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NOTES ON THE BREEDING OF LARGE LAKE TANA BARBS (*LABEOBARBUS* SPP.) IN NATURE AND LABORATORY

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ABSTRACT: The largest lake species flock within cyprinids comprises of 16`Lake Tana (Ethiopia) *Labeobarbus* species differing in ecology, morphology, and sites of reproduction. Most of them migrate to rivers for spawning and head down to the lake after spawning. During 2005−2015, species composition of Lake Tana barbs was studied in five rivers and their tributaries including those so far not sampled. Our observations revealed that several species, earlier considered to spawn in the lake, were found to be riverine spawning. Results confirm the occurrence of spatio-temporal segregation in barbs' reproduction as previously reported as a mechanism enhancing reproductive isolation between closely related Lake Tana barbs. However, considerable overlap both in space and time of spawning in different barb species recorded in this study indicates that spatio-temporal segregation is far from complete. Breeding behavior of Tana barbs was also studied in nature and in the laboratory. Species-specific differences were revealed in the pre-mating behavior of different barb species. It is proposed that barbs participate in reproductive isolation enhancing the effects of space-temporal segregation.

Key words/phrases: Breeding behavior, *Labeobarbus*, Lake Tana, Reproductive isolation, Spatio-temporal segregation.

INTRODUCTION

Lake Tana, the largest lake in Ethiopia, is situated at the north-western Ethiopian highlands at an altitude of 1830 m above sea level. The surface area of the lake is large (3156 km^2), maximum length – 84 km, maximum width – 66 km, with mean and maximum depth of only 9 and 14 m, respectively. The lake contains about half of the freshwater resources in Ethiopia. Seven large permanent rivers contribute about 95% of the total annual inflow, the remaining inflow is provided by small seasonal rivers. The main tributary rivers are Gilgel Abbay and Megech flowing into the

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southern and northern part of the lake, respectively, and Gumara and Ribb flowing into its eastern part. The only surface outflow from the lake is the Blue Nile River, with the mean annual discharge comprising about 8% of the Blue Nile flow.

The analysis of sedimentary sequence revealed that Lake Tana desiccated several times. The last desiccation period when the lake dried out for some time was at Late Pleistocene (after 18,700 cal BP), simultaneously with the desiccation of Lakes Victoria and Albert. This period was followed by the appearance of the papyrus swamp and shallow water at the central part of the lake between 16,700 and 15,100 cal BP. During this period the lake was fed by seasonal stream inflows, remained closed and evaporation caused its water to become relatively saline. The lake returned to the freshwater state about 14,750 cal BP, when it began to overflow to the Blue Nile River. Further, Lake Tana underwent several small regressions associated with Younger Dryas and arid event at 8,200 cal BP (Lamb *et al*., 2007).

The relatively recent desiccation period and isolation from the Blue Nile R. basin by the Tissisat waterfall (45 m high) have likely predetermined the low diversity of the lake’s fish community. Greenwood (1976) described the ichthyofauna of the lake as truncated, i.e., very poor in species and families. Nowadays, four bony fish families occur in the lake: Cichlidae, Clariidae, Balitoridae, and Cyprinidae. The Nile tilapia, *Oreochromis niloticus*, is the sole cichlid species inhabiting the lake. Catfish family Clariidae is represented by *Clarias gariepinus*. A single representative of Balitoridae family - *Afronemacheilus abyssinicus*, is a rare, narrow-range endemic species (Prokofiev and Golubtsov, 2013). The family Cyprinidae is the most diverse in the lake. It is represented by *Varicorchinus beso*, four species of Garra (*G. dembecha, G. dembeensis, G. regressus*, and *G. tana*) (Stiassny and Abebe Getahun, 2007), three small diploid barbs g. *Barbus* (*B. paludinosus* (=*B. pleurogramma*), *B. tanapelagius* and *B. humilis*) (de Graaf *et al*., 2000; Eshete Dejen *et al*., 2002), and sixteen forms/species of large hexaploid barbs belonging to genus *Labeobarbus* (=*Barbus*) (Nagelkerke and Sibbing, 2000). To a large extent, the diversity of Lake Tana cyprinids is a result of explosive sympatric speciation. Two species of *Garra, G. regressus* and *G. tana*, one species of small barb, *B. tanapelagius*, and fourteen species of large barbs, g. *Labeobarbus*, are endemic to the lake and likely have originated within the lake. The sympatric cyprinids differ in ecology and morphology. Thus, *Garra* species differ in the intestine length (Stiassny and Abebe Getahun, 2007), a character which clearly indicates differences in food preferences. The endemic small barb, *B. tanapelagius*, is a zooplanktivorous fish, whereas other small barb species are mainly benthic feeders. Small barbs also differ in morphology: head and body shapes and proportions, eye sizes and mouth positions (Eshete Dejen *et al*., 2002). However, the most spectacular variability is manifested by the *Labeobarbus* species. They drastically differ in ecology. The absence of obligate piscivorous fish, typical of the Blue Nile basin, allowed eight *Labeobarbus* species to become piscivorous, which is unusual for cyprinids ecological adaptation. The remaining large barbs became bentivorous, zooplanktivorous, detritus and plant feeders and omnivorous (de Graaf *et al*., 2008). They also drastically differ in morphology: head and body shapes and proportions, mouth positions, eye diameters, gut lengths, etc. (Nagelkerke and Sibbing, 2000).

