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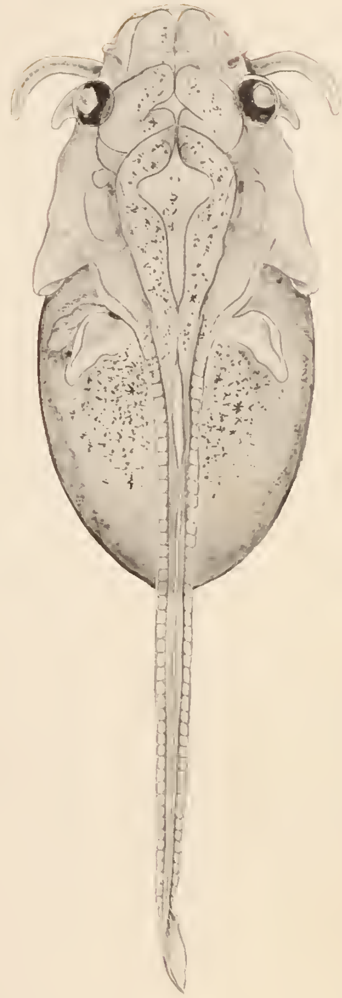

By PHILIP B. ARMSTRONG, M.D.
College of Medicine at Syracuse
State University of New York
AND *Marine Biological Laboratory*
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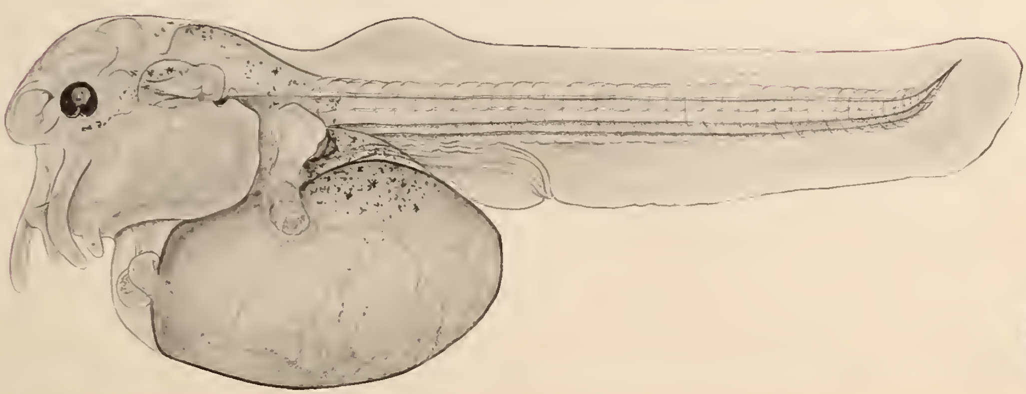

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MANUFACTURED IN THE UNITED STATES OF AMERICA

STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

Ictalurus nebulosus, the brown bullhead or horned pout, is generally distributed throughout the Eastern and Central United States from Maine and the Dakotas on the north into the Gulf States on the south. It has been introduced successfully into streams and ponds on the West Coast and into the waters of several European and Far Eastern countries.

Under favorable conditions adult bullheads attain a length of a foot or more with occasional specimens 18 inches in length weighing 3 to 4 pounds. When crowded they are much smaller. *Ictalurus nebulosus* is very tenacious of life, surviving under conditions which will not support other native fishes. It is commonly found in small ponds, lakes, and sluggish streams and does well even in a muddy habitat. The adults are frequently caught on a drop-line during the daytime but are more active at night. The eyes are small but the olfactory and cutaneous senses are highly developed. They are omnivorous, the diet depending on what is available. The adults are readily collected in a baited minnow trap with an adequate entrance and with reasonble care will survive well in aquaria.

The reproductive habits of *Ictalurus nebulosus* have been observed in both natural surroundings and in aquaria. The eggs are usually deposited in a prepared nest, an open shallow excavation among weeds, or a burrow excavated by the adults under the roots of a water plant which will support the roof of the nest. A common site for such a burrowed nest is horizontally for as much as three feet into the bank of the stream or pond. The bullhead resembles the wren in selecting a nesting site, taking advantage of protected locations in pails, stovepipes, rubber tires, cinder blocks—or under boards, stones, or other articles thrown by chance into the water. Rarely are the eggs laid in an open site without preparation. Depending on the nature of the nest, the floor is invariably some firm material—coarse sand, gravel, or stone. When the nest is found in the muddy bottom of a pond, it is located where the mud is a shallow layer on a sandy or gravel bottom to which the nest is excavated. Drainage tiles provide attractive nesting sites for bullheads. Tiles are particularly useful in ponds supporting a heavy growth of weeds in which it is difficult to locate the natural nesting sites. The tiles should be distributed separately around the margin of the pond in two or three feet of water, their locations indicated by a suitable marker.

The eggs are adhesive when laid and form a spongy pale yellow mass which adheres to the floor of the nest. Each egg through its chorionic membrane has adhesion discs with several adjacent membranes which sometimes show up on the surface of the chorionic membranes when they are torn from each other (see figure of Stage 20) . The micropyle is located as the center of a stellate structure seen in some membranes (Stage 1 *et al.*). When the eggs are laid in individual drainage tiles it is frequently necessary to gently scrape the egg mass free of the tile to which it adheres. If the floor of the nest is sand or gravel, grains of sand and small pebbles are found adhering to the egg mass. There are from several hundred up to a thousand and more eggs in a single egg mass. The adults, particularly the male, guard the eggs. Frequently an observer can flush the adult off a nest by wading in a pond a few yards off shore, locating as closely as possible the point at the bank from which the adult emerges. Close inspection of the bank at this point will usually reveal the opening of the nest. The collector can reach into the bur-

row, and by careful exploration the egg mass, which is soft and yielding, can be obtained. It must be handled gently. (Sometimes a second fish is flushed from the nest and rushes past the collector's arm as he attempts to find the egg mass.)

The adults work over the eggs very actively (Breder, 1935), fanning them with their fins, taking them in their mouths and ejecting them, and in general handling them rather roughly. Apparently almost constant agitation is required to assure normal development of the eggs, since eggs removed to still water usually will die within a day or so. Two simple methods were devised for handling the eggs; either will usually give normal development of the major share of the eggs of a cluster. The cluster may be placed in a seven-inch finger bowl in tap water flowing from a rubber tube of which the lower three inches rests on the bottom of the finger bowl directed to give a swirling flow of the water in the bowl. There should be a flow of water adequate to agitate the eggs slightly but not to carry them with the current. A constant flow of water can best be obtained through a petcock without a washer. In a washered outlet, the flow reduces as the washer swells. The second method is illustrated in Figure 1, which shows a strainer to which a metal tube is soldered in such a position that the water flows from below through the egg mass resting in the strainer. The egg masses should be examined at least twice a day and any dead eggs removed, since these quickly develop mold which extends to the adjacent eggs and kills them.

The laying season starts about the same time in central New York State and on Cape Cod, Massachusetts—i.e., about the middle of May. The latest that newly laid eggs were found on Cape Cod was July 28. Adults in aquaria will lay spontaneously, with the opening and closing dates of the spawning somewhat later than with fish in the wild. Females early in the winter carry eggs which in appearance and size are close to maturity. We induced spawning and obtained fertilized eggs out of season by injecting into a pair of bullheads crushed pituitaries from ripe female alewives, but additional work must be done to standardize the procedures if uniform success is to be achieved.

The eggs measure about 3 mm. in diameter. Those from the same egg mass are remarkably uniform in size but there are differences in size from one egg mass to another. The subchorionic space is wide and accounts for about one-third of the diameter of the egg. The chorionic membrane is transparent, naked, and relatively thin for the size of the egg. The membrane can be removed readily without injury to the developing embryo merely by tearing the membrane with fine forceps. After the blastopore is closed, the embryos survive in running tap water without any special treatment; in fact, they are less susceptible to death than if left in the membranes where the mechanics of aeration are more difficult. The embryos hatch spontaneously in Stage 43 and will swim actively on the floor of their container. In the earlier swimming stages, the embryos can be kept in an open dish with a gentle stream of water circulating through the dish. In the more advanced stages, they become negatively geotropic and are lost from an open dish, but they can be kept in a battery jar closed with a strainer fitted with a tube through which water can be introduced from above (Figure 2). The strainer must be

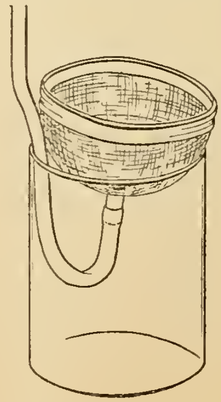

FIGURE 1

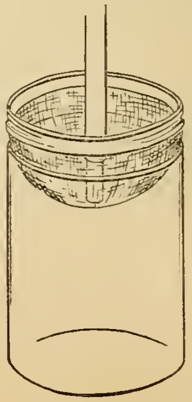

FIGURE 2

of such a size as to fit snugly in the top of the battery jar.

The embryos leave the nests and seek food when the yolk has been absorbed. For a few days they swim as a compact school in shallow water guarded by the adults which swim about below the school. After a few days the school breaks up and the larvae disappear into deeper water.

To identify stages of the embryos, it is advantageous to examine them with transmitted and with reflected light. Light transmitted through the embryo from a white substage mirror is especially effective in bringing out the details of pigmentation. The developing blood vessels and circulation are revealed best by the use of a regular substage mirror. In lighting the embryo from above, strong illumination, such as that from a zirconium lamp, is required to bring out the finer details. Also all available features of the developing embryos, both morphological and physiological, should be taken into consideration for accurate identification of stages.

