Master's thesis:

Diet assessment of commercially important fishes in Lake Tanganyika using stable isotope techniques in combination with stomach analysis

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Preface

This is a 60 ECTS experimental master thesis, made in collaboration with Aarhus University and the Tanzania Fisheries Research Institute (TAFIRI), funded by the DANIDA Fellowship Centre. The construction of this assignment took place over the course of 10 months, February to November 2017, and is structured in the form of a scientific report. The report aims to gather information relevant to the sustainable management of Lake Tanganyika, specifically the fisheries taking place in the lake, and in particular to help construct an estimate of the pelagic food web transferring energy from the primary producers to the fisheries. In order to do this, data was collected in February-April 2017 as part of the 'Projections of Climate Change Effects on Lake Tanganyika' (CLEAT) project, and compared to historical data available from the lake.

The report is structured to first give readers an insight in the biological system of Lake Tanganyika, the importance of the fishery to the local populace, as well as theory behind the methods used to analyze the collected data. After this, the collected data is analysed and discussed in relation to the sustainable management of the lake.

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Abstract

Lake Tanganyika is a large rift valley lake in the Albertine Rift in eastern Africa, bordering on Tanzania, Zambia, Burundi, and the Democratic Republic of Congo. The pelagic fishery in the lake, which is largely made up of the three species *Lates stappersii, Limnothrissa miodon* and *Stolothrissa tanganicae,* is a hugely important source of food and income, supplying approximately 25-40% of the animal protein consumed in the riparian nations, and employing roughly one million people. Catch per unit effort is declining in the lake, and there are disagreements as to the primary underlying cause, with overfishing and climate changes being the most popular hypotheses. Because of this, models that account for both fisheries pressure and climate changes are being developed as part of the CLEAT project. This study aids in this endeavor, by establishing a pelagic food web for use in this modelling process, along with estimations of fish growth, zooplankton densities and current limnological variables.

The limnological variables, temperature and oxygen saturation, showed a shallowing of the mixed depth, consistent with earlier reports on the rate of shallowing, and a general warming of the upper layer of the lake, also consistent with climate change induced warming reported earlier.

Counts of primary increments on otoliths from the three species indicated approximately linear growth at the life stages examined, but these linear models were found unlikely to explain growth for the entire growth range of any of these fish; Von Bertalanffy growth seemed more appropriate for this. Back calculated hatch dates based on these counts suggested continuous, rather than restricted, spawning periods.

A stomach analysis of *L. stappersii* showed a change in diet composition throughout the life cycle of the fish, shifting from copepods, to fish larvae supplemented by shrimps, to whole fish prey as the fish grows. Stable carbon and nitrogen isotope values from fish muscle were largely consistent with this, but more interestingly, showed no major differences among the fish species when size was accounted for, suggesting that fish diet is a function of size, rather than species, likely because more palatable prey is consumed as soon as the fishes size allows for this. Further, the stomach analysis indicated that shrimps were a relatively unimportant source of food for the *L. stappersii*, despite earlier studies claiming the fishes have the ability to sustain themselves entirely on shrimps, possibly indicating selective feeding on the likely more palatable fish larvae or whole fish prey when available. Fish below 80mm total length were likely to consume primarily copepods, supplemented by shrimps. Around 80mm a shift to primarily feeding on fish larvae, still supplemented by shrimps and/or copepods, was observed. In the case of *L. stappersii*, the fish grow large enough to consume whole fish when they reach a total length of 250mm, after which whole fish, namely *S. tanganicae*, compose the majority of the food consumed. These results should help to strengthen further modelling and management, by giving a recent estimation of carbon flow from the primary producers to the pelagic fishery in the lake, as well as new knowledge on the growth pattern of these species.

Resume

Tanganyika søen er en stor ferskvandssø i den Albertine Rift i øst-afrika, som grænser på Tanzania, Zambia, Burundi og Den Demoratiske Republik Kongo. Det pelagiske fiskeri i søen, som primært er udgjort af de 3 arter *Lates stappersii*, *Limnothrissa miodon* og *Stolothrissa tanganicae*, er en meget vigtig kilde til både indkomst og føde i de ripariske nationer, idet det bidrager med omtrent 25-40% af den animalske protein i de omkringliggende lande, samt beskæftiger en million mennesker. Fangsten af fisk i søen dalende, og der er uenighed om den primære drivkraft bag denne nedgang, med klimaforandringer og overfiskeri værende de mest populære hypoteser. På grund af dette, er modeller som kan tage højde for både fiskeritryk og klimaforandringer ved at blive udarbejdet som del af CLEAT projektet. Dette studie søger at bidrage til disse modeller ved at opbygge en model for det pelagiske fødenetværk, samt at estimere fiskenes vækst, densiteten af zooplankton, og aktuelle limnologiske variable.

De limnologiske variable, temperatur og iltmætning, viste en tendens til reduktion af den blandende dybde af epilimnion, samt en opvarming af søen, konsistent med tidligere estimeringer at rater af opvarmning og styrkelse af søens stratificering.

Tællinger af primære inkrementer i otolither fra de tre fiskearter viste at fiskene voksede tilnærmelsesvist lineært i de undersøgte størrelseskategorier, men viste også at lineær vækst næppe var en passende antagelse for alle fiskenes livsstadier; her lod Von Bertalanffy vækst til at beskrive væksten bedre. Tilbageberegninger af klæknings-tidspunkter baseret på disse tællinger indikerede kontinuær reproduktion, snarere end begrænsede gydningsperioder.

En maveanalyse udført på *L. stappersii* viste en ændring i fødesammensætning gennem fiskens liv, skiftende fra fokus på copepoder til fiskelarver suppleret af rejer, til hele fisk som fisken vokser. En stabil carbon og nitrogen isotop analyse baseret på muskelvæv fra fiskene gav det samme billede, men mere interessant, indikerede at størrelse forklarede mere af forskellen end art. Dette tyder på at fiskene spiser de mest værdifulde fødeemner så snart fisken når en størrelse som gør disse tilgængelige. Maveanalysen viste yderligere at rejer var en relativt lille del af kosten hos *L. stappersii*, selvom tidligere studier havde fundet at den kunne ernære sig udelukkende på disse, når mere energirigt bytte ikke var til stede. Dette indikerer også selektiv fødesøgning, hvor fiskene spiser de mere energirige fiskelarver, eller hele fisk, når dette er muligt. Fisk under 80mm spiste primært copepoder, skiftende til et fokus på fiskelarver suppleret af rejer som de når de 80mm. I *L. stappersii*s tilfælde blive fiskene store nok til at kunne spise hele fisk omkring en total længe på 250mm, hvorefter hele fisk, navnlig *S. tanganicae*, udgør det meste af deres kost. Disse resultater skulle styrke yderligere modellering og forvaltning, ved at give et mere nutidigt indblik i vejene som carbon kan tage fra primærproducenterne til det pelagiske fiskeri, samt ny viden om fiskenes vækstmønstre.

Introduction

Natural resources across the globe are under increasing pressure, owing to a mixture of expanding population sizes, with proportional increases in demand of said resources, as well as the growing problem of climate changes affecting the various ecosystems providing these resources (Lake Tankanyika Strategic Action Programme (LT-SAP) 2012). One of these pressured resources is fish stocks, being heavily exploited in both marine and freshwater systems (Srinivasan et al., 2010, Allan et al., 2005). Overfishing may deplete stocks on its own, but these problems are further confounded by climate changes, which may alter ecological factors such as carbon flow, reproductive timings and available habitat ranges (Verburg 2003, O'Reilly 2003).

While most research regarding sustainable fisheries practice has been directed towards the oceans, freshwater systems may be equally vulnerable, if not more so, to the effects of overfishing and climate change. In addition to facing the same problems as the ocean's fish stocks; fish being caught before maturation or in too great numbers, eutrophication and subsequent lack of oxygen (Boqiang et al., 2006); lakes can also be isolated, making it harder for species to naturally re-immigrate after a mortality-event.

Lake Tanganyika

Lake Tanganyika is a large rift valley freshwater lake, located in the Albertine Rift in eastern Africa, bordering on Tanzania, Zambia, Burundi, and The Democratic Republic of Congo. It is the worlds longest lake at 676 km. With its average width of 50 km, it spans an area of 32,600 km^2, contains 18,900 km^3 of water, and extends to a maximum depth of 1470 m, mean depth of 570 m, causing it to rank second in terms of volume, and sixth in terms of area, when compared to other lakes across the globe. It has two major inflows: the Ruzizi river and the Malagarasi river, as well as a primary outflow; the Lukuga River. The outflow of the lake only accounts for ~9% of the inflow, the remaining water being lost by evaporation (Edmond 1993). The lake consists of three primary basins: the central, northern and southern basins, formed approximately 9-12, 7-8 and 2-4 million years ago, respectively (Cohen 1993).

The water of the lake has a retention time of approximately 5500 years. The water is also alkaline, having a pH range of 9 in the epilimnion, decreasing to 8.3-8.5 in the hypolimnion (Wever et al., 2005). The lake is oligotrophic, with nutrient concentrations in the epilimnion of ~0.6 µmol l^{-1} for nitrogen, and ~0.05 µmol l^{-1} for phosphorous. Concentrations of soluble reactive phosphorous (SRP) was found to be below detection limits (<0.01 µmol l^{-1}) in the northern basin, based on 1998 data (Järvinen et al., 1999), while Harmon (1974) documented PO_4 values between 0.17-6.67 µmol l^{-1}.

Besides its impressive size, Lake Tanganyika also host an impressive number of species, ranking as one of the top spots for aquatic biodiversity on a global scale (Groombridge & Jenkins 1998). The lake has been estimated to contain more than 1,500 species, with roughly 600 species being endemic to the lake (Coulter 1991). Most famous of these might be the Tanganyika cichlids, which are exported for use in aquariums throughout the

world. The lake is home to approximately 245 species of cichlids, the majority of which live in the litoral zone (LT-SAP 2012). The lake provides a host of ecosystem services such as transport, drinking water and tourism, but it is especially important as a source of animal protein from its unusually productive pelagic fishery, with a production potential of 165.000 to 200.000 tons annually (LT-SAP 2012, O'Reilly 2003).

The lake's abundance of species is, however, not reflected in its relatively species-poor pelagic system (Coulter 1991). In terms of phytoplankton, Lake Tanganyika is neither exceptionally diverse or species-poor, compared to other tropic lakes (Coulter 1991), being home to about 116 species (Hecky & Kling 1981), the only remarkable feature being the relative importance of the *Chrysophycae*, which is not found in other lakes under similar conditions.

The zooplankton community in Lake Tanganyika consists of relatively few groups: protozoa, copepods, shrimps, jellyfish and fish larvae (Hecky & Kling 1981, Narita et al., 1986, Coulter 1991). The protozoans are unusually abundant in the lake, particularly the ciliate *Strombidium*, and likely fulfill the role of linking the primary production and the higher consumers (Yasindi & Taylor 2010), as other taxa of microzooplankton, such as rotifers, are absent in the lake, leaving only the protozoa and copepod nauplii in the 10-50µm size category (Hecky & Kling 1981, Coulter 1991). The crustaceans, ie. the copepods and shrimps, compose the majority of the zooplankton biomass, despite the absence of cladocerans in the lake. The copepods are composed of three species: the calanoid *Tropodiaptomus simplex*, and the cyclopoids *Mesocyclops aequatorialis* and *Tropocyclops tenellus*. The copepod biomass varies extensively over the year, being higher in the wet season. It is dominated by the two larger copepods, *T. simplex* and *M. aequatorialis*, with *T. simplex* being relatively more abundant at the start of the wet season, switching to dominance of *M. aequatorialis* just prior to the dry season (Mulimbwa et al., 2014a). The shrimps are of the family Atyidae, and the lake is believed to be home to some endemic species of *Limnocaridina* (Coulter 1991). Sadly, as of yet, no proper determination-key has been developed for the shrimps of the lake, and determination of species is not routinely carried out, so no assessment of individual species' relative importance is available. The jellyfish, *Limnocnida tanganicae*, appear abundant in the lake (Sarvala 1991). Their importance in the food web of Lake Tanganyika is still poorly understood, although there is no indication that the pelagic fish eat them, which means they may be a "dead end" when considering carbon flow from primary production to the fisheries. Salonen et al., (2012) used a stable isotope analysis (see later section) to determine the trophic level of the jellyfish, and found them to be at the level of the clupeid, *Stolothrissa tanganicae*, suggesting a diet of zooplankton, likely protozoans and copepods, as the jellyfish grows.

The pelagic fish species are also limited in number: the two planktivorous clupeids, *Stolothrissa tanganicae* and *Limnothrissa miodon*, and the four piscivorous *Lates* species, *Lates mariae, L. microlepis, L. angustifrons* and *L. stappersii*. Of these species, only *L. stappersii* is considered purely pelagic, in contrast to the other species, which utilize the in-shore areas for spawning (Coulter 1991). The clupeids appear in the pelagic zone

from 55-100mm Total Length (TL), whereas the *Lates* species appear to remain in the near-shore until they reach a TL of 180mm (Coulter 1976, FAO 1978). *L. stappersii*, while not having a litoral phase, does however venture to the near-shore areas during its second year, presumably to feed on the maturing clupeids.

The clupeid species both utilize the near-shore area in their early life, although *L. miodon* spends more time here before leaving to the pelagic at a TL of approximately 100mm, compared to 55mm for *S. tanganicae* (Coulter 1991, FAO 1978). They also have different estimated maximum sizes, with *S. tanganicae* growing to 100mm, and *L. miodon* having been recorded as large as 170mm (Eccles 1992). *S. tanganicae* appears to feed primarily on phytoplankton in its juvenile life stages, shifting to zooplankton, namely *T. simplex* about the same time they move to the pelagic (Chèné 1975). Chèné (1975) also concluded that *S. tanganicae* feeding is unselective and filtration-based, whereas Marlier (1957) suggested selective feeding on shrimps when available. Mulimbwa (2014a) found that clupeid cohorts appeared to fare better when copepods were abundant, indicating that they might be an important food source. However, some strong cohorts were also found even when copepods were scarce, showing that the clupeids must be capable of utilizing other food sources as well when necessary. *L. miodon* feeding is similar to *S. tanganicae* in earlier years, although more diverse, however it shifts to feeding on *S. tanganicae* as it reaches 58-115mm (Coulter 1991, Bashirwa unpublished data). Differences in feeding by young *S. tanganicae* and *L. miodon* is largely explained by their differences in distribution (FAO 1978). The clupeids are schooling fish, and have a marked diurnal migration, staying near the bottom of the epilimnion during daytime, and coming near the surface during nighttime. This movement would appear to be related in part to the movement of their copepod prey, which aggregate in the upper layer of the lake at dusk (Vuorinen et al., 1999, Coulter 1991). Light stimulates the schooling behaviour exhibited by the clupeids, and Coulter (1991) hypothesizes that this is in response to the presence of predators, namely *L. stappersii*, who feeds intensively on the clupeids during these migrations (Coulter 1981).

The *Lates* species can be divided into two categories. The litoral demersal species, *L. mariae* and *L. angustifrons*, and the pelagic predator species, *L. microlepis* and *L. stappersii*: *L. mariae* and *L. angustifrons* are benthic species, occupying progressively deeper water with size, after leaving the near-shore area, (Coulter 1976), but migrating to the surface to feed on clupeids at night when these are seasonally abundant (Coulter 1991). Throughout the Lates' juvenile phases, their diet consists mainly of the copepod *T. simplex* in the near-shore area, but they grow increasingly piscivorous as they mature and move to the deeper water, with the majority of their diet being composed of benthic cichlids and macroinvertebrates, when the clupeids are not seasonally abundant (Coulter 1991). *Lates microlepis* is the top predator in the lake, reaching sizes towards 930mm. Its distribution is closely linked with that of its primary prey, the clupeid *Stolothrissa tanganicae* (Chapman et al., 1976). Like *L. mariae* and *L. angustifrons,* it feeds primarely on *T. simplex* in its earliest life stages, shifting to feeding on juvenile clupeids inshore, and then later the mature clupeids and juvenile *L. stappersii* in the pelagic (Coulter 1991). All life stages of *Lates stappersii* can be found in the pelagic waters. The juvenile stages appear

to live alongside the pelagic stages of *S. tanganicae* (Chapman & Van Well 1978). The adult stages aggregate more than the other *Lates*, and are often found at depths of 20-30m at night (FAO 1978). In their juvenile stages they feed primarely on the *Mesocyclops* copepods and atyid shrimps in the pelagic, switching to a more piscivorous diet from a TL of 130mm, their main food sources being *S. tanganicae* and shrimps (Ellis 1978). They have an estimated maximum size of 450mm. *Lates stappersii* differentiates from *L. microlepis* in its apparent ability to subsist purely on shrimps even as an adult (Coulter 1991, Pearce 1985).

The Value of Lake Tanganyika and its fishery

While its high degree of biodiversity does possess some ethical value, Lake Tanganyika also provides more tangible benefits for the people living in its basin. Through its size, it is capable of not only acting as a near inexhaustible source of drinking water, but also as a corridor for transportation. Due to the beauty of the lake and the surrounding areas and national parks, tourists are also drawn to this region of Africa, which injected more than three billion USD into the local economy in the years 2008-2009 (LT-SAP 2012).

Aside from these already tangible benefits, Lake Tanganyika also hosts an unusually productive freshwater fishery. While fishermen also catch the cichlids in the lake, the vast majority of the catch is composed of the three pelagic species; the clupeids *Stolothrissa tanganicae* and *Limnothrissa miodon*, and the piscivorous *Lates stappersii* (Fig. 1). Together these species make up 95% of the pelagic catches, estimated 165.000 to 200.000 tonnes annually, and have been estimated to supply between 25% and 40% of the animal protein consumed in the bordering countries, employing approximately one million people, supplying food and livelihood to more than ten million people (Mölsä et al., 1999, Kimirei & Mgaya 2007, LT-SAP 2012).

