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Food resources of Lake Tanganyika sardines

Metabarcoding of the stomach content of *Limnothrissa miodon* and *Stolothrissa tanganicae*

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Abbreviations

COI – cytochrome c oxidase subunit 1 gene

EBFM – Ecosystem-Based Fisheries Management

ITCZ – Intertropical convergence zone

LM – *Limnothrissa miodon*

LT – Lake Tanganyika

Mya – million years ago

N – nitrogen

P – phosphor

PCR – polymerase chain reaction

PCoA – principal coordinate analysis

SDG – Sustainable Development Goals

SL – standard length

ST – *Stolothrissa tanganicae*

Summary

The sardines from Lake Tanganyika in Africa are an important food source for millions of people living around the lake. Stocks of *Stolothrissa tanganicae* and *Limnothrissa miodon* have been declining over the years due to overfishing, climate change and other anthropogenic factors. It is important to have ecosystem-based fisheries management (EBFM) to avoid overfishing in the lake. To establish EBFM, it is vital to assess the biology and ecological interactions of the fish. The Lake Tanganyika sardines are an important link between zooplankton and larger fish within the pelagic food web. This study conducted a metabarcoding technique on the stomach content of the sardines to examine their prey item composition. It investigated whether the prey composition in the stomach varied between sardine species, location, season, sex and length. The results showed significant differences in prey composition between species. *Limnothrissa miodon* had a more diverse diet compared to *S. tanganicae*. A significant difference was also found between locations and seasons, probably due to the limnology of the lake and the spatial and temporal variability of both biotic and abiotic factors. The diet composition varied with varying standard length for *S. tanganicae* but not for *L. miodon*. Genetic material from multiple phyla was found in the stomachs of the sardines, including cnidarian DNA. Future changes in population dynamics of both the sardines and their prey due to overfishing, climate change or other anthropogenic factors could impact the whole pelagic ecosystem. It is important to identify these dynamics and establish international EBFM plans.

Samenvatting

De sardienen van het Tanganyikameer in Afrika zijn een belangrijke voedselbron voor de miljoenen mensen die rond het meer leven. Het visbestand van *Stolothrissa tanganicae* en *Limnothrissa miodon* is de afgelopen decennia afgenomen door overbevissing, klimaatverandering en andere antropogene factoren. Het is belangrijk om een ecosysteem gebaseerd visserijbeheer (EBFM) te hebben om overbevissing tegen te gaan. Om een EBFM op te stellen, is het essentieel om de biologie en de ecologie van de vis te bepalen. De sardienen van het Tanganyikameer vormen een belangrijke schakel tussen zoöplankton en grotere vissen in het pelagische voedselweb. Deze studie gebruikte een metabarcodingstechniek op de maaginhoud van de sardienen om hun mogelijke prooisoorten te identificeren. Het onderzocht variatie in de maaginhoud tussen sardiensoort, locatie, seizoen en standaard lengte. De resultaten toonden een significant verschil in samenstelling van prooisoorten tussen de sardiensoorten. *Limnothrissa miodon* had een gevarieerder dieet vergeleken met *S. tanganicae*. Een significant verschil werd ook gevonden tussen locaties en seizoenen, waarschijnlijk als gevolg van de limnologie van het meer en de ruimtelijke en temporele variabiliteit van zowel biotische als abiotische factoren. De samenstelling van het dieet varieerde met variërende standaardlengte voor *S. tanganicae* maar niet voor *L. miodon*. DNA van meerdere phyla werd gevonden in de magen van de sardienen, inclusief DNA van neteldieren. Toekomstige veranderingen in de populatiedynamiek van zowel de sardienen als hun prooi als gevolg van overbevissing of klimaatverandering kunnen het hele pelagische ecosysteem beïnvloeden. Het is belangrijk deze dynamieken te identificeren en internationale EBFM-plannen op te stellen.

1 Introduction

1.1 Understanding Ecosystem-Based Fisheries Management

Freshwater habitats contain 42% of the known fish species. They are one of the most threatened ecosystems worldwide (Lynch et al., 2017). Forty percent of the global fish catches originate from inland fisheries (both catch and aquaculture) (Lynch et al., 2016). It is crucial to have an effective management plan to avoid overexploitation of the living resources (Pikitch et al., 2004). Sustainable fisheries are an important element for sustainable development. They contribute to food security, sustainable livelihoods and poverty mitigation (Diz et al., 2017). In the past, fisheries relied mostly on Single-Species Management, where the focus was on the protection of a single species. Ecosystem-Based Fisheries Management (EBFM) considers ecosystem interactions and focusses on the management and conservation of the entire ecosystem (Pikitch et al., 2004; Plisnier et al., 2018). An EBFM acquires knowledge on ecosystem processes, avoids degradation of the ecosystem, limits irreversible changes of the ecosystem and establishes and maintains a sustainable socio-economic relationship (Pikitch et al., 2004). In order to apply an EBFM, eco-trophic models of the ecosystem have to be acquired and refined (Pikitch et al., 2004).

Freshwater lakes and their dynamics are often very variable and unpredictable. Several factors could improve the understanding of the ecosystem dynamics. One of them is the insight in the hierarchical relationship of key organisms. Nutrient loading and the energy transfer system determine lake productivity. However, the food web interactions determine the fulfilment of this potential (Carpenter, 1988). In pelagic ecosystems, producers and consumers both influence the structure and productivity of the food web. The nutrient source, mixing and other abiotic factors determine the potential biomass. Consumers determine the actual biomass and structure of the pelagic food web (Carpenter, 1988). Previous studies show that an increase in piscivores decreases planktivore abundance, a rise in planktivores impacts the zooplankton biomass and composition and a rise in zooplankton reduces phytoplankton biomass due to trophic cascades (Scheffer et al., 2005). In oligotrophic lakes, the effect of the consumers is most powerful at the phytoplankton and herbivorous level of the food chain. The strong consumer effect moves up to the piscivorous level when the eutrophy of the lake increases (Carpenter, 1988).

The sardines of Lake Tanganyika are both ecologically and economically important species. However, not much is known about their feeding habits. It is important to analyse the diet composition of the sardines, and its spatial and seasonal variability. A diet analysis will improve the understanding of their role in the ecosystem and the food web. These findings will be vital to establish EBFM.

A detailed and accurate analysis of the diet composition of species within the ecosystem is vital to establish aquatic food-web interactions (Albaina et al., 2016). There are several techniques to identify the diet of a species. The traditional method is visual by looking at the morphology of organisms found in the stomach content. This technique requires taxonomic experts on the prey taxa found and is very time-consuming. Additionally, some prey individuals are too degraded by digestion to be identified (Jakubavičiute et al., 2017). Another approach to identify the diet of organisms is to apply high throughput sequencing technologies on the stomach content. One of these technologies is DNA metabarcoding (Albaina et al., 2016). In this technique, one or few DNA regions (barcodes) for each specimen within a sample are amplified and sequenced. It is very sensitive and can identify traces of DNA within an environmental sample or the stomach content (Albaina et al., 2016; Vamos et al., 2017). Metabarcoding is not fully ready to estimate the relative abundance of an organism within a sample. Analysis of relative abundances by metabarcoding should be approached carefully. Estimates of the relative abundance are more accurate within samples with a limited number of taxa (Albaina et al., 2016). Metabarcoding is able to identify even small and fragile species within stomach samples that were not detected visually. In a study by Albaina et al. (2016), metabarcoding showed that the European sardine (*Sardina pilchardus*) feeds on planktonic larvae of echinoderms, a previously undetected prey item (Albaina et al., 2016). Metabarcoding has a few disadvantages. It only provides the prey species ingested by the predator at a given moment, not an average of the diet. This is especially important since diets can vary according to spatial and temporal variables (Churchill et al., 2015; Jakubavičiute et al., 2017). Furthermore, sequences that result from metabarcoding also require a reference database. A complete, accurate and precise database is needed to identify species found within a sample (Albaina et al., 2016; Yang et al., 2017). Several errors could occur during the process of metabarcoding, for example due to the trade-off between the discriminatory factor and barcode amplification efficiency (Albaina et al., 2016). These errors could reduce the quality of the DNA and the detection of possible prey species (Albaina et al., 2016; Jakubavičiute et al., 2017). A method to detect the trophic level of an organism is the stable isotope analysis (isotopes $\delta^{13}C$ and $\delta^{15}N$). The ratio of stable isotopes found in the tissue of the predator reflects the ratio of stable isotopes present in the prey (Churchill et al., 2015). This technique gives a general idea of the prey species consumed by the predator. The isotope $\delta^{13}C$ additionally gives an idea of the origin of the prey and where primary production takes place. The isotope $\delta^{15}N$ is often enriched from one trophic level to the next and is used to estimate the trophic level of an organism (Churchill et al., 2015). A disadvantage of this technique is that the ratio of the isotopes can easily vary within taxa. Similar ratios may represent similar trophic levels or niches, but different diets and specific prey species cannot be identified by using isotopes (Churchill et al., 2015). A stomach content analysis (e.g. metabarcoding) can identify prey species up to a lower taxonomic level than the stable isotope analysis (Churchill et al., 2015). The best technique to determine trophic

relationships and diet compositions is to combine the different techniques and compare the results (Albaina et al., 2016; Jakubavičiute et al., 2017; Churchill et al., 2015).

This thesis will focus on the metabarcoding technique used on the stomach content of two sardine species in Lake Tanganyika.

1.2 Lake Tanganyika

1.2.1 Geology and geography

Lake Tanganyika, situated in the East African Rift System (Fig. 1), is the second oldest and second deepest lake in the world (Coulter, 1991). It is surrounded by four countries. A large proportion of the eastern section of the lake belongs to Tanzania, the western part to D.R. Congo. Burundi lies at the northern end and Zambia on the south. Arab traders called the lake 'Sea of Uniamesi'. The first map of the lake made by J.J. Erhardt, reached Europe in 1855. Although it was believed for some time that the lake was a relict Jurassic sea, Lake Tanganyika was probably never part of any sea or ocean (Coulter, 1991; Wilson et al., 2008).

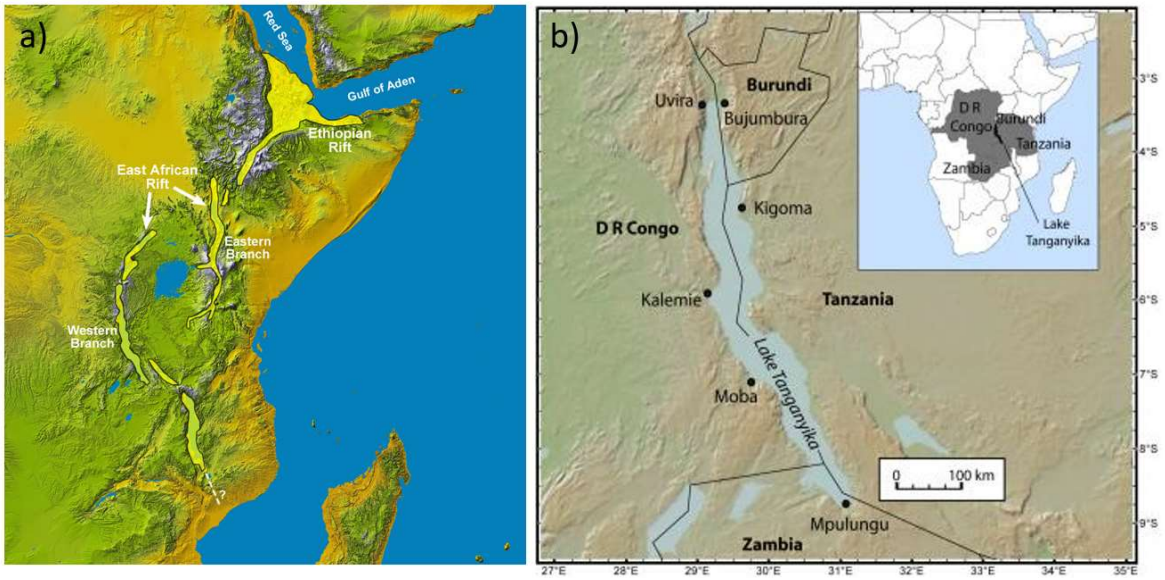

Figure 1: Images of a) The East African Rift (Wood and Guth, n.d.) and b) Lake Tanganyika geographical location (Tierney, 2010).

The East African Rift divides the African continent into the Somalian plate to the east and the African plate to the west (Coulter, 1991). Rifting gave rise to the African Great lakes like Lake Victoria, Lake Malawi and Lake Tanganyika. The rift basins for both Lake Tanganyika and Lake Malawi were formed approximately 25 million years ago (Mya) when the western branch of the East African Rift System, the Albertine Rift, started to break (Danley et al., 2012; Plumptre et al., 2007). Lake Tanganyika itself originated around 9-12 Mya and is situated in a deep and narrow trough 773 m above sea level (Nkotagu, 2008; Plisnier et al., 1999). It is 650 km long, on average 50 km wide and at its maximum 1410 m deep (Plisnier et al., 1999). The volume of the lake is 18,940 km^3, which means that it holds 15% of the world's total freshwater

volume originating from lakes (Langenberg at al., 2008). Lake Tanganyika is oligotrophic, chlorophyll a production in surface pelagic waters is low while light transparency is high (Coulter, 1963; Plisnier et al., 1999; O'Reilly et al., 2003; Naithani et al., 2011). The lake has a stagnant anoxic and H_2S-charged monimolimnion below 200 m. This means that three-quarter of the lake's water volume contains an inadequate amount of dissolved oxygen to support life (Beadle, 1974). The lake is divided into three basins. The shallow and narrow Kigoma basin in the north, the central Kungwe basin and the Kipili basin to the south (Plisnier et al., 1999; Langenberg et al., 2008). These basins have been connected and disconnected throughout the ages, depending on the water level. 106 thousand years ago was the last time the basins were disconnected (Danley et al., 2012). The coastline is mostly precipitous, except for the northern and southern end. The coast is generally rocky with stony or sandy beaches (Beadle, 1974). There are only a few small rivers that form the relatively poor drainage system of the lake. This is due to the high mountain ranges which form a barrier. The major inlets are the Rusizi river in the north and the swampy Malagarasi river in the east. The major outflow of the lake is the Lukuga river in the west which flows to the Congo basin (Beadle, 1974; Nkotagu, 2008). However, the main source of water loss is evaporation (Beadle, 1974).

1.2.2 Limnology and seasonality

Lake Tanganyika lies on the migration path of the Intertropical convergence zone (ITCZ). This causes the lake to experience a wet and dry season. The short dry season stretches from June to August and the long rainy season from September to May (Danley et al., 2012). At the beginning of the dry season, south-east trade winds cause a movement of warm surface waters towards the northern end of the lake (Langenberg et al., 2008). From there, cold deep water currents flow back to the south. In the south, these deep water currents rise to the surface. The cold deep water layer is nutrient rich. With the rise or upwelling of these waters, there is also an upwelling of nutrients in the south. The trade winds coincidence with the coldest annual temperature. Cold air temperatures combined with the upwelling of cold water lead to the cooling of surface water in the south. It creates a greater mixing and so increases nitrogen (N) and phosphor (P) concentrations (Plisnier et al., 1999). N and P concentrations control phytoplankton growth, biomass and species composition and their increase results in an increase of phytoplankton biomass (Plisnier et al., 1999; Xu et al., 2010). In September, at the end of the dry season, the strong winds cease and the upwelling in the south stops. The metalimnion continues to oscillate over several months until it returns to a horizontal position. During these months, there is an increase in water movements. The oscillations cause a secondary upwelling in the north around October-November. Deep nutrient-rich water rises to the surface and causes phytoplankton blooms (Plisnier et al., 1999). It was first thought that these phytoplankton blooms were caused by an increased nutrient input due to land surface runoffs caused by the heavy rains during the wet season. A delay between the increased rainfall

and the algal blooms suggested that the secondary upwelling played a more important role (Plisnier et al., 1999). Other studies suggested that the Ruzizi river in the far shallow and narrow northern end of the lake may affect its physical and chemical properties to a greater extend (Langenberg et al., 2008). 30% of the total riverine input in Lake Tanganyika originates from the Rusizi River. The water of this river is heavily loaded, has a high density and a low temperature. It was generally accepted that the river inflow in the lake descents to the hypolimnion (Vandelannoote et al., 1999). Vandelannoote et al. (1999) found that the inflow of the Rusizi actually mixes with the epilimnion, not the hypolimnion. Rusizi deposits could increase inorganic N and alkalinity offshore from the river mouth, which contributes to the total nutrient concentrations in the north (Vandelannoote et al., 1999). Rainfall influences the river flow of the Rusizi and its sediment load. The river flow increased during and after the wet season and decreased during and after the dry season (Vandelannoote et al., 1999). However, according to Coulter (1991) riverine input has a limited influence on the pelagic ecosystem due to the long water turnover time based on riverine inflow (around 1000 years). The oscillations at the beginning of the wet season probably cause strong surface waves in the south which are locally known as 'Chimbanfula', meaning digging for rain. From February until May, the lake remains relatively calm (Plisnier et al., 1999).

1.2.3 Biodiversity

Lake Tanganyika has a high biodiversity, with over 2000 aquatic fauna and flora species of which at least 700 are endemic (Nkotagu, 2008). Most species occur in the littoral (0-10 m) and sub-littoral (10-40 m) zones. The alternating sandy and rocky substrates provide various isolated habitats that encourage divergence and speciation (Beadle, 1974; Nkotagu, 2008). The benthic environment (20-200 m) has an upper mud layer of organic material. Many fish and invertebrates (e.g. insect larvae, molluscs and ostracods) live in the benthic zone. They feed on organic detritus from the higher water layers (Beadle, 1974). The pelagic environment (100-200 m) is relatively poor in species richness (Nkotagu, 2008). Cichlid fish represent the largest proportion of endemic species in Lake Tanganyika (Nkotagu, 2008; Van Steenberge et al., 2011). Over 255 species of cichlids occur in Lake Tanganyika of which 250 are endemic (Snoeks, 2000). They show an exceptional adaptive radiation which has intrigued biologists for decades and became the topic for many ecological and evolutionary studies (Kocher et al., 1993; Clabaut et al., 2007; Irisarri et al., 2018). There are 145 species of non-cichlid fish, divided into 21 families (Kawanabe et al., 1997). The non-cichlid fish, molluscs and crustaceans of Lake Tanganyika show a high diversity in morphology and behaviour (Nkotagu, 2008).

1.2.4 The pelagic ecosystem

As mentioned before, the pelagic environment of Lake Tanganyika is relatively poor in species diversity. Several studies reported phytoplankton species of the phyla Diatomea, Cyanophyta, Chlorophyta, Chrysophycea, Cryptophycea, Euglenophyta, Protozoa and Peridineae. None of

these are endemic. Endemism in phytoplankton is rare. This is probably a result of the swift dispersal of both their living cells and rest stages (Coulter, 1991). The pelagic zooplankton community is relatively simple and contains species of shrimps, copepods and medusae. The copepods include the calanoid species *Tropodiaptomus simplex* and the smaller cyclopoid species *Mesocyclops aequatorialis* and *Tropocyclops tenellus* in the north and *Microcyclops cunningtoni* in the south. There are several species of freshwater shrimp within the genera *Limnocaridina* and *Macrobrachium*. The single Cnidaria species is the medusa *Limnocnida tanganjicae* (Sarvala et al., 2003). The zooplankton community experiences a high predation pressure. The high predation pressure together with a low seasonality explains why the zooplankton community is relatively simple, a trait often found in tropical lakes (Coulter 1991). The pelagic fish community of Lake Tanganyika involves two clupeid species, four centropomid species and several cichlid species (Shirakihara et al., 1992; Koblmüller et al., 2014). They are all endemic and show an offshore fish community not seen in other East African lakes since these other lakes are generally dominated by Cichlids (Coulter, 1991; Kawanabe et al., 1997). The planktivorous sardines, both of the family *Clupeidae*, consist of *Limnothrissa miodon* and *Stolothrissa tanganicae*. The four *Centropomidae* or *Latidae* species (Perciformes) that can occur in the pelagic zone are *Lates stappersii*, *L. microlepis*, *L. angustifrons* and *L. mariae* (Coulter, 1991; Shirakihara et al., 1992; Kawanabe et al., 1997). The largest known cichlid *Boulengerochromis microlepis* is a deep pelagic species within the tribe *Boulengerochromini* (Koblmüller et al., 2014). There are also multiple other cichlid tribes in the pelagic zone, which mainly consist of deep water species. These species belong to the tribes *Bathybatini* (*B. fasciatus*, *B. leo* and *B. minor*), *Hemibatini*, *Trematocarini, Cyprichromini* and *Benthochromini* (Koblmüller et al., 2008; Kirchberger et al., 2012).

The structure and productivity of the pelagic food web in Lake Tanganyika has a highly efficient carbon transfer from primary producers to fish production. The rate of carbon transfer is comparable with that of the most efficient marine fisheries (Coulter, 1991). Lake Tanganyika is an oligotrophic lake and hence has a limited amount of nutrients. The microbial food web plays an important role within these nutrient-poor conditions. The large amount of organic matter discharged by phytoplankton during primary production is processed by heterotrophic bacteria. The increased biomass of picoplankton has a positive effect on the total biomass of phytoplankton under oligotrophic conditions (De Wever, et al., 2007). The amount of nutrients and plankton in the lake shifts strongly within time and space (Langenberg et al., 2008). The phytoplankton community of Lake Tanganyika is able to rapidly take up nutrients and use them for growth. This strategy is needed in a lake with irregular patterns of mixing and upwelling (Järvinen et al., 1999; Langenberg et al., 2008). The autotrophic organisms, mainly phytoplankton, show a high seasonal and spatial variability in biomass. During the dry season, an increase in diatom biomass is observed in the north of the lake. This increase is caused by

the oscillations of the thermocline and mixing of the water. In the south, it is mainly the autotrophic picoplankton and small eukaryotic phytoplankton which increase in biomass during the dry season. Large phytoplankton does not seem to increase in the south during the dry season (De Wever et al., 2007). In the north, a higher mixing zone to euphotic zone ratio, differences in N:P ratio and changes in the availability in iron probably cause the difference in phytoplankton biomass when compared to the south (De Wever et al., 2007). However, other researchers suggest that the higher phytoplankton biomass in the northern basin is caused by the rather shallow environment and the nutrient inflow from the Rusizi river (Langenberg et al., 2008). Cyanobacteria can create dense algal blooms in Lake Tanganyika, probably when the pH is high (ca. 9) with the resulting low concentration of free carbon dioxide, and/or during a shortage of nitrogen. These cyanobacterial blooms seem to consist generally of species from the genus *Anabaena*. This genus and other cyanobacteria are more efficient in acquiring CO_2 in warm climates when compared to other algae (Salonen et al., 1999). They can produce cyanotoxins which can be harmful for other organisms (Kimambo et al., 2019).

