## SOME EMBRYONIC AND LARVAL STAGES OF THE WINTER FLOUNDER.

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The height of the spawning season of the winter flounder, *Pseudopleuronectes americanus* (Walb.), in the vicinity of Woods Hole, Mass., is reached at what is usually the coldest time of the year, most frequently during February. The material on which this paper is based was acquired during the period extending from January 28 to February 23, 1921, the spawn being gathered from Bowen's pond, Poket, and Waquoit. These are small arms of the sea superficially quite lacustrine in appearance and of rather low salinity, due to a considerable influx of fresh water. The fish were taken in small fyke nets set at a depth of about 8 feet through breaks in the ice. The temperatures and salinities of these spawning grounds during the period of observation, which probably give a fair indication of the average variation, were as follows:

Locality.	Tempera- ture ° F.	Specific gravity at 60° F.
Bowen's pond.	33-37	1.011-1.020
Poket.	32-37	1.010-1.011
Waquoit.	35	1.016-1.022

Possibly the temperatures dropped a little lower at times when no records were taken.

On February 23 the entire catch of one fyke net (85 specimens) was examined in detail and 24 per cent was found to be ripe, 37 per cent spent, 33 per cent partly spent, and 6 per cent immature. These data would seem to indicate that the season was well under way and about to wane, which was substantiated by statements of the men and subsequent observations. A few examples of the younger fish were always found along with the mature fish, apparently following them to the spawning grounds, although sexually immature and unable to partake of the activity.

These fish had fed sparingly if at all. Seven were completely empty, and of the remainder very few contained more than mere traces of a yellowish white paste destined only to become offal. The volumetric percentages of the various items of the stomach contents were as follows: Gastropods (*Haminea solitaria-?*), I + per cent; amphipods (sand fleas), 8 - per cent; prawns (small), trace; mud, II - per cent; yellowish white paste, 80 per cent. The yellowish paste might well have been the remains of food taken before migration to the spawning grounds, as the peristalsis of fishes in winter is usually extremely slow.

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FIG. 274.-Egg of winter flounder, Pseudopleuronecles americanus.

- a. Unfertilized egg.
  b. Egg with blastoderm of two cells.
  c. Egg with blastoderm of four cells.
  d. Egg with blastoderm of eight cells.
  e. Egg with blastoderm of many cells.

- f, Embryo in early stage of differentiation. g, Embryo further differentiated. Note small sphere simi lar to an oil globule.
- k, Embryo in an advanced stage of differentiation.
  i, Egg about to hatch.

In the latitude of New York this species is angled for in the fall and sometimes as late as the middle of December. From then on it usually ceases to take the hook until it is next taken in late February, the anglers' belief being that the flounders lie dormant in the mud during the coldest weather. Along the New England coast the fall season closes earlier and the fishes do not reappear to the anglers much before the middle of March or later. Correlating this with the preceding study of the stomach contents it is evident that these fish feed little, if at all, during the period of sexual activity.

The spawning act in the confinement of the hatchery tanks was invariably performed at night, most frequently between the hours of 10 p. m. and 3.30 a. m. The details of this are fully described in Copeia for January 22, 1922.<sup>1</sup>

Embryology.—The eggs of Pseudopleuronectes americanus are minute, adhesive, and demersal. They have a modal diameter of 0.81 mm. and vary from 0.71 to 0.86 mm. Due to their adhesive nature, they are frequently more or less distorted, in some cases being quite ovoid; but it is doubtful if the deformed eggs produce normal larvæ. Laid along a ruler, as fish-culturists measure, the eggs run 31 to the linear inch. The blastodisk (fig. 274a) is large and of a pale amber color, while the yolk is colorless. The surface of the yolk is finely tuberculate, and the egg membrane resembles fine grain leather in texture. The spermatozoons average about 0.030 to 0.035 mm. in total length.

