NOTES ON THE EMBRYOLOGY AND LARVAL DEVELOPMENT OF FIVE SPECIES OF TELEOSTEAN FISHES

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Contribution from the United States Fisheries Biological Station, Beaufort, N. C.

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INTRODUCTION.

The work of which the results are embodied in the present paper comprises observations on the eggs and larvæ of five species of teleosts, viz, *Cyprinodon variegatus*, *Lucania parva*, *Kirtlandia vagrans*, *Gobiosoma bosci*, and *Ctenogobius stigmaticus*. This work was carried on at the Beaufort, N. C., station of the United States Bureau of Fisheries during the summer of 1914. It was undertaken in pursuance of a general plan of the Bureau to secure a record as complete as possible of the time of spawning and of the embryological and larval development of fishes common in these waters.

It is not the purpose of this paper to discuss at length the embryological development of each species, but rather by means of illustrations and descriptions to afford a ready means of identifying eggs or larval fishes at any time during embryological and larval life. The eggs of the three species first named above are very typical. The study of their development adds nothing essentially new to our knowledge of the embryology of teleosts. The eggs of *Gobiosoma bosci* are characterized by a small yolk sphere and a relatively large amount of protoplasm. This condition is emphasized still further in the eggs of *Ctenogobius stigmaticus* in which the yolk sphere is exceedingly minute and the quantity of protoplasm relatively enormous. The disparity of yolk in proportion to the quantity of protoplasm present in these eggs leads to some interesting deviations, during the process of gastrulation and the differentiation of the embryo, from the course followed by the more typical teleostean eggs.

Observations were made exclusively on living material. The eggs of each species were fertilized and hatched in the laboratory. Males ripe for stripping were rarely taken. In nearly all cases fertilization followed the maceration of the testes of the male in the water into which the eggs were stripped.

CYPRINODON VARIEGATUS. SHEEPSHEAD MINNOW.

Spawning.—The spawning season of this species, which is very abundant in the brackish waters of North Carolina, continues throughout the summer. According to records kept by Mr. S. F. Hildebrand, director of the station, gravid females were taken in Mullet Pond as early as April 17. The ovaries of these females contained, in addition to the mature ova, immature ova of at least two different stages of development, thus suggesting the probability that more than one brood is produced during the season.

On May 4 young ranging from 6 to 12 mm. in length were present in considerable numbers. During the month of August, when the following observations on the eggs and young of this species were made, young ranging from 6 to 30 mm. and over in length were present in great abundance. It is obvious therefore that the spawning season continues from April until late summer.

Females ripe for stripping taken in August spawn relatively few ova, while immature ova in various stages of development are still present in the ovaries. Many females which could not be stripped when taken yielded a relatively small number of mature ova after being kept in an aquarium for several days. The presence in the ovaries of ova in various stages of development and the relatively small number ripe for spawning at the same time during late summer seem to indicate that these fish spawn repeatedly during the season.

Eggs.—The mature unfertilized ova (fig. 1) are spherical in form and 1.2 to 1.4 mm. in diameter. Their specific gravity is slightly greater than that of sea water and
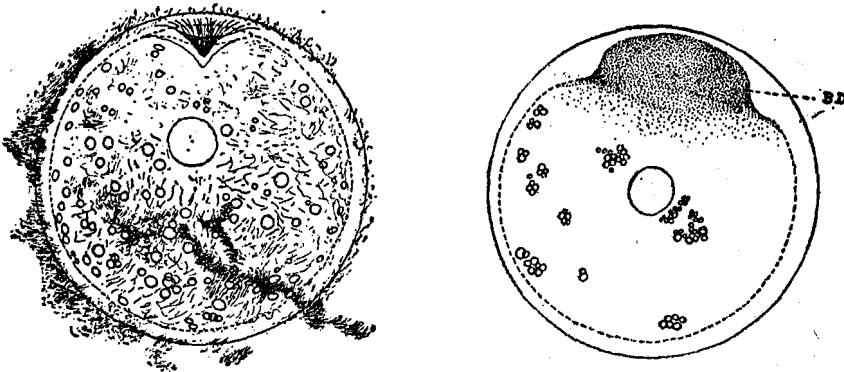
CYPRINODON VARIEGATUS.

FIG. 1.—Mature unfertilized egg. × 33.   Fig. 2.—Egg with fully developed blastodisc (BD).

they adhere in clumps, being held together by a tangle of very minute adhesive threads. They are yellowish in color and highly translucent. The egg membrane is thick and horny. Between it and the delicate vitelline membrane there is a perceptible perivitelline space. The large micropyle appears as a conspicuous cone-shaped depression in the egg membrane which also causes a slight indentation in the surface of the yolk. Scattered over the surface of the yolk are small groups of minute oil globules. The single large oil globule contained in the yolk sphere normally rests at the upper pole.

Blastodisc.—The quantity of protoplasm contained in these eggs is relatively large. Before fertilization the protoplasm is disposed in a layer of uniform thickness investing the yolk. After fertilization has taken place this layer of protoplasm becomes concentrated at one pole of the yolk sphere to form the blastodisc. The protoplasm being coarsely granular in appearance, the "streaming" movements toward the pole of the blastodisc which occur during the process of concentration may be readily observed. These "streaming" movements have been well described by Ryder [a] in the eggs of the cod and more recently by other investigators in the eggs of other species of teleosts.

[a] Ryder, J. A.: Embryography of osseous fishes. Report, United States Fish Commission, 1882, p. 455–605.

The fully developed blastodisc (fig. 2, BD) forms a thick protoplasmic cap, circular in outline, covering one pole of the yolk sphere. It is apparently of nearly uniform thickness throughout the central area and thins out very abruptly near the periphery. At the periphery it fades away almost imperceptibly into the very thin layer of protoplasm which remains at the surface of the yolk.

After fertilization has taken place the egg membrane becomes slightly expanded and the perivitelline space becomes more apparent. As the blastodisc becomes differentiated the egg also becomes somewhat more transparent.

Segmentation.—Segmentation takes place in a manner quite typical for the eggs of teleosts. The first act of cleavage occurs about one and one-half hours after fertilization. The second occurs less than 30 minutes after the first. The first and second cleavage planes cut the blastodisc meridionally and at right angles to each other. The first four blastomeres are usually approximately equal in size and quite symmetrical. The cleavage furrows cut deeply into the blastodisc and the blastomeres show a decided
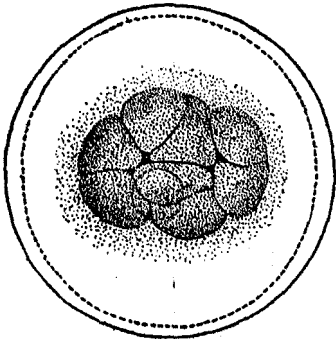

CYPRINODON VARIEGATUS.

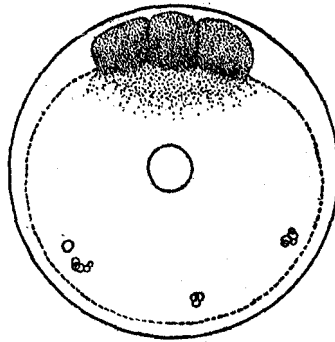FIG. 3.—Egg with blastodisc of 8 cells. FIG. 4.—Egg with blastodisc of 8 cells, optical section.

tendency to assume a spherical form. During the 2-cell stage the axis of the blastodisc at right angles to the first plane of cleavage is noticeably elongated. During the 4-cell stage the two axes are again approximately equal.

