



# CONDUCTING FISH STUDIES ON LAKE KIVU AND REINFORCEMENT OF PLANKTON CAPACITIES

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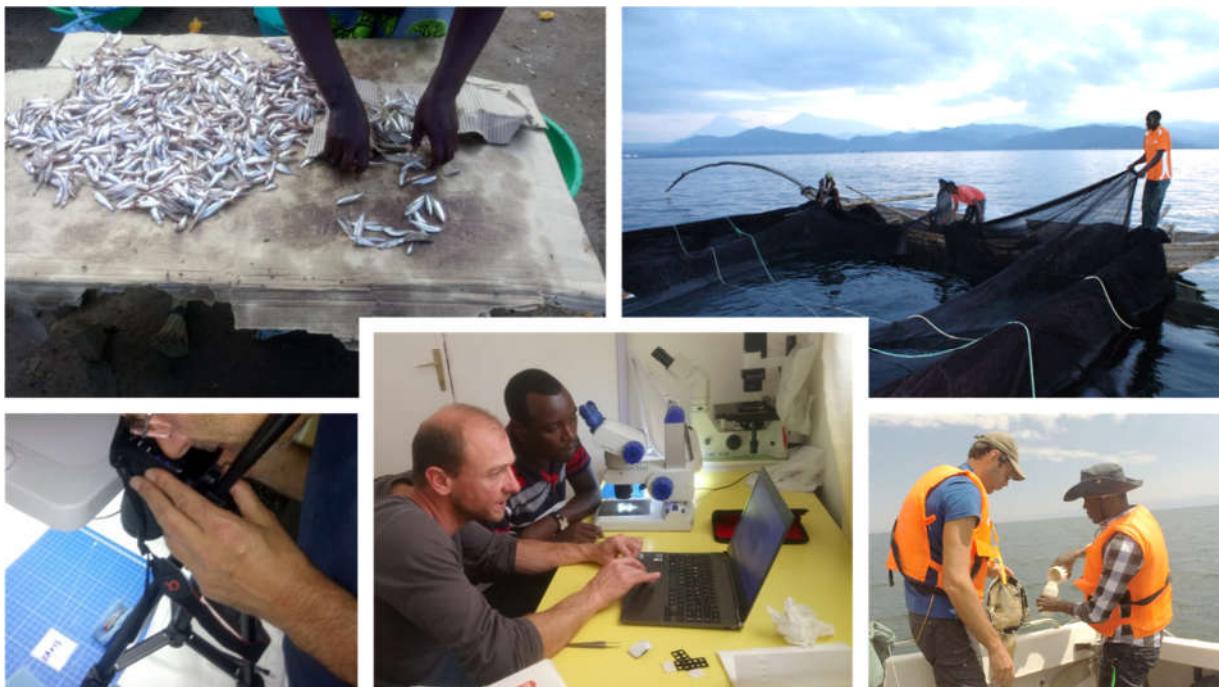
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## CONDUCTING FISH STUDIES ON LAKE KIVU AND REINFORCEMENT OF PLANKTON CAPACITIES

### FINAL REPORT



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# Summary

## **Background**

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In 2012, the Lake Kivu Monitoring Program initiated a biological baseline on the fish and plankton communities of Lake Kivu to serve as a reference for future changes, consecutive to global warming, local human impacts, and methane gas extraction. The “Fish & Plankton study” carried out between 2017 and 2019 aimed to complement this baseline, as well as to conduct new research on the Lake Kivu ecosystem and to provide reinforcement of LKMP staff capacities. Research and training activities were initiated in 2017 by French, Swiss and Belgian experts in collaboration with LKMP, for the study of 3 biological compartments in Lake Kivu: plankton, macroinvertebrates and fish.

## **Main outcomes**

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A new HPLC system was installed at LKMP laboratory in Rubavu under the supervision of the experts. The equipment is fully operational, with the capacity to provide reliable data on phytoplankton in the framework of the monitoring program of Lake Kivu. The staff has been trained not only in the analysis of marker pigments in the phytoplankton extracts, but also in the processing of the pigment concentrations to obtain reliable estimates of chlorophyll *a* concentration, and of the biomass of the phytoplankton classes. Further analyses of these data shall reveal their dependence to environmental variables, including weather data (available from the AWS off Gisenyi, installed during the EAGLES project). However, a valid comparison with earlier data, for instance to assess changes that may occur from the operation of the gas extraction plants, will not be possible if regular monitoring of all relevant variables is not ensured.

A better knowledge of littoral macroinvertebrates biodiversity is required to serve as a reference for future changes, but also to determine fish food availability. The analysis of macroinvertebrate diversity and abundance, together with their distribution and decrease according to depth, provided a valuable insight of the general quality of Lake Kivu. The results obtained in Gisenyi in 2017 using a standardized procedure, showed 43 different taxa (dominated by Diptera Chironomidae), some taxa being associated to good ecological value (Ephemeroptera *Povilla* and Trichoptera *Ecnomus*). These results were still difficult to compare with other African lakes or with previous data in Lake Kivu, given the disparity of sampling methods. During this study, LKMP technicians were trained on macroinvertebrates sorting and identification at the genus level for some taxa.

Fish studies mainly focused on the acquisition of complementary biological and ecological knowledge on the two main pelagic species in Lake Kivu: Sambaza (*Limnothrissa miodon*) and *Lamprichthys tanganicanus*. The following biological and ecological features were addressed: fecundity, weight-length relationship, age structure, growth rates, food habits and trophic position. A special emphasis was granted to the competitive interactions between the two species, using stable isotope analyses to get insight of the trophic niche overlap. Sampling performed during the present study from fishing crews confirmed the predominance of *L. miodon* in the pelagic catches. The recently introduced *L. tanganicanus* occupies mostly the littoral habitat, although some individuals are sometimes captured in pelagic zones. The results obtained on compared biology and ecology of both species are consistent with previous studies: *L. miodon* is a highly reproductive, fast-growing and short-living species, whereas *L. tanganicanus* shows lower fecundity rates, slower growth and longer lifespan. Both species may compete for food when they live in the same habitats, but this trophic competition seems to be restricted to the inshore zone where both species cohabit.

Pelagic fish stock was assessed in 2018 throughout the entire Lake Kivu using hydroacoustics. Between 2012 and 2018, the total fish biomass showed a high variability in the pelagic zone of Lake Kivu, ranging between around 1'000 to more than 4'000 tons with important spatial, seasonal and interannual variations. In 2018, the total fish biomass increased in all basins and reached a maximum over the period 2012-2018. This assessed biomass is consistent with previous studies of pelagic fish stock carried out in the 90's and in 2008. At a large temporal scale, the pelagic fish stock seems to be stable, the total amount being similar between FAO 90's evaluation, 2008 ones and present surveys. In parallel with the pelagic biomass survey, fisheries data should be gathered from fishermen in order to assess the fishing effort together with the total monthly catches. There is only a few data and knowledge about fisheries status and actual fishing effort in Lake Kivu, since the FAO project Isambaza at the end of the 80's. A survey of fisheries was set up in 2011 and conducted for 2 years during the EAGLES project along the Rwanda shore of the Lake. Conversely, fisheries yield in DRC are still unknown, and statistics still need to be set up in a similar manner in both countries. Lake-wide fishery management is recommended but is presently impossible as long as fisheries statistics are not established in a similar way in both Rwanda and DRC.

For the future, the proposition is to globalize the monitoring of fish, macroinvertebrate and plankton communities and to add new sampling sites distributed all over the lake area to ensure a strong and robust knowledge of biodiversity and biomass production, and to allow the disentangling of the effects of gas extraction platform from other local drivers (water pollution, shore and inflows artificialization, etc.). To reinforce the capacity building of Rwanda and DRC managers, we recommend LKMP to continue a straight collaboration with the multidisciplinary international team of experts. The monitoring should be pursued with a site by site approach, doing in parallel all measurements to ensure a better understanding of the lake functioning from primary production to fish biomass and fisheries.

## **Recommendations by theme**

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1. We first recommend the continuation of phytoplankton sampling and the measure of limnological variables (including nutrients) twice a month at least at the monitoring site off Gisenyi. In the future, it will be necessary to differentiate the changes brought about by plant operation from those due to other environmental changes, and this will not be possible if data collection is interrupted for long periods of time.
2. Zooplankton is obviously a key link in the food web, depending on phytoplankton production and on predation by planktivorous fish. In the perspective of developing a predictive model of fish production (see below), good data on zooplankton composition and abundance, thus allowing good estimates of zooplankton biomass and production, will be necessary.
3. We propose the development of a process-based or data-based model, with the objective of linking primary production and fish production, allowing prediction of fisheries yield in a context of environmental change and of large-scale methane exploitation.
4. It would be also important to acquire additional knowledge about benthic macroinvertebrates composition in other areas of Lake Kivu, using standardized procedure, both in Rwanda and DR Congo. This will serve as a reference to appreciate the effects of environmental and climatic changes, together with the effects of methane gas extraction. Macroinvertebrates should therefore be added in the monitoring program in Lake Kivu.
5. Complementary surveys on *L. miodon* and *L. tanganicanus* distribution and trophic positions should be carried out in other basins in collaboration with DRC, given the disparity of the lake functioning between basins. We recommend complementing some of the biological and ecological key features of *L. miodon* and *L. tanganicanus* in different basins and over seasons: length-weight relationships, growth curves, fecundity analysis. Additional training of LKMP staff will be necessary so that they become self-reliant.
6. For future monitoring of pelagic fish stock (*L. miodon*) in Lake Kivu, we would highly recommend to further carry on the annual hydroacoustic campaigns over the entire lake (all basins) during the dry season to encompass newly recruited cohort. Due to the lack of expertise of LKMP on hydroacoustics analyses after Alice Muzana's departure, complementary training should be provided to LKMP staff in order to become self-reliant on calibration, maintenance of equipment, echogram reading and data analysis.



7. We recommend conducting fisheries surveys over the whole lake, in parallel with annual hydroacoustic surveys, both in Rwanda and DRC. The methodology proposed during the EAGLES project should be applied and complemented.
8. Additional knowledge should be acquired on fish diversity and species distribution according to depth and habitat in Lake Kivu using standardized sampling with multimesh gillnets, coupled with genetical analyses.
9. Finally, we suggest updating the genetic structure and effective population size of *L. miodon* populations for the rational and sustainable exploitation of this natural resource in Lake Kivu.

## Introduction

Lake Kivu is one of the East African great lakes, located at the border between the Republic of Rwanda and the Democratic Republic of the Congo (DRC). It contains high amount of methane ( $\sim 55\text{--}60 \text{ km}^3$ ) and carbon dioxide ( $\sim 300 \text{ km}^3$ ) dissolved in its deep waters, with a reported high risk for local populations in case of release (Nayar, 2009). The gas also represents an opportunity for energy production in this region, which can benefit this available energy source for its economic development. The methane gas exploitation started in 2008 with the first pilot plant named KP1 (Kibuye Power 1), which stopped its production in 2016. In the last years, gas exploitation increased: Kivuwatt powerplant started methane gas harvesting in 2015, with a production capacity of 26.2 MW, and will reach 100 MW in the next years according to the Rwanda Energy Group (REG) projections.

In parallel to methane gas pilot harvesting, the biological monitoring of Lake Kivu started in 2012 with a baseline project, supported by the Belgian Technical Cooperation, which aimed to serve as a reference to assess future changes in the lake ecosystem, consecutive to methane gas exploitation, climate change and anthropization (Descy & Guillard, 2014). This first project had for objectives to gather the available scientific knowledge on the Lake, prior to intensive gas harvesting. Since then, the monitoring was continued by the Lake Kivu Monitoring Program (LKMP), with a special emphasis on plankton survey and analysis, and fish stock assessment.

The ‘biological baseline’ of Lake Kivu, along with training of local technicians and scientists of LKMP, provided information consistent with the previous studies on the lake (synthesized in Descy et al., 2012), and established a reference state for assessing changes expected from future large-scale methane exploitation. At the end of that project, recommendations were made, including the need to pursue regular sampling and data acquisition – at least monthly - from the floating platforms installed off Kibuye and Gisenyi, needed to monitor the status of the mixolimnion (or “biozone”). This data acquisition includes CTD casts down to 70 m depth, nutrient measurements on the seston, determination of phytoplankton biomass and composition, based on HPLC analysis of marker pigments, completed by examination at the microscope for identification of the most abundant taxa (genus or species). In the baseline, the fish stock assessment was a key issue, and was made by hydroacoustic surveys, carried out with equipment purchased during the project. The objective has been to follow the variations of the lake-wide stock of the main fish species, the “Tanganyika sardine” *Limnothrissa miodon* (locally called *sambaza*). So far, however, the variation of the sardine abundance could not be related to variation of plankton production, expected from a planktivore, nor to the fishing effort, given the lack of reliable fisheries statistics in both countries. Although a subsequent research project (EAGLES, Descy et al., 2015) has provided detailed data for the Rwandan fishery, highlighting that the *sambaza*

remains the main resource, data from RDC (Democratic Republic of the Congo) are still lacking for assessing the whole lake fishery yield. An additional problem is the introduction of *Lamprichthys tanganicanus*, a potential competitor of *Limnothrissa* (Masilya, 2011) for resources and habitat. Further recommendations from the “baseline” were to pursue the whole-lake hydroacoustic surveys, to collect fisheries statistics and catch per unit effort (CPUE), and to update the existing knowledge on the biology of *L. miodon*, in order to verify, among other things, the relevance of the weight – length models established a few years ago by Lamboeuf (1989) and by Kanigini (1995). As the lake productivity is driven by climatic factors (Darchambeau et al., 2014), it is expected that the fish stock and the fishery yield respond to variation of phytoplankton and zooplankton production, driven by climate variability at the regional scale. Building data bases and analysing long-term data is likely to allow identifying the processes governing the variations of the fish stock, at different time scales (seasonal, annual, possibly decadal ....), with the ultimate objective of developing predictive models of the lake’s production as responding to climate change, as well as to possible impacts of gas harvesting on the lake’s ecosystem.

The project “Fish study in Lake Kivu and reinforcement of plankton capacities” proposed additional research on fish and plankton communities in Lake Kivu, together with further training of LKMP staff on specific water compounds analyses. Several activities were initiated by Belgian, French and Swiss experts for the study of three biotic communities: plankton, macroinvertebrates and fish. After an inception report (October 2017) that detailed the implemented protocols, field sampling and training activities were carried out for two years. This final report describes the main outcomes of the studies carried out by LKMP scientists and the experts between 2017 and 2019.

The aspect “reinforcement of plankton capacities” consisted in intensive training of the LKMP staff for phytoplankton analysis, based on determination of phytoplankton marker pigments, both in Europe (at University of Liège, Belgium) and in LKMP laboratory (Gisenyi). The training of the LKMP staff was carried out in three phases. The first phase consisted in a one month stay in Belgium (October 2017), at University of Liège, with a training on the existing HPLC (High Performance Liquid Chromatography) system installed at the Unit of Chemical Oceanography (UOC), directed by Dr. A.V. Borges. In the second phase, which took place in 2018, a new system (Waters Alliance) was purchased from the company Microsep (South Africa) and installed at the LKMP laboratory at Rubavu. The system was operational within a week and several sets of samples collected in 2017 and 2018 were analysed and processed for quantitative determination of phytoplankton composition at the class level. In a third phase, in April 2019, the validation and interpretation of the data were done during a one-week stay of the scientist Eric Mudakikwa at University of Liège. The result of this stay was the setup of a data base for storing the results of the phytoplankton monitoring and the collection of data for a scientific paper on phytoplankton monitoring in Lake Kivu. This part of the project also included the acquisition and utilization of a new CTD system (Conductivity, Temperature and Depth), allowing monitoring of the main limnological variables in the surface and deep waters of the lake.



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## **Objectives**

The objective of this study is (1) to carry out extended research on fish, and (2) to provide additional onsite training on Lake Kivu actual sampling strategies and samples, both for fish and plankton monitoring, in order that LKMP and regional scientific institutions become self-reliant on more aspects as possible of the monitoring data acquisition, as well as sample and data analysis and interpretation.

More specifically, the **objectives for fish studies** are the followings:

- ✓ To analyse and interpret, in partnership with Lake Kivu monitoring Programme, recent data on fish stock estimates and to contribute to a better understanding of fish stock evolution and seasonal variations;
- ✓ To complement knowledge on biology and ecology of the exploited pelagic species *Limnothrissa miodon* and a potential competitive species *Lamprichthys tanganicanus*;
- ✓ To establish and implement a monitoring and assessment protocol for fish and fisheries in Lake Kivu, and to provide basic information for the different authorities to properly manage pelagic fisheries on the whole lake.

The objectives of the **training on biological monitoring** are the following:

- ✓ To complement the LKMP staff's capacities on actual phytoplankton sample analysis, through marker pigment method (HPLC), in the continuity of the training already received during the biological baseline study at the laboratory of Liège University;
- ✓ To improve plankton measurements, as well as related field work, with new appropriate and high-quality instruments and tools, according to biological baseline recommendations and considering the development of extraction activities to be monitored.

# **Chapter 1. Phytoplankton in Lake Kivu: reinforcement of LKMP capacities and perspectives for the monitoring**

## **1.1 Introduction**

The inception report of the project contains a literature review on the phytoplankton of Lake Kivu, with an emphasis on its recent taxonomic composition and biomass, based on the studies conducted since 2002 in the framework of different projects and finishing with the “Biological baseline of Lake Kivu”, carried out in 2012-2014 (Descy and Guillard, 2014). These data have been gathered in a data base, which can be used as a reference for the lake monitoring to evaluate potential effects of the ongoing gas extraction, since no large-scale gas exploitation existed in that period of time. Most of these data have been presented in international publications (Sarmento et al., 2006, 2012; Darchambeau et al., 2014; Rugema et al., 2019).

A detailed report on phytoplankton taxonomic composition of Lake Kivu was published by Sarmento et al. (2007). According to these authors, the most common species were the pennate diatoms *Nitzschia bacata* Hust. and *Fragilaria danica* (Kütz.) Lange Bert., and the cyanobacteria *Planktolyngbya limnetica* (Lemm.) Komárková-Legnerová and Cronberg. Additionally, the pico-cyanobacterium *Synechococcus* sp. was shown to constitute a major compartment of the autotrophic plankton in Lake Kivu, with persistently high abundances ( $\sim 10^5$  cell ml $^{-1}$ ) throughout the year (Sarmento et al. 2008). At times, under stratified conditions in the water column, the centric diatom *Urosolenia* sp. and the cyanobacterium *Microcystis* sp. may appear near the surface. Periods of stable stratification also allow the development of metalimnetic populations of Cryptophytes (*Cryptaulax* sp., *Cryptomonas* sp., *Rhodomonas* sp.) and of cyanobacteria (*Merismopedia trolleri*). Worth noting is that green algae were not abundant in the lake in 2002-2006, when the study was conducted: few species were present, as the desmid *Cosmarium laeve* and a peculiar form of *Tetraëdron minimum*, a small Chlorococcace (Stoyneva et al., 2012).

In a more complete limnological survey carried out in 2002-2004 (Isumbisho et al. 2006, Sarmento et al. 2006) phytoplankton biomass and composition were assessed combining diverse complementary techniques such as HPLC analysis of marker pigments and CHEMTAX processing (for details on the technique, see annex 1), flow cytometry, and epifluorescence and electron microscopy. Annual average chlorophyll a (Chl a) in the mixed layer was 2.2 µg L $^{-1}$  and the nutrient levels in the euphotic zone were low, placing Lake Kivu clearly in the oligotrophic range. Variations of algal biomass and composition occur at a seasonal scale, depending on variability of wind velocity and direction, as well as air

temperature and relative humidity. There is typically a dominance of diatoms during the dry season (May to September) when deep mixing occurs in the mixolimnion. During the rainy season (October to April), the water column is permanently stratified, with a seasonal thermocline located between 20 m and 30 m, with a dominance of filamentous, diazotrophic cyanobacteria and of picocyanobacteria. There can be a substantial inter-annual variability of phytoplankton biomass peaks, related to the intensity of the dry season mixing (Darchambeau et al., 2014). The dry season biomass peaks may determine the productivity of the lake for the whole year, and most likely affect consumer production and ultimately fisheries yield.

The phytoplankton studies carried out in Lake Kivu since 2002 till end of 2014 have been based on the analysis of marker pigments by HPLC: this approach has allowed continuous monitoring of the lake phytoplankton with the same method during around 12 years, which is unique for an African great lake. It was thus essential, for the lake monitoring carried out by LKMP in the framework of gas extraction, to ensure that the phytoplankton data would be collected using the same methodology, thanks to the acquisition of a HPLC system, to its installation in the Rubavu laboratory and to training of the LKMP staff on the new equipment.

## 1.2 Installation and operation of the Waters Alliance HPLC system

The unpacking and physical installation of the system took place on Monday September 10, 2018: all the system parts were assembled and training on the startup of the system was organised by Christo Frylinck, from MICROSEP, South Africa (CF hereafter), who also introduced the principles of HPLC analysis. The system consists of different parts, integrated in a single unit (see Figure 1): a binary pump, which allows mixing of different mobile phases, with a constant flow rate; an autosampler from which the acetone extracts will be injected ; a compartment with the column, in which the pigments mixed in the extracts will be separated and eluted ; and a detector, which is a photodiode array (PDA), which measures the absorbance of the separated pigments in a range of wavelengths. This allows to get the absorbance spectra of the pigments, which is their “signature”, facilitating their identification. The whole apparatus is controlled by the Empower software, which runs on the PC and allows programming the analyses and storing as well as processing the results. A printer connected to the PC was also installed, as well as a UPS, which allows keeping power on the system (at least for 10 min but in fact for as much as 30 min) in case of power cut.

The equipment conformed to the order placed by SCIMABIO, including vials for placing the extracts and guard columns, and everything worked properly.



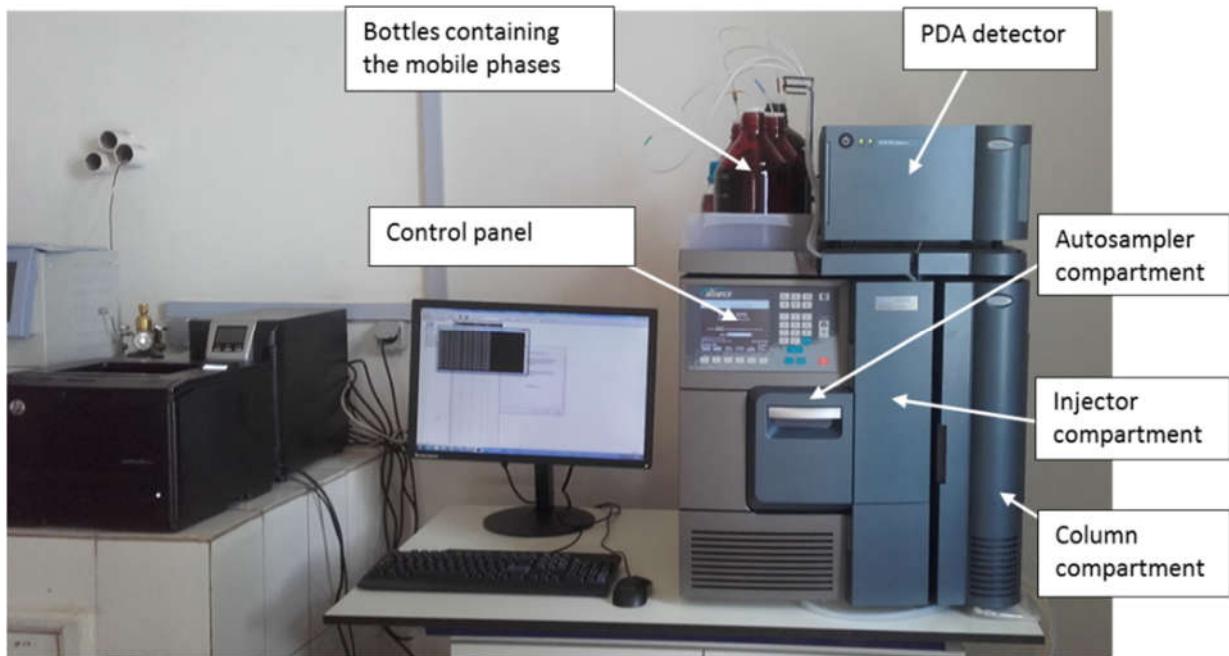


Figure 1: Picture of the Waters Alliance HPLC system installed in the LKMP lab at Cape Rubona.

On the second day, the mobile phases for pigment analysis were prepared and a first run with injection of a sample was made on the same day, using the method described in annex 1. This method had to be slightly adapted in order to achieve a critical separation between two marker pigments (lutein, marker of green algae, and zeaxanthin, abundant in cyanobacteria).

On the 3<sup>rd</sup> day, 20 phytoplankton pigment standards, purchased by University of Liège from DHI, Denmark, and transported from Belgium to Rwanda in a dry shipper by J.-P. Descy (JPD hereafter), were run on the system, in order to provide calibration curves. A list of these standards, with their concentration certified by the provider, is given in annex 2. The file also contains the calibration curves, calculated in Excel.

The calibration of the system with the standards was done on the same day and on the 4<sup>th</sup> day, and the integration and quantification of a lake sample was made with the Empower software by the LKMP personnel with the help of JPD and CF. A sample set template was developed, so that the LKMP staff would be able, in the following days, to run the Lake Kivu phytoplankton extracts collected during the months preceding the installation of the HPLC system.

During the second mission, different procedures were developed further by JPD and Bruno Leporcq (BL). Most of the samples collected in 2018 had been run on the system by the LKMP team. They were all re-processed with the help of JPD and BL, and methods for chromatogram integration and processing were refined, in order to facilitate future work by the LKMP team. More specifically, a method for exporting the data to Excel was developed by BL, and JPD wrote simplified guidelines for the use of the Alliance HPLC, from startup to

pigment processing (annex 3). The calibrations obtained with the Alliance HPLC (annex 2) were compared with those obtained with the older system located at University of Liège, Belgium (used during the October 2017 training): they were quite similar, which validates the quantification made at the LKMP lab and guarantees continued acquisition of phytoplankton data from Lake Kivu, and comparability of data in the long term.

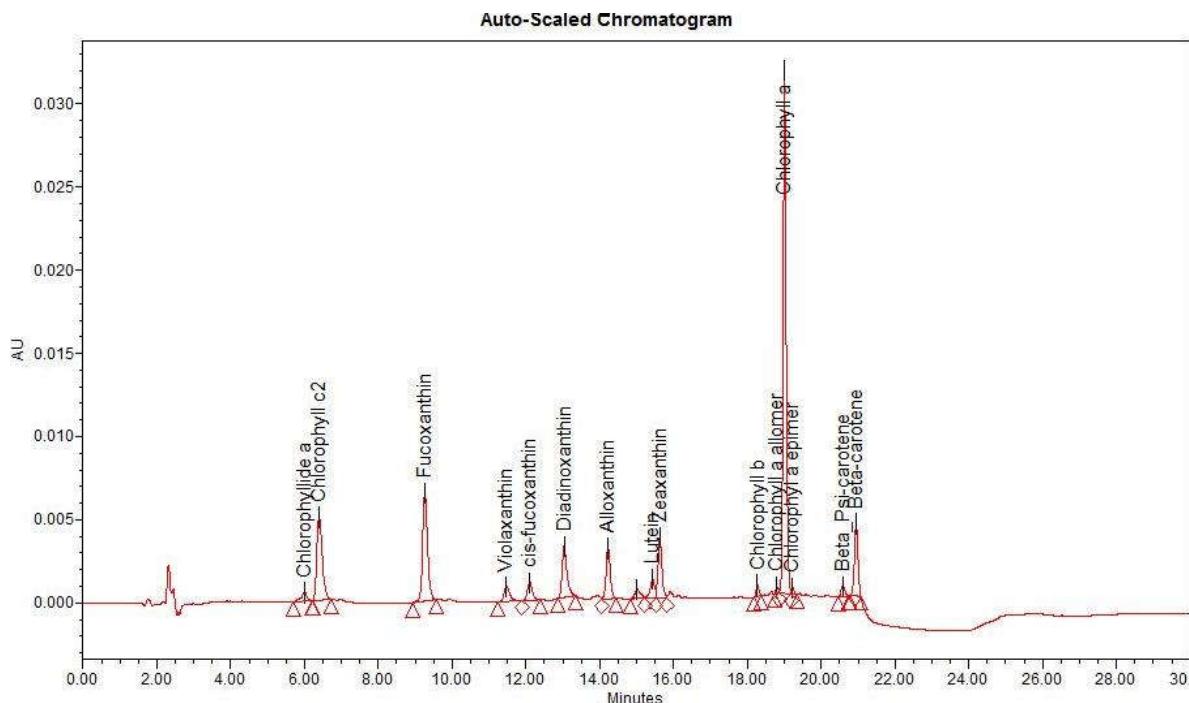


Figure 2: Example of a typical chromatogram obtained with the LKMP Alliance HPLC system, after processing for pigment identification. The major peak, with a retention time of ~19 min, corresponds to chlorophyll a, which is present in all phytoplankton groups. In this example, the sample contained mainly diatoms (with fucoxanthin and diadinoxanthin as markers), cryptophytes (alloxanthin), green algae (lutein and chlorophyll b), and cyanobacteria (zeaxanthin).

A last session was devoted to processing the pigment concentrations with CHEMTAX (Mackey et al., 1996), using the analyses of samples collected from January to March 2018. An example of results in terms of phytoplankton abundance is shown in Figure 3.

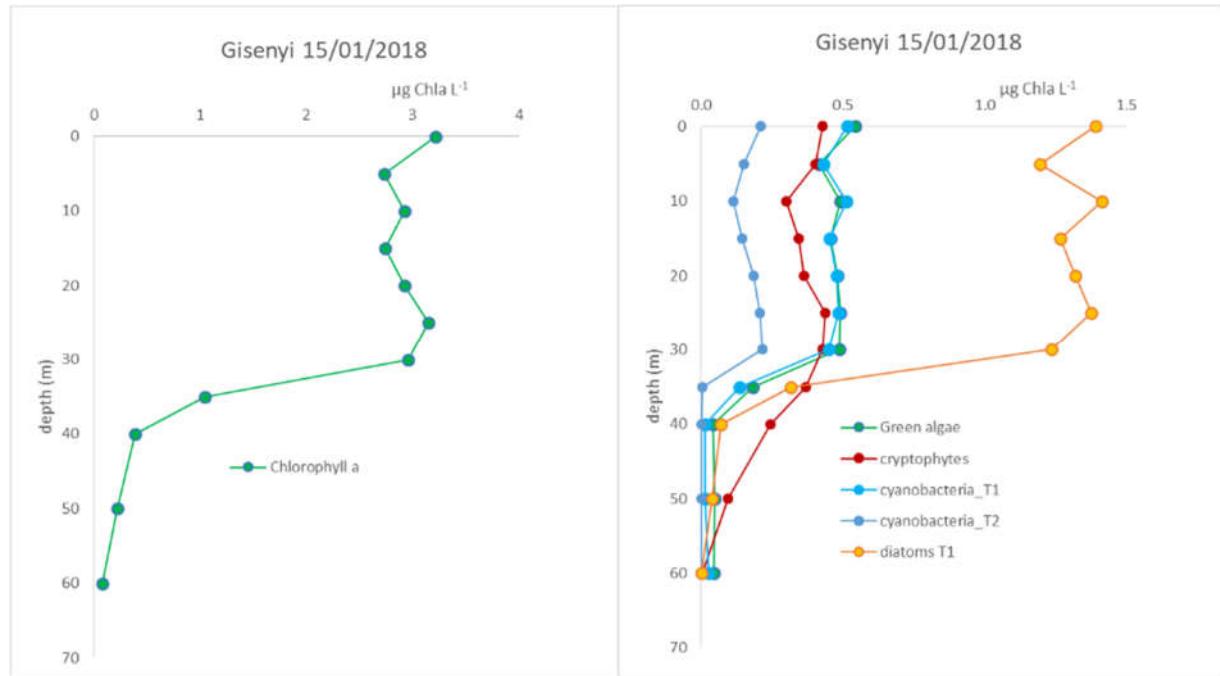


Figure 3: Vertical profiles of phytoplankton abundance in Lake Kivu, January 2018. Right: profiles of the main groups (green algae, cryptophytes, cyanobacteria type 1 & 2, diatoms type 1); Left: profile of chlorophyll a. The chlorophyll a profile suggests that the mixed layer was approximately 30 m. These results were obtained during the second mission in 2018, by Eric Mudakikwa, with the help of J.-P. Descy and Bruno Leporcq.

In conclusion, after two missions in 3 weeks in total, including installation by Microsep, training and supervision by the phytoplankton experts, the LKMP team developed the capacity to monitor the phytoplankton of Lake Kivu in an autonomous way, thanks to an HPLC system that has been working perfectly so far.

### 1.3 Setup and use of the SeaBird CTD

A new CTD was purchased on the project, essentially for making deep profiles of temperature, pH and conductivity, which may be affected by methane exploitation, as a result of re-injection of degassed water. The CTD is a Seabird SBE 19 V2 equipped with the adequate sensors, with in addition a fluorometer for chlorophyll a measurements. This conforms to the order established by F. Darchambeau in December 2016 in coordination with V. Gosselain: the objective was to equip LKMP with a sensor (the fluorometer) which was not present on the existing CTDs. It may be used for obtaining phytoplankton biomass profiles in the mixolimnion. This sensor may remain mounted on the CTD body during acquisition of a deep profile, but it will not provide meaningful results, as phytoplankton is only present in the 0-60 m layer.

The sensor operation is relatively easy: the measurements start when a switch is put on, and data are recorded within the CTD. Downloading and transferring to Excel is, however, less user-friendly. To this end, an operation procedure was written by B. Leporcq (see annex 4). An example of profile obtained with this CTD is presented in Figure 4.

