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EMBRYONIC AND LARVAL DEVELOPMENT OF AN ENDEMIC LAKE TANA BARB, *LABEOBARBUS BREVICEPHALUS* (TELEOSTEI: CYPRINIDAE)

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ABSTRACT: The present study is a description of the embryonic and post-embryonic development of Lake Tana endemic barb species, *Labeobarbus brevicephalus*, from fertilization until active feeding. Bouin's solution immersed and unfertilized egg diameter ranged from 1.63 to 1.93 mm. The first cleavage occurred within 60-minute post-fertilization at a temperature of 23.0–25.8°C. Yolk invasion was completed by gradual spreading over the germ layer at 20 h. At about 43 h, the embryo occupied more than ¾ of the egg peripheral space. The heart was beating actively and the blood circulation was visible. Yolk sac was restricted to the head region, embryos became active and exhibited continuous twitching movement. Hatching was synchronous, started 58 h + 30 min post-fertilization and was completed within 65 h at the same temperature range. Average total length of newly hatched larvae was 5.06 ± 0.16 mm (mean ± SE, n = 5). The overall embryonic and post-embryonic development of Lake Tana *L. brevicephalus* were completed in eight days time. By this time post-fertilization larva began active feeding and had well developed cartilaginous neurocranium, most of the bony elements in splanchnocranium, pigmentation on the head, eyes and body, cartilaginous pectoral fin and pectoral fin girdle with single bony element cleithrum, reduced fin fold, developed cartilaginous support and several ossified fin rays in unpaired fins. The information obtained from this study will be useful to cover gaps in early life history, first feeding and ontogeny of *L. brevicephalus* for its conservation and restoration.

Key words/phrases: Conservation, *Labeobarbus brevicephalus*, Lake Tana, Ontogeny, Post-fertilization, Yolk invasion.

INTRODUCTION

Labeobarbus brevicephalus sensu (Nagelkerke and Sibbing, 2000) (Teleostei; Cyprinidae) is one of the endemic large African hexaploid barb species composing Lake Tana barb species flock. It is the smallest maturing species in Lake Tana with size at maturity ranging from 18.8 cm to 25 cm FL (Nagelkerke and Sibbing, 2000). *L. brevicephalus* was by far the most common *Labeobarbus* species in Lake Tana. It was restricted to the sub-

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littoral and offshore pelagic areas (de Graaf *et al*., 2008).

According to Nagelkerke (1997), the non-piscivore *L. brevicephalus*'s feeding position is not completely consistent: it was predicted to be close to the benthivore group in food partitioning, but it feeds to a large extent on micro-crustaceans.

L. brevicephalus was the last species to aggregate in the river mouths during the breeding season, with highest densities in September-October. During spawning season, running females and males of the *L. brevicephalus* were abundant almost in all tributaries. Spawning took place on shallow (depth 0.10 m) gravel beds under clear water conditions in the river mouth of Wanzuma and from the river mouth to the waterfall in Ducalit tributaries of Gumara river (de Graaf, 2003).

The fast commercialization of fisheries in the last three decades in Ethiopia in general and Lake Tana, in particular has resulted in overfishing (de Graaf *et al*., 2006; Wassie Anteneh *et al*., 2012). In the case of Lake Tana, it caused a drastic decrease of the natural populations of barbs and shifted the conservation status of the majority of endemic L. Tana barbs to "vulnerable" or "endangered" status (IUCN Red list, http://www.iucnredlist.org/search). *L. brevicephalus* is a small-sized species (max. 25 cm FL) able to escape through the fishing gear and therefore absent in the catches. It therefore did not fall in the category of IUCN list. But the current situation for the larger barbs demanded an immediate development of conservation programs focusing on artificial mass rearing and restoration of Lake Tana large hexaploid barbs species.

A crucial part of conservation and restoration program for any biological species is investigations of its ontogeny. In Lake Tana, there are some studies concerning early ontogenesis for some species (*L. intermedius*: Ryabov, 2002; small large barbs; Alekseyev *et al*., 1996). We believe that the information is scanty and there is a paucity of knowledge on Lake Tana large hexaploid barbs species early life history, first feeding ontogeny and their conservation. So it is worthy to focus the investigation of early developmental stages on *L. brevicephalus* as a representative of L. Tana *Labeobarbus* fish species within the frame of future conservation and restoration programs.

The present study aimed to generate information on early life history, first feeding and ontogeny of *L. brevicephalus*, which can be utilized as a spring board for future rehabilitation efforts to save the endangered *Labeobarbus*

fish species of Lake Tana.

MATERIALS AND METHODS

Experimental station

The research was conducted in the laboratory of the Bahir Dar Fisheries and Aquatic Life Research Centre (BFLARC) in six rectangular glass aquarium free of bottom substrata (V=25 l). The water temperature in the Gumara River was 20.4–21.2°C at night (6–7 a.m.) and 20.1–24.4°C in the day (1–2 p.m.); in its tributary the Ducalit River it was 19.0–20.7°C and 23.0–27.8°C, respectively (Dzerzhinskii *et al*., 2007). The eggs and larvae were incubated under a temperature range of 23–25.8°C that corresponds to its natural fluctuations using an electrical thermo-regulating system (Tetratec HT 100 heaters). Dissolved oxygen was kept at 7–8 mg l^{-1} with continuous aeration and a time-clock was used to impose a light period of 12 h light - 12 h darkness. Glass aquarium in which egg and larvae were incubated maintained constant temperatures by an electrical regulator.

Experimental animals

During the end of the rainy season in October 2016, we caught a small-sized fish from the Gumara River flowing into Lake Tana (Fig. 1). For identification of morphotypes we used descriptions and pictures presented by Nagelkerke *et al*. (1994) and Nagelkerke and Sibbing (2000). According to these authors, morphotypes represented in our experiment are characterized as follows: (maximally 317 mm LF), deep-bodied (BD> HL) species, without a nuchal hump. The head is very short and with a straight to slightly convex dorsal profile.

They were most abundant and spawning in Gumara tributaries of Lake Tana or were ready to spawn. We judged the condition of their gonads and observed the main noticeable sign of the spawning fountain of water and silt with audible splashes produced by the female's tail movements. The spawning took place in gravel riffles beginning around 4:00 p.m.