Most of the large barb species, as well as catfish and tilapia, are objects of commercial fishery. In the last two decades, increase of anthropogenic pollution, intensification of agriculture, dam and hydroelectric power stations construction, deforestation, etc. led to the negative changes in the populations of commercial fish, especially in barb populations (Wassie Anteneh *et al*., 2012; Eshete Dejen *et al*., 2017; Goraw Goshu *et al*., 2017). The main impacts are the seasonal illegal gillnet fishery, irrigation and dam construction on the lake tributaries, which are used by barbs as the spawning sites. Given the crucial role of *Labeobarbus* spp. in the lake ecosystem, further degradation of their populations may lead to irreversible negative consequences for the lake and surrounding area.

To protect the natural populations of large barbs and prevent the collapse of the lake ecosystem, immediate actions are strongly required. The implementation of the fishery legislation of Federal Democratic Republic of Ethiopia and Fisheries Management Plan for Lake Tana (Eshete Dejen *et al*., 2017) should serve the positive effects and conserve the current state of fish populations. However, we suggest that development of restoration program especially for endangered and vulnerable barb species that are included in IUCN Red List, is needed to provide rehabilitation of the natural barbs stock. Such sort of program requires generation of massive data on the biology of target species.

The stunning pace of speciation, spectacular ecological and morphological diversity make Lake Tana barbs one of the most attractive models for evolutionary biologists, and several international groups actively study this species flock, the largest within cyprinids.

As was reported by Nagelkerke and Sibbing (1996), *Labeobarbus* spp. migrate toward rivers for reproduction in the rainy season (August-October). Thus, in the Gumara River, barbs pass long distance in a few days from the river mouth to the upstream, where they spawn mainly in the small tributaries. Riverine spawning was confirmed for six species: *L. acutirostris*, *L. brevicephalus*, *L. macrophtalmus*, *L. megastoma*, *L. truttiformis*, and *L. tsanensis* (Nagelkerke and Sibbing, 1996). Later, the riverine spawning was reported for *L. platydorsus*. For the remaining species, among them *L. nedgia*, *L. dainelli*, *L. longissimus*, *L. crassibarbis*, *L. gorguari*, and *L. surkis,* lacustrine spawning or spawning in rivers that have not been sampled was proposed (Palstra *et al*., 2004; de Graaf *et al*., 2005). Similar list of riverine spawners was reported by Dgebuadze *et al*. (1999) and Palstra *et al*. (2004). But, the studies of Nagelkerke and Sibbing (1996), Dgebuadze *et al*., (1999) and Palstra *et al*. (2004), all focused mainly on the Gumara River. When some other Lake Tana tributaries were examined, *L. nedgia* and *L. surkis,* which were considered to be lacustrine species, were found in the Ribb (Wassie Anteneh *et al*., 2013), Megech (Wassie Anteneh *et al*., 2008), and Arno River (Shewit Gebremedhin *et al*., 2012). Consequently, to renew the current situation regarding places of barbs reproductive migrations, there is strong demand to enhance further the range of rivers to be researched on *Labeobarbus* spp. composition.

According to Nagelkerke and Sibbing (1996), different riverine spawning species clearly demonstrate temporal and spatial segregations which provide reproductive isolation and prevent interspecies hybridization. Later, when more rivers were examined, spatial spawning segregation was in general rejected (de Graaf *et al*., 2005) whereas temporal one was confirmed (de Graaf *et al*., 2005; Wassie Anteneh *et al*., 2012; 2013). However, simultaneous occurrence of running barbs belonging to different species at the same time and at the same spawning site (Dgebuadze *et al*., 1999) calls into question the completeness of spatio-temporal segregation. Moreover, a female and several males of different species were recorded to comprise the same spawning aggregation (Dzerzhinskii *et al*., 2007; Shkil *et al*., 2008). Artificial hybridization of different *Labeobarbus* spp. revealed absence of post-zygotic reproductive isolation (Alekseyev *et al*., 1996; Dzerzhinskii *et al*., 2007; Shkil *et al*., 2008). Under such circumstances, in which neither temporal-spatial segregation nor post-zygotic reproductive isolation can completely prevent interbreeding, differences in breeding behavior may be instrumental in reproductive isolation. But, data on breeding behavior of large Tana barbs are very scarce (Alekseyev *et al*., 1996; Dzerzhinskii *et al*., 2007).

In 2005−2016, our group studied developmental biology of *Labeobarbus* spp. of Lake Tana to reveal ontogenetic mechanisms underlying explosive morphological diversification accompanying evolution of the Lake Tana *Labeobarbus* species flock (Shkil and Smirnov, 2015; Shkil *et al*., 2015). Though breeding biology was not a primary goal of our study, numerous data concerning this item were accumulated during our field and laboratory observations. In the current issue, we present some of them, which may be useful for *Labeobarbus* restoration program.

MATERIALS AND METHODS

The detailed analysis of migratory activities and species composition during the spawning season are beyond the scope of our research. However, to study barbs interspecies developmental variability, we searched for spawning *Labeobarbus* in August-October in the different tributaries of Lake Tana for ten years (2005−2015). The work was performed at the upstream of the following rivers: i) the Gumara River and its tributaries; the Ducalit, Kyzyn, and Chan, ii) the Ribb River and its tributaries; the Demesse and Barewanz, iii) the Chibirna River, iv) the Arno River, and v) the Enfranze River (Fig. 1). We collected fish mainly with cast net (5.65 m^2) and sometimes from the local fishermen. Each fish specimen was checked for maturity stages. Most of them were pictured and sampled for osteology, and morphological and genetic analyses. Given that cast netting is not an appropriate method for quantitative study, in the current issue we present combined data concerning presence of ripe (ready for spawning or spawning) barbs in the particular rivers only.