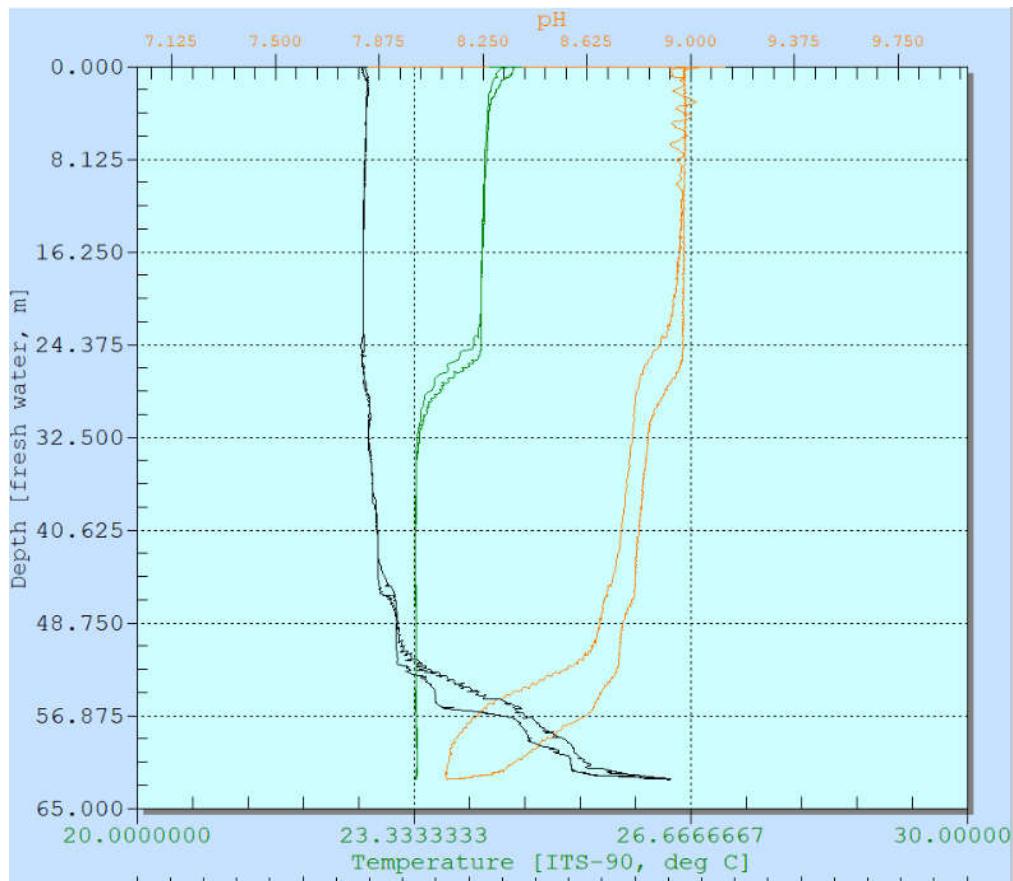


Figure 4: Vertical profiles of temperature (green lines), pH (red lines) and conductivity (blue lines) in Lake Kivu, November 2018. These results were obtained during the second mission in 2018, with the help of Bruno Leporcq.

#### 1.4 Results of the analysis of the samples collected in 2017 and 2018 and phytoplankton pigment data base.

The scientist trained on analysis of phytoplankton pigments, Eric Mudakikwa, did a stay at the Chemical Oceanography Unit, University of Liège, Belgium, April 1-5, 2019. During this stay, the results of the analyses carried out on the LKMP HPLC system were checked and validated. These results covered the period August 2017 to December 2018, when 27 sampling series in both monitoring sites were performed, with samples collected at 11 depths in the mixolimnion (0-60 m).

The data were then entered in the data base developed during the “Biological baseline of Lake Kivu”. This data base, containing all phytoplankton analyses performed since 2002, updated with the results from 2017 and 2018, now contains 5300 individual observations. An

observation consists in phytoplankton pigment concentrations for one date and one depth. From pigment concentrations, the biomass of the phytoplankton classes was calculated using CHEMTAX: these are the data which can be used for monitoring the phytoplankton variations in the lake.

In the following figures (Figures 5-8) we detailed some of the results of phytoplankton biomass and composition for the year 2018, and some relevant environmental variables. Figure 5 shows that chlorophyll a varied in an expected way (see Sarmento et al., 2006, 2012) in parallel with the depth of the mixed layer: the highest phytoplankton biomass was reached in the dry season (June-September), as the deep mixing of the mixolimnion allowed greater nutrient availability. By contrast, in the rainy season, when the lake was stratified due to the temperature gradient, nutrient limitation within the euphotic layer reduced phytoplankton growth.

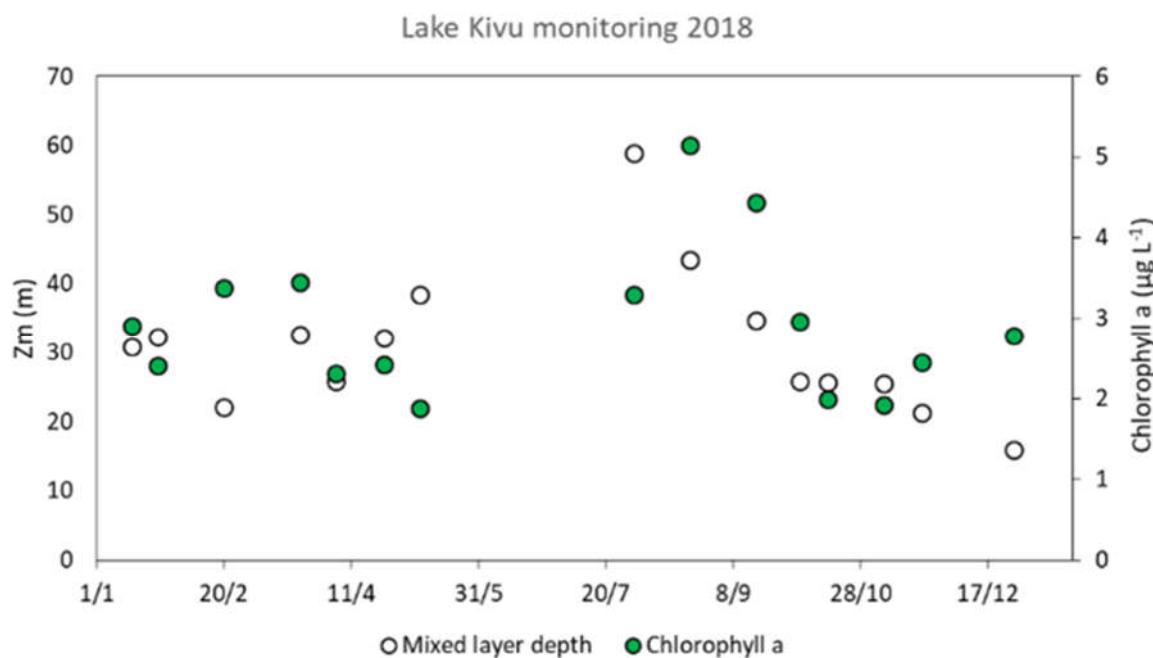


Figure 5 : Plot of chlorophyll a (i.e. total phytoplankton biomass) vs. depth of the mixed layer in Lake Kivu, 2018, off Gisenyi and Kibuye.

The next figure (Figure 6) shows diatom biomass, superimposed to the mixed layer depth. Diatom biomass doubled when Zm (and the Zm:Zeug ratio) increased. This is also an expected response to deeper mixing in the dry season, resulting in increased nutrient availability and lower exposure to light as phytoplankton is transported by vertical mixing below the euphotic zone.

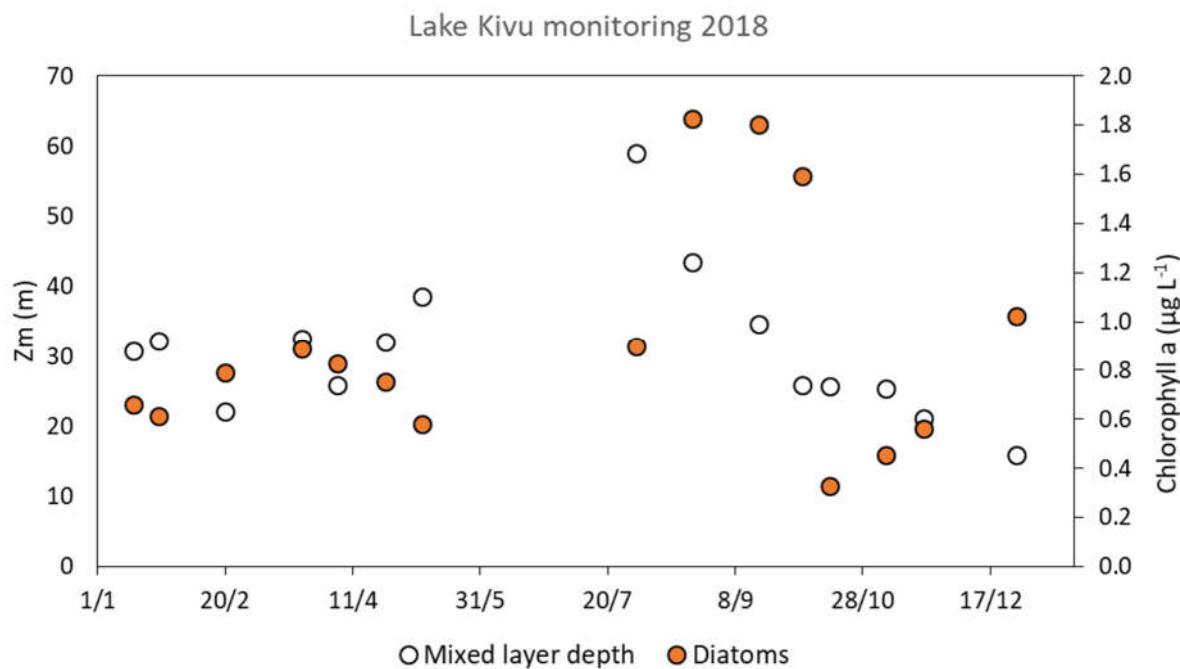


Figure 6 : Plot of diatom biomass (brown dots), expressed in chlorophyll a units ( $\mu\text{g L}^{-1}$ ), vs. the  $Z_m$  (depth of the mixed layer) in Lake Kivu, 2018, off Gisenyi and Kibuye.

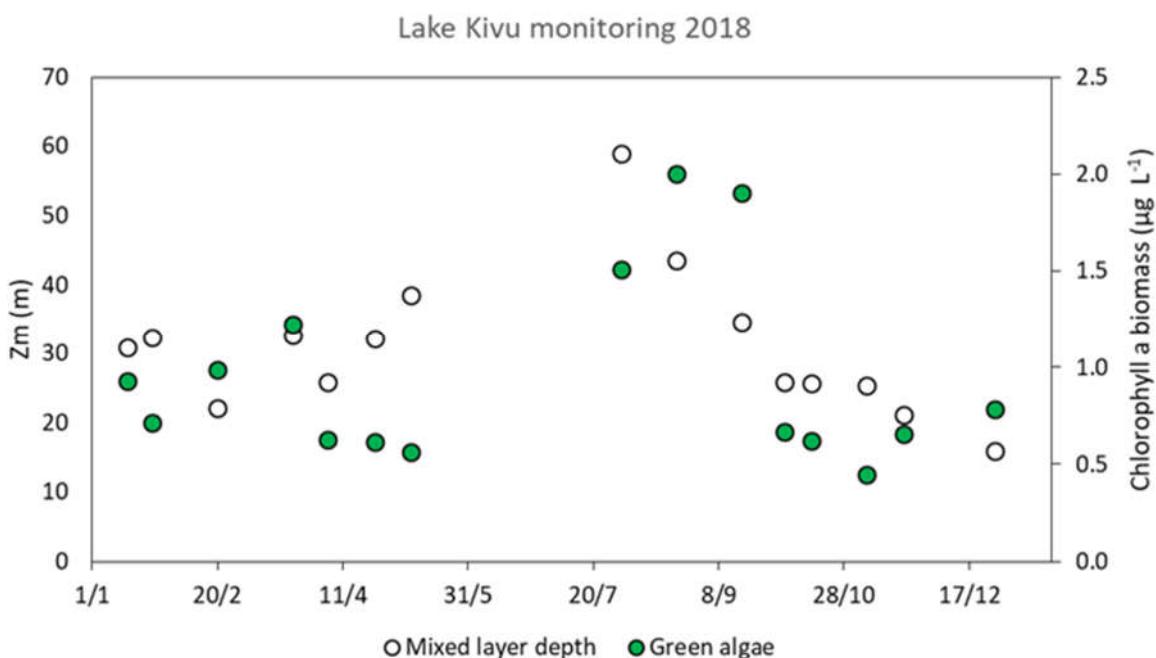


Figure 7 : Plot of green algae biomass (green dots), expressed in chlorophyll a units ( $\mu\text{g L}^{-1}$ ), vs. depth of the mixed layer in Lake Kivu, 2018, off Gisenyi and Kibuye.

To some extent, green algae exhibited a response to the seasonal changes of temperature and status of the water column (Figure 7) similar to that of the diatoms. Indeed, these green algae are non-motile forms, which need vertical mixing to remain in suspension.

They may also benefit from the greater nutrient concentration in the dry season. By contrast, cyanobacteria were better developed in the rainy season, i.e. under stratified conditions (Figure 8). The same was true for the less developed groups, Cryptophytes and dinoflagellates, which are motile and can concentrate in the metalimnion (Cryptophytes) or in the surface layer (dinoflagellates), and therefore benefit from stratified conditions.

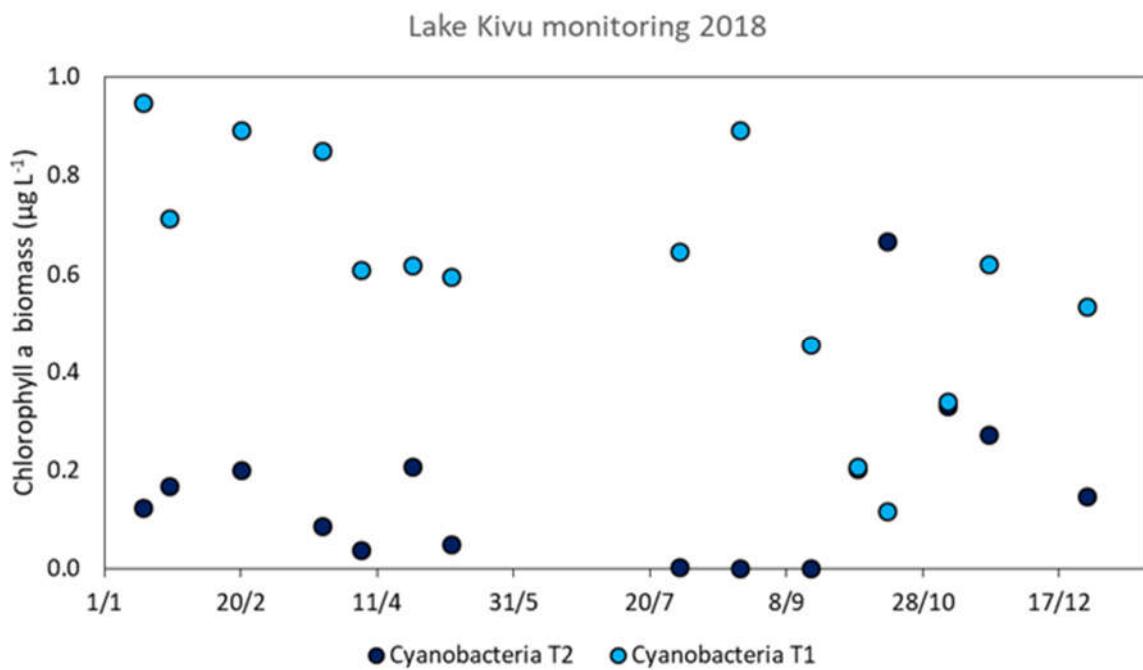


Figure 8 : Plot of cyanobacteria biomass, expressed in chlorophyll a units ( $\mu\text{g L}^{-1}$ ) in Lake Kivu, 2018, off Gisenyi and Kibuye.

In conclusion to the examination of the 2017-2018 phytoplankton monitoring data, the response of phytoplankton groups to seasonal variations in the lake mixolimnion is consistent with earlier observations (Sarmento et al., 2006, 2012; Rugema et al. 2019). This further validates the measurements carried out in the LKMP laboratory, from which estimates of phytoplankton biomass and composition were made.

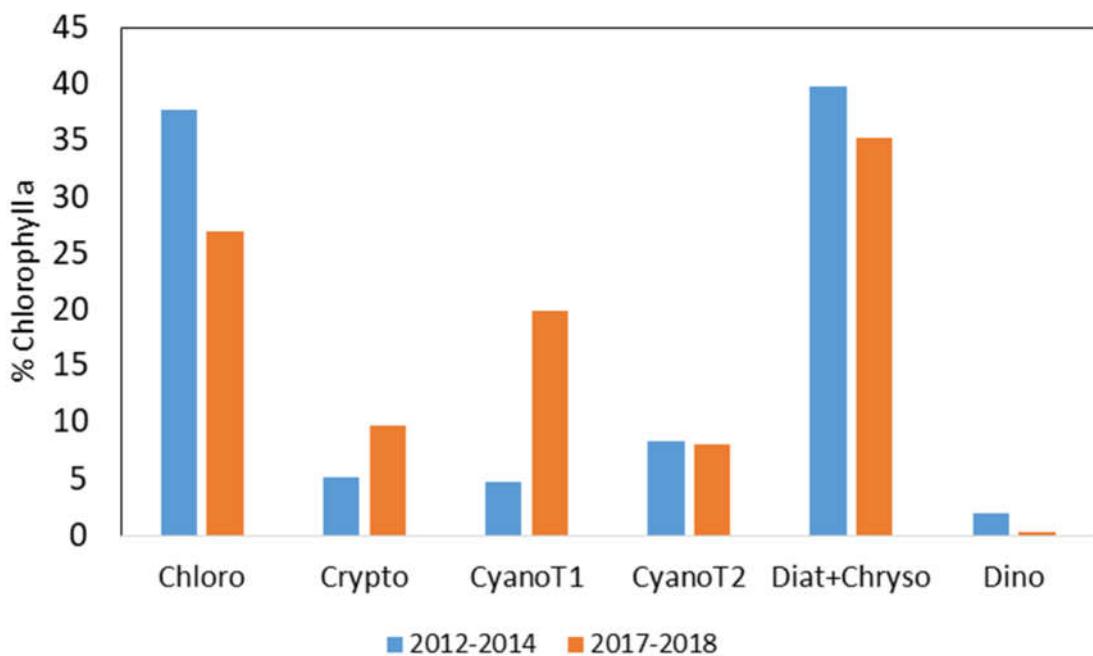
## 1.5 Data interpretation: comparison with 2012-2014

For the interpretation of the phytoplankton data, a first step consisted in building a data matrix, in parallel with the environmental data. The phytoplankton data were the chlorophyll a concentration and the biomass of phytoplankton classes averaged between 0 and 20 m, i.e. approximately the euphotic zone. The available limnological measurements from CTD casts, performed in parallel with the phytoplankton sampling, allowed to determine several mixolimnion variables at each sampling date: mixed layer depth (Zm), maximum temperature in the mixolimnion (Max\_T), minimum temperature in the mixolimnion (Min\_T), Delta\_T (Max-T – Min\_T), Secchi depth (Secchi), depth of the euphotic zone (Zeu) and the Zm

: Zeu ratio. In addition, we obtained from Prof. Wim Thiery detailed meteorological data from the Automatic Weather Station (AWS) deployed off Gisenyi on the REC platform. These data were used to determine mean daily values of air temperature (AirT), relative air humidity (RH%), solar radiation (Solrad), wind speed (WS) and rainfall (Rain).

In order to make a comparison with the 2017-2018 data, we included in the matrix the data acquired previously during the BTC Baseline, from October 2012 to September 2014 (n=53 sampling series at both monitoring sites). Then, the final data matrix contained 80 observations of limnological, meteorological and planktological variables.

The total phytoplankton biomass, as estimated by chlorophyll a concentration (Chla), was  $2.86 \pm 0.65 \mu\text{g L}^{-1}$  in 2017-2018, which is in the same range as in 2012-2014 ( $2.79 \pm 0.79 \mu\text{g L}^{-1}$ ). **The contribution of the different phytoplankton classes to chlorophyll a was broadly similar in both periods** (Figure 9): the present lake phytoplankton is still dominated by green algae and diatoms, and cyanobacteria are the third phytoplankton group. Differences between the two periods seem to appear in the proportions (see Figure 9), but they might be due to differences of coverage of the two main seasons, as the sampling periodicity varied somewhat in 2017-2018.



*Figure 9: Mean contribution to chlorophyll a of the main phytoplankton groups, as estimated from the marker pigment concentrations processed with CHEMTAX: Comparison between the monitoring of Lake Kivu in 2012-2014 and in 2017-2018. Chloro = green algae; Crypto = Cryptophytes; CyanoT1 = cyanobacteria T1; CyanoT2 = cyanobacteria T2; Diat+Chryso = diatoms + Chrysophytes; Dino = dinoflagellates.*

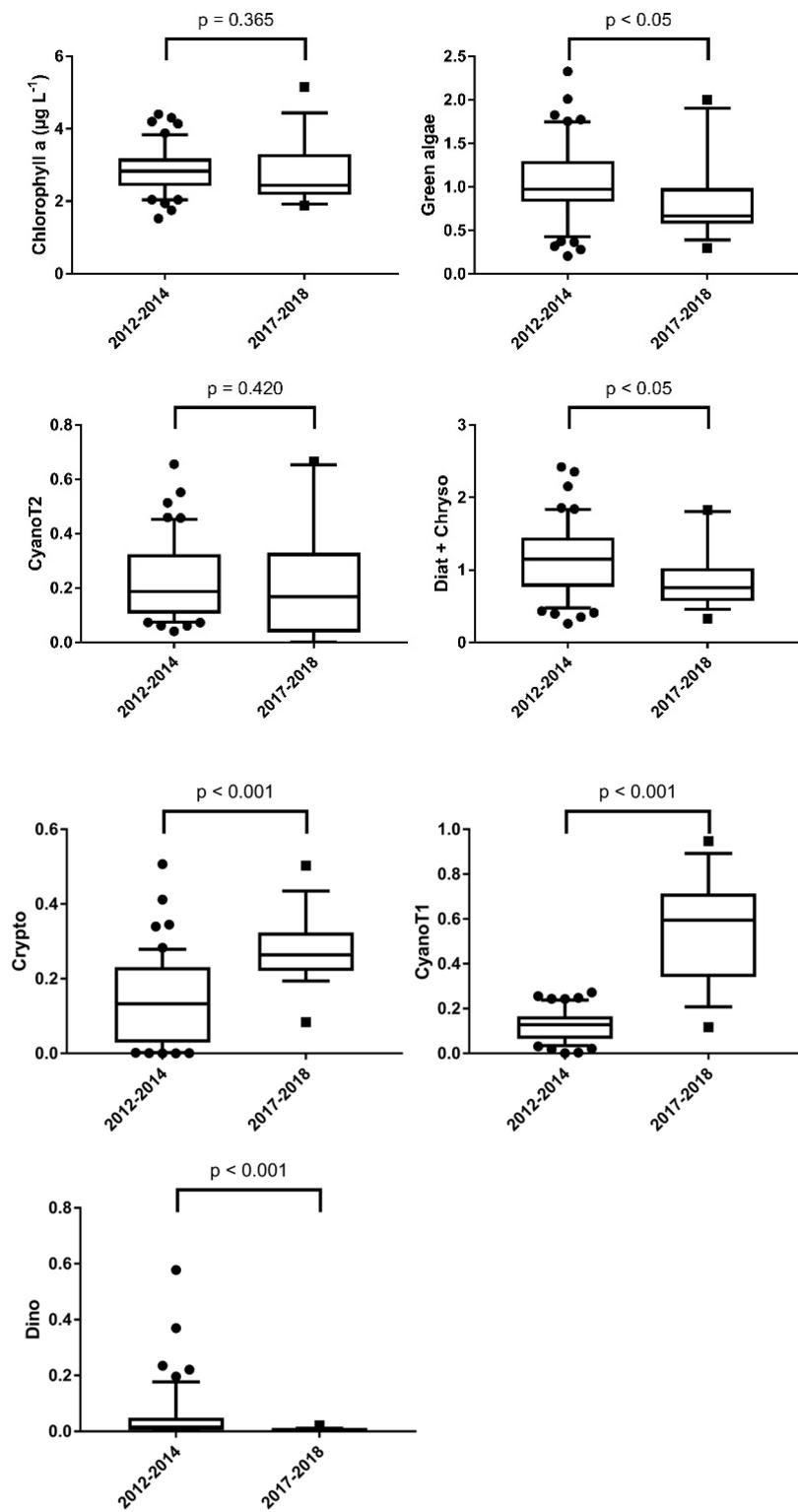


Figure 10 : Box-plots of chlorophyll a ( $\mu\text{g L}^{-1}$ ) and biomass phytoplankton groups ( $\mu\text{g chlorophyll a L}^{-1}$ ) in Lake Kivu, from the lake's monitoring off Kibuye and Gisenyi in 2012-2014 and in 2017-2018. Crypto = Cryptophytes; CyanoT1 = cyanobacteria T1; CyanoT2 = cyanobacteria T2; Diat+Chryso = diatoms + Chrysophytes; Dino = dinoflagellates. A Mann-Whitney test was applied to assess the significance of the difference between the two periods.

A more detailed comparison is presented in the box-plots of Figure 10. In short, **there was no (statistically significant) difference in chlorophyll a concentration between the 2012-2014 and 2017-2018**. By contrast, significant differences appear for some phytoplankton classes: Cryptophytes, cyanobacteria T1, and dinoflagellates. However, these differences should be considered with caution, as the sampling frequency was irregular in 2017-2018, so that the sample numbers were far from equivalent (n=27 in 2017-2018 vs. n=53 in 2012-2014). The increase of Cryptophytes and cyanobacteria in 2017-2018 might have resulted from a greater water column stability (i.e. stratification) when the samples were taken.

Subsequent data analyses, based on multivariate techniques, will allow to further examine those responses to limnological variables but also to meteorological variables.

## 1.6 Conclusion

The results presented above have demonstrated that the HPLC system installed at LKMP laboratory in Rubavu is fully operational, with the capacity to provide reliable data on phytoplankton in the framework of the monitoring program of Lake Kivu. The staff has been trained not only to the analysis of marker pigments in the phytoplankton extracts, but also to the processing of the pigment concentrations to obtain reliable estimates of chlorophyll a concentration and of the biomass of the phytoplankton classes. Further analyses of these data shall reveal their dependence to environmental variables, including weather data (available from the AWS off Gisenyi, installed during the EAGLES project). A publication of these results in an international journal should be possible soon.

However, a valid comparison with earlier data, for instance to assess changes that may occur from the operation of the gas extraction plants, will not be possible if regular monitoring of all relevant variables is not ensured. In particular, we recommend to sample phytoplankton and to measure limnological variables (including nutrients) twice a month at least at the monitoring site off Gisenyi. In the future, it will be necessary to tell apart the changes brought about by plant operation from those due to other environmental changes and this will not be possible if data collection is interrupted for long periods of time. For instance, a recent study based on the data collected between 2002 and 2015 in Lake Kivu during various projects allowed to provide evidence of a substantial change of phytoplankton composition and biomass attributable to changes in the lake's mixing dynamics, likely driven by a change in weather pattern (Rugema et al., 2019). Such a study would not have been possible without regular sampling of the lake for a dozen years, with sufficient detail on phytoplankton composition, using the same techniques.

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## Chapter 2. Benthic fauna in Lake Kivu

### 2.1 Introduction

Macroinvertebrates are a key element in the food chain of aquatic ecosystems. They integrate all the physical, chemical and biological characteristics of the environment. These organisms are especially sensible to water and substrate quality (Hynes, 1960; Woodiwiss, 1964 ; Verneaux et al., 1967 ; 1976). This study presents the results of the benthic macrofauna of Lake Kivu in the Gisenyi area in 2017. It is an evaluation of the benthic macroinvertebrates taxonomic composition. These results will be compared with old data and those from neighboring lakes.

### 2.2 Methodological reminders

This macrobenthos study was carried out in Gisenyi area (Figure 11).

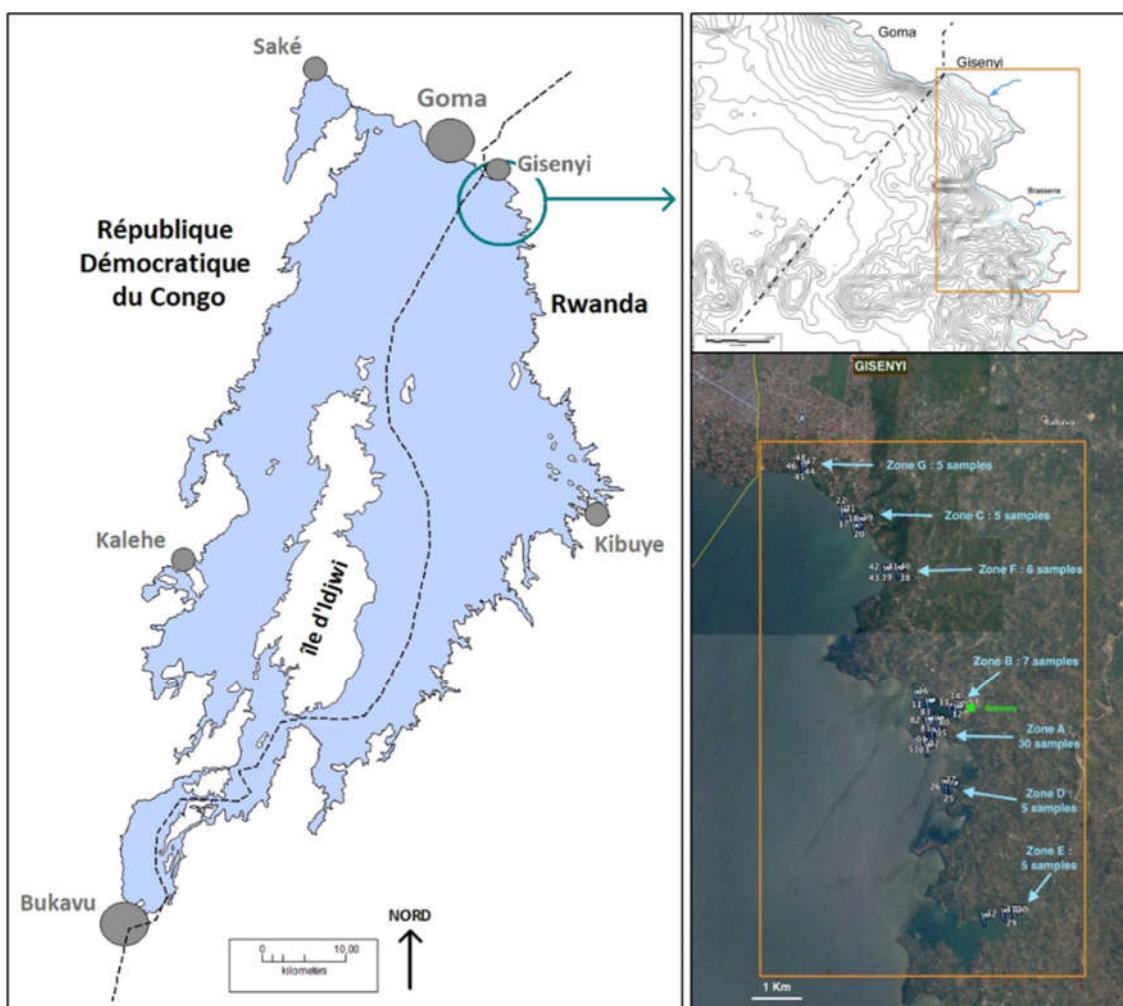


Figure 11 : Investigated area

### 2.2.1 Sampling details

Sampling was performed from June 2 to June 6, 2017, during the lake stratification period. Different samplings were done (Figure 12):

- Deep sampling (> 1.0 m) using an Eckman trap, loaded onto a motorized boat. The depth (using an echo sounder), the location (GPS) and the matrix (gravel, sand, mud,...) of each sample were recorded.
- Shore sampling (< 1.0m) with a Sürber net. The depth, the location (GPS) and the substrate (vegetation, rocks, cobbles, sand, mud,...) of each sample were recorded.



Figure 12 : Sampling using Ekman sediment trap and Sürber net

Fine sediments and sands were the most sampled substrates (Table 1). All depth ranges were sampled down to 60 m (Table 1). Sampling in the littoral zone, between 1 and 2 meters deep, gives the biogenic potential of the lake related to conductivity, calcium and nutrients (Verneaux et al., 2004). Sampling in the deep zone, on different transects between 2 and 55 meters deep, is used to characterize the taxonomic deficit on the lake. A total of 63 samples were collected.

Table 1 : Sampled substrates and depth range

	Depth range (m)								Number of samples
	0 - 1	1 - 3	3- 10	10 - 20	20 - 30	30 - 40	40 - 50	50 - 60	
Nature of sample	Fine sediments (fno)	2	2	5	5	5	6	3	31
	Sands (sab)	7	5	1	3		1		17
	Cobbles (gal)	5							5
	Blocks (blo)	1							1
	Rock (dal)	5							5
	Helophytes (hel)	4							4
	Number of samples	24	7	6	8	5	7	3	63

## 2.2.2 Sample processing

After collecting, samples were rapidly rinsed, filtered (at 250 µm) and sorted to avoid formalin fixation.

On the shore, macroinvertebrates were sorted in white plastic containers using magnifying glasses and tweezers. Finally, the macroinvertebrates were stored individually in ethanol solution (80%).



Figure 13 : Macroinvertebrate sorting

The determination of chironomid larvae required individual preparation. To dissolve the tissues and obtain soft and translucent larvae, they were placed for 8 to 12 hours in a 10% KOH (or NaOH) solution. This dissolution step can be accelerated after prior immersion of the larvae in a water bath heated to 80° C (15 minutes). To dissolve carbonates and avoid crystallization, the larvae of Chironomidae were successively transferred in distilled water (5 minutes) and in 10% HCl solution (5 minutes) and finally in distilled water (5 minutes) or 80% ethanol to remove air bubbles. After dissolution, each larva was placed in a gel (Aquamount) on a glass slide for microscopic observation and species identification (Figure 14).

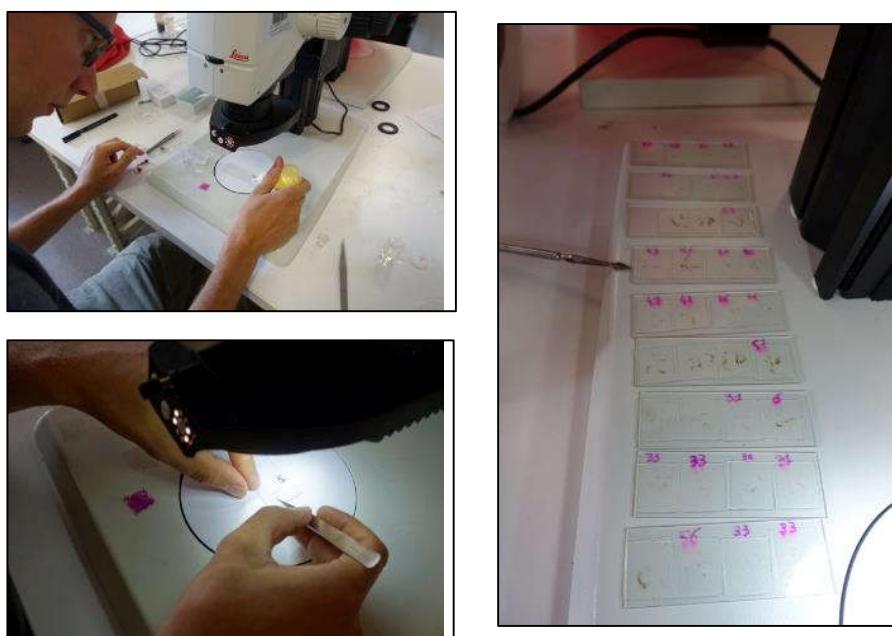


Figure 14 : Preparation of chironomid larvae.

