STUDIES OF THE EMBRYONIC DEVELOPMENT OF THE BLUE GOURAMI, *TRICHOGASTER TRICHOPTERUS* (PALLAS)

By

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INTRODUCTION

This thesis is the report of studies on the embryonic development of the blue gourami, *Trichogaster trichopterus* (Pallas). This fish was selected because of the unusual speed with which it develops, and the peculiar manner in which the embryos are incubated. This study is essentially a description of the early organogenesis of this species and a comparison of the development of this and other fishes as described in the literature.

The study of teleost embryology has become increasingly important with the use of different species of fish as experimental animals. Few persons are familiar with the development of the bony fishes because this group is not generally included in college courses in embryology. Text books usually supply only a minimum of information concerning fish ontogeny. Yet, as a teaching tool, fish embryos are most useful in clarifying certain points in the development of the vertebrates.

The embryos of several species of fishes develop at such a rapid rate that during the course of a single laboratory period several different stages of development may actually be observed in the living specimens under the microscope. The size of most fish embryos is such that great numbers may be incubated in a minimum amount of space. The fact that water is the culture medium and that most embryos are quite transparent, makes it easy to observe them.

For example, the origin of the heart structure is easily observed in the developing embryo of the teleost. Colorless blood cells can first be seen slowly moving across the yolk surface in a broad indefinite path. As differentiation continues, the cells move faster and

follow a more clearly defined course. Eventually, the blood cells show color, and walls are formed around the blood paths which connect to the pulsating heart.

The development of the brain is easily observed in most fish embryos. The constrictions and flexures which separate the regions of the brain can easily be seen and studied as they form. The teleost embryo does not exhibit the embryonic membranes, such as the allantois or amnion, which are found in the chick embryo. This lack of membranes allows the specimen to be more easily observed. However, a transparent chorionic membrane or egg shell encases the embryo of most species, and a "fertilization membrane" is formed after the sperm penetrates the unfertilized egg.

The speed at which some fish embryos differentiate is another factor which makes them an excellent teaching and research tool. Where else can one find a vertebrate that will develop from fertilization of the ovum to hatching in as little time as 22 or 24 hours? (Dadyburjor, 1951; Cooke, 1952)

The problem of ontogeny has long been a little-understood phenomenon although many persons down through the centuries have been concerned with the development of various embryos. Perhaps with a more comprehensive knowledge of fish embryology another link could be added to our chain of information about ontogeny.

The embryological and larval development of many species of fishes has been observed and recorded. These studies have included both fresh-water and marine forms. Most of these have been of interest because of their economic importance. In general, the development of all teleosts so far described is similar to that recorded by Wilson (1889) for the

sea bass, or by Solberg (1938) and Oppenheimer (1937) for Fundulus heteroclitus (Linneaus). However, each species has its own rate of development and each has certain peculiarities in the manner in which organogenesis occurs. Because of the similarity in general appearance and gross development of most teleost embryos, much of the detail and variation in the sequence of morphogenic processes have been over-looked.

One of the groups found in the bony fishes includes the so-called labyrinthine fishes or the bubble nest builders. These are of special interest because the eggs are kept at the surface of the water during their development, (either due to egg structure or to support of the bubble nest), and because of their speed of development. How can this rapid development be brought about? Is there any modification of the normal sequence of differentiation in order to permit such a fast development to occur?

In order to answer some of the above questions, the blue gourami, Trichogaster trichopterus (Pallas), was selected for this study. One of the reasons for this selection was its unusually rapid development. Only some 24 hours of incubation are required for the embryo to emerge from the chorion. This is one of the most rapid embryonic developments known among the vertebrates.

The evidence as presented in this paper shows that the embryos of the blue gourami, in general, follow the developmental pattern as described for other teleosts. The more rapid rate of cell division appears to be the best explanation for the exceptionally fast morphogenic activity. Other differences peculiar to this species or at least not described in the literature available are presented.

LITERATURE

The eggs of fishes have been observed since man first began to make a study of embryology. However, most of the available detailed descriptive studies have been made since the latter part of the nineteenth century. As a group, the teleosts follow a typical pattern in their morphogenesis; although, there are wide variations in the details of the organogenesis of the individual species.

One of the important descriptions of the embryology of a bony fish is that of Wilson (1889), who described in detail the developmental stages of the sea bass, Serranus atrárius (Linnaeus).

The sea bass is a marine fish and one of several that spawn in great numbers along the Atlantic Coast. The eggs are pelagic and measure about 1 mm. in diameter. They require approximately 75 hours to develop to the hatching stage at a temperature of 15.5° C. This incubation period is reduced at higher temperatures. The embryo is surrounded by an egg membrane or chorion which is very thin and horny. Imbedded in the yolk, but near the surface, is a single oil globule, which is always uppermost in the floating egg.

The formation of the periblast of the sea bass is described in detail by Wilson. Nuclei are derived from the marginal cells of the blastodisc, and migrate into the periblast protoplasm. Eventually the nuclei multiply, and move into the central periblast. The periblast is thought to have the physiological function of aiding in the assimilation of the yolk material.

The cells of the periphery of the blastoderm begin a centripetal growth or invagination. This growth is most rapid at the point corresponding to the dorsal lip of the blastopore of the amphibians. The

thickened mass around the yolk, formed by these in-growing cells, is known as the germ ring. The blastoderm continues to grow and encloses the yolk. According to Wilson, in the spreading of the blastoderm of the sea bass, the posterior margin or dorsal lip of the blastopore remains a fixed point, while the anterior margin and lateral margins travel around the yolk mass.

During the encirclement of the yolk by the blastoderm, an area becomes marked off in the region of the posterior margin. This area is more or less triangular-shaped and thicker than the surrounding blastoderm. It is formed by the migration or movement of cells into the area, and by the rapid proliferation of cells in the region of the dorsal lip of the blastopore. This is known as the embryonic shield and develops into the embryo proper.

In the early stages, the ectoderm over the embryonic shield becomes greatly thickened in comparison to the extra-embryonic ectoderm. Along the mid-line a thickening forms the naural keel, which as it grows deeper causes a consequent thinning of the lateral edges; until finally, there is a narrow deep keel which passes at the sides into the thin ectodermal layer.

As development continues, the keel becomes constricted from the surface ectoderm. The cells tend to line up in two parallel rows which separate to form the naural canal. Thus, the formation of the brain cavities, optic sacs and the central naural canal is accomplished by delamination in the teleosts rather than by folding as in the higher vertebrates.

By the time the naural chord has begun to form in the trunk region, the formation of the notochord and the secondary layers has also

commenced, thus adding to the thickening of the axial line. The notochord and mesoderm, etc. form first in the posterior region and then proceed foreward. When the entodermal layer is completely established, the notochord has assumed its ultimate shape, that of a cylindrical rod. The cells which make up the chords become flattened antero-posteriorly, and spread out in the transverse plane, These cells then become vacuolized, until at hatching, the protoplasm of the notochord cells is reduced to a thin peripheral layer around a central cavity.

Wilson's work is one of the most complete studies on early teleost embryology, and has become the classical pattern for subsequent investigations.

There is a great deal of variation in the degree of differentiation of the different species of teleosts at certain stages of development, i.e. closure of the blastopore, hatching, etc. When the germ ring is at the equatorial position in the sea bass, the embryonic shield is just beginning to be visible as a triangular thickened area and has moved only slightly in an anterior direction. At a similar stage, in the position of the germ ring, the embryo proper of the pointed-nosed sole, Parophrys vetula Girard, has lengthened until it extends half way around the yolk (Budd, 1940). Once past this equatorial position, the germ ring pivots at the posterior pole, which remains stationary, while the anterior edge of the ring draws away from the head end of the embryo. After completely encircling the yolk, the germ ring pinches together and closes the blastopore. The cells of the germ ring make up part of the caudal mass.

The pointed-nosed sole has a transparent pelagic egg with a diameter of 0.9 mm. Hatching occurs after 90 hours of incubation at

13.0° C.

Budd (1940) has contributed the descriptions of the embryology of five other California fishes:

1. Pleuronichthys verticalis Jordan and Gilbert, the sharp-ridged turbot, has a pelagic egg of 1.07 mm. in diameter. The chorion exhibits a hexagonal pattern that extends through the entire thickness of the membrane. Hatching occurs in 86 hours at 13.8° C.

2. Pleuronichthys decurrens Jordan and Gilbert, the California turbot, has a pelagic egg of 1.44 mm. in diameter. The germ ring forms a tight band around the yolk causing a marked constriction. The incubation period lasts one week at a temperature of 13.8° C.

3. In the mottled turbot, Pleuronichthys coenosus Girard, the eggs have a diameter of 1.38 mm. They contain no oil globules. The embryonic shield never attains any great size, because there is only a slight centripetal growth on the part of the germ ring. Differentiation does not take place to any extent until after the closure of the blastopore. Twelve days of incubation are required for hatching at a temperature of 13.8° C.

4. Artedius lateralis (Girard), a tide-pool cottid, has a demersal egg that adheres firmly to the substrate. Demersal eggs are heavier than water and fall toward the bottom. The average size of A. lateralis eggs is 1.07 mm. with much variation in the individual diameters. There is a single large oil globule which is colored a light cherry red. Kupffer's vesicle appears after 73 hours of incubation, which is proportionately later in appearing than the pelagic eggs, already mentioned, in which Kupffer's vesicle is formed by the time the blastopore closes. Hatching occurs after sixteen days of incubation at a temperature of

15.5° C. Newly-hatched larvae are well developed and swim actively about immediately after emerging from the chorion. Difficulties characteristic of embryos from pelagic eggs, such as erratic swimming and the inability to maintain an upright position occasioned by the large amount of unused yolk mass, are not exhibited.

