

Belgian Journal of Zoology

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Research article

<https://doi.org/10.26496/bjz.2024.183>

Spawning migration and habitat characteristics of *Labeobarbus* **species in the Gumara River and its tributaries, Lake Tana subbasin, Ethiopia**

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Abstract. Information on the spawning migration and habitat use of migratory fish is critical to protect and restore threatened or endangered populations. Twenty-two individuals of three species, namely *Labeobarbus platydorsus*, *L. megastoma*, and *L. truttiformis*, were implanted with radio tags to study their spawning migration in the Gumara River and its tributaries between July and October of 2018 to 2021. Fourteen individuals were found at least twice throughout their migration, eleven moved upstream up to 41.0 and 44.4 river kilometers (rkm), and three were tracked when moving downstream. The upstream spawning movement of the tagged fish lasted 9 to 27 days, and their ground speeds ranged from 0.07 to 1.50 km h-1. The habitat use of untagged *Labeobarbus* specimens in the spawning sites was also assessed using fyke nets, cast net fishing, and data from fishermen's catches. *Labeobarbus truttiformis* mainly exploited the Kizen tributary stream with gravel substrates and less turbid water for spawning. Several small-sized individuals of *L. megastoma* spawned in the Wonzuma and Dukalit tributaries. By contrast, larger-sized specimens of *L. platydorsus* and *L. megastoma* spawned in the main river channel at the riffles and the gravel/pebble size substrate. Destructive fishing using gillnet dragging, filtering, and damming/fencing has recently intensified at the spawning areas (\sim 41.0 rkm to 45.0 rkm), which likely affects spawning populations. Therefore, we recommend that fishing in the main river channel and tributaries must be banned during the months of August, September, and October to safeguard and conserve the threatened *Labeobarbus* species.

Keywords. Radio-telemetry, *Labeobarbus*, Lake Tana, migration, Gumara River.

Zelalem W., Mingist M., Anteneh W., Kibret M., Adgo E., Nyssen J., Verleyen E. & De Vocht A. (2024). Spawning migration and habitat characteristics of *Labeobarbus* species in the Gumara River and its tributaries, Lake Tana subbasin, Ethiopia. Belgian Journal of Zoology 154: 97–116.<https://doi.org/26496/bjz.2024.183>

Introduction

Cyprinidae is the most species-rich fish family in Lake Tana, comprising 17 hexaploid endemic *Labeobarbus* species (Nagelkerke & Sibbing 2000; Gethahun & Dejen 2012). Of these, seventeen large *Labeobarbus* species (Beshera *et al.* 2016; Nagelkerke & Sibbing 2000), more than half are known as riverine spawners (Gebremedhin *et al*. 2012; Dejen *et al*. 2017; Shkil *et al*. 2017). The spawning migration of these species from the lake to the rivers takes place during the rainy season between July and October. Their migration behaviour is also sequential and can be divided into three distinct periods (Palstra *et al*. 2004). The first phase involves the movement from foraging areas in the lake to river mouths. In the second phase, they travel upstream into the main channels of the rivers. In the third phase, the fish reach their spawning habitats, which are generally fast-flowing, clearwater, well-oxygenated riffles with gravel beds in tributary streams. However, larger migratory *Labeobarbus* species may also spawn in the main river channel (Palstra *et al*. 2004). The fish usually spawn after sunset (Dgebuadze *et al*. 1999; Palstra *et al*. 2004; Anteneh *et al*. 2008).

Apart from this basic knowledge on spawning behaviour, little is known about the specific habitat use of different *Labeobarbus* species. This information is, however, crucial for protecting and conserving these endemic species which are currently under threat. Indeed, during the first phase of the spawning aggregation in the river mouth, migratory *Labeobarbus* species are exposed to intensive, targeted commercial gillnet fishing, which reduces their reproductive stock (De Graaf *et al*. 2008). In addition, illegal, unregulated, and unreported (IUU) fishing also resulted in a reduction in the fish stock as indicated by a decline in the fish catch since the 1990's (Gebremedhin *et al*. 2019). More in particular, the estimated catch per unit of effort for *Labeobarbus* species in the lake was approximately 63 kg per trip in 1993. The catches declined to 28 kg per trip in 2001 and to 6 kg per trip in 2010 (Dejen *et al*. 2017). Gebremedhin *et al*. (2019) reported that the catch dropped even further to 2 kg/trip in 2016-2017.

Also well documented is the timing of the upstream spawning migration and sexual maturation differs between species (Palstra *et al*. 2004). Most have a single, short reproductive period with peak spawning between August and September, but the spawning of *L. surkis* mainly occurs between December and January. The *L. intermedius* 'shore-complex' is the only exception having an extended spawning season with sexually mature individuals occurring throughout the entire year (Nagelkerke $\&$ Sibbing 1996; Sibbing *et al*. 2005). Additionally, spawning migration patterns may also differ between different *Labeobarbus* species. For instance, *L. megastoma* is the first species to migrate, followed by *L. tsanensis*, *L. acutirostris*, and *L. trutiformis* (Palstra *et al*. 2004)*.* By contrast, *L. brevicephalus* and *L. macrophtalmus* migrate only late during the wet season for spawning (Palstra *et al*. 2004).

