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THE DEVELOPMENT AND SEQUENTIAL OSSIFICATION IN THE MARINE CATFISH, *TACHYSURUS THALASSINUS* (RUPPELL)

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ABSTRACT

The development of *Tachysurus thalassinus* is described based on experimental rearing of embryos, from early embryonic stage to complete absorption of yolk sac. The time taken for development from egg to complete absorption of yolk is thirty five days. The progressive ossification of embryonic/larval skeleton is studied with the help of alizarin - red S stained, embryonic skeleton at various stages of development. The prominent characters of the species are laid down early in the larval stages and maintained throughout the life.

INTRODUCTION

Identification of embryos of larvae does not pose any problem as far as marine catfishes are concerned, because they live in parental mouth till the post-larvae are released, on complete absorption of the yolk sac. The characters of the embryos and larvae are quite distinct for each species. These characters appear early and maintain throughout the life history.

In the following account a brief description of the development of *Tachysurus thalassinus* is given, based on embryos/larval rearing studies, along with the sequence of ossification of skeleton. With the exception of short accounts on the eggs and some of the larval stages of *Galeichthys felis* by Gudger (1916, 1918), Merriman (1940) and Ward (1957), the early development of *Arius jella* by Chidambaram (1942) and the developmental stages of *T. thalassinus* by Al-Nasiri & Hoda (1977) no other detailed information is available on the early development of tachysurids. Chidambaram (1942) reared *A. Jella* larvae in the laboratory upto the stage of complete absorption of the yolk. Based on artificially fertilized eggs, Ward (1957) gave a short account on the cleavage and early embryology of *Galeichthys felis* from the Biloxi (Mississippi) Bay. Eventhough the skeleton of several tachysurids has been described, the progressive ossification has not been studied in this group. Almost all investigations on bone formation have been limited to some specified areas like vertebral column of *Lebistes* (Mookerjee *et al.*, 1940), the caudal fin of *Thymalles arcticus* (Norden, 1961) and the opercular apparatus of *Salvelinus* sp. (Day, 1963). The present investigation brings out in detail the sequential ossification of the various bones in *T. thalassinus* at different stages of development.

MATERIAL AND METHODS

The embryos were collected from Mandapam and Calicut during the breeding seasons (April - August) of 1970 - '80. Live embryos were placed in a shallow trough, containing filtered sea water, with the

embryos resting on a piece of plastic wire netting, loosely stretched across the wide mouth of the trough, sagging in the centre. The level of sea water was just enough to cover the embryos. The sea water was changed twice daily and kept in circulation and was properly aerated. The embryos were rinsed with sea water twice daily to prevent any accumulation of debris and to ward off ciliate attack. The temperature of the water was maintained within the range of 29.5 - 31.0°C. The development of embryos was traced till the complete absorption of yolk. Many fertilized eggs, embryos and larvae at different stages of development were also collected from the mouth of gestating males and were compared with the aquarium-reared ones.

In order to study the sequential ossification, embryonic/larval skeleton was stained with alizarin red S *in toto* by following the method of Lipman (1935) with suitable modifications. After removing the yolk sac, the embryos/larvae were hardened for two weeks in 5% formalin. After sufficient hardening the specimens were rinsed in running water for 2 to 12 hours to remove the formalin. The embryos/larvae were cleared in 2% potassium hydroxide solution for 1-3 days until the entire dorsal area and the caudal peduncle became transparent. The samples were stained with alizarin red S dye solution for 1 to 3 days depending on the strength of the dye solution and the size of the embryo/larva. After staining, the samples were transferred to 1% KOH solution to remove excess dye from the flesh. After passing through 3 different steps in a solution of 2% KOH and glycerin at the ratio of 60:40, 40:60, and 10:90 for periods of 24 hours in each stage, the specimens were finally stored in pure glycerin with a crystal of thymol.

