, leaving only sufficient space in the centre to hold a tow-net. On going to these places during particular states of the ebb, which later on we were able to locate with considerable accuracy, and fixing our nets so that the water passed through them, we were able to secure practically all the material swept off a considerable area of the shore by the force of the receding tide. On favourable days, when there was no wind and the water free from suspended mud, we could actually see the various kinds of young fishes, Crustacea, &c., being carried into the net with the current. The nets were lifted up from time to time, and their contents emptied into collecting bottles. After the water had ceased running, we removed the nets and returned to the Laboratory with our captures, where they were sorted out, the fish being placed in tanks and glass aquaria. The Crustacea captured were chiefly used for feeding fish already in the tanks.

It was amongst the fish taken in this way that we obtained the majority of our *Leptocephali*. Occasionally others were captured by forcing the tow-net over the surface mud at the roots of *Zostera*, which is fairly common in some parts of our district. With everything in our favour, we could almost depend with certainty on having at least one *Leptocephalus* each time we tried for them. Sometimes we would get two, and once we captured four. At the end of June, when we ceased finding them, we had as many as fourteen *Leptocephali* living in the glass aquaria. They were then measured one by one and placed in two aquaria, which had a layer of sand on the bottom. The sizes ranged from $2\frac{6}{10}$ to $2\frac{9}{10}$ inches in extreme length, $\frac{3}{16}$ of an inch in vertical depth, from fin to fin, and about $\frac{1}{12}$ of an inch thick. They were flat, colourless, and perfectly transparent, the viscera, and the heart and its movements, being easily seen through the skin.

On being placed in the aquaria, the *Leptocephali* swam swiftly round the sides, with an undulating movement, like that of the sand-eel. They soon settled down to their new surroundings, and quickly buried themselves in the sand. The movements gone through in burrowing are exactly similar to those made by the sand-eel. The head is first directed into the sand, then by a rapid backward and forward movement of the posterior part of the body, the anterior part is forced into the sand, and finally, by a gliding motion, the posterior part disappears. During the day-time the *Leptocephali* remained hidden away in the sand. On the slightest disturbance of the water, such as would be caused by the throwing in of food, their heads would be thrust out and a rapid survey taken to ascertain the nature and position of this disturbing element. If it were food that happened to fall close to them, they would seize it without coming entirely out of the sand, and would then glide backwards into the burrow. If the food did not fall within reach, they would not venture to pursue it in daylight. On going into the tank-room at night, when all was in darkness, and suddenly flashing a light on the aquaria, it would usually be seen that the *Leptocephali* were swimming about actively, but soon retired into the sand if the light were continued.

The *Leptocephali* were kept under observation for a week or two, when all, with the exception of two, were unfortunately lost through the overflowing of the aquaria in the night, no doubt having jumped out when the jars were full. The survivors remain alive, and at the end of November measured $3\frac{7}{10}$ inches in length, one having grown three-fourths of an inch in four months and the other slightly less. The transformation of these larvæ from the *Leptocephalus* stage was not actually observed, but on June 30th they were flat, transparent, colourless *Leptocephali*; and on August 3rd had passed into young eels, having a smoky-tinted back, silvery-grey sides, and being no longer transparent.

During the earlier parts of August numbers of young eels were found under the stones on various parts of the shore, which exactly corresponded with the appearance of the transformed *Leptocephali.*

From these observations it would thus appear that the *Leptocephali* are inhabitants of the mud, and their occasional presence in surface tow-net collections is due to their having been swept out of their burrows by the strong currents, and that they are never taken in the dredge is owing to their activity. Attempts to capture them with a tow-net when they are swimming against the current usually ends in failure.

In passing, it may be noted that all the *Leptocephali* I have obtained from tow-nettings on the Lancashire coast are identical with the hemi-larval stage described by Grassi, and many of the observations agree with those made by him, and published in his paper in the Proceedings of the Royal Society already referred to.

Observations on the Habits and Food of Young Fishes.

(Andrew Scott.)

Early in May the young flat fishes hatched out under natural conditions in the sea began to appear on all the sandy shores. By allowing the water draining down the gullies, when the tide was ebbing, to pass through a tow-net, considerable numbers were captured. Many of these were kept alive in our tanks and aquaria; some were preserved for future study, and others were examined at once to ascertain the nature of the stomach contents.

These little fish, chiefly Plaice and Flounders ranging from two-fifths to three-fifths of an inch in length, were quite colourless and transparent, the stomach and alimentary canal showing clearly through the skin. Although they had assumed the flattened character of their parents and the eye had begun to move over the head, they still swam about in a vertical manner. In the course of a day or two they were observed to have considerable difficulty in maintaining the upright position, and ere long, after a few more feeble attempts to swim vertically, they settled down to a semi-sedentary life in the sand. During the day-time they remained buried, except the mouth and eyes, and could only be detected with difficulty. In the darkness they came out and swam freely on the surface of the sand. After a few weeks, when the little flat fishes had become accustomed to their surroundings, they ceased burying themselves, and simply lay on the surface. Sometimes they clung to the sides of the jars with great tenacity.

The food of these young fishes was found to consist almost entirely of Copepoda. Collections made later on, when the fish had grown to an inch and upwards in length, showed them to be feeding on *Mysis* alone.

From their peculiar structure, one would naturally expect that the flat fishes would be rather sluggish in their movements, and not at all particular as to the nature of their food. Far from this being the case, however, they pursue their food with much vigour and select a special diet, as is clearly shown when one examines the contents of their stomachs. The stomachs of the smaller ones, from one inch up to four inches in length, captured on the shores of our neighbourhood, are usually almost entirely filled with *Mysis*, a group of Crustacea that depend chiefly on the power to escape capture by making sudden leaps when approached by any moving object. The flat fish appear to be aware of this peculiarity, as they carefully stalk the *Mysis*, and when they get close up make a sudden spring, seldom failing to capture their prey. That the young flat fish prefer living to dead food can easily be seen by throwing a mixture of dead and living *Mysis* amongst them. The fish are always on the look out for food, and at once proceed to investigate any object that makes its appearance in their vicinity. If the *Mysis* swims or leaps away, then it is pursued and captured, but if it makes no attempt to escape, the fish will abandon it for a more lively prey. Of course, when the fish are hard pressed for food they may not be so particular in waiting until the object shows considerable signs of life before they capture it.

The older flat fishes, from four inches and upwards, captured on the Roosebeck Scars, usually feed on young shellfish, such as Mussels, &c., worms *(Arenicola)*, and Crabs *(Carcinus)*.

Shortly after the advent of the flat fishes, the young of the various round fishes, such as the codling, bluffin, sand-eel, herring, goby, lumpsucker, stickleback, and lesser grey mullet, began to make their appearance in regular order; each having their own manner of capturing their prey, and all, with the exception of the young mullet, feeding on *Mysis*. Even the ungainly-looking and awkwardly-swimming young lumpsuckers are able to capture *Mysis*. They swim after their prey at, for them, a rapid rate, making sudden dashes as they pass, and usually trying to seize the *Mysis* by the middle. This is probably done to prevent too much attention being bestowed upon them by their less fortunate companions, which they would otherwise be sure to receive if the prey were captured by the head or tail. It occasionally happens that the young lumpsuckers do capture the *Mysis* by one end, and before they can swallow it another lumpsucker, usually a smaller one, has seized the free extremity. It is rather an interesting sight to see the stronger one trying to shake off the weaker, but so tenaciously do they cling to their victim, that the smaller fish is frequently in danger of disappearing after the *Mysis*. Only at the last moment does it reluctantly relinquish its hold.

The last of the young fishes to appear on the shores in the vicinity of Piel, so far as has yet been observed, are the lesser grey mullet. Numbers of these fish were captured in a well-defined gully on the east side of the breakwater joining Foulney Island to the land. They were first noticed about the middle of September, and were then fully an inch in length. The stomachs, on examination, were found to be filled with vegetable food, chiefly Diatoms, *Navicula* being the prevailing species. Later on, when they had reached the length of one and a half inches, the food was found to consist of a mixture of Diatoms and Copepoda *(Tachidius)*.

There appears to be little or no difference in the food of the young fishes frequenting the shore, and no matter whether they were captured at mid-day or mid-night, the the food was always the same. On several occasions we trawled the gullies at mid-day and mid-night with a small otter shrimp net. This was found to be very successful in capturing young fish, &c.

A number of experiments were made to ascertain how far the colours of certain Crustacea protected them from falling a prey to the fishes. It was found that when semi-transparent and dark-coloured *Mysis* were put in the jars, the colourless ones were eaten before the dark. Similarly, when a large number of variously coloured *Hippolyte (Virbius) varians*, ranging from transparent to almost black were used, the transparent ones were the first to disappear. Gradually the others were captured; last of all, but very seldom, were the dark ones pursued. Are the pigmented forms less noticeable under the circumstances, or is the pigment itself distasteful?

The Plankton Work.

(Andrew Scott.)

The examination of the floating plankton collected in the vicinity of the Lancashire coast has been continued throughout the greater part of the year. A satisfactory investigation of the material, and the accurate identification of the organisms contained therein, is a matter of considerable difficulty, owing to the large quantity of debris that is nearly always present.

Areas of the sea into which the contents of large rivers flow are usually contaminated with the spoil carried off the land. This finds its way into the river either by accident or intention, and the period during which the lighter particles remain afloat depends largely upon the strength of the current that carries the material along.

Practically the whole of the Lancashire coast-line is influenced more or less by the outpouring of large rivers, such as the Dee, Mersey, and Ribble in the southern and central part, and by all the rivers flowing into Morecambe Bay in the north. Therefore, unless we can go to a considerable distance from land, our tow-net collections are almost wholly composed of vegetable debris, the land origin of which is clearly demonstrated by the presence of sporangia of ferns, seed capsules and leaves of mosses, protecting scales of leaf-buds, twigs and leaves of trees, etc.

The quantity of debris present in these local townettings is, of course, subject to weather and tidal influences. After a spell of calm, during neap tides, we occasionally get a gathering nearly free from rubbish. One or two gatherings from the vicinity of the Bar Lightship in the Mersey consisted almost entirely of the Copepod *Eurytemora*. In the summer of 1895, gatherings were taken in the Rock Channel, which contained nothing but large quantities of *Noctiluca*. This was unusually abundant throughout the southern part of our district for a few weeks, occasionally giving the water a distinct brown appearance. None of the gatherings taken in the Barrow Channel, outside of Walney Island Lighthouse, have been free from debris, and the same is true of those taken in the Ribble, off Lytham Pier, and in the neighbourhood of Nelson Buoy. Mr. Ascroft's system of placing his tow-nettings in white dishes with clean sea water, and allowing the organisms to separate out and come to the sides of the vessels, where they are secured and preserved, is a very useful one, but, unfortunately, not always practicable by our fishery officers, as their police work takes up most of their time.

After the experience gained last year, we arranged that the gatherings should be taken in more seaward positions during 1898. This has been done, but the results are very much the same as before. The weather is frequently quite unsuitable for our sailing boats to venture far from land, and on calm days rowing out to the stations is tedious and dangerous, owing to the tides.

To gain an accurate idea of the floating plankton of the Irish Sea other means will require to be adopted. The method that suggests itself as being the most convenient from all points of view, is to make use of the lightships that are anchored off various parts of our coast. There is probably sufficient current set up by the rise and fall of the tide to keep a tow-net extended. By supplying the keepers with bottles containing preservatives, tow-nets, and necessary instructions for working the nets, preserving the material collected, and the times (night or day, or both) when the collections should be taken, a more satisfactory knowledge of the plankton would be obtained. One of the men when off duty might be taken out in our steamer and shown the methods. Then the steamer could visit the lightships when convenient, say once a month, leave a fresh supply of bottles, replace worn out tow-nets, and bring back the collections taken during the interval.

The success of this method, of course, depends entirely upon (after the necessary permission has been obtained) the zeal and care of the men themselves, and the encouragement we give them.

The collecting stations which might be tried this ensuing year are the North-West, the Morecambe Bay, the Selker, and the Bahama Lightships, and if the results prove satisfactory, which no doubt they will, the system can easily be extended.

The material examined during the past year has given us results which vary very little from those already given in former Reports. The following are a few of the more noteworthy organisms observed:—

Mysis occurred in many of the gatherings throughout the year, but only a few individuals were present in each tow-netting. They were very common during the whole year in the shore pools in our neighbourhood. On September 14th, immense number of young *Mysis* were observed in the channels near Baicliff. This is undoubtedly one of the most valuable sources of food supply for young fishes.

Crangon, *Pseudocuma*, *Gammarus*, and *Eurydice* were occasionally noticed, but only one or two individuals at a time. On a warm day, when the sea is calm, numbers of *Eurydice* may be seen disporting themselves on the surface. In their movements they are not unlike the "Whirligig" beetle of the fresh-water ponds.

