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The early ontogeny of the southern mouthbrooder, *Pseudocrenilabrus philander* (Pisces, Cichlidae)

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Synopsis

The early development of the southern mouthbrooder, *Pseudocrenilabrus philander,* is documented from activation until the early stages of the juvenile period. The duration of the embryonic period is about 14 days at 25° C. Development is direct and there is accelerated exogenous feeding into the embryonic period. The pattern of development and the timing of ontogenetic events and structure formation are a reflection of both internal and external environmental conditions. During mouthbrooding, oxygen uptake is facilitated by embryonic respiratory plexuses and flapping of the pectoral fins. At the time of first release from the buccal cavity, the embryos are in an advanced state of development. The switch-over from the temporary embryonic respiratory system to the adult branchial system has occurred. The yolksac serves as a supplemental source of nutrition as the embryos develop their external food-gathering abilities. The skeletal and sensory systems are sufficiently developed to allow the young to return to the safety of the female's buccal cavity. Pigmentation may provide disruptive colouration. The rate and pattern of development of another mouthbrooding cichlid, *Oreochromis mossambicus,* is similar to that of *P. philander* despite their phylogenetic differences, and may be a consequence of similar life-history styles.

Introduction

The southern mouthbrooder, *Pseudocrenilabrus philander* (Weber, 1897), occurs in the Zambezi, Limpopo, upper Zaire and Orange River basins, in the river systems of Natal, southern Mozambique and the Okavango Swamps, as well as in the sinkholes of Namibia (Bell-Cross 1966, Trewavas 1973, Skelton et al. 1985). It is found in backwaters, shallow lagoons, swamps, small streams, rivers and springs. Although it is present in natural lakes and artificial impoundments (Balon 1974a, b), it usually confines itself to marginal, shallow regions or swampy areas. In large rivers it prefers slow-flowing regions and it also occurs in estuaries (Bruton 1980, Whitfield 1980, Loiselle 1982, see also Greenwood 1989). It is a eurytopic, generalized, riverine haplochromine which has attributes which may be similar to those of the ancestral haplochromines from which the lacustrine species, as well as some of the derived haplochromines, may have arisen (Poll 1967, Trewavas 1973, Van Couvering 1982). Of all the haplochromines, the *P. philander* superspecies is considered the most ecologically versatile (Loiselle 1982). Its generalized characteristics include its widespread distribution and its tolerance for wide fluctuations in pH, salinity, temperature and hardness (Loiselle 1982, Ribbink in Loiselle 1982).

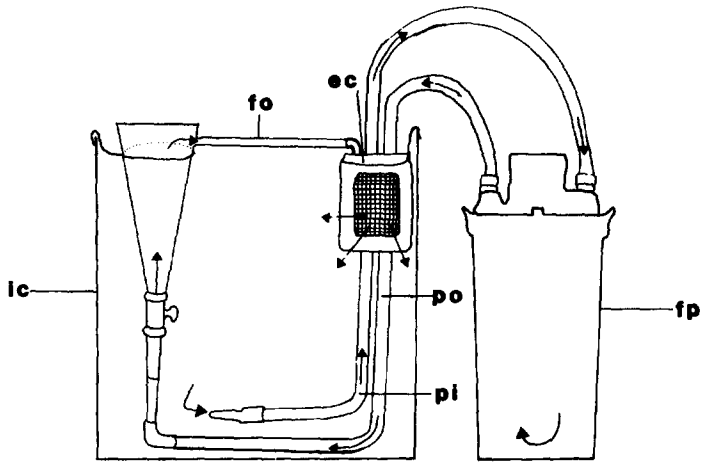

Fig. 1. Side view of the incubating system. Arrows indicate the direction of the water flow. The outflow of the water coming from the pump churned the eggs/embryos at the bottom of the funnel. The embryos swam through the funnel outflow into the embryo chamber once they had developed sufficient swimming abilities (ec = embryo chamber; fo = funnel outflow; fp = filter/pump; ic = incubating tank; pi = pump inflow; po = pump outflow). Drawing modified from Holden & Bruton (1992).

P. philander lays relatively numerous, small eggs and has a shorter incubation period than lacustrine, haplochromine species (Fryer & Iles 1972). Although *P. philander* is not threatened (Skelton 1987) some of the geographically isolated populations may be at risk (Ribbink & Twentyman-Jones 1989, Skelton 1990, de Villiers et al. 1992). Current research suggests that *P. philander* may be in some of these isolated populations an incipient species (de Villiers et al. 1992).

Several authors consider *P. philander* to have plesiomorphic features which suggest that it might be an ancestral haplochromine (Poll 1967, Trewavas 1973, Van Couvering 1982). Greenwood (1989), however, questions this conclusion, suggesting instead that at least some of the ancestral features may be neotenic. Regardless of its phylogenetic relationships, *P. philander* is regarded by all authors to be a eurytopic, riverine, generalized haplochromine species. Balon (1977) described the early development of *Labeotropheus* sp., a specialized, stenotopic haplochromine. The present paper provides a baseline for comparisons of the early ontogeny of a generalized, and possibly ancestral, haplochromine. This is especially important as adaptive radiation is not confined to adult features but also includes early life-history characteristics (Fryer & Iles 1972).

Materials and methods

The parent fish were collected from a wild population in a stream near Durban, South Africa (29°56′ S; 30°59′ E). The descriptions of the early ontogeny were derived from two spawnings of different females with the same male. The female: male ratios in the breeding tanks were 11:1 and 9:1. The methods of Balon & Flegler-Balon (1985) were followed and were adjusted for mouthbrooding species as outlined below and described in Holden & Bruton (1992).

Samples were taken from the time of activation until the juvenile period at varying time intervals depending on the rate of development (sampling intervals were more frequent during the early stages). Activation 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 ageing purposes. 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. 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 the specimens during the microscopic observations. Specimens were placed in vials for preservation and stored in a buffered formalin solution (1.8 g sodium

phosphate monobasic and 1.8 g anhydrous sodium phosphate dibasic in 1 l of 5% formalin).

The breeding and incubation tanks were housed in a temperature-controlled room with a photoperiod of 14 h light: 10 h dark and the water temperature was maintained at 25 ± 0.5° C. The eggs were removed from the female's buccal cavity immediately after spawning. The eggs and embryos were incubated in a 60 l glass aquarium with heaters which were connected to an electronic relay box. 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 (Fig. 1). The outlet of the funnels flowed through transparent plastic tubing into small plastic jars covered with fine netting, which were suspended in the incubator tank. The incubation tank was contained in a darkened enclosure in order to simulate the lower light levels expected in a female's buccal cavity. Eighteen days and two hours after activation, embryos were removed from the darkened enclosure and placed in breeding baskets in a tank with a photoperiod of 14 h light: 10 h dark.

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. Heart rate was determined by timing 10 or 20 beats with a stopwatch. Two to four counts per individual were taken and, in some cases, heart rate for more than one specimen of a given age was recorded.

Food was added to the incubating funnels (at age 8 d) in advance of the expected time of first exogenous feeding. Commercially produced fish food (Tetra Min Baby Fish Food 'E' and Liquifry No. 1) and live brine shrimp nauplii were frequently added to the funnels and specimens were removed periodically to check visually for food in the gut.

Specimens were cleared with trypsin and stained with alcian blue for mucopolysaccharides (cartilage) and with alizarin red-S for calcium phosphate (bone). 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. Embryonic lengths were measured from drawings of live specimens. As the size and shape of the yolk and yolksac changed over time it was not possible to determine yolk volume. Additionally, the ovoid shape of the eggs made a mathematical formulation to determine volume inadequate. Therefore, yolk area was measured from lateral drawings of live specimens to demonstrate yolk absorption. These data were used as an indication of relative changes in rates, sizes and/or patterns of development.

We have chosen to follow the terminology of ontogenetic events and intervals of Balon (1975, 1990), and its applicability to another mouthbrooder with a similar developmental style is discussed in Holden & Bruton (1992). The embryo period is subdivided into a cleavage, an embryo and a free-embryo phase. The cleavage phase begins with activation and ends once organogenesis begins. During the embryo phase, intense organogenesis occurs and hatching begins. Hatching of almost all the clutch members frees them from the confines of the egg envelope and marks the beginning of the free embryo phase. During the embryonic period the major

Table 1. Mean maximum and minimum diameters of *Pseudocrenilabrus philander* eggs from two clutches (STD = standard deviation).