Seven *Labeobarbus* species, including *L. acutirostris*, *L. brevicephalus*, *L.macrophtalmus*, *L.megastoma*, *L. truttiformis*, *L. tsanensis*, and *L. platydorsus* are known to spawn in the upstream part of the Gumara River and its tributaries (Nagelkerke & Sibbing 1996; Palstra *et al*. 2004; Dzerzhinskii *et al*. 2007). Except for *L. intermedius*, which is presumed to spawn in both the lake and river systems (Anteneh *et al*. 2012), the spawning locations of the remaining species are unknown. These species are assumed to be lake spawners (Nagelkerke & Sibbing 1996; Dgebuadze *et al*. 1999; Palstra *et al*. 2004; Sibbing *et al*. 2005). Nevertheless*, L. nedgia* and *L. surkis* (Anteneh *et al*. 2008; Gebremedhin *et al*. 2012; Anteneh 2013; Teshome *et al*. 2015), as well as *L. crassibarbis*, *L. gorgorensis*, and *L. longissimus* (Shkil *et al*. 2017), have been reported frequently from the river system. However, the exact location of their spawning grounds and conditions are still unclear.

Exact information on the timing of spawning migration, the exact location of the spawning sites, and habitat use of these threatened, commercially valuable species is critical for developing effective longterm conservation strategies in the Lake Tana Subbasin. In previous studies in the lake, fish migration was only estimated based on traditional population size assessments in different habitats using gillnet,

fyke net, and basket trap fishing. Such studies, however, lacked qualitative and behavioural data, including movement patterns. Moreover, the lack of tracking studies limits our understanding of the timing and extent of the movements of *Labeobarbus* species in rivers and their specific habitat use. To close these knowledge gaps, this study aims to investigate the spawning movements of *Labeobarbus* species migrating in the Gumara River with radio-telemetry focusing on (i) the traveling time to reach the spawning site and (ii) their post-spawning downstream movement. In addition, to further study habitat use for spawning, catch data from local fishermen were collected in the spawning areas.

Material and methods

Study area

Gumara River is one of the tributary rivers of Lake Tana, which is the largest lake in the north-western highlands of Ethiopia (Fig. 1). The river has a catchment area of about 1394 km². It is situated within between 11º34′41.4″ – 11º56′36″ N, and 37º29′30″ – 38º10′58″ E (Andargachew & Fantahun 2017). The river is fed by several small intermittent streams, including the Kizen, Wonzuma, Dukalit, and Chan (Palstra *et al*. 2004; Abate *et al*. 2015). The river flows 133 kilometers from the Guna Mountains to Lake Tana. Based on meteorological and remote sensing precipitation data from 1981 to 2019, the average annual rainfall in the catchment is about 1326 mm per year (Abebe *et al*. 2020). The annual temperatures range from 16°C to 32°C (Wondim 2016).

The Gumara River and its tributaries are frequented by many of the lake's upstream spawning *Labeobarbus* species (Nagelkerke & Sibbing 1996). Large boulders and cobble/gravel riffles with fast to moderate-fast flowing water in this upstream section of the study area are ideal spawning sites for *Labeobarbus* species (Nagelkerke & Sibbing 1996; Dgebuadze *et al*.1999; Palstra *et al*. 2004; Anteneh *et al*. 2012; Shkil *et al*. 2017). Fish spawning movement is thought to be interrupted by a big waterfall (locally called Durubaw, approximately 12 m high) on the main Gumara River above a small village called Wanzaye and 48.2 river kilometers (rkm) from the Gumara River mouth in the lake (Palstra *et al*. 2004).

Figure 1 – Location of the study area and tagged fish detection points in Gumara River and its tributaries.

An overview of Cyprinid's gonadal maturation stages which was adopted from Nagelkerke & Sibbing (1996) and based on De Silva *et al*. (1985) and Pet *et al*. (1996).

Sampling fish for tagging

Fish specimens were collected using multifilament gillnets with stretched mesh sizes of 10, 12, and 14 cm at the mouth of the Gumara River, where adult migratory fish aggregated. The nets were checked at 30- to 60-minute intervals to reduce mortality risk. A 2.4 m long cast net and a 4.0 m wide and 0.7 m deep fyke net were also used to catch fish in the Gumara River and its tributaries. The fyke net was placed at dusk in a relatively shallow and narrow stretch to catch upstream migrating fish. Metal rods and big stones were used to fix the fyke net in its position. The trapped fish were collected and identified at the species level. Their readiness for spawning or sexual maturation was assessed by pressing the abdomen and observing the presence of running eggs or sperm. Active and unharmed fish specimens were chosen as experimental animals for tagging.

