Chapter 6

Changing ecology of Lake Victoria cichlids and their environment: Evidence from C^{13} and N^{15} analyses

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Abstract

Eutrophication is an increasing global threat to freshwater ecosystems and the people depending on them for their livelihood. East Africa's Lake Victoria has suffered from severe eutrophication in the past decades which is partly responsible for the dramatic decline in haplochromine cichlid fish species diversity. Some zooplanktivorous and detritivorous haplochromine species recovered and shifted their diet to macroinvertebrates and small fishes. We used four formalin preserved cichlid species caught over the past 35 years to investigate whether stable isotopes of these fish are reflecting the dietary changes, habitat differences and if these isotopes can be used as indicators of eutrophication. We found that $\delta^{15}N$ signatures mainly reflected dietary shifts to larger prey in all four haplochromine species. We also observed shifts in $\delta^{13}C$ signatures (Suess corrected) that likely represent habitat differences and dietary changes. The $\delta^{13}C$ signatures tend to be heavier in haplochromines, Nile perch and *Rastrineobola argentea* caught from inshore stations compared to fish from offshore stations, indicating little horizontal dispersal of these fishes. In addition, a shift to remarkably heavy $\delta^{13}C$ signatures in 2011 was found for all four haplochromine species which might infer increased primary production and thus eutrophication although more research is needed to confirm this hypothesis. The observed temporal changes show that preserved specimens can be used to trace historic changes in fish ecology and the aquatic environment. This highlights the need for continued sampling as this information could be of essence for reconstructing and predicting the effects of environmental changes.

Introduction

Eutrophication is the enrichment of water bodies by inorganic plant nutrients (e.g. nitrate and phosphate, Lawrence *et al.* 1998). Eutrophication of freshwater ecosystems is increasingly common and is a major threat to biodiversity and to aquatic resource use by local human populations (Smith & Schindler 2009). Most eutrophication assessment methods identified increased primary production as the immediate biological response to nutrient enrichment (Ferreira *et al.* 2011); and consequently, primary productivity has been suggested to be a sensitive and accurate indicator of eutrophication (Paerl *et al.* 2003; Andersen *et al.* 2006; but see Smith 2007 and Garmendia *et al.* 2013 for exceptions). Increased primary productivity and nutrient enrichment generally result in the preferential removal and depletion of lighter ^{12}C leading to heavier $\delta^{13}C$ signatures in aquatic food chains (Schelske & Hodell 1991). Increased nitrogen pollution from runoff is reflected by heavier $\delta^{15}N$ signatures while a high N demand by primary producers can favour N-fixing cyanobacteria and consequently lighter $\delta^{15}N$ signatures (Peterson & Fry, 1987). Therefore, both carbon and nitrogen stable isotopes are sensitive to nutrient enrichment and increased primary productivity (Schelske & Hodell 1991; Cabana & Rasmussen 1996; Vander Zanden *et al.* 2005; Gu *et al.* 2006) and might therefore be useful indicators of eutrophication.

Besides being used as indicators of primary productivity and of changes in basal signatures in food webs, stable isotopes are commonly used to function as estimators of trophic position and carbon flow in aquatic ecosystems (Peterson & Fry 1987; Post 2002). The $\delta^{15}N$ signatures of consumers are typically enriched with 3-4‰ with each trophic level while the $\delta^{13}C$ signatures are similar or only slightly enriched ($\delta^{13}C$ <1‰) with each trophic level (Peterson & Fry 1987; Vander Zanden & Rasmussen 2001). Stable isotopes can also provide information on the habitat of aquatic species. In general, limnetic phytoplankton photosynthesis results in light $\delta^{13}C$ signatures whereas heavier $\delta^{13}C$ signatures are caused by benthic algae photosynthesizing within a boundary layer (France 1995; Hecky & Hesslein 1995). This phenomenon makes it possible to infer whether the prey of primary consumers has a benthic, littoral or limnetic origin (Hecky & Hesslein, 1995; Vander Zanden & Rasmussen 1999). Stable isotopes of primary consumers are also related to the habitat gradient, with light $\delta^{13}C$ and heavy $\delta^{15}N$ signatures in profundal habitats and vice versa in littoral habitats (Vander Zanden & Rasmussen 1999).

Lake Victoria has suffered from severe eutrophication in the past decades, and the shallow, inshore habitats especially have high algal biomasses and a high carbon demand by photosynthesis (Ramlal *et al.* 2001; Hecky *et al.* 2010). Based on paleolimnological analyses, changes in lower food web organisms began as early as the 1940s but accelerated dramatically through the 1960s and 1970s (Verschuren *et al.* 2002; Hecky *et al.* 2010). From the 1980s onwards, several studies showed increased nitrogen and phosphorous loadings in the lake, and these coincided with decreased water transparency and decreased oxygen levels (Mugidde 1993; Hecky *et al.* 1994, 2010; Seehausen *et al.* 1997a; Verschuren *et al.* 2002; Chapter 5 in this thesis). Most of the studies reporting eutrophication focussed on the northern part of the lake (Hecky 1993; Mugidde 1993; Mugidde *et al.* 2003); and because of the lack of regular and consistent measurements of biological productivity, paleolimnological analysis was used to provide more continuous

analysis of historical changes in ecosystem. For the Mwanza Gulf, located in the southern part of the lake, even fewer data on productivity are available (Akiyama *et al.* 1977; Shayo *et al.* 2011; Cornelissen *et al.* 2013), although other environmental variables such as dissolved oxygen (DO) levels and Secchi depth data have been measured on a fairly regular basis in the last four decades (Chapter 5 in this thesis). In addition, the Lake Victoria biodiversity crisis has been well documented for the Mwanza Gulf from the 1970s onwards (Witte *et al.* 2007). In the 1980s, populations of the introduced Nile perch, *Lates niloticus*, boomed. Together with eutrophication, this boom resulted in a major decline of cichlid species because the Nile perch is predatory on cichlids (Witte *et al.* 1992a; Seehausen *et al.* 1997a; Goudswaard *et al.* 2008). During the 1990s, the population size of some cichlid species, especially zooplanktivores and detritivores, recovered (Seehausen *et al.* 1997b; Witte *et al.* 2007; Kishe-Machumu 2012) and shifted their diet towards macroinvertebrates and to fish (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Chapter 4 in this thesis). Based on formalin-fixed, ethanol preserved cichlid specimens collected over the past 35 years, we demonstrated that the recovered species showed morphological changes that we hypothesise to be adaptive responses to the environmental changes (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]; Chapter 4 in this thesis).

