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Aulne L Batiabo Mikembi
Faculté de Sciences et Techniques, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo; Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Armel Ibala Zamba
Ecole Nationale Supérieure Agronomie et de Foresterie, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo; Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Victor Mamonekene
Ecole Nationale Supérieure Agronomie et de Foresterie, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo; Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Honest F Poaty Ngot
Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Hélène Dembe Louvinguila Tenda
Ecole Nationale Supérieure Agronomie et de Foresterie, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo; Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Joseph Vouidibio
Faculté de Sciences et Techniques, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo.

Correspondence
Aulne L Batiabo Mikembi
Faculté de Sciences et Techniques, Université Marien Ngouabi, BP : 69, Brazzaville, République du Congo; Institut National de Recherche en Sciences Exactes et Naturelles, BP : 2400, Brazzaville, République du Congo.

Diversity and distribution of fish species along the Loua River, lower Congo river basin (Republic of the Congo, Central Africa)

Aulne L Batiabo Mikembi, Armel Ibala Zamba, Victor Mamonekene, Honest F Poaty Ngot, Hélène Dembe Louvinguila Tenda and Joseph Vouidibio

Abstract
Ichthyofaunal diversity and distribution were studied in the Loua River, a small tributary of the Congo River located downstream of Pool Malebo (Republic of the Congo). The study area encompassed the entire length of the river and comprised six stations, each sampled four times over a two-year period. Fishing techniques following standardized methods utilizing dip nets and monofilament gill nets, and 17 environment variables were measured at each station. Twenty-eight fish species belonging to 19 genera and 12 families were recovered with the Alestidae and Mormyridae being the most diversified. Canonical correspondence analysis with forward selection coupled with Monte Carlo permutation tests identified current speed and distance from the river bank as accounting for 79.0% of total variance (p<0.05). Species richness increases from upstream to downstream.

Keywords: Freshwater fish, diversity, environmental variables, Loua River, Congo Basin

1. Introduction
As human populations continue to grow and impact freshwater resources, improved methods of allocating and protecting water quality become increasingly critical [1]. Long spared these impacts, central Africa is now feeling their effects although impacts from direct pollution are generally still relatively limited in extent [2-4]. Nonetheless, the consequences of human activities potentially endanger the biological integrity of central African freshwater ecosystems and the diversity of their ichthyofaunas [5].
The main goal of ecological studies of fish communities is to understand the mechanisms and processes responsible for, and similarities between, assemblages [6]. And understanding the spatial and temporal patterns in both the structure of communities, and the size and distribution of populations, is an important function of ecological studies [7]. Therefore, the monitoring of populations is essential for this understanding, and for informing management of ecosystems [8]. However, it is known that distribution and abundance of stream fishes are influenced by many factors operating at multiple scales [9]. Local-scale habitat variables, such as substrate composition, presence of pools, and the amount of available cover have all been shown to correlate strongly with fish assemblage structure [10, 11, 5]. Unfortunately, investigations of the drivers of fish community assemblages have rarely been performed in Africa [12, 13], and the few available studies have mostly been undertaken in West Africa [14-17, 12, 18, 13, 3], and less commonly in South [19] and Central Africa [20-24, 5].
To contribute to the knowledge of the diversity and ecology of the Congo basin ichthyofauna, the present study was undertaken in the Loua River basin where the diversity and the spatial distribution fish population was investigated and the main environmental variables associated with species assemblages was determined.

2. Material and methods
2.1. Study area
Fish samples were collected in the Loua River, a small right bank affluent of the lower Congo River, located downstream of Pool Malebo in the Republic of the Congo. With a main-channel

length of around 25 km, the Loua River is situated in Madibou, the 8th district of the city of Brazzaville, draining the southern part of the city.

2.2. Fish sampling

Six stations along the Loua River were sampled, from its source to its confluence with the Congo main channel (Figure 1). These were Mayitoukou (May), Makana (Mak), Chéval (Che), Eglise (Egl), Sabine (Sab) and Confluence (Con). Each station was sampled four times over a two-year period (January 2015 – December 2016), including both the dry and rainy seasons. Standardized fishing techniques utilized dip nets and an array of five monofilament gill nets of 10, 12, 15, 20 and 30 mm mesh size. At each station, dip nets were used during 30 min before setting gill nets overnight from 17 h to 7 h following [25]. Fish species were identified in the field, specimens were counted by species, measured to the nearest millimeter standard length (SL), and weighed (fresh total weight) to the nearest 0.01 g. Samples for which field identification was uncertain were preserved in 10% formalin for subsequent identification in the laboratory of the National Institute of Research in Exact and Natural Science (IRENS), Republic of the Congo. Families follow [26], and the genera and species are in alphabetical order.