The lake fishery is primarily an artisanal one, with katamaran-fishing units typically being two motor-powered canoes, each carrying two or three persons, operating a lift net. Since light stimulates the schooling behavior

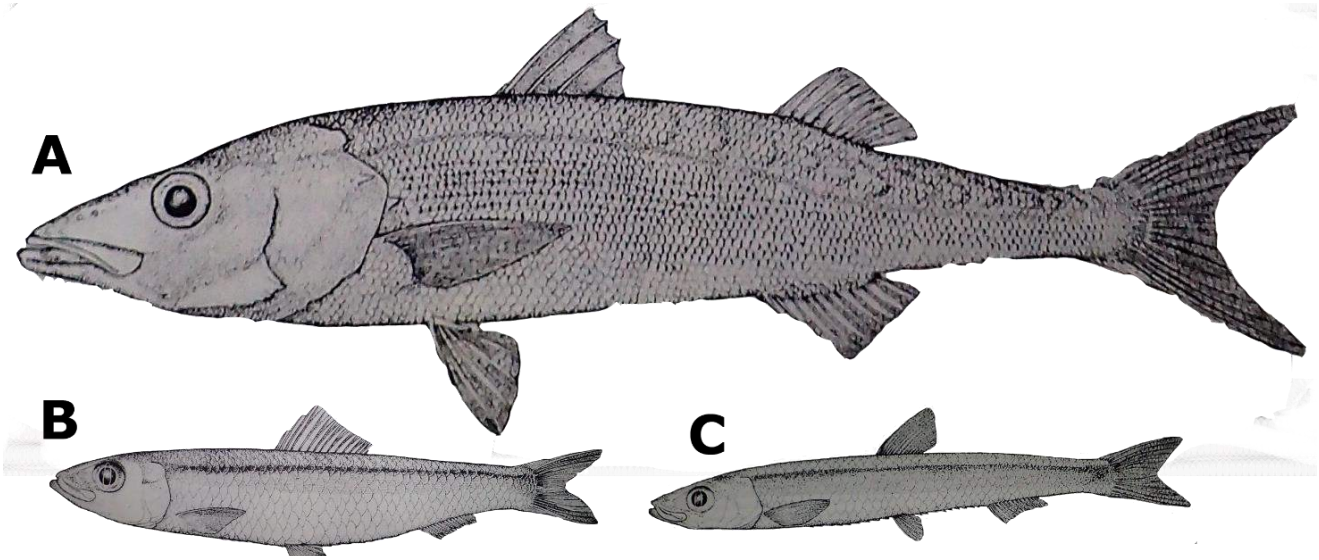


Fig. 1: The three most important species in the pelagic fishery in Lake Tanganyika. A) Lates stappersii. B) Limnothrissa miodon. C) Stolothrissa tanganicae. Pictures from Coulter 1991.

or the clupeids, fishing takes place during nighttime, using kerosene lamps to attract the clupeids. Nets are left in the water for two-three hours to allow the fish to gather on top of them, and then pulled upwards rapidly (Obs. local fishermen). Aside from the clupeids, *L stappersii* is also caught in the lift nets. It is presumably present to feed on the clupeids (Coulter 1981, Coulter 1991). Besides these lift nets, industrial purse seines also operate with a typical unit consisting of the seiner, an auxillary vessel, and three or four lamp boats luring the fish. These industrial units primarily target *L. stappersii* (Sarvala 2006a). Before the use of kerosene lamps and katamaran-fishing units became the norm, the fishermen used scoop nets termed 'lusenga' to catch the fish. Light was still used to attract fish, but rather than lamps, fire was used. This was done by burning bundled canes, termed 'matete'. Matete was, however, phased out with the introduction of kerosene lamps, likely due to practicality. As of yet, no reports have examined the influence on catches by different light sources, but one such comparison is being done as part of the Projections of Climate Change Effects on Lake Tanganyika (CLEAT) project.

While earlier reports indicate that the lake was not fished to its full potential, predicting a potential yield of 380.000 and 460.000 tonnes annually (Coulter 1991), recent research indicates that despite an increase in fisheries efforts in recent years, the total catches have been declining (Mölsä et al., 1999, Sarvala et al., 2006). This makes it harder to meet the increasing demand caused by the rapidly expanding population (LT-SAP 2012, Kimirei 2008). In response, the Lake Tanganyika Authority (LTA), was authorised to implement regulations to combat the use of illegal fishing equipment, such as beach seines and nets with mesh sizes below 8mm, as well as to increase community investment by the local stakeholders in the proper management of the lakes resources. However, this has so far proved insufficient, as catches are still declining (Van der Knaap et al., 2014). While there could be several reasons for the decline of catches, the most likely causes are overfishing, climate changes, or a mixture of these two.

Climate change

Climate change may affect fisheries yield through a plethora of indirect means. One such way is by increasing the stability of the lake stratification: warmer air temperatures increase the temperature of the mixed epilimnion, increasing the difference between epi- and hypolimnion. In lakes with little external nutrient loading, such as Lake Tanganyika, nutrients quickly become a limiting factor for growth of primary producers, and the hypolimnion becomes a vital source of nutrients. But in order for those nutrients to become available to the primary producers, mixing or upwelling events are needed. Increasing the stability of the stratification may reduce the frequency and potency of such events, thus leading to a bottom-up control of the pelagic food chain. In the case of Tanganyika, the lake is permanently stratified, and upwelling events in the northern end of the lake are tied to the shift to northwestern winds that come with the rainy season (Coulter 1991, Mulimbwa 2014ab). Evidence of increased stability of stratification in the lake was found by O'Reilly et al. (2003), who documented an increase in temperature, a decrease in wind velocity, and a subsequent increase

in stability (defined as “the work required to mix the water column to uniform density”) of 97% from 1913 to 2003. Carbon-isotope analysis of sediment cores revealed a correlation between the decrease in productivity and the regional warming. Based on the same historical data, Verburg et al. (2003) also found that since 1938, the anoxic layer had risen from a depth of 300 metres to 120 metres, indicating reduced mixing, along with a tripling of dissolved silica in the upper 50 metres, which may indicate reduced diatom production. Along with these changes, phytoplankton biomass had declined by roughly 70% since 1975, and lake transparency had increased, likely as a result. This theory is further supported by modelling by Naithani et al. (2011), who found that an increase in temperature might indeed reduce biomass of Lake Tanganyika, if not mitigated by a corresponding increase in wind velocity.

But while climate change may have reduced productivity in the lake, overfishing cannot be ruled out as the primary cause of decline just yet. Overfishing is one of the main factors pressuring fisheries across the globe. With 77% of the worlds marine fish stocks being fully exploited (FAO Fish stock assement, based on 2004 data), it is possible that inland fisheries face a similar pressure. The mechanisms of overfishing are straightforward: fish are removed from their habitat at a faster rate than they can replenish their numbers, leading to a decline in Spawning Stock Biomass (SSB), and subsequent decrease in Catch Per Unit Effort (CPUE). Aside from just removing too many fish, unsustainable fishing may also catch fish before they reach maturity, thus not only removing individuals, but also hampering future recruitment, further reducing the SSB. Sarvala et al. (2006ab) pointed out that the conclusion made by the O’Reilly and Verburg papers (2003) was based on data that might not be representative, and as such, that the connection between climate change and fisheries yield was ultimately inconclusive. He instead proposed that overfishing might be the leading cause of the declining fisheries, as along with the decrease in yield, a shift in catches to smaller sizes of fish was observed; a typical response to increasing fishing mortality. This happened along with a considerable increase in artisanal fishing

Fig. 2: A small individual of L. stappersii, likely caught with illegal fishing gear. Ruler displays centimeters.

units and effectivization of methods, also consistent with overfishing. Furthermore, it is known that illegal beach seines are employed in some degree throughout the lake (Mbonde 2017, unpublished data), as well as nets with a mesh size of less than 8mm, down to 6 or 4mm (LT-SAP 2012, Pers. Obs.). The beach seines are problematic, because not only do they catch the clupeids before they mature and migrate to the pelagic, it also destroys the litoral habitat used for reproduction of both clupeids and cichlids. The use of small meshed nets confounds this problem, also by removing fish before they have a chance to mature (Pers. Obs.)(Fig. 2).

Given the opposing viewpoints on the cause of the decline, it is difficult to enact a policy that will maximize yield within sustainable boundaries. Therefore, as a part of the CLEAT project, a model that allows accounting for both fisheries pressure and effect of climate change is being developed using the Ecopath with Ecosim software. In order to develop the best possible model, detailed knowledge is needed, not only on the link between various climatic factors and production, but also on the food web that links this production to the fisheries.

Stomach content analysis

When building a food network, information on the diet of the involved species is required. A very intuitive way to determine the diet of a species is a stomach analysis, in which the stomachs of several individuals are emptied, and their content determined. This content is then used to estimate the contribution of either carbon or dry-weight mass (DW) from all the potential food items to the consumer in question.

This method has been widely used in the past, due to its strengths: it is intuitively simple, and it provides a robust estimation of the current diet of a given population. That said, the word ‘current’ needs to be stressed in this context. While the stomach analysis is not as open to interpretation as a stable isotope analysis (see next section), it only shows what the consumers have eaten within the research period. Even the most accurate description of a consumer’s diet may not be sufficient to answer your research question, if the description is only valid for a one-month period. Consumers’ diets may change over time, either due to varying prey availability, seasonality, migrations or even large-scale events like El Niño. As such, stomach analyses may not be the proper method for research questions dealing with diet throughout the life of a consumer.

An additional factor to consider when performing a stomach analysis is the quality of the available stomachs. Not all food items are equally digestible, be it because of size or composition (Legler et al., 2010, Macdonald et al., 1982). Smaller and more easily digestible items may be broken down prior to preservation of the stomachs. This could lead to underestimation of their importance, or even to them being overlooked completely. Even if stomachs are perfectly preserved within minutes after catching the fish, there is no guarantee that differing digestive rates have not already altered the composition of the stomach; for that to be the case, one would need to be certain that the fish had eaten immediately prior to being caught.

Stable isotope analysis

Stable isotope analysis is a useful tool when attempting to construct food webs or estimate carbon flow throughout an ecosystem (O'Reilly & Hecky 2002, Zanden et al., 1999). Compared to stomach analysis, the major advantage that isotope analysis possesses is that it is time integrated, meaning it does not run the same risk of capturing an unrepresentative snapshot of a seasonal tendency in diet preference. The drawback, however, is that it can be less 'certain'; if a food item is present in stomachs, you can say with certainty that the consumers do ingest these items. Stable isotope analysis, on the other hand, is certain to be subject to interpretation. To understand this, the mechanisms of the isotope analysis must be clarified.

The premise of the isotope analysis is the comparison of ratios between the primary isotopes of carbon and nitrogen, namely ^{12}C and ^{14}N, and the rarer, heavier isotopes ^{13}C and ^{15}N. This ratio is commonly described with the δ-notation:

$$\delta^{H}X\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] * 1000$$

Where ^{H}X is either ^{13}C or ^{15}N, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively. The R_{sample} value is the ratio in the collected samples, and the $R_{standard}$ value is a known constant, such as the $^{15}N/^{14}N$ ratio of air, or the PDB standard value for $^{13}C/^{12}C$.

Consumers grow by incorporating the matter of their food items into themselves. In an ideal world, the various food items would each possess a completely homogenous isotopic ratio of both C and N, both between individuals, but also between various tissue types. Also ideally, the consumers would then consume all parts of their foods evenly, and integrate the carbon and nitrogen in their own tissue mirroring the exact C and N ratios of the food.

However, those ideal conditions are rarely, if ever, met. There can be notable variation in the isotopic ratio within a given species, even when occupying the same ecological niche. Furthermore, various tissue types cannot always be expected to display the same isotopic ratio, even within a single individual. Due to the differing rates of tissue turnover, the 'soft' tissues will reflect a shorter period within the individuals' life. That is to say, should the individual experience a change in food availability/preference, whether due to spatial or temporal changes, the tissues with a faster turnover rate will more rapidly cycle out the old tissue reflecting the prior diet, and their isotopic ratios will then more accurately reflect the current diet. This, however, does not mean that high-turnover tissues like blood plasma is the best choice for isotope analysis. One might indeed be looking for a snapshot of current diet within a population, but one might also be looking for a temporally integrated diet preference, perhaps to be certain that season, migrations or other changes are accounted for. In this case, it may be more suitable using tissue types with slower turnover rates, such as bones, scales or claws.

Further complicating the interpretation of a stable isotope analysis, comes the fact that even when the effect of various tissue types is accounted for, consumers do not integrate the heavier and lighter isotopes evenly. Throughout the trophic levels, the heavier isotopes are accumulated, because the lighter isotopes are more easily released; a process called fractionation. This causes the $\delta^{13}C$ and $\delta^{15}N$ values to increase with trophic level. However, fractionation of C and N are not equally pronounced. Typically, $\delta^{13}C$ increases by 0.5-1.0 ‰ per trophic level, whereas $\delta^{15}N$ increases by 3-4 ‰ (DeNiro & Epstein 1977, DeNiro & Epstein 1981, Minagawa & Wada 1983). Because of this difference, the $\delta^{15}N$ value is usually considered a proxy for trophic position, while $\delta^{13}C$ remains more stable throughout a food chain, making it useful for estimating diet source. One need to be wary when interpreting results thusly, however, as two individuals at the same trophic level, within the same ecosystem, may still exhibit different $\delta^{15}N$ values if the primary producers laying the foundation for their respective food chains have different $\delta^{15}N$ values. Similarly, two different primary producers may still have indistinguishable $\delta^{13}C$ values. Therefore, both isotopic values should be considered in tandem before conclusions are drawn. In the case of fish muscle tissue, which will be the focus in this report, Sweeting et al. (2007) showed that if no species specific knowledge is available, a mean $\delta^{15}N$ fractionation of 3.15 ‰ might be assumed.

In addition to the various tissue types representing different time scales, some tissue types may also show skewed values of $\delta^{13}C$ and $\delta^{15}N$. Lipids generally contain less ^{13}C, leading to more negative $\delta^{13}C$ values (Post et al., 2007). If the organisms in question contain high amounts of lipids, or if the lipid amount is very variable within a particular species, this may introduce a bias which needs to be accounted for. In such cases, it might be advantageous to perform lipid extraction on the samples beforehand. But while the lipid extraction may lessen the bias for $\delta^{13}C$ values, it can cause some additional fractionation of $\delta^{15}N$ (~0.25‰) (Sotiropoulos 2004), and should therefore not be used unless lipids are judged to present a bias. Carbonate in samples may also skew the values, because the carbon that is incorporated in carbonate is derived from the surrounding aqueous carbonate (Fritz & Poplawski 1974), rather than food items. Thus, removal of carbonate

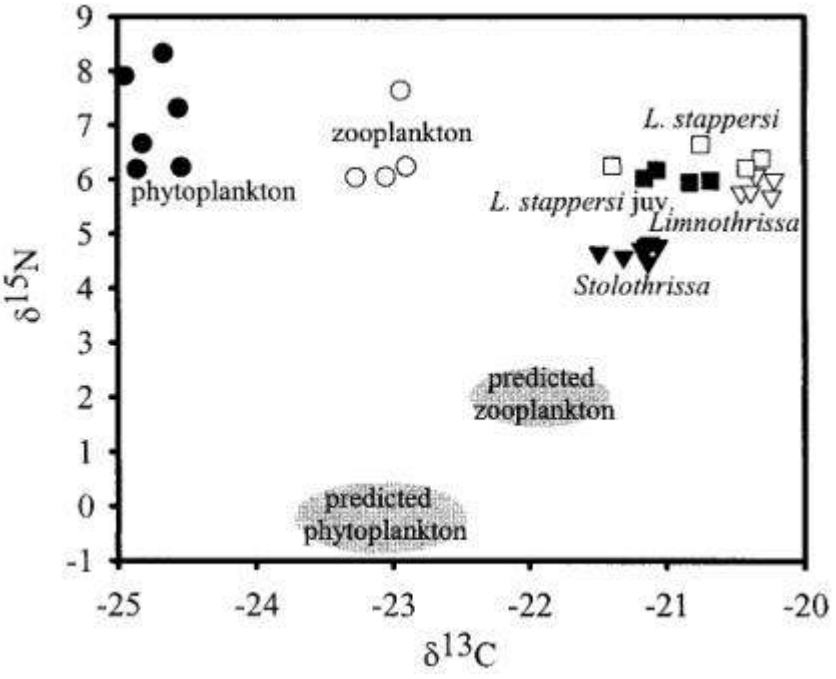


Fig. 3: Depiction of the results of a previous isotope analysis carried out on the pelagic food web in Lake Tanganyika by O'Reilly & Hecky (2002).

may be prudent when comparing individuals with varying carbonate content. Removal can be done by adding a small amount of acid to the samples, followed by drying the samples (Jacob et al.,, 2005), but similar to lipid extraction, this may alter the $\delta^{13}C$ and $\delta^{15}N$, and should not be done unnecessarily.

Once the samples are ready, lipid- or acid treated if necessary, analysis will yield a $\delta^{13}C$ and $\delta^{15}N$ value for each individual sample. When these values are plotted against each other (Fig. 3), the food chain can be visually examined, by accounting for the aforementioned fractionation.

Otolithometric analysis

Otolithometry, a sub-category of sclerochronology, deals with extracting information about fish growth, size, and other variables relating to fish ecology from otoliths (ear stones). Otoliths are calcareous structures in the inner ear, primarily composed of aragonite. They are present in the otic sacs within the inner ear, and are involved in mechanoreception of movement, thus aiding senses like balance (Panfili et al., 2002). In the case of osteichthyan fishes, three such otic sacs are present within each inner ear, leading to a total of six otoliths. The otoliths from the different otic sacs differ in shape and size, with the 'Sagitta' otolith often being the largest, and thus the most widely used. Furthermore, there is a large inter-specific variation in otolith size and shape. Some species can even be determined from otolith morphometry (Hecht 1979).

While the chemical elements in the otolith can be used to extract information regarding the life history of fish (Elsdon & Gillanders 2002), the focus of otolithometry tend to be estimation of age and growth of the individual. Because the otoliths are not subject to the same resorption of minerals as skeletal structures, unless subjected to extreme stress (Mugiya & Uchimura 1989), they can often be considered representative of the individuals life history; that is, they are continuously built throughout the life of the fish.