Copepoda—*Eurytemora*, *Acartia*, *Paracalanus*, *Temora*, *Calanus*, *Pseudocalanus*, and *Oithona*, were present more or less throughout the year, but never, as a rule, in any quantity. In a few of the gatherings from the vicinity of the Bar Lightship, *Eurytemora* was, on one or two occasions, very common. The striking difference between local and off-shore collections was clearly demonstrated by the comparative scarcity of Copepoda in-shore. As early as January 10th one-fifth part of the tow-nettings, taken by the "John Fell," five miles north of the Selker Lightship, consisted of *Calanus*, *Paracalanus*, and *Acartia*. At the same time, only a few individuals were taken in local gatherings.

At a very early stage in the life history of most fishes, Copepoda play an important part as a food supply. The majority of the young flat fishes—Plaice, Flounder, Dab,

&c.—when they first appear on the sandy shores, feed almost entirely on these minute crustaceans. The species usually found in the stomachs of the young fishes, between half-an-inch and an inch, are chiefly littoral, such as *Eurytemora*, *Ectinosoma*, *Tachidius*, and *Jonesiella*. Although the Copepods at particular stages form a food supply for the fishes, it is just possible that the fishes themselves, when they are newly hatched, may be eaten by the Copepods. Instances of this were demonstrated during our feeding experiments, and a species of Copepod *(Centropages)* was seen to capture one week old Flounders and eat them.

Other invertebrates, such as *Sagitta*, Medusæ, Ctenophora, and *Oikopleura* occurred very sparingly in the local gatherings.

The eggs and larvæ of fish were very plentiful in townettings taken in the open sea, but very few eggs and no larvæ occurred in the local collections. The first collection in which fish eggs were observed were those taken by the steamer on January 27th, at the "Top end of the Hole." None were found in local gatherings until February 4th.

Amongst the surface material collected by tow-net when the steamer was in the Clyde on April 21st, were three large fish eggs, measuring fully three millimeters in diameter. The larvæ were well developed, and hatched out a few days later, but did not survive. There was one large and one small amber-coloured oil globule present, but no space between the embryo and the egg membrane. The species of fish to which these eggs belonged is still uncertain. The only eggs corresponding to them in size are those of the Halibut; but the eggs of this fish have apparently not yet been taken in surface nets. The newly hatched larvæ had a large yolk sac and very short tail.

Larvæ of various groups of the invertebrata, including Mollusca, Crustacea, Polyzoa, Vermes, and Cœlenterata, were occasionally observed in the local collections. The floating eggs of *Alcyonium* were very common in Barrow Channel, off Roa Island, in the beginning of April, and also in the tow-nettings taken in the Clyde.

Noctiluca was very common in the collections taken in the vicinity of the Bar Lightship, Mersey, on June 24th and onwards; but was not observed in the northern part of the district until the end of August.

Ceratium tripos, *C. furca*, and *C. fusus*, occurred in many collections throughout the year.

Diatoms (*Biddulphia*, *Coscinodiscus*, &c.) were very seldom found in the local collections, but were abundant in the gatherings made by the steamer further out, in the spring months.

Experiments with Weighted Drift Bottles.

(Andrew Scott.)

In a former Report,* under the title, "The Drift Bottles and Surface Currents," in which the result of the distribution of drift bottles over the Irish Sea, and the conclusion drawn therefrom, is given, reference is made to an experiment of Mr. Ascroft's, where, instead of using small bottles containing only the post card, larger bottles were employed, but, in addition to the post card, were so weighted with sand that they floated almost entirely submerged, and of which nearly 30% were returned. It is also incidentally remarked that some of them sunk out of sight when set free, one being subsequently brought up in a steamer's trawl while fishing in the vicinity of the Bahama Lightship on the north-east coast of the Isle of

Report for 1895, Sect. II., p. 12.

Man. This is now accounted for by the following explanation. During transit some of the bottles were broken, but in order that the post cards should not be wasted, other whole bottles were obtained, the card and the same sand put in, then the whole sealed up, over-looking the fact that all bottles may not be of the same weight, each requiring careful ballasting, consequently when these bottles were thrown overboard the majority of them sank.

The result of this experiment of Mr. Ascroft's showed that weighted bottles tended to go south, thus differing from the light ones, which largely went to the north. In order to throw further light on this apparent southerly drift of weighted bottles, it was decided to give the weighted bottles a further trial, and in addition, by setting them free on the spawning grounds when the steamer was trawling for spawning fish, perhaps gain some definite information regarding the direction of the drift of the surface waters in which the embryos and larval fish usually are.

Accordingly we had a number of ordinary post cards having the same notice previously used, printed on the back, and we purchased a supply of bottles, known as "cocoa wine bottles," of about one pint capacity. These bottles were placed one by one floating in a bucket of sea-water of 1·0026 specific gravity, and then carefully ballasted with dry sand, so that when the rolled up and numbered post card was placed inside, the cork inserted, and the whole sealed up with paraffin wax, only about an inch of the neck of the bottle was above the surface of the water. They were then sent on board the fisheries steamer in batches, each batch being accompanied by forms having the numbers of the bottles, and spaces for the insertion of the position of the steamer when the bottle was set free, the date, time, state of the tide, direction of the wind, and approximate quantity of floating eggs taken in the tow-nets. The forms were filled up by Captain Wignall, and returned to me, after the bottles had been set free.

Altogether 102 bottles were made up, but one of these was broken in transit, so that during the time the steamer was trawling for spawning fish, from February to the middle of May, 101 bottles were set free, of which we have exact particulars regarding their distribution. Of this number 41 or 40·5% have been returned to Piel from various parts of the coast-line of Cheshire, Lancashire, Cumberland, and the South of Scotland.

The following table gives the position of the steamer when the bottles were let off, the place where they were subsequently picked up, and the number of days that elapsed between their despatch and recovery (see p. 34) :—

From this table it will be seen that nearly 22% of the bottles have drifted in an easterly or southerly direction after being set off, and nearly 18% have taken a northerly direction. Only one bottle, No. 60, appears to have crossed the head of the tide. Of the 40 set free in the vicinity of the Bahama ship, 12 have been returned, and of these eight have gone north to the south coast of Scotland, and four have taken a south-easterly direction, landing on the Cumberland coast. Bottles 51 and 53, set free within fifty-five minutes of each other, yielded rather peculiar results, the first going to Holyhead in seven days, and the second to Duddon in 47 days, distances of fifty-five miles and twenty miles respectively in a straight line from the point of despatch, but in exactly opposite directions.

Of the various days on which the bottles were found, only three were Sundays, and seven were Mondays. The conclusion naturally drawn from this evidence is, that the bottles were probably found almost immediately after being stranded. The shores are so regularly patrolled by the people living in the immediate neighbourhood, that there is little chance of any unusual object being long over-looked. One or two of the finders stated that the bottles came ashore during the previous tide, and one was picked up at sea, five miles from Blackpool, by a fisherman, after it had been 23 days in the water.

In all probability the bottles would float slightly higher in the water of the open sea than they did in the bucket at Piel, as the specific gravity of the water at the various stations ranged from 1·00268 to 1·0027, so that there would be little chance of their going towards the bottom of the sea till they entered the estuaries of rivers.

The table shows that out of the 101 bottles set free, fully 30·6% have been stranded on the Cheshire, Lancashire, and Cumberland coasts, and therefore the result of this experiment, even with weighted bottles in place of light ones, confirms, in a striking manner, the conclusion arrived at by Professor Herdman* when summing up the results of the first series of experiments, *i.e.*, "That the embryos of fish spawning in the deep water on the eastern side of the Isle of Man would go to supply the nurseries in the shallow Lancashire and Cheshire Bays."

Fish eggs and larvæ were found in all the tow-nettings made during the experiment.

[Table over page.

* Rep. Lanc. Sea-Fish. Lab., 1895, pp. 20—21.

No. of Bot.	Position.	Date, Time, and Tide.	Direction of Wind.	Where and When Found.		No. of D'ys
2	17 m. N.W. ½ W. from Gas Buoy, Piel ...	Feb. 21, 3-30 p.m., 3¾ h. ebb ...	N.N.W.	W. side of Walney	Mar. 1	8
3	Ditto ditto ...	,, 3-45 ,, ...	,,	Ditto	,, 1	8
5	12 m. W. from Selker Lightship	,, 4-50 ,, ...	N.	Heysham	,, 3	10
7	13 m. W. by N. from Selker Lightship ...	,, 5-20 ,, ...	,,	W. side Foulney	,, 2	9
8	13 m. West of above ship	,, 5-45 ,, ...	,,	W. side Walney	Feb. 28†	7
10	Ditto ditto	,, 6-15 ,, low-water ...	,,	Ditto	Mar. 6*	13
12	10 m. N. by W. ½ W. from Morecambe Bay Lightship	Mar. 10, 1-15 a.m., 45 min. ebb...	N.W.	Silecroft	,, 16	6
13	11 m. N.N.W. of above ship...	,, 1-30 ,, ...	,,	Ditto	,, 15	5
14	Ditto ditto	,, 1-45 ,, ...	,,	Ditto	,, 16	6
18	Ditto ditto	,, 2-45 ,, ...	,,	Ditto	,, 16	6
22	15 m. S.W. by W. from Selker Lightship ...	,, 3-45 ,, 3¼ h. ebb ...	Calm.	Haverigg point	,, 18	8
23	Ditto ditto ..	,, 4-0 ,, ...	,,	Duddon	,, 17	7
25	Ditto ditto ...	,, 4-30 ,, ...	,,	Silecroft	,, 16	6
27	Ditto ditto ...	,, 5-0 ,, ...	,,	Whitbeck	,, 16	6
31	4 m. S. from Bahama Lightship	Mar. 15, 6-30 ,, 3 h. ebb ...	S.W.	Haverigg point	Apr. 11†	20
46	5 m. E. by N. of ditto	Mar. 16, 12-45 p.m., 1½ h. fl. ...	W.S.W.	Seascale	Mar. 24	8
47	Ditto ditto	,, 1 p.m. ...	,,	Ditto	,, 24	8
50	5 m. E. by S. of Bahama Lightship... ...	,, 2 p.m. ...	,,	Nethertown	,, 21†	5

51	17 m. W.S.W. of Blackcombe	Mar. 22, 12-20 p.m., 1 h. ebb ...	W.N.W.	Holyhead	,, 29	7
53	Ditto ditto	,, 12-55 ,, ...	,,	Duddon	May 8*	47
59	13 m. S.W. by W. ¼ W. from Selker ship...	,, 2-25 ,, ...	,,	Haverigg	Apr. 20	29
60	Ditto ditto ...	,, 2-40 ,, ...	,,	Powillimount	,, 18†	27
63	19 m. W. of Blackcombe	,, 3-15 ,, ...	,,	Duddon	,, 12	21
68	18 m. ditto	,, 4-30 ,, ...	,,	Bootle	,, 11†	20
69	16½ m. ditto	,, 6-40 ,, ...	,,	Askam	,, 12	21
73	¾ m. from Bahama ship	Apr. 12, 11-30 a.m., 3 h. fl. ..	N.	E. side Wigton Bay	,, 19	7
74	Ditto ditto	,, 11-50 ,, ...	,,	Gatehouse	,, 22	10
76	½ m. E. ditto	,, 12-15 p.m. ...	,,	Garliestown	,, 20	8
80	2 m. E.N.E. ditto	,, 1-30 ,, ...	,,	Ditto	,, 20	8
81	1 m. S.S.E. of Bahama ship	,, 6-30 ,, 3½ h. ebb ...	S.W.	Wigton	,, 26	14
86	Ditto ditto	,, 7-45 ,, ...	,,	Isle of Whithorn	,, 23	11
87	Ditto ditto	,, 8 ,, ...	,,	Mull of Galloway	,, 29	17
90	Ditto ditto	,, 9 ,, ...	,,	Garliestown	,, 22	10
92	10 m. W. of Morecambe Light Vessel ...	May 19, 12-15 ,, 1¾ h. fl. ...	E.N.E.	Hoylake	June 2	14
95		,, 1 ,, ...	,,	New Brighton	,, 3	15
97		,, 1-30 ,, ...	,,	Knot-end	July 4†	47
98		,, 1-45 ,, ...	,,	Altcar	June 2	14
99		,, 2-0 ,, ...	,,	Blackpool	,, 20†	32
100		,, 2-30 ,, ...	,,	At sea 5 m. from B'kpool	,, 11	23
101		,, 2-45 ,, ...	,,	Blundellsands	,, 4	16
102	to 16 m. W. by N. of same ship	,, 3 ,, ...	,,	Altcar	,, 5*	17

* Sundays. † Mondays.

The Spawning of the Mussel *(Mytilus edulis)*.

(J. Johnstone.)

During the last year an investigation of the reproductive organs of the common Mussel has been made in relation to the period during which spawning takes place on the beds along the Lancashire sea coast, and to the histological changes accompanying the ripening and extrusion of the reproductive products. The methods employed were:—(1) The microscopic examination of the gonads of specimens taken by Mr. A. Scott from the Roosebeck scars and the beds in the Barrow Channel, and of specimens sent by the bailiffs from the Wallasey, New Brighton, and Morecambe beds; (2) the search for free-swimming and fixed larvæ on the beds themselves, and (3) the examination of the in-shore tow-nettings taken by Mr. Wright in Morecambe Bay, and by Mr. Eccles outside the estuary of the Mersey. The records of a continuous weekly series of tow-nettings, taken in the year 1895 by Mr. R. L. Ascroft, at Lytham Pier, have also afforded valuable evidence.