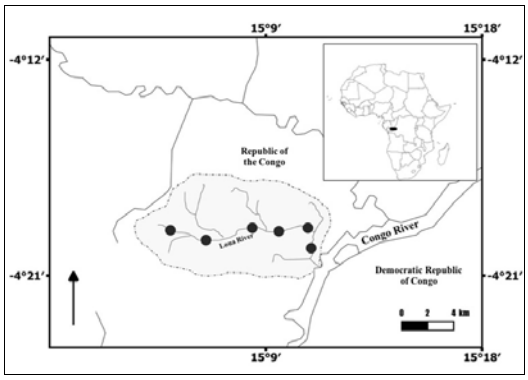

Fig 1: Loua River basin and sampling stations

2.3. Environmental data

Seventeen environmental variables were collected at each station (Table 1). Sampling protocol followed [25]. The following substrate categories were identified and scored as %: rocks; sand; mud (without wood and aquatic plants); dead wood; dead leaves; aquatic plants. For each station, values given are mean values for 5 gill-nets, 1 day, 2 seasons and 2 years.

Table 1: Physico-chemical characteristics measured. *: Mean values for 5 gill-nets, 1 days, 2 seasons and 2 years (total n = 20)

Environmental variables	Code	Stations					
		May	Mak	Che	Egl	Sab	Con
Temperature (°C)*	Temp	22.3	21.7	23.1	23.1	22.8	22.1
pH*	PH	4.9	5.6	6.1	6.1	5.9	6.1
Conductivity (µS/cm)*	Co	12.5	9.2	12	14.3	17.5	15.6
Salinity* (ppm)	Sal	13.4	12.4	13.1	14.3	14.7	15.9
Total Dissolved Solid (mg/l)*	TDS	8.9	6.7	8.1	10.2	12.5	12.1
Altitude (m)	Alt	360	327.5	298.5	282.5	267	258
Depth (m)*	Prof	0.2	0.3	0.8	1.2	1.4	1.5
Current speed (m/s)*	Vit	1.3	0.8	0.7	0.8	0.7	0.6
Distance from river bank (m)	Disb	0.3	1.1	0.4	0.3	0.4	5.5
Minimum width (m)	Larg	1.6	2.5	5	6.4	4.2	8.7
Canopy height (m)	Hcan	0	1.6	1.6	0	2.4	0
Substrate types (in %)							
Rocks	Roch	0	0	13.8	76.3	62.5	78.4
Sand	Sabl	60	65	69.5	8.7	21	7.3
Mud	Bou	19	11.2	4.7	0	0	0
Dead wood	Bmor	8.8	6.3	5.3	4.3	4.2	4.3
Dead leaves	Fmor	8.7	8.7	4.7	5	8	5
Aquatic plants	Paqu	3.5	8.8	2	5.7	4.3	5

2.4. Statistical analyses

The ecological health of the Loua River basin was evaluated at each station by calculating three ecological diversity indices [27, 28], using PRIMER version 5 [29]: Species richness S, Shannon index H', and Equitability R. Species richness (S) is the number of species represented in the catches. Shannon diversity index (H') [30] were calculated according the formula:

$$H' = -\sum_{i=1}^{S} P_i \ln P_i$$

With $P_i = n_i/N$; N being the total number of individuals obtained for all species, ni is the number of individuals of species *i* and P_i the relative abundance of species *i* in the sample. Shannon index varies between 0 and H' maximum, calculated according to the formula:

$$H'max. = \ln S.$$

The Equitability (R) [31] indicates whether individuals are equally distributed among the species of the target area, and varies between 0 and 1. It tends towards 0 when the totality of catches is almost entirely of one species, and towards 1 when all species have the same abundance within given sample. It is calculated using the formula:

$$R = H'/H'max.$$

A unimodal Canonical correspondence analysis (CCA) of fish species-environment data was undertaken using Canonical Community Ordination (CANOCO version 4.5) based on gradient length (GL > 3) of the DCCA [32]. CCA were performed to identify associations between fish assemblages and environmental descriptors potentially influencing their

distribution. Therefore, two matrices covering the six stations were constructed: (1) numerical abundance of all species collected and (2) environmental variables. Monte Carlo tests (999 permutations, $p<0.05$) were used to select environmental variables explaining variation in the fish species data. Prior to ordination, fish abundance and environmental data were transformed to better meet the assumptions of normality [11] using respectively $\log_{10}(x+1)$ and $\ln(x+1)$ or $\text{ArcSin}\sqrt{x}$ for percentages [5].