Here we use these same unique cichlid museum specimens caught at a three year time intervals from 1978 onwards, to test how the environmental and ecological changes might be reflected in the C and N stable isotopes of these fish and if they can be used as indicators of eutrophication. In addition, we investigated whether habitat and seasonal changes were reflected in these isotopes as not all fish were caught at the exact same location and period at the research transect. Kishe-Machumu (2012) showed that formalin fixation and ethanol storage had a small but consistent effect on the stable isotopes of cichlids. However, since all fish were preserved the same way, we assume that preservation effects will not influence our results.

For this study, we used two closely related zooplanktivorous species (we give abbreviations of the species names in parentheses); *Haplochromis pyrrhocephalus (pyr)*, *H. laparogramma (lap)*, the zooplankti/insectivorous species *H. tanaos (tan)* and the mollusci/detritivorous species *Platytaeniodus degeni (deg)*.

Dietary gut content analyses revealed that the species *pyr* and *lap* shifted their diet towards large macroinvertebrates such as insects, shrimps and molluscs, and to fishes during the 1990s. This diet partly changed back to zooplankton during the 2000s (Katunzi *et al.* 2003; Kishe-Machumu 2012; Chapter 4 in this thesis). The species *tan* and *deg* both showed the most pronounced diet changes towards macroinvertebrates and fish during the 2000s (Van Oijen & Witte 1996; Chapter 4 in this thesis).

There are no substantial changes over time in the sedimentary $\delta^{15}N$ of the lake based on results from three sediment cores from various locations (R. E. Hecky, unpublished data). Therefore, we expect the dietary changes to be reflected in the $\delta^{15}N$ signatures of the cichlids as was found by Kishe-Machumu (2012). However, the response of $\delta^{15}N$ in fish muscle could be more complex as shifts in basal signature in phytoplankton will be additive to possible shifts in diet to prey which might be lighter or heavier in $\delta^{15}N$. The eutrophication of the lake coincided with the increase of primary productivity, composition,

and abundance of phytoplankton in the northern part (Hecky 1993; Verschuren *et al.* 2002) as well as in the southern part of the lake (Cornelissen *et al.* 2013). The shift from diatoms to cyanobacterial phytoplankton dominance was accompanied with an increase of 2‰ in the $\delta^{13}C$ (Suess corrected) of organic matter (Hecky *et al.* 2010). This probably occurred as the higher biomass of filamentous and colonial cyanobacteria raised the demand for CO_2 relative to availability in this soft water lake (Ramlal *et al.* 2001) and also may have decreased isotopic fractionation by boundary layer effects in the larger filamentous and colonial cyanobacteria (Hecky & Hesslein 1995). Therefore, we expect $\delta^{13}C$ signatures may have shifted towards heavier values in these cichlids even without shifts in their diets, especially in inshore habitats. In any case, we hypothesized that changes in environment and trophic behaviour may be evident in the fish isotopic composition for the historic collection of haplochromine fishes from Mwanza Gulf.

Materials and methods

Fish collection

Most fishes were collected from a research transect in the northern part of the Mwanza Gulf (6-14m) on the southern coast of Lake Victoria. Fishes were caught with a bottom trawler during the period 1978-2011. The species *pyr* and *lap* were mainly caught above mud at station G (12-14m) of the transect. Selected *pyr* specimens from 1987 were from Luanso Bay (Goldschmidt *et al.* 1993), a shallow bay (3-4m) 10 kilometres south of the transect, as no *pyr* specimens caught on the transect in 1987 were preserved. The species *tan* and *deg* were mainly caught at sand/mud bottoms (Butimba and Kissenda Bay) at the opposite ends of the transect (Figure 1.1). Fishes were fixed and preserved in 4% formaldehyde (buffered with borax) and after shipment to Leiden transferred to 70% ethanol and stored at the Naturalis Biodiversity Center. A total of 273 male specimens (eight fish per year per species on average) were selected from the years 1978, 1981, 1984, 1987, 1991, 1993, 1999, 2001-02, 2006, and 2011 which is a selection from the same specimens used by van Rijssel & Witte (2013); see also Chapter 3 and Table 6.1 in this thesis.

Table 6.1 Catch locations and number of specimens per species per year.

Year	*H. laparogramma*	N	*H. pyrrhocephalus*	N	*H. tanaos*	N	*P. degeni*	N
1978	Transect	5	Transect	5	BB, NB	5	BB	5
1981	G	5	G	5	BB	5	BB, K	5
1984	G	10	G	10			BB	10
1987	G	10	Luanso Bay	10			BB, Transect	4
1991	J, P	10	J, P	10				
1993	G, H, I	10	H, I	10	I, J, K	4		
1999	Transect	6	Transect	10				
2001-2002	G, J	10	G	10	J, BB	10	J	10
2006	F-J	10	G	10	E	10	J	10
2011	F, H-J, south of G	10	F	10	J	10	F, J, K	9
Total		**86**		**90**		**44**		**53**

E-K, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; NB, Nyegezi Bay; Transect, unknown station along the transect. The location of Python Island, Nyamatala Island and Luanso Bay are indicated on maps found in Bouton *et al.* (2002b), Witte *et al.* (1992b) and Goldschmidt *et al.* (1993), respectively.