As a result of this year's observations, it has been found possible to fix approximately the date of a maximum in the spawning of the Mussel, during which a rapid and complete extrusion of the genital products, accompanied by other histological changes in the mantle and visceral mass of the animal, takes place. In the year 1898 this was found to begin about the beginning of July and last till about the beginning of August; but it is probable that the limits of this period are variable to some extent. There is, however, considerable doubt as to whether this is the only time in the year during which spawning takes place, and various observations render it at least possible that there is a secondary spawning period early in the year, and that there is a continual but slow emission of ova and spermatozoa from the time when these have accumulated in considerable quantity in the gonads, that is to say, from the beginning of April on to the beginning of the summer spawning period. And it seems certain, considering the variability observed in the ripening of the gonads, that isolated individuals may undergo complete spawning a considerable time in advance of, or later than, the date of occurrence of the maximum. However that may be, there can be no doubt that the number of larvæ resulting from spawning in the early part of the year bears a very small proportion to those produced during the maximum spawning period in the summer months.

There is still some uncertainty regarding the rate of growth of the young Mussel, and this is due probably to variations contingent on the conditions under which the adult animal spawns, and the larva undergoes its early development. Most probably in the early stages during which the young Mussel has a free-swimming existence, the development and rate of growth are fairly constant, but with the acquisition of the byssus and the fixation of the larva, the subsequent growth is dependent, to a large extent, on the situation it finds itself in, the supply of food, the extent to which the larvæ are crowded together, and on the time of year in which spawning of the parent occurred. From the observations made by Wilson,* who succeeded in artificially fertilizing eggs of the Mussel, and tracing out the early development, it appears that the larva, about 0·15 mm. in length, provided with semicircular valves, showing the first rudiment of the anterior adductor muscle, and still using the velum as a locomotive organ, is at most 12 days old. An older stage than this, with the

* J. Wilson.—"On the Development of the Common Mussel." Annua Report of the Fishery Board for Scotland for the year 1885, pp. 218—222, and Report for 1886, pp. 247—256, Plates XII.—XIV.

valves assuming an oval form, the foot developing, the posterior adductor muscle present, rudiments of four gill filaments appearing, and the velum still functional is, at the outside, a month old. Subsequently to this (0·28 mm. long) blue pigment is deposited round the margin of the shell, the foot becomes the organ of locomotion, and the larva prepares for fixation by the development of byssus gland and byssus.

Free-swimming larvæ, somewhat younger than the stage last referred to, that is, about 0·25 mm. in diameter, and provided with circular valves showing no trace of pigment, were taken by Mr. Scott in a tow-net gathering at Piel, on September 9th, last year. These Mussels were certainly less than one month old, and their appearance at this time is in accordance with anatomical observations made on specimens taken in the neighbourhood, which led us to fix July and early August as the months during which spawning took place. But the evidence given by other observations of this kind is very perplexing. Thus young Mussels with circular or oval valves ranging in size from 0·27 to 0·45 mm., and with the rudiments of four or more gill filaments present, were taken in June, 1892, fixed to various zoophytes, and assuming the age of these not to exceed a month, the time of spawning is thrown back to May or early June. An examination of the in-shore tow-nettings shewed that young Mussels of approximately this size (0·3 to 0·6 mm.) were taken in the estuary of the Ribble on January 27th, 1898, and others, varying in size from 0·2 to 0·7 mm., were found in Ulverston Channel on February 4th. Some of these had probably been already fixed and were loosened by the force of the tide, but others had all the characters of the free-swimming stages described above, and their presence at this time is only explicable on the assumption that there had been considerable retardation in the rate of growth, or that they had resulted from spawning early in the year. The early maturity of the spermatozoa is interesting in this connection. In some specimens taken in December by Mr. Scott, ripe spermatozoa, which remained alive for about 12 hours, were expressed from the mantle lobes. An attempt was made to bring about artificial fertilization, but although the eggs were found to be covered with motile spermatozoa, and in some cases the formation of the first polar body took place, the segmentation of the ovum was not observed.

The assumption that some Mussels at least may spawn early in the year is necessary to explain the presence of larvæ in January and February, if it should be found impossible to account for the presence of such by considerable variation in the early development and rate of growth; and that there is a gradual emission of spawn during April, May, and June seems probable also in view of the fact that free-swimming larvæ are to be found in May and June. But from the anatomical standpoint, such secondary spawning periods are accidental, and do not effect the statement that there is a yearly cycle of changes in the reproductive organs of the Mussel, which begins with the termination of the act of spawning sometime in the summer months, includes the gradual and continuous ripening of the gonads, and ends with a comparatively rapid extrusion of the reproductive products, leaving the animal in a "spent" condition, after which a short period of rest occurs, and the cycle of changes is repeated. The duration of the maximum spawning period we have not precisely made out, and its date is probably variable and dependent on changing meteorological conditions, but that it occurs during May, June, July, and August seems perfectly certain. This cycle of changes will now be considered.

It will be remembered that in the common Mussel the sexes are separate. Hermaphrodites, if they exist, must be extremely uncommon as, in the examination of many hundreds of specimens, none have been observed. There is a slight preponderance of males over females, the ratio in 218 specimens examined being 118 : 100 ; but it is probable this is too low, as it is hard to avoid selection in choosing the specimens for examination.* Except for a slight difference in the colour of the mantle lobes there are no external sexual characters.

The gonad (Pl. I., fig. 2) is situated in the visceral mass and mantle lobes; the organ is paired and bilaterally symmetrical, and consists of a branching tubular gland, the efferent duct of which is situated on the summit of a small papilla (Pl. I., fig. 1, *pp. gen.*), about 1 mm. in height, which lies posterior to the foot and immediately beneath the gills (*br. R.*), and can be easily seen by reflecting the latter and the mantle (*Mn. R.*). This duct is richly ciliated for some distance internally, when the ciliated wall is partially replaced by germinal epithelium. Strips of columnar ciliated epithelium (Pl. II., fig. 7, *ep. cil.*) are present along the greater length of the tubules, but the terminal portions are entirely lined by germinal epithelium. Passing inwards these tubules branch

* Since the above was written the tabulated results of the examination of the specimens sent to the Laboratory by the Bailiffs have been referred to. The portion of these records dealt with relates to the examination of a large number of Mussels taken at random from most of the Mussel beds in the district, and sent to the Laboratory in lots of a dozen each, and extends over the years 1892-96. In order to eliminate as many sources of error as may be possible, all those sheets in which the sex of one or more of the specimens is regarded as doubtful, and also those dealing with Mussels taken about the time of the "spent" period, have been rejected. This leaves a total of 821 specimens of which the sex was probably accurately determined. Of these 449 were male and 372 female, giving a ratio of males to females equal to 6 : 5 (very nearly).

repeatedly and in quite an irregular manner. Anastomoses between the various branches have been figured by Wilson, but we have not seen these. All along the course of the main branches small twigs are sent out, some no longer than their own diameter, and these grow and invade whatever portions of the body they can find room in. The gonad thus occupies no particular part of the body of the animal except that the position of the external opening is constant, but it may be conveniently said to be situated posteriorly to the digestive gland, and as ripening proceeds, to invade every part of the body, where only connective or parenchymatous tissue is found (Pl. I., fig. 2, *tub. ov.*). Simultaneously with the encroachment of the genital tubules, this tissue undergoes absorption or degeneration. The keel-like mass, the "abdomen," (Pl. I., fig. 1., *abd.*) situated medially and posteriorly, is thus completely filled up by the organ, and as maturation goes on, the whole space in the mantle lobes (Pl. I., fig. 2, *Mn. L.; Mn. R.*), between the internal and external epidermal surfaces is filled up by the branching tubules of the gonad and except for the merest trace of connective tissue contains nothing else. Long before spawning occurs these tubules have invaded almost every part of the body (Pl. I., fig. 2), even the delicate membrane forming the external wall of the pericardial cavity being overspread by them and becoming opaque. With the act of spawning the whole appearance of the animal may be changed, owing to the emptying of these tubules of their contents, and the mantle lobes may lose their thick and opaque appearance, and become thin and transparent. This is the case in the Mussels in some of the beds in the district, but more usually the mantle lobes remain thick and opaque. After spawning it is only by microscopic examination that the condition of the animal can be determined.

As indicated above, the cycle of changes in the gonads is a yearly one, or approximately a yearly one, variations due to the nature of the season doubtless taking place. This cycle of changes may be represented by four stages. In giving the dates, we refer more particularly to what was found during the year 1898.

Stage I.—The end of *July* and the beginning of *August*. The Mussel is "spent," that is, the reproductive products have been entirely extruded, and in the mantle lobes and visceral mass the genital tubules are, to a large extent, collapsed or degenerated. The state of the animal in respect of its reproductive function is one of rest.

Stage II.—*September*. The gonads have begun to invade the mantle lobes and other parts of the body. Proliferation of ova and spermatozoa from the germinal epithelium is in progress, and goes on slowly and continuously from now till early in the following year, when it becomes very active, and the mass of the gonads becomes much greater.

Stage III.—*April*. The mantle lobes have attained their maximum of thickness, and are completely filled up by the gonads. The tubules composing the latter have increased greatly in sectional area, and are completely filled up by ova or spermatozoa in female and male respectively. Proliferation from the germinal epithelium is not now so active as in the earlier part of the year, and maturation of the genital products probably goes on. Where the ova or spermatazoa are in contact with the ciliated portions of the tubules, they are probably being swept away and removed out of the body.

Stage IV.—*July* and early *August*. Spawning in the sense of a complete removal of the genital products is now in progress. There is a rapid decrease in the mass of the gonads, both in visceral mass and mantle lobes, and a corresponding increase in mass of the parenchymatous tissue lying between the tubules. Many of the branches of the tubular gonad disappear entirely, but many others, and particularly the ciliated, non-glandular portions, persist *in situ*. At the end of this period, when the animal again enters on Stage I., the parts of the body formerly occupied by the swollen genital tubules are now the seat either of a massive syncytial tissue, or of a delicate reticulum, the difference depending probably on the condition of nutrition obtaining on the bed from which the specimen was taken.

We may now consider the characters presented by the animal in the various stages referred to more particularly.

Stage I. (Pl. II., figs. 3, 4, 6).—Externally there may be nothing to indicate that the specimen under examination has spawned, although in a section, or in a cleared preparation, the difference between this stage and the preceding one is striking. The sex of the animal is determinable only with some difficulty, by the presence of stray masses of ova or spermatozoa, which have failed to be extruded. Where the germinal epithelium can be recognized, it is not very different in male and female. The area in a section of the mantle now occupied by the tubules of the gonads is so greatly reduced that, on a superficial examination, it might be thought that the latter had completely disappeared. But more careful scrutiny reveals the presence of many tubules in a collapsed condition (Pl. II., figs. 3, 4, *tub. ov.*), the walls pressed against each other by the pressure of the reticular tissue. It is, however, only the larger tubules which so persist; the finer branches have been absorbed or broken down in some way.

The space occupied in the ripe Mussel by the gonads is now filled up by a large celled parenchymatous tissue

(Pl. II., fig. 3, *ret.*) of peculiar characters. This tissue which, in the ripe Mussel, was represented by a few delicate fibres, lying wedged in between the tubules, has now increased greatly in mass, and occupies almost the whole thickness of the mantle lobes. It does not, like the corresponding tissue in the Oyster, consist of cubical or polygonal cells, with clear bodies arranged to form a coarse network, the bars of which are composed of distinct cells, but is rather a syncytium, in which cell outlines cannot be readily distinguished. The surfaces of attachment of the parts to each other are large, but occasionally delicate fibrous connecting strands may be found. The cells are multinucleated, an isolated patch may be seen to possess several nuclei, but no trace of cell walls. The cell substance stains deeply with eosin. In moderately thick sections it appears to be dotted over with small, light, circular areas, but in thinner parts these are seen to be vacuoles, possibly filled with a substance which does not readily stain.

The epidermis (Pl. II., fig. 3, *ep. ext.*) of the mantle is formed of a single layer of cells, with large nuclei and rather indistinct cell walls. On the internal face of the mantle this epidermis is ciliated. Beneath it there may be a layer of longitudinal fibrous tissue, but this is not constantly present. The reticular tissue of the interior of the mantle is connected with the epidermal layer by delicate strands, but occasionally large rounded masses may be seen lying beneath the former. The epidermis contains eosinophilous cells forming projections internally and externally. Blood vessels are abundant along the external face of the mantle (Pl. II., fig. 4, *bl. sp.*). The larger of these have a fine endothelial lining the structure of which is difficult to make out. These, as well as the interspaces of the reticulum beneath the epidermis, may be crowded with blood corpuscles.