The embryos in the same egg mass, if properly handled, show remarkable synchrony in their development. However, minor but perceptible variations do occur from mass to mass, possibly because the development of the various features of the embryo do not depend on a single common factor. Because of this, embryos will be included in the same stage in spite of some such minor variation from the pertinent illustration. In the illustrations the embryos are magnified 17 times.

Some of the special features of development are described below.

CLEAVAGE

Stages 1 through 7

Cleavage in the bullhead egg is meroblastic and meridional as is characteristic of the teleosts, the cleavages being limited to the blastodisc, not involving the yolk. The diameter of the early blastodisc is only about one-eighth the circumference of the egg. The successive cleavages follow each other at 35 to 40 minute intervals and extend well through the disc. The blastomeres formed at each cleavage are of equal size and the cell divisions at any one cleavage are closely synchronous. From the margins of the disc the protoplasm thins out abruptly and extends around the yolk as a thin continuous layer, as in *Fundulus*. There is no defined periblastic ridge such as occurs in *Serranus* (Wilson 1891). Cleavage continues to be meridional in the bullhead at least through the 32-cell stage and possibly through the 64-cell stage, but it is quite probable that in this latter cleavage some of the central cells divide horizontally.

BLASTULA

Stages 8 through 15

The subchorionic (perivitelline) space in the bullhead egg is wide and the chorionic membrane thin and yielding. This permits the formation of a high blastula which is not seen in those teleosts with narrow subchorionic spaces such as *Trutta*, *Fundulus*, and *Opsanus*. The early blastulae (Stages 8 and 9) very much resemble the morula stage of some of the invertebrates, the individual cells being large enough to give the morula effect. The blastula remains high (Stages 10 and 11) through additional cell divisions and then gradually flattens out (Stages 12 to 15) with the formation of the blastocoele which is apparent in the living egg (Stage 15). The high blastula (Stage 11) has a diameter even less than that of the one-cell stage, whereas the flattened blastula (Stage 15) has a diameter more than half again that of the one-cell stage. As the blastula begins to flatten out in Stage 12, there is the beginning formation of marginal periblast cells, the process continuing through Stage 13, resulting in the formation of roughly two rows of such cells placed at a distance from the blastoderm. These periblast cells disappear later as the blastoderm extends over the yolk.

GASTRULA

Stages 16 through 19

When the blastoderm has expanded to cover about one-fourth of the yolk, there develops, through mitotic activity, an accumulation of cells at its margin (Stage 16) resulting in the formation of the germ ring. In Stage 17 the anterior lip of the blastopore is seen as an extra accumulation of cells forming the embryonic shield which becomes more prominent in Stages 18 and 19, seen best as viewed on the horizon of the egg.

NEURULA

Stages 20 through 24

During Stages 20 to 24 the blastoderm continues to extend over the yolk. This culminates in the closure of the blastopore late in Stage 24. The blastoderm covers about half the yolk in Stage 20. The embryonic shield in this stage bears considerable resemblance to the open neural plate of the amphibia. However, true concrescence as in the amphibia does not occur. There is a median longitudinal cellular proliferation with a narrowing of the neural plate resulting in the formation of a definitive presomitic embryonic axis (Stage 21). After the formation of the embryonic axis neurulation proceeds rapidly. In Stage 22 the main divisions of the brain can be discerned, the optic vesicle is rudimentary, and the first somites appear. There follows continued growth and further differentiation of the divisions of the brain and of the optic vesicle and a continuing addition of somites in a caudal direction. In Stage 24 there is a bilateral accumulation of cells, the rudiment of the otic vesicle, at the level of the anterior hind brain.

TAIL BUD EMBRYO

Stages 25 and 26

In Stage 25 (*ca.* 14 somites) the tail bud is well defined. Around its periphery, the ectoderm is directly continuous with that of the yolk sac. The blastopore is closed, but its location is marked by a slight depression in the yolk. Although the main divisions of the brain can be discerned, the brain ventricles are not yet apparent.

With continued growth, the tip of the tail bud develops as a projection free of ectodermal continuity with the yolk sac as in Stage 26 (*ca.* 17 somites). In this stage the pericardial sac is developing and the heart rudiment forms as a small bit of tissue in the pericardial sac under the head. However, the heart rudiment does not beat when it first forms.

ORGANODIFFERENTIATION

Stages 27 through 53

This period includes development extending through to the complete absorption of the yolk. The embryos prior to Stage 43, when hatching normally occurs, were removed from the chorionic membranes for clearer observation and illustration.

In Stage 27, the heartbeat is just discernible in transmitted light. It is faint but regular. Also, slow weak contractions of the anterior somites result on gross mechanical stimulation of the embryos. No cross striations are seen in the developing muscle.

In Stage 28, the heartbeat is still faint but readily visible. Also there are spontaneous contractions of the anterior somitic muscle. The first formation of the brain ventricles is apparent in the region of the hind brain indicated by the formation of the roof of the fourth ventricle, seen in the lateral view of the embryo.

In Stage 29, muscle contractility is still limited to the anterior somites. There has been an increase in the size of the heart, but the circulation of the blood is not established until Stage 30 and at first is sluggish and principally intraembryonic. Sometimes at this stage there is seen in the lower part of the otocyst an aggregation of minute granules, the earliest indication of the otoliths.

In Stage 31 a sluggish circulation is present over the surface of the yolk sac, with the cells of the communicating blood islands moving slowly toward the venous end of the heart. There are now in each otocyst two miniscule otoliths, each composed of a number of minute granules. During Stages 32 and 33 the operculum and gill arches differentiate, but circulation through the gill arches does not appear until late Stage 35 when it is established through the first arch.

At Stage 35 the head has extended forward, carrying with it the operculum, resulting in a gap between the operculum and the first gill arch. During Stages 35 and 36 there is an open communication through the pharynx from side to side. This closes in Stage 37 by the backward growth of the operculum.

It is in Stage 37 that the first melanin pigment is seen in the eye, sparsely scattered in the upper posterior quadrant. Blood vessels form in the eye in Stage 38 with an active circulation. Also at this time a circulation is established through the liver which is located along the posterior margin of the left anterior cardinal vein lateral to the body axis.

In Stage 40, circulation is established in the maxillary and lateral mandibular barbels, in the pectoral fin and in the caudal fin, the latter by a single vessel extending posteroventrally from the aorta. Also in Stage 40, bile is present in the gut at its anterior end and movements of the lower jaw first appear.

During the period of organodifferentiation there is a progressive extension caudally of contractility of the myotomes. At Stage 29 the tail flexes laterally to the side of the head, at Stage 37 it sweeps over the dorsum of the head. Also in Stage 37, the muscles of the anterior somites are cross striated. In late Stage 41, progressive undulatory swimming occurs, the embryo commonly swimming on its side. Photokinetic response to the "off" of light occurs first in Stage 42, with a well-defined negative phototactic response demonstrable in Stage 44.

Spontaneous hatching occurs in Stage 43, with most of the embryos of an egg mass emerging in three to four hours but with delay of some until Stage 44. By the time Stage 44 is reached, there is yellow bile in all but the terminal end of the gut.

The subsequent stages are marked by an increase in size of the embryos and their appendages, development of the circulation into the fins, and an extension of the pigment to finally produce what is essentially the adult pattern. The embryos are positively geotropic until Stage 52, when some negative geotropism appears. This becomes more marked as the embryos progress into Stage 53. This negative geotropism is probably correlated with the development of the bilobed swim bladder which can be demonstrated readily on dissection of fixed embryos. Also in these late stages the embryos apparently adapt to intensities of light which earlier evoked a negative phototactic response.

At 20 to 21° C. the development described above through Stage 53 is completed in seventeen days. The table below gives the time sequence of this development.

Time Sequences,
Development of Ictalurus nebulosus

Stage	*Time*	*Stage*	*Time*	*Stage*	*Time*
1	1.2 hrs.	25	2 days	43	8 days
2	1.6 hrs.	29	3 days	46	9 days
4	3 hrs.	34	4 days	48	10 days
8	6 hrs.	37	5 days	50	12 days
14	9 hrs.	39	6 days	52	15 days
18	1 day	40	7 days	53	17 days

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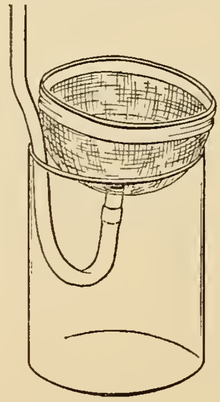

FIGURE 1

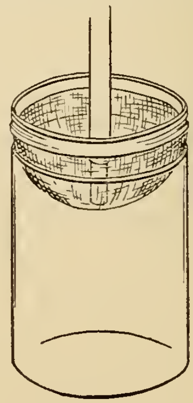

FIGURE 2

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Stages 1 through 7

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GASTRULA

Stages 16 through 19

When the blastoderm has expanded to cover about one-fourth of the yolk, there develops, through mitotic activity, an accumulation of cells at its margin (Stage 16) resulting in the formation of the germ ring. In Stage 17 the anterior lip of the blastopore is seen as an extra accumulation of cells forming the embryonic shield which becomes more prominent in Stages 18 and 19, seen best as viewed on the horizon of the egg.