Stable isotope analysis

From each fish, the right side of the epaxial muscle located dorsal of the lateral line was dissected after removal of the skin. These muscle tissue samples were then freeze-dried for 72 hours and grounded into fine powder with a pestle in an Eppendorf tube. A subsample of 1.25 mg was placed into tin cups and shipped to the University of California Davis Stable Isotope Facility for analysis. Stable isotope analysis of ^{13}C and ^{15}N was carried out with a PDZ Europa Automated Nitrogen Carbon Analyzer-Gas Solids and Liquids (ANCA-GSL) elemental analyzer interfaced to a PDZ Europa 20-20 continuous flow Isotope Ratio Mass Spectrometer (IRMS). The $\delta^{13}C$ and $\delta^{15}N$ values were expressed relative to international reference standards V-PDB (Vienna PeeDee Belemnite) and air respectively. The difference (δ) in isotopic ratio between the sample and standards was calculated as:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample} - R_{standard})/ (R_{standard}) \times 1000$$

where $R= {}^{13}CO_2 / {}^{12}CO_2$ for $\delta^{13}C$ or $R= {}^{15}N_2 / {}^{14}N_2$ for $\delta^{15}N$
and values are expressed as ‰.

Glutamic acid, nylon and bovine liver which were similar in composition as the samples being used, were used as standards. These standards were previously calibrated against National Institute of Standard Technology (NIST) Standard Reference Materials such as International Atomic Energy Agency (IAEA-N1,-N2, -N3), USGS-40 (light carbon and nitrogen isotopes in L-glutamic acid) and USGS-41 (heavy carbon and nitrogen isotopes in L-glutamic acid).

Due to deforestation and fossil fuel burning which is naturally depleted in $\delta^{13}C$, atmospheric CO_2 levels have been increasing while $\delta^{13}C$ of CO_2 has declined, especially over the past 35 years (Francey *et al.* 1999). This decrease in $\delta^{13}C$ of atmospheric CO_2 due to anthropogenic perturbations is known as the Suess effect (Keeling 1979) and has been most severe as the present day is approached (Verburg 2007). As atmospheric and aquatic CO_2 equilibrate, it was necessary to apply a Suess correction in order to compare $\delta^{13}C$ signatures of fish collected over the last 35 years according to the following formula:

$$7.7738118 * 10^{-16} * Y^6 - 1.2222044 * 10^{-11} * Y^5 + 7.1612441 * 10^{-8} * Y^4 - 2.1017147 * 10^{-4} * Y^3 + 3.3316112 * 10^{-1} * Y^2 - 273.715025 * Y + 91703.261,$$

with Y as year since 1700, as recommended by Verburg (2007). The Suess correction was subtracted from $\delta^{13}C$ values of the years 1981-2011 with the smallest correction for 1981 (-0.07‰) and the largest correction for 2011 (-1.09‰).

Statistical analysis

Differences in stable isotopes over time were tested with a One-way ANOVA and *P*-values were corrected with a sequential Bonferroni test. To test if standard length (SL) influences the stable isotopes of the fish, a Pearson correlation test was used after testing for normality with a Shapiro-Wilk test. In three out of four species (*lap*, *pyr* & *tan*), only five significant correlations between $\delta^{13}C$ and SL were found within a year. There were no significant

correlations between $\delta^{15}N$ and SL within years. Because there was no consistent trend and the correlations occurred in both positive and negative direction within a species, we decided not to correct for SL (Appendix Table 6.1). To test if the number of different catch locations per year influenced the variation in stable isotopes, we correlated this number with the standard deviation (st. dev.) of the stable isotopes per year. We applied the same method to test for seasonal effects by correlating the number of catch dates with standard variation of the stable isotopes. All statistical tests were performed with SPSS version 20.

Results

All four species showed significant changes over time in $\delta^{13}C$ ($P < 0.001$) and $\delta^{15}N$ ($P < 0.01$; Figures 6.1, 6.2). The two zooplanktivorous species (*pyr* and *lap*) showed shifts towards lighter $\delta^{13}C$ and heavier $\delta^{15}N$ during the 1990s compared to 1978 ($P < 0.05$). The species *tan* and *deg* both showed heavier $\delta^{15}N$ values in the 2000s and *deg* showed a shift towards heavy $\delta^{13}C$ values during that period. In 2011, three out of four species (*pyr*, *lap* and *tan*) shifted towards lighter $\delta^{15}N$ values ($P < 0.05$) while the $\delta^{13}C$ shifted to remarkably heavy values in all four species ($P < 0.001$; Figures 6.1, 6.2).

Haplochromis laparogramma

The $\delta^{13}C$ shifted to lighter values in 1981 compared to 1978 (though not significant, $P = 0.072$) and then in 1984 shifted back to heavier values ($P < 0.001$), similar to those of 1978. In 1987, there was again a shift towards lighter $\delta^{13}C$ values compared to 1984 ($P < 0.001$) which did not change significantly again until 1999. There was a significant shift towards heavier $\delta^{13}C$ values in the years 2006 and 2011 compared to all other years ($P < 0.001$, Figures 6.1A, 6.2A).

The $\delta^{15}N$ values increased in 1981 compared to 1978 ($P = 0.015$, not significant after sequential Bonferroni correction) but these values decreased again in 1984 ($P = 0.025$, not significant after sequential Bonferroni correction). The $\delta^{15}N$ values decreased even further in 1987 which gave the lowest $\delta^{15}N$ values compared to all other years ($P < 0.001$). All years in the period 1993-2006 showed significant higher $\delta^{15}N$ values compared to 1978 and 1984 ($P < 0.01$). The $\delta^{15}N$ values in the year 2011 did not differ from the $\delta^{15}N$ values in the period 1978-1984 (Figure 6.1A, 6.2A).