The characteristic of this stage is the enormous development of this reticulum, which has all the characters of a tissue produced by the rapid multiplication of nuclei, without a corresponding differentiation of cell bodies and walls. In some sections the space occupied by it is so great, that the appearance is almost that of a homogeneous nucleated matrix, perforated in many places.

The description given of this stage applies to the Mussels obtained from the Wallasey and Morecambe beds, the Roosebeck outer scar, and from those in the Barrow Channel. Mussels obtained from the Roosebeck inner scar have different characters in respect of the condition of the gonads at this stage, and these are probably correlated with the different conditions of nutrition obtaining on this bed, which have been commented on in a former Report.* Spawning in these Mussels leaves the mantle lobes delicate and transparent. The reticular tissue has the form of an attenuated network of large mesh, and, in consequence of this, the remains of the genital tubules are more easily seen, and a resting stage is not so conspicuous.

Stage II. (Pl. II., fig. 7; Pl. I., fig. 2).—The period of rest which follows immediately after spawning, and which is represented by Stage I., is of short duration, and as early as the beginning of September the developing gonads may be seen in the mantle. There can be no doubt that, after spawning, many of the tubules break down and disappear, but it seems quite clear that the more important branches persist in the mantle through the period represented by Stage I. Towards the end of September ripening has commenced, and proliferatiom from the germinal epithelium is in progress. At any

* A. Scott. "Mussels and Mussel Beds." Lanc. Sea-Fish. Laby. Report for 1895, pp. 21—32.

stage this process is further advanced in the male than in the female. During September the tubules are of small diameter, no greater than that of an interspace in the reticulum. The structure of the cells composing the germinal epithelium of this stage is nearly similar in both sexes. An irregular layer of cells is supported on a basement membrane. The female germinal epithelium (Pl. II., fig. 7, *ep. ger.*) can be distinguished by the large nuclei of the cells. At this stage it consists of few cells, and the first few ova separated off fill the lumen of the tubule. Proliferation is a more rapid process in the male, and in a short time the tubules are filled with cells, resulting from the divisions in the germinal epithelium. Most of these cells become spermatoblasts, and divide subsequently to form the spermatozoa, but many of those separated off at this stage become modified to form the system of supporting filaments so conspicuous at later stages (Pl. II., fig. 5, *sup. fil.*) The cell migrates towards the centre of the tubule, becomes slightly elongated in a radial direction, and its outer end becomes frayed out into one or more delicate filaments, which seem to retain some connection with the wall of the tubule. The nucleus and cell body then break down and disappear, and a group of filaments is left arranged radially in the lumen of the tubule, on which the spermatozoa formed at this time, and subsequently are arranged in rows. In the smaller and terminal portions of the tubules the whole epithelium takes part in the formation of spermatozoa or ova, according to the sex, but in the larger branches, and this is the more common appearance in the sections, the tubule possesses a longitudinal strip of columnar epithelium, cells with clear cell bodies, and with long cilia projecting into the lumen. This strip of ciliated epithelium extends round the tubule for about one-fourth or less of the circumference. It is more common in the male gonad, but is present also in the female.

The parenchymatous tissue between the tubules has much the same characters as in Stage I., but it is now arranged in a more even system of bars and is not so massive. Certain appearances, such as the difficulty of distinguishing the limits of the growing tubules in relation to the surrounding reticulum, seem to indicate that the gonads grow at the expense of this tissue, or at least absorb it during the process of growth. It was, however, impossible to demonstrate this with certainty in the preparations studied.

Ripening of the gonads proceeds slowly from now on to the end of January, and consists of a gradual increase in the width of the already-existing tubules and of the formation of side twigs. The contents of the tubules slowly increases, and it must be understood that from November onwards there is an increasing number of free and apparently mature ova and spermatozoa in the gonads. The fact that moving spermatozoa may be obtained from the mantle at the end of the year has been already referred to. Whether the genital products present in the gonads at the end of the year are fully mature and are capable of development is uncertain, but if so, the presence of free-swimming larvæ in the sea about this time may be easily explained, since ova and spermatozoa may be swept out of the lower partially ciliated tubules, and fertilization may result. But a certain time may also be required for the complete maturation of the genital products after their proliferation from the germinal epithelium.

Stage III. (Pl. II., figs. 1, 2).—About this time the mantle lobes have attained the maximum of thickness, and the gonads have invaded every part of the body unoccupied by the other organs, even the outer wall of the pericardial sac being overspread by them. The whole space between the internal and external faces of the mantle is now filled up by the gonads, and the tubules of the latter are filled, in female and male, with a dense mass of ova and spermatozoa respectively. The spermatozoa are arranged in rows on the radial-supporting filaments already mentioned, and the mutual pressure of the ova against each other in slightly later stages than this gives them a polyhedral form. The germinal epithelium is still undergoing proliferation, but this is not so active now. Where, in the male, the walls of the tubules are closely compressed together, the basement membrane can be seen as a thin, homogeneous line, on either side of which is an irregular row of large, rounded cells, with clear cell bodies and conspicuous nuclei, and internal to this the tubule is filled with a mass of radially-arranged rows of spermatozoa. The conspicuous reticular tissue present in earlier stages is reduced to the merest trace, and is present mostly at the angles formed at the junction of several tubules. It stains less intensely with eosin, but the peculiar vacuolated appearance already referred to still exists. Here and there it is present as rounded masses possessing single nuclei, but it is mostly fibrous.

As in the male, the germinal epithelium still exists in the female, and ova are still being separated off. Most of the eggs lie freely in the cavity of the tubule in contact with each other, but some are still attached to the wall by a short stalk. In some parts the wall of the tubule seems to consist of the basement membrane only.

Stage IV. (Pl. II., fig. 5).—From April onwards there is apparently little change in the appearance of the gonads. The maximum development, as indicated by the increase in mass of the tubules, the numbers of ova and spermatozoa liberated from the germinal epithelium, and the reduction of the other tissues in the mantle, was obtained in the specimens taken during April. Some taken later were, indeed, less advanced, but generally this seems to be the time at which proliferation from the walls of the tubules mainly ceases. In July, Mussels were taken in which spawning was in progress. In these the females had almost completely spawned, and in the males the gonads had undergone a great reduction in mass. A few stray eggs lay scattered over the section situated in tubules of greatly reduced diameter, one egg being present, as a rule, in the cross section of the tubule, and generally nearly filling it. Many tubules were completely empty. The germinal epithelium persisted in some of these tubules as one or more layers of small, rounded cells, but more often its structure was difficult to make out.

The reticulum so apparent in Stage I. is already formed, but in structure is more spongy than in the stage following the complete extrusion of the ova, and the spaces between the bars are about equal to those occupied by the tissue itself. These interspaces have, in many cases, a circular outline, and probably represent the former situation of ovarian tubules. The general histological characters of the tissue are similar to those described in Stage I., except that the vacuolated appearance is not so apparent.

The most striking changes have occurred in the male. In some of the specimens taken at this time extrusion of the spermatozoa has been almost completely effected, but in others the animal had been taken in the act (Pl. II., fig. 5). Here the sperm tubules have been greatly contracted in cross area, and the mass of spermatozoa (Pl. II., fig. 5) within the tubule has become correspondingly denser. The reticulum has increased enormously, and as it seems difficult to derive this tissue from the exceedingly fine fibrous network present in the last stage, it is possible that it has spread into the spaces surrounding the tubules of the gonad from other parts of the body. Round each tubule this reticulum forms a dense mass, with few or no interspaces, and its syncytial character is here strongly suggested. The nuclei are not so evident as at a later stage. Within the tubule itself the linear arrangement of the spermatozoa is very striking, but instead of being directed towards the centre these rows point towards that part of the wall lined with the strip of ciliated epithelium already described (Pl. II., fig. 5, *ep. cil.*). Often a blood space (*bl. sp.*) is situated beneath this epithelium; over the cilia and within the tubule is a space free from spermatozoa. The wall of the tubule has become indistinct from the mass of spermatozoa which seem to abut directly on the tissue of the reticulum (*ret.*) surrounding the tubule. The whole arrangement strikingly suggests the exercise of pressure on the tubule by the rapidly developing reticulum and the removal of the genital products brought into close contact with the ciliated strips of the tubules. The lower conducting portions of the gonads are entirely lined with ciliated epithelium, and once here the genital products must be speedily removed.

Briefly summarised then, the history of the yearly change in the reproductive organs of the Mussel is as follows:—(1) There is a short period of rest following spawning, and occupying some part of August and September, during which the space in the body of the animal formerly occupied by the gonads is largely filled up by a large-celled reticulum. This is followed by (2) the reformation of the germinal epithelium and a slow proliferation of ova and spermatozoa, lasting for the rest of the year. During this period the gonads increase greatly in mass, and invade the mantle and other parts of the body, displacing the reticulum formed during spawning. Succeeding this is a period

(3) of active formation of the genital products, ending late in the spring, after which ova and spermatozoa are formed less rapidly. A slow emission of these now goes on until in the summer, about June or July, the maximum spawning period of the Mussel occurs.

It is unlikely that any great proportion of the larvæ found during the course of the year are spawned otherwise than at the maximum spawning period in the summer months. We have seen that it is probable that a certain proportion are spawned during the first half of the year, but that these probably result from the spawning of isolated individuals or from the slow emission of ova and spermatozoa prior to the act of spawning proper, and the fact that this occurs does not materially affect the general statement that the Mussel spawns in the summer. For the year 1898 we have fixed this spawning period as having occurred during July, but it is probable that it is variable, and as the Committee's bye-law fixes the months of May, June, July, and August as those during which it is illegal to take Mussels from the beds in the district, it is very probable that, notwithstanding some considerable variation in the occurrence of this period, it will always fall within that close time. Probably spawning is very generally over before August begins, but of this further evidence is wanted.

Explanation of the Plates.

Plate I.

Fig. 1. Dissection of an adult Mussel to show the external opening of the gonad. The right valve has been removed, and the right gill and mantle lobe cut away close to the junction with the visceral mass and reflected upwards. Full size. α—β indicates the plane of the section shown in fig. 2.

Fig. 2. Complete transverse section through the body of an adult female Mussel passing through the pericardial cavity just posterior to the heart. Note the presence of ovarian tubules in every part of the section, the stage of development being the same both in mantle lobes and in visceral mass. The specimen was taken about the end of September, and proliferation of ova from the germinal epithelium is beginning, but the diameter of the tubules is still very small. × 5 diameters.

Plate II.

Fig. 1. Transverse section of mantle of nearly ripe female Mussel from Roosebeck outer scar, taken during March. The mantle is nearly filled with the tubules of the gonad containing many free ova. The germinal epithelium is still undergoing proliferation. × 40 diameters.

Fig. 2. Transverse section of mantle of nearly ripe male Mussel from Roosebeck outer scar. (March). The mantle is filled with sperm tubules. × 40 diam.

Fig. 3. Transverse section of mantle of female spent Mussel from New Brighton beds, taken during August. The tubules of the gonads have in many cases broken down. Two are shown in the section, one containing an egg which has not been extruded during spawning. Note the great development of the reticulum. × 200 diam.

Fig. 4. Transverse section of mantle of spent female Mussel from the beds in the Barrow Channel (August). A blood space lying immediately beneath the epidermis is shown, beneath which are two collapsed ovarian tubules. Note that

each is lined partly by ciliated and partly by germinal epithelium. × 200 diam.

Fig. 5. Transverse section of mantle of spawning male Mussel (New Brighton beds, July). A sperm tubule is shown, partly lined with ciliated epithelium. Note the great development of the reticular tissue. × 230 diam.

Fig. 6. Transverse section of mantle of completely spent female Mussel (Barrow Channel, August). × 35 diam.

Fig. 7. Portion of transverse section of mantle of female Mussel (Barrow Channel, September 30). A developing ovarian tubule is shown. Actual size = 0·568 mm. Note the proliferating germinal epithelium, and the ciliated strip of wall. × 660 diam.

Reference Letters.

abd., abdomen; *add. a.*, anterior adductor muscle of the shell; *add. p.*, posterior adductor muscle of the shell; *br. R.*, right gill; *br. L.*, left gill; *bl. sp.*, blood space; *cr. st.*, crystalline style; *ep. ger.*, germinal epithelium; *ep. cil.*, ciliated epithelium; *ep. ext.*, external epidermis of mantle; *int.*, intestine; *Mn. L.*, left mantle lobe; *Mn. R.*, right mantle lobe; *per.*, pericardial cavity; *per. gld.*, pericardial gland and auricle; *pp. gen.*, right genital papilla; *pa. d.*, right dorsal labial palp; *pa. v.*, right ventral labial palp; *ren.*, renal organ; *ret.*, reticulum of mantle; *ret. bys.*, retractor muscles of the byssus; *ret. bys. in.*, insertion of retractor of the byssus into the shell; *rect.*, rectum; *sup. fil.*, supporting filaments in sperm tubule; *st.*, tubular portion of the stomach; *sp.*, rows of mature spermatozoa; *tub. ov.*, ovarian tubule; *visc. nv.*, visceral nerve cord.

On Sea-Fish Hatching.

(W. A. Herdman.)