5. Clinocottus analis (Girard), another tide-pool cottid, has a brownish yellow egg of 1.30 mm. in diameter. Several large oil globules and scattered groups of small ones are always present. Hatching occurs after 24-30 days of incubation at a temperature of 15.5° C.

The literature on the embryology of other Pacific Coast fishes includes the description (Bolin, 1930) of the labrid fish, Oxyjulis californicus Günther. The eggs of this species are spherical, pelagic, and have a diameter of 0.74 to 0.79 mm. These eggs contain a single large oil globule which is colored orange-pink. This color is unusual in fish eggs and is therefore a valuable diagnostic character.

The germ ring is well differentiated in ten to twelve hours and Kupffer's vesicle appears when the germ ring forms an equatorial belt. An unusual occurance was the presence of two Kupffer's vesicles in about 50% of the specimens observed by Bolin.

The embryo emerges from the chorion after about 48 hours. This is the least time required for incubation of eggs, from the time they are layed to hatching, of any species described from the west coast.

The embryology of the Pacific mackerel, Pneumatophorus diego (Ayres), was described by Fry (1936). The unfertilized ovum of this fish averages 1.05 mm. in diameter. It contains a single large oil globule which is 0.26 mm. across. The perivitelline space is very narrow and may be easily overlooked. Black pigmentation appears soon after the embryo is segmented. Yellow pigment is present after the lens of the eye is

formed. The oil globule becomes pigmented with melanophores, which are always irregular in shape and arrangement. It is difficult to tell from Fry's description whether there is a cellular membrane around the oil globule in which the pigment forms, or whether the melanophores simply form a network on the surface of the droplet. Hatching in this species occurs after some 50 hours of incubation at a temperature of 18.5° C.

Although we say that the teleosts follow a typical pattern of embryological development, the same order of procedure is not followed by all of the individual species in the differentiation of certain structures. For example, the manner in which the germ ring envelops the yolk mass has been described in two different ways in the literature.

For the sea bass, Wilson described the posterior pole or dorsal lip of the blastopore as remaining in a relatively fixed position and acting as a pivot for the remainder of the germ ring. Budd (1940) described the same procedure for the germ ring migration of Parophrys vetula.

Others, i.e. Jones (1937), Solberg (1938), and Tavolga and Rugh (1947) have observed that in some species all lips of the blastopore move downward over the yolk, although the dorsal lip may be somewhat retarded in its movement.

The whitefish embryo, Coregonus clupeaformis (Mitchill), the development of which was described by Price (1934), contains no fixed oil globules by which the relative positions of the germ ring can be judged. It was concluded, however, that the closure of the blastopore in the whitefish occurs in the same manner as in the sea bass; but a slight backward growth of the dorsal lip was recognized. Price pointed out that although Wilson's statement is to the contrary, his figure 38

shows the posterior margin slightly closer to the oil globule than the earlier stage of figure 36. Thus the closure involves epiboly of the anterior or ventral lip, aided by concrescence and a slight backward growth of the dorsal lip.

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The unfertilized whitefish ovum has a diameter of 3.0 mm. There is a collection of oil droplets at the animal pole. Since the eggs are laid in November when the water is very cold, the embryo develops slowly and requires 24 hours to reach the eight-cell stage. The closure of the blastopore occurs in 22 days and four hours at a mean temperature of 3.0° C. At this time the embryo is clearly outlined and lies in a straight line over the curvature of the egg. All three primary cerebral vesicles are distinct, and the naural keel lies deeply embedded in the yolk. Eleven pairs of somites as well as the notochord have differentiated. Kupffer's vesicle has reached its maximum development. As compared with other teleosts, the embryo of the whitefish is well differentiated by the time the blastopore closes.

By the 67th day of incubation, most of the organs of the whitefish have formed. The full number of somites are present and the eyes, nose, and ears are advanced beyond the vesicular stage. The last half of the incubation period is taken up with growth and development of the organ primordia which were differentiated in the first 67 days. Price (1935) states, "In the present series, the incubation period lasted 134 days, at winter lake temperatures, slightly above freezing (1.5° C.)."

Another fish, the embryology of which shows a downward movement of the dorsal lip of the blastopore, is Fundulus heteroclitus. Phases of the embryology of this fish were described independently by Oppenheimer (1937), Jones (1939), and Solberg (1938).

Solberg (1938), described the germ ring of Fundulus heteroclitus as growing over the yolk to the opposite side from which it started. Jones (1937), stated, "Wilson's conclusion that the posterior pole or tail end of the embryo remains a comparatively fixed point, does not seem justified in the case of F. heteroclitus." While the oil droplet is consistently lateral to the embryonic axis, it is eventually covered by the downward growth of the posterior embryonic shield. The oil droplet may retard the downward progress of the germ ring, but it is completely enclosed before the formation of a distinct blastopore. Thus, there is recognized a posterior as well as an anterior epiboly.

Eggs of Fundulus heteroclitus have an average diameter of 2.0 mm., and are covered by a thick chorion from which projects adhesive strands. When incubated at a temperature of 25° C., the embryos hatch in eleven days. Oppenheimer (1937), and Solberg (1938) both describe developmental stages of F. heteroclitus so that they may be used for comparative purposes in experimental studies.

The embryology of the gizzard shad, Dorosoma cepedianum Le Sueur, has also been described (Warner, 1940). This fish has an egg that is 0.75 mm. in diameter. The chorion is adhesive. The two-cell stage is reached in one hour, and hatching takes place in 36 hours at a temperature of 26.6° C. At 16.8° C. the hatching time is extended to 95 hours. A slight backward growth of the dorsal lip of the blastopore takes place during gastrulation. One feature, peculiar to this fish, is the lack of pigmentation at hatching time. Even the eyes are devoid of pigment until three days after hatching.

Included in the group of fresh-water fishes, the embryology of which has been studied, is the common gold fish, Carassius auratus (Linnaeus). The embryology of this fish was described by Battle (1940). This Cyprinid has a spherical, pale, cream colored egg with a diameter of 1.25 mm. The surface of the chorion is of a mucilaginous nature allowing the egg to adhere to plants and other objects. Development is typical of the teleosts. The brain is clearly visible and optic vesicles are partially differentiated by the time the blastopore closes. The heart appears on the anterior surface of the yolk sac after 24 hours of incubation. Hatching occurs in 76 hours at a temperature of 25° C., but may not occur for as long as 14 days at lower temperatures.

Another fish of the fresh-water group is the bridled shiner, Notropis bifrenatus (Cope), the embryology of which has been described by Harrington (1947). The eggs of this minnow are spherical and demersal, and vary from 1.0 mm. to 1.5 mm. in size. The blastoderm completely envelopes the yolk before even the rudiment of the embryo is visible. This represents an extreme contrast to the whitefish, which is well differentiated at the time the blastopore closes. Hatching in N.

bifrenatus occurs after 56.5 hours of incubation at a temperature of 23.0° C.

The embryology of the largemouth bass, Huro salmoides (Lacépède), was described by Carr (1942). The eggs of this fish are demersal with an average size of 1.60 mm. The eggs are hatched after 47 hours of development at 23° C. to 26° C.

The early embryology of the zebra fish, Brachydanio rerio (Hamilton), was described by Roosen-Runge (1938). Much of his description was made from a study of specimens he had recorded on film. A separation of the protoplasm from the yolk begins when the cell first takes up water. This action ceases just before the first cleavage takes place. After the first mitosis another separation process commences. This consists of a streaming of the protoplasm through the yolk toward the blastodisc. At the same time a counter stream of protoplasm is moving toward the vegetal pole.

Some descriptions of early embryonic stages have been made by Moore (1944) on the minnow Notropis girardi Hubbs and Ortenburger. Although the cleavage stages were not observed, many eggs in the blastodisc stage were obtained. These developed to hatching within 24 hours after being taken from the river. The eggs are transparent and are 1 mm. in diameter. At hatching time the embryo has a large yolk sac which is usually absorbed in three days. After the yolk sac is absorbed, the larva can swim in a horizontal position.

While most work on teleostean embryology has been done on egg-laying species, the embryology of the live bearer, Platypoecilus maculatus Güenther, has been described by Tavolga and Rugh (1947). Tavolga and Rugh state, "the platyfish is a truly viviparous fish." It is used ex-

tensively in genetic studies and cancer research. The ovum averages 1.5 mm. in size and is not surrounded by a chorion. Several oil droplets are contained in the egg, and their arrangement and number varies with the individual female.

The early cleavages are not readily observed, but the compact blastula stage is easily identified. The embryonic shield is formed by a widening and thickening of one sector of the germ ring. As gastrulation continues, elongation takes place principally in the posterior region or dorsal lip of the blastopore. Thus the head of the embryo develops at the original position of the embryonic shield. This description is in direct opposition to the anterior growth of the embryo and germ ring that previously mentioned authors have described for other teleosts.

The platyfish develops extra-embryonic membranes not found in most other fishes. The pericardial sac is developed extensively in P. maculatus, and after expanding anteriorly, it folds over and covers one-fourth of the embryo. It presumably brings nutrition to the embryo. In the regression of this structure a temporary neck strap is formed. The mechanism of over growth of the platyfish membrane is essentially the same as that in the amnion of reptiles and birds.

