

# THE EMBRYOLOGY OF THE WHITEFISH, *COREGONUS CLUPEIFORMIS* (MITCHILL)

## PART I

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### INTRODUCTION

The importance of the lake whitefish as a food fish has long been recognized, and the recent decline in its population in the Great Lakes is cause for serious concern. Its artificial propagation in state and federal hatcheries has been conducted on a large scale for more than fifty years. Several investigators, Couch ('23), Leach ('23), Van Oosten ('23), Mellen ('23), Koelz ('27, '31), Wickliff ('29), Hart ('30, '31), have made studies on the rate of growth, the age at sexual maturity, breeding and food habits, migrations, and hatchery methods, of this species. However, the literature dealing with the embryology of the Whitefish is confined to two series of microphotographs of the eggs undergoing cleavage, by Leach ('23), and by Wickliff ('28), and to a short description of the unfertilized egg and cleavage stages by Mrs. Fish ('29), whose paper deals primarily with the early life history subsequent to hatching. The present series of papers, of which this is the first number, represents a general survey of the embryology of this species, from fertilization until hatching. This first paper traces the major events of segmentation and germ-layer formation and general development up through the closure of the blastopore. The later embryology and development of each of the organ systems will be treated in subsequent papers. Such a preliminary survey has been made with the hope that it will serve as a basis for further study, and that it will also yield information useful in attacking various hatchery problems relative to whitefish propagation.

### MATERIALS

The eggs of *C. clupeaformis* have a prolonged period of incubation, usually extending from the middle of November to the first week in April. Such an extended period permits the

collection of large series of eggs in closely graduated stages. During the winter of 1926-'27, a series of 803 stages was collected at the Ohio State Fish Hatchery at Put-in Bay, under the direction of Mr. E. L. Wickliff, Assistant Chief of the State of Ohio Division of Conservation. The eggs were taken at four hour intervals, day and night from one day after fertilization, November 21, 1926, until hatching time, April 5, 1927, a total of 134 days, 16 hours. Temperature readings were made at 8 A. M. and 4 P. M. daily during this period, using a Fahrenheit thermometer sensitive to fluctuations of one degree. This series is the basis for the present study, and it is now in the possession of the Department of Anatomy of the Ohio State University. The author wishes to express his appreciation to the State Department of Conservation and to the Anatomy Department for the use of this material and to Dr. Ralph A. Knouff for his constructive suggestions and criticisms of this study.

For purposes of a preliminary survey, every sixteenth stage of the eight hundred was studied, with additional ones in the early stages, making a series of fifty-five units. Those involving early cleavage, germ ring formation, the primitive streak, the formation and closure of the blastopore and the differentiation of the primary germ layers are described from surface views of whole mounts, and from serial sections, in the present paper.

Organogenesis from this point to hatching will be discussed in papers II and III of this series. This later development is shown by reconstruction drawings, which were built up from serial sections. These reconstructions indicate the general development of the brain, sense organs, cranial nerves, notochord, somites, pronephric tubules, the gut, branchial pouches and the heart.

#### METHODS

All of the eggs were fixed at the time of collection in Bouin's solution. When removed from this, the shell was tough and could easily be removed with needles. The yolk was fairly brittle, yet not so hard but that it could be sectioned satisfactorily. For most of the series, the sections were stained with iron-alum haematoxylin, and counter stained with eosin. A few embryos were stained *en toto* in Mayer's acid carmine. Greater differentiation was secured with the former method when the sections were allowed to remain in the mordant, but

not in the oven, overnight and from 24 to 48 hours in the haemotoxylin. The sections were cut ten microns thick and mounted by the water-albumen method.

For the preparation of whole mounts, the embryo was first picked off the yolk with fine needles. It was then cleared in cedar-wood oil, stained *en toto* with acid carmine, and mounted in balsam. Some of the mounts were simply cleared and not stained. In some respects, these served better for study than the stained embryos.