In the Twelfth Annual Report of the Sea-Fisheries Inspectors for England and Wales (1898), under the marginal heading "Sea-Fish Hatcheries" (p. 29), Mr. C. E. Fryer enters upon such a severe and detailed criticism of the results attained that it cannot be regarded as other than an attack upon the practice of artificial hatching of sea-fish eggs as developed in America. It is fair to say that it is the results of the hatcheries in America that are attacked, as, although Scotland and Norway are mentioned, these countries are treated very briefly, and no conclusions are drawn from the few statistics quoted. The greater part of the discussion deals, then, with Canada, Newfoundland, and especially the United States. I gather that Mr. Fryer has not himself visited the hatcheries he discusses, and that his information is entirely derived from the published annual reports—an unexceptional source, so far as it goes, but one which may usefully be supplemented by personal impressions and conversation with those engaged in the work.

I regard Mr. Fryer's paragraphs as a most useful statement, drawing attention to the present position of the hatching question from the critic's point of view, counteracting any exaggerated opinions that may have been expressed, and impressions that may have been entertained, in regard to the immediate and enormous results to be obtained from artificial operations, but requiring some criticism, some modification, and some additions before it can be accepted as a wholly satisfactory statement of the matter. For although Mr. Fryer seems rather carefully to avoid drawing any formal conclusions, he certainly, consciously or unconsciously, by his manipulation of the statistics, conveys to the reader an impression entirely adverse to sea-fish hatching. And this impression is, I believe, derived mainly from statements in regard to the difficulty and not in regard to the efficacy of the operations.

Mr. Fryer's remarks are largely directed towards removing impressions that may prevail as to the *simplicity* of artificial hatching; and here we, in common with all who have taken any part in such work, readily concur in the general conclusion that the work is sufficiently difficult and troublesome, if pains be taken to attain success. I do not think that amongst those in this country who have had to obtain in mid-winter or early spring the spawning fish or the fertilized spawn, who have had to filter the sea-water and watch day and night its purity, salinity and temperature, who have jealously guarded the embryos from danger and have tried to obtain suitable foods for the larvæ, there can ever have been any fond delusions as to the simplicity of the business. The work is hard, there are many difficulties, the operation is a delicate one, requiring constant and intelligent attention; but all that need not deter. Given suitable conditions and the right men, and the work can be successfully carried out.

While agreeing with Mr. Fryer that "simplicity" is a delusion, I must demur against an impression which his pages are liable to convey. It must be remembered that the harrowing descriptions on pp. 36 and 37 of fish perishing in the nets owing to the heavy storms, and freezing in the boats and on the cars, and of embryos killed by the million in the hatching boxes on account of the intense cold, refer to conditions on the coast of North America, and do not apply to our seas. These exceptional difficulties either do not exist or are only present in quite a minor degree for very short periods in our warmer and more protected coastal waters.

Turning now to the question of efficacy, we meet with this difficulty—Mr. Fryer apparently does not accept the turning out of millions of healthy young fry into the sea as a proof of successful hatching operations. He seems to demand a demonstration that the number of adult fish in the neighbourhood has increased, without taking into account the fishing operations that may be going on. In asking for immediate proof that the fry live to become adult fish the opponents of fish hatching are taking up what may seem a very secure, but is, I consider, a very unreasonable position. So many factors enter into the case that it is almost impossible to devise a crucial experiment, or give immediate scientific proof of a result. The demand may possibly be satisfied in any locality next year, or the answer may be delayed for 10 years or for longer; and yet the fisheries may all the time be largely benefitted by—may owe their continued existence to—the artificially hatched fry added to the population of the sea. And this may be the case *even if not a single individual out of the millions of fry set free ever lives to become adult.*

I do not think it at all probable that such unfavourable conditions ever exist, but I wish to point out that even in such an extreme case the expenditure on land and the sacrifice of life of the fry in the sea will not have been in vain. If we try to realise the struggle for existence in the sea, the toll that is taken at every period of life from the egg to the adult, but which is exceptionally heavy in young stages, a moment's reflection will show that the addition in any natural hatching area of some millions of young fish must distribute the danger of being caught by an enemy over a much larger number of individuals and so give to each a greater chance of escape. And if—as may be the case, we do not know—the naturally hatched fry are hardier or more active, then they will be the more likely to survive the perils, and will owe their continued existence and their appearance in due course somewhere as marketable fish to the presence for a time in the sea of their brethren from the hatchery.

I shall come now from these general considerations—to which Mr. Fryer does not seem to give enough attention—to some of the detailed statements in his report as to the results of hatching, and upon some aspects of which, such as the mortality in captivity, in my opinion he lays far too much stress. I cannot now go into every point upon which we may differ more or less, but shall take up his treatment of the United States statistics, as they are, perhaps, the most important, and the ones discussed at greatest length.

After quoting (p. 39) the statistics of the Cod operations at the Wood's Holl Hatchery, he admits that the figures show "successful results." He then attempts to upset this conclusion by arguing that in computing the number of eggs and fry derived from a parent fish we should take into account not merely the actual parents, the fish that spawned, but also all the others that were penned up in the spawning pond. This is surely a most unjustifiable procedure. What reason is there to think that these non-spawning fish would have spawned if left in the sea? In fact, if the object is to make a comparison between natural and artificial conditions, surely from what we know of life in the sea it is highly probable that some of the fish that spawned in captivity would, if left at large, have been destroyed by man, or their natural enemies, before they became parents. Consequently, I consider that the Superintendent of the Hatchery was perfectly right in considering as parent fish only those from which spawn was obtained, and I should remove from Mr. Fryer's table, at the foot of p. 39, the three columns headed "penned up," as giving no additional useful information.

It is also unfair to compare an average obtained by including all the fish penned up, many of which did not breed, with the total number of ova calculated as being present in one selected large breeder. The comparison can only be fairly made with the eggs in a similar number of Cod, of the same size as those in captivity, chosen at random from the sea. Even in that case the comparison would only indicate whether there was a diminution in the amount of spawn produced per captive fish. There probably is such a diminution; but, on the other hand, it is certain that a large number of possible spawners in the sea are killed by man and other enemies before or during the spawning season.

Then, again, in making a further comparison between artificial hatching and the "processes of nature," it is not right to substitute (p. 41) the number of eggs calculated to be present in the body of a parent for the actual number of eggs treated in the hatchery. This method of "counting the chickens" not only before they are hatched but even before the eggs are laid, is an ingenious method of making the proportion of fry produced in the hatchery seem very small; but I think the Superintendent's figure, 54·6 per cent. of fry to ova, will commend itself to most people as the correct calculation on the common-sense basis of so many ova supplied and so many fry produced. What would have happened to, say, 100 of the hatchery spawners if they had been left in the sea, no one can say. How many of them would live to spawn and how much spawn they would produce we do not know. The percentage of fry to parent fish in the hatchery may, under some circumstances, be small, but it may be smaller still in a state of nature.

It is curious how Mr. Fryer entirely ignores that side of the question—the destruction in nature. He makes a great point of the mortality in artificial operations, and virtually assumes that in the sea every egg in every fish will be spawned and will then hatch out. This is entirely contrary to the experience of naturalists. In addition to the destruction of the parents in the sea, and the risks of non-fertilisation, and of being cast ashore, we find at the spawning season nearly everything that has a mouth (such as Medusæ, Sagitta, Copepoda, and many fishes) feeding upon fish eggs. One of the strongest arguments in favour of the hatchery is that it protects the embryos from their natural enemies. Judging from the apparently stationary condition of the fish population in the sea, from the enormous numbers of eggs produced, and from our observations on the contents of fishes' stomachs, it is obvious that there must be a very great destruction of eggs, and embryos and larvæ under natural conditions—probably much greater than anything known in artificial operations.

Moreover, in talking (as many do) of the small scale of man's hatching work compared with what takes place in the sea, we do not sufficiently realise the meaning of what is called "the balance of nature." Millions of millions of young are produced and these same millions of millions are destroyed by natural causes, with the result that the species remains fairly constant. If now man disturbs the balance by catching some thousands of the adult fish in a district, he is only equalizing matters and endeavouring to minimise his destructive effect when he adds to that sea-area some millions of artificially hatched fry. And (theoretically at least—probably practically also, for correct theory and perfect practice must be in accord) he is the better equalizer of the disturbed balance when he uses for the hatchery the eggs of fish which would not have spawned in a state of nature—that he can do by obtaining ripe spawn from the fish trawled for market at the spawning season. This plan has been adopted in some hatcheries; and in our own experiments at Port Erin and at the Piel hatchery. It is the least expensive method of getting spawn, but it is open to several difficulties and objections. The other method is to collect the parent fish beforehand and keep them in ponds until they spawn. It is the method adopted in Norway and to a large extent in the United States, and which Mr. Fryer has tried to show results in a comparatively small number of fry per fish used.

I shall not pursue the matter further. There is no need for me to defend or expound the methods and statistics of the United States Fish Commission. Their own officials are very well able to answer for themselves, and very probably they will do so—if they think it worth while.*

I have merely gone into some of the questions raised in Mr. Fryer's article because it seemed to me that he was modifying the statistics in an unjustifiable manner, and drawing conclusions with which I could not agree, and which might have some bearing upon our procedure in this

* It may be well before leaving this part of the subject to point out that in the appendix (a Manual of Fish-Culture, based on the methods of the United States Commission of Fish and Fisheries, Washington, 1898) to their last published report, which appeared only a few months ago, the United States authorities remark in regard to their artificial propagation of the Cod:—"The unmistakable economic results which have attended these efforts warrant all the time and money devoted to them and justify the greatest possible expansion of the work." I have also more recent letters from the Commissioner and the Naturalists on his Staff making detailed statements to the same effect.

country. We are not, however, in our work here so much concerned with the actual details and difficulties of the American work which Mr. Fryer criticises, as with those general biological principles which I have emphasised above—such as the destruction of young under natural conditions in the sea, and the duty of man if he disturbs the balance of nature in a locality to do what he can to equalize matters by helping in the production and protection of the young.

With Mr. Fryer's remark (foot of p. 41) as to breeding from the larger and more vigorous parents I am in cordial agreement. If aquiculture has been long delayed as a scientific industry, it has as a consequence this advantage, that it can adopt at once principles and practices evolved gradually in the long history of agriculture and stock-raising. It labours, however, under the obvious disadvantage that so much of the result of our labour passes at once beyond our ken. We cannot control the fish throughout their life-history, we cannot yet take stock of the population of our seas. This introduces such an element of uncertainty into the problem, that, although I think we have good reason to be encouraged and to continue the work vigorously in a hopeful spirit and with an open mind, still I for one would not go so far as to say (as some have done) that marine fish-hatching had passed beyond the experimental stage; and I may refer in this connection to the somewhat fuller statement on the subject I made in last year's report,* and to my discussion there of the conditions of a crucial experiment for the purpose of testing the results of adding artificially hatched fry to the population of a circumscribed sea-area. We await with interest the result of the Fishery Board for Scotland's work in Loch Fyne.

* Report for 1897, pp. 24 and 25.

I cannot do better than conclude this section by quoting a couple of sentences from a letter received during the year from Professor M'Intosh, whom we regard as the pioneer of scientific fisheries work in this country. In speaking of marine hatcheries, he says:—"Of course such institutions are strictly experimental, and it may be some time before a decisive result is evident. Meanwhile, work them thoroughly and support them liberally." I desire to endorse that opinion.

Oysters and Disease.

(W. A. Herdman.)

In collaboration with my colleagues, Professor Boyce (Bacteriologist) and Dr. C. Kohn (Chemist), I have been working at this subject in our laboratories at University College, Liverpool, for the last three years, and interim reports upon the progress of the work have appeared in our three last Fisheries Laboratory Reports. A detailed paper, giving the full results of the investigation, has lately been laid before the Royal Society of London, and the main conclusions of that paper are as follows* :—

"1. Although our primary object was to study the Oyster under unhealthy conditions, in order to elucidate its supposed connection with infective disease, we found it necessary to study in minute detail the histology of certain parts of the body, especially the gills and mantle lobes, the alimentary canal and liver. We give figures and descriptions of these structures in both normal and abnormal conditions.

"2. We have also worked out the distribution and probable function of a minute muscle, which we believe

* Quoted from *Nature* for January 26th, 1899, p. 305.

to be the modified representative of the protractor pedis muscle of some other molluscs.

"3. A diseased condition we found in certain American Oysters very soon brought us into contact with the vexed question of the 'greening' of Oysters, and one of the first results we arrived at was that there are *several distinct kinds* of greenness in Oysters. Some of them, such as the green Marennes Oysters, and those of some rivers on the Essex coast, are healthy; while others, such as some Falmouth Oysters, containing copper, and some American Oysters re-bedded on our coast, and which have the pale green 'leucocytosis' described in our former paper to the Royal Society, are not in a healthy state.

"4. Some forms of greenness (*e.g.*, the leucocytosis) are certainly associated with the presence of a greatly increased amount of copper in the Oyster, while other forms of greenness (*e.g.*, that of the Marennes Oysters) have no connection with copper, but depend upon the presence of a special pigment, 'marennin.'