Chironomids were identified under a microscope with a magnification from x250 up to x1000. For the identification of other taxa (Ephemeroptera, Trichoptera, Crustaceans, Molluscs, etc.), a stereo microscope with a magnification from x40 up to x60 was used.



Figure 15 : Identification of chironomid larvae.

## 2.3 Results

### 2.3.1 List of macroinvertebrate taxa in Gisenyi area

The samples contained 43 taxa (Table 2). The following taxa were identified at the genus level: *Trichoptera*, *Ephemeroptera*, *Odonata*, *Diptera Chironomidae*, *Gasteropoda*, *Decapoda*. Other taxa were identified at the family level or higher taxonomic levels (order or class).

Table 2: List of determined taxa

Phylum Subphylum	Class	Order	Family	Subfamily	Tribe	Genus
Hexapoda	Insecta	Trichoptera	<i>Ecnomidae</i>			<i>Ecnomus</i>
			<i>Hydropsychidae</i>			<i>Hydropsyche</i>
			<i>Hydroptilidae</i>			<i>Orthotrichia</i>
		Ephemeroptera	<i>Baetidae</i>			( <i>Cloëon</i> )
			<i>Caenidae</i>			<i>Caenis</i>
			<i>Polymitarcyidae</i>			<i>Povilla</i>
		Odonata	<i>Gomphidae</i>			und.
			<i>Macromiidae</i>			<i>Phyllomacromia</i>
			<i>Zygoptera</i>			und.
		<i>Coleoptera</i>	<i>Haliplidae</i>			( <i>Haliplus</i> )
		<i>Hemiptera</i>	<i>Vellidae</i>			und.
		Diptera	<i>Anthomyidae</i>			und.
			<i>Ceratopogonidae</i>			und.
			<i>Chironomidae</i>	<i>Chironominae</i>	<i>Chironomini</i>	<i>Chironomus</i>
						<i>Cladopelma</i>

							<i>Cryptochironomus</i>
							<i>Cryptotendipes</i>
							<i>Dicrotendipes</i>
							<i>Glyptotendipes</i>
							<i>Goeldichironomus</i>
							<i>Kiefferulus</i>
							<i>Microchironomus</i>
							<i>Parachironomus</i>
							<i>Polypedilum</i>
					<i>Tanytarsini</i>	<i>Cladotanytarsus</i>	
						<i>Sublettea</i>	
						<i>Tanytarsus</i>	
				<i>Orthocladiinae</i>		<i>Corynoneura</i>	
						<i>Cricotopus</i>	
						<i>Psectrocladius</i>	
						<i>Pseudorthocladius</i>	
			<i>Tanypodinae</i>	<i>Procladiini</i>	<i>Procladius</i>		
					<i>Procladius holotanypus</i>		
			<i>Tanypodini</i>	<i>Tanypus</i>	<i>Tanypus</i>		
					und.		
			und.	und.	und.		
		<i>Empididae</i>				und.	
<i>Mollusca</i>	<i>Gasteropoda</i>	<i>Littorinimorpha</i>	<i>Bithyniidae</i>			<i>Gabbiella</i>	
<i>Crustacea</i>	<i>Malacostraca</i>	<i>Decapoda</i>	<i>Potamonautes</i>			<i>(lirrangensis)</i>	
	<i>Ostracoda</i>					und.	
<i>Annelida</i>	<i>Clitellata</i>	<i>Hirudinae</i>	<i>Hirudinidae</i>			und.	
		<i>Oligochaeta</i>				und.	
<i>Arthropoda</i>	<i>Arachnida</i>	<i>Hydracarina</i>				und.	

The molluscs were identified by Dr. Albrecht<sup>1</sup>. A very large majority of them were subfossils (the shells were empty and more or less degraded).

<sup>1</sup> Department of Animal Ecology and Systematics. Systematics and Biodiversity Group. Justus Liebig University Giessen. Giessen, Germany.

*Chironomidae* (Diptera) and *Oligochaeta* (Annelida) were the most abundant taxa in the samples. Both groups represented approximately 96 % of the total numerical abundance (Tab. 3).

Table 3: Relative abundance of the faunistic groups

Groups (Orders)	Abundance (%)
<i>Trichoptera</i>	1,34%
<i>Ephemeroptera</i>	0,72%
<i>Odonata</i>	0,10%
<i>Coleoptera</i>	0,02%
<i>Hemiptera</i>	0,02%
<i>Chironomidae</i>	58,59%
<i>Other Diptera</i>	0,61%
<i>Gasteropoda</i>	0,48%
<i>Decapoda</i>	0,02%
<i>Ostracoda</i>	0,40%
<i>Hirudinae</i>	0,15%
<i>Oligochaeta</i>	37,43%
<i>Hydracarina</i>	0,11%

Photos

<i>Trichoptera Ecnomus (Ecnomidae)</i>

<i>Ephemeroptera Povilla (Polymitarcyidae)</i>

<i>Odonata Phyllomacromia (Macromiidae)</i>

<i>Diptera Microchironomus (Chironomidae)</i>

<i>Gasteropoda Gabbiella (Bithyniidae)</i>

<i>Crustacea Potamonautes (Potamonautidae)</i>

### 2.3.2 Distribution of benthic fauna with depth

The benthic fauna diversity decreases with depth (Figure 16). The maximum taxonomic diversity was observed in the littoral zone, between 0 and 3 meters deep (and more in 0-1 m.). In the stratum < 1 meter deep, 35 different taxa were identified, which represent 81% of the total observed diversity. Beyond 41 meters, no fauna was observed in the samples. This clear break is related to the lack of dissolved oxygen from this depth.

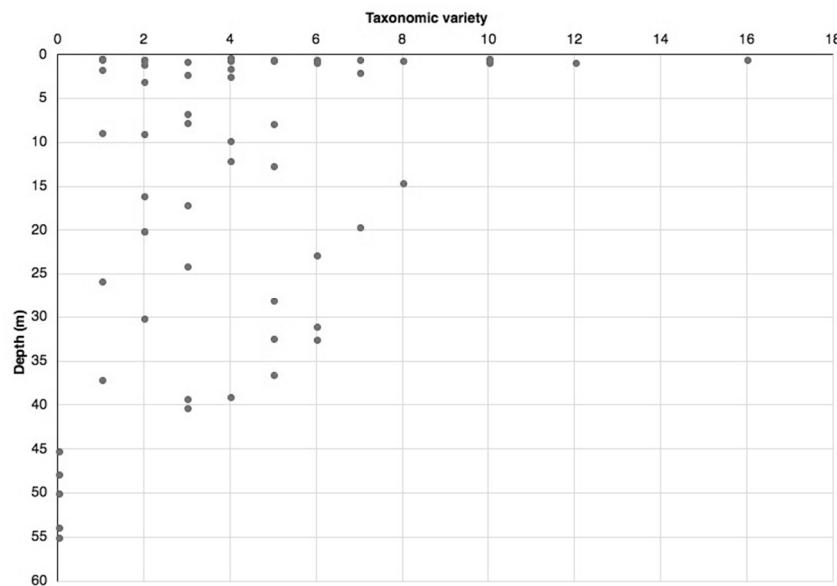


Figure 16 : Taxonomic diversity according to depth (63 samples).

The 30-40 meters stratum contains the most abundant fauna, with more than 5'000 individuals per  $m^2$  on average (Figure 17). But this is explained by only two samples collected at 32.5 and 31.0 meters deep, which showed the highest abundances with respectively 28'000 and 7'200 individuals per  $m^2$ . Beyond 40m depth, benthic fauna was virtually absent.

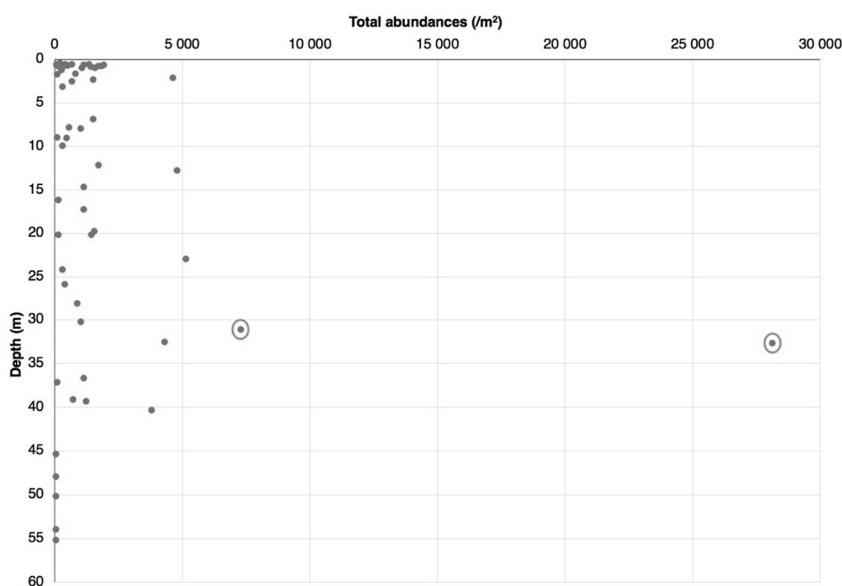


Figure 17 : Numerical abundance of macroinvertebrates ( $ind / m^2$ ) in samples according to depth (63 samples).

### 2.3.3 Occurrences and distributions of taxa

The *Oligochaeta* were found in about 60% of the samples. Then 5 genera of *Diptera Chironomidae* (*Cladotanytarsus*, *Microchironomus*, *Chironomus*, *Cricotopus*, *Procladius*) showed occurrences between 20 to 40% (Figure 18). *Trichoptera Ecnomus* was observed in 19% of the samples.

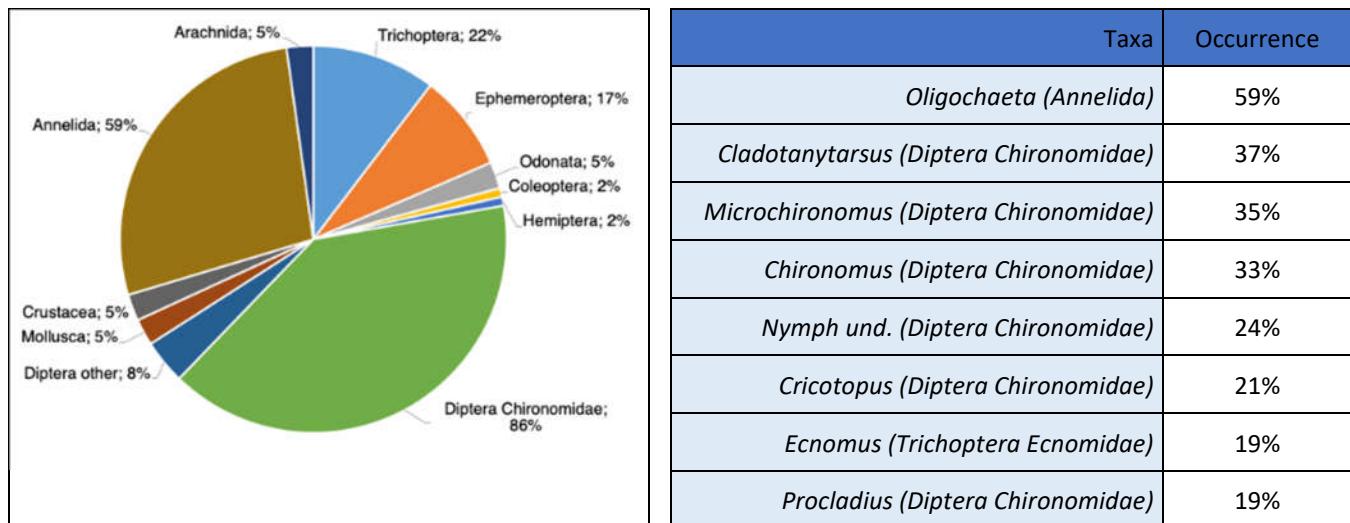


Figure 18 : Occurrences of the groups in the 63 samples and the most frequent taxa

The depth distribution of the 11 taxonomic groups shows that the *Annelids* and the *Chironomids* become very abundant in the deepest zone – for some samples (Figure 19)

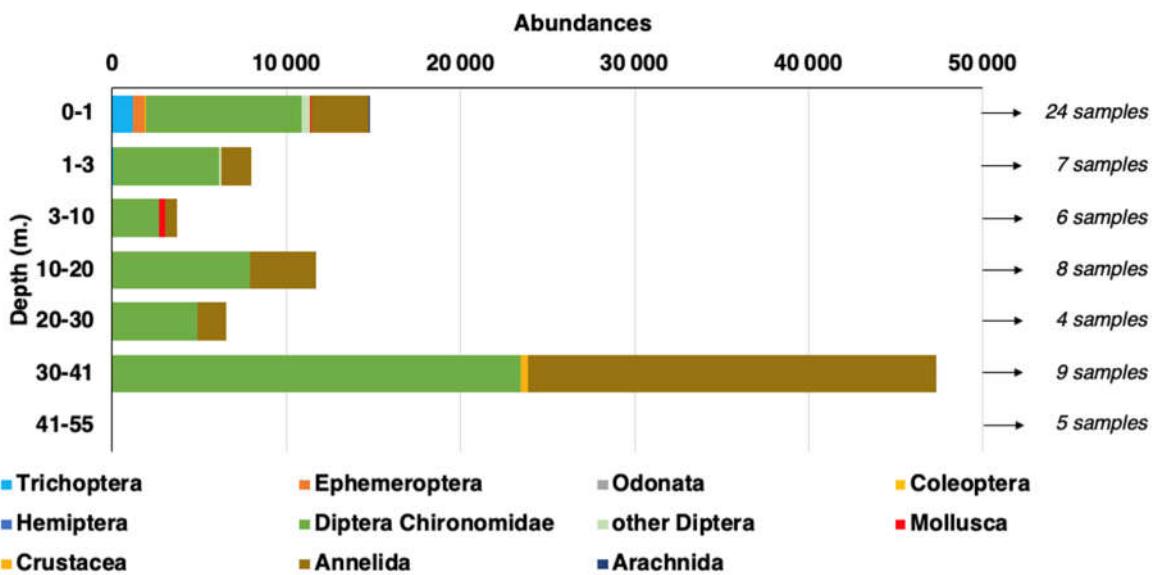


Figure 19 : Abundance of groups according to depth strata.

The groups are distributed very differently along the depth gradient (Figure 20):

- *Ephemeroptera*, *Trichoptera* and *Diptera* other than *Chironomidae* are dominant in the littoral zone between 0- and 3-meters depth;
- *Chironomid Diptera* show a wide distribution along the depth gradient, with a peak of abundance in the 30-40 meters stratum;
- *Annelids* and *Crustaceans* are much more abundant in depths between 30 and 40 meters.

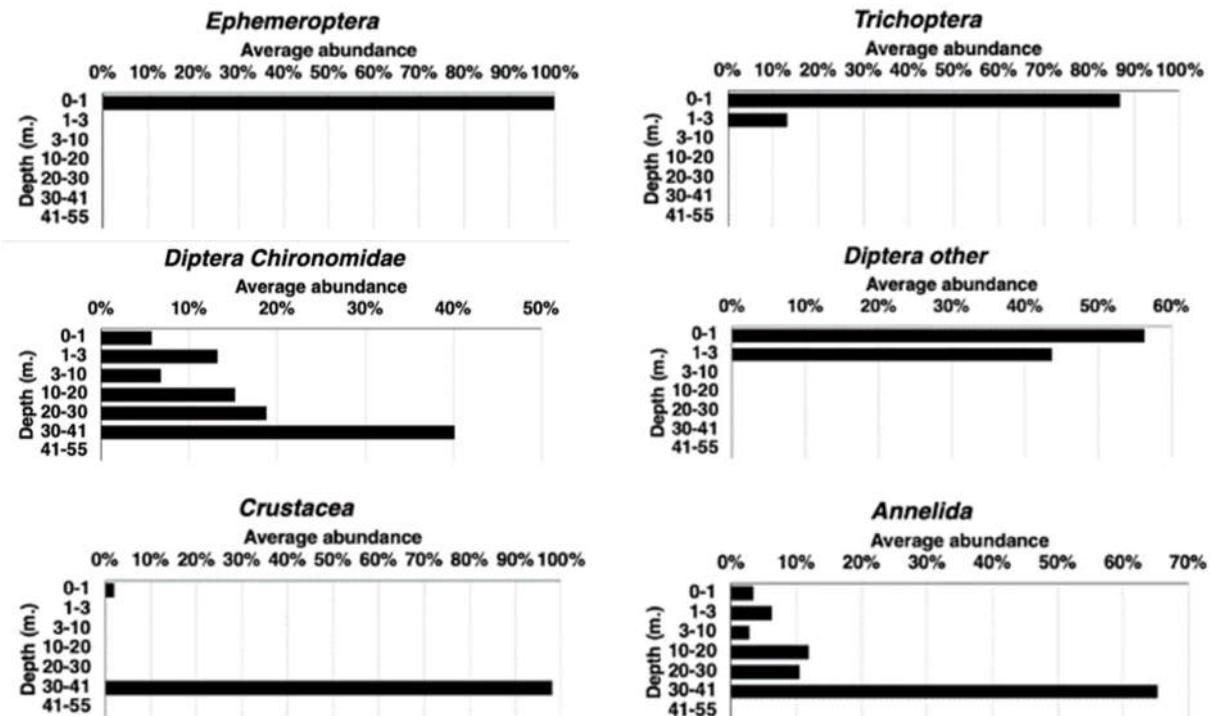


Figure 20 : Distribution according to the depth of 6 groups

Taxa distribution according to depth showed different patterns (**Erreur ! Source du renvoi introuvable.**):

- Some taxa such as *Microchironomus* occur at all depths, up to 40 m, which is the beginning of the anoxic zone;
- Other taxa, such as *Ecnomus*, *Cricotopus* or *Cladotanytarsus* are preferentially found in the littoral zone;
- The Gastropod *Gabbiella* was sampled mainly in the stratum 3-10 meters;
- *Procladius sp.* and *Procladius holotanypus* prefer strata 10-30 meters;
- *Oligochaeta* and *Chironomus* showed highest abundances around 30 to 40 meters.

The calculation of the distribution is an average of the abundances (per m<sup>2</sup>) obtained by strata.

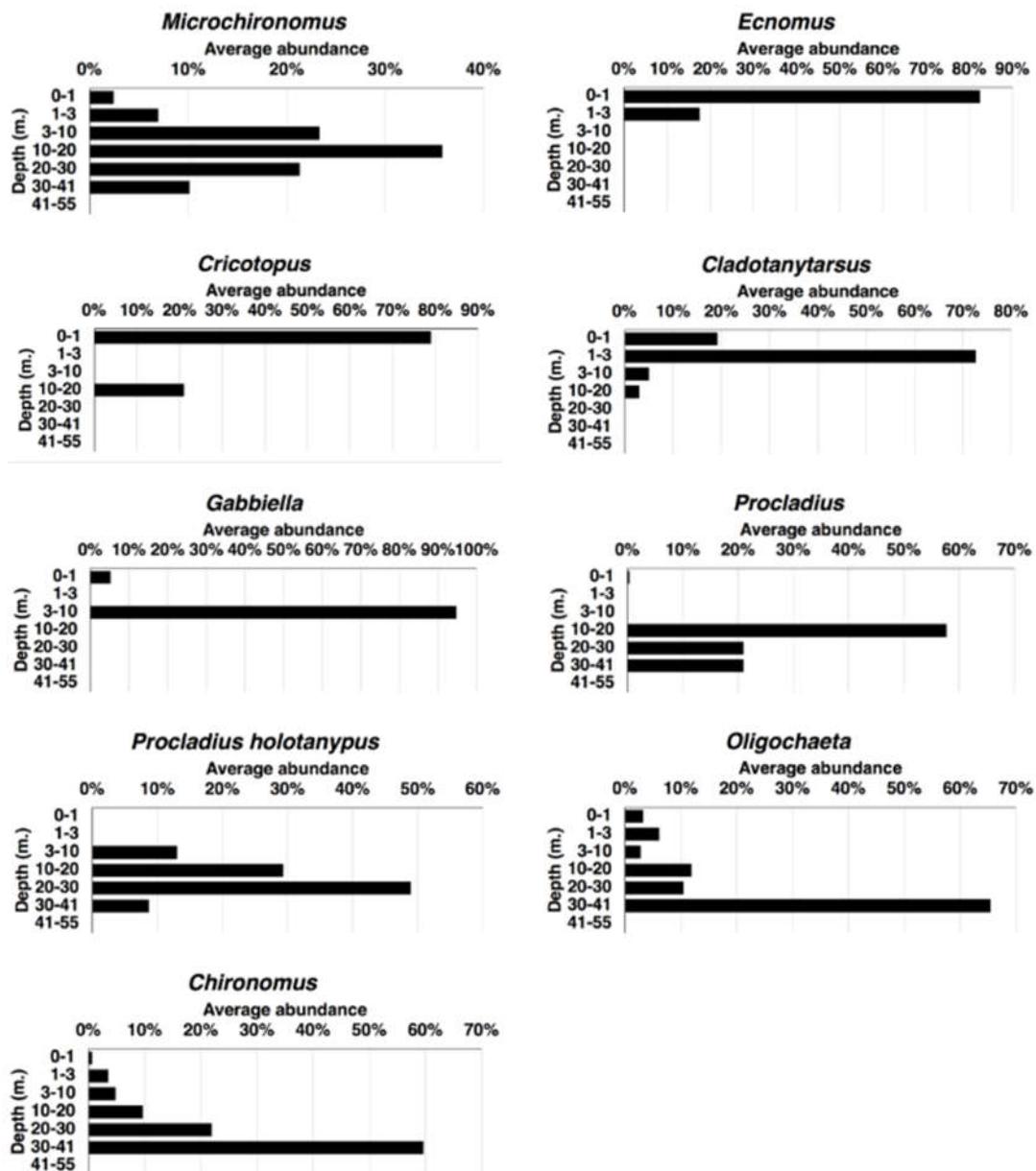


Figure 21 : Distribution according to depth of the most occurrent taxa

## 2.4 Autoecology of taxa

### 2.4.1 Diptera Chironomidae

Most Chironomidae genera observed in the samples collected in 2017 in Lake Kivu have a worldwide distribution. This does not exclude the presence of strictly African or even endemic species in Lake Kivu. For instance, some genera show an afrotropical distribution: *Cladopelma*, *Glyptotendipes*, *Microchironomus*, *Pseudorthocladius* (Armitage et al., 1994).

Most Chironomidae found in Lake Kivu are associated with standing waters. Some genera are strictly related to this environment: *Dicrotendipes*, *Goeldichironomus*, *Kiefferulus*, *Pseudorthocladius* (Armitage et al., 1994). Some species of *Procladius* are associated with deep areas of large lakes. Other genera have an affinity with running waters: *Pseudorthocladius*, *Sublettea*. The latter were found in Lake Kivu in our samples near tributaries at shallow depths.

Many genera found in 2017 are linked to fine sediment, mud and sand habitats (Armitage et al., 1994). However, some genera are related to other habitats like immerged vegetation. For example, *Parachironomus* was found in reeds and *Cricotopus* in macrophytes and algae.

Larvae of Chironomidae from Lake Kivu are mostly shredders, fine sediment and filter-feeders. But some genera are predators like *Tanypus*.

#### 2.4.2 Epheremoptera

The mayfly *Povilla* (most probably the species *P. adusta* in this region of Africa, according to de Moor et al., 2003) prefers standing waters and is abundant in many African lakes (Corbet et al., 1974). *Povilla* filter-feeds in the first larval stages but then moves on to feed on periphytic algae. It needs a suitable substrate to dig into (submerged trees, plant stems, gravel, ...) (Bidwell, 1979). Moreover, *Povilla adusta* also requires a high concentration of oxygen (Bidwell, 1979).

Conversely, the genus *Cloëon* (*Ephemeroptera Baetidae*) can withstand anoxic conditions (Bauernfeind & Soldan, 2012). It occupies all types of lentic environments (de Moor et al., 2003, Demoulin, 1956) in Holarctic, Eastern and Afrotropical regions (Bauernfeind & Soldan, 2012). The herbivorous larvae search for different microhabitats: aquatic vascular plants, mosses, submerged roots of riparian vegetation, organic debris and silty sediments (Bauernfeind & Soldan, 2012). Verbeke associated *Cloëon* with algae zones in Lake Kivu, which are also preferred by *Caenis* (*Ephemeroptera Caenidae*) (Verbeke, 1957).

#### 2.4.3 Trichoptera

The Trichoptera *Ecnomus* is limnophilic and thermophilic (Stroot et al., 1988) and lives in sands, muds (Dejoux, 1969) or in algae growing on rocks, particularly on Lake Kivu (Jacquemart, 1957). The larva is carnivorous (Stroot et al, 1988; Marlier, 1973).

The genus *Orthotrichia* mainly feeds on macrophytes and lives in very different types of aquatic environments, from small streams to large systems, such as lakes (Guenda, 1996). This *Hydroptilidae* lives in zones with vegetation located at the mouths of Lake Kivu tributaries (Guenda, 1996). This is fully confirmed by the results from the 2017 survey.

#### 2.4.4 Mollusca

The only gastropod found alive in the samples was *Gabbiella*, from the family Bithyniidae. The species likely is *Gabbiella humerosa kivuensis* (Albrecht, pers. comm. Dec. 20, 2018). *G. humerosa* has a distinct distribution in the Great Lakes region: Lake Victoria, the Victoria Nile, in Lake Kyoga, Lake Albert, Lake Edward, Lake Kivu, the upper Ruzizi and in tributaries and marshes around Lake Tanganyika (Mandahl-Barth, 1968 ; Brown, 1994 ; Van Damme & Lange, 2017). The subspecies *G. h. kivuensis* is endemic to Lake Kivu and the tributary Ruzizi River of the lake. As *Gabbiella humerosa* is widespread in the region and with no known major threats, this species is therefore considered as "Least Concern" by IUCN (Van Damme & Lange, 2017) from a conservation perspective.

#### 2.4.5 Crustacea

The crab *Potamonautes*, probably belonging to the species *P. lirrangensis*, is also called "The Malawi blue crab". It is a common and widespread species, living in Upper Congo, Central Africa and along the Rift Valley (Kivu Lakes, Tanganyika, Malawi) (Dobson, 2004; Meyer & Cumberlidge, 2011). It is listed as a "Least Concern" status by IUCN.

### 2.5 Comparison with previous data on Lake Kivu

#### 2.5.1 All macrobenthos (Verbeke 1957)

The only existing complete list of macroinvertebrates of Lake Kivu was established as part of the ecological research performed on the fauna of the large lakes of eastern Belgian Congo between 1952 and 1954 (Verbeke, 1957).

Hydrobiological research from 1952-1954 found 42 taxa, compared to 43 taxa in 2017. This apparent similarity hides many differences in taxa composition (Table 4).

Table 4: Comparative list of taxa found in Lake Kivu between 1952-1954 and 2017.

Taxa			Verbeke 1952-1954	Teleos 2017
<i>Ephemeroptera</i>	<i>Caenidae</i>	<i>Caenis</i>	+	+
	<i>Baetidae</i>	<i>Baetis</i>	+	
		( <i>Cloëon</i> )	+	+
<i>Trichoptera</i>	<i>Polymitarcyidae</i>	<i>Povilla</i>	+	+
	<i>Ecnomidae</i>	<i>Ecnomus</i>	+	+
	<i>Hydropsychidae</i>	<i>Cheumatopsyche</i>	+	
		<i>Hydropsyche</i>		+
<i>Odonata</i>	<i>Hydroptilidae</i>	<i>Orthotrichia</i>	+	+
	<i>Leptoceridae</i>		+	
<i>Coleoptera</i>	<i>Gomphidae</i>		+	+
	<i>Libellulidae</i>		+	
	<i>Macromiidae</i>	<i>Phyllomacromia</i>		+
	<i>Zygoptera</i>			+
	<i>Halophilidae</i>	( <i>Haliphus</i> )		+
	<i>Hydrophilidae</i>		+	

	<i>Dytiscidae</i>	<i>Cybister</i>		
<i>Hemiptera</i>	<i>Naucoridae</i>		+	
	<i>Veliidae</i>	<i>Microvelia</i>	+	+
	<i>Mesovelidae</i>		+	
	<i>Gerridae</i>		+	
	<i>Hydrocorisae</i>		+	
	<i>Corixidae</i>	<i>Micronecta</i>	+	
	<i>Naucoridae</i>	<i>Anisops</i>	+	
	<i>Pleidae</i>	<i>Plea</i>	+	
<i>Diptera</i>	<i>Chironomidae</i>	<i>Cladotanytarsus</i>		+
		<i>(Sublettea)</i>		+
		<i>Polypedilum</i>	+	+
		<i>Tanytarsus</i>	+	+
		<i>Pentaneura</i>	+	
		<i>Procladius</i>	+	+
		<i>P. holotanypus</i>		+
		<i>Tanypus</i>	+	+
		<i>Tanypodinae und.</i>		+
		<i>Chironomus</i>	+	+
		<i>Cladopelma</i>		+
		<i>Glyptotendipes</i>	+	+
		<i>Cryptochironomus</i>		+
		<i>Cryptotendipes</i>		+
		<i>Dicrotendipes</i>		+
		<i>Goeldichironomus</i>		+
		<i>Kiefferulus</i>		+
		<i>Microchironomus</i>		+
		<i>Parachironomus</i>		+
		<i>Corynoneura</i>		+
		<i>Cricotopus</i>		+
		<i>Psectrocladius</i>		+
		<i>Pseudorthocladius</i>		+
		undetermined		+
	<i>Ceratopogonidae</i>			+
	<i>Anthomyiidae</i>			+
	<i>Empididae</i>			+
	<i>Scatophagidae</i>			+
	<i>Ephydriidae</i>			+
	<i>Dolichopodidae</i>			+
	<i>Culicidae</i>	<i>Anopheles</i>		
<i>Crustacea</i>	<i>Decapoda</i>	<i>Potamonautes lirrangensis</i>		+
		<i>Caridina</i>		+
	<i>Ostracoda</i>			+
<i>Acari</i>				
<i>Mollusca</i>	<i>Planorbidae</i>	<i>Gyraulus</i>	+	
	<i>Bithyniidae</i>	<i>Gabbiella</i>		+
<i>Turbellaria</i>	<i>Macrostromum</i>			+
	<i>Plagiostromum</i>			+
<i>Hirudinae</i>	<i>Erpobdella</i>			+
<i>Oligochaeta</i>				+
<i>Nematoda</i>				+

The Verbeke (1957) study was a scientific exploration that aimed to provide the hydrobiological basis of large lakes. In Lake Kivu, more than 150 samples were collected all around the lake, using different methods:

- net trawls with distances of 5 to 10 meters performed close to the shore, in particular on the vegetation;

- mowing nets, for adult aquatic insects;
- ultraviolet ray traps, for nocturnal captures;
- sediment bins with a sampling area of 0,1 m<sup>2</sup>.

In 2017, 63 samples were taken from the Gisenyi area, by coupling Surber net samplings with an area of 0,05 m<sup>2</sup> and the Ekman grab with a sampling area of 0,0192 m<sup>2</sup>. The goal in 2017 was to study in a defined area the fauna of benthic invertebrates in a representative and reproducible way.

**Sampling efforts and goals were thus not similar between the two studies.** As very few samples were fully enumerated in 1952-1954, the comparison of abundances with present day samples is thus impossible.

When compiling the faunistic data of both studies (1952-54 and 2017) an overall number of 64 taxa were observed in Lake Kivu:

- 30% were identified in both studies;
- 37% were only found in 1952-1954 study;
- 35% were found only in 2017 study.

We can observe a relative similarity of genera composition for *Ephemeroptera*, *Trichoptera* and *Odonata*. The main differences are:

- the high taxonomic diversity of Coleoptera and Hemiptera found in 1952-1954, likely the result of intense search in the littoral zone;
- the high diversity in Diptera Chironomidae inventoried in 2017, explained by the exhaustive sorting of samples, the assembly and determination of all individuals.

### 2.5.2 Gastropods (Dartevelle & Schwetz, 1947)

In 1947, the study of the malacological fauna of Lake Kivu counted 9 genera of gastropods (Dartevelle & Schwetz, 1947). In 2017, *Gabbiella* was the only genus found in Gisenyi. The difference lies in the specific qualitative research of the 1947 study, supplemented by regional collections, without established protocol.

Table 5: Comparative list of Gastropods of Lake Kivu between 1952-1954 and 2017.

Taxa		Dartevelle & Schwetz 1947	Teleos 2017
Lymnaeidae	<i>Lymnaea</i>	+	
	<i>Biomphalaria</i>	+	
	<i>Afroplanorbis</i>	+	
	<i>Gyraulus</i>	+	
Planorbidae	<i>Bulinus</i>	+	
Viviparidae	<i>Viviparus</i>	+	
Bithyniidae	<i>Gabbiella</i>	+	+
Thiaridae	<i>Melanoïdes</i>	+	
Corbiculidae	<i>Corbicula</i>	+	

### 2.5.3 Diptera Chironomidae (Chrispeels, 1959)

During the explorations of Lakes Kivu, Edward and Albert from 1952-1954, Chrispeels (1959) mainly focused on the larvae of Chironomidae. This additional study on Chironomidae provided a more extensive list and indicated some local densities.

Table 6: Comparative list of Chironomidae of Lake Kivu between 1952-1954 and 2017.

Taxa			Chrispeels 1952-1954	Teleos 2017
Chironominae	<i>Chironomini</i>	<i>Chironomus</i>	+	+
		<i>Cladopelma</i>		+
		<i>Cryptochironomus</i>		+
		<i>Cryptotendipes</i>		+
		<i>Dicrotendipes</i>		+
		<i>Glyptotendipes</i>		+
		<i>Goeldichironomus</i>		+
		<i>Kiefferulus</i>		+
		<i>Microchironomus</i>		+
		<i>Parachironomus</i>		+
		<i>Polypedilum</i>		+
	<i>Tanytarsini</i>	<i>Cladotanytarsus</i>		+
		<i>(Sublettea)</i>		+
		<i>Tanytarsus</i>		+
Orthocladiinae		<i>Orthocladius</i>	+	
		<i>Corynoneura</i>		+
		<i>Cricotopus</i>	+	+
		<i>Psectrocladius</i>		+
		<i>Pseudorthocladius</i>		+
Tanypodinae	<i>Coelotanypodini</i>	<i>Clinotanypus</i>	+	
		<i>Pentaneurini</i>		+
		<i>Procladiini</i>		+
		<i>Tanypodini</i>		+

Chrispeels (1959) found 11 genera of Chironomidae during the 1952-1954 campaign, while 20 genera were enumerated in 2017 in Gisenyi. Among these taxa, 8 genera are common to both campaigns.