While working on other problems involving the use of fish embryos, the embryology or order of organogenesis of the species concerned have been described by several authors.

Such a description was made of the embryology of the cottid fish, Clinocottus recalvus (Greeley) by Morris (1951). This fish has an average egg size of 1.32 mm. and hatches in 19 to 20 days at a temperature ranging between 13.0° C. to 15.0° C.

A summary of the order of organogenesis in the Danio was made by Goff (1940).

By studying the descriptions of the embryonic development of the various species, it is observed that teleost embryology, in general, follows a broad, typical pattern but individual variations may be very great. Whether these variations are actual or only proportionate differences due to a difference in the time required for the differentiation of the embryo, needs further study.

MATERIALS AND METHODS

The blue gourami is an exotic fish belonging to the order Labyrinthici, and family Anabantidae. There are at least two varieties of gourami using the name Trichogaster trichopterus. They are the blue gourami and the three-spot gourami. The blue gourami was the variety used in these studies. Fishes in the order Labyrinthici are characterized by the presence of an auxiliary breathing apparatus called the labyrinth. This adaptation enables the fish to obtain oxygen directly from the atmosphere.

The fish which were to furnish embryos in this study were purchased as adults from a local tropical fish retail store. While being conditioned for spawning, the sexes were kept in separate tanks. A temperature between 22° C. and 26° C. was maintained for the conditioning of the adults. Usually a female would be able to spawn every two or three weeks. The rapidity with which the spawnings could take place seemed to depend on food conditions and temperature. A generous amount of live food was supplied daily. This consisted of chopped earth worms or cultivated white worms. In addition, prepared dried foods such as shredded shrimp, dried daphnia and mixed foods were offered. Approximately 500 eggs were discharged at each spawning. Two or three female fish were used alternately, while one or two males provided an abundance of sperm as needed.

Embryos were secured for study by two methods--natural spawning and stripping. For natural spawning a pair of adult fish were placed in a five gallon tank. This tank was prepared in advance and contained about seven inches of water w ich had been aged long enough for the

chlorine to have passed off as gas. The fish were separated by a glass partition and allowed to become adjusted to the tank. When their behavior indicated a willingness to spawn, the partition was removed on the afternoon preceding the day on which the embryos were needed. The male, having constructed a bubble nest, would drive the female beneath it and spawning would occur in the manner characteristic of the labyrinthine teleosts. The eggs were placed in the floating bubble nest by the male where they could be removed easily with a pipette. After the eggs were removed they were placed in finger bowls and incubated at a temperature of 23° C.

Light seemed to be a very important factor in stimulating the spawning process. On two occasions ovulation was induced at night when artificial lights were turned on.

Two to three hours are usually required for a pair of fish to complete a spawning; consequently, there was considerable variation in the stage of development between the eggs discharged at the beginning of the spawning period and those discharged last. To overcome this disadvantage, a stripping technique was employed. The same general procedure was used as described above for natural spawning. The fish were allowed to perform one or two clasps at which time a pipette was inserted in their vicinity, and a small quantity of water was removed and placed in a finger bowl. This was done in order to secure spermatozoa which the male had discharged into the water. The female was then removed from the tank and held ventral side up in wet hands. With a gentle pressing movement of the thumb and forefinger, starting anteriorly and proceding posteriorly across the abdomen, eggs were extruded. The ventral surface of the female was then dipped into the sperm charged water where the eggs floated away. This method resulted

in as high a percentage of fertilized eggs as were obtained from a natural mating.

The male was stripped for sperm on some occasions. This was done by turning the male ventral side up and applying pressure with the thumb and forefinger along the ventral line in the area between the anus and genital pore. The success of this operation was very doubtful since there was never any visible evidence of obtaining sperm as would be indicated by the appearance of a milky colored seminal fluid. The presence of the genital fluid in the male gourami is not indicated by a white line between the anus and genital pore as in the *Fundulus heteroclitus* (Jones, 1937). Because of the abundance of sperm in the water under the nest after a few claspings, stripping of the male is not absolutely essential.

Stripping of the female had some advantages over natural spawning. It allowed unfertilized eggs to be obtained for study and photographic purposes. The stripped eggs could all be fertilized at approximately the same time, and this resulted in more uniformity in the development of the eggs of a single spawning.

Photographs were taken with a Leica camera using a Micro-Ibso attachment on the microscope. Illumination was provided by a Bausch and Lomb ribbon filament lamp. Eastman's Panatomic X film for the 35 mm. camera was used. This is a fine grain film especially useful in photomicrography. Wratten color filters were very useful in photographing certain structures such as the heart and circulatory system.

Photographs were made of live embryos at five minute intervals for the first two hours after fertilization. After this period they were made at half-hour intervals until hatching. Since development is continous through both day and night, a study of this kind requires a

considerable amount of patient watching. Two spawns were observed continuously from ovulation to hatching, and specimens were collected and fixed at regular intervals (30 minutes) for later study. Eight different spawns were observed, compared and photographed. To date, several hundred photographs have been made of the embryology of this fish.

A series of time-lapse photographs was made using a Bolex 16 mm. camera with a time-lapse attachment. This series of pictures was very useful in determining the time of cleavage. It gave an accurate record of the length of time needed before various structures were formed.

The phase microscope was useful when photographing and studying early cleavage stages; however, the phase microscope was not effective on thethicker specimens in the later stages of development; nor was it effective with objectives lower than 16 mm.

THE DEVELOPMENTAL STAGES OF THE BLUE GOURAMI
TRICHOGASTER TRICHOPTERUS

Although the development of an embryo is a continuous process, it is convenient in a study of this kind to analyze the development into stages. The description of the embryology of the blue gourami, *T. trichopterus*, has been arbitrarily divided into 27 stages. These descriptions have been made at times when easily visible changes have occured; i.e., cleavage stages, germ ring, heart formation, circulation, etc. Representative photographs have been included to illustrate each stage. Incubation was at 23.0° C. and the approximate incubation time is indicated for each stage.

Stage 1. (Fig. 1) Unfertilized egg.
The unfertilized eggs of the blue gourami, *T. trichopterus*, are spherical in shape with an average diameter of 0.71 mm. They are a pale amber color and may easily be overlooked in the water. They are pelagic with a specific gravity less than that of the water.

Three distinct regions are readily observed in the unfertilized eggs. Uppermost is the large, highly refractive oil droplet approximately .22 to .28 mm. in diameter, which is embedded in the heavier yolk material. The yolk makes up the second area and is slightly flattened on the side opposite the oil droplet. The protoplasm is collected on this flattened side forming a cap. This is very different from the unfertilized ova described for other teleosts. In the sea bass (Wilson, 1889) the protoplasm covers the yolk in a thin uniform layer and collects at one pole after fertilization. The "streaming" of the protoplasm may be observed as this movement occurs. In *Fundulus heteroclitus* (Solberg, 1938; Oppenheimer, 1937) the same phenomenon occurs. The

protoplasm occupies a uniform imperceptable layer surrounding the yolk. Only after fertilization does it collect at one pole to form the blastodisc.

In T. trichopterus, the unfertilized eggs stripped from the female show this protoplasmic cap already collected on the side of the yolk opposite the oil droplet. The first step in its rapid ontogeny is thus taken before fertilization occurs.

Stage 2. (Fig. 2) Fertilized ovum. Incubation time 0 minutes. After fertilization no immediate change can be seen in the egg. Since the protoplasm is already collected into a cap, the "streaming" effect observed in the sea bass (Wilson, 1889) does not occur.

Prior to the first cleavage, the blastodisc changes its shape with the edges becoming more abrupt. A thin protoplasmic layer, extending from the blastodisc, encloses the yolk.

Stage 3. (Fig. 3) The two-cell stage. Incubation time 30 minutes. The first cleavage is complete approximately 30 minutes after fertilization at 23° C. This is a meridional cleavage which divides the blastodisc into two equal blastomeres. The blastomeres are large and well rounded. They are completely formed within four minutes after the first indication of the cleavage furrow appears.

Stage 4. (Figs. 4-A, 4-B) The four-cell stage. Incubation time 44 minutes. The second cleavage begins about 14 minutes after the first is completed. This second meridional cleavage is perpendicular to the first (Fig. 4-A) and forms a four-cell blastoderm (Fig. 4-B).

Stage 5. (Fig. 5) Eight-cell stage. Incubation time 58 minutes. The third cleavage takes place some 14 minutes after the second. All subsequent cleavages occur at approximately this same interval of time. The

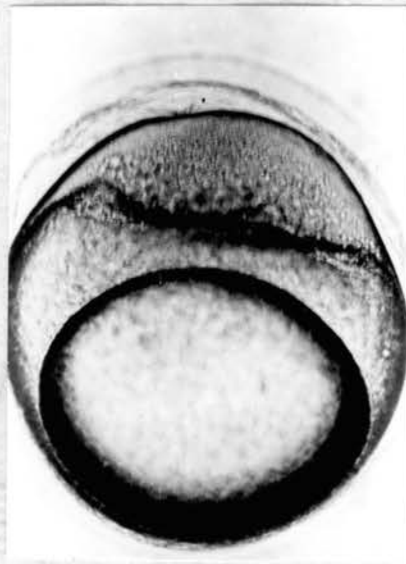

Figure 1. Unfertilized ovum. Note the protoplasmic cap and single large oil droplet.