A vertical microprojecting apparatus calibrated to 100 diameters was used in making the reconstruction drawings of the later stages. Since the sections were cut ten microns thick, each section was represented by one millimeter on the drawing. The outline of the entire embryo of the stage to be reconstructed was first made from the whole mount of that stage. After the angle at which the sections had been cut was determined and the base line drawn on graph paper, then the image of a section thrown by the microprojector was transposed to the graph paper by using a pair of dividers to mark out the limits of the part being reconstructed. Every fifth section was thus transposed and these sections form the basis for the drawings made at 100 diameters. The magnification of the finished drawing is indicated by the size of scale beneath it. By this method, the embryo may be reconstructed on any desired plane, parallel to the antero-posterior axis of the body.

#### THE DESIGNATION OF STAGES

All of the eggs in this series were taken from the same incubation jar at the state fish hatchery. They had all been fertilized at the same time and subjected to the same temperatures and oxygen content throughout the incubation period. Any wide variation in temperature to which eggs of the same age had been subjected would probably result in marked fluctuations in the relative development of consecutive stages. However, no marked breaks or gaps in the series have been discovered. It is assumed therefore that all normal eggs had developed at approximately the same rate.

With temperatures of the lake in midwinter fluctuating about a mean of 33° F., whitefish in the state fish hatchery at Put-in-Bay have over a period of years had an incubation period of from 120 to 140 days (Leach, '23, Wickliff, '28). However, some embryos in the series described by Mrs. Fish

('29) reached the hatching stage on the 61st day. No temperatures were given for this series. Obviously, different series cannot be compared on the basis of time alone.

There are other bases however that may be used. Wallich ('00) designated the age of fish embryos by thermal units. By a thermal unit (t. u.) is meant a temperature of 1° F. above 32 degrees for a period of 24 hours. A mean temperature of 36° F. for one day yields 4 thermal units. Thus, when the incubation period for any series or any particular stage in that series is given in days, and the mean daily temperatures are known, the thermal units may be readily calculated by simple addition. The thermal unit then expresses the age of an embryo as the sum of both time and temperature. Embryos in different series whose ages in thermal units are the same would be comparable. For example, embryos incubated for 131 days at 34½° F. would have been subjected to 325 thermal units and they would be the same age on this basis as other embryos incubated for only 65 days at a mean temperature of 47 degrees. Assuming that this relationship between time and temperature holds within certain limits the thermal unit then may be used as a basis for making comparisons between embryos of different series.

The age in thermal units is given for every stage of whitefish embryos described in this series, together with the age in days. For post-cleavage stages, the length of the embryo and the number of somites are also designated. Thus there are four means of denoting the various stages in this series which may serve in making subsequent comparisons.

In the drawings, figure numbers correspond to the serial stage number from which they are taken.

#### DESCRIPTION OF EARLY CLEAVAGE STAGES THE UNFERTILIZED EGG

The unfertilized whitefish egg is approximately 3 mm. in diameter. (See Fig. 1a, Plate I.) It is round, almost transparent when alive, and free to turn in the perivitelline space beneath the shell. The shell is smooth, tough in texture, and is held turgid by the enclosed perivitelline fluid. The perivitelline space is less evident in unfertilized eggs than in eggs subsequent to fertilization. There is a collection of many oil globules at the animal pole of the egg. These are conspicuous

during the early segmentation stages, and underlie the developing germinal disc.

Notwithstanding the fact that a micropyle has been described for the eggs of the salmon (Doyere, '50), for the trout (Henneguy, '88), the carp, the pike, and for various other teleosts, I have failed to discover any indication of such a structure in the whitefish egg.

A thin layer of protoplasm of uniform thickness completely surrounds the yolk.

#### EARLY SEGMENTATION STAGES

*Four-celled Stage.*—Since the fixation of eggs as originally collected did not begin until twenty-four hours after fertilization, the events concerned with fertilization and the early cleavage cannot be described from this material. Mr. Wickliff, in the fall of 1927, however, secured eggs from the same source and under similar conditions, taken three hours after fertilization. These eggs were in the four-celled stage. (See Fig. 1b, Pl. I.) Judging from what is known in other species, there must have occurred a "streaming of protoplasm" toward the animal pole, for there is present in these eggs at that pole a rounded disc of protoplasm, lenticular in character. This disc of protoplasm tapers off rapidly at its edge, and continues over the surface of the yolk in an extremely thin layer. The first two cleavage planes are meridional and at right angles to each other. They cut deeply into the blastodisc but do not extend into the yolk.