Three genera were only found during the 1952-1954 research: *Orthocladius* (*Rheorthocladius*) identified on a single location (at Cyangugu, in the Bukavu Basin, south of the lake); *Pentaneura* located on a single location in the Ishungu Basin (southeast of Idjwi Island); *Clinotanypus* was found at two locations in Kibuti Bay (west side of the lake) and east of Idjwi Island. These three genera display a very narrow distribution on the lake and were not reported close to Gisenyi.

Twelve genera were only found during Gisenyi 2017 surveys (

Table 7).

*Table 7: Distribution of Chironomidae found only in Lake Kivu in 2017 and not in the 1957 Verbeke campaign.*  
*(Ref : 1 : Copeland et al., 2012 ; 2 : Eggermont & Verschuren 2003b ; 3 : Eggermont et al., 2008 ; 4 : Eggermont & Verschuren 2003a ; 5 : Chrispeels, 1959 ; 6 : Verschuren, 1997 ; 7 : Eggermont et al., 2005 ; 8 : Harrison, 2004 ; 9 : Freeman et Cranston, 1980)*

Lakes and regions	Lake Tanganyika (RDC, Bur, Tanz., Zamb.)	Lake Kariba (Zim.)	Lake Chilwa (Mal.)	Lake Chad (Chad)	Lake Edouard (RDC, Uga.)	Lake Albert (RDC, Uga.)	Lake Naivasha (Ken)	Afro-tropical	Eastern Africa	Central Africa	Southern Africa	Sub-Saharan Africa	Western Africa
Bibliographical references	1 ; 2 ; 3 ; 4	1	1	1	5	5	6	1	7	1	1 ; 8	9	1
<i>Cladopelma</i>	X									X		X	
<i>Cryptochironomus</i>	X	X	X							X		X	
<i>Goeldichironomus</i>													
<i>Kiefferulus</i>	X	X	X	X				X					
<i>Microchironomus</i>	X								X				
<i>Parachironomus</i>	X		X					X					
<i>Cladotanytarsus</i>	X	X	X					X					
<i>Sublettea</i>													
<i>Tanytarsus</i>	X				X	X			X	X		X	
<i>Corynoneura</i>	X												
<i>Psectrocladius</i>	X					X					X		
<i>Pseudorthocladius</i>								X					



Almost all the genera inventoried in 2017 in Gisenyi and absent in 1952-1954 have been found in the Great Lakes region, in different stretch of water more or less close to Lake Kivu. The presence of these genera in the littoral or deep zone of Lake Kivu is therefore normal.

In the 1952-1954 study, *Chironomidae* larvae were counted from 11 samples (between 19 and 92 meters deep) distributed all over the lake and not only in the Gisenyi bay. Therefore, the average density was 185 individuals per m<sup>2</sup>, ranging from 40 to 430 ind/m<sup>2</sup>.

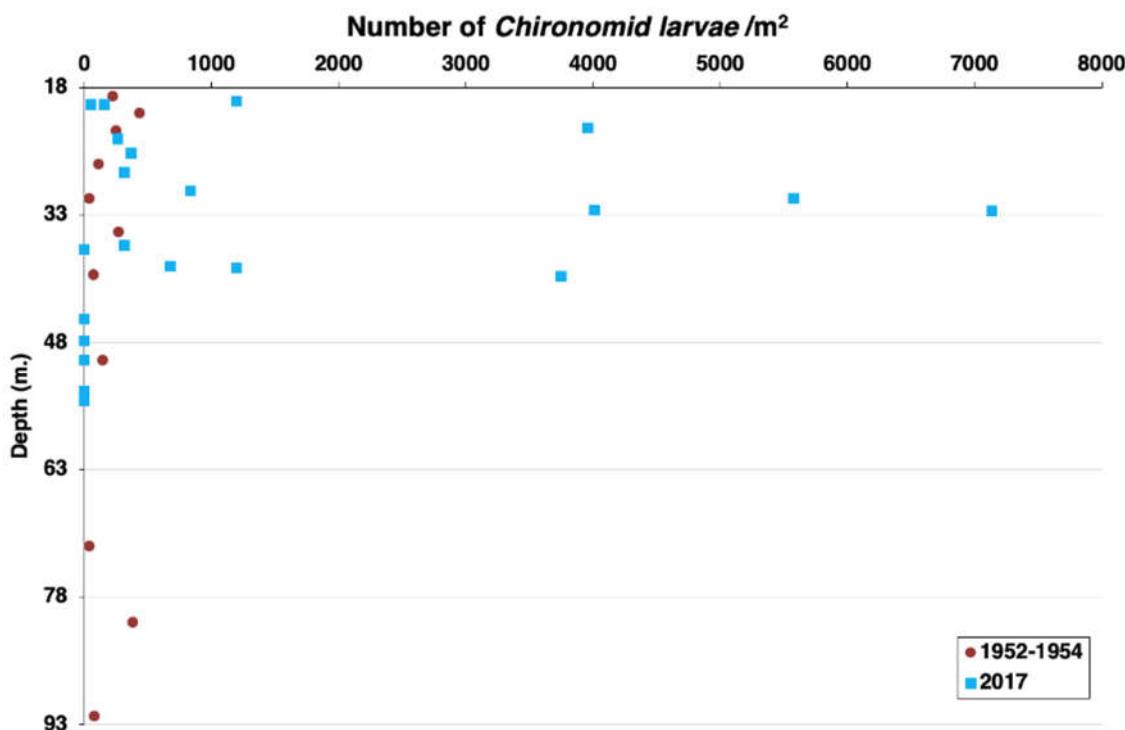


Figure 22: Distribution according to depth of the Chironomid larvae in 1952-1954 and in 2017. No density exists below 19 meters in 1952-1954.

The comparison between the data from 1952-1954 and 2017 according to the densities per depth indicates (Figure 22):

- mean density at 20-, 30- and 40-meters depths are much higher in 2017 (2.5 to 26 times higher in 2017);
- the 50-meter stratum no longer contains *Chironomidae* larvae in 2017.

However, this comparison is difficult because the sampling locations are not identical. In 1952-1954, no data were available on Gisenyi, and *Chironomidae* were observed below 50 metres in Bukavu Bay (in the south basin), where the lake operates differently. The comparison of macroinvertebrates densities should thus be interpreted cautiously.

## 2.6 Comparison with other nearby lakes

The macroinvertebrate sampling protocols implemented in the different studies in lakes of East Africa differ from each other (in terms of material used, number of points or sampled locations, depth zones sampled, taxonomic determination levels, ...). **It is therefore difficult to compare the varieties and densities of the macrobenthos.**

### 2.6.1 Comparison of generic variety

In the different studies, the Chironomids larvae have not always been determined at the generic level (or partially). **The comparison of the total number of genera of Lake Kivu benthos in Gisenyi with the Great Lakes varieties is therefore not appropriate.** It is thus necessary to compare the varieties without the Diptera Chironomids data (Figure 23).

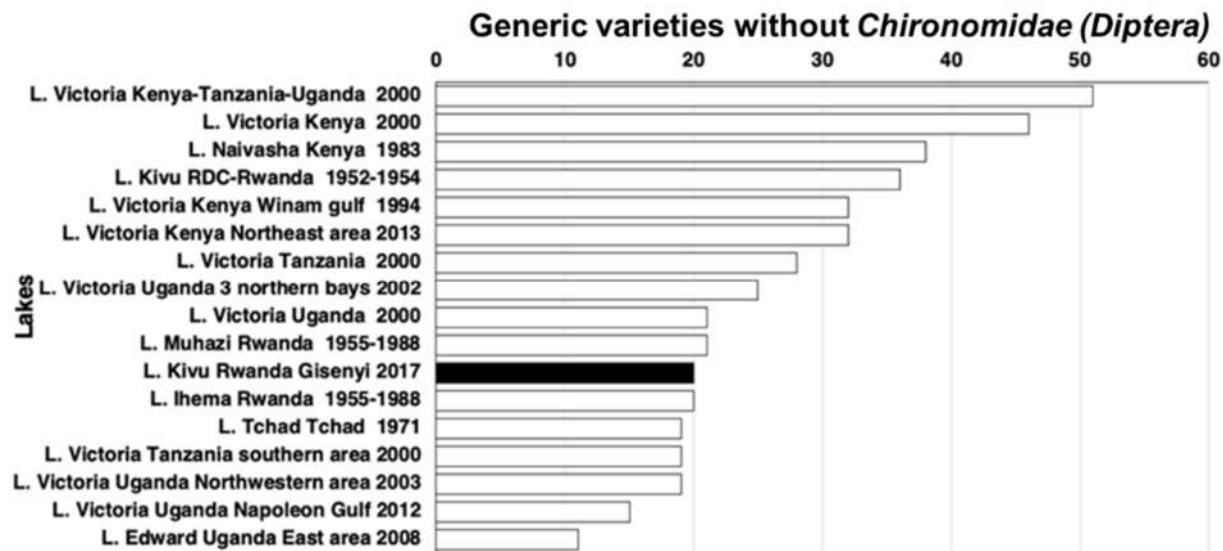


Figure 23: Generic varieties of macro-invertebrates without Chironomid larvae of lakes of East Africa. (according to Wakwabi et al., 2006; Verbeke, 1957; Clark et al., 1989; Sekiranda et al., 2004; Muli et al., 2001; Orwa et al., 2015; Lévéque et al., 1979; Plisnier, 1990; Mwambungu, 2010; Mwebaza-Ndawula et al., 2005; Magezi et al., 2012; NaFIRRI, 2008).

The position of Lake Kivu in Gisenyi in 2017 is quite low. With 20 taxa (without the Chironomids), the variety is far from the best levels recorded on Lake Victoria in 2000 (51 genera), Lake Naivasha in 1983 (38 genera) or Lake Kivu in 1954 (all the lake).

### 2.6.2 Comparison of Chironomid densities

There is only scarce data on benthic invertebrate densities in the literature, however some densities of *Chironomidae* larvae exist for Lake Edward (Verbeke, 1957; NaFIRRI, 2008) and Lake Tanganyika (Eggermont et al., 2008) (Figure 24).

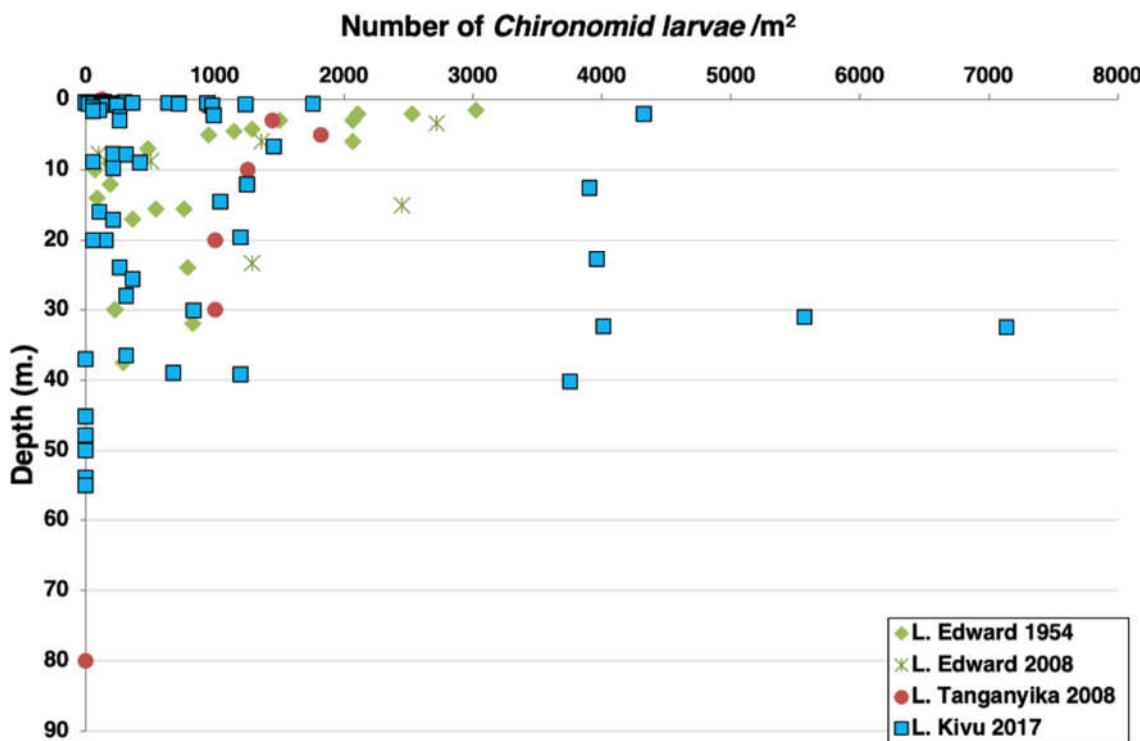


Figure 24: Distribution according to depth of the Chironomid larvae in Lakes Edouard, Tanganyika and Kivu (Verbeke, 1957; NaFIRRI, 2008; Eggermont et al., 2008), and mean numerical abundances according to depth strata.

The comparison of the data from the 3 lakes shows that the highest density ranges were found in Lake Kivu in 2017. However, when considered by depth zones, the perspective may be different.

While the average density (for all depth strata) is relatively similar in the 3 lakes, around 1'100 larvae per m<sup>2</sup>, important differences between strata appear:

- the average density in the littoral benthic zone (0 to 5 m) of Kivu (863 ind./m<sup>2</sup>) is significantly lower than the other lakes (1828 ind./m<sup>2</sup> for L. Edwards 1954; 2721 ind.m<sup>2</sup> for L. Edwards in 2008; 1401 ind./m<sup>2</sup> for L. Tanganyika, 2008) ;
- the average density in the stratum 5-20 meters is similar (755 ind./m<sup>2</sup>) to that of the other lakes (527 for L. E. 1954 ; 1103 for L. E. 2008 ; 1130 ind./m<sup>2</sup> for L. T. 2008) ;
- the average density in the stratum 20-40 meters is higher (2183 ind./m<sup>2</sup>) than in the other two lakes (472 for L. E. 1954 ; 1288 for L. E. 2008 ; 1004 ind./m<sup>2</sup> for L. T. 2008).

In 2017, the observed density of Chironomids larvae in the demersal zone of Lake Kivu (i.e. above the anoxic zone) was therefore remarkable and was not found in the neighboring lakes.

## 2.7 Conclusions and perspectives

### 2.7.1 *Outcome of the study*

This study provides a first overview since 1954 of the benthic invertebrate fauna of Lake Kivu in the Gisenyi area. Because macrobenthos plays an important role in the transformation of matter, and this at different levels of the food chain, the examination of this fauna is essential to monitor the ecological health of the lake. In the area of Gisenyi, the fauna is represented by 43 different taxa, dominated by *Diptera Chironomidae*.

The quality or ecological conservation of Kivu macroinvertebrate communities is difficult to evaluate. It is also difficult to assess its temporal evolution and to compare it with data from other Great Lakes, because the methods applied across different lakes are not standardized and because in 2017, only a restricted area of Lake Kivu was sampled. The taxonomic variety registered in Gisenyi in 2017 is quite high, but far behind the highest values observed in the region (Lake Victoria for example). This could be possibly explained by the genesis of the lake, its age, its physico-chemical characteristics and its hydrographic position.

Among the lake fauna sampled in 2017, some taxa are demanding with regard to the ecological quality of the lake floor (Chutter, 1972, Hilsenhoff, 1982, Sekiranda et al., 2004, Verneaux et al., 2004, Eggermont et al., 2008). They thus indicate a fairly good general quality or environmental conservation:

- The Ephemeroptera *Povilla* (*Polymitarcyidae*),
- The Trichoptera *Ecnomus* (*Ecnomidae*),

And to a lesser extent:

- The Ephemeroptera *Caenis* (*Caenidae*),
- The Odonata *Phyllomacromia* (*Macromiidae*) and *Gomphidae*,
- *Dicrotendipes* (*Chironomi Chironomidae*),
- *Cladotanytarsus* (*Tanytarsini Chironomidae*),
- *Procladius* (*Tanypodinae Chironimidae*).

Below 40 meters depth, the benthic macrofauna was totally absent in the sampled area. This absence can be explained by anoxic conditions of the water below 40 m, which is related to the presence of methane and CO<sub>2</sub>.

Also, in terms of density (of *Diptera Chironomidae* in particular), few data are available on nearby lakes. Anyway, the average density in the littoral zone seems to be much lower in Kivu than in other lakes. Conversely the density seems higher for depths between 20-40 meters compared to other lakes. This is observed here on only a few samples.

The high density of *Chironomidae* larvae - in some samples of the demersal zone just above the oxygen-free zone - seems therefore to be a specificity of Lake Kivu. This characteristic may be related to the importance of the microbial loop on this oxygenated stratum situated right above the chemocline, where methanotrophic bacteria might be abundant.

### 2.7.2 Continuation of the training in 2020

Following up the June 2017 training, which focused on sampling procedures and sorting Gisenyi's macrobenthos, a training session is planned for the beginning of 2020. At this occasion, all samples taken in June 2017 and identified in the laboratory will be given to the LKMP (alcoholic pill bottles and glass slides with cephalic capsule of *Chironomidae*).

At this training session:

- the preparation and assembly of Diptera larvae will be reminded and the assembly of the cephalic capsules will be practiced;
- other taxa, *Trichoptera*, *Ephemeroptera*, *Gasteropods*, other insects, Annelids, ... will be presented;
- based on this material, species determination sessions will be organized using stereo microscopes with a magnification and microscopes of the LKMP with help of appropriate literature.

### 2.7.3 Outlook

In order to extend the knowledge on benthic macrofauna, their spatial and bathymetric distributions, and to appreciate the temporal evolution, the present study of Gisenyi area should be carried on. It is also important to acquire knowledge about the fauna of other areas of Lake Kivu, both in Rwanda and DR Congo. Therefore, a standardized sampling protocol should be defined and applied in different areas all over the lake. The results of this global study could be compared with the available literature. Finally, such a designed monitoring would allow a better understanding of the ecological conservation of Lake Kivu.

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## **Chapter 3. Pelagic fish stock estimate from hydroacoustic surveys**

### **3.1 Introduction**

*Limnothrissa miodon* was introduced in 1959 in Lake Kivu and colonised the pelagic waters, in the absence of other planktivorous fish. The pelagic area of Lake Kivu is mainly occupied by this species, as already shown by several studies (Kaniningini et al., 1999; Masilya, 2011; Guillard et al., 2012).

The production of *L. miodon* pelagic fishery was first studied 30 years ago by Lamboeuf (1991) using hydroacoustics. Since then, new stock estimations were carried out from 2008 (Guillard et al., 2012) to 2018. Several surveys were performed in the framework of the biological baseline between 2012 and 2014 (Muzana & Guillard, 2014; Descy et al., 2015) and have shown that the estimated biomass for the whole lake fluctuated over years and seasons with the highest tonnage in April 2013 during the rainy season while the lowest one was obtained in July 2012 during the dry season. In this study, according to the project, the 2018 survey will be presented and compared with previous surveys (2012, 2013, 2014, 2015 and 2016) regarding acoustic metrics and the estimated biomass, the protocol being similar to previous surveys. More exhaustive comparisons, notably with the previous surveys will be investigated in a scientific paper (in preparation). It is worth to note that during this project, surveys were done using the equipment bought during the Baseline project (Descy et al., 2015). Alice Muzana (LKMP) was trained to calibrate the sounder, to plan the surveys, to use the sounder, to calibrate the equipment and to analyse data. She left the LKMP and scientist Eric Mudakikwa received during his short stay in UMR CARRTEL a training lesson about data analysis. Alice Muzana's departure did not allow to go as far as expected in data analysis. A preliminary check of all the available dataset was necessary to be sure that the data were reliable.

### **3.2 Material and methods**

*The lake was divided into four large basins (Figure 25) as done in Guillard et al. (2012). The inshore area (zone with a depth less than 50 m and close to the main banks or the banks of islands) in Lake Kivu is small, less than 10 % of the lake surface and was not considered in this analyse focusing only on the pelagic fish stock. The 2018 survey was carried out in July during the dry season. Data were recorded during daytime for both safety reasons and correspondence with historical surveys (FAO 1992; Guillard et al., 2012). Routes (*

Figure 25) were performed in a zigzag shape to optimize the time spend and the volume sampled. Some transects were shortened due to period of strong winds and for logistic reasons.

The echosounder was a Simrad EK60 operating at a frequency of 70 kHz. The echosounder was composed of a split-beam transducer, with a half-power beam angle of 11° at -3 dB beaming vertically. A GPS acquired boat positions. The echosounder ping rate was set to 5 emissions by second and the pulse-length was fixed at 0.256 ms. Sounder calibration was carried out according to the standard protocol of Foote et al. (1987). Survey was performed at a mean speed of 8 km.h<sup>-1</sup>, the transducer being vertically attached along the boat at a depth of 0.5 m. Acoustic data were analyzed using Sonar5-Pro software (Balk and Lindem, 2006) in order to calculate total acoustic fish biomass by depth layers and size distributions, using -66 dB for individual targets (Sed-threshold) and -60 dB for echo-integration data (Amp-threshold) according to Parker-Stetter et al. (2009).

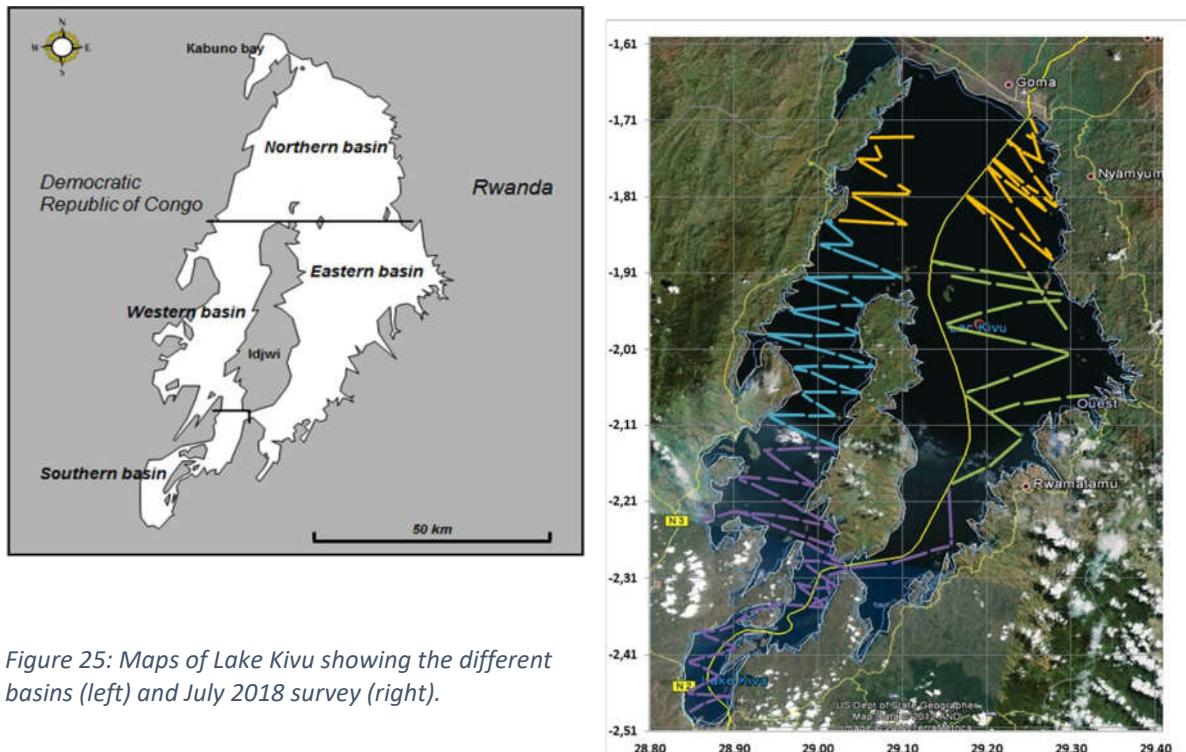


Figure 25: Maps of Lake Kivu showing the different basins (left) and July 2018 survey (right).

Data were analysed by vertical layers of 15 m, to be consistent with Lamboeuf (1991) and Guillard et al. (2012): surface - 15 m, 15 - 30 m, 30 - 45 m, 45 – 60 m. Single-echo detections, defined by 0.8 – 1.6 relative pulse width, a one-way beam compensation less than 3 dB, and a maximum phase deviation of 0.3, were used to build tracked fish (Balk and Lindem, 2006), with three echoes of the same target, separated by a maximum of one missing ping within a 0.3 gating range. From each tracked fish, the averaged Target Strength (TS, dB) (MacLennan et al., 2002) was calculated in the linear domain, then giving the layer-specific TS frequency distribution in 2 dB classes and thus, mean TS. The Elementary Sampling Distance Unit (ESDU) was fixed to 1'000 m, according to Muzana & Guillard (2014). Previous studies as Lamboeuf (1989) and Guillard et al. (2012) showed the absence of fish below 60 m, due to the

lack of oxygen. Therefore, data were analysed down to this limit. Layers were merged for total biomass estimations. Data were expressed in acoustic energy reflected per unit of area ( $S_a$ ,  $m^2.ha^{-1}$ ) (MacLennan et al., 2002). Tracked fish sizes were calculated by basin and gathered in two size classes, according to the biology of *L. miodon* (Kaniningini, 1995). To obtain the average acoustic biomass for each basin, arithmetic means of  $S_a$  values were calculated. In Lake Kivu, the arithmetic mean can be considered as an unbiased estimate of the average, given that the effort is distributed in a homogeneous way, without initial statistical assumption (Smith, 1990). From  $S_a$  data, the fish density by surface (pa: number of fish .  $ha^{-1}$ ) (Simmonds and MacLennan, 2005) was calculated for each basin and for each size class, using the basin-average TS by class using  $\sigma$ , the backscattering cross-section. Thus, the proportion of fish  $P_{ij}$  in each size class  $i$  was calculated for each basin  $j$ , as well as the arithmetic mean of TS, in the linear domain, for each size class and basin ( $TS_{ij}$ ) and the arithmetic mean of  $S_a$  values by basin ( $S_{aj}$ ).

To transform fish densities in weight, TS was converted into fish size according to the equation defined by Love (1971) and the mean individual fish fresh weight of each size class  $i$  and basin  $j$  ( $W_{ij}$ , g) was calculated using the size-weight relationship from T the commun equation of Carlander (1969):

$$W_{ij} = 0.055 L_{ij}^{2.27} \quad \text{where } L_{ij} \text{ is the mean fish length (cm) of size class } i \text{ for basin } j.$$

The mean fish biomass by basin ( $W_j$ , g. $ha^{-1}$ ) was then calculated :

$$W_j = \sum_{i=1}^n \frac{S_{aj} \cdot P_{ij} \cdot W_{ij}}{TS_{ij}}.$$

The biomass ( $kg. ha^{-1}$ ) was then analysed by basin (North Rwanda, North Congo, West, East and South) and for the lake Kivu in its globality over the period 2012-2018.

### 3.3 Results

#### 3.3.1 Analysis of 2018 survey

The  $S_a$  values were estimated by basin and for the whole lake, based on the mean value of all the  $S_a$  (Table 8).

Table 8 : Mean  $S_a$  value (acoustic energy) by basin in Lake Kivu during the 2018 survey

	Mean Sa ( $\text{m}^2.\text{ha}^{-1}$ )	IC 95%
<b>North-Rwanda</b>	1,395	0,331
<b>North-Congo</b>	1,089	0,274
<b>East</b>	0,892	0,177
<b>West</b>	1,586	0,434
<b>South</b>	1,542	0,389
<b>Kivu</b>	1,329	0,171

The fish size distributions by basin are similar (Figure 26). The mode is the same for the four basins and can be estimated to a mean size close to 9 cm.

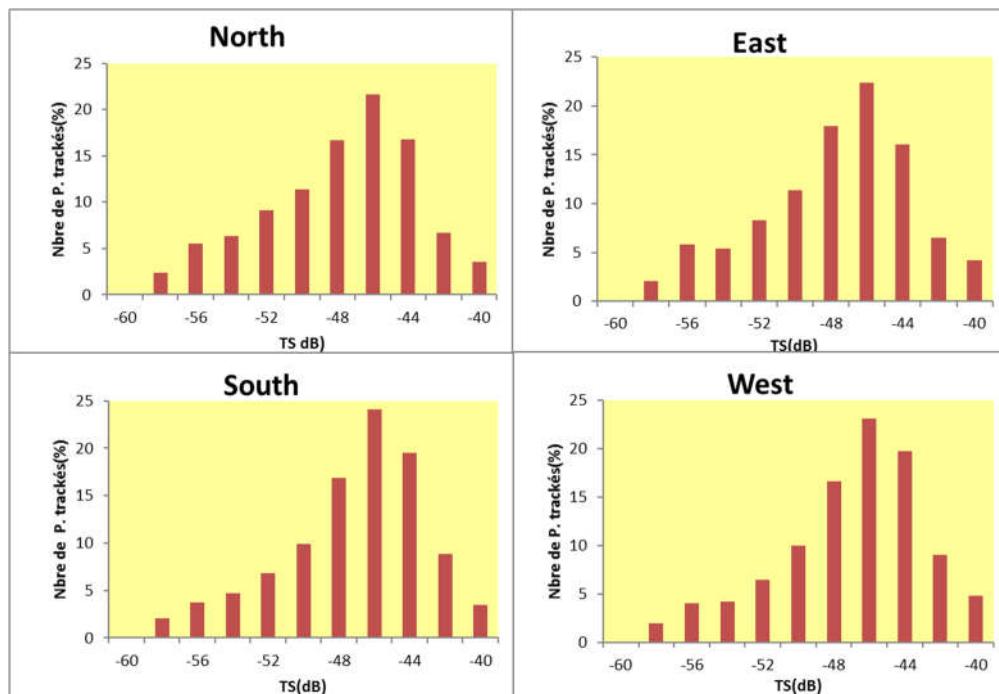


Figure 26: Fish size distributions in the four basins of Lake Kivu

The biomass estimates by basin and by size class were summarized in Table 9. In 2018, the fish stock estimate in Lake Kivu was 4067 tons, which is slightly under the previous values (Guillard et al., 2012).

Table 9: Biomass ( $\text{kg.ha}^{-1}$ ) estimated in 2018 by basin and by size class (TS limit set to -42 dB; small fish < 11 cm; big fish > 11 cm)

	North Rwanda	North RDC	East	West	South	Lake Kivu
Biomass small fish ( $\text{kg. ha}^{-1}$ )	14.91	14.67	11.23	21.68	19.93	17.45
Biomass big fish ( $\text{kg. ha}^{-1}$ )	1.55	0.60	0.64	0.99	1.07	0.90
Biomass ( $\text{kg. ha}^{-1}$ )	16.45	15.27	11.87	22.66	21.00	18.35
<b>Total biomass (T)</b>	<b>740</b>	<b>763</b>	<b>1069</b>	<b>725</b>	<b>204</b>	<b>4067</b>

### 3.3.2 Comparison with previous surveys

#### 3.3.2.1 Seasonal and spatial variations

The goal of the initial survey schedule initiated in the Eagles project (Muzana & Guillard, 2014) was to determine if there is an optimal season to monitor fish using hydro-acoustics. This approach is based on the seasonal variability of biological parameters, in our case mainly fish behaviour, reproduction or migration, which can affect fish detectability to the method. Surveys were initially scheduled based on the hydrological seasons and their impact on fish behaviour in 2013 and 2014. In 2013, surveys have been performed in January, April, July and October. In 2014, the same seasons were sampled, excepted in October. During these surveys, the whole lake was not sampled at each campaign, especially in 2013, when only the Rwanda was done. The same fish distributions in the water column were observed in 2013 and in 2014 and were similar to 2008 distributions, with no clear pattern (Figure 27).

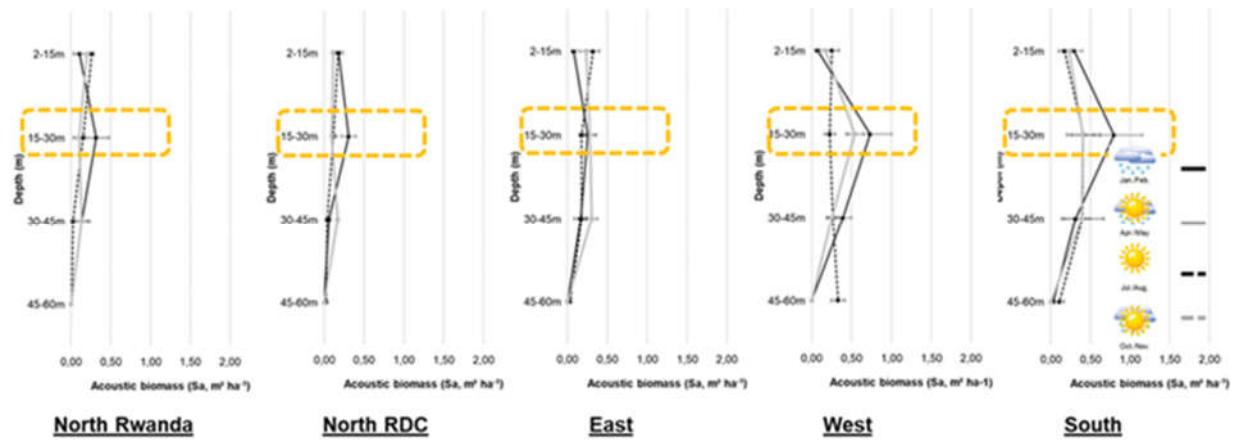


Figure 27 : fish distributions according to depth in the different basins of Lake Kivu in 2014

Size distributions were also analysed: an evolution of the size distribution, i.e. the proportion of smaller fish vs bigger ones (Figure 28), can reflect the reproduction pattern. The two peaks of fish reproduction have been described to occur from August to October and from March to May (Kaniningini et al., 1999).

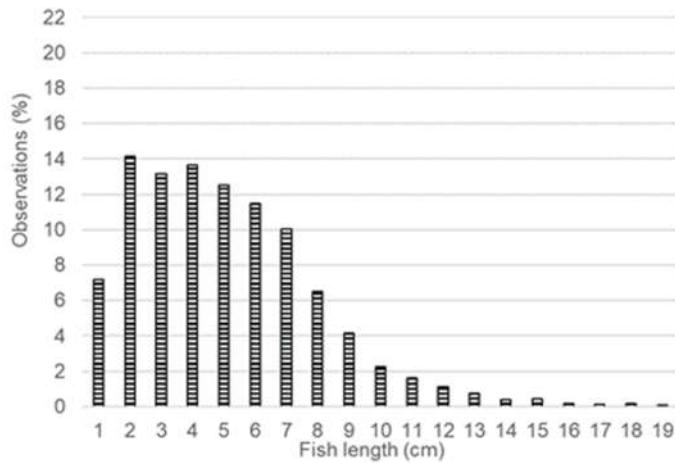


Figure 28 : Example of fish distributions in the North basin in July 2014 showing a bimodal distribution and a high proportion of small fish.

