NOTES ON THE HABITS, MORPHOLOGY OF THE REPRODUCTIVE ORGANS, AND EMBRYOLOGY OF THE VIVIPAROUS FISH GAMBUSIA AFFINIS

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INTRODUCTION.

Gambusia affinis (Baird and Girard), according to Smith (1907), "is found along the coast from Delaware to Mexico and reaches inland as far as Illinois. In North Carolina it is excessively abundant in the lowlands, in swamps, ditches, creeks, and also in the open waters of the rivers." [a] It is known primarily as a fresh-water species, but occurs also in brackish water. Early in July and again on August 1, 1912, a considerable number of these fish were taken along the swampy borders of Mullet Pond, Shackleford Banks. The specific gravity of the water in which they were taken on August 1 was 1.0081. This reading, however, does not represent the normal specific gravity of the water along these swampy borders, as considerable rain had fallen during the preceding 12 hours. At the time the above reading was taken, water from the central part of Mullet Pond showed a specific gravity of 1.0106. On July 24, 1912, a single specimen was caught in the seine in the terrapin pens at the Beaufort Laboratory. This was a large female, bearing mature, unfertilized eggs. The water in these pens is salt, only a very little fresh water entering through small pipes from an artesian well.

Twenty-four of the fish taken in Mullet Pond on August 1 were transferred to sea water in a small aquarium, where they remained for a period of 10 days. At the end of this time 1 was found dead. The remaining 23 were apparently in a normal condition; they had, however, lost much of their pigment and their tissues had become slightly transparent.

During the entire month of July, 1912, these fish were present in abundance in a brooklet emptying into Beaufort Harbor just east of Beaufort. The water in this brooklet is supplied largely by springs. It was reddish brown with organic matter and contained considerable débris. Most of the fish used in this study were taken in this brooklet.

The generic name *Gambusia* is derived from the name "Gambusina," commonly used in Cuba, which means "small" or "of no importance." While of no commercial value, this species has an important economic worth. It feeds largely on insects and insect larvæ. Wherever it inhabits waters in which mosquitoes breed the mosquito larvæ constitute its principal food. The introduction of these fish into the natural

[a] Smith, H. M.: Fishes of North Carolina. North Carolina Geological and Economic Survey, vol. II, p. 153.

waters as well as into artificial ponds, aquatic gardens, etc., in mosquito-infested regions may, therefore, play an important rôle in the extermination of these pests. Experimental work of this kind already undertaken in New Jersey suggests that the plan of combating mosquitoes by the introduction of *Gambusia* and other fishes with similar habits is entirely feasible.

As was pointed out by Seal [a] (1908), *Gambusia* and the related genus *Heterandria* possess certain habits and characters which render them superior to all other fishes as mosquito destroyers. As suggested by their common name, top minnows, they feed at the surface. Being of small size they readily find their way into shallow waters which are inaccessible to larger fishes. *Gambusia affinis* is often found in large numbers in water less than an inch in depth. Furthermore, it habitually searches for food among the vegetation and débris along the borders of pond or stream. In Mullet Pond it is rarely found in the open water, but is present in abundance among the marsh grasses along the swampy borders, where it not only finds food but is also protected from larger fishes.

The small size of this species, its viviparous habits, and its hardy nature ought to render its introduction and maintenance in new waters comparatively easy. It thrives under a wide range of conditions. Furthermore, the young, being brought forth in an advanced stage of development, are not subjected to many of the dangers which beset the young of oviparous fishes.

The breeding season continues during the spring and summer, several broods being produced during the season. Seal [b] (1911), observing these fish in captivity, has demonstrated that two or more generations may be born in a summer.

The adult females vary greatly in size, ranging from 3 to 6.5 centimeters in length. The males are relatively fewer in number and smaller than the females. The adult males range from 1.8 to 3 centimeters in length. Nearly all of the adult females taken by the writer during July, 1912, carried either mature ova or embryos.

The present investigation was carried on at the United States Fisheries Laboratory at Beaufort, N. C., during the summer of 1912.

REPRODUCTIVE ORGANS.

FEMALE.

Ovary.—The ovary is located in the abdominal cavity just beneath the air bladder and dorsal to the posterior portion of the intestine. It opens directly into the urogenital sinus, which communicates with the exterior through the urogenital aperture just posterior to the anal opening. It is a paired tubular organ, but, unlike the ovary of many teleosts, it is not bifurcated and has no distinct median wall. The left side of the ovary is always shorter than the right. (Pl. XVI, fig. 7.) This disparity in the length of the two sides of the ovary is due to the position of the stomach, which is located in the left side of the abdominal cavity. When distended with mature ova or embryos, the ovary fills the greater part of the abdominal cavity beneath the air bladder and causes considerable distension of the abdominal walls. At the left the ovary in this distended

[a] Seal, William P.: Fishes in their relation to the mosquito problem. Bulletin Bureau Fisheries, vol. XXVII, 1908, p. 831–838.

[b] Seal, William P.: Breeding habits of the viviparous fishes *Gambusia holbrookii* and *Heterandria formosa*. Proceedings of the Biological Society of Washington, vol. XXIV, p. 91–96.

condition presses forward against the stomach, while at the right it extends anteriorly alongside the latter organ. (Pl. XVI, fig. 8.)

