

A life-history approach to the early ontogeny of the Mozambique tilapia *Oreochromis mossambicus* (Pisces, Cichlidae)

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The early ontogeny of *Oreochromis mossambicus* (Peters) was followed from the time of egg activation until the juvenile period. Development is direct and consists of an embryonic period of approximately 15 days. The embryonic period can be divided into a cleavage, an embryonic and a free-embryonic phase. A detailed developmental description is given and the relationship between the early ontogeny and the early life-history pattern is examined. The terminology used for the last step of the free-embryonic phase and a possible truncated larval period is discussed.

Die vroeë ontwikkeling van *Oreochromis mossambicus* (Peters) word gevolg van die oomblik van eieraktivering tot die jongvisstadium. Die ontwikkeling is direk en bestaan uit 'n embrionale periode van ongeveer 15 dae. Die embrionale periode kan verdeel word in die selklowingsfase, die embrionale fase en die vry-embriofase. 'n Gedetailleerde beskrywing van die ontwikkeling word gegee en die verwantskap tussen die vroeë ontwikkeling en die patroon van die vroeë leefwyse word ondersoek. Die terminologie wat vir die finale stap van die vry-embriofase en 'n moontlik verkorte larwale tydvak gebruik word, word bespreek.

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Historically, embryological and larval research has been conducted using mainly preserved specimens. This research has centred largely on producing fate maps and explaining developmental changes using sectioned material, thereby often producing a disjointed, discontinuous overview. The early ontogeny of an organism is, however, a dynamic, ever-changing process. Information collected in the laboratory, therefore, needs to be related to the ecology and ethology of the animal in its natural environment. This approach has been adopted in some fish developmental studies, such as the work of McElman & Balon (1979, 1980), Paine & Balon (1984a, 1984b) and Cunningham & Balon (1985). The majority of this research has been conducted on north temperate species. Only a few comprehensive studies of the development of African fishes have been completed, e.g. Fishelson (1966) and Balon (1977) on cichlids and Haigh (1989) on cyprinodontids.

As the Mozambique tilapia, *O. mossambicus* (Peters 1852) (Pisces, Cichlidae), is easily bred in captivity, it was chosen for this project. The deposition of clutches of numerous eggs which can be fertilized by the same male assures ample numbers of genetically similar offspring that are incubated under identical conditions.

O. mossambicus exhibits a high degree of plasticity, both phenotypically and in its life-history style. Its natural distribution ranges from southern Kenya along the inland and coastal regions of south-eastern Africa southwards to the eastern Cape, South Africa (Philippart & Ruwet 1982). They are found in riverine, lacustrine and estuarine habitats as well as in the ocean and are capable of tolerating wide fluctuations in water temperature and pH (Trewavas 1983).

The ability of an organism to utilize and survive environmental flux is largely dependent on the life-history style that it has evolved. Several alternative life-history patterns have been described, such as r/K-selection (MacArthur & Wilson 1967), altricial/precocial (Nice 1962; Balon 1981) and maintenance/dispersal phenotypes (Geist 1989). Certain suites of

characters define the different life-history styles. A particular life-history pattern is not necessarily useful for all types of animals or circumstances. Because of its emphasis on early ontogenetic and reproductive characteristics, the altricial/precocial life-history pattern was utilized for this study. Precocial characteristics of the early life history of a species are small numbers of large, highly nutritious eggs, well-developed, large young at first exogenous feeding, direct development and extensive parental care. In contrast, the suites of characters of altricial animals include numerous small eggs, smaller and less developed embryos at first exogenous feeding, indirect development and less parental care.

The parent fish used in this study were obtained from a population that inhabited a stable and relatively predictable environment (Mill Farm Dam) in the eastern Cape, South Africa. The population as a whole expressed precocial life-history traits of fast growth rates, long life, delayed maturation at a high mass, low spawning frequency and relatively low fecundity (James & Bruton 1992).

The aim of this study was to follow the development of *O. mossambicus* in its entirety, and to explore how the unfolding ontogenetic events related to each other. An additional objective was to relate how the pattern and rate of development of *O. mossambicus* reared in the laboratory under simulated natural conditions reflects the ability of this species to cope with the unique conditions of its expected natural habitat. The relationship between the ontogenetic events, the evolution of the life-history style and the reproductive guild of this species was also examined.

Materials and Methods

Laboratory set-up and procedure

The incubation tanks were housed in a controlled-temperature room with a photoperiod of 14 h light : 10 h dark and the water temperature was maintained at $25 \pm 0.5^\circ\text{C}$.

The eggs and embryos were incubated in a 60 l glass aquarium. Aquarium heaters were placed in the tank and connected to an electronic relay box (custom made by Labotec Natal Ltd). Temperature control was maintained via a contact thermometer which was connected in series to the heaters through the relay box. A simulated mouthbrooding action was obtained by connecting steep-sided separating funnels to the outflow of a Fluval 102 outside filter/pump (Figure 1). The outlet of the funnels flowed through transparent plastic tubing into small plastic jars covered in fine netting which were suspended in the incubator tank. In order to obtain a low light level, the tank was placed in a darkened enclosure.

A successful spawning was obtained using one male with six females and it was this ratio which was used throughout the project. Activation time was considered to be the midway point between the time of first egg deposition and the last sperm uptake by the female and was used for age determination. The descriptions of early ontogeny were derived from individuals from six different spawnings of three females. It was necessary to use live and preserved specimens which were sampled at predetermined and frequent intervals. The methods of Balon (1985) were best suited to this study and his procedures were therefore followed, as outlined below.

Samples were taken from the time of activation until the juvenile period at varying time intervals depending on the level of development and the importance of the ontogenetic events. Often more than one individual at a time was removed. The specimens were placed in a large depression slide and positioned under a Nikon SMZ-10 stereo-zoom microscope with a Microflex HFX-II photomicrographic attachment. Photomicrographs were taken and drawings were made using a drawing tube attachment. A fibre optic light source (Fi L151) was used for reflected light in order to avoid overheating of the specimens during the microscopic observations. Specimens were placed in vials for preservation and stored in a buffered formalin solution (1,8 g each of

sodium phosphate monobasic and anhydrous sodium phosphate dibasic in 1 l of 5% formalin).

Treatment of live and preserved specimens

Subsamples from clutches were taken and weighed on a Sauter electronic, analytical balance accurate to 0,0001 g. The eggs were then placed in a drying oven set at 75°C.

The heart rate was determined by timing 10 or 20 beats with a stopwatch. Older, active specimens were anaesthetized with a 1 ppt solution of MS222 (tricaine methane sulfonate). A few drops were added to the depression slide after recording behavioural and morphological information such as eye, jaw and fin movements and heart rate. Embryos were removed from the darkened enclosure and placed in cloth-meshed breeding baskets in a tank with a photoperiod of 14 h light : 10 h dark no sooner than 14 d, 16 h after activation. Notes were taken on the behaviour of the embryos in their simulated environment. Although the information obtained under these artificial conditions may not be a direct reflection of natural events, it does indicate the level of the morphological and behavioural development of the offspring at that point in its ontogeny.

Food was added to the incubating funnels in advance of the expected time of first exogenous feeding. Commercially produced fish embryo food (Tetra Min Baby Fish Food 'E' and Liquifry No. 1) and live brine shrimp nauplii were added to the funnels and jars at regular intervals and specimens were removed periodically to check for food in the gut.

Specimens were cleared with trypsin and stained with alcian blue for cartilaginous mucopolysaccharides and with alizarin red-S for calcium phosphate. The procedure used was that of Potthoff (1984) with some slight modifications. Because alcian blue solutions have the potential to decalcify bone, some specimens were stained with alizarin red-S only and compared with the double-stained specimens.

Composites of the developmental illustrations were derived from slides, drawings and cleared and stained specimens. Some measurements were taken from drawings and slides of live specimens. Total embryonic lengths and the area of the yolk sac were determined from drawings using a computer digitizer. These data were used as an indication of relative changes in rates, sizes and/or patterns of development and are not to be taken as absolute values for a given time or interval. The terminology of ontogenetic events follows that of Balon (1981). The embryonic period has been divided into three phases: cleavage (C), embryonic (E) and free-embryonic (F). The superscript numeral refers to the step within the phase and the second numeral is the accumulative step from the time of activation (e.g. E¹4 refers to the first step in the embryonic phase and the fourth step since activation). The age of the specimens was denoted as days : hours after activation, and indicates the beginning of the sampling time when the specimen was removed from the incubator or the breeding basket. Activation is used as defined by Balon (1985, p. 20). The age is also expressed in temperature units (TU = degree-days or temperature units) and was calculated by multiplying age (hours/24) by temperature (25°C). The main source of information used for naming blood vessels was Balon (1985). Skeletal structures

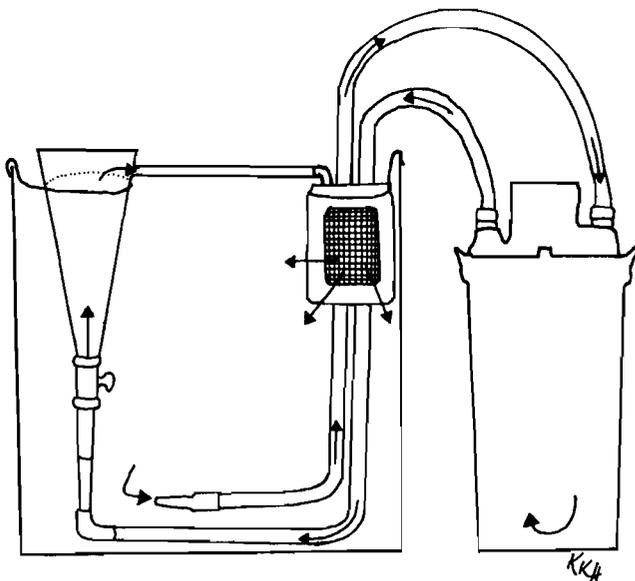


Figure 1 Side view of the incubation system. Arrows indicate the direction of the water flow.

were named according to Balon (1985) and Cunningham & Balon (1985).

Ideally, if the ontogeny of several individuals could be followed from the time of activation until the juvenile period, a more accurate account of the developmental rates and patterns would have emerged and individual variation could have been measured. However, the effects of removal from and return to the incubators during the sampling regime would add bias to the results. More sophisticated equipment which would allow continual observations without disturbance to the animal could help to solve this problem. Two other problems encountered were the subjectivity of the researcher in determining the important developmental boundaries, and, in the case of *O. mossambicus*, the nature of the opaque eggs which limits visibility, especially prior to hatching.

Results

Steps in the early development of *Oreochromis mossambicus*

Embryonic period 00:00–11:00 (TU = 0 to 275)

Cleavage phase 00:00–01:11 (TU = 0 to 37)

The fertilized eggs had an ovoid shape with the vertical axis slightly longer (mean = $3,04 \pm 0,20$ mm) than the horizontal axis (mean = $2,19 \pm 0,16$ mm). The vegetable pole was marginally broader than the animal pole. Table 1 and Table 2 show the mean dimensions and the wet and dry weights of the eggs of different clutches, respectively. The large, yellow yolk was opaque with a uniform consistency.

Table 1 Mean maximum and minimum lengths of *O. mossambicus* eggs (*SD* = standard deviation)

Clutch number	Sample size (<i>n</i>)	Mean max length (mm)		Mean min length (mm)	
		length	<i>SD</i>	length	(<i>SD</i>)
3	12	3,09	0,15	2,20	0,09
4	7	3,10	0,08	2,14	0,03
4,2	3	3,03	0,02	2,18	0,06
6	12	2,80	0,19	2,02	0,13
6,2	10	3,22	0,11	2,32	0,11
6,3	4	3,14	0,03	2,40	0,05
Total	48	3,06	0,20	2,21	0,16

Table 2 Wet and dry weights of *O. mossambicus* eggs. The weights were determined by weighing subsamples of the clutch and dividing the total weight by the number of eggs in the subsample

Clutch number	Wet weight (g)	Dry weight (g)
3	0,0088	0,0041
4	0,0079	0,0032
4,2	0,0091	0,0052
6,3	0,0102	0,0058

No obvious oil globules were present. The envelope was translucent and had a slightly dimpled texture.

*C*¹ 00:00–00:01 *Bipolar differentiation, egg envelope hardening and the formation of the perivitelline space occurred in this step.*

One hour after activation (TU = 1) a one-celled blastodisc had formed at the animal pole. The perivitelline space was visible only above the cytoplasm. The micropyle had a cone-shaped configuration (Figure 2a).