NEURULA

Stages 20 through 24

During Stages 20 to 24 the blastoderm continues to extend over the yolk. This culminates in the closure of the blastopore late in Stage 24. The blastoderm covers about half the yolk in Stage 20. The embryonic shield in this stage bears considerable resemblance to the open neural plate of the amphibia. However, true concrescence as in the amphibia does not occur. There is a median longitudinal cellular proliferation with a narrowing of the neural plate resulting in the formation of a definitive presomitic embryonic axis (Stage 21). After the formation of the embryonic axis neurulation proceeds rapidly. In Stage 22 the main divisions of the brain can be discerned, the optic vesicle is rudimentary, and the first somites appear. There follows continued growth and further differentiation of the divisions of the brain and of the optic vesicle and a continuing addition of somites in a caudal direction. In Stage 24 there is a bilateral accumulation of cells, the rudiment of the otic vesicle, at the level of the anterior hind brain.

TAIL BUD EMBRYO

Stages 25 and 26

In Stage 25 (*ca.* 14 somites) the tail bud is well defined. Around its periphery, the ectoderm is directly continuous with that of the yolk sac. The blastopore is closed, but its location is marked by a slight depression in the yolk. Although the main divisions of the brain can be discerned, the brain ventricles are not yet apparent.

With continued growth, the tip of the tail bud develops as a projection free of ectodermal continuity with the yolk sac as in Stage 26 (*ca.* 17 somites). In this stage the pericardial sac is developing and the heart rudiment forms as a small bit of tissue in the pericardial sac under the head. However, the heart rudiment does not beat when it first forms.

ORGANODIFFERENTIATION

Stages 27 through 53

This period includes development extending through to the complete absorption of the yolk. The embryos prior to Stage 43, when hatching normally occurs, were removed from the chorionic membranes for clearer observation and illustration.

In Stage 27, the heartbeat is just discernible in transmitted light. It is faint but regular. Also, slow weak contractions of the anterior somites result on gross mechanical stimulation of the embryos. No cross striations are seen in the developing muscle.

In Stage 28, the heartbeat is still faint but readily visible. Also there are spontaneous contractions of the anterior somitic muscle. The first formation of the brain ventricles is apparent in the region of the hind brain indicated by the formation of the roof of the fourth ventricle, seen in the lateral view of the embryo.

In Stage 29, muscle contractility is still limited to the anterior somites. There has been an increase in the size of the heart, but the circulation of the blood is not established until Stage 30 and at first is sluggish and principally intraembryonic. Sometimes at this stage there is seen in the lower part of the otocyst an aggregation of minute granules, the earliest indication of the otoliths.

In Stage 31 a sluggish circulation is present over the surface of the yolk sac, with the cells of the communicating blood islands moving slowly toward the venous end of the heart. There are now in each otocyst two miniscule otoliths, each composed of a number of minute granules. During Stages 32 and 33 the operculum and gill arches differentiate, but circulation through the gill arches does not appear until late Stage 35 when it is established through the first arch.

At Stage 35 the head has extended forward, carrying with it the operculum, resulting in a gap between the operculum and the first gill arch. During Stages 35 and 36 there is an open communication through the pharynx from side to side. This closes in Stage 37 by the backward growth of the operculum.

It is in Stage 37 that the first melanin pigment is seen in the eye, sparsely scattered in the upper posterior quadrant. Blood vessels form in the eye in Stage 38 with an active circulation. Also at this time a circulation is established through the liver which is located along the posterior margin of the left anterior cardinal vein lateral to the body axis.

In Stage 40, circulation is established in the maxillary and lateral mandibular barbels, in the pectoral fin and in the caudal fin, the latter by a single vessel extending posteroventrally from the aorta. Also in Stage 40, bile is present in the gut at its anterior end and movements of the lower jaw first appear.

During the period of organodifferentiation there is a progressive extension caudally of contractility of the myotomes. At Stage 29 the tail flexes laterally to the side of the head, at Stage 37 it sweeps over the dorsum of the head. Also in Stage 37, the muscles of the anterior somites are cross striated. In late Stage 41, progressive undulatory swimming occurs, the embryo commonly swimming on its side. Photokinetic response to the "off" of light occurs first in Stage 42, with a well-defined negative phototactic response demonstrable in Stage 44.

Spontaneous hatching occurs in Stage 43, with most of the embryos of an egg mass emerging in three to four hours but with delay of some until Stage 44. By the time Stage 44 is reached, there is yellow bile in all but the terminal end of the gut.

The subsequent stages are marked by an increase in size of the embryos and their appendages, development of the circulation into the fins, and an extension of the pigment to finally produce what is essentially the adult pattern. The embryos are positively geotropic until Stage 52, when some negative geotropism appears. This becomes more marked as the embryos progress into Stage 53. This negative geotropism is probably correlated with the development of the bilobed swim bladder which can be demonstrated readily on dissection of fixed embryos. Also in these late stages the embryos apparently adapt to intensities of light which earlier evoked a negative phototactic response.

At 20 to 21° C. the development described above through Stage 53 is completed in seventeen days. The table below gives the time sequence of this development.

Time Sequences,
Development of Ictalurus nebulosus

Stage	*Time*	*Stage*	*Time*	*Stage*	*Time*
1	1.2 hrs.	25	2 days	43	8 days
2	1.6 hrs.	29	3 days	46	9 days
4	3 hrs.	34	4 days	48	10 days
8	6 hrs.	37	5 days	50	12 days
14	9 hrs.	39	6 days	52	15 days
18	1 day	40	7 days	53	17 days

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PLATE I – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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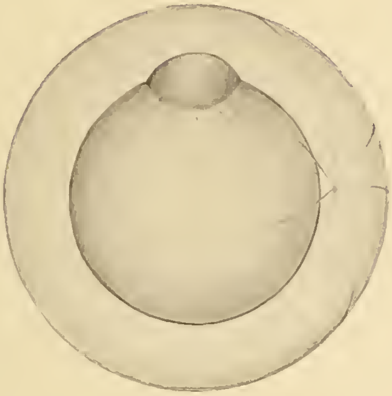

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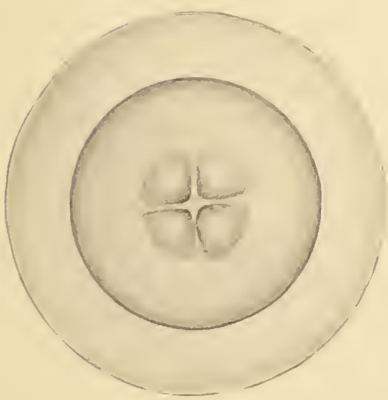

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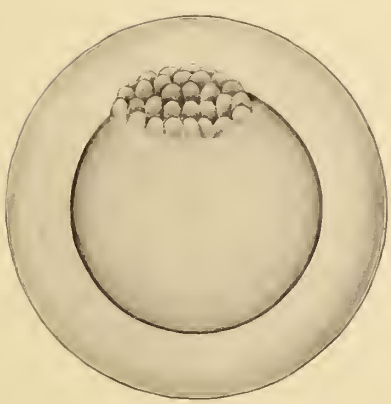

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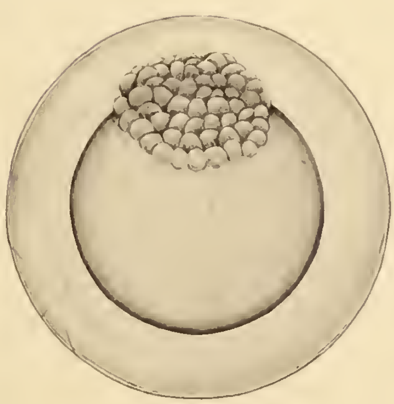

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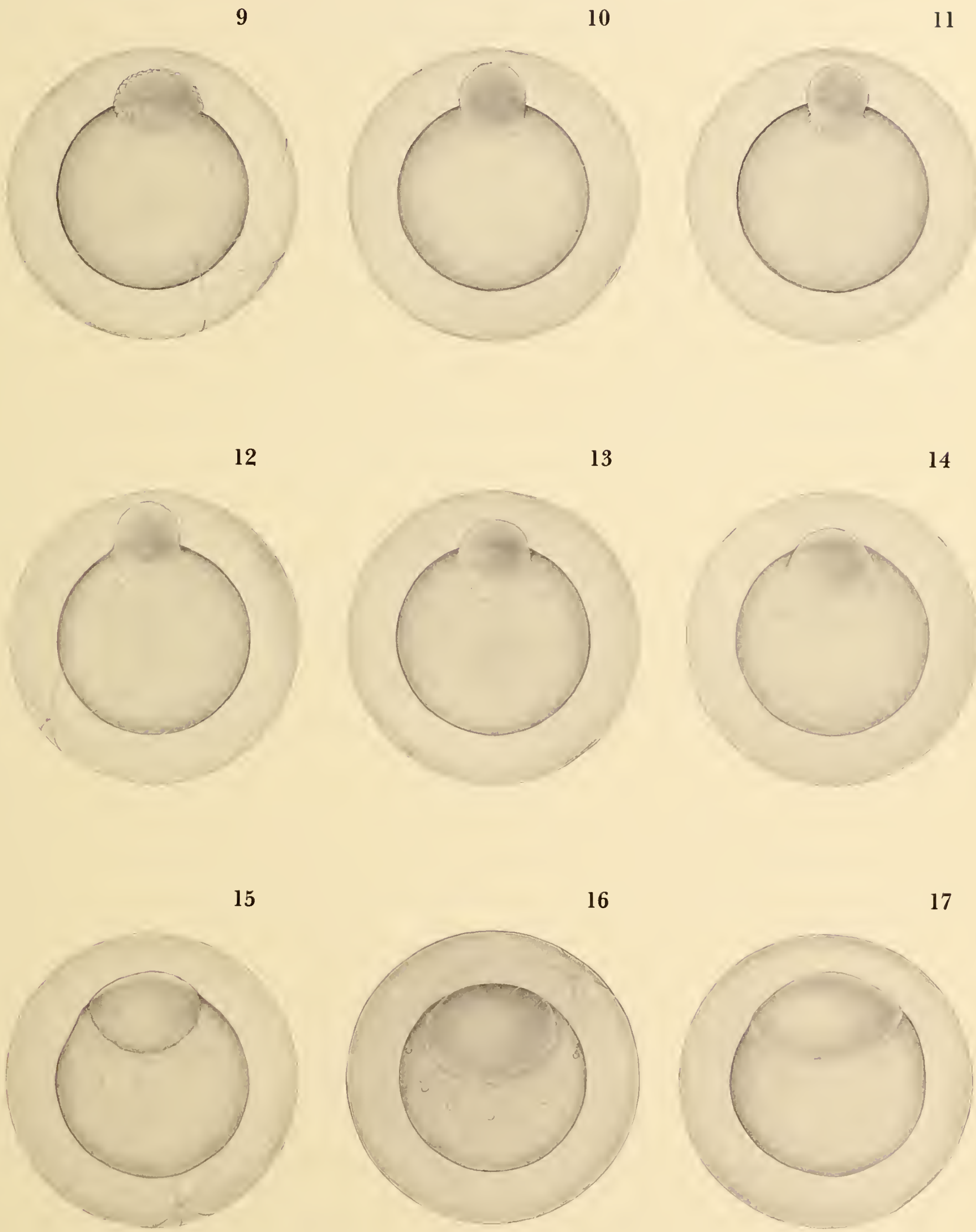