Fish catches at the spawning sites

Monthly catch data were obtained based on several approaches. At the spawning habitats, fish catch data were collected from fishermen, or based on catches using cast net and fyke net fishing. In addition, at the Gumara River mouth catch data were obtained from fish collections on Sundays using multifilament gillnets with stretched mesh sizes ranging from 12 to 16 cm. Upstream in the Gumara River, catch data were obtained from catches of fishermen using long stick scoop nets with stretched mesh sizes of 8 to 10 cm, and hooks. The catch data were subsequently combined into monthly catches to quantify the migration of fish to the spawning habitats. Additionally, the number of fish species that visited a tributary stream was determined by catching specimens that returned from spawning streams to the Gumara River channel after midnight by setting up a fyke net facing upstream. The maturity stage of the captured specimens at each spawning site was determined by employing the gonadal developmental stages classification for *Labeobarbus* species as outlined in Nagelkerke & Sibbing (1996) (Table 1).

Biological information and track data for radio tagged *Labeobarbus* species in Gumara River and its tributaries. Abbreviations in the Fish ID: pla=*L. platydorsus*; meg=*L. megastoma*; and tru=*L. truttiformis*. Capture sites: Gunk=Gumara River near Kizen steam; GuRM=Gumara River at the river mouth; Gunw=Gumara River near Wonzuma stream; Kizn=Kizen stream.

Fish tagging and tracking

This study was conducted during the rainy seasons in 2018, 2019, 2020, and 2021, from July to October, which is an ideal period for most species of *Labeobarbus* to migrate for spawning in the area (Palstra *et al*. 2004; Anteneh *et al*. 2012). Four sites were used for specimen collection and tagging of targeted fish species. The first site was at the lake-river junction (i.e., the mouth of the Gumara River at Lake Tana). The specimens tagged at the river mouth were used to investigate the upstream movement of fish and the time to reach the potential spawning grounds. Moreover, samples taken from the Kizen (39.7 rkm), Wonzuma (43.5 rkm), and Dukalit (44.2 rkm) tributary streams, as well as the Gumara River channel (Fig. 1) close to those stream mouths, were used to investigate (i) their final upstream spawning destination, (ii) the time required to reach those destinations, and (iii) their downstream movement to the lake. These upstream sites were selected based on the spawning behaviour as described in previous studies (Nagelkerke & Sibbing 1996; Dgebuadze *et al*. 1999; Palstra *et al*. 2004; Anteneh *et al*. 2012; Shkil *et al*. 2017), and the assumption that the downstream habitats have muddy substrates, which may not be suitable for the riverine spawning *Labeobarbus* species. The specific spawning habitats of the tagged specimens were identified by observing the fish during their overnight stays at each putative spawning riffle habitat.

Twenty-two adult running individuals (fishes with a large amount of sperm or egg extruded by pressing the abdomen) were collected from reported riverine spawners with unknown specific spawning habitats (Table 2). In this tracking experiment, a river kilometer (rkm) was used as the distance measuring unit

for fish movement, which started from the mouth of the Gumara River to the upstream fish destination, and from the spawning area back to the lake.

Collected fish were placed in a tank and visually inspected to check if their post-capture movement was not impeded. Based on their swimming activity, healthy individuals were selected for tagging with a radio transmitter with a unique frequency (ATS Inc, USA, ranging between 150.110 to 150.662 MHz). The specimens were then immersed in a solution of 2-phenoxyethanol with lake/stream water (0.4 ml/L) for about 30 to 60 seconds until they lost balance. The duration of anesthesia by 2-phenoxyethanol solution was determined during preliminary trials before the start of the tagging experiment. Each fish was taken out of the anesthetic solution, and the Fork length (FL) to the nearest 0.1 cm was measured using a measuring tape. The specimens were grouped into their respective developmental stage following Nagelkere & Sibbing (1996) (Table 1). The body weight (in grams) was measured with a digital balance. The fish was moved from the anaesthetic bath to a container filled with stream/lake water for surgery. The area of needle insertion was sterilized using a povidone-iodine antiseptic solution before tagging. A 2 to 3-cm incision was made between the pelvic girdle and the anal fin in the abdomen area. Transmitters of the model F1215 (weighing 13 grams) and the model F1225 (weighing 17 grams), which contain a lithium battery that lasts 202 to 362 days and 280 to 500 days, respectively, were utilized for larger fish. Small-sized transmitters (model F1170), weighing 4 g and having a battery life of 189 to 441 days, and model F1415, weighing 0.75 g and having a Silver Oxide battery life of about 24 days, were implanted in fish with a body mass ratio or tag ratio (tag weight/fish body weight) of 2.5 % or less (Table 2). The incision was closed with two or three single interrupted sutures with sterile Ethicon 3/0 catgut on a 24-mm triangular cutting and resorptive suture material. Finally, the stitched incision was swabbed with Vetbond (tissue adhesive) to aid wound closure (Hegna *et al*. 2019). The surgery took about 3 to 6 minutes.

After surgery, the specimens were transferred to a 40-litre holding tank and held in the tank for about 5 to 10 minutes until they restored their balance and normal swimming capacity. After demonstrating full recovery and spontaneous swimming activity, the tagged individuals were released at the capture site. A scanning receiver (Advanced Telemetry Systems (ATS) model R410) was used to track the movement of the tagged fishes. Tracking was conducted using an antenna mounted on a car or boat (Browning Model BR-150-S), along with both a three-element handheld Yagi antenna (Model F152-3FB 15144) and a five-element handheld Yagi antenna (Model F150-5FB 130853). These antennas enabled the detection of tags within a range of approximately 250 to 350 meters.

