THE EMBRYOLOGY AND LARVAL DEVELOPMENT OF THE GOLDFISH (CARASSIUS AURATUS L.) FROM LAKE ERIE

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INTRODUCTION

Osburn (1901) noted that the common goldfish (*Carassius auratus* L.) had escaped from cultivation in some parts of Ohio and as early as 1888 was reported by Henshall as "not rare in the canal basin near Elmwood, Hamilton County." The species has become quite common in Lake Erie in shallow parts, especially bays and creek mouths (Greeley, 1928). It has been reported by fishermen off the north shore at Port Stanley where it is said to attain a length of 14 to 15 inches. The description of its early development has not, however, been included by Mrs. Fish (1932) probably because it is not an indigenous species.

The goldfish and other Cyprinidae have long been used by geneticists, and also by embryologists, for specialized studies in fish development. However, other than the account by Khan (1929) and the illustrations by Dr. F. J. Myers in the volume by Innes (1936) an adequate description of the general features of its embryonic and larval development is lacking. The following account is an attempt to fill this gap since the goldfish is becoming increasingly important not only from the standpoint of the ornamental fish trade, and as an experimental test animal, but also because of its ability to adapt itself successfully to environmental conditions in Lake Erie.

MATERIALS AND METHODS

All of the eggs for this study were obtained from two sources, Squaw Harbor, South Bass Island, Ohio, in the summer of 1938, and the Moore Water Gardens, Port Stanley, Ontario, in the spring of 1939. The latter provided stages from spawning through early cleavage, since in the breeding cages it was possible to obtain eggs very shortly after they had been deposited on the roots or leaves of any convenient floating aquatic plant such as *Potamogeton*, *Valisneria*, or *Chara*.

All observations were made on living material which was

later fixed in Bouin's Fluid and preserved in 70% alcohol for future reference. The eggs were brought into the laboratory while still attached to water plants and small sections of the latter bearing eggs were transferred to finger bowls containing lake water. To simulate natural conditions some were hung over the float in bottles lowered to a depth of six to twelve inches, while others kept in a semi-dark room were subjected to strong sunlight for short periods daily. No discernible differences were evident between those reared under the two conditions.

Larvae after the first five to seven days were kept in an aquarium in aerated water. Food consisted of plankton taken by a fine net from the adjacent region of the lake, and finely ground rice flour.

SPAWNING

Fearnon (1931) states that goldfish begin breeding in their second year and while they may continue to reproduce for six or seven years they yield the maximum number of eggs in their third and fourth years. Khan (1929) found that spawning usually begins early in the spring and occurs at frequent intervals from April to August over a period from 7 a. m. to 10 a. m. Innes (1936) noted that spawning usually starts at daybreak and lasts till mid-afternoon. From early spring it may be repeated every few weeks until early August but the first spawn of the season is the largest. At Squaw Harbor eggs were obtained in segmentation stages about 8 a. m. to 9 a. m. as late as August 17th, 1938. The capsules of the eggs are of a mucilaginous character and adhere readily to aquatic plants to which they are usually attached singly, rarely in twos or threes and at intervals of one-half to one inch.

EMBRYONIC DEVELOPMENT

Characteristics of the egg

The eggs of the goldfish are spherical pale cream-colored globules, 1.25 to 1.46 mm. in diameter, and slightly flattened along the margin of attachment. When first laid the whole surface is adhesive, but this quality is lost as soon as they have become "water hardened" and attached to aquatic plants. Like other fish eggs, those of this species are telolecithal, the heavier yolk lying beneath the cap-like whitish protoplasmic blastodisc which covers the upper surface (fig. 1). A narrow perivitelline space (.1 mm.) separates the yolk sphere from the heavy structureless egg capsule. The cream-colored yolk is rather unique since it appears to be coarsely granular with a rather dense fluid matrix

containing sparsely scattered transparent oil droplets (.01 to .05 mm. in diameter). The latter have a tendency to disappear during later stages of development.