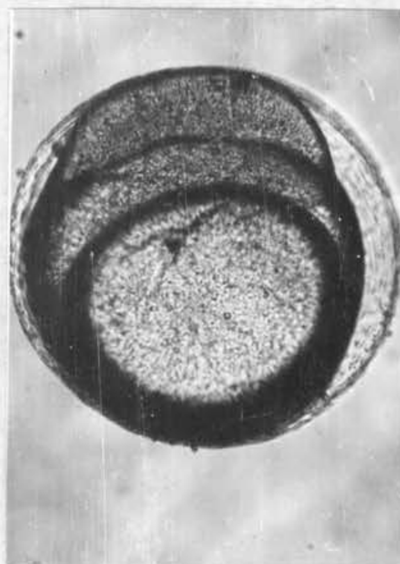

Figure 2. Fertilized ovum. The protoplasmic cap is more rounded and separate from the yolk.

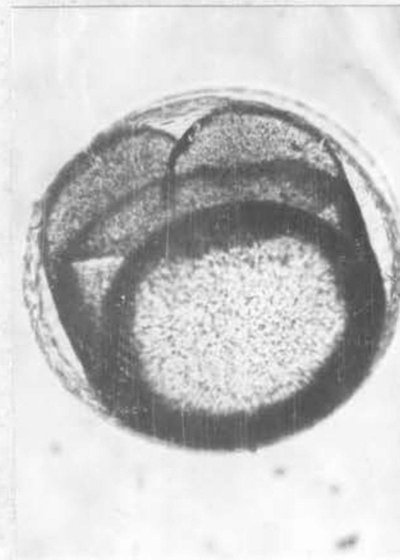

Figure 3. Two-cell stage. Note the three distinct regions; oil droplet, yolk mass, and protoplasm.

PLATE I

planes of the third cleavage are double and parallel to the first. This causes an elongation of the axis of the second cleavage plane. It has been suggested that the long axis of the eight-cell blastoderm may become the axis of the future embryo (Oppenheimer, 1937). No attempt was made in this study to determine if this were true for the blue gourami.

Stage 6. (Fig. 6) Sixteen-cell blastoderm. Incubation time 1 hour 13 minutes. At this stage the blastoderm loses the symmetry which characterized previous cleavages. With each successive cleavage the individual blastomeres become smaller and arranged in an irregular, flattened layer that is one cell thick.

During this period of development, the cells in the center pull away from the central periblast forming a space called the segmentation cavity. The central periblast is the thin layer of protoplasm which lies next to the yolk and connects the peripherial cells at their bases. It is formed when the cleavage furrows fail to cut entirely through the blastoderm during cleavage. This phenomenon can not be observed and photographed very satisfactorily in the living embryos, but it has been described in detail by Wilson (1889).

Stage 7. (Fig. 7) Thirty-two-cell stage. Incubation time 1 hour 28 minutes. The thirty-two-cell stage is reached after one hour and twenty-eight minutes of development. It is the result of the fifth cleavage. The blastomeres are becoming increasingly smaller, and the peripherial cells are breaking down to form the periblast.

Stage 8. (Figs. 8-A, 8-B) Many-cell blastoderm. Incubation time 2 hours 20 minutes. The developing blastoderm has regained its symmetry in this stage. It forms a well-rounded dome on the surface of the yolk. The size and shape of the blastoderm at this time is strikingly similar

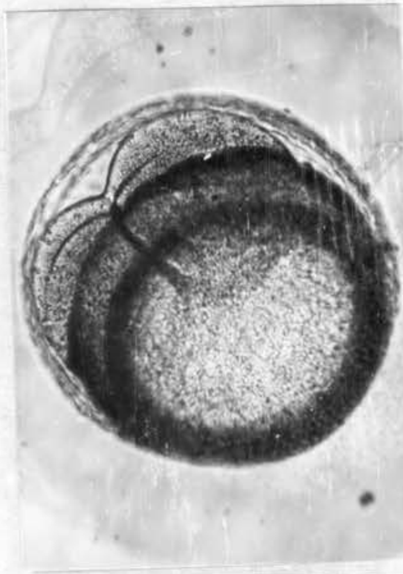

Figure 4-A. Four-cell stage. Note the starting of a second cleavage plane perpendicular to the first.

Figure 4-B. Four-cell stage, second cleavage completed.

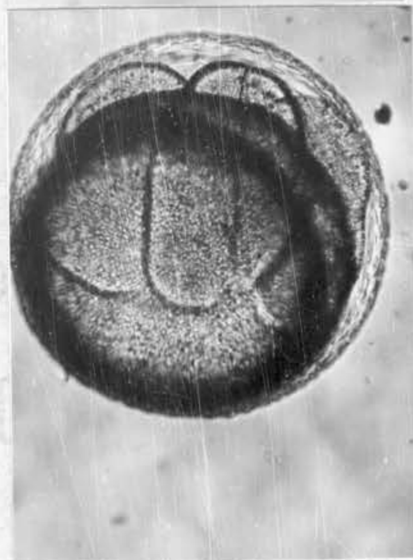

Figure 5. Eight-cell stage, ventral view.

PLATE II

to that of the blastodisc (Fig. 2) and that of the unfertilized egg (Fig. 1); however, the cellular structure of the blastoderm can easily be seen (Fig. 8-B). This is the early blastula stage.

The cellular nature of the peripheral periblast is not visible in the living embryo except in the marginal region surrounding the blastoderm. However, it has been described by Wilson (1889) and Price (1934a) based on observations of stained sections. The peripheral cells have indistinct boundaries at the place where they join the thin protoplasmic layer surrounding the yolk. This peripheral protoplasm concentrates into a ridge and receives nuclei from the adjoining cells of the blastoderm. This forms a syncytial periblast. The nuclei will eventually migrate to the central periblast. The ultimate fate of the periblast nuclei is not known, but they are thought to have a physiological function in the absorption of the yolk by the developing embryo (Wilson, 1889).

Stage 9. (Fig. 9) Flat blastula. Incubation time 3 hours 15 minutes. As mitosis continues, the resulting cells of the blastoderm become smaller. The dome-like shape of the blastoderm changes with the cap of cells becoming flattened on the surface of the yolk. This stage is reached just prior to the start of gastrulation.

Stage 10. (Figs. 10-A, 10-B, 10-C) Early gastrula. Incubation time 4 hours 30 minutes. As the blastoderm continues to enlarge and expand, the cells in the center thin out and the outer or peripheral edge becomes thickened (Fig. 10-C). This thickened area is called the germ ring. It is formed by a rapid growth and proliferation of marginal cells which turn under centripetally (involution). This thickening occurs around the entire periphery of the blastoderm. Wilson (1889) gave a detailed description of this phenomenon.

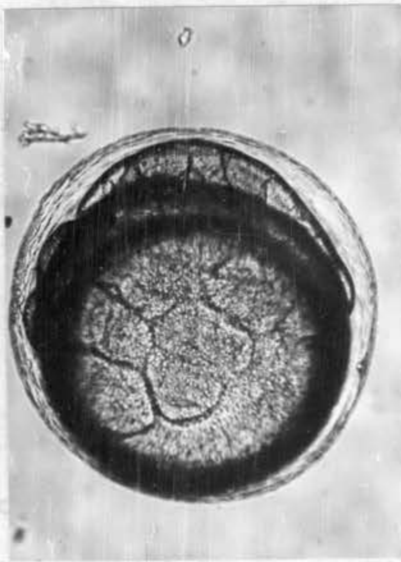

Figure 6. Sixteen-cell stage, ventral view.

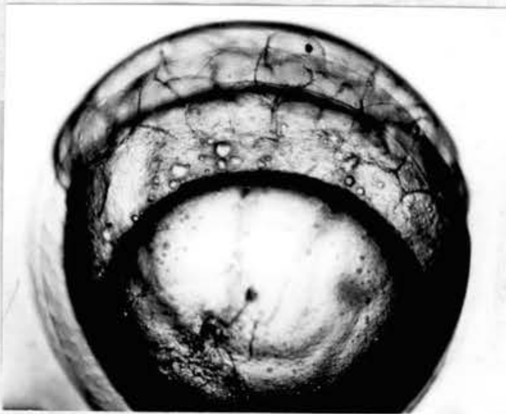

Figure 7. Early blastula. Note the open peripherial cells and the decrease in size of the individual blastomeres.

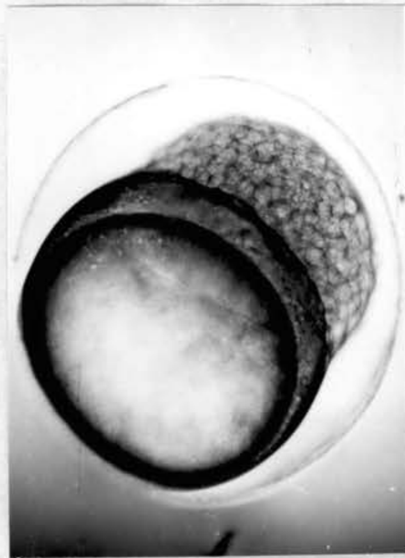

Figure 8-A. Blastula. Blastoderm conposed of many cells. Note the relative positions of oil droplet, yolk mass, and blastoderm.