Clutch	n	Mean max. length (mm)	STD	Mean min. length (mm)	STD
1	22	2.20	0.273	1.59	0.124
2	23	2.50	0.073	1.70	0.072
Total	45	2.33	0.238	1.65	0.113

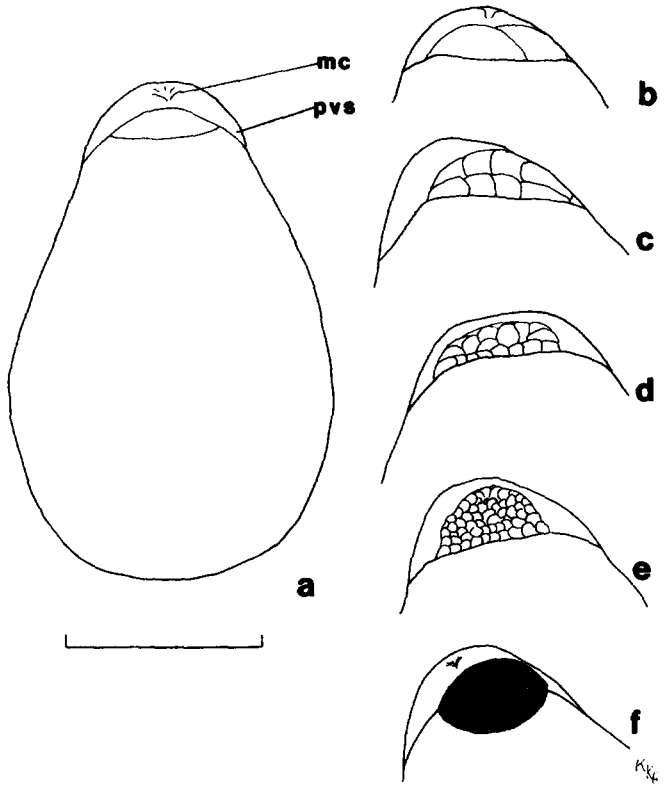

Fig. 2. Cleavage phase: a – age 00:01; b – age 00:02; c – age 00:04; d – age 00:05; e – age 00:07; f – age 00:11 (mc = micropyle; pvs = perivitelline space). Scale = 1 mm.

or exclusive source of nutrition is endogenous via the yolk. Once the yolk has been absorbed and the nutritional requirements are met by exogenous sources, the young fish is considered to be either a larva or a juvenile. In the case of a mouthbrooder, which has direct development, the young fish retain a large yolksac once first exogenous feeding begins and an interval of mixed feeding exists. The level of development at this time is not that of a juvenile and there is no larval period. We, therefore, have chosen to call this the free-embryo phase (for an expanded argument on the terminology see Holden & Bruton 1992).

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. 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 sources of information used for naming blood vessels and skeletal structures were Cunningham & Balon (1985) and Holden & Bruton (1992).

Results

Description of development

The eggs of *P. philander* are ovoid with the longitudinal axis longer than the transverse axis and the animal pole narrower than the vegetal pole. The yellow, opaque yolk is of uniform consistency without oil globules. Table 1 lists the mean maximum and minimum diameters of eggs from two clutches.

Embryo period

Cleavage phase 00:00-01:12. During the first hour, the cytoplasm forms a one-celled blastodisc and the perivitelline space becomes visible at the animal

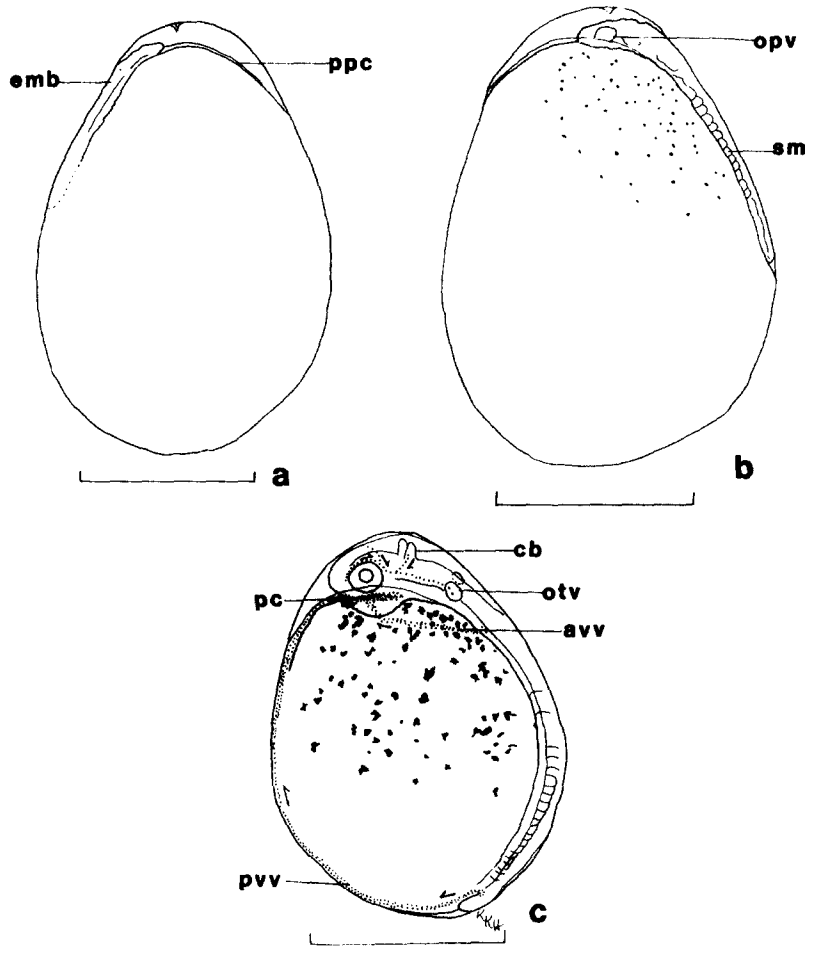

Fig. 3. Embryo phase: a – age 01:02; b – age 02:12; c – age 03:00 (avv = anterior vitelline vein; cb = cerebellum; emb = embryonic shield; opv = optic vesicle; otv = otic vesicle; pc = pericardial cavity; ppc = presumptive pericardial cavity; pvv = posterior vitelline vein; sm = somites). Scales = 1 mm.

pole (Fig. 2a). By age 00:02 (TU = 2) first division takes place and the resultant two cells extend well above the yolk (Fig. 2b). At age 00:03 (TU = 3) there are four cells, and at age 00:04 (TU = 4) the eight cells (Fig. 2c) divide into 16 cells. The first horizontal division occurs at age 00:05 (TU = 5) (Fig. 2d) and within the next hour there is a 64-celled blastodisc (see Fig. 2e). In some cases the blastodisc lies skewed to one side of the yolk. By age 00:11 (TU = 11), there are innumerable cells of undetermined size (Fig. 2f). Between the ages of 00:12 and 00:13 (TU = 12, 13) the periblast forms below the lip of the blastodisc.

At age 00:14 (TU = 14) the periblast becomes broader and more obvious and epiboly begins. By age 01:02 (TU = 27) the embryonic shield lies to one side of the yolk and a layer of less dense, translucent cells is at the opposing side. Over the next several hours, the translucent area becomes a thin layer over the apex of the yolk and the blastodisc extends to the equator.

Embryo phase 01:12-04:23. At age 01:12 (TU = 38) swelling in the anterior region of the embryonic shield and a faint line along its axis indicate that organogenesis has begun. The presumptive pericardium forms a narrow, transparent chamber along the dorsal surface of the yolk (Fig. 3a). After eight hours (TU = 46), rudimentary optic vesicles form elliptical bulges and at least eight somites are present. Melanophores occur along the yolk adjacent to the embryonic body. Body pigments occur on the