Unlike the ovary of many oviparous teleosts, the ovary of *Gambusia* is not lobulated and contains relatively few ova. In the same ovary may be found ova in various stages of development, ranging from almost microscopic dimensions to a diameter of 1.8 millimeters attained at maturity. A considerable number of ova apparently reach maturity at the same time. These being fertilized give rise to a brood of young. After the birth of this brood, another lot of ova reach maturity, and, being fertilized, give rise to a second brood. Thus, perhaps, all the ova required to produce the several broods which are born during a spring and summer may be present in the ovary at the beginning of the season.

The larger females usually give rise to a larger brood of young than do the smaller ones. The average number of embryos contained in the ovaries of females 5 to 6 centimeters in length, based on a limited number of counts, was found to be 33. The maximum number removed by the writer from a single female was 76. The number of embryos contained in the ovaries of the smaller females ranges from 2 or 3 to about 20.

In females of this species taken in the Potomac River early in June, 1912, Smith [a] found the average number of embryos contained in the ovary to be 100. This average is considerably greater than the maximum number observed by the present writer. This difference is probably due to the fact that the broods observed by Smith were the first broods produced during the season, while those observed by the present writer were the second or later broods. As suggested by Smith, the first brood of the season is probably considerably larger than the later broods.

The ova of *Gambusia* have no investment of their own save a delicate vitelline membrane. Each ovum is inclosed in a separate cellular follicle which is attached to a central rachis (Ryder) by a slender stalk. Running longitudinally in the central rachis are a pair of vascular trunks from which smaller blood vessels arise and pass out along the stalks of the follicles. These smaller blood vessels break up into capillaries which radiate in all directions over the follicular walls. These follicles were described by Ryder (1885) as follows: "The ovarian follicles of *Gambusia* containing mature ova or fœtuses are built up internally of flat or squamous polygonal cells of pavement epithelium, and externally of a network of multipolar fibrous connective tissue cells and minute capillary blood vessels with cellular walls, which radiate in all directions over the follicle. From the point at which the main arterial vessel enters it, this vessel, together with its accompanying vein and investment of fibrous tissue, constitutes the stalk by which the follicle and its contained naked ovum is suspended to the main arterial trunk and vein." [b]

In an earlier paper, Ryder [c] (1882) has furthermore described a minute aperture in the follicular membrane near the stalk of the follicle which he has designated "the follicle pore." Through this pore, he believes, the spermatozoa enters the follicle.

I was able satisfactorily to observe such a pore in the follicular membrane in only a few instances. I have no reason, however, to doubt the presence of a follicular pore

[a] Smith, H. M.: The prolificness of *Gambusia*. Science, vol. XXXVI, n. s., no. 920, 1912, p. 224.

[b] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 147.

[c] Ryder, John A.: A contribution to the embryography of osseous fishes, with special reference to the development of the cod (*Gadus morrhua*). Report of the U. S. Fish Commission, 1882, p. 461.

in all the ovarian follicles. Without assuming the presence of an aperture in the follicular walls, it would be difficult to understand how the spermatozoa could come in contact with the ova.

I can not agree with Ryder (1785), however, that "the ovary itself seems to have no exterior investment, so that the follicles lie directly within the abdominal cavity, the young fishes upon the completion of their development rupture them and escape into the latter, and from thence through the abdominal pore into the outer world." [a] The ovary, as stated above, is a tubular organ which opens directly into the urogenital sinus. When distended with advanced embryos, the exterior walls of the ovary are very tenuous. The young fishes do not, however, break out into the abdominal cavity, but pass out of the ovary directly through its opening into the urogenital sinus, thence to the exterior. There is no aperture leading directly from the abdominal cavity to the exterior. Furthermore, examination of the ovary of a female immediately after she has given birth to a brood of young shows the walls of the ovary intact. No ruptured ovarian follicles communicate with the cœlom. When the exterior walls of the ovary are dissected off, the ruptured ovarian follicles are found in place in a somewhat shrunken condition.

It may not be amiss at this point to call attention to an error which appears in the recent paper by Seal (1911) referred to above. "The ova of a full-sized *Gambusia* are," he says, "when fully developed, about an eighth of an inch in diameter, transparent and nonadhesive. Each one is held, apparently, by a thread of membrane to a central nucleus, the character of which could only be determined by microscopic observation. The young fish can be seen fully formed, their eyes moving as they turn around in the egg." [b]

That the author quoted above has mistaken the ovarian follicle for the egg is obvious. The embryo is developed at the surface of the egg, which has no investment of its own save the vitelline membrane. When the yolk has been absorbed by the embryo there remains no trace of the egg. The young fish is then inclosed in the ovarian follicle which is suspended to the central rachis by the structure referred to in the above quotation as "a thread of membrane holding the egg to a central nucleus."

In the paper quoted above (p. 93), Seal describes the extrusion of the young as follows: "They are expelled one at a time and the ejection of each fish is so rapid that they appear as though shot out with some force. This, however, might be due to the bursting of the follicle and the uncoiling of the fish as it is released from restraint. * * * The follicles are undoubtedly ruptured at the moment of extrusion, whether inside or out I have never succeeded in observing, but it appears the more probable that it is inside."

