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Congo basin: From carbon to fishes

COBAFISH

E. VERHEYEN, W. WILLEMS, K. MARTENS, A.V. BORGES, F. DARCHAMBEAU, T. LAMBERT, J.-P. DESCY, S. PETROVIC, S. BOUILLON, C. TEODORU, D.X. SOTO, C. COCQUYT, J. C. TAYLOR, J. SNOEKS, E. DECRU, J. BAMPS, T. MOELANTS, L. ANDRÉ

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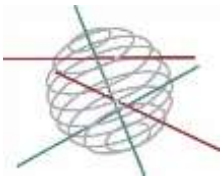

Atmosphere and terrestrial and marine ecosystems

FINAL REPORT

Congo basin: From carbon to fishes

COBAFISH

SD/AR/05A

Promotors
Erik Verheyen, Royal Belgian Institute of Natural Sciences
Alberto V. Borges, University of Liège
Steven Bouillon, KU Leuven
Christine Cocquyt, Botanic Garden Meise
Jos Snoeks, Royal Museum for Central Africa

Authors
Erik Verheyen, Royal Belgian Institute of Natural Sciences
Wim Willems, Royal Belgian Institute of Natural Sciences
Koen Martens, Royal Belgian Institute of Natural Sciences
Alberto V. Borges, University of Liège
François Darchambeau, University of Liège
Thibault Lambert, University of Liège
Jean-Pierre Descy, University of Liège
Sandro Petrovic, University of Liège
Steven Bouillon, KU Leuven
Cristian Teodoru, KU Leuven
David X. Soto, KU Leuven
Christine Cocquyt, Botanic Garden Meise
Jonathan C. Taylor, Botanic Garden Meise
Jos Snoeks, Royal Museum for Central Africa
Eva Decru, Royal Museum for Central Africa
Jolien Bamps, Royal Museum for Central Africa and Royal Belgian Institute of Natural Sciences
Tuur Moelants, Royal Museum for Central Africa
Luc André, Royal Museum for Central Africa

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Avenue Louise 231
Louizalaan 231
B-1050 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be

Contact person: Georges Jamart
+32 (0)2 238 36 90

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COORDINATOR:

P1. Erik Verheyen, Royal Belgian Institute of Natural Sciences, Operational Direction Taxonomy and Phylogeny – Vertebrates, Vautierstraat 29, 1000 Brussels, Belgium, erik.verheyen@naturalsciences.be

OTHER PARTNERS:

- **P2.** Alberto Borges, Université de Liège, Chemical Oceanography Unit, Institut de Physique (B5a), B-4000 Liège, alberto.borges@ulg.ac.be
- **P3.** Steven Bouillon, KU Leuven, Department of Earth and Environmental Sciences, Celestijnenlaan 200E, B-3001 Leuven, steven.bouillon@ees.kuleuven.be
- **P4.** Christine Cocquyt, Botanic Garden Meise, christine.cocquyt@botanicgardenmeise.be
- **P5.** Jos Snoeks, Koninklijk Museum voor Midden-Africa, Unit Vertebrates,Tervuren, jos.snoeks@africamuseum.be

AUTHOR(S):

E. Verheyen[1], W. Willems[1], K. Martens[1], A.V. Borges[2], F. Darchambeau[2], T. Lambert[2], J.-P. Descy[2], S. Petrovic[2], S. Bouillon[3], C. Teodoru[3], D.X. Soto[3], C. Cocquyt[4], J. Taylor[4], J. Snoeks[5], E. Decru[5], J., Bamps[5], T. Moelants[5], L. André[5]

Numbers in superscript refer to partner institution (1: Coordinator, 2: Partner 2, etc)

PROJECT WEBSITE(S): http://www.congobiodiv.org/nl/projecten/cobafish

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ACRONYMS and ABREVIATIONS

BBPf: Belgian Biodiversity Platform
$bSiO_2$: biogenic silica
C: carbon
cDOM: chromophoric dissolved organic matter
CH4: methane
CHEMTAX: program for estimating class abundances from chemical markers
CO_2: carbon dioxide
CSB: Centre de Surveillance de Biodiversité
DGCD: Belgian Development Cooperation
DIC: dissolved inorganic carbon
DNA: Deoxyribonucleic acid
DOC: dissolved organic carbon (concentrations expressed in mg L^{-1})
DOM: dissolved organic matter
DSi: dissolved silicon
EA-IRMS: elemental analyzer – isotope ratio mass spectrometry
FAO: Food and Agriculture Organization
***F*CH_4**: CH_4 flux across the air-water interface
***F*CO_2:** CO_2 flux across the air-water interface
fDOM: fluorescent dissolved organic matter
GHG's: greenhouse gases
HPLC: High Performance Liquid Chromatography
IUCN: International Union for the Conservation of Nature
JRC: Joint Research Centre
***k*:** gas transfer velocity
LM: light microscope
MAB: Man And Biosphere
MOB: methane-oxidizing bacteria
N: Nitrogen
N_2O: Nitrous oxide
O_2: Oxygen
pCO_2: partial pressure of CO_2
POC: particulate organic carbon (concentrations expressed in mg L^{-1})
R: pelagic community respiration
SEM: scanning electron microscope
Si: silicon
$SUVA_{254}$: specific ultraviolet absorbance of DOM at 254 nm, positively correlated with the degree of aromaticity of DOM.
S_R: Spectral ratio. Inversely correlated with the average molecular weight of DOM.
TA: total alkalinity (in mmol kg^{-1})
TSM: total suspended matter concentration (in mg L^{-1})
WWF: World Wide Fund for Nature
$\delta^{13}C_{DOC}$: stable carbon isotopic composition of DOC, used as a tracer of DOM sources.
ΔCH_4: air-water gradient of CH_4
ΔCO_2: air-water gradient of CO_2

SUMMARY

Context

The Congo River harbours the richest known fish species diversity on the African continent. Its fish fauna also represents a major source of proteins for the riparian human population. Despite of this, the ecology, dynamics and ecosystem functioning of the Congo River remain poorly understood.

The overall goal of the COBAFISH project is to link terrestrial inputs, primary producers (algae and aquatic macrophytes), macro-invertebrate and fish biodiversity to ecosystem dynamics and functioning in the Congo River in order to delineate factors that drive species and trophic biodiversity of fishes.

Objectives

To achieve this overall goal, COBAFISH addresses four key questions:

(1) How diverse are fish communities in two sub-catchments of the Congo River (Lobilo and Lomami) in terms of biodiversity and functional/trophic diversity?
(2) Which factors can be identified in regulating this diversity?
(3) To which extent do fish communities in these river systems depend on autochtonous (aquatic) primary production or on lateral allochtonous (terrestrial) production?
(4) What is the importance of seasonal flood events on global ecosystem functioning?

Main conclusions

Three extensive field campaigns were carried out (December 2012, September 2013, and March 2014). Three areas were thoroughly sampled for aquatic biogeochemistry, fish, aquatic macro-invertebrates and diatoms. Samples taken in the Lobilo River, which drains water from the UNESCO Man and Biosphere (MAB) Yangambi Reserve, represent the near-pristine condition, whereas the samples collected in the Lower Lomami River represents a human impacted area with different land-use patterns and vegetation. This allowed us to sample in stream water with different characteristics according to the features of the surrounding area/vegetation/land use.

Primary production and phytoplankton biomass were highest in the Congo River maintream, and negligible in black water streams such as the Lobilo. Green algae (both chlorophytes and streptophytes) dominated in the mainstem in high waters, whereas diatoms dominated in falling waters; cryptophytes and cyanobacteria were more abundant but still relatively low in the falling water period, both in the tributaries and in the main channel. Phytoplankton biomass and production was higher than previously reported in tropical river channels, and net phytoplankton growth occurred in the Congo River mainstem, whereas in other tropical rivers phytoplankton production mainly takes place in the floodplain lakes. This is due to generally low total suspended matter (TSM) values in the Congo and the relative shallowness of river channels that allows net phytoplankton production in the mainstem unlike in other deeper and more turbid tropical rivers such as the

Amazon. Pelagic community respiration (R) was overwhelmingly higher than primary production indicating a net heterotrophic status that is fuelled by allochthonous carbon inputs from terrestrial or wetland origin. Indeed, dissolved organic matter (DOM) in the Congo River and its tributaries mainly originates from terrestrial sources. A greater phytoplanktonic development was observed during low flow periods in the mainstem Congo River, but without any effect on the concentration and the composition of DOM. This can be related to the fact that phytoplankton exudates are very labile and rapidly assimilated by bacteria as shown in tropical lake waters such as Kivu (refer to results from the BELSPO EAGLES project). The data presented here strongly suggest that the autochthonous production is very limited and/or is not enough to significantly contribute to the DOM pool. Yet, CO_2 emissions to the atmosphere in three COBAFISH study sites and elshewhere in the Congo basin were higher up to an order of magnitude than R. This indicates that the dissolved CO_2 is laterally imported either from soils or wetlands. Within the Congo and across 12 African rivers dissolved CO_2 and CH_4 concentrations correlated to wetland coverage in the catchement, suggesting that the lateral inputs from wetlands are as important (if not more) that terrestrial soils. Indeed, hydrological connectivity is much higher in wetlands than non-flooded uplands where the CO_2 from soil respiration is usually released to unsaturated soil horizons and can be directly emitted back to the atmosphere and not be transported laterally into rivers. Finally, the comparison of the Congo and the Amazon reveals that CO_2 levels are similar in the river channels but CH_4 concentrations are three to four times higher in the river channel of the Congo than in the Amazon. This seems to be related to differences in seasonal water level changes that are lower in the Congo than in the Amazon, allowing the development of extensive and permanent floating macrophyte meadows along the mainstem and major tributaries that promote the transfer of CH_4 directly to river channels. Floating macrophytes in the Amazon are present in the floodplains but not along the river banks due to stronger currents and higher depths.

The total number of fish species found was 132. Lomami harboured a slightly higher number of species (81) than the other two rivers. The Lobilo clearly had the highest number of unique species (31) more than double the number in the two other systems, hence illustrating its special character, that also showed in the physio-chemical parameters. For most fish groups, taxonomic problems were encountered, which illustrates the poor knowledge of the ichthyofauna of the Congo basin. Some of these were tackled during the project. A revision of the *Distichodus antonii* assemblage, resulted in the removal of one species from the list of Congo species and the discovery of two new species. For this group and for the genus *Citharinus* new identification keys were compiled. Another illustration of the poor knowledge of the fish fauna of the region studied is the relatively low identification success compared to other barcoding studies, as a result of many discrepancies between morphology-based species identifications and the different lineages recognized by this molecular technique. In addition, clear indications were found for the presence of cryptic species and hybridization events in the area.

One of the most interesting results of the combination of morphological and molecular techniques was found in *Enteromius*. In this genus of small carp-like fishes, 23 genetic lineages (COI, mtDNA) were found within four species as identified based on the literature available. Subsequent morphological analyses revealed most lineages to be also morphologically different, suggesting they might represent distinct species, almost all new to science.

No indication was found for genetic population structuring for the fishes in the three systems. Multivariate analyses of fish occurrence data confirmed the separation of the Lobilo from the two other systems, but also differences in species composition between the three expeditions became apparent. In a preliminary analysis with a forward selection procedure, pH and temperature came out as important variables with uncorrelated contributions to fish distribution.

The general picture resulting from the stomach analyses is that of a dominance of omnivorous species in all rivers, followed by invertivores, illustrating again the mainly generalist character of the fish fauna of the region. However, one of the main findings is that the majority of the species in some way are dependent on the terrestrial environment for food. Indeed, 61% of fishes representing 37% of species had eaten prey of terrestrial origin.

Analysis of the aquatic foodweb using both gut content analyses and stable isotope analyses allows us to conclude that (i) terrestrial invertebrates comprise a surprisingly large contribution to the diet of many fish species, highlighting the importance of riparian vegetation and habitats not only as a refuge but also to provide access to terrestrial food sources, and (ii) despite the low aquatic primary productivity, the isotope data indicate a wide diversity of food sources can contribute and suggest that at least part of the fish community is specialized towards certain basal food sources. A significant number of invertebrate and fish samples show particularly low $\delta^{13}C$ signatures, which we currently hypothesize to reflect a contribution of methane-derived carbon via methanotrophic bacteria.

Finally, the environmental monitoring data obtained from two stations near Kisangani (one located on the mainstem Congo River, one on the Tshopo River) at two-weekly intervals is ongoing and will be continued outside of the COBAFISH project through an agreement with the CSB/ University of Kisangani. This forms an important longer-term commitment and is leading to an unpredecentedly large dataset on riverine biogeochemistry in this part of the world, currently covering 4 full years of data acquisition.

Output for sustainable development

Present day total anthropogenic CO_2 emissions are estimated at 9.4 PgC (10^{15} gC) yr^{-1}, part of which accumulates in the atmosphere at a rate of 4.2 PgC yr^{-1}, while oceans and land equally absorb ~2.6 PgC yr^{-1}. This terrestrial sink of anthropogenic CO_2 motivated the United Nations' "Reducing Emissions from Deforestation and forest Degradation" (REDD) program, which aims at mitigating climate change through reducing emissions of greenhouse gases (GHGs) and removing GHGs through enhanced forest management in developing countries. The latter is of particular relevance for Sub-Saharan African countries, which host significant tropical rain forest cover. Our estimates of CO_2 and CH_4 emissions from rivers in Central Africa and in the Congo show that the emissions of these GHGs from inland waters strongly offset the terrestrial carbon sink. We recommend that emissions from rivers are better quantified in a systematic fashion for a thorough accounting to the United Nations Framework Convention on Climate Change (UNFCCC) in the frame of the national inventory reports and in the frame of REDD. For the particular case of Congo, our data acquired in the chosen three sites in COBAFISH do not encompass the full range of aquatic biogeochemistry and in particular GHG emissions, hence, we also recommend large scale basin wide quantification of GHG emissions from inland waters (rivers and lakes).

Gut content analysis indicates that most of the fish have food sources from riparian vegetation. We recommend in case of forest clearing for land usage that a riparian vegetation fringe is accommodated to maintain food sources for fish and for sustainable fisheries management.

Keywords

Congo basin, Congo River, fish ecology, biodiversity, foodwebs, diatoms, biogeochemistry, greenhouse gases, fisheries.

1. INTRODUCTION

1.1 Context

The COBAFISH project aims to yield a better understanding of interactions between biodiversity and the functioning of the Congo River ecosystem, which is the basis for future studies on ecosystem services in the context of environmental and climate change. Moreover, the obtained results are intended to result in baseline data that will enable future studies on the impact of human-induced changes (including climate changes) on loss of biodiversity and related ecosystem services.

The overall objective of COBAFISH is to contribute to the description of the biodiversity and functional/trophic diversity of fish communities in the Congo River and to investigate the factors that structure fish biodiversity. To achieve these goals, the project builds on recent and past data series gathered in the field, on fish collections mainly from the late1940s and early 1950s in the Lobilo River and more recent observations made in the Lomami River during two expeditions (2009 & 2010) carried out by the project team in the context of the Congo2010 project.

Fishes represent one of the major protein sources for riparian human populations in the Congo River basin. COBAFISH puts the participating Belgian researchers in a unique position to investigate the second largest river system in the world. Because the international scientific community is extremely interested to initiate research projects on the Congo River, this project would provide a basis on which partners involved in this project could claim a leading role should research initiatives at European and international levels be launched. This would in particular be the case for calls on cooperation and development with African countries that might arise in the European Commission Seventh Framework Program, for instance in relation to Observatory for Sustainable Development and Environment of the Joint Research Centre (JRC), or in the frame of the 'Millennium Development Goals', more specifically 'Food Security Targets' of the EU-AFRICA Strategic Partnership.

To achieve the operational objectives, we organized three field campaigns to acquire new data on several aspects such as limnology, biogeochemistry, biology and ecology.

Knowledge and instruments (databases) developed by COBAFISH will allow to help local decision makers and policy makers at regional (ICCN) and international (IUCN & FAO) to develop science-based management strategies towards the sustainable exploitation of fish stocks in the Congo River basin.

1.2 Objectives

The **four main operational objectives** of COBAFISH are:

(1) Evaluating the diversity of fish communities (biodiversity, taxonomic diversity and functional/trophic diversity) in two sub-catchments of the Congo River (Lobilo and Lomami). Our working hypothesis is that diversity of fish communities will be different in these two sub-catchments, based on a limited preliminary data-set.

(2) Identifying the factors that regulate this diversity. Our working hypothesis is that the possible regulating factors are: i) availability and origin of organic matter; ii) environmental physical and chemical conditions.

(3) Determining to which extent fish communities in these river systems depend on autochthonous (aquatic) primary production or on lateral allochthonous (terrestrial) production.
(4) Determining the importance of seasonal flood events on ecosystem functioning. Our working hypothesis is that sources of carbon that flow through the food-web up to higher trophic levels change seasonally.

1.3 Expected outcomes

WP 1: Carbon sources, cycling and aquatic metabolism

D.1. Data-set, analysis and synthesis of carbon stocks and isotopic signatures in the terrestrial littoral, the water column and in the sediments
D.2. Data-set, analysis and synthesis of aquatic metabolic rates
D.3. Data-set, analysis and synthesis of dissolved and biogenic silica isotopes

WP 2: Aquatic floral and faunal diversity

D.4. Data-set, analysis and synthesis of phytoplankton taxonomic composition
D.6. Inventory of macro-invertebrate communities biodiversity (collections)
D.7. Inventory of ichthyofauna biodiversity (collections)
D.8. Inventory of ichthyofauna biodiversity (DNA barcoding)
D.9. Ichthyofauna diversity Indices (identification tools and conservation)

WP 3: Ecosystem trophic structure (foodweb analysis)

D.10. Evaluation of aquatic food web structure
D.11. Evaluation of the support for faunal communities (allochtonous versus autochthonous)

WP 4: network integration and coordination

D12. Reporting and dissemination of results

2. METHODOLOGY AND RESULTS

2.1 Scientific methodology

WP 1: Carbon sources, cycling and aquatic metabolism

Task 1.1: Relative contributions of allochthonous and autochthonous production to aquatic C reservoirs (KUL, ULg)

The periodic monitoring program at Kisangani of several biogeochemical variables in the Congo River and in one of its tributaries, the Tshopo, started in December 2012, and is on-going at two-weekly intervals. Partners 2 and 3 have committed to continuing this monitoring in collaboration with CSB-UNIKIS in an effort to provide a long-term dataset on riverine geochemistry for this section of the Congo River basin. The monitoring involves the characterization of biogeochemical variables by quantitative measurements of different carbon reservoirs (sediment organic C, particulate and dissolved organic carbon (POC, DOC) in the water column), by fluorescence signals from the different components of the dissolved organic matter (cDOM and DOM excitation-emission fluorescence matrix), and by stable isotope measurements on both dissolved inorganic carbon (DIC), POC and DOC. In addition, stable O and H isotope measurements are conducted on these water samples as well as on monthly precipitation samples (rain gauge installed at the UNIKIS campus in 2013), in collaboration with the International Atomic Energy Agency (Vienna); the resulting data are part of the GNIR and GNIP networks (Global Network for Isotopes in Rivers and Precipitation, respectively), and publically accessible.

To characterize the terrestrial end-member, samples of surface soils and litter were collected, and aquatic macrophytes and periphyton were sampled where present. The information base gathered here provides a critical contribution to the work on foodweb structure and resource used by fish communities (Task 3.2), and is highly complementary to the work on aquatic primary production and net community metabolism (Task 1.2). A recently started Marie Curie postdoctoral project (AQUAHYDRO) will process COBAFISH foodweb samples to explore the use of hydrogen stable isotope ratios in further distinguishing terrestrial *versus* aquatic contributions to the aquatic foodweb.

Finally, data from geographical information systems (GIS) on land-use, soils and vegetation (Global Land Cover 2000 database of Africa, Woods Hole Research Center pantropical national level carbon stock dataset, Global Lakes and Wetlands Database, Global Lithological Map, Soil Map of Africa), as well as meteorological (WorldClim – Global Climate Data) and morphological (HYDRO1K global hydrologic data set) data were linked to field-based data riverine physical and -chemical variables, organic matter composition and processing in a representative range of smaller-scale sub-basins with a more uniform vegetation and land-use. These datasets allow us to delineate sub-catchments and correlate their characteristics with biogeochemical data in the aquatic system.

Task 1.2: Aquatic primary production, respiration, and net community metabolism (ULg, KUL)

In order to determine the fate of autochthonous carbon inputs, the level of allochthonous production, the net community metabolism, and the impact of the latter

on the exchange of CO_2 with the atmosphere, we carried out a series of process rate measurements:

Net plankton primary production based on short-term (2h) deliberate ^{13}C-tracer experiments at various light intensities; plankton community respiration based on short-term (24h) dark consumption O_2 incubations; exchange of CO_2 between surface waters and the atmosphere. The latter was also compared to the exchange of CH_4 that usually has lower exchange rates than CO_2 but much higher global warming potentials (Denman et al. 2007).

These process rates are compared to the isotopic Si mass balances (as proxy of diatom production, cf. Task 1.3) and interpreted with ancillary measurements of ecological and biogeochemical variables such as water discharge, light availability (water transparency and total suspended matter (TSM) concentration), inorganic nutrient (nitrate, nitrite, ammonia, phosphate, silicate) concentrations, POC and DOC concentrations, fluorescence and isotopic signatures (Task 1.1), bacterial community composition (flow cytometry), phytoplankton community composition (Task 2.1), O_2 levels and CO_2 concentrations.

Data acquired in the frame of COBAFISH were integrated to other data-sets acquired in the Congo river mainly from the Congo2010 expedition and the three cruises from a FNRS funded project (TransCongo) for a larger description at the scale of the basin. Similarly, the Congo data were put into perspective with a joint analysis across 12 Sub-Saharan bassins mainly acquired in the frame of ERC StG AFRIVAL (involing partners 3 and 2), as well as with a comparison with data from the Amazon acquired by Gwenaël Abril (University of Bordeaux) who served in the Follow-up Committee of COBAFISH.

Task: 1.3: Identification of Si sources as nutrients and the contribution of diatoms to primary production (MRAC, NBGB)

This task aimed at assessing the sources and the seasonality of Si and at quantifying the seasonal diatom production in the watershed. Due to recurrent technical issues with the new mass spectrometer installed at the ULB, all samples collected during these fieldtrips still await further analyses (Task 1.2).

WP 2: Aquatic floral and faunal diversity

Task 2.1: Qualitative and quantitative diversity of aquatic primary producers (ULg, NBGB)

Abundance and diversity of the phytoplankton assemblage were evaluated by a combination of microscope observations and pigment analyses. Variations in abundance of phytoplankton taxa were studied in relation to the abiotic parameters (e.g. light availability, residence time and nutrient levels, Task 1.2).

The analysis of marker pigments by high performance liquid chromatography (HPLC) was used to determine the taxonomic composition of the phytoplankton assemblage at the class level using the CHEMTAX software (e.g. Descy et al. 2005). This approach allowed screening numerous samples, which will be complemented by microscopic analyses for determining composition at lower taxonomic level (species or genus). The approach combining marker pigment analysis and identification of the main phytoplankton taxa (Sarmento & Descy, 2008) allowed to identify functional groups (Reynolds et al., 2002) closely related to environmental factors such as light, nutrient supply, environmental variability, …). Bulk periphyton samples collected from

different substrates were also analysed by the same technique, allowing a global assessment of the algal flora (including cyanobacteria).

The dominant diatom species in the phytoplankton and benthos have been identified and enumerated on a semi quantitative basis. Phytoplankton samples were collected with a plankton-net (10 µm mesh size); epiphytic samples were collected from submerged parts of aquatic plants; epipsammic and benthic samples were collected from the bottom of the rivers and streams. After cleaning with peroxide; the material was mounted in Naphrax ® (RI: 1.71). The obtained permanent microscopic slides were studied using an Olympus BX51 light microscope equipped with differential interference contrast. Additional observations with a scanning electron microcopy were undertaken for taxonomical purposes. All diatom taxa have been determined up to genus level. Identification up to species level is problematic as no literature is available for the Lomami and Lobilo watershed and is even limited for D.R. Congo. Many species are unknown to science or resemble existing taxa needing in-depth investigation.

Task 2.2: Trophic diversity of macro-invertebrate communities (RBINS)

We established biodiversity, densities and biomass of macro-invertebrate communities in the stations where fish community structure were evaluated. Macro-invertebrates were at the taxonomic level required for their classification into Functional Feeding Groups (FFGs). The trophic niche of representatives of the different macro-invertebrate FFGs was established by stable isotope analysis (see Task 3.2). Furthermore, fish stomachs of selected fish species were analyzed and compared with standing densities/biomass of various macro-invertebrate FFG's/ taxa collected in the field.

The obtained stable isotope data link the macro-invertebrates to lower trophic levels, while the diversity/ density/ biomass assessments are used to describe extant levels available to fish, and the fish stomach analyses were used to link the macro-invertebrates to the higher trophic levels (see Task 3.1).

Replicate samples for macro invertebrates were taken near the points of standardized fish sampling: CPUE (catch per unit effort) samples were taken with a hand net in vegetation, grab samples (PONAR) for meiobenthos and larger molluscs, standardized transects along nearby shorelines were carried out.

Task 2.3: Qualitative and quantitative diversity of fish communities (RMCA, RBINS)

2.3.a. Fish sampling, morphology based identifications (RMCA)

During the project, two food web stations on the Lobilo River situated in the Yangambi Biosphere Reserve and two food web stations on the Lower Lomami River were intensively sampled for fishes. For this, we used standardized gill net sampling with monofilament gill nets (8, 10, 12, 15, 20, 25 and 30 mm mesh size k.t.k.) from 17h to 06h on each of the selected sampling points.

Additional sampling with fykes, dip nets and purchase from local fishermen was included for the fish biodiversity survey. A representative sample of all species collected at each of the sampling stations was preserved in 10% formalin. Stomachs of the most abundant species and species with the highest biomass were injected with 10% formalin to be available for possible stomach content analysis. Fin-clips were taken of all sampled species with a representative of at least two specimens of each species for each sampling point for DNA-analysis and COI barcoding. A

detailed set of limnological, biogeochemical and ecological variables will also be collected at each of the sampling points (see WP1).

Correct identification of fish species is of prime importance for the fish biodiversity section of the research project. Species for which no identification keys are available were compared with type- and other specimens housed at the RMCA. For specific taxonomic cases, analysis of morphological data with multivariate [Principal Component Analysis (PCA)] as well as univariate (Mann-Whitney U test) methods were used (see Snoeks 2004).

2.3.b. Use of DNA barcodes to facilitate species separation for cryptic species (RBINS)

During the sampling fin clips were taken from all different species. DNA was extracted and sequenced to barcode those species. More reliable and faster species identifications because of link between specimen and sequences will be made available to (1) Fish Barcode of Life Initiative FISHBOL (http://www.fishbol.org/), (2) fish specimen and sequence data are ready to be entered in the http://jemu.myspecies.info/dna-barcoding-selected-congolese-vertebrates website initiated to disseminate biodiversity data to stakeholders, with the possibility to identify species on the basis of mitochondrial DNA sequences against EMBL and/or the specimen collection of the RBINS and the RMCA; and (3) a web portal that was developed by the Belgian Biodiversity Platform to make all biodiversity, biogeochemical and cartographical gathered during the Congo2010 expedition available for the international scientific community.

WP 3: Ecosystem trophic structure (food web analysis)

A general assessment of the primary producer community was made to assess the relative biomass of micro- and macrophytes in the rivers systems.
Data on presence and abundance of microphytes and macrophytes in the fish diet was inferred from stomach content analyses, and isotope signature of the micro- and macroflora that complement these data. The trophic information gathered by the density study of the macro-invertebrate communities (Task 2.2) allowed to quantify the relative importance of the various functional feeding groups (FFG's).

Macro-invertebrate specimens were classified into scrapers/grazers, consuming algae and associated material; shredders, consuming leaf litter or other CPOM (Coarse Particulate Organic Matter), including wood; collector-gatherers, collecting FPOM (Fine Particulate Organic Matter) from the stream bottom; collector-filterers, which collect FPOM from the water column using a variety of filters; and predators, which feed on other consumers (Naiman and Bilby, 2001, Monakov 2003). A potential sixth category, other, includes omnivores, or simply do not fit into the other categories. This approach allowed us to determine the origin of carbon and nutrients within the macro-invertebrate trophic levels. Isotope analyses were carried out on selected macro-invertebrate specimens (see Task 3.2).

Due to its importance for resolving trophic links and origin of food for fishes, a specific task was entirely dedicated to fish gut content analyses (Task 3.1). The isotopic signature of the various components of the food web (terrestrial litter, aquatic macrophytes, phytoplankton, epiphytic algae, macro-invertebrate consumers classified into FFG's and main fish species) gained from Task 3.2 allows depicting and evaluating the importance of the various trophic links between components.
Isotopic data coupled with in situ estimates of primary producers (Task 1.2), macro-invertebrate functional feeding groups (Task 2.2), fish gut contents (Task 3.1), and

indirect methods for estimating consumer production were used in a foodweb analysis for both tributaries of the Congo River.

Task 3.1: Fish stomach content analysis (RMCA, RBINS, NBGB)

The stomach of the fishes of the third expedition from which a sample for stable isotopes was taken was injected with formaldehyde to ensure that the stomach content was preserved. In the lab, the stomach was removed and the content was divided in different prey categories.

Task 3.2: Stable isotope-based foodweb analysis (KUL)

Sampling for isotope studies of trophic structure included analyses of $\delta^{13}C$ and $\delta^{15}N$ signatures of potential organic matter sources (see Task 1.1.), as well as combined $\delta^{13}C$ and $\delta^{15}N$ analyses on invertebrates and fish fauna. For the latter, a representative set of fish species were selected (see Task 2.3) whereby multiple specimens from each selected species (to the extent possible) were analysed in order to asses intra-population variations. Within the framework of a recently project, (a subset of) COBAFISH samples are also being analysed for $\delta^{2}H$.

2.2 Results

WP 1: Carbon sources, cycling and aquatic metabolism

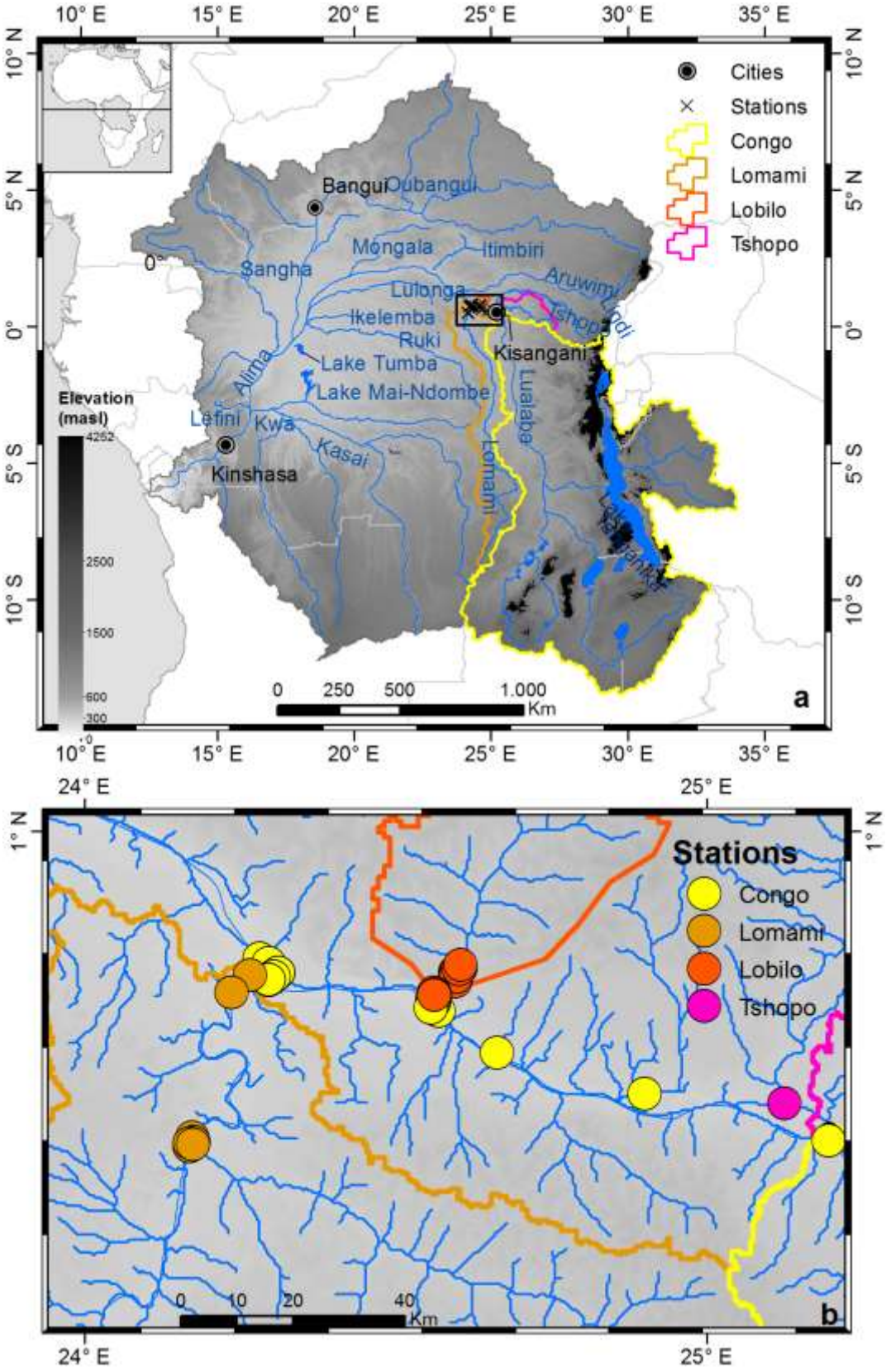


Fig 1: (a) Map of the Congo Basin showing the drainage area of the river studied in this project. (b) Zoom on the stations sampled during the different COBAFISH cruises.