As the third act of cleavage occurs one axis of the blastoderm again becomes distinctly longer than the other. The eight blastomeres thus formed are at first quite symmetrical, but before the fourth act of cleavage occurs much of the symmetry of the blastoderm is lost and the arrangement of the cells becomes quite irregular (fig. 3). Viewing the blastoderm of eight cells from the surface, the cells appear distinctly outlined peripherally. Viewed in optical section from the side, however, the marginal cells appear somewhat constricted at the base but are not entirely cut off peripherally (fig. 4). They remain continuous with the thin layer of protoplasm which invests the yolk.

As segmentation continues beyond the 8-cell stage the arrangement of the cells becomes increasingly less symmetrical. A typical blastoderm of 16 cells is illustrated in figure 5. A less symmetrical blastoderm of the same stage in an egg of *Lucania parva* is illustrated in figure 21. The blastoderm is now approaching a circular outline and becomes more nearly circular as segmentation advances.

Formation of the periblast.—As segmentation advances the blastoderm becomes distinctly dome-shaped and the segmentation cavity becomes apparent beneath its central area. The thin layer of protoplasm at the surface of the yolk becomes concentrated at the periphery of the blastoderm to form a somewhat flattened ridge. This ridge of protoplasm gives rise to the periblast (fig. 6, PB). While the periblast is becoming differentiated nuclei become apparent in it. As observed by Agassiz and Whitman,[a] these nuclei are doubtless derived from the marginal cells of the blastoderm. The periblast is relatively broad and deep. The periblast nuclei are relatively numerous and easily observable in the living material.

During the earlier stages in the differentiation of the periblast the cells at the periphery of the blastoderm remain continuous with it. As segmentation advances farther the peripheral cells of the blastoderm become completely cut off from the periblast. A thin sheet of protoplasm now advances centripetally beneath the segmentation cavity.

Formation of the germ ring and differentiation of the embryo.—The germ ring arises during the later stages in the differentiation of the periblast as an apparent thickening
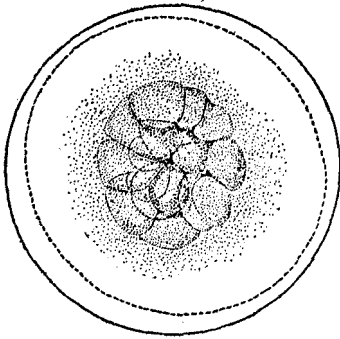

CYPRINODON VARIEGATUS.

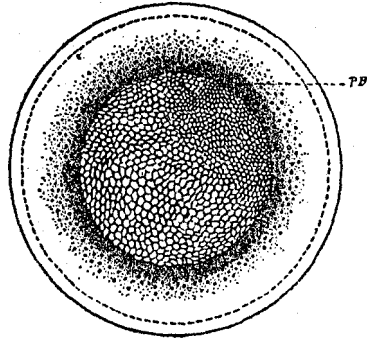FIG. 5.—Egg with blastoderm of 16 cells.   FIG. 6.—Egg with blastoderm in late cleavage stage; PB, periblast.

of the peripheral area of the blastoderm. (A late stage in the differentiation of the germ ring in an egg of *Lucania parva* is illustrated in figure 22.) This apparent thickening is due primarily, as observed by Götte,[b] to the thinning of the central area of the blastoderm and secondarily to the ingrowth (invagination) of the marginal cells. The part played by invagination can not be satisfactorily studied in living material. For a detailed discussion of the rôle of invagination in the formation of the germ ring and the embryonic shield the reader is referred to Wilson's valuable paper on the embryology of the sea bass.[c]

As the central area of the blastoderm becomes thinner its under surface becomes distinctly concave. The subgerminal cavity between the blastoderm and the central periblast is now closed in on all sides by the germ ring.

Before the germ ring is completely differentiated it becomes apparent that invagination begins earlier and advances more rapidly at one pole than round the rest of the

a Agassiz and Whitman: On the development of some pelagic fish eggs. Proceedings, American Academy of Arts and Sciences, vol. 20, 1884.

b Götte, A.: Der Keim des Forelleneies. Archiv. f. Mikr. Anat., 1873.

c Wilson, H. V.: The embryology of the sea bass (*Serranus atrarius*). Bulletin, United States Fish Commission, vol. IX, 1889, p. 209-277.

periphery of the blastoderm. From this pole, which is the posterior or embryonic pole of the blastoderm, a broad tongue of cells several layers in depth grows forward into the subgerminal cavity. While the germ ring is becoming differentiated the blastoderm gradually increases in size by centrifugal growth. After the germ ring is completely differentiated the growth of the blastoderm round the yolk continues more rapidly than in the earlier stages. The broad tongue of cells growing forward from the germ ring at the posterior pole of the blastoderm becomes longer and gradually assumes a roughly triangular form. In this manner the embryonic shield becomes distinctly outlined. As the embryonic shield increases in size a thickening representing the axis of the future embryo occurs along its anteroposterior axis. As soon as this linear thickening occurs an embryonic and an extra-embryonic area may be distinguished within the embryonic shield. Figures 7 and 8 illustrate two successive stages in the differentiation of the embryonic shield and the embryonic axis. The linear thickening advances anteriorly from the posterior pole of the blastoderm but the differentiation of the embryonic axis begins in the head region and advances posteriorly. The embryonic area soon
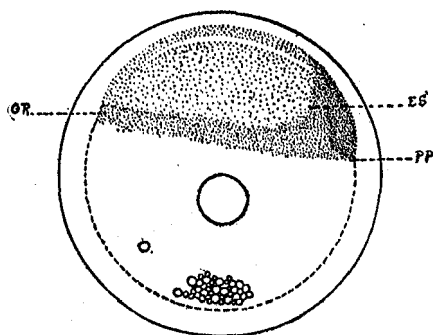

CYPRINODON VARIEGATUS.

FIG. 7.—Egg with blastoderm showing germ ring, GR, fully differentiated and an early stage in differentiation of embryonic shield, ES; PP, posterior pole of blastoderm.
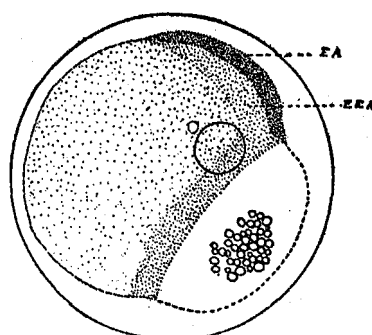
FIG. 8.—Egg showing later stage in differentiation of embryonic shield; EA, embryonic area; EEA, extra-embryonic area.

becomes broader in the anterior or head region than in the posterior region. In surface view the embryonic area now presents a more or less regular spatulate form. During the formation of the embryonic shield and the differentiation of the embryonic axis the growth of the blastoderm round the yolk advances very rapidly. Before the embryo is well differentiated (fig. 8) the blastoderm covers more than three-fourths the surface of the yolk. Before segmentation of the embryo becomes apparent the blastoderm has grown completely round the yolk and the blastopore is closed (fig. 9).

The closure of the blastopore occurs less than 24 hours after fertilization. At this time the embryo extends less than halfway round the circumference of the yolk. It is relatively short and thick and somewhat irregular in outline. There is as yet no evidence of pigmentation and the embryo is almost transparent.

