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DEVELOPMENT OF EGGS AND YOLK-SAC LARVAE OF YELLOWFIN MENHADEN

By JOHN W. REINTJES

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ABSTRACT

Fertilized eggs were obtained by manually mixing ova and sperm of yellowfin menhaden (*Brevoortia smithi*). Rearing was done in February 1960 at Indian River, Florida. Descriptions and illustrations are given for the developmental stages of the embryo and larva, through absorption of the yolk. Temperature and salinity observations are included.

DEVELOPMENT OF EGGS AND YOLK-SAC LARVAE OF YELLOWFIN MENHADEN

By JOHN W. REINTJES, *Fishery Research Biologist*
BUREAU OF COMMERCIAL FISHERIES

The menhadens, genus *Brevoortia*, inhabit the coastal waters of the western Atlantic Ocean from Nova Scotia to central Argentina and support the largest commercial fishery in the United States, yet their early developmental stages are little known. Kuntz and Radcliffe (1917) described developing eggs, yolk-sac larvae, and older larvae identified as Atlantic menhaden (*B. tyrannus*). Based on their descriptions, Atlantic menhaden eggs and larvae have been reported from Chesapeake Bay (Pearson, 1941), Long Island Sound (Perlmutter, 1939; Wheatland, 1956; Richards, 1959), and Narragansett Bay (Herman, 1959). Eggs, tentatively identified as Atlantic menhaden, were obtained off the North Carolina coast in November and December, 1956 and 1957 (Reintjes).[1] In 1957 eggs were hatched in the laboratory, but the larvae died after the yolk sac was absorbed.

Menhaden eggs and larvae were reported from plankton collections made off the south Atlantic coast of the United States during three cruises of the motorship *Theodore N. Gill* (Reintjes, 1961), but no identification to species was made. Although the foregoing observations provided a description of eggs and larvae and information on their distribution, some question remained as to whether these actually were menhaden.

The absence of spawning, or running-ripe, fish in the landings has precluded mechanical fertilization and rearing of the eggs and yolk-sac larvae for the identification of Atlantic menhaden (*B. tyrannus*) and Gulf menhaden (*B. patronus*), the two species of principal commercial importance. The occurrence of spawning yellowfin menhaden (*B. smithi*) in the landings of a gill-net fishery at Sebastian, Fla., made possible the distinction of eggs and yolk-sac larvae of this species from those of other clupeoid fishes. Development of embryos and larvae was followed and described from the time of fertilization until absorption of the yolk.

The procedures of the work were: (1) obtain ripe ova and sperm from freshly caught yellowfin menhaden, (2) effect fertilization by mixing the sex products, (3) hold fertilized eggs in a suitable environment at known temperature during development, (4) remove and preserve examples of developing eggs and larvae, (5) observe the properties of eggs and the behavior of early larvae, and (6) collect planktonic eggs and larvae concurrently for comparative material.

MATERIALS AND METHODS

Beginning in November 1959 weekly samples of adult yellowfin menhaden were obtained from gill-net landings at Sebastian, Fla., to follow maturation of ovaries and testes. Each sample consisted of about 100 fish taken at random from the catch. Free-flowing milt was observed from cut testes in mid-December, and on January 11, 1960, several females in the sample extruded ova when pressed firmly. Each week thereafter, the number of fish apparently ready to spawn increased. On February 8, approximately one-fourth of the females and all of the males appeared ready to spawn.

On February 12, a temporary field laboratory was set up in a small dockside building at Sebastian, Fla. Equipment included compound and dissecting microscopes, thermometers, salinometers, small dip nets, one-half-meter plankton nets, an assortment of glass preparation bowls and polyethylene containers, and pens with nylon-net compartments. Other than the pens, no other equipment of special construction was used.

For rearing purposes, two pens, or enclosures, were constructed, following the design of the blue crab shedding floats, or live-cars, used throughout the Chesapeake Bay and middle Atlantic region

[1] Eggs and yolk-sac larvae of Atlantic menhaden. Unpublished manuscript. U.S. Bureau of Commercial Fisheries Biological Laboratory, Beaufort, N.C.