In view of the fact that the ruptured ovarian follicles are found in place in the ovary after the young fishes are extruded, it is obvious that the rupturing of the follicle occurs not only within the body of the parent but within the ovary. The young fish is, doubtless, uncoiled as soon as it leaves the follicle. This uncoiling could, therefore, add little to the force with which the young fish is extruded. The rapid escape of the

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 148.

[b] Seal, William P.: Breeding habits of the viviparous fishes *Gambusia holbrookii* and *Heterandria formosa*. Proceedings of the Biological Society of Washington, vol. XXIV, p. 93, 1911.

young fish, if, as is usually the case, it comes out head foremost, may be readily explained by the tapering form of its body and by its own swimming movements. That some force is necessary, however, for the extrusion of the young is evidenced by the perceptible contractions of the muscles of the abdominal walls of the parent just before the young is extruded.

MALE.

Modified anal fin.—The male members of the species may be readily recognized by the modified anal fin, which functions as an intromittent organ. The third, fourth, and fifth rays of this fin are enlarged, greatly elongated, and variously curved. All of the rays are composed of segments. The diameter of each segment is slightly greater at the ends than in the middle. Thus each ray shows a series of slight circular ridges. These ridges are most prominent on the third ray, which is the largest of the elongated rays and has a slight backward curve near its proximal end. The distal portion of this ray bears a row of short, pointed spines on its anterior aspect, while posteriorly it is fringed, a short distance from the tip, by a dentate ridge apparently in the fin membrane.

The proximal portion of the fourth ray has a gentle forward slope until it comes into close proximity with the third. From this point the former ray extends distally parallel with the latter. The fourth ray is slightly longer than the third. Its distal portion is divided, the two divisions diverging for a short distance and again coming in contact with each other at the tip. The anterior division bears a few very small spines anteriorly. The posterior division bears a considerable number of short, slender spines posteriorly a short distance from the tip. The proximal ones of these spines are arranged in two groups of three spines each. The fifth ray makes a short, sigmoid flexure at its proximal end and then extends distally parallel with the fourth. Near its distal end it makes another slight sigmoid flexure and terminates in a small hammer-shaped enlargement which interlocks with a slightly recurved hook on the posterior division of the distal portion of the fourth ray. The third, fourth, and fifth rays of the anal fin are bound together by the fin membrane. The fifth ray may be brought forward at one side of the fourth until it comes into close or immediate proximity with the third. In this manner a groove or tube is formed through which the milt is transmitted into the genital aperture of the female. The first two and the last five rays of the anal fin are somewhat modified but not elongated.

The rays of the modified anal fin are illustrated in figure 2, plate XVI. Figure 3, plate XVI, shows the distal portion of the three elongated rays drawn in detail under higher magnification.

Mechanism controlling anal fin.—The modified anal fin is controlled by a powerful muscle which is inserted on the proximal ends of the rays of the anal fin and has its origin on the modified hæmal spines of the first three caudal vertebræ and a similar process projecting ventrally from the fourth to the last abdominal vertebra. This muscle stands in an almost vertical position and is so large that it causes a perceptible bulging of the body walls just above the vent. (Pl. XVI, fig. 1.)

The process projecting ventrally from the fourth to the last abdominal vertebra has a slight forward slope. The modified hæmal spines of the first three caudal vertebræ project forward into the abdominal cavity in an almost horizontal position. The first hæmal spine is nearly straight, having a slight downward curve near its distal end.

The second makes a slight forward curve near its origin. From this point a somewhat flattened forked process extends posteriorly, one prong of the fork passing on either side of the third hæmal spine. These two prongs terminate in footlike enlargements in the muscles of the anterior caudal segments. The third hæmal spine makes a somewhat stronger forward curve near its origin than the second. From its proximal portion a short, flattened, keel-shaped process extends posteriorly. The distal ends of these three hæmal spines are connected by a narrow band of cartilage. (Pl. xvi, fig. 2, HS.)

The interhæmals are correspondingly larger in the male than in the female and are embedded in the large muscle controlling the modified anal fin. The one articulating with the third ray of the modified anal fin is greatly enlarged and articulates loosely with the two anterior processes on which the muscle has its origin. (Pl. xvi, fig. 2, IH.)

The mechanism controlling the modified anal fin projects anteriorly into the abdominal cavity to such an extent that the space allotted to the air bladder becomes somewhat restricted. Consequently, the latter organ is relatively shorter and occupies a more oblique position in the male than in the female.

Ryder [a] (1885) has given us a brief description of the modified anal fin of *Gambusia* and the mechanism by which it is controlled, which is in many respects erroneous. A comparison of Ryder's description with the description given above will not be attempted in this paper. The former description, published more than a quarter of a century ago, was obviously not the result of an exhaustive study.

Testis.—The testis, like the ovary, is a paired tubular organ and is not distinctly divided. (Pl. xvi, fig. 4.) It is located in the abdominal cavity dorsal to the posterior portion of the intestine and just anterior to the large muscle controlling the anal fin. The testis does not extend as far anteriorly as does the ovary, but, like the latter organ, the left side of the testis is shorter than the right.