Period of Incubation

Khan (1929) found the hatching period to vary from forty-six to fifty-four hours at 84° F. Innes (1936) reports a hatching period of from four to fourteen days according to temperature. At 70° to 75° F. five to seven days are required. No attempt was made to rear the eggs from Squaw Harbor at a constant temperature, but those kept in the laboratory with an air temperature ranging from 18.5° C. to 29.5° C. hatched in three to four days. Eggs placed in containers and reared in the lake water which was subject to less radical temperature variations (24° C. to 28° C.) required only sixty-four to seventy-two hours. At Port Stanley, following the late cleavage stages, eggs were subjected to a constant temperature of 25° C. and hatching took place in seventy-six hours.

Cleavage Stages

A few minutes after fertilization the blastodisc contracts and forms a white dome on the upper surface of the yolk (fig. 1). In approximately half an hour the first cleavage furrow, a vertical meridional one, appears (fig. 2) forming two large and approximately equal blastomeres. Fifteen minutes later the second cleavage furrow also a vertical meridional one appears at right angles to the first (fig. 3). Following this, cleavage becomes irregular and in two hours' time the blastoderm is composed of a mass of relatively small but distinct cells (fig. 4). After four hours' development the cells have become relatively smaller owing to rapid cell division, and the margin of the blastoderm has begun to extend slightly farther over the yolk (fig. 5). In each cell the cytoplasm shows a tendency to radiate from the nucleus.

Investment of the yolk by the blastoderm

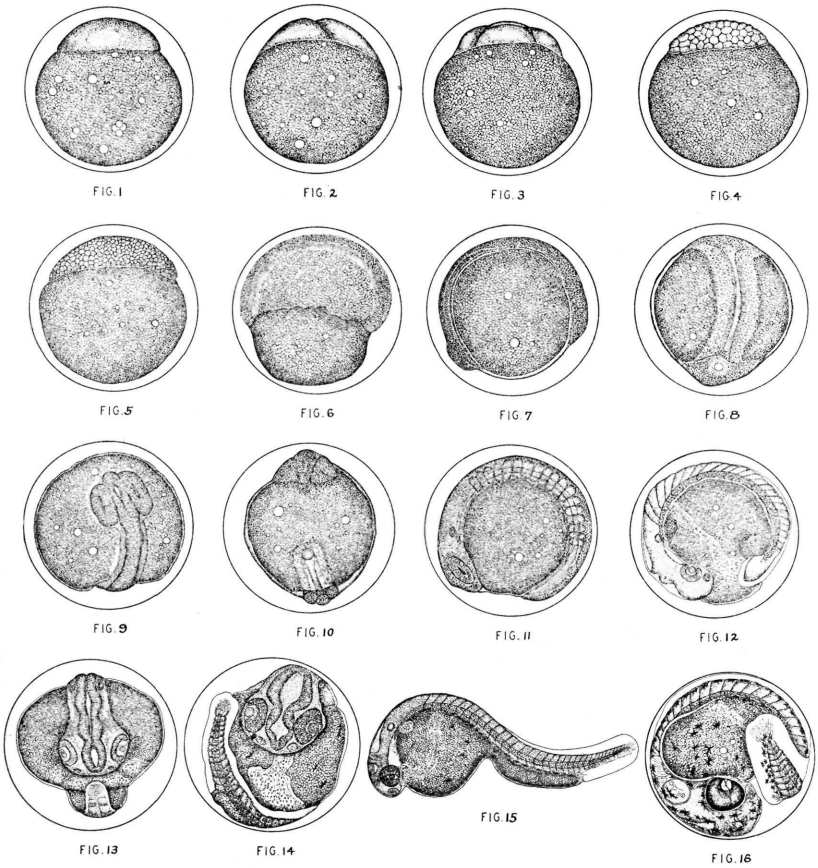
The blastoderm becomes thinner along its free edge and now commences to grow ventrally to extend over the yolk sphere. By seven hours' of incubation, it has reached the equator of the egg (fig. 6). The advancing rim of the blastoderm stands slightly above the surface level of the yolk. In the nine hours' stage the blastoderm has completely encircled the yolk with the exception of a small spherical blastopore surrounding a plug of yolk.

Differentiation of the Embryo

Ten hours.—The surface view gives no indication of the embryonic axis aside from a slightly opaque area of undifferentiated tissue passing anteriorly along one radius from the margin of the blastopore.