The movement of fish was tracked using a car or boat during the day, followed by on-foot tracking during day and night towards the spawning grounds up to a place called Durubaw (at 48.2 river kilometers, Fig. 1). The handheld Yagi antenna was held vertically to detect the position of a fish from a distance. Following detection, the strongest signal obtained at the lowest gain was used to estimate the position of the tagged fish, and the locations of the fish were determined by bi-angulation from markers on the riverbanks and marked using a Geographical Positioning System (GPS) with an accuracy of 3 m. Fish were tracked three to five days per week, and once the fish approached the presumed spawning area, tracking was conducted day and night until the fish returned downstream. The ground distance moved (i.e., the difference between each detection point) was calculated in rkm and recorded for each tagged fish. The time difference between the two detection points was used to calculate the specimens' ground speed in the river or stream between the first and second detection points, assuming the fish travelled in a straight line and with a constant speed between the recorded positions (Zarada *et al*. 2019). Visual observations were additionally carried out to identify the spawning activity of fish without tags.

Physical and chemical characteristics of the water bodies to which fish migrated for spawning were measured during the spawning seasons of 2018 to 2021. The parameters measured included water

The travelled distance and the ground speed used by upstream and downstream moving radio-tagged *Labeobarbus* species in the Gumara River and its tributaries.

* migrated to the Kizen tributary.

depth, current speed, concentration of dissolved oxygen, temperature, turbidity, and substrate type and composition. Dissolved oxygen, temperature, and conductivity were measured in situ using a YSI ProDSS (digital sampling system) multi-meter probe. Because measuring the depth at the high flooding time was difficult in the main river, it was estimated using a combination of measurements with a meterstick when the water level was lower and a reference point in the landscape. Flow velocity and turbidity in the Nephelometric Turbidity Unit (NTU) were measured using a Geopacks advanced stream flow meter and a Turbidity meter model AL450T-IR, respectively. The type and size of the substrate were assessed when the water level was low. Substrate types were assessed using a caliper at each point of the presumed spawning habitat. Particles less or equal to 2.0 mm were classified as silt/clay/sand, between 2.1 and 16.0 mm in size as gravel, between 16.0 and 64.0 mm as pebble, and between 64.1 and 256.0 mm as cobble (Wentworth 1922).

Data analysis

The Shapiro-Wilk test was used to check for normality, and Levene's test to test for equality of variance. The Wilcoxon rank test was applied to examine the variation in fish length data between spawners in rivers and streams. The Kruskal-Wallis test was used to test for significant differences in ground speed among tagged fish. Wilcoxon's two-sample test was also used to compare the difference in ground speed in the home range between upstream and downstream directional movements. One-way ANOVA was also applied to compare the physicochemical variables of the Gumara River and selected tributary streams and between species. Tukey's Honestly Significant Difference (Tukey's HSD) posthoc test for pairwise comparisons was used when significant differences $(p<0.05)$ were found. Data of fish habitat use, comprising the measured physical and chemical parameters, were statistically explored in a principal component analysis (PCA), using the factoextra package, to illustrate the patterns in habitat use of the species in their preferred sites. All statistical analyses were performed in R 4.0.2 (R Core Team 2020).

Results

Fish movement for spawning

In this study, a total of 22 individuals belonging to three *Labeobarbus* species (*L. megastoma*, *L. platydorsus*, and *L. truttiformis*) were tagged with radio transmitters. Out of these, 16 were tagged for upstream migration toward spawning habitats, while the remaining 6 were tagged for downstream migration back to the lake (Table 2 for details). Fourteen (63.6%) of the tagged fishes were tracked while moving in the river and tributary streams (Table 3).

Upstream movement

Among the fish specimens tagged at the Gumara River Mouth (GuRM) in 2019 and 2021, only five (55.6%) were detected at least two times at different locations when moving to the presumed spawning habitats, while we failed to detect the others (Table 2). The tagged fish with code meg332 was located for the first time at 6.1 rkm in the Gumara River and moved up to 42.0 rkm at speeds ranging from 0.07 to 1.45 km h-1. This fish stayed for about four days between 41.5 to 42.0 rkm, which is one of the presumed spawning grounds for the species. Some fish tagged at the GuRM (e.g., meg152 and tru582), stayed for days in the river mouth, while meg523 left the site within a day and was found at 2.3 rkm the day after being released. In their upstream movement, fish typically rest for hours up to several days in deeper areas with lower water currents. As a result, their upstream migratory activity required 9 to 27 days (with an average of 18.6 days) to reach the spawning habitats. The upstream fish movement was slow when the fish approached the spawning habitats.

The individuals with code pla602 and meg621 moved up to 42.1 rkm and 43.6 rkm with speeds ranging from 0.3 to 0.7 kmh-1 and 0.11 to 0.65 kmh-1, respectively (Table 3). These two fish stayed almost in the same location at a place called Megenagna (at 41.5 rkm), which is thought to be a spawning location and a frequented fishing spot for local fishers. The other fish, meg641, moved up to 44.4 rkm at speeds ranging from 0.24 to 0.37 kmh-1. Before being captured by fishers, this fish stayed for a day in the nearby area with a pebble/cobble substrate, which is potentially a spawning site.