At a temperature of 69° F. the first cleavage took place at about 21/4 hours after fertilization (fig. 274b). Eggs when isolated from their companions showed flat marks on the membrane where others had been adhering, some presenting the appearance of fairly regular polyhedrons. Such pressure marks on the membrane appear not to affect the development in any way and, being purely mechanical, are not shown in the sketches, as eggs isolated from the start are perfectly spherical. The further cleavages followed after the typical manner of teleostian eggs. By the time 24 hours had passed the blastoderm was broken up into a high number of cells (fig. 274 c to e). By the end of the third day differentiation had begun and the eggs appeared as shown in figure 274j. At six days the differentiation had reached the stage shown in figure 2749, primitive segmentation having commenced and the cephalic region having become more recognizable as such. It might be noted that in many of the eggs a small sphere similar to the oil globules in pelagic ova was observed, and in a few several such globules were present in a small cluster. Such a structure is indicated in figure 2749. Eggs beyond this stage failed to show this peculiarity. The embryo was a pale amber of the same tint as the unfertilized blastodisk, and the "oil globule" was colorless. Figure 274h shows the appearance at nine days. The embryo was well differentiated, and chrome yellow chromatophores were sprinkled over the body, as indicated by black These were punctulate and difficult to see by transmitted light, but they stood dots. out prominently on a dark field. Figure 274i gives the appearance at 15 days. The chromatophores presented a similar appearance at this stage, but there was a noticeable concentration of them as a vertical band in the caudal region. The heart could be seen beating in slow rhythm, but no other motion was noted. The cephalic region appeared

<sup>&</sup>lt;sup>1</sup> Breder, C. M., jr.: Description of the Spawning Habits of *Pseudopleuronecles americanus* in Captivity. Copeia, January 22, 1922, No. 102, pp. 3-4. New York.

to be finely tuberculate. At 15 days hatching had commenced, although comparatively little activity on the part of the embryo had been observed at any time while in the egg.

Larval development.—On hatching the larva appeared as in figure 275a. In some individuals the eyes were entirely unpigmented, while in others chrome yellow pigment similar to that present on other parts of the body was present in the iris, a type of ocular pigmentation developed by all the larvæ within a few days. Later the pupil became generally tinged with greenish, and by the time the larva reached the stage shown in figure 275 b and c, 19 days, the pupil had become black and the iris presented a metallic greenish iridescence. The eyes were directed slightly forward and downward, the mouth was large and functional, and the yolk was absorbed at this stage. The entire animal was perfectly symmetrical as yet. The pigment had become a little darker, approaching a light orange. None of the larvæ survived for more than 22 days.

Preserved material which had been collected at Boothbay Harbor, Me., by Supt. E. E. Hahn near the end of the same season was also examined, and it was found that the development had been quite similar to that of the material studied at Woods Hole. Mr. Hahn succeeded in holding some larvæ until the twenty-seventh day after hatching. The chief advancement beyond that condition shown in figure 275 b and c was one of size. These examples, which were preserved in formalin, averaged about 5 mm. in length, while those found to be living at the end of 22 days at Woods Hole averaged about 4.5 mm., which is a trifle over that of the example illustrated by figure 275 b and c. The larvæ were held in Chester jars, the mouths of which were covered with a double thickness of cheesecloth. It was difficult to find a screening which would hold the fishes in and at the same time allow smaller food organisms to pass through and permit a sufficient change of water. The cloth employed was far from satisfactory, but fine bolting cloth was found to be entirely unsuitable, as it clogged too readily with diatoms and fine débris. A similar difficulty was reported from the Boothbay Harbor station.

In 1885, under the name of *Pleuronectes americanus* Walb., from material studied at Newport, R. I., during July and August, Agassiz and Whitman<sup>2</sup> described and illustrated the development of what they believed to be this species. It is now apparent, however, that these splendidly rendered illustrations and carefully written descriptions refer to some species of a very different habit. Aside from the fact that the illustrations do not agree with those accompanying the present paper, which have been based on material definitely known to be *P. americanus*, it is evident, since their material was pelagic and was taken in midsummer, that their work was a study of the development of another fish. It is doubtful, indeed, if the material was of a Pleuronectid nature at all, principally owing to the long gut shown in their plates, which is highly unusual for members of this group. In a previous paper Prof. Agassiz<sup>3</sup> illustrates more advanced stages of some flounder which he also identified as this species, but it is thought that his sketches represent some other form, although the insufficiency of data presented therewith precludes any very conclusive statement at present.

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<sup>&</sup>lt;sup>2</sup> Agassiz, Alexander, and C. O. Whitman: Studies from the Newport Marine Laboratory. XVI. The Development of Osseous Fishes. I. The Pelagic Stages of Young Fishes. Memoirs of the Museum of Comparative Zoology at Harvard College, Vol. XIV, No. 1, Part I, 56 p., XIX pls. Cambridge, 1885.

Agassiz, Alexander: Development of the Flounders. Proceedings, American Academy of Arts and Sciences, Vol. XIV; p. 1-25, pls. Boston, 1879.



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