*Eight-celled Stage.*—Stage No. 1, O. S. U. series. Incubation period: 1 day; 8 t. u. (See Fig. 1c, Pl. I.) In the egg that is figured, the blastodisc is somewhat longer than broad, with the eight or more blastomeres arranged roughly as four pairs, one behind the other. In an egg of this pattern, the first cleavage furrow must have been transverse to the long axis of the figure and through its center. The second cleavage furrow would then be at right angles to the first, along the antero-posterior plane. In the rounded blastodisc of the four-celled stage as figured, however, no antero-posterior lengthening can be distinguished. Since the first eggs of the series have already undergone these first two divisions, any attempts to identify the first cleavage planes with the antero-posterior plane of the embryo must be reserved for later study on living material. With regard to the third division, in

Fig. 1c, there must have been two transverse furrows, one through each pair of the first four cells, which by subsequent growth have produced an elongated blastodisc.

The serial sections of this stage were cut transversely, each section passing through two blastomeres. These cells are separated by a very fine membrane which extends deeply into the blastodisc, almost to the yolk. At the periphery of the blastodisc, the blastomeres continue into a progressively thinner layer of superficial protoplasm, which extends over the yolk. The zone of transition between the edge of the blastodisc and the superficial layer of protoplasm over the yolk constitutes the early periblastic ridge as described for *Serranus* by Wilson. The astral rays of the mitotic figures are very conspicuous, extending nearly to the boundaries of the cell. All eight cells are actively undergoing mitosis. The planes of the spindles in various cells which are in the metaphase condition indicate that the succeeding or fourth cleavage furrow will be parallel to the second, as described above, and at right angles to the third. This will produce four more or less distinct rows of cells and increase the breadth of the blastodisc, tending to restore its rounded outline, as in Fig. 4, Pl. I.

All eight-celled blastodiscs are not elongated as described above, although probably about three out of every four eggs at this stage show this pattern. Some were observed in which the blastodisc was distinctly circular and the cells were disposed symmetrically about a central point. In these, it is assumed that the first two furrows had been meridional and at right angles to each other, but the third cleavage furrow was completely circular and located midway between the center and the periphery of the blastodisc. This tendency of the third furrow to be circular is seen with all its modifications. Indeed, the third cleavage furrow in Fig. 1c, separating the third and fourth pairs of cells describes an arc of such a circle. It is well known that such variations in cleavage patterns are determined by varying rates of growth of the cells and by the relative pressures which they mutually exert upon one another. In eggs in which the cells are concentrically disposed, the fourth cleavage furrow is apparently meridional. The resulting cleavage pattern accompanied by some irregular growth of the cells is illustrated by Fig. 2, Pl. I.

In succeeding stages, the bilaterality of the blastodisc is no longer apparent. Fig. 3, Pl. I, is approaching the 32-celled

condition, and the blastodisc shows a decided tendency to become rounded up. By Stage No. 8, the blastodisc is completely rounded.

*Stage No. 8.* O. S. U. series—Incubation period, 2 days, 4 hours, 15 t. u. Transverse sections through this stage (see Fig. 8, Pl. II) reveal that, while the cells of the blastoderm are comparatively large, they have been reduced through cleavage to about one-fourth the diameter of cells of Stage No. 1. The blastodisc is four cells deep through its center but shallower at its margins. The cells at the surface are slightly broader than deep and closely joined at their edges, representing the initial stage in the formation of the epidermic stratum. The deeper lying cells are loosely arranged with spaces between them in the central portion of the blastodisc. These scattered spaces represent the segmentation cavity of a type similar to that described by Kopsch ('04) for the trout, and differs from the single continuous ventral one as described by Wilson for the sea bass. The early periblastic ridge at the margin of the blastodisc is prominent. The marginal blastomeres which form the ridge are prolonged into the protoplasm covering the yolk, to form the central periblast. The latter is an extremely thin layer of protoplasm lying immediately beneath the blastodisc. It is not separated from it by the segmentation cavity as shown in Wilson's figures 17 and 18 of *Serranus*. The yolk mass underlying the central periblast at this stage contains many scattered, irregularly shaped darkly pigmented bodies or granules which may constitute a syncytium of yolk nuclei, but more probably they are simply yolk granules. These are not to be confused with the syncytium of nuclei formed later in the central periblast from cells migrating inward from the periblastic ridge.