Eggs and milt were obtained from wild spawners caught by cast-net at spawning sites in Gumara River during the end of the rainy season (October 2016) at dusk (17:21) that corresponds to its breeding period. The fish were selected according to the typical morphological characteristics of sexual maturity, and based on these characteristics, two females and two males with 165–215 mm fork length (LF) and 59–134 g total weight were chosen for artificial reproduction. The females were selected by external characteristics according to the swollen purple abdomen and bigger size (Fig. 2a), and the

males were chosen according to the white colour and high fluidity of milt when the abdominal region was squeezed and smaller in size (Fig. 2b).

Fig. 1. Aerial photography of the sampling sites Gumara and Ducalit River (Source: Google Earth, 2018).

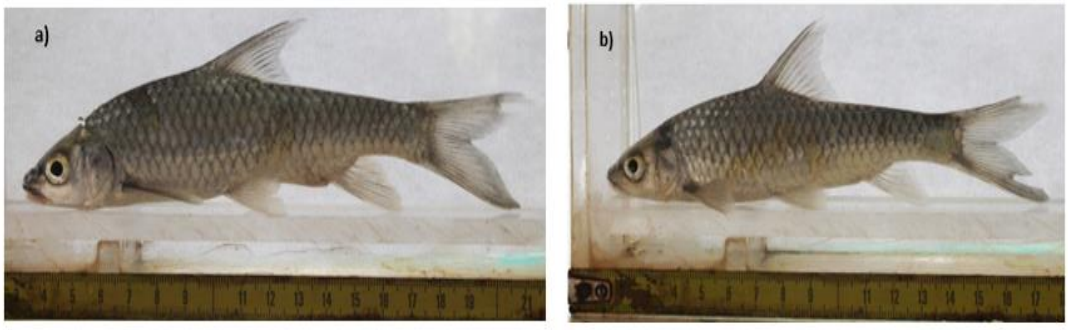

Fig. 2. Lake Tana *Labeobarbus brevicephalus*: (a) Female; (b) Male.

Fertilization

Artificially fertilized (via dry method) eggs from wild spawners were transferred to the indoor aquarium (350–400 eggs per aquarium) and reared under standard conditions as described in Dzerzhinskii *et al*. (2007).

Incubation of eggs and yolk sac larvae

Eggs from each progeny were incubated at the same conditions in a separate aquaria for comparison. Dead eggs were removed every time from each aquarium. After hatching, larvae of each offspring were distributed into three separate aquaria with about 150 larvae per aquaria. Every day, 5–10 l of rearing water was replaced with water taken from Lake Tana filtered and UV-treated within 24 hours.

Characterization of the stages of development

In the present study, the *L. brevicephalus* developmental stages were divided into oocytes, embryonic, post-embryonic, larval and post-larval development. The terminologies used for the developmental stages are based on embryonic developmental studies and are with some modification in line with earlier embryonic developmental studies in order Siluriformes and other fish species' orders (Cardoso *et al*., 1995; Kimmel *et al*., 1995; Olaniyi and Omitogun, 2014). The stages used in this study are explained as follows:

Oocyte stage: A stage of unfertilized matured egg characterized with spherical or ovoid shaped structure and largely filled yolk content. It is not regarded as part of embryonic development stage when not fertilized.

Embryonic stage: The next stage occurs inside the chorion when the oocyte is fertilized and ends in hatching.

Post-embryonic stage: This period of development starts immediately when the larvae hatches out of chorion until the completion of yolk absorption.

Larval stage: A stage characterized by nutritive contributions of the yolk sac and the stage ends when the larva becomes capable of exogenous feeding.

Post-larval stage: A stage characterized by autonomous feeding.

Sampling and staining

Five fertilized oocytes were randomly sampled into Petri dish (n = 500). Sampled eggs were observed every 30 minutes until hatching and after hatching, larvae were sampled twice a day for observation. Oocyte, embryonic and larval developmental stages were studied right from unfertilized matured oocytes to fertilization and hatching with the aid of stereomicroscopes (euromex Holland). After observations, sampled oocytes were immersed in Bouin's fluid for six hours. Six hours later, the eggs were thoroughly washed with a large amount of tap water and 70% ethanol until the yellow colour disappeared. Lastly, eggs were stored in 70% alcohol. The

Bouin's solution used was prepared as follows: Picric acid, saturated aqueous solution - 75 ml; Formalin, 40% aqueous solution - 25 ml; Acetic acid, glacial - 5 ml (Carson and Hladik, 2009). Larvae specimens were fixed in 10% neutral formalin solution and stained for skeletal structures in Alizarin red and Alcian blue (Webb and Byrd, 1994).

Image capture and analysis

Embryonic and larval development were observed by taking digital images of live and fixed samples. Bouin's fluid fixed embryos were carefully removed after tearing the egg shells due to the opaqueness of the membrane enclosing the egg. Picture was taken by stereomicroscopes (euromex Holland) with an attached digital camera Canon EOS 1100D (72 dpi resolution) and analyzed with Canon Digital Photo Professional Software (Version 3.10.0.0). The Lidocaine injection B.P. Lox*2% (NEON) was used to immobilize the embryos and larva. Measurements of average egg diameter between larger and smaller were done by ocular micrometer (40x magnification) before fixation. Larval total length (TL - length from the tip of snout to the end of the caudal fin; Crawford, 1986) were measured by Matrix electronic digital calipers (precision of measurements = 0.01 mm). The average mean TL was calculated and recorded. Data were statistically analyzed by using statistical analysis software IBM SPSS Statistics v22. Data of eggs from each progeny were compared using Student t-test with $\alpha = 0.05$.

RESULTS

There was no significant difference ($p>0.05$) among the progenies from the two pairs of reproducers in development rate ($n = 500$). The post-larval stage begins immediately upon absorption of the yolk sac and was characterized by autonomous feeding. Summarized embryonic development stages are presented in Table 1.

Table 1. Brief descriptions of the embryonic developmental stages of Lake Tana *Labeobarbus brevicephalus*.