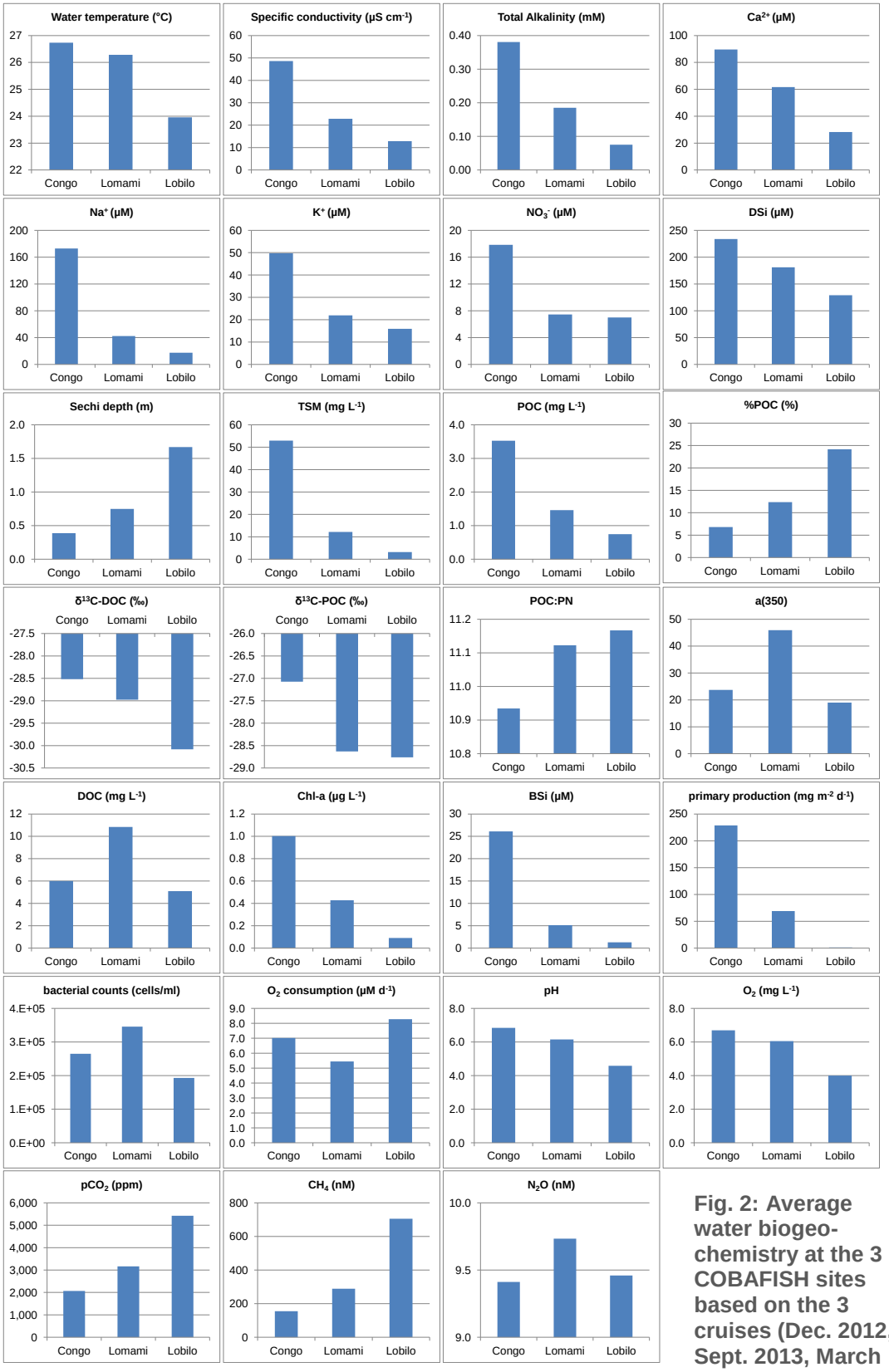


Fig. 2: Average water biogeo-chemistry at the 3 COBAFISH sites based on the 3 cruises (Dec. 2012, Sept. 2013, March 2014)

The COBAFISH project investigated the biogeochemistry and aquatic ecology of 3 river bassins, the Lobilo River located close to Yangambi, the Lomami R. and the Congo R. upstream of the confluence of the Lomami (Fig.1). These three rivers are very different in size, the Congo being the largest and the Lobilo the smallest, with catchment area spanning three orders of magnitude and freshwater discharge spanning two orders of magnitude (Table 1).

Table 1: Morphological, hydrological and catchment characteristics of the three COBAFISH study sites (Congo, Lomami and Lobilo).

	Congo	Lomami	Lobilo
Drainage area (km²)	1,035,637	116,215	1,424
Mean slope (°)	0.93	0.19	0.05
Discharge (m^3 s^{-1})	8,654	1,215	44
Catchment land cover (%)			
Dense and mozaic forest	48	71	100
Grasslands, woodlands and shrublands	41	28	0
Catchment lithology (%)			
Unconsolidated sediments	16	61	58
Siliciclastic sedimentary rocks	9	27	0
Metamorphics	72	12	42
Evaporites and volcanic	3	0	0
Dominant soil types (%)			
Ferralsols	35	78	100
Cambisols	20	0	0
Acrisols	22	22	0

The land cover is also very different. In the Congo, the catchment is equally covered by forest and savannah; the Lobilo is exclusively covered by forest; the Lomami shows an intermediary land cover between both. Due to this different land cover, the Congo and Lomami have biogeochemical characteristics of white water rivers, and the Lobilo of black water rivers (Fig. 1). Soils in the Congo are highly heterogeneous, with a slight dominance of ferralsols while in the Lomami and the Lobilo, ferralsols represents 78 % and 100 % of soil types, respectively.

The forest cover on the Lobilo can explain the lower water temperatures than the other two rivers (Fig. 2). The Lobilo is characterized by low conductivity, total alkalinity (TA), major elements (Ca^{2+}, Na^{+}, K^{+}) and dissolved silica (DSi) than the other two rivers (Fig. 2), due to lower rock weathering related to lower slope and higher forest cover (Table 1). Additionally, the Congo has highly soluble volcanic and evaporite rocks (Table 1). The Lobilo is also characterized by lower NO_3^- concentrations (Fig. 2), and by higher water transparency (Secchi depth) due to lower total suspended matter (TSM). The particulate organic carbon (POC) content of the Lobilo is lower, but the contribution of mineral phase to TSM is lower as indicated by higher %POC (Fig. 2). The higher contribution of soils compared to direct vegetation inputs to the TSM pool in the Congo compared to the Lobilo is also reflected in the lower POC:PN ratio in the Congo (Fig. 2). The higher carbon stable isotope ratio of dissolved organic carbon (δ^{13}C-DOC) and of POC (δ^{13}C-POC) in the Congo compared to the Lobilo (Fig. 2) is due to higher contribution of C4 vegetation to DOC and POC in the Congo and to a denser forest cover in the Lobilo (shading effect). The absorption coefficient at 350 nm (a(350)) of dissolved organic matter (DOM) followed the pattern of DOC concentration (Fig. 2) with higher values in the Lomami than the Congo due to higher forest cover, but surprinsingly lower DOC values in the Lobilo. This is probably related to a higher contribution of ferralsols (Table 1) in the Lobilo that leads to a strong soil DOC adsorption (Davis 1982, Kaiser

and Zech 2000, Lucas et al. 2012). Furthermore, there was a correlation between DOC and flooded dense forest at the catchment scale that was highest in the Lomami (Fig. 3). In all rivers DOC concentrations were higher during the high flow period (2012 campaign) and decreased during the first low flow period occuring in march (2014 campaign) (Fig. 4a). DOM composition was investigated through two proxies derived from the absorption properties of DOM, namely the specific ultraviolet absorbance of DOM at 254 nm ($SUVA_{254}$), positively related to the degree of DOM aromaticity (Weishaar et al., 2003), and the spectral slope ratio (S_R), inversely related to the average DOM (MW) (Helms et al., 2008). Both parameters exhibited little variation regarding the range of variability that can be observed in natural freshwaters (Jaffé et al., 2008, Spencer et al., 2012). Thus, $SUVA_{254}$ values were elevated in all stations and were associated with low S_R values during the two field campaigns (Fig. 4c and 4d), indicating that DOM was dominated by aromatic compounds of high MW. Along the rivers, DOM aromaticity and MW increased in the order Congo < Lomami < Lobilo. Overall, the DOM composition in the Congo Basin is dominated by aromatic compound of high molecular weight (MW). The highly aromatic character of DOM in tropical ecosystems (especially in rivers draining forest basins) compared to temperate and Arctic freshwaters was recently illustrated in a large scale studies including African large rivers (Lambert et al., 2015). The elevated $SUVA_{254}$ and low S_R values all together indicate that DOM is mainly derived from terrestrial sources freshly transported towards the aquatic ecosystem. An importance source of DOM appears to be the flooded forest. This is suggested by the good correlation between DOC and the extent of flooded dense forest, and is also consistent with the well-documented role of wetland areas in delivering great quantity of aromatic DOM in freshwaters despite their limited extent at the catchment scale (Hanley et al., 2013; Mann et al., 2014; Lambert et al., 2016).

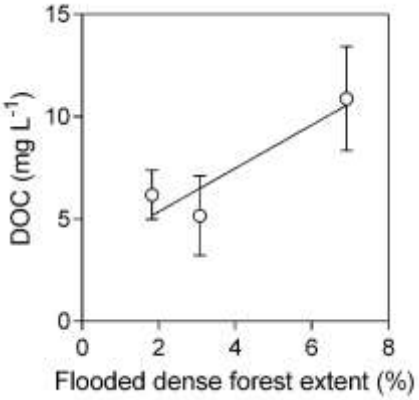


Fig. 3: Correlation between the average DOC concentrations measured in the three studied sites and the extent of flooded dense forest at the catchment scale

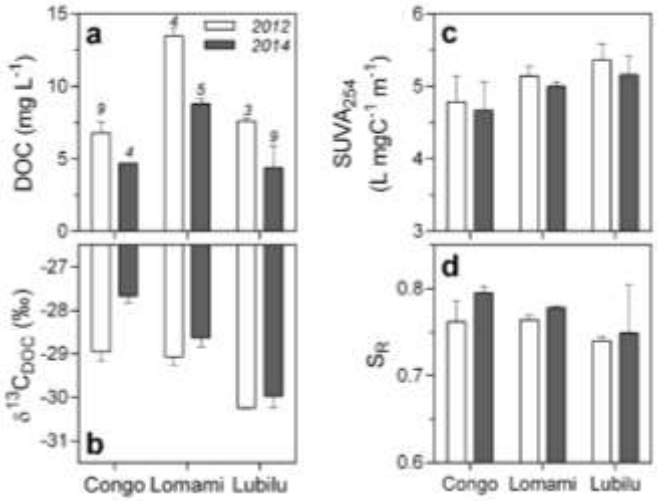


Fig. 4: (a) DOC concentrations, (b) stable carbon isotopic composition, (c) DOM aromaticity and (d) DOM molecular weight in the studied sites during the 2012 (white bars) and 2014 (black bars) campaigns.

Primary production was extremely low in the Lobilo and higher in the Congo, due to a higher phytoplankton biomass in the Congo as indicated by chlorophyll-a (Chl-a) and biogenic silica (BSi) (Fig. 2). The low primary production in the Lobilo is probably due to low light conditions due to shading by forest cover and also due to very low pH values (Fig. 2). Bacterial counts generally followed DOC concentrations while O_2 consumption only showed modest changes with tendency towards higher O_2

consumption in the Lobilo. Nevertheless, O_2 and CH_4 concentrations and the partial pressure of CO_2 (pCO_2) were distinctly higher in the Lobilo than in the Congo. Nitrous oxide showed very modest changes and was highest in the Lomami.

Task 1.1: Relative contributions of allochthonous and autochthonous production to aquatic C reservoirs (KUL, ULg)

Monitoring of water biogeochemistry at Kisangani (Congo and Tshopo)

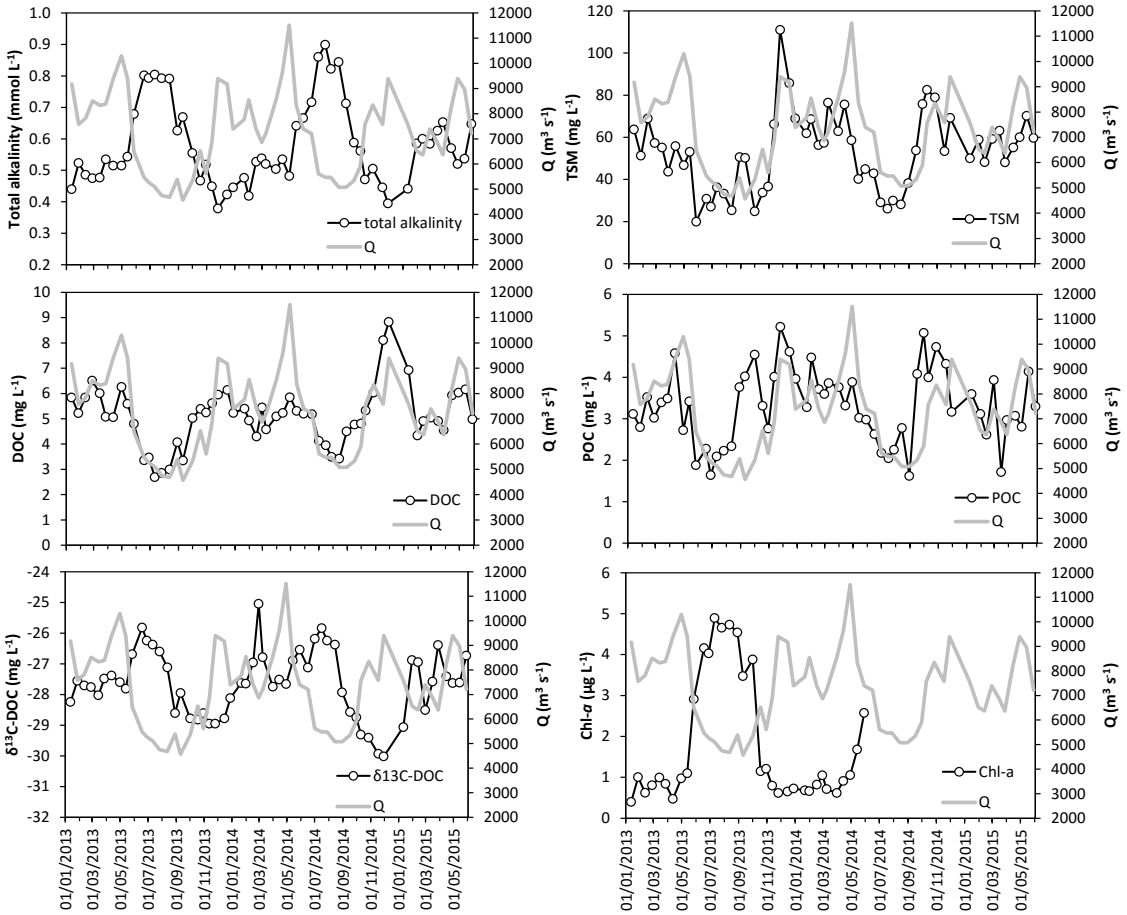


Fig. 5: Seasonal variations of biogeochemical variables and freshwater discharge (Q) in the Congo River at Kisangani.

The fortnightly monitoring at Kisangani of several biogeochemical variables in the Congo River and in one of its tributaries, the Tshopo, started in December 2012, and is on-going. Most of the biogeochemical variables in the Congo (Fig. 5) and the Tshopo follow regular seasonal variations that are either positively or negatively related to freshwater discharge. This can be explained by the fact that during high-water, the main water flows occur through superficial soils while during low-water a more important fraction of the water flow has passed through deeper parts of the soils, with an increased contribution from groundwaters (i.e. baseflow). This explains the higher conductivity and TA during low-water with flow of water from deeper parts of soils (groundwaters enriched in ions from rock dissolution). Also, the marked increase of TSM and the less marked increase of POC are related to transport during high-water of particles from superficial soils. During high-water DOC increases due to mobilization by surface runoff of organic exudates from superficial soils, while during low waters groundwaters have lower DOC content with a more refractory nature as indicated by the increase of δ^{13}C-DOC. The δ^{13}C-POC on the other hand does not show a marked seasonal variation (not shown). During low-water, residence time

also increases and lower dilution from decreased water inputs to rivers, which might explain the increase of Chl-*a* during low-water as phytoplankton accumulates. However, it is not excluded that the decrease of TSM and DOC during low-water might also contribute to better light conditions and also explain the increase of chl-*a*.

The Congo River was enriched in DOC (5.2±1.3 mg L^{-1}, n = 72) compared to the Tshopo River (3.9±0.9 mg L^{-1}, n = 72) (Fig. 6a). Overall, DOC concentrations followed the water level fluctuations in both rivers, being higher during high flow periods relative to low flow periods. The relationship between DOC and stream water was however stronger in the Congo River than in the Tshopo River (Fig. 6). Likewise, changes in DOM composition were more marked in the Congo River than in the Tshopo River (Fig. 6b-d). Thus, $\delta^{13}C_{DOC}$ values in the Congo River were highly variable (-27.8±1.1 ‰, min = -30.0 ‰, max = -25.0 ‰). Lowest values were observed during high discharge events, and were associated with elevated DOC concentrations and DOM of high aromaticity (high $SUVA_{254}$) and high MW (low S_R). $\delta^{13}C_{DOC}$ increased after peak discharge, coinciding with decreasing DOC concentration and DOM of lower aromaticity and MW. The seasonal pattern of DOM in the Tshopo River was more complex and varied from one year to another. Thus, $\delta^{13}C_{DOC}$ values showed limited variability (-29.8±0.5 ‰, min = -30.7 ‰, max = -28.3 ‰). Significant increases during low flow periods were only observed in March and August 2014, while no changes were detected in August 2013 and 2015. DOM aromaticity and MW were also relatively stable. A slight decrease in $SUVA_{254}$ and associated increase in S_R coincided with the small increase in $\delta^{13}C_{DOC}$ values. Overall, DOM in the Thopo River was more aromatic (4.7± 0.3 mgC L^{-1} m^{-1}) and characterized by higher MW (0.75±0.04) than in the Congo River ($SUVA_{254}$ = 4.5± 0.5 mgC L^{-1} m^{-1}, S_R = 0.77±0.04).

The seasonal changes in DOM concentration and composition are more important in the Congo River than in the Tshopo River. The large variation in $\delta^{13}C_{DOC}$ values in the Congo River at Kisangani along the hydrological cycle can be related to a shift in the source of DOM mobilized in the upper part of the basin due to differences in water routing during the hydrograph. Decreasing $\delta^{13}C_{DOC}$ signatures that occurred with increasing water discharge during high flow periods has been attributed to the mobilization of fresh DOM from superficial soil horizons in wide variety of catchments (Neff et al., 2006; Sanderman et al., 2009; Lambert et al., 2011; Bouillon et al., 2012). Inversely, highest $\delta^{13}C_{DOC}$ values during low flow periods reflect the deepening of water flow paths and the subsequent mobilization of more degraded and less aromatic DOM from deeper soil horizons, as evidenced by the decrease of $SUVA_{254}$ and parallel increase of S_R values. The less marked changes in the Tshopo River imply a strong connection with superficial terrestrial DOM sources along the hydrological cycle. This is likely due to the very small fluctuations of water level that are typically less than 10 cm (Fig. 5).

The differences observed between the Congo and Tshopo (Table 2) can be related to differences in land cover (Fig. 8) or lithology (Fig. 9). The watershed of the Tshopo consists exclusively of forest (dense and mosaic) while the one of the Congo upstream of Kisangani is constituted of 47% of forest and 46% of savannah (woodlands, shrublands and grasslands). The unexpected lower DOC concentrations in the Tshopo relative to the Congo can also be explained by the dominance of ferrralsols in the Tshopo basin (100% of the basin).

As noted elsewhere in the Congo basin (Seyler et al., 2006 ; Bouillon et al., 2014), conductivity, total alkalinity, TSM are lower and conversely %POC is higher in basins with a higher forest cover. Also, the higher values of δ^{13}C-POC and δ^{13}C-DOC in the in the Congo compared to the Tshopo can be attributed the C4 plants in the

grasslands and savanna compared to the C3 plants present in the forest. However, surprisingly, the POC and DOC concentrations are lower in the Tshopo than the Congo, since based on the land cover the reverse would have been expected (Bouillon et al. 2014).

Table 2: Average ± standard deviation of biogeochemical variables on the Congo and the Tshopo.

	Congo	Tshopo
Total alkalinity (mmol L^{-1})	0.6±0.2	0.3±0.1
TSM (mg L^{-1})	53.5±20.0	11.8±9.7
POC (mg L^{-1})	3.4±0.9	1.3±0.9
DOC (mg L^{-1})	5.2±1.4	3.7±0.8
%POC (%)	6.5±1.5	13.7±6.8
δ^{13}C-POC (‰)	-27.0±0.6	-29.7±0.8
δ^{13}C-DOC (‰)	-27.8±1.1	-29.7±0.5

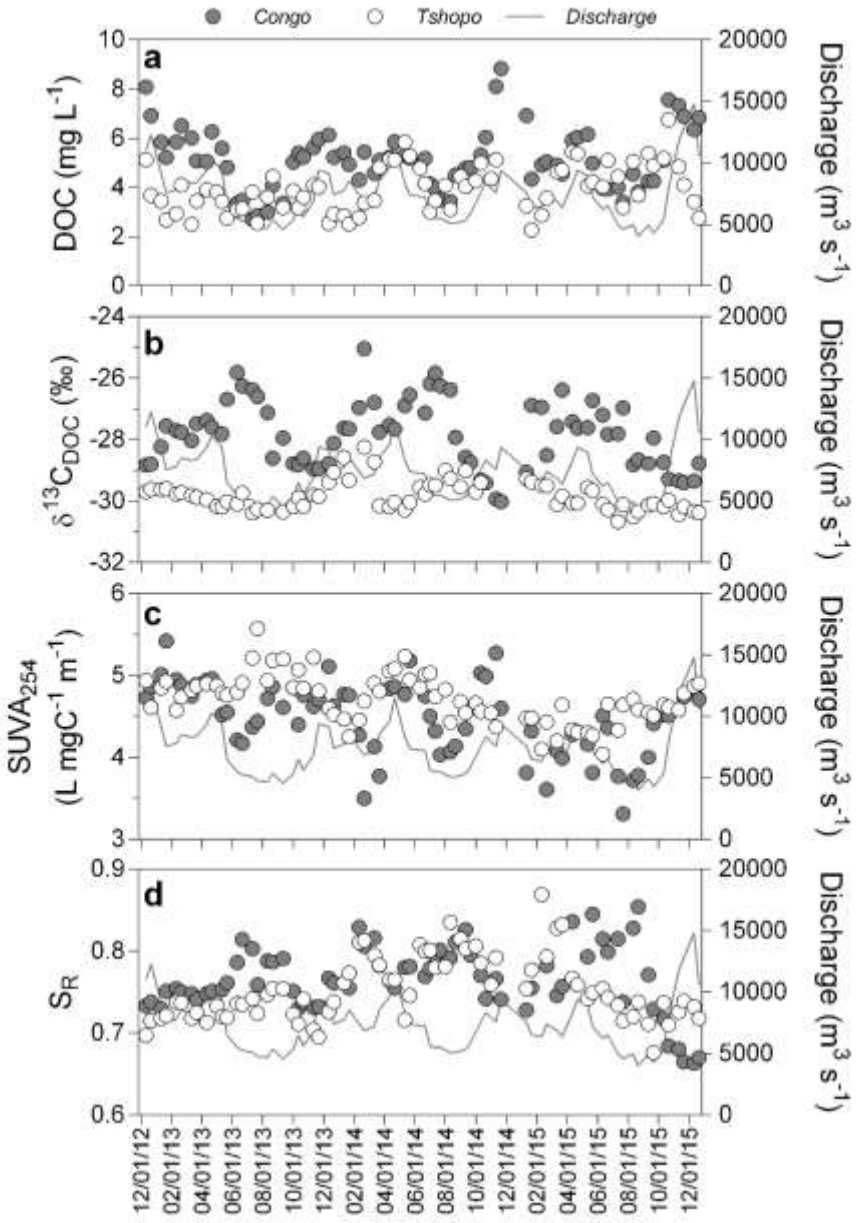


Fig. 6 Temporal variability of (a) DOC concentrations, (b) δ13CDOC, (c) SUVA254 and (d) SR in the Congo and the Tshopo rivers during the monitoring. The water discharge fluctuation in the Congo River is plotted in the background.

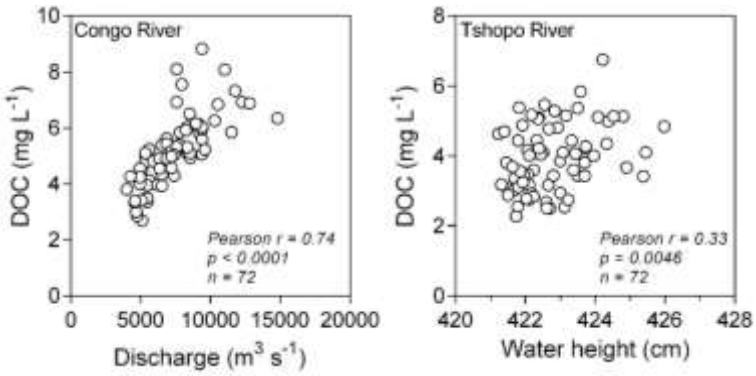


Fig. 7 Correlation between water discharge (Congo River) or water height variation (Tshopo River) and DOC concentrations.

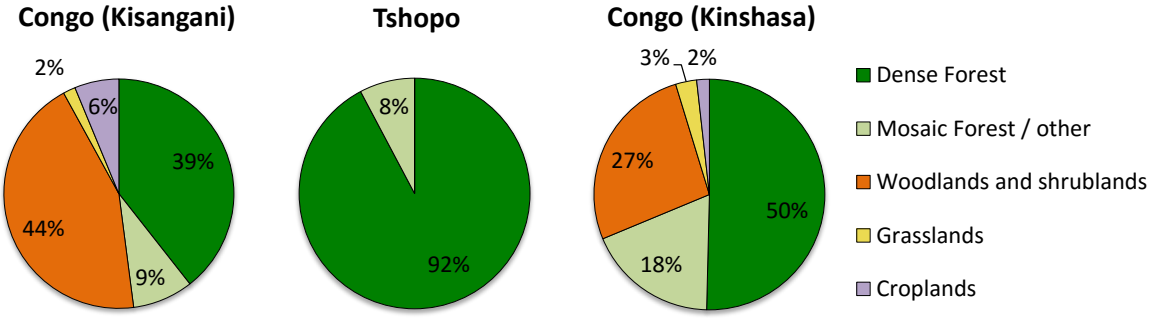


Fig. 8: Land cover on the catchment of the Congo River upstream of Kinsangani, and of Kinshasa and of the Tshopo, based on Mayaux et al. (2014).

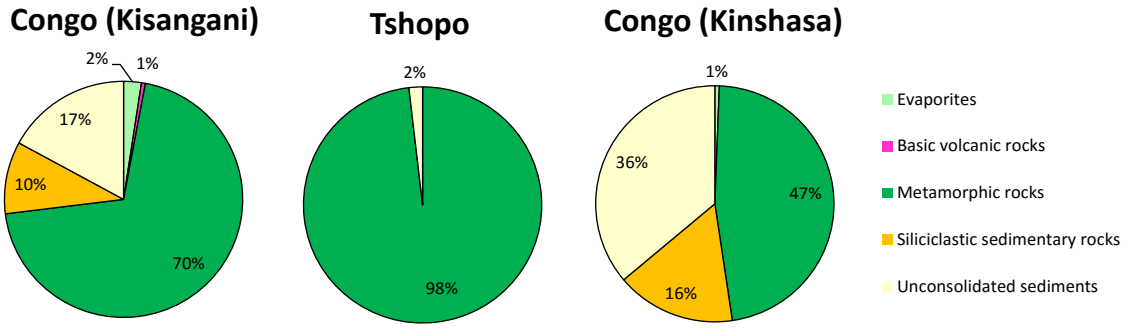


Fig. 9: Lithology on the catchment of the Congo River upstream of Kinsangani, and of Kinshasa and of the Tshopo, based on Hartmann & Moosdorf (2012).

The higher conductivity and total alkalinity in the Congo than the Tshopo can be explained by the differences in lithology. The catchment of the Tshopo is almost exclusively (98%) constituted of metamorphic rocks (granite, gneiss, micaschiste) (Fig. 9) (Nkounkou & Probst 1987) that undergo the lowest chemical erosion among major rocks outcropping on continents (Amiotte Suchet & Probst 1993).

The catchment of the Congo upstream of Kisangani has a lower proportion of metamorphic rocks (70%) and a higher proportion of siliciclastic sedimentary rocks (10% mainly as sand stone) and unconsolidated sediments (17% as sand and clays) that are more soluble than metamorphic rocks. Further, the catchment of the Congo upstream of Kisangani also contains evaporites (2%) and volcanic rocks (1%) that highly soluble. Indeed, the Virunga region that is rich in volcanic rocks (including basalts) has been shown to be a hotspot of chemical weathering (Balagizi et al. 2015).

Comparison of the Congo at Kisangani and Kinshasa

Kisangani is situated 1700 km upstream of Kinshasa, and in-between the Congo River drains the "Cuvette Centrale Congolaise" that is the second largest wetland area in the World after the Amazon. This is apparent in the changes in land cover upstream of Kisangani and Kinshasa with a reduction of savannah cover from 44% to 27% and a concomitant increase in forest cover (Fig. 8). The water of the "Cuvette Centrale Congolaise" have low total alkalinity, TSM and POC, and are extremely rich in DOC (Laraque et al. 2009; Bouillon et al. 2014). Hence, the inputs from the "Cuvette Centrale Congolaise" lead to a dilution of POC, TSM and total alkalinity, and a marked increase in DOC, when comparing composition at Kinshasa with Kisangani (Table 3). Also, the relative contribution of DOC to total organic carbon strongly increases from Kisangani to Kinshasa.

Fluxes were calculated from concentrations and average discharges. All of the fluxes (TSM, DOC, POC, TA) increase at Kinshasa compared to Kisangani owing to the increase of flow by a factor of five (Table 4). Yet, fluxes of TSM, POC and total alkalinity only increase by a factor of 2, whether the fluxes of DOC increase by a factor of nearly 10. This is obviously related to the relative increase of concentrations (Table 3) and the influence of the "Cuvette Centrale Congolaise".

Table 3: Comparison of concentrations of several variables on the Congo at Kinshasa and Kisangani (COBAFISH data for 2013-2014). Data for Kinshasa are from Coynel et al. (2005) and Wang et al. (2013).

	Congo at Kisangani	Congo at Kinshasa
Total alkalinity (µmol L^{-1})	568	175
DOC (mg L^{-1})	5.2	10.6
POC (mg L^{-1})	3.4	1.7
TSM (mg L^{-1})	53.8	26.3
DOC/(DOC+POC) (%)	61	86

Table 4: Freshwater discharge (Q) and fluxes at Kisangani and Kinshasa.