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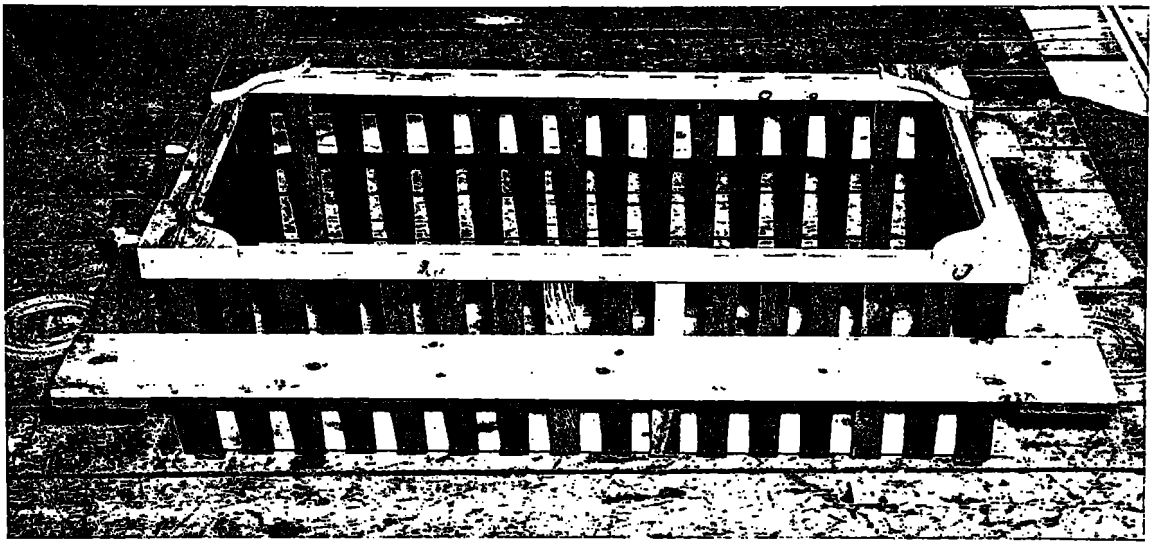

FIGURE 1.—Pen used to confine yellowfin menhaden eggs during development.

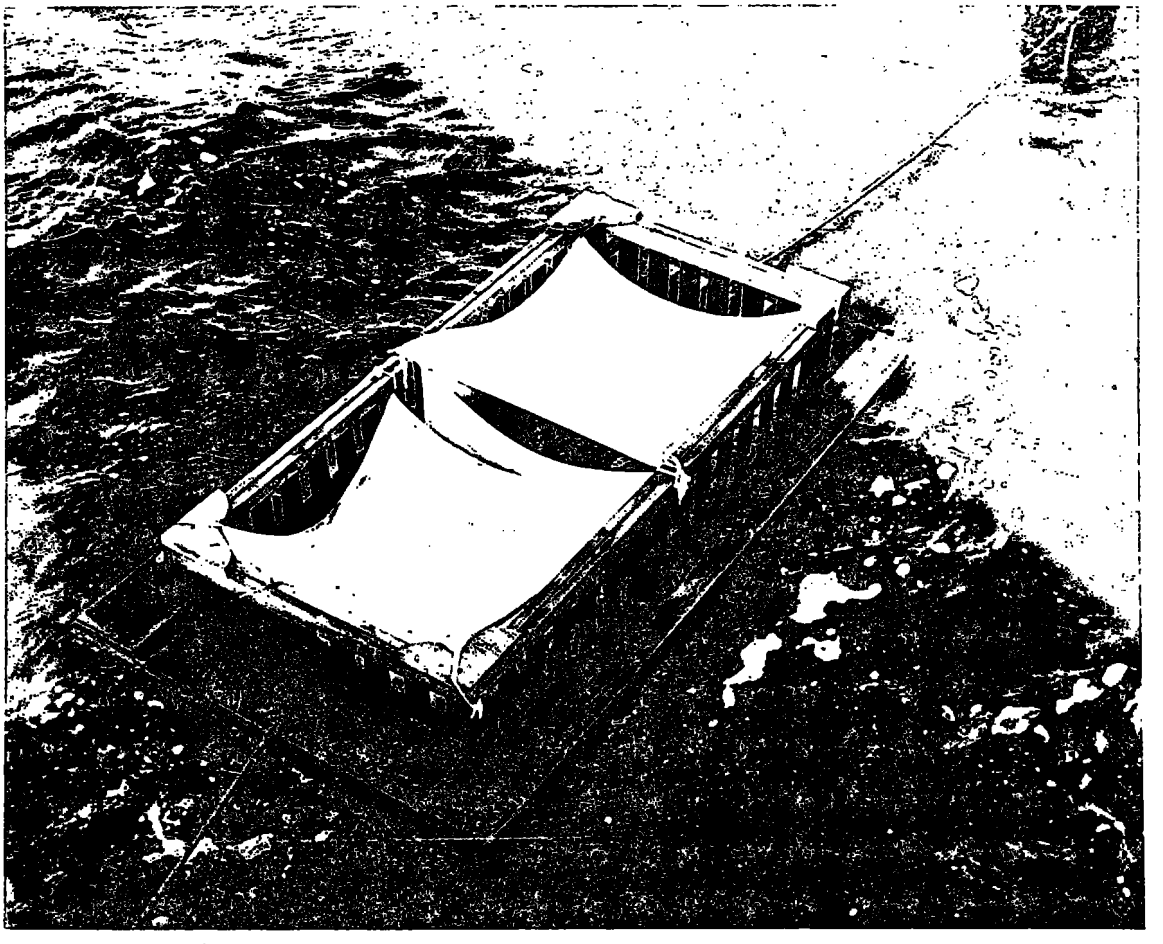

FIGURE 2.—Pen with nylon-mesh compartments floating in Indian River, Fla.