Developmental stage	Phase	Time after fertilization (min)	Main events
I	Unfertilized oocyte	0	Single, opaque, demersal, spherical and yellowish in colour (Fig. 3A)
II	Fertilized egg	30	Egg transparent, concentration of yolk at the centre, with increase in perivitelline space (Fig. 3B and C)
III	Animal and vegetal poles	60	Formation of embryonic disc; distinct vegetal pole; pigmentation of animal pole (Fig. 3D*)
IV	2 cell stage	90	First mitotic cleavage, meroblastic division into two equal size blastomeres (Fig. 3E)
V	4 cell stage	120	Second mitotic cleavage, 2 × 2 array of blastomeres (Fig. 3F)

Developmental stage	Phase	Time after fertilization (min)	Main events
VI	8 cell stage	148	Third mitotic division, resulting into two parallel rows of four blastomeres each, 2 × 4 array (Fig. 3G)
VII	16 cell stage	173	Fourth cleavage, formation of sixteen blastomeres; 4 × 4 array (Fig. 3H*)
VIII	32 cell stage	202	Fifth cleavage, formation of 32 irregularly arranged blastomeres (Fig. 3I*)
IX	Early morula stage	262	Cleavage stage where blastomeres continue to divide synchronously and form a mulberry-like cluster (morula) at animal pole resulting into 64 blastomeres (Fig. 3J)
X	Late morula stage	294	Further cell divisions led to numerous uncountable blastomeres. The blastodermal cells were smaller than the previous stage (Fig. 3K)
XI	Early blastula	420	The cleavage resulted into numerous blastomeres and the blastoderm was still in high (thick) stage (Fig. 3L)
XII	Late blastula	480	Spherical shape, flat border between blastodisc and yolk (Fig. 3M)
XIII	Pre-early gastrula stage	535	Dome shaped structure (Fig. 3N)
XIV	30% epiboly†	690	The brownish thickened margin of blastoderm covered the yolk (about ¼ of the yolk sphere) (Fig. 3O)
XV	Mid gastrula	840	Distinct germinal ring occupied a large part of the yolk (Fig. 3P)
XVI	Late gastrula	990	Blastoderm covered more than 80 percent of the yolk, embryonic shield at the animal pole and optic rudiment were clearly visible. Head and tail rudiment visible (Fig. 3Q)
XVII	Neurula	1200	Notochord became visible, 7 somites appeared, auditory and optic bud developed, a body formed into C-shape. The yolk was attached between tail and head (Fig. 3R)
XVIII	Somite	2010	Brain and spherical optic lens formation. Embryo started occasional movement Start of heart beating) (Fig. 3T)
XIX	Tail-free stage	2580	Embryo occupied more than ¾ of the egg peripheral space. The heart was beating actively and the blood circulation was visible. Otic vesicles with the early emergence of two (2) tiny otoliths, yolk sac was restricted to head region, embryo became active and exhibited continuous twitching movement (Fig. 3U)
XX	30 minutes before hatching	3000	Embryo continuously beat the eggshell vigorously by the caudal region especially around the middle part of the body (Fig. 3X)
XXI	Hatching	3030	Embryo ruptured the egg shell, larva emerged out (Fig. 4D)

* Bouin's fluid immersed larvae picture

†Epiboly is a cell movement that occurs in the early embryo, at the same time as gastrulation.

Live unfertilized eggs of *L. brevicephalus* are demersal, spherical, adhesive, with a smooth, clear and spherical chorion, and with yellow homogeneous yolk. Unfertilized eggs had a diameter range of 1.63 mm to 1.93 mm. Immediately after fertilization and contact with water, egg diameter increased up to 2.50–3.03 mm. The egg size drastically increased until late morula, and then it remained equal in size until ¼th of the yolk sphere were covered by a brownish thickened margin of blastoderm (Fig. 3k). A decrease in egg diameter was observed during mid and late gastrula stages which gradually increased and became more or less uniform until hatching (50 h + 30 min) (Fig. 7).

Yolk invasion was completed by gradually spreading over the germ layer at 18 h after fertilization (Fig. 3l). The notochord could be clearly seen 20.5 hours after fertilization, with four identifiable somites (Fig. 4c), auditory and optics bud formation being almost complete. Cephalic to caudally progressive increase of somites block in the embryo resulted in maximally in 32 somite blocks at 30 h (Fig. 4e). During late gastrula stage rudimentary head and tail were formed and differentiated from the yolk (Fig. 3k).

Twenty eight hours after fertilization, the caudal region detached from the yolk mass and became free. The embryo exhibited bulb-like shape with bigger head and short stout tail. By this time, the embryo showed slow wriggling movement restricted to the head region by stretching the somite block (12 wriggling/minutes). As the time elapsed, the wriggling movement increased to 21 wriggling/minute with synchronized wriggling movement of head and tail region which was accomplished at 29 h. At 30 h the embryo did 360° anti-clockwise rotational movement in 52 seconds inside the chorion (Fig. 4e).

As the embryonic development was completed, the growing embryo occupied the entire previtelline space; it exhibited frequently vigorous twitching movement by lashing the tail against the chorion. The twitches and lashings became progressive, stronger and frequent. These facilitated the hatching. The heart beat frantically at an average rate of 85 beats/min (Fig. 4f).