The size distributions have generally shown increasing proportion of small fish in July. These juvenile fish were born after the March-May spawning season and constitute then the new cohort. So, the surveys realised in July-August take into account this new cohort.

Looking at biomass by basin, from the two acoustic metrics, Sa and TS, no clear pattern in the seasonal evolution was shown (Figure 29)

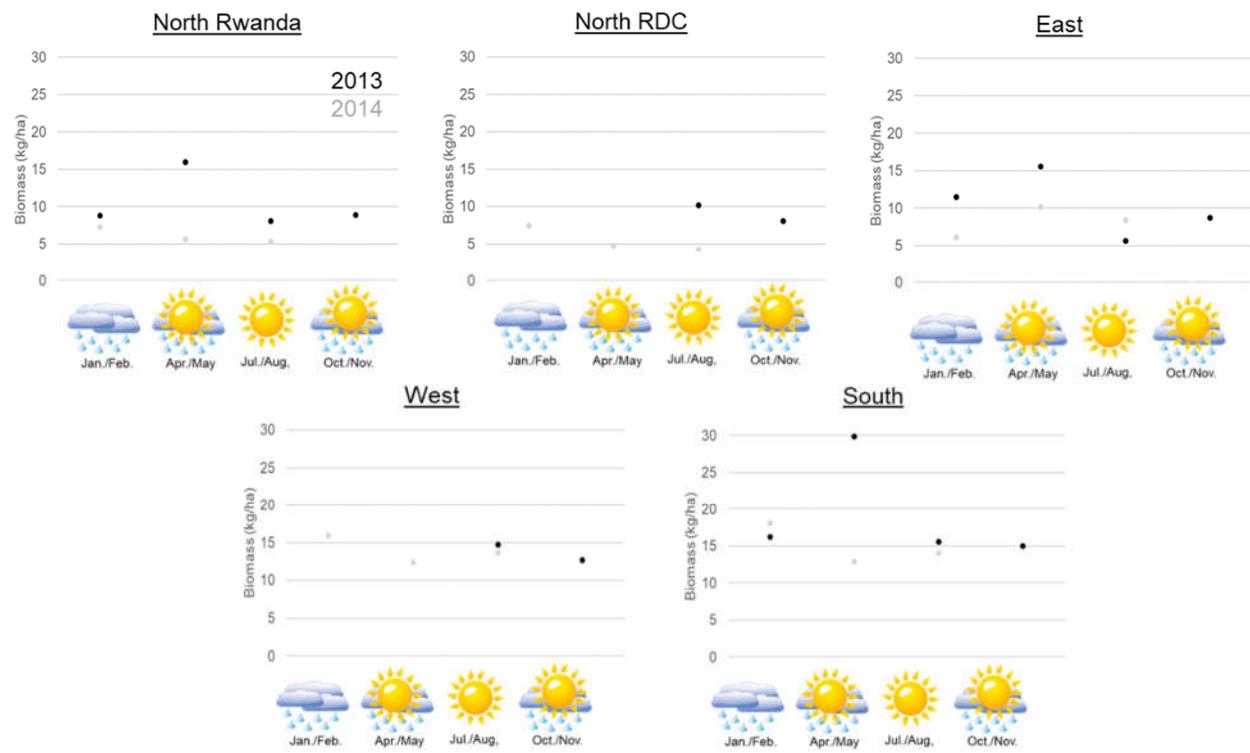


Figure 29 : Fish biomass by basin and by season in 2013 and 2014.

### 3.3.2.2 Interannual variations

To compare the biomass estimates among years, only the surveys performed in July and August were analysed. All the basins were surveyed, except in 2012 and 2015, when only the Rwanda part of the lake was sampled (Figure 30). The cover ratio are shown in table 10. As the degree of coverage was higher than 6, the results could be considered as representative (Aglen, 1983).

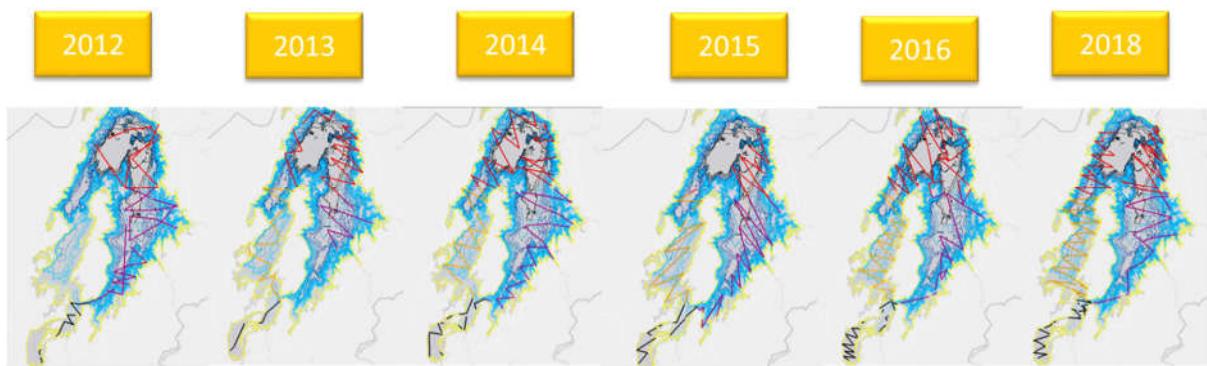


Figure 30 : Tracks performed during July-August hydroacoustic surveys from 2012 to 2018

Table 10 : Cover ratio of hydroacoustic regarding each year monitored

	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>2015</b>	<b>2016</b>	<b>2018</b>
Cover ratio	7.0	6.8	6.8	8.9	11.6	12.2

To summarize, the estimated biomass for the whole lake Kivu during the period 2012-2018 varied from year to year, with an increase of the stock (Figure 31).

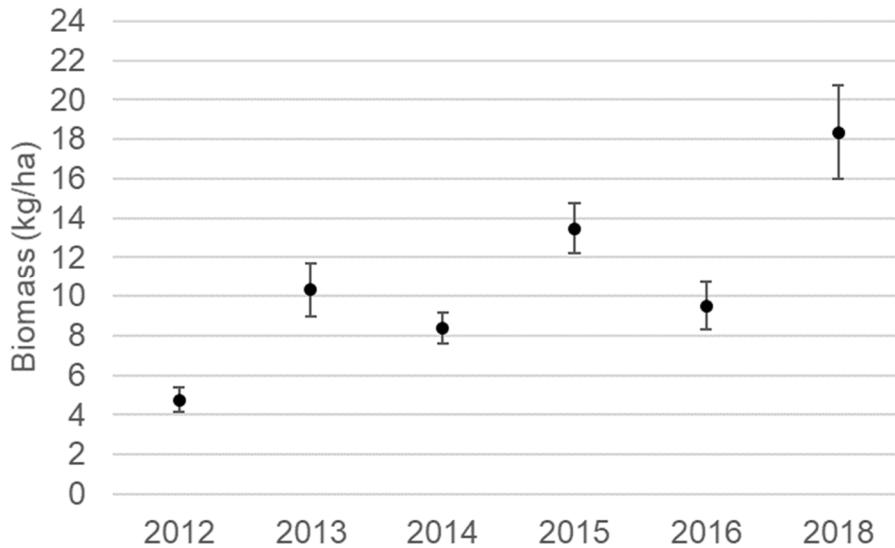


Figure 31 : Biomass ( $\text{kg.ha}^{-1}$ ) of Lake Kivu by year with confidence interval at 95%

### 3.4 Discussion

The 2018 survey was performed during daytime, as those conducted in the 80's (Lamboeuf, 1991), the ones done by Guillard et al. (2012) with the same protocol, and as those performed during the Baseline project (Muzana & Guillard, 2014). This allows to compare the fish stock estimation in 2018 with previous ones. Data recording was performed by the LKMP team, Alice Muzana being the scientist in charge of this part of the project. Her departure has conducted to train Eric Mudakikwa to analyse recent data (2017 and 2018) but also to check all the database to ensure data reliability. This task was not in the initial project and was very time consuming.

The occurrence of *L. tanganicanus* is low in the pelagic area (Masilya, 2011) and so it cannot significantly change the *L. miodon* stock estimated in 2018. As shown in previous studies (Guillard et al., 2012; Muzana & Guillard, 2014), the fish density varied according to seasons and to basins, with higher value in the South basin. The deepest layer, 45-60 m and the one from 0 to 15 m were the layers with the lowest densities. In 2018, the size distributions were mainly unimodal, representative of only one fish species without marked cohorts. The percentage of juvenile fish, larger than 1-2 cm and smaller than 6 cm, did not vary much

according to basins and were around 30-35% of the total population, which is similar to results from Guillard et al. (2012).

Fish distribution patterns between seasons show that July and August can be considered as the best season to perform the hydroacoustic surveys. Indeed, considering that the main spawning season occurs in March-May (Kaningini 1995), the new generation of fish will appear in the data during high dry season.

The 2018 survey led to an estimate of total fish stock of 4067 tons in July which is close to the results found in July 2008 by Guillard et al. (2012) and in a similar range as those published by Kanningini et al. (1999).

At a global scale, the biomass by basin increased over the period 2012-2018, although lower values were observed in 2014 and 2016, which is presumably due to lower recruitment these years. Clupaeide are known to have strong variations over years (Yáñez et al, 1992; Toresen et al, 2019). The lower global values are mainly consecutive to lower values in North basin. However, at the same time, the East basin, the larger one, showed an increase of the biomass. Fish migration from one basin to another one, as a function of hydrological conditions, could explain these variabilities. An analysis of environmental conditions at a whole scale during this period could give some clues to explain the observed variations.

Data from the hydroacoustic series confirm at a large temporal scale the stability of *L. miodon* population in Lake Kivu, but high variation from one year to another. This high variation can be mainly due to recruitment variability, well known on stock of fish from the same family, Clupeidae. These variations are due to environmental process at large scale (Fréon et al., 2008; Lluch-Belda et al., 1992) and that the fisheries can be considered as sustainable so far. Of course, data on the number of fishermen and the caught by effort unit are necessary to confirm this assumption. Unfortunately, although it was initially planned to record fisheries statistics during this project, such recent data are not available. As underlined by Gozlan (2008), this fish introduction can be considered as a successful one, as far as the fishery is concerned, and the evolution over years confirm the stability. In the future, changes in environmental data due to climate change and gas exploitation can affect fish reproduction and/or behaviour and/or food web and then fish stock. An annual survey using the same protocol and with reliable data (using calibration procedure) allows to look at variability on long time scale.

### 3.5 References

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## **Chapter 4. Compared Ecology and Biology of two possibly competing species**

### **4.1 Introduction**

This chapter reviews new studies on *Limnothrissa miodon*, which is the main pelagic species in Lake Kivu since its introduction, although the more recently introduced *L. tanganicanus* (presumably also consecutive to introduction from Lake Tanganyika) has become abundant. However, this latter species seems to be still scarce offshore, being mostly restricted to coastal areas (Masilya, 2011; Paris et al., 2013; Descy et al. 2015).

The main objective of this chapter is to provide updated data on biological and ecological features of *L. miodon* and *L. tanganicanus*, two possibly competing species in Lake Kivu. The following traits were studied at two contrasted sampling occasions (dry / rainy seasons) and compared to previous studies:

- Length-weight relationships
- Growth
- Fecundity
- Diet (gut contents) and trophic position (stable isotopes analysis).

### **4.2 Fish sampling**

Fish samples were collected from Lake Kivu in Gisenyi and Kibuye sites. Two sampling campaigns were organized by the experts in December 2017 and July 2018. These campaigns aimed to collect fish from fishermen of the 2 sites, to get samples of muscle, otoliths and gonads, collect juveniles of both species, and collect stomach contents to analyse fish preys.

After the first sampling campaign carried out by Scimabio's experts and LKMP in December 2017 (2 sites: Gisenyi and Kibuye), LKMP continued fish collection from fishermen in January, February, March, April, June, August, October and November 2018 in Gisenyi only, following the same protocol. In July 2018, a similar sampling campaign was performed in both sites with the experts. Therefore, a total of 10 sampling campaigns occurred between December 2017 and November 2018, among which 8 campaigns were only performed in the Gisenyi area and 2 campaigns in both sites.

Sampling sites were described by their distance to the shore (inshore/offshore), the water depth, and the nearshore bathymetry (bay or deep area). At each sampling occasion, about 2 kg of fish were collected in the **inshore** (< 100m from the littoral) and **offshore** areas (more than 1 km away from the littoral, >150m depth) from two fishermen crews (or 4

fishermen crews when the 2 sites where sampled). Given the bathymetry of the lake (Figure 32 and Figure 33), shallow littoral habitats are absent or restricted to a narrow band close to the shore. In July 2018, additional sampling was carried out close to the shore to collect juveniles of both species (**edge** sampling; Figure 32 and Figure 33).

All fish collected from fishermen were sorted by species. *L. miodon* and *L. tanganicanus* were individually measured and weighed and the following tissues were extracted on 30 individuals / species / zone: otoliths, muscle, stomach and gonads (for mature females).

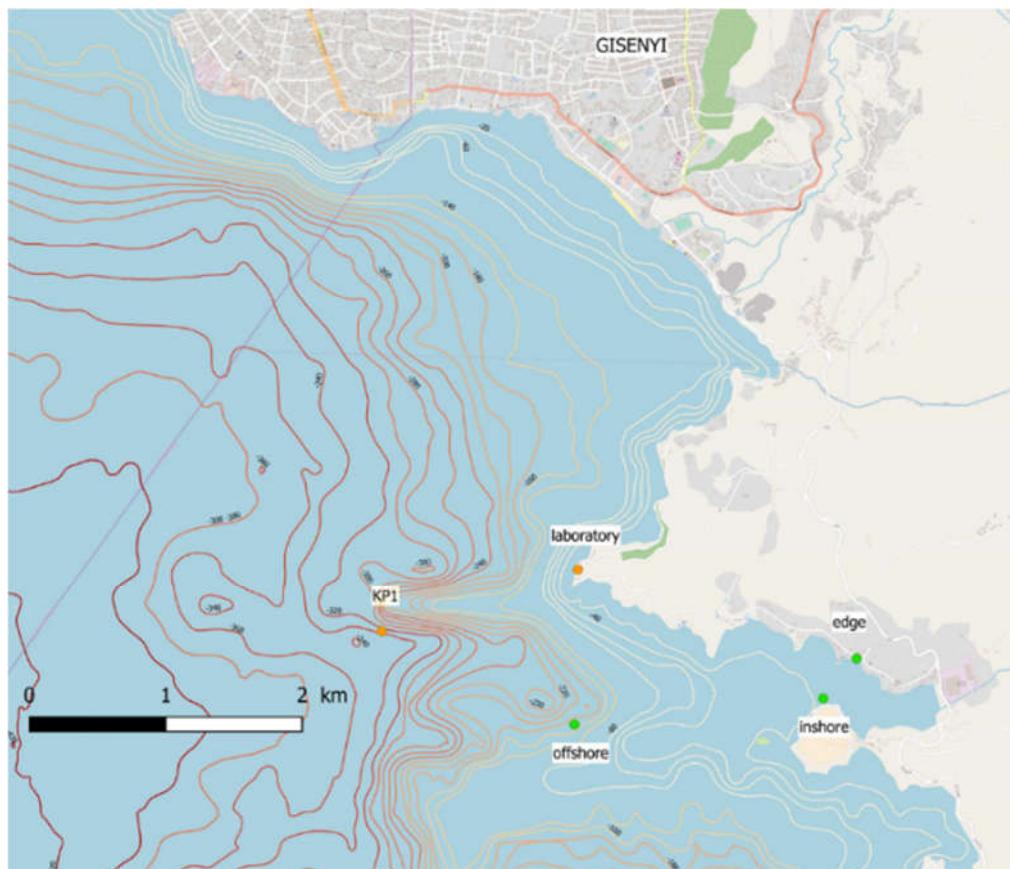


Figure 32 : Location of the sampling sites (green dots) in Gisenyi in July 2018

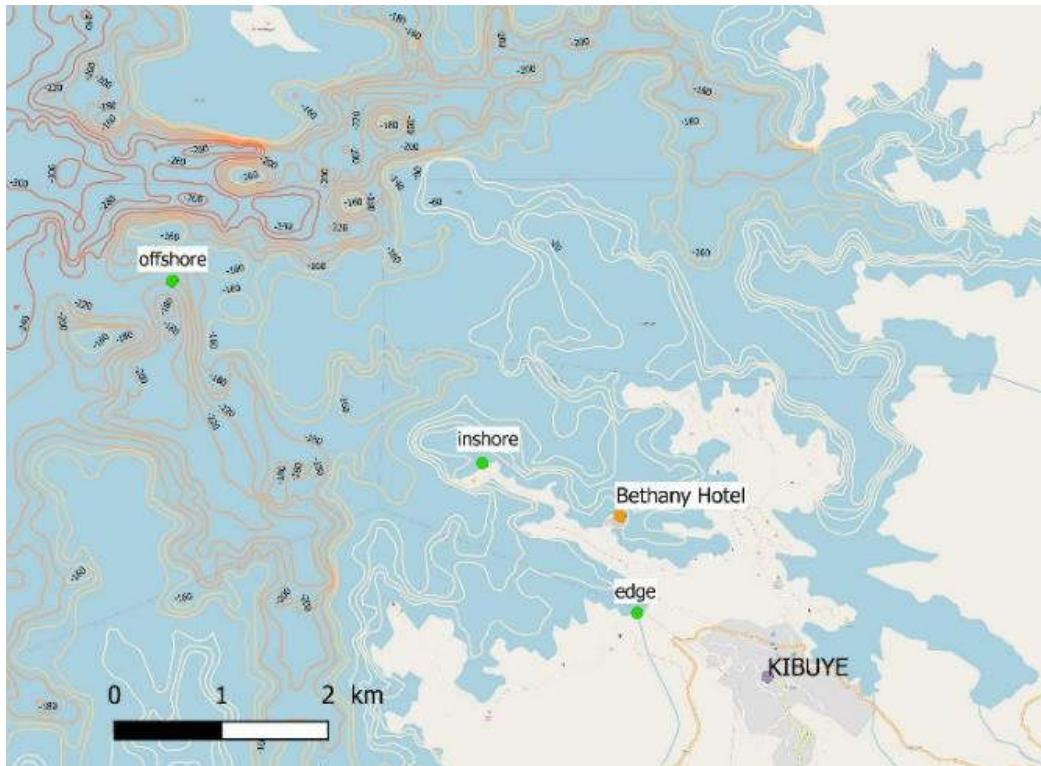


Figure 33: Location of the sampling sites (green dots) in Kibuye in July 2018

### 4.3 Length-weight relationships

#### 4.3.1 Principle

The relationship between length ( $L$ ) and weight ( $W$ ) provides useful information on fish condition and can be compared for a single species between lakes, within a lake (among sampling sites) and on a temporal scale between years or within a year (seasonal variations). This relationship is usually described as a two-parameters power function:

$$W = aL^b$$

where  $a$  and  $b$  are constants. If  $b = 3$ , then the species shows isometric growth, which means that it grows without changing its shape or density. If  $b > 3$ , fish condition tends to increase with size, larger fish becoming “plumper” (Blackwell et al., 2000; Ogle, 2013).

The relationship can be then linearised after log-transformation (natural logarithm) of the two variables (Beckman, 1948):

$$\log W = \log a + b \log L$$

Analyses were performed using least square regressions with  $W$  as the dependant variable.  $a$  and  $b$  were calculated for each species in a global model, then spatial (site effect) and seasonal effects were added and tested as covariates.

### 4.3.2 Sample collection

The data required to assess the length-weight relationship were obtained by the measurements of the total length ( $\pm 1$  mm) and weight ( $\pm 0.01$  g) of captured fish, at each of the 10 sampling occasions. Data were first cleaned, after removing the records where measures of weight or length were missing, and the records with a measured log weight less than -0.5 (Ogle, 2013). Outliers were removed after plotting log data (Froese, 2006). Finally, a total of 5'401 fish (3'798 *L. miodon* and 1'603 *L. tanganicanus*) were used for the length-weight analysis. A description of the analysed fish samples is provided in Fig. 34 and annex 5.

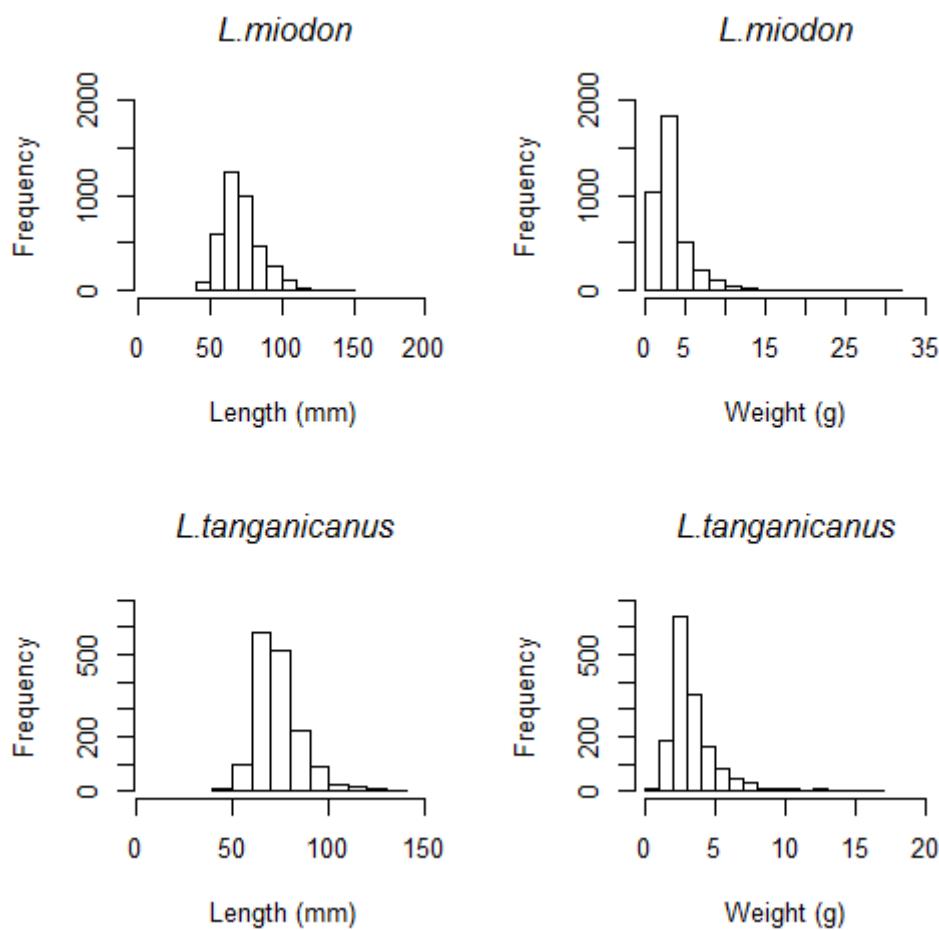


Figure 34 : Distribution of the length and weight of *L. miodon* (above) and *L. tanganicanus* (below) species. Fish were captured in Lake Kivu between December 2017 and November 2018.

### 4.3.3 Results

Length-weight models for *L. miodon* and *L. tanganicanus* showed a tight fit to the transformed data (see Figure 35,  $R^2 = 0.98$  and 0.95 respectively). Both species exhibit allometric growth (i.e. coefficient  $b \neq 3$ ), with an exponent parameter  $b$  ranging between 3.22 and 3.26 for *L. miodon* and between 2.91 and 2.97 for *L. tanganicanus* (with 95% confidence).

The logarithmic relationships between weight ( $W$ ) and length ( $L$ ) can be summarised as follows:

$$(eq. 1) : \log(W) = -12.81 + 3.24 * \log(L) \text{ for } L. miodon$$

$$(eq. 2) : \log(W) = -11.50 + 2.94 * \log(L) \text{ for } L. tanganicanus$$

Using these models, it was assessed that the mean weight of a 100 mm *L. miodon* varied from 8.3 to 8.4g and from 7.5 to 7.7g for *L. tanganicanus*.

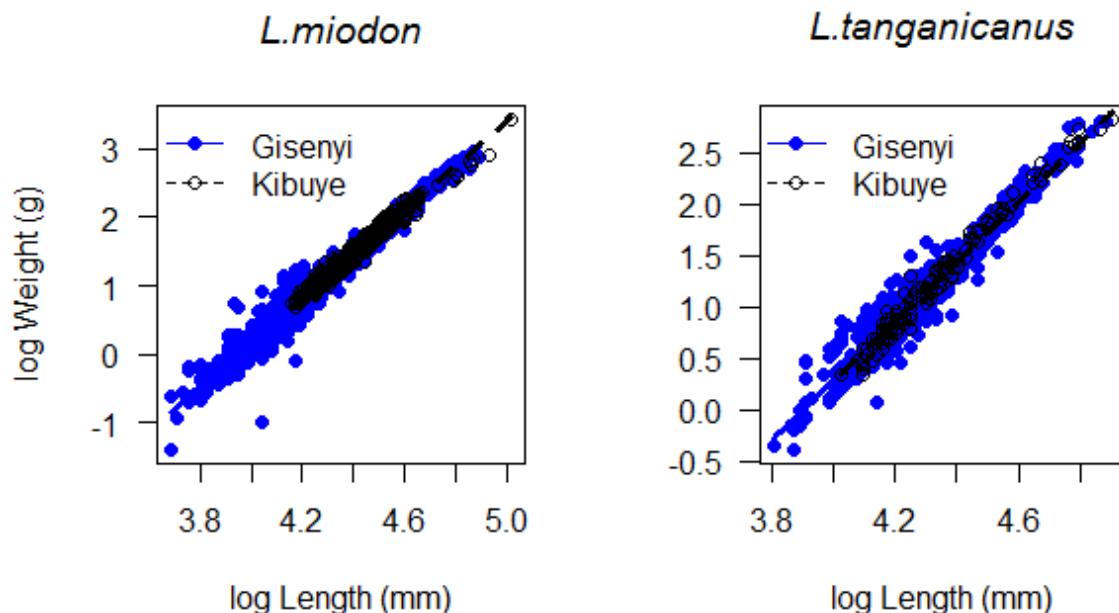


Figure 35: Natural log total length and weight of *L. miodon* (a) and *L. tanganicanus* (b) from L. Kivu. Samples were collected in littoral and pelagic zones of Kibuye and Gisenyi, at different occasions from December 2017 to November 2018\$

#### Comparison with previous studies:

Different length-weight relationships were found in the literature. Lamboeuf (1989) proposed the following equation:  $W \text{ (g)} = 0.055 * L \text{ (cm)}^{2.27}$ . An updated equation was established by Kaningini (1995) on a large sample of *L. miodon* in Lake Kivu (approx. 20'000 ind.):

$$\log(W) = -4.5 + 2.7 \log(L) ; R^2 = 0.8765, N=19'665.$$

Lastly, Fishbase (Froese & Pauly, 2019) provides Length-Weight Relationship for *L. miodon* from Bayesian approach:

$$a = 0.00741 \text{ (0.00414 - 0.01327)}, b = 2.98 \text{ (2.83 - 3.13)}, \text{ with Total Length in cm.}$$

We observe important discrepancies between the models, and in particular between  $b$  parameters. The reasons are unknown. This might be due to sample size variability, local differences in available food resource (i.e. Bukavu Bay may differ from Gisenyi area),

differences in protocol not known, as wet or dry fish, or to a trophic change in the pelagic zone of the Lake since 1995, leading to plumper adults compared to juveniles.

For *L. tanganicanus*, Masilya (2011) obtained the following relationship:

females:  $\text{Log}(W) = 2.92 \text{ Log}(L) - 4.92$  and  $R^2 = 0.92$

males:  $\text{Log}(W) = 2.82 \text{ Log}(L) - 4.72$  and  $R^2 = 0.94$

These results are consistent with those observed in the present study (eq. 2). We did not find any difference between sex in WL relationships neither on the slope (ANOVA,  $p=0.70$ ) nor on the intercept (ANOVA,  $p=0.44$ ), on a subset of data including information on sex ( $n=630$ ).

Bayesian length-weight relationships from Fishbase (Froese et al., 2014) provided approximate values for a and b parameters with large confidence intervals [ $a=0.00437$  (0.00162 - 0.01178),  $b=3.14$  (2.91 - 3.37)], probably due to scarce information on biology and ecology of this species.

## 4.4 Age determination and growth analysis

### 4.4.1 *Introduction*

Age determination of tropical fish species requires the analysis of otoliths, which are small calcified structures of the inner ear of fish. Otoliths are formed by the accretion of calcium carbonate and protein matrix alternating on a daily cycle. In most fish species, it is possible to count the daily increments, giving a proxy of age (Cochrane, 1984; Marshall, 1987; Pannella, 1971; Panfili et al., 2009). Bony fish have three pairs of otoliths called *sagittae*, *lapilli* and *asterici*. The *sagittae* are most often used for ageing and other analyses because they are the largest, earliest formed and easiest to extract of the three pairs.

The procedure for otolith preparation differs according to the size and the shape of the otoliths, which is inherent to each fish species. In this study, *sagittae* otoliths of *L. miodon* and *L. tanganicanus* were extracted and analysed, other pairs of otoliths being too small to be observed and removed. The procedures for otolith preparation was achieved for *L. miodon* and *L. tanganicanus* and is described below.

### 4.4.2 *Otolith extraction*

Otoliths were extracted in LKMP laboratory, according to species-specific procedures (Annex 6), given the difference in otoliths shapes and sizes between the two species. *L. miodon*, like other pelagic clupeids, have small otoliths in proportion to their size, it is therefore necessary to make a longitudinal section of the head before otoliths extraction. Conversely, otoliths of *L. tanganicanus* are larger and can be easily extracted after scalping

the head and removing the brain (see Annex 6 for further details of the procedures). After extraction, otoliths were stored in eppendorf tubes at ambient air until analysis in France.

#### 4.4.3 Otolith preparation of *L. miodon*

##### ➤ Otolith picture

Otoliths (*sagittae*) of *L. miodon* were photographed under a dissecting microscope (LEICA S9i with built-in camera CMOS 10mPixels) and using LEICA Application X Software (LAS X) (Figure 36). The distal side was preferred to the proximal side (side with *sulcus acusticus*). To reduce otolith opacity and improve the sharpness of image outlines, otoliths were immersed in Ethanol 70%. After the picture was done (Figure 37), the otoliths were quickly rinsed into water in order to stop the ethanol-induced dissolution of chalky layers.



Figure 36 : Otolith photography under LEICA dissecting microscope

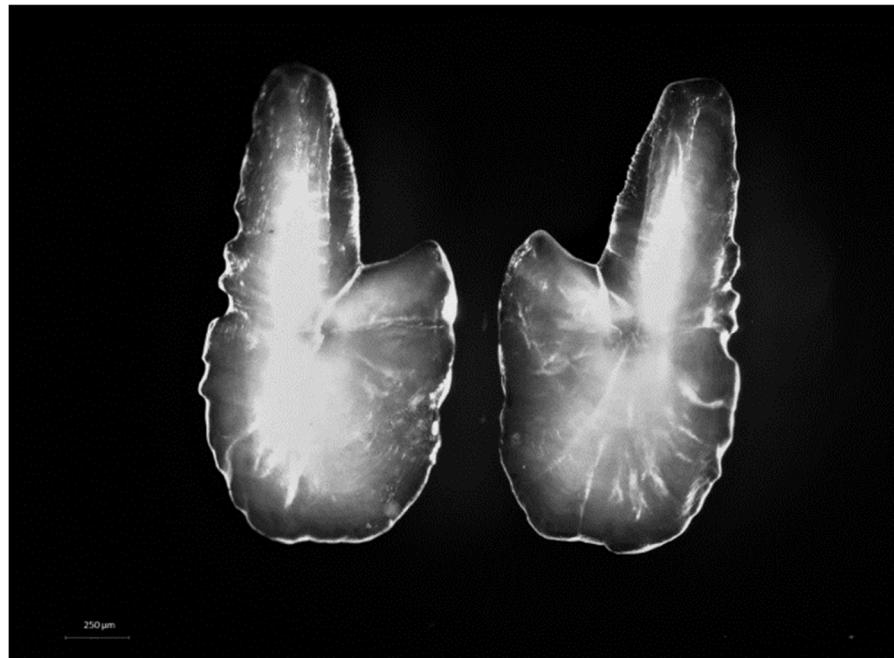


Figure 37 : Pictures of left and right otoliths of *L. miodon* observed in distal view

#### ➤ Otolith embedding

As otolith of *L. miodon* become translucent in epoxy liquid resin (Araldite®2020 bicomponent, HUNTSMAN), the bottom of the embedding molds (SPI Supplies Brand Silicone Embedding Molds; size: 7mm x 3mm; 3mm deep) was coloured using a black marker. The resin, which is toxic when breathed in, was prepared under extractor hood by mixing 10 mL of component A and 3.5 mL of component B of Araldite, taking care to remove bubbles. Then, each mold was 1/3 filled with liquid resin, and remaining bubbles were removed using a needle. The silicone mold was then placed in laboratory oven at 40°C for 3 hours, so that the resin starts hardening.

After 3 hours in oven, the mold plate was removed and cooled at ambient temperature. Right otoliths were individually placed in, distal side up, using a dissecting microscope (Figure 38, Figure 39). Left otoliths were either used for pre-tests or conserved for later analysis if right otoliths were not readable (in case of abnormal vaterite otoliths or if otolith preparation did not allow to count daily increments). The molds were then half-filled with resin. The otoliths were turned over to remove the bubbles which might be stuck below. The otoliths were then repositioned distal face up, and molds were filled with resin until the resin forms a dome shape over the mold. The mold plate was then put in the oven at 40°C for 48h.