Soon after the closure of the blastopore relatively large melanophores appear sparsely scattered on the surface of the embryo and throughout the extra-embryonic blastoderm. Yellow chromatophores appear somewhat later. The latter never become numerous in the extra-embryonic blastoderm but soon become more numerous on the

embryo than the melanophores.   Figure 10 is an attempt to illustrate the distribution of chromatophores on the embryo and in the extra-embryonic blastoderm about 48 hours after fertilization.   No attempt is here made to distinguish between black and yellow chromatophores.   The embryo is now segmented throughout and circulation is established.   The chromatophores in the extra-embryonic blastoderm show a marked tendency to become aggregated along the larger blood vessels.

As development advances the yolk mass becomes materially reduced.   At 72 hours after fertilization (fig. 11) the embryo appears relatively large and plump.   The posterior portion of the body is free from the yolk and moves freely within the egg membrane.

As the time of hatching approaches the yolk mass may be reduced to half its original volume.   The embryo is well developed and exhibits a characteristic distribution of
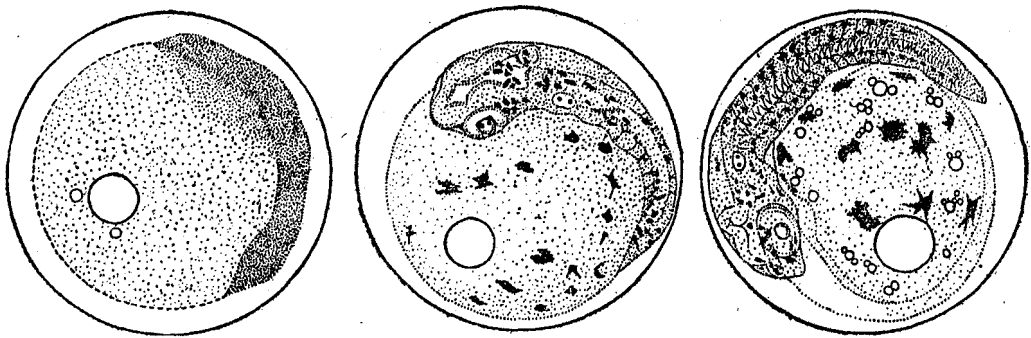
CYPRINODON VARIEGATUS.

FIG. 9.—Egg showing an early stage in   FIG. 10.—Egg 48 hours after fertilization.   FIG. 11.—Egg 72 hours after fertilization.
    differentiation of embryo.

chromatophores.   It remains relatively short and plump.   Its length usually does not exceed the circumference of the egg.

Larval development.—Incubation at laboratory temperature occupies five to six days.   The newly hatched larvæ (fig. 12) are approximately 4 mm. in length and relatively plump.   The yolk sac remains relatively large but the head is not deflected.   The dorsal fin fold has its origin relatively far posteriorly.   Both dorsal and ventral fin folds are continuous.   The depth of each fold is less than half the depth of the body posterior to the vent.   The vent is located at the posterior margin of the yolk sac.   The larva is slightly yellowish in color and the posterior half of the body is marked by lighter and darker vertical bands.

At five days after hatching (fig. 13) the yolk is almost completely absorbed.   The larvæ are now 5 mm. or over in length.   The head has become bluntly pointed and the depth of the body has somewhat increased.   The general color remains slightly yellowish and the vertical bands are somewhat more conspicuous than in the preceding stage.

Figure 14 illustrates a young fish 9 mm. in length.   At this stage many of the characters of the adult are already apparent.   The body is relatively slender, however, and the back is not yet elevated.   The vertical bands characteristic of the species are present but not fully developed.

Young fish 12 mm. in length (fig. 15) exhibit practically all of the diagnostic characters of the species. The back is becoming strongly elevated and the depth of the body is proportionally greater than in the preceding stage. The caudal fin still
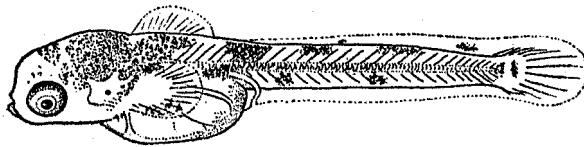
FIG. 12.—Newly hatched fish, actual length 4 mm.

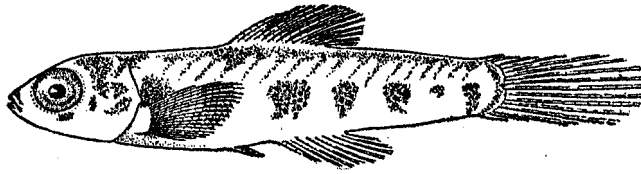FIG. 13.—Larval fish 5 days after hatching, actual length 5 mm.
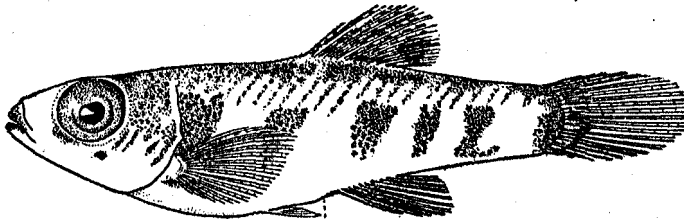
FIG. 14.—Young fish 9 mm. in length.

FIG. 15.—Young fish 12 mm. in length.

CYPRINODON VARIEGATUS.

remains more rounded than in the adult. The coloration is quite characteristic, although the general color is lighter and the light vertical bands are more conspicuous than in the adult.

LUCANIA PARVA. RAINWATER-FISH.

Spawning.—The spawning season of this species, like that of *Cyprinodon variegatus*, continues throughout the summer. According to the records kept by Mr. Hildebrand, females ripe for spawning were taken in Mullet Pond on April 17. He also observed at this time that in addition to mature ova the ovaries contained immature ova in various stages of development and suggested the probability that more than one brood is produced during the season. Young ranging from 15 to 19 mm. in length were taken on May 25. During the latter half of July, when the present study was begun, this species was still spawning freely, although a few of the females taken were entirely spent. The ovaries of many of the females gravid with mature ova still contained immature ova in more than one stage of development. Females ripe for spawning were taken in consid-

erable numbers as late as August 15.   During the latter half of August very few females with mature ova were taken.   Young ranging from 10 to 30 mm. and over in length were present in abundance throughout July and August.

Eggs.—The mature unfertilized ova (fig. 16) are spherical in form and 1.1 to 1.3 mm. in diameter.   Their specific gravity is slightly greater than that of sea water and they
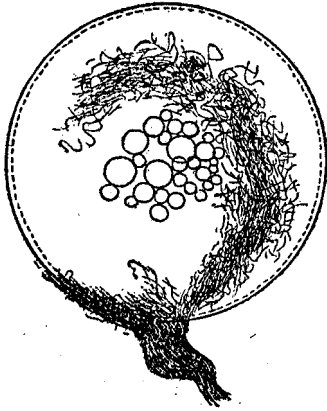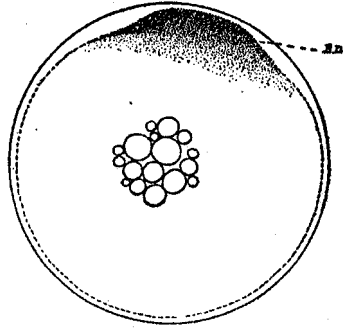
LUCANIA PARVA.