		Kisangani	Kinshasa	Ratio
Q	(m^3 s^{-1})	7,454	37,047	5.0
TSM	Tg yr^{-1}	12.9	30.7	2.4
POC	TgC yr^{-1}	0.8	2.0	2.4
DOC	TgC yr^{-1}	1.3	12.4	9.7
Total alkalinity	TgC yr^{-1}	1.5	2.1	1.4

Stable isotope signatures of primary producers

Overall, stable isotope signatures of terrestrial primary producers all corresponded to C3 plants, for which average $\delta^{13}C$ signatures of -32.3 ± 2.3 ‰ and -29.8 ± 1.6 ‰ were measured during the first two field campaigns (n=44 and 15, respectively). These are relatively low values for C3 vegetation, but consistent with expectations for tropical rainforests where $\delta^{13}C$ values are often low due to high precipitation and 'understory effects' (i.e. the contribution of ^{13}C-depleted soil-respired CO_2 which is re-assimilated by plants, Kohn 2010). The only C4 plants sampled were the Hippo Grass, *Vossia cuspidata*, from patches growing along the river banks.

As expected these grasses have typical C4 signatures of -13.0 ± 1.0 ‰ (n=16). Aquatic macrophytes showed ^{13}C signatures within a relatively narrow range (-30.3 ± 1.9 ‰ and -29.9 ± 3.0; n=31 and 8 for the first two field campaigns) with 2 exceptions, i.e. a *Ranunculus* sp. from the Lobilo River with highly ^{13}C-depleted signature (-44.5 ‰) and an unidentified macrophyte from the Lomami with a low value of -37.6 ‰. It must be noted here that the macrophyte data presented include data from both submerged and floating species (e.g., *Eichhornia crassipes*), whereby the latter type derive their CO_2 from the atmosphere and therefore have signatures similar to those of terrestrial C3 vegetation.

Task 1.2: Aquatic primary production, respiration, and net community metabolism (ULg, KUL)

A very comprehensive data-set of phytoplankton composition in the Congo River was acquired along a 1700 km stretch (Kisangani-Kinshasa) in the mainstem during high water (December 2013) and falling water (June 2014). A total of 164 samples for phytoplankton analysis were collected in the main river, in tributaries and one lake. Based on marker pigment concentration by high performance liquid chromatography (HPLC) verified by microscopic examination, green algae (both chlorophytes and streptophytes) dominated in the mainstem in highwaters, whereas diatoms dominated in falling waters; cryptophytes and cyanobacteria were more abundant but still relatively low in the falling water period, both in the tributaries and in the main channel (Fig. 10).

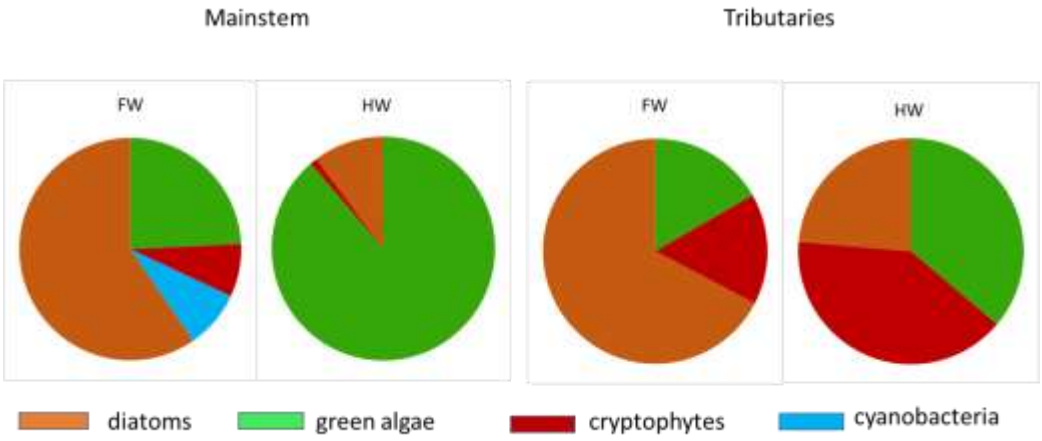


Fig. 10: Average relative contribution to chlorophyll a of the main phytoplankton groups during the sampling campaigns of December 2013 (high water, HW) and June 2014 (falling water, FW)

Phytoplankton biomass and production was higher than previously reported in tropical river channels, allowing net growth within the mainstem in many sites, whereas in other rivers phytoplankton production mainly occurs in the floodplain lakes. This is due to generally low TSM values in the Congo and to its relative shallowness that allows net phytoplankton growth in the mainstem unlike other deeper and more turbid tropical rivers such as the Amazon. Pelagic community respiration (R) was overwhelmingly higher than primary production (Fig. 11) indicating a net heterotrophic status that is fuelled by allochthonous carbon inputs from terrestrial or wetland origin. Yet, CO_2 emissions to the atmosphere in three study sites and elshewhere in the Congo basin were up to an order of magnitude higher than R (Fig. 12). This indicates that the dissolved CO_2 is laterally imported

either from soils or wetlands. Indeed, within the Congo basin, there was a correlation between the wetland fraction of the catchment surface and the concentration of GHGs (Fig. 13). The pCO_2 and CH_4 values were positively related to wetland fraction, while $\%O_2$ and N_2O were negatively related to the latter. This is in line with findings in the Amazon basin where CO_2 emissions from wetland lakes and river channels have been attributed to organic C from wetlands (Engle et al. 2008; Melack and Engle 2009; Abril et al. 2014) that also sustain intense CH_4 evasion (Melack et al. 2004).

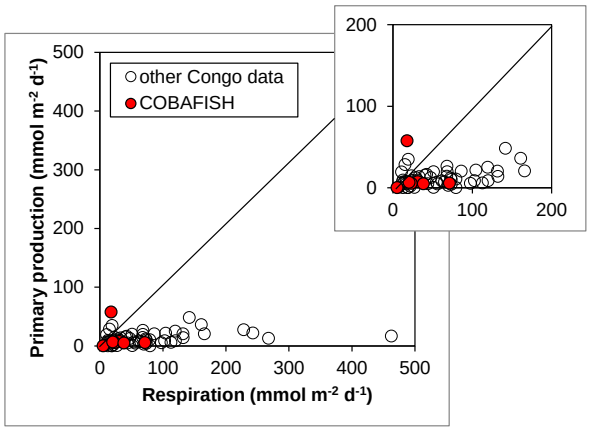


Fig. 11: Primary production versus pelagic respiration in the three COBAFISH sites and in the rest of the Congo basin based on data gathered during CONGO2010 and the three TRANSCONGO cruises.

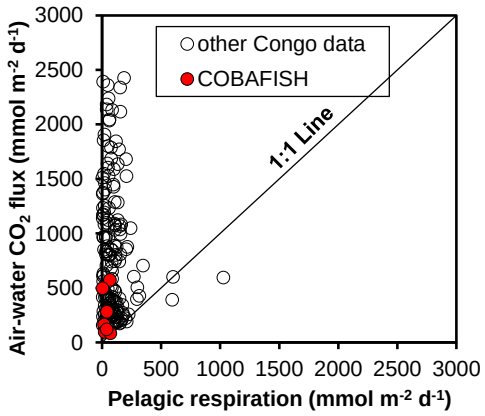


Fig. 12: Air-water CO_2 fluxes versus pelagic respiration in the three COBAFISH sites and in the rest of the Congo basin based on data gathered during CONGO2010 and the three TRANSCONGO cruises.

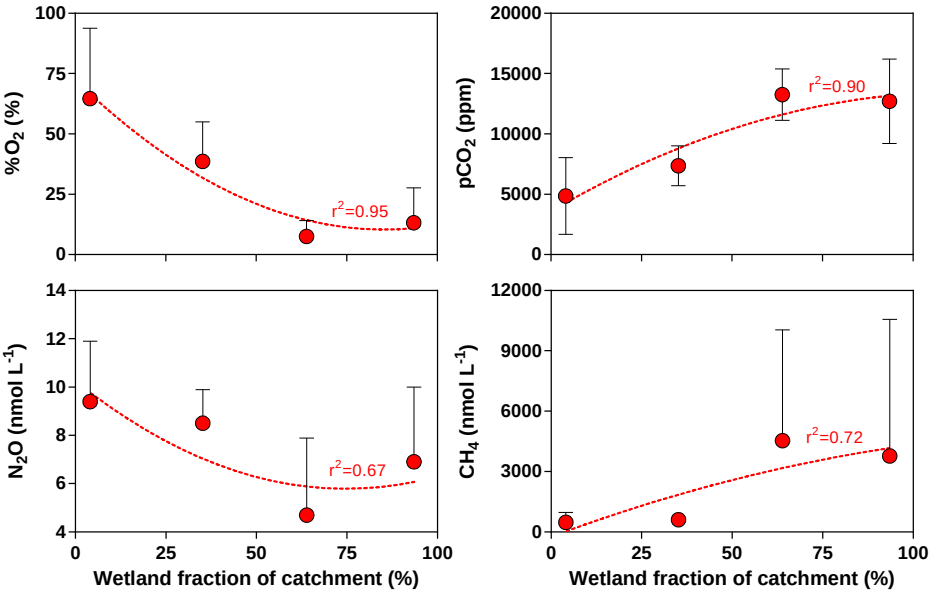


Fig. 13: Wetland presence drives the pattern of GHGs and O_2 in the Congo River. pCO_2 (ppm), CH_4 (nmol L^{-1}), N_2O (nmol L^{-1}) and $\%O_2$ (%) in 46 rivers of the Congo basin as function of wetland fraction of catchment surface (%). Data were bin-averaged by intervals of 25%. Two extreme CH4 values (>25000 nmol L^{-1}) were removed from the analysis. Error bars correspond to the standard deviation on the mean. Lines correspond to a second degree polynomial fit.

Comparison of greenhouse-gases of the Congo with other African basins and with the Amazon

The COBAFISH biogeochemistry data has been integrated into a larger data-set from the Congo basin, that was used for a comparison of greenhouse-gases (CO_2, CH_4, N_2O) across different African basins (Fig. 14) and with the Amazon (Fig. 15).

A relationship between CH_4, pCO_2 and oxygen saturation level ($\%O_2$) with wetland fraction was found across seven river systems in Africa (Fig. 13), consistent with findings in the Amazon basin where CO_2 emissions from wetland lakes and river channels have been attributed to organic C from wetlands (Engle et al. 2008; Melack and Engle 2009; Abril et al. 2014) that also sustain intense CH_4 evasion (Melack et al. 2004). However, basins that are virtually devoid of wetlands such as the Tana (<0.5% of the catchment) were still found to be sources of CO_2, although admittedly lower than other African rivers. This suggests that part of the CO_2 emissions from African rivers is also partly sustained by non-flooded biomass. Combined pCO_2 and CH_4 data were compiled in the Amazon (n=136) and Congo (n=208). Data were aggregated into mainstem (MS), large and small tributaries (T>100 m and T<100 m width, respectively). The pCO_2 in the Amazon mainstem was significantly higher than in the Congo mainstem, but pCO_2 values were not significantly different in large and small tributaries (Fig. 15). The CH_4 in the mainstem, large and small tributaries were significantly higher in the Congo than in the Amazon (Fig. 15). The median CH_4 in the Congo was three to four times higher than in the Amazon, for mainstem/small tributaries and large tributaries, respectively.

Several hypotheses can explain the different behavior of CH_4 in the Amazon and Congo river channels:

- The Congo flooded wetland is in majority flooded forest. In the Central Amazon, flooded forest accounts for 80% of flooded wetland, and the remaining 20% corresponds to temporary and permanent lakes. Floodplain lakes are characterized by high gas transfer velocity values, that promote the evasion of CH_4 to the atmosphere and water oxygenation that will favor bacterial CH_4 oxidation.
- Local upland runoff is the main source of the wetland water in the Congo, and not flooding by riverine overflow as in the Amazon. This unidirectional flow pattern will promote the transport of the CH_4 produced in the flooded forest towards the small and large river channels of the Congo, unlike the Amazon where during rising water and high water, the water transport is from the river channels towards the wetlands.
- The Congo wetlands are mostly permanently flooded unlike the Amazon floodplains that are seasonally flooded. Permanently flooded wetlands are known to be stronger CH_4 emitters and presumably CH_4 producers than seasonal flooded wetlands.
- In the Congo, floating macrophytes (mainly *Vossia cuspidata*) commonly occur along channel edges and within channels, and form large meadows in streams, rivers and mainstem, in all types of waters (white and black). Floating macrophytes are known to host high CH_4 production and emission that will be directly delivered into the Congo river channels. This does not occur in the Amazon where floating macrophytes are mainly present in floodplain lakes and do not occur in large tributaries and the mainstem due to strong currents, and are absent in black waters. The CH_4 released by floating macrophytes in the Amazonian wetland lakes will be lost locally by evasion to the atmosphere and CH_4 oxidation (see above), and not transported to the river channels.

All these differences are related to the smaller water height variations in the Congo mainstem (3-4 m) compared to the Amazon (10-12 m). In the Congo basin that straddles on the equator, the dry season on the Northern part of the basin is compensated by the rainy season on the Southern part of the basin, and vice-versa, leading to a regulation of seasonal water height variations

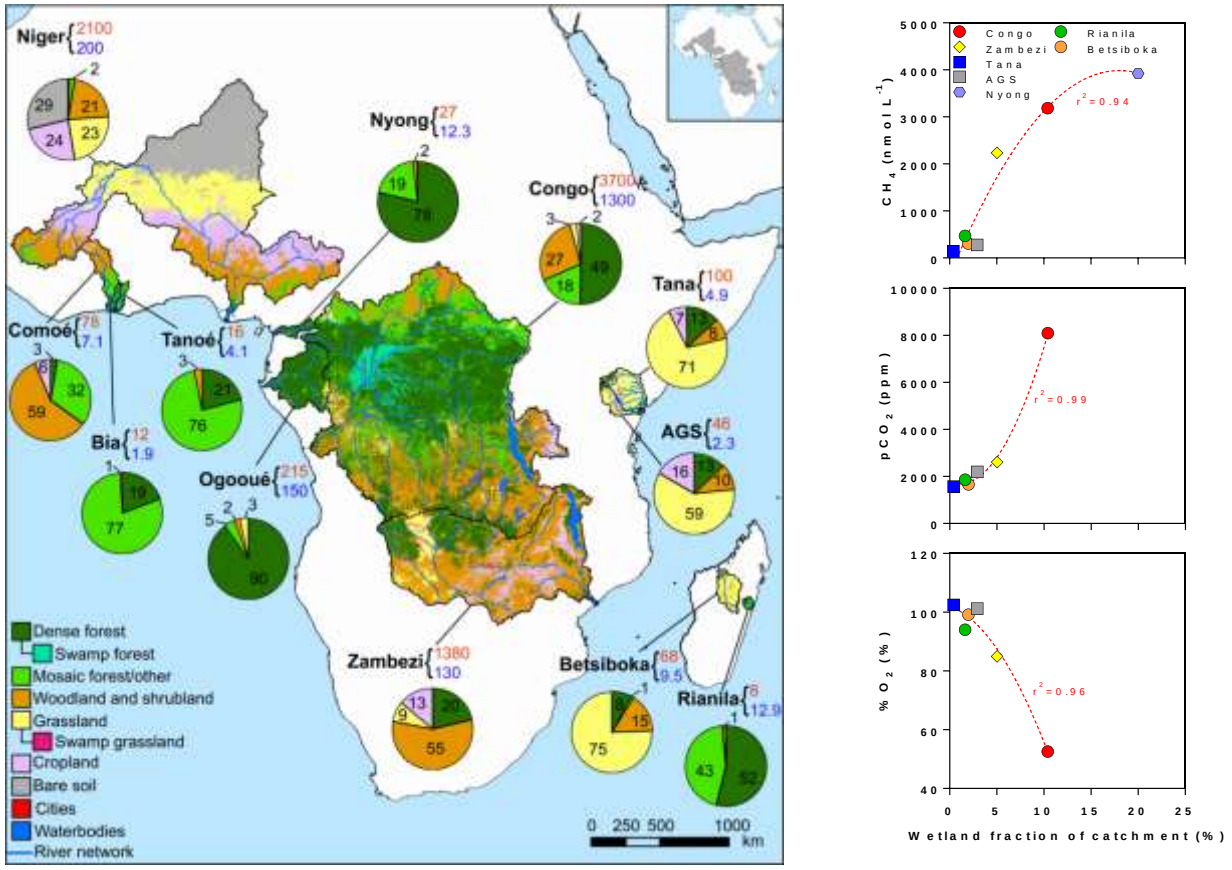


Fig. 14: Catchments and land cover of the 12 African rivers were GHG data have been compiled. The red numbers correspond to the catchment surface area (10^3 km^2) and the blue numbers correspond to the annual freshwater discharge (km^3 yr^{-1}). pCO_2 (ppm), CH_4 (nmol L^{-1}) and $\%O_2$ (%) as a function of the wetland fraction of catchment surface (%) in seven African rivers. Only the data-sets that capture spatial variations were included in the analysis (excluding fixed time-series in the mainstem of rivers). AGS = Athi−Galana−Sabaki River

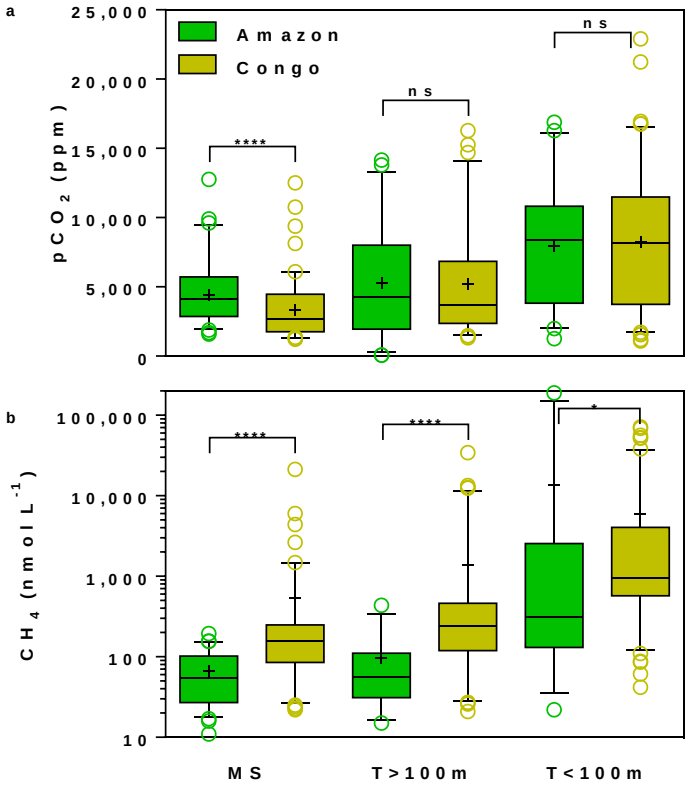


Fig. 15: Box and whisker plots of pCO_2 a) and CH_4 b) in the Amazon and Congo. The box spans the interquartile range (25-75 percentiles), whiskers correspond to 5-95 percentiles, horizontal bar to median, cross to average, and circles to outliers. Differences were tested with a Mann Whitney test at 0.05 confidence interval level, where ** corresponds to p<0.0001, * to p=0.0278, and ns to not significant. MS = mainstem. T>100m = large tributaries. T<100m = small tributaries.**

Task: 1.3: Identification of Si sources as nutrients and the contribution of diatoms to primary production (MRAC, NBGB)

The geochemical cycle of silicon (Si) is closely linked to the carbon cycle and has recently been the subject of growing attention. On the Earth's surface, the Si cycle is chemically controlled by weathering processes and biological activities that are both terrestrial (phytoliths in plants) and aquatic (mainly diatoms). The biological fixation of dissolved Si under the form of biogenic silica ($bSiO_2$) initiates the biological cycle of Si and links the silicon and carbon cycles.

The role of ecosystems on the continental biogeochemical cycle of Si is still poorly understood and quantified and the relative importance of diatoms in this "hidden" continental cycle remains unclear. Knowledge of the biogeochemical Si cycle is of great concern here since Si is a key nutrient for diatoms, CO_2-consumers in oceans and continental aquatic and terrestrial biota (Tréguer and Pondaven 2000). We previously showed that Si-isotopes could be used to better constrain Si fluxes in the Congo River (Cardinal et al. 2010), highlighting their potential as tracers of continental processes. Silicon isotope fractionation during uptake by diatoms has recently been used as a proxy for diatom production (Hughes et al. accepted) - this fractionation being both species- and temperature-independent (SPSD2 CLIMLAKE project; Alleman et al. 2005). It has been shown that export of diatoms out of the water column (e.g., by sedimentation) could represent an important fraction of the production (>80%) in the Congo Basin that is not taken into account by direct $bSiO_2$ measurement (Hughes et al. 2011). This export has been quantified by measuring the imprint of diatoms growth on the isotopic composition ($\delta^{30}Si$) of the dissolved silicon (Hughes et al. 2011).

Biological processes dominated the seasonal variations of the dissolved $\delta^{30}Si$ ratio that are superimposed on a constant abiotic $\delta^{30}Si$ value of +0.70% +/- 0.05% throughout the year. The measured biogenic silica concentration is less than the amount required to explain the monthly variations of dissolved $\delta^{30}Si$ signatures. We

use these signatures and a Rayleigh isotopic fractionation model to calculate that 82% +/- 7% of the diatoms produced each month would be exported out of the water column, probably through settling in the Malebo Pool or further upstream. The uptake of dissolved silicon by diatoms during low water flow periods could explain the absence of the dilution effect observed for the other major elements. Annual Si export to the estuary is 1.17 $x10^{10}$ mol yr^{-1} in the form of biogenic silica and 2.2$x10^{11}$ mol yr^{-1} in dissolved Si form, with a mean dissolved δ^{30}Si of +0.96% 6 0.27%. Phytoliths make only a minor contribution to the annual biogenic silica flux, and dissolved Si fluxes predominate over biogenic Si fluxes. Complementary samplings were taken during the expeditions in the Congo river, the Lomami and the Lobilo. They were prepared for analysis in the lab. Unfortunaly the new mass spectrometer broke down.

WP 2: Aquatic floral and faunal diversity

Task 2.1: Qualitative and quantitative diversity of aquatic primary producers (ULg, NBGB)

Despite the dominance of terrestrial inputs in the aquatic organic matter reservoirs, autochtonous primary producers (i.e. by phytoplankton, periphyton and macrophytes) may have a disproportionally important role in sustaining higher trophic levels (invertebrates and fish), as suggested by a limited number of studies in other tropical river systems and floodplains (e.g., Hamilton et al. 1992, Lewis et al. 2001).

Diatom diversity

Samples for diatom investigation were collected in the Lobilo, Lobaye, Lomami and Congo River during the COBAFISH expeditions in November 2012 (12) and September 2013 (142). Some additional samples (63) were taken by M. de Haan and D. Van den Broeck during a COBIMFO field campaign in the region of Yangambi in October/November 2013 from small rivers and streams. All samples received a CCA herbarium number and are deposited in the herbarium of the Botanic Garden Meise, Belgium (BR).

Diatom composition of the studied samples taken in the Lomami River and the Congo River during the COBAFISH expeditions resembles very well the composition of the samples taken during the Boyekoli Ebale Congo 2010 expedition. Both rivers are sediment loaded and have a brown colour in contrast to the black coloured small acid rivers (pH below 5), such as the Lobilo River samples during the COBAFISH expeditions and the Lilanda and Baombo Streams sampled in 2010, all located in the vicinity of Yangambi in an almost pristine tropical lowland rainforest at the edge of the MAB reserve. These acid waters have a diatom flora characterised by the dominance of *Eunotia* taxa (Fig. 16) and many unknown species, while the Lomami and Congo River have more cosmopolitan taxa. Therefore, before analysing the samples, taxonomic studies are essential, resulting in the description of new species, e.g. *Cavinula lilandae* (Cocquyt et al. 2013), *Surirella ebalensis* (Cocquyt & Taylor 2015), *Surirella congolensis* (Cocquyt & Taylor 2015), *Gomphenema grande* (Karthick et al. 2016) a species closely related to *Gomphonema zairensis* described in the 1990's from the Tshopo River near Kisangani, and *Eunotia leonardii* and *Eunotia fuseyi* (Taylor & Cocquyt 2016). Several manuscripts on other *Eunotia* taxa, a typical component of the acid black waters, are in preparation, some of these taxa resemble species described from the Amazon basin, thus requiring a thoroughly study where observations using a Scanning Electron Microscope is indispensable. The same applies for the small

Naviculoid taxa which in-depth studies were suspended till the new scanning electron microscope (SEM) was installed at the Botanic Garden Meise. The magnification of the old SEM was insufficient for the taxonomic needs nowadays. The problems with the identity of *Navicula fuerbornii* var. *africana*, described by Foged in 1966 from Ghana, are solved and the manuscript is published in Fottea (Taylor et al. 2016). Besides all these problems within the diatoms mentioned, it became clear that local researchers in DR Congo were not familiar at all with the recent tendencies in diatom taxonomy which started at the end of the 20th century and is characterized by splitting and the descriptions of many new genera. Therefore we started to work on a manual, including 91 diatom genera illustrated with, besides LM and SEM micrographs, original drawings indicating the differentiating characteristics for each genus. The book will appear in the series Abc Taxa, funded by the Belgian Development Cooperation.

A total of 300 diatom taxa (intact valves, broken or part of valves are not taken into account) were observed in the studied samples. A preliminary list of the diatom genera and number of taxa (species and infraspecific taxa) observed in 40 samples from the Lomami River, the Congo and some tributaries are given in Table 6. The Lobaye, Lohulu and Lulu rivers are tributaries of the Lomami River located on the left bank of the Congo River; the Isaloe, Libongo, Lilanda/Baombo and Lobilo rivers located in the MAP reserve near Yangambi on the right bank. As the number of samples for each river is different, for example only 3 for the Congo River compared to 16 for the Lomami River, is it part of the explanation why the species richness in the Lomami is higher than in the Congo River. The saturation curve of diatom genera of the Lobilo, Lobaye, Lomami and Congo River and of these four rivers together (Fig. 17) shows that in all these rivers the number of diatom cells observed during the investigation is not enough to cover the total diatom diversity, even for the Lomami with 4000 cells.

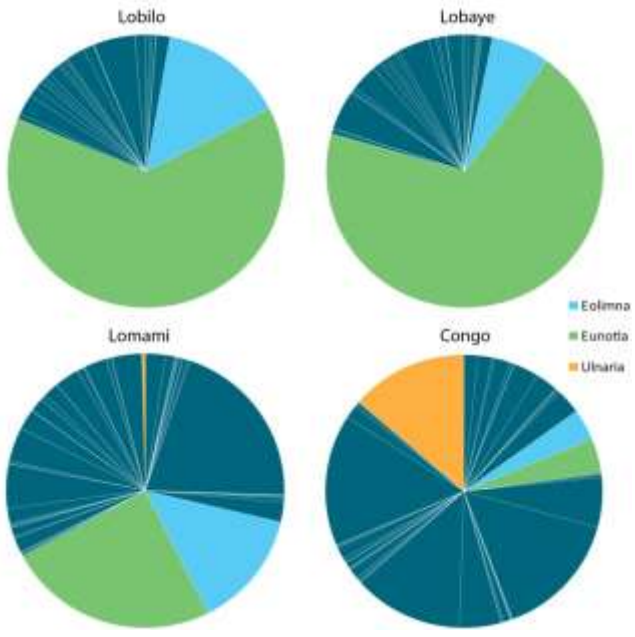


Figure 16. Pie diagrams of the relative abundances (%) of genera in the rivers Lobilo, Lobaye, Lomami and Congo.

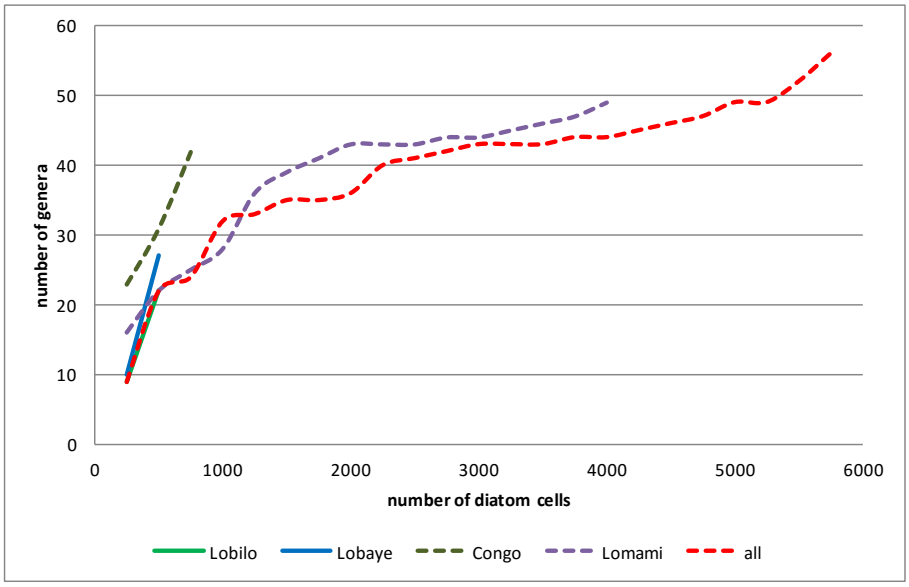


Figure 17. Saturation curve of the diatom genera in the rivers Lobilo, Lobaye, Lomami and Congo River; x-axis: number of diatom cells counted; y-axis: number of genera.

The lower species richness observed in the tributaries is due to the acid environment in these waters, which are always characterized by low diatom diversity and by the richness in desmids (Chlorophytes) not studied in the project. For comparison with the fish and invertebrate data only results for the diversity and uniqueness of the studied river ecosystems of the Lobilo, Lobaye, Lomami and Congo River are given in Table 5. The diatom results are derived from the semi quantitative investigation where 500 valves were enumerated for each slide studied. It concerns only samples taken during the COBAFISH expeditions. A total of 57 genera are observed in the four ecosystems. No unique genera are observed in the Lobilo and Lobaye as diatoms can be easily transported by the water current from the acid tributaries into the black waters of the major tributaries and the Congo River itself. The reported genera from the Lomami and Congo River ecosystems are thus representing not only their own diatom flora but also cells supplied by smaller tributaries. The relative portion of each genus is represented in Figure 1 for the same four river ecosystems. *Eunotia*, characteristic for acid waters (Cholnoky 1968), is dominant in the Lobilo and Lobaye ecosystems (63.9 and 69.4% respectively) and of decreasing importance in the black waters of the Lomami (24.6%); in the Congo River the relative abundance is less than 5%. *Ulnaria* (*Synedra* pro parte), on the other hand, is absent in the two acid rivers, but reaches up to 13.7% in the Congo River. Most of the taxa belonging to this genus live in alkaline waters (Cholnoky 1968).