*C*² 00:01–00:22 *This step began with the first cleavage, continued with germ ring and periblast formation and ended with the flattening of the blastodisc just prior to epibolic movement.*

By age 00:02 (TU = 2), the first division of the cytoplasm had occurred (Figure 2b). The second division had been completed by age 00:03 (TU = 3) and took place vertically. The distal margin of the blastodisc was close to contacting the dorsal surface of the egg envelope (Figure 2c). At age 00:04 there were eight cells of similar size, and division still appeared to be on the meridional plane (Figure 2d). By age 00:05 (TU = 5), there were 32 cells and horizontal division had taken place (Figure 2e). During the next 2 h, cleavage continued such that the perivitelline space was filled by the large-celled blastodisc of undetermined cell number. At ages 00:15 to 00:17 (TU = 15 to 18) the blastodisc had flattened and consisted of innumerable cells of extremely small dimensions (approximately 0,023–0,028 mm in diameter). From age 00:18 (TU = 19) until the end of this step the periblast was visible as a transparent band around the circumference of the yolk below the lip of the blastodisc (Figure 2f).

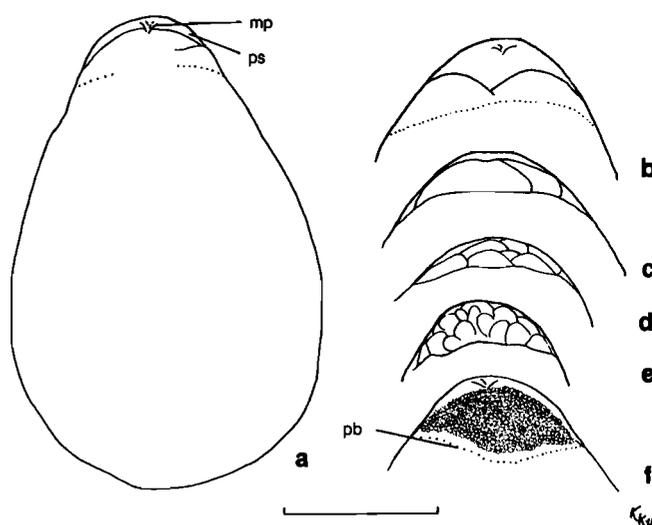


Figure 2 Lateral views of steps *C*¹ and *C*²: a – age 00:01 (TU = 1), b – age 00:02 (TU = 2), c – age 00:03 (TU = 3), d – age 00:04 (TU = 4), e – age 00:05 (TU = 5), f – age 00:18 (TU = 19) (mp = micropyle, pb = periblast, ps = perivitelline space). The total number of blastomeres is not visible in all the drawings owing to egg orientation. Scale = 1,0 mm.

C³ 00:22–01:11 This step began with epiboly. The embryonic shield formed and differentiation at its anterior end marked the end of this step.

By age 00:22 (TU = 23), individual cells were no longer distinguishable and the blastodisc was greatly flattened. The lip of the blastodisc extended approximately 10–15% down over the yolk. Cells were aggregating to form the embryonic shield. By age 01:11 (TU = 37), a ridge could be seen along one side of the blastodisc which extended to almost the equator and was in contact with the envelope wall along its dorsal aspect. Slight vertical and lateral swelling in the anterior region of the embryonic shield was an indication that neurulation had begun.

Embryonic phase 01:11–04:16 (TU = 37 to 117)

E⁴ 01:11–02:12 At the beginning of this step neural plate development began as slight swellings and continued until distinguishable fore-, mid- and hindbrain components, as well as optic vesicles were visible. At least 17 pairs of somites, four neuromeres and the presumptive pericardial cavity were formed. Body and yolk sac melanophores appeared.

At the beginning of this step, the embryonic shield was obvious as a thickening along the dorso-lateral margins of the yolk and the presumptive pericardial cavity was visible as a thin, transparent chamber extending along the dorsal surface of the yolk (see Figure 3a). The leading edge of the periblast had reached the yolk equator and the tail mound had begun to form. Over the next 9 h the length of the embryonic shield ranged between 1,5 to 1,7 mm with its posterior end slightly above the margin of the descending periblast. An excised specimen at age 01:19 (TU = 45) consisted of aggregations of small cells of equal size which

gave shape to the following structures but did not form any clear-cut divisions or boundaries. A fine line along the body axis indicated that some notochordal development had begun. Somite formation was noted as simple cell aggregations without clear-cut definition.

Definite somites were visible from age 01:20 (TU = 46) until the end of this step. The initial counts were 10 to 13 pairs with at least 14 to 17 pairs by the end of the step. The anterior somites were clearly defined but the postanal ones were indistinguishable as separate units because they were not as yet fully developed (Figure 3d).

The first appearance of pigments occurred at age 01:21 (TU = 47). There were two dark spots in the body tissue just posterior to and above the presumptive optic vesicles and pale, fibrous melanophores were present laterally on the yolk sac near the head region. At age 02:00 (TU = 50), the optic vesicles appeared as lateral outpockets from the slightly differentiating fore- and midbrain (Figures 3b & c). The development of the presumptive pericardial cavity progressed throughout this step until it extended posteriorly along the body axis to the hindbrain. Dorsally it extended to about the middle of the optic vesicle. By age 02:03 (TU = 53) constrictions in the brain made the prosencephalon (forebrain), the mesencephalon (midbrain) and the rhombencephalon (hindbrain) distinguishable. The optic vesicles were clearly separate from the brain (Figure 3d). Four to five neuromeres were forming. The head extended farther forward over the dorsal surface of the yolk and was almost in contact with the egg envelope. About 66% of the yolk was covered by the enveloping cells. Body pigmentation had extended slightly along the body axis at the yolk sac junction behind the head. The yolk sac pigmentation extended posteriorly and ventrally and covered a larger area. Aggregations of melanophores formed dark, dense regions on the dorso-lateral regions of the yolk sac.

The mean total length of embryos between the ages of 01:20 and 02:03 was 1,9 mm. During the entire step there was a gradual increase in embryo length of 0,5 mm and little change in yolk area.

Step summary: The basic body form has been established and serves as substratum for further tissue and organ development.

E⁵ 02:12–03:00 This step began with the first heartbeat. Other events included the formation of the eye lenses, the heart-tube, the otic capsules and otoliths, first muscle contraction, further brain differentiation, elongation and separation of the tail, and anastomisation of blood islands and the anterior vitelline veins.

The first heartbeats were recorded from two specimens as 86 and 98 beats/min at age 02:12 (TU = 63) but no blood flow was detected. Eye lenses had formed. The optic ventricle had formed in the centre of the mesencephalon. Differentiation of the hindbrain into the cerebellum and the medulla oblongata had begun (Figures 4a & b). The configuration of the melanophores on the yolk sac became stellate in addition to circular. Body movements were first recorded at age 02:16.

At age 02:20 (TU = 71), the tail was free from the yolk-sac and active muscular contractions occurred. The heart

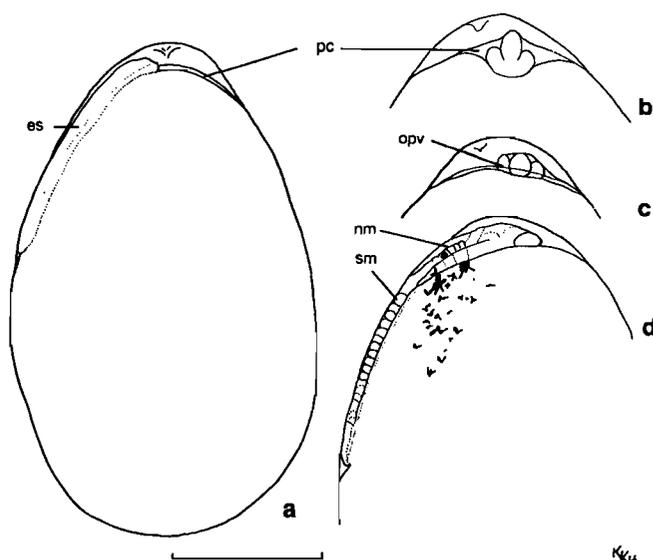


Figure 3 Step E⁴: a – lateral view at age 01:13 (TU = 39), b – frontal view at age 02:00 (TU = 50) showing the lack of differentiation of the optic vesicles, c – frontal view with slight rotation at age 02:00 showing optic vesicles in a more advanced stage than in b, d – right lateral view at age 02:03 (TU = 53) (es = embryonic shield, nm = neuromeres, opv = optic vesicles, pc = presumptive pericardial cavity, sm = somites). Scale = 1,0 mm.

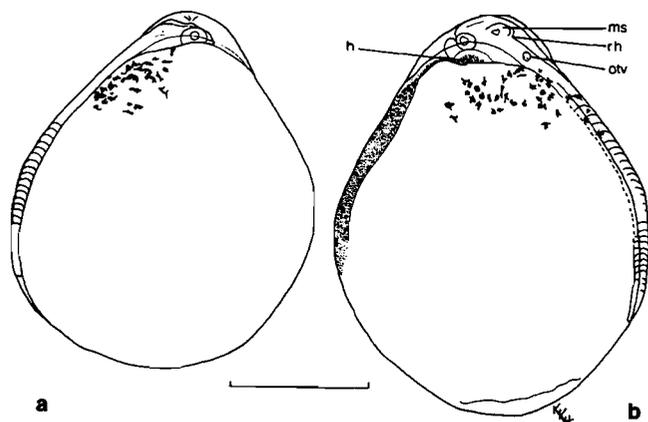


Figure 4 Step E²⁵: a – right lateral view at age 02:12 (TU = 63), b – left lateral view at age 02:20 (TU = 71), the stippled area on the left of the yolk sac shows an area which contained blood cells in which there was no blood movement (h = heart-tube, ms = mesencephalon, otv = otic vesicle, rh = rhombencephalon). Scale = 1,0 mm.

was a thinly-walled tube. A few blood cells at a time entered the heart-tube from a wide, pinkish region on the yolk sac (Figure 4b).

Some haemoglobin was present in the blood. Most of the circulatory system was still undetectable except in areas of high blood concentration or extremely strong flow. Blood flow movement was seen along the pathway of the anterior vitelline veins and at the junction of the pre-anal plexus and the yolk sac, but no distinct vessels were visible. There was no visible blood flow in the body but some vessel development must have occurred in the cardinal vein and the pre-anal plexus as evidenced by the yolk sac circulation. There were two cavernous areas at the ventral pole of the yolk sac which were full of blood. Various indentations and channels were forming on the yolk sac surface but they were not distinguishable as distinct entities. Hemispherical division of the prosencephalon, mesencephalon and the cerebellum had occurred. Otic capsules were first seen at this time and were already well developed. Two otoliths were present on each side. The distance from the posterior margin of the eye to the anterior margin of the otic capsule was 0,31 and 0,43 mm in two individuals. The visceral cavity was visible between the body proper and the yolk sac. Melanophores were present along and perpendicular to the body axis in the region of the first five pairs of somites (Figure 4b). Somites extended almost to the tip of the elongated tail. Pigment cells were present above the visceral cavity and further ventral migration of melanophores had occurred on the yolk sac. The egg envelope burst easily during handling. Throughout this step, the heart rate was very erratic, ranging from 86–151 beats/min. Mean embryo length was 2,9 mm ($n = 3$). There was a substantial increase (0,7 mm) in length between the last specimen of the former step and the first individual in this step. There was no growth and very little change in yolk sac area within this step.

Step summary: Rudimentary organs have developed, especially in neural, respiratory and circulatory structures. A basic

communication system (the brain and parasympathetic nervous system) and primitive transport and gaseous exchange systems (vitelline, finfold and body vascularization) have been established.

E³⁶ 03:00–03:20 Strong body circulation became evident at the beginning of this step. Numerous head vessels and the ventral median finfold respiratory plexus developed. Eye pigmentation began, the heart-tube started differentiating, the posterior vitelline vein branched and some haemoglobin was produced. In the last few hours hatching began and the posterior tip of the notochord was flexed dorsally.

An additional inner ring (the semicircular canal) was forming in the otic capsule ventrally and along the sides but not yet completed dorsally. The mean distance between the eye and the otic capsule margin was 0,27 mm ($n = 6$, range = 0,20–0,32 mm). Yolk sac pigmentation became denser and in some areas the melanophores aggregated such that individual cells were not distinguishable. Pigmentation extended more ventrally on the yolk sac surface. Changes in body pigmentation were minimal with a slight increase of cell proliferation along the body axis and above the visceral cavity. Darkening around the periphery of the optic capsules and along the circumference of the lenses was first noted half-way into this step. The mesencephalon extended more posteriorly and elongation of the prosencephalon anteriorly made the distinction between these two brain components more striking. The optic ventricle became tear-drop shaped and obvious. Lobular development in the mesencephalon gave it the appearance of consisting of three separate components and there were six neuromeres in the hindbrain. At age 03:12 (TU = 88) the egg envelope had a cloudy and ragged appearance and at age 03:15 (TU = 90) hatching began (Figure 5c). Some individuals were still contained inside the envelope, some had either their tail and/or head protruding and others were completely free. In the hatched individuals it could be seen that flexion of the notochord had begun and the spinal cord extended posteriorly beyond the notochord tip. The gastrointestinal tract was a simple thick-walled tube with a fine central line but no lumen. The presumptive anus protruded slightly below the ventral median finfold, effectively separating it into a pre- and postanal component (Figure 5c).