(Wharton, 1954). The pens were made of juniper boards and cedar slats (fig. 1). The dimensions were as follows: 18 inches high, 62 inches long, and 32 inches wide, with a 5-inch flange on all four sides. The flange regulated the submergence depth of the pen and provided stability.

Compartments, made of woven-mesh nylon netting, were used to confine the fertilized eggs and larvae within the pens. Two compartments were made of mesh with an average opening of 0.5 mm., and two compartments were made of mesh with an average opening of 1.0 mm.[2] All seams reinforced with nylon binding tape. The compartments were enclosed, except for access along one side (fig. 2). Because the access slit gaped during rough weather and allowed debris to enter and eggs to escape, a plastic slide fastener later was added. Each pen held two compartments that were kept in place by a tie at each corner.

Yellowfin menhaden used in the fertilization trials were obtained from gill-net catches made within sight of the temporary field laboratory. Usually, the nets were set at dusk and picked up about an hour later. Ripe females with extruded ova, or greatly distended abdomens, were removed and set aside while the net was being recovered. Males producing milt with viable sperm commonly occurred in the catch. Ripe females, on the other hand, were rare, for only 25 gravid females were found among approximately 4,000 fish examined. For all attempted fertilizations, fish were dead less than two hours.

Fertilization was accomplished by mixing ova and sperm obtained by pressing the sides of the fish, or by cutting open the ovary or testes to free the mature sex products. The ova and sperm were mixed "dry," i.e., without sea water. Dockside water was filtered through cotton to remove organisms including fish eggs and then added. The criterion of fertilization was the formation of a wide perivitelline space.

Fertilized eggs were placed in the mesh compartments of the floating pens and in glass or polyethylene containers in the laboratory. Samples of the developing embryos were removed periodically from the floating pens and placed in the laboratory containers. Development was observed with a microscope, and samples were removed and preserved in 5 percent formalin.

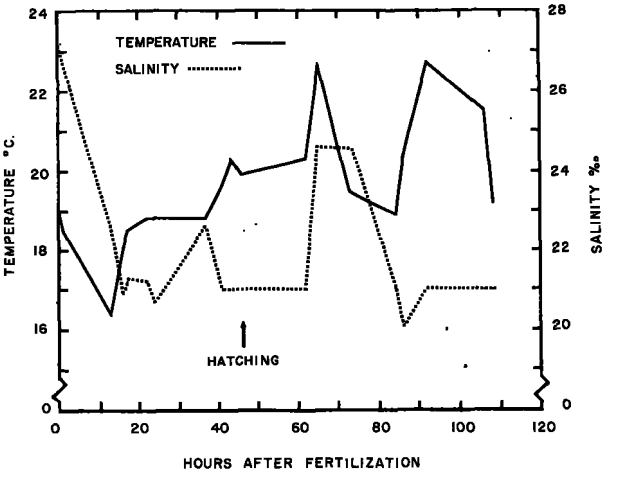

FIGURE 3.—Fluctuations of temperature and salinity during the development of eggs and yolk-sac larvae of yellowfin menhaden.

The time required for development of the embryo was recorded as age-in-hours from manual fertilization and for the yolk-sac larva, from the time of hatching. The water temperature of Indian River, immediately adjacent to the dock and rearing floats, and of the culture bowls was recorded at infrequent intervals during development (fig. 3). The observed temperature ranged from 16.4° C. to 22.7° C., with a mean of 19.6° C.

Salinity was determined at each temperature observation. The observed salinity ranged from 20.1°/oo to 27.2°/oo, with a mean of 22.1°/oo.

Plankton collections of yellowfin menhaden eggs and larvae developing under natural conditions were obtained in the Indian River. Ten-minute tows were made with a half-meter net in the vicinity of the gill-net fishing grounds near Sebastian Inlet, and at one mile intervals for a distance of 6 miles north and 12 miles south of the inlet. Developing eggs from the plankton collections were used for the photographs of several stages not obtained during the development of artificially fertilized eggs. Although the size and appearance were similar to eggs of known origin, there were slight differences that are without adequate explanation. However, the identity of the planktonic eggs was assumed because of structural similarities and the concurrence of spawning yellowfin menhaden in the immediate vicinity.

[2] One hundred percent Dupont nylon pattern No. 109 (0.5 mm.) and pattern No. 1400 (1.0 mm.).

DESCRIPTION OF FERTILIZATION TRIALS

Sixteen manual, or artificial, fertilizations were attempted to obtain developing embryos from positively identified yellowfin menhaden. A single female and several males were used in the first trial. The ova were removed by dissection, divided into three lots, and those in each lot mixed dry with milt from a separate male. Fifteen minutes later, filtered dockside water (salinity 20.5°/oo, and temperature 20.1° C.) was added to each container. An hour later, approximately 90 percent of the eggs in one lot were fertilized. The other two lots contained so few fertilized eggs, perhaps because of less viable sperm, that they were discarded. Development was arrested after several hours during early cleavage. Whether the failure to develop was due to decomposition, stagnation, or immaturity of ova or sperm could not be determined.

