Report on the Spawning of the Common Sole (*Solea vulgaris*) in the Aquarium of the Marine Biological Association's Laboratory at Plymouth, during April and May, 1895,

With preliminary remarks on some of the morphological conclusions that may be drawn from the study of the early embryological history of this form.

By

Gerard W. Butler, B.A.

I. Introductory.

From April 3rd to May 17th of this year I occupied a table at the Plymouth Laboratory, to study the embryology of Teleosteans. As some of the fish in the flat-fish tank were known to be spawning, a net was fitted to the overflow channel into the adjoining tank. By the kind permission of the Director I examined this net daily, and, as a rule, a number of times a day, so that I obtained a pretty complete record of the spawning of the fish in this tank during the period mentioned.

Four or more species spawned during this period,* but the point most worthy of record is the breeding for the first time in the Plymouth Aquarium, and perhaps for the first time in captivity, of the common sole.

* Fertilized eggs of the Plaice (*Pl. platessa*) 2 mm. in diameter were obtained on April 2nd, 4th, 7th, 12th, and some of these hatched out in 10–12 days. Eggs about 1·5 mm. in diameter, apparently those of the "Merrysole" (*Pl. microcephalus*) were obtained unfertilized on April 19th, and fertilized on the nights of May 5th, 8th, and 10th. Some of these hatched out on the 5th day, the water temperature being 13° C. Smaller eggs, also without oil globule, varying in diameter from ·98 mm. to 1·15 mm., and thus answering to the unfertilized eggs of the flounder obtained from different fish, but possibly including eggs of some other flat-fish besides *Pl. flesus*, were obtained repeatedly during April and first half of May, but only one or two fertilized eggs were seen. Attempts to artificially fertilize flounder eggs resulted in nothing beyond the irregular segmentation of some of the eggs. Probably this was due to the only male available not being in proper condition.

II. Dates and Times of Spawning of the Sole.

I obtained unfertilized eggs of the sole on April 3rd and 7th, but on April 12th I found fertilized eggs for the first time. Then, again, on April 20th and 21st there were only unfertilized eggs. From this time onward, however, fertilized eggs were found during the rest of my stay at the Laboratory, sometimes on two consecutive days, sometimes with one day's, sometimes with two days' interval; and unfertilized eggs were the exception. Thus fertilized sole eggs were obtained on April 23rd, 25th, 26th, 28th, May 1st, 2nd, 4th, 7th, 8th, 10th. Then on May 11th, 12th, and 13th there were only a few eggs each day, of which the majority were unfertilized, and then again a plentiful batch of fertilized eggs on May 16th.

The time of day at which spawning occurred seemed to get earlier as the weather got warmer. Thus, during the last week of April, the eggs would be in the first segmentation (two blastomere) stage between 6 and 7 p.m., which, according to subsequent observations, would point to their having been spawned about, or rather before, 4 p.m.; but later on the egg-laying would begin about noon. On one occasion, when it began about 11.30 a.m., it was not ended before 2 p.m., which is not so surprising, since, as will be explained later, the eggs seem to be shed one at a time.

III. On the Appearance of the Ovaries during the Spawning Season.

On May 15th, as it seemed desirable that the state of the ovaries under these known conditions should be studied, it was decided to sacrifice one of the females, of which there were a fair number spawning, and preserve the ovaries for histological study.

I first tried whether any ripe eggs were to be obtained from the living fish, but without success; and on opening the dead fish there seemed to be no quite ripe eggs in the cavity of the ovary tube. This, it may be remembered, was a day on which none of the other fish spawned, though they did on the next day. Judging by the number of fish spawning in the tank and the number of eggs spawned, the number of eggs ripening each day must have been small proportionately to the eggs in the ovary, which is not, of course, surprising, if the spawning is destined to be kept up, on the average every other day, for a period of three months or so. The ova were of all sizes. The largest and most transparent ones, presumably those most nearly ripe, were distributed singly among those less ripe over the whole laminar surface of the ovary, and did not seem to be confined specially to one region of the ovary. However, the third

quarter or so of each ovary (reckoning from the head end backwards) was dotted over with small bloodspots, answering to Holt's description of the spent sole.* I presume, therefore, that this region of the ovary was that from which, in the first three weeks or month of the spawning season, the majority of the eggs had been derived.