Age estimation is made possible by the increments visible when viewing otolith cross-sections under large magnification (in the case of very small otoliths, increments may even be visible without cutting the otolith). These exist in various 'resolutions', from the primary increments allowing for resolution of days, to the annual increments used when determining age of perennial fish (Fig. 4). Alongside these, seasonal increments at the resolution of months may also be present, in addition to discontinuities caused by stressors or changes in life history. Due to the many possible types of increments present, knowledge on what governs the increment deposition, and whether the increments even represent the desired time scale, is critical if one is to draw usable conclusions from the increments.

The mechanisms responsible for increment formation, as well as the terminology used when describing them, differs somewhat between the various increment types. While the seasonal increments appear to be caused by some seasonality in the environment of the fish, and the discontinuities may be caused by a sudden stressor such as a change in habitat, the more widely used primary and annual increments may have more complex factors governing the increment formation (Panfili et al., 2002).

In the case of primary increments, the increments are caused by the otolith being composed of sequential layers of mineral-rich and mineral-deficient (matrix-rich) zones (Watabe et al., 1982). These zones are dubbed L- and D-zones respectively, for 'Light' and 'Dark', which becomes apparent when the otolith is viewed under transmitted light. The factors that control the formation of these increments are not entirely known, and appears to vary between species, with photoperiod and temperature seeming the most important (Panfili et al., 2002).

The annual increments are termed 'opaque' and 'translucent' zones, rather than L- and D-zones, but are similar in them being composed of sequential matrix-rich and mineral-rich zones (Beckman & Wilson 1995). When viewed under transmitted light, the opaque zone appears dark, while the translucent zone appears bright. While the structure of these increments is similar to the primary increments, the mechanisms governing the deposition must necessarily differ, as they would otherwise be deposited daily. Opaque zone deposition generally takes place in spring and summer (Beckman & Wilson 1995), although the deposition period is both delayed and prolonged in higher latitudes (60-90 ^{O}N).

Because of the uncertainty regarding the exact mechanisms behind increment formation, it can be hard to determine whether primary increments are truly formed daily; indeed, one would need to confirm this by monitoring increment formation along a temporal gradient in one or more cohorts of any given species, or even population. While no such studies are available for the fish in question, the primary increments in *S. tanganicae* and *L. miodon* otoliths have previously been assumed to represent daily increments (Kimura 1995,

Fig. 4: Otolith from Limnothrissa miodon, showing visible primary increments.

Pakkasmaa Unpublished data) based on their similarity to daily increments in other clupeids. The diurnal vertical migration carried out by the clupeids further lends credibility to the idea that their primary increments represent daily depositions, as migrations are considered one of the exogenous factors that may influence increment formation (Panfili et al., 2002).

Methodology

To serve the aim of this study (see next section), this report will utilize the following methods:

Zooplankton counts will be used to estimate the density of prominent zooplankton species in the pelagic zone of Lake Tanganyika, granting a clearer view on the food availability for the pelagic fish community.

Limnological variables will be measured at various depths, at several stations, in the northern end of the lake to give an image of the physical environment and productivity of the lake.

Stable Isotope Analysis will be used to create an overview of the structure of the pelagic food web in Lake Tanganyika, including the trophic position of the fish species in question. This will be supplemented by a stomach analysis on *L. stappersii*, giving non-interpretive view on food items that are definitively consumed. Together, these should provide a robust estimate of the trophic relationships between the pelagic fish and zooplankton species.

In addition to the construction of said food web, the growth rates of the three fish species will be estimated using otolithometry, which will also allow for back-calculation of hatch dates, which will be used in describing the spawning-pattern of the three species (seasonal or continuous).

Aim of the study

Due to the increasing population in the riparian nations surrounding Lake Tanganyika, demand for animal protein is increasing as well. However, the catches of the important pelagic species in the lake are declining, which will inevitably lead to shortage of this desired food item in the years to come. Therefore, now more than ever, knowledge of the lakes ecosystem that will allow for proper management of the natural resources is needed. The CLEAT project is an multidisciplinary project, aiming to ensure sustainable management of Lake Tanganyika, accounting for the expected climate changes.

This study aims to: construct a food web for the important pelagic species and their food items, along with density estimates of these food items, in order to assist further modelling of the pelagic ecosystem in Lake Tanganyika as a part of the CLEAT project; and improve knowledge on the growth and spawning pattern of the three commercially important fish species, *L. stappersii, L. miodon* and *S. tanganicae*, to aid in decision making and proper management.

Methods

Study area

Data collection was based in Kigoma (LTK, Fig. 5), Tanzania, a city adjacent to Lake Tanganyika, between the 3rd of February and 29th of April 2017. During this period, 2 trips were taken southwards in the lake to Mahale Mountain National Park (LTM), on the 23rd-24th of February and the 27th-29th of April, where supplementary samples were taken at the LTM main station. Additional samples were taken along the nine stations (LT1-LT9) along the transect between LTK and LTM during the second trip.

During these two trips, limnological variables were examined at all 11 stations. Oxygen and temperature were measured with a YSI EXO2 Multi-Parameter Water Quality Sonde, lowered to ~110m depth. Water samples were collected with a 7.4 liter Limnos water sampler at every 20 meters for the upper 100m water column, and every 10m for 100-200m water column at the main stations; and at 0, 10, 20, 40 and 60 meters at the transect stations. The water was filtered through 0.7µm glass fiber filters, and kept in 10ml vials of 90% (v/v) acetone. These vials were placed in a Branson Sonicator for 15 minutes to disrupt the cells, kept in a freezer at -4 °C overnight, before being re-sonicated. Chlorophyll *a* was then determined spectrophotometrically at 665 nm wavelength.

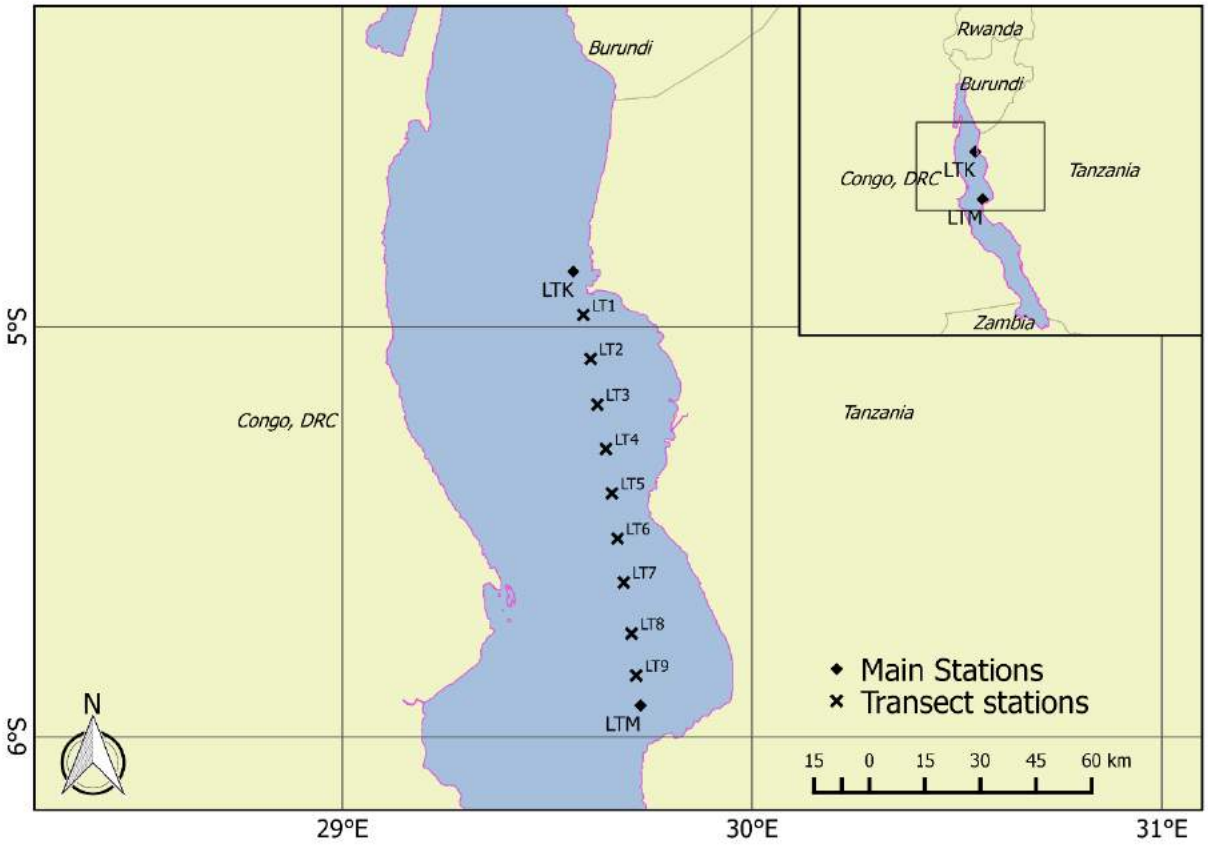


Fig. 5: Map of the research area on Lake Tanganyika. The main stations LTK and LTM are located at Kigoma and Mahale, respectively.

Sampling procedure

Each month was divided into two sampling periods of roughly two weeks each, starting in late February. Sampling of fish was conducted continuously throughout these sampling periods by buying *L. stappersii*, *L. miodon* and *S. tanganicae* from the local fishermen in the morning as they arrived at the Kigodeco, Kibirizi and Katonga landing sites in Kigoma.

The fish were caught by local fishermen during nights, and brought to the lab on the subsequent mornings. They are lured to the surface with light, and caught mainly using lift nets, though some individuals may have been caught by scoop nets. Once in the lab, the fish were sorted into species and divided into size groups based on total length: *Lates stappersii* was divided into individuals between 80 and 130mm, between 130 and 250mm, and above 250mm; *Limnothrissa miodon* were divided into individuals shorter and longer than 110mm; *Stolothrissa tanganicae* were divided into individuals shorter and longer than 80mm. For the clupeids, these size categories were chosen tentatively based on the length found in the catches, aiming to divide what was considered to be 'large' and 'small' individuals. In the case of *L. stappersii*, they are considered piscivorous from a TL of 250mm, and the other size categories were selected to see if diet showed any signs of changing prior to this shift. Supplementary individuals of *L. stappersii* shorter than 80mm were taken from frozen samples caught on the 30th of August 2016 (henceforth Sampling period 0), due to this size group being absent in catches during the data collection period (obs. local fishermen). That being said, a single catch containing *L. stappersii* individuals down to 34mm was obtained in sampling period 3; likely stemming from illegal fishery with a small mesh size (Fig. 2).

In addition to the fortnightly fish sampling in the Kigoma region, five individuals of each size group of *S. tanganicae* were obtained from the fishermen near LTM during the first trip along the transect.

Zooplankton samples were collected by vertical hauls with zooplankton nets of 40µm and 500µm mesh size, with diameters of 30cm and 45cm, respectively. Samples were taken from 60m and 120m with both nets, hauled at approximately 0,25 m s^{-1} for the 40µm net, and approximately 0,5 m s^{-1} for the 500µm net. A total of four zooplankton samplings were conducted at the LTK main station, two of these including a nightly sample (later than 21:00). Due to difficulties securing nets, the third trip only sampled copepods with the 40µm net, and the fourth trip sampled only shrimps with a newly constructed net of approximately 500µm mesh size, mounted on a 500µm filter. Aside from the zooplankton caught with net hauls, seven large shrimp individuals, ~2cm (henceforth 'Large Shrimp'), unlike those caught in the zooplankton hauls, were found as bycatch from the same fish purchase containing the very small *L. stappersii*.

For comparative purposes, periphyton samples were collected in the littoral zone, from rocks at ~10m depth by the stony reefs near Kigoma.

Stomach analysis

Stomachs were analyzed for all size groups of *L. stappersii*, aiming for five for each size-group/sampling-period, by emptying the stomachs into a petri dish, and doing a count of all present prey items. Prey items were sorted into copepods, nauplii, shrimps, fish larvae, and fish. Stomach analysis was not conducted on *S. tanganicae* and *L. miodon*, as the quality of the stomachs available was deemed too low; nearly all stomachs (>95%) were empty, and the ones that weren't mainly contained unidentifiable pulp.

The counts of prey items from the stomachs were converted to estimated Dry Weight (DW) contributions in the following way: for copepods, the median dry weight of individuals of the *Eudiaptomus* genus as given in the 'Zooplankton in lakes – processing of samples' field guide provided by Aarhus University was used for all individuals; for nauplii, the median weight from the same field guide was used; for shrimps, a length-DW relationship for the summer generation of *Neomysis integer* (Aaser 1993, unpublished MSc thesis) was applied to the average length measured on 180 shrimps collected during the zooplankton samplings; for fish larvae, 36 individuals judged to be in good condition were removed from the stomachs, dried along with the isotope samples (see next section) and weighed. Their average weight was then used for each individual; for fish, a length-weight relationship was established for the combined clupeid species based on the length and weight of 47 individuals, and used to estimate the fresh weight of fish found in stomachs - DW was then estimated as 20% of this weight, based on previous experiences (Pers. Obs, Grønkjær).

Stable isotope analysis

For the fish tissue samples needed for the stable isotope analysis, up to five individuals within each species-size category were haphazardly selected from the caught fish within each sampling period, provided the fish were available from the local fishermen. For larger *L. stappersii* muscle tissue was removed from the upper lateral portion of the tail, to minimize risk of bones being present in the sample (Fig. 6). For *L. miodon* and *S. tanganicae*, as well as the smaller *L. stappersii*, muscle was extracted from the lateral area, and bones were removed from the sample manually if present. For the very small *L. stappersii* caught in sampling period 3, the

Fig. 6: Procedure for removal of fish muscle tissue in A) L. miodon and B) L. stappersii

head was removed, and the remaining body was used. The extracted muscle tissue was dried at 60 °C for at least 24 hours on Teflon plates, and kept in Eppendorf tubes for transport back to Denmark. For the potential food item samples, copepods were collected from the 40µm net hauls, shrimps were collected from the 500µm net hauls, and both were dried at 60 °C for at least 24 hours on Teflon plates, after which they were kept in Eppendorf tubes for transport back to Denmark. Fish larvae were collected from stomachs (same 36 individuals used to estimate average larvae weight), and dried individually along with the seven large shrimps, following the same procedure as the fish and zooplankton samples. Periphyton samples were scraped off stones found in the littoral area from ~10m depth, and similarly dried and carried to Denmark.

In Denmark, the dried fish and large shrimp samples were ground in an agate mortar, wiped down with 96% ethanol between each sample. All samples were then dried again at 30°C for 2 days in open Eppendorf tubes, and kept in a sealed container with silica gel. Approximately 0.3 mg of each of the twice dried samples were packed in tin cups, and analyzed using Isotope Ratio Mass Spectrometry (IRMS), along with 'Gel A' standards, with a known $\delta^{13}C$ of -21.81‰ and $\delta^{15}N$ of 5.4‰. The 0.3 mg was chosen because previous tests using Gel A revealed this to be the least biased weight, with lower weights losing accuracy in $\delta^{15}N$, and higher weights drifting in $\delta^{13}C$. The Gel A standards were spread out between tissue samples, with standards occurring between every nine to ten samples.

After the analysis, the standards were used to calibrate the data, using the following procedure: for every separate day of analysis, the Gel A standards were isolated, and both $\delta^{13}C$ and $\delta^{15}N$ were plotted as a function of running number (the order in which the samples were analyzed). Points that were judged to be outliers were excluded, and a linear regression was fitted to the remaining points. The slope of this regression was assumed to be the drift occurring throughout the day, and the difference between the intercept and the known $\delta^{13}C$ or $\delta^{15}N$ were assumed to be the deviation. Both were then used to correct the tissue sample values for the corresponding days.

For visual comparison, estimated fractionation values of 0.75‰ for $\delta^{13}C$, and 3.15‰ for $\delta^{15}N$ were subtracted from the mean values for all Species-Size categories of fish, which were then plotted along with mean values for potential food items.

Otolithometric analysis

Sagitta otoliths (pairs, if available) were extracted during sampling period 4 and 5 from the same fish used for isotope samples, aiming for five otoliths from each Species-Size category. The otoliths were transported to Denmark, where they were mounted on microscope slides.

Pictures were taken using the NIS-Elements software, utilizing a Nikon DS-FI1 camera mounted onto a microscope, using transmitted light. Primary increments were subsequently counted manually on these photos. When areas of any given otolith proved unreadable, increment number in the obscured area was

estimated by assuming the increment width within the area was the average of the increment widths in the areas prior to and just after the obscured area.

Beyond what was assumed to be the daily increments, sub-daily increments were found in the otoliths of the smaller size categories of all three species (Fig. 7). Differentiation of these were made based on consistency and periodicity (Dougherty 2008).

Statistical analysis

For the isotope analysis, comparisons between groups were done partly by visual comparison of an isotope biplot, and partly through comparison of standard ellipses (Batschelet 1981) and Standard Ellipse Area (SEA), which can be considered the standard deviation of bivariate data. The SIBER package (Jackson et al., 2011) was used in R v. 3.4.1 to calculate Standard Ellipses, and Standard Ellipse Overlap between groups.

Linear models, ANOVAs, ANCOVAs and Tukey post-hoc tests, used for otolithometric- and stomach data were carried out in R v. 3.4.1. The Von Bertalanffy growth model (Bertalanffy 1957) fitted to the Otolithometric data was carried out using Excels Solver function, restricted by the estimated maximum lengths for each fish species mentioned in the introduction.

For extrapolation of limnological data along the transect, Surfer v. 6.01 was used, using the Kriging gridding method, which is commonly used for such transect data, as it gives less weight to clusters in overall prediction (Cressie 1990).