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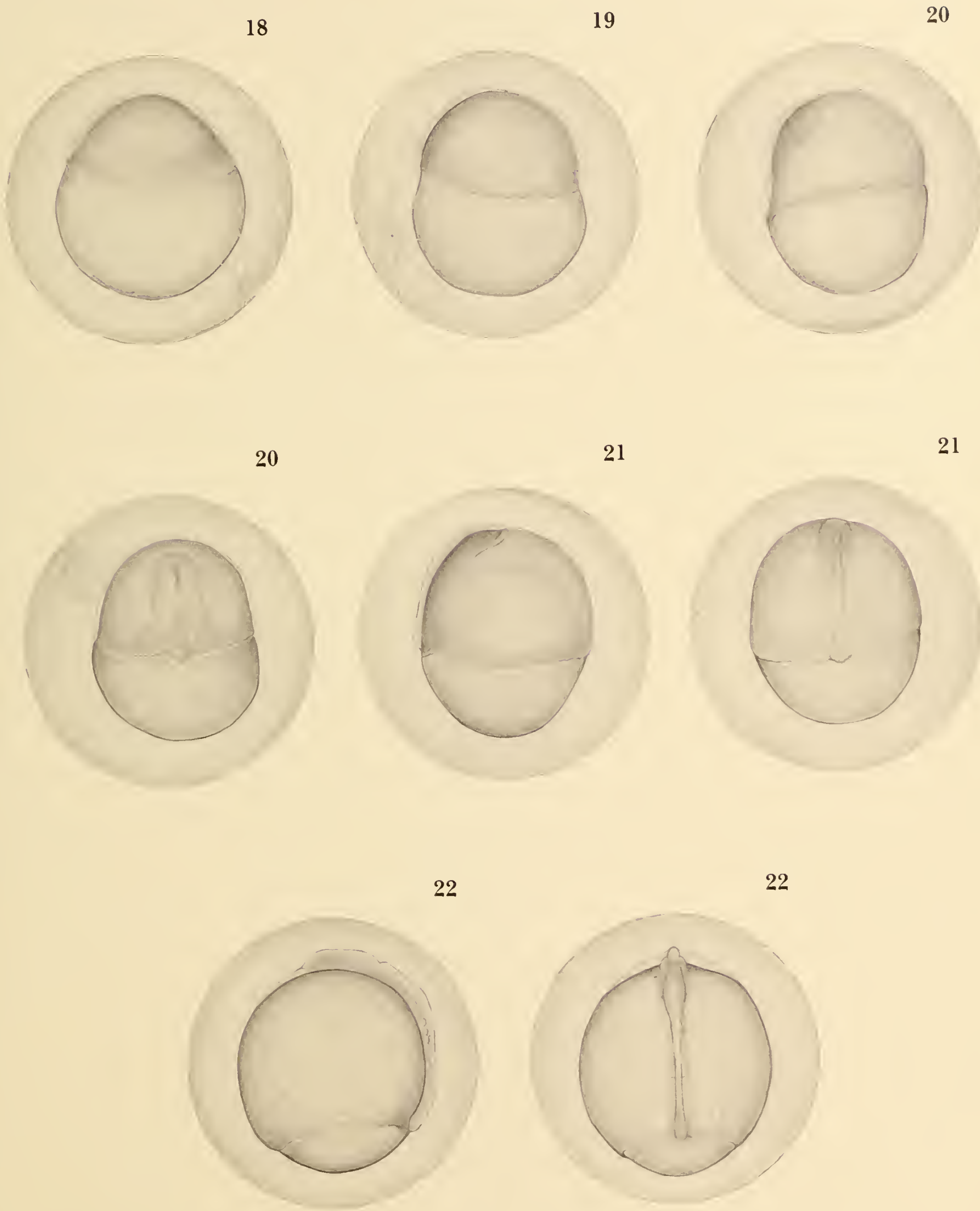

PLATE IV – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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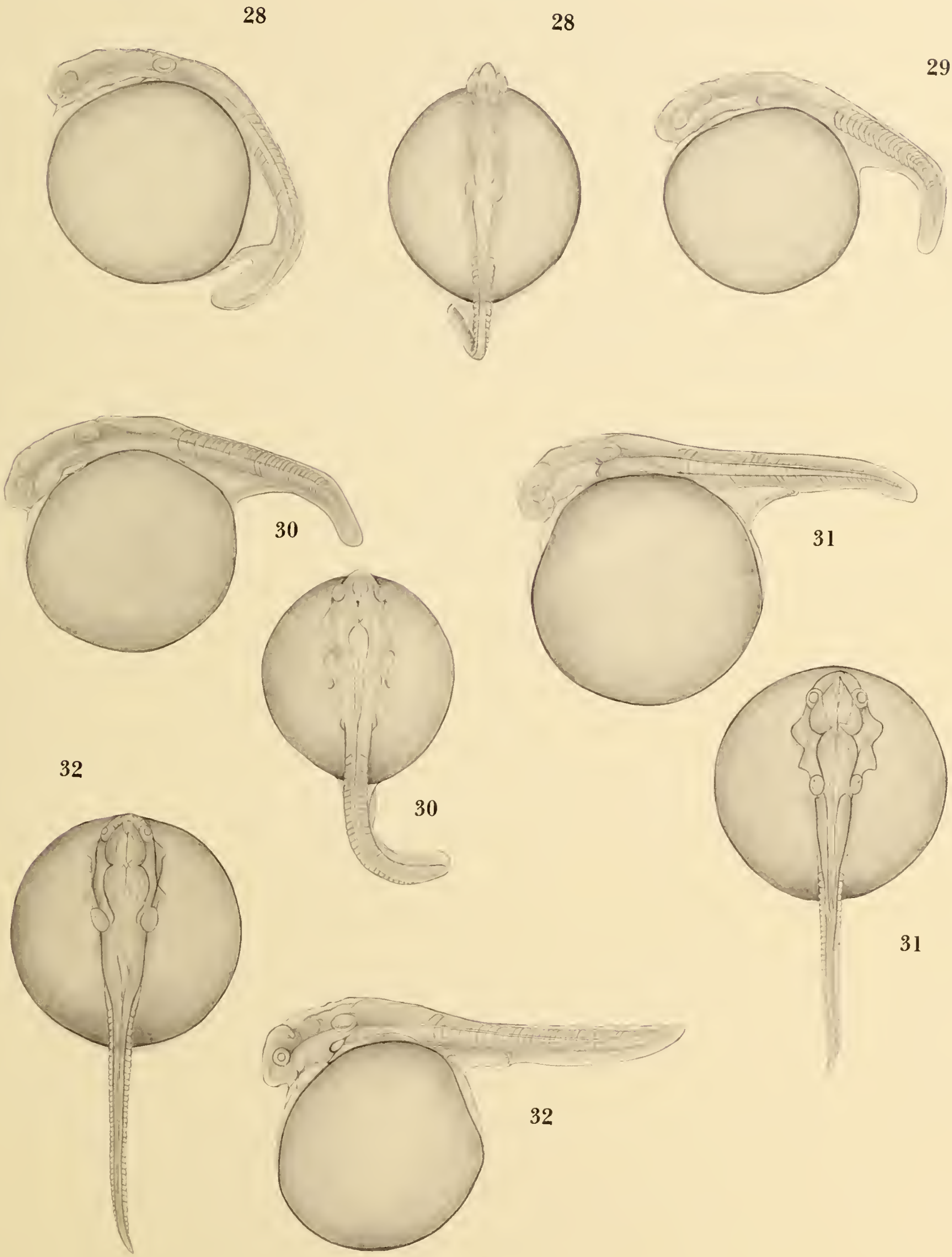
PLATE V – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*