The spermatozoa are contained in spermatophores, which are rounded or spherical bodies, 0.1 to 0.2 millimeters in diameter. (Pl. xvi, fig. 5.) The walls of the spermatophores are exceedingly delicate. If the spermatophores are ruptured under the microscope, the spermatozoa may be seen to escape freely even though they are still immature and inactive. The spermatozoa are comparatively large. Each one is composed of a comparatively large, elongated, slightly curved and bluntly pointed head, a middle piece which is nearly as long but more slender than the head, and a long flagellate tail. (Pl. xvi, fig. 6.)

In most of the spermatophores observed, the spermatozoa were inactive and apparently curved around a small, bubble-like body, thus forming a more or less complete ring. When the spermatophores were broken many of the spermatozoa were released from this curved position and freed from the small, bubblelike body. The tails, however, still retained a slight curve. The heads of the spermatozoa may be readily observed in the spermatophores under moderately high magnification. They are closely aggregated but show no regular arrangement. While no spermatophores were observed in the genital organs of the female, it is highly probable that the spermatozoa are transmitted from the male to the female in these bodies.

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings of the U. S. National Museum, vol. VIII, p. 143, 144.

EMBRYOLOGY.

OVUM.

The mature ovum is a spherical body having a diameter of about 1.8 millimeters. It has a gold-yellow color and, being heavily laden with yolk, is quite opaque. It is invested by no distinct egg membrane such as invests the eggs of most of the oviparous fishes, but is covered only by a thin, vitelline membrane. Beneath the vitelline membrane the entire surface is more or less completely covered by oil globules of unequal size and distribution. (Pl. XVII, fig. 1.)

BLASTODERM.

The ova are fertilized within the ovarian follicles. Unless the time of fertilization can be controlled, it becomes difficult to secure the earliest stages of development. The earliest stages which were secured after fertilization showed a small blastoderm in the many-cell stage. This blastoderm appears as a small, almost circular cap of cells which is slightly elevated above the surface of the yolk. (Pl. XVII, fig. 2, B.) The distribution of the oil globules is not disturbed during the process of cleavage and numerous globules may be observed through the blastoderm.

As the blastoderm increases in size, the cleavage cavity becomes plainly visible. The germ ring is never well defined, but appears as a slight thickening of the periphery of the blastoderm. The cleavage cavity, as observed through the overlying blastoderm, soon assumes a somewhat triangular outline. The blastoderm becomes slightly elongated along the axis, which becomes the future axis of the embryo. At the side of the cleavage cavity on which the thickened area at the periphery of the blastoderm is broadest, the blastoderm becomes thicker and more opaque. This area is symmetrically divided by the long axis of the blastoderm and, inasmuch as it gives rise to the embryonic shield, may be recognized as the posterior pole of the blastoderm. This area increases in size and distinctness until the embryonic shield is well outlined. (Pl. XVII, fig. 4.)

DIFFERENTIATION OF THE EMBRYO.

From the posterior pole of the embryonic shield a narrow thickened area grows anteriorly. This thickened area alone represents the embryonic area, while the thinner lateral areas represent the extra-embryonic area of the embryonic shield. (Pl. XVII, fig. 4.) The embryonic area continues to grow anteriorly over the cleavage cavity and becomes gradually enlarged at the anterior end. In this manner the head of the future embryo becomes outlined.

While the embryonic area is becoming differentiated the blastoderm spreads rapidly over the yolk until the latter is completely covered. The progress of the growth of the blastoderm over the yolk could not be observed satisfactorily, partly because the germ ring is not well defined and not easily observed on this very opaque egg and partly because not all the desired stages of development could be secured. A careful study of the stages available, however, seems to indicate that the differentiation of the embryo of *Gambusia* takes place in a manner which is quite typical for teleosts.

LATER DEVELOPMENT.

After the formation of the embryonic area the embryo soon becomes well outlined. Plate XVIII, figure 5, illustrates a stage at which the tail bud has already grown out and the anlage of the neural axis is apparent throughout the entire length of the embryo. The optic vesicles are well formed and from 3 to 4 somites are already apparent.

Plate XVIII, figure 6, represents an embryo in which the divisions of the brain are becoming distinctly outlined. The auditory vesicles and from 12 to 14 somites are already present. At this stage the heart is becoming differentiated as a simple curved tube. The heart soon begins to pulsate, and a circulation is set up over the surface of the yolk. This circulation is at first slow and irregular but soon becomes very vigorous.

The growing embryo lies in a groove in the surface of the yolk and is inclosed only by the ovarian follicle. As development advances the ovarian follicle increases in size and becomes increasingly vascular. The space between the egg and the follicle becomes filled with a transparent fluid. Thus the embryo lives in a fluid medium. Although the ovarian follicle becomes highly vascular, a placental or pseudoplacental relationship such as exists in the selachians or even in some of the viviparous teleosts is not suggested. The embryo develops no structures which would seem to be adapted to absorb nourishment from a fluid medium. Furthermore, no fæces of any kind are ever observed in the follicle. The abundant yolk supply in the egg is, doubtless, sufficient to supply all the food material required by the embryo.