Constrictions and a slight left to right twist in the heart-tube were an indication that chamber development was underway. The circulation of blood through the body was pronounced at the beginning of this step as a strong flow in the dorsal aorta. Within 4 h, flow in vessels of the head region and the pre-anal plexus was also noted. The anterior vitelline veins followed an arc-shaped path across the dorso-lateral surface of the yolk sac. Blood from the pre-anal plexus collected in the subintestinal vein, which then entered the left side of the yolk sac into the posterior vitelline vein (see Figures 5a & c). A cavernous area on the ventrum of the yolk sac served as a collection site for the blood from the posterior vitelline vein. At age 03:10 (TU = 85), the posterior vitelline vein branched before entering the ventral, cavernous regions of the yolk sac and branching vitelline vessels continued flowing dorsally from there towards the heart-tube (Figures 5a & b). Blood islands and anastomosing

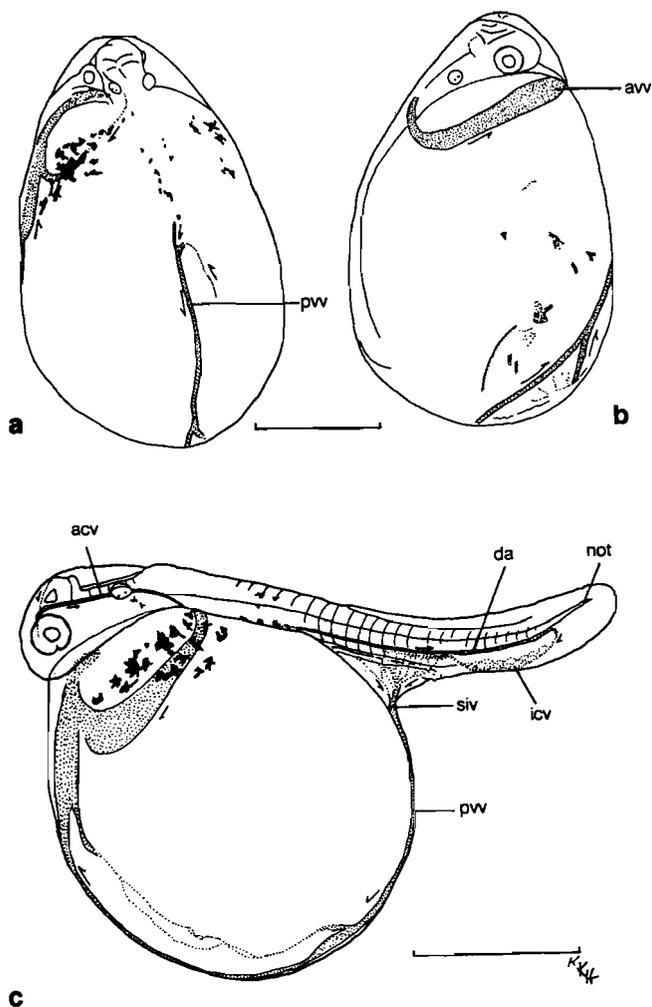


Figure 5 Step E³6: a & b – dorsal and right lateral view at age 03:10 (TU = 85) showing the early stages of development of the vitelline respiratory plexus, c – left lateral view at age 03:15 (TU = 90) of a newly hatched individual (acv = anterior cardinal vein, avv = anterior vitelline vein, da = dorsal aorta, icv = inferior caudal vein, not = notochord, pvv = posterior vitelline vein, siv = subintestinal vein). Scales = 1,0 mm.

veins were more evident. The heart was slightly S-shaped. By the end of this step there was extensive head and finfold circulation. Blood flowing ventrally between the forebrain and the midbrain joined with the dorsal flow from a vessel behind the eye. From there, a common vessel followed along the base of the mesencephalon and emptied into the anterior cardinal vein which continued posteriorly behind the otic vesicle before bending ventrally to enter the anterior vitelline vein (Figure 5c). The postanal plexus was fed by the dorsal aorta which formed a single, caudal loop. The inferior caudal vein followed the distal margin of the postanal finfold, curved dorsally and rested below the dorsal aorta where it fanned out into the pre-anal plexus. Most of the flow between the two ventral finfolds was through the inferior caudal vein which collected blood from most of the minor vessels of the postanal plexus. There were a few minor veins forming a junction between the two finfold networks but they crossed over at a more posterior point than that of the inferior caudal vein. The pre-anal plexus was a complex network of fine vessels which flowed into

the subintestinal vein which then emptied into the posterior vitelline vein. The posterior vitelline vein branched at the ventral pole of the yolksac into several smaller vessels to form a complex network which continued in a dorso-anterior direction over the surface of the yolksac. The blood from these vessels converged to form a collective, sheet-like flow prior to entering the heart-tube. The vitelline plexus was difficult to see in detail except in the large vessels discussed above.

As in the previous step, the heart rate was inconsistent. The first counts during this step averaged 125 beats/min ($n = 3$) from one clutch while an individual from another batch measured 143 beats/min. In the last sample of this step a markedly higher heart rate was recorded from three individuals (mean = 197 beats/min). The range of heart rates throughout this step was 90 to 205 beats/min; the lowest value was taken from a specimen aged 03:09 that appeared to be retarded in its development.

The length of an embryo measured during this step was 3,4 mm. There was a slight increase in length (0,5 mm) and no change in yolk area from the previous step.

Step summary: The major emphasis was on the establishment of simple circulatory and respiratory systems, especially in the temporary embryonic respiratory organs. There was a marked increase in activity, especially in tail movement, throughout this step.

E⁴ 03:20–04:16 The development of the hepatic vitelline network, the branchial arteries and arterial flow into the pre-anal plexus began. The pectoral fin anlage formed. Almost all individuals had hatched by the end of this step.

Hatching continued throughout this step. Pigmentation began to extend from the periphery of the optic capsules and the lenses to include the overall surface of the eye, giving it a peppery appearance. The mean distance between the otic capsule and the eye was 0,18 mm ($n = 4$, range = 0,15–0,22 mm). The pectoral fin anlage was apparent at the onset of this step as a small mound of tissue (Figure 6a). By the end of this step, it had developed into a semi-circular structure which extended dorsally to the middle of the notochord.

In the head, several vessels had formed loops in the brain region. Between the lateral hemispheres of the mid- and hindbrain an artery extended dorsally and proceeded posteriorly along the top of the brain before bending ventrally behind the cerebellum (Figure 6b). In one specimen, a vessel was noted above the notochord which extended posteriorly to mid-body. From that point a fine vessel flowed ventro-laterally. Early in this step some very fine vessels were noted in the area of the four gill arches (Figure 6b). By age 04:09 (TU = 109), branchial vessels, which curved in a dorso-posterior direction, were noted in three of the four simple gill arches. A fourth vessel was noted between the branchial vessels and the eye.

In the ventral finfold plexus there was an increase in the complexity of the finer vessels of the network and the major vessels became broader. Ventral to the dorsal aorta, a vessel fanned out into the pre-anal plexus, but the major contribution to the network was from the postanal veins.

Several veins on the upper left side of the yolksac at the

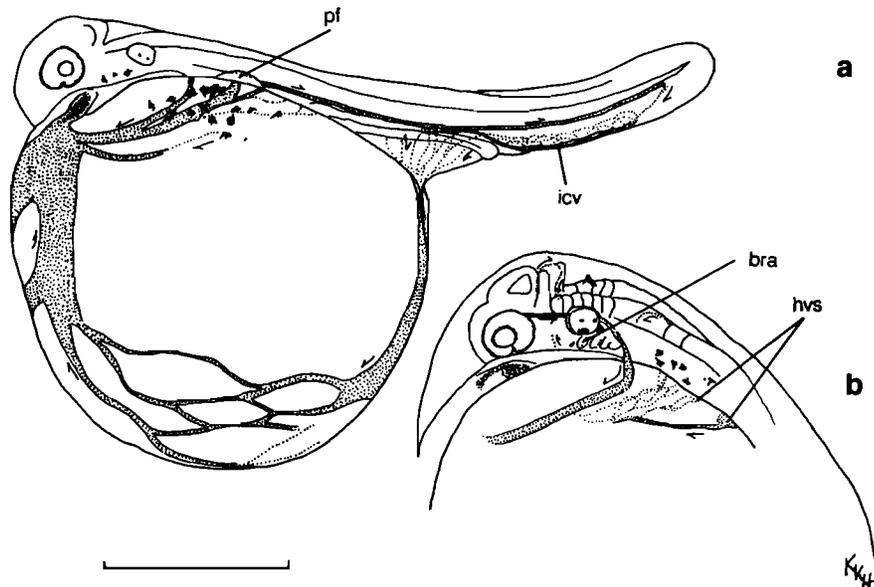


Figure 6 Step E⁴: a – lateral view at age 03:20 (TU = 96), b – lateral view at age 03:22 (TU = 98) showing head, hepatic and branchial circulatory development (bra = branchial arteries, hvs = hepatic vitelline system, icv = inferior caudal vein, pf = pectoral fin). Scale = 1,0 mm.

beginning of this step signified the start of the hepatic vitelline system (Figures 6a & b). Several small veins and one major vein left the body posterior to the anterior cardinal vein and ventral to the pectoral mound. Some of the smaller veins joined the anterior vitelline vein while others formed junctions with the major hepatic vein. In one specimen the veins entered to the right of the body. Throughout this step, the hepatic vitelline network became increasingly more complex. Eventually, all specimens had venous networks entering the yolksac on both sides of the body. The pattern of the vessels was not symmetrical and showed variation amongst the individuals. In the majority of the cases, the network on the left side consisted of numerous fine veins which formed an intricate pattern. On the right side, the veins were usually broader but less numerous. As the complexity of the dorso-lateral vitelline veins increased, it was assumed that several blood vessels associated with the gastrointestinal system were contributing to the network (such as the hepatic and mesenteric veins), but it was not possible to identify specific vessels. The posterior vitelline vein was a single vessel which branched into a complex network of veins on the ventrum of the yolksac. From this network one major vitelline vein continued anteriorly and was fed by several smaller veins. The anterior vitelline veins were also branched and formed their own networks. Blood from five major vessels fed by the networks described above (right and left anterior veins, right and left dorsolateral veins and the main vein of the subintestinal plexus), converged to form a sheet-like flow into the sinus venosus (Figures 7a–c & 8a). The lateral areas of the yolksac appeared devoid of any blood flow but blood islands were visible. In some of the areas where blood accumulated, the yolksac had a cavernous appearance.

Mean total length was 4,1 mm ($n = 4$, range = 3,8–4,4 mm). This indicates slight growth both within this step and between this and the previous step. There was no change in yolksac area. The heart rate from the first sample of this step (175 beats/min, $n = 2$, range = 174–176) dropped markedly

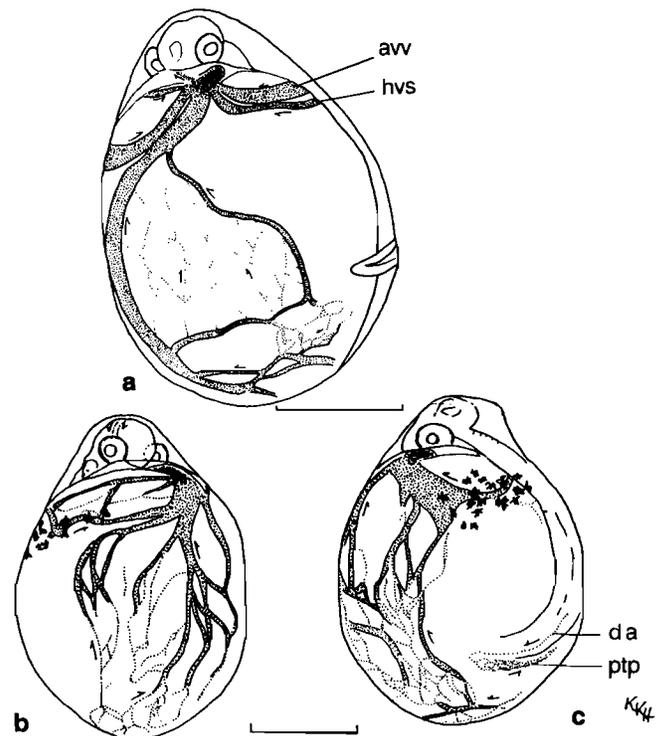


Figure 7 Step E⁴: Frontal and lateral views of unhatched individuals showing the vitelline plexus: a – age 03:22 (TU = 98), b & c – age 04:02 (TU = 102) (avv = anterior vitelline vein, da = dorsal aorta, hvs = hepatic vitelline system, ptp = postanal plexus). Scales 1,0 mm.

from that recorded for the last sample of the previous step (197 beats/min $n = 3$, range = 190–205), increased slightly then decreased again in the last sample to a mean of 145 beats/min, ($n = 3$, range = 140–150).