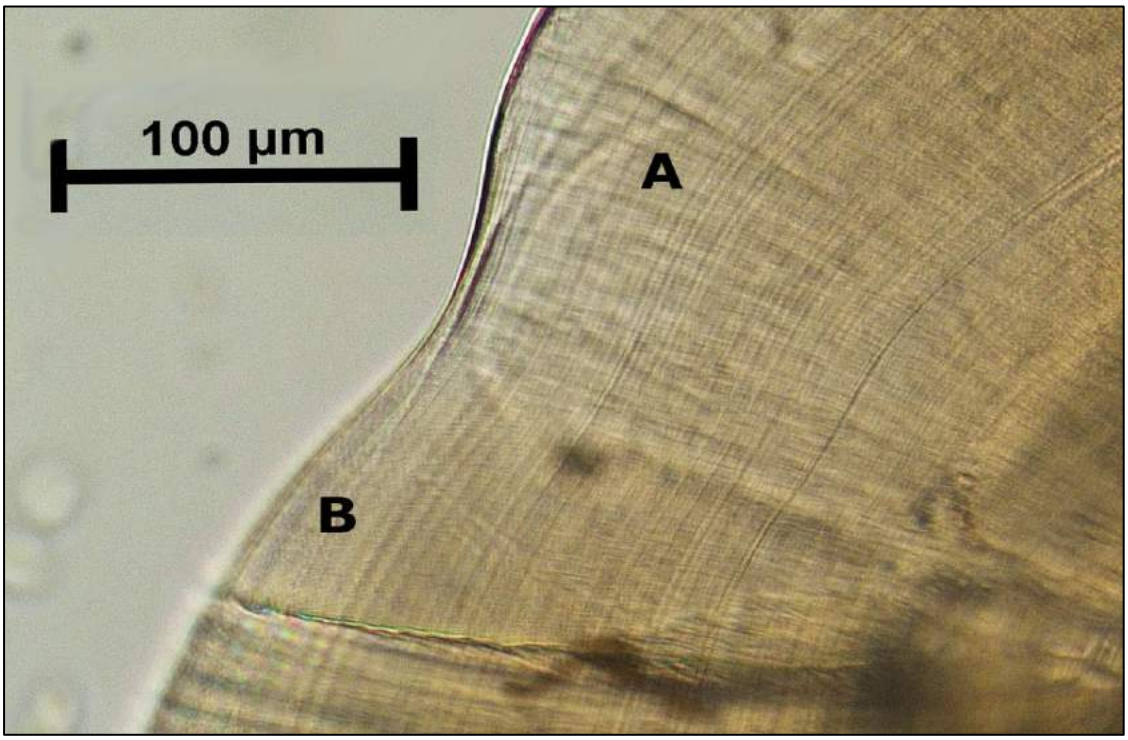


Fig. 7: An otolith of a L. stappersii individual of 60mm length, showing both A) Sub daily increments and B) daily increments.

Results

Based on the 47 zooplankton hauls conducted throughout the sampling periods, the densities of copepods, shrimps and jellyfish in the lake were estimated (Tab. 1). Copepod density was based on hauls with the 40µm net, which caught negligible amounts of shrimps and jellyfish, while the shrimp and jellyfish densities was based on the 500µm net hauls, containing negligible amounts of copepods. It was judged that, due to the inconsistent nature of these samplings, no robust statistical tests could be carried out with confidence, since sampling frequency was too irregular; for instance, only two nightly samples being collected, and most hauls from the same depths were collected at separate stations.

Copepod densities varied widely, finding anywhere between 17 and 237 individuals m^{-3}, with no apparent differences between depth, station, or time of sampling. In the case of the jellyfish, there appeared to be no differences between the 60 and 120m hauls either, indicating an even spread throughout the water column, with densities ranging from 0.5 to 7 individuals m^{-3}. For the shrimps, however, there seems to be a trend of higher densities in the 120m hauls for the daily samplings, with the distribution being more even in the two nightly samples, implying that the shrimps spend the daytime in the deep waters, migrating to the surface at night. Densities of shrimp also varied greatly, being as low as 0.1 individuals m^{-3}, and as high as 30.5 individuals m^{-3}.

Measurements of oxygen saturation, temperature and Chl *a* was mapped against depth, and distance along the transect sailed between Kigoma and Mahale in isopleth plots (Figs. 8 & 9). The oxygen and temperature plots reveal a notable oxy- and thermocline around 50-70 meters depth for the length of the entire transect in both February and April. Below this depth, temperature falls from ~27 ^{0}C at the surface to below 25 ^{0}C, while the oxygen saturation falls from >90% to <20%. This indicates a mixed depth of roughly 60 meters.

Looking at the Chl *a* plots along the transect, the primary production does not appear to be restricted to specific depth, rather, Chl *a* is seen clustered at primary production hotspots. In the February transect these peaked at a Chl *a* concentration of 2.1 µg L^{-1}, just below the surface, and at ~20m depth, with concentrations above 1.0 µg L^{-1} occurring at 60m depth near LT7. In the April transect, Chl *a* peaked at 1.8 µg L^{-1} around 40m depth between LT6 and LT8.

Table 1: Densities of zooplankton species in individuals m^{-3} based on zooplankton net hauls

			Densities					
			Copepods		Shrimps		Jellyfish	
Date	Time	Station	60m	120m	60m	120m	60m	120m
07-02-2017	Day	LTK	235.8	145.2	0.1	3.7	4.4	1.6
07-02-2017	Night	LTK	69.8	39.6	1.5	0.4	1.0	0.8
16-02-2017	Day	LTK	233.9	125.4	1.3	6.8	7.0	7.5
16-02-2017	Night	LTK	100.0	237.7	28.4	30.5	2.7	2.1
24-02-2017	Day	LTM	17.0	155.6	0.3	22.5	0.5	0.6
14-03-2017	Day	LTK	69.8	89.6	0.9	1.5	2.5	0.5
07-04-2017	Day	LT1	88.7	-	12.4	-	-	-
08-04-2017	Day	LT2	81.1	-	6.5	-	-	-
09-04-2017	Day	LT3	133.9	-	-	-	-	-
10-04-2017	Day	LT4	133.9	-	-	-	-	-
11-04-2017	Day	LT5	205.6	-	-	-	-	-
12-04-2017	Day	LT6	171.7	-	-	-	-	-
13-04-2017	Day	LT7	103.7	-	-	-	-	-
14-04-2017	Day	LT8	122.6	-	-	-	-	-
08-04-2017	Day	LTM	132.0	-	-	-	-	-

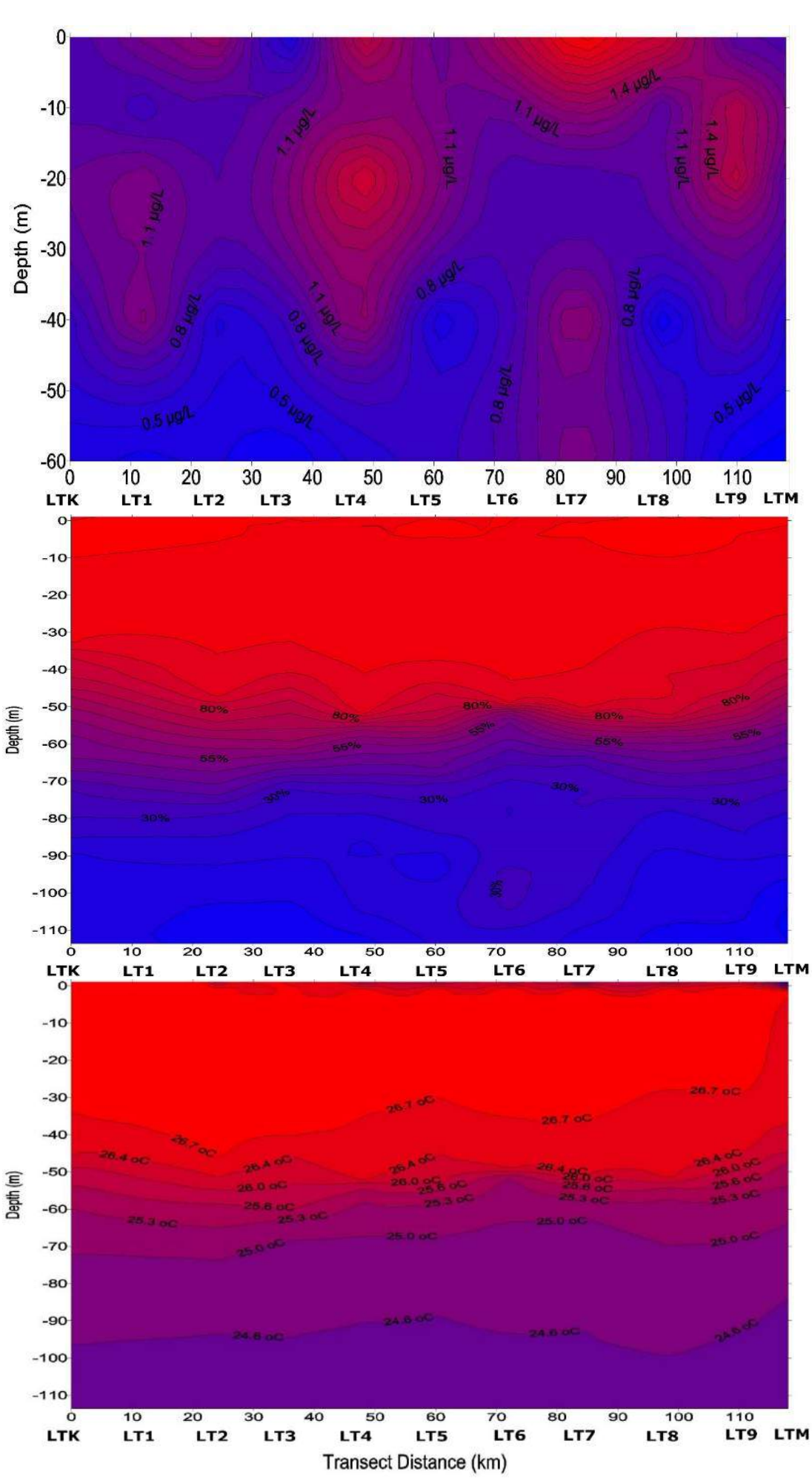


Fig. 8: Isopleth plots from February of the variables: A) Chl a (µg/L), B) Oxygen Saturation (%) and C) Temperature (^{O}C)

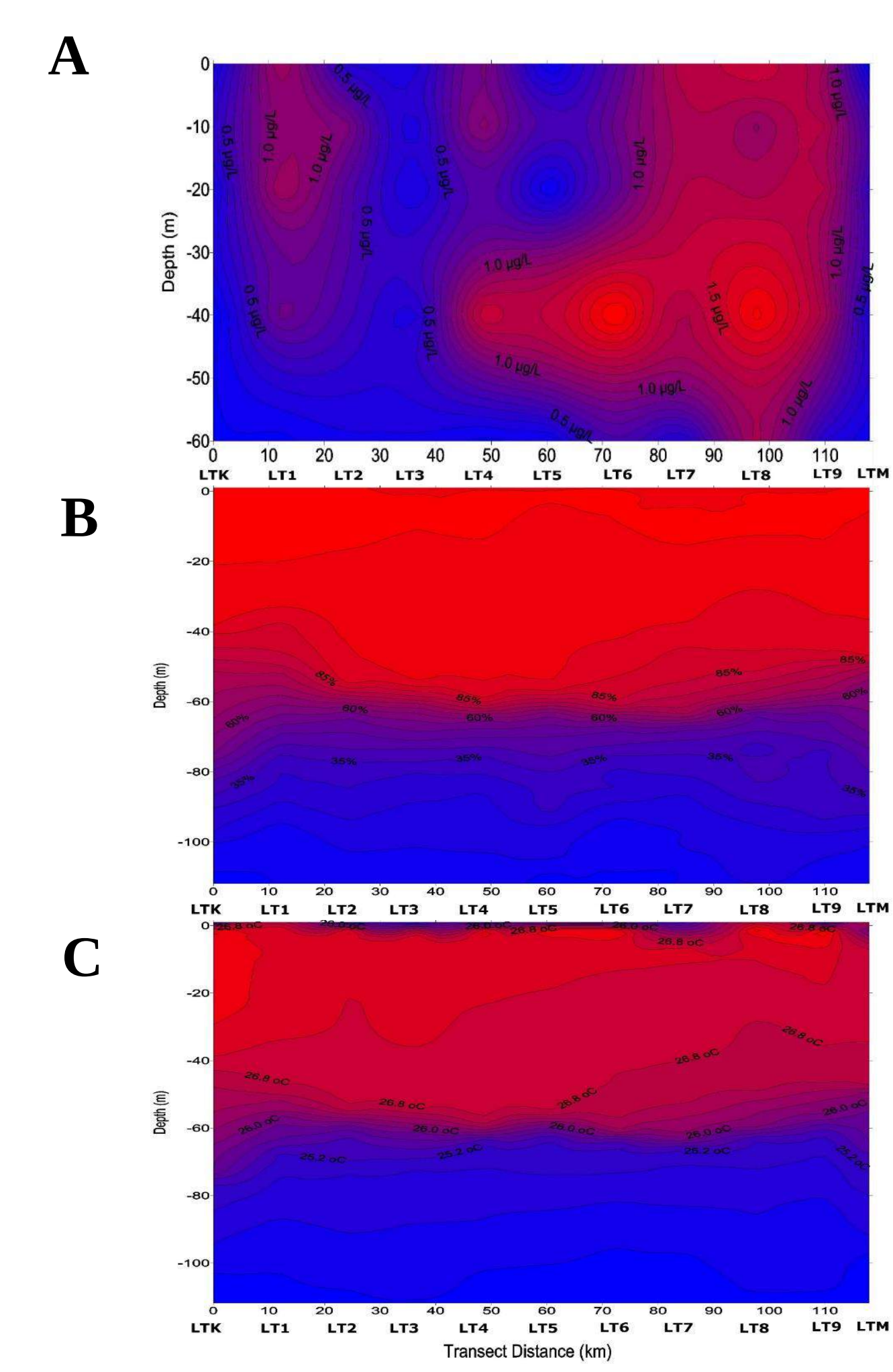


Fig. 9: Isopleth plots from April of the variables: A) Chl a (µg/L), B) Oxygen Saturation (%) and C) Temperature (°C)

Stomach analysis

The average DW found for individual fish larvae was 2.2 mg with a standard error of 0.30 mg, based on the 36 individuals taken from the stomachs. The measured shrimps had a mean length of 1.7 mm, with a standard error of 0.042, leading to an average weight of 0.018 mg per individual. A power regression explaining clupeid wet weight as a function of length (Fig. 10) was found to provide a good fit for the available data (R^2=0.98).

Fifty-five non-empty stomachs were examined, spread across the four size categories of *L. stappersii* (Tab. 2). Empty stomachs were discarded, and have not been considered in this analysis. In the stomachs of the smallest *L. stappersii* individuals, below 80 mm, copepods composed the majority of the prey, being dominant both numerically and in terms of DW contribution. Shrimps were still eaten, but in smaller numbers compared to the other size groups. It should be noted, however, that these fish were caught in August 2016, whereas the individuals from the other size groups were caught in February and March 2017.

The intermediate sizes did not display any apparent differences in dietary preferences, both eating primarily fish larvae and shrimps. While shrimps were numerically dominant, the fish larvae contributed ~80% of the DW on average in these size groups.

The largest individuals, longer than 250mm, showed a diet consisting almost exclusively on fish larvae and fish, consistent with their assumed piscivorous tendencies. It is worth noting that the fish prey was all found in three stomachs, in which they composed ~100% of the DW contribution, one of these filled to the point of literally bursting upon handling. Despite only being found in a third of the stomachs, whole fish prey made up 92% of the DW in this size category.

One-way ANOVAs explaining DW of the various prey items as a function of size category found significance for all prey items: copepods, $F_{3,51}$=26.31, p<0.001; nauplii, $F_{3,51}$=10.3, p<0.001; shrimps, $F_{3,51}$=4.99, p=0.004; fish larvae, $F_{3,51}$=5.205, p=0.003; fish, $F_{3,51}$=3.813, p=0.015.

Tukey post-hoc tests were conducted for all the above-mentioned ANOVAs: for copepods and nauplii, the smaller than 80mm size category was significantly different from all other size categories (p<0.001). Similarly, in the case of fish prey, the longer than 250mm size category was significantly different from all other size categories (0.02<*p*<0.04). For fish larvae, significant differences were found only between the largest size category, and the smallest (p=0.002) and second smallest (p=0.02) size categories. Shrimps were significantly different for the 130-250 size category and the <80 (p=0.01) and >250 (p=0.04) size categories.

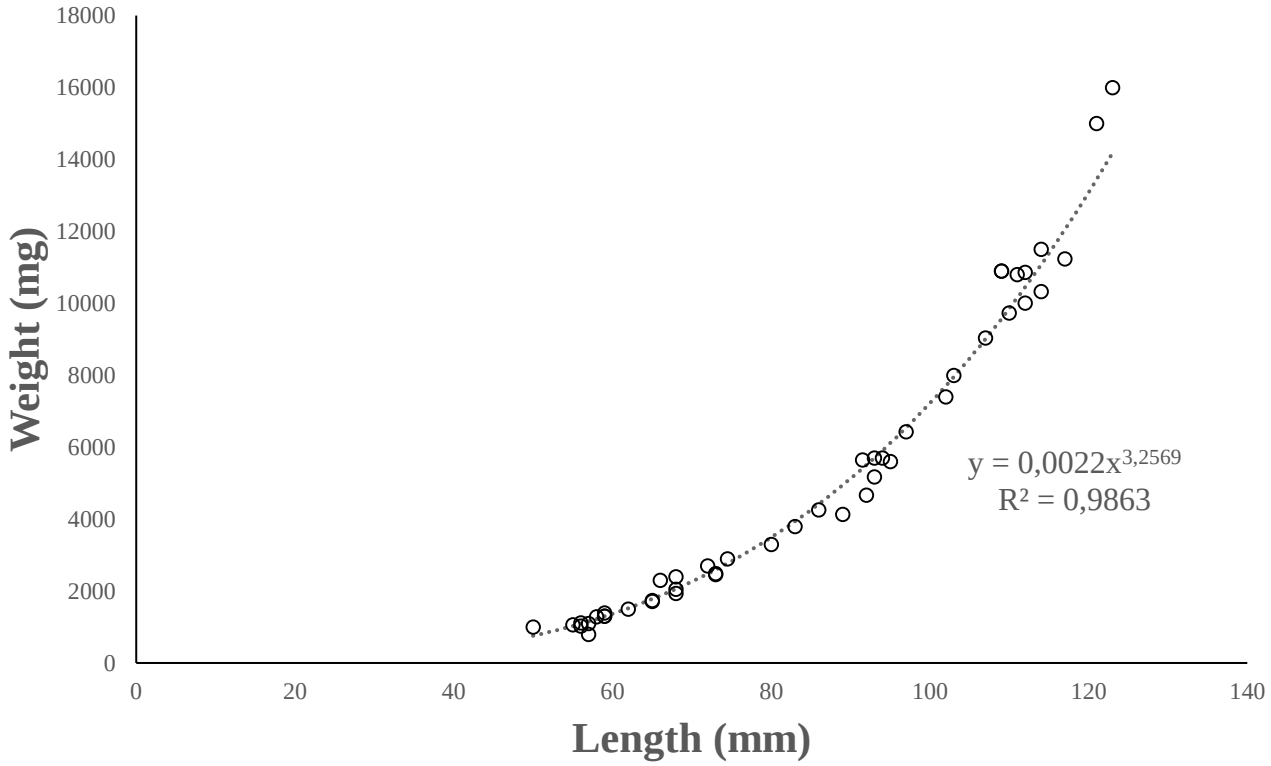


Fig. 10: Length-Weight relationship for pooled individuals of S. tanganicae and L. miodon.