The zooplankton species of *Tropocyclops* and *Limnocaridina* probably feed on picocyanobacteria and/or larger nitrogen-fixing cyanobacteria. Other larger zooplankton species possibly have a more mixed diet of different phytoplankton or zooplankton species (Sarvala et al., 2003). The zooplankton community in the north is dominated by *Cyclopoida* and medusae (Kurki et al., 1999; Langenberg et al., 2008). Here, an increased stratification causes a reduced nutrient loading. This process favours smaller cyclopoid species (Langenberg et al., 2008). The northern area is rather shallow which favours the polyp stages of *Limnocnida tanganjicae* (Kurki et al., 1999). The amount of calanoids in the south is larger than in the north and is probably similar to the amount of Cyclopoida (Kurki et al., 1999; Langenberg et al., 2008). In the south, the number of diatoms increases during upwelling. A higher concentration of diatoms could favour the growth of the larger Calanoids and the planktivorous *M. aequatorialis* (Langenberg et al., 2008). The total abundance of copepods near Mpulungu in the south is low when compared to the north. In the south, shrimps are the dominant zooplankton in contrast to the north where the shrimp biomass is lower (Mannini et al., 1999). The south has a higher primary production than the north due to several environmental factors (e.g. dept water mixing, N:P ratio and iron concentration) (De Wever et al., 2007). A higher phytoplankton biomass corresponds with an increased biomass of zooplankton species like the copepod *Tropodiaptomus simplex* (Narita et al., 1986). These findings support the higher total zooplankton biomass found in the south compared to the north (Langenberg et al., 2008). The majority of the zooplankton migrates diurnally. During the day, they stay at 50-120 m dept. At night they rise to the surface above 10 m (Beadle, 1974). Copepod species in the south tend to have a stronger vertical migration pattern than in the north. Possibly due to an increased predation pattern by planktivorous fish (Langenberg et al., 2008). Zooplankton species are

presumably not able to perform a top-down control on phytoplankton biomass by grazing on them. A potential increase in the biomass and body size of zooplankton is limited due to a high predation pressure by other zooplankton and fish (Järvinen et al., 1999).

The fluctuations in zooplankton occurrence are influenced by the lake's hydrophysical and biological variability. There are daily vertical migrations, a patchy horizontal distribution and a high seasonality in zooplankton biomass (Mölsä et al., 1999). The pelagic clupeid and centropomid fish species are adapted to this highly variable and non-predictable food source and show an r-selected life history. They have a high reproduction, early maturity, high mortality and a short lifespan (MacArthur and Wilson, 1976; Stearns, 1976; Marshall, 1993). This makes the clupeids and the zooplankton important items in the food chain of Lake Tanganyika (Beadle, 1974). The clupeid species are key species of the pelagic food web. They link the planktonic and piscivorous level (Coulter, 1991). The diet of *L. stappersii* varies spatially and seems to depend mainly on the food availability rather than their life stage (Mannini et al., 1999). It feeds on copepods, shrimps and clupeids. *Limnothrissa miodon* lives in its early stages in inshore waters, outside the feeding grounds of *L. stappersii.* Shrimps are the main prey item of *L. stappersii* in the south. In the north, their diet is more heterogeneous (Mannini et al., 1999). Large *L. miodon* also prey on *S. tanganicae.* Both *L. stappersii* and *L. miodon* seem to have a strong top-down control on *S. tanganicae* in the south. This explains the relative low abundance of *S. tanganicae* in the south when compared to the north (Mannini et al., 1999). The large cichlid *B. microlepis* occurs in several habitats within Lake Tanganyika. It is a very mobile species and lives in both shallow and pelagic environments. This top predator feeds on insect larvae, molluscs, crustaceans and other fish (Koblmüller et al., 2015). *Lates microlepis* is also a top predator in the pelagic zone and feeds on clupeids and *L. stappersii* (Coulter, 1991). The *Bathybates* species *B. fasciatus, B. leo* and *B. minor* are piscivorous and feed on clupeid species. Pelagic species from the genus *Trematocara* feed on phytoplankton, several invertebrate species and fish larvae (Kirchberger et al., 2012).

In general, the north seems to be dominated by Cyclopidae, Clupeids and medusae where the south is dominated by calanoids, shrimps and *L. stappersii* (Kurki et al., 1999).

1.3 The clupeid species

Worldwide, 197 species of Clupeidae exist. Of these species, 27 are African riverine species from the Dorosomatinae subfamily (Kmentová et al., 2018), including the two clupeid fish species that occur in Lake Tanganyika, *Limnothrissa miodon* [Boulenger, 1906] and *Stolothrissa tanganicae* Regan 1917 (Fig. 2) (Whitehead, 1985; Wilson et al., 2008; Betancur et al., 2017). They probably evolved from a common ancestor that lived in the ancient Congo River System (Coulter, 1991). Wilson et al. (2008) believed that the Lake Tanganyika sardines are descendants of a herring group (Pellonulinae) that colonised West Africa during a marine

incursion around 100-35 Mya. The Pellonulinae diversified and colonised Lake Tanganyika at approximately 7.6 Mya (95% reliability interval: 2.1–15.9 Mya) (Wilson et al., 2008).

Figure 2: Clupeids caught on Lake Tanganyika (©Charlotte Huyghe).

1.3.1 *Stolothrissa tanganicae*

The *Stolothrissa tanganicae* (Greek, stole + thrissa: suit + shad; tanganicae: Tanganyika) is also known as the Lake Tanganyika sprat. The species is endemic to Lake Tanganyika and does not occur in other African lakes (Whitehead, 1985). Boulenger (1919) reported *S. tanganicae* in the Lukuga River near Albertsville (present Kalemie), but it is believed that these specimens were taken from the lake itself near Kalemie (Kullander and Roberts, 2011). The average standard length of *S. tanganicae* is 70 mm and the maximum standard length is 100 mm (Whitehead, 1985; Plisnier et al., 2009). The maximum age of *S. tanganicae* is probably 1.5 years (Plisnier et al., 2009).

1.3.1.1 Ecology of *Stolothrissa tanganicae*

Stolothrissa tanganicae reproduces throughout the year but is believed to spawn more frequently in the first six months of the year or at the end of the dry season during August and September (Mulimbwa et al., 2014). They probably use offshore pelagic waters as spawning grounds (Mulimbwa and Shirakihara, 1994; Mulimbwa et al., 2014). After fertilisation, the egg size increases and the eggs start to sink (Coulter, 1991). They hatch after 24-36 h at a depth of 75-150 m. After hatching, the larvae swim upward (Coulter, 1991). In the south where the anoxic water layer begins at a lesser dept, the larvae could hatch in anoxic water. This can reduce their survival (Coulter, 1991). When juveniles reach a length of 10 mm, their pigmentation increases and they start schooling (Coulter, 1991). The juvenile schools move more inshore until the individuals are approximately 50 mm long. They then return to deeper pelagic waters (Coulter, 1991). The females reach maturity around 75 mm and males around 64 mm length (Coulter, 1991).

The adult planktivorous *S. tanganicae* lives in large schools (Plisnier et al., 2009). They live in more offshore waters in comparison with *L. miodon* (Phiri and Shirakihara, 1999) and seem to

be more specialised for a pelagic life (Coulter, 1991). During the day, schools stay below 60 m dept. At night, schools rise to 8-15 m (Plisnier et al., 2009). These diurnal migrations may be caused by feeding behaviour and/or strong predation pressure by *Lates* species (Coulter, 1991). At dusk, *S. tanganicae* moves upward to feed on zooplankton. At dawn, they move back to deeper and darker waters to escape from visual predators (Coulter, 1991). *Stolothrissa tanganicae* shows a weak genetic differentiation. There is no evidence of population structures or genetic differentiation by environmental adaptation within Lake Tanganyika (De Keyzer et al., 2019).

Stolothrissa tanganicae appears in larger numbers in the north in comparison to the south. This can be explained by the limnological variability of the lake and the predator-prey interactions between *L. stappersii* and *S. tanganicae,* or their life cycle as explained before (Coulter, 1991; Plisnier et al., 2009). In the south, the oxygenated water layer is deeper. *Lates stappersii* needs more oxygen than the clupeids. *Stolothrissa tanganicae* can hide from *L. stappersii* by swimming in deeper oxygen poor water. This indicates that *S. tanganicae* in the south has fewer escaping options when compared to the north where the oxygenated water layer is less deep (Plisnier et al., 2009). The abundance of *S. tanganicae* increases in the south when the region becomes more favourable due to planktonic blooms. When seasonal upwelling causes a plankton bloom in the south, *S. tanganicae* is able to rapidly migrate to the nutrient rich waters. Coulter (1991) reported that their schools could travel 20 km each day and fishermen noted that they can swim against currents (Plisnier et al., 2009). During these blooms, the water transparency decreases. *Lates stappersii*, just like *S. tanganicae,* is a visual predator. This *Lates sp*. is negatively affected by decreased visibility (Plisnier et al., 2009).

1.3.1.2 Food sources of *Stolothrissa tanganicae*

Stolothrissa tanganicae is a visual predator. During the day, the zooplankton community remains at a greater dept to avoid predation. They rise to the surface at dusk and remain there during the night. *Stolothrissa tanganicae* feeds mainly at dusk and at dawn, when the zooplankton is close to the surface and when there is still enough light to hunt. They can also be found closer to the surface during phytoplankton blooms and cloudy days as the water visibility decreases (Coulter, 1991). Coulter (1991) reports that juveniles of *S. tanganicae* feed mainly on phytoplankton including the diatoms *Nitzschia* and *Navicula* and the peridinid *Gymnodinium* (Coulter, 1991). The Lake Tanganyika Research Project (unpublished, 1998) suggests that juveniles have planktonic nauplii as their main food source (Mulimbwa et al., 2014). When *S. tanganicae* moves to deeper water, they probably switch to their adult diet. Adults feed on copepods (mainly the calanoid *Tropodiaptomus simplex)* and the larger shrimps (*Limnocaridina spp.*) (Coulter, 1991; Plisnier et al., 2009). Some Researchers found only sporadically shrimps in the stomachs of *S. tanganicae*, while others reported that it was their major food source (Coulter, 1991). Coulter (1991) tries to explain this variation by the

patchiness of shrimp schools. He suggests that *S. tanganicae* feeds preferentially on shrimps when encountering a school of shrimps. However, since Copepoda are more numerous, *S. tanganicae* feeds more on copepods (Coulter, 1991). Whitehead (1985) reported that *S. tanganicae* feeds on prawns, shrimps, copepods, chironomids, diatoms and algae (Whitehead, 1985).

1.3.2 *Limnothrissa miodon*

Limnothrissa miodon (Greek, limne + thrissa: Swamp + shad) (original name: *Pellonula miodon*) is also known as the Lake Tanganyika sardine. It is endemic to Lake Tanganyika but is successfully introduced in three other African lakes (Whitehead, 1985; WoRMS, 2019). The average length of *L. miodon* in Lake Tanganyika is around 100 mm and its maximum length is 130 mm (Whitehead, 1985; Eccles, 1992). Their maximum age is three years (Coulter, 1991).

1.3.2.1 Ecology of *Limnothrissa miodon*

Limnothrissa miodon reproduces throughout the year, with seasonal peaks in reproduction (Marshall, 1993). In Lake Tanganyika, *L. miodon* spawns mainly in the rainy season, from November to May. Peaks in spawning are also reported from August to October in the northern basin (Mulimbwa et al., 2014). This period could vary from year to year depending on the food availability. A long period of sufficient food supply seems to support spawning (Mulimbwa et al., 2014). *Limnothrissa miodon* spawns in shallow, littoral waters (Coulter, 1991; Marshall, 1993). The eggs hatch on the bottom. The larvae of *L. miodon* are similar to the larvae of *S. tanganicae* (Coulter, 1991). The juveniles start forming schools when they have a body size of around 10 mm. Juveniles from 15 to 40 mm length occur close to the shore (Coulter, 1991). As the juveniles grow older, they move gradually to deeper, pelagic waters (Marshall, 1993). In Lake Tanganyika, the males of *L. miodon* mature around 64 mm and females around 75 mm length (Marshall, 1993).

L. miodon lives in large schools. Compared to *S. tanganicae*, *L. miodon* lives in more inshore waters (Whitehead, 1985; Marshall, 1993; Phiri and Shirakihara, 1999). They are more numerous on shelves than on steep bottoms (Coulter, 1991). *Limnothrissa miodon* is especially abundant in inshore pelagic waters at the end of the dry season, from August to October (Phiri and Shirakihara, 1999). Their dense schools show a daily vertical migration between 20 and 40 m (De Vos et al., 1996; Hauser et al., 1998). The schools of *L. miodon* are size-specific and temporally stable. These size-specific schools are the result of non-random association, either by assortative grouping or by favouring their original school. The fish within the schools disperse at night to feed (Hauser et al., 1998). There are genetic microgeographical differences when looking at the schools of *L. miodon*. No genetic differentiation is detected on a larger geographical scale. This could indicate that *L. miodon* within Lake Tanganyika has no population structure (Hauser et al., 1998).

L. miodon has been introduced in several natural and artificial lakes. These include Lake Kivu (D.R. Congo and Rwanda), Lake Kariba (Zambia and Zimbabwe) and Lake Cahora Bassa (Mozambique) (Marshall, 1993; Isubisho et al., 2006). *L. miodon* has traits which facilitate successful introduction: maturation at a small size, high fertility and the opportunity to reproduce at any moment throughout the year (Isubisho et al., 2004). A low fish diversity is a favourable environmental condition to successfully introduce *L. miodon* in another East African lake. Lake Kivu and Lake Kariba had an open niche for a planktivorous fish species (vacant niche hypothesis by Simberloff, 1995) (Isubisho et al., 2004).

L. miodon was voluntarily introduced in Lake Kivu around 1959. Verbeke (1957) suggested to introduce a planktivorous fish in the lake to fill the vacant pelagic niche and to have more organic matter converted into edible fish (de Iongh et al., 1983). From 1958 to 1960, thousands of *L. miodon* and *S. tanganicae* fry were taken from Lake Tanganyika and stocked in Lake Kivu. In 1974, *L. miodon* was recorded in the southern part of the lake and in 1976 all over Lake Kivu (de Iongh et al., 1983). *Stolothrissa tanganicae* was never observed during field expeditions. This species was probably unable to adapt to the environmental conditions (de Iongh et al., 1983). The introduction of *L. miodon* in Lake Kivu had a large impact on its zooplankton community. Before the introduction, the zooplankton community was dominated by Copepoda and Cladocera. After the introduction, there was a trend towards smaller body sizes among the zooplankton community due to predation pressure. The larger Cladocera species, including the efficient grazing species *Daphnia curvirostris,* disappeared and made way for small-bodied taxa (Isubisho et al., 2004; Isubisho et al., 2006). In recent years, *L. miodon* in Lake Kivu is in interspecific competition for food resources with the Tanganyika killifish *Lamprichthys tanganicanus*. The planktivorous fish *L. tanganicanus* was probably accidentally introduced from Lake Tanganyika into Lake Kivu and started to appear in commercial fish catches around 2006 (Masilya et al., 2011).

Lake Kariba is a man-made reservoir between Zambia and Zimbabwe (Balon and Coche, 1974). *Limnothrissa miodon* was introduced successfully in Lake Kariba between 1967 and 1968. This lake had a vacant niche for a pelagic planktivorous fish. In 1969, *L. miodon* became an established fish species in Lake Kariba (Chifamba, 2000). Here the catch per unit effort of *L. miodon* is influenced by temperature, rainfall and total river inflow (Chifamba, 2000). *L. miodon* escaped from Lake Kariba and invaded the artificial Lake Cahora Bassa through the Zambezi River. *Limnothrissa miodon* individuals caught in Lake Kariba and Lake Cahora Basse are often only half the size of the individuals caught in Lake Tanganyika and Lake Kivu (Chifamba, 2000).

1.3.2.2 Food sources of *Limnothrissa miodon*

Limnothrissa miodon is just as *S. tanganicae* a visual predator. They feed mainly at dusk and dawn when the zooplankton community is closer to the surface and when there is still sufficient light to hunt (Coulter, 1991). In Lake Tanganyika, *L. miodon* has a more varied diet than *S. tanganicae*, probably because of its more littoral lifestyle. Food is more available and generalised inshore (Coulter, 1991; Marshall, 1993). The main diet of *L. miodon* includes cyclopoids, calanoids, atyid shrimps, insect larvae and juvenile clupeids (de Iongh et al., 1983; Coulter, 1991). Coulter (1991) reported that large *L. miodon* around 1 year of age (100 mm length) move to the pelagic zone and become mainly piscivorous. They feed on *S. tanganicae*. Bashirwa (FAO, unpublished) reported that *L. miodon* with a size of 58-115 mm feed on *S. tanganicae* with a size of 27-65 mm (Coulter, 1991). This could influence the stocks of *S. tanganicae*. Cannibalism within *L. miodon* in Lake Tanganyika is minimal since the younger and smaller individuals live closer to the coast than the older and bigger ones (Coulter, 1991). In lake Kivu, de Iongh et al (1983) reported that the juvenile littoral *L. miodon* (30-70 mm) feed mainly on Copepoda, Cyanophyta and Rotatoria. Adult pelagic *L. miodon* (65-110 mm) feed mainly on Copepoda and Cyanophyta while littoral individuals feed mostly on Copepoda, Microcystis, chironomid pupae, insect larvae and juvenile clupeids. (de Iongh et al., 1983). Marshall (1993) noted that there is a high degree of cannibalism within *L. miodon* in Lake Kivu. 20 % of larger adults feed on juvenile clupeids. *Limnothrissa miodon* in Lake Kivu acts as a top predator which are otherwise absent (Marshall, 1993). *Limnothrissa miodon* in lake Kivu feeds in the late afternoon and digests during the night. It eats again at sunrise and digests afterwards (de Iongh et al., 1983). In Lake Kariba, smaller Cladocera species (*Bosminopsis deitersi, Bosmina longirostris and Ceriodaphnia cornuta*) are the main prey item of *L. miodon*, followed by immature Copepoda (nauplii, copepodites and *Diaphanosoma excisum*) and Rotifera. Their diet also includes other crustaceans, aquatic insects and phytoplankton (Mandima, 1999). The catches of *L. miodon* in Lake Kariba fluctuates in accordance to the food availability. The abundance of *L. miodon* seems to be in relation to the river inflow. Food availability apparently has a greater impact on the abundance of the sardines than predation or fishing pressure (Mandima, 1999). Food availability has an impact on the size at maturity, fecundity and the juvenile survival of sardines (Mandima, 1999). *Limnothrissa miodon* in the Cahora Bassa reservoir feeds on all crustacean species present in the lake (Gliwicz, 1986). In this lake, lunar cycle related fluctuations in zooplankton abundance seems to be the result of varying sardine feeding efficiency. *Limnothrissa miodon* feeds more intensively on zooplankton during the full moon compared to the new moon period (Gliwicz, 1986).

1.4 Fisheries: opportunities and threats

1.4.1 Fisheries

Lake Tanganyika holds historically one of the most efficient and productive pelagic fisheries in the world (Nkotagu, 2008). The main goal of the local fishery is to nourish the communities

surrounding the lake (Coulter, 1991). Up to 40% of the local protein intake originates from fish (Naithani et al., 2010). They often combine this with subsistence agriculture, especially in the rainy season. Fish that are not eaten are sold for money. Cichlids are often caught as ornamentals for the worldwide aquarium trade (Coulter, 1991; Mölsä et al., 1999). Commercial fishing started in the lake around 1958 in the northern region and around 1962 in the southern region (Coulter, 1970). The fish are traded on local markets and transported to remote towns (Nkotagu, 2008). Lake Tanganyika has the second largest inland fishery in Africa and it provides resources for the whole East-Central African region (Mölsä et al., 1999). The annual yield of the Lake Tanganyika fisheries was 165 000-200 000 tons in the 1990's (Mölsa et al., 1999). A survey from 2011 showed that there are 93 214 active fishermen on Lake Tanganyika. The processing of the fish catches at the landing site of the boats directly employs 38 765 people and the trading employs 23 090 people, most of them women (Petit and Shipton, 2012). The main species caught in Lake Tanganyika are *Lates spp*. and clupeid species (Coulter, 1991). The clupeid species or sardines are called 'ndagala' in DR Congo and Burundi, 'dagaa' in Tanzania and 'kapenta' in Zambia. They comprise 65 % of the total commercial fish catches in weight (Fig. 3). 30 % of the catches are *L. stapersii* which are locally called 'Mukeke' or 'Migekuba'. The remaining catches mainly consist of the other *Lates* species (Mölsä et al., 1999; Van der Knaap et al., 2014).

Figure 3: Fishing and drying of ndagala in D.R. Congo (©Charlotte Huyghe).