Step and phase summary: Further complexity in the temporary embryonic structures was the main morphological fea-

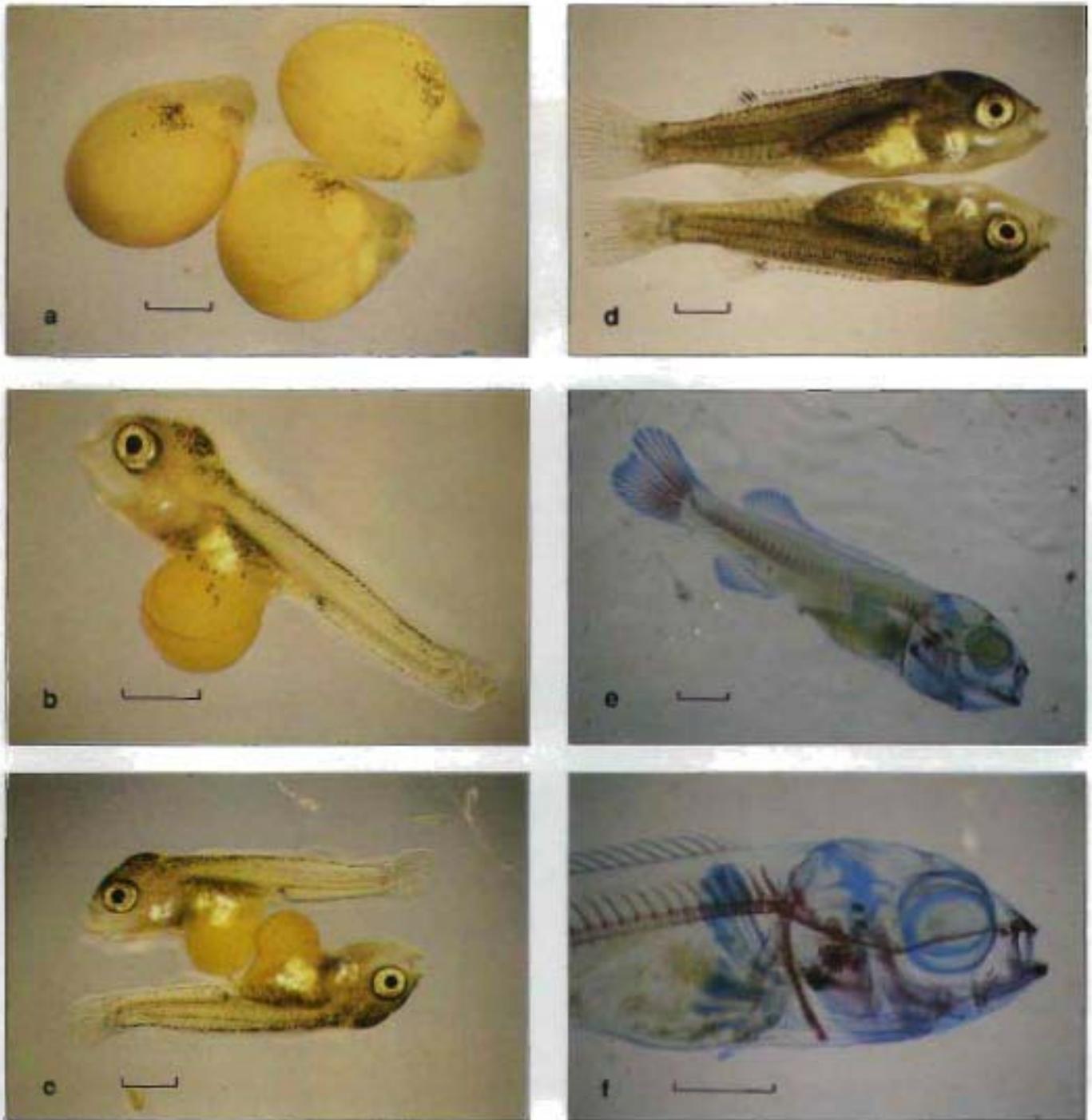


Figure 8 a – right lateral (left), dorsal (top) and right frontal (bottom) views of unatched individuals at age 04:09 showing the vitelline respiratory plexus; b – at age 10:00; c – at age 11:12 and d – at age 14:12 showing rapid yolk sac enclosure and pigmentation development; e – at age 15:00 and f – at age 15:15 showing skeletal development of juveniles, illustrating the degree of calcification and the differentiation of the fins. Scales = 1,0 mm.

ture of this step. The formation of some basic structures of adult respiratory organs had begun, i.e. branchial arteries. The digestive and sensory organs (except for the eyes) were simple but the brain and nervous system were well developed. Growth throughout the embryonic phase occurred gradually (Figure 9a) and the yolk sac area decreased slightly (Figure 9b). The overall trend in cardiac contractions was an increase in beats/min (Figure 10). Heart rate decreased at the end of this step and remained consistent into the initial stages of the next step. This plateau in heart rate encom-

passed the boundary between the embryonic and free-embryonic phases.

Free-embryonic phase 04:16–15:00 (TU = 117 to 375)

Because most of the specimens had hatched at the beginning of this step, it was considered to be the beginning of the free-embryonic phase.

F¹ 8 04:16–08:04 The head became free from the yolk sac. Intersegmental vessels and the caudal circulatory network

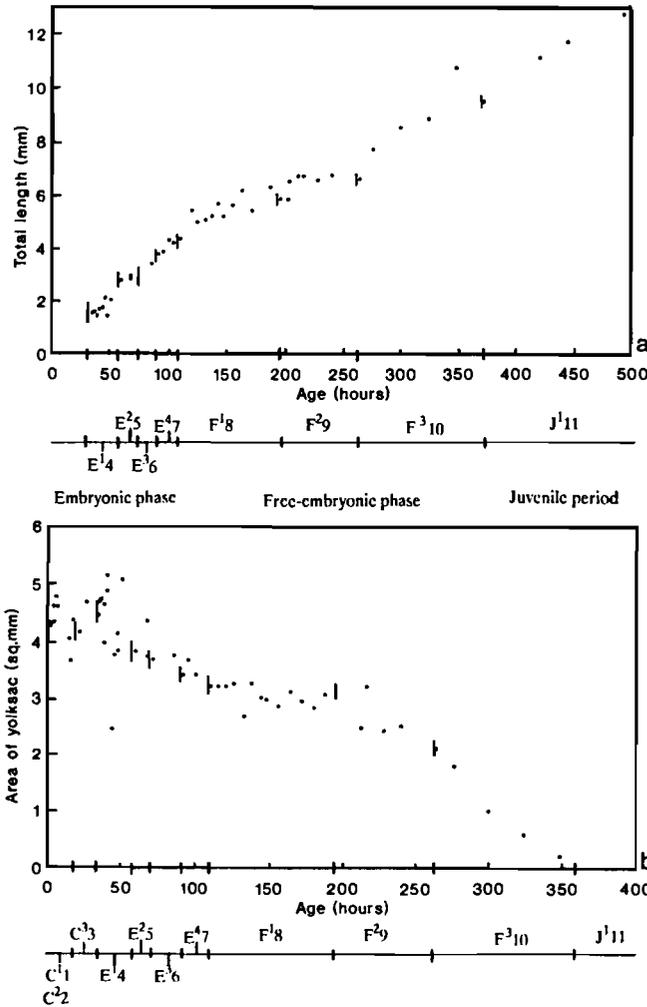


Figure 9 Growth in total length (a) and decrease in yolk sac area (b) by steps and hours after activation of *Oreochromis mossambicus* embryos. The vertical lines represent step boundaries.

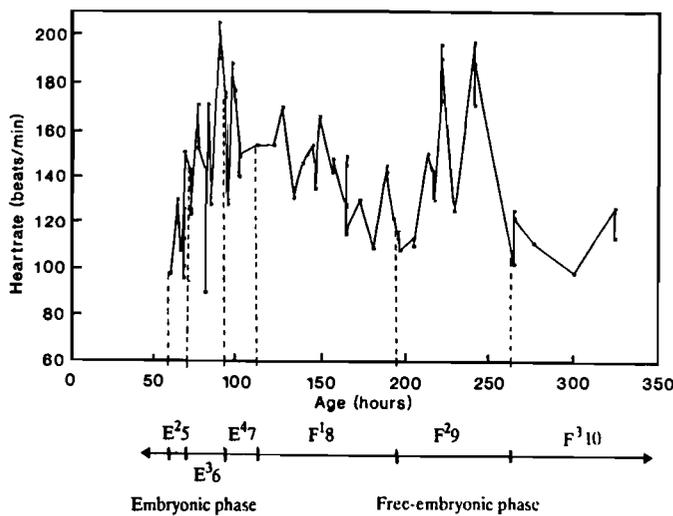


Figure 10 Heart rate by steps and hours after activation of *Oreochromis mossambicus*. The vertical bars represent step boundaries.

the anastomosing profundal caudal vein prepared to replace the inferior caudal vein. The heart chambers began to differentiate and take up their final position. Blood flow was first seen in the gill filaments and the pectoral fins. The vitelline plexus reached maximum development. First chondrification occurred in axial and appendicular skeletal components. Some dermal bone differentiation began. The mouth opened and jaw, pectoral fin and peristaltic movements were noted. The stomach, the spleen and the gall-, swim- and urinary bladders differentiated. Fin differentiation began. Iridocytes and retinal pigment were present.

At the beginning of this step anterior intersegmental vessels had formed amongst the first eight pairs of somites (Figure 11a). A small inverted U-shaped vessel lay between the eye and the gill arches. A second caudal loop (the urostylar artery and vein) had formed ventral to the notochord and extended almost to its tip. At age 05:13 (TU = 139), a third caudal loop formed over the hypural area at a 90° angle to the notochord and the main urostylar branch. At this time, both the pre- and postanal finfold plexii reached a maximum degree of development and complexity (see Figure 11b). The network of the postanal finfold resembled lacunae owing to the profuse inundation of the vessels. In both finfolds, it was difficult to distinguish between veins and other tissues. Several hours later gill filaments were visible as semicircular pouches on the gill arches where blood was seen to flow dorsally (Figure 11b). Although the posterior intersegmental veins probably emptied into the postanal plexus at an earlier age, their pathway into the finfold network was clearly visible.