Figure 38 : Right otoliths of *L. miodon* (distal side up) placed in molds filled with epoxy resin

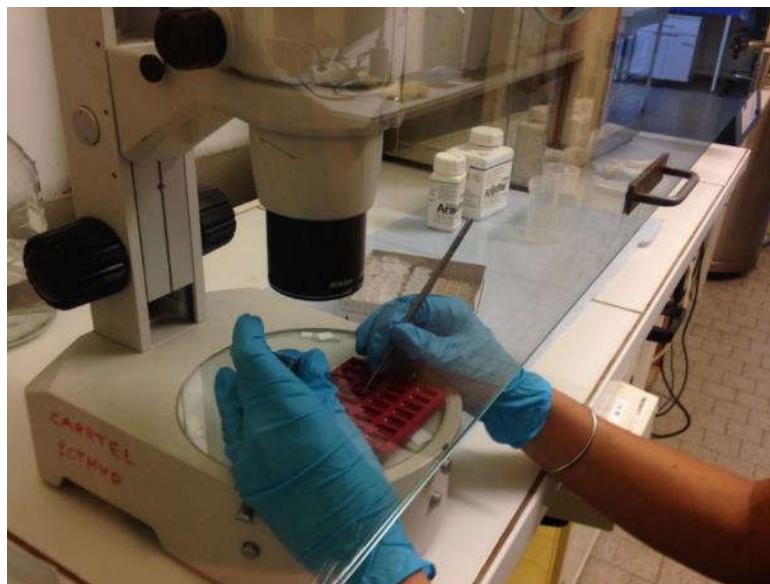


Figure 39 : Otolith positioning in molds under a dissecting microscope

#### ➤ Otolith preparation

Preparation usually requires sectioning or grinding the embedded otolith along a particular plane. Three main planes may be used: sagittal, frontal and transverse (Figure 40). The sagittal plane can be used for small-sized fish for which both the core and the edge of the otolith can be reached easily (Panfili et al., 2009). For otoliths with a concave-convex shape, the transverse section plane is recommended. After several tests, *sagittae* otoliths were observed in the sagittal plane, according to the method proposed by Kimura (1995) on *L. miodon* of Lake Tanganyika. Grinding was chosen (Figure 41) instead of sectioning because of the small size of otoliths. The following steps were followed:

- grinding by hand with wet abrasive paper, from coarser to finer grit (PSA G600 and G1200), until the approximate position of the nucleus is reached. Proceed with random movements to avoid systematic scratches and otolith removal. Use a microscope to regularly check the progress and ensure that the nucleus has not been passed ;

- polishing by hand (with protecting gloves) to remove the scratches. Use a polishing disc and alumina powder  $\text{Al}_2\text{O}_3$  ( $3 \mu\text{m}$ ) diluted in water (Figure 41). Proceed with random movements and use a microscope to regularly check the otolith ;
- repeat the same process (grinding and polishing) on the other side of the otolith until obtaining a thin slice (about  $300 \mu\text{m}$ ).

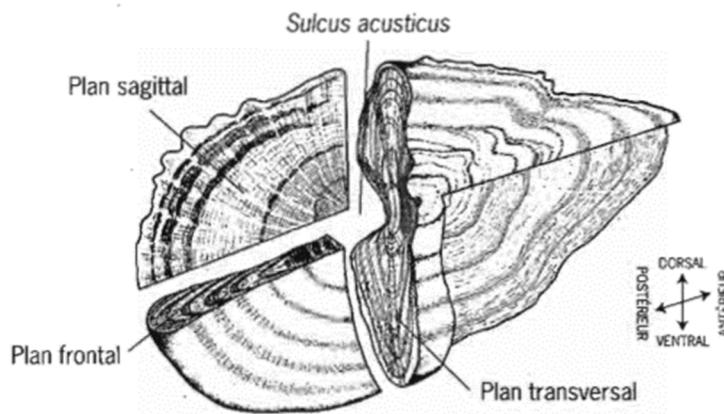


Figure 40 : The 3 different planes used in otolithometry (Panfili et al., 2002)



Figure 41 : Grinding and polishing of a thin slice of otolith

#### ➤ reading of daily increments

The thin slides were observed under a microscope (Olympus BX40, x40 magnification), and pictures were taken using a camera AxioCam MRc Olympus and Axio Vision Rel. 4.7 Software. It was not possible to get appropriate focus in the sagittal plane, to simultaneously

observe all the rings. It was necessary to take several pictures along the longer axis and to assemble the pictures. The rings were then counted from the nucleus till the edge of the otolith (Figure 42 ; Kimura, 1995).



Figure 42 : Picture of a ground and polished sagittae otolith of *L. miodon*. The black arrow indicates the axis used for daily increment counting.

Pictures that were taken along the same axis were then uploaded on Hugin software. This software helped assembling the pictures in sequence and by pairs, to build panoramic pictures (Figure 43). Some checkpoints were automatically placed on the pictures, other ones should be manually placed.

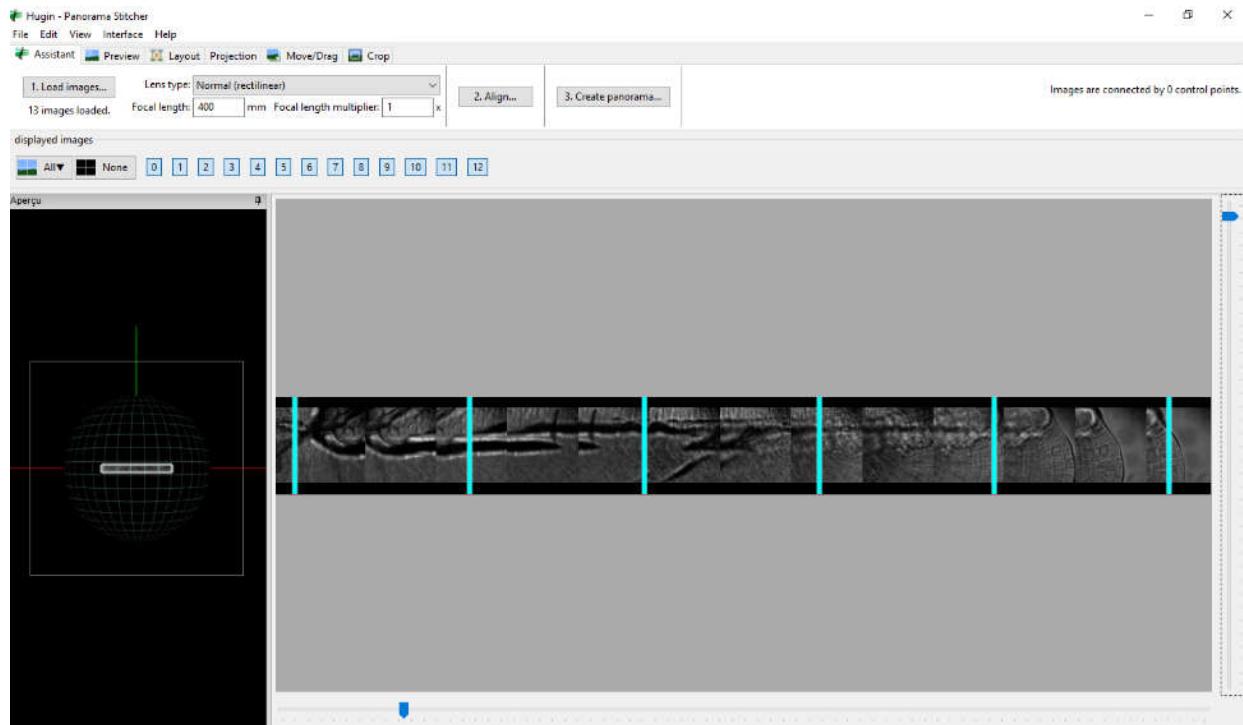


Figure 43 : Otolith pictures after upload in Hugin software (Picture M. Hautier)

After picture assembling, the final picture was opened using Image J software (Figure 44), and a segment was drawn along the longer axis of the otolith, to count the dots placed on each daily ring. The rings were counted two times by a single operator; beyond a difference of 20 rings between the two successive counting operations, a third counting was done. Otoliths were rejected and considered as unusable when more than 10% of the profile was unreadable. Below 10%, the number of rings was assessed considering an average interval length between two consecutive strings before and after the unreadable zone.

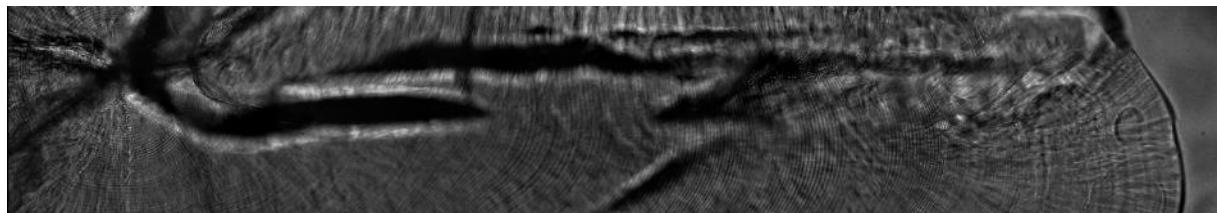


Figure 44 : Picture assembling of a sagittae of *L. miodon* along the main axis (Picture M. Hautier)

#### 4.4.4 Otolith preparation of *L. tanganicanus*

For *L. tanganicanus* otoliths, the procedure differed on a few points, due to the specific shape of otoliths: the sagittal plane was finally selected for this species after several tests of different preparation protocols in the transverse and frontal planes. Before preparation, each otolith was first observed under a stereomicroscope to check the absence of cracks or breaks. The orientation was adjusted so that the proximal side was up (Figure 45).

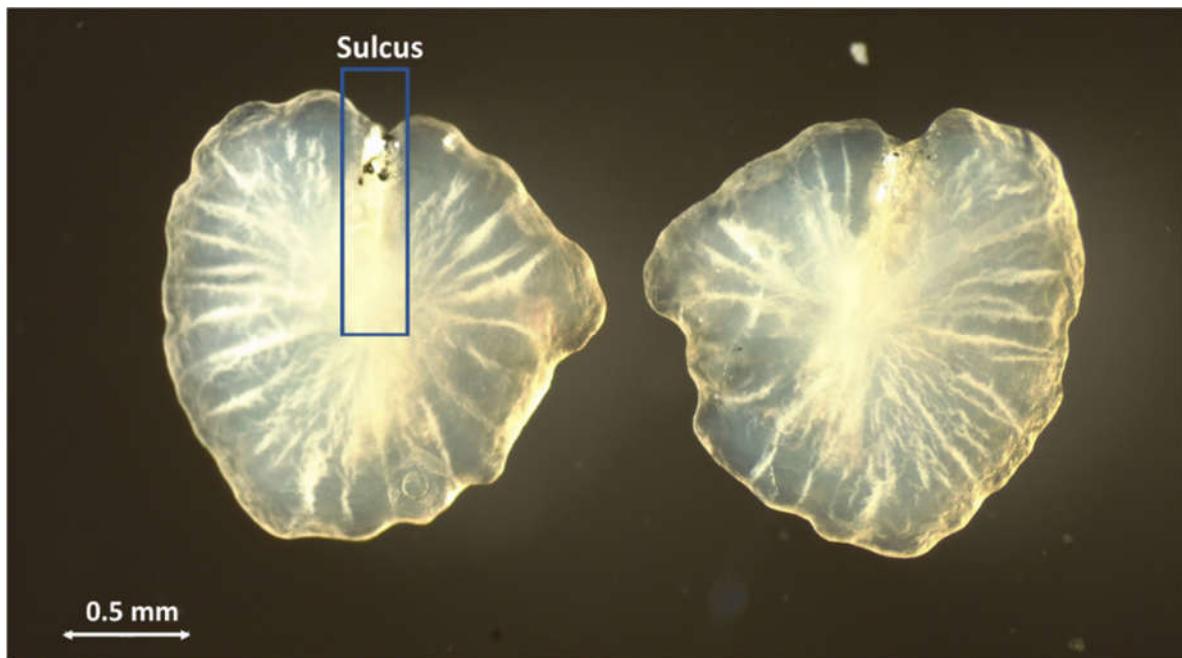


Figure 45 : Pictures of left and right otoliths of *L. tanganicanus* observed from proximal side (Picture M. Hautier)

The resin (Crystalbond™, Ted Pella Inc.) was heated on a hot plate at 200°C, until it became liquid and easy to handle. A small drop of resin was placed in the middle of a thin blade, which was also laid on the hot plate. The size of the drop should be smaller than the otolith diameter, so that the otolith was stuck on the distal side, but the glue should not cover the otolith edge (better for the reading). The otolith was carefully taken using tweezers after plunging the tip in the resin, then the otolith was placed on the drop.

Before the resin had cooled down, the operator checked the otolith position under a stereomicroscope. The otolith should be as flat as possible, and in the same position as on Fig. 2.4. The otolith adjustment should be done quickly, since the Crystalbond hardens in a few seconds. If needed, the slide was re-laid on the hot plate to keep the resin viscous enough.

After complete cooling, the otolith was ground on a wet fine abrasive sheet (2400 grit sandpaper), moving the thin slide in the shape of a figure eight. The blade should remain parallel to the working plan for a regular grinding. Several go-and-return were required between the grinding workstation and the stereomicroscope to make sure not to miss the nucleus. As a prudent measure, the grinding was stopped when the first daily rings appeared. Otoliths were then polished on a disk of felt and alumina powder dissolved in water, to remove the stripes produced by grinding. An otolith was correctly ground and polished when at least 90% of the daily rings were visible.

In the case of thick otoliths, the grinding-polishing procedure was repeated on the other side of the otolith. The slide was then re-laid on the hot plate, the operator turned over the otolith and controlled that it was well positioned before starting to grind.

After the procedure was completed, several pictures were taken on a same axis under a x40 magnification (Olympus BX40 microscope), using Axio Vision Rel. 4.7 software. The focus was adjusted between two consecutive pictures. The pictures were then merged using Hugin software (Figure 46). Then, each reconstituted picture was read from the nucleus until the edge of the otolith, following the same procedure as for *L. miodon*.

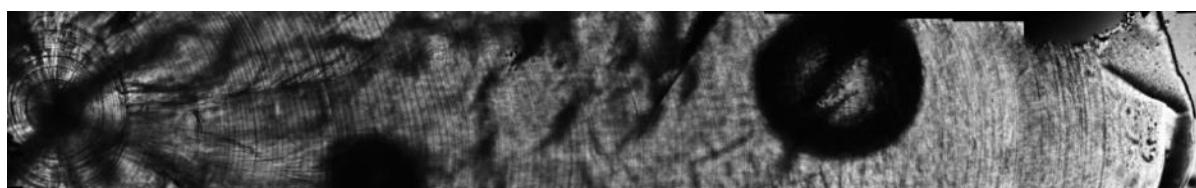


Figure 46 : transect of otolith of *L. tanganicanus*, after merging several pictures (Picture M. Hautier)

#### 4.4.5 Datasets and statistical analysis

A total of 108 otoliths of *Limnothrissa miodon* were analysed (mostly right otoliths, left otoliths were only analysed when right otoliths were not readable, or for additional age validation). Fish lengths (total length) ranged between 19 and 128 mm (mean  $\pm$ SD = 77.7  $\pm$  26.8 mm) and all size classes were analysed. Most fish (n=73) were sampled in December

2017, both in Gisenyi and Kibuye and in inshore/offshore habitats. The remaining 35 fish were sampled in July 2018; most of them were juveniles collected close to the shore in Kibuye and Gisenyi area. The dataset includes fish from both sexes. Due to the very long process required for each otolith preparation and reading (at least 2-3 hours / otoliths), it was not possible to increase the dataset. However, we considered that a sample of 100 individuals was consistent enough for an analysis of growth and was conformable to literature (Panfili et al., 2009).

**60 otoliths of *L. tanganicus*** were also analysed (mean  $\pm$ SD = 63.6  $\pm$ 31.3 mm). The fish originated from Kibuye and Gisenyi and were sampled in Dec 2017 and July 2018. The sample size was smaller than for *L. miodon* for technical reasons, given the long process required to set up the protocol, and the reading uncertainties on several otoliths which were excluded from analysis.

For each species, a standard Von Bertalanffy growth model (Allen, 1966) was fitted to the data, according to the following equation:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

Where  $t$  is time,  $k$  is the growth rate,  $L_t$  is length, and  $L_\infty$  is the asymptotic length at which growth is zero.  $t_0$  is the theoretical age at which the fish would have zero size.

Statistical analyses were then performed with R studio (RStudio Team, 2015), using FSA (Ogle et al., 2019) et nlstools packages (Baty et al., 2015). Effects of sex and sampling site were tested using the model selection procedure described by Ogle (2013).

#### 4.4.6 Growth of *L. miodon*

A Von Bertalanffy growth model was shown to consistently adjust to the dataset, although the lack of large individuals ( $> 120$  mm) did not allow to provide a reliable estimate of the asymptotic length ( $L_\infty$ ). The model adjustment was slightly better after removing individuals captured close to the shore (during juvenile sampling) with size  $> 32$ mm (n=9). This choice was justified by the objective to produce growth model for pelagic *L. miodon*. These individuals showed important growth variability compared to the pelagic fish, 6 out of them being slow-growing fish. Anyway, the model parameters do not change significantly (Figure 47).

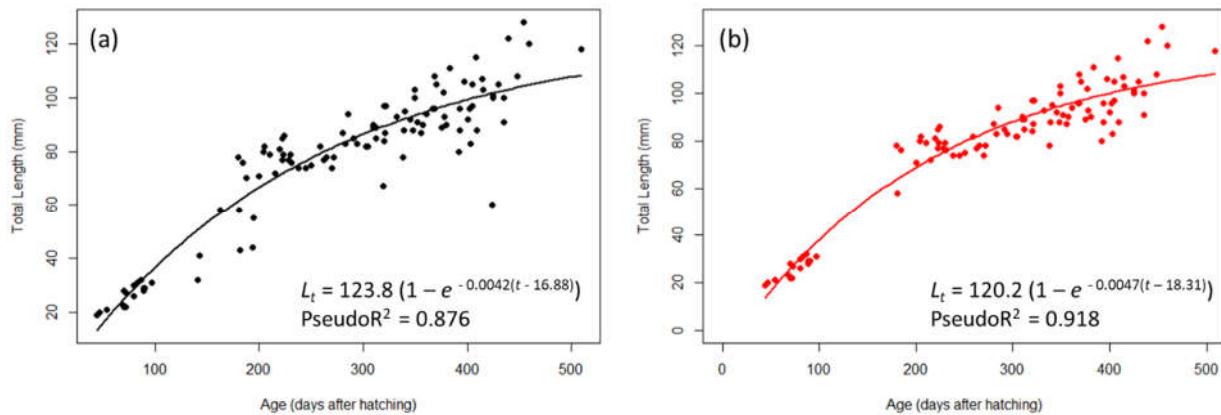


Figure 47 : Growth curve of *L. miodon* using standard Von Bertalanffy model, considering: (a) all the dataset; (b) after removal of slow-growing individuals captured close to the shore

Using the growth model fitted on Figure 47b, predictions were made on *L. midon* size at 6 and 12-month old:

$$6 \text{ months} : \quad L = 64.4 \text{ mm [IC95\% } 62.2; 67.4]$$

$$12 \text{ months} : \quad L = 96.5 \text{ mm [IC95\% } 94.8; 98.0]$$

The growth of *L. miodon* did not vary between Gisenyi and Kibuye (Figure 48), only intercept  $t_0$  differed between the two sites (Anova,  $p=0.007$ ), the two other parameters ( $L_\infty$  and  $K$ ) being independent from fish location. This difference should most likely reflect a bias inherent to the analysed sample, rather than a real growth difference at the juvenile stage between the two sites.

To test for any difference between sexes (Figure 48), the 20 juveniles ( $L_t < 40$  mm) were randomly assigned a sex, since sex determination was not proceeded for immature fish. This step was mandatory so that the Von Bertalanffy growth model would fit to the data. We observed that the females have a larger asymptotic mean length ( $L_\infty$ ) than the males (Anova,  $p = 0.002$ ). According to this model, the mean total length of a 1-year old female will be 98 mm instead of 92 mm for a male. These results should be confirmed with further otoliths analyses.

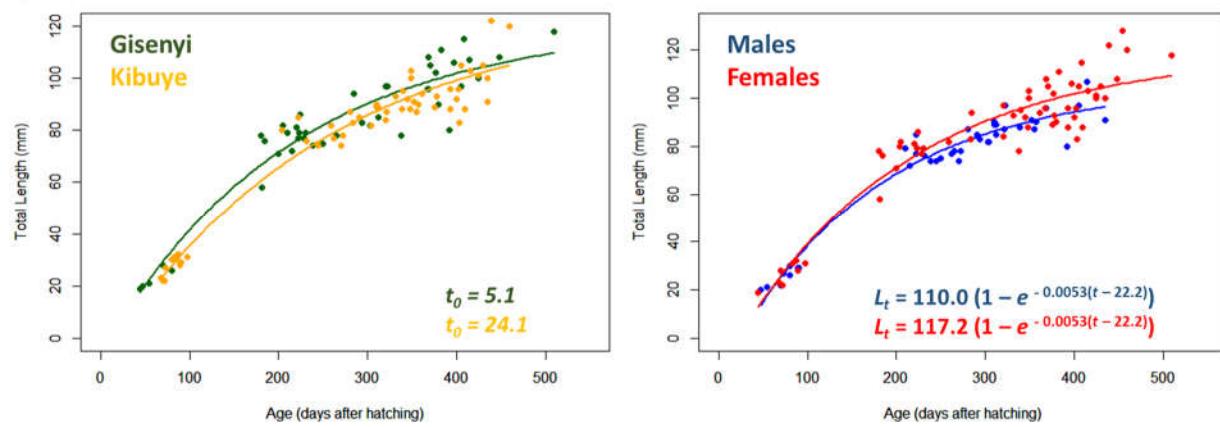


Figure 48 : Growth curve of *L. miodon* according to sampling location (left) and sex (right) using standard Von Bertalanffy model

The operator effect was also tested. 30 otoliths of *L. miodon* were blind-read by 2 different technicians. The number of daily increments did not differ strongly between operators, ranging between 0.5% and 4.3% (mean 2.0%). This difference represents a maximum of 12 days for a fish, which age ranges between 356 and 368 days. Therefore, **we can assume that changing operators would not significantly affect the results, provided that any new operator receives a specific training first.**

#### 4.4.7 Growth of *L. tanganicanus*

The reduced number of otoliths analysed only allowed a first drawing of the Von Bertalanffy curve (Figure 49). There is high uncertainty in the asymptotic length, since only 8 individuals longer than 100 mm could be analysed.

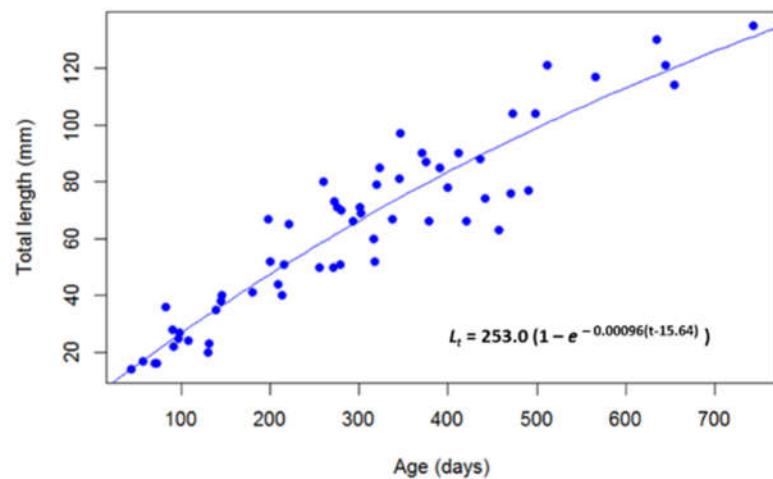


Figure 49 : Growth curve of *L. tanganicanus* using standard Von Bertalanffy model

#### 4.4.8 Comparison with literature

The growth of *L. miodon* in African lakes has been widely studied. Conversely, no reference was found on the growth of *L. tanganicanus*.

- *L. miodon* in Lake Kivu

The growth of *L. miodon* in Lake Kivu was previously studied by Spliethoff et al. (1983) and Kaningini (1995), using length-frequency distributions. The model equations are presented on Figure 50. The corresponding sizes for 6-month-old fish appear very similar for all 3 models. Overall, the growth model obtained from otolith analyses in the present study is very close to Spliethoff et al. (1983) model; Kaningini (1995) obtained larger asymptotic length, which might be consecutive to the capture of larger individuals in the southern basin.

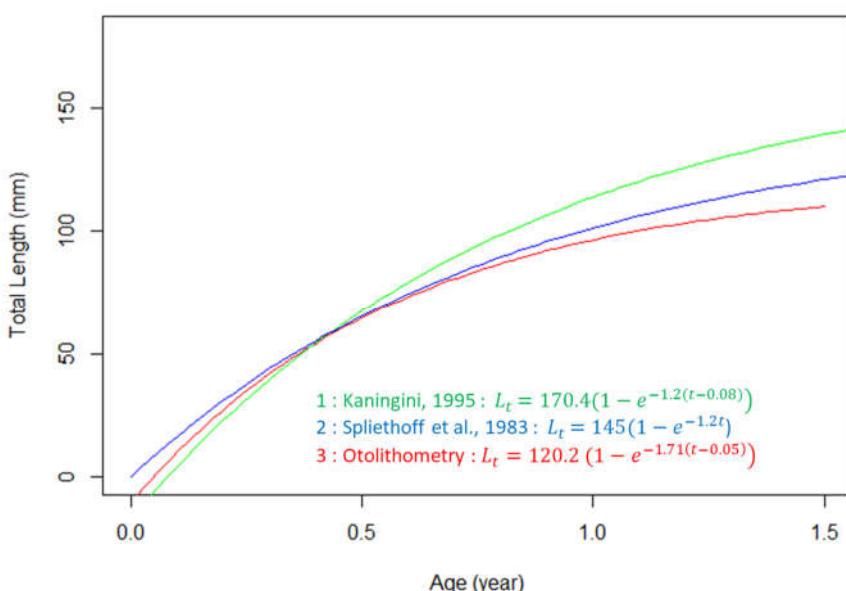
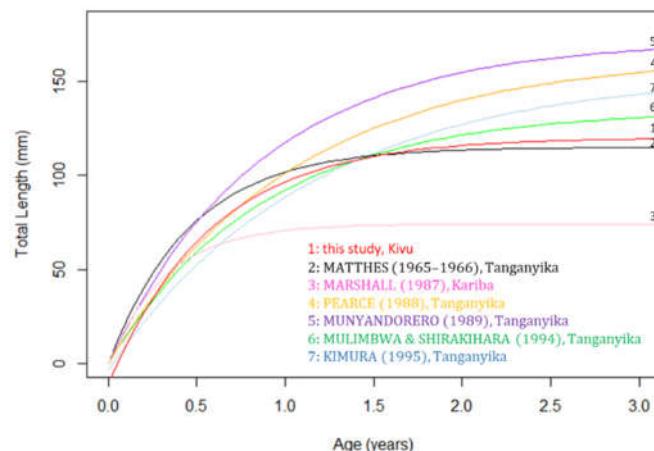


Figure 50 : Von Bertalanffy growth models of *L. miodon* in Lake Kivu

- *L. miodon* in other African lakes

Most growth studies on *L. miodon* were carried out on Lake Tanganyika. We observe similar growth patterns between all models during the first 6 months (Figure 51): the growth rate ( $K$ ) in Lake Tanganyika ranged between 0.862 and 2.16. The growth rate was similar in Lake Kivu ( $K = 1.71$ ).



Authors	Von Bertalanffy parameters		
	$L_{\infty}$ (mm)	K (per year)	$t_0$
This study	120.2	1.71	0.05
MATTHES (1965–1966)	115	2.16	-
MARSHALL (1987)	74.2	3.048	-
PEARCE (1988)	164	0.96	-
MUNYANDORERO (1989)	172	1.15	-
MULIMBWA & SHIRAKIHARA (1994)	135	1.15	-
KIMURA (1995)	155.4	0.862	0.022

Figure 51 : Von Bertalanffy growth models of *L. miodon* in other African lakes

Some discrepancies appear between models for older fish. It is generally considered that the growth parameters may vary between locations and between years (Kimura, 1995). This can partially explain the observed differences. However, models on Lake Tanganyika generally show higher  $L_{\infty}$  values than on Lake Kivu. Conversely, in Lake Kariba,  $L_{\infty}$  remains very small. The underlying factors affecting growth of *L. miodon* are not known, although they generally depend on food availability, predation, water temperature and/or fisheries.

## 4.5 Fecundity analysis

### 4.5.1 Methodology

Part of the female gonads, which were sampled, weighed and frozen during the campaign of December 2017, were analysed using the following methodology (Figure 52).

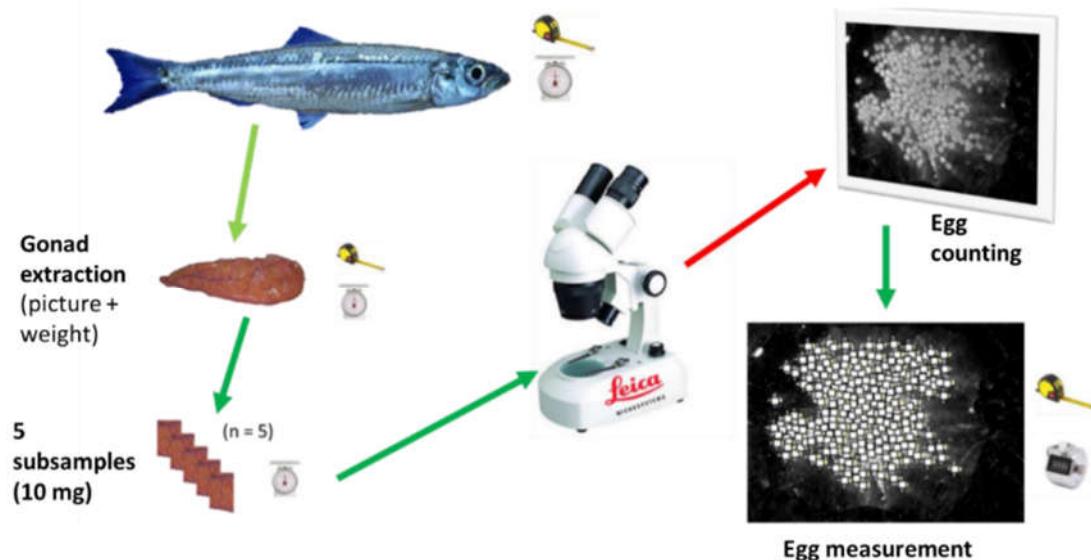


Figure 52 : Procedure for gonad analysis (source: Reveret, 2018)

Due to the high fecundity of *L. miodon*, five subsamples of each gonad were extracted, weighed (approx. 10-20 mg each) and observed under a stereomicroscope. Then all oocytes were isolated and counted. Oocyte diameters were also measured on a few samples. Gonads of *L. tanganicanus* females were analysed following the same methodology. Then, from egg counts in each subsample, the total egg number was assessed, together with the standard deviation. Due to the lower fecundity of *L. tanganicanus*, an exhaustive counting was performed for some individuals. A total of 30 and 39 female gonads of *L. miodon* and *L. tanganicanus* were analysed respectively (Table 11).

The gonadosomatic index (GSI, expressed in %) was also calculated as the ratio between gonad weight and total weight of the fish after gonad removal, at each sampling occasion (Table 11). GSI is a good indicator of gonad development, in absence of clear ovarian and testicular stages of development.

Table 11 : Sample sizes for fecundity and gonadal development analysis

Date	Effective for GSI analysis (females)		Effective for egg counting	
	<i>L. miodon</i>	<i>L. tanganicanus</i>	<i>L. miodon</i>	<i>L. tanganicanus</i>
déc.-17	73	21	10	9
janv.-18	54	8		
févr.-18	50	11		
mars-18	42	19		
avr.-18	37	10		
juin-18	32	12		
juil.-18	80	34		
août-18	8	15	3	12
oct.-18	18	12	8	10
nov.-18	34	15	9	8
Total	428	157	30	39

#### 4.5.2 Results and interpretation

*L. miodon* fecundity ranged between 7'149 and 37'838 (mean  $\pm$  SD = 19'660  $\pm$  7545, n=30) for female length ranging between 6.6 and 12.4 cm. This observed fecundity is similar to previous studies: McIntyre (2005) analysed fecundity of *L. miodon* from Lake Tanganyika (11.3-13.5 cm SL), and counted between 16'235 and 51'403 eggs. In the same lake, approximately 55'000 eggs were counted for a 140 mm individual (Matthes, 1965–1966, in Muderhwa and Matabaro, 2010). In Lake Kivu, 51'146 eggs were counted for a 121 mm female by Kaningini et al., 1999.

A significant positive correlation with fish size was observed (Spearman's rank correlation test, p<0.001; Figure 53). When extrapolating the egg number from this model for a 140 mm female, 43'095 eggs would be estimated, which is consistent with Matthes (1965-66).

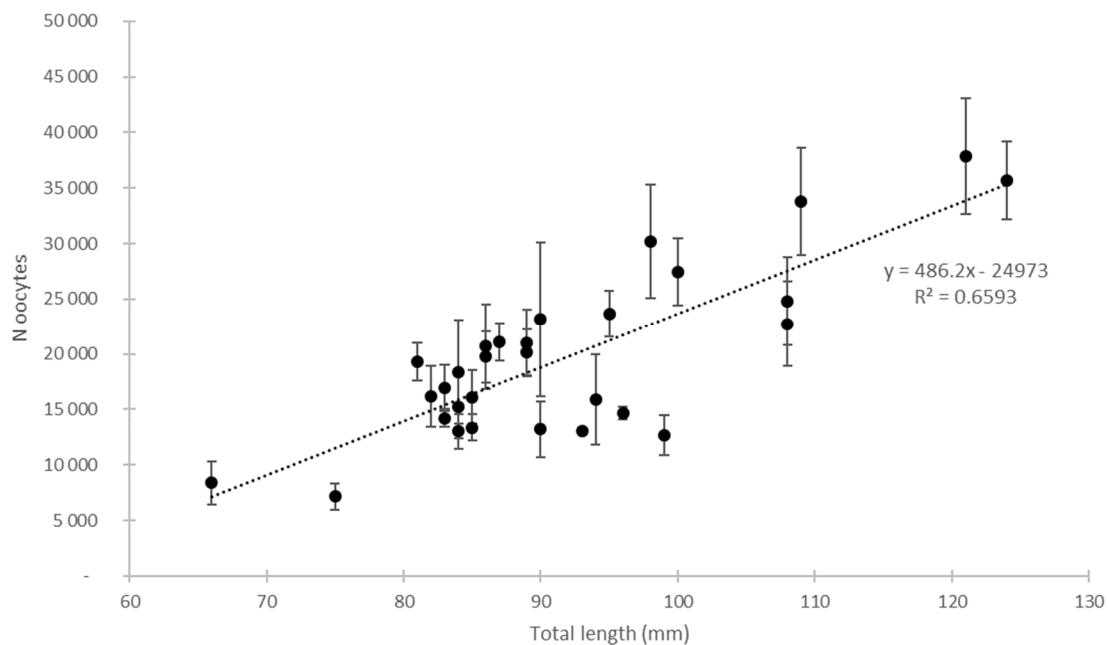


Figure 53 : Relationship between fecundity (number of oocytes) and fish size for *L. miodon*

*L. tanganicanus* fecundity was much lower and ranged between 58 and 450 oocytes (mean =  $193 \pm 95$ ). We did not observe any significant correlation between the number of oocytes and female sizes (Spearman's rank correlation test,  $p=0.38$ ).