Results

The egg

The egg of *Tachysurus thalassinus* is spherical in shape, golden yellow in colour, translucent and measures, on average, 14 mm in diameter. It is heavily yolked and, when dropped in water sinks rapidly to the bottom. The perivitelline space is narrow in the unfertilized egg, but becomes wider in fertilized egg (Fig. 1). The chorion (the egg membrane or zona radiata) is thin, transparent and the surface of the egg shell appears like a sieve. The yolk is yellow, dense and granular. The blastodisc is narrow and transparent. The vitelline membrane is thin, transparent and is easily ruptured. The freshly spawned eggs stick together in a cluster by means of a mucous substance. After fertilization the eggs get separated and remain in the mouth of the male parent. The perivitelline space in the embryonic area is wider than in other regions of the egg.

Early development

As in most species of teleosts, *T. thalassinus* displays a meroblastic type of cleavage, in which the segmentation is confined to the disc of protoplasm mounted on the yolk. The following developmental stages were obtained by rearing the embryos in the laboratory and also by observations on the eggs and embryos, in different stages of development, collected from the field.

Stage I : Examination of ten eggs in the blastula stage showed that the blastodisc is well formed and berry-like in appearance. It measures 1 mm in diameter at the centre of the embryonic area (Fig.2).

Stage II : The segmentation cavity is present at this stage. The blastula spreads over a wider area as a white patch over the yellow yolk surface and the cell layers are fragile (Fig. 3).

Stage III. The embryonic shield is well advanced in development and is seen as a slight thickening over the yolk. It is pear - shaped, with excessive thickening at the pointed end (Fig. 4).

Stage IV (8 mm embryo): The embryo is barely visible to the naked eye. The brain is a three vesicle structure with no specific indication of the cerebral hemispheres and cerebellum. The mid and hind brain at this stage, appear to be in the form of neural groove. The optic cups make their appearance and olfactory placods are also visible. The pulsating heart is visible. Two of the gill buds are noticed at this stage. No barbels are seen and the mouth appears as a crescent shaped depression ventrally, far behind the eyes. A fin fold is seen, beginning dorsally immediately behind the head and gradually broadening out posteriorly to mark the future adipose dorsal, well demarcated from the caudal fin by a notch. The caudal fin is pointed and the tip of the notochord is directed upwards. No fin rays are noticed in any of the fins. On the ventral side, the caudal fin fold extends upto the anus, where it is broader than in the dorsal part. The anterior gill bud is covered by the opercular flap while the posterior gill bud is open to the exterior. The alimentary tract is a straight tube extending from the mouth to the anus. At the anterior part of the gut is seen a small dorsal pouch, the future air bladder, still connected to the alimentary tract by a wide opening. The enbryo lies horizontally over the yolk mass. The tail is now separated from the yolk sac, and twitching movements of the tail and trunk are noticeable. The embryo is devoid of any pigmentation at this stage.

A pair of blood vessels orginates from the ventral part of the head, runs close together anteriorly for a short distance and then diverges outwards, skirting the embryo along the margins of the blastoderm. The flow of blood in these vessels is towards the heart. In the posterior region of the embryo another pair of blood vessels, omphalomesenteric vessels, makes their appearance as faint channels giving off small lateral branches. Under low magnification, the slow circulation of blood from the heart, is visible in the omphalomesenteric vessels (Figs. 5 and 6).

Stage V (16 mm embryo) : The embryo appears embedded in the yolk. The most important change noticed at this stage is the pigmentation of the retina. A small number of melanophores appear along the dorsal surface of the head. Rolling movements of the eye are noticed at this stage. Four gill buds are clearly seen, each containing efferent-branchial vessels. The pectoral fin buds are present as leaf-like structures immediately dorsal to the developing omphalomesenteric vessels. Two to three fin rays are visible in the pectoral fin buds. The pulsating heart shows occasional pauses for 2 to 5 seconds. The urostyle of the embryo is curved upwards and the caudal fin is rounded and has 4 to 6 fin rays. Anterior part of the median fin fold is marked off from the continuous median fin fold by a constriction and it is slightly thickened with one or two fin rays. Mouth is inferior with the gape extending till eye. The anterior part of the tubular alimentary canal is dilated to form the future stomach. Rudiments of the maxillary barbels are present but no mandibular barbels are noticed at this stage. There are lateral flexion and extension of the body and tail region.