PLATE III

At one point the germ ring is thicker than elsewhere (Fig. 10-A). Here invagination is most rapid causing a tongue of cells to protrude into the segmentation cavity. This marks the beginning of gastrulation. This point on the germ ring may be called the dorsal lip of the blastopore, since it corresponds to a similar structure in the developing amphibian embryo. The invaginating tongue of cells is the beginning of the embryonic shield from which the embryo proper will develop. The invaginating tip of the embryonic shield marks the anterior end of the future embryo, while the dorsal lip of the blastopore marks the posterior end.

At the same time the embryonic shield is differentiating, the germ ring is moving down--over and around the yolk.

Stage 11. (Fig. 11) Equatorial germ ring stage. Incubation time 5 hours 30 minutes. The germ ring continues to expand over the surface of the yolk, and in this stage it is in an equatorial position. It retains its thickness in the region of the embryonic shield, while the rest of the germ ring, the extra-embryonic part of the ring, grows thin; and the cells become flattened. The ring produces a slight constriction in the surface of the yolk as it moves along.

The embryonic shield has become more elongate indicating the axis of the future embryo.

Stage 12. (Fig. 12) Yolk plug stage. Incubation time 6 hours 10 minutes. At this stage the germ ring almost encircles the yolk except for a small area at the upper end of the oil droplet. The constriction imposed by the germ ring forces the oil droplet into an elipsoidal shape, with the small end forming the yolk plug.

The embryonic shield continues to elongate and becomes thicker in

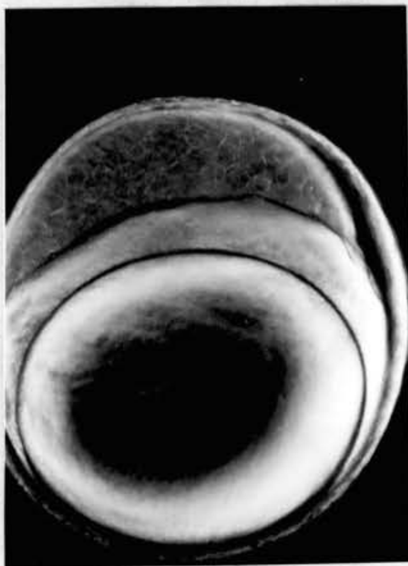

Figure 8-B. Blastula. (dark field photo). Note the individual cells are still distinguishable.

Figure 9. Flat blastula. Blastoderm just prior to gastrulation.

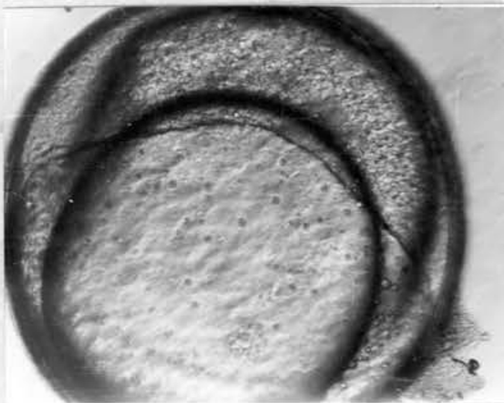

Figure 10-A. Early germ ring. Note the thickened ring area on the left of the photo.

PLATE IV

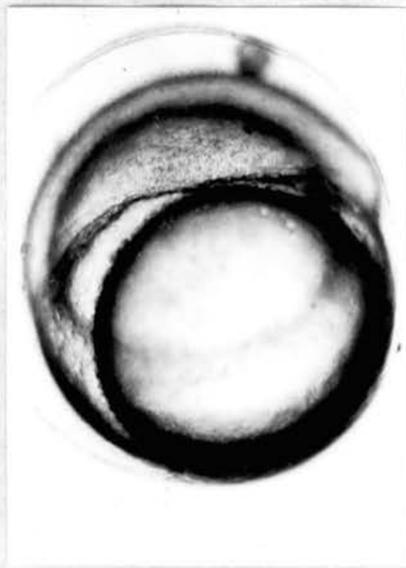

Figure 10-B. Germ ring. View of dorsal surface.

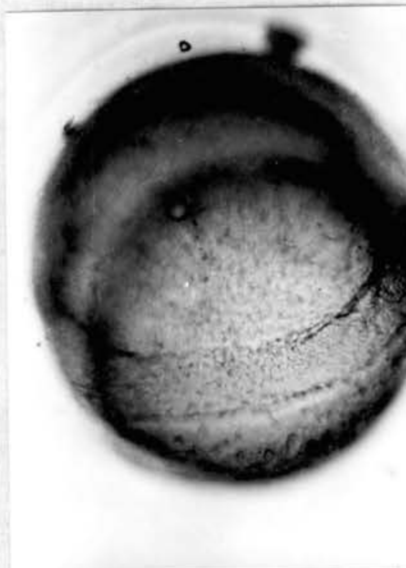

Figure 10-C. Germ ring. View of the ventral surface of the same egg as in figure 10-B. Note the thickness of the germ ring.

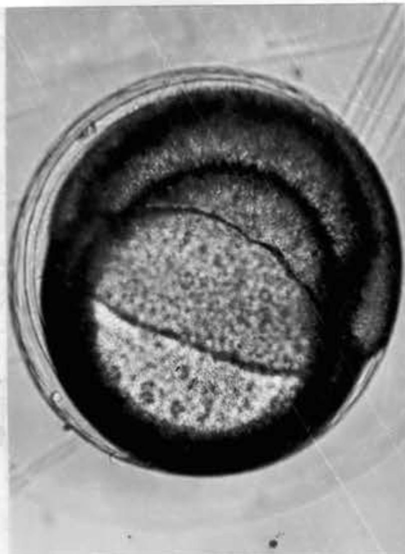

Figure 11. Equatorial germ ring. Note the embryonic shield extending perpendicular from the germ ring.

PLATE V

the central axis. This added thickness causes it to press down into the yolk material.

Stage 13. (Figs. 13-A, 13-B) Closure of the blastopore. Incubation time 7 hours 50 minutes. After seven hours and fifty minutes of development, the closure of the blastopore takes place. This is a period of much activity. By proper light manipulation the embryonic shield may be seen (Fig. 13-A) as a narrow darkened streak extending from the dorsal lip of the blastopore around the curvature of the yolk perpendicular to the germ ring. (Note the V-shaped indentation on the top where the embryonic shield presses into the yolk) In Fig. 13-B, the microscope was focused through the egg onto the shield on the lower side. These photographs show that the embryonic shield has encircled approximately half of the yolk mass.

Stage 14. (Figs. 14-A, 14-B) Embryonic shield. Incubation time 9 hours. The embryonic shield grows anteriorly and becomes thicker dorsoventrally along the median line. Another structure, the neural keel, makes its appearance and is destined to form the notochord and central nervous system. The keel continues to grow and press downward into the yolk mass forming a V-shaped indentation (Fig. 14-A). The lateral portions of the embryonic shield become thinner where they join the extra-embryonic membrane.

The embryonic shield shows a marked bulge in the anterior region (Fig. 14-B). This is the cephalic area of the developing embryo, and the lateral expansion is caused by the solid mass of cells of the optic primordia.

Stage 15. (Fig. 15) Somite formation. Incubation time 10 hours 30 minutes. Early in the differentiation of the embryo, a solid rod of

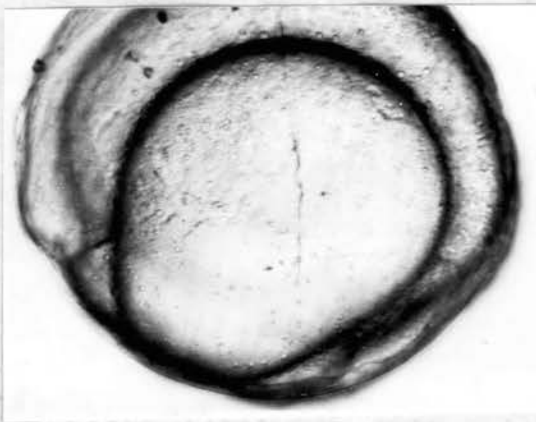

Figure 12. Yolk plug, lateral view. Note the embryonic shield (upper left).

Figure 13-A. Closure of the blastopore. Note the dorsal lip of the blastopore indicated by arrow.

Figure 13-B. Closure of the blastopore. This is the same embryo as figure 13-A with the microscope focused on the ventral surface.

PLATE VI

cells appears along the center line of the embryonic axis. This structure is known as the notochord and lies next to the yolk mass. On both sides of the notochord, mesodermal somites begin to differentiate in the anterior region, and continue to form posteriorly. An undifferentiated mass of cells is visible in the caudal region (Fig. 15).

The neural keel continues to develop and the cephalic region attains considerable dorsoventral thickness and pushes out into the perivitelline space. The optic vesicles become more distinguishable and may be seen as flattened lateral expansions of the cephalic region.

Stage 16. (Figs. 16-A, 16-B) Neurula. Incubation time 12 hours. By continued growth and differentiation, the embryo moves around the yolk until both head and tail regions may be seen at the same time (Fig. 16-A). The forebrain and optic vesicles are still somewhat flattened and adhere very closely to the underlying periblast (Fig. 16-B). The notochord may be seen in the tail region as a solid rod of cells, and the neural keel lies dorsal to the notochord as a solid structure. Undifferentiated mesoderm extends laterally on both sides of the notochord and will be used in the formation of the somites.