3. Results

3.1. Species composition

Twenty-eight fish species belonging to 12 families and 19 genera are listed (Table 2). Among families collected, Alestidae and Mormyridae (n = 5 species each), Cichlidae, Clariidae and Mochokidae (n = 3 species each) are the five most represented. The remaining families are poorly represented ($1 \leq n \leq 2$).

Table 2: List of species collected, their code and abundance

Families and species	Code	Stations					
		May	Mak	Che	Egl	Sab	Con
Mormyridae							
Campylomormyrus rhynchophorus (Boulenger. 1898)	*Carh*	0.0	0.0	40.0	0.0	0.0	60.0
Marcusenius moorii (Günther. 1867)	*Mamo*	0.0	0.0	88.9	0.0	0.0	11.1
Mormyrus rume Valenciennes. 1847	*Moru*	0.0	40.0	20.0	0.0	40.0	0
Mormyrops boulengeri Pellegrin. 1900	*Mobo*	0.0	0.0	90.0	0.0	0.0	0.1
Petrocephalus simus Sauvage. 1879	*Pesi*	0.0	0.0	0.0	0.0	0.0	100
Cyprinidae							
Labeo cyclorhynchus Boulenger. 1899	*Lacy*	0.0	4.8	16.7	4.8	23.8	50.0
Raiamas christyi (Boulenger. 1920)	*Rach*	1.9	10.9	30.8	5.1	10.9	40.4
Alestidae							
Brycinus bimaculatus (Boulenger. 1899)	*Brbi*	0.0	0.0	0.0	0.0	0.0	100
Brycinus imberi (Peters. 1852)	*Brim*	0.0	0.0	0.0	0.0	0.0	100
Bryconaethiops yseuxi Boulenger. 1899	*Brys*	0.0	33.3	0.0	0.0	0.0	66.7
Micralestes acutidens (Peters. 1852)	*Miac*	0.0	13.3	0.0	0.0	0.0	86.7
Micralestes humilis Boulenger. 1899	*Mihu*	0.0	17.4	0.0	0.0	0.0	82.6
Citharinidae							
Citharinus congicus Boulenger. 1897	*Cico*	0.0	0.0	0.0	0.0	0.0	100
Distichodontidae							
Distichodus fasciolatus Boulenger. 1898	*Difa*	0.0	0.0	0.0	0.0	0.0	100
Claroteidae							
Parauchenoglanis punctatus (Boulenger. 1902)	*Papu*	27.8	38.9	11.1	11.1	11.1	0.0
Schilbeidae							
Schilbe intermedius Rüppell. 1832	*Scin*	0.0	0.0	0.0	100	0.0	0.0
Clariidae							
Clarias angolensis Steindachner. 1866	*Clan*	44.4	0.0	22.2	0	22.2	11.1
Clarias gabonensis Günther. 1867	*Clga*	0.0	0.0	50.0	50.0	0.0	0.0
Clarias platycephalus Boulenger. 1902	*Clpl*	0.0	0.0	66.7	0.0	33.3	0.00
Mochokidae							
Synodontis contractus Vinciguerra. 1928	*Syco*	33.3	0.0	0.0	66.7	0.0	0.0
Synodontis greshoffi Schilthuis. 1891	*Sygr*	20.0	60.0	0.0	20.0	0.0	0.0
Synodontis nigriventris David. 1936	*Syni*	0.0	0.0	33.3	0.0	0.0	66.7
Aplocheilidae							
Aphyosemion schioetzi Huber & Scheel. 1981	*Apsc*	100	0.0	0.0	0.0	0.0	0.0
Cichlidae							
Ctenochromis polli (Thys. 1964)	*Ctpo*	0.0	25.0	0.0	12.5	0.0	62.5
Hemichromis elongatus (Guichenot. 1861)	*Heel*	0.0	0.0	30.0	40.0	30.0	0.0
Hemichromis stellifer Loiselle. 1979	*Hest*	0.0	71.4	28.6	0.0	0.0	0.0
Anabantidae							
Ctenopoma nigropannosum Reichenow. 1875	*ctni*	0.0	0.0	100	0.0	0.0	0.0

3.2. Variation of ecological diversity indices

Diversity indices, including species richness (S), Shannon index (H'), Shannon maximum index (H' max). and Equitability (R) were calculated for each station (Table 3). With 30 specimens and three species, station 1 (May) was the least diversified, and station 3 (Che) was the most diversified with 96 specimens and 14 species. However, values of Equitability were highest 0.87 in station 4 (Egl) and lowest 0.64 in station 1.