IV. The Act of Spawning.

At the time of spawning the soles came to the very front of the tank close to the glass, so that on a number of occasions I had a good view of the process.

The soles lay about on the bottom apparently indiscriminately, here one by itself, there two, three, or more near together. One of them would from time to time move leisurely to another place, and in passing by or over one of its companions, would evidently take notice of it, as by feeling it with the under side of its head, but this never led to anything of the nature of pairing, such as some have imagined might occur in the case of the sole; for the fish would again move on and continue the spawning process elsewhere, apparently regardless of the exact position of its fellows, and preoccupied with its own share in the operation. Doubtless, however, such recognitions in passing are the outward sign of the instinct whereby the fish assemble at the spawning time, so that eggs and spermatozoa may rise together in the water, and fertilization take place.

In spawning the sole lay on the bottom of the tank, and raising its head, brought it down again with force. This act involved a certain agitation of the hinder regions of the body also, which was perhaps as important as the more conspicuous movement of the fore part in assisting the expulsion of the ova or spermatozoa, but the appearance was as if the fish desired to create a splash of sand by the downward movement of its head. The movement was quite different from that by which soles commonly cover their upper side with sand, and had not that effect.

The eggs appear to be shed one at a time, each as the result of one of the movements just described. It seemed to me that this movement wafted the egg tailwards; at least a fresh egg commonly appeared above the tail of a fish after each of the head splashes described.

I never actually saw the exit of either ova or spermatozoa from a fish, but if the eggs are shed singly in the manner described, and the spermatozoa in correspondingly small numbers, one could, perhaps, hardly expect to; and I think anyone who saw the eggs slowly rising

* E. W. L. Holt. "North Sea Investigations" (contd.), *Journal of Mar. Biol. Ass. of U. K.*, vol. ii. (N.S.), p. 371.

towards the surface from within an inch or two of the ground, and fresh eggs taking their place to the accompaniment of the movements described, would draw the conclusions that I have.

In considering how it is that the fish come to the front of the tank, and will spawn undisturbed while you are just on the other side of the glass, instead of, as one might have expected, retreating to the farther side, one must remember that the fish now spawning have been five years or so in the aquarium, and that thus they are not only more or less tame generally, but have probably come to consider the window border of the tank floor as their place of assembly *par excellence;* for it is to that side that they are impelled by the common craving of hunger as feeding time approaches, and on that side that they at all sorts of times tend to linger, from that milder motive of curiosity about us strange creatures in the air-tank on the other side of the glass.

V. General Remarks on the Development.

On the three occasions on which I tried, I failed to obtain eggs from the living fish, and thus I never witnessed the process of fertilization so as to time the development from the very beginning, as I should have liked. Perhaps I should have succeeded, had I captured the fish as soon as they began to spawn. However, I obtained eggs one hour before the first formation of the protoplasmic disc at the lower side of the egg, and two and a half hours before the first segmentation.

The rapid streaming of the protoplasm between the large yolk spheres of the lower part of the egg (which spheres became temporarily transformed into cones pointing downwards), to form the disc, was a very interesting sight. What I saw fully bears out the late George Brook's explanation* of Kuppfer's account of the phenomenon in the herring's egg.

Segmentation was repeatedly followed and sketched, such sketches agreeing essentially as to direction of the segmentation furrows with Wilson's figures of the segmentation stages of the sea bass; but I saw no nuclei in the living egg, except in the "parablast" at a much later stage.

The intervals between the segmentations decreased markedly at first, but a limit was soon reached. The rate of development, of course, varies considerably with the temperature of the water, whether one considers particular stages, or the whole time before hatching.

* G. Brook, "The Formation of the Germinal Layers in Teleostei," *Trans. Roy. Soc. of Edinburgh*, vol. xxxiii. part i. (for 1885–6), publ. 1887.

As to the latter, a very healthy batch of eggs, spawned on April 28th, hatched out in numbers on the seventh day, while eggs spawned about a week later, and from thence onwards, hatched out on the fifth day, when the temperature of the water was between 13° and 14° *C.* in the daytime. Both these times are considerably quicker than that of eggs studied by Cunningham, when the water was colder.