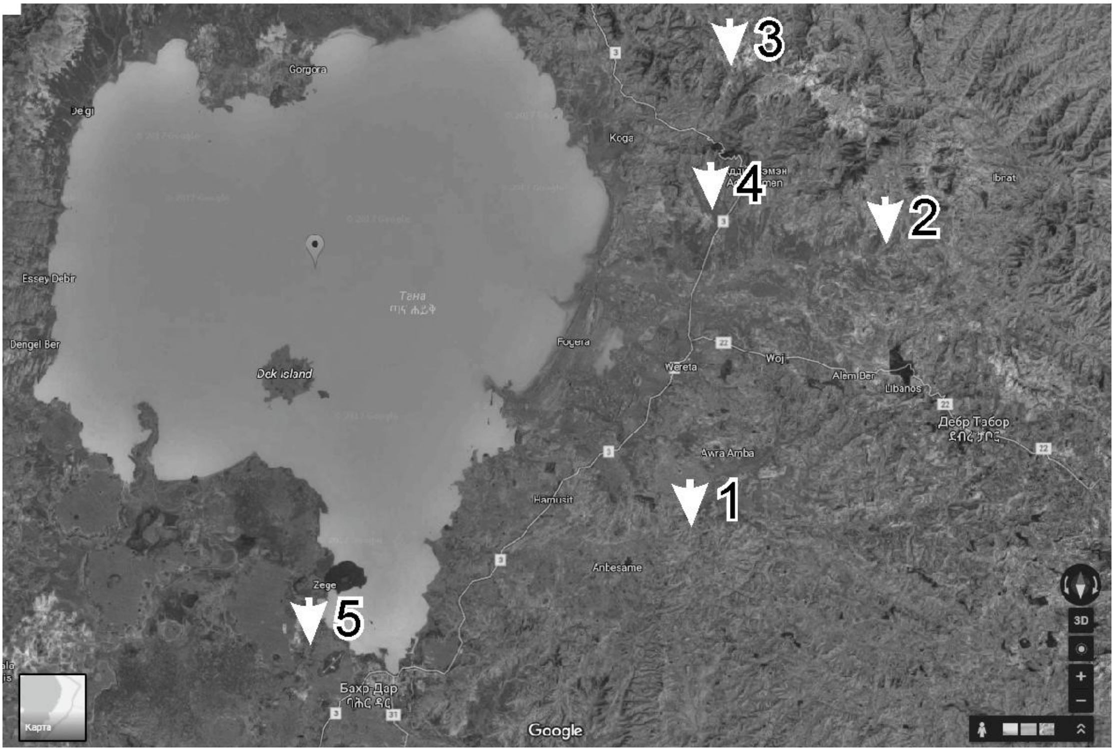

Fig. 1. Catching localities: 1. Gumara River and tributaries; 2. Ribb River and tributaries; 3. Arno River; 4. Chibirna River; 5. Enfranze River.

The main goal of our field activity was to obtain fertilized eggs from different species of Tana barbs. For this goal, we caught running barbs at the spawning sites and made artificial breeding via “dry method”. To catch the running individuals, cast netting was used. This method provides the minimal loss of gonadal products and diminishes fish damage. Usually, several males were caught beforehand and kept alive. If a running female was absent and it was impossible to keep males alive, the milt was collected into the specimen tube and stored in fridge (t=+4°C) or on ice for one-two days. To collect the milt, anal fin and caudal peduncle of male should be (were) dried with a cloth. Then, anal fin should be directed to the specimen tube, and the researcher carefully pressed fish belly in the direction from pectoral fin to pelvic one. To avoid milt’s desiccation during the storage, it is possible to add small amount (5−10% of total amount) of 0.01 M phosphate buffer saline (PBS). After the stripping procedure, males can be (were) returned to the nature. The captured female should be processed immediately after catching. The water from anal fin and caudal peduncle of female should be removed carefully with a dry cloth. Then, female’s anal fin should be directed to the dry container with perforated walls (we used Petri dish equipped with the mesh extending the wall). Eggs can be obtained via the careful stripping in antero-posterior direction. After the stripping, female may be moved back to the nature. The sufficient amount of milt must be added to the batch immediately after a female was stripped. Gonadal products were mixed carefully with a feather. Then, small amount of water should be added into the batch and mixed with it. Finally, the container should be placed into the stream. When fertilized, eggs lose the adhesiveness, they can be transferred into the ordinary plastic bottle (1.5−2.0 l) equipped with an air stone connected to the air pump, and transported to the laboratory for further incubation. This method was used by our group many times. Thus, we obtained progenies from different *Labeobarbus* spp. and reared many of them up to maturity under artificial conditions: in indoor aquaria and in open-air ponds (Table 1). However, the declining of barb populations, as well as transformation and extinction of the spawning sites due to the human activities, make the catching of spawning fish more and more difficult. These circumstances along with the presence of artificially rearing barb stock in Bahir Dar Fisheries and Other Aquatic Life Research Center (BFALRC) encouraged us to develop the laboratory methods of barb breeding.

Table 1. List of crossings obtained in nature and reared in aquaria and artificial ponds in 2005−2015.

Species	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
L. intermedius	1		5	2	3	2			1	2	4
L. brevicephalus	3		2	2	5	5	3		2	2	10
L. megastoma	3					1	4		2		4
L. platydorsus									2	1	1
L. truttiformis	1			1				1			1
L. crassibarbis									2	1	1
L. macrophtalmus	1				2						
Hybrids	3	8		5			3		2		

For laboratory breeding, we collected ripe *L. intermediu*s males and females from open air ponds, where they were reared, and transferred into the indoor aquaria system with permanent aeration, circulating and filtering water obtained from Lake Tana, room temperature (20−22°C), and natural light/dark regime. Males and females were kept separately in glass aquaria (V=200 l) for one week.