Table 3: Ecological diversity indices. N: number of specimens; S: Species richness; H': Shannon index; H'max.: Shannon maximum index; R: Equitability.

N°	Stations	Code	S	N	H'	H'max.	R
1	Mayitoukou	May	3	30	0.70	1.10	0.64
2	Makana	Mak	11	42	1.94	2.40	0.81
3	Cheval	Che	14	96	1.84	2.64	0.70
4	Eglise	Egl	7	21	1.70	1.95	0.87
5	Sabine	Sab	7	37	1.49	1.95	0.76
6	Confluent	Con	20	165	2.14	3.00	0.71

3.3. Fish communities and environmental variables

Canonical correspondence analysis (Figure 2. a-b) indicates that the first two axes (55.5% and 38.4%, respectively) express 93.9% of cumulative variance in species data. Forward selection and Monte Carlo permutation (999 iteration) confirm that the contributions of axis 1 and 2 to data arrangement is significant (F = 2.99; p = 0.003). This test also identifies two environmental variables as accounting for 79.0% of total variance explained by the 17 variables: current speed (41.0%) and distance from bank (38.0%), and that these variables have significant (P<0.05) influence on the distribution of fish species in the Loua River.

Three Groups of sampling sites are distinguished in relation to both CCA Axes 1 and 2 (see Figure 2.a): Group A, represented by habitats close to the source (May); Group B, habitats composed of intermediate stations (Mak, Che, Egl and Sab); and Group C, composed of a single station (Con) situated to the confluence of the Loua River with the Congo River. The main fish species found in Group A, positively correlated with Axis 1 are *Aphyosemion schioetzi* and *Clarias angolensis*. Group B, negatively correlated with Axis 2, is mainly composed of *Clarias gabonensis*, *C. platycephalus*, *Ctenopoma nigropannosum*, *Hemichromis elongatus*, *H. stellifer*, *Labeo cyclorhynchus*, *Marcusenius moorii*, *Mormyrops boulengeri*, *Mormyrus rume*, *Parauchenoglanis punctatus*, *Raiamas christyi*, and *Synodontis greshoffi*. Group C, negatively correlated with Axis 1 and positively correlated with Axis 2, is distinguished by an assemblage including *Distichodus affinis*, *Brycinus bimaculatus*, *B. bimberi*, *Bryconaethiops yseuxi*, *Campylomormyrus rhynchophorus*, *Citharinus congicus*, *Ctenochromis polli*, *Micralestes acutidens*, *M. humilis*, *Petrocephalus simus*, *Shilbe intermedius*, *Synodontis contractus*, and *S. nigriventris*.

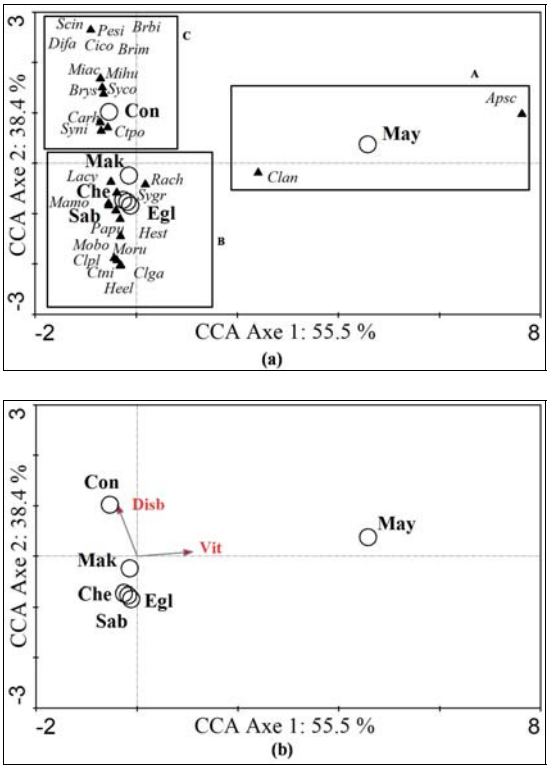

Fig 2: CCA ordination of species, stations and the two forward selected environmental variables. **A**: biplot of stations and species; **B**: biplot of stations and environment variables. ▲: species; O: stations.