FIG. 16.—Mature unfertilized egg. × 35.   FIG. 17.—Egg showing early stage in formation of blastodisc (BD).

are held together in loose clumps by a tangle of coarse adhesive threads.   They are very slightly yellowish in color and almost transparent.   The egg membrane is relatively thick and horny.   A small perivitelline space is apparent but not conspicuous.   The micropyle is relatively small.   The yolk sphere contains a group of oil globules of unequal size, varying from 12 to 20 in number, which normally rests at the upper pole.
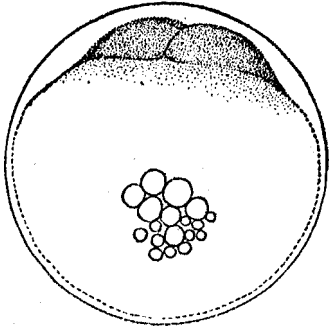
LUCANIA PARVA.

FIG. 18.—Egg with blastoderm of 2 cells.   FIG. 19.—Egg with blastoderm of 4 cells.
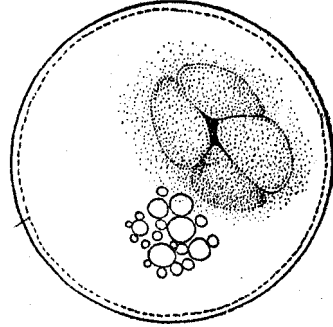
Embryology.—The eggs of this species develop in a manner quite typical for teleosts. The course of their development conforms essentially to the course of development as outlined above for the eggs of *Cyprinodon variegatus*.   The embryology of this species will, therefore, be discussed but briefly and with reference to the above discussion of the embryology of the former species.

The quantity of protoplasm contained in these eggs is relatively less than that contained in the eggs of the former species and early development advances somewhat more rapidly. The blastodisc is well developed one hour after fertilization. An early stage in the development of the blastodisc is illustrated in figure 17. It is now lenticular in form, but before cleavage occurs it becomes of nearly uniform thickness throughout
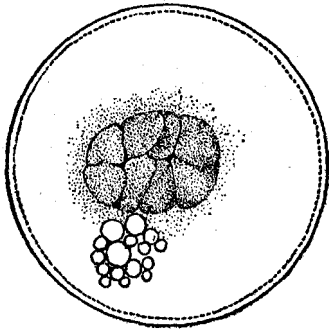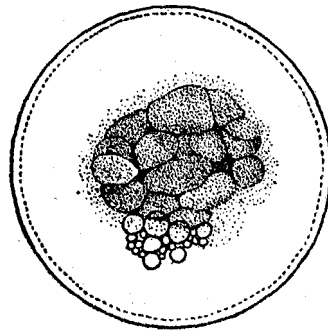
LUCANIA PARVA.

FIG. 20.—Egg with blastoderm of 8 cells.   FIG. 21.—Egg with blastoderm of 16 cells.

the central area and thins out abruptly near the periphery. The first act of cleavage usually occurs within one and one-fourth hours after fertilization. A typical 2-cell stage is illustrated in figure 18. Successive stages of cleavage are illustrated in figures 19 to 21.

Later cleavage advances relatively rapidly and at 13 hours after fertilization the germ ring is completely differentiated (fig. 22, GR). Figure 23 illustrates a late stage in
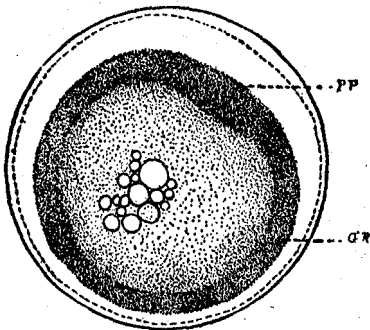
LUCANIA PARVA.

FIG. 22.—Egg with blastoderm showing germ   FIG. 23.—Egg with blastoderm showing advanced stage
ring (GR) fully differentiated; PP, posterior   in differentiation of embryonic shield; EA, embryonic
pole of blastoderm.   area; EEA, extra-embryonic area. GR, germ ring.

the formation of the embryonic shield in which the embryonic axis is already differentiated. The blastoderm now covers more than half the surface of the yolk.

At 24 hours after fertilization (fig. 24) the embryo is well differentiated and the blastopore is closed. At this stage the length of the embryo is less than half the circumference of the yolk sphere and is relatively more slender than the embryo of *Cyprinodon variegatus* at a corresponding stage.

The later development of the embryo advances relatively slowly. Incubation at laboratory temperature occupies seven to eight days. Before the close of the second

day of incubation the embryo becomes segmented throughout and circulation is established. An attempt is made in figures 26 and 27 to illustrate the course of the larger blood vessels in the extra-embryonic blastoderm 68 hours after fertilization.

A late stage in the development of the embryo is illustrated in figure 29. It is now relatively large, its length exceeding the circumference of the egg. The yolk mass is materially reduced and the embryo is free to move within the egg membrane.
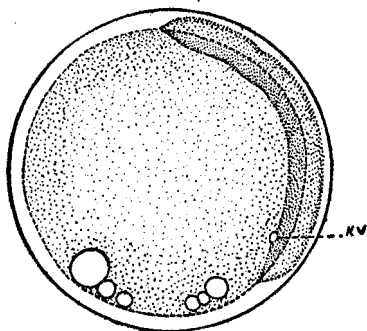
LUCANIA PARVA.

FIG. 24.—Egg 24 hours after fertilization; KV, Kupffer's vesicle.   FIG. 25.—Egg 48 hours after fertilization.
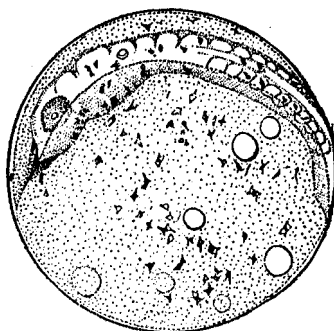
Pigmentation.—The eggs of *Lucania parva* afford very favorable material for the study of the development of chromatophores. While the embryonic shield is becoming differentiated, cells which may be recognized in the living material by their refractile properties proliferate from its inner margin and from the inner margin of the germ ring and become sparsely scattered over the blastoderm. These cells are irregular in outline and usually send out a relatively small number of slender protoplasmic processes. They
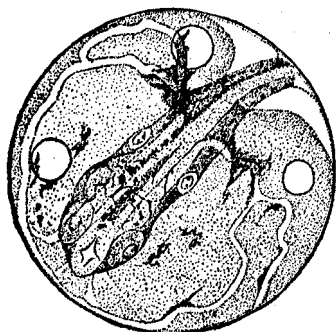
LUCANIA PARVA.

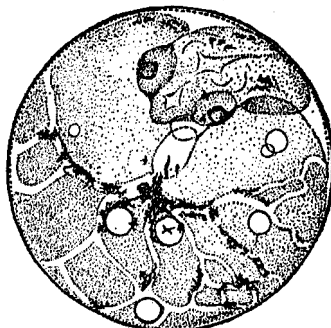FIG. 26.—Egg 68 hours after fertilization, viewed from the dorsal aspect of the head of the embryo.   FIG. 27. Egg 68 hours after fertilization, viewed from the anterior aspect of the head of the embryo.

undergo slow ameboid movements which involve form changes of the cell body rather than marked extension and retraction of protoplasmic processes. These cells at first appear isolated. Before the embryonic axis is well differentiated many of them are apparently connected by their protoplasmic processes and form a syncytial network which involves the entire extra-embryonic area of the blastoderm. Some of the ameboid cells still remain isolated.

Soon after the closure of the blastopore, i. e., about 24 hours after fertilization, melanin granules arise in some of these ameboid cells. These granules first appear in the central region of the cell, i. e., in proximity with the nucleus, and gradually push out toward the periphery or into the protoplasmic processes. Under high magnification the movements of these granules may be readily observed. They are apparently determined by the movements in the cytoplasm.