Different fishing techniques are used in Lake Tanganyika (Fig. 4). Most of them use light to lure the fish to the boat during the night. The traditional method is the use of a scoop net or 'lusenga'. The fish, mainly clupeids, are attracted to the boat at night. The lusenga is then plunged into the attracted school. Subsistence fishermen stay near the shore and use hand lines, gill nets, fishing baskets and beach seines. Beach seines are one of the most important fishing methods, although they have been banned all over Lake Tanganyika (Coulter, 1991; Petit and Shipton, 2012). The nets hang vertically in the water. Schools of different fish species are caught while dragging the net on both ends to the shore. The catch is large compared to lusenga fishing. This technique is used throughout the year and mainly in the south, in Tanzania and

Zambia. Another technique is the catamaran lift net. In this method, a lift net is lowered between canoes and lights above the net are used to attract clupeid schools. They are caught when the net is pulled up. This method is mainly used in the north because the strong winds in the south make this fishing method less efficient. In the south, they use the 'chiromila'. A boat with light attracts clupeids. A net which forms a quarter of a sphere is then drawn by other boats around that boat to catch the fish. For industrial fishing, larger purse seines are used with industrial boats (Coulter, 1991). A survey in 2011 showed that gill nets are the major fishing gear (51 011 of which 32.4 % are illegal), followed by lift nets (5 331, 25.5 % illegal), beach seines (1 778, all illegal) and purse seines (978, 10.6 % illegal) (Petit and Shipton, 2012).

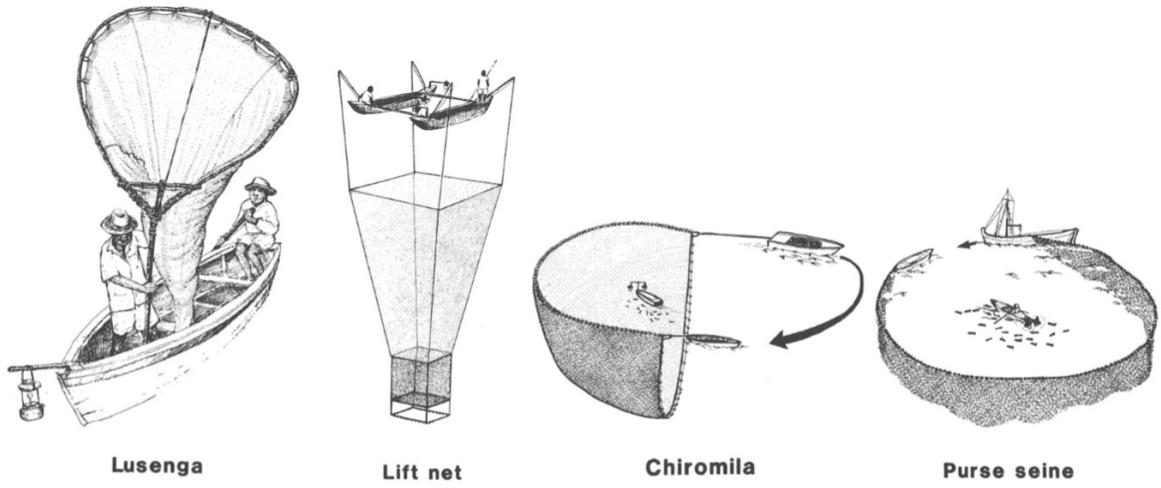

Figure 4: Fishing techniques at Lake Tanganyika, including a lusenga hand net, lift net, chiromila and purse seine (Coulter, 1991).

The Great Lake Region, which include Rwanda, Uganda, Burundi and D.R. Congo, experienced many years of conflict. In the 1990s, the region experienced a decay of the states caused by the neglect of certain provinces, the increasing autocratic governments and exploitation of the local communities. There have been wars and genocides caused by various militia which triggered large displacements of local people (Khadiagala, 2017). Many people who lived at the coast of Lake Tanganyika fled their homes. Boats and motors were taken away during raids on local communities. People stopped fishing in the lake (Van der Knaap et al., 2014). In 2006, the region surrounding Lake Tanganyika became relatively stable. Refugees were assisted to return back to their homes. The villages became populated again. The UN and NGOs distributed fishing gear among the communities to help the people feed themselves (Van der Knaap et al., 2014). Large quantities of monofilament gillnets were handed out in the region which triggered a commerce in cheap fishing nets. Fishing nets became available at low prices on local markets, which caused an increase of the local fisheries all over the lake. The number of illegal fisheries increased. The gillnets are not very size or species selective and pose a threat to all fish species in Lake Tanganyika (Van der Knaap et al., 2014). The major threat to offshore

fish stocks are lift nets that are used on juvenile *L. stappersii* and *S. tanganicae* (Petit and Shipton, 2012). Beach seines harm fish stocks and the benthic environment. It destroys cichlid nests (e.g. from *B. microlepis*) and captures juvenile *L. mariae* (Petit and Shipton, 2012). Mosquito nets, distributed in Tanzania to control malaria, are often used as fishing nets (McLean et al., 2014). In the north, scoop nets made from mosquito nets harm *L. miodon* stocks (Petit and Shipton, 2012). A large percentage (10-27%) of the nets used are illegal (Petit and Shipton, 2012).

Over the past 15 years, fishing capacity has increased strongly in Lake Tanganyika and fish stocks are under huge fishing pressure (Van der Knaap et al., 2014). This probably caused the decrease in fish stocks, especially in the north where the fishing pressure in relation to the length of the coast is higher when compared to the south (Mulimbwa et al., 2014). Several large fish species are sold less frequently on local markets in the north. The price of the fish has increased (De Keyzer et al., unpublished; Van der Knaap et al., 2014). On some local markets in Uvira (DR Congo), people sold frozen fish from boxes originating from China because these were less expensive than the local fish (personal observation). In 2008, the Lake Tanganyika Authority (LTA) was established. The Convention on the Sustainable Management of Lake Tanganyika was implemented by the LTA to provide an international management system for Lake Tanganyika (Petit and Shipton, 2012). The current fish management policies between the four countries surrounding the lake are not consistent. Formalisation of the LTA in national fishing policies is one of the issues. The establishment of co-management policies and harmonising the management of fisheries between the countries will be very important for the future of the Lake Tanganyika fisheries (Petit and Shipton, 2012).

1.4.2 Pollution

Another threat to Lake Tanganyika is pollution. Evidence suggests that the lake is already polluted by pesticides, sulphur oxides (originating from the burning of fossil fuels and industrial processes), heavy metals and hydrocarbons (Plisnier et al., 2018). Polluting sources include industrial waste, domestic waste, agriculture (pesticides and fertilisers), mining and boats (Plisnier et al., 2018). The human population around the lake is rapidly increasing. This increase together with the increasing urbanisation could intensify the risk of pollution (Plisnier et al., 2018). There have been large changes in land-use and -coverage of the areas surrounding Lake Tanganyika over the last decades (Ekaka Azanga et al., 2016). These changes were frequently a result of anthropogenic deforestation and fires. Forests, woodlands, grasslands and wetlands are cleared to establish farmlands, plantations, houses and industries. The wood and timber from the cleared forests are used as firewood (Ekaka Azanga et al., 2016). These changes in land use contribute to an increased soil erosion and runoff of polluted sediments into Lake Tanganyika (Ekaka Azanga et al., 2016). An excess of sediment load in the lake can affect local organisms in several ways. It can reduce the light penetration in the water which in turn will

reduce visibility and the photosynthetic rate of phytoplankton. Sediment can form a layer over benthic algae and reduce the morphological complexity of the environment. It can reduce the nutritional value of detritus and physically damage organisms by abrading their body surface or by interfering with their filtering mechanism. Sediments can act as a nutrient/contaminant sink or source which could disturb the nutrient dynamics of the whole lake (Cohen et al., 1993). Another pollution risk could rise in the future: exploitation of fossil hydrocarbons (Verheyen, 2016). D.R. Congo and Tanzania have signed a memorandum of understanding in 2016 to explore and produce hydrocarbons in Lake Tanganyika (Ng'wanakilala et al., 2016). Lake Tanganyika is a closed ecosystem with a retention time of around 7000 years. If an oil spill would occur, the recovery of the lake ecosystem could take millennia (Verheyen, 2016). Appropriate infrastructure to respond to such a disaster is lacking in the area. A rapid response to an oil spill by transporting the needed equipment to the lake is almost impossible due to logistics, expensiveness and troublesome movement of the infrastructure (Verheyen, 2016). These oil spills can occur by accident when extracting or transporting oil. In Nigeria, thousands of oil spills have been reported. Sabotage is a threat that can result in oil spill, as frequently observed in the Niger delta (Verheyen, 2016).

1.4.3 Global warming

Global warming is another possible threat to Lake Tanganyika (Naithani et al., 2011; Plisnier et al, 2018). Increasing temperatures can cause biodiversity loss, a decline in fish stocks, a decrease in agricultural productivity, an increase in invasive species and an increase of water-borne diseases (Plisnier et al, 2018). Studies have tested several climatic scenarios to predict the effects of global warming on Lake Tanganyika (Naithani et al., 2011). Increased air temperatures will cause the water temperature to rise. This will lead to a decrease of the thermocline dept and an increase the stability of the water column (Naithani et al., 2011). The mixing and upwelling of deep nutrient rich water from the hypolimnion will be limited. (Naithani et al., 2011; Plisnier et al., 2018). For Lake Tanganyika, declining high winds seem to be the dominant factor that affects the decreased mixing and upwelling of nutrients (O'Reilly et al., 2003; Naithani et al., 2011; Plisnier et al., 2018). A change in mixing and upwelling will cause a change in primary production which could affect the fish stocks and biodiversity (O'Reilly et al., 2003; Naithani et al., 2011). If there is a limitation in nutrients, the primary production will drop, which might impact the fish yield (O'Reilly et al., 2003). O'Reilly et al. (2003) reported that since the mid-1900s, the carbon stable isotopes showed a trend towards more negative values. These carbon stable isotopes are an indicator for primary production. A decrease of carbon isotope records in sediment cores indicated in this case a decrease in primary productivity (O' Reilly et al., 2003). O'Reilly et al. (2003) attribute the recent decrease of fish yield in Lake Tanganyika to this decrease in primary production saying, "Carbon isotope records in sediment cores suggest that primary productivity may have decreased by about 20%,

implying a roughly 30% decrease in fish yields." Sarvala et al. (2006) question these causalities. They suggest that the intensification of the fisheries is the dominant factor determining the decrease in fish yield (Sarvala et al., 2006). However, Cohen et al. (2016) notes that declines in fish stocks have started way before commercial fisheries at Lake Tanganyika commenced (Cohen et al., 2016).

2 Aim

The aim of this study is to investigate the exact food source of the Lake Tanganyika sardine species by using a metabarcoding technique on their stomach content. Until now, studies on the diet of the Lake Tanganyika sardines involved a microscopally visual examination of the stomach content. This study will identify the prey taxa and examine whether there is a variation in prey between the two sardine species (*Stolothrissa tanganicae* and *Limnothrissa miodon*), locations (north: Uvira (D.R. Congo) and Bujumbura (Burundi); central: Kalemie (D.R. Congo); south: Sumbu and Mpulungu (Zambia)) and seasons (wet and dry season (April and September)). The results will indicate if the feeding on certain prey taxa varies between basins (north, central and south) and if the season has an influence. It will illustrate whether the sardines are specialized feeders or generalized feeders. This experiment could help future studies to investigate food webs and ecosystem interactions within Lake Tanganyika for EBFM.

3 Material and Methods

3.1 Sampling

Individuals from *Stolothrissa tanganicae* and *Limnothrissa miodon* were captured at five sites along the north-south axis of Lake Tanganyika (LT) (Fig. 5). These sites were situated in the northern end of the lake in the Kigoma basin (Bujumbura in Burundi and Uvira in DR Congo), central in the Kungwe basin (Kalemie in DR Congo) and on the southern end in the Kipili basin (Mpulungu and Sumbu in Zambia). In each of the three basins, a total of 20 specimens from both species were obtained (Table 1). This means that for Bujumbura, Uvira, Sumbu and Mpulungu, 10 specimens of both species were sampled at each site. At Kalemie, 20 specimens of both species were collected. These were sampled in April 2018, in the wet season. In Uvira, an additional 10 specimens of each species were sampled in September 2018 during the dry season to investigate whether there could be a difference in food preference between seasons.

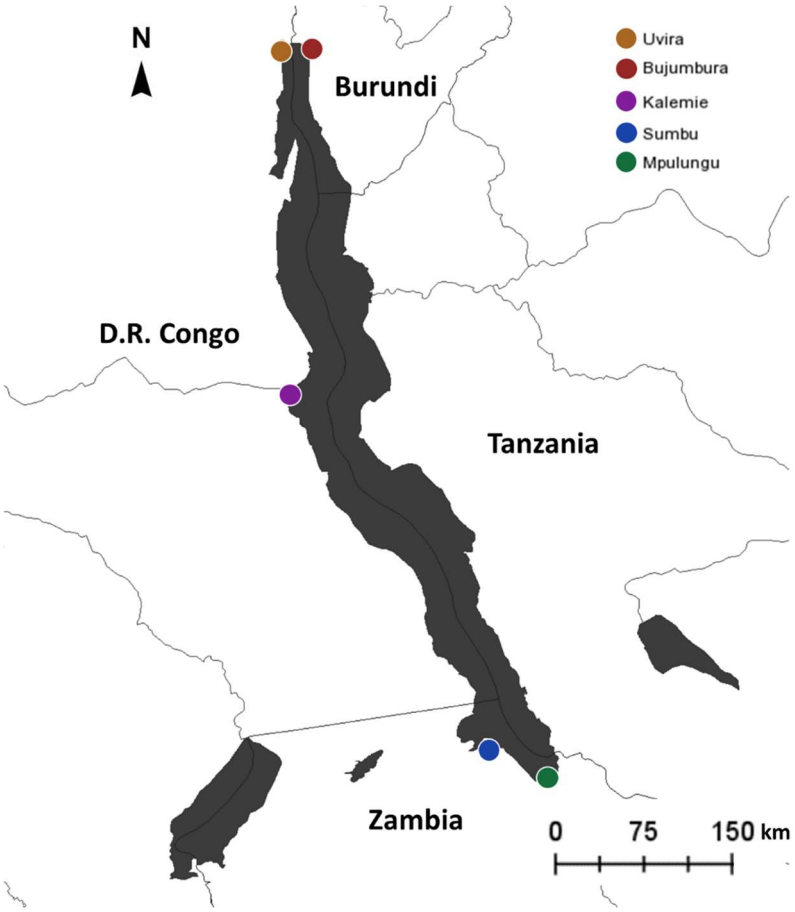

Figure 5: Sampling sites of L. miodon and S. tanganicae in Lake Tanganyika. Northern region: 1. Uvira (D.R. Congo), 2. Bujumbura (Burundi); central region: 3. Kalemie (D.R. Congo); southern region: 4. Sumbu (Zambia), 5. Mpulungu (Zambia) (Map made with Simple Mapper (http://research.amnh.org/pbi/maps/)).

Table 1: Samples of clupeids by species, location, basin, date, season and number of individuals (n).

Species	Location	Basin	Date	Season	n
S. tanganicae	Uvira (Experimental fishing)	north	15 September 2018	Dry	10
S. tanganicae	Uvira (Plage Igalundu)	north	12 April 2018	Wet	10
S. tanganicae	Bujumbura (Nyamgari Plage)	north	10 April 2018	Wet	10
S. tanganicae	Kalemie (Kamko/Lumbu)	central	12 April 2018	Wet	20
S. tanganicae	Sumbu	south	12 April 2018	Wet	10
S. tanganicae	Mpulungu	south	12 April 2018	Wet	10
L. miodon	Uvira (Experimental fishing)	north	15 September 2018	Dry	10
L. miodon	Uvira (Plage Igalundu)	north	12 April 2018	Wet	10
L. miodon	Bujumbura (Nyamgari Plage)	north	10 April 2018	Wet	10
L. miodon	Kalemie (Kamko/Lumbu)	central	12 April 2018	Wet	20
L. miodon	Sumbu	south	12 April 2018	Wet	10
L. miodon	Mpulungu	south	12 April 2018	Wet	10

Sampling was carried out under various circumstances. In the northern and central basin, specimens were sampled by local institutes. This was the case in Bujumbura with the University of Burundi, in Uvira with the Centre de Recherche en Hydrobiologie and in Kalemie with the Université de Kalemie. In the southern basin, the samples were obtained by researchers on a field expedition. Fresh samples were obtained at the fisheries market or bought directly from the fishermen. The samples from Uvira for the dry season were sampled by experimental fishing. At night, a drift net (gill net) was used to collect *L. miodon* at a depth of 50 m (coordinates: 3°25'07.5"S 29°09'21.4"E). *Stolothrissa tanganicae* was obtained from fishermen who used lift nets and lamps suspended between pirogues to catch them in the early morning around 100 m (coordinates: 3°23'22.2"S 29°10'01.9"E). Only adult individuals (> 7.0 cm) were included. This was impossible in some cases due to a shortage of specimens. In these cases, the largest specimens from the batch were chosen. All specimens were stored in absolute analytical grade ethanol.

3.2 Dissection

Before dissection, each specimen was morphologically identified to species level. The body of *S. tanganicae* is more slender than the body of *L. miodon*. The eyes of *S. tanganicae* are also smaller compared to *L. miodon* who has an eye width as large as the length of the head behind the eye. *Stolothrissa tanganicae* has a shorter maxilla blade which does not reach forward to the hind tip of pre-maxilla in contrast to *L. miodon* (Whitehead, 1985) (Fig. 6). In dorsal view, the snout of *S. tanganicae* is narrow and concave where the snout of *L. miodon* is broad with tampering sides, and not concave. The distinct silver stripe at the lateral flank has some

variation in width when looking at *S. tanganicae*, while *L. miodon* has a straight silver line (Whitehead, 1985).

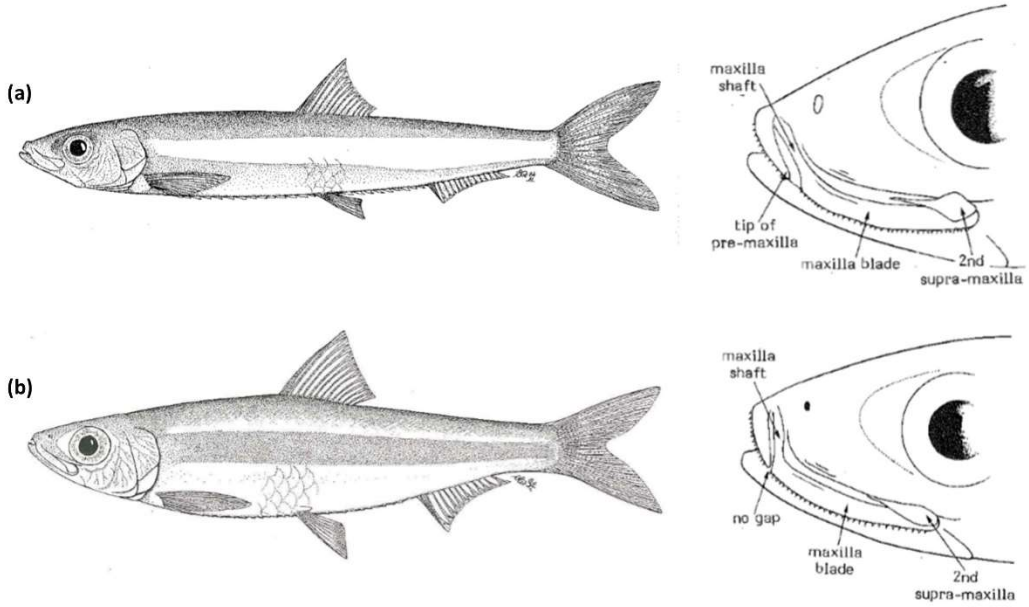

Figure 6: Morphology of a) Stolothrissa tanganicae and b) Limnothrissa miodon (Whitehead, 1985).

After species identification, specimens were labelled and measured. After extracting a fin clip for the genetic identification of the individual, the abdominal cavity was carefully opened (Fig. 7). The sex was identified by inspecting the gonads. The female ovaries are rounded on a cross-section. The two ovaries are yellow to orange and are strongly connected at the oviduct. The male testes are more triangular on a cross-section. They are milky-white and are not firmly connected to the duct (Fig. 7). The sex was identified by looking at the gonads. The female ova are rounded on a cross-section. The two ova are yellow to orange and are strongly connected at the oviduct. The male testes are more triangular on a cross-section. They are milky-white and are not strongly connected at the duct (Fig. 7). To extract the stomach, the digestive tract was cut at the oesophagus and just behind the pyloric caeca. The stomach of each specimen was stored on 100 % ethanol at 5 °C. Later, the stomach content was extracted and used for metabarcoding. In the cases where there was no stomach content, stomach juice and remnants of prey on the stomach wall was used for metabarcoding.

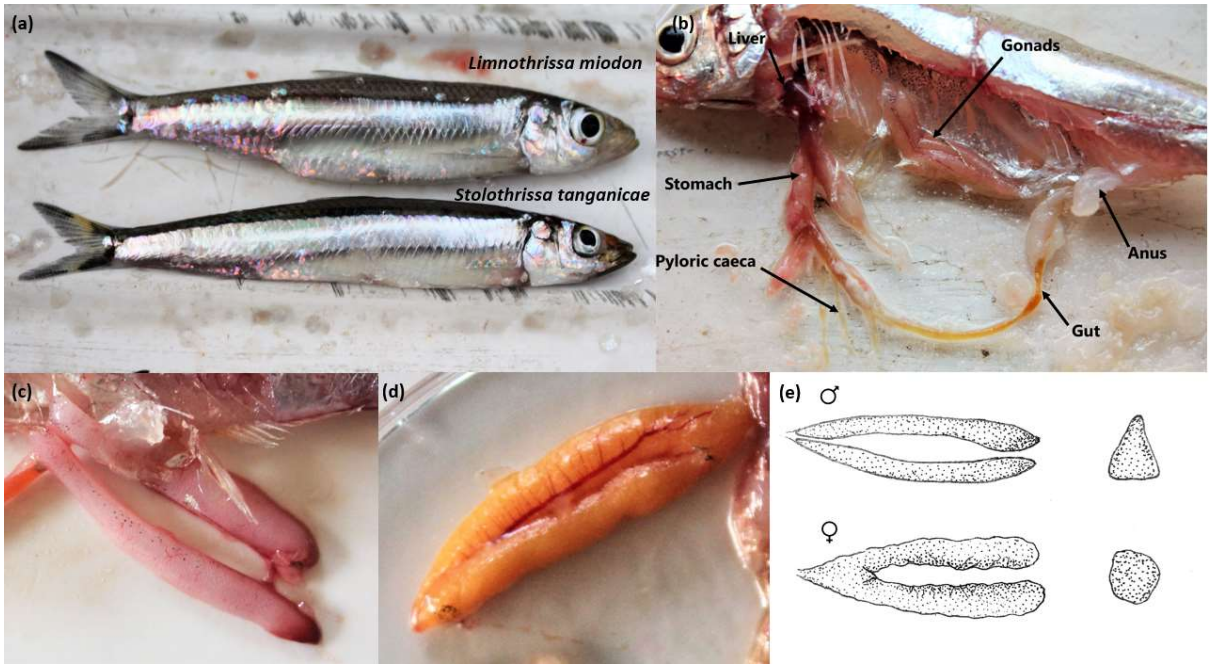

Figure 7: a) Freshly caught L. miodon and S. tanganicae; b) dissection of a Lake Tanganyika sardine; c) male testes; d) female ova; e) gonads and their cross-section (©Charlotte Huyghe).