Four females were used in the second trial. Ova were removed by dissection, mixed "dry" with milt, and 15 minutes later, dockside water was added (27.2°/oo, 19.0° C.). The apparent success of fertilization varied from 40 to less than 10 percent. Two lots of eggs were placed in the floating pen anchored off the end of the dock where salinity was 26.7°/oo and temperature 18.5° C. The remaining two lots were placed in containers in the laboratory. Twelve hours later, eggs in the laboratory containers had failed to develop beyond early cleavage and showed signs of decomposition. Samples of eggs from the pens appeared normal, although in one compartment, few ova were fertilized. This trial furnished most of the developing embryos and yolk-sac larvae used for the descriptions.

DESCRIPTION OF EGG

Living eggs showed an iridescent, glasslike transparency, with little or no color in the yolk. Iridescence disappeared when the material was placed in formalin, but the chromatophores were retained and accentuated as the developing embryo and yolk became clouded. The following description is based on preserved material.

The egg is spherical and has a resilient, transparent membrane. Under magnification of 100 diameters or more, the membrane surface is marked with fine, short lines that form no discernible pattern. The yolk is segmented, contains a single oil globule, and is pale yellow. The oil globule is near the vegetative pole and floats uppermost throughout development. Coarse granulation of the yolk appeared to be characteristic of eggs not fully matured.

Comparative measurements showed the planktonic eggs to be slightly larger than those obtained artificially (table 1). Fertilized eggs in the plankton, similar in appearance and structure to those artificially fertilized, were assumed to be from yellowfin menhaden. Whether the artificially fertilized eggs had not reached maximum size because of immaturity, or whether naturally spawned eggs swell to a greater size could not be determined. Eggs, ranging from approximately 1.0 to 1.1 mm. in diameter, developed a fertilization membrane and perivitelline space; however, the very low fertility and the failure of most eggs to develop beyond the earliest stages of cleavage indicated that these ova had not reached maturity.

TABLE 1.—*Measurements of yellowfin menhaden eggs, in millimeters*

Item	Planktonic eggs (N=200)		Artificially fertilized eggs (N=50)	
	Range	Mean	Range	Mean
Fertilized egg	1.21–1.48	1.34	1.15–1.30	1.22
Perivitelline space	.33– .50	.42	.34– .46	.39
Yolk	.77–1.04	.90	.77– .95	.86
Oil globule	.05– .18	.13	.07– .16	.13

Developing yellowfin menhaden eggs from the plankton were buoyant, floating just beneath the surface film. Unfertilized eggs rested on the bottom in still water. Artificially fertilized eggs formed a layer above the unfertilized eggs, floating off the bottom with the slightest disturbance.

DEVELOPMENT OF THE EMBRYO

In discussing the development of yellowfin menhaden eggs the following three stages are used (Ahlstrom and Counts, 1955):

Early—from fertilization to closure of the blastopore.

Middle—from closure of the blastopore to the time that the separating tail begins to curve laterally away from the embryonic axis.

Late—from the time the tail curves away from the embryonic axis to the time of hatching.

EARLY-STAGE EGG

The perivitelline space developed and widened within 15 minutes after ova and sperm were mixed in sea water. If the ova and sperm were mixed in the absence of water, the perivitelline space was not readily apparent until after sea water had been added. Unfertilized and fertilized eggs from the same lot, one hour after the sex products were mixed, are shown in figures 4 and 5.

Early cleavage was rapid, and a layer of cells was formed by the 7-hour stage (fig. 6). Continued cell division resulted in the formation of a dome-shaped blastodermal cap on the yolk (fig. 7), after 12 hours. Eggs collected from the plankton (fig. 8) showed the blastodermal cap covering nearly one-third of the yolk. These late blastula were estimated as 14 hours old.

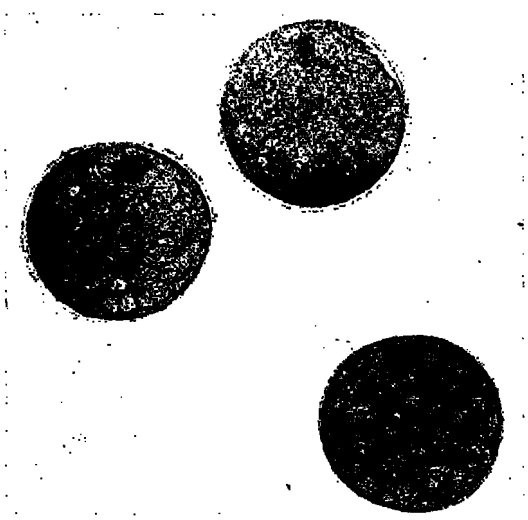

FIGURE 4.—Unfertilized egg of yellowfin menhaden.