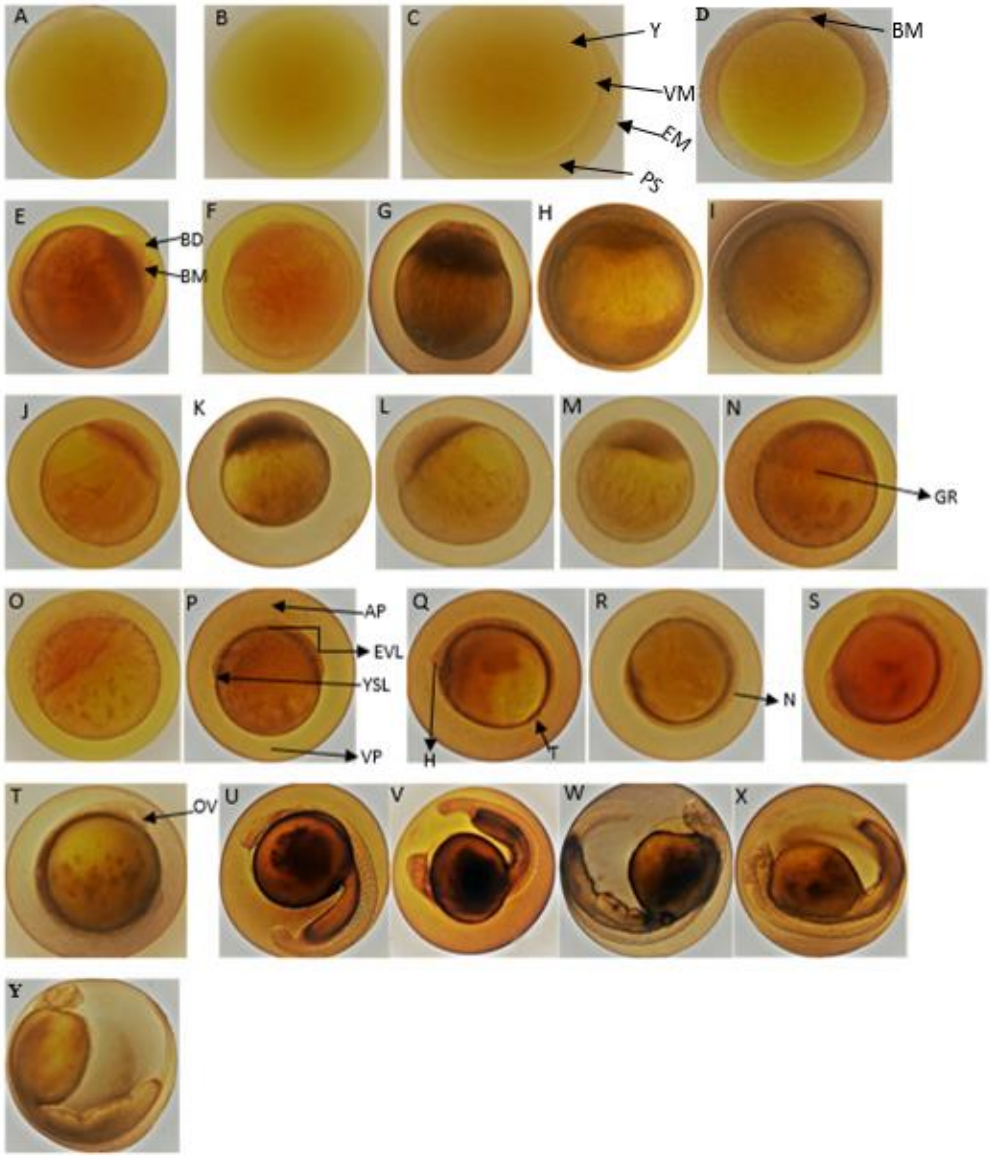

Fig. 3. Early stages of development of Lake Tana *L. brevicephalus* (from cleavage to the formation of embryo's body (2B-Y)). (A) unfertilized egg, (B) Fertilized egg, (C) Fertilized egg (30 min), (D) Animal and vegetal poles (1 h), Cleavage: (E) 2 blastomeres (1 h 30 min, 23.8°C); (F) 4 blastomeres (2 h, 24.1°C); (G) 8 blastomeres (2 h 28 min, 24.8°C); (H) 16 blastomeres (2 h 53 min, 25.0°C); (I) 32 blastomeres (3 h, 22 min. 25.8°C); (J) Early morula stage (4 h 22 min. 23.8°C); (K) Late morula stage (4 h 54 min, 24.1°C); (L) Early blastula (7 h, 23.8°C); (M) Late blastula (8 h, 24.1°C); (N) Pre-early gastrula stage (8 h 55 min, 25.8°C); (O) 30% epiboly (11 h 30 min, 25.8°C); (P) Mid gastrula (14 h, 25.8°C); (Q) Late gastrula (16 h 30 min, 25.8°C); (R) Neurula (20 h, 25.8°C); (S) Embryo with 5 septum (23 h, 25.8°C); (T) Somite (33 h 30 min, 25.8°C); (U) Tail-free stage (43 h, 25.8°C); (V) Embryo with 35 somite blocks (43 h 30 min, 25.8°C);

(W) 1 hour before hatching (49 h 30 min, 25.8°C); (X) 30 minute before hatching (25.8°C) and (Y) Last minute hatching (50 h 29 min, 25.8°C).

(EM - Egg membrane, VM - Vitelline membrane, PS - Perivitelline space, Y - Yolk, BD - Blastoderm, BM - Blastomere, GR - Germinal ring, AP - Animal pole, EVL - Enveloping layer, VP - Vegetal pole, YSL - Yolk syncytial layer, H - Head portion, T - Tail portion, YS - Yolk sac, N - notochord, OV - otic vesicle). Magnification: ×40.

After a few seconds pause, this movement suddenly culminated in a violent jerk where the fully developed embryo ruptured the eggshell, and the hatchling emerged, tail first (Fig. 4g). Hatching occurred 50 h + 30 min - 58 hours after fertilization at water temperatures 25–26°C. Newly hatched larvae were transparent, straight, elongated and gradually flattened towards the tail with tapering tail tip inside the membranous sheath and characterized by the presence of an almost round yolk sac (Fig. 5a). The total length of newly hatched larvae were 3.3 mm (3.1–3.7 mm; S.D. = 0.2; n = 5). The hatchlings had unpigmented eyes, closed mouth, and tiny head. The oval-shaped yolk sac (approximately 40% of the total body length) was yellowish-brown (Fig. 5a). The numerous myomeres at the dorsal region gave it a bright appearance. The newly hatched larvae could not swim and lay at the bottom of the glass aquarium and tried to hide in any refuge they could find, usually beneath the remnants of the chorionic membrane. Within 58 h approximately 40% of the larvae had hatched, whereas 50% of them hatched after 64 h. After 2.4 days of fertilization, 90% had hatched and the larvae gathered in clusters on the substratum, and periodically underwent rapid tail movements, which moved them haphazardly on the aquarium bottom. After 4.2 days of fertilization larvae possessed darker colour and they were clustered together on the glass floor, with their anterior section of heads adhering to each other or individually adhering to the sides of the aquarium. The larvae adhered to other larvae, dead eggs or the edges of the aquarium glue. The larvae were at every instant agitated and separated from a cluster, they moved rapidly around on the glass floor until they adhered to a cluster of larvae again.