Oocytes of *L. miodon* showed similar sizes within a gonad and amongst females (range = 160 – 460  $\mu\text{m}$ ; mean =  $313 \pm 63 \mu\text{m}$ ), see Figure 54 left). This was not the case for *L. tanganicanus*; for this latter species, gonads both contain large mature oocytes (from 2 to 4 mm) and small immature ones (Figure 54 right). Some mature oocytes were observed in all sampled females.

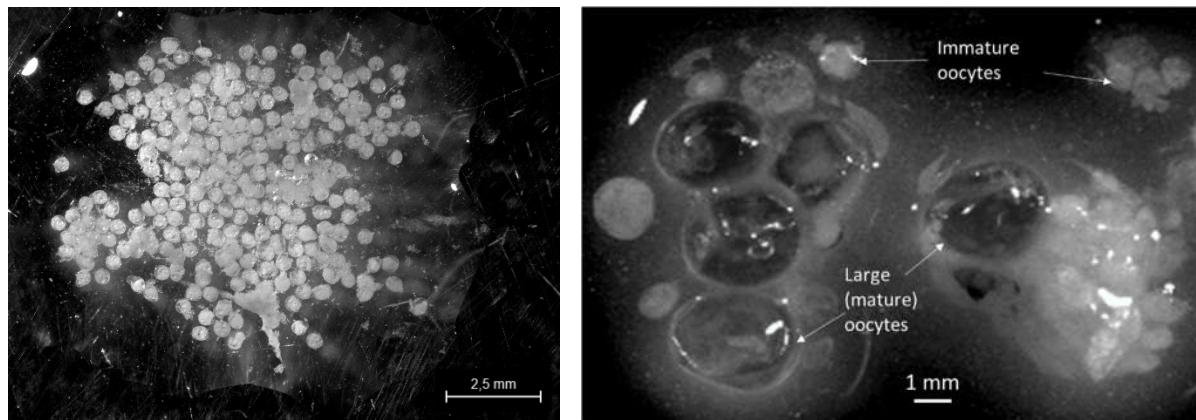


Figure 54 : homogeneous oocytes sizes for *L. miodon* (left) and different stages of oocytes development in *L. tanganicanus* (right). Pictures A. Reveret.

Oocytes diameter distribution was measured for one *L. tanganicanus* (total length = 85 mm, weight = 5.58 g, n= 75 oocytes; Figure 55).

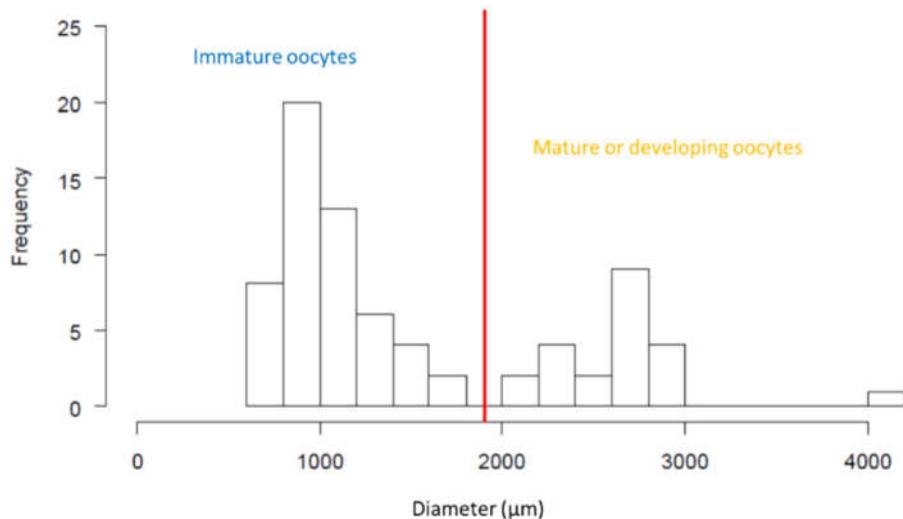


Figure 55 : Egg size distribution of a mature female *L. tanganicanus* (TL = 85 mm)

Among the 75 counted eggs, a major part 71% (53 oocytes) were immature, with diameter close to 1 mm. The 29% remaining eggs were developing or mature oocytes, whose diameter was > 2 mm.

The gonadosomatic index of *L. miodon* females analysed over time showed significant differences between sampling occasions (Kruskal-Wallis test, p<0.001). Higher GSI were observed between August and December, which may correspond to a period of gonad maturation (Figure 56). Conversely lower values were observed from January to April (mean GSI<2%).

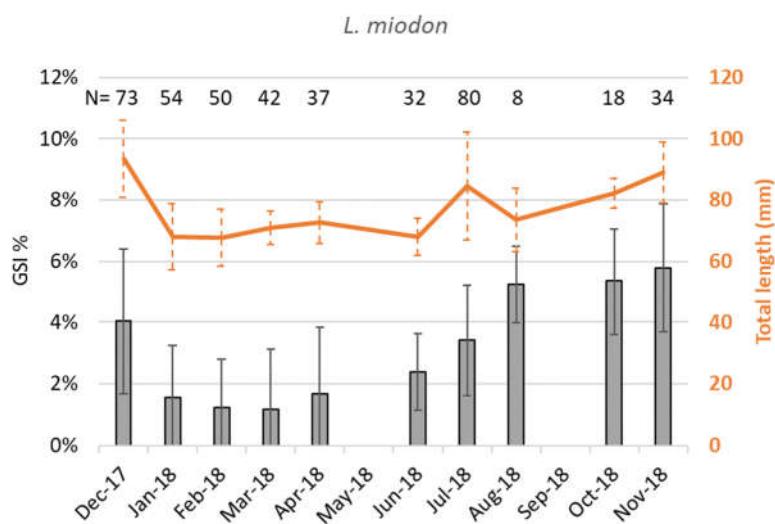


Figure 56 : Gonadosomatic index in females *L. miodon* between December 2017 and November 2018.

For *L. tanganicanus*, the GSI values did not show any obvious trend over time (Figure 57) but significantly differed among sampling occasions (Kruskal-Wallis test,  $p=0.002$ ). All females contained mature or developing oocytes, which may indicate a continuous spawning over time.

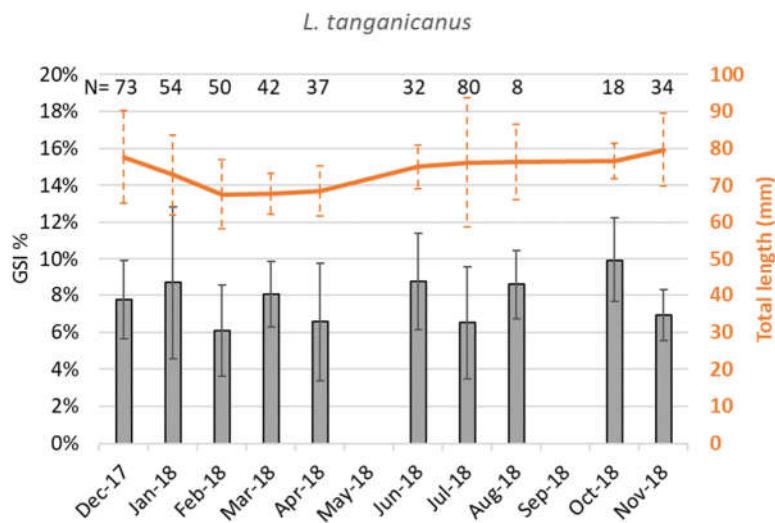


Figure 57 : Gonadosomatic index in females *L. tanganicanus* between December 2017 and November 2018

These results highlight two distinct reproductive strategies between *L. miodon* and *L. tanganicanus*: the former produces high number ( $\times 10'000$ ) of small-sized oocytes with a peak of reproduction which seems to occur at the beginning of the rainy season (Sept-Oct to December). This result is not really consistent with the peak of small pelagic fish (3-4 cm) observed in July 2018 from hydroacoustics data. However, there is no well-defined spawning season in *L. miodon* and GSI peak may vary between years (Mannini et al., 1996; Mulimbwa et al., 2014). In Lake Tanganyika, the interannual variations of the GSI peak in females *L. miodon* seems to be explained by the availability of copepods in the preceding months (Mulimbwa et al., 2014). Further analyses of *L. miodon* fecundity in Lake Kivu should help understanding the variability of the main spawning season.

Conversely, *L. tanganicanus* gonads of mature females contain a few dozen of large-sized oocytes; the females seem to spawn throughout the year. Size at maturity cannot be assessed from the collected data. Indeed, we did not sample enough small-sized fish to determine a median size for sexual maturity.

## 4.6 Stomach content analysis

### 4.6.1 Sampling and stomach analysis

During the sampling campaigns carried out in December 2017 and July 2018, some stomachs of *L. miodon* and *L. tanganicanus* were extracted and preserved in ethanol 96%. In the laboratory, prey items were sorted by taxa (determination at the class or family level)

under a dissecting microscope LEICA. Excess of water was quickly removed with absorbent tissue. Then, the volume of each prey group was measured with a precision of 0.025 ml using the displacement method (Hyslop, 1980): a hypodermic syringe was inserted in a graduated Eppendorf tube full of water, the water level was adjusted to the graduation, then the volume of each prey item was estimated as the volume of displaced water.

#### 4.6.2 Results of the first sampling campaign

Over 30 *L. miodon* sampled in the inshore area of Gisenyi in December 2017, only 1 stomach contained a Diptera larvae (Simuliidae); in the offshore area, 11 individuals over 30 contained fish scales.

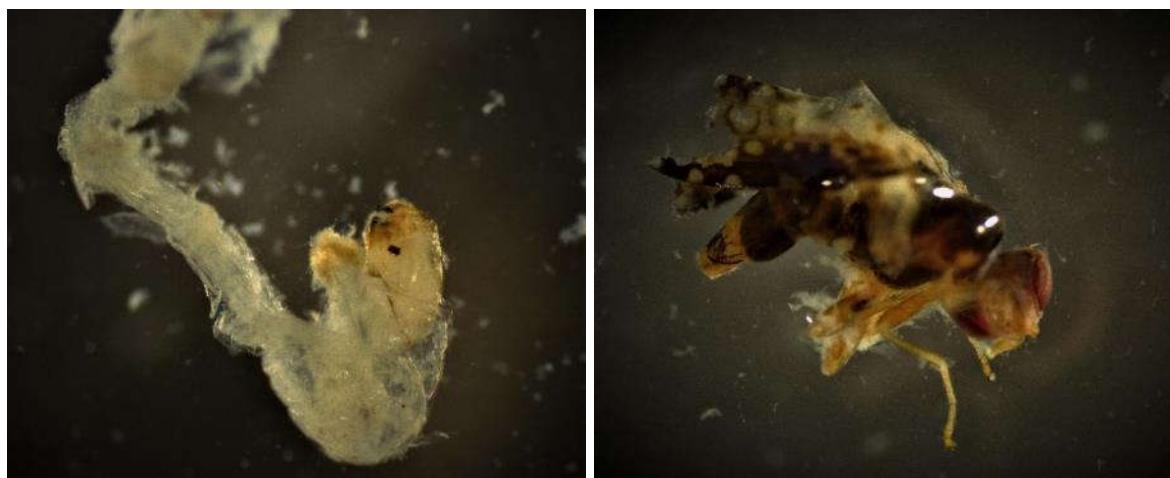


Figure 58 : Left : Example of aquatic prey (Chironomidae, Diptera) ; right : terrestrial prey (Hymenoptera)

Over 40 *L. tanganicanus* collected close to Gisenyi, 11 contained entire preys or prey fragments. Terrestrial insects (Ephemeroptera, Hymenoptera, Diptera) were found in 5 stomachs. Six stomachs contained aquatic larvae, mainly Chironomidae, and fish bones.

Table 12 : Number of prey items observed in fish stomachs (Gisenyi area)

	Aquatic preys				Terrestrial preys				Total stomach contains
	Diptera larvae		Fishes		Diptera	Hymenoptera	Ephemeroptera	Terrestrial unidentified	
	Chironomidae	Simuliidae	Scales	Fishbones					
<i>L. miodon</i>	0	1	11	0	0	0	0	0	60
<i>L. tanganicanus</i>	5	0	4	3	1	1	1	3	40

All stomachs of fish collected from the Kibuye area were empty. The digestion probably continued during fish transport to LKMP lab (at least 3 hours conserved in ice boxes before being analysed). However, the timing of sampling was probably the major hypothesis for the

high observed vacuity rate. *L. miodon* have diurnal feeding habits, with higher feeding activity in the morning or evening (De longh et al., 1983). So, sampling before sunrise is probably not optimal for diet analysis. Therefore, samplings in July 2018 were performed early in the night, 2-3 hours after sunset.

#### 4.6.3 Results of the second sampling campaign

Stomach contents of 96 fish were analyzed in July 2018: 67 *L. miodon* and 29 *L. tanganicanus*. The samples were fairly distributed between the two study sites (Figure 59).

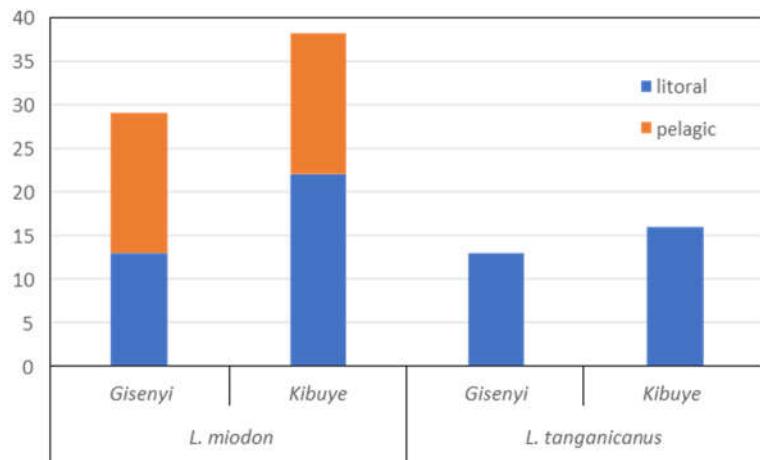


Figure 59 : sample origins

Among 96 analysed fish, 16 showed empty stomachs (Figure 60; vacuity rate = 18% for *L. miodon* and 14% for *L. tanganicanus*). Moreover most stomachs of *L. miodon* were almost empty (51% categorized “-“ on Figure 60).

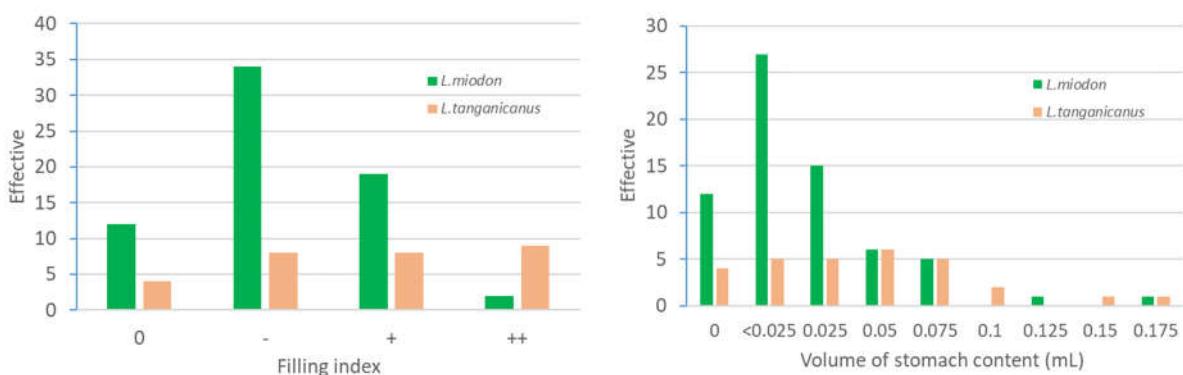


Figure 60 : (left) stomachs filling (“0” for empty; “-“ for almost empty, “+” means that the stomach contains few preys, ++ means that the stomach contains several preys; (right) volumes distribution of the whole gut contents

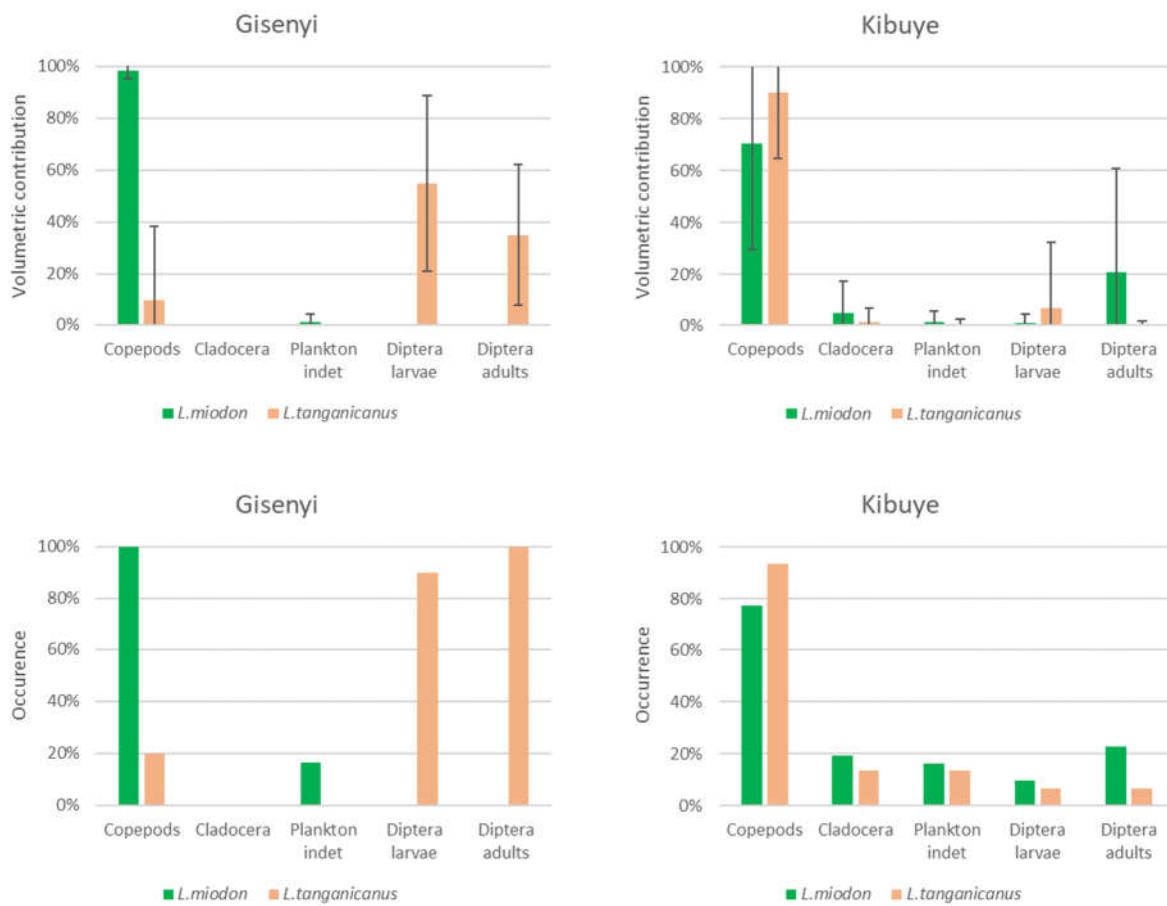


Figure 61 : Mean  $\pm$  SD volumetric percentages (up) and occurrence (down) of the main prey taxa found in the stomachs of the 2 fish species, collected in the inshore areas of the 2 sites.

At both sites, *L. miodon* preyed preferentially upon zooplankton, copepods representing >65% of the volumetric content of stomachs. Benthic preys represented only a small proportion of preys, and were relatively rare. Gut contents of *L. tanganicanus* were more variable between sites, and more pelagic-oriented in Kibuye (90% of the gut volume was composed of copepods) than in Gisenyi, where dipterans (adults and larvae) made up to 90% of the gut contents. Overall, fish scales were uncommon in July 2018 for both species.

Based on gut content data, *L. miodon* and *L. tanganicanus* rely on similar food sources in Kibuye, while the competition for food seems much more limited at Gisenyi.

#### 4.6.4 Comparison with previous studies

The obtained results are consistent with Masilya (2011) who observed similar diet for *L. miodon* and *L. tanganicanus* in Bukabu bay (Figure 62). More insects were observed in the stomachs of both species captured close to the shore in comparison with pelagic fish, which diet was mainly composed of zooplankton.

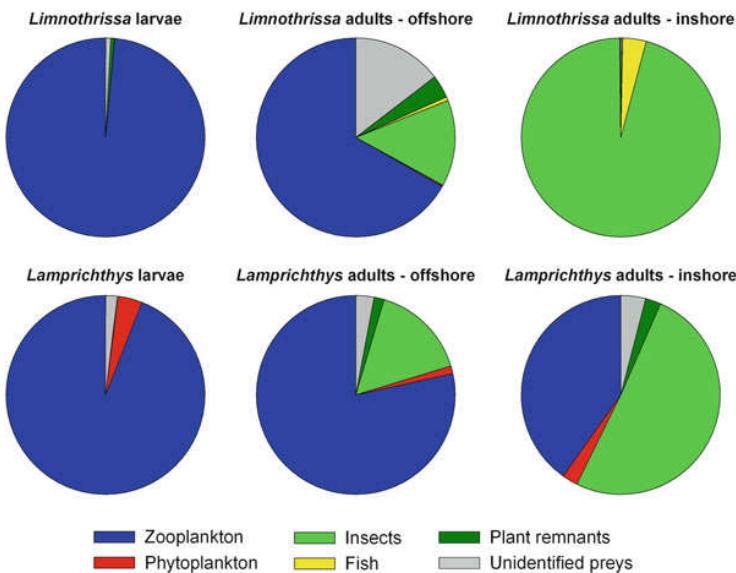


Figure 62 : Summarized results of the diet of *L. miodon* and *L. tanganicanus* in Lake Kivu (source: Snoeks et al., 2012, in Descy et al., 2012)

Yet, gut contents only represent a snapshot of consumed preys and are highly sensitive to sampling times of the day and seasons. If indeed, the operational shift from sampling gut contents from early morning (Dec 2017) to early night (July 2018) decreased the number of found empty stomachs, trophic competition on the long-run between both species is better investigated using markers that integrated the food acquisition and assimilation over longer periods of time. For that matter, stable isotope analyses (SIA\_C&N) were performed on fish bulk tissues at 6 different dates from Dec 2017 to July 2018.

Shall patterns of trophic competition observed by gut contents be confirmed by SIA, the isotopic composition of fish tissues shall be more segregated at Gisenyi as compared to Kibuye, with a high contribution of the zooplanktonic food items at the latest site.

## 4.7 Stable isotope analysis (SIA)

### 4.7.1 Principle

The basics of SIA is that the isotopic composition of a consumers' tissues retains that of the food sources that mostly contributed its biomass. In other words, SIA predicts the trophic origin of the food items from which tissues were built (assimilation) while gut contents track ingestion (Fry, 2006; Perga & Gerdeaux, 2005). Provided the potential food items are also sampled, and reveal to be sufficiently isotopically different, then the relative proportion of the different food sources/habitats to the fish biomass can be assessed using linear mixing models (Vander Zanden & Rasmussen, 1999). Also, because SIA is relatively cheap and fast-processing, it allows dealing with a large amount of individuals, and thereby to really quantify the specialization, competition, or the trophic segregation, between species, or populations (Matthews & Mazumder, 2004). Depending on the considered element (C or N mainly), the relationship between the isotope composition of the preys and the consumers' tissue occurs

according to an offset (i.e. isotopic fractionation factor) that depends on the considered element (0.8‰ for C, 3.4‰ for N on average, Vander Zanden & Rasmussen, 2001) but also on environmental conditions (standard deviation observed for C = 1.5 ‰, N=2.0 ‰; Sweeting et al., 2007). Recent improvements in mixing models now allow to account for such uncertainties in the trophic fractionation factors, by solving mixing equations within a Bayesian framework (Parnell and Inger, 2013). Trophic competition between species and populations can be quantified using metrics of isotopic overlap (i.e. Similarity and Nestedness, Cucherousset et Villéger, 2015).

#### 4.7.2 Sampling design

278 Individuals from both species (*L. miodon* and *L. tanganicanus*) were collected at the two stations (Kibuye and Gisenyi). Individuals were caught in the littoral (0-5 m depth), inshore (5-40 m depths) and offshore zone (> 40 m depths, *L. miodon* only as *L. tanganicanus* stay inshore). The sampling effort covered the whole rainy season at Gisenyi, while sampling was only conducted in Dec 2017 and July 2018 for Kibuye (Table 13). Additional samples were collected during the dry season (August-October 2018); the related analyses are ongoing.

Table 13. Summary of fish sampled for stable isotope analysis.

Species	Station	Dates	Group	Number of sampled individuals
<i>L. miodon</i>	Gisenyi	Dec-2017	1	30
		Feb-March 2018	2	30
		April 2018	3	15
		June-July 2018	4	38
<i>L. tanganicanus</i>	Kibuye	Dec-2017	1	30
		June-July 2018	4	29
	Gisenyi	Dec-2017	1	22
		Feb-March 2018	2	15
		April 2018	3	7
		June-July 2018	4	24
	Kibuye	Dec-2017	1	16
		June-July 2018	4	22

The fish dorsal muscles were dissected and frozen in LKMP lab. Samples were transported in isothermal containers back to France.

In July 2018, potential fish food items were sampled in order to provide estimates of endmembers for mixing models at both sites (table 13). Macroinvertebrates were sampled using a Surber in the littoral zone and an Eckman grap in deeper areas. Zooplankton was sampled using vertical net trawls (200 µm-mesh size) from a boat. Terrestrial insects were manually collected. Littoral algae were scrubbed from their support from littoral areas. All samples were frozen after collection and transferred to France in isothermal bottles. In INRA laboratory, all samples dedicated to SIA were freeze-dried, grounded to powder and weighted in tin capsules. SIA were conducted at the SINLAB, new Brunswick, Canada

*Table 14. Summary of sampled prey items for SIA (July 2018).*

End-members	Gisenyi	Kibuye
Littoral algae	7	1
Macroinvertebrates (from 0-40m depths)	8	9
Terrestrial insects	5	2
Zooplankton	6	2

#### 4.7.3 Isotopic composition of prey species

Figure 63 emphasizes the average (+/- standard deviation) C and N isotope compositions of prey items sampled on July 2018. Littoral macroinvertebrates were enriched in  $\delta^{13}\text{C}$  (higher  $\delta^{13}\text{C}$ ) as compared to other prey items, consistently with the  $\delta^{13}\text{C}$  values observed for the sampled littoral algae (-18 to -16 ‰, data not shown). In contrast, benthic macroinvertebrates sampled inshore had the lowest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , which is consistent with the values observed for deep particulate organic matter in Lake Kivu (-34 to -30 ‰) by Morana et al. (2015b). Overall, the isotope composition of benthic macroinvertebrates decreased along depth (Figure 64), as a typical pattern of macroinvertebrates switching from periphyton, to sedimenting phytoplankton and methanotrophic bacteria from the littoral to the inshore habitats (Grey, 2016). The isotopic composition of zooplankton stands in between these two extremes, with values consistent with those observed for epilimnetic POM (-25 to -22 ‰) by Morana et al. (2015a). Terrestrial preys had  $\delta^{13}\text{C}$  values close to those of zooplankton. Altogether, prey items could be segregated by their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Permanova, prey type effect,  $F_{3,31}=7.7, p=0.001$  and  $F_{3,31}=10.8, p=0.001$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively), and there were no significant differences between sampling sites (Permanova, site effect,  $F_{1,31}=0.5, p=0.43$  and  $F_{3,31}=3.7, p=0.05$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively). The fish isotope composition (pooled species) clustered close to the littoral endmember, anticipating that they

greatly forage in the shallow depth areas. These high values of  $\delta^{13}\text{C}$  for both fish species precludes any important contribution of methanogenetic carbon to the food web leading to both fish species.

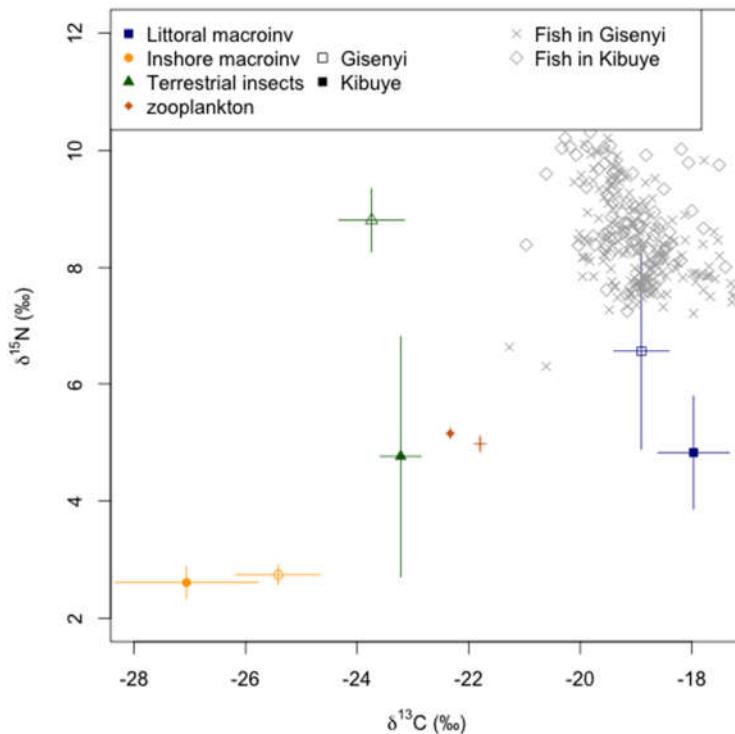


Figure 63.  $\delta^{13}\text{C} / \delta^{15}\text{N}$  biplot of potential prey items for *L. miodon* and *L. tanganicanus*.

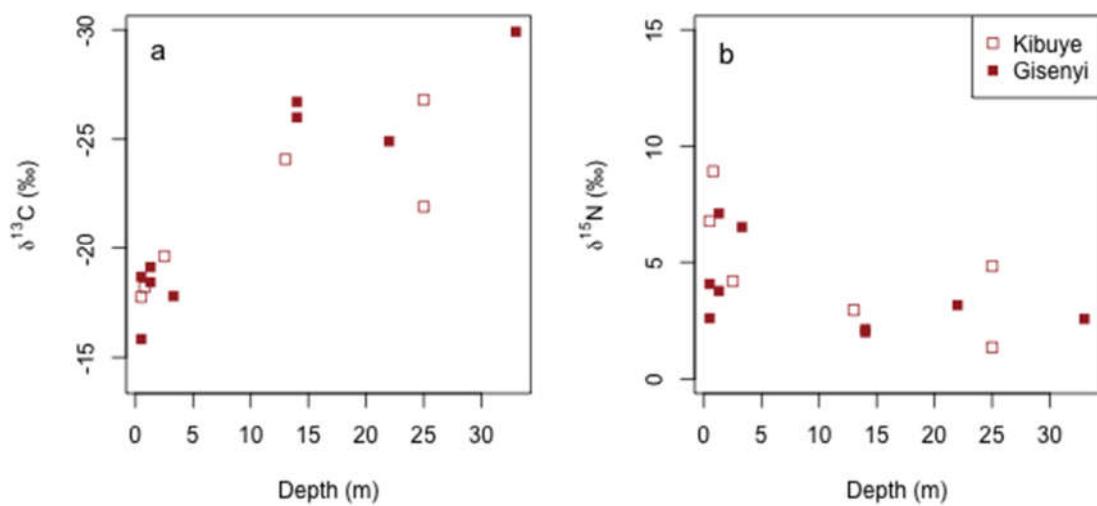


Figure 64. Relations between a)  $\delta^{13}\text{C}$  and b)  $\delta^{15}\text{N}$  and sampling depths for benthic macroinvertebrates

A previous SIA was conducted in 2008 in the southern Basin and these isotopic data were compared to ours. Despite a discrepancy in sampling sites and seasons, the isotope composition of prey items were quite comparable between studies (table 15). Thereby, and

despite the lack of sampling anterior to July 2018, we considered in the following work, that the isotope composition of prey items was relatively constant over time.

*Table 15. Comparison of the C and N isotope composition of potential prey items between the Masilya's study (2011) in the Southern Basin of Lake Kivu and this study (July 2018, data pooled between the two sampling sites)*

	Zooplankton	Littoral macroinvertebrates		Benthic macroinvertebrates (inshore)	
	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<b>Masilya, 2011</b>					
(Southern Basin)	-23.2 ± 1.1	7.2 ± 1.3	-17.1 ± 3.8	4.6 ± 2.1	ND
This study	-22.1 ± 0.9	5.1 ± 0.2	-18.4 ± 1.1	5.7 ± 2.6	-26.2 ± 2.05
					2.6 ± 0.4

#### 4.7.4 Assessment of preys contribution to the fish biomass

Bayesian mixing models were run to assess the relative contributions of potential prey items to the biomass of both species (*L. miodon* and *L. tanganicanus*) at both sampling sites. Introduction of priors increases the accuracy of Bayesian models: herein, terrestrial preys were very unfrequently observed in gut contents, and thereby were not likely to be an important contributor to the fish biomass. Only littoral and inshore macroinvertebrates, along with zooplankton, were introduced as potential prey items for both species. Besides, there are some uncertainties about the values for trophic fractionation factors in tropical aquatic ecosystems (Kilham et al., 2009), so we voluntarily introduced large standard deviation for those in the models in order to decrease the conclusions sensitivity to values assigned to trophic fractionation factors.

Considering all species, seasons and sites together, the best fit led to littoral macroinvertebrates being the dominant prey items (60%, SD=15%), zooplankton the second most important (25%, SD=15%) and inshore macrobenthos the least important (17%). Overall, isotopic insights led to a higher estimated contribution of littoral macroinvertebrates as compared to gut contents. Such an apparent discrepancy between methods can arise from two main reasons. First, macroinvertebrates are more nutritionally rewarding than zooplankton. Thereby, although zooplankton may dominate gut contents by abundance and volume, macroinvertebrates C and N are preferentially allocated to biomass in fish. Then, although predation on zooplankton is important, chironomid nutrients over-contribute to anabolic processes in fish. Second, gut content estimates are biased according to the sampling time of the day and fish may feed preferentially on zooplankton during the day (right before fish were collected for gut contents analysis) but capture emerging macroinvertebrates in the

night. Altogether though, the consistently high  $\delta^{13}\text{C}$  values for fish at all seasons and sites contradicted a dominant contribution of pelagic preys, along with an important contribution of inshore macroinvertebrates that may thrive on methanotrophic bacteria.

The distribution of contributions of littoral macroinvertebrates and zooplankton yet showed an important variability between individuals (Figure 65). The flatter distribution for *L. tanganicanus* suggested that the species is more trophically plastic than *L. miodon*. Distributions are not unimodal for any of the species and sites, suggesting distinct trophic preferences between sub-populations or age groups within the same species. Because we observed no significant differences in the contribution of prey items between sites, data from Gisenyi and Kibuye were pooled in the following analyses.