In the course of a few hours after the appearance of the first melanin granules, yellow pigment granules arise in some of the ameboid cells. Like the melanin granules, the yellow pigment granules arise in the central region of the cell and later push out toward the periphery. The movements of these granules are apparently identical with those of the melanin granules.

The phenomena involved in the development of chromatophores could not be as satisfactorily observed on the embryo as in the extra-embryonic blastoderm. Pigment arises in the chromatophores on the embryo simultaneously with the appearance of pigment in the chromatophores in the extra-embryonic blastoderm. Furthermore the
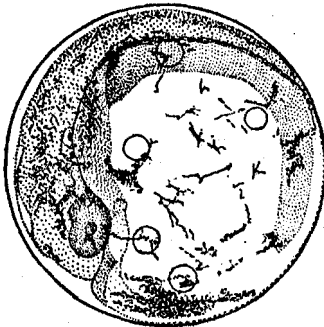

LUCANIA PARVA.

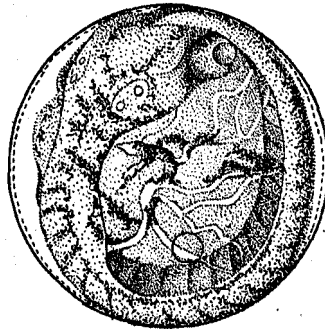Fig. 28.—Egg 90 hours after fertilization.   Fig. 29.—Egg just before hatching.

chromatophores on the embryo, during the early stages of development, are cells of essentially the same character as those in the extra-embryonic blastoderm. It is quite probable that they arise in the same manner. Figure 25 is an attempt to illustrate the distribution of chromatophores on the embryo and the extra-embryonic blastoderm about 44 hours after fertilization.

After circulation becomes well established the majority of the chromatophores in the extra-embryonic blastoderm become aggregated along the larger blood vessels. The distribution of chromatophores along the blood vessels in the extra-embryonic blastoderm 68 hours after fertilization is illustrated in figures 26 and 27.

As indicated above, the pigment granules arise in the central region of the cell and gradually push out toward the periphery. Until pigment is present in all parts of the cell the parts free from pigment remain clear. Even after pigment has been present in all parts of the cell it may become concentrated in the central region leaving the peripheral region clear. In many instances as the pigment becomes concentrated isolated granules or groups of granules remain far out in the protoplasmic processes. The concentration and redistribution of pigment granules is obviously not due to ameboid movements of the cells but to movements of the pigment granules in the

cytoplasm. This conclusion is in full accord with the findings of Franz (1908) in larvæ of *Pleuronectes platessa.*[a]

As development advances ameboid movements of the chromatophores become less apparent. No conclusive evidence of ameboid movement of chromatophores was secured during the later stages of embryonic development or in newly hatched fishes.

Larval development.—The newly hatched larvæ are 4.5 to 5 mm. in length. The yolk sac remains large but the head is not deflected. The dorsal fin fold has its origin relatively far posteriorly. Both dorsal and ventral fin folds are continuous. The depth of each fold does not exceed half the depth of the body posterior to the vent. The vent is located at the posterior margin of the yolk sac. The color is light yellow and quite uniform.

At seven days after hatching (fig. 31) the larvæ have grown to a length of approximately 6 mm. The yolk is completely absorbed. The head is slightly depressed and
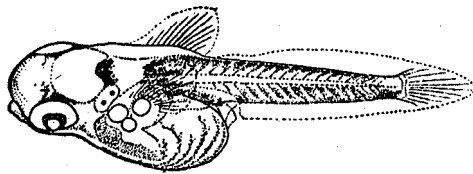

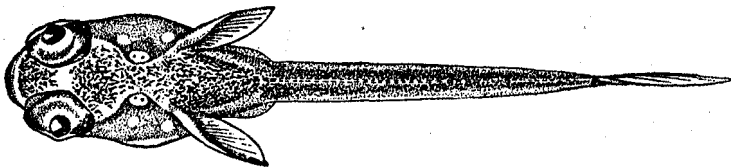FIG. 30.—Newly hatched fish, actual length 4.5 mm.

FIG. 31.—Larval fish 7 days after hatching, actual length 6 mm.

LUCANIA PARVA.

the depth of the body is somewhat greater than in the preceding stage. The color remains light yellow.

The young of this species assume the general appearance of the adults relatively early. Young 15 to 20 mm. in length show many of the diagnostic characters of the species. The larger young taken in July and August were almost identical in appearance with the adults.

KIRTLANDIA VAGRANS. ROUGH SILVERSIDE.

Spawning.—During the latter half of July and the first week in August a few females of this species ripe for spawning were brought into the laboratory. The great majority of the females taken during this period were already spent. The height of the spawning season obviously occurs earlier in the summer.

Eggs.—The mature unfertilized ova are spherical in form and 0.8 to 1 mm. in diameter. They are slightly heavier than sea water and are held together in clumps by a tangle of adhesive threads, a small tuft of which arises from the membrane of each egg. They are slightly yellowish in color and almost transparent. A small perivitelline

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[a] Franz, V.: Die Struktur der Pigmentzelle. Biologisches Zentralblatt, vol. 28, p. 536–543.

space is apparent between the egg membrane and the vitelline membrane. The micro-pyle is relatively small. The yolk sphere contains a group of oil globules of unequal size, varying from about 8 to 15 in number, which normally rests at the upper pole.

Embryology.—The embryological development of these eggs conforms in all essential respects to the course of development as above outlined for the eggs of *Cyprinodon*
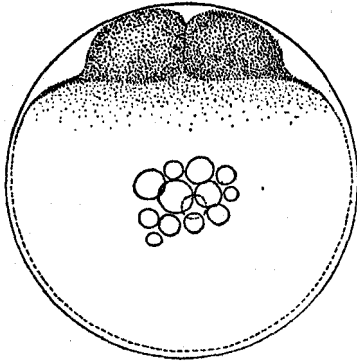
KIRTLANDIA VAGRANS.

FIG. 32.—Egg with blastoderm of 2 cells. × 50.   FIG. 33.—Egg with blastoderm of 4 cells.
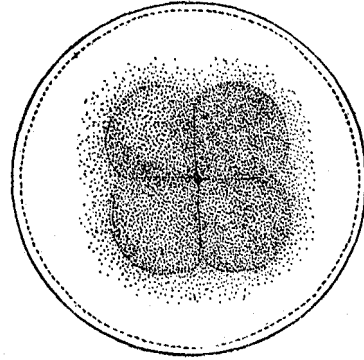
variegatus. A detailed discussion of the embryology of this species would therefore be superfluous. Successive stages in the process of cleavage and the differentiation of the embryo are illustrated in figures 32 to 37. Early development advances somewhat more rapidly than in the eggs of *Cyprinodon*. The embryo is well differentiated less
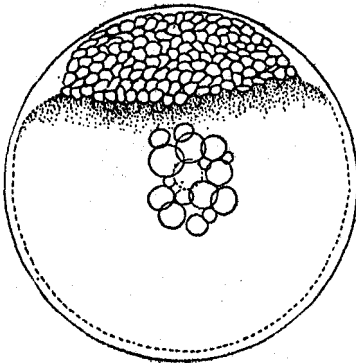
KIRTLANDIA VAGRANS.
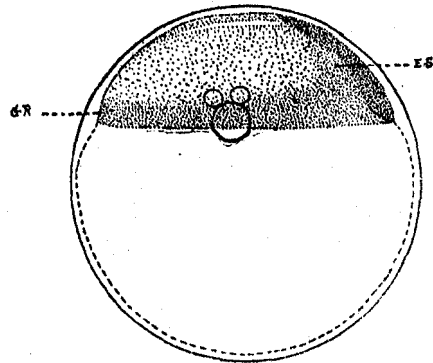
FIG. 34.—Egg with blastoderm in advanced cleavage stage.