*Stage No. 16.* O. S. U. series—Incubation period 3 days, 12 hours, 30 t. u. The blastodisc is somewhat more prominently raised up on the yolk, and the outlines of the individual blastomeres are indistinct on the surface, owing to rapid cell division. Transverse sections of this stage, (Fig. 16, Pl. II) show the blastomeres reduced to about one-fourth their former diameter in Stage No. 8. They form a blastoderm of from eight to ten cells deep. The spaces of the segmentation cavity between cells are less conspicuous. The epidermic stratum which was forming in Stage No. 8 is clearly defined as a superficial layer of somewhat flattened cells. This stage corresponds closely to

that figured by Kopsch at the end of segmentation in the trout.

*Stage No. 32.* O. S. U. series—Incubation period, 6 days 4 hours, 42 t. u. *The formation of the germ ring and subgerminal cavity.* The egg is rapidly approaching the end of the segmentation phase of its development. Many more cells are now shown in a single cross section than in previous stages, there being as many as two hundred in sections through the center of the blastoderm. The cells are correspondingly smaller in diameter. Evidently segmentation is occurring rapidly. The blastoderm spreads out over the yolk surface more than in earlier stages, and accordingly it is thinner, being only from six to eight cells deep. The epidermic stratum is a clearly differentiated layer of cells at this stage.

The periblastic wall contains clusters of nuclei of cells which have probably migrated into it from the margin of the blastoderm and subsequently lost their cell walls, in the manner described by Wilson, pp. 216–217, for *Serranus*. These nuclei have reached the center of the periblast as indicated by their presence in that region. The central periblast is now a syncytium of scattered nuclei which continues uninterruptedly into the periblastic wall and the cortical protoplasm over the yolk. The early periblastic ridge has largely disappeared. The marginal cells of the blastoderm lying above the periblast are closely packed together to form a wreath-shaped thickening, the germ ring, which is clearly seen from surface views of whole mounts. (see Fig. 32, Pl. I). The blastoderm has become greatly arched over the curvature of the egg.

As a result, a single continuous segmentation or subgerminal cavity lies prominently between the central periblast and the overlying blastomeres, to one side of the center and just within the germ ring. This segmentation cavity is apparently formed here as a larger space than in the previous stage, simply as the result of the crowding of the cells at the periphery of the blastoderm. It is probably confluent with the smaller spaces scattered elsewhere between the cells of the cross section. If this be true, the isolated spaces in cross-sections of earlier stages may actually be confluent and thus be regarded as essentially homologous to this single continuous subgerminal cavity which arises at this stage. As the cells of the blastoderm are reduced in size through repeated divisions and crowded together by the overarchng of the blastoderm, the spaces



between them are thereby diminished. These spaces are finally reduced to the vanishing point in cross-sections of the next stage, No. 48. The sole remaining space is a typical sub-germinal cavity.

*Stage No. 48*—Incubation period, 8 days, 20 hours; 60 t. u.

*The growth of the blastoderm; the primitive entoderm*—The growth of the blastoderm around the yolk continues rapidly after the completion of the germ ring in the previous stage. In the present stage, (see Fig. 48, Pl. I), the blastoderm envelops approximately one-third the yolk, and the germ ring is plainly visible at its periphery. The subgerminal cavity appears from the surface view as a translucent, circular area within the cap of cells. In cross-sections, this cavity extends broadly beneath the blastoderm. It has clearly defined limits which extend to the marginal cells of the germ ring from within. The blastoderm is more elevated above the periblast than in *Serranus*, but the marginal cells of the germ ring are similar to those in Wilson's figures 46 and 47, Pl. XCIV. The subgerminal cavity is slightly eccentric, due to the rapid proliferation of cells on one side of the germ ring to form a deeper lying mass of undifferentiated cells, the embryonic bud. (See Fig. 48, Pl. II). In this area the cells are compact and the blastodisc is approximately twice as thick as elsewhere. The embryonic bud is destined to give rise to the embryonic shield and its posterior margin will constitute the dorsal lip of the future blastopore.