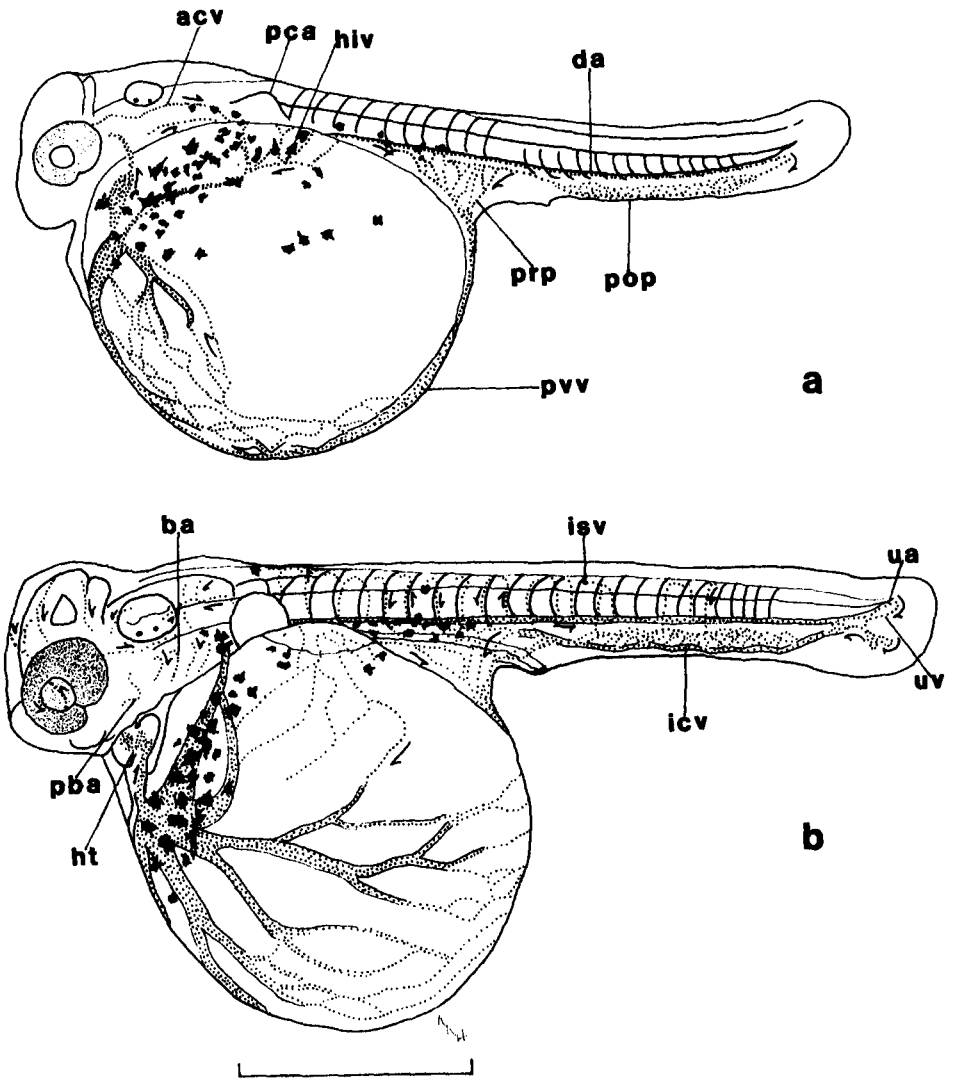

Fig. 4. a – Embryo phase: age 04:03; b – Free-embryo phase: age 05:11 (acv = anterior cardinal vein; ba = branchial arteries; da = dorsal aorta; hiv = hepatic/intestinal vitelline system; ht = heart-tube; icv = inferior caudal vein; isv = intersegmental blood vessels; pba = pseudobranchial artery; pca = pectoral fin anlagen; pop = postanal respiratory plexus; prp = pre-anal respiratory plexus; pvv = posterior vitelline vein; ua = urostylar artery; uv = urostylar vein). Scale = 1 mm.

first few somites at age 02:00 (TU = 50) (Fig. 3b). Six hours later (TU = 56), otic vesicles and a heart-tube are visible and the number of somites increases to 17. By age 02:09 (TU = 59) one otolith per side is present, eye lenses form, body melanophores extend to two thirds the body length and undercutting of the tail mound begins. Constrictions in the anterior region make the three major components of the brain distinguishable (the prosencephalon, mesencephalon and rhombencephalon) and cardiac contractions of 115 beats min^{-1} were recorded for one specimen.

By age 03:00 (TU = 75), two otoliths per side are present, pigment cells extend half-way down over the yolksac and the cerebellum is distinguishable. Blood flow follows a circular route around the eyes and empties into the anterior cardinal veins which flow into the anterior vitelline veins (Fig. 3c). Blood from the pre-anal finfold enters the posterior vitelline vein which flows along the ventral surface of the yolksac where it branches into smaller vessels

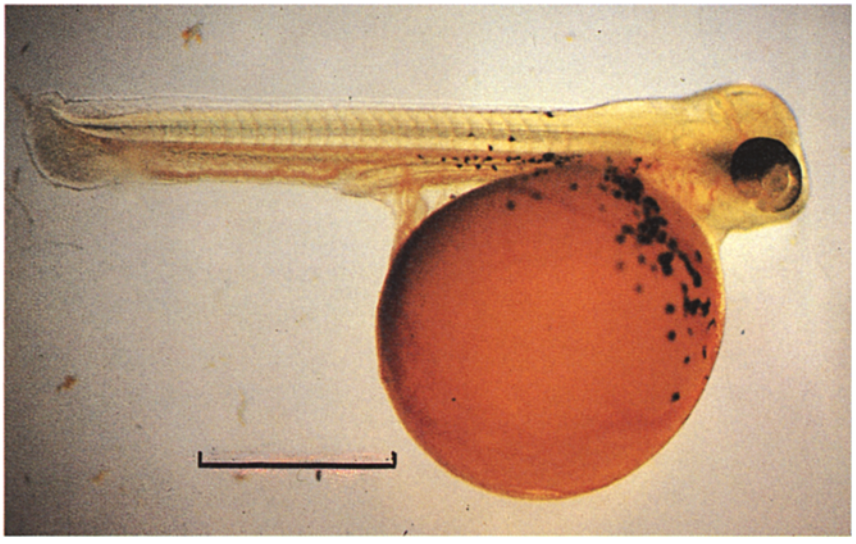

Fig. 5. Free embryo at age 05:05 from right side. Scale 1 mm.

and continues dorsally to the heart-tube. The tail is undercut from the yolksac and body muscle contractions occur.

Three hours later (TU = 78), the posterior vitelline vein branches shortly after it enters the yolksac, the heart-tube begins twisting and the dorsal aorta is visible. At age 03:11 (TU = 86) a transparent chamber is visible in the mesencephalon, the yolksac melanophores are larger and there are 26–27 somites. The dorsal aorta forms a loop in the tail region where it flows anteriorly into the inferior caudal vein. Twelve hours later (TU = 99), hatching begins and the extent of vascularization is more obvious. Additional vessels around the eyes and in the brain region form loops before emptying into the anterior cardinal veins. The inferior caudal vein runs along the distal margin of the postanal finfold. A rudimentary respiratory network is present in the tissue of both the pre- and postanal finfolds. The pre-anal finfold receives blood from the vessels of the postanal finfold and from an artery running posteriorly above the presumptive visceral cavity. The dorsal aorta has a second caudal loop and a third one is forming.

At age 04:03 (TU = 103) pigment extends along the body/yolksac interface and spreads ventrally on the yolksac. The eyes are shaded gray and pectoral fin anlagen are present. Veins enter the yolksac from the right and left sides of the embryo in the vicinity of the presumptive visceral cavity and join the anterior vitelline veins (Fig. 4a). This indicates the beginning of the hepatic and intestinal vitelline system. The left vessels are larger than those entering on the right side (see Fig. 5).

Free-embryo phase (04:23-14:00). By age 04:23 (TU = 124) almost all the embryos have hatched. The head is free from the yolksac and the longitudinal axis is straighter. The entire surface of the eye is lightly and evenly pigmented, the pectoral fin anlagen begin to rotate perpendicularly to the body line, and labyrinth formation in the dorsal regions of the otic capsules begins. Four branchial arteries are visible, the urostylar artery and vein are slightly anterior to the tip of the notochord, and intersegmental vessels in the trunk area form single loops. The density of vessels on the median finfold increases and their pattern is more complex. Blood from the posterior, anterior and hepatic/intestinal vitelline systems forms a sheet-like movement prior to entering the heart-tube. The hepatic/intestinal vitelline veins on the left side form a more complex pattern than on the right. Six hours later (TU = 130), mesenchyme aggregations form along the dorsal and caudal finfolds, the pseudobranchial artery lies anterior to the branchial arteries, vascularization on the lateral portions of the yolksac occurs and internal folding in the foregut is evident. The intersegmental