I found that even the particularly healthy batch of eggs above referred to, in which the mortality all through had been small for teleost eggs, sank to the bottom, as noted by Cunningham, half a day or so before hatching, and less healthy eggs ceased to float a day or two earlier. For this reason it seems to me that the plan of keeping the eggs, which I adopted primarily for my own convenience in studying and preserving different stages at short intervals, would be more suited to these eggs than the usual narrow-mouthed, wide gauze-bottomed hatching jars. The plan I refer to is that of the plain wide-mouthed glass beaker, with a safety siphon; the form of the latter used being not the sand filter bottom, but that with a glass funnel covered with gauze, which I found being used in the laboratory. A small jet of water, if directed so as to strike the glass side of the vessel a little above the surface of the water, and at a small angle to both the glass and the horizon (pointing downwards), seemed sufficient to keep the eggs circulating, since the surface water being made to revolve, any egg on the surface must soon come within reach of the water coming down the side of the glass, and is then driven gently downwards. With this adjustment the eggs, having small buoyancy, tend to collect on the gauze entrance to the exit funnel; but this can, if necessary, be corrected by making a second small jet of water, from a tube carried beneath the surface, gently play across the gauze mouth of the funnel.

The advantages of this type of vessel over the usual hatching jar to the embryologist are obvious, while to the practical fish hatcher it is a consideration that he can easily keep the glass bottom clean and free from dead eggs by the use of a dipping tube, so that when the eggs sink before hatching, they have not to lie on a bed of putrid eggs. Moreover, the apparatus can be at once moved into any light for inspection, and will work with comparatively little water.

VI. Preliminary Remarks on certain Morphological Conclusions to be drawn from a Study of these Eggs.

I have for some three or four years been one of those who are impressed with the strength of the case for the "Concrescence Theory," and with the lamentable waste of time in futile researches and discus-

sions on such subjects as the morphology of the notochord and the mesoblast, &c., into which some of those have drifted who have failed to avail themselves of this morphological anchor, with all that follows its acceptance; and I have, consequently, been on the *qui vive* for any evidence which might serve to prove the point definitely to its gainsayers, although feeling, personally, that the study of the living Elasmobranch blastoderm for days and weeks, and of the Teleostean egg for hours (supplemented if necessary by sections) gives ocular demonstration of concrescence.

Now the sole egg, I believe, is capable of giving such proof. The curious aggregates of small oil globules, characteristic of the sole egg, are well known, but I am, I believe, the first who has had the good fortune to study the early developmental history under favourable conditions, and thus to recognize that if suitable eggs be selected, and isolated, and carefully sketched with the camera at short intervals, these oil aggregates, which are at first distributed mainly in a zone below the equator, can (*a*) before the formation of the embryonic ring, be used as fixed points, and serve to show, at least within a small angle, the relation of the plane of symmetry of the embryo to the first cleavage planes, and (*b*) after the first formation of the ring, when they become involved in those relative movements of different parts of the egg, which are usually spoken of as epibolic gastrulation and concrescence, (by those who accept the "Concrescence Theory") may be used, so to speak, as floats whereby to follow these movements.

Having noticed this fact, I thought I could not make better use of the splendid material by the kindness of the Director so freely placed at my disposal, than by concentrating my attention chiefly on that early period of developmental history (the first two days or first day and a half, according to whether the eggs are hatched in seven days or five) during which these and other problems of fundamental morphological interest are to be studied.

I hope shortly, when I have supplemented my serial camera-sketches of different stages of the same living eggs, by sections of corresponding stages from my preserved material, to publish something fuller on the above two points, and on other matters, such as the structure of the egg, the morphological relations of the disc, the parablast and the yolk, and gastrulation. For the present, I may state that:—

I.—The plane of symmetry of the embryo does not bear one and the same fixed relation in all eggs to the first segmentation plane. Thus out of eleven eggs I found that in three the plane of symmetry of the embryo coincided with the first segmentation plane, in four with the second segmentation plane, and in four with a plane bisecting the angle between these.

I bring this forward essentially as a *negative* conclusion; as evidence, I mean, that in this form, at least, the axis of symmetry does not always coincide with the first cleavage plane, or always with the second, as some have suggested. When I speak of "coincidence," I, of course, merely mean that the directions of the planes appear to coincide. To be on the safe side, I will only assert that the planes said to coincide were not more than 15° apart, though I believe that in the case of some eggs it is possible to reduce this angle.

II.—The axial part of the embryo is formed by concrescence of the embryonic ring, or lip of gastrula mouth, from in front backwards.