A tongue of primitive entoderm extends anteriorly from the embryonic bud toward the center of the blastoderm. At this stage, it reaches almost to that point. The periblastic layer has become fully developed, and retains the features described in the previous stage. Throughout the remainder of the embryonic period, the periblast remains unmodified, as the covering of the yolk beneath the embryo.

*Stage No. 64*—Incubation period, 11 days, 12 hours, 82 t. u. The blastoderm is flattened out as a sheet of cells enveloping the upper half of the yolk sphere, with the germ ring lying in the equatorial plane. (See Fig. 64, Pl. I). In some whole mounts of this stage, the blastoderm is translucent, at least in the extra-embryonic portions and the yolk surface beneath is clearly visible. While the embryonic shield is hardly visible from surface views, cross-sections show that it is definitely established. In the posterior end of the shield,

an undifferentiated caudal mass of cells arises abruptly, with its thickest portion in the axial line. Just anterior to the caudal mass, the shield extends laterally to end abruptly at its margins. In cross-sections at this level, (see Fig. 64, Pl. II), beneath the epidermic stratum lies the ectodermal layer, several cells deep, underlaid by a two-rowed cell layer of primitive entoderm. The entoderm extends forward as a tongue of cells, as described for the previous stage, No. 48, but now underlies the entire shield, thus obliterating the subgerminal cavity. In the central axial portion, the entoderm from either side fuses into an undifferentiated line of cells, which represents the first step in the formation of the notochord. As the anterior end of the embryonic shield, the two layers of the entoderm lose their identity and appear as a single layer which fuses with the overlying ectoderm, a condition which represents the first step in the formation of what Wilson termed the neurenteric streak. (See Wilson, Fig. 52).

*Stage No. 80*—Incubation period, 14 days, 4 hours, 93 t. u. The embryonic shield becomes clearly visible on the whole mount at about Stage 72, but is figured for the first time in the drawing of this stage, Fig. 80, Pl. I. The somewhat triangular area represents the thickened embryonic portion of the blastoderm, in contrast with the lateral extra-embryonic portion which extends as a thin sheet of blastoderm over the curvature of the yolk to end in the germ ring at its margins. The bluntly rounded apex of the shield has extended forward from the condition described in Stage 64 and now clearly marks off the anterior end of the embryo.

The so-called neurenteric streak, which was beginning to form in the previous stage, is now clearly visible from the surface view, as a median longitudinal opaque area which extends from the anterior end of the embryonic shield backward to the notochord. It marks the location of the neural chord in the head end of the embryo.

From its incipient condition in Stage 64, the notochordal area has grown forward from the caudal mass and occupies the posterior third of the embryonic area. It appears in surface views of whole mounts as a light streak in the axial line. It is still an undifferentiated cell mass, fused laterally with the entoderm.

The extra-embryonic portion of the blastoderm extends laterally and anteriorly beyond the embryonic shield to the

germ ring as mentioned above, and it now encloses two-thirds of the yolk. In this extension of the blastoderm, the direction of growth is difficult to determine. There are no definitely placed oil globules as there are in *Serranus*, by which to judge the relative position of the germ ring. In *Serranus*, in *Salmo*, and in various other teleosts, it has been shown that the position of the caudal mass and hence that portion of the germ ring which constitutes the dorsal lip of the blastopore is relatively fixed. In such cases, extension takes place by way of the anterior margin of the germ ring growing around the yolk. However, in *Hemichromis*, in which the egg is oblong, McEwen ('30) describes also a backward growth of the dorsal lip of the blastopore. Thus all portions of the germ ring take part in the closure of the blastopore by their migration toward the point of closure. In spite of Wilson's statement to the contrary, p. 222, the posterior end of the sea bass embryo in his Fig. 38, Pl. XCII, is somewhat closer to the oil globule than it is in his Fig. 36 of an earlier stage. The difference is not marked however. Thus there is some backward growth of the dorsal lip of the blastopore, although it is slight in comparison to the migration of the anterior margin of the germ ring over more than half the yolk surface to finally meet the dorsal lip at the point of closure. In the Whitefish, the closure of the blastopore occurs in much the same way as in *Serranus*, involving mostly epiboly of the anterior or ventral lip, supplemented by some concrescence and by a slight backward growth of the dorsal lip.