As observed in the tagged fish and from fyke net catches, the final migration to their spawning grounds in the tributary streams frequently started at dusk. Those fish may return to the main river at dawn on the same day or stay longer. For example, the tagged male at Kizen stream, tru602, stayed for two days in the spawning area, which is 1.73 km above the Gumara-Kizen confluence. By contrast, the two female *L. truttiformis,* tru662 and tru411, moved up to 1.48 km and 1.67 km, respectively, and returned during dawn on the same day. The ground speed of fish in the tributary stream ranged from 0.20 to 1.50 km h^{-1} $(\text{mean} \pm SD = 0.79 \pm 0.46 \text{ km h}^{-1})$, while in the Gumara River, it ranged from 0.01 to 2.59 km h⁻¹ (mean $\pm SD$) $= 0.62 \pm 0.58$ km h⁻¹) but this difference was not statistically significant ($p = 0.345$).

Downstream movement

Among the tagged fish ($n = 6$) in 2018 and 2021, three individuals moved downstream and reached Lake Tana, while fishers probably caught the others. The fish with codes pla473 and pla211 which were released at 39.7 rkm, completed the downstream movement in seven days, while meg253, which was released at 43.5 rkm, completed the journey in sixteen days. The ground speed for downstream movement (mean \pm SD = 0.98 \pm 0.86 kmh⁻¹) was not significantly different from the upstream movement speed (mean \pm SD = 0.55 \pm 0.39 kmh⁻¹) in their home range (Wilcoxon test, *p*=0.193).

Based on the pooled tagged fish data, the net average ground speed for *L. megastoma* (0.61 ± 0.57 kmh⁻¹), *L. platydorsus* (0.92 \pm 0.87 kmh⁻¹), and *L. truttiformis* (0.61 \pm 0.37 kmh⁻¹) was not significantly different among species.

Migratory fish species at spawning sites

During the spawning seasons of 2020 and 2021, 275 *Labeobarbus* individuals from eleven species (132 from our captures and 143 from fishermen's catches) were collected at the spawning location (Table 4). Among the collected fish specimens, 90.2% (n = 248) were classified at stage VI, with the remaining 4.0%, 3.6%, and 2.2% corresponding to stages IV, VII, and V, respectively. The fish specimens at the spawning sites encompassing stages IV to VII reached lengths from 12.3 cm to 71.4 cm FL. The lengths of the fish differed significantly between individuals being collected in the main river (mean $= 45.0 \text{ cm}$) and in the streams (25.3 cm) (Wilcoxon signed-rank test, $p \le 0.001$). Males of the same species were generally smaller than females (Fig. 2) in the spawning area, and the ratio of females to males in the spawning population was 0.57.

Across the three months of the spawning season, the number of fishes caught was highest in September (59.9%), and lowest in August (20.3%) and October (19.8%) (Fig. 3). The high number of individuals collected in September was in line with our hypothesis used to develop the tracking experiment, namely that fish mainly migrate in August and reach the spawning area in September, thereby increasing the spawning population.

Habitat characteristics

The measured physicochemical parameters during fish movements and at the spawning habitats are summarized in Tables 5–6, and Fig. 4. An overall monthly increase in temperature was observed from July to October in the river and tributary streams (Fig. 5). The water temperature in the Gumara River varied from 18.5 to 24.0°C, and in the tributary streams from 17.9 to 25.0°C. This variation was however not statistically significant. Moreover, neither monthly variation nor species preference for a specific

Figure 2 – Boxplots displaying the length distribution of spawning males and females of *Labeobarbus* fish species in the spawning water bodies, Gumara River and its tributary streams.

The number of individuals collected from each fish species at the Gumara River and its tributary streams, the sex ratio (female (F) to male (M)), and the range (mean \pm SD) of the measured fork length (FL (cm)) in the spawning sites.

* The *p*-value indicates the significance level of difference in the size of fish specimens spawning in the river and tributary streams.

temperature at their detection point was significantly different. By contrast, conductivity ranging from 74.3 to 152.0 µS/cm in the Gumara River was significantly different compared to values measured in the tributary streams (ranging from 83.6 to 128.9 µS/cm). Similarly, turbidity in Gumara River (ranging from 18.5 to 915.0 NTU) was significantly different compared to values measured in the streams (ranging from 4.1 to 121.5 NTU). Only turbidity showed a significant difference between the respective spawning grounds of the different species (Table 5). The depth, with a range of 0.43 to 2.80 m at the spawning site in Gumara River, differed significantly from that in tributary streams (range 0.18 to 1.80 m). Dissolved oxygen levels in the Gumara River varied from 5.2 to 8.5 mg/L, while water speed ranged from 0.10 to

Figure 3 – Number of male and female individuals caught in Gumara River and its tributaries at the spawning sites.

Mean±standard deviation (SD) of measured physicochemical parameters during fish spawning movement at Gumara River and its tributary streams from 2018 to 2021. The right column displays the results of a one-way ANOVA test comparing the values between the different water bodies.