Stage 17. (Figs. 17-A, 17-B) Differentiation of the tail region. Incubation time 12 hours 50 minutes. At this stage most activity centers in the differentiation of the nervous system and the continued growth of the tail region. Somite formation has progressed posteriorly (Fig. 17-A). The notochord and neural chord are still solid structures with well defined somites formed on either side. The cephalic region of the neural chord has thickened dorsoventrally, and it is pushing down into the yolk causing the yolk mass and oil globule to assume a flattened appearance. The optic vesicles, which form as evaginations of the

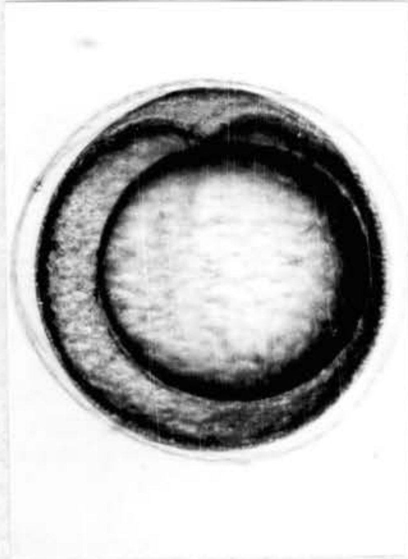

Figure 14-A. Embryonic shield. Note the neural keel pressing down into the yolk.

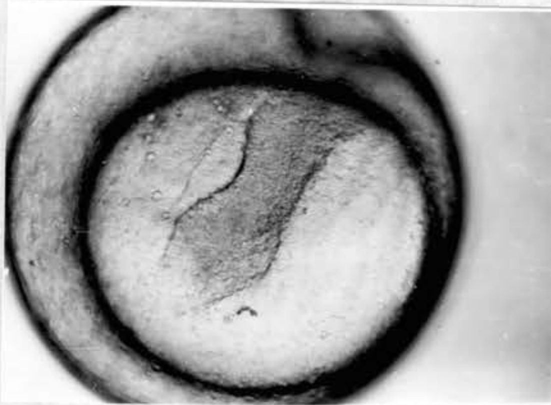

Figure 14-B. Embryonic shield, ventral view. Note the optic vesicle primordia.

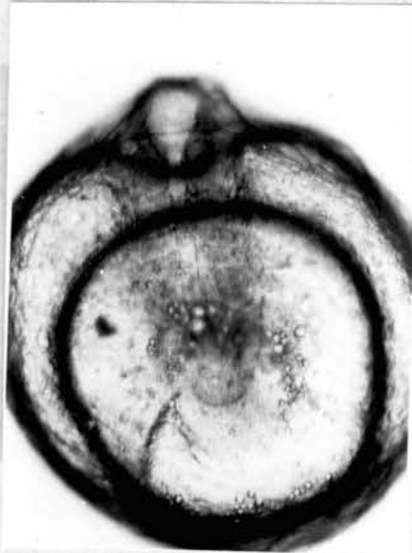

Figure 15. Somite formation, ventral view through oil droplet and yolk. Note the somites, notochord, and enlargement of the optic vesicles.

PLATE VII

mid-brain, have been partially separated from the brain by constrictions (Fig. 17-B).

Stage 18. (Figs. 18-A, 18-B) Optic cavity formation. Incubation time 13 hours 20 minutes. At this stage the cavities begin to form in the optic vesicles. The optic vesicles are still broadly connected to the brain, but the amount of separation is increasing. In the teleosts, the nervous system and associated sense organs form from solid cell masses. The cavities then form by a process of vacuolization (Oppenheimer, 1937). In other vertebrates the central nervous system forms as a hollow tube by folding.

Stage 19. (Fig. 19) Optic cup formation. Incubation time 14 hours 30 minutes. After fourteen and one-half hours of development the eyes begin to form their characteristic cup-shape. This is brought about by the outer layer of cells pushing down into the optic cavity, much in the same manner as one would push one side of a rubber ball in against the other. The lens soon forms as a thickened area in the epidermis.

Stage 20. (Fig. 20) Early pigmentation. Incubation time 15 hours. The first pigmentation may be seen after 15 hours of development. Large stellate melanophores appear along the lateral margins of the embryo. The cephalic region continues to expand and develop with the regions of the brain becoming more distinct.

Stage 21. (Figs. 21-A, 21-B) The heart formation. Incubation time 16 hours 45 minutes. At this time the heart may be seen as a round tube extending laterally from under the left eye (Fig. 21-A). As yet, the blood vessels and blood cells have not formed; thus, the circulation has not started. The position of the heart under the left eye is slightly different than that recorded for some other species. In most other

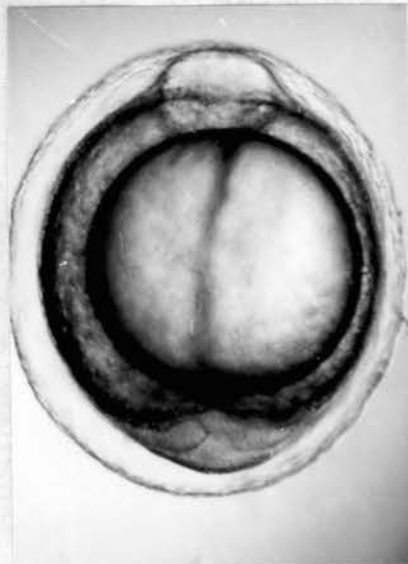

Figure 16-A. Neurula, ventral view. Note the notochord, neural keel, and undifferentiated mesoderm in the tail region (6 o'clock).

Figure 16-B. Neurula, lateral view. Note the optic vesicles (7 o'clock).

Figure 17-A. Tail formation. Note notochord, neural chord, and somites in the tail.

PLATE VIII

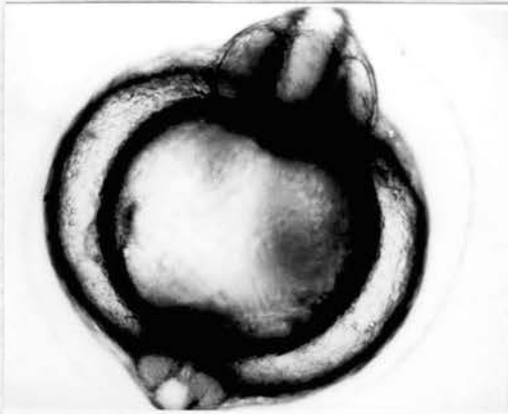

Figure 17-B. Tail formation, same embryo as in figure 17-A. Note optic vesicles and brain development.

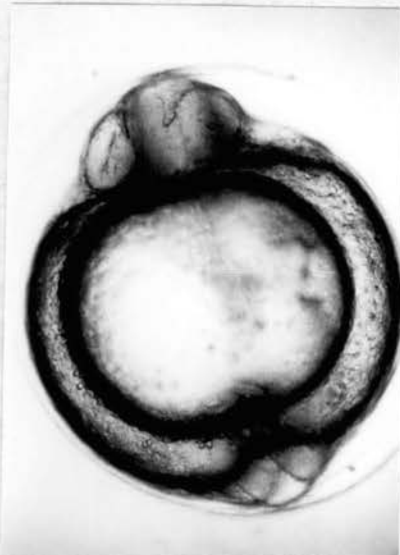

Figure 18-A. Optic cavity formation.

Figure 18-B. Optic vesicles.

PLATE IX

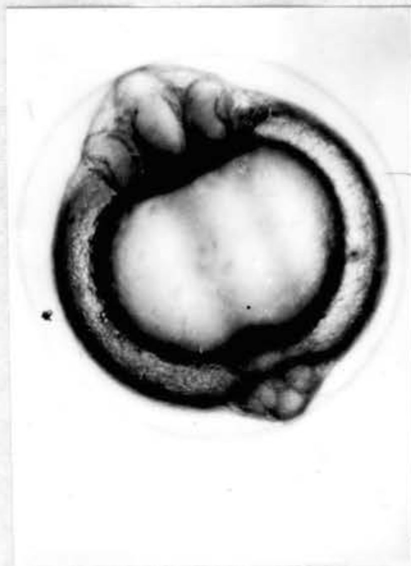

Figure 19. Optic cups.

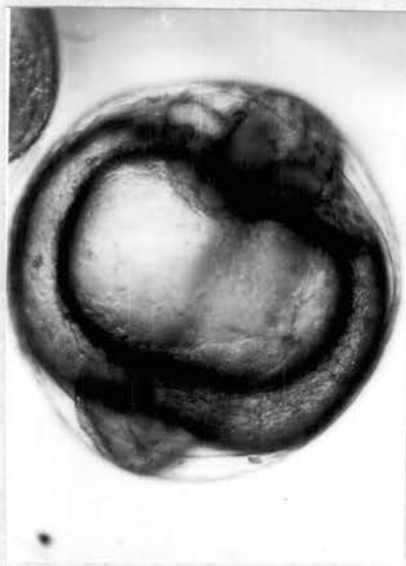

Figure 20. Early pigmentation. Note the melanophores along the sides of the embryo.