*Stage No. 96*—Incubation period, 16 days, 20 hours, 97 t. u. This stage is marked by the further growth of the blastoderm around the yolk, advancing to a *large yolk plug stage*. The embryonic shield has increased to 2.0 mm. in length. This increase in length is due partly at least to a slight backward extension brought about by a concrescence of the lateral lips of the blastopore. The line of this concrescence would correspond to the primitive streak of other forms and is marked by a thickened mass of cells referred to in Fig. 96b., Pl. II, as the caudal mass. Kupffer's vesicle is seen for the first time in this stage. It appears on the floor of the caudal mass. Its walls are formed by a single layer of regularly arranged cells. Just anterior to this vesicle, the notochord arises, possessing a distinct outline and a compact shape. Its anterior end extends two-thirds the distance from the caudal mass to the anterior

end of the embryo. The lateral mesodermal plates are now readily distinguished from the surface ectoderm dorsally, from the notochordal area medially and from the entodermal cells ventrally (See Fig. 96c. Pl. II). The ectoderm is now greatly thickened over the entire embryonic shield, as compared to the extra-embryonic portion. In the anterior end of the embryonic area, this ectodermal thickening is very marked, forming the neural keel. Above it, dorsally, a shallow median neural furrow appears, into which dips the epidermic stratum. This furrow is transitory, and disappears in a slightly later stage, No. 112. At this level the primitive entoderm forms a single cell layer beneath the neural keel.

*Stage No. 112*—Incubation period, 19 days, 12 hours, 103 t. u. Length of embryo: 2.32 mm. (See Fig. 112, Pl. I and II). By this stage, the neural keel has undergone a marked downward growth into the yolk mass. As it continues to thicken and grow deeper, the lateral portions of the neural keel grow thinner, as the result, according to Wilson, of cell migration. Thus in surface views the so-called neurenteric streak of the embryo projects anteriorly from the original dorsal lip of the blastopore as a long narrow opaque area on the surface of the yolk, of almost uniform width throughout its length, but with greater depth at the anterior end. The neural furrow, mentioned in the previous stage, is shown in the surface view here, but it disappears with this stage. It fades out progressively from behind forward. The cells of the neural keel are the same in appearance throughout, not yet being stratified nor differentiated. The optic primordia make their first appearance at this time, as solid cell masses.

The outline of the notochord is visible from the surface view. Its anterior end extends two-thirds the distance from the caudal mass to the anterior end of the embryo. In the posterior region, its cells are rounded up and may be easily distinguished from the nerve cord, the mesodermal masses, but somewhat less distinctly from the entoderm. Farther forward, however, it is progressively less differentiated. The mesodermal plates, described in Stage 96 as having separated from the adjacent layers, are now divided into two parts: the one more mesial which later forms the dorsal mesodermal plates; and the outer part, which later forms the lateral mesoderm, the intermediate cell mass and its derivatives, and the Wolffian ducts. (See Fig. 112, Pl. II; also, Swaen and Brachet,

Fig. 66, Vol. 16, '00). In the middle region of the embryo, the dorsal mesoderm is for the first time composed of a solid rosette of cells which represents the somite. There are apparently three pairs of somites present at this stage. The lateral mesoderm is likewise a solid mass of cells. On its mesial border is a single line of cells which later splits off from the remainder of the mass to form the intermediate cell mass. At the level of the somites, the entoderm lies over the periblast as an unfolded sheet of cells, one layer thick. This stage is distinguished by the *narrow yolk plug*. The blastopore is almost closed at this time.