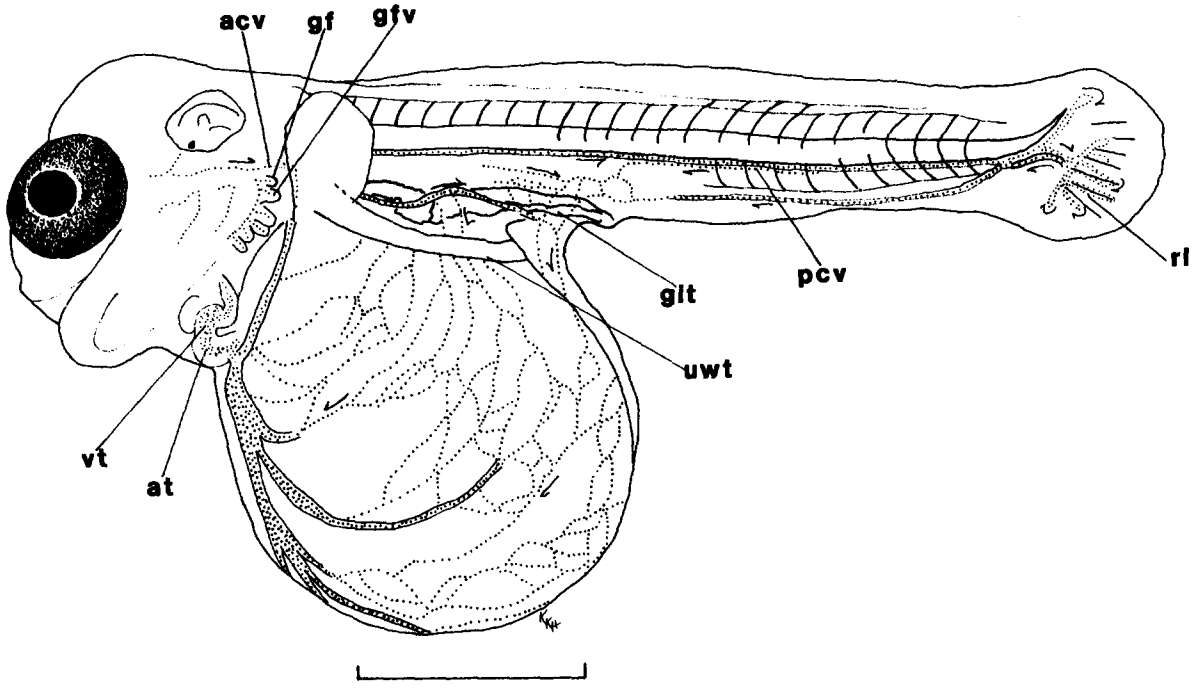

Fig. 6. Free-embryo phase: age 06:23 (acv = anterior cardinal vein; at = atrium; gf = gill filaments; gfv = gill filament blood vessels; git = gastro-intestinal tract; pcv = profundal caudal vein; rl = radial loop; uwt = unidentified white tissue; vt = ventricle). Scale = 1 mm.

vessels extend to the end of the caudal peduncle and the intersegmental veins empty into the postanal plexus (Fig. 4b, 5).

By age 05:23 (TU = 149) a thickened area of white tissue lies along the dorsal rim of the yolksac and constrictions form along the gastro-intestinal tract. Constrictions of the heart-tube make the sinus venosus, the atrium, the ventricle and the bulbous arteriosus distinctive as separate rudimentary compartments. The nares are well developed and the mouth is open but does not move. The first three gill arches are pouch-like with some filaments present. There is a reduction in the central regions of the postanal respiratory plexus. The yolksac respiratory plexus reaches maximum development and the pattern of the veins is symmetrical on both sides. The trabeculae appear as two pale blue lines which fork around the anterior tip of the notochord. Meckel's cartilage, the angulo-articular, the gill arches, the pectoral fin anlagen and the process of the opercle retain some alcian blue stain. The tissue of the caudal finfold is striated; a precursor to ray formation. The cleithrum appears as a thin, transparent line.

At age 06:05 (TU = 155) the eyes, including the lenses, are darkly pigmented and contain iridocytes. Pigment cells appear above the mid-brain, and dorsal body melanophores extend from posterior of the rhombencephalon to mid-body. The shape of the yolksac is oval and mesenchymal aggregations form in the caudal finfold and the pectoral fin buds. Circulation begins in the gill filaments. Five radial loops are present in the developing caudal circulatory system. The inferior caudal vein lies along the ventral body line and the respiratory plexus occurs only in the posterior regions of the postanal finfold. Muscular development and coiling begins in the gastro-intestinal tract which contains a lumen but is not open at the vent. The gall bladder is visible on the right side. Jaw movements begin. Chondrification begins in the ceratohyal, the interhyal, the hyo-symplectic, the palatoquadrate and along the ventral, distal rim of the otic capsule.

At age 06:23 (TU = 174) the pigment cells above the brain are more extensive and stellate-like. Melanophores extend along the ventral body line into the caudal finfold. The pectoral fin bud lies at its final position perpendicular to the body axis and

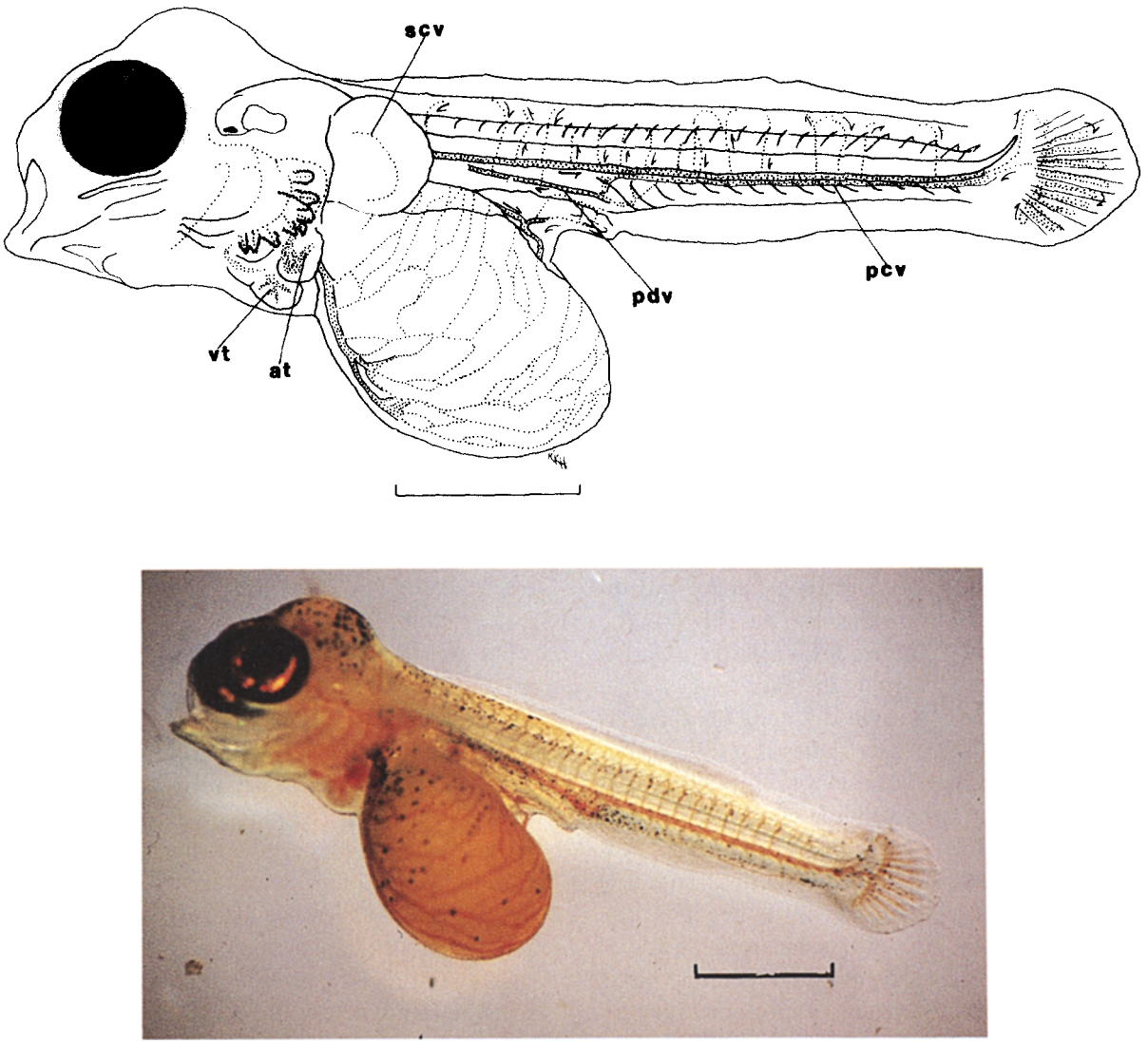

Fig. 7. Free-embryo phase: age 07:21 (at = atrium, pcv = profundal caudal vein; pdv = posterior cardinal vein; scv = subclavian vein; vt = ventricle). Scales = 1 mm.

constrictions along the caudal peduncle indicate the beginning of finfold differentiation, especially between the caudal fin and the median finfolds. Four rudimentary actinotrichia exist in the caudal fin and six radial loops are present. The profundal caudal vein is present in the anterior regions of the trunk and all that remains of the postanal plexus is the inferior caudal vein (Fig. 6). The anterior vitelline vein flows along the anterior margin of the yolksac.

By age 07:06 (TU = 181) the profundal caudal vein extends almost to the end of the caudal peduncle and there is a large reduction in the number of vessels in the pre-anal finfold. The atrium lies dorsal to the ventricle, where muscular development is visible. The subclavian veins form a single loop in the pectoral fin buds. Two branchiostegal rays are present, the posterior region of the angulo-articular begins to elongate, a jagged area forms dorsal to Meckel's cartilage and the maxilla appears as a thin line. All the above structures retained some alcian stain. Other skeletal structures beginning to chondrify are the four ceratobranchials, the anterior tip

of the basibranchial copulae, the sclera, the anterior rims of the otic capsules and the occipital arches. There are two upper and two lower pharyngeal teeth. The anterior ends of the trabeculae are flattened, and fusion for the formation of the ethmoid plate begins. In the pectoral fin buds, the coracoid-scapula begins to chondrify and takes on a triangular shape, and the tissue in the outer margins is striated. Arcualia are present and the hypural region contains three pale blue plates.