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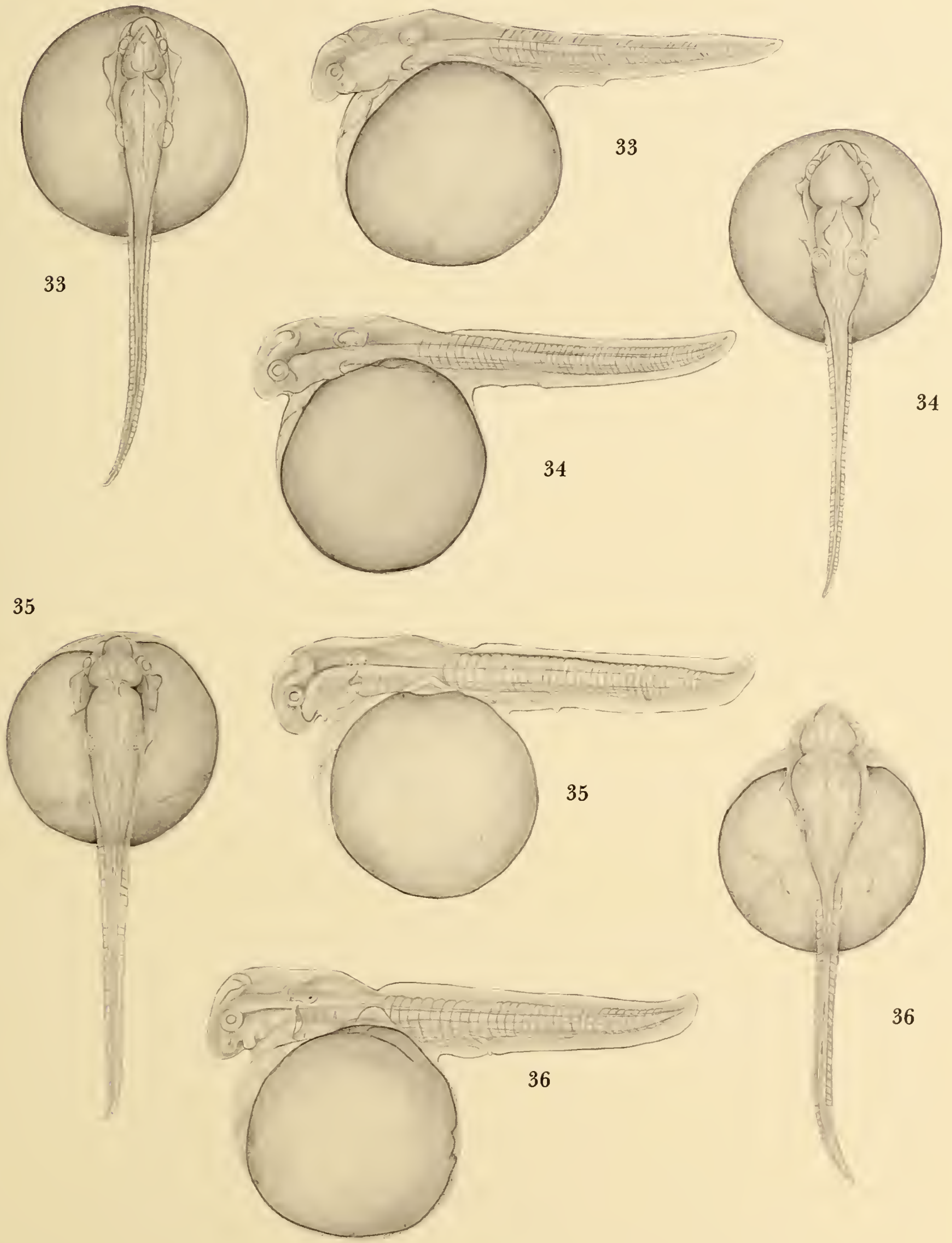

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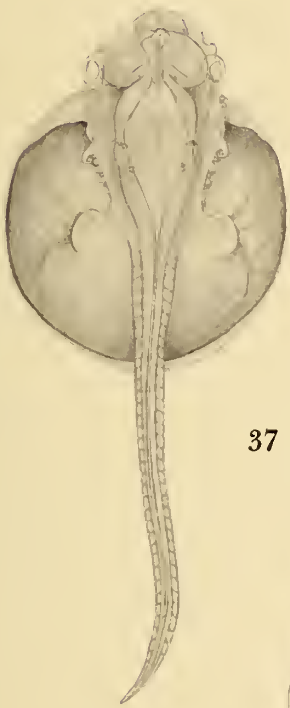

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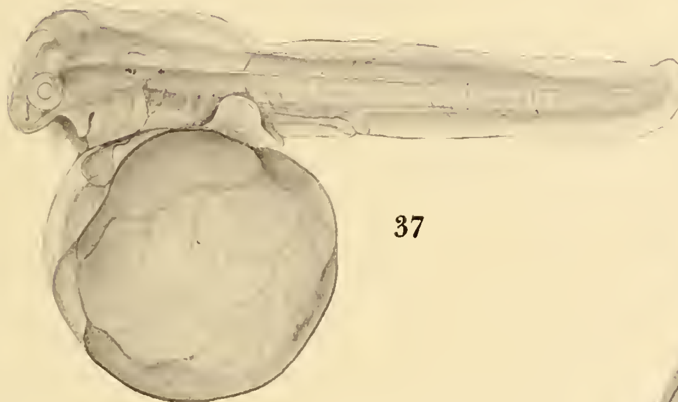

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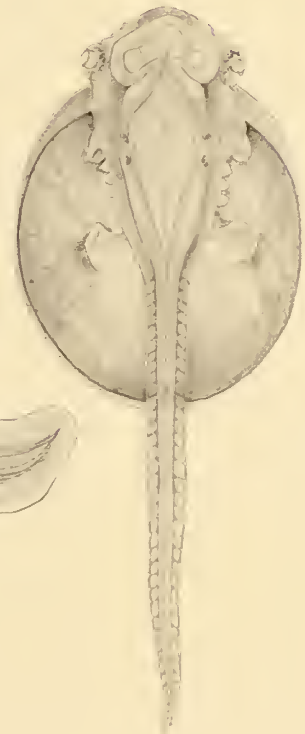

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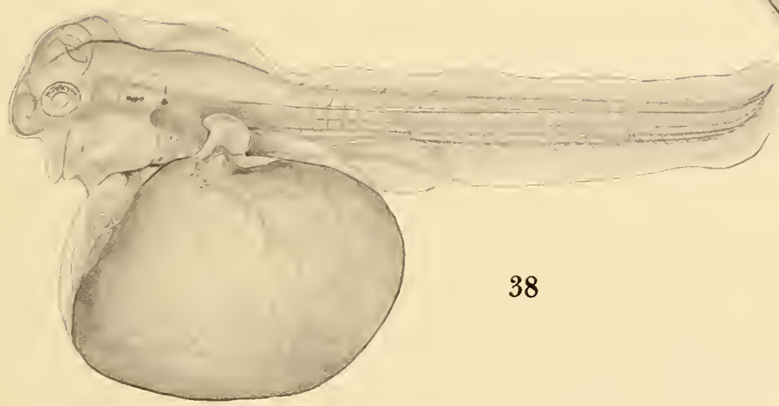

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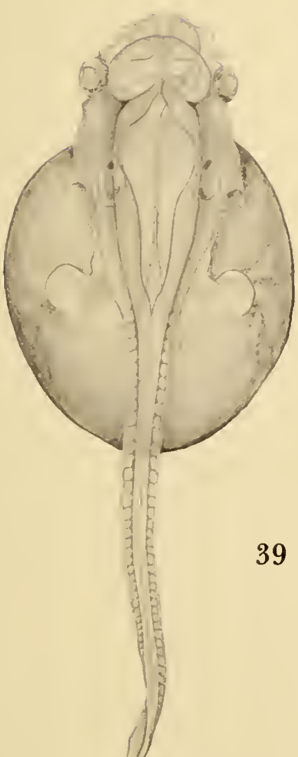

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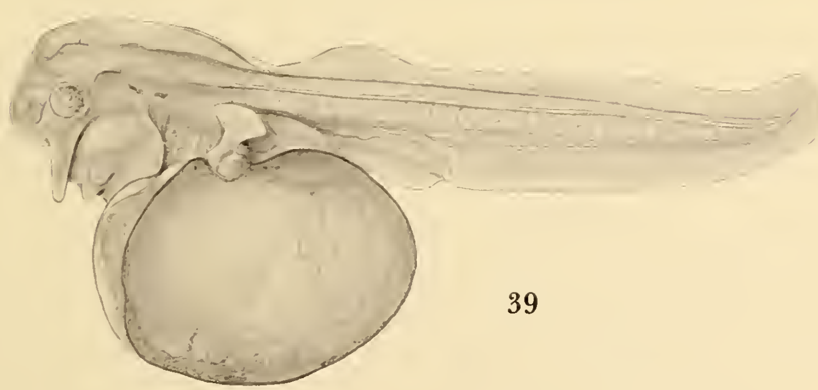

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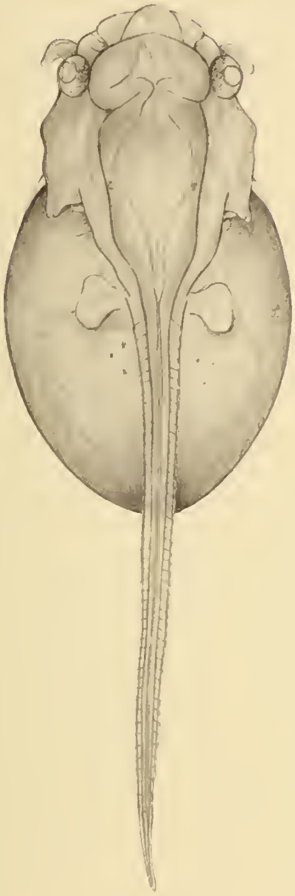