3.3 Metabarcoding stomach content

The DNA from the stomach content was extracted with the Nucleospin® Tissue Kit (Macherey-Nagel™, Ref.: 740952.250). For each sample, the stomach content was transferred to a micro-tube with 180 µl T1 buffer and 25 µl Proteinase K to pre-lyse the sample. After incubating it overnight at 56 °C, the samples continued to be lysed by adding 200 µl B3 buffer and heating it for 10 min at 70 °C. To adjust the DNA binding conditions, 210 µl 96-100% ethanol was added. The mixture was loaded into the Nucleospin® column and spun down in a collection tube at 11 000 x g for 2 min. The silica membrane was washed first with 500 µl BW buffer (Guanidine hydrochloride 36-50 %, 2-propanol 20-50 %) and then with 600 µl B5 buffer and spun down each time for 1 min at 11 000 x g. The column was centrifuged one last time for one min at 11 000 x g to dry the silica membrane and remove the remaining ethanol. The Nucleospin® tissue column was placed into a 1.5 ml microtube, 100 µl of BE elution buffer (5 mM Tris/HCl, pH 8.5) was added and it was incubated at room temperature for 30 min to elute the highly pure DNA. After spinning it down for one minute at 11 000 x g and transferring the DNA from the micro centrifuge to a 96 well plate, the DNA samples were ready for the first Amplicon PCR.

A 313 bp region of the cytochrome c oxidase subunit I (COI) gene was amplified to identify the species found in the stomach of the sardines. The first amplicon polymerase chain reaction (PCR) had a total reaction volume of 23.5 µl for each sample on the plate. The reaction mix contained 12.5 µl MyTaq™ Mix (Bioline, Ref.: BIO-25041), 0.5 µl of both forward (mlCOIintF-GGWACWGGWTGAACWGTWTAYCCYCC, 20 µM; Leray et al., 2013) and

reverse (jgHCO2198-TAIACYTCIGGRTGICCRAARAAYCA, 20 µM; Leray et al., 2013) primer, 7 µl of H_2O and 3 µl of the DNA template. A "Touchdown" PCR program was performed. The PCR profile had first 16 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s with the annealing temperature dropping each cycle with 1°C, and extension at 72 °C for 30 s. The second part of the PCR profile consisted of 25 cycles of denaturation at 95 °C for 30 s, annealing at 46 °C for 30 s and extension at 72 °C for 30 s (Leray et al., 2013).

Following PCR, a DNA cleanup and purification was performed using the Agencourt® AMPure beads kit (CleanNA, Ref.: CPCR-0050). 36 µl of Agencourt® AMPure beads / 20 µl of PCR product for each sample was added and mixed. After 5 min, the samples were placed on a magnetic plate for 5 min to separate the beads from the solution. 50 µl of the clear solution was removed and 200 µl of 70 % ethanol added for one minute to wash the beads. After removing the ethanol, 200 µl of 70 % ethanol was added for a second time which was removed completely after one minute. The samples were removed from the magnetic plate and the beads with the remaining DNA suspended in 40 µl of 1x elution buffer (Qiagen kit) and incubated for two min. After putting the samples back on the magnetic plate for one min, 30 µl of the clear end product was transferred to a new plate.

After cleanup, the Index PCR was carried out. In an Index PCR, different combinations of forward and reverse primers were used for each DNA template to give it a unique primer barcode. This was used to identify the origin of each DNA strand and to recognize the DNA from each sample after pooling all the samples together. The total reaction volume for each sample on the plate was 20 µl. Each sample contained 10 µl of Mytaq™ Mix, 0.5 µl forward primer (5 µM), 1 µl reverse primer (2.5 µM), 7.5 µl H_2O and 1 µl DNA template. The PCR profile had an initial denaturation of 30 s at 98 °C, followed by 15 cycles of denaturation at 98 °C for 10 s, annealing at 51 °C for 30 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min (Lange et al., 2014).

When the PCR was finished, the PCR product was cleaned using the Agencourt AMPure beads kit. The procedure was the same as the one described above after the first Amplicon PCR.

To check whether the samples contained enough DNA, the DNA quantity was measured and a gel electrophoresed was performed. Quant-it™ PicoGreen® ds DNA Assay kit (Molecular probes® by life technologies™, Ref.: P11496) was used for quantification. For each sample, 99 µl of 1x TE buffer, 100 µl of 1x Quant-it and 1 µl of template DNA was used. Standards were added using Lambda stock (100 µg/mL in TE buffer) instead of DNA template (Addendum, Table B1). The program i-control and the Tecan plate reader were used to measure the quantity of the DNA. With the results from our DNA and the standards, the DNA concentrations of the samples were calculated. For the gel electrophoresis, a 1.5 % agarose gel (1.05 g agarose/ 70 ml TE buffer (10 mM Tris-HCl and 1 mM EDTA) and 5 µl green fluorescent dye) was used.

After comparing the concentrations of DNA in our samples with the gel results, it was decided for which sample there was not enough high-quality template available. For these samples, the two PCRs were repeated. This was the case when the concentration was below 2.0 ng/µL and/or when the band on the gel was unclear or absent. The procedure was performed again starting from the first amplicon PCR. Only this time, 0.25 µM of magnesium chloride (MgCl) was added during the first PCR. Adding magnesium increases the activity of Mytaq^{TM} DNA polymerase during PCR. This causes primers to bind more easily to the target DNA, at the cost of lowering the specificity of annealing. When all samples had a sufficient concentration of DNA, the samples were pooled together with a mass of 20 ng DNA for each sample. The volume of DNA template necessary to add the right amount of DNA mass to the pool was calculated using the concentrations of each template acquired by the quantification. The library was sequenced using MiSeq (PE 3000, Illumina, inc.).

3.4 Sardine species identification

To verify species identification based on morphology, the mitochondrial COI gene was sequenced. DNA was extracted from fin clips following the standard sorting out procedure. First, SSTNE with a pH of 9.5 was prepared. In this example, 1 l of SSTNE will be prepared. To 800 ml of distilled stirring H_2O, 17.532 g NaCl, 6.055 g Tris, 400 µl 0.5 EDTA, 76.08 mg EGTA, 78.49 µl spermidine (Sigma-Amdrich S2626) and 52.2 mg spermidine (Sigma-Aldrich 85590) were added. Distilled water was added until the volume reached 900 ml. In order to adjust the pH to 9.5, 1 M NaOH can be added. Distilled water could be added to reach a total SSTNE volume of 1 l. The mixture was autoclaved and stored at room temperature. 300 µl SSTNE, 30 µl 10 % SDS solution, 10 µl proteinase K (10 mg/ml) and the fin clip (~1 mm^2) was placed into an Eppendorf tube. This mixture was incubated at 55 °C for three hours. The samples were turned over every 30 min. Afterwards, 1 µl of Ribo shredder RNase blend (Westburg, tano. RS12500) was added to the mixture and incubated at 37 °C. After 60 min, 240 µl of 5 M NaCl was added, mixed and incubated around 3 °C for 10 min. The samples were removed from the fridge and spun down at 20 000 g for 15 min. 300 µl supernatant was transferred to a new tube and 300 µl isopropanol was added. The samples were stored on -20 °C overnight. The next day, the samples were centrifuged at 20 000 g for 15 min. A small pellet became visible in the tubes. The supernatant was discarded and 800 µl ethanol was added. It was upended around at room temperature for two hours. After two hours, the samples were spun down and the supernatant was completely discharged. The pellet was resuspended in 50 µl of 5 mM Tris (pH 8-8.5) or 0.1 x TE and stored around 3 °C.

The first PCR was conducted following Handy et al. (2011). The total reaction volume for each sample was 25 µl. Our reaction mix contained 12.5 µl Mytaq^{TM} Mix, 1 µl "Handy" primers (20 mM, FishCOILBC-TCAACYAATCAYAAAGATATYGGCAC and FishCOIHBC-deg-ACTTCYGGGTGRCCRAARAATCA; Handy et al., 2011), 9.5 H_2O and 2 µl template DNA.

The PCR profile had an initial denaturation at 95 °C for 60 s, followed by 40 cycles of denaturation at 95 °C for 15 s, Annealing at 55 °C for 15 s and elongation at 73 °C for 10 s, and a final elongation at 72 °C for 5 min (Handy et al., 2011). The PCR product was cleaned using the Agencourt AMPure beads kit as described before.

The second PCR was conducted using the Big Dye Terminator sequencing protocol. The total reaction volume of the PCR for each sample was 10 µl. The reaction mix contained 1 µl Ready Reaction Premix, 1.5 µl 5x BigDye Sequencing buffer, 1 µl Primer (3.2 µM), 4.5 µl H_2O and 2 µl template. The following PCR protocol was used. The initial denaturation temperature was 95 °C and lasted for 1 minute. Then, 25 cycles followed with in each cycle consisting of 10 s of denaturation at 95 °C, 5 s of annealing at 50 °C and 4 s of extension at 60 °C. After that, the PCR was paused at 4 °C.

PCR products were cleaned up using CleanDTR beads (CleanNA, GC Biotech). 10 µl of beads and 42 µl of 85 % ethanol were added to each PCR product and mixed. Next, the plate with the samples were placed on a magnetic plate for 5 min. After removing the supernatant, 100 µl of 85 % ethanol was added to wash the beads. After two min the supernatant was removed and 100 µl of 85 % ethanol was added for a second time. After two min, the supernatant was completely removed. The plate was removed from the magnet and 40 µl of H_2O was added. After an incubation at room temperature for 5 min, the plate was placed back on the magnetic plate and incubated for 5 min on room temperature. 30 µl of clear solution was transferred to a new plate and stored at -20 °C and later sequenced. The quality and quantity of the DNA templates were verified by using gel electrophorese as described before.

3.5 COI sequencing of zooplankton species

Two zooplankton species from LT were sequenced at the COI gene as a reference for the metabarcoding of the stomach content. These concerned the Calanoida *Tropodiaptomus simplex* and the Cnidaria *Limnocnida tanganjicae* (Fig. 8).

The zooplankton was sampled in the pelagic zone close to Uvira (coordinates: 3°25'51.2"S 29°10'36.4"E) on 1 September 2018 at a depth of 0-60 m using a zooplankton net with a mesh size of 100 µm. They were stored on ethanol and later identified using a microscope and using the identification guide for pelagic zooplankton in LT written by Vahtera et al. (unpublished, 2002).

Zooplankton DNA was extracted using a Nucleospin® Tissue Kit following the previous instructions. Each DNA purification sample contained 10 individuals of the same species to have a sufficient amount of DNA. In total, 3 samples (of which two contained *T. simplex* and one contained *L. tanganjicae; Addendum, Fig. B1, B2 and B3*) were used.

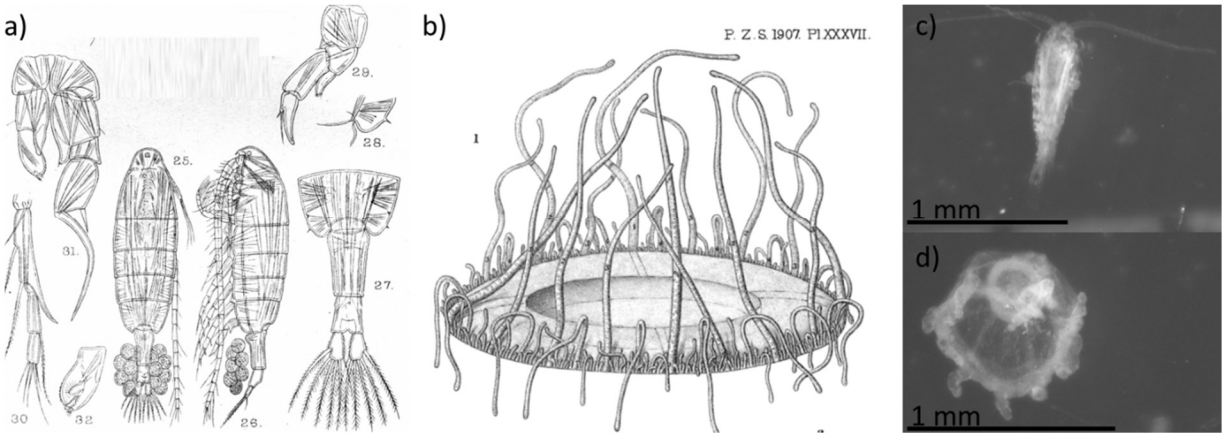

Figure 8: the zooplankton species that were sequenced including illustrations of a) T. simplex (Sars, 1909) and b) L. tanganjicae (Günther, 1907) and microscopic pictures of c) T. simplex and d) L. tanganjicae (©Charlotte Huyghe).

Different PCR techniques were tried out on the samples to investigate which method worked best on the zooplankton used in this study. On the *T. simplex* samples, two different PCR reaction mixes were used per sample. The first reaction mix contained 10 µl MyTaq^{TM} Mix, 1 µl forward primer (ZplankF1_t1, 5'-TCTASWAATCATAARGATATTGG-3', 20 µl, Prosser et al., 2013), 1 µl reverse primer (ZplankR1_t1, 5'-TTCAGGRTGRCCRAARAATCA-3', 20 µl, Prosser et al., 2013), 7 µl H_2O and 1 µl DNA template. The second reaction mix was composed according to the amplicon COI PCR of Leray et al. (2013) as described before. Two reaction mixes were also used on the sample of *L. tanganjicae*. The first reaction mix contained 10 µl MyTaq^{TM} Mix, 1 µl forward primer (Jelly F, 5'-GGTCAACAACAAATCATAAAGATATTGG-3', 20 µl), 1 µl reverse primer (Jelly R, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', 20 µl), 7 µl H_2O and 1 µl DNA template. The second reaction mix was composed according to the amplicon COI PCR of Leray et al. (2013) as described before. All samples with different reaction mixes followed the same gradient standard PCR program. First, initial denaturation occurred at 95 °C for 1 minute followed by 35 cycles. Each cycle had a denaturation at 95 °C for 15 s, annealing for 15 s and extension at 72 °C for 10 s. The final extension was at 72 °C for 5 min. For each initial sample (TS1, TS2 and LT1), 8 subsamples for each different reaction mix were made. These 8 subsamples had different annealing temperatures during the PCR (42.1 °C, 43.4 °C, 44.8 °C, 46.2 °C, 47.7 °C, 49.1 °C, 50.5 °C and 51.8 °C). These differences in primers and annealing temperatures were carried out during the experiment to investigate which primer and annealing temperature worked best for the zooplankton samples used. To investigate which combinations worked best, a gel electrophorese was carried out with all samples. The samples with the annealing

temperature of 49.1 °C or 50.5 °C contained a sufficient amount of DNA. Only these samples were used for the following procedure.

After the PCR cleanup with an Agencourt AMPure beads kit, a Big Dye Terminator sequencing protocol was followed as described before. The samples were cleaned using CleanDTR beads and sequenced.

3.6 Data filtering and statistical analysis

Reads from the metabarcoding were quality checked, filtered and aligned using Qiime v1.9.1 (Caporaso et al., 2010) in Nephele v1.8 (Weber et al., 2018). OTU's with less than 53 reads were excluded since the dataset was too big. The remaining OTUs were identified using the Barcode of Life Data system (BOLD, www.boldsystems.com; Ratnasingham and Hebert, 2007) (Addendum, Table B2). OTUs that identified as *S. tanganicae*, *L. miodon*, contamination (i.e. human DNA, marine fish) or non-Animalia were excluded from the dataset. The dataset was filtered, transformed and analysed according to the following instructions by using Calypso v8.68 (Zakrzewski et al., 2017). Samples with less than 10 read counts and OTU's with less than 0.01 relative abundance over all samples were removed. The remaining data was total sum scaled (TSS), cumulative sum scaled (CSS) and log transformed. This accounted for the sparse sequencing count data as discussed by Paulson et al. (2013). A rarefaction curve was made for each location to investigate whether the sample size of remaining individuals was sufficient for the analysis of the data.

The statistical analysis was conducted using Calypso v8.68 (Zakrzewski et al., 2017) and R (R Core Team, 2018; RStudio Team, 2016). The packages used for R were vegan (Oksanen et al., 2019), MASS (Venables and Ripley, 2002), devtools (Wickham et al., 2019) and ggord (Beck, 2019). The prey item diversity was assessed using the Shannon diversity index (Shannon, 1948). The significance between treatments (i.e. sardine species, location, season, standard length SL and sex) were tested using a multivariate ANOVA (PERMANOVA). A principal coordinate analysis (PCoA) was made to assess the similarity in stomach content between specimens. A bar chart was made using the relative presence or absence of prey species in the sardine stomachs per location and season for both sardine species. These bar charts showed the species up to order level.

The sequences from the sardine species identification were aligned and analysed using geneious v10.2.2 (https://www.geneious.com). A phylogenetic tree was made to compare the sequences to each other and to group genetically similar individuals. The sequences were used to identify the sardine species of each specimen using BOLD. Individuals that were identified differently with the genetic method compared to the morphological method, were morphologically re-examined.

The sequences that resulted from the zooplankton sequencing were aligned and quality checked using geneious v10.2.2. Good quality DNA sequences were added to BOLD. The sequences were used as a reference for the prey sequences found in the stomach of the sardines.

4 Results

4.1 Sardine species identification

Sequencing of the 313 bp region of the COI gene of the sardines indicated a morphological misidentification of a few individuals. 14 % of the specimens morphologically identified as *Stolothrissa tanganicae* were genetically identified as *Limnothrissa miodon* and 10% of the specimens morphologically identified as *L. miodon* were genetically identified as *S. tanganicae* (Addendum, Fig. B4). These individuals were morphologically re-examined. Most of them were morphologically misidentified, except for two specimens. It was unclear to which species these two individuals belonged and they were excluded from the dataset.

4.2 Stomach content

A total of 57 out of 140 stomach samples remained to be analysed after filtering (controls excluded; Table 2). A rarefaction curve of the locations and species showed an inadequate remaining sample size for most locations (Table 2; Addendum, Fig. B5). The rarefaction curve of a dataset with a sufficient sample size should reach an asymptote. It was almost impossible to create a rarefaction curve for *S. tanganicae* from Uvira during the dry season, Bujumbura and Sumbu, and for *L. miodon* from Uvira during the dry season, Bujumbura, Sumbu and Mpulungu. Of the created rarefaction curves, only Uvira (ST: n= 7, LM: n= 10) during the wet season and Mpulungu for *S. tanganicae* (n= 13) seemed to reach an asymptote. The other locations did not show any trend towards an asymptote.

Table 2: Remaining number of stomach samples after sequencing and filtering.

	Uvira Dry	Uvira Wet	Bujumbura Wet	Kalemie Wet	Sumbu Wet	Mpulungu Wet
S. tanganicae	4	7	0	6	3	13
L. miodon	3	10	3	6	1	1

A significant difference was found in the stomach content between *S. tanganicae* and *L. miodon*. This was the case when looking at all samples (PERMANOVA, F-value = 6.32, $p < 0.001$; Table 3). The samples from the wet season showed also a significant difference in stomach content between sardine species (PERMANOVA, F-value = 6.20, $p < 0.001$). A PCoA plot showed no distinct clustering of the two sardine species (Fig. 10b). The samples from Uvira from both seasons also showed a significant difference of the stomach content between species (PERMANOVA, F-value = 3.20, p=0.020). A PCoA plot showed that both species clustered apart, especially on the y-axis (y-axis: 18 %) (Fig. 10a).

There was a significant difference in stomach content between locations over all samples (PERMANOVA, F-value = 4.62, $p < 0.001$; Table 3). In the wet season, the stomach content was significantly different between locations for both species together (PERMANOVA, F-value = 4.20, $p < 0.001$), for only *S. tanganicae* (PERMANOVA, F-value = 3.03, $p < 0.001$) and for only *L. miodon* (PERMANOVA, F-value = 3.27, $p < 0.001$). A PCoA plot showed a

clear clustering of the individuals from Uvira. For *S. tanganicae* the clustering was on both axes (x-axis: 30 %; y-axis: 23 %) (Fig. 10c) and for *L. miodon* mainly on the y-axis (y-axis: 14 %) (Fig. 10d). The *L. miodon* specimens from Kalemie were clustered on the x-axis (x-axis: 24 %). The other locations were spread (Fig. 10c, 10d). A comparison of the stomach content showed that the Uvira specimens were significantly different from all other locations (Pairwise test, $p < 0.050$; Table 4). When the specimens from Uvira were excluded from the dataset, all locations and basins were still significantly different from each other (PERMANOVA, F-value = 1.96 and $p = 0.007$ for location, F-value = 1.92 and $p = 0.018$ for basin).

For the samples from Uvira, a significant difference was found in the stomach content between seasons (PERMANOVA, F-value = 6.84, $p < 0.001$; Table 3). The PCoA plots of *S. tanganicae* (x-axis: 45 %) (Fig. 10e) and *L. miodon* (x-axis: 41 %) (Fig. 10f) both showed a distinct clustering of the wet season and dry season individuals. This clustering was especially clear on the x-axis.