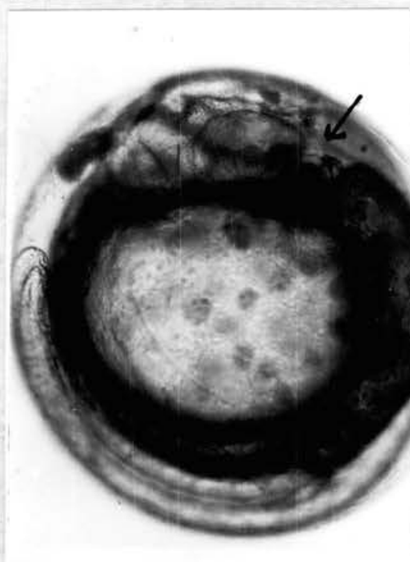

Figure 21-A. Heart. Note the heart as an open tube under and posterior to the left eye, indicated by arrow. Note the cavity in the forebrain.

PLATE X

instances described, the heart projects forward from under the head and lies on the surface of the yolk (Carr, 1942:67; Solberg, 1938:18). Figure 21-B shows an embryo of approximately the same stage as Figure 21-A, but one in which the pigmentation has failed to develop. This lack of pigmentation makes the heart and brain easily visible.

At this stage the lens of the eye is well defined and the cavities of the brain are easily observed. Melanophores have become more scattered over the yolk.

Stage 22. (Fig. 22) Circulation. Incubation time 17 hours 30 minutes. The heart beat first starts after about 17 to 18 hours of development. At first the beat is slow and irregular, but it soon becomes a steady pulsation. The first indication of circulation is the formation of blood islands on the surface of the yolk. (Note that the oil droplet, although it is easily visible, is enclosed in a thin layer of yolk material; and this in turn is covered by a layer of protoplasm). From these groups of cells the blood vessels and blood are formed (Solberg, 1938:18). The blood cells move across the yolk in a postero-anterior direction. At first their movement is slow and halting, and they follow a broad path which leads to the open posterior end of the heart. As development continues and blood cells increase in number, the speed of their movement increases and the path becomes definite and narrow (Fig. 22). Eventually walls are formed and vitelline vessels connect with the heart.

Stage 23. (Fig. 23) Fin buds. Incubation time 23 hours. The pectoral fin buds become visible after 22 to 24 hours of incubation. They are seen as rounded projections along the lateral margins of the embryo.

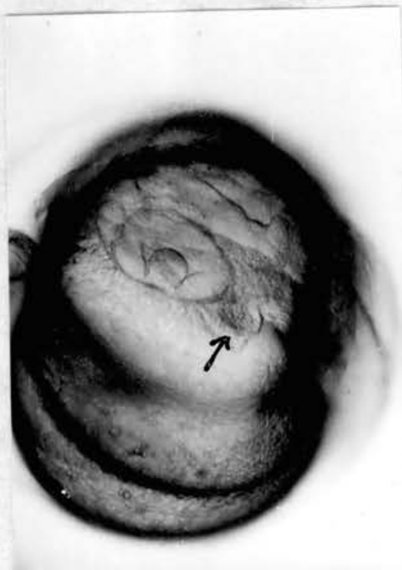

Figure 21-B. Heart, in an unpigmented embryo. Note the position of heart, also the lens of the eye and brain cavities. A dorsolateral view.

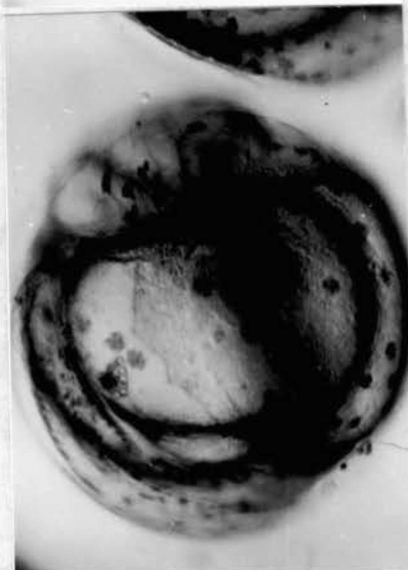

Figure 22. Circulation. Note the broad blood path across the yolk surface.

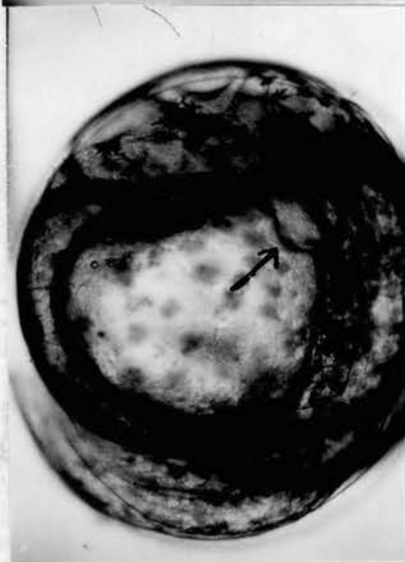

Figure 23. Fin bud.

PLATE XI

Stage 24. (Fig. 24) Hatching. Incubation time 25 hours. Hatching occurs in the blue gourami, T. trichopterus after 24 to 26 hours of development. The embryo breaks the chorion with a lashing movement of the tail and escapes into the water.

Stage 25. (Fig. 25) 28-hour larva.
The most apparent change in the embryo, after it hatches and is known as a larva, is the reduction in the amount and shape of the yolk mass. At hatching (Fig. 24), the yolk mass is well rounded and the embryo's body is still curved around this large amount of yolk material. However, soon after hatching the body becomes more elongate; and the yolk material is not only reduced in amount, but also assumes the elongate shape of the embryo proper. The larva stays at the surface of the water and floats in a vertical position. This position is due to the buoyancy of the large oil droplet and yolk mass which tends to float the larva with the head up. When disturbed the larva falls to the bottom of the container and immediately tries to return to the surface of the water by a struggling spiral movement.

Stage 26. (Fig. 26) 45-hour larva.
The larva of this stage has used up still more of the yolk material. The body shape is more elongate and straight. The somites in the most posterior part of the tail are still not completely differentiated. The pectoral fins differentiate into thin, transparent, leaf-like organs. The larva of this stage has not taken in food due to the lack of complete differentiation of the internal viscera. The yolk supply is still sufficient to furnish proper nourishment.

Stage 27. (Fig. 27) 72-hour larva.
The larva at this age has used up most of the yolk material with only a

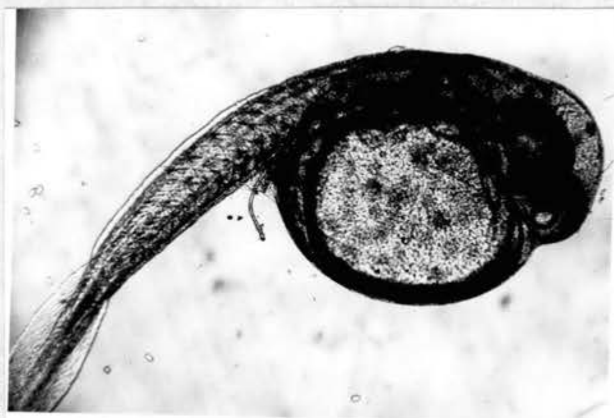

Figure 24. Twenty-five hour embryo just after emerging from the chorion.

Figure 25. Twenty-eight hour larva.

PLATE XII

small amount of the oil droplet remaining. The pectoral fins are well differentiated and are used in propelling the tiny fish. Due to the decrease in size of the yolk mass and the bilateral arrangement of the remaining oil droplet and yolk, the larva can assume a normal horizontal position and is said to be "free-swimming". The digestive tract has become well developed and feeding has begun. At this stage the modified, filiform pelvic fins have not differentiated.

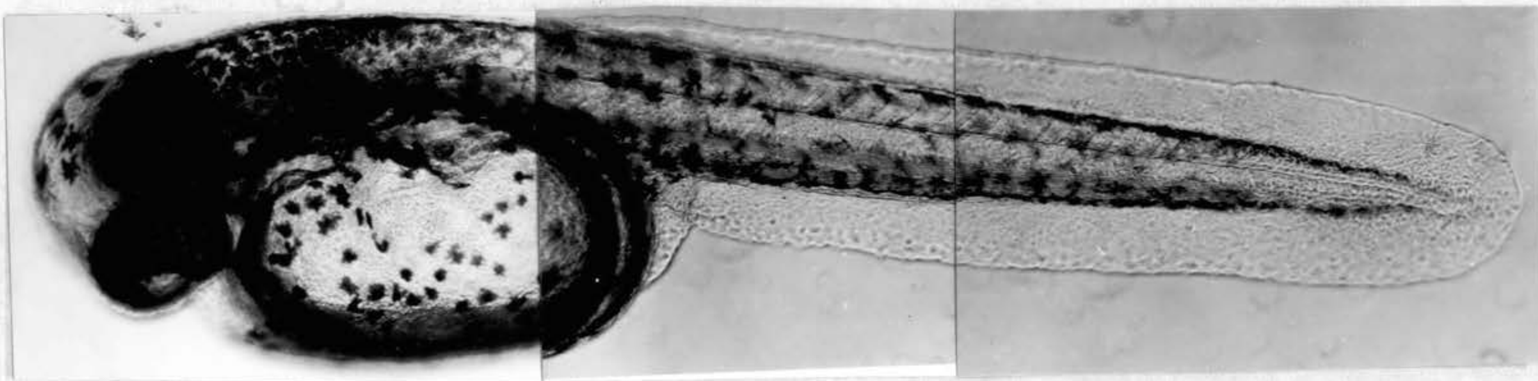

PLATE XIII

Figure 26. Forty-five hour larva.