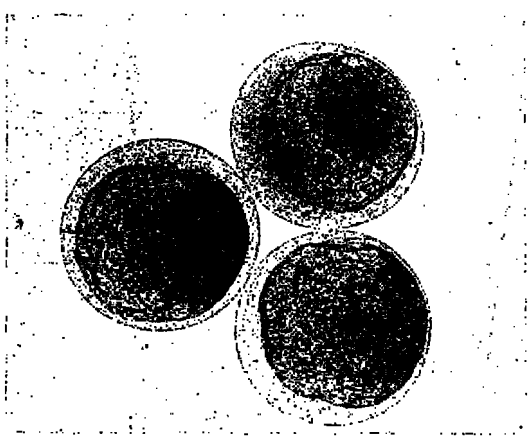

FIGURE 5.—One-hour stage with perivitelline space.

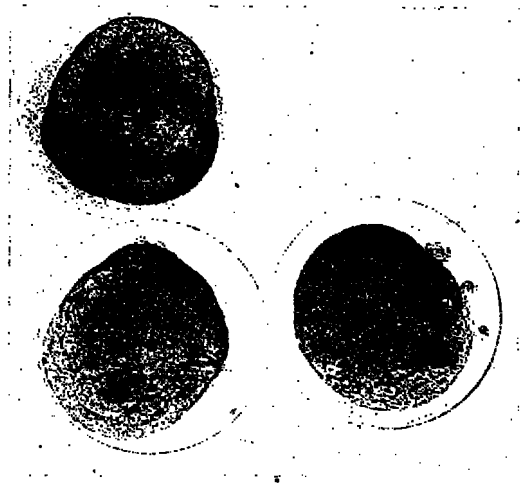

FIGURE 6.—Seven-hour stage with early cleavage.

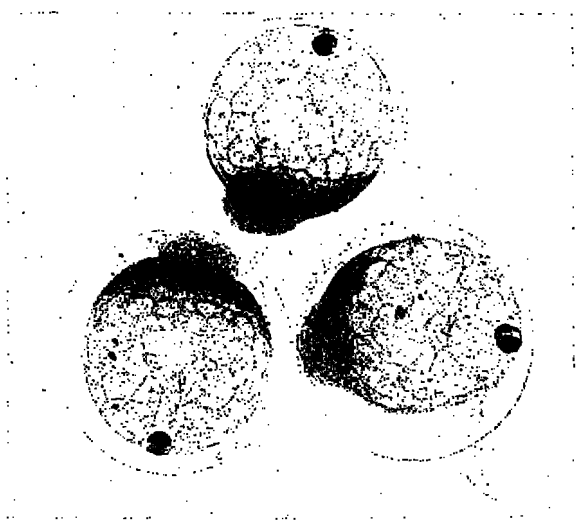

FIGURE 7.—Twelve-hour stage with blastodermal cap.

Some of the early stages showed yolk diffusion into the perivitelline space (fig. 9). This was assumed to be due to mechanical rupture of the yolk membrane during the handling and preservation of the eggs, since yolk encircled by the blastoderm in later stages did not appear to be ruptured (figs. 10, 11, 12, and 15).

At the late blastula stage the blastodermal cap, now known as the embryonic shield (fig. 9), had developed. The early embryo could be seen as a medial thickening of the shield. The peripheral cells continued to spread over the yolk surface.

The early neurula marked the end of the early-stage egg (fig. 10). The developing embryo, with a discernible head and several myomeres, became

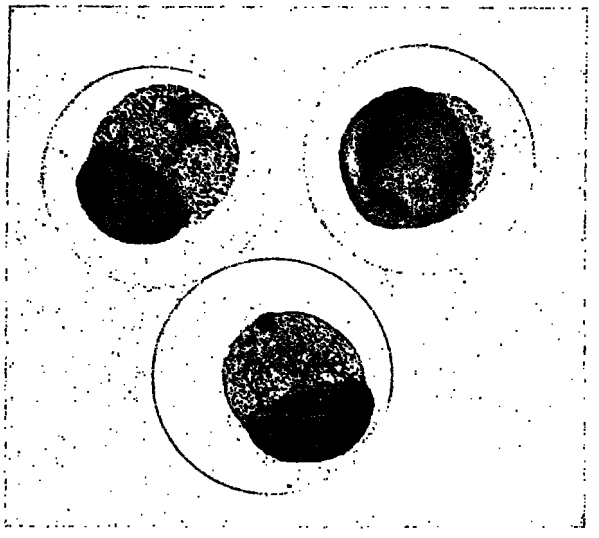

FIGURE 8.—Circa 14-hour stage with blastodermal cap.