By age 06:12 (TU = 163), blood flow was detected in some of the gill filaments (Figure 12a). All four of the heart components were distinguishable as constrictions along the S-shaped heart-tube. Intersegmental vessels were present in all but the last few somites and the arteries formed loops dorsal to the spinal column. The caudal network had increased in complexity with up to four radial loops in the hypural region (Figure 12a). A large loop between the urostylar vessels and the ventral loops, was present in all of the specimens but the direction of the flow differed; sometimes the blood flowed dorsally and at other times it flowed ventrally. The level of complexity of the caudal network was similar in all the specimens but the pattern and direction of flow was not. As the ventral finfold became more differentiated and the postanal plexus began to decline, the inferior caudal vein was positioned closer to the dorsal aorta, especially in the anterior-most region. The inferior caudal vein continued flowing anteriorly beyond the pre-anal finfold and emptied into the anastomosing posterior cardinal vein. The vitelline plexus had reached its maximum level of development and complexity, and completely covered all areas of the yolk sac. The dorso-lateral surface of the yolk sac consisted of a finer network of veins than that at the ventral aspect which contained a more gross network of larger vessels. The anterior vitelline vein had migrated forward and was visible along the anterior edge of the yolk sac when viewed from the side. Although in most cases the yolk sac plexus was symmetrical, some individuals had a finer network on the left side. At age 06:20 (TU = 171) the

were formed. The ventral finfold plexus reached maximum levels of development and declined by the end of this step as

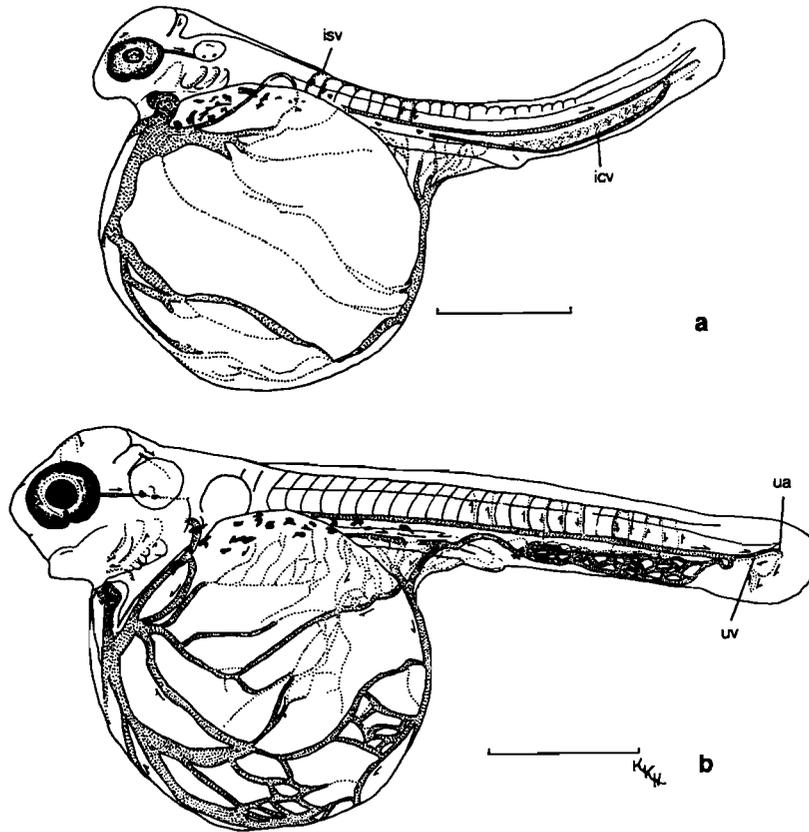


Figure 11 Step F¹8: a – age 04:16 (TU = 117), b – age 05:18 (TU = 144) (icv = inferior caudal vein, isv = intersegmental vessel, ua = urostyler artery, uv = urostyler vein). Scales = 1,0 mm.

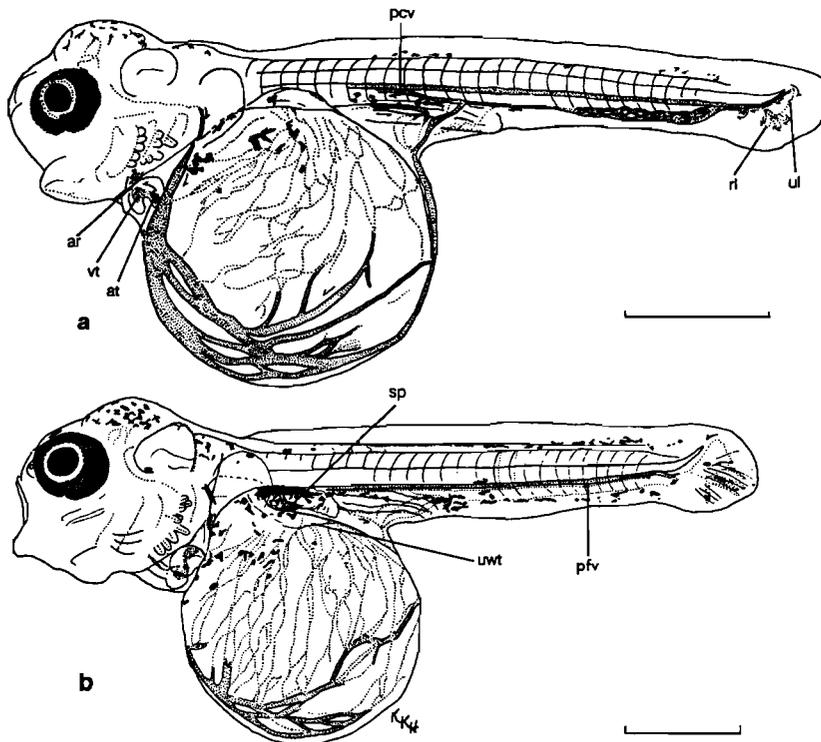


Figure 12 Step F¹8: a – age 06:12 (TU = 163), b – age 07:20 (TU = 196) (ar = arteriosus, at = atrium, pcv = postcardinal vein, pfv = profundal caudal vein, rl = radial loop, sp = spleen, ul = urostyler loop, vt = ventricle, uwt = unidentified white tissue). Scales = 1,0 mm.

subclavian vein was a single arched vessel in the pectoral fin. At age 07:12 (TU = 188) a marked reduction in the finfold plexus was noted. The replacement of the inferior caudal vein by the profundal caudal vein was underway. The

profundal caudal vein was formed from the posterior segmental veins as they emptied into the postanal finfold plexus. The anastomosing profundal caudal vein lay ventral to the dorsal aorta and extended anteriorly from the last

somite to about mid-way along the postanal finfold. By age 07:20 (TU = 196) all that remained of the postanal plexus was the strongly flowing profundal caudal vein and a much reduced inferior caudal vein (Figure 12b). The pre-anal plexus was also markedly reduced. Fine capillaries were present in the brain below the skin surface. Gill filaments were present on the first four ceratobranchials. Muscular development had begun in the ventricle which lay ventro-anteriorly to the atrium (Figure 12b). The segmental arteries and veins formed a two-tiered system in which loops and horizontal veins were formed dorsal to the notochord and at the junction of the dorsal finfold and the somites. Blood flowed through fine vessels in and around the developing gastrointestinal tract. Up to 10 radial loops had formed in the caudal fin and in one instance there were two loops associated with one ray. The subclavian vein had an additional arch.

Mesenchymal aggregations in the ventral area of the caudal finfold were noted at age 04:20 (TU = 121). Cleared and stained specimens up to age 06:00 (TU = 150) retained blue colouration in their tissue. The first indication that chondrification had begun was at age 05:13 (TU = 139). Some alcian blue uptake occurred around the ventral and lateral aspects of the developing jawline and in the presumptive actinosal plates. The cleithrum was a thread-like, transparent structure. Five hours later, alcian stain was noted in the ceratohyals, Meckel's cartilage, in the region of the otic capsules and on the articulation process of the presumptive operculum. The angulo-articula and Meckel's cartilage appeared as one elongate structure which curved ventrally at its posterior end. Ventral to the palatoquadrate, the hyo-symplectic extended postero-ventrally to the otic capsule. The unfused trabeculae extended posteriorly and curved around the sides of the notochord. The parachordals were not clearly distinguishable but a transparent bar which curved dorso-anteriorly joined the presumptive basal plate to the floor of the otic capsule. Two ceratobranchials were visible as simple loops but had not retained any alcian stain. By age 06:04 (TU = 154) the ceratohyals articulated with the hyo-symplectic. The trabeculae flattened into the ethmoid plate anteriorly. Chondrification had begun in varying degrees in all the above four structures, as well as in the palatoquadrate and the angulo-articula (Figure 13a). The occipital arches extended dorsally from the parachordals. Further development of the branchial skeleton had occurred but individual skeletal structures were not distinguishable. The anterior margin of the otic capsules was stained. The presumptive maxilla was visible as a fine line in the tissue. The scapula-coracoid had a broad, triangular shape and the actinosal plate consisted mainly of a semicircular band; both were pale blue. The tissue around the outer margins of the pectoral fins and the caudal finfold was striated.

By age 06:20 (TU = 171) two branchiostegal rays had retained some alcian stain. The ceratohyals broadened ventrally and differentiation of the interhyals had begun. The ventral hypohyals had begun to chondrify and were more advanced in their development than the dorsal ones. Chondrification had begun in all the ceratobranchials. Hypobranchials had not developed but the ventral point of the first three ceratobranchials, where they articulated with the basibranchial copulae, was broadened. The basibranchial

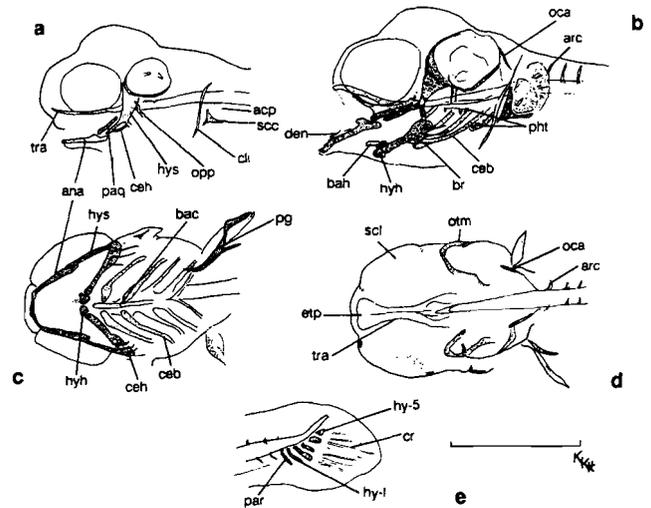


Figure 13 Step F¹⁸: Skeletal development and chondrification. a – left lateral view of the head at age 06:04 (TU = 154); b – left lateral, c – ventral and d – dorsal views of the head at age 07:12 (TU = 188); e – caudal fin at age 07:12. The degree of alcian blue uptake in the individual structures is represented by the stippled areas (acp = actinosal plate, ana = angulo-articula, arc = arcualia, bac = basibranchial copulae, bah = basihyal, br = branchiostegal rays, ceb = ceratobranchial, ceh = ceratohyal, cli = cleithrum, cr = caudal ray, den = dentary, etp = ethmoid plate, hyh = hypohyal, hys = hyo-symplectic, hy-1 = hypural 1, hy-5 = hypural 5, oca = occipital arch, opp = opercular process, otm = otic capsule margin, paq = palatoquadrate, par = parhypural, pg = pectoral girdle, pht = pharyngeal teeth, scc = scapula-coracoid, scl = sclera, tra = trabeculae). Scale = 1,0 mm.

copulae and cartilage were a single structure which extended posteriorly between the ceratobranchials and was slightly stained in the anterior tip. Two upper pharyngeal teeth per side were present. The trabeculae were in contact with the parachordal plate but whether or not fusion had occurred was not established. The posterior margin and a bar in the central regions of the otic capsule, were slightly stained. The sclera formed a pale blue band around the eye. The operculum had begun to fan out from the ventral point of its articulation process. In the pectoral girdle, two to three actinotrichia had begun to chondrify. The hypurals had begun to differentiate into four distinctive structures. Three actinotrichia had begun to form and chondrify adjacent to the two middle hypurals.

At age 07:12 (TU = 188) the palatoquadrate curved proximally to form a wing-shaped plate. Dorsal to Meckel's cartilage, the dentary had begun to form and contained three minute teeth. The basihyal was faintly visible in the branchial skeleton. The upper pharyngeal teeth consisted, on each side, of a single anterior tooth and clumps of three to four teeth of varying lengths which were positioned posteriorly. All the teeth were transparent and pointed in a slightly posterior direction. The lower pharyngeal teeth were also in clusters of three to four teeth of varying lengths on each side, but with anterior pointed tips. The actinosal plate had begun to differentiate into a dorsal and a ventral component (Figures 13b–d). Twenty-nine neural and 19 haemal arcualia had begun to develop and curved towards each other but had

not joined distally. Chondrification had begun in all the arcualia but it was more predominant in the posterior ones. Five hypurals were evident with the bases of the first, second and third closely associated (Figure 13e). A parhypural was faintly visible. Eight caudal actinotrichia were forming. All of the above caudal structures were stained blue to some degree.