4. Discussion

In the Loua River, a right bank affluent of the lower Congo River, we observed a total of 28 species, of which all are characteristic of the Congolese province [33]. Alestidae and Mormyridae dominated the composition of the ichthyofauna, and these families have already been reported as among the most diversified fish groups in the Congo River basin [34]. The presence study is one of the few ecological investigations of fishes performed in the Congo River basin and complements the short list of the similar studies recently undertaken in the Inkisi [24] and Lefini Rivers [5], respectively a left bank affluent of the Congo River in the Democratic Republic of the Congo (DRC), and a large right bank affluent in the Republic of the Congo (RC).

The Loua River basin is located in the Lower Congo Rapids freshwater ecoregion [35, 36], an ecoregion that receives the full brunt of untreated sewage, sediment, and industrial chemical pollutants from Brazzaville and Kinshasa, capitals located on opposite sides of the Congo River in the region of Malebo Pool. Given the high threat level to the region, the Lower Congo Rapids have been identified by [35] as an endangered ecoregion. Paradoxically, in view of its location passing through southern Brazzaville, in the present study, the three ecological diversity indices (S, H' and R), calculated based on species abundance, revealed that in all habitats sampled in the Loua (Groups) (Figure 2a), the H' (Group A: 0.70; Group B: mean = 1.74; Group C: 2.14) and R (Group A: 0.64; Group B: mean = 0.79; Group C: 0.71) indices are approaching their maximum values, respectively H' maximum (1.10; 2.23; 3.00) for H' and 1 for R (Table 3). According to [37] and [28], when all species of a community are in abundance and the environment in good ecological health, H' and R indices approach their maximum values. This is the case for the distribution of species abundance in the Loua river basin, despite a weak evenness observed at Mayitoukou (R = 0.64), principally explained by the slight dominance of *Aphyosemion schioetzi* in the catches. Indeed, except for some market gardening activities around Mayitoukou station, few other human activities currently exist along the course of the Loua River.

According to [38], the physical conditions found throughout a watercourse, from upstream to downstream, induces a response from biological communities, with a progressive change according to the capacities of species to adapt to environmental conditions and available food resources. This longitudinal zonation is accompanied by an increase in species richness through increasing habitat heterogeneity and volume. This standard observation of species richness increasing downstream [15-17, 19-21, 39] is also reported here for the Loua river (see Figure 2.a).

Waters of the Loua River basin, mostly (± 70%) bordered by a gallery forest, are in general acidic (4.9 ≤ pH ≤ 6.1) with a low conductivity (9.2-17.5 µS/ cm) which is probably due to the presence of organic matter (dead leaves, dead wood and mud). Similar results were reported by [5] (respectively 5.1 ≤ pH ≤ 6.1 and 5.9-9.9 µS/ cm) in the Lefini River basin, bordered by an important gallery forest. It is well known that forest rivers are often characterized by ion-poor waters, acidity (pH < 7) and low conductivity [40].

According to the CCA (Figure 2.b), current speed (41.0%) is the most important variable for fish distribution in the system. This variable is strongly correlated with Group A represented by the habitat close to the source, differing significantly from the other two habitat groups (p<0.05). In addition to this

particularity, the proximity of Mayitoukou (May) station to the source, undoubtedly contributes to its low fish diversity (3 species vs. > 7), principally represented by *Aphyosemion schioetzi*. According to [38], the short spring zones corresponding to narrow brooks are populated essentially by small species (such as *Enteromius spp.*, Cyprinodontiformes, and small alestids). Some other authors such as [41] and [42] have mentioned the importance of velocity on fish distribution in China, respectively in the Chishui River and in a headwater stream section of Lijiang River.

The distance from river bank (38.0%), characteristic for the Group C habitat composed of the confluent station (Con), represented the second most important variable for fish distribution in the Loua River. This station was most diversified (Figure 2.a), probably because of its wide channel width (minimal width = 8.7 m) providing various micro-habits and its proximity to the Congo River main channel. According to [43] habitat heterogeneity and diversity of available trophic resources increase with area, thus offering more available niches and consequently favouring the existence of a large number of species.

5. Conclusion

The present study contributes to a better understanding of fish communities in the Congo basin, by providing data on fish diversity and distribution from the Loua river, one of its small right bank tributaries. Alestidae and Mormyridae are the two most diversified families.

The high value of Shannon and Equitability indices in all habitats indicates that the Loua basin is in good ecological health. The velocity and distance from the river bank have been shown to have a significant influence on the distribution of fish assemblages. In addition, the traditional observation according to which species richness increases from the upstream to the downstream, is observed in the Loua river.

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