1.68 m/s. These values did not show a significant difference compared to those observed in the tributary streams, where dissolved oxygen levels ranged from 5.83 to 7.94 mg/L and water speed ranged from 0.05 to 0.91 m/s. In the stream spawning habitat, gravel was the predominant substratum type, whereas the main river channel was characterized by a dominance of gravel/pebble substratum.

In the PCA analysis, using both the measured physicochemical variables and spawning habitat use data, the initial two components account for 37.5% and 21.1% of the variance in the datasets, respectively. PC1

Figure 4 – Biplot of the first and second axes of a principal component analysis of the physicochemical variables showing the habitat preference of spawning species. Each ellipse represents 95% confidence intervals and captures 80% of the data in each species. Abbreviation: $D\hat{O} = \text{Dis solved Oxygen}$.

Results of ANOVA comparing the measured physicochemical parameters (mean \pm SD) at the detection point of the radio-tagged fish species in the spawning habitats at Gumara River and its tributaries.

Figure 5 – Boxplots showing the monthly differences in temperature (a) and water turbidity (b) in the Gumara River. The boxes represent the interquartile range, and the points outside the whiskers are outliers. The bold horizontal line within each box represents the median.

is positively correlated with increasing water turbidity, grain size, and water depth, while it is negatively correlated with increases in conductivity and water temperature. Water velocity is weakly but positively correlated with dissolved oxygen, along the second PC axis. Temperature and conductivity displayed a pronounced negative correlation with dissolved oxygen (Fig. 4). While there is some overlap in habitat use among the three species, the PCA results revealed spatial variation, particularly distinguishing *L. truttiformis* from *L. megastoma* and *L. platydorsus*. *Labeobarbus truttiformis* predominantly spawned in less turbid and shallow tributary streams which are characterized by a small grain size substratum.

Discussion

Fish movements

The spawning migration of *Labeobarbus* species in Lake Tana initiates in June, with fish gathering at the river mouth as the water becomes more turbid and increases in volume (Nagelkerke & Sibbing 1996). The data collected from the tagged fish at the river mouth in the present study indicate that they

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remain in aggregation near the river mouth for days to weeks. This period of aggregation could serve as an adaptation phase, allowing the fish to acclimate to in-flowing river water which is characterized by a high sediment load and relatively low temperature. Moreover, during this pre-spawning high flooding and aggregation phase, migratory *Labeobarbus* species may synchronize their reproductive cycle as frequently observed in migratory river-spawning fish species elsewhere (Bailly *et al*. 2008). Periods with high flow most likely favour the subsequent upstream migration of fish for two fundamental reasons. First, fish migration in flooded river water is guided by the water current as documented in studies such as Jonsson (1991), albeit it may be hindered by increased turbidity. Secondly, the elevated water level allows fish to reach spawning habitats that were inaccessible during the dry season.

In the first few weeks of July, individuals caught at the river mouth with gillnets were predominantly at stages IV and V, with only 31.6% (n = 29) being primarily males at stage VI. However, the patterns were different during late July and early August, when 93.3% of the specimens ($n = 71$) were at stage VI. The presence of more mature individuals in the aggregated population suggests that the majority of upstream migratory *Labeobarbus* species started their migration primarily in August, following the peak flooding which is characterized by a heavy load of sediment and plant material fragments. This observation also confirms previous findings that migratory *Labeobarbus* species move upstream after periods of intense flooding (Dgebuadze *et al*. 1999; Palstra *et al*. 2004; Anteneh *et al*. 2008).

The timing of upstream fish migration depends on various environmental factors, yet water temperature and river discharge are consistently identified as key influencing factors (Fox *et al*. 2000; Zhang *et al*. 2020). Water temperature is important as it has profound effects on the physiology, behaviour, and ecology of fish (Pankhurst & Porter 2003; Christensen *et al*. 2020). Changes in temperature have been shown to act as a trigger for the upstream movement of temperate fish species (Pankhurst & Porter 2003; Binder & Mc Donald 2008; Binder *et al*. 2010). For instance, a study on various cyprinid species, including the common barbel (*Barbus barbus*) and nase (*Chondrostoma nasus*), revealed that the start of migration coincided with an increase in water temperature and a decrease in discharge (Melcher & Schmutz 2010). Nevertheless, the environmental cues triggering spawning migration, such as water temperature, may vary among species. For some species, migration distinctly starts at a specific environmental threshold, while for others this initiation occurs within a wider range of environmental characteristics (Benitez & Ovidio 2018). In tropical water bodies, however, temperature has not been identified as the most important factor for initiating fish migration (Bizzotto *et al*. 2009). In tropical regions, river discharge appears to be the most critical factor initiating upstream fish migration, as a higher water volume and more turbid water provide a better protection against predation (Bizzotto *et al*. 2009; Jonsson 1991). This was also the case in the migratory *Labeobarbus* species from Lake Tana studied here because increased water levels resulting from higher precipitation in the upper catchment led to alterations in fish movement towards the tributary stream and changes in the duration spent in the spawning area. Similarly, the transition from July to August, marked by an increase in temperature but probably more importantly a reduction in high flood-driven turbidity, may favor the upstream migration of *Labeobarbus* species (Fig. 5).