"We are able, in the main, to support Prof. Lankester in his observations on Marennes Oysters; but we regard the wandering amœboid granular cells on the surface of the gills as leucocytes which have escaped from the blood spaces, and have probably assumed a phagocytic function.

"5. We see no reason to think that any iron which may be associated with the marennin in the gills, &c., is taken in through the surface epithelium of the gill and palps, but regard it, like the rest of the iron in the body, as a product of ordinary digestion and absorption in the alimentary canal and liver.

"6. We do not find that there is any excessive amount of iron in the green Marennes Oyster compared with the colourless Oyster, nor do the green parts (gills, palp, &c.) of the Marennes Oyster contain either absolutely or relatively to the colourless parts (mantle, &c.) more iron than colourless Oysters. We therefore conclude that there is no connection between the green colour of the 'Huîtres de Marennes' and the iron they may contain.

"7. On the other hand, we do find by quantitative analysis that there is more copper in the green American Oyster than in the colourless one; and more proportionately in the greener parts than in those that are less green. We therefore conclude that their green colour is due to copper. We also find a greater quantity of iron in those green American Oysters than in the colourless; but this excess is, proportionately, considerably less than that of the copper.

"8. In the Falmouth Oysters, containing an excessive amount of copper, we find that much of the copper is certainly mechanically attached to the surface of the body, and is in a form insoluble in water, probably as a basic carbonate. In addition to this, however, the Falmouth Oyster may contain a much larger amount of copper in its tissues than does the normal colourless Oyster. In these Falmouth Oysters the cause of the green colour may be the same as in the green American Oyster.

"9. By treating sections of diseased American Oysters under the microscope with potassium ferrocyanide and various other reagents, we find that the copper reactions correspond in distribution with the green coloration; and we find, moreover, from these micro-chemical observations that the copper is situated in the blood-cells or leucocytes, which are greatly increased in number. This condition may be described as a green leucocytosis, in which copper in notable amount is stored up in the leucocytes.

"10. We find that an aqueous solution of pure hæmatoxylin is an extremely delicate test for copper, just as Macallum found it to be for iron.

"11. Experiments in feeding Oysters with weak solutions of various copper and iron salts gave no definite results, certainly no clear evidence of any absorption of the metals accompanied by 'greening.'

"12. Although we did not find the *Bacillus typhosus* in any Oysters obtained from the sea or from the markets, yet in our experimental Oysters inoculated with typhoid we were able to recover the organism from the body of the Oyster up to the tenth day. We show that the typhoid bacillus does not increase in the body or in the tissues of the Oyster, and our figures indicate that the bacilli perish in the intestine.

"13. Our experiments showed that sea-water was inimical to the growth of the typhoid bacilli. Although their presence was demonstrated in one case on the twenty-first day after addition to the water, still there appeared to be no initial or subsequent multiplication of the bacilli.

"14. In our experiments in washing infected Oysters in a stream of clean sea-water the results were definite and uniform; there was a great diminution or total disappearance of the typhoid bacilli in from one to seven days.

"15. The colon group of bacilli is frequently found in shell-fish as sold in towns, and especially in the Oyster; but we have no evidence that it occurs in mollusca living in pure sea-water. The natural inference that the presence of the colon bacillus invariably indicates sewage contamination must, however, not be considered established without further investigation.

"16. The colon group may be separated into two divisions: (1) those giving the typical reactions of the colon bacillus, and (2) those giving corresponding negative reactions, and so approaching the typhoid type; but in no case was an organism giving all the reactions of the *B. typhosus* isolated. It ought to be remembered, however, that our samples of Oysters, although of various kinds and from different sources, were in no case, so far as we are aware, derived from a bed known to be contaminated or suspected of typhoid.

"17. We have shown also the frequent occurrence, in various shell-fish from the shops, of anaërobic spore-bearing bacilli giving the characteristics of the *B. enteritidis sporogenes* recently described by Klein.

"18. Consequently, as the result of our investigations, and the consideration of much evidence, both from the Oyster-growers' and the public health officers' point of view, we beg to recommend—

"(*a*) That the necessary steps should be taken to induce the Oyster trade to remove any possible suspicion of sewage contamination from the beds and layings from which Oysters are supplied to the market. This could obviously be effected in one of two ways, either (1) by restrictive legislation and the licensing of beds only after due inspection by the officials of a Government Department, or (2) by the formation of an association amongst the Oyster-growers and dealers themselves, which should provide for the due periodic examination of the grounds, stores, and stock by independent properly qualified inspectors. Scientific assistance and advice given by such independent inspectors would go far to improve the condition of the Oyster beds and layings, to re-assure the public, and to elevate the Oyster industry to the important position which it should occupy.

"(*b*) Oysters imported from abroad (Holland, France, or America) should be consigned to a member of the 'Oyster Association,' who should be compelled by the regulations to have his foreign Oysters as carefully inspected and certified as those from his home layings. A large proportion of the imported Oysters are, however, deposited in our waters for such a period before going to market that the fact of their having originally come from abroad may be ignored. If this period of quarantine were imposed upon all foreign Oysters a great part of the difficulty as to inspection and certification would be removed.

"(*c*) The grounds from which Mussels, Cockles, Periwinkles are gathered should be periodically examined by scientific inspectors in the same manner as the Oyster beds. The duty of providing for this inspection might well, we suggest, be assumed by the various Sea-Fisheries Committees around the coast."

NOTE ON OCCURRENCE OF IRON AND COPPER IN OYSTERS.*

By CHARLES A. KOHN, B.Sc., Ph.D.

'The investigations of Professors Herdman and Boyce on the life conditions of Oysters, which have been in progress since 1895, have pointed to the desirability of ascertaining the quantities of iron and of copper they may contain under either normal or abnormal conditions.

'Two points of interest have arisen in this connection. In the first place the relation of iron to the greenness of the healthy French Oyster (Huître de Marennes); and secondly, the extent to which copper is responsible for the pale green colour of American and other Oysters, a diseased condition accompanied by a leucocytosis discovered and especially studied by Herdman and Boyce. The presence of minute quantities of copper and of iron

* Quoted from Report of Oyster Committee to Brit. Assoc., 1898.

as normal constituents of all Oysters has been shown by the analytical data obtained.

'The results recorded have been made at Professor Herdman's request, and have proceeded side by side with his investigations. Now that these are completed, a summary of the work from a more purely chemical standpoint may be of interest, especially since the occurrence of these metals—copper and iron—either from the point of view of the origin of colouration or the cause of poisoning has from time to time been the subject of discussion.

'*The Analytical Method Employed.*—Electrolytic methods of analysis were adopted both for the determination of iron and copper: these methods, I have already shown,* possess marked advantages for the estimation of minute quantities of metal, especially if derived from organic matter, for they are quite free from any prejudicial influences traces of organic matter may exert, such as arise when volumetric or colorimetric methods are employed. In each determination the bodies or gills only of six or more Oysters were carefully washed, dried between filter paper to remove as much adherent moisture as possible, and then carefully dried in porcelain dishes in the air bath at 100° C. When this drying was as complete as possible, the Oysters were heated in the air bath until thoroughly carbonised, the carbon carefully burnt off over the free flame, and the residue finally ignited in a porcelain crucible. Special care was taken to exclude dust during both the drying and the ignition. The ash was then thoroughly extracted with a mixture of 25 c.c. hydrochloric acid and 25 c.c. sulphuric acid (1 : 2) on the water bath, and the resulting solution filtered and

'* Brit. Assoc. Rep., 1893, p. 726.

concentrated. The residue was free from both copper and iron. The acid solution obtained was electrolysed for copper with the usual precautions, a spiral of fine platinum wire weighing about 5 grme. being employed as the cathode. The iron was determined in the residual solution, after neutralisation with ammonium hydrate, &c., acidifying with a few drops of oxalic acid solution, and boiling with ammonium oxalate: 4 grme. of the oxalate were added in each case, the precipitated calcium oxalate (which is quite free from iron) filtered off and thoroughly washed and the resulting solution electrolysed, the metallic iron being also deposited on a spiral of platinum wire. A blank experiment with all the reagents employed was made, and the amount of metal found (0·002 grme. iron) deducted in each case. Also the deposited metal, both iron and copper, was dissolved off the electrode by acid, the solution obtained tested by the ordinary reagents and the spiral re-weighed, as a check upon the determinations, since the quantities found were extremely small.

'The Green Colour of French Oysters, "Huîtres de Marennes," and the Presence of Iron in Oysters.—The early observations of Dumas (1841) and of Berthelot (1855) showed that the green colour of "Huîtres de Marennes" is not due to chlorophyll, and that although every Oyster contains a certain very small amount of copper in its blood in the form of "hæmocyanin," as determined by Fredericq, the green colour of the French cultivated Oyster is not due to this metal. Ray Lankester* in 1886 confirmed the latter statement, and states in his investigation on the histological condition of the colour that there is neither copper nor iron in the refractory blue pigment "marennin" of the coloured portions of the

* Quart. Journ. Micros. Sci., 1886, 26, 71.

Oyster. Berthelot, however, suggested that the green colour was due to iron, and more recently Chatin and Muntz† have extended and corroborated this statement.

'From their analytical results these observers conclude that both the green and the brown colourations of various types of French Oysters are due to the presence of iron, and that the depth of colour bears a close proportion to the quantity of iron contained. The colourations are chiefly apparent in the gills, but extend also to the labial palps and parts of the alimentary canal. Chatin and Muntz base their conclusions in the first place upon the fact that they find considerably more iron in the gills than the rest of the body of green Oysters; and secondly, upon the occurrence of a larger quantity of iron in the gills of green than of white Oysters.

'Appended are some of their results, to which I have added a column, showing the ratio of the iron in the body, minus gills, to that contained in the gills.

Oyster.	Colour.	Iron per 100 parts of dried organic matter.		Ratio of II. : I.
		I. Gills.	II. Rest of body.	
Cancale	White	0·0379	0·0241	1·57
Arcachon	Pale green	0·0605	0·0357	1·69
Marennes	Very green	0·0702	0·0318	1·21
Cancale	Brown green ...	0·0804	0·0476	1·69
Sables d'Olonnes ...	Very dark brown green	0·0833	0·0436	1·91

'The relative proportion of iron in the gills hardly bears out the conclusions arrived at; it is the same in pale-green and brown-green Oysters, and in both, but little greater than in the white. On the other hand, the total

† Compt. Rend., 1892, 118, 17 and 56.

iron, both in the gills and in the rest of the body, shows a marked increase, apparently corresponding to the depth of the colouration. The iron was determined in these experiments by potassium permanganate, but the absolute quantities of metal found are not stated. The calculation of the results per 100 parts of dried organic matter is apt to be misleading. In my own experiments it was not found possible to get anything approaching constant weights in this way, and the results are entirely out of accord with those of Chatin and Muntz.

'The following table gives the quantities of iron found in French as compared with American Oysters, three pairs of gills being analysed in each case.

'These figures show conclusively that there is more and not less iron in the gills of the white American Oysters than in the French, and this irrespective of the basis on which the result is calculated. The ash is undoubtedly the most reliable factor to calculate on, provided the Oysters are carefully washed before drying, which was always done: the result per pair of gills (or Oyster) is most in accord with this, and has the advantage of being an easy and in many respects useful basis.

—	Huîtres de Marennes.	American.
Gross body weight, after drying between filter paper	3·8 grms.	6·5 grms.
Weight dried at 100° C. for six hours ...	0·52 ,,	1·02 ,,
Weight of ash	0·0940 ,,	0·1140 ,,
Weight of *Iron* found	0·0003 ,,	0·0008 ,,
Ratio of Iron found:—		
(1) Calculated per pair of gills	1 to	2·7
(2) ,, on gross body weight... ...	1 to	1·56
(3) ,, at weight at 100° C.	1 to	1·36
(4) ,, on ash	1 to	2·2

'The relative quantities of iron present in the gills as compared with the rest of the body were next determined in French, Dutch, and American Oysters. Six Oysters, or the gills of six Oysters, were analysed in each case with the following results:—

Six Oysters.	Weight of Iron found in mgrme.		
	French.	Dutch.	American.
Gills	0·6	0·4	2·3
Bodies minus gills	1·2	1·5	1·7
Weight of ash of gills	0·1880	0·0217	0·0294
,, ,, of bodies minus gills ...	0·5980	0·1125	0·1240
% Iron on ash. Gills	0·32	1·85	7·82
,, ,, Bodies minus gills ...	0·20	1·33	1·37
Ratio of Iron in gills to Iron in rest of body.			
Calculated per Oyster	1 : 2	1 : 3·75	1 : 0·74
,, on ash	1·6 : 1	1 : 4·1	5·7 : 1

'From these figures it is evident that the gills of the French Oysters do not contain an excessive quantity of iron such as might account for their colour. Calculated per Oyster the gills contain less iron than the rest of the body, except in the American Oyster; calculated as a percentage on the ash the reverse is the case. The proportionate quantity of iron in the gills as compared with the rest of the body is somewhat greater in the French Oysters than in the Dutch, but much less than in the American.