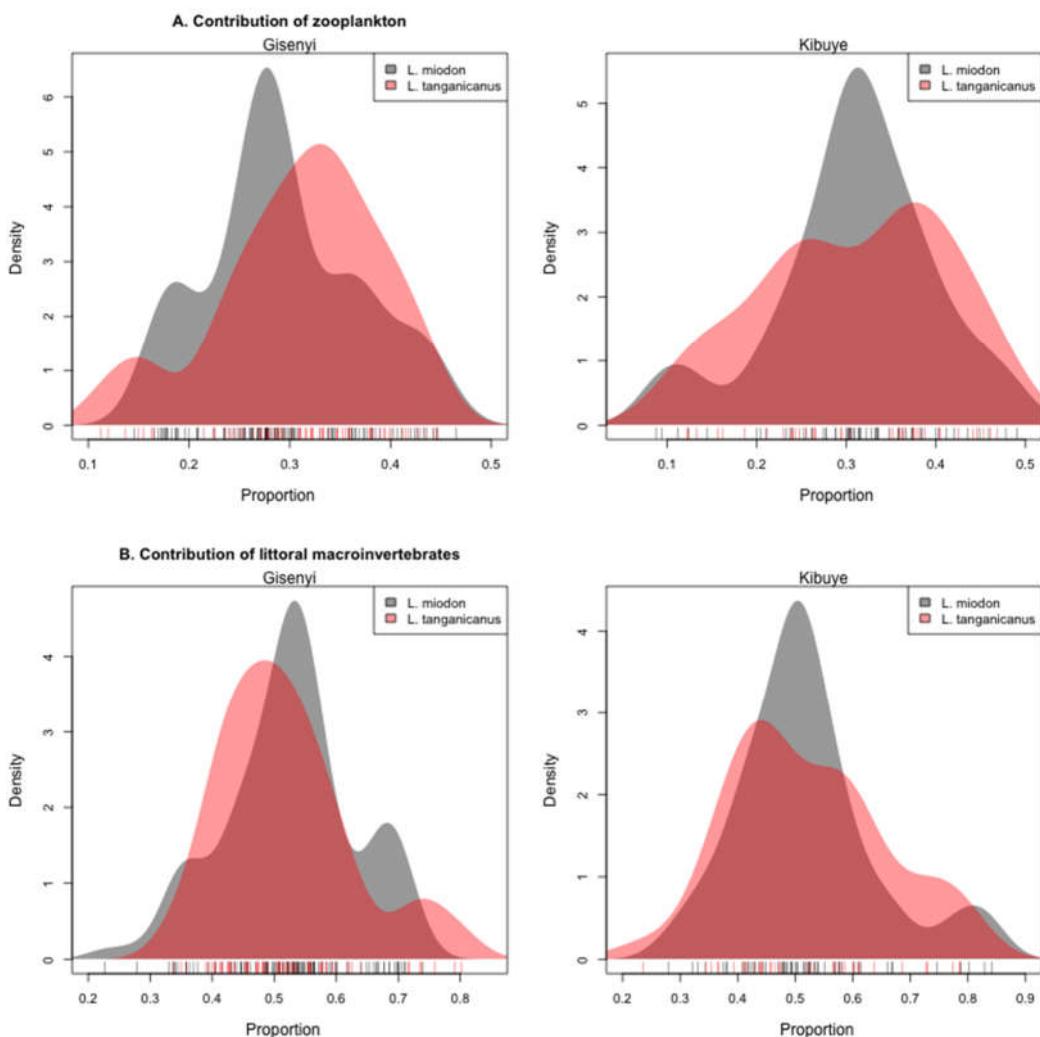


Figure 65. Distribution of the estimated contribution of zooplankton and littoral macro-invertebrates for all individual fish (*L. miodon* and *L. tanganicanus* at Gisenyi and Kibuye).

Further analyses of the estimated contributions of prey items revealed temporal changes in the contribution of littoral versus pelagic prey items (Figure 66, PERMANOVA season effect  $F_{3,277}=3.7$ ,  $p=0.01$ ). For both *L. miodon* and *L. tanganicanus*, the contribution of

littoral macroinvertebrates increased similarly (PERMANOVA species effect  $F_{1,277}=0.1$ ,  $p=0.75$ ) from 40 to 55% along the rainy season, while the zooplankton contribution decreased.

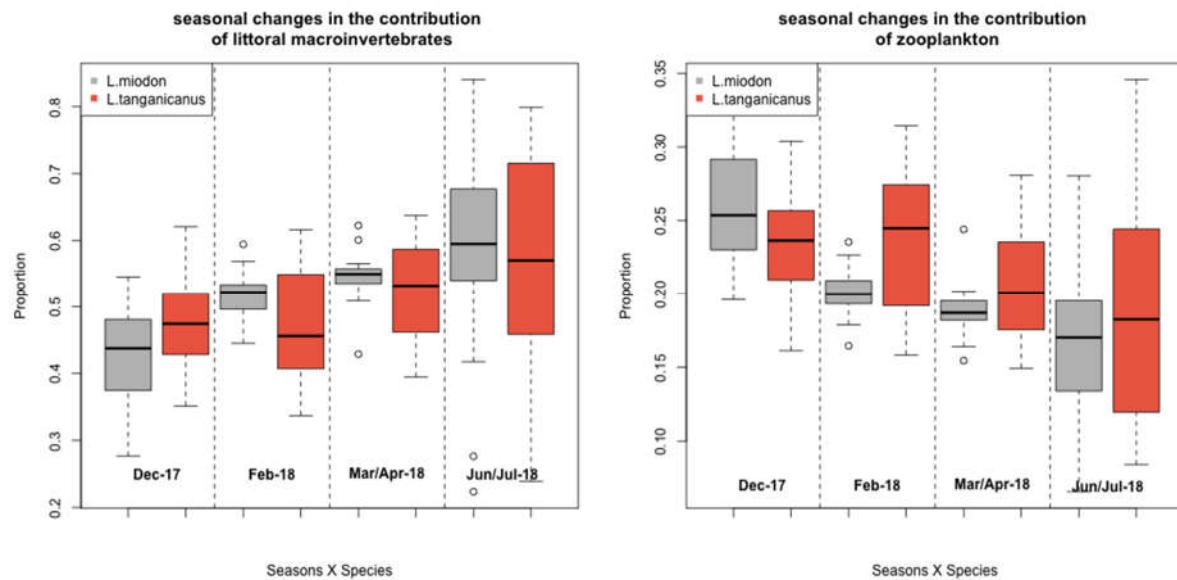


Figure 66. Temporal changes in the contribution of littoral versus pelagic prey items over the rainy season.

Besides, both fish species showed ontogenetic diet changes, juveniles below 65 mm being highly relying on littoral preys, shifting at maturity to a more-pelagic oriented diet (Figure 67a, PERMANOVA Size effect :  $F_{1,277} = 140.2$ ,  $p=0.001$ , species effect :  $F_{1,277}=0.19$ ,  $p=0.72$ ). Thereby, juveniles essentially inhabit the littoral habitats and move to the inshore and offshore pelagic habitats at maturity (Figure 67b), leading to a trophic segregation within both species according to age groups (Figure 67c).

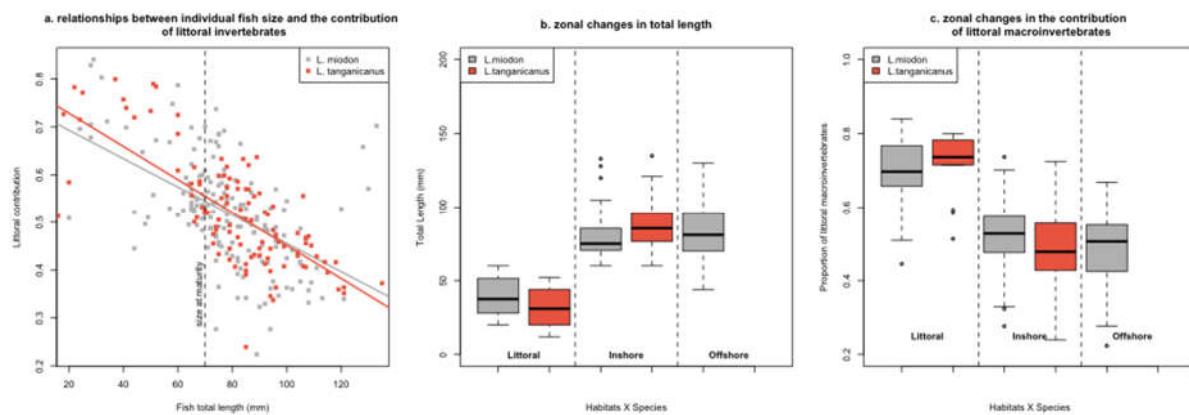


Figure 67. Ontogenetic dietary shifts. A. Relationships between preys contribution to fish biomass and fish individual size. B. Distributions of individual fish size within capture zones. c. Trophic segregation between habitats.

#### 4.7.5 Trophic competition between *L. miodon* and *L. tanganicanus*

Both species have wide isotopic niches covering the littoral to the pelagic foraging areas (Figure 68, Villeger & Cucherousset, 2015), that of *L. miodon* being even larger than *L. tanganicanus* (Indicator for richness : Irac=16.4 and 10.6 respectively). The divergence of individuals within their isotopic space was yet similar (IDiv=0.72 and 0.69 respectively) but the dispersion and evenness of individuals was higher for *L. tanganicanus* (IDis=0.27 vs 0.34), confirming the greater plasticity of this species. 55% of the total isotope surface was the overlap of the isotopic niche of both species, highlighting that more than half of individuals competed for the same food sources, while the isotopic niche of *L. tanganicanus* was almost totally embedded in that of *L. miodon* (Indicator for nestedness: INes=0.88, while 1 quantifies a perfect overlap), revealing that the colonizing species now occupies the trophic niche of the endemic *L. miodon*.

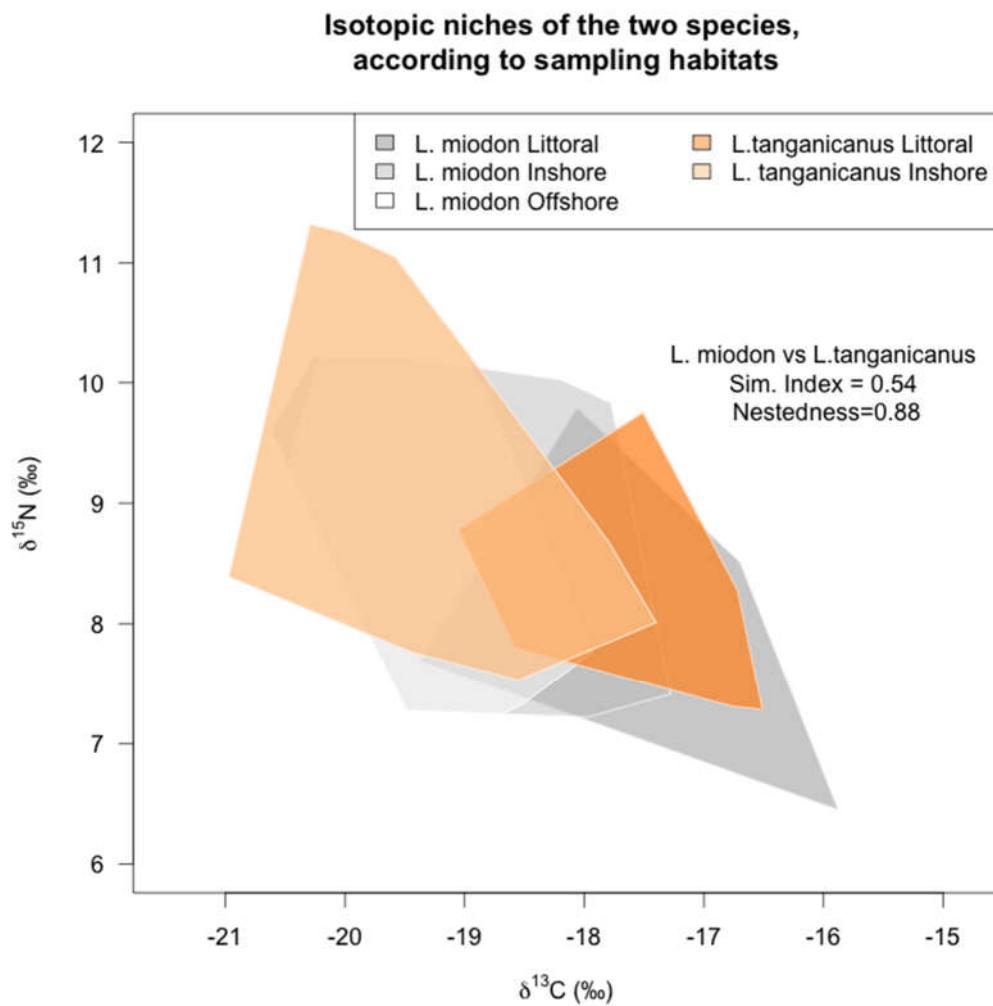


Figure 68. Comparison of the isotopic niche of both species. \*ISim (isotopic similarity) = ratio between the volume of the intersection and the volume of the union of the two groups of organisms in the stable isotope space. \*\*INes (isotopic nestedness): ratio between the volume of the intersection and the minimal volume filled by a group (Cucherousset & Villéger, 2015)

#### 4.8 Conclusions

The isotope composition of prey items analyzed in this study appears to be in the same range as previous studies in Lake Kivu (Masilya, 2011) and in other African lakes (Lake Albert, Campbell et al., 2005; Lake Tanganyika, Sarvala et al. 2003, Campbell et al. 2008). *L. miodon* and *L. tanganicanus* show wide and similar trophic areas, mainly composed of benthic macroinvertebrates and zooplankton, which is roughly congruent with gut contents analyses and with previous studies in the southern basin of Lake Kivu (Masilya, 2011). However, although zooplankton was highly predominant in the analyzed stomachs, this prey only represented 25% of the total prey contributions using stable isotopes, which is far below the contributions of planktonic items for *L. miodon* and *L. tanganicanus* (about 60% and 40% respectively) reported by Masilya (2011). Such a difference can be due to our ‘coastal’ sampling. More pelagic sampling may have provided different results, with a possibly higher contribution of planktonic items.

The high niche overlap confirms the potential competition for food in the area where both species occur. However, *L. tanganicanus* catches drastically collapse from inshore to offshore areas, where *L. miodon* is almost the sole species. *L. tanganicanus* is more linked to littoral habitat than *L. miodon*. The latter has been described to move from littoral to pelagic habitats during ontogeny, which was confirmed in the present study by the negative correlation between littoral prey contributions and fish size. The competition between the two species can occur during the larvae stage, which has never been studied. These results are congruent with Masilya (2011).

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## **Chapter 5. Synthesis, discussion and recommendations**

### **Reinforcement of plankton capacities**

The HPLC system installed at LKMP laboratory in Rubavu is fully operational, with the capacity to provide reliable data on phytoplankton biomass and composition in the framework of the monitoring program of Lake Kivu. The staff has been trained not only in the analysis of marker pigments in the phytoplankton extracts, but also in the processing of the pigment concentrations to obtain reliable estimates of chlorophyll *a* concentration and of the biomass of the phytoplankton classes. Technical guidelines have been provided for a correct use of the system and of the software for processing the analytical data.

The undergoing analysis of these data shall reveal their dependence to environmental variables, including weather data, which drive the behaviour of the water column of the lake at different time scales. A draft of a publication is currently being written based on the data collected in 2018 and 2019.

### **Guidelines for the plankton monitoring and recommendations**

A valid comparison with earlier data, for instance to assess changes that may occur from the operation of the gas extraction plants, will not be possible if regular monitoring of all relevant variables is not ensured. In particular, we recommend sampling phytoplankton and measuring limnological variables (including nutrients) twice a month at least at the monitoring site off Gisenyi. In the future, it will be necessary to differentiate the changes brought about by plant operation from those due to other environmental changes. This will not be possible if data collection is interrupted for long periods of time. For instance, a recent paper based on the data collected between 2002 and 2015 in Lake Kivu during various projects allowed to provide evidence of a substantial change of phytoplankton composition and biomass attributable to changes in the mixing dynamics of the lake, likely driven by a change in weather pattern (Rugema et al., 2019). Such a study would not have been possible without regular sampling of the lake for a dozen years, with sufficient detail on phytoplankton composition, using the same techniques. That paper also illustrates that, if the abundance of phytoplankton classes is adequate for routine monitoring of the lake's ecosystem, assessing the cause(s) underlying a change will require more detailed information on the phytoplankton composition at the genus or species level. The approach combining class abundance and further identification of dominant taxa has been used successfully for the assessment of lake ecological status (Sarmento & Descy, 2008). Therefore, we recommend conserving, at least once a month, a decanted sample taken in the mixed layer, and preserved with formalin, in

order to enable further identification of the main phytoplankton taxa by microscopy. Examination of these samples should be made by an expert in phycology, whenever necessary.

Although this aspect was not included in the present project, sampling zooplankton has been carried out at both sites, and data on zooplankton abundance and biomass in the 0-60 m layer have been collected since 2012. Zooplankton is obviously a key link in the food web, depending on phytoplankton production and of predation by planktivorous fish. In the perspective of developing a predictive model of fish production (see below), good data on zooplankton composition and abundance, thus allowing good estimates of zooplankton biomass and production, will be necessary. Therefore, the validation of the zooplankton data by an expert, for example by re-examination of preserved samples, is important. The same validation step would be useful in the framework of a second phase of the project.

#### Main outcomes of the macroinvertebrate study and recommendations for the monitoring

The extensive research carried out on Lake Kivu during the fish and plankton studies provided additional knowledge on the structure and functioning of the biological communities (phytoplankton, macroinvertebrates and pelagic fish). If some aspects of the lake ecosystem have been studied for long and some biological indicators are part of the ongoing monitoring, other compartments like benthic macrofauna have not been documented in detail since 1954. The results obtained in Gisenyi in 2017 using a standardized procedure, showed 43 different taxa (dominated by Diptera Chironomidae), some taxa being associated to good ecological value (Ephemeroptera *Povilla* and Trichoptera *Ecnomus*).

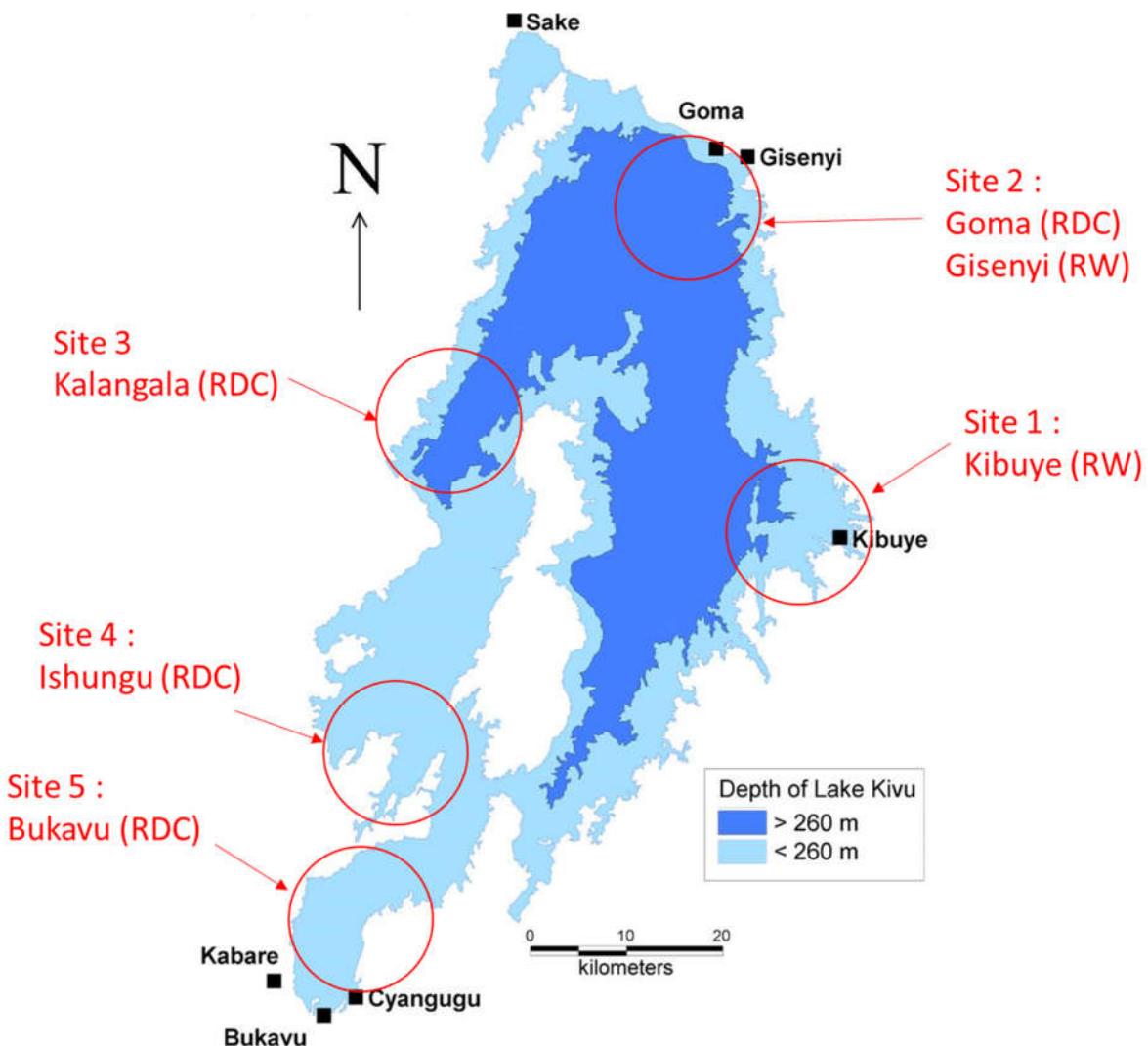
The quality of benthic macrofauna communities is difficult to assess given the absence of a multi-site sampling approach and the lack of comparable data in other tropical systems due to disparities in sampling protocols. However, comparisons with old data and with other large African lakes based on the listed species prefigure an important taxonomic diversity in Lake Kivu, although restricted to the oxygenated (<40m) layer. Given the fundamental role of macroinvertebrates in organic matter decomposition as well as being important food items for littoral fish communities, this compartment should be further studied in other areas in Lake Kivu (both in Rwanda and in DRC), using the same standardized procedure. Moreover, macroinvertebrates are good integrators of any change in environmental variables, although they are restricted to the inshore area (< 50m depth). A multi-site approach in Lake Kivu will allow defining regional indicators based on specific taxa identified to the generic level and information on their species traits (Lawrence et al., 2010), to serve as reference and to study effects of environmental change, climate change and/or impacts of methane gas extraction. Macroinvertebrates should therefore be added in the monitoring program in Lake Kivu.

### A lack of global approach on fish community and fishery

In aquatic environment, fish are integrative organisms, being sensitive to any disruption in the lake ecosystem functioning. A change in fish community and/or a decrease in catches may have potential important consequences for human population and lake biodiversity, especially when the observed change is irreversible. Therefore, fish and fishing should represent a major part of the monitoring effort. Unfortunately, the actual situation of the monitoring shows a partial vision of the ecological status of fish community and fishing activities: first fishery statistics are not yet available and crucial information related to fishers expectations are lacking, making it difficult to define what is a sustainable fishing in Lake Kivu. Secondly and perhaps the most important things, the present fish biodiversity, the species representativeness in the fish community and species distribution within the lake are unknown.

*L. miodon* is the most abundant pelagic fish, and cichlids are the most common family in the littoral zone. Hydroacoustic surveys have provided assessments of fish biomass in pelagic zone, and the present study allowed to better know the growth, the reproduction and feeding behaviour of *L. miodon* and *L. tanganicanus*. However, high uncertainty remains regarding the genetic intra- and interspecific diversity and the potential new species discovered in 2012, the observed differences between the lake basins. Another question is the evolution of fish population after a few years of Kivuwatt gas extraction in Kibuye bay. Currently, the respective effects of climate change, gas extraction, and other local drivers (water pollution, shore and inflows artificialization, overfishing,...) on fish community and fishing are still difficult to disentangle. To address those important questions, a global approach is needed for the fish monitoring. Coordinated campaigns associating physico-chemistry, macroinvertebrates, plankton and fish sampling should be performed basin by basin all over the lake, with or without the presence of gas extraction platforms. A link between the water column and shore characteristics with the fish community and fishing activity is highly recommended to better understand the consequences of human impact on fish diversity and density. To do this, we propose that at least 5 sampling sites, representative of the main basins, should be investigated and monitored: in the north the Gyseni/Goma arear, in the west the Kibuye area, in the east the Kalangala area and in the south the Ishungu basin and the Bukavu basin. To facilitate the organization and the distribution of the field work, two sites per year could be sampled in this way. Thus, each site would be repeated every two or three years, which corresponds to the natural turnover of the lake biocenosis.





For each site, it is recommended to jointly organize physico-chemistry, plankton, macroinvertebrate and fish sampling. For fish, the hydroacoustic survey should be done in parallel of the gillnets setting. For gillnet approach, all lake compartments (littoral habitat, pelagic and demersal zone) should be simultaneously sampled using a standardized protocol (CEN - EN 14757, 2015). All fish caught will need to be photographed, stored and prepared for further genetic and systematic analyses. For fisheries statistics, the fishers located around the sampling site should be contacted to accurately estimate their catches and interests. To build the capacity of local people (LKMP, ISP Bukavu and other local Universities), it is recommended to form a multidisciplinary team made up of African managers and external experts. This team would be responsible for field sampling, data analysis, interpretation and publication. Close international exchanges and training stays could also be organized.

Theme by theme, the following proposals can be detailed:

#### Modelling primary and fish production

As mentioned during the final workshop, an added value of data collected by LKMP since the beginning of the BTC baseline would be to use them for developing process-based or data-based models, with the objective of linking primary production and fish production, in order to predict fisheries yield in a context of environmental change and of large-scale methane exploitation.

The best option would be to develop a deterministic, process-based model, with the potential to assess the effects of environmental changes on the food web, including those related to methane harvesting and changes in weather pattern due to climate change. In the framework of the EAGLES project, the data acquired since 2002 (Ishungu, DRC) or since 2005 (Gisenyi, Rwanda) have been gathered in a limnological data base and a phytoplankton pigment data base. Both data bases, implemented with newly acquired data, not only allow investigation of the changes that have occurred in the lake mixolimnion (see e.g. Rugema et al., 2019), but also provide an excellent basis for model development. For instance, modelling studies were carried out during the EAGLES project, and some key steps have demonstrated the capacity of existing lake models to simulate the water column physical status of Lake Kivu, as driven by meteorological data (Thiery et al., 2014 a, b). Despite the application of an existing phytoplankton model, the efforts to simulate nutrient fluxes from diffusion and vertical mixing, which drive primary and secondary production as well as the oxygen budget of the lake's mixolimnion, should be pursued. Even though it is still imperfect, the modelling application demonstrated that an uncontrolled CH<sub>4</sub> exploitation at industrial scale in Lake Kivu would result into a dramatic shift of phytoplankton communities, and a rapid decline of ecosystem services including fish productivity and fisheries.

#### Monitoring of the biology and ecology of *L. miodon* and *L. tanganicanus*

Previous studies on pelagic fish composition in Lake Kivu pointed out that *L. miodon* has remained the prevailing species in the pelagic zone (e.g. Paris et al., 2013; Descy et al., 2015), representing more than 75% of the total fish catches in Lake Kivu (Descy et al., 2015). Sampling performed during the present study from fishing crews confirmed the predominance of *L. miodon* in the pelagic catches. The recently introduced *L. tanganicanus* occupies mostly the littoral habitat, although some individuals are sometimes captured in pelagic zones. The results obtained on compared biology and ecology of both species are consistent with previous studies from Kanningini (1995) and Masilya (2011) in the Bukavu Basin and the southern part of the lake. *L. miodon* is a highly reproductive (20-40'000 oocytes for a mature female), fast-growing and short-living (approx. 1.5 years) species, whereas *L. tanganicanus* shows lower fecundity rates (x100 oocytes), slower growth and longer lifespan (2 years or more). *L. miodon* was described to spawn close to the shore, then young-of-the-year move from littoral to pelagic habitats during ontogeny (Spliethoff & De Jongh, 1981), which was

confirmed in the present study by the negative correlation between littoral prey contributions and fish size. The competition between the two species could thus mainly occur during the larvae stage, before migration of *L. miodon* towards pelagic areas. Although both species may compete for food (mostly macroinvertebrates and plankton) when they live in the same habitats, this trophic competition seems to be restricted to the inshore zone where both species cohabit. However, the present study only focused on Gisenyi and Kibuye areas and cannot be extrapolated to the whole Lake. Complementary surveys on *L. miodon* and *L. tanganicanus* distribution and trophic positions should be carried out in other basins in collaboration with DRC, given the disparity of the lake functioning between basins. These surveys could also provide samples to continue the biological monitoring initiated in the present study. We recommend complementing some of the biological and ecological key features of *L. miodon* and *L. tanganicanus* in different basins: length-weight relationships (sampling of large individuals, analysis of season variability), growth curves refining with additional otolith analyses, fecundity analysis over time. In particular, fish growth survey is of great interest in the context of potential future ecological shift and climate change.

#### Assessment of pelagic fish stock and implementation of fisheries survey in Lake Kivu

Hydroacoustic techniques are a reliable and appropriate method to study pelagic fish stock over time in Lake Kivu. Indeed, in this lake, the littoral zone (< 50m depth) represents a small area and around 98% of the lake surface is pelagic. Moreover, *L. miodon* is the main species in the pelagic area, this was shown in several studies in Bukavu Basin and the southern part of the lake (Kaniningini, 1995; Masilya, 2011) and around Kibuye by Paris et al. (2013). As observed during the present study, this species mainly occupies the 15-30m layer and to a lesser extent the 30-45m layer, which is consistent with the use of hydroacoustics. Between 2012 and 2018, the total fish biomass showed variability in the pelagic zone of Lake Kivu, ranging between 1'000 and 4'000 tons with important spatial, seasonal and interannual variations. This variability is common in population dynamics of annual species like *L. miodon*, which can show high stock variations between years, depending on recruitment success. The assessed biomass appeared rather low compared to 2008 (5'000 tons in February and 6'000 tons in July; Guillard et al., 2012), and compared to the late 80s /early 90s hydroacoustic campaigns (3'445 to 5351 tons; Lamboeuf, 1991). However, in 2018, the total fish biomass increased in all basins and reached a maximum over the period with 4'000 tons. At a large temporal scale, the fish stock seems to be stable from the first monitoring surveys done by FAO.

Fish size distribution showed a majority of fish ranging between 3 and 8 cm in the pelagic area. During the high dry season, *L. miodon* size distribution displayed bimodal distributions with a first peak at 2-3 cm, which characterized the young-of-the-year, and a second peak between 5 and 6 cm. The dry period is therefore more relevant to conduct hydroacoustic campaigns, to get comparable data and encompass the newly recruited cohort. For future monitoring of fish stock in Lake Kivu, we would highly recommend carrying on the

annual hydroacoustic campaigns over the entire lake (all basins) during the dry season. Due to the lack of expertise of LKMP on hydroacoustics analyses after Alice Muzana's departure, complementary training should be provided to LKMP staff in order to become self-reliant on calibration, maintenance of the equipment, use of it, and echogram reading and data analysis. Anyway, additional support from experts will be necessary for statistical analyses and interpretation.

In parallel with the pelagic biomass survey, fisheries data should be gathered from fishermen in order to assess the fishing effort together with the total monthly catches. Little is known about fisheries status and actual fishing effort in Lake Kivu, since the FAO project Isambaza at the end of the 80's. A survey of fisheries was set up in 2011 and conducted for 2 years during the EAGLES project in 5 districts along the Rwandan shore of the Lake (Descy et al., 2015). In 2012 and 2013, more than 700 tons of *L. miodon* were caught by fishermen in Rwanda. Conversely, fisheries yield in DRC is still unknown, and statistics remain to be set up in a similar manner in both countries. Lake-wide fishery management is recommended but is presently impossible as long as fisheries statistics are not established in a similar way in both Rwanda and DRC.

Fish production may strongly fluctuate depending on variations in the regional climate that drives plankton productivity. Previous fishery surveys also showed seasonal variations of the catches that may be related to resource-dependent variations in the sardine stock and recruitment, rather than resulting from overexploitation. Higher catches at the beginning of the rainy season, followed by reduced catches in the dry season months, may be due to a greater abundance of large zooplankton following the phytoplankton peak, thus allowing growth to the young fish. Given the present knowledge regarding planktonic resource variations, mostly driven by climatic factors, fish production should be better linked to plankton availability, using data on weather, limnology, plankton and fisheries statistics. Therefore, we recommend conducting fisheries surveys over the whole lake, in parallel with annual hydroacoustic surveys, and relating the data from both surveys. Such data will be useful to achieve predictions of the fish yield, and for determining management objectives in order to ensure sustainable fishery (Lorenzen et al., 2016).

#### Fish biodiversity and intraspecific diversity

In a review on fish diversity and fisheries in Lake Kivu, Snoeks et al. (2012) reported poor fish fauna in Lake Kivu with 29 species comprising 15 endemic haplochromines. Experimental fishing using standardized multimesh gillnets (CEN - EN 14757, 2015) could provide comparable data of fish population structure between basins and over time. The approach was applied in Kibuye bay in 2012 and showed a higher fish diversity than expected, especially in littoral habitats, with potentially new undescribed haplochromine species (Paris et al., 2013; Guillard & Richard, 2017). Therefore, additional knowledge should be acquired on fish species distribution according to depth and habitat in Lake Kivu using standardized sampling, coupled with genetic analyses of Cichlids.

Moreover, it would be of great interest to have an update of the genetic structure and effective population size of *L. miodon* populations for the rational and sustainable exploitation of these natural resources in Lake Kivu. Thirty-four years after its introduction, *L. miodon* populations showed less genetic diversity than expected, suggesting that high post-introduction mortality and various demographic factors reduced the effective population size to tens rather than thousands of individuals (Hauser et al., 1995). Same authors emphasized that the reduced mtDNA diversity found in the *L. miodon* of Lake Kivu may therefore have some significance for the long-term persistence of the species in the lake. Twenty years after this previous study, and about 55 years after the first introduction of the species, genetic differentiation should have occurred in Lake Kivu on a very small geographical scale, even if fish exhibit large capabilities of migration, as observed in Lake Tanganyika (Hauser et al., 1998).

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## **Appendix**

- Annex 1: Estimation of Cyanobacteria Biomass by Marker Pigment Analysis
- Annex 2: Standards and calibration curves
- Annex 3: Guidelines for the use of the Alliance HPLC system
- Annex 4: Standard operating procedure for Sea Bird data extraction
- Annex 5: Tutorials for head dissection of *L. miodon* and *L. tanganicanus*
- Annex 6: Detail of the dataset for length / weight analyses