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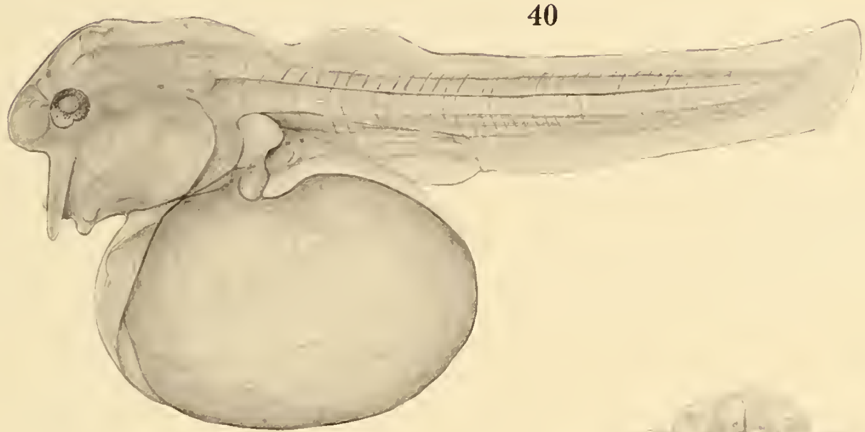

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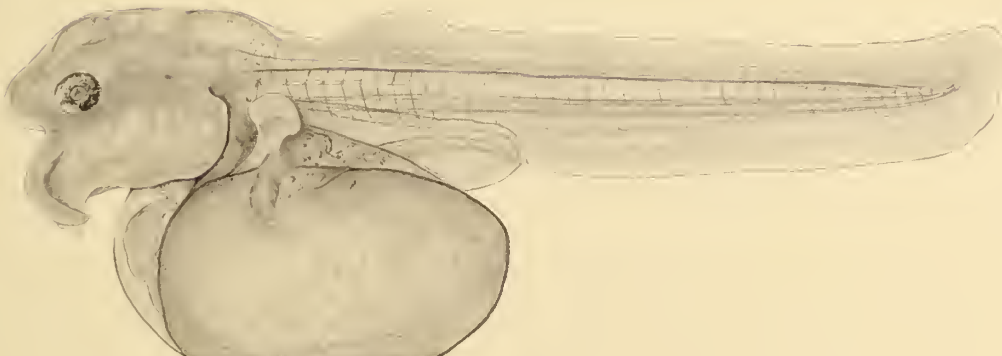

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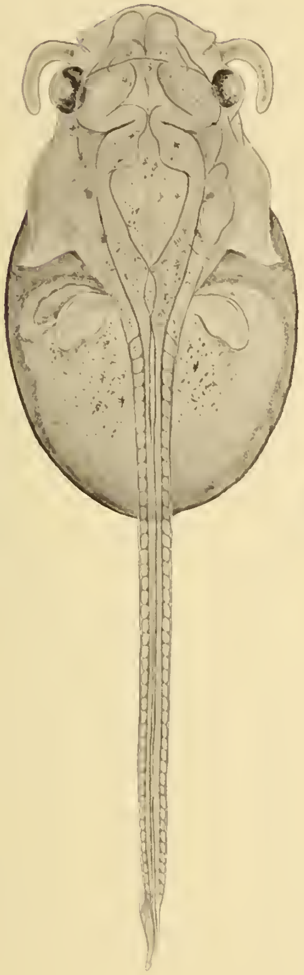

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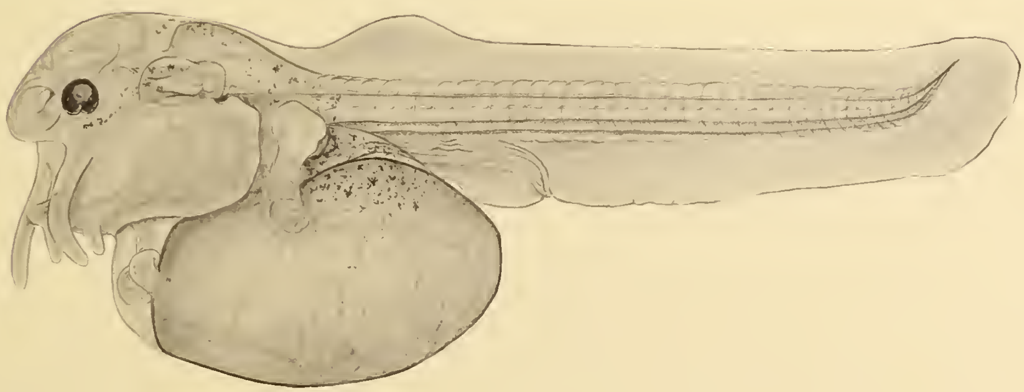

PLATE X – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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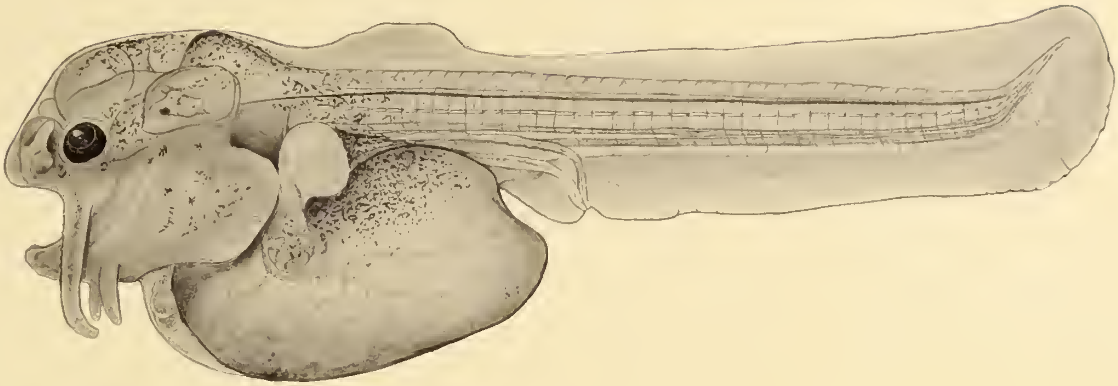

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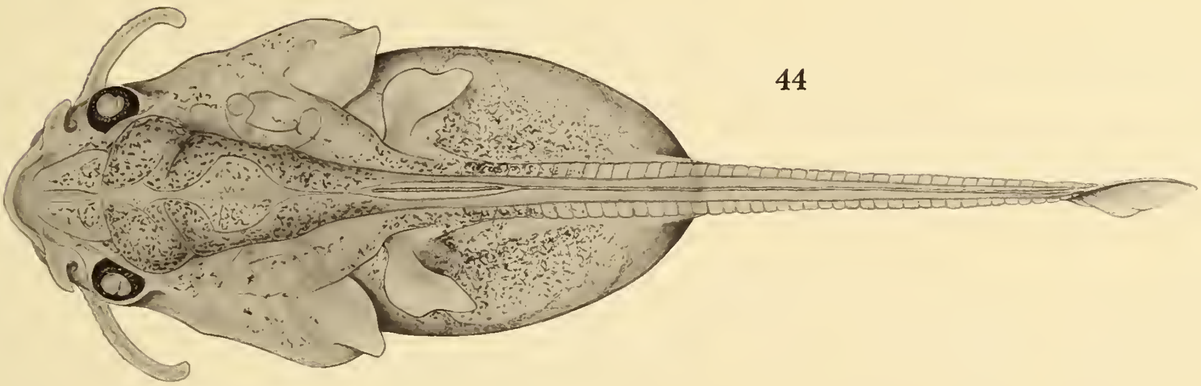

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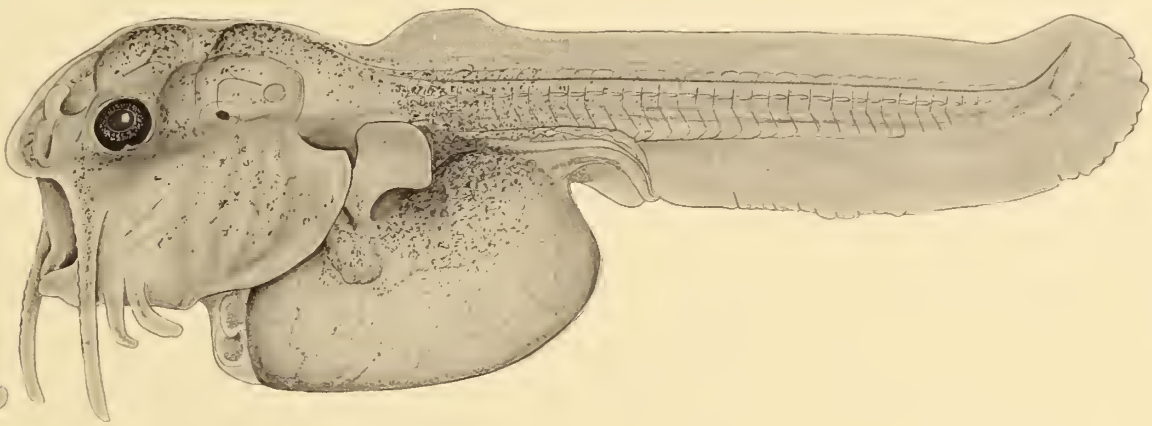