By 04:20 (TU = 121) the head was free of the yolksac. The mesencephalon and the cerebellum extended dorsally giving the head a more rounded configuration. The mesencephalon began folding posteriorly. The cerebellum and the medulla oblongata were discernible. Division into the diencephalon and the telencephalon had occurred and the nasal channels had opened. The curvature of the body axis had decreased, resulting in a straighter head and trunk alignment but the tail remained dorsally curved. The otic capsules reached their final position near the ventro-posterior margin of the cerebellum. Iridocytes were present in the eyes. A narrow line along the presumptive jawline at age 05:01 (TU = 126) marked the external opening of the buccal cavity and the initial formation of the mouth. The eyes were black and retinal pigmentation was pale at age 05:13 (TU = 139). Up to this time body pigmentation was confined to a few melanophores along the body axis dorsal to the pre-anal finfold and along the dorsal margins of the somites. The body axis was straight and the yolksac markedly reduced. At age 05:18 (TU = 144) the eyes were golden and the retinas were darkly pigmented. Between ages 06:00 (TU = 150) and 07:00 (TU = 175) stellate-like melanophores had developed above the forebrain, the midbrain and the medulla oblongata. A few iridocytes were present over the swimbladder, the visceral cavity and along the lateral flanks. The mouth had formed, jaw and pectoral fin movement was noted and the urinary bladder became visible. The gall bladder had begun forming. The pectoral fins had rotated and lay perpendicular to the body axis and posterior to the operculum which covered the third gill arch. At age 07:20 (TU = 196) one individual had a yellow substance present in the gastrointestinal tract. The oesophagus was a thick-walled, muscular tube with two distinct regions. The remainder of the digestive tract consisted of a thin-walled tube which narrowed posteriorly; the stomach and hindgut were separated by a slight constriction. The convolutions of the gastrointestinal tract went from left to right and ascended slightly to run ventral to the notochord. The spleen had formed and an unidentified thick white tissue lay along the rim of the yolksac and the visceral cavity (Figure 12b). A fine, transparent thread-like exudate extended out of the anus of one specimen at age 08:00 (TU = 200) and three others at age 08:03 (TU = 203) which indicated that the gut lumen was open at the anus. Peristaltic movements had begun. The swimbladder was visible above the visceral cavity as a small, ovoid chamber with thick walls and a slit-like lumen. The ventral finfold was reduced and differentiation had begun in the caudal finfold. Pigmentation extended along the dorsal and ventral body line and a few melanophores had formed on the roof of the pericardium. The pigments on the dorsal surface of the head had begun to aggregate. The tissue covering the visceral cavity was

heavily pigmented with brown, green and yellow melanophores as well as iridocytes. The eyes were profuse with iridocytes and a few were present in the opercular area. A few melanophores were present along the presumptive caudal fin rays (Figure 12b). Throughout this step the dorsal finfold increased and reached a maximum size by the end of this step.

As in all the previous steps, heart rate fluctuated without any apparent pattern. However, there was a general trend towards a decrease in the heart rate (Figure 10). The largest increase in embryo length (1,9 mm) of any previous step was recorded. There was no change in yolksac area (Figure 9).

Step summary: The main ontogenetic events occurred in the circulatory, respiratory and digestive systems. The basic skeletal components were formed. The ventral finfold and yolksac respiratory plexii, reached maximum development as the permanent adult respiratory structures increased in complexity. Skeletal development was predominantly in the suspensorium, the hyoid arches, the pectoral girdle and the caudal fin skeleton.

F² 08:04–11:00 The inferior caudal vein was replaced by the profundal caudal vein and the finfold network disappeared. There was a marked decline in the yolksac plexus and the secondary gill lamellae were formed and vascularized. There was increased dermal bone differentiation. The neural and haemal arches fused at their distal ends, and caudal fin lepidotrichia and proximal pterygiophores in the median fins were formed.

The inferior caudal vein was no longer present at the beginning of this step. The profundal caudal vein emptied directly into the posterior cardinal vein. The pre-anal plexus consisted of a few vessels supplied with blood from a vein leading from the junction point of the profundal caudal vein and the posterior cardinal vein. These pre-anal finfold veins fed into one large vein which flowed anteriorly and supplied blood to the digestive system. The veins of the vitelline plexus formed a symmetrical pattern over the entire surface of the yolksac (see Figures 14 & 8b). The veins leaving the body were of a uniform thickness and emptied into one major collecting vessel along the ventrum of the yolksac. The anterior vitelline vein was no longer present and it appeared that the common cardinal vein entered the duct of Cuvier directly. The second and third ceratobranchials had double gill filaments. By age 08:12 (TU = 213), secondary lamellae began to form on the gill filaments of the second and third ceratobranchials. Twelve hours later, blood vessels had formed in the secondary gill lamellae. The tissue above the anterior half of the visceral cavity was covered in iridocytes and yellow pigments. The pre-anal finfold had disappeared. The anal, dorsal and caudal fins began to take shape. The gastrointestinal tract was strongly convoluted (Figure 14). By age 10:04 (TU = 254), iridocytes extended posteriorly over the visceral cavity to the anus and ventrolaterally over the yolksac. A few pigment cells were present along the notochord and there was an increase in melanophores above the medulla oblongata and the first few somites. The heart had reached its final position. Reduction of the yolksac gave it the appearance of a sphere extruding from the stomach of the embryo. Differentiation in the

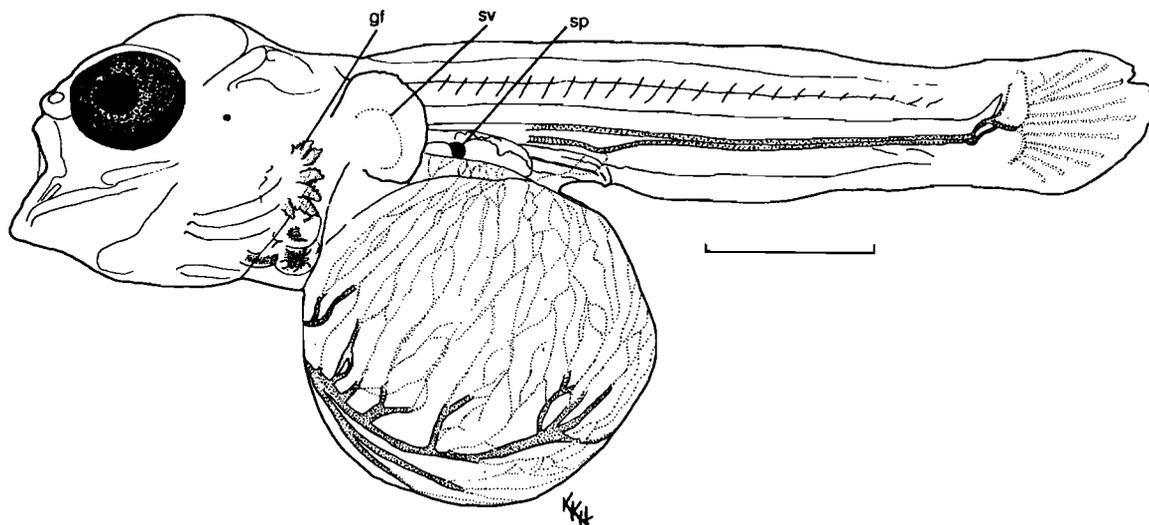


Figure 14 Step F²9: Specimen at age 09:00 (TU = 225) (gf = gill filament, sp = spleen, sv = subclavian vein). Scale = 1,0 mm.

median finfolds had begun posteriorly, making the caudal finfold markedly distinctive. The development of the digestive system made it possible to distinguish between the oesophagus, the stomach, the intestines, the spleen and the gall bladder. Pouch-like projections were visible along the lining of the stomach and the intestine.

As with the last few specimens of the former step, there was a transparent thread-like exudate extending out of the anus in the first specimen of this step. This exudate was not noted at any other time. There was no food visible in the digestive tract of live specimens during this step. Examination of cleared/stained specimens revealed the presence of an unidentified ovoid-shaped object, thought to be a planktonic crustacean, in the digestive tract of embryos from age 08:17 (TU = 218) onwards. Initially there was only one zooplankton present but with time a gradual increase in numbers was noted. From age 09:00 (TU = 225), a granular material was present in the digestive tract. At age 10:04 a round red object resembling an otolith and a blue structure were present in the gut.

Embryos in the incubator tank were relatively inactive and responded passively to the inflow at the mouth of the funnel where most of them were gently tossed around. Initially embryos in the incubator cups were also inactive and lay passively in clusters on the bottom of the cup. By age 10:23 (TU = 274), several individuals swam inefficiently around the bottom of the cup or momentarily in the water column, but the majority were still clustered at the bottom. When food was added to the funnels and the cups, the embryos did not appear to be feeding. Throughout this step, peristalsis was noted and the production of the yellow substance increased. Initially the yellow substance was present only in the foregut, but with time it was also noted in the hindgut and eventually several specimens expelled it through the anus while under observation.

At the beginning of this step, the hypobranchials had begun to differentiate and the gill filament rays had begun to chondrify. Gill rakers were visible. The basihyal was present and flattened anteriorly. Four branchiostegal rays were present. The dentary had taken up some alcian stain and extended posteriorly. The angulo-articula had begun deve-

loping processes at the articulation point with the palatoquadrate. The premaxilla was present as a transparent, ragged, fine line. The maxilla curved posteriorly at the dorsal end. The dorsal outline of the suboperculum had stained blue. The margin and the outer walls of the otic capsules, as well as some of its internal structures, had begun to chondrify. The blue-stained occipital arches extended anteriorly towards the otic capsules. Various processes extended dorsally from the neurocranial floor and connected with the otic capsules. The parachordals extended anteriorly between the trabeculae but were not stained. The ethmoid cartilage extended dorsally towards the paraphyseal bar. The epiphyseal bar and the anterior and posterior orbital bars had all taken up some alcian stain. A foramen had developed in the upper portion of the scapula-coracoid and the posterior region had begun to elongate. At age 08:17 (TU = 218), the tips of all of the pharyngeal teeth had taken up alizarin stain. The upper pharyngeal teeth consisted of two anterior teeth and a cluster of 10 to 12 posterior teeth per side. The pharyngeal bones (the fused fifth ceratobranchials) contained clusters of 10 to 12 teeth per side. The parachordals extended posteriorly where they enclosed the notochord ventrally and laterally. The floor of the otic capsule dipped ventrally to form the chamber for the sacculus. The supracleithrum was a transparent, thread-like bone extending anteriorly at an angle of 45° from the cleithrum. Fusion of the neural and haemal arches had begun posteriorly while the anterior arches remained separate. Hypurals one to three were fused at their bases. There were six actinotrichia and six double-segmented lepidotrichia present in the caudal finfold.

At age 09:00 (TU = 225) alizarin stain was retained in the branchiostegal rays, the dentary, the tips of the anterior dentary teeth, the maxilla, and the operculum process. The saggital otolith had a pink tinge.

At age 09:12 (TU = 238) two teeth had formed on the premaxilla, the ascending premaxillary process extended dorso-posteriorly towards the paraphyseal bar and four radials had begun to form from the actinosal plate. There were three mesenchymal rays in the pectoral fins and the tissue of the median finfolds was striated. By age 09:20 (TU = 246), most of the neural arches had fused and over half

had developed spines. All except the first few anterior haemal arches were fused. Three hours later, proximal pterygiophores had begun to form and chondrify along the base of the dorsal finfold; the middle ones were more advanced in their development.

By age 10:04 (TU = 254), elements of the branchial skeleton had become more distinguishable. The epibranchials and three hypobranchials had all differentiated. The basibranchial cartilage consisted of two separate components. The first section extended posteriorly from the hypohyals to the third hypobranchial and the second component lay between the fourth ceratobranchial and the lower pharyngeal bone. The dentary had begun to encircle and calcify around Meckel's cartilage. Six dentary and four premaxillary teeth were calcified. The maxilla flattened dorsally and both it and the premaxilla had taken up some alizarin stain. The hyo-symplectic was constricted at its articulation point with the interhyal and a furrow had formed in the dorsal region. The outer walls of the otic chamber were blue and five inner compartments were visible. Differentiation had begun in the operculum and the suboperculum. Calcification had begun along the margins of the parasphenoid, around the anterior tip of the notochord, and along the occipital arch. The paraphyseal bar was thickened at its junction with the anterior orbital bar which extended ventrally to join up with the ethmoid cartilage. The posttemporal lay between the supracleithrum and the posterior wall of the neurocranium, and the cleithrum had begun calcifying. Three ribs were visible. The notochord was slightly pink in the regions of the haemal arches with an increased intensity at the points of contact between the arches and the notochord. The urostyle had begun calcifying. One epural was present. Calcification in the 16 caudal rays indicated that lepidotrichia were forming. There were up to four segments in the caudal fin rays. Five proximal pterygiophores had begun to form in the anal finfold and dense mesenchymal aggregations were present in the centre of both median finfolds. The heart rate was erratic but peaked at the end of the step (Figure 10). There was no growth and the yolksac area remained constant (Figure 9).

Step summary: Marked changes have occurred in the digestive, respiratory and circulatory systems, all of which appear to be close to completion. All the adult skeletal structures were present. Differentiation and chondrification was most pronounced in structures associated with feeding and locomotion.

F³10 11:00–15:00 First exogenous feeding occurred at the onset of this step. Ossification of cartilage bone, median, caudal and pectoral fin lepidotrichia and vertebral development began. The swimbladder filled and pelvic fin buds were formed. Enclosure of the yolksac and finfold differentiation was almost completed. Neuromasts and cupola formed along the caudal peduncle. Melanophores were present in all the fins. The switch from endogenous to exogenous nutrition was completed at about age 15:00 (TU = 375), thus marking the end of the embryonic period and the beginning of the juvenile period.