Fish migration is not limited to a specific time of the day. However, many fish species show a preference for migrating upstream during twilight and darkness (Jonsson 1991; Zhang *et al*. 2020). Our observations suggest that the *Labeobarbus* specimens studied tend to migrate predominantly during nighttime, supporting these assumptions from other studies. This nocturnal migration pattern probably serves as an adaptation to avoid visual predators (Ibbotson *et al*. 2011), but it may also be a strategy to evade human disturbances, particularly fishing activities, as observed in the Gumara River.

During their upstream movement, the tagged fish consistently selected locations to rest in relatively deep water and habitats with low water speed. They moved swiftly in sections with higher flows. Consequently, the upstream spawning movement ended after reaching the spawning grounds between approximately 41.5 rkm to 44.4 rkm within one to four weeks. Shkil *et al*. (2017) proposed that some fish may migrate upstream to the Chan tributary junction, approximately 47.6 rkm. However, this could not be confirmed from our observations and fish may encounter challenges due to the presence of large stones and intense turbulence in the rapids.

Upstream movement requires energy (Brodersen *et al*. 2008). Certain species, such as salmon, do not feed during spawning migration and use their energy reserves to ensure successful migration (Newton *et al*. 2018). To conserve energy, fish use existing refuges, such as vegetation or boulders, to avoid strong currents (Brodersen *et al*. 2008). While the energy requirements during spawning migration of *Labeobarbus* species remain unknown, it is reasonable to assume that energy reserves are used when the fish are confronted with a high energy demand for migration during food shortages in high river flow conditions. The tagged fish preferred to migrate when the water velocity decreased and they remained in sheltered places or deeper pools for hours or even days. In the absence of refugia, tagged *Labeobarbus* species tended to occupy shallow depths in habitats with lower water currents and typically situated towards the side of the river channel. This behaviour was particularly observed during periods when the highest flooding occurred. The fish may therefore employ this strategy to maximize movement while conserving energy.

In lotic systems, the flow of headwater currents often changes due to variability in the amount of precipitation, which requires fish to adapt their movement accordingly. Consequently, when current velocity increases, upstream migrating fish exhibit higher ground speeds (Brodersen *et al*. 2008). During up- and downstream movement, migratory fish search for deeper and slow-moving water columns to rest and generally use shelters created by undercut structures, natural embankments, and boulders. Comparing migration speeds among species in lotic systems can be challenging due to variations in migration paths. However, in this experiment, assuming that fish movement occurred in a straight line, the ground speed was not significantly different between the three tagged species.

Fish at the spawning grounds

Eleven *Labeobarbus* species were collected in the spawning area above the Gumara River near Kizen, ~ 40 rkm to 45 rkm (Table 4). *Labeobarbus longissimus* was abundant in the tributary streams, Kizen and Dukalit, and this species migrated to the spawning ground in early August. Local fishers call this species "Gesgash", which means early visitor/migrant. In previous studies, *L. longissimus* was not reported as a riverine spawner and was considered to be a lake spawner (Nagelkerke & Sibbing 1996; Anteneh *et al*. 2012; Sibbing *et al*. 2005). However, this species was recently reported in the Gumara River (Shkil *et al*. 2017). The apparent absence or infrequent capture of this species during previous surveys can be attributed to its early arrival and departure from the spawning habitats which is typically completed in early September. Indeed, during this period, a relatively high volume of water in the river channel may hinder catching fish and hence the failure to detect this species. Moreover, in the present study, cast net fishing was used which may have facilitated the collection of specimens as compared to approaches used in other studies. Other species, such as *L. truttiformis*, *L. megastoma*, *L. crassibarbis*, *L. intermedius*, and *L. platydorsus* were found at the spawning sites from the second week of August onwards. In previous studies, *L. crassibarbis* was considered to be a lacustrine spawner (Nagelkerke & Sibbing 1996; Sibbing *et al*. 2005), but in the current study, several individuals were found (Table 4) in Gumara River at the spawning sites. Only a few running individuals of the late spawner *L. macrophtalmus* (Palstra *et al*. 2004) and the other species, *L. nedgia* and *L. gorgorinesis*, were present in our samples and those from fishers at Gumara River. These species were also reported to be riverine spawners in earlier classifications by Shkil *et al*. (2017), which is in line with our findings. Another late-spawning species, *L. brevicephalus*, which is relatively small-sized compared to other *Labeobarbus* species in the lake*,* was observed in the tributary streams starting from late September when the water volume and

turbidity declined. This is consistent with the findings in Palstra *et al*. (2004). However, despite their small size, the number of individuals belonging to *L. brevicephalus* was low in the catches of fishermen and in our survey, and it was even lower than the numbers reported in Palstra *et al*. (2004).