Average DW found in non-empty stomachs showed a consistent trend of increased consumption as the *L. stappersii* increased in size (Fig. 11). This plot otherwise revealed the same trends mentioned above, with a shift in major dietary component from copepods, to fish larvae, to whole fish as the fish grow. A point of note is that comparing the intermediate sizes with the largest size, the larger size consumes more than twice as many fish larvae, despite these being found to make up only 8% of its consumed DW, meaning that although the fish start taking in whole fish prey, it does not stop feeding on fish larvae, rather, it increases the consumption of these as well.

Table 2: The total count, DW proportion, frequency of occurence (FO) for various food items for all size categories of L. stappersii.

	<80			**80-130**			**130-250**			**>250**		
	#	**DW %**	**FO %**	**#**	**DW %**	**FO %**	**#**	**DW %**	**FO %**	**#**	**DW %**	**FO %**
Copepoda	3912	92	1.00	-	-	-	-	-	-	-	-	-
Nauplii	256	0	0.55	-	-	-	-	-	-	-	-	-
Shrimp	16	1	0.09	2078	8	0.88	3022	6	1	118	0	0.55
Fish larvae	1	7	0.09	218	92	0.94	426	94	0.89	463	8	0.66
Fish	-	-	-	-	-	-	-	-	-	15	92	0.33
Number of prey counted		4185			3448			2296			596	
Total DW found in stomachs (mg)		32			500			958			12383	
Number stomachs counted		11			16			19			9	
Average number of prey in stomachs		380			216			121			66	

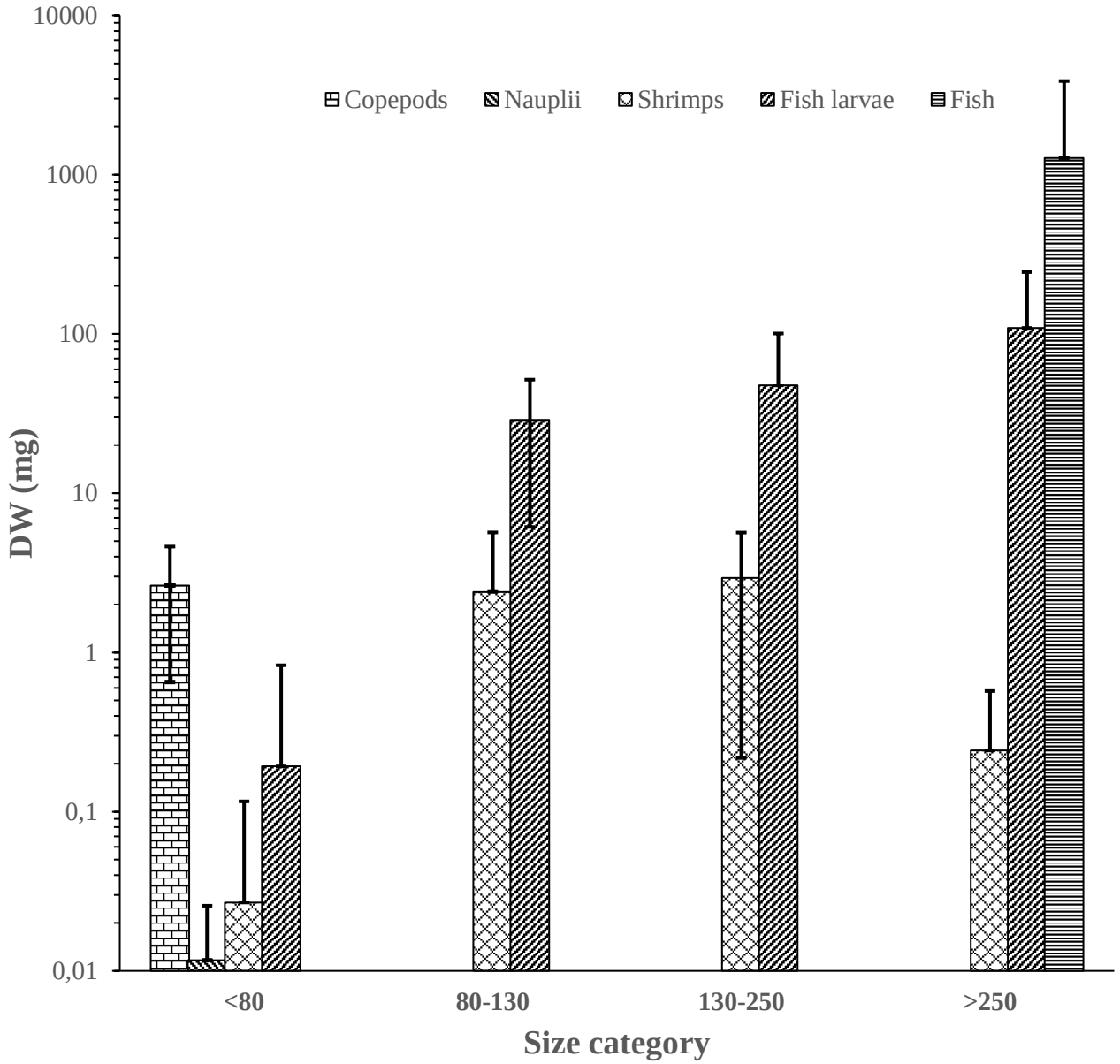


Fig. 11: Grouped Bar chart showing average DW of the various food items found in non-empty stomachs for all size-categories, plotted on a logarithmic axis. Error bars indicate Standard Deviation.

Isotope analysis

Isotope samples were collected from a total of 191 fish (Tab. 3), and supplemented with 161 samples of potential food items (Tab. 4). Gel A correction equations used to correct for bias or drift during analysis can be found in Appendix 1.

Of the 352 isotope samples, 7 were corrupted during isotope composition determination, and omitted from further analysis. The remaining samples were fitted in a ^{13}C and ^{15}N biplot for visual comparison (Fig. 12). It is readily apparent that there is a large spread for most groups, with the exception of copepods and shrimps. To check if the spread was the result of the different sampling times, a similar plot was made for only copepods and shrimps, divided into month of sampling (Fig. 13), which showed no trend between the months.

A notable observation is that the periphyton group is largely separated from all the other groups in terms of $\delta^{13}C$, making it an unlikely part of the pelagic food chain. Additionally, the group with the lowest $\delta^{15}N$ value was the shrimps, rather than the copepods. Fish larvae were found at a similar $\delta^{15}N$ value to copepods.

Fish groups had a large overlap in standard deviation, but looking at the mean values, are found largely in the order expected: lowest values of $\delta^{15}N$ was found in *S. tanganicae* smaller than 80 mm, while the individuals longer than 80 mm was around the same value as *L. miodon* smaller than 110 mm. The intermediate *L. stappersii* sizes, 80-250 mm, was found to have a $\delta^{15}N$ value slightly lower than the *L. miodon* individuals longer than 110 mm. Above these, we have the *L. stappersii* individuals longer than 250 mm, expected to be the top predators in this food chain.

The final group, *L. stappersii* smaller than 80mm appears slightly eschewed from the other fish groups, having a $\delta^{15}N$ value between that of the larger individuals of *L. stappersii* and *L. miodon*, as well as having a $\delta^{13}C$ value nearly 1.5 ‰ higher than the other fish species. This group consists of individuals from both August 2016 and

Table 3: The number of isotope and otoliths (in parenthesis) samples extracted from each Species-Size group.

	No. of isotope (and otoliths) samples in sampling periods						
Species-Size	**30.08.2016**	**10.02.2017-28.02.2017**	**01.03.2017-15.03.2017**	**16.03.2017-31.03.2017**	**01.04.2017-13.04.2017**	**14.04.2017-25.04.2017**	**Total**
Lates stappersii							
<80	20	-	-	6 (6)	-	-	26 (6)
80-130	6 (6)	5	5	5	-	-	21 (6)
130-250	-	5	5	5	-	5 (5)	20 (5)
>250	-	5	5	5	3	6 (5)	24 (5)
Limnothrissa miodon							
<110	-	5	5	5	5 (3)	5 (5)	25 (8)
>110	-	5	5	5	-	5 (5)	20 (5)
Stolothrissa tanganicae							
<80	-	10*	5	5	5 (2)	5 (5)	30 (7)
>80	-	10*	5	5	-	5 (5)	25 (5)
Total	26 (6)	45	35	41 (6)	13 (5)	31 (30)	**191(47)**

** Of the 10 S. tanganicae sampled in each size group in sampling period 1, half were collected from fishermen near Mahale.*

March 2017. Considering only the individuals from March 2017 results in the '*L. stappersii* <80 2017' group (Fig. 12), which had an isotope signal almost identical to that of *L. stappersii* between 130 and 250mm, however, the sample size of this group is only 6 individuals, of which whole headless bodies were used, rather than only muscle tissue.

Subtracting the standard fractionation values of 3.15 ‰ for $\delta^{15}N$ and 0.75 ‰ for $\delta^{13}C$ placed the fish means within the copepod, shrimp and fish larvae groups (Fig. 14). Specifically, the *L. stappersii* and the large *L. miodon* appear within the range of the fish larvae, while the *S. tanganicae* and smaller *L. miodon* seem closer to the shrimps, at least in terms of $\delta^{15}N$ value. It should be noted, however, that the fractionation values subtracted here are generalizations, and they may not be completely accurate for this particular system.

Standard ellipses were calculated for each of these groups (visualized in Appendix 2), and the overlap of these ellipses were estimated for each group as a fraction of non-overlapping area (Tab. 5). These overlaps revealed a high degree of overlap between the various fish groups. Namely, the intermediate *L. stappersii* sizes shared more than 60% of their Standard Ellipse Area (SEA). For *L. miodon*, the size group longer than 110 mm shared a large overlap with these intermediate *L. stappersii*, being noticeably more similar to these than to the smaller *L. miodon* (0.33-0.41 vs 0.14). These smaller *L. miodon* are in turn very similar to *S. tanganicae*, especially the larger individuals of these. The food items had little overlap, both amongst the other food items, and compared to the fish, the largest being between the large shrimps and the *S. tanganicae* smaller than 80 mm.

Table 4: Isotope samples taken from various potential food items

	No. of isotope samples in sampling periods					
Food items	**10.02.2017-28.02.2017**	**01.03.2017-15.03.2017**	**16.03.2017-31.03.2017**	**01.04.2017-13.04.2017**	**14.04.2017-25.04.2017**	**Total**
Pelagic Food items						
Copepods	19	5	-	30	-	54
Shrimps	20	5	-	20	-	45
Giant shrimps	-	-	7	-	-	7
Fish larvae	-	-	16	20	-	36
Litoral food items						
Copepods	-	-	-	-	4	4
Shrimps	-	-	-	-	-	-
Epiphytes	-	-	-	-	15	15
Total	39	10	23	70	19	**161**

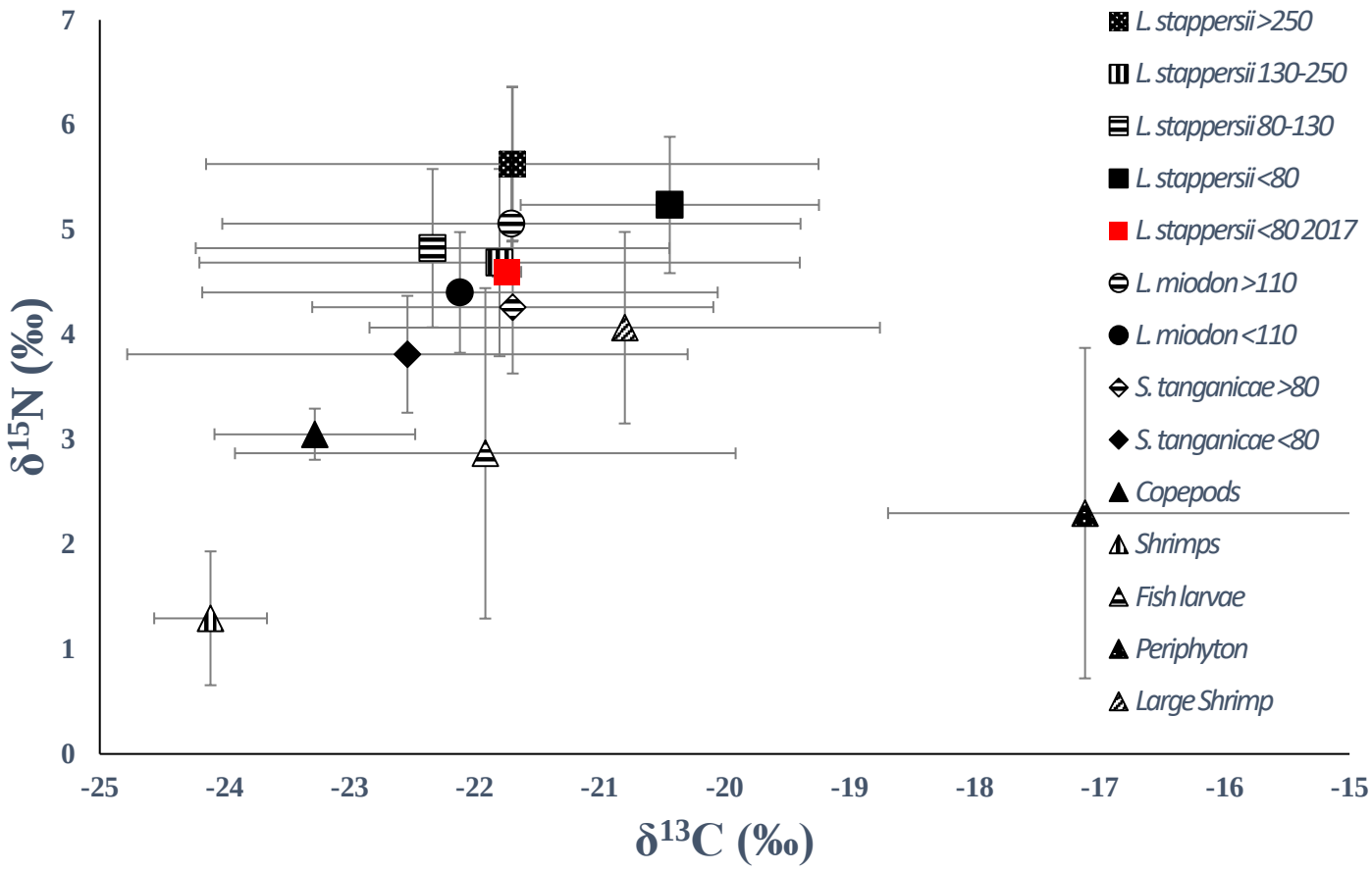


Fig. 12: Isotope biplot, showing the mean isotopic values for each Species and Size Group. Error bars indicate Standard Deviation.

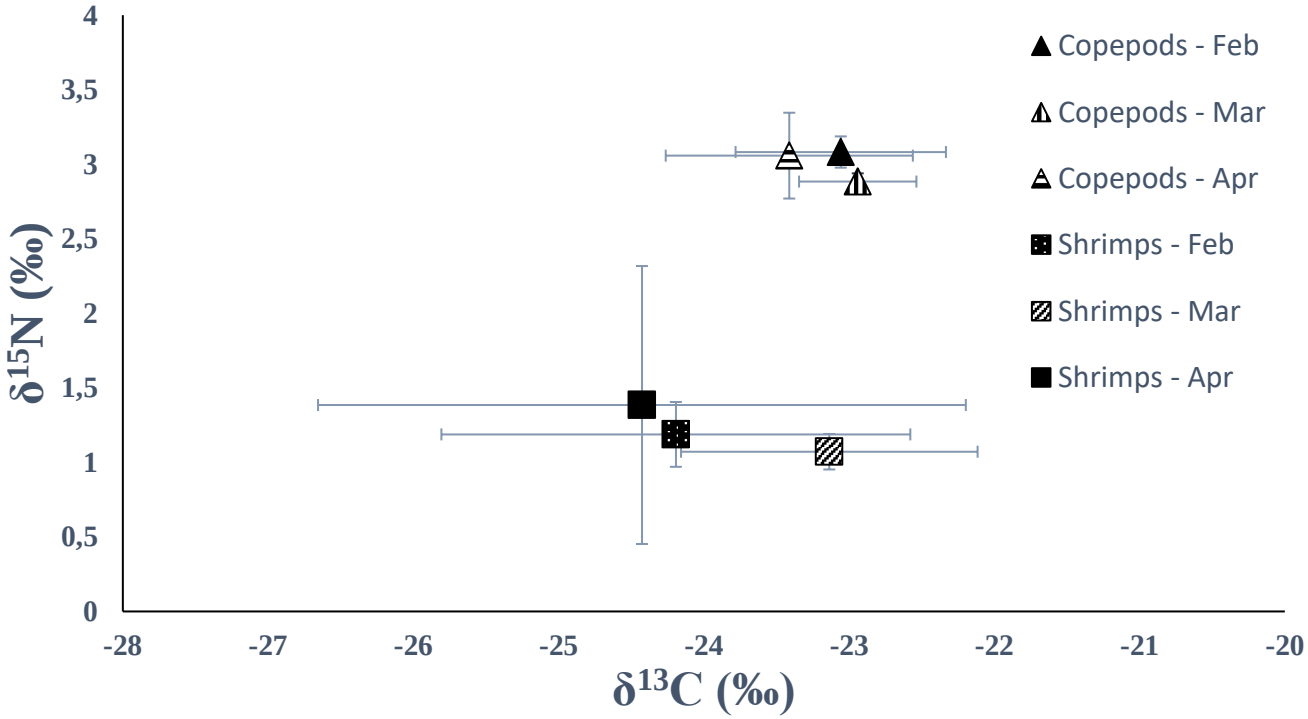


Fig. 13: Isotope biplot, showing mean isotopic values for copepods and shrimps, divided into the three months of field sampling, February, March and April. Error bars indicate Standard Deviation.