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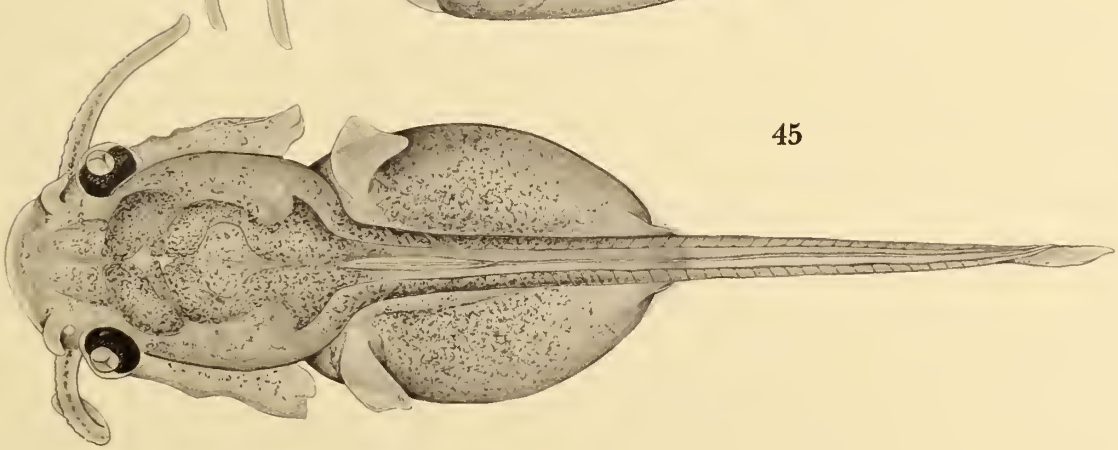

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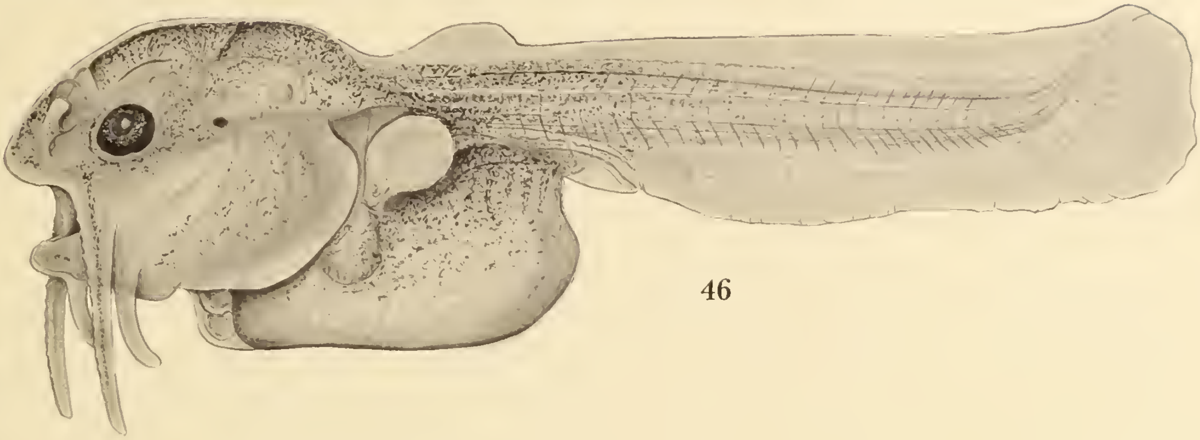

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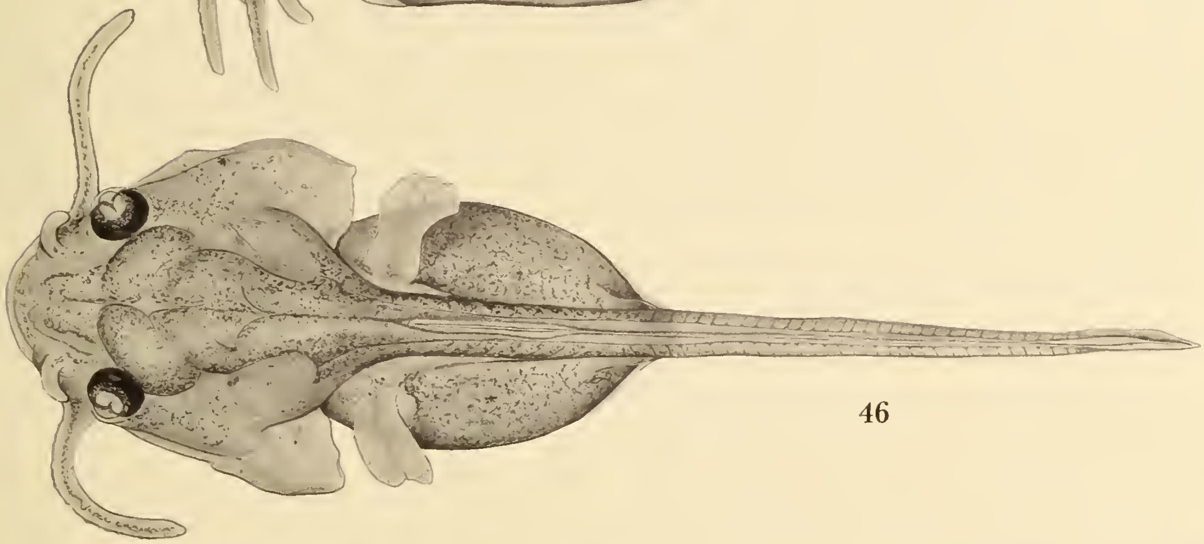

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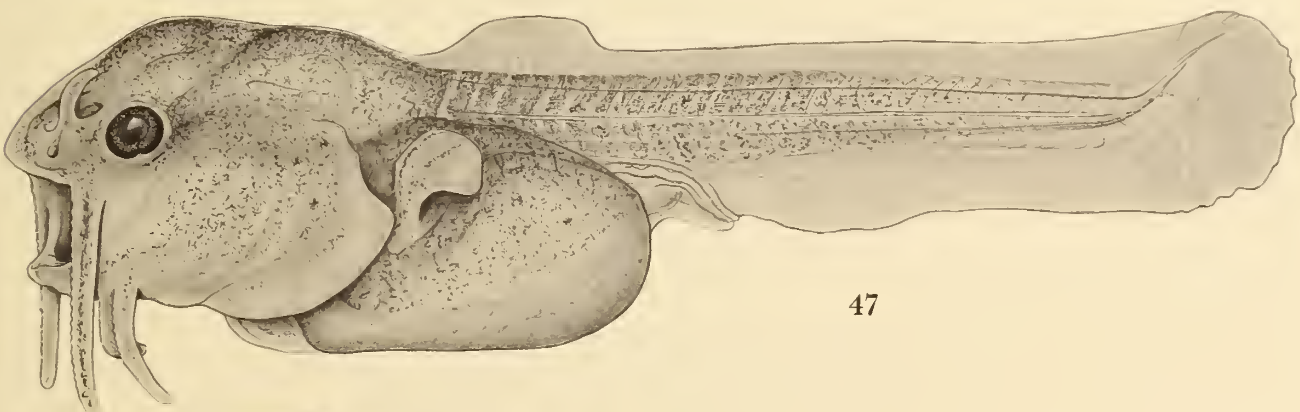

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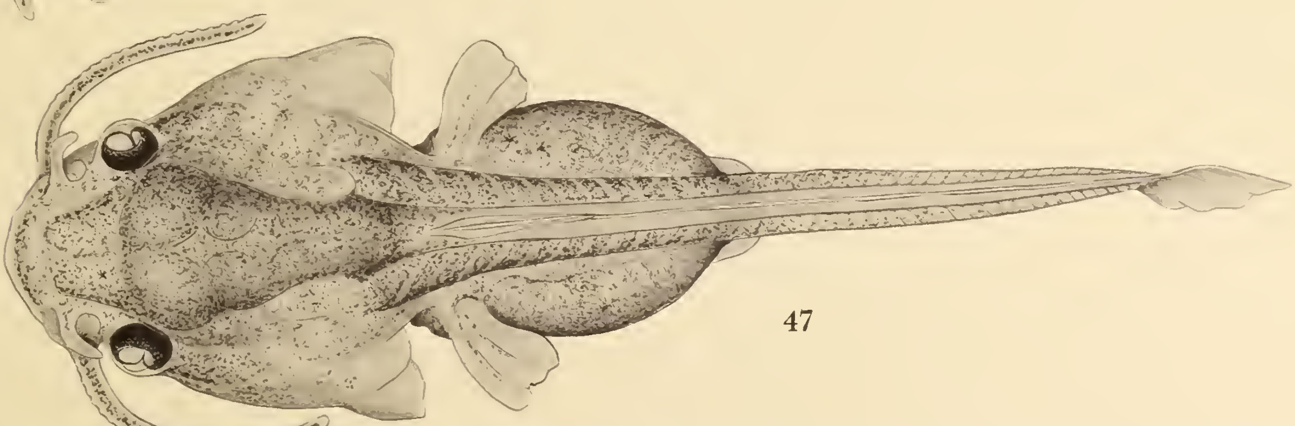