The stomach content of the sardines depended on the standard length (PERMANOVA, F-value = 2.25, $p = 0.015$; Table 3). The effect of the standard length (SL) depended on the sardine species (PERMANOVA, F-value = 1.98, $p = 0.044$; Table 3). When looking at both species separately, the stomach content depended on the standard length for *S. tanganicae* (PERMANOVA, F-value = 3.88, $p < 0.001$) but not for *L. miodon* (PERMANOVA, F-value = 0.93, $p = 0.50$). The difference in stomach content between sexes was not significant (PERMANOVA, $p = 0.056$).

Table 3: Bonferroni adjusted PERMANOVA of the variables species, location, season and standard length of the sardines and their interaction. Significant values are underlined.

Variable	Df	Sums of Sqs	Mean Sqs.	F. Model	R^2	Pr (>F)
Species	1	1.67	1.67	6.32	0.08	<u>0.001</u>
Location	2	2.44	1.22	4.62	0.12	<u>0.001</u>
Season	1	1.81	1.81	6.84	0.09	<u>0.001</u>
Standard Length	1	0.59	0.59	2.25	0.03	<u>0.015</u>
Species:Location	2	0.98	0.49	1.85	0.05	<u>0.010</u>
Species:Season	1	0.03	0.03	0.12	0.00	1.000
Species:Standard Length	1	0.52	0.52	1.98	0.03	<u>0.044</u>
Location:Standard Length	2	0.59	0.30	1.13	0.03	0.287
Season:Standard Length	1	0.27	0.27	1.01	0.01	0.427
Species:Location:SL	1	0.21	0.21	0.79	0.01	0.615
Species:Season:SL	1	0.16	0.16	0.60	0.01	0.843
Residuals		11.10	0.26		0.54	
Total		20.38			1.00	

Table 4: Pairwise ANOVA, Benjamini adjusted, between Locations. Significant values are underlined.

Locations	F. Model	R2	p. value	p. adjusted
Uvira vs. Bujumbura	4.461	0.199	0.004	0.010
Uvira vs. Kalemie	6.285	0.189	0.001	0.003
Uvira vs. Sumbu	6.514	0.255	0.001	0.003
Uvira vs. Mpulungu	8.295	0.222	0.001	0.003
Bujumbura vs. Kalemie	1.737	0.118	0.049	0.082
Bujumbura vs. Sumbu	2.426	0.327	0.140	0.175
Bujumbura vs. Mpulungu	1.654	0.099	0.183	0.203
Kalemie vs. Sumbu	2.068	0.129	0.030	0.060
Kalemie vs. Mpulungu	1.726	0.067	0.085	0.121
Sumbu vs. Mpulungu	1.353	0.078	0.210	0.210

The prey species diversity varied per location and season (Fig. 9). The mean Shannon diversity index over all samples was 1.71. The overall diversity of prey species was lower for *S. tanganicae* (Shannon index = 3.49) than for *L. miodon* (Shannon index = 4.16). The sardine species, location, and season all had an influence on the stomach content of the sardines. A bar chart (Fig. 11) showed differences in the presence of prey taxa in the stomachs between all these variables. Arthropoda species were the main prey items found over all samples (67 % presence in the stomachs), especially from the order of Diptera (31 %), Calanoida (10 %), Cyclopida (10 %) and Decapoda (9 %). The orders Lepidoptera (5 %), Anostraca (2 %), Ephemeroptera (1 %) and Amphipoda (<1 %) were less represented. In some samples, especially from Uvira in the wet season, Cichliformes species (Chordata) were strongly represented (27 %). Less represented were Limnomedusae (Cnidaria, 4 %), Siluriformes (Chordata, 1%), Artiodactyla (Chordata, <1 %) and Ascaridida (Nematoda, <1 %). The Artiodactyla consists of *Sus scrofa* (pig, 98.51 % DNA match) and the Siluriformes of *Auchenoglanis occidentalis* (96.43 % DNA match) (Fig. 11).

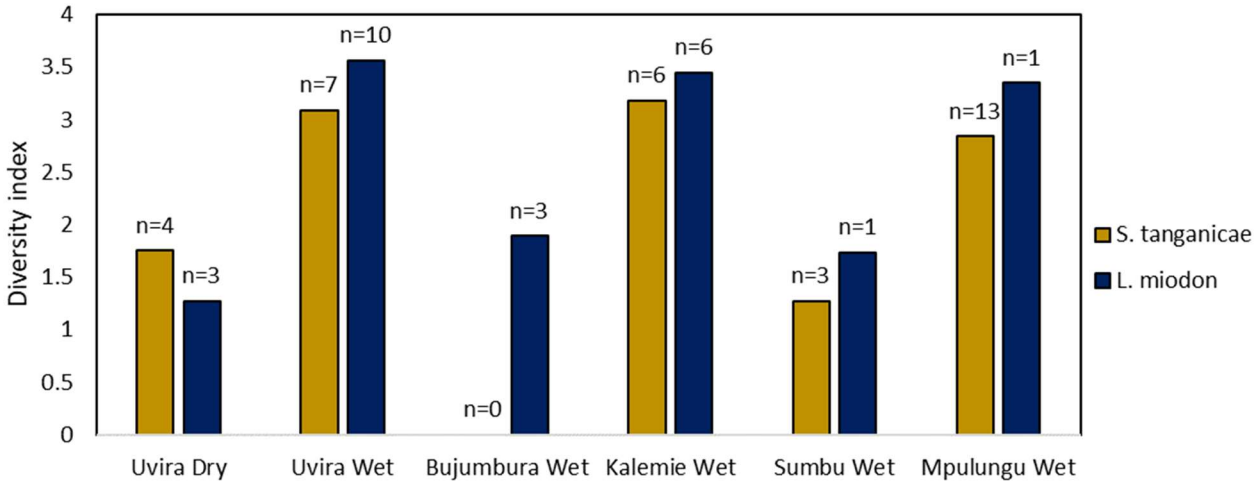

Figure 9: The Shannon-Wiener diversity index for all individuals (n) of each sardine species per location and season.

The DNA sequences that resulted from the sequencing of the zooplankton species *Limnocnida tanganjicae* and *Tropodiaptomus simplex* were compared to the prey item dataset (Addendum, Table B3). One cnidarian OTU was present in the stomach samples. It was identified as *L. tanganjicae* using the sequences from this study (98.2 % DNA match). 35 % of the stomachs contained DNA of *L. tanganjicae.* There were two OTU's found in the stomach of the sardines that had a high DNA match with the sequenced *T. simplex* (96.63 % and 98.83 % DNA match). The OTU with the highest similarity to *T. simplex* occurred in 42 % of the stomach samples. The OTU with the lower similarity to *T. simplex* occurred in 29 % of the samples.

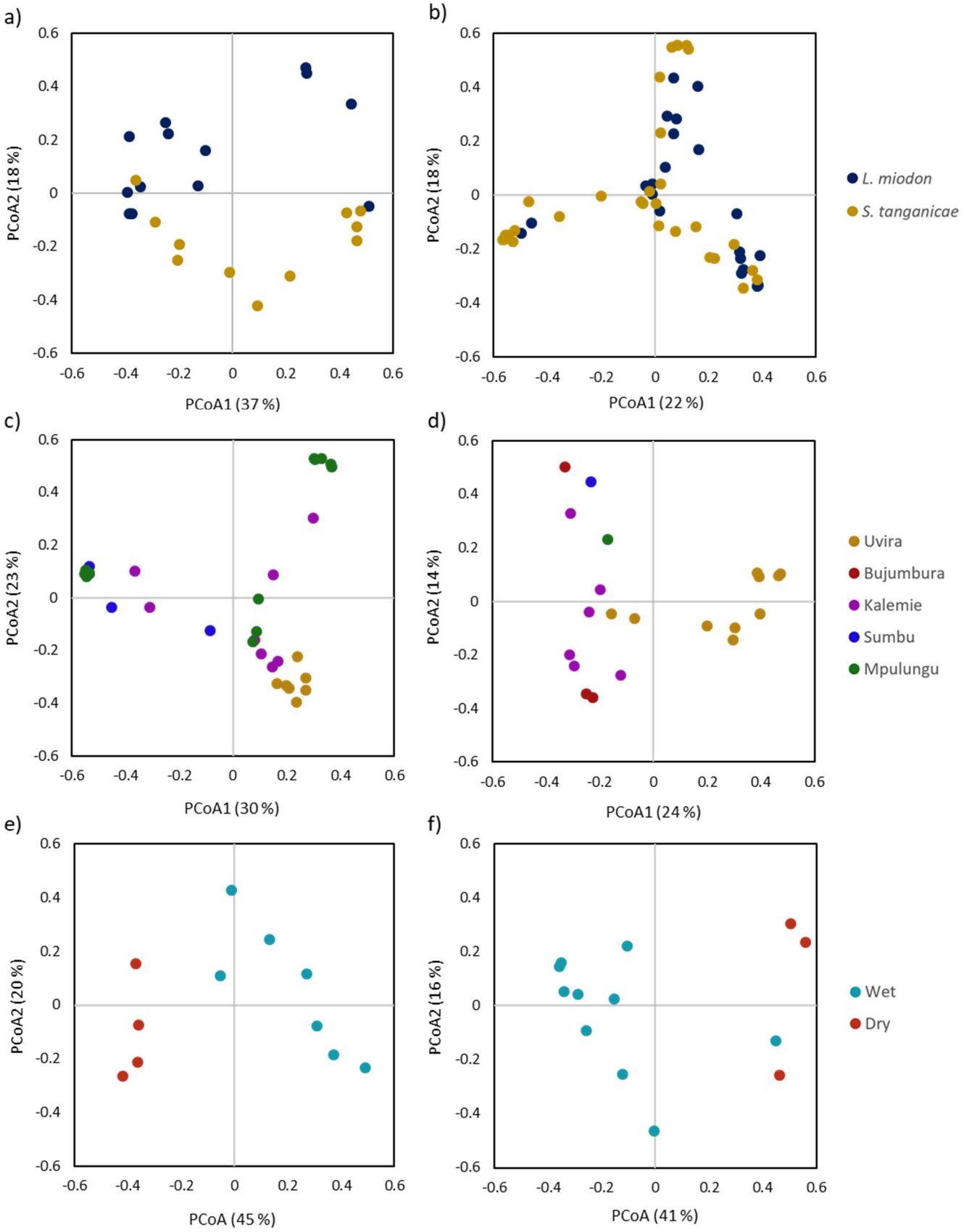

Figure 10: PCoA of S. tanganicae and L. miodon from a) Uvira in both seasons and b) all locations in the wet season. PCoA of all locations (north: Uvira and Bujumbura; central: Kalemie; south: Sumbu and Mpulungu) in the wet season of c) S. tanganicae and d) L. miodon. PCoA of Uvira in both seasons (wet and dry) of e) S. tanganicae and f) L. miodon.

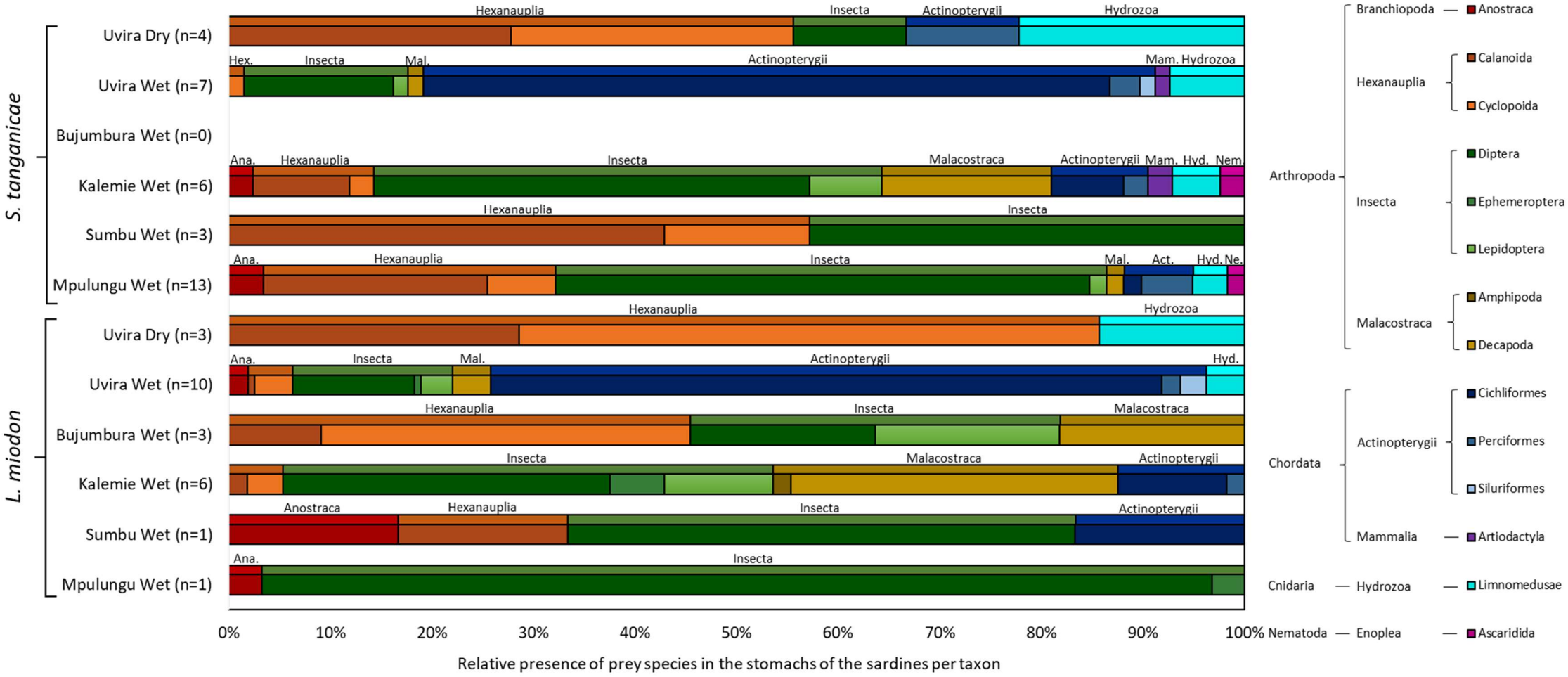

Figure 11: relative presence of prey species found in the stomachs per sardine species (S. tanganicae and L. miodon) per location (north: Uvira and Bujumbura; central: Kalemie; south: Sumbu and Mpulungu). The species are grouped within order level.

5 Discussion

Metabarcoding proved to be a valuable technique for identifying the stomach content of *Stolothrissa tanganicae* and *Limnothrissa miodon*. It showed significant differences in prey item composition between both sardine species, location (north, central and south) and season (wet and dry). Some prey taxa were known prey items of the Lake Tanganyika sardines, while others have never been reported before.

5.1 Experimental design

In this study, 40.7 % of the sequenced stomach samples could be used for the analysis. The other samples did not contain enough prey DNA. During the dissections, it became clear that several sardine stomachs were empty. Most fish, especially *S. tanganicae*, were caught by local fishermen during the night. However, the sardines feed mostly at dusk and dawn (Mgana et al., 2014). It would be an improvement if the fish are caught around this time or just after since most of their stomach content won't be fully digested. It would be good to immediately put the sardines in separate tubes filled with ethanol to avoid any cross-contamination between specimens. Defining the location of sampling and the distance to the coast would also be an improvement. The pelagic zooplankton composition closer to the coast could differ from offshore zooplankton community.

In this study, several locations had a limited number of remaining samples that could be used in the analysis due to an insufficient amount of prey DNA or filtering. A rarefaction curve of each location showed an inadequate number of samples for several locations, especially for Sumbu and Bujumbura. These two locations each had a total of 3-4 samples for both species together. This could have biased the results. The abundance and diversity of the taxa found in these samples could be unrepresentative for the general stomach content of the sardines. Some taxa that are rare could be present and other more abundant taxa could be absent by chance.

Some DNA sequences found in the stomach samples after PCR included DNA from the European plaice (*Pleuronectes platessa*), European brown shrimp (*Crangon crangon*) and *Magelona mirabilis* (Polychaeta). These examples are all marine species which do not occur in Lake Tanganyika (World Register of Marine Species WoRMS, 2019). Anostraca DNA (fairy shrimp, Arthropoda-Branchiopoda) was present in several samples from multiple locations. Until now, no studies mention the occurrence of Anostraca in Lake Tanganyika (Coulter, 1991). Amphipoda DNA (Arthropoda-Malacostraca) was only found in one stomach sample of *L. miodon* from Kalemie. There are no records of amphipods living in Lake Tanganyika. The occurrence of these taxa is probably a result of contamination during the labwork or due to low DNA quality, leading to misidentification.

During PCR, contamination can occur. There are three major sources of contamination (Dennis Lo, 1998). Carryover contamination occurs with PCR products from earlier amplifications. DNA from earlier amplifications can be present in high numbers. Another source could be cloned DNA within the laboratory. Sample to sample contamination is a third source (Dennis Lo, 1998). In the laboratory were the experiments from this study were conducted, carryover and sample to sample are probably the main sources of contamination (since no DNA is cloned). This laboratory was also used for the genome analysis of marine species. It is feasible that some contamination of our samples by marine species occurred since the blank samples also contained DNA from these species. A lab investigation found the possible source of these contaminants. The primers used for the COI metabarcoding were probably contaminated by an earlier study on the food sources of the European plaice. The laboratory was also used for studies on Anostraca. However, no Anostraca DNA was present in the blank samples. Nonetheless, Anostraca normally don't live in large freshwater lakes such as Lake Tanganyika and prefer ephemeral pools, high mountain lakes or saltwater lakes (Dumont, 2009). The Anostraca DNA found in the samples is probably a result of contamination. A measurement to decrease the contamination risk is to use a sample preparation, pre- and post-PCR area which are physically separated from each other. No material, including lab books, should be handled between them (Dennis Lo, 1998). In future studies, it would also be good to replicate one stomach sample several times to estimate the reliability of the results (Ficetola et al., 2016).

A good reference database is vital for metabarcoding. Not many possible prey species from the area of sampling were present in the COI database of BOLD. Most of the prey specimens were only identified up to order level. The sequencing of possible prey taxa (*Tropodiaptomus simplex* and *Limnocnida tanganjicae*) proved to be useful in this study. It would be beneficial for future studies to sequence more possible prey species from Lake Tanganyika. The accuracy of the prey item identification will improve when the reference database is updated with local species. It will also be possible to identify the prey item down to a lower taxonomic level.

Determining whether *L. miodon* consumes *S. tanganicae* or the other way around was impossible in this study since the risk of sample to sample contamination was very high. DNA from both species was present in all samples. Cannibalism within species was also impossible to observe. It would be good to visually identify the stomach content prior to metabarcoding. This could give an idea of the life stages of the prey taxa consumed. Metabarcoding was proven useful in this study since cnidarians were never found before in the stomach of the Lake Tanganyika sardines by using visual techniques. In future studies it would be good to combine several techniques to determine the food sources, including metabarcoding, visual analysis and stable isotope analysis.

5.2 The effect of species, location, season and body size

The stomach content of the two sardine species were significantly different from each other. The prey diversity was higher in the stomach samples of *L. miodon* compared to *S. tanganicae*. De Iongh et al. (1983) stated that the composition of the pelagic *L. miodon* stomach content resembles the composition of the pelagic plankton community. This is probably the case for *S. tanganicae*. Both species are pelagic but *L. miodon* lives closer to the shore in comparison to *S. tanganicae* (Phiri and Shirakihara, 1999). The pelagic zone is less diverse when compared to the littoral zone (Coulter, 1991). The most diverse habitat in Lake Tanganyika is the rocky littoral environment (Van Steenberge et al., 2011). Pelagic fish may feed on littoral prey due to their mobility (Schindler et al., 1996). It is possible that *L. miodon* migrates close enough to the littoral zone to feed on littoral zooplankton or that some littoral species occur or drift off to the pelagic zone. In Lake Kivu, the diet composition of *L. miodon* depended on their inshore-offshore migrations (de Iongh et al., 1983). This could also be true for *L. miodon* within Lake Tanganyika. If this is true, *L. miodon* is able to feed on both littoral and pelagic species while *S. tanganicae* is restricted to a pelagic diet. Authors like Marshall (1993) and Coulter (1991) also mention a more varied diet of *L. miodon* compared to *S. tanganicae*. *Limnothrissa miodon* could be an important link between the littoral and the pelagic food webs.

This study showed that the feeding habit of the Lake Tanganyika sardines is linked to their location. There was a significant difference in stomach content between locations and basins for both species in the wet season. Especially Uvira was significantly different from all other locations within the same season. The difference between basins can be attributed to a variation in species composition between the north and the south of Lake Tanganyika. Kurki et al. (1999) noted that the north is more dominated by cyclopoids, clupeids and medusae while the south is dominated by calanoids, shrimps and *L. stappersii*. The zooplankton composition varies in response to the environmental differences across depths and sites. The composition of the pelagic zooplankton community has been matched with the stomach content of *L. miodon* before (de Iongh et al., 1983). A study on the gut content of the sardines showed no significant difference in microbiome composition between locations and suggests that both sardine species migrate throughout Lake Tanganyika (Aerts, 2019). A genomic study on *S. tanganicae* shows no population structure and also suggests lake wide migrations of the sardine (De Keyzer et al., 2019). The stomach content shows what was eaten most recently. A detailed map of zooplankton distribution and the analysis of the stomach content of the sardines could give an estimate of the daily migrations of the sardines. A stable isotope analysis indicates which food the individual has consumed and assimilated in their tissue over a longer period of time. The type of ecosystem, the species, the growth of the organism and the used tissue determine the length of that period which can vary from days to months (Pasquaud et al., 2010). The stomach content analysis and the stable isotope analysis together will help to better understand the

migration pattern of the Lake Tanganyika sardines. Until now, only a study on the otolith elemental composition of the sardines was able to retrace the catch area of the fish to some extent. The elemental differences in water composition between locations was reflected in the otolith composition of the sardines from that area (Sako et al., 2005). Analysing the stomach content of sardines could also be used to identify their catch area if it would be possible to have a clear and detailed picture of zooplankton distribution over the lake throughout the year. Metabarcoding of the stomach content could be used as a control to monitor the fishing locations of the sardines that are sold.