Eleven to twelve hours.—The axis of the fish embryo is visible as a narrow but rather high transparent ridge extending forward from the blastopore, nearly encircling the yolk (figs. 7 and 8), and bounded on either side by the lateral margins of the embryonic shield. The future head region appears as a flat somewhat oval expansion. By twelve

hours three to four mesodermal somites have appeared and the notochord is differentiated as a narrow band of cells lying next to the yolk substance.



(Drawings by camera lucida)

Fig. 1. Recently fertilized egg.
Fig. 2. First cleavage division. 30 mins.
Fig. 3. Second cleavage division. 45 mins.
Fig. 4. Many-celled blastoderm. 2 hours.
Fig. 5. Advanced blastoderm. 4 hours.
Fig. 6. Yolk mass half overgrown by blastoderm. 7 hours.
Fig. 7. Early embryo, lateral aspect. 11 to 12 hours.
Fig. 8. Early embryo, dorsal aspect. 11 to 12 hours.
Fig. 9. Later embryo with optic vesicles, dorsal view. 15 hours.
Fig. 10. Later embryo with optic vesicles, ventral view. 15 hours.
Fig. 11. Embryo of 18 somites. 17 hours.
Fig. 12. Embryo of 25 somites. 24 to 27 hours lateral aspect.
Fig. 13. Embryo of 25 somites, dorsal aspect. 24 to 27 hours.
Fig. 14. Embryo of 32 somites. 45 hours.
Fig. 15. Embryo of 32 somites removed from egg capsule. 45 hours.
Fig. 16. Advanced embryo, preparatory to hatching. 65 hours.

Fifteen hours.—Although the blastopore has not yet closed, marked changes have taken place in the anterior axial region (figs. 9 and 10) of the embryo. The brain is clearly visible and the optic evaginations are somewhat oval extending posteriorly from the primary cerebral lobe. Eight to ten somites are present. The embryonic body appears as a thickened ridge and passes by a thin narrow margin to the portion of the blastoderm now one or two cells in thickness, which spreads over the yolk.

Seventeen hours.—The tail reaches almost around to the anterior limit of the head (fig. 11), but the embryo is still adherent to the yolk sac at both ends. Eighteen somites have been differentiated. Auditory vesicles as well as olfactory pits have appeared. The notochord extends as a solid rod of cells from the auditory vesicle to the undifferentiated caudal mass.

Twenty-four to twenty-seven hours. The embryonic body has increased in length, the tail process reaching almost to the anterior limit of the head. Both the head and tail are freed from the yolk surface. A narrow fin fold surrounds the tail and extends forward dorsally to the mid body region (figs. 12 and 13). Distinct contractile movements are evident. Twenty-five somites have been differentiated, but no pigment is visible. The brain is composed of three cerebral lobes, the posterior (rhombencephalon) being elongate with a diamond-shaped ventricle. The optic vesicle has been converted into an optic cup into which the lens has sunken. The notochord is visible as a vacuolated rod extending from the level of the auditory vesicle to the undifferentiated tissue in the core of the tail. The heart has appeared on the anterior surface of the yolk sac as a flattened tube and is pulsating irregularly. The ducts of Cuvier traverse the yolk, passing ventrolaterally from the level of the first few mesodermal somites to the posterior end of the heart. The dorsal aorta is also visible in the trunk region.

Forty-five to fifty hours.—The embryo now forms more than a complete circle around the circumference of the yolk. Its tail lies either to the right or left of the head and is often bent at an angle across the front of the latter. It undergoes rhythmic movements and swims feebly when the capsule is removed with a needle. Small scattered melanophores have appeared on the outer surface of the eye, larger stellate ones over the head and the yolk sac and along the dorsal portion of the body and the primitive intestine (figs. 14 and 15). The brain lobes have increased in size. The yolk sac which has been spherical now consists of an anterior spherical to oval division and a posterior cylindrical portion which terminates at the anus. The pectoral fins appear as fleshy folds lying over the yolk sac at the level of the auditory vesicle. The typical teleostean circulation has been set up at this time. Size measurements are as follows: Total length (head-tail), 2.5; standard length, 2.4; length to vent, 2.0; length of head, 0.70; snout, 0.10; diameter of eye, 0.25; greatest depth before vent, 1.0 millimeters. Myomeres 20 to 22 to vent plus 10 to 12 behind.