Haplochromis pyrrhocephalus

The $\delta^{13}C$ shifted towards lighter values in 1981 and 1984 ($P < 0.01$) and then shifted back to heavier values in 1987, comparable to those of 1978. It must be noted that specimens of *pyr* caught in 1987 came from the Luanso Bay, a shallow bay (3-4m) 10 kilometres south of the transect which might be the cause of the relatively heavy $\delta^{13}C$ values. During the period 1991-2001, there is again a significant shift towards lighter isotopes compared to 1984 ($P < 0.05$). In 2006, the $\delta^{13}C$ shifts to heavier values similar to those of 1984 and just as in *lap*, the $\delta^{13}C$ values of 2011 were the heaviest compared to all other years ($P < 0.001$, Figures 6.1B, 6.2B).

The $\delta^{15}N$ values increased in 1981 as well as in 1984 ($P < 0.001$) compared to 1978. As in *lap*, there was a decrease in 1987 in $\delta^{15}N$ values compared to 1984 ($P < 0.001$). The $\delta^{15}N$ values increased again in the period 1993-2006 compared to 1984, as was seen for *lap*. In 2011, the $\delta^{15}N$ values decreased to the level of 1981 and 1984 but $\delta^{15}N$ values were still significantly higher than in 1978 ($P < 0.001$, Figures 6.1B, 6.2B).

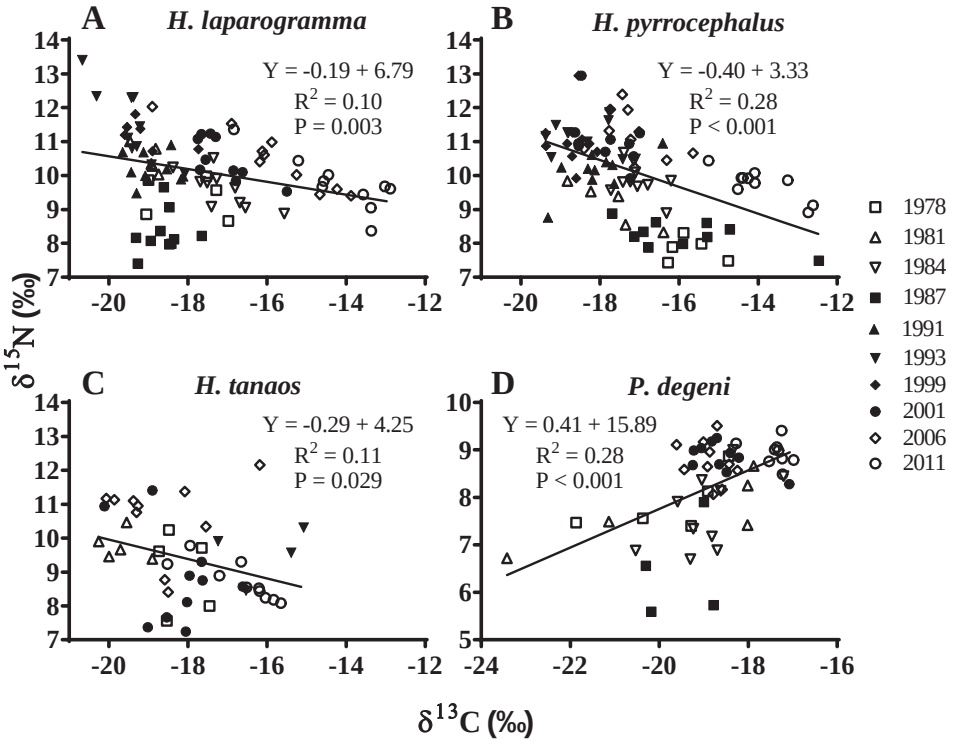

Figure 6.1 The Suess corrected $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of the four cichlid species (A) *H. laparogramma*, (B) *H. pyrrhocephalus*, (C) *H. tanaos* and (D) *P. degeni* per year. Linear regression lines, their slopes, R-squared and *P*-values are depicted for each species as a whole.

Haplochromis tanaos

As seen in *lap* and *pyr*, the $\delta^{13}C$ shifted towards lighter values in 1981 ($P = 0.016$, not significant after sequential Bonferroni correction). In 1993 (no data available for 1984 and 1987), there is a shift to heavier $\delta^{13}C$ values compared to 1978 and 1981 ($P < 0.01$). In 2001 and 2006, there is a shift back to lighter $\delta^{13}C$ isotopes ($P < 0.001$) similar to those of 1978. As was found in *lap* and *pyr*, the $\delta^{13}C$ values of 2011 were the heaviest compared to all other years ($P < 0.01$) but do not differ significantly from $\delta^{13}C$ values in 1993 (Figures 6.1C, 6.2C). The $\delta^{15}N$ values only increased in 2006 and these were significantly higher than $\delta^{15}N$ values from 1978, 2001 and 2011 (Figures 6.1C, 6.2C).