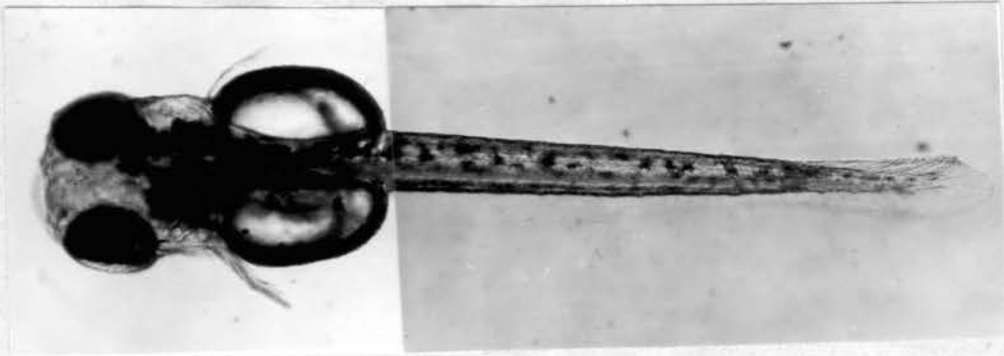

Figure 27. Seventy-two hour larva.

Firuge 28. Abnormal Embryo.
Note lack of pigmentation,
enlarged pericardial sac, and
abnormal brain region.

PLATE XIV

DISCUSSION

There exists some doubt concerning the valid scientific name of the blue gourami. In fact, there is no adequate description of this fish (Meyers, personal correspondence). A similar fish commonly known as the three-spot gourami has been described and called Trichogaster trichopterus (Pallas). The blue gourami differs from this fish chiefly in color and origin. It is found naturally only on the Island of Sumatra, while the three-spot gourami is found in India, the Malay Peninsula, and Indo-China. Innes (1948; 384) suggests that the blue gourami which he calls Trichogaster sumatranus is a color variation of the three-spot gourami, and that there is only one species.

It has been suggested (Meyers, personal correspondence) that the blue gourami may be a geographic race or subspecies of Trichogaster trichopterus. However, since the modern icthyological literature does not recognize any species of gourami of the three-spot group other than T. trichopterus, it seems justifiable to use this name for the blue gourami at the present time.

Another study of the embryology of T. trichopterus has been made by Ellinor H. Behre and is to be published soon. By correspondence it is learned that there are some differences between the observations of that investigation and the ones presented here. The main conflicts appear to concern the size of the egg and the structure of the oil droplet. Behre records an egg diameter of .87 mm., while the data reported here show a size of .71 mm. She also reports numerous small oil droplets in the egg yolk, while the eggs observed here show only a single large oil droplet that equals about one-third of the total egg volume.

In as much as the paper by Professor Behre has not been published

and the present author has been unable to observe her material, comparisons in regard to other details can not be made at this time. The author does not know whether Dr. Gehre studied the "blue" or the "three-spot" variation of T. trichopterus. One is justified, however, in asking whether there can be this much natural variation in a single species. Are their embryological differences an indication that there are two distinct species in this group of fishes?

This study has shown that the blue gourami embryo develops in much the same manner as other described teleostean embryos, yet it does differ from the other descriptions at certain points.

The unfertilized eggs of the blue gourami appear to be different from those of the other fishes described in the literature. In the blue gourami (eggs removed by stripping and unfertilized) there is present a well-rounded protoplasmic cap which can not be distinguished from the blastodisc of the fertilized egg and is easily confused with the many-celled blastoderm. In other descriptions the protoplasm of the virgin ovum is located in a thin layer around the yolk and collects at one pole after fertilization takes place, sometimes producing a visible "streaming" movement when the blastodisc is forming. The protoplasm's accumulation in the unfertilized egg does not seem to be a factor in the fourami's rapid development, since the first cleavage does not occur until 30 minutes after fertilization. Some other species with longer development time have their first cleavage within the first 30 to 40 minutes (Roosen-Runge, 1938).

The position of the heart is another observed difference. In most other descriptions of fish embryos, the heart forms as a tube extending forward from under the head, and lying on the surface of the yolk (Carr, 1942: 67; Solberg, 1938: 18). In the blue gourami the heart extends

laterally from under the left eye, where it connects to the vitelline vein. This unusual position of the heart has also been observed by the author in the Betta splendens Regan embryo, which is another member of the order Labryinthici.

The study of the embryology of this fish gave no clues to changes that would account for its rapid rate of development other than the rate of mitotic cleavage, which is materially faster than that of many other forms described. Other of the labryinthine fishes [Betta splendens; Macropodus opercularis (Linnaeus)] observed by the author (Ingersol and Jones, 1949) tend to develop rapidly and have a relatively short mitotic cycle.

As previously referred to in the literature section of this thesis, there is a question as to the movement of the germ ring and the subsequent closure of the blastopore. While it was beyond the scope of this study to make a detailed investigation of this phenomenon, it should be pointed out that two general procedures of germ ring migration have been described by the various workers. Only two investigators (Price, 1937: 97; Jones, 1937:31-32) have suggested that this difference exists. Does the germ ring move across the yolk from one end to the other, or does the dorsal lip of the blastopore remain in a stationary position and the rest of the ring move around the yolk pivoting from this point? The evidence presented in the literature indicates that both processes have been observed in teleostean development with the blastopore closure being effected by concrescence.

In the development of the blue gourami embryo epiboly occurs throughout the germ ring. To support this conclusion it is noted that the oil globule is relatively fixed and is always uppermost in the floating egg.

The protoplasm is collected on the lower surface of the yolk and opposite to the oil droplet. As the germ ring forms, it moves upward over the yolk on all sides. At the time of the closure of the blastopore, the protoplasm has encircled the yolk except for a small area on the upper most surface of the oil globule (Fig. 13-A). If the dorsal lip of the blastopore remained at the position it occupied when the germ ring was first formed, the closure of the blastopore would take place on the side of the oil globule instead of the uppermost surface of it.

When culturing one group of embryos for observation, a situation occurred which needs some explanation. This group of embryos failed to develop pigmentation (Fig. 21-B; Fig. 28). The culturing medium used in the culture dishes was aged water that had been stored in clean bottles. Water from one particular bottle produced this abnormal effect which was uniform and repeated in other spawnings. Some of the affected embryos were transferred to fresh water soon after hatching, and these then became pigmented and continued to develop normally. Embryos left in the contaminated culture water after hatching, in addition to the lack of pigmentation, developed a greatly enlarged pericardial sac (Fig. 28), which in many cases stretched the heart into a straight tube. Other deformities occurred such as malformation of the head and brain and lack of development of the tail. Also noticable was one or more abnormal body flexures.

The contaminating substance in the culture water has not been isolated although some preliminary experiments have been made. This will be the object of future study. The significance of this phenomenon, as far as this study is concerned, lies in the fact that it enabled photographs to be made of structures that would ordinarily be difficult to observe if the embryos were normally pigmented.

Observations of many deviations in the development of fish embryos seemingly exposed to similar environmental conditions, such as the one described above, indicate that the factors which influence the development of fish embryos are many and varied; i.e. how does temperature affect the rate of development? It is generally thought that higher temperatures increase the development rate within certain limits. However, one group of fishes, the soil breeders of the Fundulopanchax group, develop faster at 21° C. than at a temperature of 26.5° C. (Klee, 1951). For the blue gourami our data indicate that lower temperatures extend the incubation period. The minimum and maximum temperature limits of embryonic activity for this species were not determined.

All of the fertile eggs of a single blue gourami spawn hatched at approximately the same time (25 hours). However, eggs of Aphyosemion sjoestedti (Loennberg) that are fertilized at the same time and incubated under identical conditions may hatch from one month to several months apart (Girard, 1951). What factors can cause so much variation in development under the same environmental conditions?

These and other questions should certainly demand our attention and an adequate study might give more meaning to the descriptive stages of the presently described teleostean species.

SUMMARY

Because of the increased use of fish embryos as experimental animals, and because of their value as a teaching tool, this descriptive study of the embryology of the blue gourami, Trichogaster trichopterus (Pallas), was made. The blue gourami was selected for the study because of the unusual speed with which it develops.

The egg of this fresh-water fish is approximately 0.71 mm. in size and is characterized by a large single droplet of oil, which is embedded in the heavier yolk material, but near the surface. This oil droplet is always uppermost in the floating egg. A cap-like protoplasmic disc is collected at the side opposite the oil globule in the unfertilized egg.

The first cleavage takes place in about 30 minutes after fertilization, and subsequent cleavages occur at intervals of from 12 to 15 minutes, at a temperature of 23° to 24° C. In the blue gourami the germ ring moves around the yolk on all sides instead of pivoting from a stationary dorsal lip of the blastopore as recorded by Wilson (1889).

The heart appears on the anterior surface of the yolk sac and under the left eye. It first appears after about 16 hours of development. Pigmentation occurs on the head and melanophores are scattered over the yolk sac when the embryo has incubated 15 hours.

Hatching is accomplished by a lashing movement of the tail which ruptures the chorion after about 24 to 26 hours of incubation. The newly-hatched larva continues to be nourished by the yolk mass for about three days, after which it is "free-swimming" and able to feed.

With the above exceptions, the embryonic development of the blue gourami follows the basic pattern of teleostean embryology described by

Wilson (1939) for the sea bass. The mitotic cycle of 12 to 15 minutes which is relatively more rapid than that reported for other forms, is the only factor observed which might account for the rate of organogenesis characteristic of this animal.

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