At age 07:21 (TU = 197) the yolksac is tear-shaped, pigmentation extends posteriorly above the notochord and there is pale yellow colouration along the back. Mesenchymal tissue is present in the median finfolds where some differentiation occurs. There are 11 actinotrichia in the caudal fin and nine radial loops. The inferior caudal vein has disappeared and the profundal caudal vein flows directly into the posterior cardinal vein (Fig. 7). The anterior vitelline vein is no longer visible and secondary lamellae develop on the gill filaments. The gall bladder is dark green in color and the spleen is visible. Coiling of the gastro-intestinal tract is considerable but distinctive chamber development is not evident. The swimbladder contains thick walls with a narrow lumen. A fourth branchiostegal ray is forming, the premaxilla is a thin line, differentiation of the presumptive hypobranchials begins at the ventral ends of the ceratobranchials and the lateral ethmoid extends dorsally from the ethmoid plate. One anterior and four clumped posterior pharyngeal teeth per side take up alizarin red-S stain in their tips. There are 3–4 lower pharyngeal teeth per side. The outer walls, some internal structures and the posterior rim of the otic capsules begin to chondrify. The opercle takes on a fan-shaped configuration and both it and the dorsal rim of the subopercle are blue. The 27 presumptive neural arches vary from pale blue to transparent in colour from the anterior to the posterior. Some presumptive haemal arches are barely visible.

At age 08:05 (TU = 205) two dentary teeth are present. There is an increase in the number of pharyngeal teeth, all of which have begun to calcify. Double filaments form on, and chondrification begins in, the gill filaments of the first three ceratobranchials. The fourth epibranchial begins differentiating and the actinosal plate retains alcian stain.

By age 08:23 (TU = 224) pigment cells extend all along the dorsal surface to the tail, and finfold differentiation is more obvious. A constriction divides the gastro-intestinal tract into a larger anterior and a narrower posterior portion. Processes form on the dorsal end of the maxilla. The premaxilla has two teeth and a dorso-posterior process. The palatine portion of the palatoquadrate begins to differentiate and chondrify. Gill rakers are present on all four ceratobranchials. Fusion of the trabeculae and the parachordals occurs. The epiphyseal bar, the paraphyseal bar, the supracleithrum and the preopercle begin forming. The opercle process begins calcifying and the full complement of five branchiostegal rays is present. There are three pectoral and 15 caudal actinotrichia. The tissue of the dorsal and anal finfolds is striated.

At age 09:08 (TU = 233), 10 dorsal and four anal mesenchymal rays are present. Slight calcification begins in the tips of the dentary teeth, the dorso-anterior portion of the dentary, the branchiostegal rays, the rim of the subopercle and the anterior tip of the notochord. The parasphenoid and the post-temporal begin to form but are not calcified. All except the anterior nine neural arches are fused. Most of the haemal arches are fused, with the posterior-most ones forming haemal spines. In the actinosal plate four radials begin differentiating. Seven of the 15 caudal lepidotrichia show some degree of calcification. The pre-anal respiratory network has disappeared and blood flows directly from the profundal caudal vein into vessels around the gastro-intestinal tract. The anterior end of the gastro-intestinal tract contains internal, finger-like projections.

At age 10:00 (TU = 250) pigmentation occurs along the lateral flanks, especially in the anterior, and extends to the tail and along most of the caudal lepidotrichia (Fig. 8a). Blood flows into the second lamellae of the gill filaments and along the pectoral actinotrichia. The blood vessels of the yolksac are relatively equal in width and form a fine network. There is a noticeable increase in the degree and occurrence of calcification. Some of the new structures which begin calcifying are the premaxillary teeth, the articulation points of the angulo-articular and

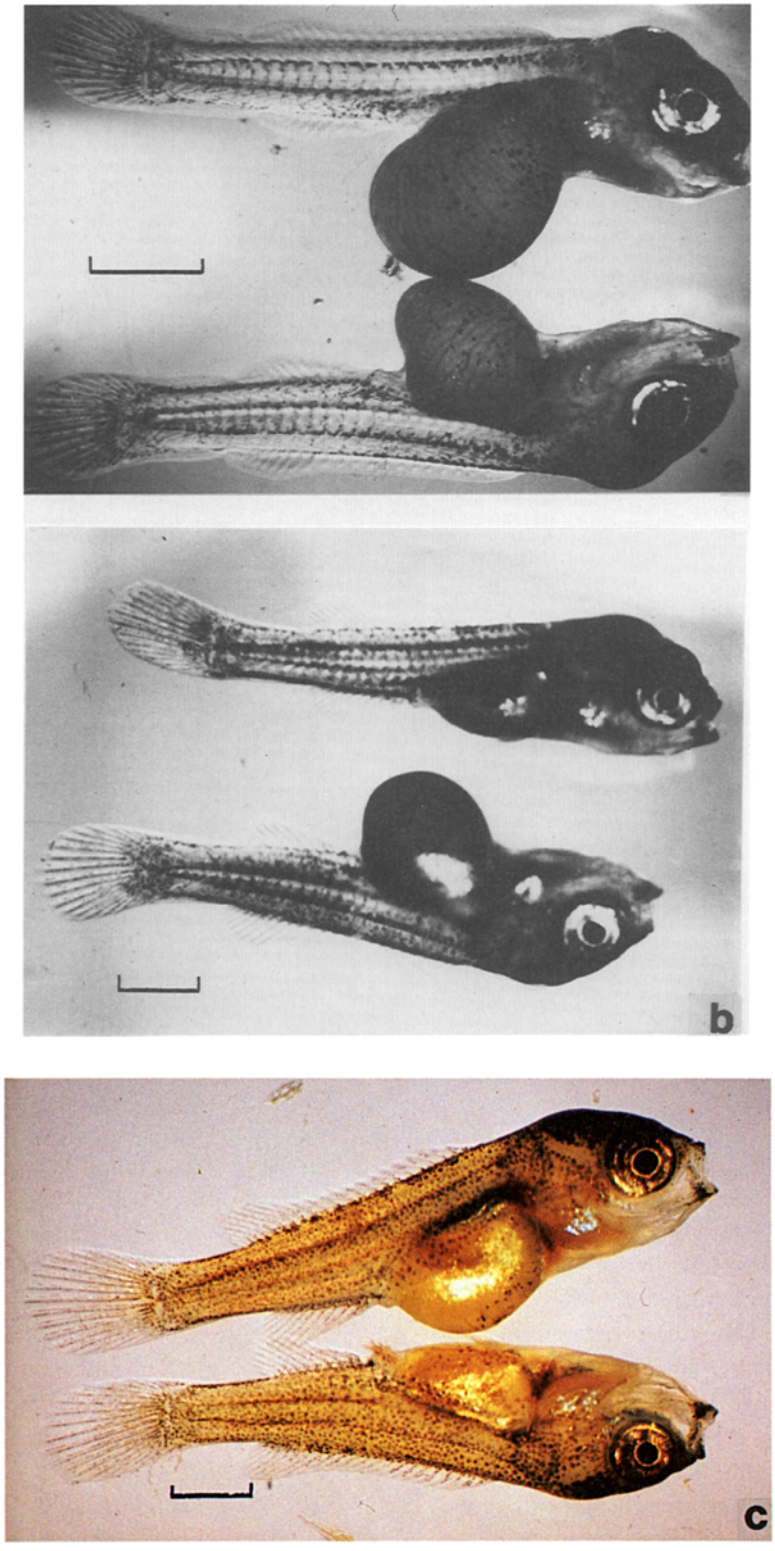

Fig. 8 a – age 10:00; b – age 12:00; c – juvenile at age 14:00. Note the variation in the size of the yolksac and the rapid decline in yolksac size over time. Scales = 1 mm.