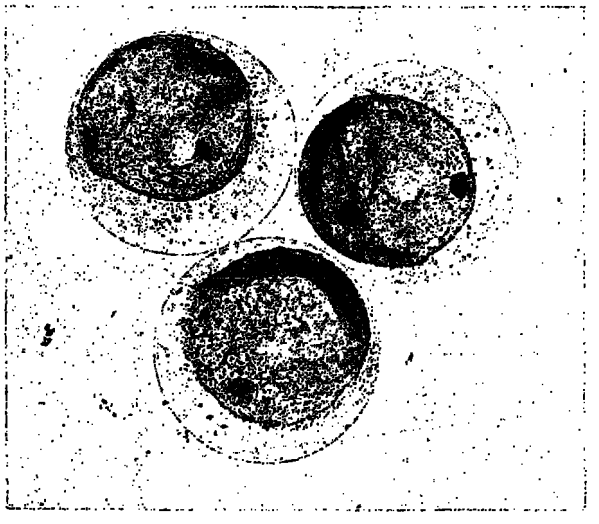

FIGURE 9.—Sixteen-hour stage with embryonic shield.

visible about the time of blastopore closure. Artificially fertilized eggs were not sampled at this stage. Eggs estimated at the 24- and 30-hour stages were obtained from plankton collections made during the rearing studies. Particles adhered to the surface of artificially reared eggs, probably due to the absence of water movement in the culture bowls. Eggs from the plankton were clean by comparison.

The early-stage eggs showed little pigmentation. A few small chromatophores were scattered over the surface of the yolk, but none was apparent on the blastula or early neurula.

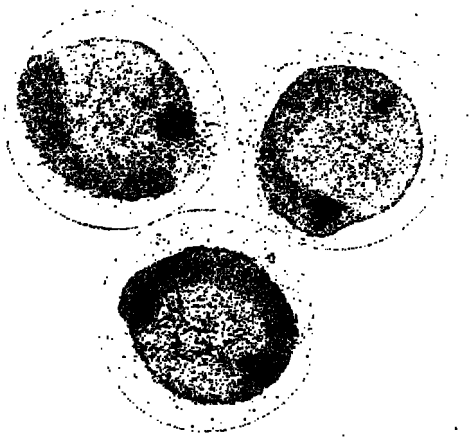

FIGURE 10.—Circa 24-hour stage from plankton with early neurula.

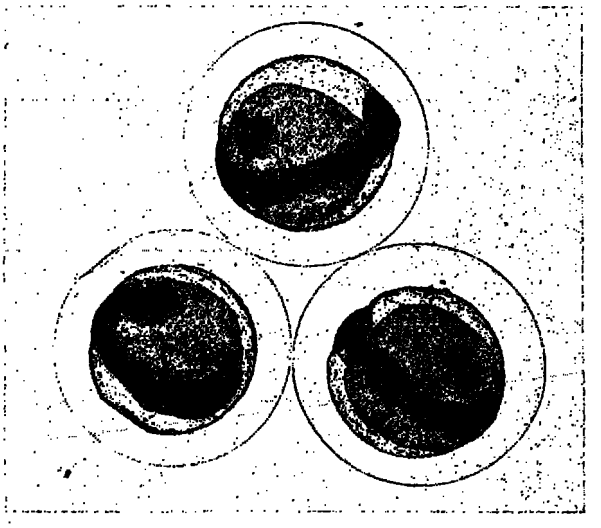

FIGURE 11.—Circa 30-hour stage from plankton with late neurula.

MIDDLE-STAGE EGG

The developing embryo encircled two-thirds of the yolk. Myomeres were visible along most of the embryo, the head was well-defined, and the optic lobes appeared as lateral expansions (fig. 11). The late neurula was raised above the yolk as a cylindrical embryo and not as a mere thickening of the embryonic shield (fig. 12). At the end of this stage, the tail had become separated from the yolk and was curved laterally away from the embryonic axis (fig. 13). This occurred 40 hours after fertilization. Small chromatophores developed on the yolk, and several appeared along the embryo, usually just posterior to the head.

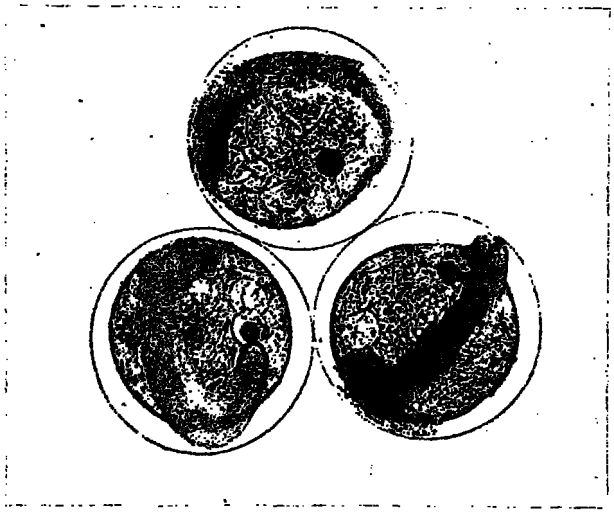

FIGURE 12.—Thirty-eight-hour stage with early embryo.

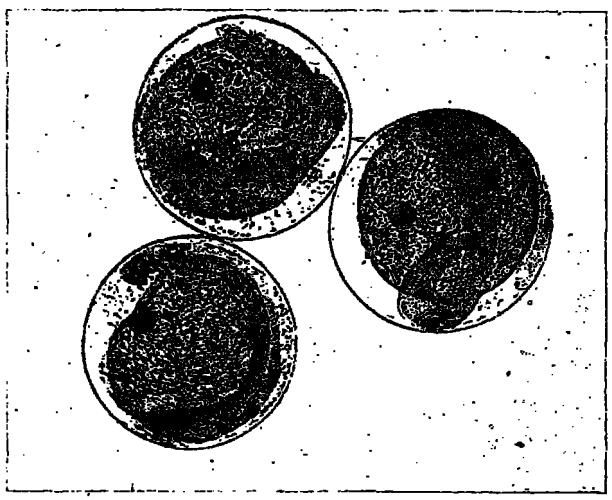

FIGURE 13.—Forty-one-hour stage with tail separating from yolk.