Then, a female was transferred into the separate aquarium (140 x 70 x 100 cm), in which the bottom was covered with gravel (grit diameter=0.5−2.5 cm), permanent aeration, natural light/dark regime (11/13 h), t= +21-22°C, and permanent bottom stream were provided by two immersed water pumps. The air stones were located near the immersed water pumps to provide high oxygenation of the outlet stream. The aquarium was also equipped with digital camera, which permanently recorded a video. Three days later, two running males were transferred into the aquarium with a female. As a result, barbs performed spawning in the subsequent three days. Then, fish were returned back to the pond. The fertilized eggs were collected from the gravel.

RESULTS AND DISCUSSION

Breeding in nature: timing, locations, and behavior

Gumara River

The following species were caught in the main stream of the Gumara River: *L. brevicephalus*, *L. intermedius*, *L. megastoma*, *L. macrophtalmus*, *L. crassibarbis*, *L. platydorsus*, *L. truttiformis*, *L. tsanensis*, *L. acutirostris*, *L. nedgia*, *L. gorgorensis*, and *L. longissimus*. In this list, species are positioned in the decreasing order of occurrence frequency. *L. brevicephalus* was the most abundant species; *L. longissimus* was caught only sporadically. We visually observed the spawning of *L. brevicephalus*, *L. intermedius*, *L. megastoma*, *L. crassibarbis*, *L. platydorsus*, and *L. truttiformis* at the shallow gravel beds (less than 0.5 m deep) in the main stream of the Gumara. The remaining species likely prefer to spawn at the deeper sites of the river.

In comparison with earlier data (Nagelkerke and Sibbing, 1996; Palstra *et al*., 2004), list of fish migrating into the Gumara River comprises additionally *L. crassibarbis*, *L. nedgia*, *L. gorgorensis*, and *L. longissimus*. From these, *L. crassibarbis* was common at the beginning of October in 2011 and 2012 as well as in the middle of September in 2013. *L. nedgia* was often caught in October 2011. Earlier, the rare appearance of *L. gorgorensis* and *L. crassibarbis* in the Gumara was reported by Dgebuadze *et al*. (1999), one specimen of *L. nedgia* was caught by Abebe Ameha (2004).

The spawning activity of barbs in the main stream of the Gumara is not limited by the end of the rainy season. Ripe barbs were observed in the dry season, also. In 2010, we collected several mature *L. intermedius* and *L. brevicephalus* specimens near the Ducalit mouth in April. These findings indicate that the resident *Labeobarbus* spp. (i.e., *L. intermedius*) breed in the rivers throughout the year. Earlier, the reproduction throughout the year for *L. intermedius* was described by de Graaf *et al*. (2005) and Wassie Anteneneh *et al*. (2008).

Palstra *et al*. (2004) reported reproduction of *L. brevicephalus*, *L.* megastoma, *L. macrophtalmus*, *L. acutirostris*, and *L. truttiformis* in the Gumara tributaries: Kyzyn. Ducalit, and Wanzuma. We observed *L. brevicephalus*, *L. intermedius*, *L. megastoma*, and *L. truttiformis* in the Ducalit; *L. brevicephalus*, *L. intermedius*, and *L. macrophtalmus* in the Kyzyn; and *L. brevicephalus*, *L. intermedius*, *L. megastoma*, *L. truttiformis*, *L. gorgorensis*, and *L. longissimus* in the Chan. However, only one species, *L. brevicephalus*, regularly use these tributaries for spawning. The remaining species entered small tributaries only sporadically, after rainfall, when water level was drastically increased.

Ribb River

In the main stream of the Ribb River, we collected *L. megastoma*, *L. intermedius*, *L. brevicephalus*, *L. nedgia*, *L. tsanensis*, and *L. truttiformis*. Earlier, these six species were reported as dominant in the Ribb and its seven tributaries sampled by Wassie Anteneh *et al*. (2013). In the tributaries we examined (i.e., the Demesse and Barewanz), we did not find *L. tsanensis* and *L. truttiformis*, whereas other species were present. In accordance with our observations, *L. megastoma* entered both tributaries. In the Barewanz, this species occupied downstream and middle reach, in the Demesse it was grouped near the mouth only. *L. intermedius* and *L. brevicephalus* were distributed in both tributaries, but their density was much higher in the Demesse. In the main stream of the Ribb River as well as in downstream of the Demesse and Barewanz, we visually observed the breeding of *L. megastoma*. Spawning of *L. intermedius* and *L. brevicephalus* was visually detected in the Ribb, near the Demesse mouth, and in the Demesse tributary. Notably, in the Demesse upstream, *L. intermedius* and *L. brevicephalus* with different stages of maturity were collected simultaneously. Most fish were mature and ripe. However, males and females with gonadal stage II-III were present also. This finding indicates that barbs may miss out the spawning season.

Arno River

Shewit Gebremedhin *et al*. (2012) reported *L. brevicephalus*, *L. intermedius*, *L. nedgia*, and *L. tsanensis* to be the dominant species in the Arno River. In our samples, *L. brevicephalus* prevailed, whereas other species were rarely collected. It is worthy to note that sometimes we collected fish with unusual morphology, which was intermediate between those of *Labeobarbus* sp. and *V. bezo*. Also, mature *V. bezo* specimens with “normal” morphology were present simultaneously with *Labeobarbus* spp. In sum, these facts indicate putative interbreeding between these species. Earlier, as a rare phenomenon, hybrids between *V. bezo* and *Labeobarbus* sp. were reported from Lake Tana by Nagelkerke and Sibbing (1996).