The elimination of dark, particulated faeces and the presence

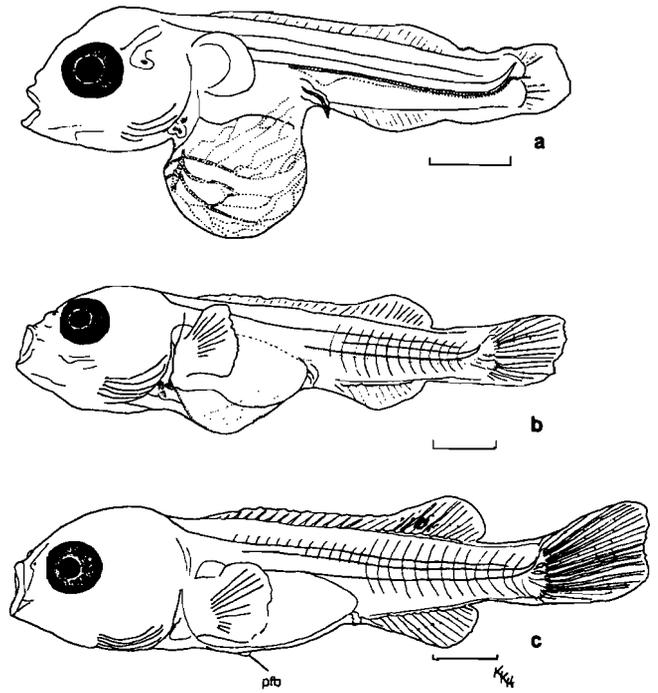


Figure 15 Step F³10: a – at age 11: 00 (TU = 275), b – at age 12:12 (TU = 313), c – at age 14:12 (TU 363). Note the rapid decrease in the yolksac as the body wall descends and the differentiation of the fins (pfb = pelvic fin bud). Scales = 1,0 mm.

of large quantities of zooplankton in the intestinal tract of the specimen aged 11:00 (TU = 275) were indications that exogenous feeding had begun. The yolksac was spherical and large relative to body length, and the vitelline plexus formed a uniform network over the entire yolksac surface (Figure 15a, see also Figure 8c). At age 12:12 (TU = 313) the yolksac was still relatively large and bulged below the ventral body line (Figure 15b), but by 13:12 (TU = 338) a marked reduction of both the yolksac and the embryonic finfold was noted. By age 14:12 (TU = 363) the yolksac was visible as a slight extension ventral to the body line and the vitelline plexus consisted of a few vessels, a pinkish area, or simply as channels without any flow (Figures 15c & 8d). In most specimens all that remained of the yolksac could be seen through a narrow gap between the opposing sides of the descending body wall tissue (Figure 16a). Remnants of the yolksac were present in the visceral cavity. This marked the end of endogenous feeding and was the termination of mixed nutrition.

At the beginning of this step embryos had begun to swim in the water column of the funnel. Within 27 h a large proportion of individuals were free-swimming in the upper portions and upwards along the walls of the funnel and some had made their way through the outflow tube into the cups. By age 12:12 the embryos appeared to be feeding and by age 13:12 they were actively pursuing brine shrimps in both the funnels and the cups. By that time most of the individuals had swum into the cups.

At the beginning of this step fine, thread-like melanophores extended laterally along the flanks of the body, and the dorsal regions of the head were heavily pigmented. A few pigment cells were present along the posterior base of

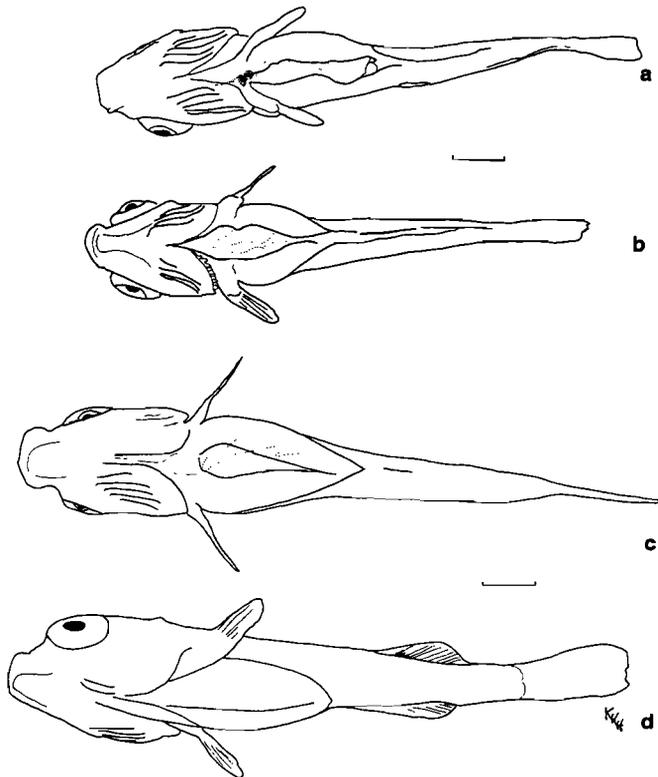


Figure 16 Ventral views showing migration of the body wall over the yolk sac; a – at age 14:12 (TU = 363), b – at age 15:12 (TU = 388), c – at age 15:00 (TU = 375), d – at age 16:18 (TU = 419). Scales = 1,0 mm.

the dorsal finfold (see Figure 8c). The walls of the swim-bladder were thinner and the lumen had begun filling with air.

Dechondrification in the central regions and calcification along the margins of the hypurals, the interhyals, the ceratohyals, the hyo-symplectic and the palatoquadrate were evident. The third and fourth pharyngobranchials had begun to calcify. There were three teeth on the second pharyngobranchial and the posterior clumped teeth were arranged in rows on the third and fourth pharyngobranchials. Other structures which had begun calcifying were the pharyngeal bone, the midline of the urohyal, the lepidotrichia of the pectoral fins, the bases of the neural and haemal arches, the urostyle and the anterior tip of the notochord. Faint pink banding on the notochord indicated that vertebral development had commenced. The maxilla had fused with the premaxilla. A cartilaginous bar extended antero-ventrally beyond the junction of the paraphyseal bar and the anterior orbital commissure, where it almost touched the anterior tip of the palatine. Development of the parietal had begun as evidenced by two opposing bones forming along the dorsal roof of the neurocranium; one bone extending posteriorly from the epiphyseal bar and another bone extending anteriorly from the dorso-posterior wall of the neurocranium. The preoperculum had begun forming and lateral line pores were present along its dorsal rim. Posterior constrictions of the median finfold made the shape of the differentiating dorsal and anal fins obvious. Mesenchymal rays had formed in the median fins and aggregations of mesenchyme along the leading edges of the descending body wall were the first

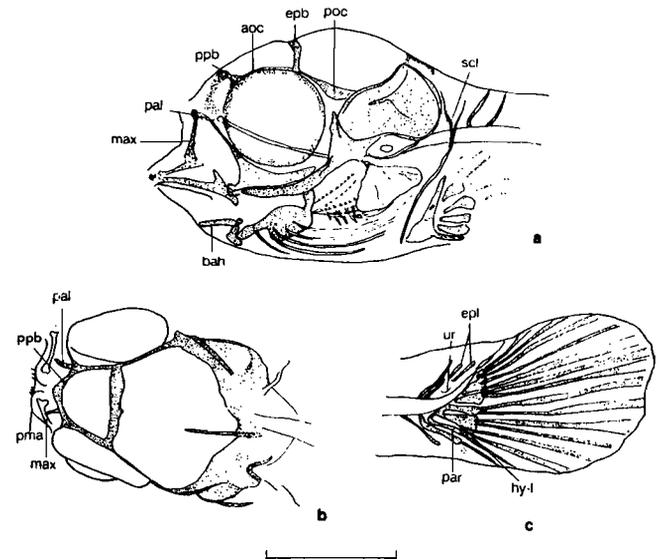


Figure 17 Step F³10: Skeletal development at age 11:01 (TU = 276); a – lateral view of head, b – dorsal view of head, and c – lateral view of caudal fin (aoc = anterior orbital commissure, bah = basihyal, epb = epiphyseal bar, epl = epural, hy-1 = hypural 1, max = maxilla, pal = palatine, par = parhypural, pma = premaxilla, poc = posterior orbital commissure, ppb = paraphyseal bar, scl = supracleithrum, ur = uroneural). Scale 1,0 mm.

signs of pelvic fin formation. Figure 17 illustrates the skeletal development at age 11:01.

Over the next three and one half days increased development and calcification was noted in the oro-nasal area. The vomer was calcified and had a ragged appearance along the ventro-anterior surface. Eight ribs were present. The vertebrae centre at the extremities of the notochord were distinguishable and had begun to calcify. The postcleithra and one uroneural had formed and begun calcifying. Two epurals were present. Distal pterygiophores and up to 28 rays, some with two segments, had formed in the dorsal fin, and in the anal fin there were up to 12 lepidotrichia, some with two segments. There were three spines and 10 rays present in the anal fin. The proximal pterygiophores extended farther into the body tissue and were positioned between the haemal and neural arches. Calcification had begun in the median fin rays. The caudal fin had up to 22 lepidotrichia with up to five segments. The pectoral fin had 12 rays with up to three segments. All the fin rays extended to the distal margins of their respective fins. The pelvic girdle was an undifferentiated fine line with flaps of striated tissue extending from it. There was a concentration of pigments along three of the dorsal fin rays which denoted the beginnings of the characteristic 'tilapia spot'. A few melanophores were present along some of the anal and pectoral fin rays, along the distal membrane of the dorsal fin, and throughout the caudal fin. The entire body was covered in pale, stellate melanophores (Figure 8d). Some green and yellow colouration, as well as iridocytes, were also noted. At least 12 neuromasts with cupolae were present along each side of the caudal peduncle. At age 14:12 (TU = 363) white rings separated the developing vertebrae which were slightly concave.

There was a marked decrease in the heart rate (Figure 10),

an increase in growth (4,1 mm) and a comparative decrease in yolksac area (Figure 9).

Step summary: By the end of this step all that remained of temporary embryonic structures was a remnant of yolk within the visceral cavity. All adult structures were present except for the gonads. Skeletal development was predominantly in the structures relating to locomotion, thus increasing swimming efficiency.

Juvenile period (J¹11) 15:00–?

The initial stages of the juvenile period are presented below. By age 15:00 (TU = 375) finfold differentiation was complete and the development of neuromasts with cupolae along the lateral line extended up to and included the head region. Demarcation and ossification of individual vertebral centra was obvious (Figures 8e & f). Spike-like processes had formed on the anterior and posterior corners of the vertebrae.

The yolksac was visible ventrally through a narrow gap (see Figures 16b & c). In specimens aged 16:00 and older a large vein ran anteriorly along the dorsal surface of the spinal cord. This vein received blood from the caudal network and the intersegmental vessels. Many of the lepidotrichia in the tail had double loops and an intricate caudal pattern of vessels had developed. By age 16:03 (TU = 403), in most specimens, the opposing sides of the descending body wall tissue met, completely enclosing the visceral cavity and any remaining yolk (see Figure 16d). By age 18:12 blood vessels were noted at the bases of the dorsal

and anal fins, and by age 19:12 (TU = 488) these vessels formed loops which extended partially up individual lepidotrichia. One row of scales had developed along the midline of the caudal peduncle. Over the next three days scales had extended anteriorly to the posterior margin of the operculum and covered the trunk and tail region in all but the dorsal and ventral areas. Strong circulation existed in both median fins with the vessel loops extending along the lepidotrichia towards the distal fin margins. Pigmentation of the 'tilapia spot' was complete and there were three faint vertical bars along the body, directly posterior to the head. Green and yellow melanophores and iridocytes were profuse over the entire body.

Discussion

The life-history model of Balon (1981) is a useful tool for organizing data generated from this type of research. It allows the researcher to order the events of development into natural, meaningful intervals rather than arbitrary stages. Whether or not the boundaries between the developmental steps are saltatory or gradual is an issue to be addressed in another paper. The results of this study suggest that the early ontogeny of *O. mossambicus* is direct with accelerated feeding into the embryonic period. The embryonic period is divided into a cleavage phase (1 day, 11 h), an embryonic phase (3 days, 5 h) and a free-embryonic phase (10 days, 8 h). The juvenile period begins at 15 days. Table 3 summarizes the ontogenetic events from activation until the beginning of the juvenile period.