'Clearly, therefore, there is no connection between the green colour and the quantity of iron present. This result is quite in accord with Ray Lankester's observation that his "marennin" is free from iron as well as from copper.

'Both the gills and bodies of Oysters contain a small quantity of iron, which is evidently normally present, the gills containing a somewhat larger amount in proportion to the total quantity of mineral matter present.

'Finally, the total iron in a variety of Oysters was determined in order to ascertain the normal quantity present. These data, which are tabulated below, show a fairly constant proportion of iron per Oyster, from 0·15 to 0·36 mgrme., or from 0·18 to 0·65 per cent. on the ash.

Total Iron present in Oysters.

Variety of Oyster	Number Analysed.	Total Iron grme.	Weight of Ash.	Mgrme. Iron per Oyster.	Percentage of Iron on Ash.
'Huîtres de Marennes'	6	0·0018	0·7860	0·30	0·23
Dutch	6	0·0009	0·1393	0·15	0·65
American	5	0·0018	0·2791	0·36	0·64
Colne	10	0·0020	1·0938	0·20	0·18
Deep Sea	2	0·0064	1·5017	0·32	0·43
Falmouth	6	0·0016	0·4534	0 27	0·35

'In considering the variations in quantity, the very small amounts of metal present must be borne in mind.

'It may be added that although Carazzi has attributed the green colour of French Oysters to iron taken up from the mud of the Oyster-park or "claire," experiments on feeding Oysters with very dilute solutions of iron salts (0·02 to 0·01 per cent.) carried on in conjunction with Prof. Herdman produced no green colouration whatever. The only result was a certain amount of "browning" throughout the Oyster, the gills being no more affected than the rest of the body. More recently Carazzi has shown that Oysters fed with similar dilute iron solutions acquire a pale yellowish colour in certain parts (branchial epithelium and the œsophageal mucous membrane), and that in these parts microscopic tests show the presence of granules of iron. The actual meaning of these results can hardly be recognised without quantitative data.

'*The Presence of Copper in Oysters.*—Fredericq has shown that a certain small amount of copper is present normally in the hæmocyanin of the blood of crustaceans and molluscs. The quantity thus present in Oysters of different origin is fairly constant as shown in the following table: it varies from 0·25 to 0·66 mgrme. per Oyster, or from 0·30 to 1·18 per cent. on the ash.

Variety of Oyster.	Number Analysed.	Total Copper grme.	Weight of Ash.	Mgrme. Copper per Oyster.	Percentage Copper on Ash.
'Huîtres de Marennes'	6	0·0024	0·7860	0·40	0·30
Dutch	6	0·0015	0·1393	0·25	1·08
American	5	0·0033	0·2791	0·66	1·18
Colne	10	0·0036	1·0938	0·36	0·33
Deep Sea	2	0·0069	1·5017	0·34	0·46

'0·4 mgrme. per oyster may be taken as an average, a quantity slightly greater than the average iron (0·26 mgrme.). The calculated percentages on the ash show greater variations, due to the very considerable differences in the total quantities of mineral salts present, and it is probably to this last factor that the popularly recorded differences in taste of the various kinds of oysters is really due. Certainly the minute quantities of copper and iron present cannot account for them.

'The copper was also determined in the gills and in the bodies minus gills of French, Dutch, and American Oysters, with the following results:—

Determination of Copper.

Six Oysters.	French 'Huîtres de Marennes.'	Dutch.	American.
Gills only	Trace	0·8	1·7
Bodies minus gills...	1·4 mgrme.	1·4	3·3

'These data show conclusively that the green colour of the gills of French Oysters is also in no way connected with the copper present.

'Quantities of copper greater than those recorded point to abnormal conditions. Such have been found to occur with certain Falmouth Oysters, and in an especially interesting manner with the green leucocytosis of American and Falmouth Oysters—the diseased condition referred to above.

'*Falmouth Oysters.*—The presence of relatively large quantities of copper in Falmouth and other Cornish Oysters has been repeatedly associated with their bluish-green colour. Dr. T. E. Thorpe* states that these Oysters, the colour of which, both in character and distribution, is quite different from that of the Marennes Oysters, contain on the average about 1·3 mgrme. of copper per Oyster. This large proportion is, Dr. Thorpe says, "obviously caused by the mechanical retention of cupriferous particles." On relaying they lose their colour, and the quantity of copper present becomes normal, 0·4 mgrme. per Oyster.

'Six Falmouth Oysters, the bodies of two of which were of a distinct arsenic-green colour, were dried at 100° C., and then digested with water and subsequently with dilute hydrochloric acid. The extract contained about half the total copper present, showing that the metal is partially,

* "Nature," 1896, p. 107.

at any rate, mechanically retained on, or in the body of the Oyster, probably as a basic carbonate.

'The analytical results were as follows:—

Six Oysters.	Copper.	Iron.	Wght. of Ash.	Mgrme. Copper per Oyster.	Mgrme. Iron per Oyster.	Per cent. Copper on Ash.	Per cent. Iron on Ash.
Extract with dilute acid ...	0·0097	0·0024	0·2272	1·62	0·40	4·22	1·06
Oysters ...	0·0114	0·0016	0·4534	1·90	0·27	2·51	0·35
Total ...	0·0211	0·0040	0·6806	3·52	0·67	3·10	0·59

'The total copper present is almost nine times the normal quantity, and about half of this is easily removed by dilute acid. It is quite likely that the remainder is partially or wholly simply entangled in the food passages of the Oyster, and that the green colour may be due to some other cause than this mechanically retained copper, as suggested by Herdman.* Mr. G. C. Bourne, indeed, regards it as due, in some Falmouth Oysters, to a green desmid upon which the Oysters feed in quantity.

'The occurrence of copper under such conditions is due to the locality, and may quite possibly attain injurious proportions, for the Oysters were obtained from a creek which is locally supposed to bring down copper, and the mud of which was found by Thorpe to contain 0·148 per cent. of copper. Normal sea-water contains such an excessively small quantity of copper that it was not found possible to detect its presence, even electrolytically, in a litre of sea-water, after concentration.

'The *green leucocytosis* already referred to was first noticed by Herdman and Boyce in American Oysters

* "Nature," 1897, p. 366.

which had been relaid near Fleetwood. The colour manifests itself in patches and streaks of pale green on the mantle, in engorgements of the blood vessels and in masses of green coloured leucocytes in the heart. The leucocytes are apparently all amœboid wandering cells, comparable to the colourless corpuscles of the blood of higher animals, and the colouration coincides with their distribution.

'The six greenest and six whitest of 120 of these Oysters were chosen for analysis; also a quantity of the greenest portions of the greenest Oysters was selected from another batch, and compared with the corresponding portions of the whitest Oysters. The iron was not determined in the latter comparison, owing to contamination of metal in cutting.

'The following were the results obtained :—

Oysters.	Copper.	Iron.	Ash.	Mgrme. Copper per Oyster.	Mgrme. Iron per Oyster.	Per cent. Copper on Ash.	Per cent. Iron on Ash.
Green ...	0·0158	0·0091	1·1450	2·63	1·52	1·38	0·79
White ...	0·0042	0·0036	1·0948	0·70	0·60	0·38	0·33
Greenest parts	0·0033	—	0·0780	—	—	4·23	—
Whitest parts	0·0009	—	0·0452	—	—	1·99	—

'The excessive quantity of copper in the selected green Oysters is 3·75 times that in the white calculated per Oyster, and 3·63 times calculated on the ash. In the selected parts the total copper present calculated on the ash is high in both cases, and the green parts again show a marked excess in the proportion of 2·1 to 1. The copper and iron in the white specimens are about normal, but the increased quantity of iron in the green is marked, being 2·5 times that of the former. Still there is relatively a large excess of copper as compared with iron in the green Oyster, as is evident from the analyses, the ratio being 1·1 : 1 for the white and 1·8 : 1 for the green.

'It is to be concluded, therefore, that the green colour of these Oysters is coincident with the distribution of the excessive quantity of copper present, and that the copper is in consequence to be regarded as the cause of the colour. The histo-chemical investigations of Boyce and Herdman have amply confirmed this conclusion.

'Further, this leucocytosis is not accompanied by a mere redistribution of copper, but by an absolute increase of the amount present in the body.

'The deposition of copper in this manner is regarded by Boyce and Herdman "as a degenerative reaction, due to a disturbed metabolism, whereby the normal copper of the hæmocyanin, which is probably passing through the body in minute amounts, ceases to be removed, and so becomes stored up in certain cells." The change is comparable in kind to the accumulation of iron in pernicious anæmia.

'The increased quantity of iron present may also be due to abnormal conditions of life, but a more accurate localisation of the normal iron of the Oyster is necessary before this can be decided.

'This green leucocytosis has been observed by Herdman and Boyce in other Oysters, including those of Falmouth, and it is likely to be the real cause of their colour; a colour therefore due to copper as previously supposed, but accompanied by a diseased condition. Whether the presence of copper in the water facilitates in any way the development of the disease has not been determined; experiments made on keeping Oysters in very dilute saline copper solutions give no affirmative results beyond a certain amount of post-mortem green staining.

'*Manganese* was found to be present in several of the varieties of Oysters analysed. Its detection is readily effected in the electrolytic method of analysis as it separates at the anode as peroxide. Colne Oysters contained 0·14 mgrme. per Oyster—a rather smaller quantity than the iron found.'

Mussel-beds and Mud-banks.

By R. L. Ascroft.

On the Lancashire and Cheshire coasts it is often noticed that Mussel-beds are situated on banks of mud. Mussels require, in the earliest shelled stage, some hard substance to attach themselves to, such as stone, gravel, hardened sand (such as Sabellaria tubes, for instance), shells, such as Cockles or Mussels, wooden piles, and the bottoms of boats. As they grow up and increase in size (when located where there is little wave action), through their excreta and the mud settling amongst them they are inconvenienced, and to escape being buried a lengthening of their cables, or byssus, takes place, and so they lift themselves. This process, repeated time after time, raises the Mussels a great height above their original location, and the bed of mud increases accordingly.

The greatest depth that I have heard of occurred in the River Ribble, where a bed of gravel was bare in the channel a little above Lytham, when a strike of Mussels occurred on it, and in the course of three years the Mussels accumulated a bank of mud ten feet in depth—there being no wave action to affect it.

At St. Annes-on-the-Sea, below the pier, there is a bed of gravel on which a strike of Mussels occurs every two years. During the two years growth the mud gets to a depth of two feet, and then the heavy seas reach the bed and roll the Mussels off like a carpet, and I have seen them strewn along the beach at high-water mark as far as Lytham Pier, a distance of four miles, during the same tide.

Under the refreshment rooms at Southport Pier is a foundation composed of stone on which Mussels strike. They then accumulate mud until their cables, or byssus, reaching down to the stones, get too weak, and they are then washed away. The same thing occurs in the Rock Channel, and on the great scars at Heysham.

In the case of St. Annes, and doubtless in the other cases also, if the seaward edge of the scar was protected by piles or posts, and the Mussels so rendered less liable to be broken into by the sea, they would then, in all probability, be able to grow to a marketable size.

The researches of Mons. Viallanes at Arcachon, in the South of France, have proved the power of Mussels in comparison with Oysters to separate food and refuse (sand, &c.) from the water. By his experiments he shows that for one quart of water filtered by a French or native Oyster 18 months old, a Portuguese Oyster of the same age filtered $5\frac{1}{2}$ quarts, and a middle-sized Mussel 3 quarts. The excreta respectively were 0·199 gramme, 1·075 grammes, and 1·768 grammes per 24 hours. So that in proportion to the numbers covering the same area of ground the Mussel will deposit three times as much material as a Portuguese, and eighteen times as much as a French or native Oyster.

There is no doubt but that the rate of growth is to a great extent proportional to the food consumed, so that it is clear that the Mussel has a much greater quickness of growth than a native Oyster.

The accumulation of mud (other conditions being the same) is much greater where brackish water flows at times over the bed, and it is a well known fact that Mussels in such a place thrive and grow much more quickly than those in pure sea water, no doubt through the large quantity of food brought down by the rivers. So much is this the case that every year Mussels are removed from the scars on the seaward side of the River Wyre below Fleetwood and placed in the same river above Fleetwood to have the benefit of the brackish water.

The number of young Mussels (eggs and embryos) given off by their parents must be in quantity simply enormous. At Southport during the autumn, if a rope's end is allowed to hang in the water for the space of a month you find it at the end of that time coated with Mussels, like a swarm of bees hanging on a bough.

Boats, buoys, and all wrecks or posts near low-water mark are smothered with them. These objects form natural bouchots from which the young Mussels might well be removed for cultivation elsewhere.

Report on the Physiology of Colour-Change in Hippolyte and other Marine Crustacea.

By F. W. Keeble, M.A., and F. W. Gamble, M.Sc., Owens College, Manchester.