Chibirna River

In the upstream of Chibirna River we collected *L. intermedius*, *L. brevicephalus*, *L. truttiformis*, and rarely *L. nedgia*. One of the local fishermen reported that the period of the presence of *Labeobarbus* spp. in this river is very short. Fish usually come during the night time and escape from the upstream early in the morning. He also remarked that he caught mature *L. surkis* at the vegetation area located downstream.

Enfranze River

In the Enfranze River *L. brevicephalus*, *L. intermedius*, *L. nedgia*, *L. gorguary*, and *L. surkis* were observed. Several *L. brevicephalus* specimens collected in this river had unusually high dorsal spine (Fig. 2). Since within *L. brevicephalus* such dorsal spine was unique for those inhabiting the Enfranze River, we propose that they could be residential barbs. Individuals of *L. gorguary* were observed only sporadically. In accordance with information obtained from local fishermen and BFALRC staff members, the period of occurrence of *L. gorguary* in the Enfranze River is very short, one or two days per year. However, during this period *L. gorguary* is abundant. Local fishermen also reported the occurrence of mature *L. surkis* in the grass area in the middle stream of the Enfranze River.

Given that our activity was focused on the mature representatives of *Labeobarbus* spp., which spawned or were completely ready to spawn, the results obtained allow us to conclude that all the above mentioned species use rivers as spawning sites. Thus, our findings expand the list of riverine spawners (Table 2) and presents doubt about the putative obligate lacustrine reproduction for some species of the *Labeobarbus* spp. of Lake Tana.

Fig. 2. *L. brevicephalus* from Enfranze R. with normal (a) and extremely high (b) dorsal fin spine.

Table 2. List of riverine spawning species.

Species	**Gumara R.**	**Ribb R.**	**Arno R.**	**Chibirna R.**	**Enfranze R.**
L. brevicephalus	+	+	+	+	+
L. intermedius	+	+	-	+	+
L. nedgia	+	+	-	+	+
L. truttiformis	+	+	-	+	-
L. megastoma	+	+	-	-	-
L. tsanensis	+	+	-	-	-
L. macrophtalmus	+	-	-	-	-
L. gorgorensis	+	-	-	-	-
L. longissimus	+	-	-	-	-
L. crassibarbis	+	-	-	-	-
L. platydorsus	+	-	-	-	-
L. acutirostris	+	-	+	-	-
L. surkis	-	-	-	-	+
L. gorguary	-	-	-	-	+

The observations also lead us to conclude that Tana barbs have mechanisms of pre-mating isolation, but the isolation is not complete, in which our long-term data support and complement previously obtained results (Palstra *et al*., 2004; de Graaf *et al*., 2005; Wassie Anteneh *et al*., 2013). Usually different species demonstrated pronounced temporal segregation since they reached spawning sites at different times. For example, in the Gumara River near the Ducalit tributary, at the end of September - beginning of October, numerous *L. megastoma* individuals come first followed by mass of *L. brevicephalus*. In a short time, massive invasion of *L. intermedius* is observed. Simultaneously, the representatives of *L. crassibarbis*, *L. platydorsus*, and *L. truttiformis* begin to run downstream from the spawning sites located upstream. Individuals of *L. macrophtalmus*, *L. acutirostris*, and *L. tsanensis* are met at the end of October, when ripe representatives of other species start to migrate downstream. More or less similar situation was observed in the Ribb River.

Differences in the timing and sites of spawning usually are accompanied by disparities in the reproductive behavior. In 2010, we observed *L. megastoma* spawning in the main stream of the Ribb River. Numerous ripe males of *L. megastoma* gathered at the rapids in the main stream during day time. However, females were not present in which they occupied places not accessible for cast net fishing. We got only one ripe female during day time at the mouth of the Demesse tributary. Numerous *L. intermedius* and *L. brevicephalus* (ripe males and unripe females) and unripe males of *L. nedgia* were present in the main stream of Ribb River at the same time. Similar composition of barbs species was observed in the Demesse River, but in a drastically different ratio. We caught numerous ripe males and unripe females of *L. intermedius*, several unripe *L. nedgia*, several ripe males of *L. brevicephalus* but only one *L. megastoma* male.

The situation changed drastically after sunset. *L. intermedius* and *L. brevicephalus* began to spawn in the mouth and downstream of the Demesse tributary. In the main stream of the Ribb River, ripe males of *L. megastoma* occupied gravel place in one river branch. They displayed obvious competition for the sites. Other species of large barbs were not present at that gravel site, but were present in the neighboring branch of the river, as well as upstream and downstream. Ripe *L. megastoma* females came to the gravel site from the downstream in the midnight. In the gravel site, ripe female moved slowly and "tested" the ground taking gravel into the mouth. Several (5−15) *L. megastoma* males formed the spawning group, they followed the female hardly competing for her. However, males did not follow female for a long distance, usually after 7−10 m they came back to their site. It was the female that selected a place for spawning. Spawning in the same group repeated several times with small breaks for 5−10 minutes. Then, the female left the gravel place. Males, which participated in spawning, tried to occupy their sites again. Examination of spawned individuals revealed that females didn't cast out all eggs in one visit of spawning site. In many spawned individuals ovary contained a lot of mature eggs. Spawned males also retained milt. Consequently, these findings indicate intermittent spawning of *L. megastoma*. Similar pattern of spawning of *L. megastoma* at the same place was observed in 2011, 2013, and 2015, and in the Barewanz tributary in 2010. In August 2010, Wassie Anteneh *et al*. (2013) also observed large aggregation of ripe *L. megastoma* males waiting for females in the Melo River, tributary of the Ribb. Interestingly, aggregations of ripe males waiting for females and competing for them were described also for the African cyprinid *Labeo capensis* (Winker *et al*., 2012).