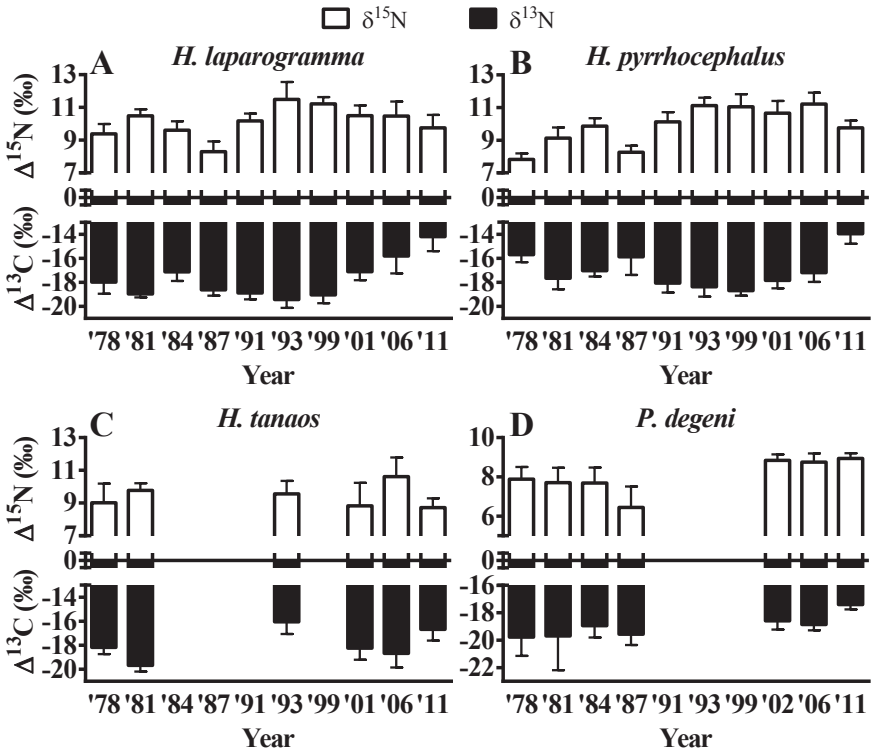

Figure 6.2. The average and standard deviation of Suess corrected $\delta^{13}C$ and $\delta^{15}N$ stable isotopes of the four cichlid species (A) *H. laparogramma*, (B) *H. pyrrhocephalus*, (C) *H. tanaos* and (D) *P. degeni* per year. The sample size of 1978 and 1981 of all species was 5, the sample size of all other years was 10, except for *H. laparogramma* 1999 (N = 6) and *H. tanaos* 1993 (N = 4).

Platytaeniodus degeni

The $\delta^{13}C$ values do not differ from each other during the period 1978-1987. There is a shift to heavier $\delta^{13}C$ from 1978 to 2002 ($P = 0.038$, not significant after Bonferroni correction). The $\delta^{13}C$ values of 2006 do not differ from other years except for 2011 which has, like in the other three species, the heaviest $\delta^{13}C$ values compared to all other years ($P < 0.05$, Figures 6.1D, 6.2D).

The $\delta^{15}N$ values do not differ in the years 1978-1984 and, as was found for *lap* and *pyr*, 1987 had the lowest $\delta^{15}N$ compared to all other years ($P < 0.01$). The years 2002-2011 did not differ from each other and all had significantly higher values compared to the years 1978-1987 ($P < 0.01$, Figures 6.1D, 6.2D).

Effect of catch location

Fish from multiple catch locations showed a higher within-year variation in $\delta^{13}C$ than fish caught in years with fewer catch locations (four different species combined, Spearman correlation, r = 0.422, *P* = 0.014). Since each catch location had a different depth, this means that fish caught in years with multiple catch locations were also caught from different depths. All four species showed positive (mostly non-significant) correlations between the number of catch locations per year and the st. dev. of $\delta^{13}C$. There was one significant correlation for *lap* (r = 0.851, *P* = 0.002) and an almost significant correlation for *deg* (r = 0.732, *P* = 0.061) between the number of catch locations per year and the st. dev. of $\delta^{13}C$ (Table 6.2).

The relation between the number of catch locations and the amount of within-year variation in $\delta^{15}N$ was less clear and showed no significant correlations. The number of catch locations per year showed a nearly significant positive correlation with the st. dev. of $\delta^{15}N$ for *lap* but a nearly significant negative correlation for *pyr* (Table 6.2).

Table 6.2 Pearson correlations per species between the number of catch locations, catch dates per year and the st. dev. of $\delta^{13}C$, $\delta^{15}N$. Significant values are depicted in bold.

Species	Number of catch locations / dates	N	$\delta^{13}C$		$\delta^{15}N$	
			r	***P***	**r**	***P***
H. laparogramma	Locations	10	**0.851**	**0.002**	0.597	0.068
	Dates	10	0.574	0.083	0.524	0.12
H. pyrrhocephalus	Locations*	10	0.055	0.879	-0.624	0.054
	Dates	10	-0.129	0.723	0.203	0.573
H. tanaos	Locations	6	0.147	0.781	0.514	0.297
	Dates	6	0.297	0.568	-0.037	0.945
P. degeni	Locations	7	0.732	0.061	0.285	0.536
	Dates	7	**0.770**	**0.043**	0.605	0.15

* Indicates Spearman correlations.

Effect of catch date

There was a significant positive correlation between the number of catch dates and the st. dev of $\delta^{13}C$ per year for *deg* (r = 0.77, *P* = 0.043) and an almost significant positive correlation for *lap* (r = 0.574, *P* = 0.083). There were no significant correlations between the st. dev. of $\delta^{15}N$ and the number of catch dates per year (Table 6.2).

Discussion

Stable isotope changes through time

This study shows how dietary shifts are reflected in the stable isotopes of formalin fixed Lake Victoria cichlids. The increase of $\delta^{15}N$ values through time of all four species concurs with the reported shift in diet to larger prey for all four species.

Although the species shifted their diet already in 1987 (Chapter 4 in this thesis), there was no increase but a decrease in $\delta^{15}N$ values in that year. Stomach and gut content analysis

revealed that the diet of the zooplanktivores consisted for a large part of detritivorous shrimps and detritus (Chapter 4 in this thesis), which explains the low $\delta^{15}N$ values. Campbell *et al.* (2003a) reported that *Caridina* (shrimps) had substantially lower $\delta^{15}N$ and $\delta^{13}C$ than zooplankton in Napoleon Gulf in northern Lake Victoria which is in agreement with our results. Though stomach and gut contents were not analysed for *deg* in 1987, based on their low $\delta^{15}N$ values and the dramatic increase of shrimps in the Mwanza Gulf during that time (Goudswaard *et al.* 2006; Chapter 4 in this thesis), it is likely that this species had shifted to a diet similar to that of the zooplanktivores.