At this stage a pair of additional blood vessels, the vitelline vessels, is observed in the posterior part of the body for supplying blood to the posterior region and also over one half of the surface of the yolk sac. Of the two anterior blood vessels, the right vessel disappears at this stage and the left vessel

occupies a more median position in the anterior region, which extends over the yolk sac. Several lateral branches are given off from this main vessel to the surface of the yolk. This is the main collecting vessel in the embryonic circulation. Just behind the pectoral fin bud, a small protuberance can be seen richly spplied with blood vessels, which is the rudiment of the future liver (Figs. 7,8 and 9).

At this stage of development, the lateral parts of the centra of the first 27 vertebrae are ossified. The base of the parasphenoid is also slightly calcified. The dentaries are the only ossified elements of the jaw. The ossification starts from the anterior margin of the dentaries. The opercles develop their characteristic shape, the jagged ventral margin indicates a downward progression in ossification. The cleithra are the only ossified parts in the pectoral girdle.

Stage VI (25 mm embryo) : There is a remarkable increase in the size of the head, with a large midbrain and very prominent eye balls. The retina is fully pigmented and the eye ball is with an open choroid fissure. Rolling movement of the eye balls can be seen at intervals. The mouth is now in a more terminal position and the external nares are well developed, the posterior opening having a valve. The alimentary canal is separated into the stomach and a single coil of intestine. The air bladder is separated from the stomach except for a small tubular connection. The dorsal fin is separated from the median fin fold and a long and broad adipose dorsal fin is seen at this stage. The caudal fin is round, with a few more additional fin rays. Some 10 to 12 fin rays (appearing as thickening) are now seen in the ventral fin fold, which is still continuous with the caudal fin. Pectoral fins are well developed with fin rays and the pectoral spines make their appearance as a thick first fin ray. On dissection of the embryo, the liver is seen as a light reddish elongated organ in the anterior part of the abdominal cavity. The highly vascularised reddish kidney occupies the entire body cavity of the embryo. The embryo at this stage is slightly recurved and the caudal portion of the body turns towards the right side of the embryo. Occasional twitching movement of the body continues.

The heart occupies a position just below the gillbuds and it pulsates 90 to 100 times per minute. The anterior median vessel and the posterior pair of blood vessels are very prominent but the middle pair of blood vessels is not very clear at this stage. The anterior vessel ramifies over the yolk on the lower part of the yolk sac and the posterior pair of vitelline vessels over the lower and posterior parts of the yolk sac. The former collects the blood from, and the latter supplies blood to the yolk sac. The hepatic and renal portal systems are clearly visible (Fig. 10).

The maxilla, dentary, the circumorbital ring bones, quadrate, interopercle, preopercular and opercular bones are fully ossified. All the 5 branchiostegal rays make their appearance at this stage and the hyoid arch and urohyal are partly ossified. Basioccipital, exoccipital, parasphenoid, mesethmoids and alisphenoids of the cranium show calcification. Sagitta, asteriscus and otoliths are well ossified. Nasal bones are found to be partly ossified. The lateral walls of the pterotic, sphenotic and frontal are fully formed while the supratemporal is partly ossified. Coracoid and clavicle are well ossified. All the vertebrae as well as the neural and haemal arches are ossifed. Typus of the weberian ossicles are clearly visible and a few ribs are formed at the anterior part of the body. The basal portion of the dorsal, pectoral and anal fin rays are now ossified. The caudal fin rays are ossified at its

basal portion, hypurals too are ossified at this stage.