Sixty to sixty-five hours.—The embryo has increased considerably in size occupying most of the perivitelline space. Rhythmic movements

occur freely within the egg capsule (fig. 16). Heavy stellate melanophores are massed over the head, the yolk sac, along the dorsolateral musculature, and ventrally over the region of the intestine and ventral musculature. The eye has increased in size and has become densely pigmented. The auditory vesicle is now enlarged and thin-walled, containing two otoliths and the rudiments of semi-circular canals. The dorsal fin fold extends forward to the level of the first somite. The pectoral fins are now free from the yolk surface and are composed of a fleshy base bearing a membranous tip. Removed from the capsule the embryo now resembles the newly hatched larva. Total length 4.4; standard length, 4.2; length to vent 2.9; length of head, 0.78; snout, 0.17; diameter of eye, 0.3; greatest depth before vent, 0.95 millimeters. Myomeres, 21 to 22 to vent plus 11 to 12 behind.

DEVELOPMENT OF THE LARVA AND POST LARVA

Newly-hatched larva (fig. 17.) The larva frees itself by violent lashing movements of the tail which eventually rupture the egg capsule. Total length, 4.5; standard length, 4.3; length to vent, 3.0; length of head, 0.8; snout, 0.17; diameter of eye, 0.3; greatest depth before vent, 0.95 millimeters. Myomeres, 21 to 22 to vent plus 11 to 12 behind.

Pigmentation consists of stellate melanophores located around the jaws, on the dorsal surface of the head, and the whole dorsal aspect anteriorly to the caudal fin. The pigment spots are fewer on the sides of the head and body at the level of the lateral line. There is a heavy subsurface mass of them dorsal to the gill arches, and a double to triple series on the dorsal surface of the intestine extending beyond the vent to the tip of the notochord. The yolk sac bears large scattered melanophores more especially anteriorly. Swimming movements are somewhat restricted owing to the mass of yolk material. The larva shows a positive thigmotropism, adhering to the aquarium walls or any fragments of plants. The heart is differentiating into chambers although it is still almost vertical in position at the anterior end of the yolk sac. Five aortic arches are present. The continuous median fin fold with its preanal portion has already been adequately described by Grimm (1937). The cerebral hemispheres, pineal body, mid brain (optic lobes), cerebellum and medulla oblongata are all clearly distinguishable.

5.8 millimeter stage. (fig. 18). Age 1½ to 2½ days. Total length, 5.8; standard length, 5.2; length to vent, 4.1; length of head, 1.3; snout, 0.3; diameter of eye, 0.35; greatest depth before vent, 0.85 millimeters. Myomeres, 22 to vent plus 12 behind. The embryo shows a distinct reduction in the size of the yolk sac which has now become almost tubular due to its greater absorption anteriorly.

Pigmentation resembles that of the recently hatched larva except for an increase in density especially in the eye, and the appearance of heavy masses of yellow pigment spots (xanthophores) along the dorsal musculature and over the head. The mouth has opened and an opercular membrane is growing posteriorly over the gills. The air bladder is partially inflated. The gut is a simple tube dilated to some extent just behind the air bladder. The heart owing to the absorption of the yolk material now assumes an axis parallel with the median longitudinal

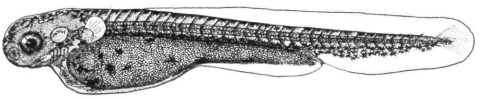
axis of the body, by the dorsal migration of the posterior end in an arc of approximately 30°.