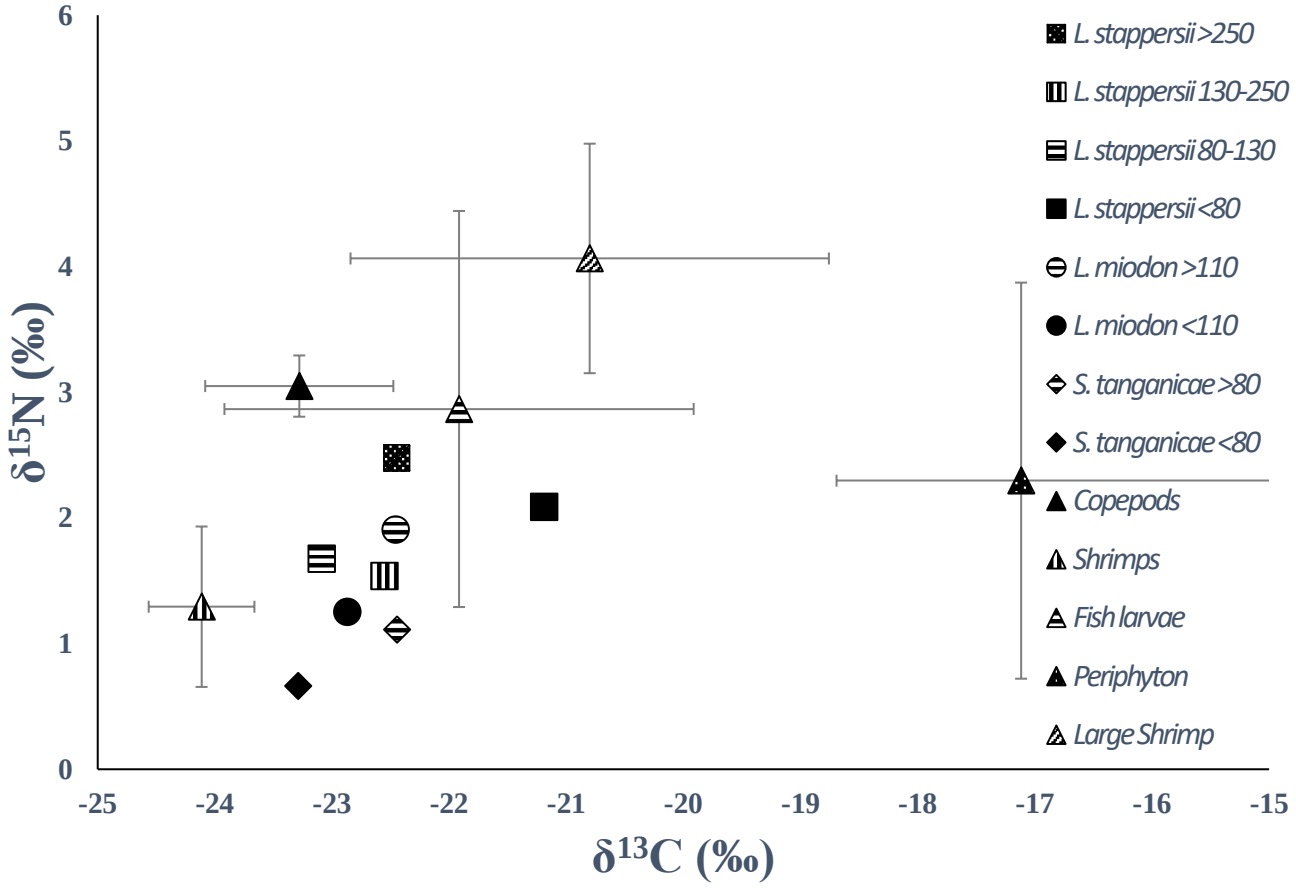


Fig. 14: Isotope biplot, showing mean isotopic values for all Species and Size Groups, with the generalized fractionation values of 0.75‰ $\delta^{13}C$ and 3.15‰ $\delta^{15}N$ subtracted for the fish means. Error bars indicate Standard Deviation.

Table 5: Overlap of Standard Ellipse Area (SEA) between all groups, as a fraction of non-overlapping area. Values above 0.2 are bolded.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
1: *L. stappersii* <80	*NA*												
2: *L. stappersii* 80-130	0.18	*NA*											
3: *L. stappersii* 130-250	**0.22**	**0.64**	*NA*										
4: L. stappersii >250	0.19	**0.26**	0.13	*NA*									
5: *L. miodon* <110	0.08	**0.44**	**0.58**	0.01	*NA*								
6: *L. miodon* >110	**0.33**	**0.33**	**0.41**	**0.21**	0.14	*NA*							
7: *S. tanganicae* <80	0	0.11	0.14	0	**0.24**	0	*NA*						
8: *S. tanganicae* >80	0.07	**0.27**	**0.39**	0	**0.57**	0.03	**0.40**	*NA*					
9: Copepods	0	0	0	0	0	0	0	0	*NA*				
10: Shrimps	0	0	0	0	0	0	0	0	0	*NA*			
11: Large Shrimps	0	0.03	0.05	0	0.08	0	0.16	0.13	0	0	*NA*		
12: Fish larvae	0	0	0	0	0	0	0	0	0	0	0	*NA*	
13: Periphyton	0	0	0	0	0	0	0.001	0	0	0	0.01	0	*NA*

Otolithometric analysis

Forty-seven otoliths were collected (Tab. 3), all from individuals also used in the stable isotope analysis. Of these collected otoliths, only 36 were judged to be readable. The remaining otoliths were either cracked, mishandled, or simply unreadable.

Based on the catch date and estimated age, hatch dates were estimated for all examined individuals (Fig. 15). Most of the examined fish were hatched between summer 2016 and early spring 2017. There does not appear to be confined hatch periods, instead it would appear that spawning is somewhat continuous throughout the examined period.

There also appears to be a relation between TL at capture, and hatch date, with longer individuals stemming from earlier cohorts. This trend is consistent across all species (apart from the *L. stappersii* caught in August 2016), which may indicate similar growth rates for all three species.

For each species, a general linear model was fitted, explaining the size as a function of the estimated age from the count of primary increments (Fig. 16). The slopes of these regressions provide an estimate of growth in mm/day for each species in the examined range of sizes. These slopes revealed an approximate growth of 0.65 mm day^{-1} for *L. stappersii*, 0.37 mm day^{-1} for *L. miodon* and 0.26 mm day^{-1} for *S. tanganicae*. It should be noted that while these slopes appear to fit the observed relationship well, they are linear regressions, and in the case of *L. stappersii* and *S. tanganicae* they indicate a hatch length of >40mm. As such, even though the regressions explain most of the variation in the observations, they may not be representative of the earlier life stages. Aside from these linear models, Von Bertalanffy growth models (Bertalanffy 1957), which are reminiscent of power functions describing declining growth as the individual nears its maximum size, fitted to the observed data are shown as well. The Von Bertalanffy fittings estimated growth parameters k=0.002 for *L. stappersii*, k=0.005 for *L. miodon*, and k=0.01 for *S. tanganicae*, and T_0 (age at length 0 mm) as -21.5 for *L. stappersii*, 57.9 for *L. miodon* and -23.6 for *L. miodon*, when restricted to S_{max} (maximum sizes) of 450mm for *L. stappersii*, 170mm for *L. miodon* and 100mm for *S. tanganicae*. Testing for difference in the linear regression slopes, it was found that *L. stappersii* was significantly higher than both *L. miodon* (ANCOVA, $F_{1,21}$=8.71, p=0.008) and *S. tanganicae* (ANCOVA, $F_{1,22}$=17.9, p<0.001). The difference between *S. tanganicae* and *L. miodon*, however, was not significant (ANCOVA, $F_{1,17}$=3.2, p=0.09)

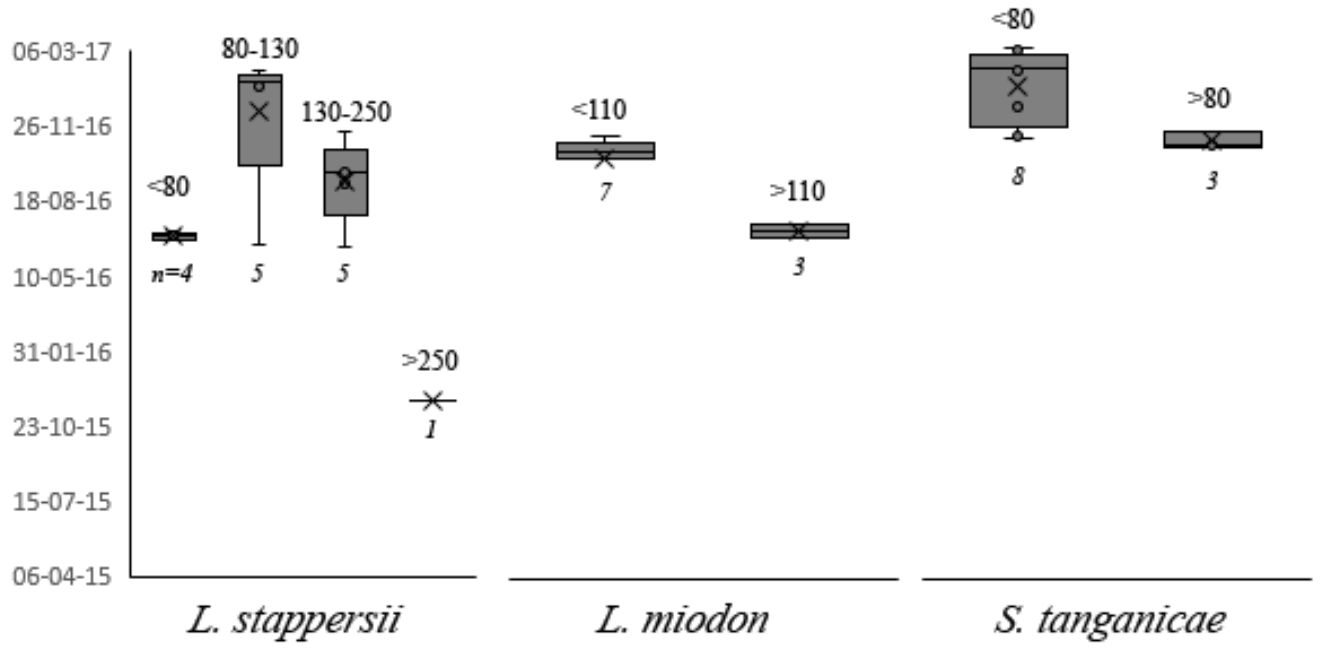
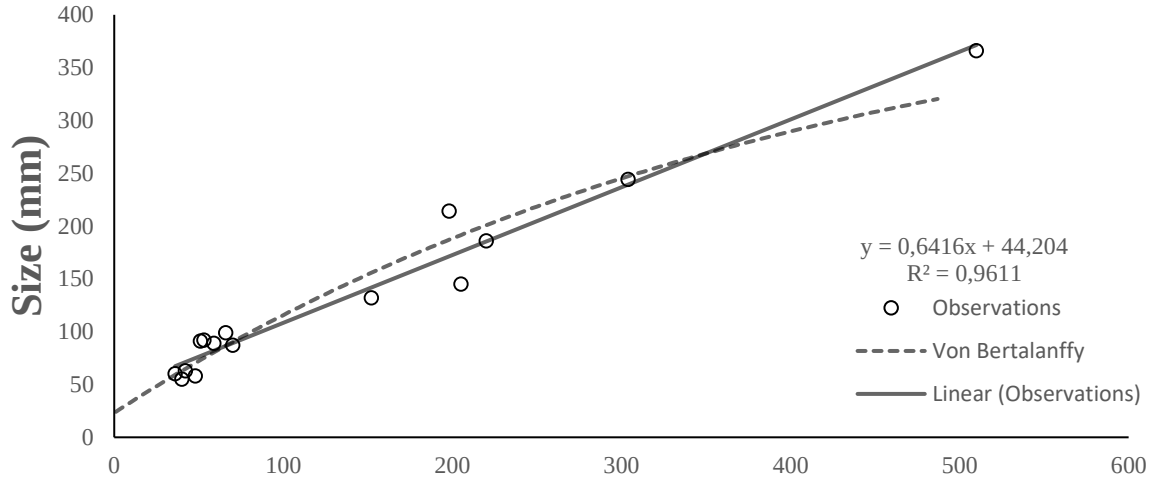
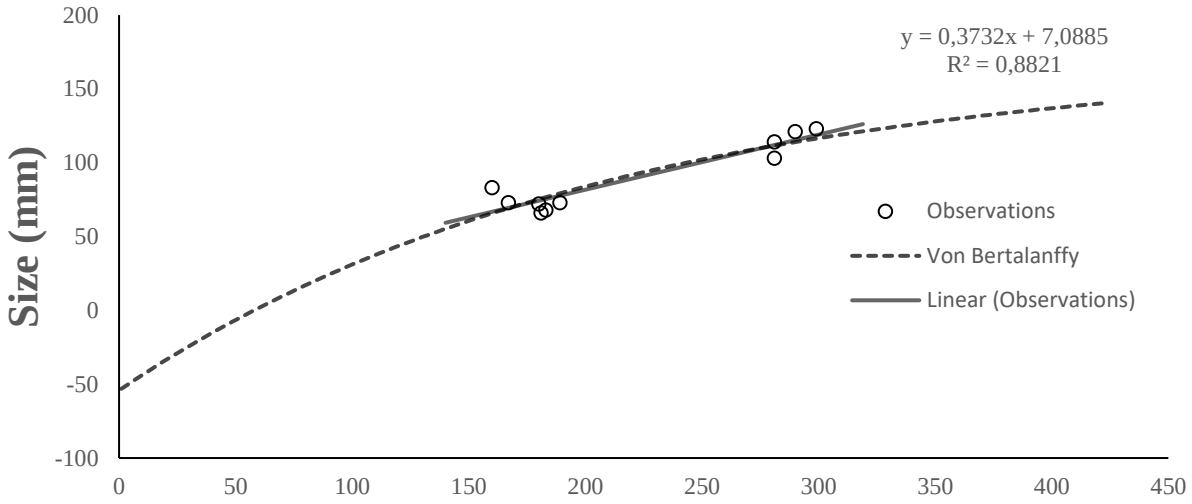


Fig. 15: Box plot of back-calculated hatch dates, based on catch dates and estimated ages. Whiskers indicate minimum and maximum values, boxes indicate upper and lower quadrants, as well as median. Crosses indicate means. Note that the L. stappersii individuals smaller than 80mm were caught in August 2016.

Lates stappersii



Limnothrissa miodon



Stolothrissa tanganicae

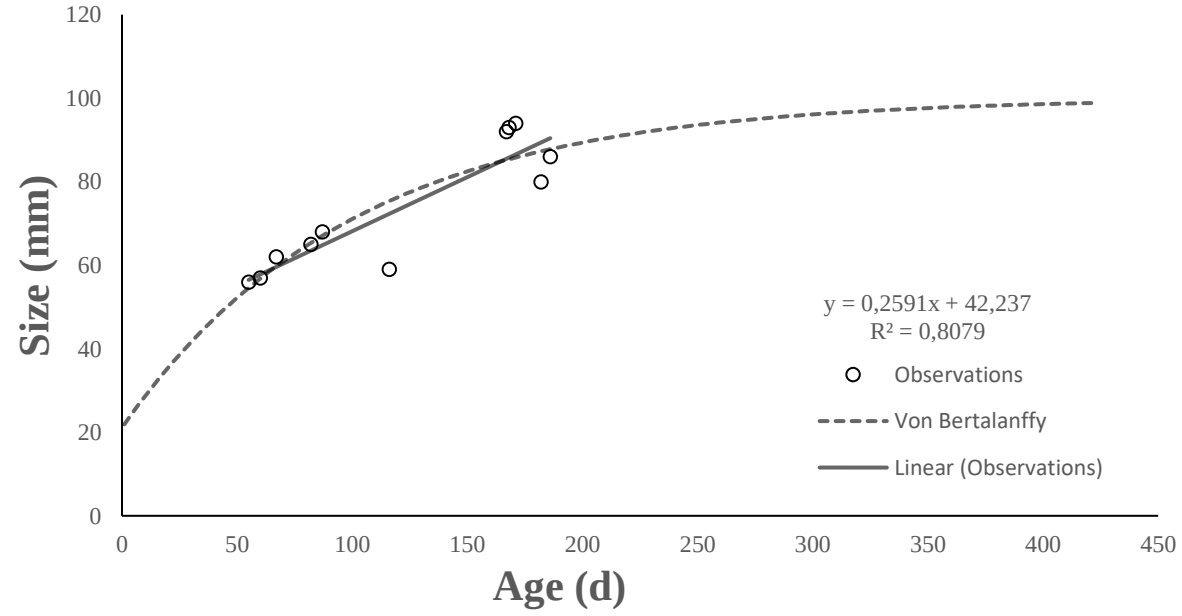


Fig. 16: Length-at-age graphs for all the fish species, showing the length observations as a function of the ages estimated from the daily ring counts. Linear regressions are shown, and Von Bertalanffy growth models fitted to the observations are also shown.