Although all three tagged *Labeobarbus* species spawn on a gravel/cobble bottom substrate, some spatial segregation between these species and also between other taxa was observed. *Labeobarbus truttiformis* primarily spawned in tributary rivers like Kizen and particularly in habitats with a substrate consisting of relatively small grains (gravel). Spawning *L. brevicephalus* and *L. longissimus* were also observed in tributary rivers, and particularly in Dukalit and Kizen, but seemed to prefer habitats characterized by substrates with larger grains. In contrast to these three species and with the exception of a few small-sized (< 25cm FL) *L. megastoma* individuals, both *L. megastoma* and *L. platydorsus* spawn in the main river channel between \sim 41.5 and 44.4 rkm on gravel/pebble substratum and not in the tributary rivers. These findings support previous studies (Palstra *et al*. 2004 and Shkil *et al*. 2017) in which these *Labeobarbus* species were reported to likely spawn in the main channel of Gumara River. The substantial presence (16.4%) of *L. crassibarbis* specimens observed in the catches of the fishers near the spawning ground of *L. megastoma* and *L. platydorsus*, suggests that also this species may use similar spawning habitats in Gumara River. In contrast, the catch data from both the river and stream habitats revealed a relatively high prevalence of the small-sized species *L. brevicephalus*, *L. tsanensis*, and *L. longissimus* in the shallower tributary streams, which suggests a body size-related difference in site selection between the river and streams (Fig. 6). This body size-related site selection is most likely due to differences in water depth, turbidity, and grain size of the substrates between the tributary streams and the main river channel. In Gumara River, the gravel/pebble spawning habitats for migratory *Labeobarbus* species have a patchy distribution and are affected by sedimentation and flow regimes. Over the four-year study period, it was observed that sedimentation and fluvial scour affected the substratum, leading to profile changes in some spawning habitats in both the tributary streams and Gumara River. These profile alterations may impact the accessibility of suitable and adequate spawning grounds for the fish (Montgomery *et al*. 1996).

Although many factors contribute to the ongoing decline of the population size of *Labeobarbus* species in Lake Tana, targeted fishing using gillnet dragging /filtering, and fencing and damming techniques at the spawning sites could be one of the most destructive practices observed in the Gumara River and

Figure 6 – The length distribution of migratory fish caught at the spawning sites in Gumara River and its tributary streams.

its tributaries. We therefore recommend that fishing activities at the spawning sites particularly the hotspots between 41 and 45 rkm, should be prohibited from August to October. This measure is likely to be critical for the conservation of these species, and the promotion of sustainable fisheries in the lake.

Conclusions

Despite the limited number of individuals tagged in this tracking experiment, our results unequivocally confirm that *Labeobarbus* species undertake yearly migrations to their spawning habitats and that this upstream movement takes at least a week. As the fish approach these spawning sites, their movement speed slows down, and they aggregate and occupy sites characterized by a deeper water column and relatively slow water currents during the day. They enter the riffle for spawning, typically from dusk to midnight. Male tagged individuals spent three to four days in the spawning locations, most likely waiting for a running mate. The three tagged fish species strongly prefer gravel/pebble size substrates. However, the spawning activity of *L. truttiformis* was specifically associated with gravel-sized bottoms in the tributary streams, and mainly in the Kizen stream, in sites with an average depth of 0.68 m and a water current of 0.5 m s⁻¹. The specific preference of this species for this stream can be attributed to the higher availability of suitable spawning habitats, the lack of barriers, and hence a good accessibility of this stream which is relatively long, and has a relatively high water volume compared to other tributaries in the basin.

This radio telemetry experiment represents the first attempt to study the migratory patterns of *Labeobarbus* species in Lake Tana. However, open-access fishing, fencing, or other obstructions to fish movement and removal of the tagged fish specimens complicated our study. In addition, manual tracking proved to be labour-intensive and time-consuming, and this is particularly the case in rivers like Gumara with no river-side walkways. For future studies using radio telemetry, we recommend exploring the implementation of fixed stations for detecting fish movement at particular distances upstream, or by using receivers mounted on drones for aerial tracking over rivers and tributaries.

Some species, such as *L. macrophtalmus*, which were categorized as riverine spawners in previous studies, were only represented by a single specimen in our catch data. This suggests that the population size of this species has declined, which emphasizes the urgency to take immediate management and conservation actions to protect this species, as well as other riverine spawning fish species.

Ethics statement

The Ethical Committee of Bahir Dar University, College of Agriculture and Environmental Sciences, approved the study. The care and use of animals followed all applicable international, national, and institutional guidelines.

Acknowledgements

The authors thank the Belgian Development Cooperation (VLIR-UOS) for funding this research through the Inter-University Cooperation of Flemish and Bahir Dar Universities (IUC-BDU). Bahir Dar University's VLIR-UOS project staff are acknowledged for the support and logistics provided. Two anonymous reviewers and the editor are thanked for providing constrictive and insightful comments and suggestions to improve the manuscript. We thank Dr. Belay Abdissa from Bahir Dar Fishery and Other Aquatic Life Research Center and Dr. Melaku Bogale from Bahir Dar University for their technical help.

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Belg. J. Zool. 154: 97–116 (2024)

Manuscript received: 10 August 2022 Manuscript accepted: 28 June 2024 Published on: 9 July 2024 Branch editor: Luc Brendonck