6.8 millimeter stage (fig. 19). Age 7 to 8 days. Total length, 6.8; standard length, 6.1; length to vent, 4.7; length of head, 1.45; snout, 0.3; diameter of eye, 0.52; greatest depth before vent, 1.0 millimeters. Myomeres, 22 to vent plus 12 behind. The most conspicuous change, while the fish grows from 5.8 millimeters to 6.8 millimeters, takes place in the absorption of the yolk material, which is now represented by a few granules midway between the pectoral fin and the anus. The mouth has enlarged and the lower jaw moves rhythmically. The posterior end of the notochord has become bent upward slightly and rudiments of caudal fin rays are evident in the fin fold among the melanophores below the curved notochord. The outline of the fin itself however remains somewhat truncate. Fin rays are not evident elsewhere although a concentration of mesenchyme occurs at the region of the future dorsal. The operculum is well developed and a few gill filaments protrude beyond its posterior margin. Melanophores on the dorsal surface of the head are beginning to round up and others have appeared on the ventral margin of the operculum and on the dorsal surface of the air bladder. Dense yellow pigment is present all over the surface of the body. The lateral line has become more distinct. The alimentary canal is a straight tube enlarged anteriorly. The liver, reddish yellow in color, appears as a triangular mass on the ventral surface immediately posterior to the heart.

7.9 millimeter stage (fig. 20). Age 15 to 18 days. Total length, 7.9; standard length, 7.0; length to vent, 5.3; length of head, 1.65; snout, 0.35; diameter of eye, 0.71; greatest depth before vent, 1.20 millimeters. Myomeres, 22 to vent plus 12 behind. Melanophores about the head region have rounded up but over the rest of the body are stellate. A new row has appeared marking the lateral line. The yellow pigmentation is heavy and the general body surface is taking on an iridescent appearance. The caudal fin has forked into dorsal and ventral lobes supported by unbranched fin rays. The dorsal fin region is marked by an elevation of the fin fold and the appearance of elongated melanophores among the embryonic rays. The anal fin is indicated by an accumulation of mesenchyme and melanophores in the median fin fold which has become slightly lobed a short distance behind the anus. Embryonic fin rays have appeared in the pectorals. The otoliths have become almost as large as the lens of the eye. The operculum completely covers the gills. The air bladder is partially divided into two chambers. Minute spherical partially pigmented elevations are present on the lips.

9.4 millimeter stage (fig. 21). Age 22 to 23 days. Total length, 9.4; standard length, 8.3; length to vent, 6.4; length of head, 2.4; snout, 0.52; diameter of eye, 0.85; greatest depth before vent, 1.7 millimeters. Myomeres, 22 to vent plus 12 behind. The organism is becoming noticeably more opaque and has taken on a greenish yellow to fawn coloration. Aside from the margin of the operculum and adjacent to the fin rays, the melanophores are no longer stellate but contracted into spherical spots which are concentrated chiefly above the lateral line. The caudal fin shows little advancement over the 7.9 mm. stage, but the dorsal now

bears nine true rays. The median fin fold is beginning to disappear dorsally between the dorsal and caudal fins and between the caudal and anal fins ventrally. The anal and pectoral fins have developed distinct rays. The pelvic fins have appeared as minute lateral fleshy protuberances from the body wall midway between the pectorals and the anus.



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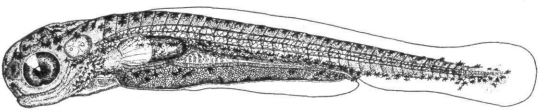

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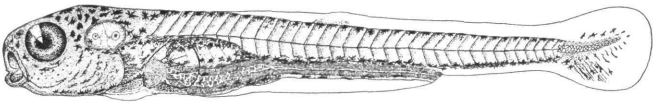

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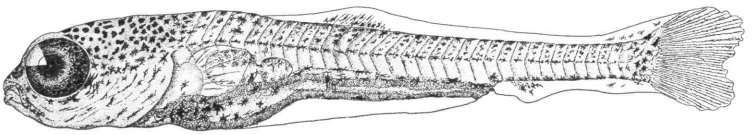


FIGURE 20

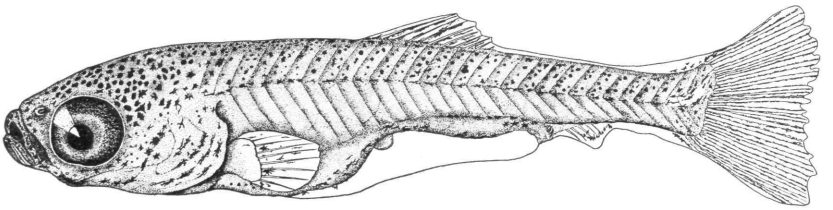


FIGURE 21

(Drawings by camera lucida)

Fig. 17. Newly-hatched larva. 4.5 mm.
Fig. 18. 5.8 mm. stage. 1+ to 2+ days.
Fig. 19. 6.8 mm. stage. 7 to 8 days.
Fig. 20. 7.9 mm. stage. 15 to 18 days.
Fig. 21. 9.4 mm. stage. 22 to 23 days.