The samples from Uvira showed a significant difference in stomach content between the wet and the dry season. The diversity in the wet season was higher than the dry season. The zooplankton composition in the lake shifts between seasons due to nutrient upwellings and algal blooms (De Wever et al., 2007). An algal bloom was observed several days prior to the sampling of the dry season specimens (personal observation). This could have been a result of the secondary upwellings (Plisnier et al., 1999). The algal bloom could have caused a rise in zooplankton biomass. Since the sardines are visual predators (Isumbisho et al., 2006), it is possible that the sardines favour certain prey items when they have the choice. In Lake Kivu, the introduction of *L. miodon* probably caused the disappearance of a large *Daphnia sp.* and the decrease in body size of another cladoceran species (Isumbisho et al., 2006). In this study, the sardines of Uvira fed to a greater extend on copepods and medusae during the dry season compared to the wet season. The sardines could prefer the larger calanoid *Tropodiaptomus simplex*, other copepods and medusae when they are abundant enough. When these zooplankton groups become less abundant, the sardines have to be less selective and more opportunistic in their feeding behaviour. This could explain the decreased prey diversity in the stomachs during the wet season.

Over all samples, diet composition depended on the standard length of the sardines. However, the standard length was correlated with the sardine species. When only looking at one species, *L. miodon* showed no correlation between stomach content and standard length while the stomach content of *S. tanganicae* did depend on their standard length. When the body size of an individual increases, the mouth width often also increases. The sardines will be able to consume lager prey when they are larger themselves.

5.3 Prey composition

DNA from the cnidarian species *Limnocnida tanganjicae* (Cnidaria-Hydrozoa) was found in several stomach samples from *S. tanganicae* and *L. miodon*. The DNA sequence found in the stomach had a high match with the sequenced *L. tanganjicae*. It is safe to say that the cnidarian DNA found in the stomach of the sardines was not a result of lab contamination. Clupeid species are visual predators (Coulter, 1991). Consumption by accident seems unlikely. This suggests

that both clupeid species feed on *L. tanganjicae* medusae, something that was never observed before in Lake Tanganyika (Kurki et al., 1999; Salonen et al., 2012). Dumont (1994) mentioned a strong vertical migration of *Limnocnida* in Lake Tanganyika. He noted that *Limnocnida* swarms stayed below sardine schools during the day and rose to the surface at night. He tried to explain the cause of this behaviour, either attributing it to predator avoidance or light evasion. No species were known to feed on *L. tanganjicae*. Dumont (1994) suggested benthic decapods as their predators. However, no evidence has been found and some observations contradict this idea (Salonen et al., 2012). Predation by the Lake Tanganyika sardines might explain the daily vertical migration pattern of *L. tanganjicae*. Predation on medusae has already been documented for marine clupeids like *Sardinops sagax* and *Opisthonema oglinum* (Ates, 1988). It is important that the stomachs of fresh fish are examined for visual identification. The gelatinous material of medusae is easily destroyed by preservation or digestion which might explain the lack of previous evidence of predation on *L. tanganjicae* by *S. tanganicae* and *L. miodon* (Purcell and Arai, 1997). Medusa DNA was more abundant in the stomach samples from the northern basin, especially in the dry season. Kurki et al. (1999) found that the macrozooplankton community in the northern end of the lake is predominated by medusae which could explain this finding. A few days prior to sampling of the dry season specimens, an algal bloom was observed near Uvira. At dawn, the medusae could be seen near the surface between the algae (personal observation).

Calanoida and Cyclopoida DNA (Arthropoda-Hexanauplia) was present in the samples of both species and all locations. However, Cyclopoida DNA was absent in the *L. miodon* samples from Sumbu and Mpulungu (southern basin). As mentioned before, the number of samples originating from Sumbu and Mpulungu is quite low. More stomach samples with sufficient prey DNA are needed to determine whether Cyclopoida are a prey item of *L. miodon* in the southern basin. In the wet season, calanoids seemed to be more present in the samples from the southern basin. Cyclopoida DNA occurred mainly in samples from the northern basin. The presence of Calanoida in the south is higher than in the north while Cyclopoida dominate the north (Kurki et al., 1999; Langenberg et al., 2008). In Uvira, calanoids and cyclopoids were more present in the dry season compared to the wet season. The upwelling in the north during the dry season and the observed an algal bloom a few days prior to sampling could explain this increased presence (Langenberg et al., 2008). The calanoid DNA most likely originates from *Tropodiaptomus simplex* and/or a highly related species since it showed a high correlation in DNA sequence with the *T. simplex* individuals sequenced in this study.

Diptera DNA (Arthropoda-Insecta) was present in stomach samples from almost all locations for both clupeid species, with an exception for *L. miodon* from Uvira in the dry season. The number of Diptera taxa present in southern samples was higher when compared to northern samples. The abundance of Diptera in central samples was less or similar to the southern

samples. De Iongh et al. (1983) observed clupeids feeding on chironomids (Diptera) in Lake Kivu. Chironomid larvae are often important food sources in aquatic ecosystems (Armitage et al., 1995). When chironomid pupae are mature, they rise to the surface. During this time, chironomid larvae are susceptible for predation (Oliver, 1971). The observed chironomid prey of clupeids in Lake Kivu included species of *Microchironomus*, *Procladius*, *Cladotanytarsus*, *Kiefferelus* and Tanypodinae (de Iongh et al., 1983). *Microchironomus spp.* occur in relatively deep water up to 64 m in Lake Tanganyika. They are consumed by several *Xenotilapia* species that live in deeper waters. Some *Xenotilapia spp*. that live closer to the shore feed mostly on Tanytarsini which occur mainly in the littoral zone (Gysels et al., 1997). Eggermont et al (2008) observed several chironomid species in the sandy bottom of a bay in Kigoma (Tanzania) at 0-80 m dept. These included 13 species of Chironominae (seven Chironomini and six Tanytarsini), one species of Orthocladinae and two species of Tanypodinae. No chironomid larvae were found at a depth of 50-80 m (Eggermont et al., 2008). They also found that in their study area, chironomid larval distribution was linked to the density of macrophytes (Gross et al., 2002; Eggermont et al., 2008). Macrophytes are generally not abundant in the littoral zone of Lake Tanganyika. There are a few exceptions including a shallow region with small islands near Mpulungu (Coulter, 1991). If chironomids are indeed more abundant in regions with a higher macrophyte density, this could explain the higher abundance of Diptera in the stomachs of the sardines near Mpulungu.

There was also other DNA from the class of Insecta was present in the stomach samples. Ephemeroptera DNA (mayfly) occurred in several *L. miodon* samples from Uvira, Kalemie and Mpulungu in the wet season. Mayflies occur in Lake Tanganyika (Coulter, 1991). Other fish species in the lake, like the benthopelagic cichlid, *Lobochilotes labiatus,* prey on Ephemeroptera (Kohda and Tanida, 1996). Of the two clupeid species, only *L. miodon* consumed Ephemeroptera. *Limnothrissa miodon* occurs closer to the shore when compared to *S. tanganicae,* which could explain this difference (Coulter, 1991). DNA from Lepidoptera species was present in several samples from Uvira, Bujumbura, Kalemie and Mpulungu and in samples of both clupeids. Several Lepidotera species occur in and around Lake Tanganyika (Coulter, 1991).

Decapoda DNA (Arthropoda-Malacostraca) was almost completely absent in the stomach samples from the south. Shrimp DNA was only detected in the stomachs of *S. tanganicae* from Mpulungu. This was unexpected, since the zooplankton composition in the south is dominated by shrimps (Kurki et al., 1999). Kurki et al. (1999) and Mannini et al. (1999), note that shrimps are very abundant in the pelagic zooplankton community in the south. They also mention that they are important prey for both clupeid species. Why they are underrepresented in this study is unclear. Coulter (1991) noted that shrimps do not often occur in the stomach of sardines. He attributes this finding to the possible patchiness of shrimp schools. Sardines have to encounter

such a school to be able to feed on them, which is probably not always the case (Coulter, 1991). Several authors noted the presence of shrimps in the southern zooplankton community of Lake Tanganyika throughout the year. Their abundance peaks at the end of the dry season (Kurki et al., 1999). Kurki et al. (1999) noted that the abundance of shrimps in the stomachs of *L. stappersii* from Mpulungu is high all year round in contrast to the north where *L. stappersii* feeds mainly on copepods. Perhaps *L. stappersii* is in a strong competition for food with the clupeids in the south during months with less shrimps. However, our results are more likely a consequence of the low sample size of the southern sardines, as shown by the rarefaction curve. Apart from the south, shrimp DNA was present in samples from Bujumbura and Uvira in the wet season.

DNA from multiple fish species (Chordata-Actinopterygii) was present in the stomachs of the Lake Tanganyika sardines. They probably feed extensively on their eggs, larvae, fry and juveniles that occur in the pelagic zone. DNA fragments of several cichlid species were found in the stomach of both sardine species from several locations. Some of the cichlid genera with a high DNA match occur in the pelagic environment like *Boulengerochromis*, *Bathybatini* and *Cyprichromini* (Koblmüller et al., 2008; Kirchberger et al., 2012; Koblmüller et al., 2014). Some species with a higher match did not occur in the pelagic environment. Cichlids have ill-defined species boundaries. It is difficult to identify them correctly with only a DNA match to a reference database (Breman et al., 2016). It would be better to combine several techniques to identify the cichlid species. Cichlid species from Lake Tanganyika should be identified using morphospecies and species complexes (Breman et al., 2016). There was much cichlid DNA present in the stomach samples of both sardine species from Uvira in the wet season. It is unclear what caused this strong presence of cichlid DNA in the stomachs. The spawning of cichlids coincided with the sampling of the sardines. Sampling took place around the fourth quarter of the lunar cycle, when the intensity of the moonlight is low. At this time, a peak in larval dispersal of some cichlid species (e.g. *Cyprichromis leptosome*) occurs to avoid visual predation (Watanabe, 2000). It is possible that the increased density of cichlid larvae in the water column explains their high presence in the stomachs of the sardines. Perciformes DNA was present in samples of both sardine species from several locations. Both *Lates stappersii* and *Lates microlepis* occur in the pelagic zone (Coulter, 1991). Juveniles of *L. stappersii* occur in the northern basin of the lake. The southern basin is dominated by adult *L. stappersii*, which are predators of the sardines (Kurki et al., 1999). *Lates microlepis* is a top predator in the pelagic zone, predating on both clupeid species (Coulter, 1991). It is plausible that the sardine species feed on the perciform eggs or juveniles. Coulter (1991) noted that *L. stappersii* juveniles with a length of several millimetres occur in the zooplankton of the pelagic zone. The other *Lates spp.* juveniles stay more inshore (Coulter, 1991). Siluriformes DNA was only found in samples from Uvira in the wet season for both clupeid species. The highest DNA match with the BOLD

reference system was *Auchenoglanis occidentalis* (96.4 %). *Auchenoglanis occidentalis* is a benthic catfish from the family Bagridae and occurs in rivers and lakes throughout Africa. This species has been reported to occur in Lake Tanganyika up to a depth of 20 m and up to 140 m in the south (Coulter, 1991). They have the capacity to strongly adapt to different environments and ecological circumstances. However, no proof exists that these fish, or their eggs, occur in the pelagic zone of Lake Tanganyika (Coulter, 1991). Several Siluriformes species live in the deep waters of the lake, some of them scavenging on dead fish from the pelagic zone. Only one species has been described that moves occasionally to the pelagic zone during the night to prey on clupeids, *Dinotopterus cunningtoni* (Coulter, 1991; van Zwieten et al., 2002).

Pig DNA (*Sus scrofa*) was found in two *S. tanganicae* samples originating from Uvira and Kalemie. It is possible that this DNA originated from domesticated pigs. Pig is consumed in the Uvira region, although not so much as other local livestock (Katunga and Muhigwa, 2014). In aquacultures worldwide, animal waste is used as a fish feed. In Africa, pig manure is used in polyculture of the cichlid *Oreochromis niloticus* and the catfish *Clarias gariepinus* (Prinsloo and Schoonbee, 1986). However, there are currently no records of active aquaculture near Uvira or Kalemie. It is feasible that pig waste was used by fishermen to lure schools of *S. tanganicae* to their nets. It is more plausible that pig waste was discarded into the lake by fishermen or though faeces. Nematoda DNA was present in the *S. tanganicae* samples from Kalemie and Mpulungu. Both free living and parasitic nematodes occur in Lake Tanganyika (Coulter, 1991). It is unclear whether the DNA found in the stomach samples concerned parasites of the sardines or its prey items, or if it were prey items themselves.

5.4 Implications for trophic relationships

Data from the stomach analysis can help to investigate trophic relationships and estimate the trophic level of the sardines (Pasquaud et al., 2010). Our study indicated that the Lake Tanganyika sardines are mainly pelagic plankton feeders. Some of the DNA found in the stomach (e.g. pig DNA) would suggest that the sardines could also be scavengers or filter feeders. However, no previous evidence of scavenging behaviour of *S. tanganicae* and *L. miodon* exists. Chéné (1975) suggested that the Lake Tanganyika sardines at least partially filter feed. He noted that the prey present in the stomachs is very diverse and included very small copepods. He mentioned that the gill-rakers of *S. tanganicae* had characteristics found in filter feeders (Chéné, 1975). Since there is no further evidence to support this theory, it is probable that these prey items were ingested by accident. The sardines are an important link between plankton and species of higher trophic levels. The Lake Tanganyika sardines seem flexible in their prey choice. Factors influencing the diversity and amount of zooplankton indirectly influence the feeding habits of the sardines (de Iongh et al., 1983). Nutrient upwelling, moon phase and human input for example, might influence their stomach content. *Stolothrissa tanganicae* had a less diverse diet compared to *L. miodon*. The prey taxa of *S. tanganicae*

comprised mainly Copepoda (dominated by Calanoida) and Diptera (most likely larval stage). They also preyed on cichlids and medusae. In lesser extent, they fed on Decapoda, Perciformes, Lepidoptera (larvae) and Nematoda. *Limnothrissa miodon* also fed mainly on Copepoda (dominated by Cyclopoida) and Diptera (larvae). Cichlids, medusae, Decapoda and Lepidoptera (larvae) were also part of their prey composition. *Limnothrissa miodon* rarely fed on Ephemeroptera, Perciformes and Siluriformes. It is important to note that this study only shows the Animalia prey species of the sardines. Both clupeids may also feed extensively on algae or species from other biological kingdoms. Juvenile sardines for example feed on phytoplankton like *Nitzschia spp.* and *Navicula spp.* (Coulter, 1991).

5.5 Potential trophic cascades

It is hard to predict the future dynamics of the Lake Tanganyika sardine populations. Several biotic and abiotic factors affect the sardines directly or indirectly through trophic interactions. Trophic bottom-up cascading effects can have a profound impact on the sardines. It is still unclear what will happen to the seasonal nutrient upwelling when global warming intensifies. A decline in nutrients will have a negative influence on the primary production in the pelagic ecosystem. The zooplankton biomass, including copepods, medusae and shrimps, will decline when the phytoplankton biomass decreases. Copepods for example depend on primary producers as a food source (Sarvala et al., 2003). Changes in land use and pollution by heavy metals can cause a decrease in the number and diversity of insects. Mayflies are overall more sensitive to heavy metals than chironomids (Winner et al., 1980). Additionally, sedimentation, pollution and ground exploitations will cause a decrease in macrophytes. Insect larvae often depend on macrophytes. A decrease in macrophyte biomass and diversity will negatively affect the number and diversity of insect larvae. The sardines seem to feed extensively on the larval stages of insects. These bottom-up trophic cascades will have an impact on the zooplankton and fish diversity of Lake Tanganyika. Fish feeding on these zooplankton species will have less food sources. Planktivorous fish species and their predators will decrease in numbers due to this trophic cascade if there are no alternative food sources or if the competition is too high. The Lake Tanganyika sardines have a wide range of potential prey species. They will be able to feed on alternative prey when the abundance of one or several prey species declines to some extent.

It is difficult to predict the effects of eutrophication and global warming on the pelagic ecosystem of the lake. Eutrophication increases the nutrient load of an aquatic ecosystem. An increase of nutrients, especially phosphor, has a strong effect on the food web of freshwater ecosystems (Smith, 2003). It causes a shift in its composition and structure (Smith et al., 2006). An increase in phytoplankton could cause a depletion of oxygen in the hypolimnion and an increased turbidity (Colby et al., 1972). The Lake Tanganyika sardines are visual predators and need oxygen rich water. If the density of algae increases, it could hinder the foraging capacity

of the sardines. Algal blooms will be more intense and occur more frequently in warm and eutrophic conditions. These algae blooms are often dominated by harmful Cyanobacteria that release toxins in the water (Smith et al., 2006). An increase of nutrients due to pollution and an increase in temperature could cause large phytoplankton blooms in the normally oligotrophic Lake Tanganyika. Cyanobacterial blooms have already been reported in Lake Tanganyika (Kimambo et al., 2019). They produce cyanotoxins that cause massive fish kills in African Lakes (Kimambo et al., 2019). Due to a top-down effect, algal growth will be higher in lakes dominated by planktivorous fish compared to lakes dominated by piscivorous fish (Smith, 2003).

Global warming and eutrophication can also have an effect on other trophic interactions and could cause a regime shift. Jellyfish blooms could become a big threat to the Lake Tanganyika ecosystem when eutrophication, warming and overfishing continues. There are global cases of massive jellyfish blooms following an increase of eutrophication and temperature (Purcell et al., 2007). In contrast to fish species, jellyfish can tolerate low oxygen concentrations created by potential algal blooms (Møller and Riisgård, 2007). They can feed extensively on zooplankton which results in a decline of zooplankton biomass. Their top-down control on zooplankton species inhibits the overall grazing intensity of the zooplankton on algae (Møller and Riisgård, 2007). The top-down trophic cascade intensifies the effect of oxygen depletion and eutrophication (Møller and Riisgård, 2007). This study showed that the Lake Tanganyika sardines are the potential predators of the Lake Tanganyika jellyfish *Limnocnida tanganjicae*. The sardines may be able to inhibit possible *L. tanganjicae* blooms to some extend by top-down control. However, the number of sardines decreases due to overfishing (Mulimbwa et al., 2014) and they are less tolerant towards oxygen depletion (Plisnier et al., 2009). A top-down trophic cascade following a decrease in the number of sardines, could enhance jellyfish blooms. These medusae prey on copepods and other zooplankton (Salonen et al., 2012). A rise in medusae biomass could increase the competition with other species that feed on copepods. *L. tanganjicae* also feeds on fish eggs (Dumont, 1994). An increase in medusae within Lake Tanganyika can pose a threat to the abundance of fish from several trophic levels. Lake Tanganyika could experience a regime shift if warming, eutrophication and overfishing continues to rise (Scheffer and Carpenter, 2003).

Pollutants like heavy metals, petroleum hydrocarbons and chlorinated hydrocarbons (pesticides) are another threat for the Lake Tanganyika sardines. Pollution can cause a decrease in the growth rate of fish eggs and larvae (Von Westernhagen et al., 1988). Heavy metals can have several effects. Mercury and cadmium disrupt osmoregulation while copper can inhibit feeding behaviour of the larvae (Von Westernhagen, 1988). Petroleum hydrocarbons could anesthetise fish embryos and larvae. Chlorinated hydrocarbons reduce the amount of viable hatch within the gonads of the fish (Von Westernhagen, 1988). Both a regime shift and

pollutants will reduce the number of larvae and eggs of both the sardines and their prey. Copepods, shrimps, medusae and fish are important food sources of the Lake Tanganyika sardines. The sardines will be able to feed on alternative food sources if one declines. They will have less alternatives if several food sources decline or disappear as a result of global warming or other anthropogenic factors. More studies are needed to better understand the influence of all these factors on the sardines and other species within the ecosystem.

6 Conclusion and future perspectives

Metabarcoding of the stomach content proved to be a useful tool in determining the prey item composition of the Lake Tanganyika sardines. The prey items of the adult sardines included mainly species of copepods, insects, shrimps, fish and medusae. Traces of the Lake Tanganyika medusa *Limnocnida tanganjicae* were never found before in the stomachs of the sardines. The feeding habit of the Lake Tanganyika sardines varied according to their species, location and season. *Limnothrissa miodon* had a more diverse diet compared to *Stolothrissa tanganicae*. Sardines from the northern part of the lake fed more on copepods, cichlids and medusae while sardines from the southern part fed mostly on insects and calanoids. In Uvira, at the northern end of Lake Tanganyika, the prey item composition of the sardines varied between the wet and dry season. The sardines consumed mainly cichlids in the wet season while feeding mostly on copepods in the dry season. For *S. tanganicae*, the prey composition varied with standard length. These findings can help to understand the trophic relationships and future dynamics of the sardines, their prey and predators. More studies are necessary to develop a clear understanding of the food web and species interactions of the sardines and other economically important fish species. The information of this study and other studies should be implemented in future Ecosystem-Based Fisheries Management plans. The Lake Tanganyika sardines and other species probably migrate across borders. It is important that all countries surrounding Lake Tanganyika work together to establish these management plans. Collaboration between countries to establish EBFS is vital to provide sustainable fisheries for the local people. The Sustainable development goals (SDG) includes no poverty, zero hunger and partnerships for the goals. International sustainable fisheries will be vital in achieving these SDG's.