Table 3 Step summaries of the early ontogeny of *O. mossambicus*

Step	Age (days:hours)	Step summary
C ¹	00:00–00:01	Bipolar differentiation; perivitelline space formation; hardening of egg envelope
C ²	00:01–00:22	Cleavage; germ ring formation; flattening of blastodisc
C ³	00:22–01:11	Beginning of epiboly; formation of embryonic shield
E ¹ 4	01:11–02:12	Neurulation; organogenesis; formation of fore-, mid- and hindbrain, optic vesicles, at least 17 pairs of somites, pericardial cavity and neuromeres; first pigmentation
E ² 5	02:12–03:00	First cardiac and muscle contractions; initial blood flow; formation of eye lenses, otic capsules, and heart-tube; simple vascularization of the vitelline plexus - anterior vitelline veins, blood islands; hemispherical division of the brain; elongation and separation of the tail
E ³ 6	03:00–03:20	Strong head and body circulation; branching of the posterior vitelline vein; twisting of the heart-tube; some haemoglobin; first eye pigmentation; simple median finfold plexus; hatching begins
E ⁴ 7	03:20–04:16	Hepatic vitelline network and branchial arteries develop; arterial flow into the preanal plexus; pectoral anlagen; hatching of almost all individuals
F ¹ 8	04:16–08:04	Formation of caudal finfold network, intersegmental and pectoral fin vessels and heart chambers; blood vessels in gill filaments; maximization of median and vitelline plexii; anastomoses of profundal caudal vein for replacement of the inferior caudal vein; decline of median finfold network; head free of yolksac; chondrification of skeletal structures; peristalsis; jaw and pectoral fin movements; differentiation of stomach, spleen, and gall-, urinary- and swimbladder; beginning finfold differentiation; first iridocytes and retinal pigments
F ² 9	08:04–11:00	Replacement of the inferior caudal vein by the profundal caudal vein; disappearance of finfold respiratory network; marked decline in vitelline plexus; formation and vascularization of secondary gill lamellae; formation of dermal bones; fusion of neural and haemal arches; formation of caudal lepidotrichia and proximal pterygiophores of the median fins
F ³ 10	11:00–15:00	Mixed exogenous and endogenous feeding; ossification of chondroid bone, median, caudal and pectoral lepidotrichia; formation of vertebral rings and pelvic fin buds; filling of swimbladder; finfold differentiation; enclosure of yolksac almost complete
J ¹ 11	15:00–?	Complete yolksac enclosure; ossification of vertebrae; vascularization of dorsal and anal fins; squamation

As this research was conducted in accordance with the basic premises of the life-history model proposed by Balon (1986), we have also adopted his terminology for developmental intervals. However, in the last step of the embryonic period (in *O. mossambicus* between ages 11 and 14 days) we have some reservations in accepting that a young fish so far advanced in its development can still be considered an embryo, albeit a free-embryo. It could even be questioned that the interval between hatching and first exogenous feeding is an embryonic period (Flegler-Balon 1989). First exogenous feeding has been emphasized both as a point for interspecific comparisons of developmental levels and as a major boundary between the embryonic and larval periods (Balon 1981, 1986; Noakes & Balon 1982; Flegler-Balon 1989). The initial stages of the larval period may, however, be a time of mixed feeding, thus obscuring the boundaries between the two periods. In direct development, the boundary between the embryonic and juvenile periods is the onset of exogenous feeding. In some cases, the interval of mixed nutrition and the persistence of temporary organs or structures is of a short duration, resulting in a truncated larval period; or the larval period may be eliminated. In mouthbrooders, when hatching or release coincides with the onset of exogenous feeding, this event marks a direct switch from the embryonic into the juvenile period (Noakes & Balon 1982). When present, a large yolk sac is indicative of the importance of endogenous feeding and therefore implies that the young fish is still an embryo. In these cases, there is extended mixed feeding into the embryonic period (Balon 1990, 1991).

In the case of some bearers, i.e. *Labeotropheus* spp. (Balon 1977), where there is no recall into the buccal cavity, release and first exogenous feeding are coincidental, and the newly released young are juveniles. With *O. mossambicus* the situation is different in that the female releases and recalls the young into the buccal cavity and the young may not necessarily have attained the juvenile level of development at the time of first release. According to Vaas & Hofstede (1952) and Bohrer (1953), the time of first release for *O. mossambicus* is between 11 and 14 days. In our case, if first release were to occur at 11 days, which is the time of first exogenous feeding, the embryos would not have been sufficiently developed to be considered juveniles (see Figures 15 & 17). If first release time had been at 14 days the level of development would have been close to that of a juvenile. The presence of the large yolk sac at age 11 days implies that the endogenous food source may still be a primary source of nutrition. This level of development could, therefore, be considered to be embryonic and is indicative of accelerated exogenous feeding into the embryonic period (Balon 1990, 1991). However, there is no evidence of what proportion of the nutrient requirements is endogenous (i.e. yolk) or exogenous. By the time of final release between 14 and 22 days (Vaas & Hofstede 1952) the young are juveniles. Further evidence on the primary source of nutrition and the level of development at first release of *O. mossambicus* is required to clarify this issue.

Assuming that first exogenous feeding and first release are coincidental (at age 11 days), it could be argued that the interval of mixed feeding is a truncated larval period of

short duration. On the other hand, there are no larval structures to be remodelled. Because some embryonic structures are still present (e.g. the yolk sac respiratory plexus and the median finfold), the stage of development reached is that of an embryo. There are numerous fish species with recognized larval periods which do not have specific larval structures but do have embryonic ones which do not require major remodelling, as in typical larvae, e.g. many guarders (see Noakes 1991).

The difficulties of defining first metamorphosis and the characteristics of a typical fish larva have been addressed. Just, Kraus-Just & Check (1981) outlined three basic criteria for general chordate first metamorphosis, and Youson (1988) added three additional criteria with reference to fish ontogeny. These include changes in non-reproductive structures, occupation of different ecological niches, morphological changes triggered by external/internal cues, a marked change in form, dissimilarity to the adult phenotype and cessation of growth. Considering all these criteria, *O. mossambicus* does not develop a larval form in its ontogeny and neither do many of the species in which the presence of a larval period is commonly accepted.

The age of final release in *O. mossambicus*, between 14 and 22 days (Vaas & Hofstede 1952; Bohrer 1953), coincides closely with the transition into the juvenile period on an eco-morphological and an eco-ethological level. In the case of *O. mossambicus*, and possibly other mouthbrooding cichlids with a recall of the young, if a time of release is one of the criteria for determining the beginning of the juvenile period, the age at final, not first release is a more appropriate criterion. The interval between the age at first release (which is coincidental with first exogenous feeding) and the age of final release could thus be considered a truncated larval period and the larval period would, therefore, be vestigial. This does not preclude the possibility that the onset of the juvenile period may occur before final release. A particular level of ontogenetic development should be the criterion for the beginning of the juvenile period and not the state of parental care. In this study, a prolongation of the mouthbrooding period beyond 15 days would be an example of an overlap between the juvenile period and final release.

The young of *O. mossambicus* do not develop any temporary larval structures and thus have no metamorphosis. Direct development from the embryonic condition into the adult condition while still feeding endogenously seems to be characteristic of this species but some clarification is required regarding the proper terminology for the interval of mixed feeding.

Are we not simply dealing with a problem of semantics? The important issue is that, regardless of whether one chooses to call the level of development in question a larval or an embryonic period (or to call the young a larva or a free-embryo), the evolutionary trend towards greater specialization remains the same. As Flegler-Balon (1989) states 'The decision whether the larval period begins with hatching or with first exogenous feeding does not make the actual development of any particular species more or less direct'. *O. mossambicus* is intermediate in its position between the typical guarding cichlids and the more specialized mouth-

brooders such as *Labeotropheus trewavasae* and *Cyphotilapia frontosa* in which the released young are fully differentiated adult phenotypes (Balon 1990; Noakes 1991). Development is direct in *O. mossambicus*, and the acceleration of exogenous feeding while a large source of endogenous food is available does produce larger and better developed young when parental care ceases. This effectively results in a decrease in vulnerability and a greater chance of survival for the juveniles. Part of the confusion regarding terminology has been created by the representative species used for the life-history model being at the extremes of a range (i.e. guarders and very specialized bearers). The intermediate situation is not represented and possibly does not fit the model as well as the examples stated. The young of species with recall behaviour are probably less advanced in their level of development than those of species without recall (Noakes 1991). Although the time of first release is unknown, the level of development of the embryos of *O. mossambicus* in this study at first exogenous feeding was less advanced than that of the more specialized *Labeotropheus trewavasae* young (Balon 1977) in which first exogenous feeding is coincidental with first release.

O. mossambicus belongs to the reproductive guild C.1.3, i.e. mouth brooders without buccal feeding (Balon 1990). Within the Cichlidae, this species lies between the hole nesters, such as *Tilapia rendalli*, and the mouth brooders with buccal feeding, such as *Cyphotilapia frontosa*.

Components of the environment which affect the ability of fish in the early developmental stages to survive are predation pressure and oxygen and food availability. The ability of *O. mossambicus* to tolerate these major environmental influences is reflected in both its reproductive and developmental styles. Assuming that first release occurs at about age 11 days, most of the embryonic period of *O. mossambicus* takes place while the embryos are within the buccal cavity of the female, which reduces the risk of predation pressure. The crowded and assumed low oxygen conditions within the female's mouth have made it necessary for the embryos to develop a complex temporary respiratory organ on the ventral finfolds and the yolk sac. Oxygen uptake may also be facilitated by the flapping of the pectoral fins which creates a water current over the vitelline plexus (Fishelson 1966).

During the last intervals of the embryonic period, a new and different situation arises. Oxygen is not in short supply for the newly-released, mobile embryos in shallow, sandy, wave-washed nursery areas. In habitats of dense vegetation, oxygen availability could be low, especially at night, but this deficiency is overcome by the respiratory function of the large, well-vascularized yolk sac. The yolk sac serves a second purpose of supplying sufficient nutrition until the period of mixed feeding ends. The nurseries of *O. mossambicus* are commonly in well-vegetated areas that offer an abundance of food for the newly released young (see Trewavas 1983). Predation, however, is a serious consideration and three adaptive features have evolved to overcome this threat. Firstly, there is recall of the offspring into the female's buccal cavity whenever a threatening situation arises. Secondly, the level of development of the free-embryos is relatively well advanced at this stage and, consequently, so is their ability to avoid predators. Plants in the

nurseries provide cover and camouflage.

The co-evolution of this species and its external environment is clearly reflected in the ecomorphological and eco-ethological characteristics of its reproductive and developmental life-history style. The ecomorphological contributions from the adult include an egg with a high carotenoid and nutritional content for increased utilization of oxygen and rapid development and growth, respectively, (see Balon 1991 for information about the oxidative role of carotenoids). Eco-ethologically, the act and duration of mouth-brooding, release and recall, and the ability of the mother to move into appropriate nursery areas is an adaptation to predatory, nutritional and oxygen risks. In the offspring, ecomorphological adaptations include the presence of well-developed temporary respiratory organs to cope with low oxygen levels in the buccal cavity. Rapid overall development is also advantageous.

Phenotypic plasticity in the early life-history style and the reproductive guild of *O. mossambicus* is an important factor in the ability of this species to colonize and invade different habitats. Their invasive capabilities go beyond successful translocations in southern Africa (de Moor & Bruton 1988) as they are internationally one of the most successful natural and man-aided fish invaders (Bruton 1986). The early life-history traits which exhibit a high level of plasticity that allows successful invasions are numerous. The variable release time allows an extended interval of parental care which could increase the duration of benign conditions that mouthbrooding provides. On the other hand, if environmental conditions are unsuitable, the female could brood the young until more favourable conditions are experienced. Another alternative would be for the female to move to a more suitable habitat. The persistent vitelline plexus supplements the nutritional and oxygen requirements of the newly released embryos. This is of particular importance in sub-optimal habitats and could result in an extended embryonic period. Conversely, in optimal conditions the last step in the embryonic period could be shortened. These heterochronic shifts in duration or timing of ontogenetic events not only allow increased plasticity for the individual or the population, but can alter the life-history styles of successive generations if the shifts are persistent over time. Heterochronic shifts owing to environmental influences are an important factor in the formation of an ecophenotype (Balon 1985; Bruton 1989).

In conclusion, the early ontogeny of *O. mossambicus* consists of an embryonic period with three phases lasting approximately 15 days. First release may be coincidental with first exogenous feeding and recall of the young into the female's mouth may extend into the juvenile period. Further research in the field is necessary to confirm these ideas about the level of ontogeny during incubation. Phenotypic and life-history plasticity, which is well documented in the adult period, appears to be characteristic of the embryonic period as well.

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