The smallest among the *Labeobarbus* spp.s, *L. brevicephalus*, displays different reproductive behavior as compared with *L. megastoma*. We observed spawning activity of *L. brevicephalus* many times in different rivers. As a rule, *L. brevicephalus* spawns during the dark time, but spawning was also recorded during the day time. Usually *L. brevicephalus* bred in the mouth and downstream of small tributaries, as well as at the shallow (2−10 cm deep) gravel riffles in the main stream of big rivers. In all cases, a shoal of males aggregated downstream in shallow places. Sporadically some of them passed into the spawning site for a short time, and then returned to a shoal. Females entered the shoal and then rushed toward the gravel place. If a female entered a small tributary, it travelled only a short distance, usually no more than 10 m. During the trip, a female was followed by 5−10 males. Then, the female spawned several times in a short period (10−20 min) and came back downstream to a deeper place. Males disorderly came back to a shoal. Usually, spawned female lacked eggs completely, while spawned males could participate in the next spawning group.

We did not observe spawning activities of other species in detail. However, the composition of our catches and visual observations at the Gumara River revealed that *L. crassibarbis*, *L. platydorsus*, and *L. truttiformis* form homonomic spawning groups composed by a female and one-three males. Sometimes, one or two males belonging to different species (mostly *L. intermedius*) followed the group. During the day time groups stood in the deepest places of the Gumara River. After sunset, the group entered the spawning site (more than 0.5 m deep), a gravel riffle in the main stream of the Gumara River, and spawned. Examination of spawned females indicated the intermittent spawning in these species.

So, we may conclude that temporal segregation as well as differences in reproductive behavior provide the pre-mating isolation between different *Labeobarbus* spp. However, sometimes it was observed that there was simultaneous spawning activity of different species at the same spawning site. Simultaneous breeding of *L. intermedius*, *L. megastoma*, and *L. truttiformis* in the Ducalit River was described in detail by Dzerzhinskii *et al*. (2007). Moreover, in 2008, ripe females and males of *L. intermedius, L. megastoma*, *L. truttiformis*, *L. acutirostris* as well as ripe males of *L. macrophtalmus*, *L. platydorsus*, and *L. gorgorensis* were collected at the same time and at the same gravel bed (about 50 m^2) in the main stream of the Gumara. We did not observe directly the spawning between different species, but the maturity state of fish and catch compositions (one-two females and several males belonging to different species) both indicate the possibility of the interspecies breeding.

Breeding under laboratory conditions

Our experiments demonstrated that the laboratory-reared fish can be stimulated to spawn in the aquarium without hormonal injection, if the conditions resemble the natural spawning sites as nearly as possible. Another important factor is the correct selection of the spawners with the appropriate physiological state.

For comparative developmental analysis, we pictured artificially reared fish and checked their maturity state twice per year: in March-April and October-November. It was revealed that fish reared in open air ponds become ripe at the same time as wild ones, at the end of rainy season. In males and females, the overall coloration became darker and white stripes appeared on the lips and first rays of pectoral and pelvic fins. Most of the males displayed asperity on the frontal surface of the head. The pressing of male’s belly resulted in milt emission. The belly of females became more rounded.

For the induction of natural spawning, the female should be transferred to the breeding aquarium alone, as the presence of active and ready for spawning males can disturb her and cause negative effects. Males were transferred to the breeding aquarium on the third day after the female and began to demonstrate the pre-mating behavior almost at once. The records obtained from the camera allowed us to describe in detail the nuptial behavior of *Labeobarbus* spp. in the aquarium.

When females and males were kept in the aquarium, their behavior was more or less similar. They actively moved in mid-water and upper water layer, fed on an artificial food, and tried to jump out of aquarium during the night time. The females transferred one by one into the breeding aquarium displayed disparity in behavior. Some of them did not change their behavior, remained mainly in the upper water layer and tried to escape from the aquarium. As was revealed later, these individuals were not ready to spawn. However, several females displayed significant behavioral changes. They remained mainly in the bottom layer and faced to the outlet stream from the water pumps. Those females sporadically touched the gravel with fins and belly and tested it by taking it into the mouth.

Males transferred into the breeding aquarium displayed nuptial behavior. They communicated with female and competed with each other. Males’ nuptial behavior comprised several distinguishable elements: i) active movement around the female; ii) approaching to the female’s genital pore; and iii) snuggling to female accompanied by the whole body-quivers (Fig. 3). The spawning was performed on the gravel bottom. Both fish, female and male, faced the stream of water coming from the pumps. The male began to press the female and quiver. For several seconds, the female lay down on the gravel and began the whole-body quivering, too. The male moved back, and its genital pore reached the same level with the female’s genital pore. Both spawners lifted up their caudal peduncle and dorsal fin

(Fig. 4). The most vigorous quivering was observed near the base of caudal peduncles and anal fin. However, such active movement did not result in changes of fish position. Finally, barbs spawned with appearance of a mixed cloud containing eggs, sperm, gravel, and sand. The cloud was moved back by the stream and began to settle. Immediately after the spawning, males began to eat the eggs actively, whereas female seemed to try to banish them from the batch. The spawning was repeated several times. The developmental stage analysis of eggs obtained from the aquarium gravel revealed that fish spawned minimum six times (Fig. 5). The most intensive spawning occurred during the evening time between 17.00−22.00.