Stage VII (30 mm embryo): The prominent feature at this stage is the complete coiling of the embryo, with the caudal fin resting on its head. There is considerable pigmentation over the dorsal surface of the body, especially over the dorsal and pectoral fin regions. The brain has differentiated into the five secondary divisions. The cerebral hemispheres and the optic lobes assume the adult shape. The cerebellum is seen as a thin flap immediately cepahlic to the roofless fourth ventricle. The auditory placodes are clearly visible in the region opposite the myelencephalon. All the barbels are clearly visible. Mouth is terminal in position. The alimentary tract is well differentiated into the stomach and the intestine which is looped twice before reaching anus. The adipose dorsal fin is comparatively large and separate from the caudal fin. All the fin rays of the caudal fin are developed and the tip of the urochord is turned upwards. The caudal fin is forked. The anal fin is separated from the caudal fin, the fin rays being seen as corrugations in the anal fin lobe. Pelvic fin buds are also evident, with a few rays. The pectoral and dorsal fins are well developed and well differentiated. The first ray is thickened as the prospective spine. Small ossicles are noticed in the lateral line.

The embryo constantly makes twitching movements of the caudal portion along with slight jerking movements of the whole body. Rolling movements of the eye and slight gasping movements of the jaws are also noticed. At intervals, the embryo flips the tail from side to side. The heart beats are 78 to 90 per minute. The head of the embryo is richly vascularised. The two posterior vitelline vessels unite to form a single vessel near the anus and run towards the heart as a single vessel. The yolk sac is richly vascularised, especially at its ventral and posterior parts, by the anterior blood vessel and the posterior vitelline vessels.

In the cranium, all the bones associated with the opercular assembly are well calcified. All the 5 branchiostegal rays are well ossified and the lateral extremities of the lateral ethmoid showed slight ossification. Supraoccipital crest is well formed, the pterygoids, supratemporal, sphenotic and pterotic are partly calcified. The quadrate, urohyal and hyomandibular bones show calcification except at their articulation facets. The first few vertebrae fuse together to form the 'complex vertebra'. All the bones of the weberian apparatus like trypus, claustrum and scaphium are ossified. Almost all ribs commence ossification. Hypurals are well ossified and 36 caudal fin rays are calcified except for their extremities. Alisphenoid and nasal bones are partly ossified. Spines and fin rays of dorsal and pectoral fins are ossified in their basal portions. All the bones of the pectoral girdle are well calcified at this stage of development. All the 19 anal fin rays are ossified in the basal portions. Eleven of the anterior lateral line ossicles are calcified and partial calcification is seen in a few more ossicles. All the three bones of the circumorbital ring, such as antorbital, suborbital and postorbital are calcified.

Hatching: Before hatching, the embryo shows pronounced jerking movements of the head and tail. These movements along with the activity of the proteolytic hatching enzymes (Ishida, 1951) possibly exert the necessary pressure on the chorion to cause a small rupture in the region of the yolk sac. The embryo starts wriggling inside the egg membrane. Then gruadually the yolk sac starts protruding through this rupture (Fig. 11). At 5 to 10 minute intervals the embryo

makes jerky movements. Once the yolk sac has completely emerged from the egg membrane, the embryo makes sudden lashing movements of the tail, and the tail also comes out of the egg membrane and gradually the whole embryo discard the egg membrane. It takes two hours for the hatching process to complete. About half an hour after hatching, the larva starts swimming slowly in the water with the yolk sac on its under surface. The tail of the newly hatched young one is curved upwards (Fig. 12).

In a just-hatched larva all the adult bones are ossified eventhough the sphenethmoid and the anterior part of the frontal bone show only the beginning of calcification. All the other bones of the cranium are well ossified. Premaxillae show fine teeth and parasphenoid and orbitosphenoid make their appearance. Exoccipitals, basioccipital, epiotic lamellae, post-temporal and parasphenoid bones are almost fully ossified at this stage. Prevomerine band of teeth, ectopterygoid and additional toothed plates are fully formed with the characteristic shape of the species. The lower jaw bones are fully ossified and the hyomandibular arch and the branchial arches are also calcified. The whole opercular assembly is fully formed, and the weberian apparatus shows calcification in all the bones associated with it. All the fin rays are fully ossified except for the dorsal and pectoral spine tips. The pelvic fin rays are partly ossified. All caudal rays and ribs are ossified at this stage.