Table 5. Number of genera in each river system (α-diversity) and the amount that is only encountered in that river (unique). For each river the number of shared genera with each other river is given and, the percentage which refers to the share of the total amount of genera of each river represented. The β-diversity represents total number of genera that are unique to each of the ecosystems.

river	α-diversity	unique genera	unique genera	Lobilo			Lobaye			Lomami			Congo		
		#	%	#	%	β	#	%	β	#	%	β	#	%	β
Lobilo	22	0	0				17	77.3	5	22	100	0	14	63.6	8
Lobaye	27	0	0	17	63.0	10				27	100	0	22	77.8	5
Lomami	50	7	14	22	44.0	28	27	54.0	23				36	72.0	14
Congo	43	7	16	14	32.6	29	22	51.2	21	36	83.7	7			
total genera	57														

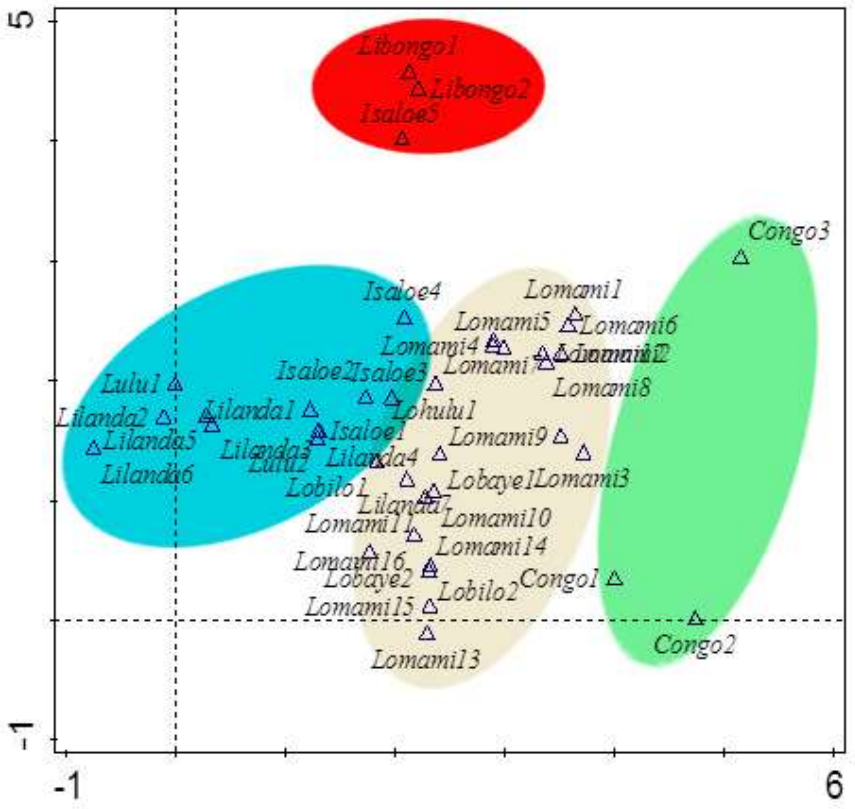


Figure 18. First and second axis of the DCA analysis, after square root transformation, for the samples from the rivers Congo, Lomami, Lobaye, Lulu, Lohulu, Lobilo, Lilanda, Isaloe and Libongo, with the distribution of the samples.

Table 6: List of the diatom genera and number of taxa (species and infraspecific taxa) observed in all samples studied from the Lomami River and three of its tributaries, the Lobaye, the Lulu and the Lohulu, from the Congo River, and from some rivers in the MAB reserve near Yangambi: the Lobilo, the Lilanda (including Baombo Stream), the Isaloe and the Libongo.

genus	Lomami	Congo	Lobaye	Lulu	Lohulu	Lobilo	Lilanda	Isaloe	Libongo
Achnanthes	4	4					1	2	
Achnanthidium	9	5	3			1	1	4	
Actinella							1		
Adlafia	4	1	4		2		2	1	
Amphora	1								
Aulacoseira	6	5	1		2			3	
Bacillaria	1								
Caloneis	3	2					1		
Capartogramma	1	1	1		1				
Cavinula	1					1		2	1
Chamaepinnularia	1								
Cocconeis	3	2	1						
Craticula	5							4	2
Cyclotella	3	2	1		3				
Cymbella	4	2							
Cymbopleura				1			1		
Desmogonium	1			1		1	1	1	
Diadesmis	1								
Diploneis		1							
Discotella	1	1			1				
Encyonema	3	1	1						
Encyonopsis	1		1			1		2	
Eolimna	2	1	1	1		1	1	1	1
Eunotia	15	4	7	6	4	6	12	12	6
Fallacia	2	2	1		1		2	2	
Fistulifera	1								
Fragilaria	2	1							
Fragilariforma			1	1		1	1	1	
Frustulia	4		2	3	2	5	6	3	2
Geissleria	4	3	1					1	
Gomphonema	8	10	2		1	2	1	1	
Gyrosigma		1							
Halamphora		1							
Hantzschia								1	
Hippodonta	2	1							
Humidophila	3	2	3	2			2	2	1
Luticola	3		1		2	1	2	3	
Mayamaea	5	1	2			2		1	
Melosira								1	
Navicula	21	15			3	1	5	8	3
Neidium	6		1	2	2	2	1	4	1
Nitzschia	16	32	1		2	1	1	1	
Nupela	3	2	1			2		4	2
Orthoseira	1	1				1	1	1	
Pinnularia	20	4	7	2	6	3	6	4	3
Placoneis	6		2		1	1	3	2	
Planothidium	4	1	1			1			
Pleurosira		1							
Pseudostaurosira		1					1		
Rhoicosphenia	1								
Sellaphora	9	5	1		1	3	3	3	1
Simonsenia		1							
Stauroneis	8	2					2	1	1
Staurosira	1	1	1						
Staurosirella	1	1							
Stenopterobia	3	1	1			1	1	1	
Stephanodiscus	1	1							
Surirella	2	3	1				1	1	
Thalassiosira	1	1							
Trybionella		1			1				
Ulnaria	3	6							
Urosolenia	1						1		
Total taxa	212 (n=16)	134 (n=3)	52 (n=2)	21 (n=2)	36 (n=1)	40 (n=2)	63 (n=7)	77 (n=5)	24 (n=2)

The results of the microscopic analyses of diatom counts, expressed as relative abundances, from 40 samples taken in the Lomami River and the Congo River, and in some acid watershed of smaller rivers and streams were analysed with ordination techniques using the programme CANOCA. A Detrended Correspondance Analysis (DCA) was performed as the data have a gradient of 10.0 SD units long. The eigenvalues of the first two axes are 0.4418 and 0.1358 respectively explaining 20.32 % and 2.86 % of the variation, after square root transformation of the dataset (Fig. 18). Four groups can be distinguished. The “pristine” acid rivers and streams (Lilanda, Lulu, Isaloe upstream near it source) are all located at the left side of the graph (Fig. 18, blue) and are characterised by *Desmogonium*, *Eunotia* and *Fragilariforma* (Fig. 19). The samples from the Congo River are located at the opposite side of the first axis (Fig. 18, green) and are characterized by, among others, *Gyrosigma*, *Pleurosira*, *Staurosira* and *Staurosirella* (Fig. 19). The samples from the Lomami River are situated in between (Fig. 18, light-brown), having more diatoms in its waters supplied by the acid tributaries. The samples from the mouth of the Libongo and Isaloe are also grouped (Fig. 18, red); these samples are strongly impacted by human pollution.

Task 2.2: Trophic diversity of macro-invertebrate communities (RBINS)

To establish biodiversity, densities and biomass of macro-invertebrate communities in the stations where fish community structure are evaluated. This requires the identification of macro-invertebrates at the taxonomic level that is required for their classification into Functional Feeding Groups (FFGs). The trophic niche of representatives of the different macro-invertebrate FFGs are established by stable isotope analysis (see Task 3.2), and the fish stomachs of selected fish species were analyzed and compared with standing densities/biomass of various macro-invertebrate FFG’s/ taxa collected in the field. The obtained stable isotopes are used to link the macro-invertebrates to lower trophic levels, while the diversity/density/biomass assessments will describe extant levels, available to fish, and the fish stomach analyses will link the macro-invertebrates to the higher trophic levels (see Task 3.1).

Table 7: Overview of samples, showing abundance (% of samples in which taxon is present) and number of species (conservative estimate) for 2010 Boyekoli Ebale Congo (181 samples), 2013 (67 samples) and 2014 (53 samples) COBAFISH expeditions. Only aquatic taxa are shown.

	2010 Abundance (%)	# species	2013 Abundance (%)	# species	2014 Abundance (%)	# species
Oligochaeta	9	3	7.5	2	7.5	2
Hirudinea	5	2	0	0	0	0
Mollusca (Gastro. + Biv.)	21	6	22	4	15	3
Crustacea-Decapoda	44	3	28	3	40	3
Crustacea-Others	19	3	15	2	4	1
Coleoptera-adults	29	9	63	5	57	10
Coleoptera-larvae	20	?	45	6	34	5
Heteroptera (adults)	41	25	52	16	72	11
Odonata (larvae)	56	15	57	10	51	10
Diptera (larvae)	42	6	81	5	64	5
Ephemeroptera (larvae)	37	9	66	5	34	5
Plecoptera (larvae)	1	1	25	1	19	2
Trichoptera (larvae)	13	5	46	4	43	3

Whereas the samples of the 2010 Boyekoli Ebale expedition were sorted earlier, the samples from the COBAFISH September 2013 and March 2014 expeditions, 67 and 53 samples respectively, were sorted and split into large taxonomical units. An overview is given in Table 7.

Based on relative high abundance and species diversity, identification efforts focused on Heteroptera. Therefore almost all available literature was collected and a preliminary ID-guide was made. A total of 31 taxa has hitherto been identified (see Table 8).

In addition a checklist of all species of aquatic and semi-aquatic heteropterans (Nepomorpha and Gerromorpha) for the DRC is compiled, based on our data as well as literature data. At present a total of 248 taxa (218 species) is known to occur in DR Congo. This checklist is currently transformed into a draft manuscript with provisional title: “Semi-aquatic (Gerromorpha) and aquatic (Nepomorpha) Heteroptera of the Democratic Republic of Congo, new records and a country checklist” and will be a useful tool for scientists working on aquatic heteropterans in central Africa, as it will not only act as a faunistic list, but will also enhance accessibility of the very scattered literature through an extensive reference list.

Table 8: Systematic overview of species of Heteroptera, present in DR Congo-samples.

Belostomatidae
Apassus ampliatus (Bergroth, 1890) Polhemus, 1995
Apassus grassei (Poisson, 1937) Polhemus, 1995
Apassus nepoides (Fabricius, 1803) Amyot & Serville 1843
Hydrocyrius columbiae Spinola, 1850
Hydrocyrius rectus Mayr, 1863
Limnogeton scutellatum Mayr, 1863
Corixidae
Micronecta spec.
Gerridae
Limnogonus (Limnogonoides) intermedius Poisson, 1941
Naboandelus sp.
Hebridae
Hebrus n.(?) spec.
Helotrephidae
Esakiella spec.
Esakiella hungerfordi/hutchinsoni-complex
Mesoveliidae
Mesovelia vittigera Horvath, 1895
Naucoridae
Macrocoris spec. (?*nigropunctatus africanus* Poisson 1949)
Naucoris spec. 1
Naucoris spec. 2
? *Neomacrocoris*
Nepidae
Laccotrephes spec.
Ranatra emaciata emaciata Montandon, 1907
Ranatra parvipes vicina Signoret, 1880
Notonectidae
Anisops (Micranisops) apicalis apicalis Stål, 1855
Anisops (Anisops) sardea sardea Herrich-Schäffer, 1850
Anisops (Anisops) (nov.?) spec.
Enithares sobria sobria Stål, 1855
Neonychia congoensis (Hungerford, 1946) Hungerford, 1950
Pleidae
Paraplea piccanina (Hutchinson, 1929) Esaki & China, 1928
Paraplea pullula (Stål, 1855) Esaki & China, 1928
Veliidae
Angilia (Adriennella) conradsi Poisson, 1950
Microvelia spec.
Rhagovelia infernalis (Butler, 1876) Lundblad, 1936
Xiphoveloidea spec.

Furthermore, all Plecoptera were identified as representatives of *Neoperla*, the only taxon of stoneflies in tropical Africa. Representatives of decapod crustaceans and molluscs were identified by Dr Sammy De Grave and Dr Bert Van Bocxlaer respectively. Three species of decapod Crustacea were present in the material (2010 and COBAFISH expeditions): *Potamonautes* cf. *lirrangensis* (Rathbun, 1904), a freshwater crab, *Macrobrachium sollaudi* (De Man, 1912) and *Caridina togoensis* Hilgendorf, 1893, both freshwater shrimps. Concerning molluscs, only the COBAFISH-material is treated here and consists of three species of Bivalvia and at least 14 species of Gastropoda (see Table 9).

Table 9: Systematic overview of species of Mollusca, present in COBAFISH-samples.

BIVALVIA		
	Unionidae	
		Coelatura aegyptia gabonensis (Küster, 1862)
	Sphaeriidae	
		Pisidium sp.
		Eupera ferruginea (Krauss, 1848)
GASTROPODA		
	Ampullariidae	
		Lanistes congicus (Boettger, 1891)
		Lanistes cf. *nsendweensis* (Dupuis & Putzeys, 1901)
		Lanistes cf. *ovum* (Peters, 1845)
	Achatinidae	
		Achatina ?
	Bithyniidae	
		Gabbiella cf. *kisalensis* (Pilsbry & Bequaert, 1927)
	Lymnaeidae	
		Lymnaea (Radix) natalensis (Krauss, 1848)
	Planorbidae	
		Biomphalaria sp.
		Bulinus forskalii (Ehrenbarg, 1831)
		Ceratophallus natalensis (Krauss, 1848)
		Ferrissia sp. (*F. burnupi* (Walker, 1912) or new sp.)
		Gyraulus cf. *costulatus* (Krauss, 1848)
		Melanoides nsendweensis (Dupuis & Putzeys, 1900)
		Melanoides tuberculata (Müller, 1774)
		Lentorbis cf. *junodi* (Connolly, 1922)

Analyses of abiotic data revealed that there are no significant differences in values between the March and September expeditions, i.e. there is no seasonality effect. But although there is a significant difference in temperature, pH, conductivity and O_2-concentration between the rivers Lomami and Lobilo, no differences could be found in either richness (no. of taxa) nor abundance (no. of individuals) between both rivers. Furthermore, the results of CCA and DCA did not reveal any differences between both rivers, or between the two different macrophyte habitats either. This is most probably a result of the high-level taxonomic units that are used and results could be improved by identifying all organisms to genus/species level.

The Ostracoda of the Congo River were studied in the framework of a non-EU postdoc allocated by Belspo to Dr Janet Higuti (12 months). The title of this postdoc project is ": A comparative analysis of the biodiversity of ostracoda (crustracea) in the Congo river (Africa) and Amazon river (South America) catchments". Ostracoda from the pleuston living in and on *Eichhornia crassipes* roots and amongst stands of Vossia cuspidata were compared. Eichhornia crassipes is native in the Amazon and invasive in the Congo River; *V. cuspidata* is native in the Congo River.

We used data from ostracods associated of *E. crassipes* and *V. cuspidata* of the same sampling period of both river catchments to compare the ostracods fauna. The localities sampled in the Amazon floodplain and Congo River catchment are generally located close to cities or villages. Thirteen localities were selected for each of the river

catchments (Figure 20). In each locality, two replicate samples of each aquatic macrophyte (*E. crassipes* in Amazon, *E. crassipes* and *V. cuspida* in Congo) were taken. *Eichhornia crassipes* individuals of similar size were hand collected and were placed in plastic buckets to remove the ostracods; roots were separated from the leaves and were washed in the bucket. The residuals were filtered in a hand net (mesh size c 160 µm). Ostracods in *Vossia* stands were collected directly by moving the handnet through the rooted plants for c 5 minutes. Ostracods were killed by adding 97% ethanol to the wet residual; all samples were washed again in the lab and were transferred to fresh ethanol (70%). Samples were sorted under a microscope stereoscopic. Specimens were identified using valves (Scanning Electron Microscopy) and appendages (soft parts dissected in slides with light microscopy).

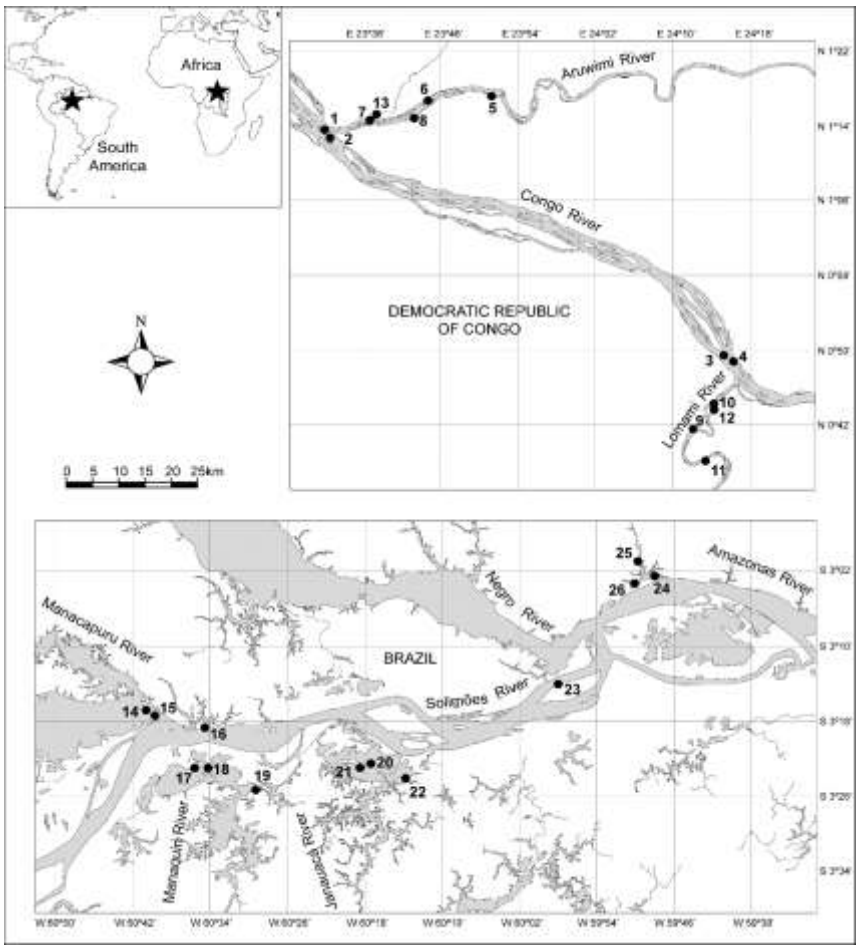


Fig. 20. Sampling sites in the Congo and Amazon basin

The environmental variables measured in the field included water temperature (°C) and dissolved oxygen (mg L^{-1}) (oxymeter-YSI), pH and electrical conductivity (µS cm^{-1}) (YSI 63). We recorded 26 species of ostracods associated to *E. crassipes* in the Amazon floodplain and 41 in the Congo River catchment, distributed by 32 species in *E. crassipes* and 27 in *V. cuspidata*. The highest diversity and abundance were recorded in the Congo River. The result of Principal Coordinates Analysis (PCoA), used to evaluate the (dis)similarity between different catchments, showed significant differences in species composition. The dispersion homogeneity test (PERMDISP) showed no significant differences in the variability of the composition of species of ostracods (beta diversity) within Congo River and Amazon floodplain. Ostracod communities in the Congo and Amazon were dissimilar, indicating *E. crassipes* roots in the Congo River basin were colonized by African ostracods. It appears that local ostracod faunas have adapted to exploit the opportunities presented by these floating invasive Eichhornia, as

diversity of ostracod communities is higher in the invasive *Eichhornia* than in the native *Vossia*. The detailed results are provided in Higuti and Martens 2016, see Annex 1:)

Task 2.3: Qualitative and quantitative diversity of fish communities (RMCA, RBINS)

The Congo River basin hosts the fish species richest diversity on the African continent and is second only after the Amazon in terms of species richness (Lundberg et al., 2000). Yet, no attempt has been made for a regional review or synthesis. Furthermore, the factors that regulate the diversity of fish remain to be established in the Congo River.

a. Fish sampling, morphology based identifications (RMCA)

Gosse (1963) made a first compilation of the ichthyofauna around Yangambi and listed about 15 species for the small, right bank, affluents (incl. the Lobilo River) of the Congo. Existing RMCA collections for the Lobilo contain about 50 fish species (collections made between 1946-57 and in 1989 mainly).

In contrast to the area around Yangambi, there is no literature available on the ichthyofauna of the Lomami River. In addition to the newly-collected specimens during the project's expeditions, also collections from the PhD study of Tuur Moelants and from the large Congo-2010 expedition were identified.

Fishes were collected during the three Cobafish expeditions. During the first expedition, that took place in November 2012, 670 specimens were collected. We refer to this expedition with the collection number B2-44. We collected 1371 specimens during the second expedition. This expedition took place in September 2013 and is referred to as B3-30. The last expedition was organized in March 2014, during which 768 specimens were collected. The collection number for the last expedition is B4-16. In total, 2809 specimens were collected.

All these specimens were morphologically identified based on literature, a comparison with available collections and thanks to the expertise of other ichthyologists. The number of taxonomic levels in all three systems is very similar (Table 10). The Lomami has the highest species diversity and also evenness, meaning it is a diverse and rich system. The number of species in the Lobilo and Congo is very similar (77 vs. 75). The list with all fish species found in each river is provided in the 'List of species found in the Cobafish area studied' (see Annex I)

Table 10. Number of taxa at different taxonomic levels, and diversity indices for each system studied.

	# Ordos	# families	# genera	# species	H (Shannon-Wiener)	Evenness
Lobilo	6	15	35	77	3,3425	0,7695
Congo	7	14	35	75	3,2174	0,7455
Lomami	6	14	36	81	3,5044	0,7975

The representation of all families in each system is given in Figure 21.
In each system, the most dominant families (i.e. represented by the most species) are Mormyridae, Alestidae, Distichodontidae. These three families are also the most diverse in the number of genera. Most of the families captured are represented in all three of the systems. Exceptions are: Amphiliidae (1 species) and Citharinidae (2 species) have only been captured in the Lomami, while Anabantidae (3 species) have not been captured in this system; Channidae (1 species) and Hepsetidae (1

species) have only been captured in the Lobilo; and Malapteruridae (1 species) and Mastacembelidae (1 species) have only been captured in the Congo main stream. No Clariidae have been captured in the Congo main stream, although the same fishing techniques have been applied in all systems.

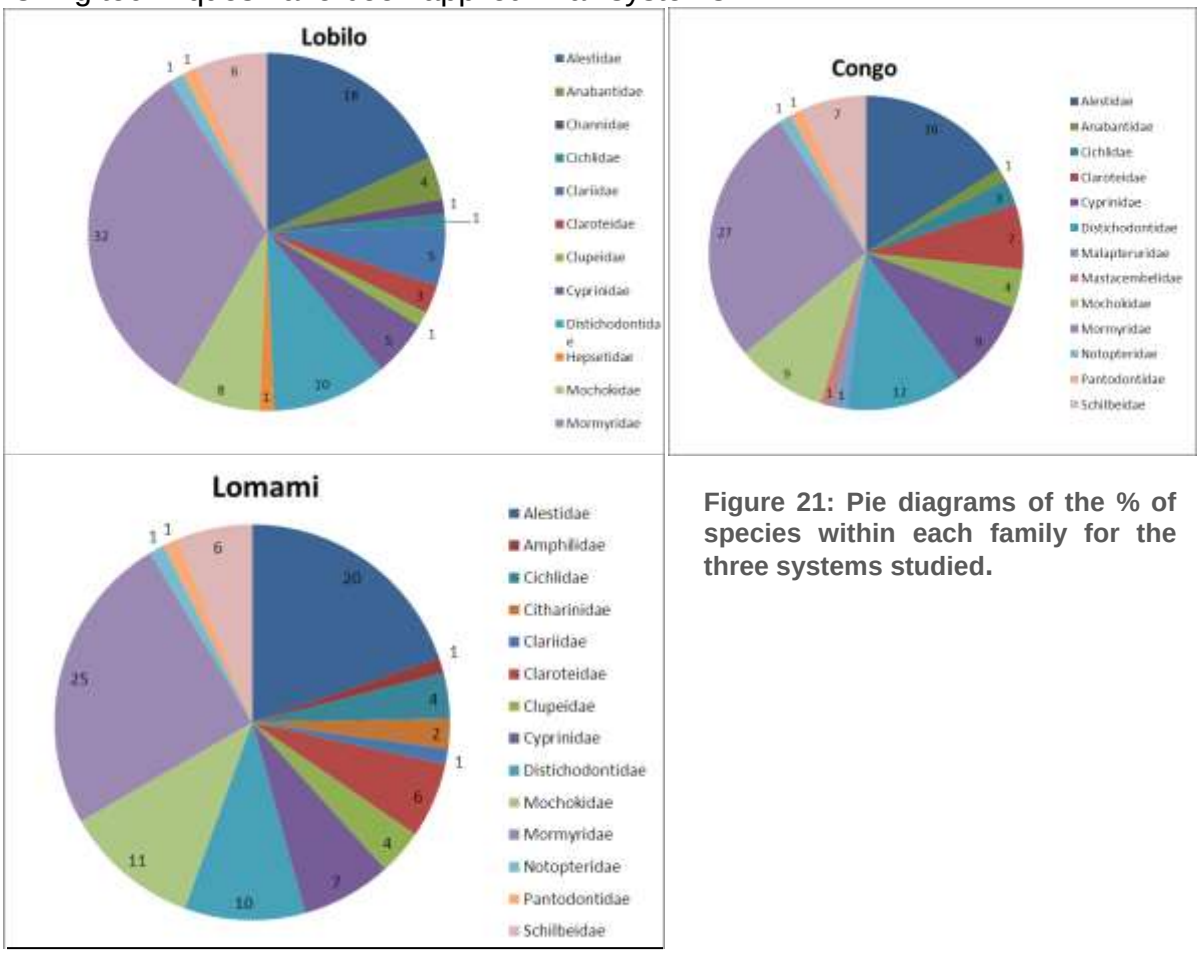


Figure 21: Pie diagrams of the % of species within each family for the three systems studied.

During the project, 29 species (22,0% of the total diversity) have been caught in all three systems (Table 11). Clearly, the Lobilo contains the highest number of unique species (not captured in the other two systems), which could be due to the fact that the Lobilo is a smaller affluent with different environmental conditions. The Congo and Lomami hence share the most species (and have thus also the lowest β-diversity).

Table 11: number of species in each river (α-diversity), and the number and percentage only encountered in a certain river (unique). In addition, for each set of two rivers, the number of shared species is given (#), its percentage (%) and the absolute species turn over between the two systems (β-diversity).

Site/River	#species (α)	unique species	unique species	Lobilo			Congo			Lomami		
		#	%	#	%	β	#	%	β	#	%	β
Lobilo	77	31	40,3				34	44,2	84	41	53,2	76
Congo	75	15	20,0	34	45,3	84				55	73,3	46
Lomami	81	14	17,3	41	50,6	76	55	67,9	46			
total # species in region (γ-diversity)	132											
species present in all rivers:	29											
% of total # species	22,0											

To evaluate to which extent the species collected represent the total biodiversity in the selected sites, we calculated species richness curves. The shapes of these curves indicate that the sampling effort has been insufficient to detect all species in the area (Fig. 22). The saturation curve of the Lobilo (smallest affluent) however starts to level off, meaning that a higher percentage of the total diversity in this river is covered.

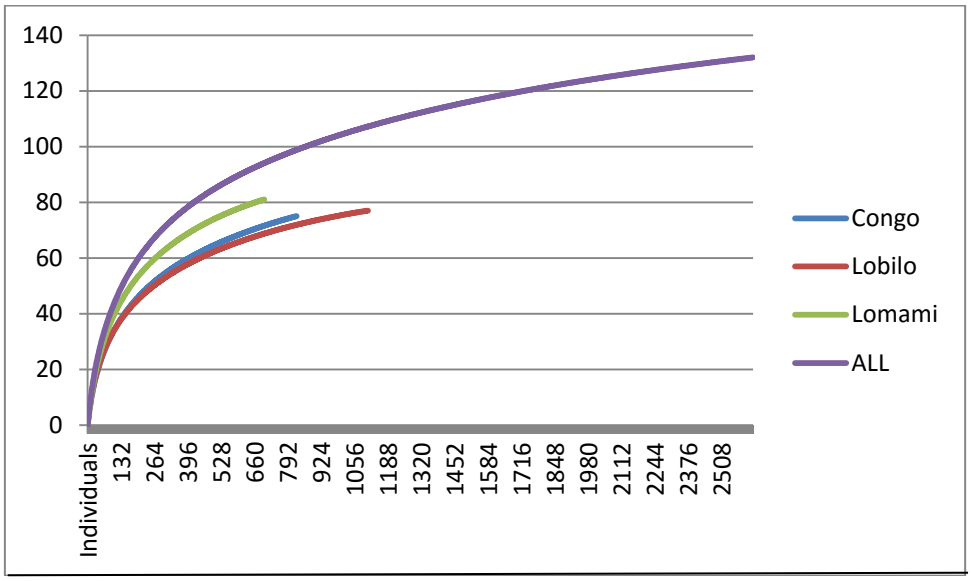


Figure 22. Estimated species richness curve with increasing sampling effort (# of specimens) for the different river basins).

During our study, many taxonomic problems were encountered, not all of which could be dealt with. Below we report on the numerous cases we did study with the proper morphometric methodology. For some, molecular analyses were included as well.

In order to have an overall view of the fishes of the area, we worked with Tuur Moelants (then PhD student), who examined the ichthyofauna in the Kisangani area, comparing the ichthyofauna up- and downstream of the Wagenia rapids. Data from the Cobafish field expedition 2012 were included in the results of his PhD thesis. This inventory provided a first basis of the ichthyofauna of the Lomami and Congo main stream, which are part of the study region of the Cobafish project. This study reported for the first time the presence of *Synodontis dorsomaculatus* and *S. flavitaeniatus* in the study area; the former from the Congo basin just up-and downstream of the Wagenia Falls and from the Lomami River, and the latter from the Loboya.

A taxonomic revision was made of the *Distichodus antonii* assemblage from the Congo basin (Moelants et al., 2014), a group of large, striped *Distichodus* species with a brownish-greenish colour pattern. Eight meristics and 28 measurements were taken on a total of 126 specimens. The results confirmed *D. atroventralis, D. antonii*, *D. fasciolatus* and *D. langi* to be valid species, even though the latter for long time has been considered to be a possible junior synonym of *D. antonii*. Reports of *D. mossambicus* from the Congo basin were found to be based on misidentifications. Additional discriminating characteristics between these species were discovered and redescriptions and an identification key have been provided, making identifications in the future much easier. In the project area studied, *D. atroventralis, D. antoni* and *D. fasciolatus* occur, as well as *D. lusosso* and *D. sexfasciatus,* which are large also

striped *Distichodus* species, but which were already easily distinguished from the others.

The remaining problems of the *D. atroventralis* complex were analysed separately. Two new species were found, which in contrast to *D. atroventralis*, do not occur in the area (Moelants et al., in press).

Also the genus *Citharinus* has been revised (Moelants et al., in prep.), for which a total of 122 specimens was examined using nine counts and 32 measurements. Surprisingly, *Citharinus* displays a remarkably stable taxonomy, as all six species were found to be valid. Again, the detection of additional discriminating characters and the identification key make future identifications easier. Three of the six species of genus, *C. congicus, C. gibbosus* and *C. macrolepis* occur in the area studied.

In collaboration with Tuur Moelants and Eva Decru (then PhD student), a DNA barcoding study (COI, mtDNA) was executed on the ichthyofauna of the north-eastern part of the Congo basin, including the project area, to evaluate the fish diversity on a genetic basis (Decru et al., 2015a). The identification success was low compared to other barcoding studies, as a result of many discrepancies between morphology-based species identifications and the different lineages recognized by this molecular technique. This is largely due to the fact that the ichthyofauna of the Congo basin is still underexplored. Identification keys and detailed information to distinguish the different species are often lacking, and the distributions are often not sufficiently known. In this study, two or three genetic lineages were found within each of the ten (out of 160) species from which we had more than one sample, indicating the possible presence of cryptic species. Conversely, in four cases, two morphologically distinct species clustered into the same genetic lineage, which could be due to introgression after hybridization. The DNA barcodes confirmed the existence of previously known identification problems within certain genera such as *Enteromius*, *Clarias* and *Labeo* (see below) but also confirmed species delineations that were already suggested by morphological studies in *Bryconaethiops* and *Distichodus*. For *Bryconaethiops,* the validity of *B. macrops* and *B. microstoma* (both occurring in the Cobafish study area) was questioned as the two could only be distinguished from each other by their number of caudal peduncle scales (10 vs. 12). The DNA barcoding study revealed a genetic distance of 10.5% between both taxa, confirming their validity. The other case concerns *Distichodus teugelsi*. In the Itimbiri, Aruwimi, Lobaye and Lomami rivers, specimens have been found that are very similar to *D. teugelsi* in terms of their basic colour and the number of scales around the caudal peduncle (16). However, they had dark spots on the flanks along the longitudinal line, a melanin pattern not found in *D. teugelsi*, but present in *D. decemmaculatus*. The latter species, however, has 20 caudal peduncle scales. The barcoding results indicate that the specimens indeed appear to be conspecific with *D. teugelsi*. As such, this species appears to display a much more variable colour pattern than previously reported.

Further research has been executed for some of the taxonomic problems discovered during this DNA barcoding study. Despite the fact that the genus *Clarias* was revised in 1986 by Teugels, species identification remains challenging, and some taxonomic problems persist. Two groups were found that resemble *Clarias pachynema*: *Clarias* sp. “pachynema very long barbels” found in the Lomami, and *Clarias* sp. “pachynema long barbels and pectorals”. Both groups differ from *C. pachynema* a.o. by their longer maxillary barbels; the latter species also by the longer pectoral fins. Further research is necessary to resolve this confused taxonomy.