Discussion

For the zooplankton data, only the shrimps exhibited the expected tendency of migrating to the surface near dusk, and even for these shrimps it cannot be concluded with certainty, due to the lack of statistical tests. Copepods and jellyfish appeared randomly distributed, both spatially and temporally, with no clear pattern. This does not fit well with the expectations (Coulter 1991), stating that the clupeids migrate to the surface at night to feed on the aggregating copepods (Mgana et al., 2014). Narita (1986) noted that the clupeids tend to aggregate below the surface around dusk, but dispersing after sundown. It is possible then, that the nightly samples taken were taken after the dispersal, thus missing the aggregated timeframe. Due to the lack of closing nets, it was not possible to get a more detailed resolution of abundance in the vertical dimension, which may have served to illuminate zooplankton densities throughout 24-hour periods more clearly, but the large inter-day variation of shrimps and copepods could suggest that these zooplankton are found aggregated, and density would then likely be a question of whether such a zooplankton cluster is hit. It is also difficult to compare these current estimated densities with historical densities, as sampling procedure and nets used have not been standardized. Indeed, it is possible that the mesh sizes used were not optimal for the zooplankton species sought. Further studies may benefit from developing a standardized sampling method. Considering this, along with the lack of robust statistical tests, conclusions based on these density estimates should be drawn with caution.

The oxygen saturation and temperature isopleth plots reveal a definitive oxy- and thermocline around 60m depth, indicating that this is the depth of the mixed layer. This continues the trend of a shallowing oxycline reported by O'Reilly et al. (2003), where mixing depth was estimated to be ~80m depth, with an estimated rate of shallowing of 1.3 m $year^{-1}$. As such, these data support the hypothesis that climate changes increase the stability of the lakes stratification, leading to reduced mixing depth. The temperatures found also follow the trend of warming described by O'Reilly et al. (2003), as well as Verburg et al. (2003), with temperatures at 110m depth found to vary between 24.4-24.6 ^{O}C, compared to the 24 ^{O}C found in 2000. While this difference is small, it was consistent along the transect, and did not vary noticeably between February and April. O'Reilly et al. showed a rate of increase in temperature at 150m of 0.1 ± 0.01 ^{O}C pr. decade since 1913, which fits well with this increase, when considering the difference in depth. Since the warming documented here is below the current metalimnion, such warming would be expected to weaken the lake stratification, if not matched by a proportionate warming of the lake surface temperature. Tierny et al. (2010) used the TEX_{86} proxy method, based on sediment cores acquired near Mahale, to estimate earlier lake surface temperatures. Their estimated surface temperature in 1900 was 23-24 ^{O}C, increasing by 2 ^{O}C to 25-26 ^{O}C by the year 2000. These data show a further increase, with temperatures now reaching 27 ^{O}C at the surface. This increase is notably larger than the increase below the thermocline, which supports the theory that climate changes are increasing the stability of the lakes stratification, as proposed by O'Reilly et al. (2003) and Verburg et al. (2003).

Chl *a* data was available for the upper 60m of the water column, and shows no signs of being restricted to a narrow depth range, with concentrations even peaking at 40m depth in April. Rather, it seems that primary production takes place at hotspots of locally abundant Chl *a*. While there is a difference in the location of the hotspots between February and April, it is not certain what causes this. It may be caused by baseline variation or flow patterns, but a likely explanation is that it is the result of upwelling events. Upwelling events caused by shifts in wind is the primary source of nutrients in the lake (Coulter 1991), and this would also explain why the Chl *a* concentration is not highest near the surface, where irradiation would be higher. Considering this, when comparing the isopleth plots for Chl *a* and temperature in April, Chl *a* concentration is highest near LT8, where the thermocline is also less pronounced. This more gradual thermocline could indicate that colder waters, carrying nutrients, has indeed risen locally. The hotspot depths also serve to demonstrate how clear the water is in Lake Tanganyika, allowing primary production to occur as deep as 60m, compared to, for instance, Lake Victoria, which has a Secchi depth 0.9-2.4m (Lung'aiya et al., 2001).

Stomach analysis

It should be noted once again, that the stomachs used in this analysis were all non-empty. This was done because of the sheer amount of stomachs being empty, which was a large majority (Pers. obs.). Therefore, when drawing conclusions based on these data, it should be kept in mind that these data do not describe how much food is consumed by the various size groups of *L. stappersii*, rather, they describe the composition of what is consumed. As such, they can be used to estimate the importance of various prey items compared to each other, but cannot estimate total consumption or predation pressure on the prey items in question. To do this, the average mass of consumed prey per individual would have had to be multiplied by the ratio of non-empty to empty stomachs.

The fact that a stomach analysis could not be performed on the clupeid species underlines another caveat under circumstances such as these: if stomachs cannot be preserved immediately after capture, post-mortem digestion is likely to affect the composition of the stomachs at the time of analysis. The stomachs used in this study were bought directly from the fishermen. The fishermen catch fish continuously throughout the night, so the fish may have been lying aboard a boat from anywhere between one and ten hours, at temperatures above 20 ^{O}C. The absence of usable clupeid stomachs is then likely the result of the content being digested before acquisition of the fish was possible. Furthermore, since prey of varying size and composition are likely digested at different rates, the stomachs that were used might present a bias for larger prey. Such effects were documented by: Legler et al. (2010), who found that digestion rate of fish larvae decreased with increasing prey size, and increased with temperature; and Macdonald et al. (1982), who documented differences in digestion rates of differing prey types. In this case, it may lead to an overestimation of fish larvae, and an underestimation of the importance of shrimps and copepods, copepods in particular, which should be kept in mind when interpreting the results.

The problem is confounded by the added variable of 'time spent out of water' for the fish. If the fish were all caught at the same time, one might be able to correct for this bias, but since the fish are caught at varying times throughout the night, it becomes impossible to distinguish between compositions resulting from diet, and those resulting from digestion rates. Further stomach analysis done in similar environments would do well to find a way to preserve the stomachs quickly after capture.

With this disclaimer in mind, it becomes more striking, however, that copepods were found to compose so large a proportion of the diet in the *L. stappersii* smaller than 80 mm, especially when compared to the other size groups where they were absent. While it should be noted that these fish were caught in August 2016, the same possible bias would also apply to this time period, as there is little temperature variation throughout the year in the area. One possible explanation is that these fish were caught just prior to the fishermen returning to land, and as such, spent little time out of water before being frozen. Another explanation is that these fish simply ate very large quantities of copepods, but the copepods that were present were relatively well preserved, so it is likely that only little digestion had taken place. Either way it is reasonable to assume that the contents of these stomachs are representative of their size group.

The same confidence, however, cannot necessarily be applied to the analysis of the other size groups, where the size bias of prey might be acting. As a personal observation, a large number of shrimps in these stomachs showed signs of advanced decomposition. As such, while no copepods were found, it cannot be ruled out that they were present. Despite this, copepods are still unlikely to meaningfully impact the average DW composition in any of the larger size groups, due to the sheer size difference in these prey items, with shrimps contributing roughly 3 times more DW than copepods per individual, and fish larvae contributing more than 300 times as much DW pr. individual. Considering the number of shrimps and fish larvae found in the stomachs of the intermediate size groups, and the fish found in the largest size group, copepods are likely negligible.

Assuming then, that copepods are indeed unimportant, the diet of the intermediate size groups must then be composed primarily of shrimps and fish larvae, as the results show. Based on average DW contribution, it appears that fish larvae make up 80% of the DW, while shrimps account for the remaining 20%. Given their respective sizes, this seems reasonable, but it cannot be ruled out that shrimps are underestimated. As mentioned above, a large number of shrimps were severely decomposed, and in the cases of some stomachs, shrimps had to be counted based on the number of eye pairs remaining. Coulter (1991) and Pearce (1985) indicated that *L. stappersii* was different from other *Lates* species because of its ability to subsist on shrimps as an adult when fish prey was scarce. This seems to conflict with the findings presented here, where even the large number of shrimps appears to be out-shadowed by the fish larvae found in stomachs. There are two likely explanations for this: first off, shrimps may very well be underestimated in this stomach analysis, and contribute more than estimated to the DW consumed; secondly, *L. stappersii* may be selectively feeding on more palatable fish larvae, because these are present. In this case, a decline in available fish prey would cause

it to focus more on hunting shrimps, leading to a higher uptake. Based on personal observations during the counting, the second option seems more likely. While many shrimps were very digested, the eye pairs appeared to be very resistant to this decomposition, and counts of eye pairs are therefore less likely to underestimate number of shrimps. A similar trend was observed in marine fishes (The Resource Ecology and Ecosystem Modelling Program, stomach analysis manual). It is still possible that many eye pairs were also completely digested prior to the analysis, so supplementary stomach analysis' on better preserved stomachs, or alternatively, knowledge on decomposition rates of these specific prey items, could serve to confirm this.

In the case of the largest size category, fish larvae and fish prey appeared to be the primary food items, collectively making up almost the entirety of the DW. Fish prey, despite being present in only a third of the stomachs, made up 92% of the DW. It is likely that fish prey is the primary food item once the *L. stappersii* reach this size, as even a single fish will out-shadow even large amounts of fish larvae and shrimps. Most stomachs still contained more DW from fish larvae than from fish prey, but this is caused by the low frequency of occurrence for the fish prey. When considering why the frequency is so low, a possible explanation could be a bias in the catching method. The *L. stappersii* were caught along with the clupeids using lift nets, and it is assumed that the *L. stappersii* are present here to feed on the schooling clupeids. It is possible that individuals who have already fed leave this zone, meaning the fish that are caught are the ones still hunting for food, i.e. the ones who have not yet eaten fish prey. As these data cannot account for such a bias, further studies would be needed to confirm if this is the case.

Isotope analysis

The isotope analysis largely revealed the expected overall structure of the food web, starting with the smallest *S. tanganicae* individuals, gradually getting enriched in both $\delta^{13}C$ and $\delta^{15}N$, ending with the largest *L. stappersii* individuals. There were, however, some signals that bear mention: A clear outlier in the isotope biplot is the periphyton, being isolated in terms of $\delta^{13}C$ value, and with very low degree of overlap in SEA with the other groups. This fits the expectations, as the periphyton was collected in the littoral zone at a depth of ~10 meters, whereas the other prey items, as well as the fish, were collected in the pelagic. The periphyton was therefore not expected to be a part of the food chain for these fish.

Fig. 17: Picture from zooplankton hauls showing two copepods (upper) and one shrimp (lower).

Another disparity between the expected and observed relationship between groups is the signals of copepods and shrimps: copepods were approximately 2‰ higher for $\delta^{15}N$ value and 0.8‰ higher for $\delta^{13}C$, which would be consistent with copepods preying on shrimps. As the sample size is fairly large, and standard deviation is small, this relationship is likely accurate. That being said, copepods preying on shrimps in the lake seem

very unlikely, given their relative sizes (Fig. 17). Just looking at the relationship, it is not possible to tell whether copepods unexpectedly enriched in $\delta^{15}N$, or if shrimps are unexpectedly depleted in $\delta^{15}N$. Both copepods and shrimps were assumed to be planktivorous, feeding on phytoplankton and proto-zooplankton (Sarvala 1999, O'Reilly 2002, Yasindi & Taylor 2010). Considering the $\delta^{15}N$ for the periphyton, a primary producer, lying at 2.3‰, with the shrimps lying at 1.3‰, it seems more likely that the shrimps are lower than what would be expected. The question then becomes: what do the shrimps eat? A possible hypothesis could be that they utilize a different pool of particulate organic matter (POM), compared to the copepods, perhaps migrating below the mixed layer to forage. This would fit with the observations of Hecky and Fee (1981), where they noted that even accounting for the high rate of primary production, fisheries yield was unusually high in the lake, indicating either an unusually high trophic efficiency, or additional sources of energy, perhaps found in the deep water. Another isotope analysis gathering POM from several discrete depths may serve to answer this. Detailed knowledge of the depth distribution, stretching deeper than 120m, of the shrimps, as well as the copepods, would help establish any difference in feeding patterns between the two, and could also give an indication of how deep the shrimps are capable of migrating. Based on the results found in this report, shrimps appear to favor lower depths during day, having higher densities between 60 and 120m than between 0 and 60m, although this cannot be concluded with certainty, due to the lack of statistical tests.

The fish larvae were found at 2.8‰ $\delta^{15}N$, almost identical to the value of the copepods. This is not consistent with fish larvae feeding on copepods, as was expected. Rather, it would imply a similar trophic level. The fish larvae are also roughly 1.5‰ higher in terms of $\delta^{13}C$, making similar diets unlikely. It is possible that the fish larvae consume some ratio of phytoplankton or proto-zooplankton, but isotopic signals for the potential prey species would be needed to be certain. Further studies regarding the diet of the copepods, shrimps and fish larvae might be advantageous for future management, as these groups appear to form the basis of the pelagic food chain.

A notable deviation from the expected signal is the *L. stappersii* smaller than 80 mm. These were expected to be in the range of the smaller *L. miodon* and *S. tanganicae*, but instead, they share a larger SEA overlap with the 3 larger *L. stappersii*, and an especially high overlap with the large *L. miodon*. In addition to their unexpectedly high $\delta^{15}N$ value, their $\delta^{13}C$ value is also highly isolated from the other fish. This is unlikely to be explained by fractionation, and as such, it is inconsistent with them feeding on any of the sampled potential food items. As no other likely food items were discovered during sampling, the most likely explanation for this is the discrepancy in sampling date between these smaller *L. stappersii* and the other fish. Of the 26 small *L. stappersii* examined, only 6 were from the field work period, the remaining 20 being frozen samples collected in August 2016. It is possible that the isotopic signal of the prey items has changed since then. This is supported by the stomach analysis: the eleven non-empty stomachs examined for *L. stappersii* smaller than 80 mm was from the same individuals used in this isotope analysis. These stomachs showed a strong dominance of

copepods as a food source for these particular individuals, despite the isotopic signal of this group being inconsistent with feeding on copepods. Additional samples from copepods or other fish from August 2016 would be needed to verify, but it is a likely explanation that the copepods isotopic signal has changed since then, perhaps coinciding with the switch from dry season to wet season, and the shift from dominance of the copepod *M. aequatorialis* to *T. simplex*.

When accounting only for the *L. stappersii* individuals smaller than 80mm caught in March 2017, only six individuals remain, with a mean isotopic value almost identical to that of the individuals between 130 and 250mm. Before any conclusions are made based on this, it should be noted that for the six individuals in question, whole headless bodies were used, whereas only muscle tissue was used for all other fish samples. Caut et al. (2009) showed that, for fish, $\delta^{13}C$ fractionation could be nearly 1‰ higher when using whole bodies, compared to muscle tissue, resulting in a total $\delta^{13}C$ fractionation of ~2.3‰, with no significant difference in $\delta^{15}N$ fractionation. Compared to the mean isotopic signal of the copepods, the March 2017 *L. stappersii* smaller than 80mm had ~1.8‰ higher $\delta^{13}C$ and ~1.5‰ higher $\delta^{15}N$, which is consistent with feeding on copepods, supplemented with shrimps and/or fish larvae. This is further supported by the results of the stomach analysis, showing the same trends (see above).

The largest *L. stappersii*, was found at the highest trophic level, as expected. Its mean was found 2‰ higher for $\delta^{15}N$ and 1‰ higher for $\delta^{13}C$, than the small *S. tanganicae*. While this does differ from the mean fish muscle fractionation of 3.15‰ $\delta^{15}N$ described by Sweeting et al., (2007), it is still well within the range of possible fractionation, as the generalized fractionation found by Sweeting had a Standard Deviation of 1.28‰. As such, these fractionation values are consistent with *L. stappersii* individuals longer than 250 mm feeding on the smaller *S. tanganicae* individuals. The small *L. miodon* individuals, whilst having a notable overlap of SEA with the small *S. tanganicae*, does not fit as well as a prey of the *L. stappersii*, being only ~1.4‰ and ~0.5‰ lower for $\delta^{15}N$ and $\delta^{13}C$, respectively. While still within possible ranges of fractionation, especially due to the considerable variance in isotopic signal of the fish species, it is likely that *L. stappersii* primarily feed on the *S. tanganicae* individuals, supplemented by *L. miodon* individuals. A hypothesis could be that *L. stappersii* does not selectively feed on *S. tanganicae*, rather, it opportunistically feeds on clupeids it encounters, if they are of a size possible for it to consume. The reason that *S. tanganicae* then seems to compose more of its diet would be because *S. tanganicae* is not only more abundant in the lake (Coulter 1991, Plisnier 2009), it also enters the pelagic waters at a smaller total length (Coulter 1991, FAO 1978), making it more readily available as potential prey.

The large *L. miodon* and the intermediate *L. stappersii* sizes all share considerable SEA overlap, and are also found visually similar in the biplot, and likely have similar diets. Their position in the biplot would appear to indicate a diet of fish larvae and copepods, being ~2‰ higher than both for $\delta^{15}N$, similar to fish larvae for $\delta^{13}C$, and ~1.5‰ higher than copepods for $\delta^{13}C$. This is supported for the *L. stappersii* by the stomach analysis

showing a strong dominance of fish larvae derived DW. No copepods were found in the stomachs of intermediate *L. stappersii* sizes, however, and considering the sizes of copepods and shrimps, copepods appear unlikely to contribute meaningfully to a diet once fish larvae become available. Additionally, shrimps were found in the stomachs of the intermediate *L. stappersii*. It is possible that these fish do indeed have a diet that consists mainly of fish larvae, supplemented by shrimps, leading to a slightly lower value for both $\delta^{15}N$ and $\delta^{13}C$, than would have been found, had their diet consisted purely of fish larvae.

The large *S. tanganicae* shared a sizable overlap of SEA with the smaller *L. miodon*, and likely share similar diets. As these species are very similar, and have access to the same food items, it seems reasonable that they would have similar diets at similar sizes, which also fits well with the assumed diet described by Coulter (1991), as the *L. miodon* may have not yet reached a size capable of preying on the juvenile *S. tanganicae*. Collectively, these two groups are within the 80-110mm size range, with the *L. miodon* individuals generally being slightly bigger. These groups also share a large overlap with the intermediate *L. stappersii* and large *L. miodon*, being separated by only ~0.5‰ $\delta^{15}N$. Their isotopic signal is therefore also consistent with a diet consisting mainly of fish larvae, supplemented by copepods and/or shrimps. A slightly larger preference for shrimps compared to the larger fish might explain the lower $\delta^{15}N$ value, but given the variance for the groups, this difference seems trivial, and may indeed be explained by the variance.