FIG. 35.—Egg with blastoderm showing germ ring (GR) fully differentiated and an early stage in differentiation of embryonic shield (ES).

than 20 hours after fertilization. At 40 hours after fertilization (fig. 38) the embryo is relatively large. It is segmented throughout and circulation is well established. A few chromatophores are now apparent on the anterior region of the body. A few hours later a relatively small number of melanophores arises also in the extra-embryonic blastoderm.

Figure 39 illustrates an egg shortly before hatching. The length of the embryo now exceeds the circumference of the egg. The yolk mass is materially reduced and the embryo moves freely within the egg membrane. Pigmentation has not increased materially and the embryo remains relatively transparent.

Larval development.—The incubation period, at laboratory temperature, occupies six to seven days. The newly hatched larvæ (fig. 40) are approximately 5 mm. in length.
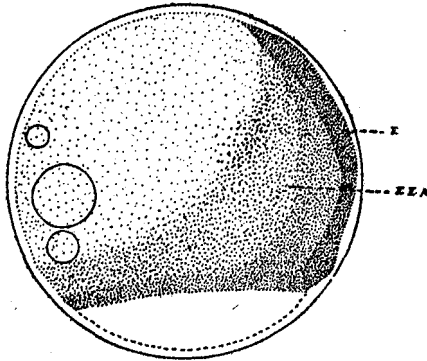

KIRTLANDIA VAGRANS.

FIG. 36.—Egg showing an early stage in differentia-
tion of embryo (E); EEA, extra-embryonic area of
embryonic shield.
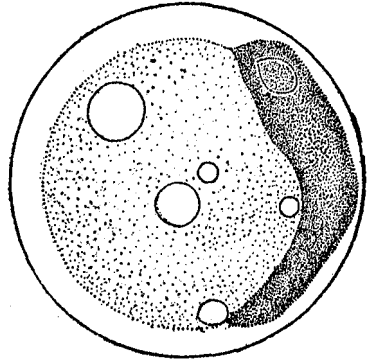
FIG. 37.—Egg showing later stage in differ-
entiation of embryo.

The yolk sac is relatively small. The dorsal fin fold has its origin just posterior to the head. Both dorsal and ventral fin folds are continuous. The depth of each fold does not exceed half the depth of the body at the vent. The vent is located near the posterior margin of the yolk sac. The newly hatched larvæ are highly transparent. A few pig-
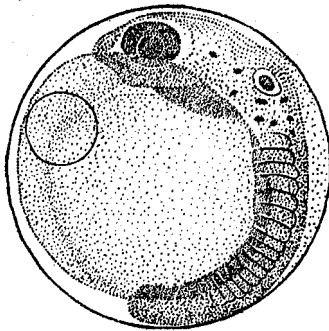

KIRTLANDIA VAGRANS.

FIG. 38.—Egg 40 hours after fertilization.
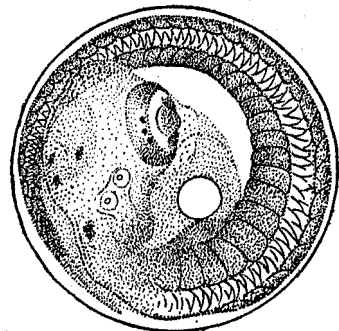
FIG. 39.—Egg just before hatching.

ment spots occur on the dorsal aspect of the head and in a line at the base of the ventral fin fold.

Figure 41 illustrates a young fish 9 mm. in length. The body is relatively slender. The soft dorsal and anal fins are becoming differentiated. A few pigment spots remain scattered on the dorsal aspect of the head and a dark longitudinal line extends along the side of the body. The silvery character of the side is not yet apparent.

Figure 42 illustrates a young fish 11 mm. in length.   At this stage the young exhibit some of the characters of the adult.   The characteristic number of rays are present in the soft dorsal and anal fins.   The spinous dorsal is not yet fully differentiated.   The
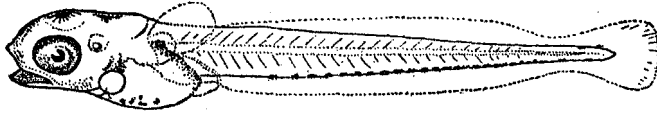

FIG. 40.—Newly hatched fish, actual length 5 mm.

FIG. 41.—Young fish 9 mm. in length.

FIG. 42.—Young fish 11 mm. in length.

KIRTLANDIA VAGRANS.

color remains lighter than that of the adult.   The sides are becoming distinctly silvery, but the pale green on the back, characteristic of the species, is not yet apparent.

GOBIOSOMA BOSCI. NAKED GOBY.

Spawning.—This relatively obscure species was found spawning in Mullet Pond throughout August.   The majority of the females taken during this period were already spent.   Only occasionally was one found ripe for stripping.   Obviously the height of the spawning season was past.

Eggs.—The mature unfertilized ova (fig. 43) are approximately spherical in form and about 0.5 mm. in diameter.   They are yellow in color and opaque.   Their specific gravity is greater than that of sea water.   In the ovary they are attached to a central rachis by a thick peduncle composed of bundles of minute hairlike threads and inserted in the egg membrane.   When stripped from the female they remain aggregated in a compact clump.   The egg membrane is comparatively thin and closely applied to the vitelline membrane.

As soon as fertilization has taken place the egg membrane begins to expand and gradually assumes an elliptical form.   When fully expanded the major axis is 1.2 to 1.4 mm. in length, the minor axis 0.5 to 0.7 mm.   The point at which the peduncle is inserted remains at one pole of the major axis.   The egg remains located near one pole of the major axis where it lies in a cavity the volume of which is somewhat greater than its own. The wall of this cavity is indicated by a dotted line in figure 44.   It is indicated also at a later stage in figure 48.

Embryology.—The volume of protoplasm is considerably greater in proportion to the volume of yolk in these eggs than in the more typical teleostean eggs described above. In the unfertilized egg the protoplasm is disposed in a layer of uniform thickness investing

the yolk sphere. After fertilization has taken place this layer of protoplasm becomes concentrated in a typical manner to form the blastodisc (fig. 44, BD). The protoplasmic movements involved in the process of concentration can not be satisfactorily observed by reason of the opacity of the yolk. The fully differentiated blastodisc is relatively thick and covers a relatively larger area of the surface of the yolk than is the case in the more typical eggs described above. It thins out gradually toward the periphery and
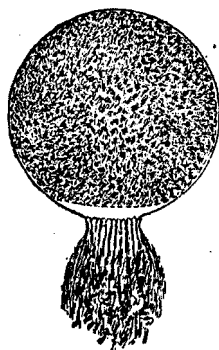
GOBIOSOMA BOSCI.
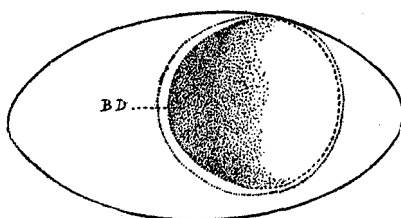
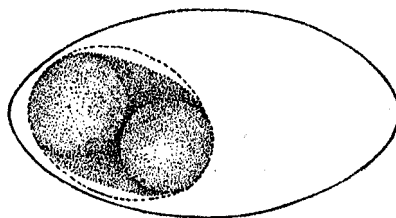FIG. 43.—Mature unfertil-   FIG. 44.—Egg with fully developed blastodisc   FIG. 45.—Egg with blastoderm of 2 cells.
    ized egg. × 50.   (BD).

fades away almost imperceptibly into the thin layer of protoplasm which remains at the surface of the yolk.