Another taxonomic puzzle concerns the cichlid species *Tylochromis labrodon, T. lateralis* and *T. variabilis.* Some differences were described between the former two, e.g in the morphology and dentition of the lower pharyngeal jaw, in the post-temporal latero-sensory canal (closed vs. open), and in the pectoral fin length. However, when identifying our specimens, discriminating between the two species was not obvious. In addition, both species resemble *T. variabilis*. A morphological comparison of the three species revealed no differences, and open and closed latero-sensory canals were found in specimens of all three species (Musschoot, pers. comm.). Therefore, the *Tylochromis* specimens occurring in the Cobafish study area are tentatively identified as a member of the *T. lateralis*-complex.

Also some species of *Enteromius* from the north-eastern part of the Congo basin*,* a genus for which the taxonomy of the Congolese species is still largely understudied, have been examined into more detail. In this study (Van Ginneken et al., in press), an integrative approach combining barcoding and morphometrics was applied. Additionally, members of the *E. miolepis/eutaenia* complex from throughout the Congo basin have been examined. The study revealed an unexpected high cryptic diversity, as no less than 23 genetic lineages (COI, mtDNA) were found within what was initially identified as only four distinct species based on the literature available. Subsequent morphological analyses revealed most lineages also to be morphologically different from each other, suggesting they might indeed represent distinct species, almost all new to science. As only a part of the Congo basin and a subset of the species diversity within *Enteromius* was examined, it appears that the species richness of *Enteromius* in the Congo basin is considerably underestimated. This observation may also apply to other regions in Africa where *Enteromius* occurs. Within the specimens sampled during the COBAFISH expeditions, three different genetic clades were found: one clade with samples initially identified as belonging to the *E.* cf. *miolepis* complex from the Lobilo and Lomami and two clades initially identified as *E. brazzai.* A follow-up study (Van den Bogaart, 2015) on the clades within *E. brazzai* revealed further morphological differences. One of the clades from the Lobilo most probably represents the 'real' *E. brazzai,* while the other probably is a new species.

In collaboration with Eva Decru, several other taxonomic issues have been addressed. One of these was the morphological revision of the Congolese *Hepsetus* species based on 13 counts and 36 measurements on 158 specimens. This revision revealed that in the Congo basin there are three instead of only one species present. The species occurring in the region of the Cobafish project is *H. microlepis* and not *H. odoe* as previously assumed (Decru et al. 2015b).

A revision of the Congolese *Brycinus imberi* has been executed (Bulteel, 2015). *Brycinus imberi* is described from the Zambezi, but widespread throughout the Congo basin. This study combined morphology (31 measurements and 17 counts on 242 specimens) and genetics (mtDNA, D-loop and ncDNA myh6). The results revealed the existence of a new small species, the specimens of which were initially considered juveniles of *B. imberi.* This species is widespread in the Congo basin and can most easily be distinguished from *B. imberi* by differences in premaxillary dentition. The study also confirmed that *B. imberi* indeed occurs in the Congo basin, hence invalidating the assumption that the species occurring in the Congo basin is not conspecific with *B. imberi*, described from the Zambezi. In the Cobafish study area, *B. imberi* as well as the new species occur sympatrically.

Because of the large variability in colour pattern found within *Synodontis decorus* (types with rounded spots) including its synonym *S. vittatus* (types with longitudinal bands), a detailed morphometric study was undertaken including 27 measurements and two counts of 68 specimens Danadu et al, in prep.). Three

different colour patterns were distinguished: 'banded', 'spotted' and 'uniform'. Banded specimens were found only in the Upper Congo, spotted specimens only in the Lower and Middle Congo. Uniform specimens were rare and only found in the Middle Congo. In the Uele River exceptionally only uniform specimens were found. These different colour patterns are interpreted as intraspecific variation as none of the morphometric variables revealed to be significantly different between colour pattern types. The results also confirmed the synonymy of *S. vittatus* with *S*. *decorus*. In addition, a striking resemblance in colour pattern was noticed between small *S. decorus* and *S. nummifer* specimens, both occurring in the Cobafish study area. However, the latter lack the typical branched maxillary barbels of *S. decorus* and the horizontal black band in-between the two caudal-fin lobes. Moreover, the number of mandibulary teeth is larger, 12 (rarely 8) to 19, in *S. nummifer* (vs. 0 to 10 in *S. decorus*). Finally, while *S. decorus* has 2 to 4 vertical dark brown/black bands in the caudal fin regardless of size, in *S. nummifer* this number increases with size, with larger specimens having approximately 7-8 bands.

Micralestes humilis, M. sardina and *M. stormsi* are all three reported from the study area, but are difficult to distinguish from each other. A morphological study revealed *M. humilis* and *M. sardina* to be synonyms (Cuypers, 2014). *Micralestes stormsi* remains a distinct species, but diagnostic characteristics (differences in body depth, caudal peduncle length and dorsal fin base length) are only few and overlapping.

As the DNA barcoding study revealed three genetic groups within *Brachyptersius altus,* one of which occurring in the Lomami, we examined whether these genetic groups also differ morphologically. One of these groups (from the Aruwimi) revealed to be *B. pseudonummifer*, a species that greatly resembles *B. altus,* and the validity of which has been questioned. Additional discriminating characteristics between both species were found, facilitating future identifications. The second genetic group (from the Itimbiri) was clearly conspecific with *B. altus*. The third genetic group, appears to be a mixture of *B. altus* and *B. pseudonummifer* based on morphology. The specimens of the Lomami within the latter group do, however, resemble *B. altus.* These complex patterns could be the result of possible hybridization events between both closely-related species.

The most species-rich family in the study area is the family of the Mormyridae. However, the taxonomy of species and genera within this family is problematic, with many synonymizations of genera and species in the past. A study has been executed on the species of the genera *Hippopotamyrus* and *Cyphomyrus* using morphometric techniques, including 29 measurements and ten meristics (Degryse, 2014). Among these species, six were found in the study area. The distinction between a few species proved to be troublesome, with two possible cases of synonyms (*C. discorhynchus* with *H. aelsbroecki* and *H. macroterops* with *H. psittacus*). In addition, amongst *H. ansorgii* and *H. wilverthi*, evidence was found for a possible new species. A new identification key was made, facilitating identifications in the future. This study also had implications on the generic level, as the results confirmed the position of the species *C. discorhynchus*, *H. macrops*, *H. psittacus*, *H. wilverthi* and *Pollimyrus plagiostoma* within the genus *Cyphomyrus* and suggested the inclusion of *H. aelsbroecki*, *H. macroterops* and *P. tumifrons* as well.

Because of difficulties in identifying the small-scaled species of *Marcusenius* (Mormyridae), a revision of the Congolese species of this group has been executed. We found *M. bentleyi* to be a synonym of *M. leopoldianus.* Three specimens, from the Lomami, Itimbiri and main stream near Kisangani, were identified as *M. leopoldianus.* As *M. leopoldianus* was only reported from more downstream parts of the Middle Congo (Sangha River, Lakes Tumba and Mai Ndombe) the new

specimens represent an important range expansion of the species. As the DNA barcoding study (Decru et al., 2015a) revealed that *M. monteiri* and *M. stanleyanus* share the same haplotype for COI, both species were examined morphologically. No morphologic differences were found except for tooth morphology (unicuspid vs. bicuspid). However, some specimens were detected displaying both tooth shapes. Further genetic research with nuclear markers is planned.

During the identification processes, several specimens were discovered, resembling *Marcusenius moorii* (large-scaled *Marcusenius*) but differing mainly by their straighter head profile, higher number of anal fin rays, and lower dorsal fin/anal fin length ratio. These specimens belong to a new species that is recently discovered in the Ruiki River (Sullivan, pers. comm.), and will be described in the near future. The detection of these specimens in the COBAFISH study area results in a large expansion of the distribution area of this new species.

For the genus *Labeo,* despite the two existing revisions (Reid, 1985; Tshibwabwa,1997), many species delineations remain unclear, and identification problems persist. Of the species with papillary lips, three are reported from the Middle Congo basin and are possibly present in the area studied: *L. altivelis*, *L. lineatus* and *L. weeksii.* However, a detailed morphometric study revealed the latter to be a synonym of *L. altivelis*. *Labeo altivelis* and *L. lineatus* can be distinguished by their number of lateral line scales (33-37 vs 32-35) and branched dorsal fin rays (12-14 vs 11), the absence vs. presence of a caudal spot in small specimens (< 165 mm SL) and in allometric differences in the shape and size of the dorsal fin (Van Steenberge et al., 2016). Amongst the species with plicate lips, members of the *L. forskahlii* group are notoriously difficult to identify and distinguish from each other. The last few years, many attempt have been made to disentangle their taxonomy. However, the matter remains very complex and unravelling their taxonomy will still take some time. However, we ascertained that *L. cylindricus* does not occur in the Congo basin, and that all *L. cylindricus* specimens identified from the Congo basin actually belong to *L. annectens* (Decru et al., in prep.).

Studies have also been executed on the African tigerfish. Three species within the genus are currently known from the Congo basin: *Hydrocynus goliath, H. vittatus* and *H. forskahlii.* The first species is easily recognized by e.g. its larger number of teeth (14 or more vs. 12 in the upper jaw). The latter two are however difficult to distinguish as they only display some minor differences in colour pattern and a small difference in the position of the dorsal fin. A genetic study (Goodier et al., 2011) suggested that *H. forskahlii* does not occur in the Congo basin, and that at least three distinct genetic clades were found within *H. vittatus* from the Congo basin. A preliminary morphological study confirmed that *H. forskahlii* indeed does not occur in the Congo basin, but no clear groups could be identified within the *H. vittatus* complex. The DNA barcoding study (Decru et al., 2015a) also revealed the existence of three genetic groups within the samples of the north-eastern part of the Congo basin, but no morphological differences were detected between specimens of these lineages. Thus, despite the several indications of cryptic diversity within *H. vittatus,* we were not able to distinguish morphologically distinct groups.

Fish occurrence data (grouped by area) were analyzed with Principal Component Analysis (PCA) to explore differences between river systems.

The first obvious observation is that the Lobilo has a fish fauna different from the two others. In addition, the fish composition of the first expedition differs (higher values generally on the second axis for all rivers) from those of the two other expeditions (Fig 23). Furthermore, sites with low species richness cluster on the positive part of the second axis, diverging into two lines of species-rich assemblages

on the negative part but with a different species composition. On the third axis, generally lower values are found for the third expedition.

Linking to WP1, we studied the relationships of fish occurences and environmental (physicochemical) parameters. If both sets of data were not taken at the same site, then physicochemical parameters were taken from the site nearest to the locality where fish were caught. Multivariate analyses (Canonical Correlation Analysis, Redundancy Analysis) were used to evaluate the correlation between the ecological parameters and the fish distribution. Environmental variables with missing data were excluded.

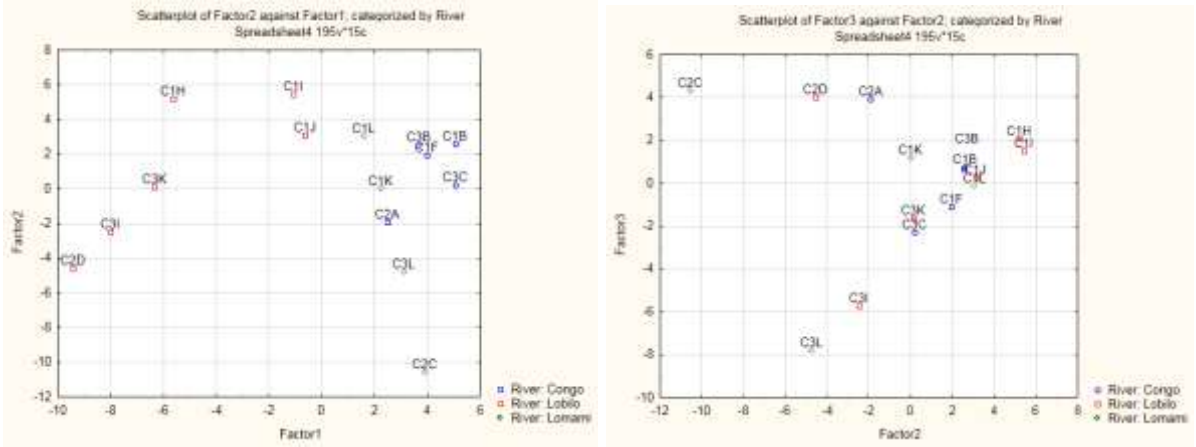


Figure 23: Position of the sampling areas on first and second axis (A) and the second and third axis (B) of a PCA on fish occurrences. Each area has a unique code with numbers refering to expeditions and colours to river systems.

We found no correlation (p=1.000 with Monte-Carlo permutation test) between the fish distribution matrix (based on abundancies on all sampling sites) and the variation in ecological parameters. A Forward Selection revealed only pH, water temperature, N_2O and δ^{13}C-POC to be uncorrelated variables that significantly influence fish species distribution. Of these pH, temperature and δ^{13}C-POC resume best the environmental impact of the set of highly correlated variables (Fig. 24), while N_2O is most probably unimportant because it is present only in low concentrations and average differences between the three sites are close to the detection limit (Fig. 2). Further analyses are planned to explore both data sets more thoroughly.

As a kind of control, analyses were conducted not excluding the variables but the fish sampling sites with missing data. These analyses demonstrated no important influence of the missing variables (secchi disk depth, %PN, DOC, δ^{13}C-DOC, Chl-a, POC/Chl-a, NO_3^-, total nitrogen, SRP and total phosphorus) on fish distribution (results not illustrated).

The CCA analysis (Fig. 24) reveals a clear separation between the sites on the Lobilo and those of the Congo and Lomami on the first axis, mainly due to a lower temperature and pH in the Lobilo (Fig. 2). There is no clear separation between sites of the Congo and Lomami, but for both systems, the sites from the first expedition (high water levels) have generally lower values on the second axis.

Plotting the individual species on the result of the CCA analysis illustrates the exclusive or focal occurrence of the species in certain areas (Fig. 25). For example, the species on the upper left corner are typical for the Lomami at the time of the second expedition, while the species on the right are exclusivly or mainly found in the Lobilo.

Further analyses have been done exploring patterns in the Congo and Lomami, hence excluding the Lobilo, and on individual river systems to examine distribution patterns among different sampling sites within the same system. No

differential structuring was between the Congo and the Lomami, nor within individual systems, except for the separation of the sites of the first expedition (already observed in the previous analyses).

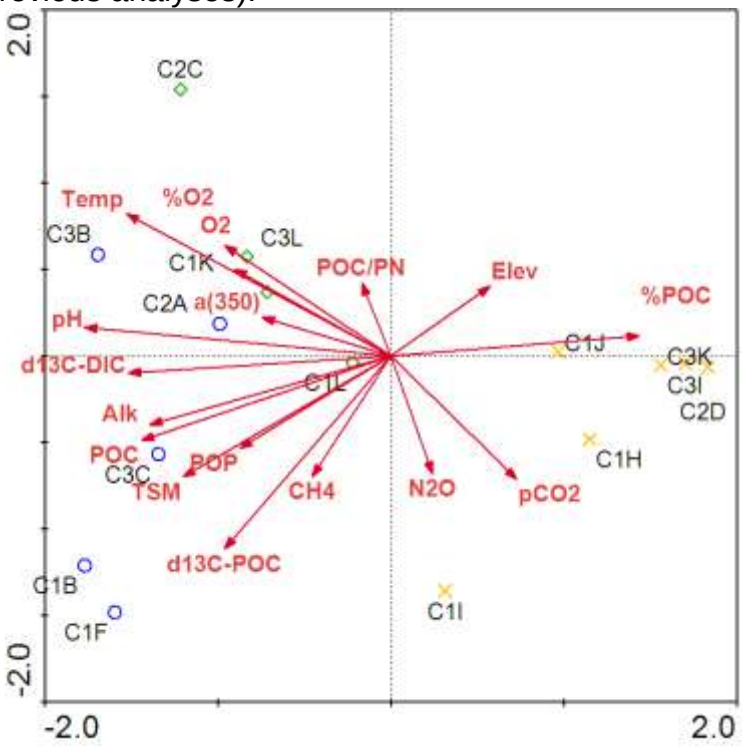


Figure 24. CCA analysis exploring the correlation between fish occurrences and environmental parameters. ×= Lobilo, ◇= Lomami, ○= Congo.

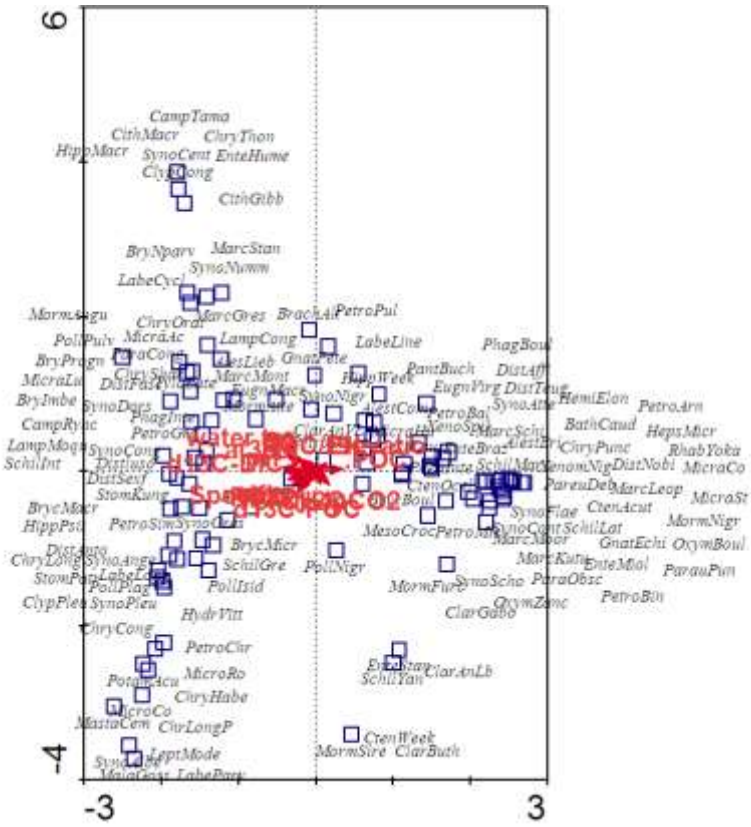


Figure 25. CCA analysis exploring the correlation between fish occurrences and environmental parameters. Same analysis as in Figure 24 but with position of fish species plotted.

b. Use of DNA barcodes to facilitate species separation for cryptic species (RBINS)

The biological sampling requires species diagnoses for a fauna that is overall not well documented. For the fishes of the Congo basin, partners RBINS and RMCA have compiled a database that links a number of mitochondrial cytochrome oxidase sequences with almost 200 reliable species names. These sequences are also linked to a specimen collection that is available for every species that has been described (or is being described) for Kisangani and its wide surroundings, as well as the Lower Congo. By employing DNA sequences as taxon 'barcodes', we have facilitated and validated the identification of the fishes collected in this study. This is feasible as our previous work demonstrated that in this fauna mitochondrial DNA sequences allow species-level assignments. Because of the limited available budget, this strategy was mainly used for species that are difficult to identify on the basis of their morphology alone. For juvenile specimens that were collected during these surveys, this approach provides a reliable, cost-effective and accessible solution to the current problem of species identification.

We realize that the obtained DNA barcodes are most likely to provide potentially useful information for groups that are already well studied, hence the importance of the existing - though undoubtedly incomplete database. To assess the putative conspecificity of species that are not yet present in our collections, we have sequenced several presumed conspecific specimens per sampling site. In collaboration with the taxonomic experts (MRAC, Snoeks and collaborators) the identification of already known species was accelerated by this procedure, while species new to science will be easier to describe. Pending their description, and their abundance/importance in the sampling, these Molecular Taxonomical Units will be used as proxies for species in biodiversity analyses.

During each expedition finclips for genetic barcoding were taken from several specimens divided over the different species found.(Muscle tissue was taken as wel from these specimens for stable isotope analysis). During the first expedition 327 finclips were taken, 360 during the second expedition and 247 during the third one.

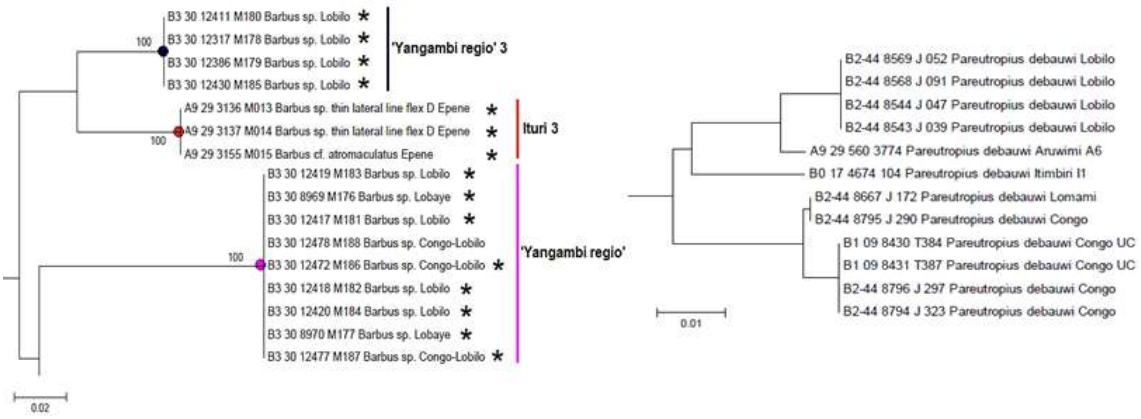


Figure 25: Left panel: *Enteromius* specimens routinely identified as *Barbus cf. atromaculatus* appear to contain several putatively undescribed species, each of which occurring in its own river system; Right panel: Genetic variation detected among geographically separated populations identified as *Pareutropius debauwi* is unusually large for a single species.

In total, 620 specimens collected during the COBAFISH expeditions were successfully sequenced. They are added to the dataset generated during the PhD theses of Eva Decru and Tuur Moelants who worked on fish faunas from neighboring regions. Through this combination of datasets, we were able compile no less than

1440 sequences for the fish species from the study areas and surrounding watersheds.

By adding sequences from earlier projects, we were able to verify whether species identifications during these different projects were done in a coherent way. The obtained datasets allowed us to discern several taxonomic issues, two of which are shown in Figure 25.

For those species, for which we had a number of sequences from the different systems, we constructed haplotype networks in order to examine the intraspecific genetic variation (illustrated in Figures 26-28 as examples). For most of these species, we did not find population structuring, meaning that there is no genetic isolation of the populations in the different systems.

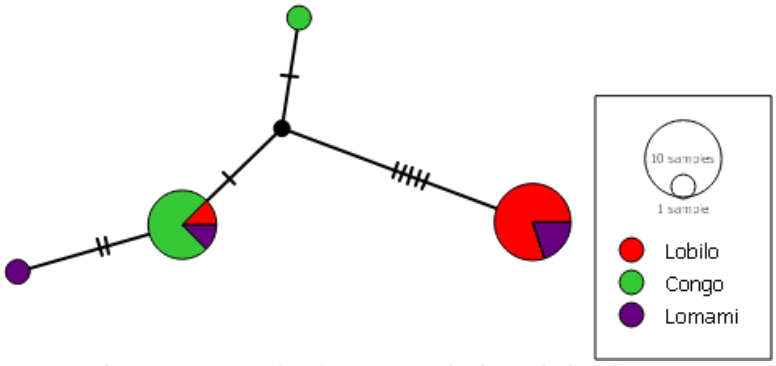


Figure 26: Haplotype network of *Petrocephalus christyi.*

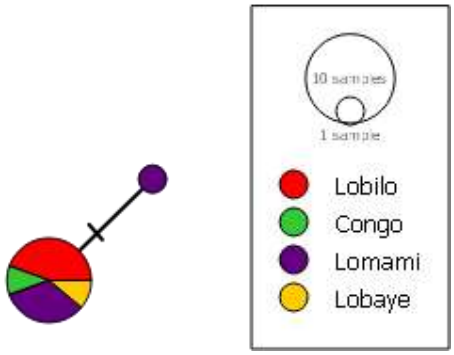


Figure 27: Haplotype network of *Marcusenius schilthuisiae*

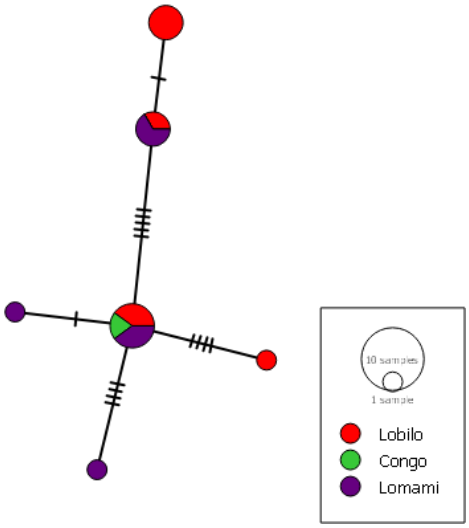


Figure 28: Haplotype network of *Schilbe marmoratus.*

WP 3: Ecosystem trophic structure (foodweb analysis)

Workpackage 3 addressed the carbon flow through the entire food web, from organic carbon over primary producers (phytoplankton, periphyton, macrophytes) and primary consumers (macro-invertebrates) to the fish communities. As such, it relies on the results of the tasks in WP1 and WP2 and integrates them.

Task 3.1: Fish stomach content analysis (RMCA, RBINS, NBGB)

In a first analysis on stomach contents, a total of 317 stomachs from 27 species from the Kisangani region were analysed with classic stomach analysis (Moelants, 2015). The most important results were that the majority of fish species were opportunistic in their food choice, consuming both aquatic and terrestrial invertebrates and plant material, and that more than 60% of the fish species consumed terrestrial invertebrates, confirming the importance of riparian sources to the diet of freshwater fishes of a Central African tropical forest.

In a next step, we studied the trophic ecology of the fish species collected during the third expedition (Van De Walle, 2015). During this approach, the stomach contents and stable isotopes of 246 specimens were analyzed.

We calculated the relative contribution of terrestrial versus aquatic food sources for each fish species. The figures below summarize the main findings for the most important species. The categories distinguished are: animal material of aquatic origin (Dier aqua), plant material of aquatic origin (Plant aqua), animal material of terrestrial organ (Dier ter), plant material of terrestrial origin (Plant ter) and unidentifiable food items (detritus, insect material such as legs, wings,..) (Rest). These results confirm our earlier findings that an important proportion of the food sources is of terrestrial origin. The figures below provide the relative percentage of the amount for each prey category that we detected for different species (Figures 29-33). Abbreviations of the fish species names in these figures are provided in annex.

We clustered (Ward's method) fish species on the basis of the proportions of the dry weight of the different prey categories in their stomachs (Figure 34). We detected six groups: (1) herbivores with a preference for terrestrial plant material, (2) piscivores, (3) omnivores with a large portion of terrestrial material, (4) *Megaloptera* eaters, (5) insectivores/invertivoresand (6) omnivores/insectivores with a large amount of detritus.

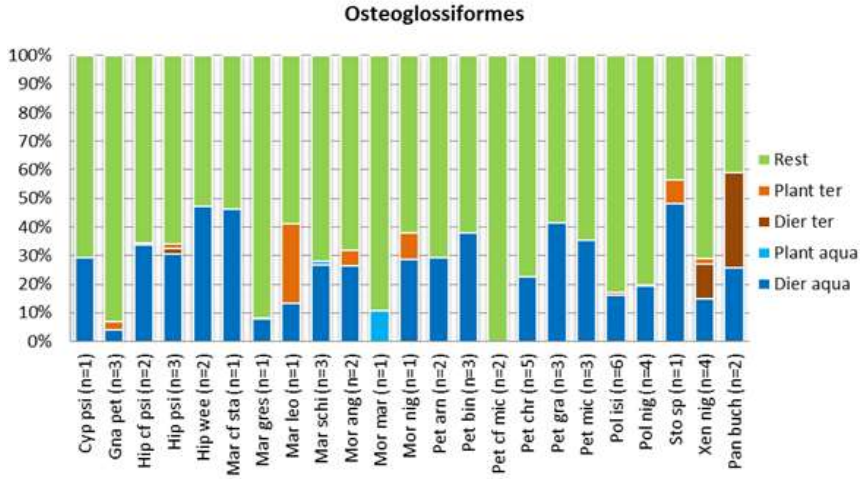


Figure 29: Relative contribution of the different prey categories in the Osteoglossiformes (Van De Walle, 2015)

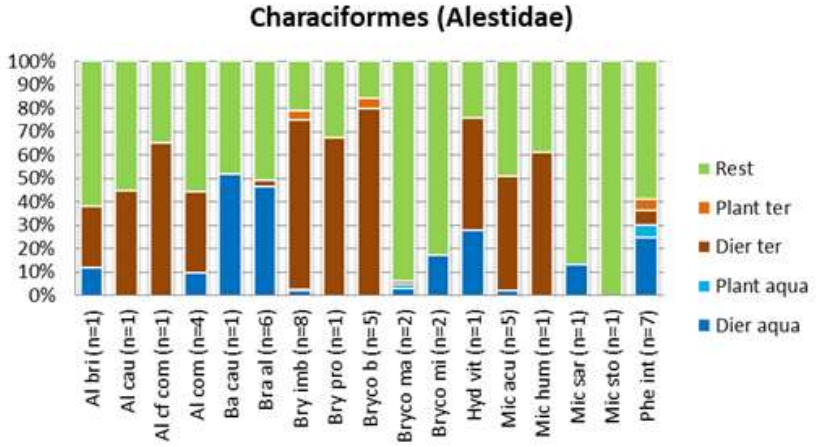
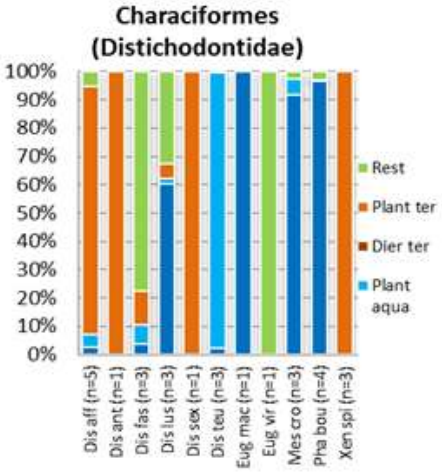
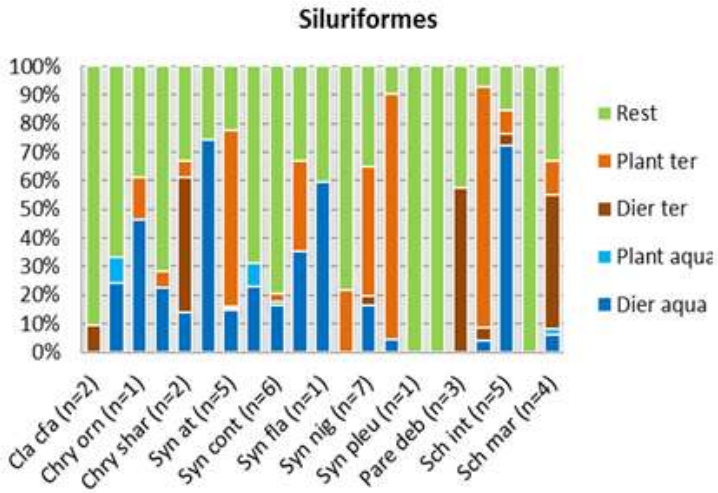


Figure 30: Relative contribution of the different prey categories in the Characiformes (Alestidae) (Van De Walle, 2015)





Figures 31-32: Relative contribution of the different prey categories in the Characiformes (Distichodontidae) & Siluriformes (Van De Walle, 2015)

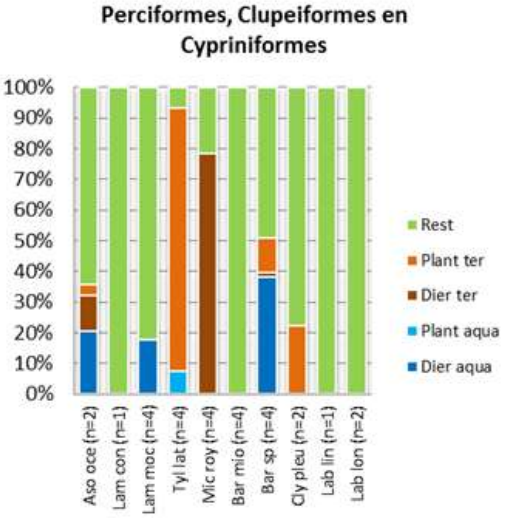


Figure 33
Relative contribution of the different prey categories in the Perciformes, Clupeidormes and cypriniformes (Van De Walle, 2015)

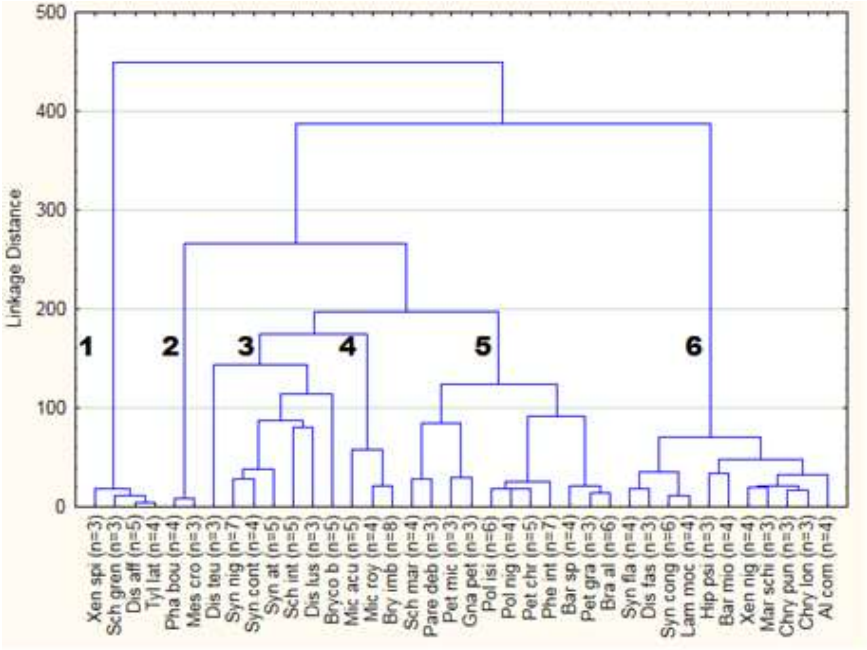


Figure 34: Result of a Cluster Analysis on the stomach contents data (Ward's Method) (Van De Walle, 2015)

In a further step, we broadened the scope of the study and allocated all species found in the study area to one of five main trophic groups, based on the stomach content analyses or on literature data, and on extrapolations thereof for those species for which no stomach content data were available. The proportion of the different trophic groups (diet preferences) has then been calculated for every system (Tables 12-13).