FIGURE 14.—Forty-six-hour stage with late embryo.

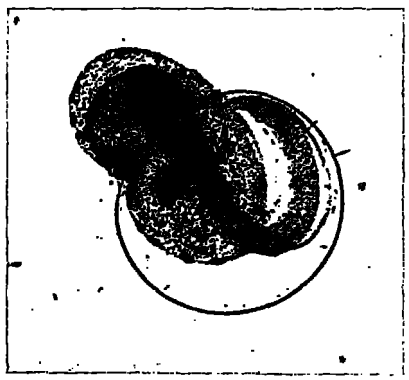

FIGURE 15.—Forty-six-hour stage hatching.

LATE-STAGE EGG

The embryo had grown so large that the tail, free of the yolk, fell just short of touching the head (fig. 14). The somites were visible except near the end of the tail. The embryo was very active, exhibiting convulsive movements at frequent intervals. The tail was outlined with a distinct finfold. Pigmentation generally was limited to one to three small chromatophores along the tail and three to eight in the head or anterior region. Pigmentation of embryos was variable, and no pattern or concentration of chromatophores was discernible. Hatching occurred with the rupture of the external membrane, and the larva emerged, head first (fig. 15), 46 hours after fertilization.

YOLK-SAC LARVA

Yellowfin menhaden, like many other fishes with pelagic eggs (Ahlstrom and Counts, 1955), hatched in a relatively undeveloped condition. The mouth had not formed, and the eyes were unpigmented. Fin rays had not developed, and the pectoral fin buds were not visible. However, the anus had formed and was discernible as a tube passing through the finfold.

The early larva (figs. 16 and 17) floated ventral side up, with the yolk and oil globule uppermost, except during brief, convulsive swimming. During initial swimming, the larva oriented dorsal side up and then, in a head-down position, would move towards the bottom. Body movement would stop after a few seconds and the larva would turn ventral side up and float towards the surface. Such behavior was most marked during the first 24 hours. As the larva grew and the yolk diminished, swimming increased and by 48 hours was

nearly continuous. Even during brief periods of rest, vertical stability was maintained with the dorsal surface up.

Measurements of larvae are given in table 2. The larvae nearly doubled in length during the absorption of the yolk; however, 80 percent of this increase occurred during the first 27 hours.

The late larva continued to lengthen slightly after the 27-hour stage (fig. 18). Between 40 and 60 hours the most apparent change was the shrinking yolk sac (figs. 19 and 20). At the 62-hour stage, eye pigment developed, and the mouth opened (fig. 19). Swimming was continuous and directed as the larva moved across a 6-inch culture bowl with apparent ease. It constantly counteracted the bouyancy of the yolk by swimming in a head-down position. Prior to the development of eye pigment, larvae appeared randomly distributed in the culture bowl and did not react to the approach of the pipette used to collect samples. After the appearance of pigment, larvae oriented away from the source of light and swam from the approaching pipette.

Pigmentation of yolk-sac larvae was limited to widely spaced, small chromatophores along the sides and on the finfold. The chromatophores appeared as faint speckling at a magnification of 20 × and as distinct structures at 100 ×.

FIGURE 16.—Newly hatched larva 2.8 millimeters long.

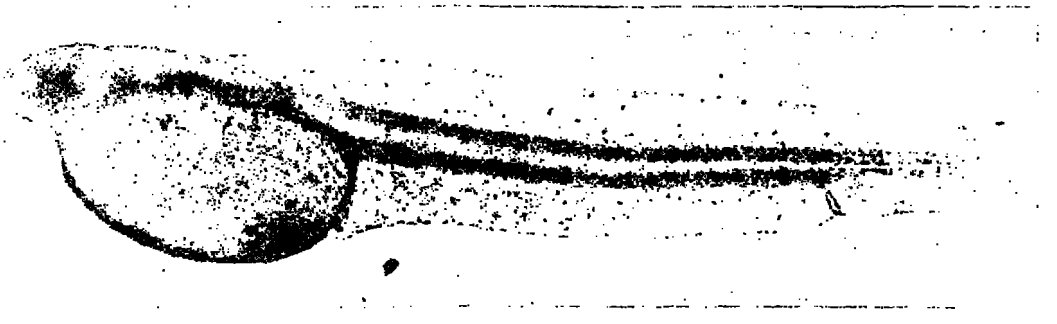

FIGURE 17.—Sixteen-hour larva 4.0 mm. long.