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Addendum

A Risk assessment

Sampling took place in Uvira, D.R. Congo during August and September 2018. Uvira is situated in the South-Kivu province. At the time of travelling, this area is marked as dangerous and unstable due to the presence of several militia. The necessary precautions should be taken. Travelling should always be done by vehicle in the company of local partners. Apart from travelling to the airport, Travellers should stay within Uvira. No unnecessary risks should be taken. Another risk concerned health. Several diseases occur in the area including malaria. Around the first of August 2018, a new Ebola outbreak occurred in the Ituri and North-Kivu provinces. Several precautions against sickness should be taken. The required vaccinations should be taken before departure. Malaria pills should be taken during the whole trip and protection against mosquitos is vital. Hands should be regularly washed and disinfected. The food should be inspected and well cooked. When sampling at the lake during the night, the travellers should check on the presence of hippos or crocodiles to avoid physical damage.

The experiments were conducted in the laboratory in Belgium. A lab coat and gloves need to be worn at all times in the lab to limit exposure to dangerous chemicals and to avoid contamination of the samples. No smoking, drinking and eating are allowed in the lab. Hands are washed regularly, and the lab coat should only be worn within the lab. During dissections, sharp objects like scissors were used together with a flame to disinfect the utensils. The sharp utensils and the fire need to be treated with care to avoid injury.

The Globally Harmonised System of Classification and Labelling of Chemicals (GHS, H- and P-phrases) or the older Risk and Safety Statements (R- and S-phrases) should be followed during the experiments. The following substances are the ones that pose health risks during the experiments of this study. Ethanol is highly flammable, causes skin, eye and respiratory irritation, and is toxic to aquatic life (H 225-315/320-335-401). It should be kept away from sources of ignition, breathing of the substance should be avoided and when in contact with the eyes, they should be rinsed continuously (P 210-261-305/351/338). The Nucleospin® Tissue Kit to extract DNA contained several chemicals safety hazards. Guanidine hydrochloride (in B3 and BW) is harmful if swallowed and irritating to the eyes (Risk phrases R22-36). Suitable eye protection should be used and when it comes in contact with the eyes, they should be rinsed with water (Safety phrases S26-39). Isopropanol (BW) is flammable and when vaporous it may cause drowsiness and dizziness (S10-67). It should be kept away from sources of ignition (S16). Proteinase K can be irritating to the eyes, respiratory system and skin, and could cause sensitisation by inhalation (R 36/37/38-42). Inhalation of the dust and contact with the skin should be avoided. Suitable protective clothing should be worn and when in contact with the

eyes, the eyes should be rinsed with water (S 22-24-26-36/37). The TE buffer contains Tris and EDTA. Tris or tris(hydroxymethyl) aminomethane is irritating to the eyes, respiratory system and skin (R 36-37-38). Suitable protective clothing should be worn and when in contact with the eyes, they should be rinsed immediately (S 26-36). EDTA or Ethylenediaminetetraacetic acid can be very irritating to the eyes (H 319). When in contact with the eyes, they should be rinsed continuously for several min and contact lenses should be removed (P 305/351/338). Quant-itTM PicoGreen® ds DNA reagent used to quantify the DNA, binds to nucleic acids and should be treated as a potential mutagen and handled with appropriate care. The green fluorescent dye of the gel electrophorese is flammable and can cause severe eye damage (H 227-320). It should be kept away from sources of ignition and in case of fire, dry sand, dry chemicals or alcohol-resistant foam should be used. Protective clothing, gloves and glasses should be worn and the skin should be washed after handling. When in contact with the eyes, they should be rinsed and contact lenses need to be removed. When eye irritation persists, medical advice should be found. The substance needs to be stored in a cool ventilated place and it needs to be disposed according to the regulations (P 210-264-280-305/351/338-337/313-370/378-403/235-501). For the extraction of the fish DNA from the fin clips, EGTA, spermidine and RNase is used. EGTA can be harmful when swallowed (H 303). Spermidine can cause severe skin burns and eye damage (H 314). Protective clothing, eye protection and gloves should be worn. When in contact with the eyes, they should be rinsed continuously and contact lenses should be removed. When swallowed, the poison center or a doctor should be called (P 280-305/351/338-310). RNase may cause allergy or asthma symptoms or breathing difficulty when inhaled (H 334). Breathing of the substance should be avoided and respiratory protection should be worn in case of inadequate ventilation. When inhaled, the victim should be moved to fresh air. If he experiences respiratory symptoms, the poison center should be called (P 261-285-304/340-342/311).

All substances used should be disposed in the appropriate waste bin. The machines used during the experiments should be treated with care to avoid damage to the machine and physical damage to the person handling it. During the data analysis and writing, ergonomic guidelines should be followed to avoid health issues like back problems. These can be avoided for example by a correct position of the laptop and the chair.

B Tables and figures

Table B1: Preparation standards for picogreen DNA quantity control.

Nr standard (n)	Lambda stock (µl)	Tube n-1 (µl)	TE buffer (µl)	Quant-it (µl)
1	2.0	0.0	198.0	100.0
2	0.0	100.0	100.0	100.0
3	0.0	100.0	100.0	100.0
4	0.0	40.0	160.0	100.0
5	0.0	40.0	160.0	100.0
6	0.0	0.0	100.0	100.0

Table B2: the selected OTU's found in the stomach content of both sardine species with their reference taxonomic unit from BOLD with the highest COI sequence match.

Fylum	Class	order	Family	Genus	Species	COI sequence similarity (%)	Origin of reference sequence
Arthropoda	Branchiopoda	Anostraca	Branchinectidae	*Branchinecta*	*lindahli*	88.99	Early-Release
Arthropoda	Hexanauplia	Calanoida	Diaptomidae	*Tropodiaptomus*	*simplex*	98.83	This study
Arthropoda	Hexanauplia	Calanoida	Diaptomidae	*Tropodiaptomus*	*simplex*	96.63	This study
Arthropoda	Hexanauplia	Cyclopoida	Cyclopidae	*Mesocyclops*	*longisetus*	86.86	Published
Arthropoda	Hexanauplia	Cyclopoida	Cyclopidae	*Tropocyclops*	*prasinus*	82.26	Private
Arthropoda	Insecta	Diptera	Agromyzidae	*Chromatomyia*	*nigra*	83.33	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*		89.58	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus*	*circumdatus*	90.18	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Cladotanytarsus*		89.5	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Cladotanytarsus*		89.84	Early-Release
Arthropoda	Insecta	Diptera	Chironomidae	*Cladotanytarsus*		89.35	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Cladotanytarsus*		90.44	Private
Arthropoda	Insecta	Diptera	Chironomidae	*Microtendipes*		89.37	Early-Release
Arthropoda	Insecta	Diptera	Chironomidae	*Paratanytarsus*		88.96	Private
Arthropoda	Insecta	Diptera	Chironomidae	*Paratanytarsus*		88.39	Private
Arthropoda	Insecta	Diptera	Chironomidae	*Paratanytarsus*		88.69	Private
Arthropoda	Insecta	Diptera	Chironomidae	*Paratanytarsus*		89.58	Private
Arthropoda	Insecta	Diptera	Chironomidae	*Stempellinella*	*cf. leptocelloides*	89.12	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Stempellinella*	*edwardsi*	88.78	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Stempellinella*	*fimbriata*	88.89	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Stempellinella*	*edwardsi*	89.8	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Tanytarsus*	*inaequalis*	90.34	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Tanytarsus*		89.37	Early-Release
Arthropoda	Insecta	Diptera	Chironomidae	*Tanytarsus*		93.33	Published
Arthropoda	Insecta	Diptera	Chironomidae	*Tanytarsus*		87.94	Published
Arthropoda	Insecta	Diptera	Chironomidae			92.68	Private
Arthropoda	Insecta	Diptera	Chironomidae			90.84	Private
Arthropoda	Insecta	Diptera	Chironomidae			83.33	Early-Release

Arthropoda	Insecta	Diptera	Chironomidae			92.83	Private
Arthropoda	Insecta	Diptera	Chironomidae			89.14	Early-Release
Arthropoda	Insecta	Diptera	Chironomidae			93.04	Private
Arthropoda	Insecta	Diptera	Chironomidae			92.75	Private
Arthropoda	Insecta	Diptera	Chironomidae			91.13	Private
Arthropoda	Insecta	Diptera	Chironomidae			88.3	Private
Arthropoda	Insecta	Diptera	Chironomidae			88.89	Private
Arthropoda	Insecta	Diptera	Chironomidae			90.11	Private
Arthropoda	Insecta	Diptera	Chironomidae			89.62	Private
Arthropoda	Insecta	Diptera	Chironomidae			89.26	Published
Arthropoda	Insecta	Diptera	Chironomidae			93.26	
Arthropoda	Insecta	Diptera	Culicidae	*Anopheles*	*MBI29*	84.08	Private
Arthropoda	Insecta	Diptera	Culicidae	*Anopheles*	*homunculus*	82.14	Published
Arthropoda	Insecta	Diptera	Drosophilidae			82.28	Early-Release
Arthropoda	Insecta	Diptera	Limoniidae	*Teucholabis*		82.56	Private
Arthropoda	Insecta	Diptera	Muscidae	*Coenosia*	*comita*	88.89	Published
Arthropoda	Insecta	Diptera	Sepsidae	*Sepsis*		84.1	Published
Arthropoda	Insecta	Diptera	Sepsidae	*Sepsis*		82.56	Published
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	*Austrophlebioides*		83.63	Early-Release
Arthropoda	Insecta	Lepidoptera	Geometridae	*Euphyia*	*altivagans*	84.07	Private
Arthropoda	Insecta	Lepidoptera	Geometridae	*Euphyia*	*altivagans*	84.07	Private
Arthropoda	Insecta	Lepidoptera	Nymphalidae	*Napeogenes*	*sodalis*	82.41	
Arthropoda	Insecta	Lepidoptera	Nymphalidae	*Napeogenes*	*sodalis*	86.11	Published
Arthropoda	Insecta	Lepidoptera	Nymphalidae	*Napeogenes*	*sodalis*	84.26	Published
Arthropoda	Insecta	Lepidoptera	Pyralidae	*Etiella*	*zinckenella*	83.61	Private
Arthropoda	Malacostraca	Amphipoda	Eusiridae			83.16	Private
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.04	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*fukienense*	87.13	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.96	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.09	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	86.55	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	86.55	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.63	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	86.76	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	86.84	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*fukienense*	84.5	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.96	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*asperulum*	85.12	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*crenulatum*	85	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	86.26	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	85.25	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*fukienense*	85.38	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idae*	84.8	Published
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Macrobrachium*	*idella*	87.21	Private
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Altolamprologus*	*calvus*	98.21	Private
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Aulonocranus*	*dewindti*	96.13	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Aulonocranus*	*dewindti*	93.75	Published

Chordata	Actinopterygii	Cichliformes	Cichlidae	*Bathybates*	*fasciatus*	88.99	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Bathybates*	*fasciatus*	87.99	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Bathybates*	*graueri*	95	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Bathybates*	*fasciatus*	90.18	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Benthochromis*	*tricoti*	97.92	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Boulengerochromis*	*microlepis*	86.9	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Boulengerochromis*	*microlepis*	86.61	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Callochromis*	*macrops*	87.2	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Callochromis*	*pleurospilus*	99.4	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Callochromis*	*pleurospilus*	96.73	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Callochromis*	*pleurospilus*	90.48	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Chalinochromis*	*cyanophleps*	86.61	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Cyprichromis*	*leptosoma*	95.24	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Julidochromis*	*regani*	97.62	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Lamprologus*	*meleagris*	85.07	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Lepidiolamprologus*	*elongatus*	97.62	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Neolamprologus*	*cylindricus*	94.29	Early-Release
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Neolamprologus*	*cylindricus*	93.58	Early-Release
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Neolamprologus*	*brichardi*	86.97	Private
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Telmatochromis*	*vittatus*	98.21	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Telmatochromis*	*vittatus*	97.02	Published
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Xenotilapia*	*spiloptera*	96.13	
Chordata	Actinopterygii	Cichliformes	Cichlidae	*Xenotilapia*	*spiloptera*	96.73	Published
Chordata	Actinopterygii	Perciformes	Latidae	*Lates*	*niloticus*	92.56	Published
Chordata	Actinopterygii	Siluriformes	Claroteidae	*Auchenoglanis*	*occidentalis*	96.43	Published
Chordata	Mammalia	Artiodactyla	Suidae	*Sus*	*scrofa*	98.51	Private
Cnidaria	Hydrozoa	Limnomedusae	Olindiidae	*Limnocnida*	*tanganjicae*	98.2	This study
Nematoda	Enoplea	Ascaridida	Kathlaniidae	*Cruzia*	*morleyi*	87.54	Early-Release

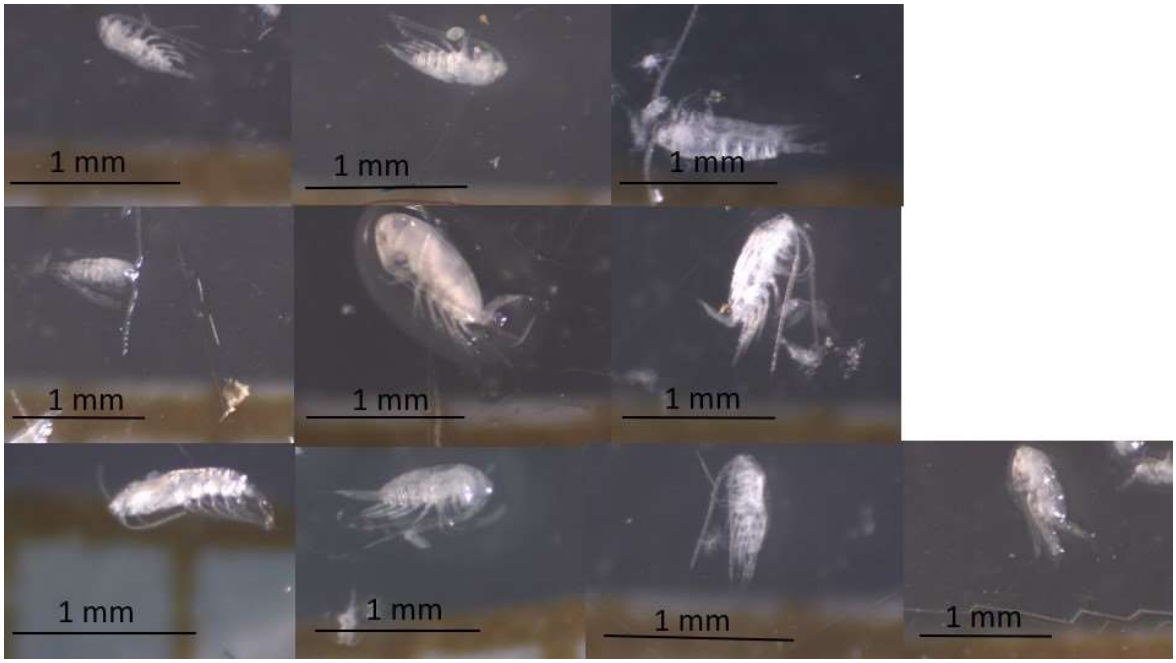

Figure B1: Individuals of Tropodiaptomus simplex used for prey sequencing, all individuals combined within TS1.

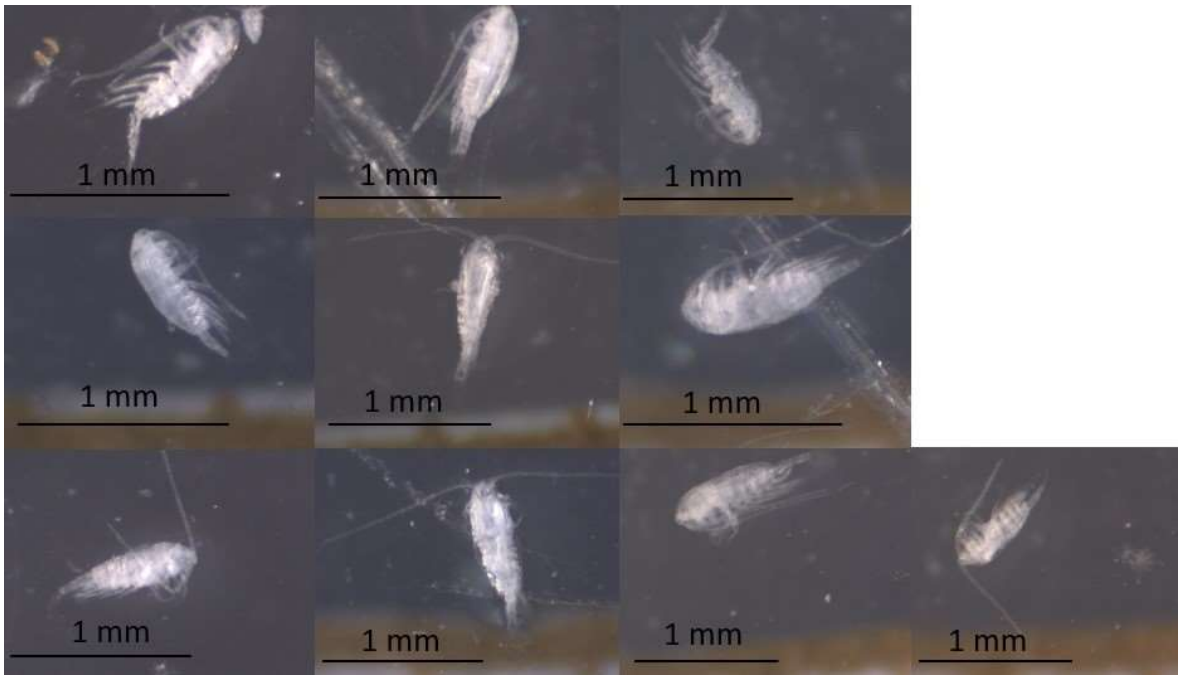

Figure B2: Individuals of Tropodiaptomus simplex used for prey sequencing, all individuals combined within TS2.

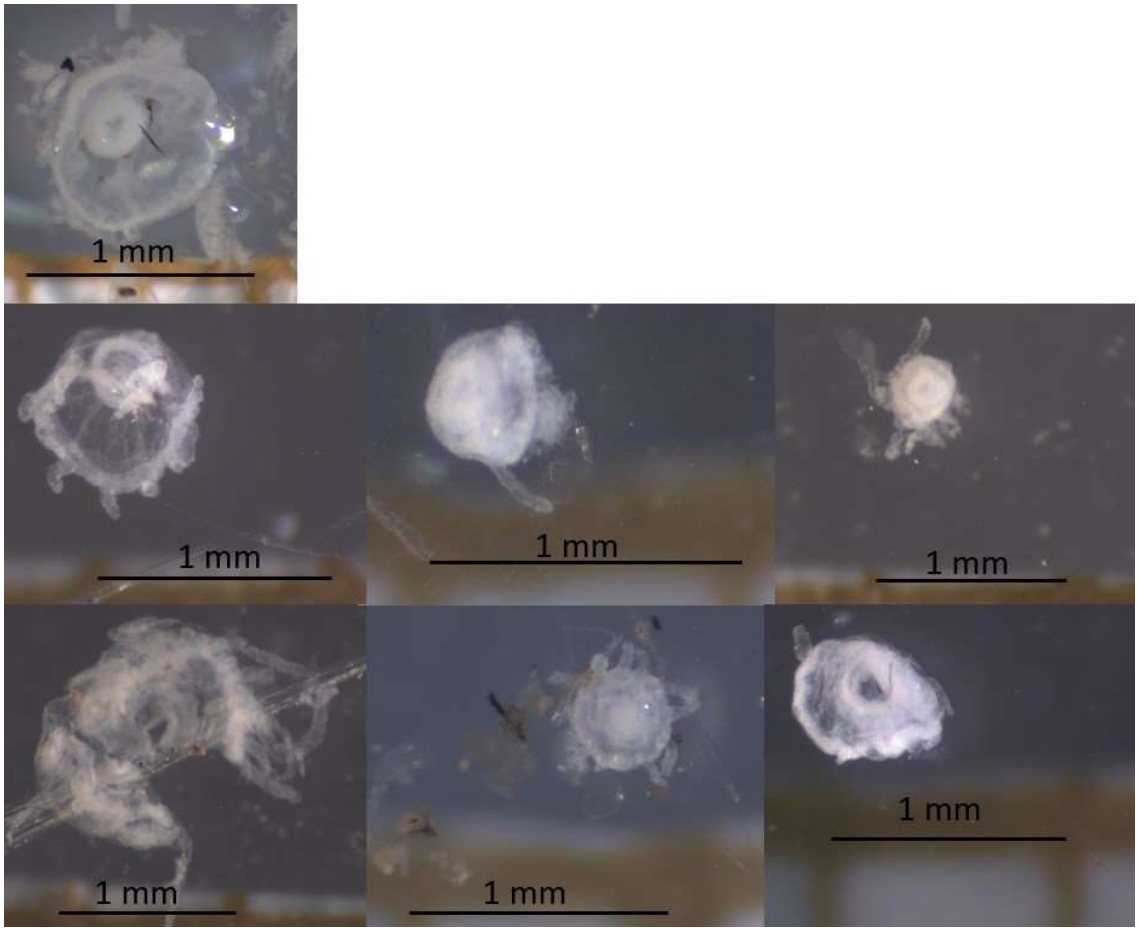

Figure B3: Individuals of Limnocnida tanganjicae used for prey sequencing, all individuals combined within LT1.

Table B3: Sequences of the zooplankton species Tropodiaptomus simplex and Limnocnida tanganjicae with different primers and annealing temperatures during the first PCR.