Table 12. Percentage of specimens per trophic group and per river system.

	%Plankton	%Herb	%Invert	%Omni	%Pisci	%unknown
Lobilo	0,0	3,2	32,3	61,0	2,5	1,0
Congo	0,8	3,9	46,1	44,4	1,8	2,9
Lomami	0,9	4,7	25,5	66,3	1,6	1,0

Table 13. Percentage of species per trophic group and per river system.

	%Plankton	%Herb	%Invert	%Omni	%Pisci	%unknown
Lobilo	0,0	6,5	23,4	58,4	7,8	3,9
Congo	1,3	10,7	25,3	52,0	6,7	4,0
Lomami	1,2	12,3	22,2	54,3	4,9	4,9

The general picture is of a dominance of omnivorous species in all rivers, followed by invertivores, illustrating again the mainly generalist character of the fishfauna of the region. In terms of abundancies, omnivores dominate clearly in the affluents, while there is a small predominance of invertivores in the Congo main channel.

Seasonal variation was studied in *Brycinus imberi* (Boyen, 2015). No strong seasonal signal was detected, but significantly more aquatic plant material and less Formicidae were found in specimens captured during the second Cobafish expedition (primary low water season) than in specimens from the first and third (secondary flood and low water seasons) (Figure 35).

With Trophlab, the trophic level of 27 species from the Lomami and Maiko rivers was calculated (Carmen, 2014). The average trophic level was for each species slightly higher in specimens from the Maiko than in specimens from the Lomami. The average trophic level was significantly lower for rapids than for less fast flowing waters.

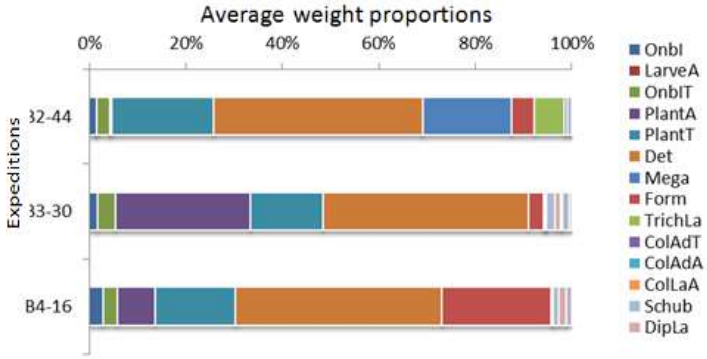


Figure 35: Mean weight proportions of each food category found in the stomachs of *Brycinus imberi*, sorted by expedition (B2-44, first expedition (November 2012), B3-30 second expedition (September 2013) , B4-16 last expedition (March 2014). (Boyen, 2015) Legend: Onbl: Unknown Insect, LarveA: Aquatical larvae, OnbiT: unknow Insect from a terrestrial, PlantA: aquatic plants, PlantT: terrestrial plants, Det: Detritus, Mega: Megaloptera; Form: formicidae, TrichLa: Trichoptera larvae, ColAdT: Coleoptera Adult terrestrial, ColAdA: Coleoptera Adult aquatic, ColLaA: Coleoptera Larvae aquatic, Schub: scales, DipLa: Diptera Larvae

Task 3.2: Isotopic foodweb analysis (KUL & RMCA)

Stable isotope ratios (SI) of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulphur ($\delta^{34}S$) are frequently used in food web studies, and recently the potential of hydrogen stable isotope ratios ($\delta^{2}H$) in differentiating dietary source contributions (e.g. terrestrial vs. aquatic) to aquatic foodwebs has been demonstrated (Soto et al. 2013). Their utility is based on the fact that the isotope composition can differ between primary producers, and because it undergoes either little or a predictable change as it is passed on to consumers. Hence, the SI composition of a consumer is a reflection of its food source. $\delta^{13}C$ is particularly useful to determine the main sources of nutrition because of the relatively small (0-1‰) discrimination from food source to consumer. $\delta^{13}C$ signatures differ strongly between terrestrial C3 and C4 plants (~-27‰ for C3; ~-12‰ for C4), and aquatic primary producers can be expected to vary in $\delta^{13}C$ signatures depending on taxa and sites. In particular, $\delta^{13}C$ data on dissolved inorganic carbon from sites across the Congo basin (unpublished data from field campaigns between 2009 and 2015) show large contrasts between different river types, ranging overall between -27.6 ‰ and ~0 ‰. This range reflects differences in weathering types (carbonate vs. silicate dominated, geogenic inputs) and metabolic balance, but implies that local aquatic producers will show a similarly wide range of variations across sites.

$\delta^{34}S$ and $\delta^{15}N$ have been used in conjunction with $\delta^{13}C$, as these can also vary between aquatic and terrestrial producers in freshwater and marine environments. However, the potential of $\delta^{34}S$ is limited in freshwater ecosystems, and our own data from freshwater foodwebs studies in the Zambezi basin indicated that this proxy has no added value, and was hence not further considered in the context of COBAFISH. While $\delta^{13}C$ generally provides information on the base of food chains, $\delta^{15}N$ is more useful as indicator of trophic positions, as it undergoes a much higher discrimination between trophic levels. Hence, the use of $\delta^{13}C$ and $\delta^{15}N$

combined gives information not only on diet sources but also on some of the main features of a food web such as individual and population trophic position, trophic length, omnivory, diet overlap, and generalized versus specific feeding.

All bulk carbon and nitrogen stable isotope measurements ($\delta^{13}C$, $\delta^{15}N$) on foodweb components collected during the three main COBAFISH field campaigns have been finalized. Additionally, during one of the "Transcongo" field campaigns (an FNRS funded project led by ULG), fish samples were also collected at various sites along the Congo River to complement the COBAFISH dataset. Jointly, these represent > 1500 samples of primary producers, invertebrates, and a wide variety of fish species. During the 3rd field campaign and the Transcongo sampling, efforts were concentrated on fish sampling. A summary of these data is presented in Tables 14-18. While $\delta^{2}H$ analyses were initially not foreseen in COBAFISH, a recently funded Marie Curie postdoctoral project (AQUAHYDRO) and our own investments in analytical infrastructure upgrades now allows to perform these analyses in-house, and AQUAHYDRO foresees to process the majority of COBAFISH samples for $\delta^{2}H$. Preliminary data on a first set of samples are discussed below as a brief indication of their potential.

Terrestrial invertebrates

Terrestrial invertebrates showed a wide range of $\delta^{13}C$ values but are clustered in two distinct groups, whereby one group derives its C mainly from C3 vegetation (with resulting $\delta^{13}C$ signatures of -27.1 ± 2.3, n=32 and -27.9 ± 1.5 ‰, n=22) and a second group relying mainly on C4-derived C ($\delta^{13}C$ of -13.1 ± 1.6, n=10, and -15.4 ± 2.1, n=15). It is interesting to note that the latter group consists of several taxa (Orthoptera, Hemiptera, Tabanidae, Coleoptera) for which other species can be found in the C3 group, suggesting species-specific feeding niches for some of these groups (i.e. localized feeding in C4 grass habitats or C3-dominated habitats).

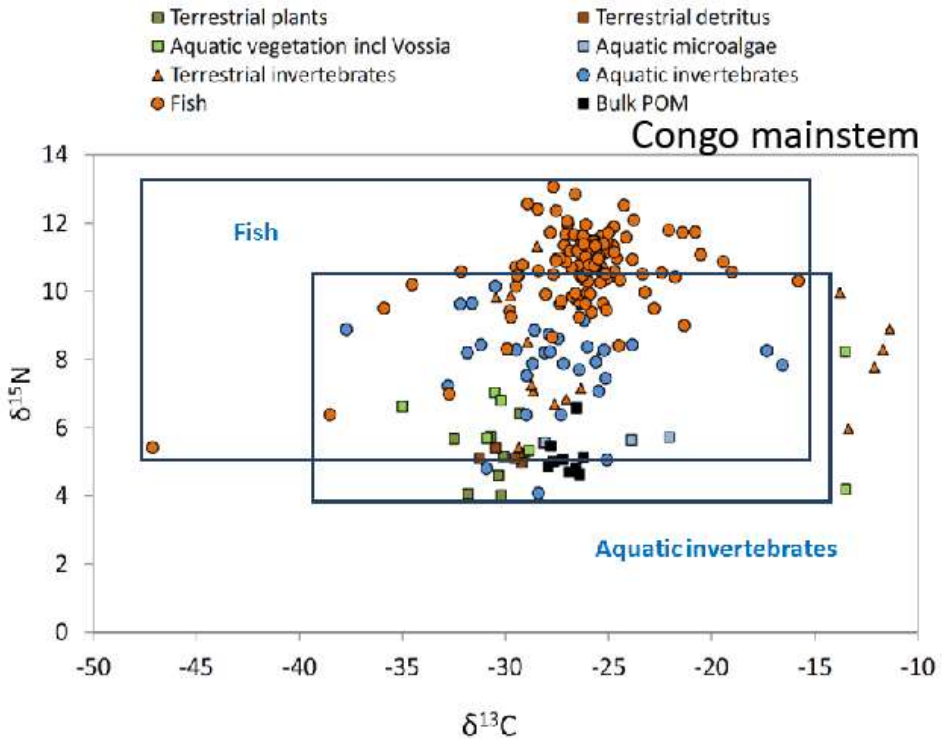


Figure 36: Combined $\delta^{13}C$-$\delta^{15}N$ data for foodweb samples from the mainstem Congo River collected in November 2012.

Aquatic invertebrates

Turning to the aquatic consumers, the dataset collected show a surprisingly wide range of $\delta^{13}C$ (Tables 14-16), ranging overall between -45.3 and -19.2 ‰ across the different field campaigns. While preliminary data from the CONGO2010 expedition had already shown a wider range of isotope signatures than could be explained by merely terrestrial C sources, the variability found in the COBAFISH2012 dataset far exceeded our expectations. A similarly high variability was encountered in fish communities, with $\delta^{13}C$ signatures between -47.1 and -12.1 ‰ across the different sites and field campaigns. Combined $\delta^{13}C$ and $\delta^{15}N$ for the mainstem Congo River in November 2012 are illustrated in Figure 36.

Table 14: Summary of $\delta^{13}C$ and $\delta^{15}N$ signatures measured on different ecosystem components collected in the Lobilo, Lobaye, Lomami and mainstem Congo River during the COBAFISH-1 field campaign (November-December 2012).

	$\delta^{13}C$ ± stdev	*n*	$\delta^{15}N$ ± stdev	*N*
Terrestrial C3 (all sites combined)	-32.3 ± 2.3	44	3.3 ± 2.2	44
Terrestrial detritus (all sites combined)	-30.6 ± 1.6	22	3.8 ± 1.5	22
Vossia sp. (all sites combined)	-13.1 ± 1.0	15	5.5 ± 2.6	15
Aquatic macrophytes (all sites) excl. 2 samples	-30.3 ± 1.9	31	4.6 ± 2.6	31
Ranunculus sp., Lobilo	-44.5	1	10.3	1
Aquatic macrophyte, unid., Lomami	-37.6	1	6.2	1
Aquatic microalgae (peri/epiphytes)	-33.0 to -21.5	23	4.2 to 5.2	23
Terrestrial invertebrates “C3 group”	-27.1 ± 2.3	32	7.9 ± 1.8	32
Terrestrial invertebrates “C4 group”	-13.1 ± 1.6	10	7.7 ± 1.6	10
Bulk POM, Lobilo	-28.3 ± 0.5	3	6.6 ± 0.4	3
Bulk POM, Lobaye	-29.0	1	4.7	1
Bulk POM, Lomami	-28.2 ± 0.3	4	4.7 ± 0.5	4
Bulk POM, Congo River	-27.1 ± 0.6	9	5.2 ± 0.6	9
Aquatic invertebrates, Lobilo	-41.4 to -20.9	10	7.4 to 10.2	10
Aquatic invertebrates, Lobaye	-31.1 to -26.1	12	6.3 to 9.8	12
Aquatic invertebrates, Lomami	-29.8 to -28.6	10	6.4 to 8.9	10
Aquatic invertebrates, Congo River	-37.7 to -23.9	26	4.8 to 10.1	26
Fish, Lobilo	-38.3 to -24.5	82	9.3 to 15.7	82
Fish, Lomami	-31.6 to -24.6	125	6.6 to 13.3	125
Fish, Congo River	-47.1 to -15.8	118	5.4 to 13.1	118

The lowest invertebrate $\delta^{13}C$ values were found in both Anisoptera and Zygoptera larvae, as well as in Chironomidae. Very low $\delta^{13}C$ signatures were also found during the Congo2010 expedition in a number of Odonata larvae and Coleoptera. The lowest $\delta^{13}C$ in fish were found in individuals of *Synodontis congica, Pollimyrus cf. plagiostoma, Petrocephalus christyi, Marcusenius* sp. and *Synodontis contracta*. Within the fish communities, highly ^{13}C-enriched signatures similar to those of C4 vegetation (i.e., *Vossia*) were consistently found in *Distochodus*

antroventralis and *D. fasciolatus*, suggesting an almost complete reliance on *Vossia*-derived C, either directly or via terrestrial invertebrates that feed within *Vossia* patches.

Despite the extremely wide range of fish $\delta^{13}C$ values at most sites and sampling seasons, average or median $\delta^{13}C$ signatures of fish show only minor shifts between the four sampling sites (e.g., for the first sampling season: -26.3 ± 3.5 ‰ for the Congo mainstem, -27.9 ± 1.4 ‰ for the Lomami, and -28.6 ± 2.5 ‰ for the Lobilo), *i.e.* a 2 ‰ gradient between the Lobilo and mainstem Congo River.

Considering the differences in $\delta^{13}C$-DIC between these sites (-25.0 ± 0.3 ‰ for the Lobilo, -21.7 ± 1.1 for the Lomami, and -13.2 ± 1.5 ‰ for the mainstem Congo River), this suggests that overall, *in situ* aquatic primary production is unlikely to play a major role in sustaining fish at the community level across these sites – although it does not include a possible role for phytoplankton in the mainstem Congo River where $\delta^{13}C$ signatures of phytoplankton are expected to overlap with those of terrestrial C sources. While the majority of fish and invertebrate data are consistent with an important role for terrestrial support (either through aquatic or through terrestrial invertebrates), the extremely low $\delta^{13}C$ signatures found in some aquatic consumers call for alternative C sources.

Table 15: Summary of $\delta^{13}C$ and $\delta^{15}N$ signatures measured on different ecosystem components collected in the Lobilo, Lobaye, Lomami and mainstem Congo River during the COBAFISH-2 field campaign (September 2013).

	$\delta^{13}C$ ± stdev	*N*	$\delta^{15}N$ ± stdev	*N*
Terrestrial C3 (all sites combined)	-29.8 ± 1.6	15	11.5 ± 5.0	15
Vossia sp.	-12.0	1	9.4	1
Aquatic macrophytes (all sites)	-29.9 ± 3.0	8	8.0 ± 2.2	8
Biofilm/filamentous algae	-30.0, -20.1	2	6.7, 14.4	2
Terrestrial invertebrates “C3 group”	-27.9 ± 1.5	22	9.1 ± 3.2	22
Terrestrial invertebrates “C4 group”	-15.4 ± 2.1	9	8.9 ± 1.9	9
Aquatic invertebrates, Lobilo	-34.0 to -23.6	11	9.7 to 16.5	11
Aquatic invertebrates, Lobaye	-45.3 to -20.7	11	5.2 to 10.4	11
Aquatic invertebrates, Lomami	-31.5 to -19.2	28	3.2 to 12.0	28
Fish, Lobilo	-40.9 to -24.0	101	9.7 to 16.0	101
Fish, Lomami	-41.4 to -21.9	103	6.9 to 14.8	103
Fish, Lobaye	-34.6 to -15.6	71	8.5 to 14.5	71
Fish, Congo River	-40.2 to -21.8	46	9.1 to 15.3	46

Table 15 : Summary of $\delta^{13}C$ and $\delta^{15}N$ signatures measured on different ecosystem components collected in the Lobilo, Lobaye, Lomami and mainstem Congo River during the COBAFISH-3 field campaign (March 2014)

	$\delta^{13}C$ ± stdev	*N*	$\delta^{15}N$ ± stdev	*n*
Aquatic invertebrates, all sites	-40.4 to -22.6	16	0.3 to 13.7	16
Fish, Lobilo	-34.1 to -21.2	97	10.9 to 15.4	97
Fish, Lomami	-36.4 to -26.0	80	8.0 to 13.7	80
Fish, Congo River	-39.1 to -20.6	58	7.8 to 14.0	58

Table 16: Summary of $\delta^{13}C$ and $\delta^{15}N$ signatures measured on different fish species collected during an FNRS/ERC funded “Transcongo” field campaign.

	$\delta^{13}C$ ± stdev	*N*	$\delta^{15}N$ ± stdev	*n*
Fish, all sites	-37.4 to -12.1	269	8.1 to 14.8	269

Three hypotheses can be explored which could lead to such ^{13}C-depleted values.

First, low phytoplankton $\delta^{13}C$ signatures are expected at some of the sampling sites based on the $\delta^{13}C$-DIC data - if we assume a shift of -20‰ between $\delta^{13}C$ values of DIC and phytoplankton, then the latter would have signatures of approximately -47, -45, -42 and -33 ‰ in the Lobaye, Lobilo, Lomami and mainstem Congo River, respectively. Even though the absence of a relationship between $\delta^{13}C$-DIC and overall community $\delta^{13}C$ signatures between sites suggests a marginal role for phytoplankton, it does not exclude the possibility that some species rely more heavily on phytoplankton-derived C at certain sites through pathways where key intermediate species are highly selective. This hypothesis seems unlikely to hold, however. Highly ^{13}C-depleted signatures in consumers were also observed in the mainstem Congo River – which has higher Chl a concentrations and somewhat higher productivity but, here, expected $\delta^{13}C$ signatures are not very ^{13}C-depleted and within the same range as terrestrial C sources. On the other hand, an extensive study of phytoplankton abundance and productivity along the Congo River and tributaries (Descy et al., 2016) demonstrated that phytoplankton abundance and productivity in the mainstem is low and is unlikely to be able to sustain fish foodwebs substantially.

Secondly, certain species could rely extensively on aquatic macrophyte-derived C. While 2 macrophyte samples indeed show very low $\delta^{13}C$ signatures (a *Ranunculus* sp. from the Lobilo with a $\delta^{13}C$ value of -44.5 ‰ and an unidentified macrophyte from the Lomami with a low value of -37.6 ‰), it seems at first sight unlikely that macrophytes would be responsible: neither of the two ^{13}C-depleted macrophytes was found in the mainstem Congo River, where the most ^{13}C-depleted values were found in consumers.

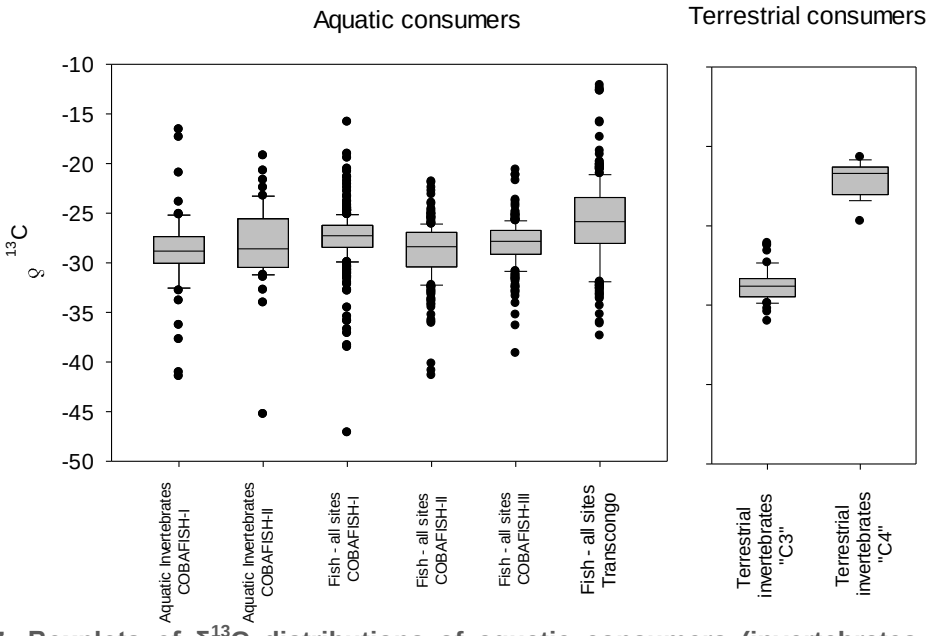


Figure 37: Boxplots of $\delta^{13}C$ distributions of aquatic consumers (invertebrates and fish for different field campaigns, all sites pooled) and terrestrial consumers (split into a C3- and C4-dependent cluster).

Thirdly, an increasing number of studies have highlighted the potential role of CH_4 based C in aquatic foodwebs (e.g., Jones et al. 2008, Jones & Grey 2011, Sanseverino et al. 2012). CH_4 produced in aquatic sediments is highly ^{13}C-depleted and the majority is oxidized by methane-oxidizing bacteria (MOB) at the oxic/anoxic interface. Under certain conditions, these MOB can form a substantial contribution to specialized feeders (e.g. Chironomidae larvae where the burrow walls are the

oxic/anoxic interface where MOB thrive, see Grey et al. 2004). While the contribution of CH_4-derived C to aquatic foodwebs has mainly been demonstrated in lakes or floodplain lakes (e.g., Sanseverino et al. 2012), it has also been demonstrated to occur in lowland river systems (Trimmer et al. 2009). Interestingly, such low $\delta^{13}C$ signatures have recently also been found in a number of invertebrate taxa in wetlands of the Zambezi river (Sven Kaehler, Rhodes University, personal communication). In addition, unusually depleted $\delta^{13}C$ signatures have also been observed in the suspended particulate organic C (POC) pool a number of tributaries of the central Congo basin during 2 recent Transcongo field campaigns ($\delta^{13}C$ as low as -39‰, Figure 38) and appear to be linked to very low POC/PN ratios and unrelated to phytoplankton (considering extremely low Chl a concentrations and very high POC:Chl a ratios in these samples, see Descy et al. 2016), suggesting a large contribution of methanotroph biomass in such blackwater systems.

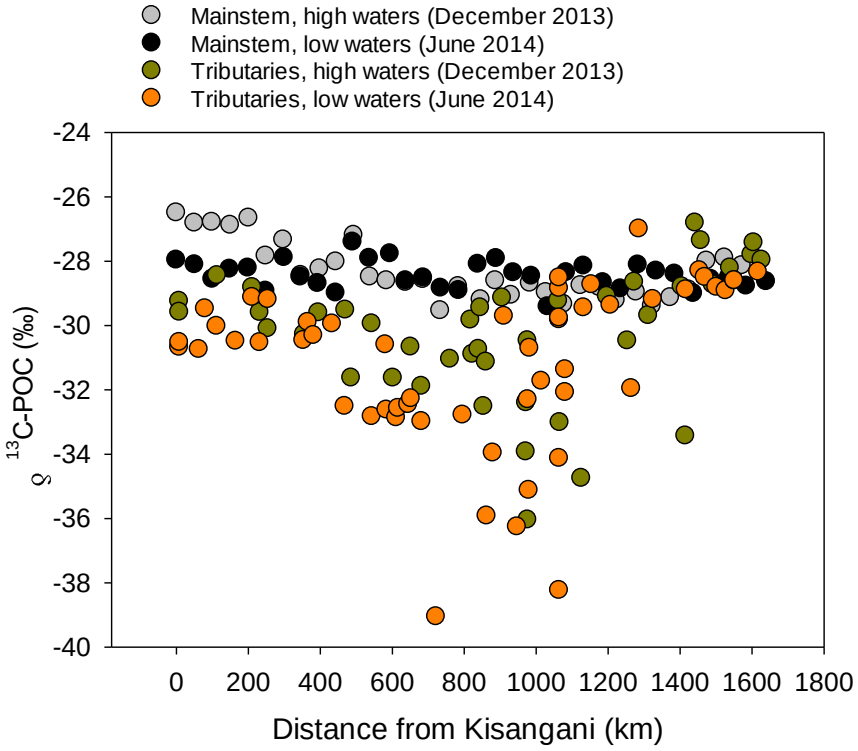


Figure 38: $\delta^{13}C$ values of suspended particulate organic carbon (POC) along the Congo mainstem and main tributaries during both high water and low water conditions (data from the TRANSCONGO project).

At this stage, we cannot unambiguously confirm the reason for some of the unusually low $\delta^{13}C$ values in consumers, but we are in the process of analysing hydrogen stable isotope ratios ($\delta^{2}H$), which are expected to form an ideal tracer to distinguish these sources (e.g., DelVecchia et al. 2016) due to (i) δD values of aquatic producers generally being much lower than those of allochthonous inputs such as leaf litter, and (ii) strong isotope fractionation during microbial CH_4 production. These analyses have only just been made feasible – given the complex sample preparation involved (steam equilibrations with 2 waters of known isotopic composition to account for exchangeable H in organic tissues) and the time needed to secure funding for the required instrument upgrades ~40 k€. All samples from the first COBAFISH field campaign for the mainstem Congo River have now been pre-processed (lipid extraction to remove the effect of variable concentrations of ^{2}H-depleted lipids), and a first series of samples has been analyzed for non-

exchangeable δ^2H. As illustrated in Figure 39, these data show some promising patterns:

(i) aquatic and terrestrial plants are relatively well separated, as expected, in the δ^2H plane, even if $\delta^{13}C$ signatures are quite similar. A more complete analysis of the samples will hopefully confirm this pattern.

(ii) The subset of fish specimens analysed all had relatively similar $\delta^{13}C$ signatures (with one exception), but show a wide spread in δ^2H signatures, suggesting a variable contribution of aquatic and terrestrial resources despite the similarity in $\delta^{13}C$.

(iii) Additionally, we need to properly account for the proportion of tissue H derived from environmental water in each trophic step – which is relatively high in aquatic organisms (20-50%). This is so called the trophic compounding effect. If we take the measured water hydrogen isotopes values in the Congo mainstem at Kisangani during 2013-2015 (-1.1 ± 4.2 ‰, n=69), the δ^2H values of potential diet for the sampled consumers will be much lower with an approximate change of 10-40 ‰ for the potential fish diet δ^2H. Thus, the fish specimen with extremely low δ^2H value is not consistent with expections for a consumer relying on aquatic primary production. Although this needs confirmation from the more complete dataset, this indirectly supports our conclusions above that CH_4-derived carbon offers a more likely explanation for some foodweb components than a reliance on aquatic primary production.

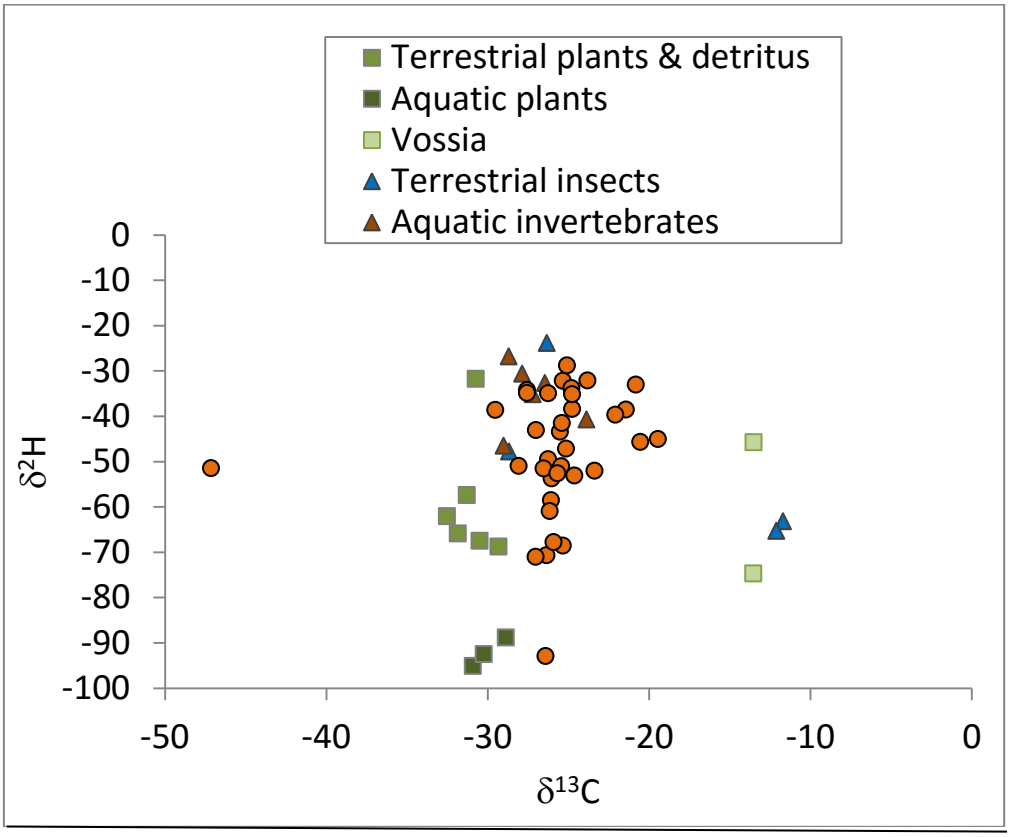


Figure 39: Combined $\delta^{13}C$-δ^2H data on a preliminary subset of samples from the mainstem Congo River collected in November 2012.

On the basis of the C and N isotopes, we compared the isotopic niche of 36 species using Siber ellipse areas (Figure 40). For each species an ellipse was calculated, covering all the isotopic values found in the specimens of that species.

For all species, we compared the sizes of the ellipse surfaces and the relative overlap between the different ellipses (see Table 17).