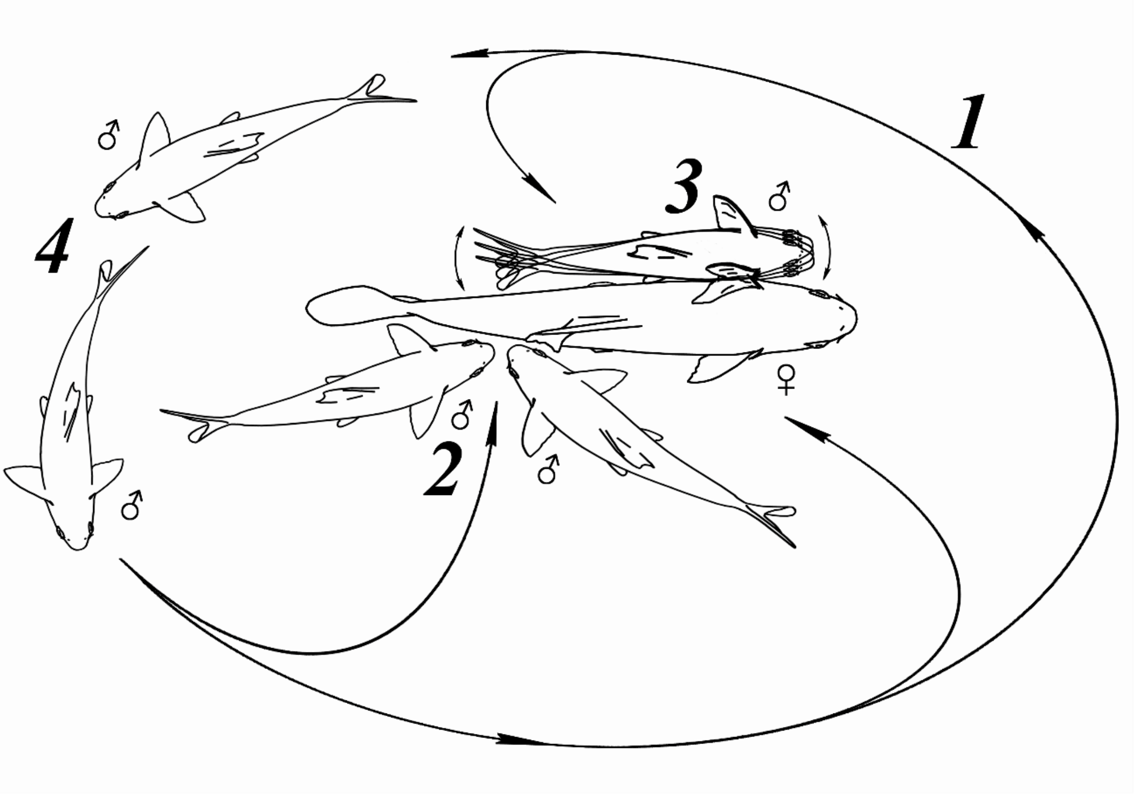

Fig. 3. Elements of the pre-mating behavior of *L. intermedius* males 1 – swimming trajectories around the female, marked by the solid lines with arrowheads; 2 – approaching to the female’s genital pore; 3 – snuggling to the female and body quivering; 4 – agonistic behavior between males.

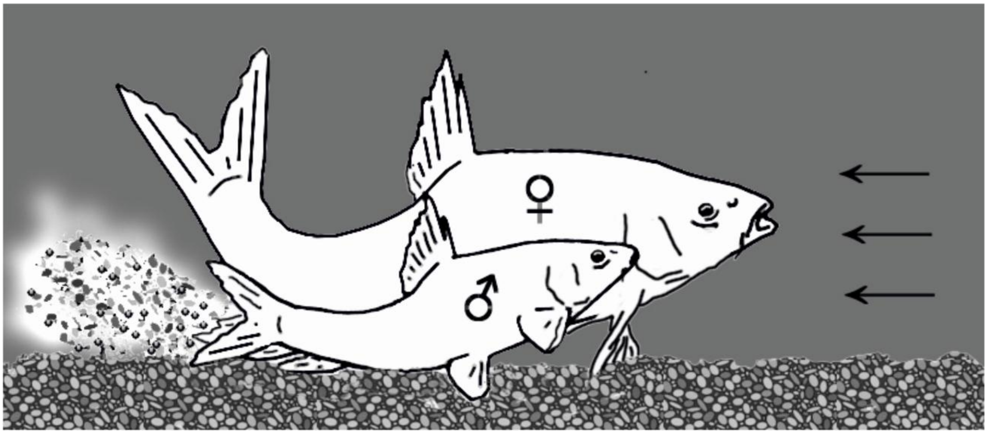

Fig. 4. *L. intermedius* spawning under laboratory conditions. The picture is based on a shot taken from the night-time filming of the spawning. Note the extremely high lifted caudal peduncle of the female and the cloud of eggs, sperm, and gravel behind the spawners. Arrows indicate the direction of the artificial stream.