*Stage No. 128*—Incubation period, 22 days, 4 hours; 108 t. u. Total length of embryo: 2.96 mm.; eleven pairs of somites; closure of the blastopore. In contrast to the previously described stage, very marked cephalization has occurred at this time, as seen in both the surface view drawing, Fig. 128, Pl. I, and in the reconstruction drawing of this stage (Fig. 128, Pl. II). Three primary cerebral vesicles have developed. The neural keel in the head region has continued its downward growth and lies half imbedded in the yolk. Its depth in the brain-forming region is at least twice that of its depth in the middle portion of the spinal cord region of the embryo. There are no cerebral ventricles present at this stage however, and the optic outgrowths are still solid cell masses. The cells of the neural keel are no longer indifferently placed, but are becoming rearranged into two parallel rows which separate in Stage 144, to form the cavity of the neural canal. This rearrangement of cells takes place first in the prosencephalon and continues backward to the middle region of the neural cord. The optic anlagen which appeared in Stage 112 have greatly enlarged, and in the reconstruction drawing are shown as projecting prominently from the sides of the prosencephalon. They are now separated externally from the brain by a fissure which appeared in Stage 120 and which now extends from above downwards and from behind forwards, leaving only the ventral portion connected with the prosencephalon. This connection is the optic stalk.

Immediately posterior to the anlage of the eye is a mass of head mesoderm, of mesenchymatous appearance. While it appears here as a distinct mass, its later disposition has not been thoroughly traced. There is but little evidence of the existence later of head somites, and none of segmentally arranged

head cavities. A wing of lateral mesoderm extends along the sides of the body from the anterior end of the paired somites at the level of the rhombencephalon forward to the middle portion of the head region. It spreads out laterally on the yolk surface, but since the keel sinks so deeply into the yolk, the mesoderm appears high on the side of the head. A longitudinal furrow appears in this same region on the dorso-lateral surface of the head at the angle between the neural keel and lateral mesoderm. It is probably the so-called sensory furrow described by Wilson in *Serranus*, and as such is the *ánlagen* of the auditory pit and lateral line sense organs.

The notochord anterior to the caudal mass is two or three cells deep. Its cells are interlocked and are approaching a period when their vacuolation begins. Anteriorly the notochord is clearly separated from adjacent areas. It extends with the flattened layer of entoderm beneath it forward to the level of the midbrain. This position is retained hereafter.

Eleven pairs of mesodermal somites are present, as shown in the drawing. Kupffer's vesicle, which appeared in Stage 96, has now reached its maximum development in the floor of the caudal mass, and from this time on, it begins to diminish in size. The blastopore is completely closed over on the surface, and its position is marked by a narrow indentation or furrow in the tail region.

#### SUMMARY

From the foregoing descriptions, it is seen that the Whitefish, in its early development up to the closure of the blastopore, is typically teleostean. The 3 mm. egg with its capsule, perivitelline space, and the concentration of oil globules at the animal pole agrees closely with eggs of other species, although a micropyle is apparently absent. The formation of the blastodisc and the cleavage of the blastomeres follows closely the pattern described by other writers for the trout, the sea bass, and other species, and may be regarded as typical of teleocithal eggs. The early segmentation cavity, by virtue of the fact that it is interspersed between scattered cells of the blastoderm, resembles that of the trout, but differs from that of *Serranus*. It may probably be regarded as a characteristic of the *Isospondyli*.

The growth of the blastoderm over the yolk, the formation of the germ ring, the periblast and the primitive entoderm,

the delineation of the embryonic shield and within it, the differentiation of the three primary germ layers, all proceed in much the same manner as previously described for other Teleosts.

However, the relative degree of development attained by the whitefish at the time of the closure of the blastopore is of special interest. In this series, this event has occurred by Stage 128, when these eggs have been incubated for 22 days and have been subjected to 108 thermal units. In terms of days, these eggs have completed just  $\frac{1}{6}$  of their incubation period, but  $\frac{1}{6}$  of the incubation period in terms of thermal units. The higher proportion of the latter is due to the fact that, during the first part of the incubation period, the temperature of the lake water is comparatively high, and the ratio of thermal units to days is consequently higher than later in the season, when the daily temperatures descend to around 33° F. It is seen then that the blastopore closes within the first sixth of the incubation period in point of time and within the first third of this total period on the basis of both time and temperature (thermal units). At this stage, the embryo is clearly outlined, lying in a straight line over the curvature of the egg, with a length of about 3 mm., which is just one-fourth its hatching length. Within the body of the embryo, all three primary germ layers have undergone considerable differentiation. Derived from the ectoderm, the three primary brain lobes are distinct, and the neural keel lies deeply imbedded in the yolk. The optic primordia are now borne on broad optic stalks. The mesoderm has given rise to a mass of head mesoderm, to the notochord which now extends forward its full length to end beneath the midbrain, and to eleven pairs of somites, or about  $\frac{1}{6}$  of the hatching number. Kupffers' vesicle has reached its maximum development. The entoderm is undergoing rapid changes, preparatory to the development of branchial pouches.