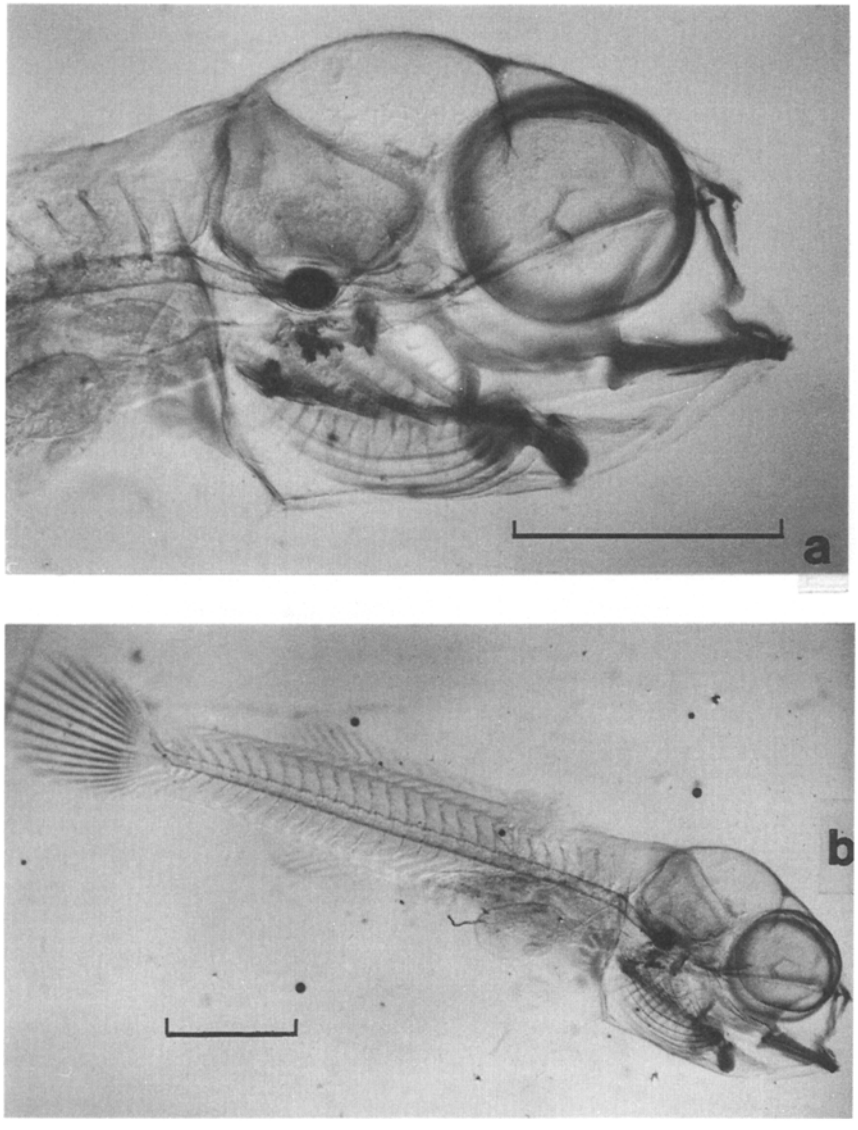

Fig. 9. Skeletal development at age 11:00. This age is coincidental with first exogenous feeding and release, and illustrates the level of development of the structures associated with feeding (a) and locomotion (b). Scales = 1 mm.

the palatoquadrate, the cleithrum, the parasphenoid, the rim of the preopercle and the sagittal otoliths. Slight alizarin red-S uptake occurs along the margins of the central notochord and the urostyle. Vertical lines in the central regions of the notochord indicate the beginning of vertebral development. One epural and uroneural are present. Spinal formation on the haemal and neural arches begins. The pectoral fins are fan-shaped with scalloped margins. There are eight, six and 19 anal, pectoral and dorsal actinotrichia, respectively. Median fin proximal pterygiophores are barely visible. The central lepidotrichia in the caudal fin have three segments. Peristalsis begins. There is a yellow substance in the hindgut and a thin, transparent exudate extends out of the open lumen of the gastrointestinal tract. Over the next 24 h several of the specimens had this colourless exudate and at ages 10:03 (TU = 253) and 10:22 (TU = 273) dark particulate material was found in the exudate of two embryos. Thus, first exogenous feeding begins between ages 10:00 and 11:00.

At age 11:00 (TU = 275) iridocytes are present on the gill covers and the descending body wall (Fig. 8b), and the embryos take a pale blue/green colouration. All that remains of the median finfolds is a narrow fold between the caudal fin and the presumptive dorsal and anal fins. Pelvic fin anlagen form along the flanks of the descending body wall. From this age onwards individual variation becomes more apparent and there are marked differences in the degree of yolksac enclosure (Fig. 8b–c).

By 12:00 (TU = 300) there are two radial loops of vessels along some of the caudal lepidotrichia. Marked increases in calcification occur at this time. The premaxilla and the maxilla are joined at their dorsal ends by a calcifying plate-like structure. Dechondrification occurs in the central areas of the hyo-symplectic and the palatoquadrate. Calcification begins along the margins of these two structures, in the central regions of the ceratohyals and in the urohyals, the hypohyals, the pharyngeal bones and the pharyngobranchials. In the neurocranial region, alizarin uptake occurs in the bases of the occipital arches, in the parasphenoid up to its junction with the ethmoid plate, the tip of the notochord along the neurocranial floor and in all three otoliths. The vomer is rough and ridged on its ventral surface. Along the notochord the first and the seventeen posterior-most vertebrae, the first two neural arches, the posterior neural/haemal arches and the uroneural are all calcified to some degree. Staining occurs along the dorsal rim of the preopercle where three pores begin forming. All neural and haemal spines are present. There are three hypurals that retain some alizarin and a parhypural is present. The pelvic basipterygium appears as an undifferentiated blue line. The pectoral lepidotrichia begin segmenting. Figure 9 illustrates overall skeletal development of a specimen at age 11:00. All but one embryo congregated at the bottom of the separating funnel with their heads oriented towards the inflow of the water. Some embryos have large, bulbous, highly vascularized yolksacs. In others a remnant of the yolksac with a few blood vessels is visible ventrally through a narrow gap between the descending body walls (Fig. 8b).

By age 13:01 (TU = 362), finfold differentiation is almost complete and the embryos begin to resemble juveniles. In most specimens the yolksac does not extend below the ventral contour and what little remains of the yolksac can be seen through a narrow gap on the ventral surface of the embryo. Blood elements in fine vessels flow on the yolksac remnant. Melanophores occur along the pectoral and dorsal lepidotrichia. Many of the embryos are free-swimming in the water column of the funnel and some make their way into the cups at the end of the outflow tubes.

Juvenile period 14:00 +

By 14:00 (TU = 350) melanophores form along the anal lepidotrichia and pink chromatophores appear. All the vertebrae are distinguishable as individual units and fin differentiation is complete (Fig. 10). Calcification begins in the orbital commissures, the vomer, the postcleithra and in the dorsal, anal and pectoral lepidotrichia. Anterior distal pterygiophores begin forming in both median fins. The pelvic basipterygium begins differentiating and there are four pelvic actinotrichia. Chondrification begins on the neurocranial roof. Some dorsal and anal lepidotrichia have two and three segments, respectively, and the caudal lepidotrichia have up to five segments. The full complement of median lepidotrichia and spines is reached (D XIV-10, A III-8). Midlateral neuromasts extend anteriorly along the caudal peduncle to the posterior of the yolksac.

By 15:21 (TU = 397) neuromasts extend to the top of the head and scales begin to form on the caudal peduncle. The outer walls of the otic capsules, the roof of the neurocranium, the pterygiophores and the pelvic lepidotrichia begin calcifying. By age 17:00 (TU = 425) circulation begins in the median fins and, within 24 h, at least two and up to four rows of scales form along the caudal peduncle. By this time, even though the yolksac does not protrude ventrally, there is still a gap between the descending body walls of some specimens. A female that had been left to incubate her eggs was observed releasing and recalling the young to her buccal cavity 17 to 18 days after spawning.