Later development

The curved tail of the embryo is found to straighten one to two hours after hatching. The total length of the larva at this stage is 35 mm, the head length 8.6 mm and the eye diameter 2 mm. The yolk sac is a rounded structure extending from the lower part of the heart to the anus (Fig. 13). All the 3 pairs of barbels are well developed, pigmented and are slowly lashing in the water. After hatching, there is dense pigmentation over the entire surface of the body. It is intense on the dorsal part of the head, at the base of the spine and at the tip of the caudal fin. The pigments are stellate structures in the initial stage and gradually the branches of the stellate structure meet together to give a uniform colour. The barbels are greyish in colour and the whole body is covered by melanophores. In the newly hatched larva the liver is seen as a small protuberance in the anterior region of the yolk sac and it is richly vascularised.

After this stage there is very little structural changes in the larva, except growth in size, change in colour and the gradual diminution in size of the yolk sac (Fig. 14). The yolk is absorbed within 15 days after hatching, leaving a small slit covered with a thin membrane in the midventral line of the young one, extending from the heart to the anus (Fig. 15). Gradually this slit also disappears and only a midventral line is seen. The larva is found to feed on small planktonic organisms present in the water. At the time of the complete absorption of the yolk sac, the larva is 76 mm in total length, 15 mm head length and 4 mm eye diameter. This is the stage at which the young ones emerge out of the parent's mouth to lead a free life. The young ones under observation moved freely, fed on the flesh of bivalves and crustaceans, and continued to be active in the aquarium.

Discussion

Chidambram (1942) stated that in *Arius jella*, the hatching took place on the 30th or 35th day after the egg was laid, and from the time of hatching it took another 30 more days for the complete absorption of yolk. During the present observation on *T. thalassinus* the development from the early embryonic stage to hatching took only 16 to 17 days. Even-