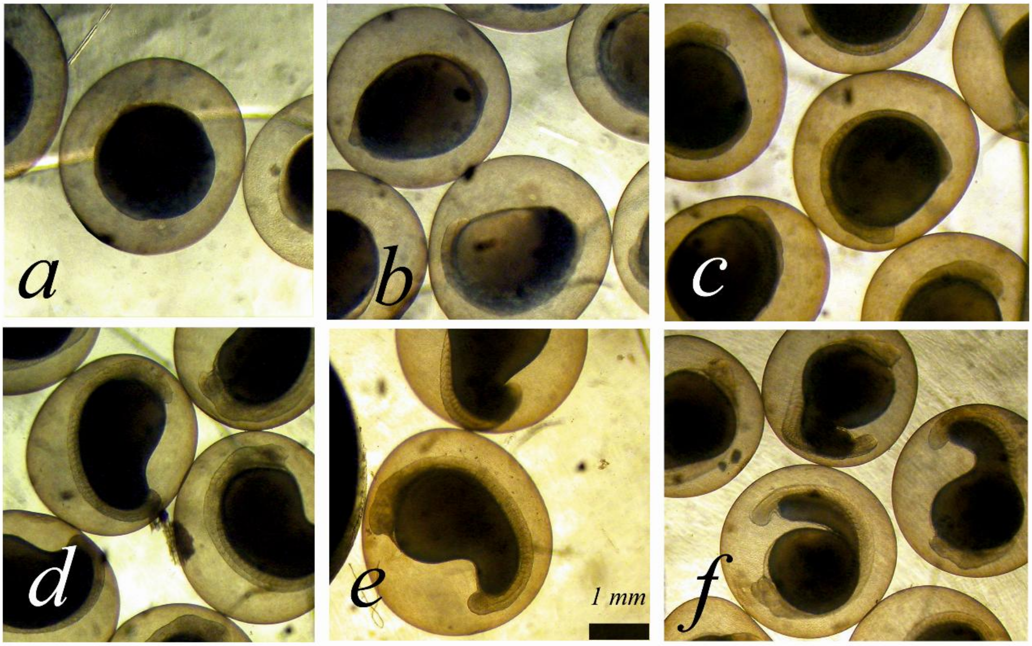

Fig. 5. Embryonic developmental stages of the eggs revealed simultaneously in the gravel after the *L. intermedius* spawning under laboratory conditions: a – 60%-epiboly (16-20 hours post fertilization – hpf); b – tail bud developing (20-24 hpf); c – beginning of segmentation (24-29 hpf); d – Kuppfer's vesicle's appearance (29-34 hpf); e and f – progressive extension of the tail rudiment and first spontaneous movements (34-40 and 40-46 hpf, respectively). The scale is uniform for all photos. The approximate age of the eggs was estimated in accordance with the averaged scale of *L. intermedius* embryonic development rate drawn from data of literature (Ryabov, 2002).

Successful spawning of *L. intermedius* in the laboratory indicates the correct set of conditions, prepared for the process, and justifies our knowledge about the reproductive biology of this species. Earlier, to study reproductive behavior, similar approach was used in the spawning stimulation of hybrids of a silver bream (*Blicca bjoerkna*) and rudd (*Scardinius erythrophthalmus*) via the imitation of natural spawning site conditions in the experimental environment (Nzau Matondo *et al*., 2013). Our experiments did not just prove the possibility of large African barb breeding in laboratory conditions without hormonal treatment, but also allowed to describe the nuptial behavior of fish, which, in some cases, is technically difficult to observe in nature. The described behavior can be considered as quite close to natural one since some elements of nuptial behavior of *L. intermedius* under the laboratory conditions strongly resembled those observed in the natural breeding. Thus, in all cases we observed females to “test” the gravel. This fact allows us to suppose that a female plays a key role in the selection of the spawning place. Males of all the species, whose spawning was observed, displayed a competition for the female. The final stage of nuptial behavior, a spawning, proceeded in the same manner in different species. Females excavate gravel during the spawning. Such behavior along with the adhesive property of eggs seems to provide better gluing of eggs into the gravel and their subsequent settlement on the bottom. Given the observed post-spawning eating of eggs by males in aquarium, we noted that rapid settlement along with the burying of the eggs by a female increases the survival of eggs, protecting them from being eaten by males and other predators.

Cyprinids, in general, have some common patterns in their courtship and reproductive behavior (Turner, 1993; Anna Mercy *et al*., 2003). These elements can be seen in the pre-mating behavior of *L. intermedius* described in our experiment. Circling movements of males around females and touching the females’ abdomen were reported for cyprinids of the genus *Puntius* (Vincent and Thomas, 2008), *Danio aequipinnatus* (Kharbuli *et al*., 2004), *D. rerio* (Spence *et al*., 2008), bitterlings *Acheilognathus signifer* (Baek *et al*., 2004), silver bream *Blicca bjoerkna* (Poncin *et al*., 2010), and many others. Body quivering of both males and females was registered in mating behavior of bitterlings *Acheilognathus signifer* (Baek *et al*., 2004) and silver bream *Blicca bjoerkna* (Poncin *et al*., 2010). Agonistic behavior of males is also very common for cyprinid species, and it can be either related to the territorial defense (Baek *et al*., 2004) or not (Spence *et al*., 2008).

It is worthy to note the rather complex courtship ritual of *L. intermedius* males, which differs from that demonstrated in nature by *L. megastoma* males (see above). This divergence can be the result of the differences in environmental conditions (aquarium and river), otherwise this could be associated with species-specific differences, promoting to some extent the reproductive segregation. This finding supports the hypothesis that patterns of courtship behavior may function as a mechanism of ethological isolation among closely related fish species (Colgan, 1996).

For large African barbs, pre-mating behavior is probably the only way to assess and select the proper partner for spawning (Dgebuadze *et al.*, 1999). Vision, although important for the mate choice of many fish species, e.g. cichlids, cannot play significant role in such assessment because of the extremely turbid water in the spawning rivers and the lack of discriminative signs in coloration of different barb species (Dgebuadze *et al.*, 1999). Chemoreception helps to find ripe females ready for spawning, but it can't determine whether a potential partner is conspecific or not and, consequently, cannot provide reproductive isolation between the species (Zworykin *et al*., 2006). Thus, our preliminary findings of differences and similarities in the pre-mating behavior of different barb species may contribute to the understanding of the mechanisms underpinning reproductive isolation of closely related sympatric species, and show the perspectives of further investigations required in this direction.

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