Cleavage occurs essentially as in the eggs above described. The volume of protoplasm being relatively greater, however, the cleavage furrows become deeper and the early blastomeres become more widely separated and show a more marked tendency to become spherical in form. After the first act of cleavage is completed the first two
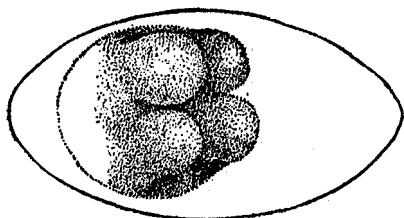

GOBIOSOMA BOSCI.

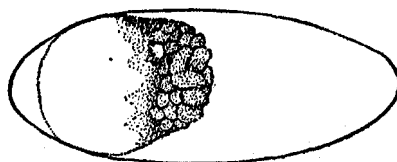FIG. 46.—Egg with blastoderm of 4 cells.   FIG. 47.—Egg with blastoderm of many cells.

blastomeres, in surface view, appear circular in outline. The same tendency is apparent also in the 4-cell stage. After the second act of cleavage is completed the first four blastomeres stand out in perspective (fig. 46) as more or less isolated rounded elevations. As cleavage advances the blastoderms exhibit a greater degree of irregularity than is observed in the more typical teleostean eggs. Figure 47 illustrates an egg four hours after fertilization. The blastoderm is now circular in outline but remains relatively thick.

As cleavage advances the blastoderm becomes distinctly dome-shaped and a small cleavage cavity becomes apparent. The periblast appears relatively thick, but can not be satisfactorily observed in the living material by reason of the opacity of the yolk. The phenomena involved in the formation of the germ ring and the early differentiation

of the embryo also are somewhat obscured in the living material. As far as may be observed, however, these stages conform in all essential respects to the corresponding stages above described in the egg of the *Cyprinodon variegatus*.

The later development of the embryo advances rapidly. At 48 hours after fertilization (fig. 48) the embryo is well formed and already shows six to eight somites. At 60
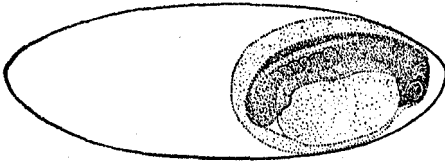

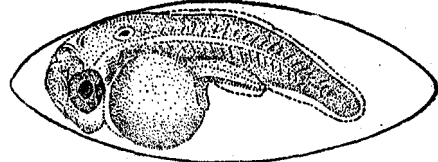FIG. 48.—Egg 48 hours after fertilization.
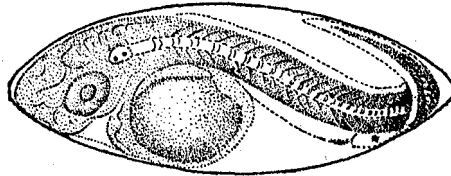
FIG. 49.—Egg 60 hours after fertilization.

FIG. 50.—Egg 96 hours after fertilization.

GOBIOSOMA BOSCI.

hours after fertilization (fig. 49) the embryo has increased materially in size. Its length exceeds three-fourths the length of the major axis of the egg membrane. The yolk mass remains opaque, but the embryo is highly transparent. No pigment is as yet apparent.
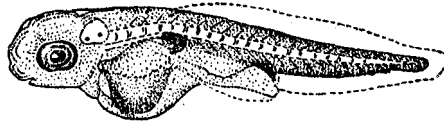

FIG. 51.—Newly hatched fish, actual length 2 mm.
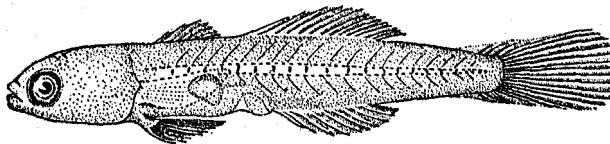
FIG. 52.—Larval fish 3 mm. in length.

FIG. 53.—Young fish 10 mm. in length.

GOBIOSOMA BOSCI.

As the time of hatching approaches (fig. 50) the length of the embryo exceeds the length of the major axis of the egg membrane and the tail becomes bent upon itself. The embryo remains highly transparent, only a few small pigment spots appearing in proximity with the vent.

Larval development.—The incubation period at laboratory temperature occupies approximately five days. The newly hatched larvæ (fig. 51) are approximately 2 mm. in length and almost transparent. The remaining yolk mass is opaque. The air bladder is already apparent at the posteriodorsal aspect of the yolk mass. The dorsal fin fold has its origin relatively far posteriorly. Both dorsal and ventral fin folds are continuous. The depth of each fold does not exceed half the depth of the body at the level of the vent. The vent is located less than half the length of the body from the posterior end. A few small pigment spots occur just above the vent and at the base of the ventral fin fold posterior to the vent.

Figure 52 illustrates a larval fish 3 mm. in length. The yolk is completely absorbed. The body remains almost transparent. The line of pigment spots at the base of the ventral fin fold is somewhat more conspicuous than in the newly hatched larvæ.

Young fish 10 mm. in length (fig. 53) show many of the diagnostic characters of the species but remain almost free from pigment. The air bladder is still apparent microscopically. The head is gradually assuming the shape characteristic of the species. The fins are well differentiated and the sucking disc formed by the ventrals is well developed.

CTENOGOBIUS STIGMATICUS. SCALLOPFISH.

Spawning.—Like the related species, *Gobiosoma bosci*, this species was spawning in Mullet Pond throughout August. As in the case of the former species, also the majority
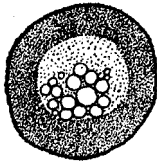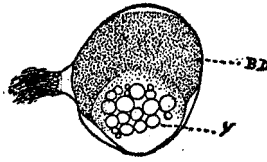

CTENOGOBIUS STIGMATICUS.

FIG. 54.—Mature un-   FIG. 55.—Egg with fully devel-   FIG. 56.—Egg with   FIG. 57.—Egg with
fertilized egg. X   oped blastodisc (BD); Y, yolk.   blastoderm of 2   blastoderm of 4
60.   cells.   cells.
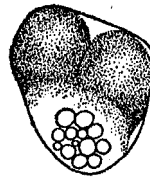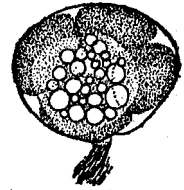
of the females taken were already spent. Obviously, the height of the spawning season occurs earlier in the summer.

Eggs.—The mature unfertilized ova (fig. 54) are very small, having an average diameter of approximately 0.3 mm., and somewhat irregular in form. They are yellow in color and highly translucent. Each ovum is attached in the ovary by a slender peduncle composed of very minute threads inserted in the egg membrane. When the female is stripped the eggs do not remain aggregated. They are only slightly adhesive. Their specific gravity is very little greater than that of sea water. The egg membrane is thin and delicate and is usually drawn out into a blunt apex at the insertion of the peduncle. Except on this side the egg membrane appears closely applied to the vitelline membrane. These eggs are characterized by a relatively enormous amount of protoplasm and very little yolk. The transparent yolk mass usually rests in the center of the mass of protoplasm, but in some eggs it occupies a somewhat eccentric position.