11.6 millimeter stage. Age 37 days. Total length, 11.6; standard length, 9.6; length to vent, 8.2; length of head, 3.1; snout, 0.68; diameter of eye, 0.95; greatest depth before vent, 2.3 millimeters. Myomeres,

22 to vent plus 12 behind. Differences from the preceding stage are principally in the greater development of the pelvic fins, in which there is an indication of rays. The preanal fin fold is reduced anteriorly, and the caudal rays are commencing to branch. The body has become more opaque, greenish yellow and iridescent in appearance.

15.7 millimeter stage. Approximate age 9 weeks. Total length, 15.7; standard length, 12.6; length to vent, 10.0; length of head, 4.0; snout, 0.8; diameter of eye, 1.4; greatest depth before vent, 4.5 millimeters. The fish now has acquired essentially the form and shape of the adult and varies from olive green or brown to orange red in color. Pigmentation is general. The body is fully scaled. Segmentation and branching of some of the fin rays may continue for some time (Grimm, 1937).

TABLE I

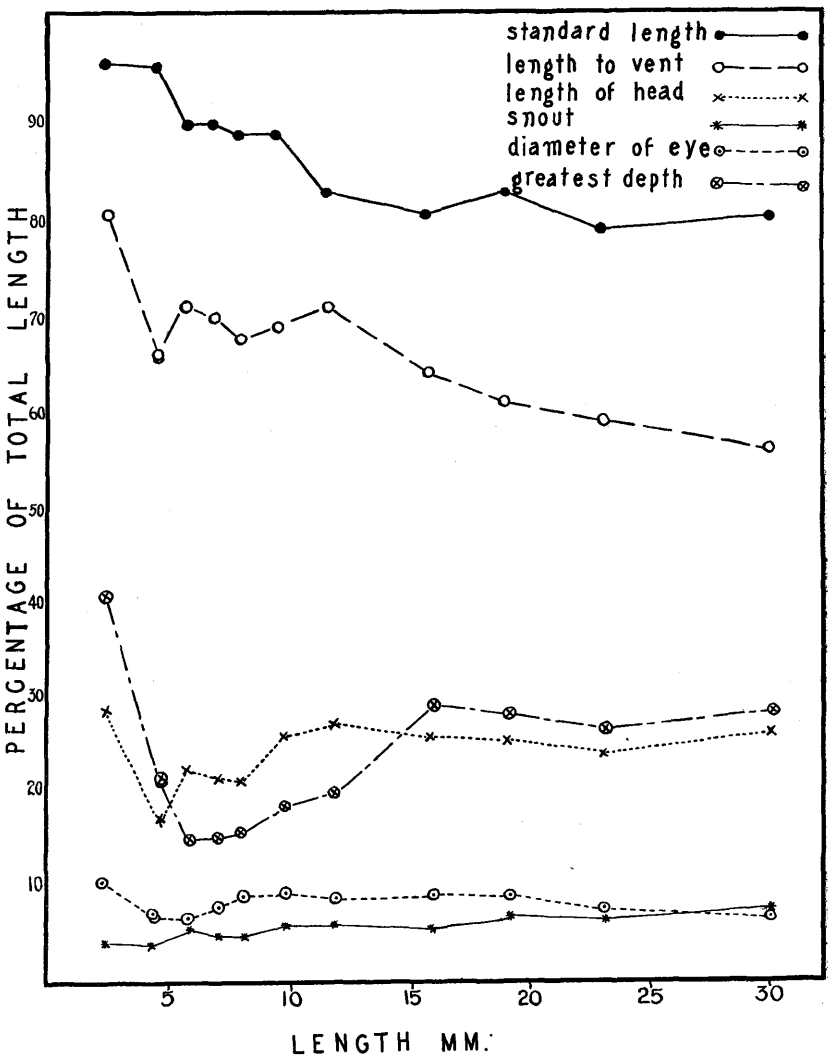
MEASUREMENTS OF GOLDFISH THROUGH PREHATCHING, LARVAL, AND YOUNG STAGES

Age	Total Length, mm.	Standard Length, mm.	Length to Vent, mm.	Length of Head, mm.	Snout, mm.	Diameter of Eye, mm.	Greatest Depth before Vent, mm.
Hours:							
45–50	2.5	2.4	2.0	0.70	0.10	0.25	1.00
60–65	4.4	4.2	2.9	0.78	0.17	0.30	0.95
76*	4.5	4.3	3.0	0.80	0.17	0.30	0.95
Days							
1½–2½	5.8	5.2	4.1	1.30	0.30	0.35	0.85
7–8	6.8	6.1	4.7	1.45	0.30	0.52	1.00
15–18	7.9	7.0	5.3	1.65	0.35	0.71	1.20
22–23	9.4	8.3	6.4	2.40	0.52	0.85	1.70
37	11.6	9.6	8.2	3.10	0.68	0.95	2.30
Weeks							
9	15.7	12.6	10.0	4.00	0.80	1.40	4.50
?	19.0	15.7	11.5	4.80	1.30	1.60	5.30
?	23.0	18.0	13.5	5.50	1.50	1.70	6.00
?	30.0	24.0	17.0	7.80	2.10	2.00	8.50

*Newly hatched larvae.

Table I gives the measurements of goldfish from 2.5 mm. (45 to 50 hours incubation) to a length of 30.0 mm. attained at the end of approximately 3½ to 4 months in an outdoor aquarium. All figures are average measurements of six specimens. The accompanying graph (graph 1) indicates the standard length, length to vent, length of head, greatest depth anterior to vent, snout, and diameter of eye, as percentages of the total length. The large initial drop of the greatest depth ratio is attributed to the absorption of the yolk substance. It will be observed

that for specimens from 15.7 mm. the percentage lines are almost parallel to one another, which would seem to indicate that only relatively minor if any changes in proportion are occurring.