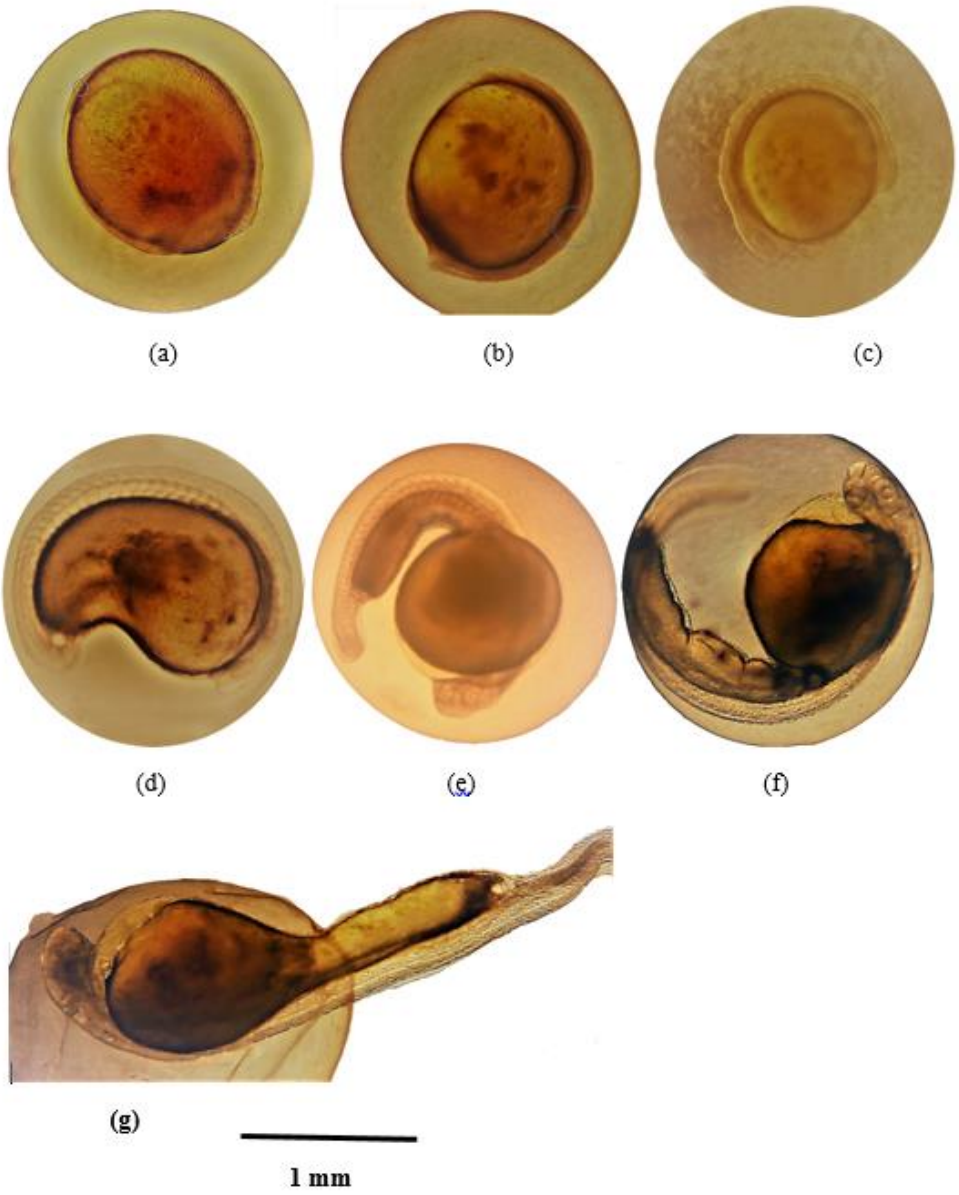

Fig. 4. Development of embryos of Lake Tana *L. brevicephalus* from the beginning of segmentation to hatching: (a) Beginning of mesoderm segmentation (17 h, 25.8°C); (b) 2 trunk somites (18.5 h, 24.1°C); (c) 4 trunk somites (20.5 h, 23.8°C); (d) 20 trunk somites (1 day 2 h 25.8°C); (e) 32 trunk somites (1 day 6 h, 25.8°C); (f) 3 h embryo just before hatching; (g) Tail first hatchling.