Blastodisc.—As soon as fertilization has taken place the protoplasm becomes concentrated to form the blastodisc (fig. 55, BD). The process of concentration can hardly be described as a "streaming" of the protoplasm toward one pole of the yolk sphere, but rather as a thinning of the layer of protoplasm on one side of the yolk and a corresponding thickening on the opposite side which pushes the yolk out into an extremely eccentric position. The fully differentiated blastodisc covers approximately half the area of the surface of the yolk. The other half remains invested by a very thin layer of protoplasm, which is continuous with the blastodisc.

Segmentation.—Early cleavage takes place essentially as in the more typical teleostean eggs, but advances more rapidly. The first act of cleavage occurs approximately 30 minutes after fertilization and the successive acts follow each other in rapid succession. Because of the disparity of yolk the figures presented by the early cleavage stages differ widely from those presented by the corresponding stages of the more typical eggs. The first cleavage plane cuts deeply into the blastodisc and divides it into two blastomeres of approximately equal volume (fig. 56). They are usually quite symmetrical but may show considerable variation. The second cleavage plane cuts the first at right angles. Figure 57 illustrates a typical blastoderm of four cells viewed through the transparent yolk. The blastomeres are quite symmetrically
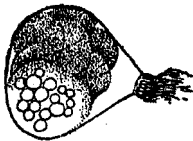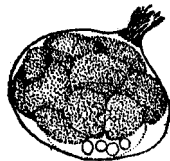
CTENOGOBIUS STIGMATICUS.

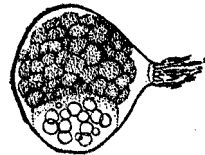| FIG. 58.—Egg with blastoderm of 8 cells.	FIG. 59.—Egg with blastoderm of 16 cells.	FIG. 60.—Egg with blastoderm of many cells.

arranged and are seen to extend beyond the periphery of the yolk. They remain continuous with the thin layer of protoplasm by which the latter is invested.

The early blastoderm usually spreads widely over the surface of the yolk, the blastomeres being arranged in a single series. Figure 58 illustrates an egg with a blastoderm of 8 cells which deviates somewhat from this condition, the blastomeres appearing heaped up at one side of the yolk. Figure 59 illustrates an egg with a very typical blastoderm of 16 cells. The cells remain in a single series and the yolk sinks deeply into the concavity of its inner surface.

As cleavage advances beyond the 16-cell stage the cells no longer remain in a single series but become heaped up on one side of the yolk. Eggs in advanced stages of cleavage are illustrated in figures 60 and 61.

During the later stages of cleavage the granular protoplasm on the surface of the yolk at the margin of the blastoderm becomes somewhat more conspicuous (fig. 61). This slightly thickened zone in the layer of protoplasm investing the yolk doubtless represents the periblast. It does not become differentiated into a well-marked ridge, however, and nuclei were not apparent in it.

As cleavage advances further the blastoderm becomes more distinctly dome-shaped, its central area becomes appreciably thinner, and the periphery advances round the

yolk. The blastoderm is now distinctly thickest at the periphery. A well-marked germ ring, however, is not apparent. The yolk mass becomes constricted at the level of the periphery of the blastoderm and is apparently squeezed up into the dome-shaped space beneath its central area, almost entirely obliterating the cleavage cavity (fig. 62). As the peripheral growth of the blastoderm advances still further the yolk is entirely engulfed and the blastopore is closed.

Differentiation of the embryo.—The closure of the blastopore occurs within six hours after fertilization. Soon after this stage is reached a distinct linear thickening of the
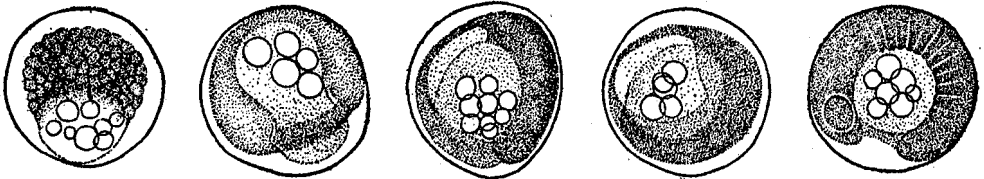
CTENOGOBIUS STIGMATICUS.

FIG. 61.—Egg with blastoderm in late cleavage stage.

Fig. 62.—Egg with blastoderm growing round yolk, shortly before closure of blastopore, 5 hours after fertilization.

FIG. 63.—Egg showing early stage in differentiation of embryo, 6 hours after fertilization.

FIG. 64.—Egg showing later stage in differentiation of embryo.

FIG. 65.—Egg showing embryo well differentiated.

blastoderm, representing the axis of the future embryo, grows anteriorly from the blastopore (fig. 63). Whether invagination of cells from the periphery of the blastoderm plays a part in the differentiation of the linear thickening could not be determined in the living material. A distinct embryonic shield was at no time apparent. As this linear thickening of the blastoderm advances anteriorly, the subgerminal cavity becomes apparent at its anterior extremity. As the differentiation of the embryonic axis advances the anterior region of the differentiated area of the blastoderm becomes dis-
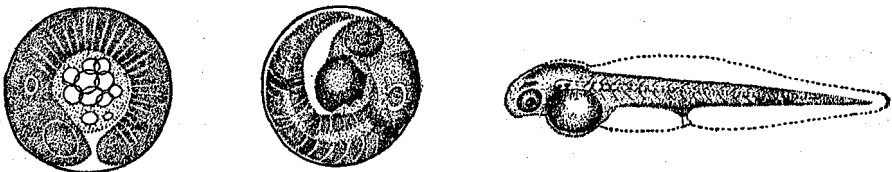
CTENOGOBIUS STIGMATICUS.

FIG. 66.—Egg 11 hours after fertilization.

FIG. 67.—Egg just before hatching.

FIG. 68.—Newly hatched fish, actual length 1.2 mm.

tinctly broader than the posterior region (fig. 64). Obviously, the differentiation of the embryo begins in the anterior or head region and advances posteriorly.

The further differentiation of the embryo advances rapidly. Within 11 hours after fertilization (fig. 65) the embryo is well formed and already shows 10 to 12 somites. At 12 hours after fertilization (fig. 66) the embryo makes almost a complete turn within the egg membrane. The posterior region of the body is already free from the yolk. Pigment is not yet present and the embryo is highly transparent. Figure 67 illustrates an egg just before hatching. The yolk mass is materially reduced. The embryo remains highly transparent but is marked by small areas of delicate pigment. It now makes more than a complete turn within the egg membrane.

Larval development.—The period of incubation at laboratory temperature occupies not over 18 hours. The newly hatched larvæ are approximately 1.2 mm. in length and exceedingly delicate. They remain highly transparent but are marked by small areas of delicate yellow pigment on the dorsal aspect of the head, just over the vent, and in a vertical band approximately halfway from the vent to the tip of the tail. Both dorsal and ventral fin folds are continuous. The depth of each fold is equal to or greater than the depth of the body posterior to the vent. The vent is located a little less than half the length of the body from the anterior end.

These larvæ being extremely delicate, it was not found possible to keep them alive in the laboratory longer than a few hours. As no recently hatched young were taken, the advanced larval stages can not be described. Young ranging from 25 to 30 mm. in length have already assumed the general appearance of the adults and present many of the diagnostic characters of the species.