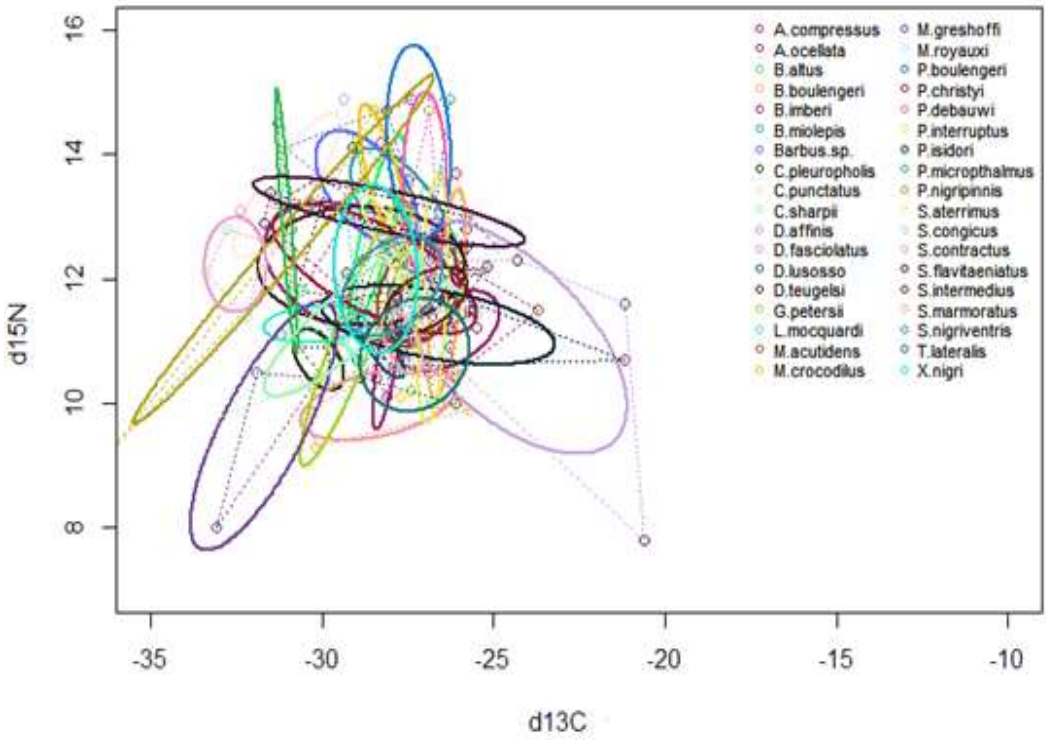


Figure 40: Isotopic niche sizes for all fish species studied (Siber Ellips Areas) (Van De Walle, 2015)

Table 17: Percentages of overlap between the Siber ellipses of the fish species studied.

% Isotopic niche overlap	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
A. compressus (1)		4	0	0	0	0	0	0	0	0	1	11	41	2	0	0	10	0	0	0	0	3	0	0	7	0	0	0	3	0	0	13	37	7	18	8
A. ocellata (2)	34		59	30	26	32	20	0	6	6	15	0	42	**86**	47	36	**89**	17	3	66	0	**93**	14	42	48	40	23	**89**	54	0	19	**91**	48	**99**	28	**85**
B. altus (3)	0	13		0	0	25	28	0	0	0	0	0	1	14	21	2	0	40	0	0	7	15	0	0	4	0	0	35	10	0	13	0	0	20	0	30
B. boulengeri (4)	0	7	0		33	10	0	0	0	0	11	4	0	9	0	0	26	0	0	51	0	12	1	40	15	0	0	0	0	0	9	38	0	3	21	0
B. imberi (5)	1	8	0	40		0	0	0	0	0	14	1	3	1	0	0	22	0	0	**57**	0	14	0	33	39	0	0	0	0	0	0	29	50	1	39	1
B. miolepis (6)	0	14	48	18	0		**58**	0	0	0	9	0	0	22	16	0	28	**74**	0	3	25	13	49	33	0	0	6	**65**	5	0	40	9	0	23	0	26
Barbus sp. (7)	0	8	48	0	0	**53**		0	9	0	1	0	0	10	13	0	0	**73**	0	0	9	6	9	0	0	0	17	43	0	0	38	0	0	2	0	25
C. pleuropholis (8)	0	0	0	0	0	0	0		0	0	0	0	0	0	9	11	0	0	9	0	0	0	0	0	0	3	0	0	18	0	0	0	0	0	0	0
C. punctatus (9)	0	2	0	0	0	0	7	0		0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	31	0	0	0	17	14	0	0	0	0	0
C. sharpii (10)	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. affinis (11)	9	28	0	**84**	**87**	37	3	0	0	0		2	0	30	7	0	**83**	8	0	**100**	4	40	26	70	70	0	0	56	15	0	20	**89**	17	50	42	21
D. fasciolatus (12)	40	0	0	7	1	0	0	2	0	0	0		8	0	18	0	0	0	2	0	0	0	0	16	0	0	0	0	0	0	0	5	0	31	0
D. lusosso (13)	30	3	0	0	1	0	0	0	0	0	0	2		1	5	3	**1**	0	0	0	0	0	0	6	0	0	0	5	0	0	0	19	7	13	9
D. teugelsi (14)	18	**84**	63	34	4	49	26	0	1	0	16	0	14		45	31	75	24	3	50	5	**82**	26	40	30	38	22	**93**	49	5	26	69	13	93	10	**80**
G. petersii (15)	0	22	43	0	0	17	15	39	0	**83**	2	20	30	21		23	9	24	4	0	3	20	4	0	16	0	0	38	42	0	10	0	0	46	3	41
L. mocquardi (16)	0	5	2	0	0	0	0	16	0	37	0	0	6	5	7		0	0	10	0	0	0	0	0	6	9	0	0	20	0	0	0	0	5	0	4
M. acutidens (17)	33	30	0	37	26	22	0	0	0	0	15	0	6	26	6	0		1	0	61	0	34	15	47	29	0	0	43	21	0	0	**93**	53	56	26	26
M. crocodilus (18)	0	3	33	0	0	32	35	0	0	0	1	0	0	5	10	0	0		0	0	4	2	2	0	0	0	7	21	0	0	13	0	0	3	0	12
M. greshoffi (19)	0	3	0	0	0	0	0	79	0	25	0	4	0	3	9	60	0	0		0	0	0	0	0	2	9	0	0	42	0	0	0	0	0	0	0
M. royauxi (20)	0	5	0	15	14	0	0	0	0	0	4	0	0	4	0	0	13	0	0		0	9	0	12	8	0	0	6	0	0	0	27	0	5	2	0
P. boulengeri (21)	0	0	16	0	0	28	12	0	0	0	1	0	0	3	4	0	0	12	0	0		0	**80**	16	0	0	10	0	0	0	16	0	0	68	0	0
P. christyi (22)	19	72	50	38	36	23	11	0	6	0	17	0	1	66	33	0	78	9	0	**96**	0		10	38	37	24	16	77	37	0	10	**92**	19	**83**	22	67
P. debauwi (23)	0	5	0	2	0	37	8	0	0	0	5	0	0	9	3	0	14	3	0	0	54	4		30	0	0	1	3	2	0	18	2	0	13	0	3
P. interruptus (24)	0	12	0	45	31	21	0	0	0	0	11	11	0	11	0	0	39	0	0	45	9	14	26		15	0	0	8	0	0	9	41	7	16	26	0
P. isidori (25)	40	31	12	38	**85**	0	0	0	0	0	24	0	53	20	22	27	54	0	1	72	0	31	0	34		0	0	9	**34**	0	0	69	71	48	49	34
P. microphthalmus (26)	0	4	0	0	0	0	0	3	10	0	0	0	0	4	0	7	0	0	1	0	0	3	0	0	0		4	0	1	0	3	0	0	0	0	0
P. nigripinnis (27)	0	13	0	0	0	8	24	0	0	0	0	0	0	13	0	0	0	21	0	0	11	11	1	0	0	20		0	0	32	14	0	0	0	0	0
S. aterrimus (28)	0	11	19	0	0	19	14	0	0	0	4	0	0	12	10	0	16	14	0	11	0	12	1	3	2	0	0		8	0	8	11	0	20	0	20
S. congicus (29)	0	25	21	0	0	5	0	**79**	0	**81**	4	0	29	24	43	65	29	0	22	1	0	22	3	0	25	4	0	31		0	0	14	5	57	4	39
S. contractus (30)	12	0	0	0	0	0	0	0	13	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0		0	0	0	0	0	0
S. flavitaeniatus (31)	0	8	27	16	0	42	45	0	20	0	5	0	0	12	10	0	**1**	33	0	0	15	6	25	15	0	13	11	31	0	0		0	0	0	0	21
S. intermedius (32)	0	17	0	30	19	**4**	0	0	0	0	9	0	0	13	0	0	52	0	0	69	0	23	1	29	20	0	0	17	6	0	0		28	27	18	11
S. marmoratus (33)	26	4	0	0	15	0	0	0	0	0	1	1	22	1	0	0	14	0	0	0	0	2	0	2	10	0	0	0	1	0	0	13		6	18	7
S. nigriventris (34)	0	37	34	4	1	20	2	0	0	0	10	0	31	35	37	12	**61**	6	0	26	52	40	14	21	28	0	0	**60**	44	0	0	52	25		12	53
T. lateralis (35)	24	14	0	43	65	0	0	0	0	0	11	37	**81**	5	4	0	37	0	0	11	0	14	0	47	37	0	0	2	4	0	0	46	**100**	16		15
X. nigri (36)	32	48	74	0	1	33	35	0	0	0	6	0	61	46	50	14	43	37	0	1	1	48	5	0	30	0	0	**89**	46	0	25	33	42	**80**	17	

A large overlap in isotope signature exists between the main trophic groups (Fig. 41). Omnivores and invertivores have the largest range; piscivores and planktivores the smallest, with piscivores having on average higher values for $\delta^{15}N$ than planktivores. Both also have a narrow range for $\delta^{13}C$.

On average, the specimens of the first expedition (B2-44, (high water) have lower values for $\delta^{15}N$ (Fig. 42).

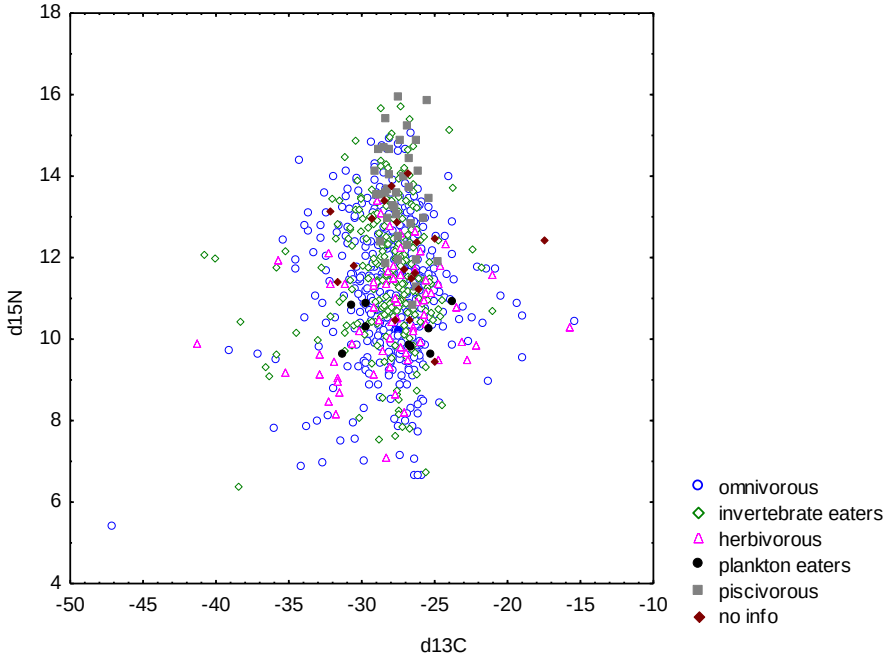


Figure 41. Scatterplot of $\delta^{13}C$ vs. $\delta^{15}N$ values for all fishes, grouped by their diet.

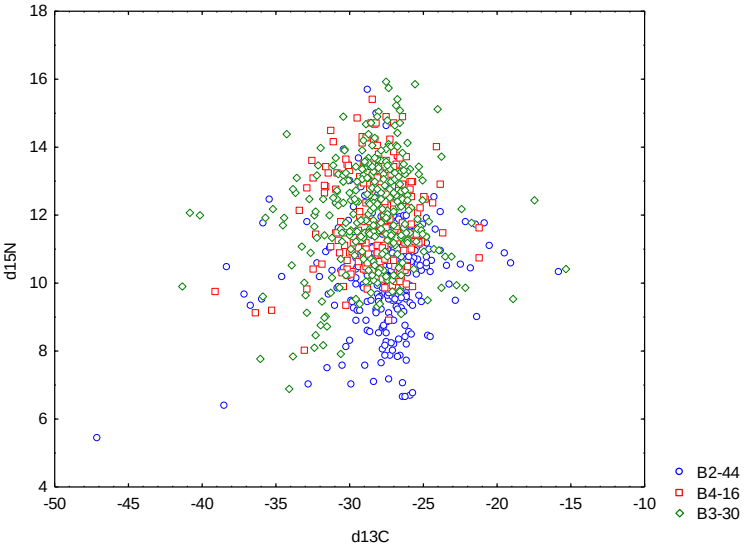


Figure 42. Scatterplot of $\delta^{13}C$ vs. $\delta^{15}N$ values for all fishes, grouped by expedition: B2-44: first expedition (high water); B3-30: second expedition (low water); B4-16: third expedition (secondary low water).

A general scatterplot by river system did not reveal clear patterns (not illustrated). However, we also compared intraspecific variation, by expedition and river system, for those species for which we had sufficient specimens. The pattern clearly differed between species. A recurrent pattern, however, was observed for the $\delta^{15}N$ values, which generally appear to be high for specimens from the Lobilo, and for specimens from the Lomami caught during the second expedition (low water). Lower values were found for specimens from the Lomami caught during the first expedition (high water). Some examples are illustrated in Figures 43 and 44.

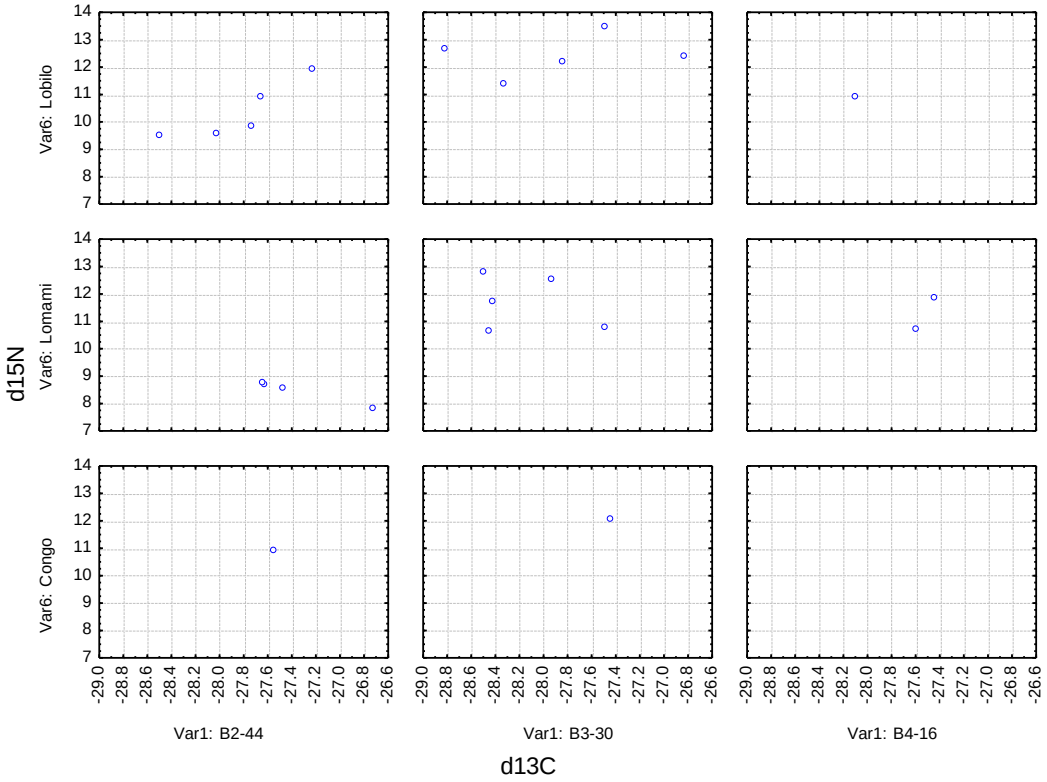


Figure 43. δ^{15}N values for *Schilbe marmoratus* arranged by expedition and river system.

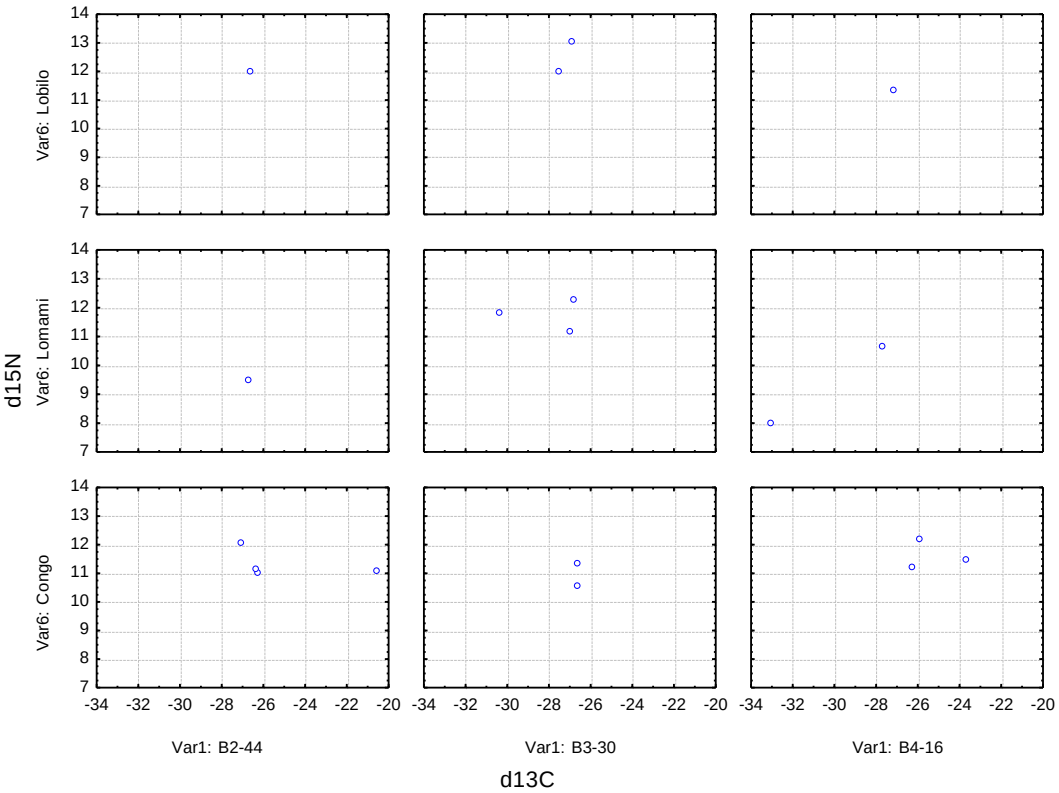


Figure 23. δ^{15}N values for *Brycinus imberi* arranged by expedition and river system.

WP4: Network integration and coordination

Task 4.1: Preliminary analysis of existing data – optimizing sampling strategy (all partners)

All available data on fish and macro-invertebrate collections from the Congo2010 expedition, including historical RMCA fish collection data, and data collected in the course of two PhD research projects, were compiled for analysis in comparison with the data collected during three field campaigns. Similarly, data on aquatic biogeochemistry and stable isotopes obtained during the Congo2010 expedition (and the 2009 “pre-expedition”) were compiled and integrated into the data analysis based on the COBAFISH field work and other sampling campaigns from parallel projects such as the FNRS funded TransCongo project.

Task 4.2: Fieldwork preparation and coordination in collaboration with local partners (RBINS)

Targeted informal meetings to plan all field sampling activities were organised by the coordinator and required contribution from all promoters. The coordinator had regular contacts with UNIKIS in relation to monitoring activities carried out by the local partners and also for the preparation of the field work, including the monitoring program.

Task 4.3: Organisation of workshops & meetings

Annual workshops were organized to discuss the results of all teams, as well as a final science and follow-up committee meeting, that was held in Brussels on November 10th, 2016 jointly with two other BELSPO-funded project on Central Africa, COBIMFO and EAGLES.

Task 4.4: Reporting and dissemination of results

Dissemination of results was done through papers and presentations (see section 5) Data was made public when published and contribute to various data banks and data bases such as BBP, FISHBASE, FISHBOL, BIOFRESH

3. POLICY SUPPORT

The COBAFISH data-set contributed to the computation of the first emissions of CO_2 and CH_4 from the Congo River network including the Cuvette Centrale Congolaise wetland. This corresponds to a total flux in CO_2 equivalents of about 0.7 PgC $year^{-1}$. This flux is quite large and has not yet been included in the national inventory from the Democratic Republic of the Congo (RDC) to the United Nations Framework Convention on Climate Change (UNFCCC), and is not accounted in the carbon budgets for the "Reducing Emissions from Deforestation and forest Degradation" (REDD) program. Nevertheless, such a flux from the rivers and wetlands is quantitatively important. For instance, based in the carbon stock of the RDC forest (40 PgC) and the annual deforestation rate (0.25%) given by UNFCCC, we can compute the carbon flux related to deforestation at 0.1 PgC $year^{-1}$. Further, the increase of the carbon sink in the overall African tropical rain forest is 0.04 PgC $year^{-1}$ (Fisher et al. 2013). The comparison of these fluxes with the emissions of CO_2 and CH_4 from the Congo River and Cuvette Centrale Congolaise wetlands, clearly show that it is essential to account emissions from inland waters when budgeting carbon fluxes in the frame of the UNFCCC national obligations. Yet, our data acquired in the three COBAFISH sites do not encompass the full range of aquatic biogeochemistry and in particular GHG emissions, hence, we also recommend large scale basin wide quantification of GHG emissions from inland waters (rivers and lakes).

The term "the silent crisis" has been adopted for the deteriorating state of the freshwater systems all over the world. In the Congo basin, protected areas are all traditionally shaped, based on terrestrial organisms and their needs; fresh water systems are not considered as important for conservation issues and human activities usually continue in an unrestricted way. This observation indicates that a change of mind is necessary and aquatic systems need to become an integral part of proteced areas. Our finding that fishes in the area (and probably in the major part of the Central Congo basin) are to a large extent dependent on terrestrial food items underscores the logic above and adds an extra dimension to the management of the aquatic ecosystem in the Congo basin. If one wants to conserve this environment, one not only needs measures for the waterbodies, but also for the riparian terrestrial environment. If not, fishes will loose a major part of their food sources and inevitably, the biomass in the basin will diminish drastically.

4. DISSEMINATION AND VALORISATION

The biogeochemical data generated by the project has been made available digitally as supplements of published papers, and the pdfs of the papers have been made available through institutional repositories such as ORBI at University of Liège (http://orbi.ulg.ac.be/) and LIRIAS at KUleuven (https://lirias.kuleuven.be/). This allows a wide dessimination of papers and the data. For instance, based on this open data acess policy, the CH_4 in the Congo basin generated by COBAFISH data were integrated in a global meta-analysis of CH_4 concentrations in rivers that was lead by the Center for Limnology of the University of Wisconsin (https://lter.limnology.wisc.edu/dataset/global-database-methane-concentrations-and-atmospheric-fluxes-streams-and-rivers).

In the framework of the participation of partners 2 and 3 in a coordinated research project (CRP, Application and development of isotope techniques to evaluate human impacts on water balance and nutrient dynamics of large river basins) of the IAEA, stable O and H isotope data on water collected during both COBAFISH field campaigns and the ongoing monitoring on the Congo and Tshopo Rivers are included in the GNIR (Global Network for Isotopes in Rivers) database, and publically accessible (http://www-naweb.iaea.org/napc/ih/IHS_resources_gnir.html). Similarly, isotope data on monthly precipitation samples are made available on in the GNIP database (Global Network for Isotopes in Precipitation; http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html). The full dataset of the biogeochemical data will similarly be made publically available upon publication (for which we aim at at least 4 full years of data acquisition).

The synthesis paper of GHGs in African rivers (Borges et al. 2015) received a fair amount of media coverage (national and international), among which:

Date	Type	Description
17-04-15	writtten press	"Une mission d'études scientifiques de l'Université de Liège dans la province du Bandundu" Agence Congolaise de Presse N°3345 p 21
21-07-15	online press	"First report on greenhouse gas emissions from African rivers" at Phys Org web site (http://phys.org/news/2015-07-greenhouse-gas-emissions-african-rivers.html)
21-07-15	online press	"Greenhouse Gas Emissions From African Rivers" at Science 2.0 website (http://www.science20.com/news_articles/greenhouse_gas_emissions_from_african_rivers-156601)
28-07-15	writtten press	"Les fleuves africains, gros émetteurs de gaz à effet de serre" Le Monde, page 5
28-07-15	television	"Un tout premier bilan des gaz à effet de serre sur les rivières africaines" RTBF, JT 20h00, 28/07/2015
30-07-15	online press	"African river study fills gap in carbon emissions tally" at SciDevNet website (http://www.scidev.net/global/water/news/african-river-study-fills-gap-carbon-emissions-tally.html)
28-10-15	writtten press	"Pourquoi le Congo produit autant de méthane" Le Soir, page 24, 28/10/2015

Numerous presentations based on COBAFISH results and activities were presented at the 1st International Conference on Biodiversity in the Congo Basin that was held in Kisangani in June 6-10, 2014 (http://congobiodiversityconference2014.africamuseum.be/). This conference was an initiative of the Consortium Congo 2010 (the University of Kisangani, the Royal Museum for Central Africa, the Royal Belgian Institute of Natural Sciences and the National Botanic Garden of Belgium) and the 'Centre de Surveillance de la Biodiversité' in Kisangani to facilitate interactions and collaborations among Congolese, Belgian and international teams and experts involved in various fields of biodiversity-related research in the Congo Basin.

5. PUBLICATIONS

5.1 Publications of the teams

5.1.1 Peer review

Partner 1

Higuti J, Martens K. 2016. Invasive South American floating plants are a successful substrate for native Central African pleuston. Biol Invasions (2016) 18:1191–1201 - DOI 10.1007/s10530-016-1061-1

Partners 1 & 5

Decru E ., Moelants T., De Gelas K ., Vreven E , Verheyen E., Snoeks J. 2015. Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. Molecular Ecology Resources (2015) doi: 10.1111/1755-0998.12445

Van Ginneken M, Decru E, Verheyen E, Snoeks J (in press.) Morphometry and DNA barcoding reveal cryptic diversity in the genus Enteromius (Cypriniformes: Cyprinidae) from the Congo basin, Africa. Biol J Linnean Society

Decru E., Snoeks J., De Gelas K., Verheyen E., Vreven E. (submitted) Species richness in the African pike Hepsetus: a perfect match between genetics and morphology. Journal of Fish Biology

Partners 2 and 3:

Abril G., S. Bouillon, F. Darchambeau, C. R. Teodoru, T. R. Marwick, F. Tamooh, F. O. Omengo, N. Geeraert, L. Deirmendjian, P. Polsenaere & A.V. Borges (2015)Technical Note: Large overestimation of pCO2 calculated from pH and alkalinity in acidic, organic-rich freshwaters, Biogeosciences, 12(1):67-78

Borges AV, Darchambeau F, Teodoru CR, Marwick TR, Tamooh F, Geeraert N, Omengo FO, Guérin F, Lambert T, Morana C, Okuku E & Bouillon S (2015) Globally significant greenhouse gas emissions from African inland waters, Nature Geoscience, 8, 637-642, doi:10.1038/NGEO2486

Borges AV, G Abril, F Darchambeau, CR Teodoru, J Deborde, LO Vidal, T Lambert & S Bouillon (2015) Divergent biophysical controls of aquatic CO2 and CH4 in the World's two largest rivers, Scientific Reports, 5:15614, doi: 10.1038/srep15614

Borges AV, S Bouillon & C Morana (2016) Methane oxidation in a large tropical river (Congo River): insights from stable carbon isotope data on dissolved methane, Aquatic Sciences, submitted

Descy JP, Darchambeau F, Lambert T, Stoyneva MP, Bouillon S, Borges AV (2016), Phytoplankton dynamics in the Congo River, Freshwater Biology, DOI: 10.1111/fwb.12851

Lambert T, F Darchambeau, S Bouillon , B Alhou, J-D Mbega, C Teodoru, F C Nyoni, P Massicotte & A V Borges (2015) Landscape Control on the Spatial and Temporal Variability of Chromophoric Dissolved Organic Matter and Dissolved Organic Carbon in Large African Rivers, Ecosystems, doi:10.1007/s10021-015-9894-5

Lambert T., S. Bouillon, F. Darchambeau, P. Massicotte & A. V. Borges (2016) Shift in the chemical composition of dissolved organic matter in the Congo River network, Biogeosciences, 13:5405–5420

Partner 4:

Taylor J.C. & Cocquyt C. (2016). Diatom research in southern and central Africa: Historical perspectives and current activities. Mededelingen van de Koninklijke Akademie voor Overzeese Wetenschappen. http://www.kaowarsom.be/documents/PDF%20BULLETIN/TAYLOR_C.pdf

Cocquyt C. & Taylor J. C. (2015). New and interesting *Surirella* taxa (Surirellaceae, Bacillariophyta) from the Congo basin (DR Congo). European Journal of Taxonomy 133: 1-15.

Cocquyt C., de Haan M. & Lokele Ndjombo E. (2016). *Eunotia rudis* sp. nov., a new diatom (Bacillariophyta) from the Man and Biosphere Reserve at Yangambi, Democratic Republic of the Congo. Phytotaxa 272(1): 73-81.

Cocquyt C., de Haan M. & Taylor J. (2013). *Cavinula lilandae* (Bacillariophyta), a new diatom species from the Congo Basin. Diatom Research 28(2): 157-163.

Cocquyt C., Taylor J.C. & Wetzel C.E. (2014). *Stenopterobia cataractarum* spec. nov. (Bacillariophyta), a new benthic diatom from a waterfall in Zambia, Africa. Phytotaxa 158: 76-84.

Karthick B., Kociolek J.P., Taylor J.C. & Cocquyt C. (2016). *Gomphonema grande* sp. nov., a new diatom (Bacillariophyta) from the Democratic Republic of the Congo, Tropical Africa. Phytotaxa 245(3): 187-196.

Taylor J.C. & Cocquyt C. Diatoms from the Congo and Zambezi Basins - Methodologies and identification of the genera. AbcTaxa 16. In press.

Taylor J.C., Cocquyt C. & Mayama S. (2016). *Navicula nielsfogedii* J.C. Taylor & Cocquyt sp. nov., a new diatom (Bacillariophyta) from tropical and sub-tropical Africa. Fottea 16(2): 201-208.

Taylor J.C., Cocquyt C. & Mayama S. (2016). New and interesting *Eunotia* (Bacillariophyta) from the Democratic Republic of the Congo, tropical central Africa. Plant Ecology and Evolution 149(3): 291-307.

Taylor J.C., Karthick B., Cocquyt C. & Lang P. (2014) *Diploneis fenestrata* spec. nov. (Bacillariophyta), a new aerophilic diatom species from Zambia, Africa. Phytotaxa 167(1): 79-88.

Taylor J.C., Karthick B., Kociolek J. P., Wetzel, C.E. & Cocquyt C. (2014). *Actinellopsis murphyi gen. et spec. nov*.: A new small celled freshwater diatom (Bacillariophyta, Eunotiales) from Zambia. Phytotaxa 178(2): 128-137.

Partner 5:

Decru, E., Snoeks, J., Vreven, E. (2015). A taxonomic evalutation of the *Hepsetus* from the Congo basin with the revalidation of *H. microlepis* (Teleostei: Hepsetidae). Ichthyological Exploration of Freshwaters, 26 (3), 273-287.

Decru, E., Vreven, E., Sadio, O., Snoeks, J. (2016). *Brycinus epuluensis*, a new species from the Epulu River (Congo basin), Africa (Teleostei: Alestidae). Ichthyological Exploration of Freshwaters, 27 (1), 49-60.

Hughes H. J., Sondag F., Cocquyt C., Laraque A., Pandi A., Andre´ L. and Cardinal D. (2011) Effect of seasonal biogenic silica variations on dissolved silicon fluxes and isotopic signa- tures in the Congo River. Limnol. Oceanogr. 56, 551–561.

Kisekelwa, T., Boden, G., Snoeks, J., Vreven, E. (2016). *Marcusenius kaninginii*, a new species of elephantfish from the Lowa River basin, Democratic Republic of the Congo (Osteoglossiformes: Mormyridae). Ichthyological Exploration of Freshwaters, 26 (4), 341-352.