The smallest fish, the *S. tanganicae* smaller than 80mm has the lowest value for both $\delta^{15}N$ and $\delta^{13}C$ of all the fish species. As it is the smallest group, alongside the *L. stappersii* smaller than 80mm, this fits the expectations. Compared to the copepods, which they are assumed to be preying upon (Coulter 1991, Mulimbwa 2014ab), they are ~0.8‰ higher in $\delta^{15}N$ and ~1‰ higher in $\delta^{13}C$. This is not quite as high as would be expected if they fed exclusively on copepods, but a possible explanation could be supplementary feeding on shrimps. Compared to the *L. stappersii* smaller than 80mm, who were also thought to feed primarily on copepods, the $\delta^{15}N$ fractionation is only about half as high for the *S. tanganicae*. This could indicate that *S. tanganicae* consume more shrimps, which have lower $\delta^{15}N$ values. Another possibility is that the fractionation values simply differ, perhaps as a result of whole bodies being used for *L. stappersii*, while muscle tissue were used for *S. tanganicae*. While Caut et al. (2009) did not find a significant difference in $\delta^{15}N$ fractionation between muscle tissue and whole bodies for fish, large variations can occur amongst species and ecosystems (Sweeting et al., 2007). More detailed knowledge of the fractionation for these specific species is needed to be sure, but differing fractionation values seems the more likely explanation.

Summarizing the above reflections, when not considering the *L. stappersii* smaller than 80mm, there seems to be a strong relation between total length and trophic level, regardless of species. The species-poor pelagic system might not allow for noticeable differences in diet between species, when examined at similar sizes. Formulated as a hypothesis: the diet of the fish in the lake is explained by size, rather by species; fish below 80mm eating primarily copepods, supplemented by shrimps, with a gradual shift to a preference for fish larvae,

still supplemented by shrimps around the 100mm size, which they maintain until they reach a size capable of preying on other fish, namely the small *S. tanganicae* individuals.

In the case of *S. tanganicae* and *L. miodon*, they never reach the size capable of eating mature fish individuals, instead simply shifting from a preference for copepods to a preference for fish larvae. In the case of *L. stappersii*, they would follow the same pattern, eventually beginning to prey on *S. tanganicae* and the smaller *L. miodon* around a total length of 250mm.

Otolithometric analysis

The growth of *L. stappersii* and *S. tanganicae* seem to be explained well by the fitted Von Bertalanffy growth models. In both cases x-intercept was quite low, being below -20. This is likely explained by the Von Bertalanffy model being a poor descriptor of very early life stages. In the case of *L. miodon*, however, the linear model seems to provide a better fit for the data present, than do the Von Bertalanffy model. This is unlikely, as fish generally do not grow linearly throughout their growth range. Furthermore, based on the similar diets of fish of similar sizes, as well as the similar hatch dates for fish of similar sizes (discussed later), there is no reason to assume that *L. miodon* would differ so markedly from the other species in terms of growth. A possible explanation to this apparent difference is that the ages of the *L. miodon* individuals have been overestimated. The counting of daily rings was found to be more difficult for *L. miodon* compared to *L. stappersii* and *S. tanganicae*, because it was more difficult to distinguish between daily and sub-daily increments (Pers. Obs, Jessen & Mgana). Counts of increments for individuals with known ages would be needed to calibrate, but this seems a likely explanation, as the mechanisms responsible for increment formation have not been clarified for any of these fish species. For all three species, length-at-age data for juveniles ranging between 0 and 80mm would help to accurately describe the manner of growth exhibited. Also for all three species, the linear models provided an excellent fit of the available data, suggesting that all three species are growing approximately linearly at these size ranges, likely the result of growth having 'evened out' as they approach their asymptotic maximum size.

Based on the back calculated hatch dates, spawning appears to be continuous throughout the year, rather than confined to a single spawning season. This fits with earlier observations on the fish of Lake Tanganyika (Coulter 1991, Mulimbwa et al., 2014ab). Since Mulimbwa et al. (2014b) did not find any solid pattern in clupeid spawning in relation to copepod abundance, it is still unknown what governs spawn timing in these fishes. Furthermore, it is possible that these species even have completely different mechanisms responsible for initiating spawning behavior.

Also based on the back calculated hatch dates, there does not appear to be any meaningful difference in hatch dates when comparing fish of similar length, even across species. This could indicate similar growth rates for all three species, as one would otherwise expect different hatch dates for fishes of similar sizes. Despite this,

the *L. stappersii* appeared to grow significantly faster than the clupeids based on the linear growth models. A possible explanation for this is that *L. stappersii* is still further from reaching its asymptotic maximum growth. If we assume that the species grow in accordance with the Von Bertalanffy growth model, growth rate should decline as individuals approach their maximum length. Most of the *L. stappersii* individuals examined were around 250mm and below. Since this species has a maximum length of roughly 450mm, these individuals have likely only reached ~55% of their maximum length. Compare this to *L. miodon*, with the largest individuals examined reaching ~130mm, being roughly 74% towards their estimated 170mm maximum; and *S. tanganicae*, where the largest individuals were slightly longer than 90mm, which is more than 90% of its expected 100mm maximum size. While the difference in growth rates of *L. miodon* and *S. tanganicae* was found not to be significant based on these data, the growth rate of *L. miodon* was slightly higher than that of *S. tanganicae*, which fits with this explanation. A comparison with the larger otolithometric dataset being developed as part of the CLEAT project may serve to confirm or reject this hypothesis, although time will not allow for it within the scope of this report.

The CLEAT Project

The aim of this report was to help ensure proper decision-making in regards to management of Lake Tanganyika and its fishery. The limnological data collected during the sampling period is consistent with the expected warming and stabilization of the lake stratification, and thus further supports the theory that climate changes are indeed altering the environment of the lake. However, this is not enough to conclude that climate changes are the main driver of the declining fisheries, as overfishing may be a larger problem still. During sample collection, caught individuals of *L. stappersii* below 40mm were found, a size far below maturation. As such, unsustainable fishery is definitely also taking place in the lake. Whether overfishing, climate changes, or a mixture of the two, is the primary driver of the decline will still require further modelling, such as the Ecopath model currently being developed as part of the CLEAT project. The diet assessment made in this report should be included in this model, giving the most recent estimate of the trophic structure of the pelagic food web. A larger exploration of the ecology of the lakes shrimps and copepods could serve to answer some of the questions that have arisen, namely what the diet of these crustaceans is composed of, and how they differ. Fish larvae were also found to be a significantly important part of the diet of many of these fish, which was not reported in the previous diet estimations. The reason for this is not known; it may be caused by other food items, such as shrimps, declining in number, leading to higher fish larvae predation. If this is the case, mortality of fish larvae would be expected to have changed, influencing recruitment.

Conclusion

Based on the stomach and isotope analyses, the major prey items in the pelagic food web appear to be copepods, shrimps, fish larvae, and whole fish prey, in accordance with expectations. There appears to be no discernible difference in diet composition amongst the three species examined when accounting for total length, indicating that diet is likely a result of size, with all three species consuming primarily copepods in the earliest life stages, shifting to a focus on fish larvae supplemented by shrimps around a total length of 80mm. The largest of the three species, *L. stappersii*, further grows until it is capable of consuming whole fish prey at a total length of roughly 250mm. The back calculated hatch dates based in primary increment count revealed no definitive difference in growth among the species.

While this report cannot conclude whether overfishing or climate change is the primary driver behind the declining catches in the lake, the limnological variables collected followed previous estimated rates of warming and shallowing in the lake, showing a shallowing of the epilimnion of nearly 20 meters since 2003, and a warming of the surface layer of 1-2 ^{O}C in the same period. This indicates that climate changes are indeed influencing the lake ecosystem. The diet compositions found in this report should strengthen further modelling, by giving a more recent estimate of how carbon flows from the primary producers to the pelagic fishery. A model incorporating both the changing climate, and the fisheries pressure, will hopefully allow for an accurate description of the forces driving the decline in this hugely important freshwater fishery.

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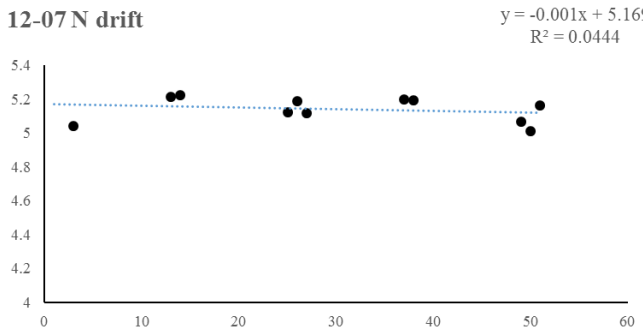
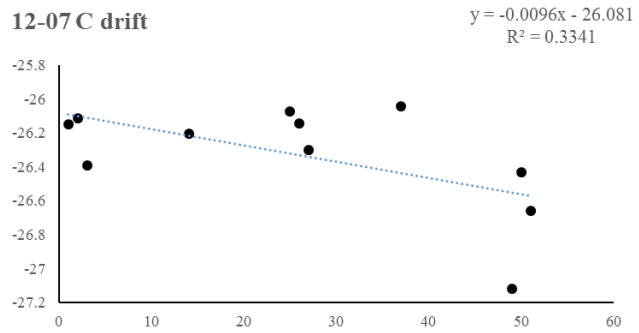
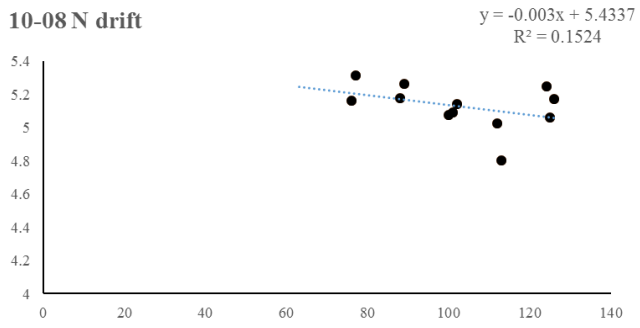
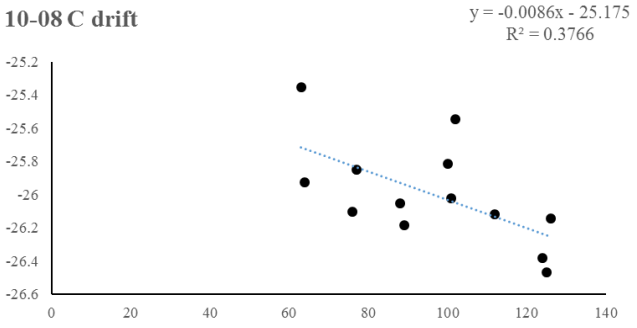
Appendix 1

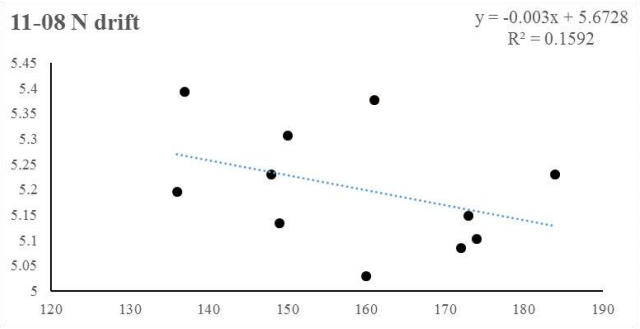
Measured Gel A values (‰) for $\delta^{13}C$ and $\delta^{15}N$ as a function of 'running number', the order in which the samples were analysed, counted cumulatively throughout the analysis. Graphs presented after removal of outliers. Regressions used in correction of data for each day of analysis.

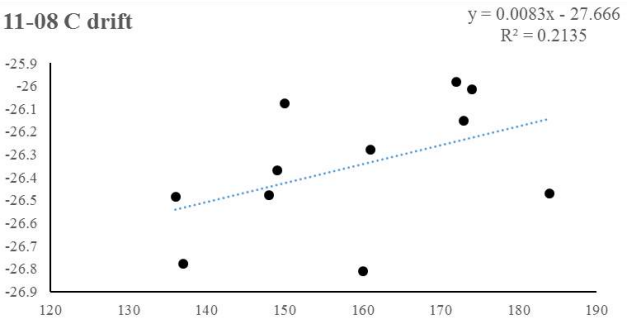


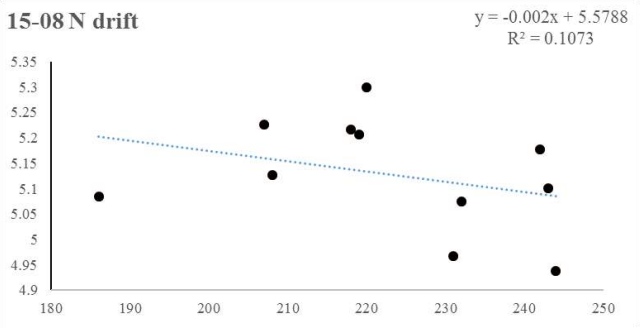


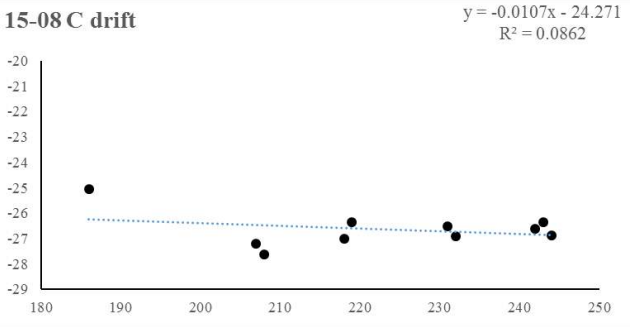






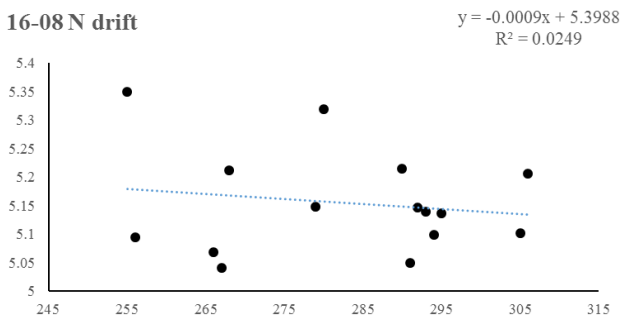


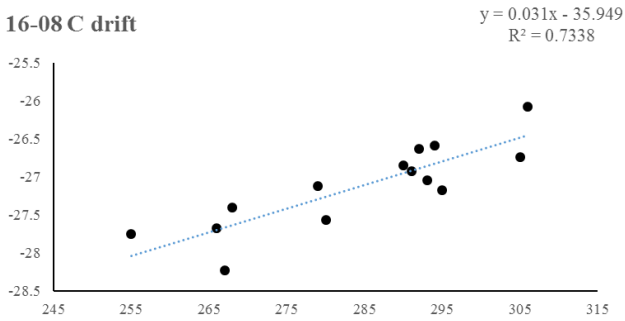


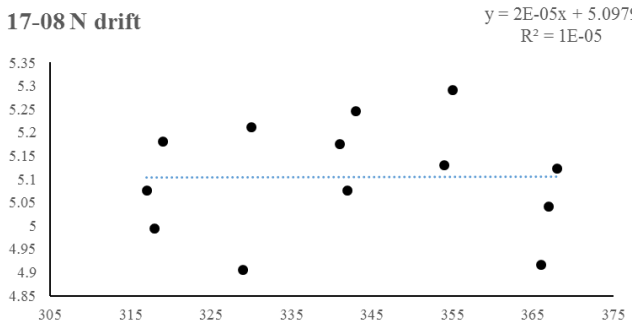


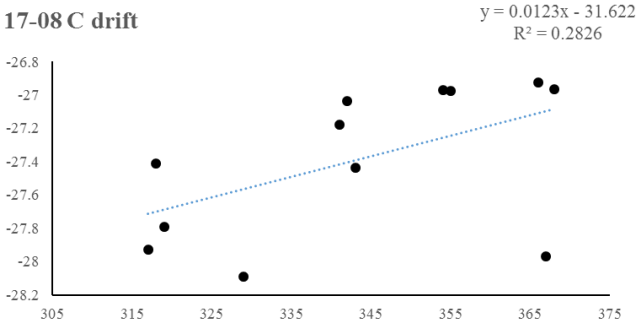
Appendix 1 – Cont.

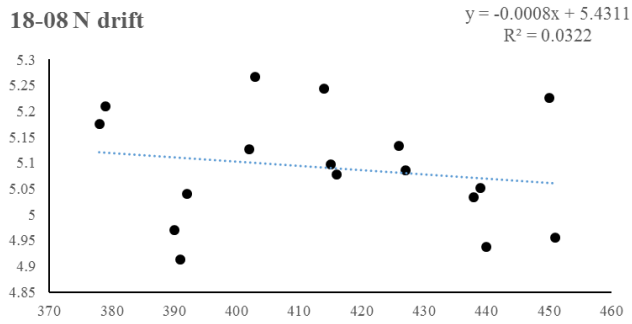
Measured Gel A values (‰) for $\delta^{13}C$ and $\delta^{15}N$ as a function of 'running number', the order in which the samples were analysed, counted cumulatively throughout the analysis. Graphs presented after removal of outliers. Regressions used in correction of data for each day of analysis.

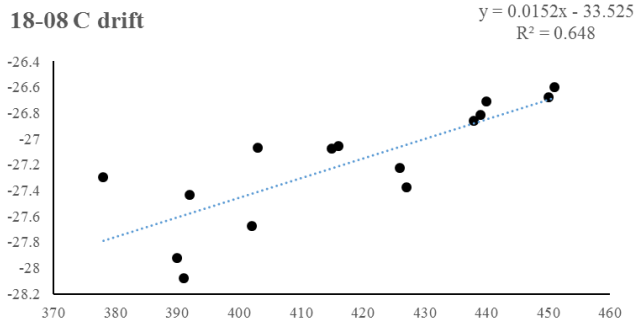












Appendix 2

Standard ellipses calculated for all groups of size categories and prey items