By the recommendation of Prof. Herdman and the permission of the Lancashire Sea-Fisheries Committee, we were allowed to utilise the Piel Laboratory for the purpose of investigating the colour-physiology of *Hippolyte varians*. We paid two short visits (from May 27th to 30th, and July 1st to 5th) in order to ascertain the resources of the Laboratory and to discover the localities in which *Hippolyte* could be obtained. As these visits were successful, it was decided to spend the long vacation at Piel, and accordingly the work was started on July 15th and carried on without interruption till Sept. 22nd., concluding with a visit Dec. 15-22. In the present Report we merely give a short account of the methods employed, since the results obtained by their means are not yet ready for publication.

Hippolyte varians is one of the few Crustacea which may be considered abundant in the neighbourhood of Piel. It keeps, for the most part, to beds of weeds below low-water mark, and hence its habits have largely to be learnt from specimens in captivity. Compared with its associates—*Idothea, Mysis, and Crangon—Hippolyte* is of a delicate constitution, and needs constant aëration or constant change of sea-water to maintain it in good health. The resources of the tank-room at Piel enabled us to overcome this difficulty. Fresh weed or the dead bodies of its fellows serve *Hippolyte* as food. With due precautions specimens may be kept under observation for ten days or a fortnight.

From the nature of the beds of sea-weed in the Barrow Channel a haul with weighted canvas tow-nets usually contains an assortment of *Hippolyte* of different sizes and colours. Shades of brown and yellow are abundant, whilst green and red are sometimes common, sometimes rare. With the large *Halidrys siliquosa* a dark brown variety is associated; among the fine Polyzoon (*Bowerbankia*) which clothes the lower parts of the *Halidrys* stems, a speckled variety of *Hippolyte* occurs: in the tide-pools of Foulney Island the green variety, and it alone, is found among the *Zostera*.

The methods employed for investigating whether there is a power of colour-change, and if so, how this power may be tested, were for the most part quite simple. One method consisted in testing the effect of light of differing intensities upon a given variety. To do this a number of jars, some of clear glass, others enveloped with muslin, others again with black cloth, were taken on board, and as soon as the nets were hauled up, the *Hippolyte* were recorded, and specimens of each variety were placed in each jar. The effects produced differed very markedly according to the time of day at which the catch was made; the result of treating a haul in this way after dark or even in waning light being very different from that obtained at mid-day.

We also directed our attention to the influence of variously coloured weeds, with the object of testing whether a brown *Hippolyte* would become green when placed with green weeds, and red when surrounded with red ones. For this purpose we had devised an apparatus which was described shortly before the British Association at Bristol.

This instrument consists of a "pressure-bottle," holding a couple of gallons of sea-water, which is discharged at a uniform rate through an escape-tube commencing with a minute opening. The water is made to circulate through a series of air-tight observation-dishes, in which the *Hippolyte* are placed. The loss of water from the pressure-bottle is made good by the entrance of air which is first drawn through a second series of observation-dishes, the water in which is changed once or twice a day only. On the whole it was found that *Hippolyte* flourishes best in the air-circulating dishes.

The influence of monochromatic light was investigated by the aid of Landolt's fluid "colour-filters." In order that the light which reaches *Hippolyte* may be of fairly high intensity and not merely equivalent to shade, we employed a mirror to reflect sunlight or the light of an incandescent lamp into the jars which were otherwise quite opaque.

The effects of direct sunlight, reflected light and scattered light upon the colour of *Hippolyte* were tested by exposing carefully recorded specimens to these influences in the open grassy space outside the Laboratory. To prevent a rise of temperature in the sea-water, we employed a circulation of water derived from the supply in the Laboratory to flow through our observation-dishes. The methods we used for investigating the effects of rise and fall of temperature, electrical stimulus and of toxic agents do not call for remark here. At night and often during the day the records (chiefly microscopical) were made by the light of an incandescent lamp.

We have arrived at the conclusion that there are two colour-phases in *Hippolyte varians*; one diurnal, the other nocturnal. The recurrence of these phases is to some extent independent of the conditions of illumination, although the colour itself may be profoundly influenced by varying the quality and intensity of the incident light, and also by other stimuli, which do not act through the eye.

The advantages which the Piel Laboratory affords for work of this kind are very considerable, and while expressing our thanks to Professor Herdman and to the Curator of the Laboratory (Mr. Andrew Scott), we wish to acknowledge our marked indebtedness to the Lancashire Sea-Fisheries Committee for the generous help which is afforded by them for the prosecution of scientific work, generosity which will surely become more widely appreciated as it becomes more generally known.

Addendum.

The species of Crustacea we worked with, have been kindly identified by A. O. Walker, Esq. From the very limited fauna of Piel shore, it may be of interest to give the list, which, however, is not quite complete.

Hippolyte varians, Leach, common, just below the level of ordinary spring tides.

Hippolyte fascigera, Gosse, a doubtful species. Almost certainly a variety of *H. varians*.

Hippolyte cranchii, Leach.
Hippolyte pusiola, Kröyer. } Less common but occurring with the foregoing.

A species of *Mysis* which gave interesting results and which occurs with *Hippolyte* has been determined as *Mysis neglecta*, G. O. Sars.

A small collection of Pycnogonida, which have no connection with the present research but which were taken among the fine red weeds of the Barrow Channel, contains the following forms :—

Ammothea echinata, Hodge.
Anoplodactylus petiolatus (Kröyer).
Pallene brevirostris, Johnst.
Nymphon gracile, Leach.

APPENDIX.

LANCASHIRE SEA-FISHERIES HATCHERY AND LABORATORY AT PIEL.

Regulations in Regard to Workers.

1°. Biologists and Students desiring to work at the Piel Hatchery should apply to the Hon. Director (Professor Herdman) who, if there is room, will allot them work places in the Laboratory in the order of application.

2°. In the absence of the Director, the Resident Assistant (Mr. Andrew Scott) will determine which places in the Laboratory workers are to occupy, and to what extent the instruments of the Laboratory (microscopes, microtomes, &c.), and the boats and collecting apparatus may be used by workers.

3°. The Aquaria in the tank house are intended for experiments in Fish Hatching and Fish Rearing, and it is only by express permission of the Director or the Resident Assistant that they may be used for private investigations.

4°. Laboratory accommodation and lodging in the house are given free of charge to those duly qualified workers or students whose applications have been accepted, and who have been assigned places in the Laboratory. Meals (breakfast, dinner, tea and supper) are provided for those working in the Hatchery at a fixed charge of 3s. per day or 15s. per week, payable to Mr. Scott.

5°. The Resident Assistant will be ready to give assistance to workers at the Hatchery, and to provide them with material for their investigations so far as it does not interfere with his routine duties and his "fisheries" work.

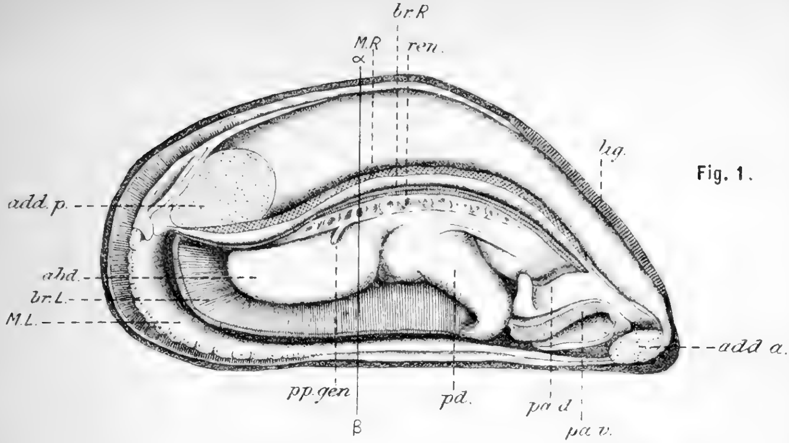
6°. All dishes, jars, bottles, tubes and other vessels in the Laboratory may be used freely, but must not be taken away from the Laboratory. If any workers desire tc make, preserve and take away collections of marine animals and plants, they must provide their own bottles and preservatives for the purpose.

7°. The fish and other specimens in the tank room are the property of the Institution and must not be used or disturbed by workers in the Laboratory.

8°. Each worker in the Laboratory is required to send a short account of his work done at the Institution, and of the results he has attained, to the Director before the 1st of December (at latest), in order that it may be entered in the Annual Report to the Sea-Fisheries Committee.

W. A. Herdman,

Honorary Director of the Scientific Work to the Lancashire Sea-Fisheries Committee.



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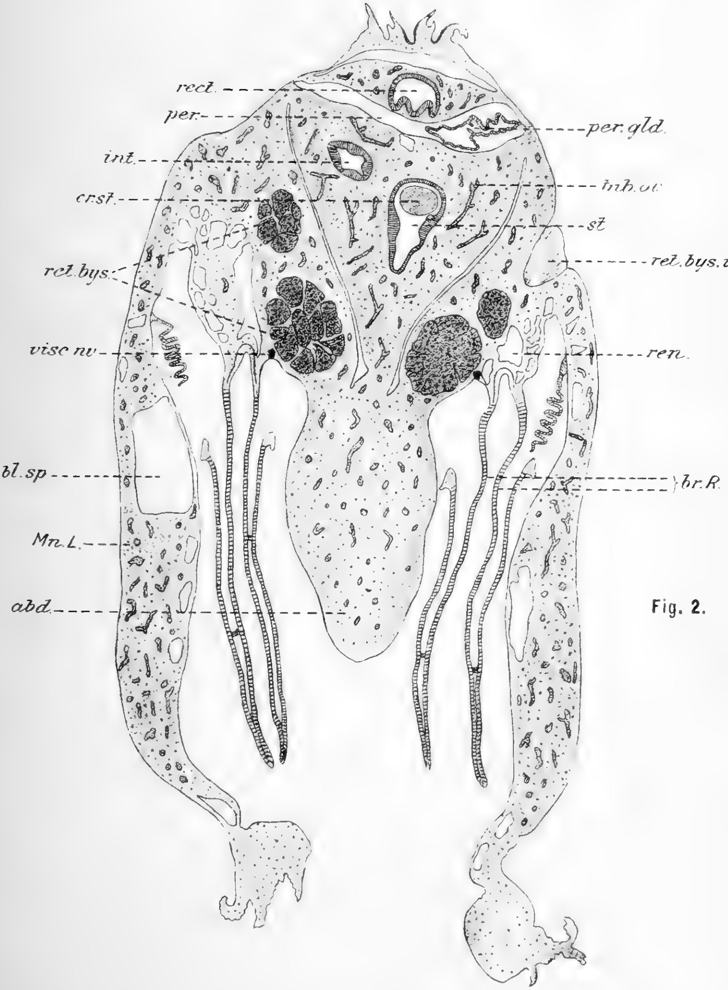

Fig. 2.

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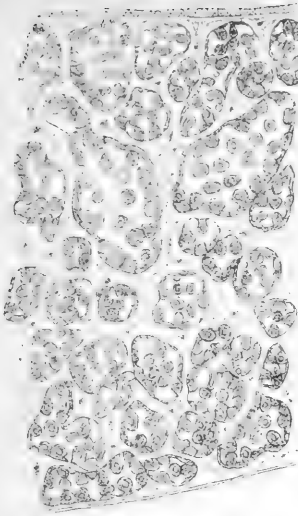

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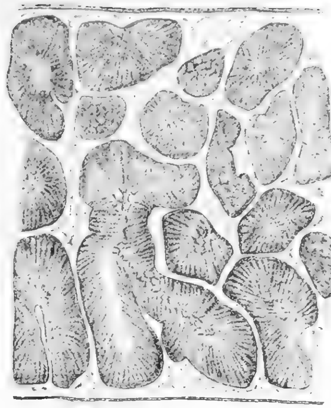

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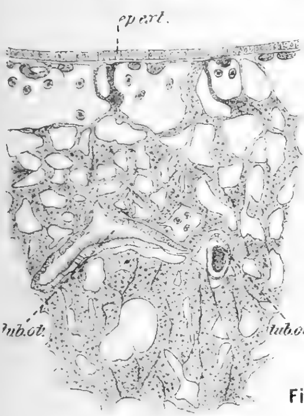
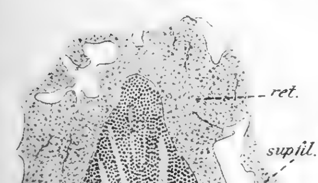


Fig. 3.

Fig. 4.



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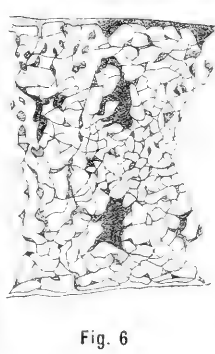

Fig. 6

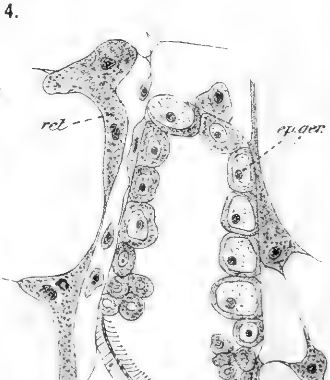

Fig. 7.

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