Graph 1. The relation between the total length of the prehatching, larval and young goldfish, and the standard length, length to vent, length of head, length of snout, diameter of eye, greatest depth before vent, expressed as percentages of the total length.

SUMMARY

This paper is the result of a preliminary study of the salient features of the embryology of the goldfish (*Carassius auratus* L.). It is based upon material obtained from Lake Erie and reared at the Stone Laboratory and at Port Stanley.

The goldfish is typical of the teleosts in its general development. The eggs which adhere to floating aquatic plants are 1.25 to 1.46 mm. in diameter and hatch in seventy-six hours at 25° C. The first cleavage takes place one-half hour after fertilization, and in nine hours the blastoderm has completely encircled the yolk with the exception of a small spherical blastopore. In twelve hours four somites are visible, in fifteen hours eight to ten. Two hours later the tail reached almost to the head, and eighteen somites have been differentiated. At twenty-four hours the somite count is twenty-five, the brain is distinctly divided into three primary lobes and the heart is beating rhythmically. Further development to hatching involves pigmentation by melanophores, enlargement of all the embryonic structures, and an elongation of the yolk sac posteriorly.

At hatching the larva is 4.5 millimeters in length, and restricted in movement by the weight of the yolk sac. By one and one-half to two and one-half days, a length of 5.8 millimeters is attained, and the yolk sac has been reduced to a narrow tubular band. Yellow pigmentation has appeared, and the standard myomere count of 22 to vent plus 12 behind is attained. The air bladder is partially inflated. At seven to eight days (6.8 millimeter stage), the yolk material has practically all disappeared. Rudiments of caudal fin rays are evident. The operculum practically covers the gills and the liver is present as a triangular mass at the anterior end of the body cavity.

The 7.9 millimeter stage (age 15 to 18 days) shows increased yellow pigmentation and reduction of melanophores to blackish spheres in the head region. The air bladder has divided into two chambers. By 22 to 23 days (9.4 millimeter stage) the organism is becoming more opaque. The pelvic fin buds have just appeared and fin rays are present in all the other fins. Pigmentation is concentrated above the lateral line.

At approximately nine weeks (15.7 millimeters) the fish has acquires scales, pigmentation is general, and the body bears essentially the adult characteristics.

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