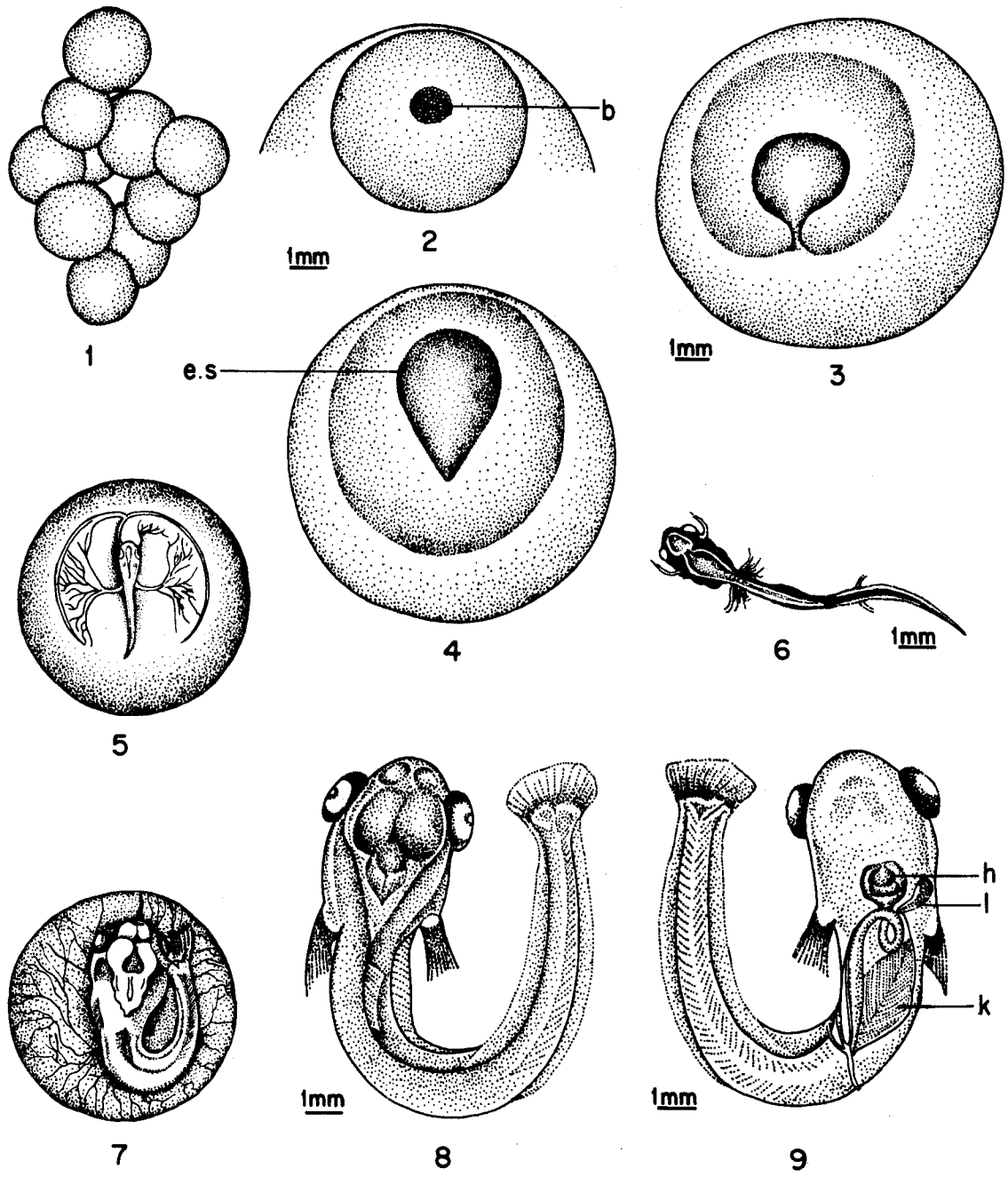

FIG. 1. 1. Eggs of *Tachysurus thalassinus*, 2. Blastula stage : b - blasto disc, 3. Blastula stage of development with the segmentation cavity, 4. Embryonic shield, 5. Early embryo (8 mm), 6. Embryo (8 mm) separated from the yolk, 7. Developing embryo (16 mm), 8. Embryo (16 mm) separated from the yolk, 9. Ventral view of dessected embryo showing h - heart 1 - liver k- kidney,