Based on stomach and gut content analysis, the species *tan* shifted its diet in 1993 from zooplankton and insects to mainly insects and fish. Although higher $\delta^{15}N$ values would be expected with a shift to larger prey, *tan* already had quite a high volume percentage of insects in their diet (8% chironomids, 5% *Chaoborus* larvae and 24% insects) before the environmental changes. Moreover, the aquatic insects and especially the decapod crustacean *Caridina* in Lake Victoria generally have lower $\delta^{15}N$ values than zooplankton, although there are exceptions among the insects (Campbell *et al.* 2003a; Ojwang *et al.* 2004) which might explain the lack of $\delta^{15}N$ increase in 1993 for *tan*. In 2006, *tan* included even more fish in their diet than in 1993 (Van Oijen & Witte 1996; Kishe-Machumu 2012; Chapter 4 in this thesis) which is reflected in the increase of $\delta^{15}N$ values as well.

More consumption of aquatic insects might also explain the lower $\delta^{15}N$ values of *pyr* compared to the closely related species *lap* from before the environmental changes. *Haplochromis laparogramma* was almost exclusively feeding on zooplankton during that time and *pyr* already included some chironomid larvae and insects next to their main prey zooplankton (Kishe-Machumu 2012; Chapter 4 in this thesis) which might have lowered their $\delta^{15}N$ values.

For one species (*lap*), we were able to perform a Pearson correlation test on the dietary contents with the stable isotopes from the same fish. However, none of the averaged volume percentages of the different food types (zooplankton, phytoplankton, detritus, insects, shrimps or fish) gave a significant correlation with $\delta^{13}C$ or $\delta^{15}N$ through time. The lack of correlation can be caused by three factors: 1) these fishes seem to be quite opportunistic regarding their food types. The studied species shifted their diet from mainly small prey (zooplankton/detritus) to a highly diverse diet containing multiple food types such as insects, fish, shrimps, detritus and phytoplankton at the time that large macroinvertebrate numbers increased in their environment (Chapter 4 in this thesis). These lower food web organisms show a high variability in their stable isotope signatures (Campbell *et al.* 2003a) which is reflected in the stable isotopes of the fish; 2) the stomach and gut contents only reflect what the fishes has been eating that day (or night) and do not always have to reflect fish's diet on the long term; 3) meteorological variability seems to be affecting the mixing depths of the Mwanza Gulf (Chapter 5 in this thesis) which have an effect on the $\delta^{13}C$ of particulate organic matter (POM) and fish and therefore interfere with stable isotope-food relationships. These three factors make direct dietary-stable isotope correlations hard to detect in these species.

Geographical variation

A larger number of catch locations correlated with a higher $\delta^{13}C$ variation. Unfortunately, the dataset we used did not allow us to detect a general trend in offshore and inshore isotopes (heavier $\delta^{13}C$ and lighter $\delta^{15}N$ values inshore vs. lighter $\delta^{13}C$ and heavier $\delta^{15}N$ offshore) as found by Hecky *et al.* (2010) and Mbabazi *et al.* (2010) in Lake Victoria and Lake Kyoga, respectively. However, these studies reported intra-lake variation on a large scale (from 1 to 150 km offshore) while our studied transect only covered 5km.

This intra-lake variation (Hecky *et al.* 2010) exhibits an inverse relationship between $\delta^{13}C$ and $\delta^{15}N$ for POM (Figure 6.3, Pearson correlation, r = -0.50, P = 0.002). This relationship shows that for every 1‰ increase in $\delta^{13}C$ (from offshore to inshore), the $\delta^{15}N$ decreases by 0.71‰ in POM. The species *pyr*, *lap* and *tan* seem to exhibit a similar trend with negative slopes of -0.40, -0.19 and -0.29 respectively (Figures 6.1A, B, C). But the slopes of these species are less steep than that of POM, and so geographic variation can only partly explain the shifts in stable isotope signatures if we assume that the relationship for POM lake-wide applies to Mwanza Gulf. The species *pyr* and *lap* did not extend their habitat to deeper water (as would be expected from the POM data and the decline of $\delta^{13}C$ in these species through the 1980s and 1990s) but rather they occupied shallower water (Seehausen *et al.* 1997b; Kishe-Machumu 2012). However, stomach and gut content analysis revealed a higher intake of chironomids, detritus and molluscs during the late 1980s and 1990s of both species (Katunzi *et al.* 2003; Kishe-Machumu, 2012; Chapter 4 in this thesis), indicating a more benthic feeding behaviour during this period. In contrast, the species *tan* did extend its habitat from shallow bays to deeper, open sublittoral areas. The species *deg* showed a positive slope (0.41, Figure 6.1D) which can only be explained by a shift to isotopically heavier prey as both $\delta^{13}C$ and $\delta^{15}N$ increase together from earlier to later years.