When compared with other species of teleosts whose embryology has been studied, it is seen from the above items that the whitefish is notably well differentiated at the closure of the blastopore.

It now remains for later papers of this series to outline the embryology of the whitefish during the remainder of the incubation period, subsequent to the closure of the blastopore.

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## LIST OF ABBREVIATIONS USED IN THE FOLLOWING PLATES

- |                                    |                               |
|------------------------------------|-------------------------------|
| b. d.—blastoderm.                  | Mesen.—mesencephalon.         |
| Bl.—blastopore.                    | n. f.—neural furrow.          |
| c. m.—caudal mass.                 | noto.—notochord.              |
| c. p.—central periblast.           | n. s.—neurenteric streak.     |
| d. mes.—dorsal mesodermal plate.   | o. g.—oil globule.            |
| ecto.—ectoderm.                    | op. prim.—optic primordium.   |
| e. e. s.—edge of embryonic shield. | op. st.—optic stalk.          |
| em. bd.—embryonic bud.             | P.—periblast.                 |
| ento.—entoderm.                    | pr. ento.—primitive entoderm. |
| ep.—epiblast.                      | Prosen.—prosencephalon.       |
| e. s.—egg shell or membrane.       | p. v. s.—perivitelline space. |
| e. p. r.—early periblastic ridge.  | Rhomb.—rhombencephalon.       |
| ep. str.—epidermic stratum.        | s. c.—segmentation cavity.    |
| g. r.—germ ring.                   | s. f.—sensory furrow.         |
| hd. mes.—head mesodermal mass.     | s. g. c.—subgerminal cavity.  |
| i. c. m.—intermediate cell mass.   | som. XI—somite eleven.        |
| K. v.—Kupffer's vesicle.           | y.—yolk.                      |
| lat. mes.—lateral mesoderm.        | y. g.—yolk granule.           |
| mes.—mesoderm.                     | y. m.—yolk mass.              |



## EXPLANATION OF PLATES

(Figure numbers correspond to the serial stage number from which they are taken.)

## PLATE I

- Fig. 1a. Unfertilized egg, with animal pole uppermost.  
 Fig. 1b. Four-celled stage, 3 hours after fertilization.  
 Fig. 1c. Blastodisc of about 8 cells, from above.  
 Figs. 2, 3, 4. Early segmentation stages, 16-32 cells.  
 Figs. 8, 16, 32. Later segmentation stages.  
 Figs. 48, 64. Formation of germ ring, and growth of blastoderm.  
 Fig. 80. Showing embryonic shield, neurenteric streak, blastopore.  
 Fig. 96. Large yolk plug stage, showing outlines of early embryo.  
 Fig. 112. Narrow yolk plug stage.  
 Fig. 128. Closure of the blastopore. Three primary brain lobes.  
 Figs. 144, 160. Surface views of subsequent development.

## PLATE II

- Fig. 8. Cross-section through center of blastodisc of Stage 8. (Drawn from slide B, first row, third section of serial mounts in O. S. U. series. Similar code is used to designate drawings of sections of other stages.) Shows early segmentation cavity, and early periblastic ridge.  
 Figs. 16, 32, 48. Cross-section of corresponding stages shown in Plate I, showing formation of subgerminal cavity.  
 Fig. 64. Cross-section through one side of embryonic shield, showing an undifferentiated notochordal area in mid-ventral line.  
 Fig. 96 B. Cross-section cut obliquely through dorsal lip of blastopore, showing Kupffer's vesicle, and the differentiation of notochordal tissue anterior to caudal mass.  
 Fig. 96 C. Cross-section more anterior than 96 B. The primitive entodermal layers are definitely separated into single layers of mesoderm and entoderm.  
 Fig. 112. Cross-section through dorsal mesodermal plate.  
 Stage 128, Reconstruction drawing, showing embryo in natural position over curvature of the yolk. This stage marks the closure of the blastopore.