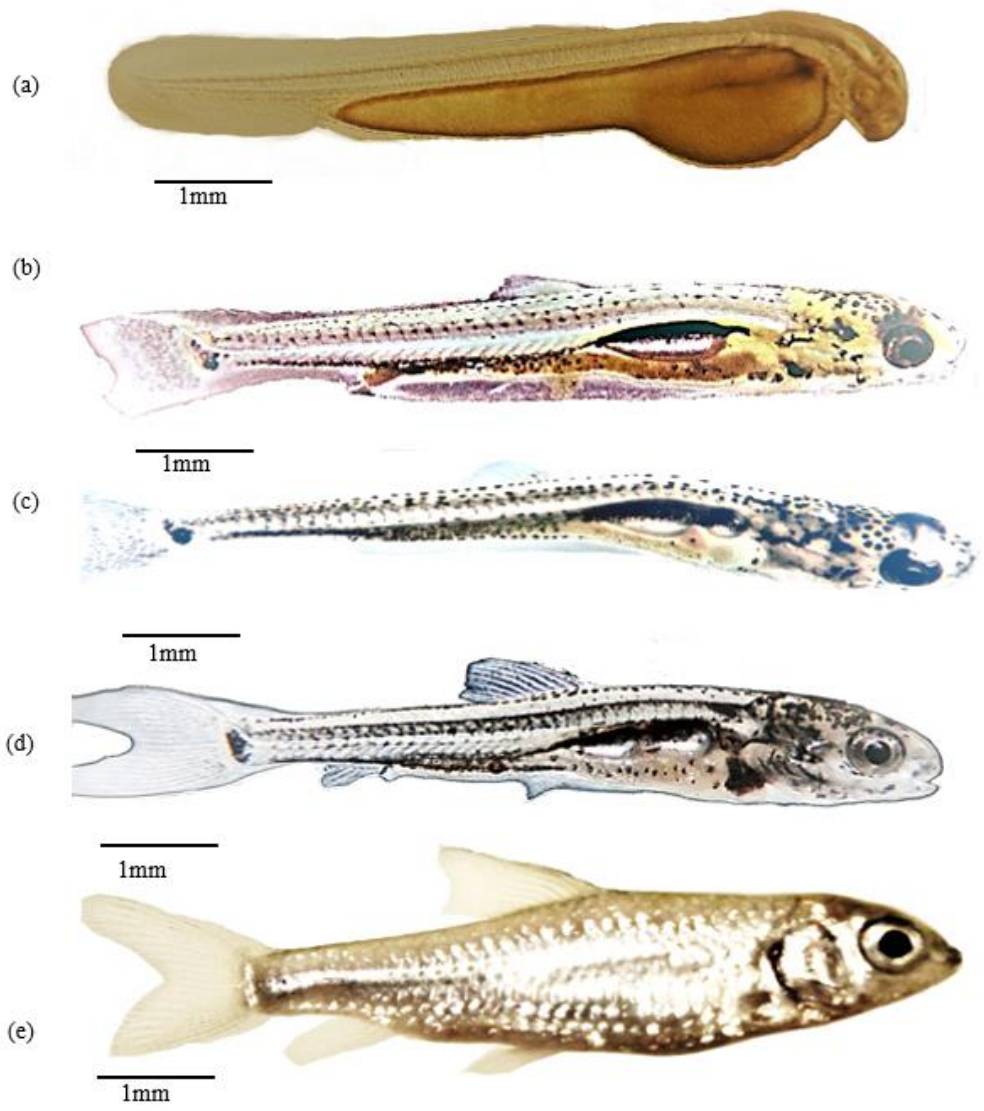

Fig. 5. The early developmental stages of Lake Tana *L. brevicephalus* at 25–26°C: (a) Newly hatched larva (age 2 days 2.5 h TL 3.3 mm; (b) 10 days larvae TL 11.4 mm; (c) 26 days larvae TL 12.3 mm; (d) 35 days larvae TL 14.8 mm; (e) 156 days larvae TL 47.2 mm.

They breathe by absorbing oxygen through the fine blood capillaries that surround the yolk sac still attached to the gut. From the head and tail region deoxygenated blood element is directed to the vessel network on the frontal part of yolk sac in v-shaped and channelled to lower part of the yolk. On the process gas exchange took place and oxygenated blood element enters in the

pouch between the yolk and its investing epidermal layer through three branched tiny capillaries and ended into the lower part of the heart and pumped.

After 2 days of hatching, remarkable developmental changes were seen on the larvae. These are: emergence of tiny knots of growing barbells, appearance of clearly shining and reflective iris (no more epicanthus covering the eyes), pectoral fin development, formation of five-gill arches with well-developed upper and lower jaw but the mouth was still kept closed.

The translucent nature of the larvae was reduced to slightly opaque. Circulation of pigmented blood could still be clearly seen within the body depth as it was interflowing inside the range of the caudal fins. Development of full functional upper and lower part differentiated pumping heart was attained. As development progressed, operculum was seen flapping the branchial arch of the larvae, mouth opened and the alimentary system became well developed by the third day.

Concerning pigmentation about 12–15 sparse tiny small, round, melanophores were noticed on the anterior part of the head and along the length of the intestinal tract spreading caudally. Pigmentation were more pronounced and condensed on the body parts as development continued. On day 4 after hatching, occurrence of nostrils, differentiation of dorsal fin, anal opening and three caudal fin rays occurred at ca. 9.2 mm TL. A few larvae 'floated' after 4.4 days of hatching, and hung vertically from the surface of the water. Some larvae immediately swam towards the surface. They avoided sinking by swimming actively and then dropped slowly.

The hatched larva completed most of its morphogenesis, and continued to grow rapidly ca. 9.6 mm TL (5 days). Addition of caudal fin rays, occurrence of pseudo-ribs (38 upper and 28 lower) and inflation of the posterior swim bladder chamber were prominent changes during this period. After 5 days of hatching, all the larvae were seen actively swimming on the upper surface and the alimentary canal was well developed and exogenous feeding was first noted with pellets of excreta. At a week old larvae the incipient dorsal fin margin partially differentiated and nineteen fin rays were readily visible on paddle-shaped caudal fin fold (10.2 mm TL). Incipient anal fin margin partially differentiated 10.3–11.4 mm TL, ca. 8 days after hatching) and completely differentiated (11.7–12.1 mm TL, ca. 13 days after hatching).

Anterior swim bladder chamber and caudal fin fork formation started at ca. 11.4 mm TL (10 days after hatching) (Fig. 6b) and caudal fin fork formation was completed with 20 branched soft rays on day 13 after hatching. Dorsal fin rays commenced branching on the eleventh day with formation of four fin rays originated over myomeres. Incipient anal fin margin partially differentiated at ca. 12.9 mm TL (23 days after hatching) and completely differentiated at 12.9–13.7 mm TL (ca. 27 days after hatching). Anal and dorsal fin rays commenced branching at 13.7–14.1 mm TL (ca. 32 days after hatching); pelvic buds formed with four soft rays anterior to dorsal fin origin at ca. 14.8 mm (35 days after hatching) (Fig. 6d). Pelvic rays fully developed at ca. 16.1–18.7 mm TL (46 days after hatching); gut commences S-shaping at 12.2 mm TL; entire fin fold absorbed at 18.0–18.9 mm TL (ca. 49 days after hatching).