Evidence for geographic and habitat variation in stable isotope signatures was also found for the zooplanktivorous cyprinid *Rastrineobola argentea* (dagaa) and the carnivorous Nile perch as well (caught on the same research transect as our study, Table 6.3). Both *R. argentea* and the Nile perch showed consistently lighter $\delta^{13}C$ values at the offshore, 12-14m deep station G compared to the shallower stations E (6-8m) and J (4-6m), which lie at the opposite ends of the transect (Figure 1.1). Nile perch showed significantly lighter $\delta^{13}C$ values at the deeper station G (ANOVA, $P < 0.05$) compared to shallower stations E and J. The $\delta^{15}N$ values were also lighter at station G than at station E (P = 0.024) and J (P = 0.08) which cannot be explained by a shift in basal signatures expected from the POM relationship (Figure 6.3), but rather must imply feeding at a higher trophic level in Nile perch occupying shallower waters. Though the small sample size did not permit us to do statistics for *R. argentea*, $\delta^{13}C$ values show a pattern consistent with the pattern found for Nile perch with lighter $\delta^{13}C$ values at the deeper station G compared to heavier values at the shallower stations E and J. But unlike Nile perch, the $\delta^{15}N$ values were heavier for the deeper station G compared to the shallower station E and J (Table 6.3). *Rastrineobola argentea* isotopes show the same geographic trend on the relatively small research transect as the POM isotopes show on a lake-wide scale. The $\delta^{13}C$ values increase from station G to J with 3.8‰ while $\delta^{15}N$ decreases with 2.4‰. The ratio of these two is -0.63 which is similar to the slope of the POM isotopes (-0.71).

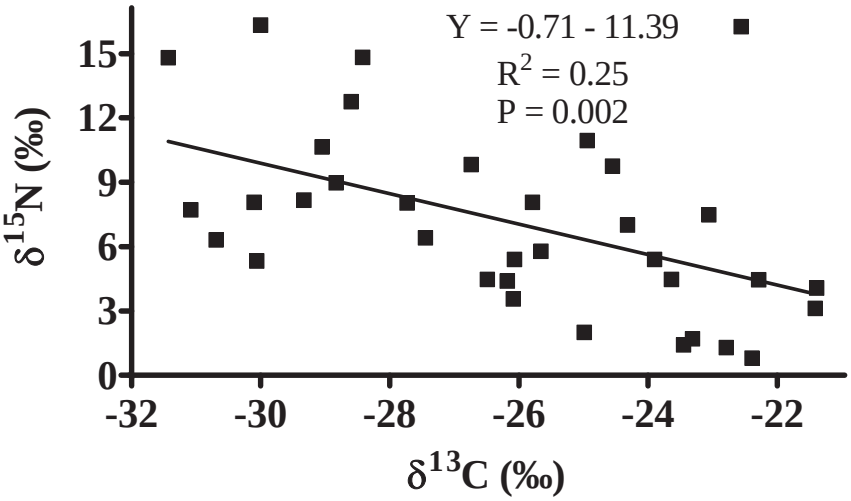

Figure 6.3 Stable isotopes of particulate organic matter (POM) collected from inshore and offshore stations along a transect from Mwanza in the south, to Port Bell in the north of Lake Victoria in October 1995; and from location V96-5MC in the middle of the lake; and from Bugaia Island in the northern part of the lake in 1995/96 (Campbell *et al.* 2003b; Hecky *et al.* 2010).

So for this species, differences between stable isotopes signatures seem to be an effect of basal signatures at base of food web per station. Nile perch isotopes, on the other hand, show an increase from station G to J of 3‰ for $\delta^{13}C$ and an increase of 1.8‰ for $\delta^{15}N$ which results in a slope of +0.6. Since this cannot be explained by changes in POM isotopes, these isotope data suggest that Nile perches are feeding on prey with a lighter isotopic composition at the deeper station G compared to the shallower stations J and E. The overall fairly low $\delta^{15}N$ values also imply that these small-sized Nile perches (<30 cm) are not entirely piscivorous yet and may feed on shrimps, insects as well as juvenile fishes (Kishe-Machumu *et al.* 2012). The relatively high $\delta^{15}N$ values of *R. argentea* suggest that these are mainly feeding on zooplankton and midge larvae (Wanink 1998).

Table 6.3 Stable isotope values of Nile perch and *R. argentea* caught at three different stations on the research transect from 25th-28th of February 2007. * Indicates significant difference of < 0.05 with values of station G (One-way ANOVA). TL, average total length.

		Nile perch				***R. argentea***			
Station	**Depth (m)**	**$\delta^{13}C$**	**$\delta^{15}N$**	**TL (cm)**	**n**	**$\delta^{13}C$**	**$\delta^{15}N$**	**TL (cm)**	**n**
J	4-6	-20.49*	8.79	19.7	4	-15.52	10.37	5.6	4
E	6-8	-21.21*	9.48*	18.9	4	-15.43	10.41	6	2
G	12-14	-23.54	6.96	15	4	-19.33	12.72	4.8	1

Although our cichlid dataset does not allow us to make within-year comparisons as depicted in Table 6.3, we suggest that a similar geographic variation in $\delta^{13}C$ isotopes might be present on such a small scale in cichlids as well. Specimens from the two closely related zooplanktivorous species *pyr* and *lap* from 1978 were caught all along the transect (specific station is unknown), while individuals of these species from 1981 were caught only at the deepest station of the transect, G (Table 6.1). Stomach and gut content analysis revealed that these fish fed mainly on zooplankton and that there was no within-species difference in volume percentages of this prey type before 1987 (Witte 1987; Chapter 4 in this thesis). This is why we consider the shift towards lighter $\delta^{13}C$ values of the two zooplanktivorous species in 1981 compared to 1978 (Figure 6.1a, b) more likely to be the result of geographic variation than a change in diet over time. The observed trend for lighter $\delta^{13}C$ values in deeper offshore water has been reported on a larger scale by Hecky *et al.* (2010). They attributed these lighter offshore $\delta^{13}C$ values to a lower offshore algal (cyanobacteria) productivity and biomass compared to inshore. Although our research transect is only 5km wide, the stable isotope data suggest that this relation might apply on a smaller scale to the Mwanza Gulf as well. This theory is supported by the findings of Kishe-Machumu *et al.* (submitted) who found heavier $\delta^{13}C$ values at the shallow station J compared to deeper stations in the Mwanza Gulf for two haplochromine cichlid species (including *H. pyrrhocephalus*).