Moelants, T., Mbadu Zebe, V., Snoeks, J. & Vreven, E. 2014 A review of the *Distichodus antonii* assemblage (Characiformes, Distichodontidae) from the Congo basin. Journal of Natural History: 48 (27-28), 1707-1735

Moelants, T., Snoeks, J & Vreven, E. (in press). *Distichodus kasaiensis* and *Distichodus ingae*, two new species in the *Distichodus atroventralis* complex (Characiformes: Distichodontidae) of the Congo basin. Ichthyological Exploration of Freshwaters.

Van Steenberge, M., Gajdzik, L., Chilala, A., Snoeks, J., Vreven, E. (2014). *Labeo rosae* (Cypriniformes: Cyprinidae) in the Congo basin: a relict distribution or a historical introduction? Journal of Fish Biology, 85, 1733-1738.

Van Steenberge, M., Snoeks, J., Vreven, E. (2016). Lingering taxonomic confusion in *Labeo* (Actinopterygii: cypriniformes: cyprinidae): correcting the records and basis of type designations for seven Congolese species. Acta Ichthyologica et Piscatoria, 46 (1), 1-8.

Vreven, E., Musschoot, T., Snoeks, J., Schliewen, U. (2016). The African hexaploid Torini (Cypriniformes: Cyprinidae): review of a tumultuous history. Zoological Journal of the Linnean Society, 177 (2), 231-305.

5.1.2 Abstracts, posters and presentations

Partners 1 and 5

Nagy, Z., Verheyen, E., Vreven, E., Sonet, G., Breman, F., Jordaens, K., Van Houdt, J., Danadu Mizani, C., Snoeks, J. 2014. Towards the DNA barcoding of the congolese riverine fish fauna. Abstracts 1st International Conference on Biodiversity in the Congo. Kisangani, Democratic Republic of the Congo, 6-10 June 2014.

Partners 2 and 3:

Bayon G, E Schefuss, L Dupont, A Laraque, AV Borges, F Darchambau, S Bouillon, G Moukangui, J- P Tathy, C Skonieczny, S Bermell, E Ponzevera, B Dennielou & L André, Neodymium isotope constraints on past hydrological variability in the Congo Basin, 6ème Réunion scientifique du Service d'Observation HYBAM, 26-30 October 2015, Cusco, Peru, oral

Bayon G, E Schefuss, L Dupont, A Laraque, AV Borges, F Darchambau, S Bouillon, G Moukangui, J- P Tathy, C Skonieczny, S Bermell, E Ponzevera, B Dennielou & L André, Neodymium isotope constraints on past hydrological variability in the Congo Basin, AGU Fall Meeting, San Francisco, California 14 December - 18 December 2015, poster

Borges AV, S Bouillon, C Teodoru, B Leporcq, J-P Descy & F Darchambeau, Inorganic and organic carbon spatial variability in the Congo River during high waters (December 2013), European Geosciences Union, General Assembly, Vienna, Austria, 27 April – 2May 2014

Borges, A.V.; Bouillon, S.Teodoru, C.Descy, J.P.; Lambert, T.Darchambeau, F. Inorganic and organic carbon spatial variability in the Congo River during high waters (December 2013) and low waters (June 2014), ASLO Aquatic Sciences Meeting February 22-27, 2015, Granada, Spain, oral

Bouillon S (2015) A synthesis of carbon cycling across contrasting African river basins. Invited keynote, Tropilakes 2015: Tropical lakes in a changing environment: water, land, biology, climate and humans. Bahir Dar, Ethiopia, September 2015

Bouillon S, E Tambwe, T Mambo, Z Kelemen, T Lambert, F Darchambeau & AV Borges, Biogeochemistry of the Congo River: annual transport fluxes and sources of carbon in the upper Congo River (Kisangani, DRC Congo), ASLO Aquatic Sciences Meeting, 22-27 February 2015, Granada, Spain, oral

Bouillon S., A. Yambélé, D.P. Gillikin, C. Teodoru, F. Darchambeau, T. Lambert & A.V. Borges, Contrasting biogeochemical characteristics of right-bank tributaries of the Oubangui River, and a comparison with the mainstem river (Congo basin, Central African Republic), European Geosciences Union, General Assembly, Vienna, Austria, 27 April – 2May 2014

Darchambeau F., Descy J.P., Leporcq B., Stoyneva M.P., Bouillon S. & A.V. Borges, Plankton diversity and metabolism in the Congo River during high waters (December 2013) and low waters (June 2014), ASLO Aquatic Sciences Meeting, 22-27February 2015, Granada, Spain, poster

Darchambeau F., S. Bouillon & A. V. Borges, First assessment of the biogeochemistry of the upper Congo River, European Geosciences Union General Assembly 2012, 22 – 27 April 2012, Vienna, Austria

Lambert T, Darchambeau F, Bouillon S, Alhou B, Mbega JD, Teodoru C, Nyoni F, & Borges AV (2015) The effect of vegetation cover and hydrological connectivity on the spatial and temporal variability of DOC and cDOM in large African rivers. ASLO Aquatic Sciences Meeting 2015; Granada, Spain.

Lambert T, F Darchambeau, AV Borges, B Alhou, J-D Mbega, C Teodoru, TR Marwick & S Bouillon. Spatial and temporal patterns of dissolved organic matter optical properties across large rivers in Africa, European Geosciences Union, General Assembly, Vienna, Austria, 27 April – 2May 2014

Lambert T., Darchambeau F, A.V. Borges, B. Alhou, J.-D. Mbega, C. Teodoru, T. Marwick, and S. Bouillon, Spatial and temporal patterns of dissolved organic matter optical properties across large rivers in Africa, The Sixth International Workshop on Soil and Sedimentary Organic Matter Stabilization and Destabilization (SOM6), October 5 - 9, 2014, Kiawa Island, USA

Marwick TR, F Tamooh, C Teodoru, AV Borges, F Darchambeau & S Bouillon, The age of river- transported carbon: new data from African catchments and a global perspective, European Geosciences Union, General Assembly, Vienna, Austria, 27 April – 2May 2014

Marwick, T. R., F. Tamooh, C. R. Teodoru, A. V. Borges, F. Darchambeau, and S. Bouillon (2014) The age of river-transported carbon: new data from African catchments and a global perspective, IsoEcol 2014, 3-8 August 2014, Perth, Australia. Oral presentation.

Stoyneva M, J-P Descy, S Bouillon, F Darchambeau & A Borges, Phytoplankton abundance and diversity in the Congo river at high and low waters, 9th Symposium for European Freshwater Sciences, Geneva Switzerland, 5-10 july 2015, oral

Vanhove D, Gillikin DP, Kelemen Z, Springer V, & Bouillon S (2015) The freshwater oyster Etheria elliptica as a tool to reconstruct climate variability across the African continent. AGU meeting, December 2015.

Yambélé A, Bouillon S, Borges AV, & Gillikin D (2013) Cycle et flux du carbone dans les rivières africaines: étude de la rivière Oubangui [Cycling and fluxes of carbon in African rivers: the Oubangui]. Congo River workshop, Brazzaville, September 2013.

Partner 4:

Boamba S.M., Cocquyt C. & Nshimba H. (2014) Etude sur la composition des diatomées phytoplanctoniques des étangs de Ngene-Ngene situés en périphérie de Kisangani. Poster presented at 1st International Conference on Biodiversity in the Congo Basin, Kisangani, D.R. Congo, 6-10 June 2014: 109-110, 205.

Cocquyt C. & Taylor J.C. (2014) Diatom diversity of some acid rivers and streams in the vicinity of Yangambi (Oriental Province, DR Congo). Lecture presented at 1st

International Conference on Biodiversity in the Congo Basin, Kisangani, 6-10 June 2014: 75-76.

Cocquyt C. (2013). De expeditie op de Congostroom in 2010. Lecture presented at Davidsfonds Zwijnaarde, 5 maart 2013, Zwijnaarde.

Cocquyt C. (2013). De expeditie op de Congostroom. Lecture presented at the Seniorenclub Eeuwige Lente, 18 september 2013, Kortrijk.

Cocquyt C., de Haan M, De Kesel A., Udar H.V. & Van den Broeck D. (2014) Cryptogam research in the Congo basin. Poster presented at 1st International Conference on Biodiversity in the Congo Basin, Kisangani, D.R. Congo, 6-10 June 2014: 154.

Lokele Ndjombo E. & Cocquyt C. (2016) First exploration of diatom biodiversity in rivers and streams in the Man and Biosphere Reserve of Yangambi, Tshopo Province, DR Congo. Poster presented at the 24th International Diatom Symposium. Québec, Canada, 21-26 Augustus 2016.

Maréchal C., Cawoy V., Cocquyt C., Dauby G., Dessein S., Douglas-Hamilton I., Dupain J., Fischer E., Fouth Obang D., Groom Q., Henschel P., Jeffrey K.J., Korte L., Lewis S.L., Luhunu S., Maisels F., Melletti M., Ngoufo R., Ntore S., Palla F., Scholte P., Sonké B., Stevart T., Stoffelen P., Van den Broeck D., Walters G. & Williamson E.A. (2014) Biodiversity conservation and management. In: de Wasseige C., Flynn J., Louppe D., Hiol Hiol F. & Mayaux Ph. (eds) The forests of the Congo Basin – State of the Forest 2013. Weyrich édition, Neufchâteau Belgium: 67-96.

Taylor G.C. & Cocquyt C. (2014) A guide to the diatom genera of tropical Africa – building capacity for diatom identification for local researchers. Poster presented at the 2nd Annual Meeting on Plant Ecology and Evolution. Louvain-la-Neuve, Belgium, 14 November 2014: 19.

Taylor J.C. & Cocquyt C. (2015). Diatom research in southern and central Africa: Historical perspectives and current activities. Mededelingen van de Koninklijke Akademie voor Overzeese Wetenschappen: in press.

Taylor J.C. & Cocquyt C. (2015). Tools for the introduction of diatoms for monitoring studies in Central Africa. Poster presented at INBAT, The International Workshop on Benthic Algae Taxonomy. Trento, Italy, 17-19 June 2015.

Taylor J.C., Cocquyt C. & van Rensburg L. (2012). Diatoms for the Congo and Zambezi sister basins – a first overview. Poster presented at the 1 Annual meeting on plant ecology and evolution (AMPEE1), 30 November 2012, Meise, Belgium.

Taylor J.C., Cocquyt C., Lang P. & van Rensburg L. (2012). The use of diatoms for monitoring river water quality in the Zambezi and Congo sister basins. Poster presented at the International Diatom Symposium, 26-31 August 2012, Ghent, Belgium.

Taylor J.C., Cocquyt C., Lang P. & van Rensburg L. (2012). The use of diatoms for monitoring river water quality in the Zambezi and Congo sister basins. Poster presented at the GAP6 Symposium (Ghent Africa Platform), 7 December 2012, Ghent, Belgium.

Partner 5:

Danadu, M.C., Vreven, E., Moelants, T., Ulyel, A.-P. & Snoeks, J. 2013. Two case studies on *Synodontis* Cuvier, 1816 (Siluriformes: Mochokidae) from the Congo basin (DRC). Abstracts International Conference of the Pan African Fish and Fisheries Association. Bujumbura, Burundi, 16-20 September 2013: 22.

Danadu, M.C., Vreven, E., Ulyel, A.-P. & Snoeks J. 2013. Problématique de *Synodontis* (Siluriformes: Mochokidae) du bassin du Congo (DRC). Abstracts

International Conference of the Pan African Fish and Fisheries Association. Bujumbura, Burundi, 16-20 September 2013: 38.

Decru E., Vreven E., Degelas K., Verheyen E., Snoeks J. (2014) A story of unexpected species diversity: the case of the african pike *Hepsetus odoe* (Bloch, 1794) (Characiformes: Hepsetidae) June 2014, Kisangani

Decru E., Vreven E., Snoeks J. (2014). The ichthyofauna of some northeastern tributaries of the congo basin: a preliminary overview. June 2014, Kisangani.

Gajdzik, L., Van Steenberge, M., Chilala, A., Snoeks, J., Vreven, E. 2013. A re-evaluation of species diversity within the *Labeo* (Cypriniformes: Cyprinidae) with papillary lips from the Congo basin. Abstracts International Conference of the Pan African Fish and Fisheries Association. Bujumbura, Burundi, 16-20 September 2013: 42-43.

Moelants, T., Mbadu Zebe, V., Snoeks, J. & Vreven, E. 2013. Towards a revision of the large-sized *Distichodus* Müller & Troschel, 1844 (Characiformes: Distichodontidae) species from the Congo basin. Abstracts International Conference of the Pan African Fish and Fisheries Association. Bujumbura, Burundi, 16-20 September 2013: 43.

Musschoot T., Boden, G., Vreven, E. & Snoeks, J. 2013. Distribution patterns of catfishes in the Congo River basin. Abstracts International Conference of the Pan African Fish and Fisheries Association. Bujumbura, Burundi, 16-20 September 2013: 20.

Snoeks J. (2014) How well do we know the fishes from the Congo basin? June 2015, Kisangani

Snoeks J., Vreven E., Dunz A., Decru E., Tchalondawa K., Katemo Manda B., Abwe E., Chocha Manda A., Van Steenberge M. (2014) Exploration into the phylogenetics of the african hexaploid barbines (Cypriniformes: Cyprinidae): a Cytb MtDNA analysis. June 2014, Kisangani

Snoeks, J., Moelants, T., Mbadu Zebe, V., Vreven, E. 2014. The large *Distichodus* species (Characiformes, Distichodontidae) from the Congo basin: taxonomy and distribution. Abstracts 1st International Conference on Biodiversity in the Congo. Kisangani, Democratic Republic of the Congo, 6-10 June 2014.

All partners

Verheyen E., Martens K., Willems W., Borgès A., Darchambeau F., Bouillon S., Teodoru C., Cocuyt C., Taylor J., Snoeks J., André L., Gajdzik L., Bamps J., Akaibe D. (2014) Congo Basin: From carbon to fishes – The COBAFISH project. Poster presented at 1st International Conference on Biodiversity in the Congo Basin, Kisangani, D.R. Congo, 6-10 June 2014: 221.

Verheyen E., Martens K., Darchambeau F., Borges A.V., Bouillon S., Teodoru C., Abrantes K., Cocquyt C ., Taylor J., Snoeks J., Moelants T., Hughes H. & André L. (February 2013). Congo basin: From carbon to fishes. Annual Scientific Report for the period 15/05/2011 to 31/01/2013, BELSPO project SD/AR/05A: 59 pp.

5.1.3: Theses

<u>5.1.3.1: Bachelor theses</u>

Partner 5:

Boyen J. 2015, Seizoenale variatie in het dieet van vissen uit het Congobekken, een case study op de Alestidae. RMCA, Promotor: Jos Snoeks

Carmen, M. 2014, Het trofisch niveau van Congovissen: een studie voor de Kisangani-regio. RMCA, Promotor: Jos Snoeks

Cuypers A. 2014, Het onderscheid tussen drie sterk gelijkende *Micralestes*-soorten in het Congobekken. RMCA, Promotor: Jos Snoeks

Van den Bogaart L. 2015, Morfologische analyse van drie genetisch verschillende groepen in *Enteromius brazzai*. RMCA, Promotor: Jos Snoeks

Weckx M. 2014, Drie genetische groepen van *Brachypetersius altus* in het Congobekken: mis-identificaties of nieuwe soorten? RMCA, Promotor: Jos Snoeks

5.1.3.2: Master theses

Partner 1 & 5:

Van Ginneken M. 2014, Morfologisch en moleculair onderzoek op enkele '*Barbus*'-soorten (Cuvier & Cloquet, 1816) van het Congobekken. RBINS, Promotor: Erik Verheyen, co-promoter: Jos Snoeks

Partner 2 & 3:

Tambwe Lukosha E (2015) Suivi biogéochimique des eaux de surface dans la partie amont du fleuve Congo et de la rivière Tshopo à Kisangani (RD Congo), M. Sc. University of Liège, 26 pp, promoter, Alberto Borges

Partner 5:

Bulteel L. 2015, A revision of the species *Brycinus imberi* (Characiformes, Alestidae) of the Congo Basin. Promotor: Jos Snoeks

Degryse S. 2013, A revision of the genera *Hippopotamyrus* and *Cyphomyrus* (Osteoglossiformes, Mormyridae) Promotor: Jos Snoeks.

Münks H. 2014, Revisie van het genus *Marcusenius* binnen het Kongobekken met 12 of meer schubben rond de staartsteel. RMCA, Promotor: Jos Snoeks

Van De Walle L. 2015, Komt alle hulp van boven? Dieetanalyse van de vissen van het centrale Congobekken.82, Promotor: Jos Snoeks

Vanstallen L. 2012, Diet analysis of the fish fauna of the Kisangani region (DRC). Promotor: Jos Snoeks

5.1.3.3: PhD theses

Partner 5:

Danadu, C., 2014, Révision des *Synodontis* (Siluriformes, Mochokidae) de la région de Kisangani (R.D.Congo). Copromoter: Jos Snoeks

Decru E. 2015, The ichthyofauna of the Central Congo basin: diversity and distribution in the north-eastern tributaries, 303 pages, Promoter: Jos Snoeks

Moelants T. 2015, Diversity and ecology of the ichthyofauna of the Middle and Upper Congo basin: a case-study in the region of the Wagenia falls (Democratic Republic of Congo). 332 pages, Promoter: Jos Snoeks

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7. REFERENCES

Abril, G. et al. Amazon River carbon dioxide outgassing fuelled by wetlands. Nature 505, 395-398 (2014).

Amiotte Suchet, P. and J.L. Probst. 1993. CO2 Flux Consumed by the Chemical Weathering of Continents: Influences of drainage and lithology. C.R. Acad. Sci. Paris, t. 317, Serie II, pp. 615-622.

Amiotte Suchet, P. and J.L. Probst. 1993. CO2 Flux Consumed by the Chemical Weathering of Continents: Influences of drainage and lithology. C.R. Acad. Sci. Paris, t. 317, Serie II, pp. 615-622.

Amundsen, P.-A., H.-M. Gabler and F.J Staldvik (1996) – A new approach to graphical analysis of feeding strategy from stomach contents data – modification of the Costello (1990) method. *Journal of Fish Biology*. 48: 607-614.

Balagizi C.M., F. Darchambeau, S. Bouillon, M.M. Yalire, T. Lambert & A.V. Borges (2015) Rivergeochemistry, chemical weathering and atmospheric CO2 consumption rates in the Virunga Volcanic Province (East Africa), Geochemistry, Geophysics, Geosystems (G-Cubed), 16,doi:10.1002/2015GC005999

Bouillon, S., Yambélé, A., Gillikin, DP., Teoduru, C., Darchambeau, F., Lambert, T., Borges A.V. 2014. Contrasting biogeochemical characteristics of the Oubangui River and tributaries (Congo River basin). Scientific Reports 4: 5402, doi: 10.1038/srep05402.

Boyen J. 2015, Seizoenale variatie in het dieet van vissen uit het Congobekken, een case study op de Alestidae. Bachelorthesis KU Leuven

Carmen, M. 2014. Het trofisch niveau van Congovissen: een studie voor de Kisangani-regio. Bachelorthesis, KU Leuven.

Cholnoky B.J. (1968). Die Ökologie der diatomeen in Binnengewässern. J. Cramer, Lehre, 699 pp.

Cocquyt C., de Haan M. & Taylor J. (2013). *Cavinula lilandae* (Bacillariophyta), a new diatom species from the Congo Basin. Diatom Research 28(2): 157-163.

Cocquyt C. & Taylor J. C. (2015). New and interesting *Surirella* taxa (Surirellaceae, Bacillariophyta) from the Congo basin (DR Congo). European Journal of Taxonomy 133: 1-15.

Coynel, A., Seyler, P., Etcheber, H., Meybeck, M and Orange, D., 2005. Spatial and seasonal dynamics of total suspended sediment and organic carbon species in the Congo River. Glob. Biogeochem. Cyc. 19, GB4019, doi:10.1029/2004GB002335.

Cuypers A. 2014, Het onderscheid tussen drie sterk gelijkende *Micralestes*-soorten in het Congobekken. Unpublished BSc thesis. KU Leuven.

Danadu Mizani, C., Vreven, E., Boden, G., Musschoot, T., Ulyel Ali-Patho, J. & Snoeks, J. Geographic variation within *Synodontis decorus* Boulenger, 1899 (Siluriformes: Mochokidae) from the Congo River basin. In prep.Davis, J. A.: Adsorption of natural dissolved organic matter at the oxide/water interface, Geochim. Cosmochim. Acta., 46, 2381– 2393, 1982.

Decru E ., Moelants T., De Gelas K ., Vreven E , Verheyen E., Snoeks J. 2015a. Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. Molecular Ecology Resources (2015) doi: 10.1111/1755-0998.12445.

Decru, E., Raats, L., Nkalubo, W., Van de Walle, L., Vreven , E. Snoeks, J. & Van Steenberge, M. in prep. A revision of the species of the *Labeo forskalii* group (Pisces: Cyprinidae) from the Congo basin.

Decru, E., Snoeks, J., Vreven, E. 2015b. A taxonomic evalutation of the *Hepsetus* from the Congo basin with the revalidation of *H. microlepis* (Teleostei: Hepsetidae). Ichthyological Exploration of Freshwaters, 26 (3), 273-287.

Degryse S. 2013, A revision of the genera *Hippopotamyrus* and *Cyphomyrus* (Osteoglossiformes, Mormyridae). Unpublished MSc thesis, KU Leuven.

DelVecchia AG, Stanford JA, & Xu X (2016) Ancient and methane-derived carbon subsidizes contemporary foodwebs. Nature Communications 7:13163; DOI: 10.1038/ncomms13163

Descy JP, Darchambeau F, Lambert T, Stoyneva MP, Bouillon S, Borges AV (2016) Phytoplankton dynamics in the Congo River, Freshwater Biology, DOI: 10.1111/fwb.12851

Engle, D.L., Melack, J.M., Doyle, R.D. & Fisher, T.R. High rates of net primary production and turnover of floating grasses on the Amazon floodplain: implications for aquatic respiration and regional CO2 flux. Glob. Change Biol. 14,369–381 (2008).

Fisher JB, Sikka M, Sitch S, Ciais P, Poulter B, Galbraith D, Lee JE, Huntingford C, Viovy N, Zeng N, Ahlström A, Lomas MR, Levy PE, Frankenberg C, Saatchi S, & Mahli Y (2013) African tropical rainforest net carbon dioxide fluxes in the twentieth century. Phil. Trans. R. Soc. B 368: 20120376, doi: 10.1098/rstb.2012.0376.

Foged N. (1966). Freshwater diatoms from Ghana. – Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter 15(1): 1-169 + 25 pls.

Gosse, J. P. 1963. Le milieu aquatique et l'écologie des poissons dans la région de Yangambi. *Annales du Musée Royal de l'Afrique Centrale, Série in-8°, Sciences Zoologiques* 116: 113-270.

Grey J, Kelly A, Ward S, Sommerwerk N, & Jones RI (2004) Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. Freshwater Biology 49: 681-689.

Hanley, K., Wollheim, W. M., Salisbury, J., Huntington, T., and Aiken, G.: Controls on dissolved organic carbon quantity and chemical character in temperate rivers of North America, Global Biogeochem. Cy., 27, 492–504, doi:10.1002/gbc.20044, 2013

Hartmann, J., and N. Moosdorf (2012), The new global lithological map database GLiM: A representation of rock properties at the Earth surface, Geochem. Geophys. Geosyst., 13, Q12004, doi:10.1029/2012GC004370

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., and Mopper, K.: Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter, Limnol. Oceanogr., 53, 955–969, 2008.

Jones RI, Carter CE, Kelly A, Ward S, Kelly DJ, & Grey J (2008) Widespread contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae. Ecology 89: 857-864

Jones RI, & Grey J (2011) Biogenic methane in freshwater food webs. Freshwater Biology 56: 213-229.

Kaiser, K. and Zech,W.: Dissolved organic matter sorption by mineral constituents of subsoil clay fractions, J. Plant Nutr. Soil Sc., 163, 531–535, 2000.

Karthick B., Kociolek J.P., Taylor J.C. & Cocquyt C. (2016). *Gomphonema grande* sp. nov., a new diatom (Bacillariophyta) from the Democratic Republic of the Congo, Tropical Africa. Phytotaxa 245(3): 187-196.

Lambert T., S. Bouillon, F. Darchambeau, P. Massicotte & A. V. Borges (2016) Shift in the chemical composition of dissolved organic matter in the Congo River network, Biogeosciences, 13:5405–5420

Lambert, T., Darchambeau, F., Bouillon, S., Alhou, B., Mbega, J- D, Teodoru, C. R., Nyoni, F. C., Massicotte, P., and A V Borges, A. V.: Landscape control on the spatial and temporal variability of chromophoric dissolved organic matter and dissolved organic carbon in large African rivers, Ecosystems, 18, 1224–1239, doi:10.1007/s10021-015-9894-5, 2015.

Lambert, T., Pierson-Wickmann, A.-C., Gruau, G., Thibault, J. N., and Jaffrezic, A.: Carbon isotopes as tracers of dissolved organic carbon sources and water pathways in headwater catchments, J. Hydrol., 402, 228–238, doi:10.1016/j.jhydrol.2011.03.014, 2011.

Laraque A, Bricquet, JP., Pandi, A and Olivry, JC., 2009. A review of material transport by the Congo River and its tributaries, Hydrological Processes, 23(22), 3216–3224.

Lucas Y., C. R. Montes, S. Mounier, M. Loustau Cazalet, D. Ishida, R. Achard, C. Garnier, B. Coulomb, and A. J. Melfi (2012) Biogeochemistry of an Amazonian podzol-ferralsol soil system with white kaolin, Biogeosciences, 9, 3705–3720.

Lundberg, J.G., Kottelat M., Smith G.R., Stiassny M. & Gill A.C., 2000. So many fishes, so little time: An overview of recent ichthyological discovery in continental waters. *Annals Missouri Botanical Garden* 87: 26–62.

Mann, P.J., Spencer, R. G. M., Dinga, B. J., Poulsen, J.R., Hernes, P.J., Fiske, G., Salter, M. E., Wang, Z. A., Hoering, K.A., Six, J., and Holmes, R. M.: The biogeochemistry of carbon across a gradient of streams and rivers within the Congo Basin. J. Geophys. Res.-Biogeo., 119, 687–702, 2014

Mayaux, P., Bartholomé, E., Fritz, S. & Belward A., 2004. A new land-cover map of Africa for the year 2000. J. Biogeogr.31, 861-877.

Melack, J.M. & Engle, D.L. An organic carbon budget for an Amazon floodplain lake. Verh. Internat. Verein. Limnol. 30, 1179-1182 (2009).

Melack, J.M. et al. Regionalization of methane emissions in the Amazon Basin with microwave remote sensing. Global Change Biol. 10, 530-544 (2004).

Moelants T. 2015, Diversity and ecology of the ichthyofauna of the Middle and Upper Congo basin: a case-study in the region of the Wagenia falls (Democratic Republic of Congo). 332 pp, unpublished PhD, KU Leuven.

Moelants, T., Mbadu Zebe, V., Snoeks, J. & Vreven, E. 2014 A review of the *Distichodus antonii* assemblage (Characiformes, Distichodontidae) from the Congo basin. Journal of Natural History: 48 (27-28), 1707-1735.Moelants, T., Nguku, J. K. & Snoeks, J. A morphometric revision of the genus *Citharinus* Cuvier, 1816 (Citharinidae, Characiformes) from Africa, in prep.

Moelants, T., Snoeks, J & Vreven, E. (in press). *Distichodus kasaiensis* and *Distichodus ingae*, two new species in the *Distichodus atroventralis* complex (Characiformes: Distichodontidae) of the Congo basin. Ichthyological Exploration of Freshwaters.

Monakov AB (2003) Feeding of Freshwater Invertebrates. Kenobi Productions, Ghent, 373 pp

Naiman RJ and RE Bilby (2001) River ecology and management: lessons from the Pacific coastal ecoregion, Springer-Verlag, 705 pp

Morana, C., Sarmento, H., Descy, J.-P., Gasol, J.M., Borges, A. V., Bouillon, S., and Darchambeau, F.: Production of dissolved organic matter by phytoplankton and its uptake by heterotrophic prokaryotes in large tropical lakes, Limnol. Oceanogr., 59, 1364 – 1375, 2014.

Neff, J. C., Finlay, J. C., Zimov, S. A., Davydov, S. P., Car- rasco, J. J., Schuur, E. A. G., and Davydova, A. I.: Seasonal changes in the age and structure of dissolved organic carbon in Siberian rivers and streams, Geophys. Res. Lett., 33, L23401, doi:10.1029/2006GL028222, 2006.

Nkounkou, R.R., Probst, J.L., 1987. Hydrology and geochemistry of the Congo river system. Mitt. Geol–Palaont. Inst. Univ. Hamburg, SCOPE/UNEP, 64, pp. 483-508.

Reid G.M. 1985**.** A revision of the African species of *Labeo* (Pisces: Cyprinidae) and a re-definition of the genus. *Theses Zoologicae* 6**:** 1-322.

Sanderman, J., Lohse, K. A., Baldock, J. A., and Amundson, R.: Linking soils and streams: sources and chemistry of dissolved organic matter in a small coastal watershed,Water Resour. Res., 45, W03418, doi:10.129/2008WR006977, 2009

Sanseverino AM, Bastviken D, Sundh I, Pickova J, & Enrich-Prast A (2012) Methane carbon supports aquatic food webs to the fish level. PLoS ONE 7: e42723. doi:10.1371/journal.pone.0042723

Taylor J.C., Cocquyt C. & Mayama S. (2016). New and interesting *Eunotia* (Bacillariophyta) from the Democratic Republic of the Congo, tropical central Africa. Plant Ecology and Evolution 149(3): 291-307.

Trimmer M, Hildrew AG, Jackson MC, Pretty JF, & Grey J (2009) Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. Limnol. Oceanogr. 54: 1541-1547.

Tshibwabwa SM. 1997. Systematique des especes africaines du genre *Labeo* (Teleostei, Cyprinidae) dans les regions ichtyogeographiques de Basse-Guinee et du Congo. Vol I et II. These de doctorat, Faculte des Sciences, Facultes Universitaires Notre-Dame de la Paix de Namur, Belgium. Presses Universitaires de Namur.

Seyler, P., Coynel, A., Moreira-Turcq, P., Etcheber, H., Colas, C., Orange, D., Bricquet, J-P., Laraque, A., Guyot, J-L., Olivry, J-C., Meybeck, M., 2006. Organic carbon transported by the equatorial rivers: example of Congo-Zaïre and Amazon basins. Land use, erosion and carbon dynamic. Lewis Publishers, CRC Press: 255-272.

Snoeks, J. 2004. - Material and methods. In: Snoeks, J. (ed). *The cichlid diversity of Lake Malawi/Nyasa/Niassa: identification, distribution and taxonomy.* Cichlid Press, El Paso, USA.: 12-19.

Spencer, R. G. M., Hernes, P. J., Aufdenkampe, A. K., Baker, A., Gulliver, P., Stubbins, A., Aiken, G. R., Dyda, R. Y., Butler, K. D., Mwamba, V. L., Mangangu, A. M., Wabakanghanzi, J. N., and Six, J.: An initial investigation into the organic matter biogeochemistry of the Congo River, Geochim. Cosmochim. Ac., 84, 614–627, 2012.

Soto, D.X., Wassenaar, L.I. and Hobson, K.A.: Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. Funct. Ecol., 27, 535–543, 2013.

Van den Bogaart L. 2015, Morfologische analyse van drie genetisch verschillende groepen in *Enteromius brazzai*. Unpublished BSc. thesis KU Leuven.

Van De Walle L. 2015, Komt alle hulp van boven? Dieetanalyse van de vissen van het centrale kongobekken.82, Masterthesis Promotor: Jos Snoeks

Van Ginneken M, Decru E, Verheyen E, Snoeks J. (in press.) Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin, Africa. Biol J Linnean Society.

Wang, Z. A., D. J. Bienvenu, P. J. Mann, K. A. Hoering, J. R. Poulsen, R. G. M. Spencer, and Holmes, R. M., 2013. Inorganic carbon speciation and fluxes in the Congo River, Geophys. Res. Lett., 40,doi:10.1002/grl.50160.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., and Mopper, K.: Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, Environ. Sci. Technol., 37, 4702–4708, doi: 10.1021/es030360x, 2003.

ANNEXES

ANNEX 1: COPY OF THE PUBLICATIONS

The annexes are available on our website:

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