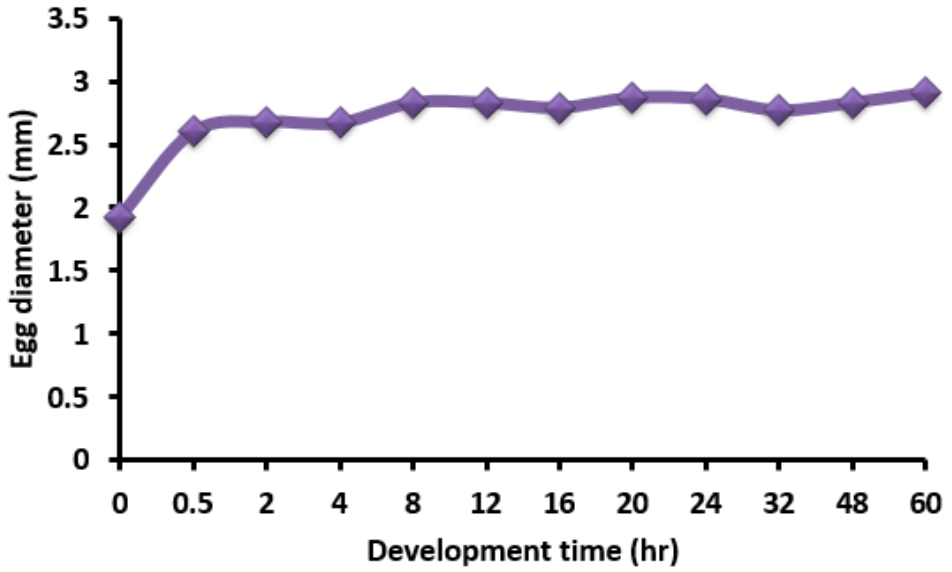

Fig. 6. Average egg diameter versus developmental timing of *L. brevicephalus*.

Pigmentation

No pigment was observed on newly hatched and day-old larval fish length 3.2 mm, first lightly pigmented occurred in the entire eye at ca. 8.1 mm TL (day 2 after hatching). At length 8.2 mm TL (3 days after hatching), eye was well pigmented, 10–16 melanophores on the dorsum of head and scattered along anterior-dorsal surface of yolk sac at ca. 9.2 mm TL (4 days after hatching). Length 12.1 mm TL (13 days after hatching): Dorsal - some pigment on

snout, more on anterior dorsum of heads, over swim bladder and two rows of pigment along the dorsal surface to the caudal area. Lateral - mid-lateral row of scattered pigment, subdermal pigmentation below auditory capsules, and along the dorsal surface of the intestinal tract. Ventral - a few melanophores on anterior and more on abdominal region, and distinct row from anus to caudal area. Length 12.3 mm TL (26 days after hatching) (Fig. 6c). Dorsal - heart-shaped pigment pattern on the dorsum of heads, 10–12 scattered pigment on the snout and two distinct, rows of pigment along dorsal surface. Lateral - more pigment behind eyes, and scattered along upper 25% of lateral surfaces and along the dorsal surface of the intestinal tract, with scattered pigment on caudal fin. Length 13.7 mm TL (30 days after hatching): Dorsal - more 'peppery,' bigger pigment on snout, the heart-shaped pattern on head faintly visible, two rows of smaller pigment along the dorsal surface to the caudal area. Lateral - mid-lateral stripe few and scattered pigments on dorsal, caudal and anal fins. Total length 20.2 mm (77 days after hatching): Dorsal pigment more, smaller scattered along the entire dorsal surface. Silvery colouration tinged on operculum, side of belly and along the length of lateral line and at caudal peduncle. This is the start of the characteristic squamation of the adult. Total length of 23.8–31.1 mm reached after 123 days of hatching. Dorsal - now triangular pigment patch on the dorsum of heads. Lateral - fine pigment extends from bottom of bows-tripe to dorsal surface, caudal spot dark, and dorsal, caudal, anal pectoral and pelvic fins lightly pigmented. Three rows of scale appeared along lateral line from caudal peduncle to supracleithrum. Total length 50 mm TL (147 days after hatching): Squamation was completed and now became similar to adults (156 days after hatching; Fig. 6e).

DISCUSSION

Research conditions in our experiment differed from those of other studies, such as Alekseyev *et al*. (1996) and Ryabov (2002) which were fully controlled rather than ambiental. The variations in temperature might have proved to be an advantage because we provided temperature, filtered water and light conditions close to those of the natural environment.

The embryogenesis of *L. brevicephalus* is a long duration, which generally agree with L. Tana *Labeobarbus* species that exhibit reproductive migration Barbel, *Barbus intermedius* (Ryabov, 2002) and African small Barbus *B. anoplus* (Cambray, 1983) and *B. trevelyani* (Cambray, 1985).

The Russian researchers worked on the spawning of small large barbs (Alekseyev *et al*., 1996) and developmental aspects of Lake Tana large barbs *L. intermedius* (Ryabov, 2002). The former study provided information on those species of Lake Tana that spawn in tributaries of Lake Tana. The latter study focused on ecological and morphological features in the development of *L. intermedius*. The present study on the embryonic and larval development of this endemic species provides additional information on larval behaviour, identification and developmental rates. Table 3 compares a few developmental stages of the two species *L. intermedius* (Ryabov, 2002) and *L. brevicephalus* and also comparisons of a few developmental traits with African small Barbus ontogeny of *B. anoplus* (Cambray, 1983) and *B. trevelyani* (Cambray, 1985) is depicted in Table 4.

Table 3. Comparison of developmental stages between Lake Tana Barbel *Barbus intermedius* and *L. brevicephalus* (from cleavage to hatching).

Developmental stages	***L. intermedius* (Ryabov, 2002)**	***L. brevicephalus* (Current)**
Cleavage		
a) 2 blastomeres	1 h 10 min, 22.2°C	1 h, 30 min, 23.8°C
b) 4 blastomeres	1 h 35 min, 23.0°C	2 h, 24.1°C
c) 8 blastomeres	2 h, 23.0°C	2 h 28 min, 24.8°C
d) 16 blastomeres	2 h 25 min, 23.0°C	2 h 53 min, 25.0°C
e) 32 blastomeres	3 h, 22.9°C	3 h 22 min, 25.8°C
f) 64 blastomeres	3 h 30 min. 24.1°C	3 h 52 min, 24.8°C
g) Stage of great-cell morula	3 h 50 min, 23.2°C	4 h 22 min, 23.8°C
h) Stage of medium-cell morula	4 h 20 min, 24.0°C	4 h 54 min, 24.1°C
i) Stage of blastula	6 h 20 min, 24.0°C	7 h, 23.8°C
j) Stage of early gastrula	8 h 35 min, 23.0°C	8 h 55 min, 25.8°C
k) Stage of late gastrula	22 h 20 min, 20.2°C	16 h 30 min, 25.8°C
l) Formation of embryo body before the closing of yolk plug	1 day, 22.0°C	18 h, 24.8°C
Segmentation		
a) Beginning of mesoderm segmentation	1 day 2 h, 20.6°C	17 h, 25.8°C
(b) 2 trunk somites	1 day 4 h, 24.0°C	18.5 h, 24.1°C
(c) 4 trunk somites	1 day 4.5 h, 24.2°C	20.5 h, 23.8°C
(d) 20 trunk somites	1day 9 h, 23.0°C	1 day 2 h, 25.8°C
(e) 32 trunk somites	2 days 2 h, 23.0°C	1 day 6 h, 25.8°C
Hatching time	70 h at 20.6°C	50 h 30 min, 25.8°C