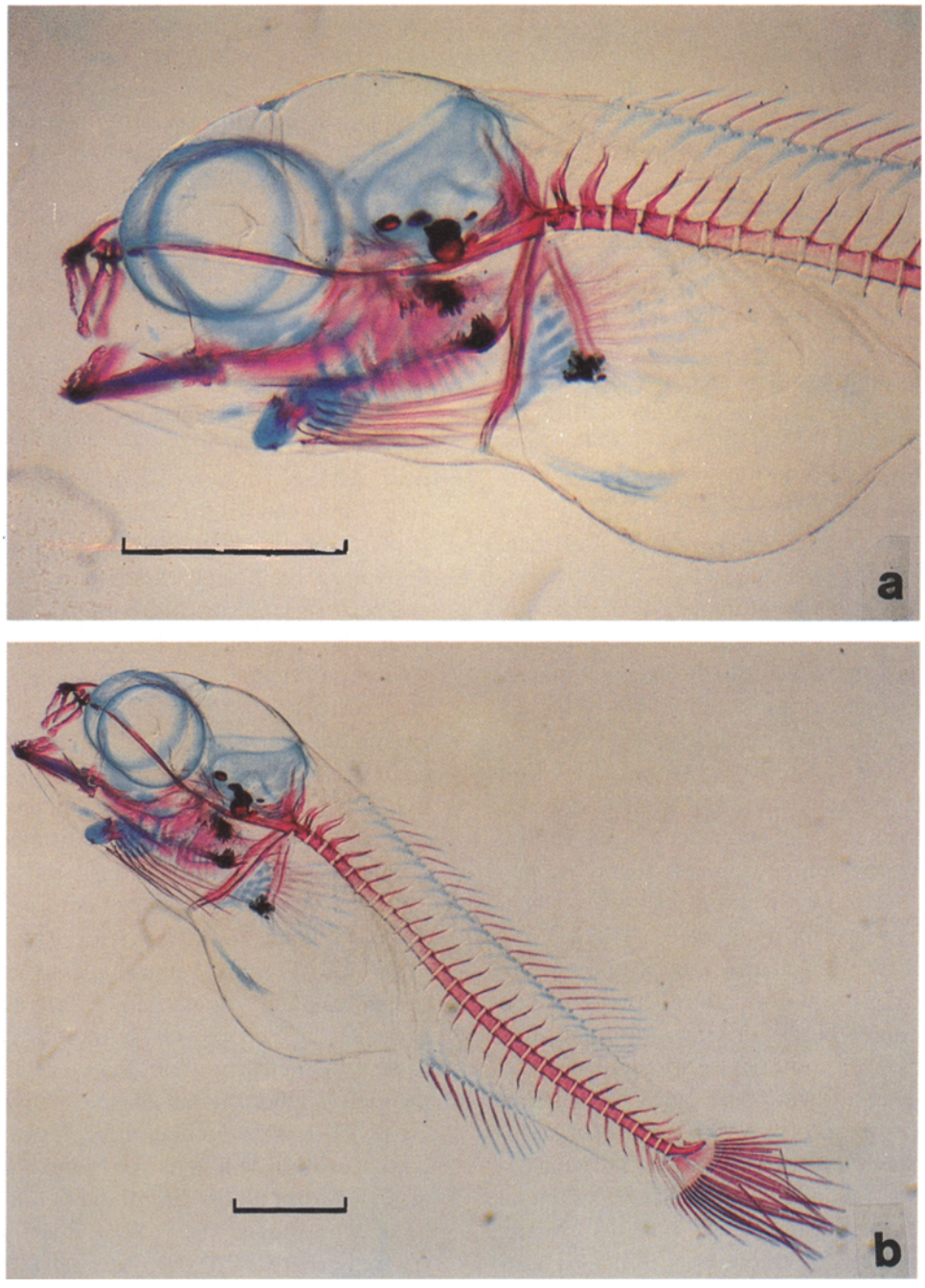

Fig. 10. Juvenile at age 14:00 depicting the advanced state of development of the skeletal system. Note the completion of finfold differentiation and yolksac enclosure, and the vertebrae as distinctive units. Scales = 1 mm.

Heart rate, growth and yolk area

Individual variation in yolk area is great, which may obscure trends over time. Yolk area changes little until halfway through the free-embryo phase (Fig. 11). Embryo length increases gradually over the same period. Yolk area decreases markedly once the embryos begin feeding exogenously but em-

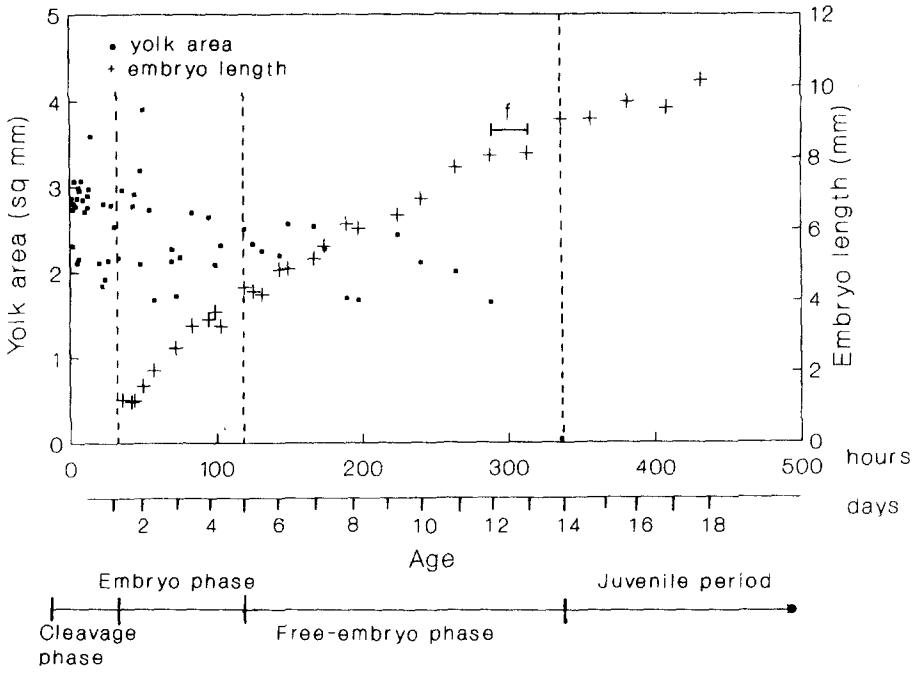

Fig. 11. Changes in embryonic length (mm) and yolk area (mm^2) of *Pseudocrenilabrus philander* over time. Vertical lines represent phase and period boundaries (f = age at first exogenous feeding).

bryonic growth continues more gradually into the juvenile period.

During the embryo period, no pattern in heart rate over age is apparent. Heart rate increases gradually and peaks at age 05:05 (Fig. 12), which coincides with hatching and a notable increase in circulatory and respiratory structures. A subsequent marked drop coincides with a reduction in the post-anal finfold respiratory plexus and an increase in the vitelline respiratory plexus (at age 05:23). This is

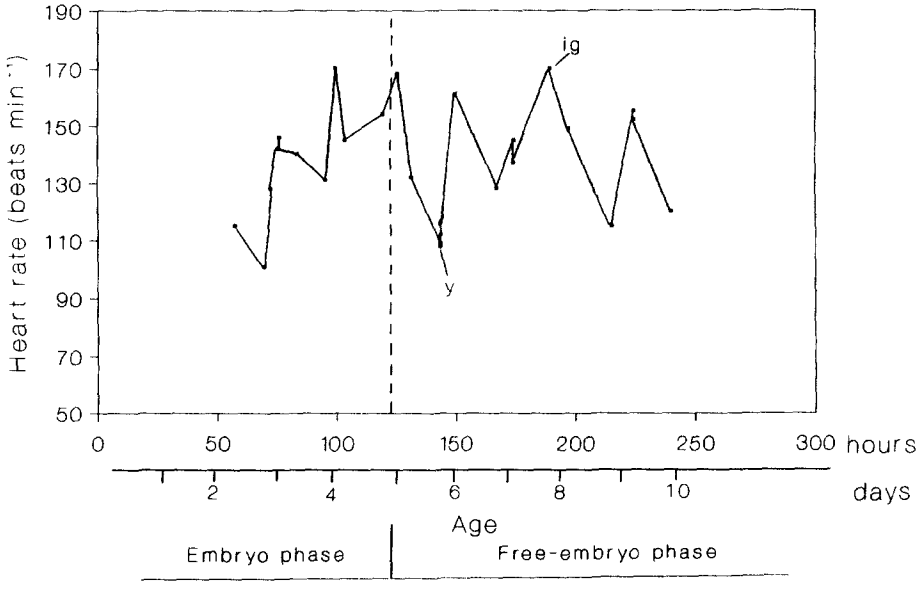

Fig. 12. Heart rate of *Pseudocrenilabrus philander* in the embryo and free-embryo phases. The vertical line represents the boundary between the embryo and free-embryo phase (ig = inferior caudal vein gone, y = maximum yolksac respiratory plexus).

followed by a gradual increase in heart rate until a peak is reached at the time that the profundal caudal vein is completed (at age 07:21). The last reading is low at age 10:00, shortly before first exogenous feeding.

Discussion

The ontogeny of fish consists of five distinctive periods; embryo, larval, juvenile, adult and senescent (Balon 1975, 1990). Within the embryo period, further divisions occur whereby the cleavage phase lasts until organogenesis begins, the embryo phase continues until most of the clutch members have hatched, and the free-embryo phase continues until the major source of nutrition is exogenous. In some cases there is a larval period or the fish develops directly into a juvenile. Exogenous feeding may begin during the free-embryo phase (Balon 1990).

P. philander develops from an embryo directly into a juvenile over a period of 14 days. The duration of the phases within the embryo period are about 1 day, 12 h for the cleavage phase, 3 days, 11 h for the embryo phase and 9 days, 1 h for the free-embryo phase. A summary of the timing of the important ontogenetic events and structures is presented in Table 2.