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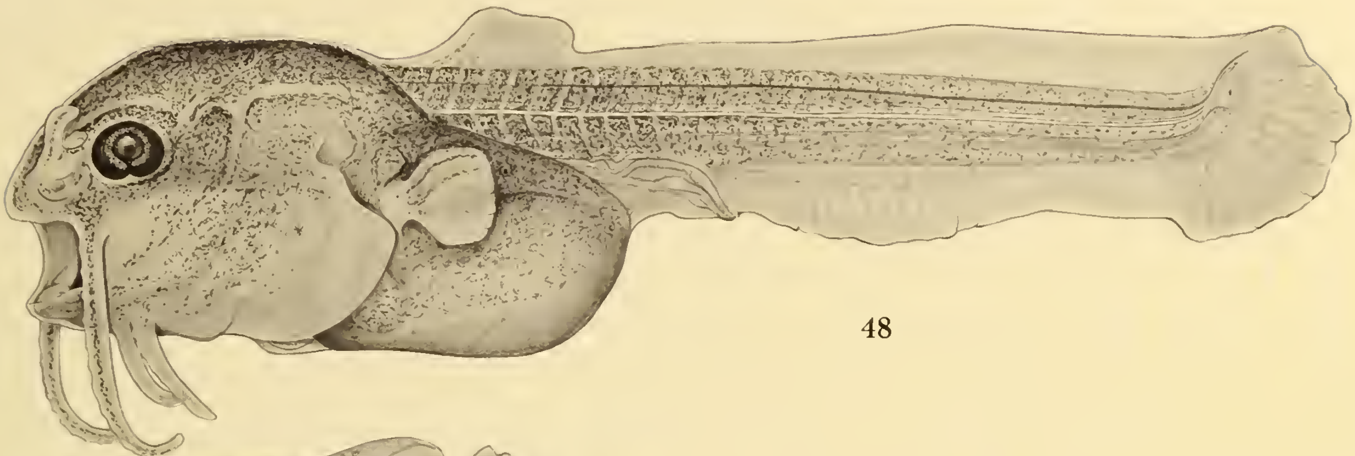
PLATE XII – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*



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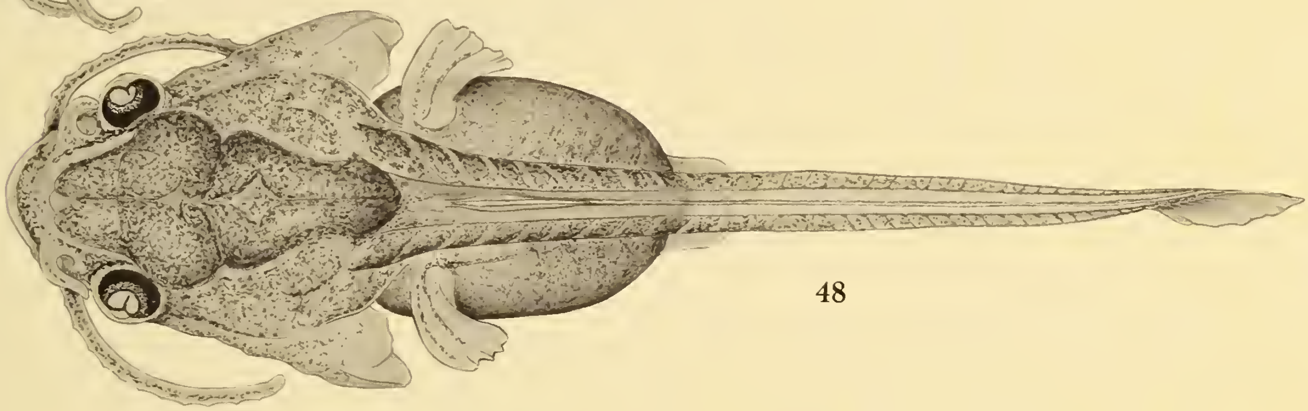

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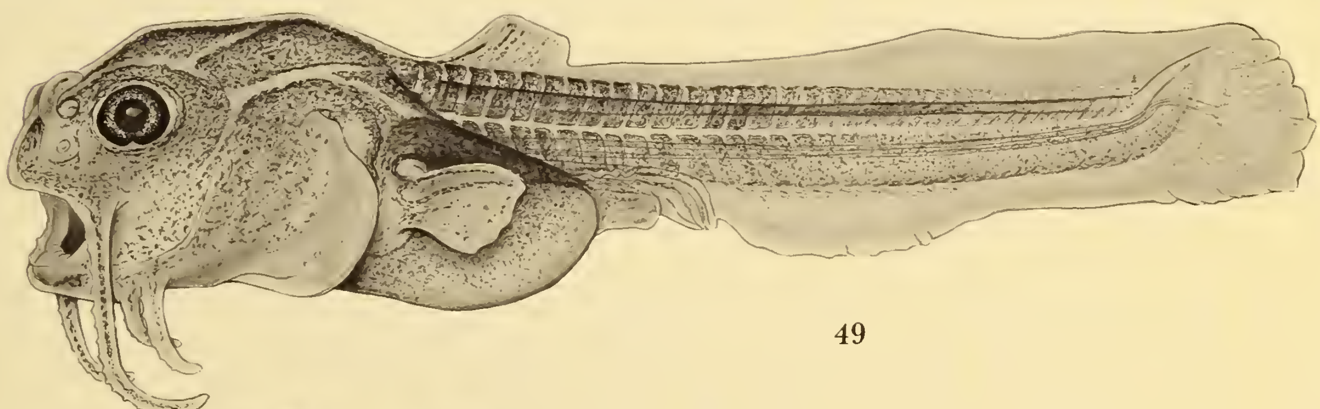

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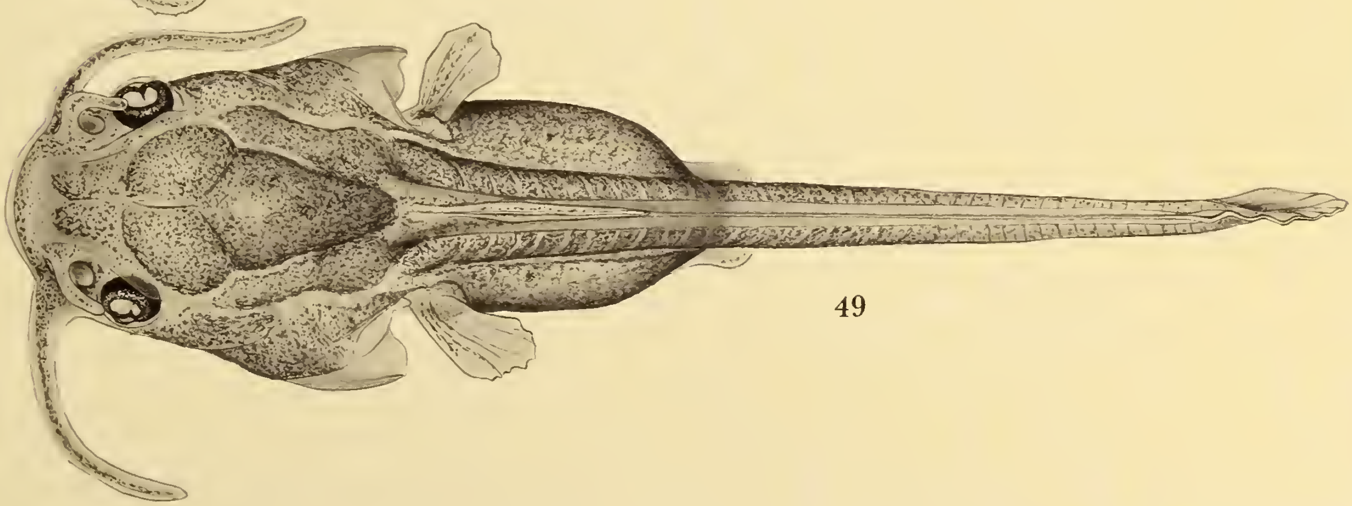

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PLATE XIII – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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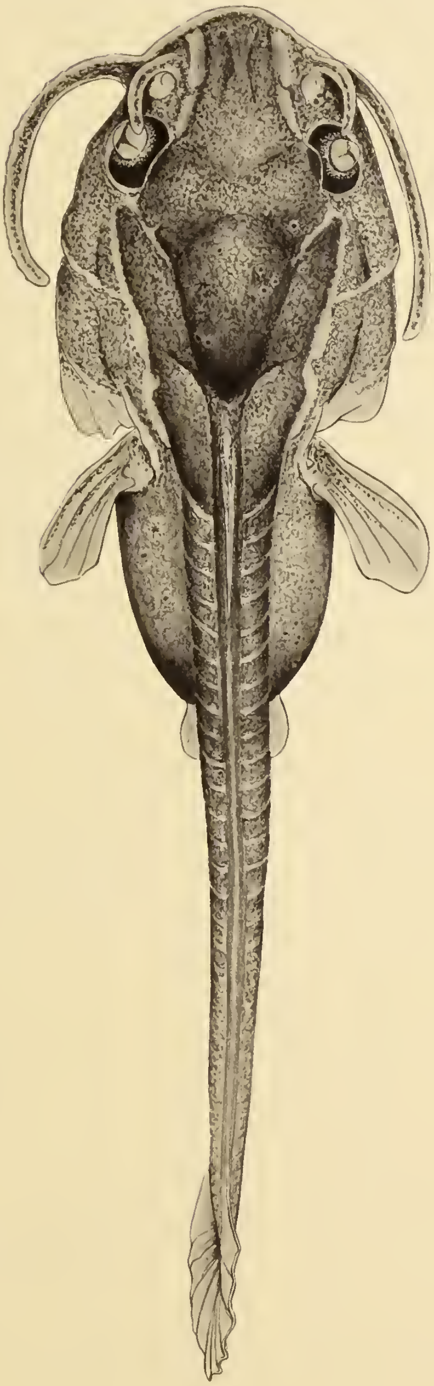

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PLATE XIV – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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PLATE XV – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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PLATE XVI – STAGES IN THE DEVELOPMENT OF *ICTALURUS NEBULOSUS*

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