It is probable, as was suggested by Ryder (1885), that "the very intricate meshwork of fine vessels which covers the follicle supplies the developing fœtus with fresh oxygen, and also serves to carry off the carbon dioxide in much the same way as the placenta or afterbirth performs a similar duty for the young mammal developing in the uterus of its parent."[a] The analogy between the intra-follicular respiration of the developing embryo of *Gambusia* and the intra-uterine respiration of the young mammal must, however, not be carried too far. The embryo of *Gambusia* develops gills which apparently become functional very early. An examination of the gills of an advanced embryo removed from the ovarian follicle, as Ryder has already observed in the paper quoted above, shows that the gill filaments are already pinnate and that the pinnæ contains loops of blood vessels. This condition of the gill filaments, as is well known, is not attained by the larvæ of many oviparous fishes for a considerable interval after hatching. Furthermore, rythmical breathing movements may be observed as the embryo lies coiled in the ovarian follicle. It is probable, therefore, that the intra-follicular respiration of the embryo of *Gambusia*, at least during the later stages of intra-ovarian life, is more nearly analogous with the respiration of adult fishes than with the intra-uterine respiration of the young mammal, the fluid in the follicle, by which the embryo is constantly bathed, being aerated by the follicular circulation.

As the embryo grows, the tail extends posteriorly partly encircling the egg. Soon, however, it bends indifferently to the right or to the left. (Pl. XVIII, fig. 8.) This bending brings the tip of the tail into proximity with the head. Consequently, as the caudal fin is developed it overlaps the face of the embryo, sometimes partly or completely covering one or both of the eyes. (Pl. XIX, fig. 9.)

[a] Ryder, John A.: On the development of viviparous osseous fishes. Proceedings U. S. National Museum, vol. VIII, p. 147.

Pigmentation begins comparatively early. Scattered pigment spots first appear on the dorsal surface, being more closely aggregated on the posterior region of the head and along the dorsal mid-line of the trunk. These pigment spots become more numerous and more closely aggregated until at birth pigmentation is almost complete.

Embryos which still retain a yolk sac of considerable size when removed from the ovary show nearly all the characteristic markings of the adult. At birth the yolk sac is completely absorbed. The newborn fish answers fairly well, except with respect to dimensions, to the diagnostic description of the species. Its color is light olive, darker dorsally than ventrally. The number of scales in the lateral and transverse series, respectively, correspond to the number of scales in these series, respectively, in the adult. The number of rays in the dorsal, anal, and caudal fins also correspond to the number of rays in these fins, respectively, in the adult. The fine dark line along the side is already present. The two or three transverse rows of dark spots on the dorsal, the dark margin on the anal, and the three or four irregular rows of dark spots on the caudal fin, characteristic of adult females, are already becoming differentiated. The dark purplish blotch on the side above the vent (absent in males) is not yet apparent. The modified anal fin of the male was not observed in newborn fishes.

The newborn fishes are 9 to 10 millimeters in length and are very vigorous. Having been protected from many of the dangers which beset the larvæ of oviparous fishes, they are now well prepared to enter upon an independent existence.

Embryos still carrying a yolk sac of considerable size, being removed from the parent, were able to swim freely in water, where they continued to live, the yolk sac being gradually absorbed. Such embryos were kept in small aquaria with occasional changes of fresh water for a period of 10 days. At the end of this time the yolk sac was completely absorbed and the young fishes were apparently in a healthy condition.

SUMMARY.

1. *Gambusia affinis* is known primarily as a fresh-water species, but occurs also in brackish water. Under experimental conditions, fishes transferred from brackish water to sea water were kept alive and apparently in a normal condition for a period of 10 days.

2. The ovary of *Gambusia* is a paired tubular organ without a distinct median wall, which opens directly into the urogenital sinus. Each ovum is contained in a separate cellular follicle in which fertilization takes place and the embryo is developed. At the completion of development the ovarian follicles which are attached to the central rachis by a slender stalk are ruptured and the young fishes are extruded directly through the urogenital aperture.

3. The modified anal fin of the male which functions as an intromittent organ is controlled by a powerful muscle which is inserted on the proximal end of the anal fin rays and has its origin on a bony process projecting ventrally from the fourth to the last abdominal vertebra and the modified hæmal spines of the first three caudal vertebræ. The third, fourth, and fifth rays of the anal fin are enlarged, greatly elongated, and variously curved, bearing short spines on the distal portions. The interhæmal which articulates with the third ray is enlarged and articulates with the two anterior processes on which the muscle controlling the anal fin has its origin.

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4. The testis, like the ovary, is a paired tubular organ. The spermatozoa are contained in the spermatophores and are probably transmitted from the male to the female in these bodies.

5. The formation of the blastoderm and the differentiation of the embryo takes place in a manner which is quite typical for teleosts.

6. As development advances, the ovarian follicle becomes highly vascular, increases in size, and is filled with a transparent fluid in which the embryo is constantly bathed. This fluid is doubtless aerated by the follicular circulation. The gills of the developing embryo apparently become functional comparatively early. During the later stages of intra-ovarian life, rhythmical breathing movements of the embryo may be observed.

7. The young are born in an advanced stage of development and show nearly all of the diagnostic characters of the species. They undergo no marked metamorphic changes after birth.