Species	Primer	Annealing T (°C)	DNA sequence (reverse)
T. simplex	NGS_COI	49.1	CCCCCCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGTAGATTTTG CAATTTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTAGGGGCGGT AAATTTTATTAGGACACTGGGAAATTTGCGAACATTTGGAATGATTTTA GACCGGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAGCAGTACTAC TCTTATTATCTTTACCAGTTTTAGCAGGGGCCATTACTATATTGTTGAC AGATCGAAATCTAAATTCAAGATTCTATGATGTTAGGGGAGGGGGGGA TCCCGGTCTTTACCAACATTTGTTTTGGTTTTTTGGCC
T. simplex	NGS_COI	50.5	CCCCCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGTAGATTTTGC AATTTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTAGGGGCGGTA AATTTTATTAGGACACTGGGAAATTTGCGAACATTTGGAATGATTTTAG ACCGGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAGCAGTACTACT CTTATTATCTTTACCAGTTTTAGCAGGGGCCATTACTATATTGTTGACA GATCGAAATCTAAATTCAAGATTCTATGATGTTAGGGGAGGGGGGGAA CCCGGTCTTTACCAACATTTGTTTTGGTTTTT
T. simplex	Zplank	49.1	GATATTGGAACCCTGTATTTAATTGCTGGGGCGTGGGCTGGAATAGTG GGTACAGGATTGAGGATAATTATTCGGATAGAGTTGGGGCAAGCGGGG TCTTTAATTGGAGAYGACCAAATTTACAATGTTGTTGTCACAGCGCATG CTTTTGTTATAATTTTTTTTATGGTGATACCAATTTTGATCGGGGGATTT GGAAATTGATTAGTGCCTTTAATATTGGGGGCGGCGGATATGGCGTTT CCTCGTATAAATAATATAAGATTTTGATTTTTATTGCCGGCATTGGTTA TATTATTATCTAGGTCTTTAGTAGAAAGAGGGGCTGGAACTGGGTGAA CTGTCTATCCCCCCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGT AGATTTTGCAATTTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTA

			GGGGCGGTAAATTTTATTAGGACACTGGGAAATTTGCGAACATTTGGA ATGATTTTAGACCGGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAG CAGTACTACTCTTATTATCTTTACCAGTTTTAGCAGGGGCCATTACTAT ATTGTTGACAGATCGAAATCTAAATTCAAGATTCTATGATGTTAGGGG AGGTGGGGATCCTGTTCTTTACCARCATTTGTTTTGATT
T. simplex	Zplank	50.5	ATATTGGAACCCTGTATTTAATTGCTGGGGCGTGGGCTGGAATAGTGG GTACAGGATTGAGGATAATTATTCGGATAGAGTTGGGGCAAGCGGGGT CTTTAATTGGAGACGACCAAATTTACAATGTTGTTGTCACAGCGCATGC TTTTRTTATAATTTTTTTTATGGTGATACCAATTTTGATCGGGGGATTTG GAAATTGATTAGTGCCTTTAATATTGGGGGCGGCGGATATGGCGTTTCC TCGTATAAATAATATAAGATTTTGATTTTTATTGCCGGCATTGGTTATA TTATTATCTAGGTCTTTAGTAGAAAGAGGGGCTGGAACTGGGTGAACT GTCTATCCCCCCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGTAG ATTTTGCAATTTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTAGG GGCGGTAAATTTTATTAGGACACTGGGAAATTTGCGAACATTTGGAAT GATTTTAGACCGGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAGCA GTACTACTCTTATTATCTTTACCAGTTTTAGCAGGGGCCATTACTATATT GTTGACAGATCGAAATCTAAATTCAAGATTCTATGATGTTAGGGGAGG YGGGGATCCTGTTCTTTACCA
T. simplex	NGS_COI	49.1	CCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGTAGATTTTGCAAT TTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTAGGGGCGGTAAAT TTTATTAGGACACTGGGAAATTTGCGAACATTTGGAATGATTTTAGACC GGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAGCAGTACTACTCTT ATTATCTTTACCAGTTTTAGCAGGGGCCATTACTATATTGTTGACAGAT CGAAATCTAAATTCAAGATTCTATGATGTTAGGGGAGGGGGGGATCCT GGTCTTTACCAACATTTGTTTTGGTTTTTCGGCCACCCCGAAG
T. simplex	NGS_COI	50.5	CCTTATCGGGAAATATTGCTCATGCAGGAAGTTCGGTAGATTTTGCAAT TTTTTCATTACATTTGGCCGGAGTAAGGTCGATTTTAGGGGCGGTAAAT TTTATTAGGACACTGGGAAATTTGCGAACATTTGGAATGATTTTAGACC GGATGCCGTTGTTCGCCTGAGCAGTTCTTATTACAGCAGTACTACTCTT ATTATCTTTACCAGTTTTAGCAGGGGCCATTACTATATTGTTGACAGAT CGAAATCTAAATTCAAGATTCTATGATGTTAGGGGAGGGGGGGATCCC GGTCTTTACCAACATTTGTTTTGGTTTTTTGGCCATCCCGAAGTCYAAR AACSGAA
T. simplex	Zplank	49.1	TGGGGCGTGGGCTGGAATAGTGGGCACAGGATTGAGGATAATTATTCG GATAGAGTTGGGGCAAGCGGGGTCTTTAATTGGAGATGACCAAATTTA CAATGTTGTTGTCACAGCGCATGCTTTTATTATAATTTTTTTTATGGTGA TACCAATTTTGATCGGGGGATTTGGAAATTGATTAGTGCCTTTAATATT GGGGGCGGCGGATATGGCGTTTCCTCGTATAAATAATATAAGATTTTG ATTTTTATTGCCGGCATTGGTTATATTATTATCTAGGTCTTTAGTAGAA AGAGGGGCTGGAACTGGGTGAACTGTCTATCCCCCCTTATCGGGAAAT ATTGCTCATGCAGGAAGTTCGGTAGATTTTGCAATTTTTTCATTACATT TGGCCGGAGTAAGGTCGATTTTAGGGGCGGTAAATTTTATTAGGACAC TGGGAAATTTGCGAACATTTGGAATGATTTTAGACCGGATGCCGTTGTT CGCCTGAGCAGTTCTTATTACAGCAGTACTACTCTTATTATCTTTACCA GTTTTAGCAGGGGCCATTACTATATTGTTGACAGATCGAAATCTAAATT CAAGATTCTATGATGTTAGGGGAGGTGGGGATCCTGTTCTTTACCAAC ATTTGTTTTGATT
T. simplex	Zplank	50.5	TTCTAGTAATCATAAGGATATTGGAACCCTGTATTTAATTGCTGGGGCG TGGGCTGGAATAGTGGGCACAGGATTGAGGATAATTATTCGGATAGAG TTGGGGCAAGCGGGGTCTTTAATTGGAGATGACCAAATTTACAATGTT GTTGTCACAGCGCATGCTTTTATTATAATTTTTTTTATGGTGATACCAAT TTTGATCGGGGGATTTGGAAATTGATTAGTGCCTTTAATATTGGGGGCG GCGGATATGGCGTTTCCTCGTATAAATAATATAAGATTTTGATTTTTAT TGCCGGCATTGGTTATATTATTATCTAGGTCTTTAGTAGAAAGAGGGGC TGGAACTGGGTGAACTGTCTATCCCCCCTTATCGGGAAATATTGCTCAT GCAGGAAGTTCGGTAGATTTTGCAATTTTTTCATTACATTTGGCCGGAG TAAGGTCGATTTTAGGGGCGGTAAATTTTATTAGGACACTGGGAAATT TGCGAACATTTGGAATGATTTTAGACCGGATGCCGTTGTTCGCCTGAGC AGTTCTTATTACAGCAGTACTACTCTTATTATCTTTACCAGTTTTAGCAG GGGCCATTACTATATTGTTGACAGATCGAAATCTAAATTCAAGATTCTA TGATGTTAGGGGAGGTGGGGATCCTGTTCTTTA
L. tanganjicae	NGS_COI	50.5	CCTTTAGCTAGTGTACAGGCTCACTCTGGTCCGTCAGTCGACATGGCCA TTTTTAGTCTTCATGCAGCTGGTGCTTCCTCCATCATGGGTTCCATGAAT TTCATCACCACTATCTTCAACATGAGAGCTCCAGGTATGACCATGGATC GTTTACCTCTTTTCGTTTGAGCTGTCTTGATCACTGCTTTCTTGTTAGTT

			CTTTCCCTTCCGGTTCTAGCTGGTGCAATCACTATGTTGCTGACTGATC GTAACTTCAACACGACTTTCTTTGACCCAGCTGGAGGAGGAGATCCCA TCCTTTATCAGCATTTGTTCTG
L. tanganjicae	NGS_COI	49.1	CCTTTAGCTAGTGTACAGGCTCACTCTGGTCCGTCAGTCGACATGGCCA TTTTTAGTCTTCATGCAGCTGGTGCTTCCTCCATCATGGGTTCCATGAAT TTCATCACCACTATCTTCAACATGAGAGCTCCAGGTATGACCATGGATC GTTTACCTCTTTTCGTTTGAGCTGTCTTGATCACTGCTTTCTTGTTAGTT
			CTTTCCCTTCCGGTTCTAGCTGGTGCAATCACTATGTTGCTGACTGATC GTAACTTCAACACGACTTTCTTTGACCCAGCTGGAGGAGGAGATCCCA TCCTTTATCAGCATTTGTTCTGATTC
L. tanganjicae	Jelly	50.5	AAGATATTGGAACACTTTATTTAGTCTTTGGCATTTTTTCAGCTATGAT CGGGACCGCTCTCAGTATGTTAATTCGCTTGGAATTATCCGGGGCCGGT GCCATGCTAGGAGATGATCAAATCTACAATGTCATTGTCACAGCTCAT GCTTTCGTCATGATCTTTTTTTTAGTCATGCCCGTTATGATGGGGGGTTT TGGTAACTGGTTTGTGCCTCTGTACATCGGAGCTCCGGATATGGCTTTC CCCAGATTGAATAACCTTAGTTTCTGATTATTACCTCCCGCTCTCTTCTT ACTMTTAGGGTCAGCTTTGGTAGAACAAGGAGCAGGTACCGGTTGAAC GGTTTATCCCCCTTTAKCTAGTGTACAGGCTCACTCTGGTCCGTCAGTC GACATGGCCATTTTTAGTCTTCATGCAGCTGGTGCTTCCTCCATCATGG GTTCCATGAATTTCATCACCACTATCTTCAACATGAGAGCTCCAGGTAT GACCATGGATCGTTTACCTCTTTTCGTKTGAGCTGTCTTGATCACTGCTT TCTTGTTAGTTCTTTCCCTTCCGGTTCTAGCTGGTGCAATCACTATGTTG CTGACTGATCGTAACTTCAACACGACTTTCTTTGACCCARCTGGAGGAG GAGATCCCATCCTTTATCAGCATTTGTT

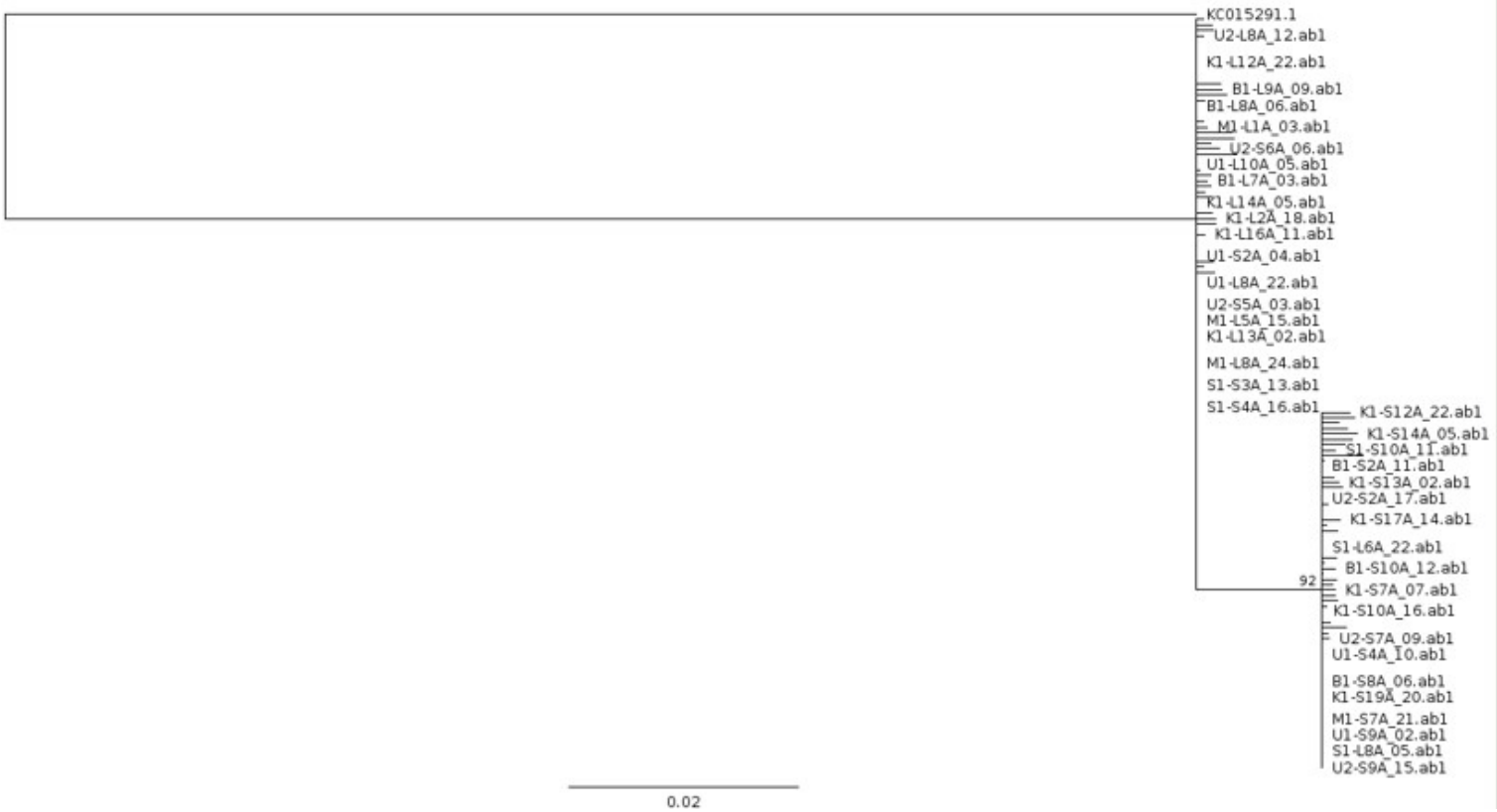

Figure B4: phylogenetic tree of some sardine specimens. The second letter in the name indicates the morphological identified species of the specimen (S: Stolothrissa tanganicae; L: Limnothrissa miodon).

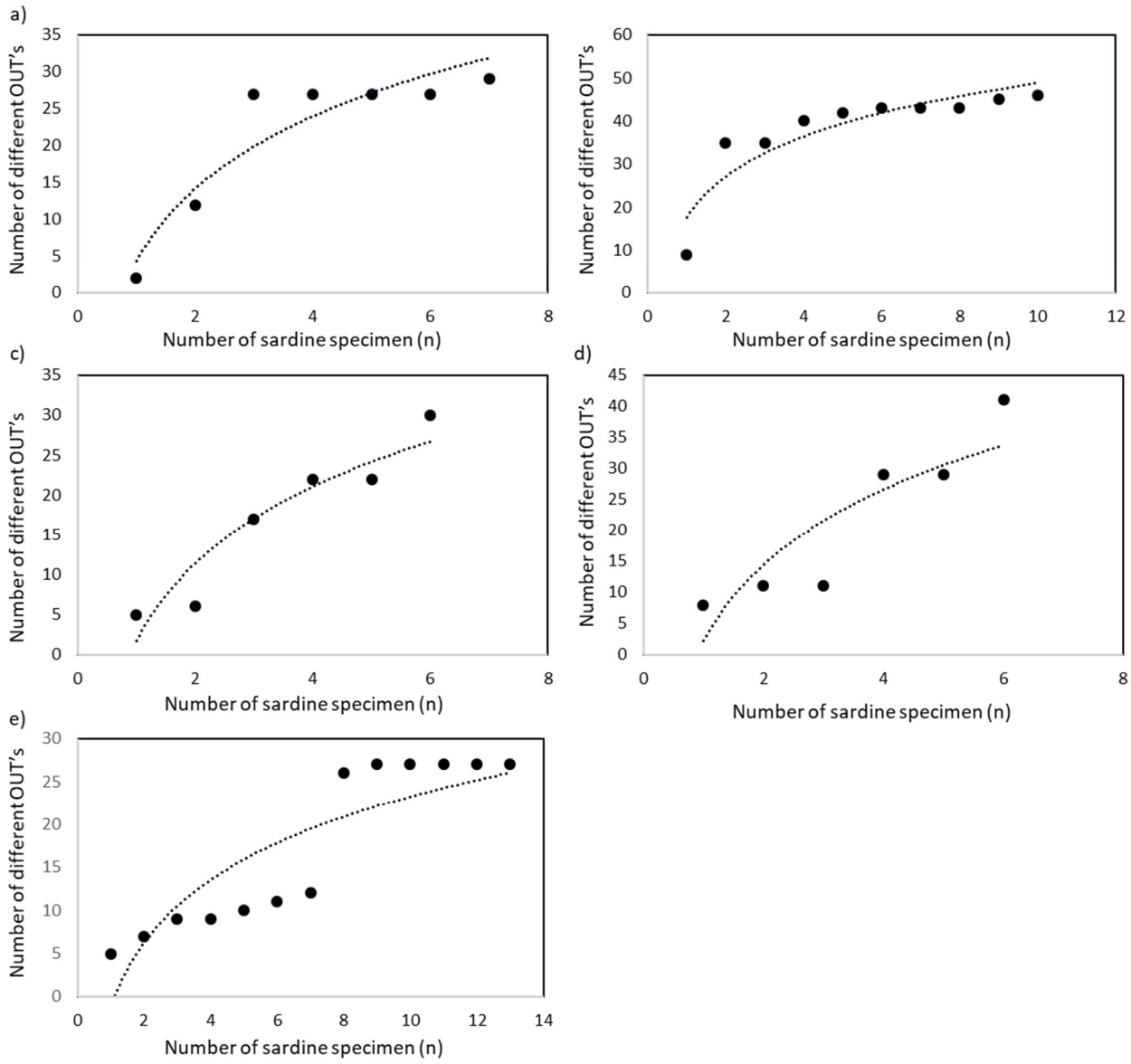

Figure B5: Rarefaction curves of locations with more than 5 individuals. Rarefaction curves of a) S. tanganicae from Uvira in the wet season, b) L. miodon from Uvira in the wet season, c) S. tanganicae from Kalemie, d) L. miodon from kalemie and e) S. tanganicae in Mpulungu.

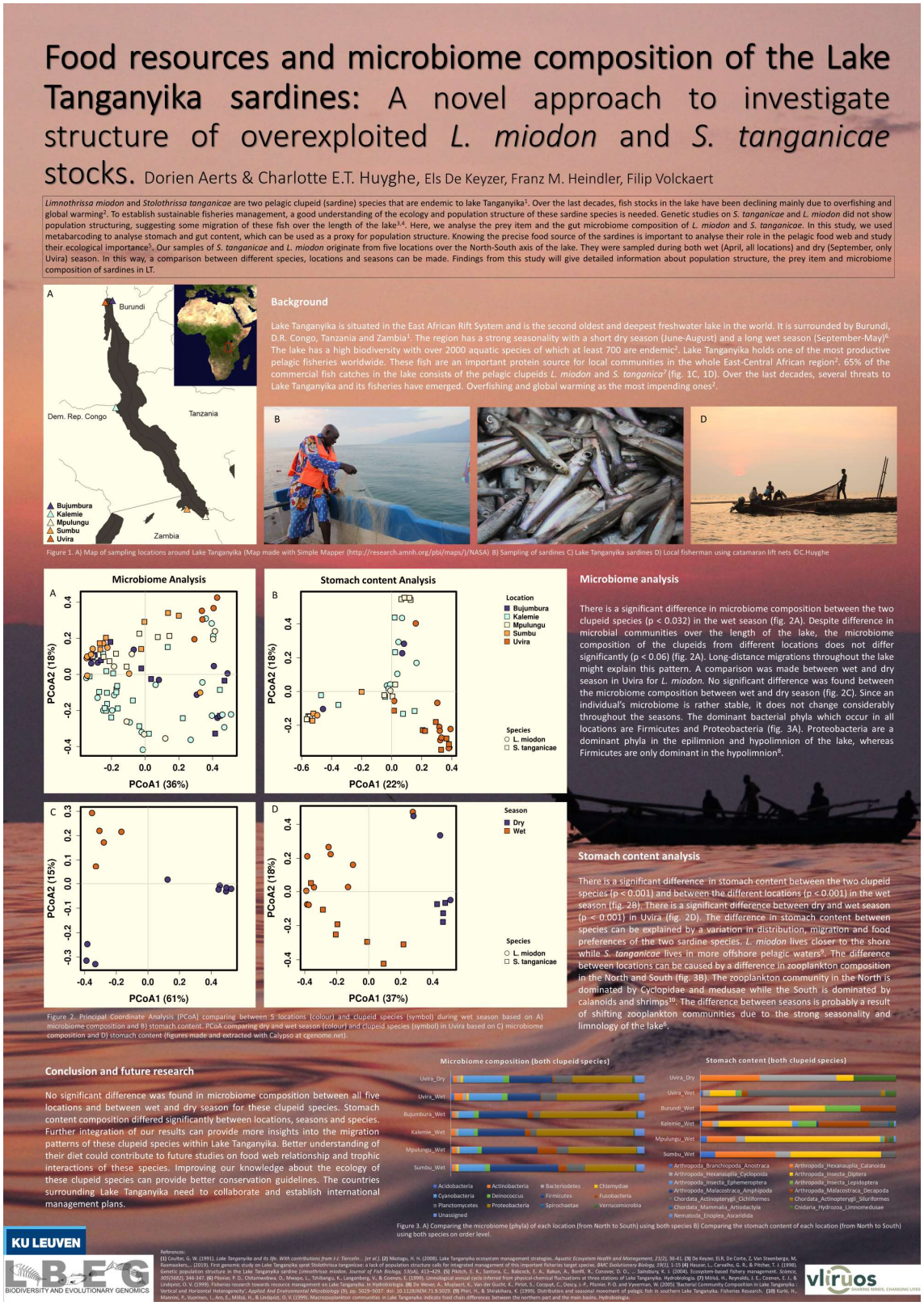

Figure C1: Personal poster for a presentation on the VLIZ marine science day 2019, in collaboration with Dorien Aerts.

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