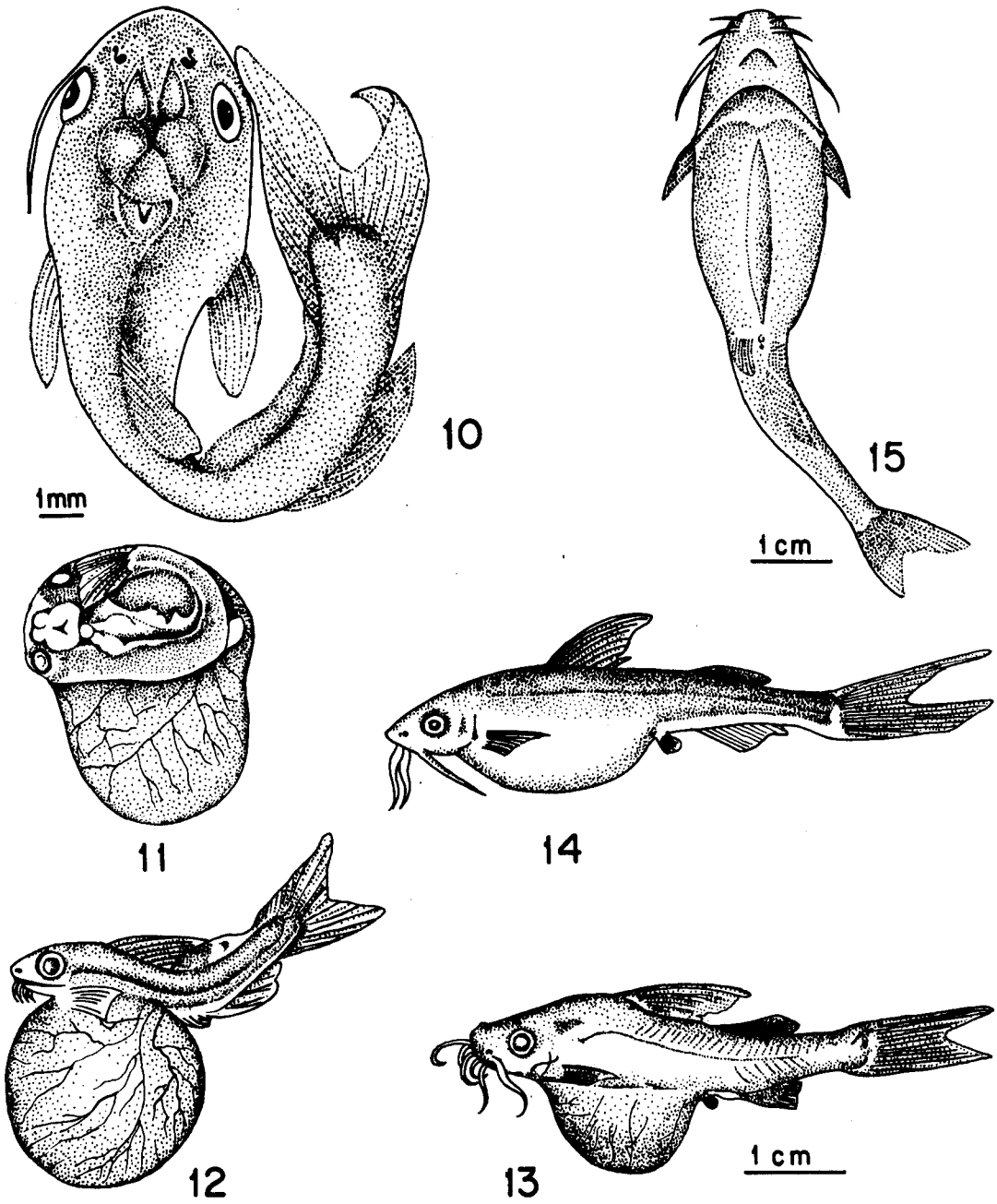

FIG. 2. 10. Embryo (25 mm) advanced stage of development, 11. Hatching - Yolk sac emerging from egg shell, 12. A newly hatched larva, 13. Larva eight days after hatching, 14. Larva twelve days after hatching, 15. Post larva - Yolk sac completely absorbed.

though the time taken for the development from fertilization to the formation of early embryo is not clearly known, it could be presumed, following Chidambaram (1942) and Al-Nasiri and Hoda (1977) that it might take about three days. In the laboratory after hatching, it took 15 days for the whole yolk to get absorbed. In short in *T. thalassinus* the period taken for the incubation is about 35 days, whereas in *A. jella* it takes about 2 months. Chidambaram (1942) observed that in *A. jella* the head of the embryo came out of the chorion during hatching, while during the present observations on *T. thalassinus* it was the yolk sac that first escaped from the egg membrane.

The embryo of *Tachysurus thalassinus* constantly opens and closes its mouth and moves the gill covers while respiring. In this species, the earliest bones to ossify are those associated with the movements of the jaw and opercles, which help in the respiratory movements. Ossification then follows in other bones, such as premaxillae, quadrates, interopercles, preopercular, hyomandibualr and all branchiostegal rays which help in the complex movements of respiration in fishes.

The bones associated with the auditory capsules also calcify early since they are important in the maintenance of balance of the embryos. All the vertebral centra get calcified in the early embryonic stage and this helps in the constant movement of the tail and trunk of the embryo even while in the egg membrane. In the early larvae only a few central caudal rays are calcified and as the embryo grows, more and more of the caudal fin rays get calcified, which helps in the constant lashing of the embryonic tail while inside the egg membrane.

The early ossification of the Weberian apparatus clearly shows that this mechanism becomes functional early in the development of *T. thalassinus*. Again, the early ossification of all the bones associated with the jaw movements can be explained on the basis of their function, as has been mentioned earlier, with the larvae feeding on planktonic organisms while they are in the parent's mouth. Since there is very little movement for the dorsal, pectoral and pelvic fins at this stage of development, they get fully ossified rather late in the larval period. In short, it is found that the bones that are formed earliest are those associated with the movement and support rather than protection.

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