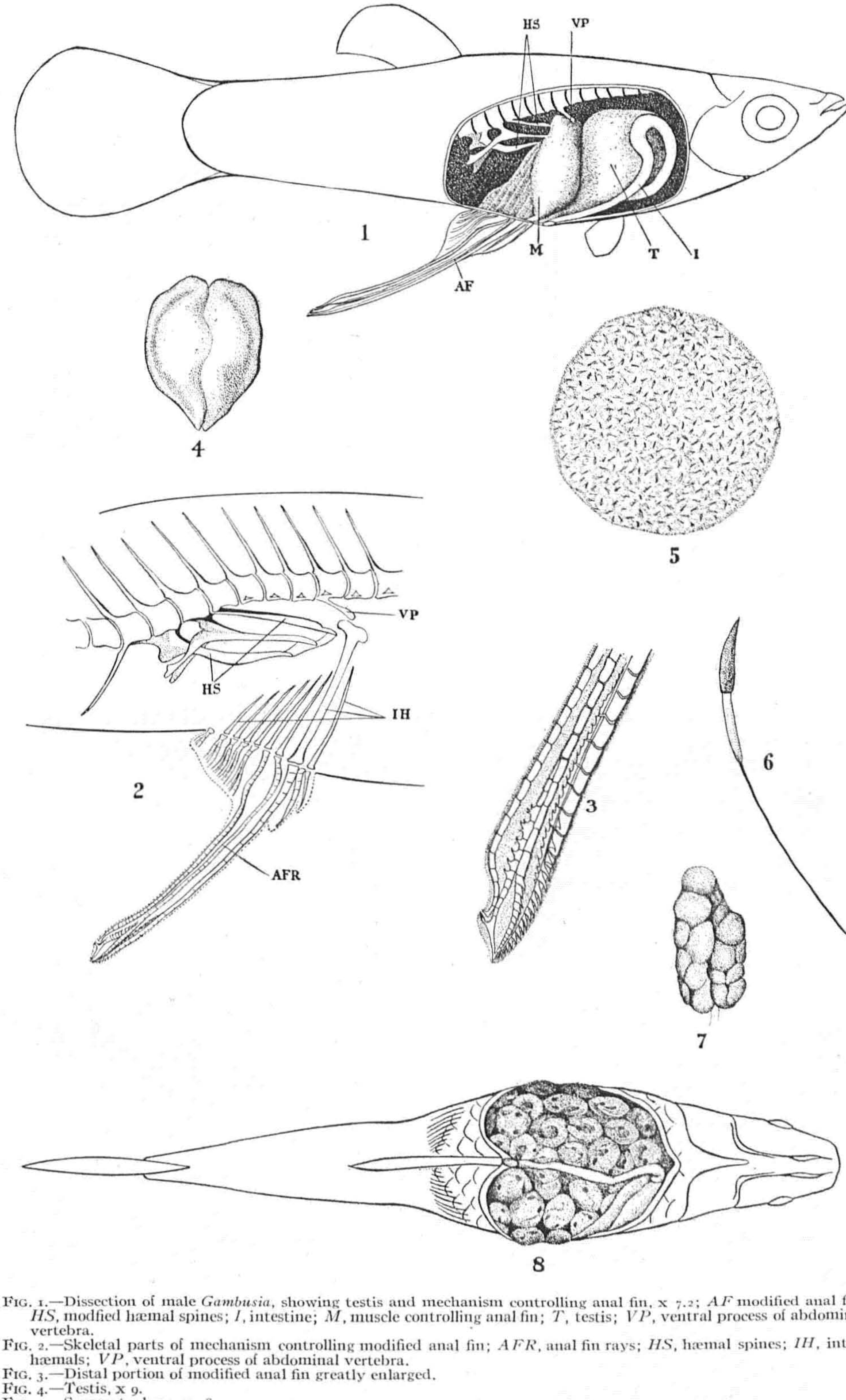
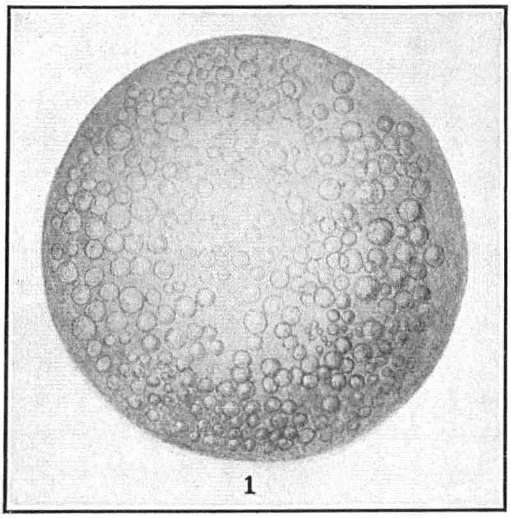
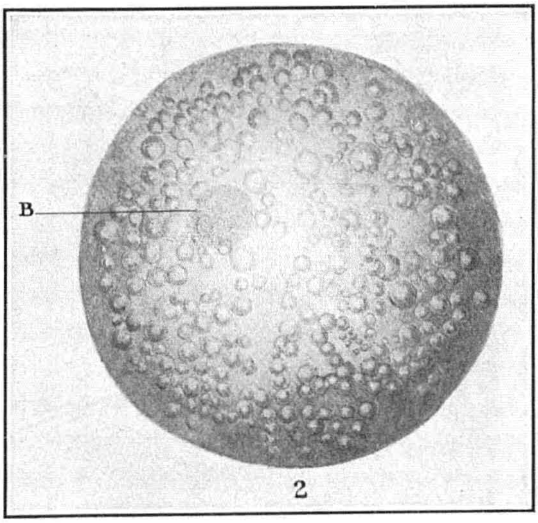
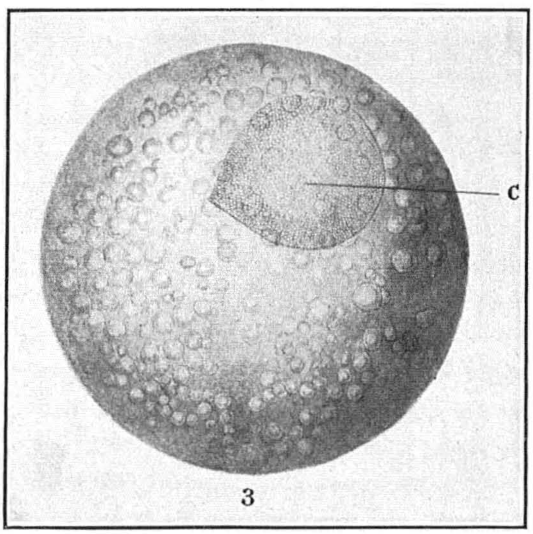