Table 2. The timing of ontogenetic events and structures in the early development of *Pseudocrenilabrus philander.*

Age (days:hours)	Ontogenetic event or structure
Cleavage phase 00:00–01:12	
00:01	bipolar differentiation; perivitelline space formation; hardening of the egg envelope
00:01–00:12	cleavage; periblast formation
00:14	epiboly begins
Embryo phase 01:12–04:23	
01:12	neurulation; organogenesis; presumptive pericardial cavity forms
01:20–02:06	optic and otic vesicles, and heart-tube formation; yolksac and body pigmentation; 17 somite pairs
02:09–03:00	cardiac and muscular contractions; two otoliths per side; formation of eye lenses, three brain components, head blood vessels, anterior and posterior vitelline veins; elongation and separation of tail; cerebellum distinguishable
03:23–04:03	hatching begins; formation of second caudal loop, hepatic/intestinal vitelline respiratory system and pectoral fin anlagen; ventral finfold circulation increases to form a network; eye pigmentation begins
Free-embryo phase 04:23–14:00	
04:23	almost all individuals hatched; head free from yolksac; canal formation in the otic capsules; formation of urostylar vessels, branchial arteries and intersegmental vessels
05:23–06:23	mouth opens; maximum yolksac respiratory plexus and decline in median finfold respiratory plexii; anastomoses of profundal caudal vein begins; formation and vascularization of gill filaments; four heart components distinctive; simple chondrification; iridocytes in eyes and retinal pigmentation; constriction and coiling of gastro-intestinal tract; gall bladder differentiates; jaw movements; caudal lepidotrichia form
07:06–07:21	atrium dorsal to ventricle; pharyngeal teeth present; inferior caudal vein replaced by profundal caudal vein; anastomoses of subclavian vein; formation of spleen, second gill lamellae and some dermal bones; yellow pigmentation; calcification of pharyngeal teeth
09:08	fusion of some neural and haemal arches; calcification of caudal lepidotrichia; formation of caudal and anal actinotrichia
10:00–11:00	blood flow into second lamellae; peristalsis; yellow substance in gastro-intestinal tract; transparent thread exudate; gut lumen open; segmented caudal lepidotrichia; first exogenous feeding; formation of pelvic fin anlagen, vertebral rings and proximal pterygiophores begin
12:00$13:00	calcification of chondroid bone, neurocranial floor, vertebral rings and hypurals begins
Juvenile period 14:00	
14:00	rib formation; segmentation of dorsal/anal lepidotrichia; finfold differentiation complete; vertebral rings distinctive as separate units; yolksac enclosure almost complete; full complement of median fin lepidotrichia; formation of distal pterygiophores; calcification of median and pectoral fin lepidotrichia
15:21	squamation; calcification in pelvic fins begins

During the cleavage phase, epiboly begins at age 00:14 and the embryonic shield forms. Neurulation and organogenesis mark the beginning of the embryo phase at age 01:12 until most of the embryos have hatched at age 04:23. During this phase, the basic body form from which rudimentary organ systems develop is established. The nervous system and the brain are relatively well developed while sensory organs (except for the eyes) are simple. The development of the temporary embryonic respiratory system is a major morphological event during this phase coupled with the inundation of blood vessels throughout the body, and the beginning of cardiac contractions. During the first portion of the free-embryo phase, permanent adult respiratory organs begin to develop and increase in complexity as the embryonic finfold plexuses decline. In the circulatory system, there is a marked increase in the vascularization of the entire embryo and differentiation of the heart-tube. The organs of the gastrointestinal tract begin to differentiate and coil. Basic skeletal development occurs, particularly in the suspensorium, the hyoid arches and the pectoral girdle. By the end of this phase, temporary embryonic respiratory structures are replaced by the permanent adult structures. Although the ventral gap between the opposing sides of the descending body walls is not completely closed, a residual yolksac lies within the visceral cavity. Except for the gonads, all the adult structures are present. There is an interval of mixed feeding from ages 10:00 to 11:00 until age 14:00. During this time a rapid change from predominantly endogenous to almost exclusively exogenous feeding occurs. The free-swimming abilities of the embryos indicate that skeletal components required for locomotion and feeding are functional. Further differentiation and calcification of the skeletal system continues into the juvenile period.

Evans (in Ribbink 1975) found that allopatric populations of *P. philander* differed in egg size and size of newly released young. The mean maximum and minimum lengths of eggs from the Durban population in her study were 2.5 and 1.8 mm, respectively. The adult fish in this study were from a Durban population and the mean egg lengths from two clutches were 2.2 and 2.5 mm by 1.6 and 1.7 mm. Embryos in the latest stages of development, when yolksac absorption neared completion, measured 8.95 mm [as in the Durban population of Evans (Ribbink 1975)]. Lengths of embryos at a similar level of development were 7.7–9.1 mm in this study.

The pattern of development and the timing of important ontogenetic events clearly reflect the environmental conditions experienced by the young southern mouthbrooder. The development of the extensive, temporary, respiratory plexuses on both the ventral finfold and the yolksac augments gaseous exchange within the buccal cavity of the female where oxygen levels are presumably low (Fig. 4) (Balon 1977). Flapping of the pectoral fins, which develop early and rapidly, creates a water current over the vitelline plexus, thus facilitating respiration (Fishelson 1966). Prior to the time of first release, between 11 and 14 days at 25° C (Ribbink 1971), the temporary respiratory structures on the ventral finfold are no longer present and the switchover to the permanent adult branchial system has occurred (Fig. 7). Higher oxygen concentrations outside the buccal cavity probably reduce the necessity for extensive additional respiratory structures. The vitelline plexus would supplement oxygen uptake should there be a deficiency. However, as there is a rapid decrease in the size of the yolksac between ages 11:00 and 14:00, and variation in yolk sac size is large, its role in gaseous exchange is probably minimal (Fig. 8). In addition, the time of first release is variable.

During the interval of mixed feeding the yolk is primarily a supplemental source of nutrition. Peristaltic movements and the expulsion of fluid through the vent just prior to release indicate that the digestive system is functional and capable of processing an exogenous food source. The remaining yolk may assure some nutritional intake (Balon 1977) while the young fish improve their food-gathering abilities.

The problem of predation pressure on the young after release, although not a consideration during the incubation period, is resolved by several characteristics of early ontogeny. The level of development of the skeletal and sensory systems is sufficient for avoiding predators by hiding or returning to the buccal cavity of the female. Pigmentation over the entire body may provide disruptive colou-

ration in the vegetated areas where the young are released (Fig. 8, 9). By the time the female ceases guarding, the young have reached a juvenile state and the skeletal system is well developed (Fig. 8c, 10).

Life-history traits also reflect the co-evolution of this species with its habitat. The carotenoid content of the yolk, and the churning of the eggs and embryos within the buccal cavity, increase oxygen utilization potential (see Balon 1991 for information about the oxidative role of carotenoids). Mouthbrooding, recall behaviour and schooling of the young fish are traits which reduce predation risk. The mobility of the brooding female allows movement into areas of optimal or preferred habitat conditions.

The rate and pattern of development of the Mozambique tilapia *Oreochromis mossambicus* (Holden & Bruton 1992) and *P. philander* are similar. The differences in the duration of the cleavage and embryo phases are 1 h and 6 h, respectively. In the free-embryo phase there is a difference in duration of 1 day, 7 hours. Hatching begins 8 h earlier in *O. mossambicus* but, with both species, continues for a period of about 24 h. Completion of the development of the digestive system followed by first exogenous feeding occurs between the ages of 10:00 and 11:00 for both species. The interval of mixed feeding and the beginning of the juvenile period differ by 1 day (age 14:00 for *P. philander* and 15:00 for *O. mossambicus*). The differences mentioned may be attributed to differing sampling times, small sample sizes (in most cases only one individual), subjectivity about the timing of events, and individual variation. Closer examination of the early development of these two species may prove otherwise, and is the subject of another study.

Several of the life-history characteristics of the two species are similar. Males build concave nests in arenas by excavating depressions in the river or lake bottom to which females are attracted for spawning (Ribbink 1971, 1975, Bruton & Boltt 1975, Bruton 1979, Trewavas 1983). Immediately after spawning, the female leaves the nest and the male attempts to spawn with other females. Females school together while brooding and the young are released in vegetated, warm shallow water (or in the case of *P. philander*, the young may also be released into deeper water; Ribbink 1975). In Lake Sibaya, young *O. mossambicus* may be released over totally barren sandy terraces (Bruton & Boltt 1975, Bruton 1979). The females continue to guard and recall the young to their buccal cavity for several days.

Several life-history traits are not shared by the two species. *P. philander* adults are smaller, the males build smaller nests, and the females produce larger eggs relative to body size, mature at smaller sizes and have smaller clutches (Ribbink 1971, 1975, Loiselle 1982, Trewavas 1983). Overlapping life-history characteristics are incubation time, interbrood time, age at maturation and size of newly released young. Since many of these traits are dependent on environmental conditions (e.g. temperature and food availability), and both these species are phenotypically plastic, it is difficult to determine the significance or the magnitude of these differences and their influence on early development.

Both species exhibit direct development with accelerated exogenous feeding into the embryonic period (Balon 1990, Holden & Bruton 1992). The southern mouthbrooder, like the Mozambique tilapia, belongs to the reproductive guild of mouthbrooders without buccal feeding (Balon 1990). It is therefore not surprising that their early development is similar, despite the phylogenetic differences between the tilapiine and haplochromine cichlids. The similar environmental conditions which the eggs and embryos are subjected to may have resulted in both species adopting the same eco-ethological and eco-morphological developmental styles (see Holden & Bruton 1992).

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