TABLE 2.—*Measurements* [1] *of yellowfin menhaden yolk-sac larvae, in millimeters*

(N=10 specimens at each age)

Hours since hatching	Total length		Distance snout to anus		Yolk-sac length		Yolk-sac width	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0	2.70–2.92	2.79	2.26–2.42	2.35	1.10–1.21	1.16	0.77–.94	0.83
16	3.85–4.18	4.03	3.24–3.41	3.32	1.10–1.21	1.16	.72–.77	.74
27	4.24–4.68	4.46	3.41–3.68	3.59	.94–1.04	1.00	.66–.72	.67
40	4.34–4.62	4.49	3.46–3.74	3.57	.77– .88	.81	.50–.60	.54
62	4.43–5.28	4.86	3.58–4.18	3.79	.38– .60	.53	.22–.39	.27

[1] Measurements of yolk-sac larvae were of preserved material. Ahlstrom and Ball (1954) estimated as much as 20 percent shrinkage due to formalin preservation. Investigators examining fresh larvae should interpret the measurements accordingly.

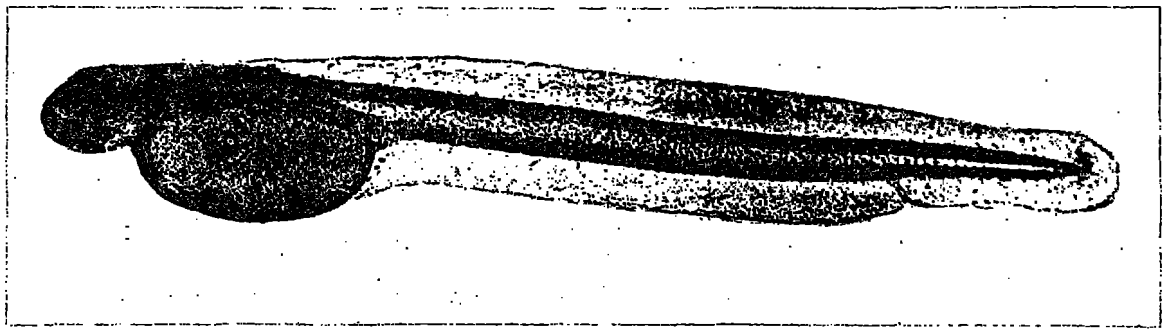

FIGURE 18.—Twenty-seven-hour larva 4.5 mm. long.

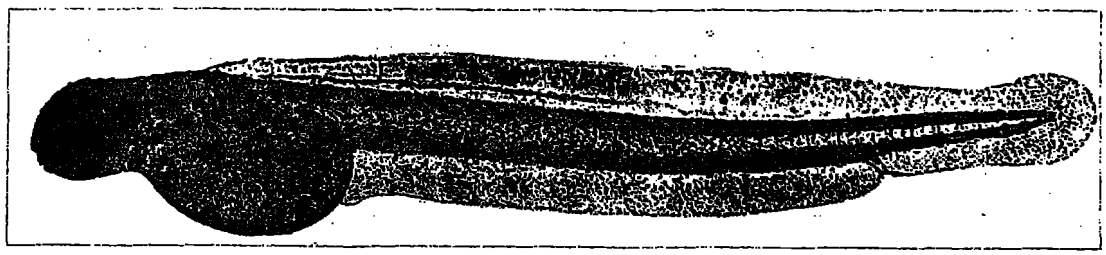

FIGURE 19.—Forty-hour larva 4.5 mm. long.

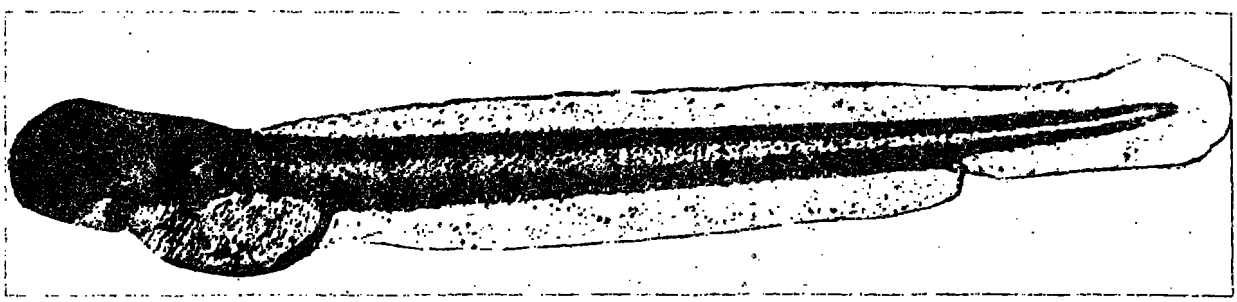

FIGURE 20.—Sixty-two-hour larva 4.9 mm. long.

The rest of the larvae died within a few hours after the 62-hour stage.

I wish to acknowledge the facilities and help furnished by Sembler Fisheries, Sebastian, Fla. Persons connected with the firm gave direct assistance during regular and trial fishing trips, plankton tow-net collections, and examination of the landings.

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