FIG. 1.—Dissection of male *Gambusia*, showing testis and mechanism controlling anal fin, x 7.2; *AF* modified anal fin; *HS*, modfied hæmal spines; *I*, intestine; *M*, muscle controlling anal fin; *T*, testis; *VP*, ventral process of abdominal vertebra.
FIG. 2.—Skeletal parts of mechanism controlling modified anal fin; *AFR*, anal fin rays; *HS*, hæmal spines; *IH*, interhæmals; *VP*, ventral process of abdominal vertebra.
FIG. 3.—Distal portion of modified anal fin greatly enlarged.
FIG. 4.—Testis, x 9.
FIG. 5.—Spermataphore, x 385.
FIG. 6.—Spermatazoon, x 3,000.
FIG. 7.—Ovary, x 4.
FIG. 8.—Dissection of female *Gambusia*, showing ovary distended with advanced embryos, x 3.6.







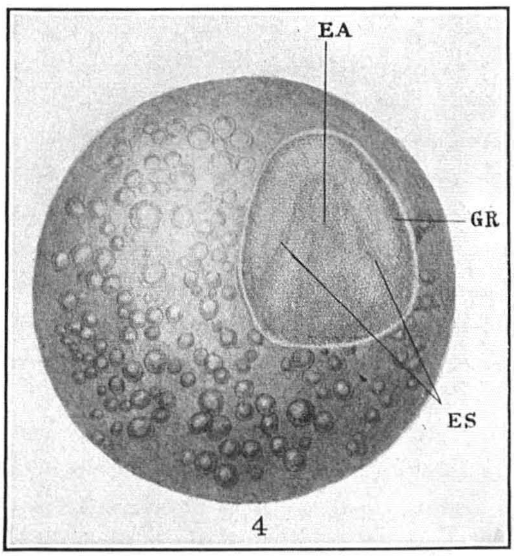
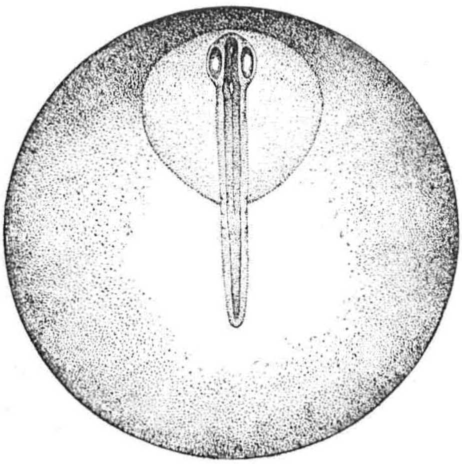


FIG. 1.—Mature ovum, x 45.
FIG. 2.—Ovum with early blastoderm, x 45; *B*, blastoderm.
FIG. 3.—Ovum with later blastoderm, x 45; *C*, cleavage cavity.
FIG. 4.—Ovum with blastoderm, showing embryonic shield, x 45; *ES*, embryonic shield; *EA*, embryonic area; *GR*, germ ring.