Table 4. Comparison of several developmental traits of *L. brevicephalus*, *B. anoplus* and *B. trevelyani*.

Developmental trait	***B. anoplus* (Cambray, 1983)**	***B. trevelyani* (Cambray, 1985)**	***L. intermedius* (Ryabov, 2002)**	***L. brevicephalus***
Eggs, demersal and adhesive	Yes	Yes	Yes	Yes
Fertilized egg diameter (mm)	1.1	1.5	3.0	3.03
Hatching time	53 h at 19–21°C 28 h at 24–25°C	67 h at 17–19°C	70 h at 20.6°C	50 h + 30 min at 25–25.8°C
Length (mm) at				

Developmental trait	*B. anoplus* (Cambray, 1983)	*B. trevelyani* (Cambray, 1985)	*L. intermedius* (Ryabov, 2002)	*L. brevicephalus*
a) Hatching	3.1	3.7	7.8	5.1
b) Yolk absorption	4.5	7.1		9.6
c) Pelvic bud formation	8.4–9.5	10.6		14.8
Larval behaviour				
a) Active upwards, passive sinking	Yes	Yes		Yes
b) Adhere to objects	Yes	Yes		Yes
c) Pelagic larvae	Some	Few		Some
d) Cluster together on the substratum	No	Yes		Yes

The diameter of unfertilized eggs of *L. brevicephalus* ranged from 1.63 to 1.93 mm and that of *L. intermedius* was 1.75. The diameter of the swelled envelope in fertilized eggs in *L. brevicephalus* was about 3.03 mm which is in agreement with the result reported by Ryabov (2002) 3.0 mm (Table 4) for *L. intermedius*. This index may vary in accordance with broodstock age, broodstock size, feed, incubation temperature, and water quality, etc. Riverine versus lacustrine reproductive strategies variation may also cause great differences among Lake Tana *Labeobarbus* fish species in egg number and their size (Table 4).

In the present study, the incubation period of *L. brevicephalus* lasted for 50 h + 30 min at a water temperature of 25.8°C. Ryabov (2002) reported 70 h incubation at a temperature of 20.6°C for *L. intermedius*. Even though incubation period was not recorded, in a different study, Belay Abdissa *et al*. (2013) reported *L. intermedius* eggs successfully hatched at ambient water temperatures at 24°C with moderate development rate and lowest mortality (12.6%). At higher temperature of 30°C, the hatching and development of larvae embryos becomes much faster but mortality is higher (61.2%). Whereas at lower temperature (18°C) the hatching and the speed of development of larvae lag behind 24 and 30°C but it shows a lower mortality rate (7.4%). This shows that temperature plays a great role in the incubation period of Lake Tana *Labeobarbus* fish species. The higher dissolved oxygen concentrations 7–8 mgl^{-1} due to aeration in the present work allowed ideal situation for the hatching process (Table 3). Hatching timing may also depend on the size of the eggs because the amount of yolk material is frequently correlated with the duration of pre-hatching development (Fig. 3) (Adriaens and Vandewalle, 2003).

Between 28 and 30 h after fertilization the embryo was actively wriggling inside the chorion. Furthermore, a well-arranged 32 somites and their synchronized contraction and relaxation might be the reason for fast

rotational movement of the embryo inside.

There is evidence of physostomous physiology in the larval stages of some fish species which are physoclistic at adults (Boglione *et al*., 2013), but *L. brevicephalus* had only physoclistous physiology. The gas pressure of the swim bladder is regulated by special tissues or glands, where there is no connection or duct between the swim bladder and the intestinal tract. The swim bladder is filled with air within 5–10 days after hatching and the larvae started to actively swim in any direction.

Pigment in the eyes and 10–16 melanophores on a body appeared at the age of 3 days after hatching (8.2 mm TL). Whereas in the case of *L. intermedius* eye pigmentation and single melanophores appeared on the 5th day (Ryabov, 2002).

Development of segmentation process in larvae of *L. brevicephalus* was faster than *L. intermedius* (Table 3) even if the incubation temperature was more or less similar. This difference may be attributed to physical and biological conditions. In the case of *L. intermedius* the experiment was carried out in the river system. The impurities transported by the water current to some extent affect gas exchange of the larvae, which leads to stress and allocation of unnecessary energy for better gas exchange. Whereas *L. brevicephalus* incubated in the controlled environment, the impurities and the oxygen stress are not in place to disturb the developmental process of the larvae. The other conditions may be genetic difference of the two species such as amount of yolk, yolk content etc. which contributed to faster or slower speed in larval development.

Perspectives on applying developmental studies in the conservation of *Labeobarbus* species flock

Labeobarbus brevicephalus experiences a lengthy sequence of ontogenetic events in embryonic and larval development. The swim bladder and upper and lower jaws were fully developed at 5–10 days' post-hatching so it is expected to start feeding the larvae at this time. Developmental studies would enable us to understand early embryonic developmental stages and first feeding of *L. brevicephalus*, which can be utilized to improve rehabilitation efforts to save the endangered *Labeobarbus* fish species of Lake Tana. The information obtained from this study will be useful to cover gaps in early life history, first feeding and ontogeny of *L. brevicephalus* and such information could be beneficial for comparative studies and as a basis for further studies on the ontogeny of *L. brevicephalus*.

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