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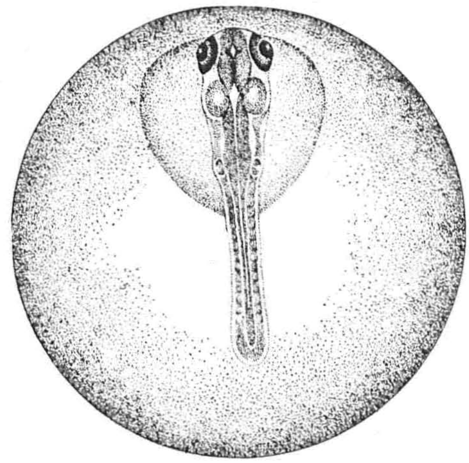

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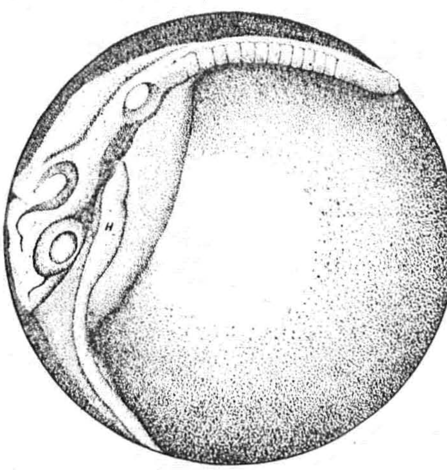

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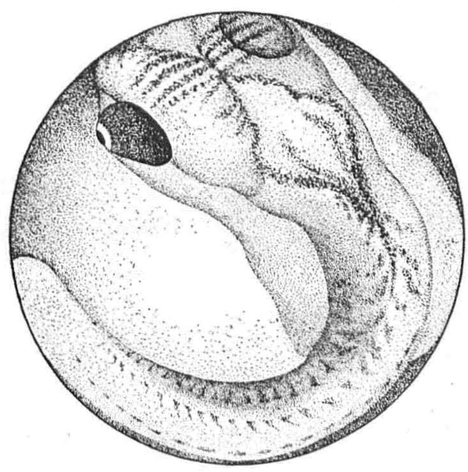

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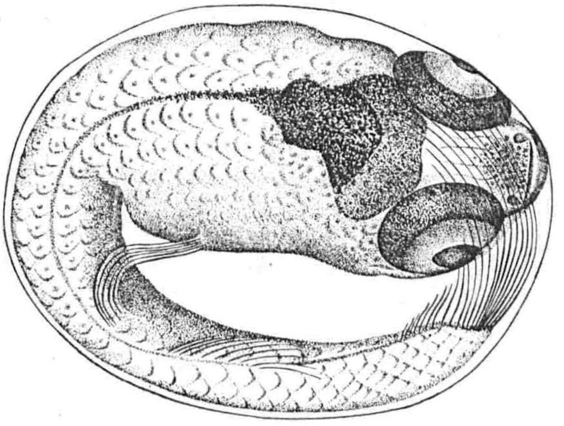
FIG. 5.—Embryo with 3–4 somites, x 50.
FIG. 6.—Embryo with 12–14 somites, x 50.
FIG. 7.—Embryo with about 12 somites, side view, x 50; *H*, heart.
FIG. 8.—Embryo with pigmentation started, inclosed in ovarian follicle, x 45.



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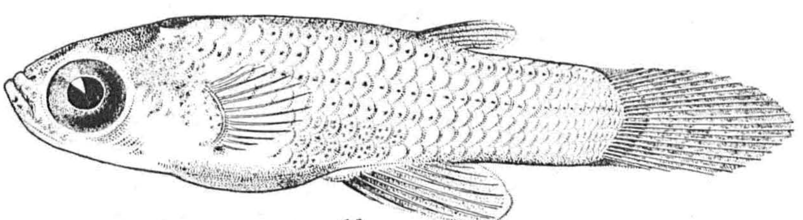

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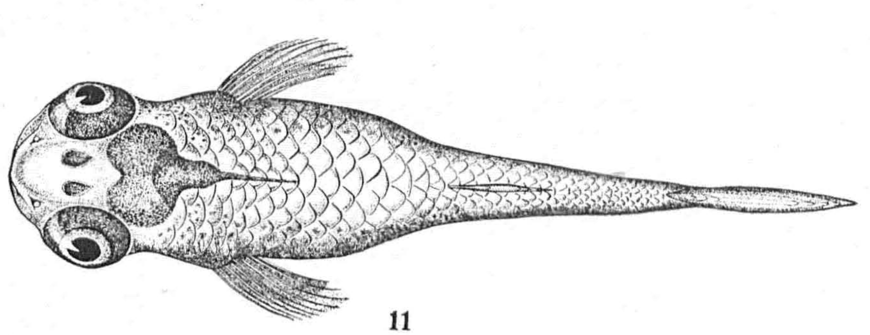

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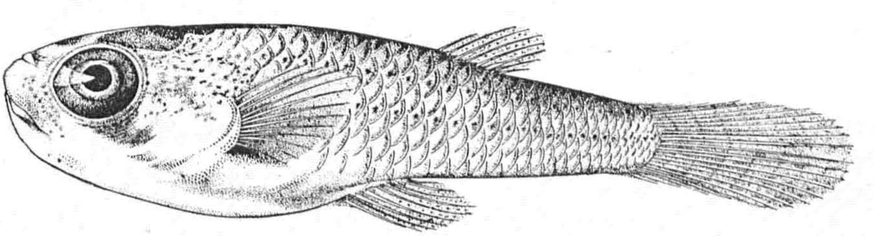

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FIG. 9.—Advanced embryo inclosed in ovarian follicle, x 30.
FIG. 10.—Embryo with yolk sac nearly absorbed, removed from ovarian follicle, x 18.
FIG. 11.—New-born fish, dorsal view, x 15.
FIG. 12.—New-born fish, side view, x 15.