This geographical variation in stable isotopes suggests also that the zooplanktivorous open water species used in this study have a limited dispersal between stations along the transect. It is known that many cichlid species are restricted by bottom types or depths but a virtual lack horizontal migration has not been reported for these open water species (Witte 1981; Witte *et al.* 2007). On the other hand, these fish have extended their habitat to shallower depths in the past decades indicating that there must be some horizontal migration but probably less than previously thought (Seehausen *et al.* 1997b; Kishe-Machumu 2012). More strikingly is the observation that, based on our limited data, Nile perch and *R. argentea* apparently show a similar habitat preference. These species are the most important commercial species in Lake Victoria since the 1980s (Ogutu-Ohwayo & Balirwa 2006; Tumwebaze *et al.* 2007). If these species are showing intra-specific habitat preference that results in reduced mobility, then this may have some major consequences for their fisheries and conservation aspects.

Seasonal variation

Primary producers are known to have within-year temporal variation in both $\delta^{13}C$ and $\delta^{15}N$ stable isotopes (Cabana & Rasmussen 1996; Post 2002). Enriched (heavy) $\delta^{13}C$ and decreased $\delta^{15}N$ values of primary producers and primary consumers have been reported during periods of stratification in temperate lakes, but to our knowledge not in tropical lakes (Quay *et al.* 1986; Zohary *et al.* 1994; Hodell & Schelske 1998; Caroni *et al.* 2012). In addition, larger consumers such as fish have long tissue turnover rates (months to years, Hesslein *et al.* 1993) and thus are their isotopic signatures representative of their diet for longer periods of time (Post 2002). This means that if there are seasonal differences in the lower food web, they will be hard to detect, especially with the dataset used in this study where we were limited to previously collected museum material.

The heavy $\delta^{13}C$ and light $\delta^{15}N$ values of 2011 found for *lap*, *pyr* and *tan* could be considered as being a seasonal effect as these fish were all caught during the warmer wet season when vertical stratification of the water column is more likely than in the cool dry season and this may lead to different availability of food resources. In contrast, comparison of these isotopic signatures from 2011 with stable isotope values from fishes caught during the wet season in the year 1999 shows that the latter actually had lighter $\delta^{13}C$ and higher $\delta^{15}N$ values. This leads us to believe that, based on our data, stable isotope signatures are a reflection of the fish's diet and location rather than season. In addition, so far, no seasonal variation in the diet of Lake Victoria cichlids has been reported (Van Oijen & Witte 1996; Katunzi *et al.* 2003; Kishe-Machumu *et al.* 2008; Kishe Machumu 2012; Chapter 4 in this thesis). Studies on seasonal variation of stable isotope signatures in Lake Victoria cichlids will provide definitive conclusions on this matter.

Signs of increased primary productivity?

Unexpectedly, the $\delta^{13}C$ values in the studied zooplanktivorous species shifted to lighter values during the 1990s where heavier values were expected due to increased demand for CO_2 and reduced isotopic fractionation resulting from the increased phytoplankton biomass (Hecky & Hesslein 1995; Hecky *et al.* 2010). However, during the 2000s and especially in 2011, there is a remarkable shift towards heavier $\delta^{13}C$ in all four species.

We hypothesize that this might be the result of increased primary productivity by phytoplankton and evidence for continued eutrophication of the lake. Recently, Cornelissen *et al.* (2013) found that phytoplankton productivity in 2009-2011 has increased in the Mwanza Gulf compared to the 1970s (Akiyama *et al.* 1977). The increase of primary productivity and a basal change of phytoplankton stable isotope signatures could be reflected in the $\delta^{13}C$ values of the fish when phytoplankton is (unintentionally) absorbed or ingested by the fish (or their prey), as has been found for several other fish species (especially during times of algal blooms, Christoffersen 1996; Smith *et al.* 2008). In case of the zooplanktivorous species (which again include mainly zooplankton in 2006 and 2011, Chapter 4 in this thesis), the preyed upon zooplankton (mainly copepods) should then feed upon cyanobacteria such as *Microcystis* and *Anabaena* and diatoms like *Nitzschia* which have replaced the original phytoplankton (mainly *Aulacoseira* [*Melosira*]) in the entire lake (Ochumba & Kibaara 1989; Hecky 1993; Kling *et al.* 2001; Verschuren *et al.* 2002) including the Mwanza Gulf (Sekadende *et al.* 2005; Cornelissen *et al.* 2013).

However, grazing experiments indicated that Lake Victoria's crustacean zooplankton (mainly cyclopoid copepods) do not control the cyanobacteria dominated phytoplankton biomass (Lehman & Branstrator 1993; Branstrator *et al.* 1998). In addition, other studies found cyanobacteria (*Microcystis*) to be toxic, nutritionally inadequate and suppressing feeding in copepods (Fulton & Paerl 1987; Demott & Moxter 1991; Demott *et al.* 1991).

On the other hand, there is a growing amount of evidence suggesting that copepods can grow and reproduce while feeding on toxic cyanobacteria (Koski *et al.* 2002; Reinikainen *et al.* 2002; Nascimento *et al.* 2008). In fact, several copepod species are known to (rapidly) adapt to increased cyanobacteria exposure enabling these zooplankters to feed upon the phytoplankton (Karjalainen *et al.* 2006; Colin & Dam 2007; Mariani *et al.* 2013).

Therefore, it is not improbable that the cyclopoid zooplankton (or cichlids) of the Mwanza Gulf partly feed upon the increased phytoplankton biomass that may have resulted in heavier $\delta^{13}C$ values in our fish. A recent stable isotope study on zooplankton caught in the Mwanza Gulf in the wet season of 2011 (same location and period as our fish) showed the same heavy $\delta^{13}C$ stable isotope values as for our fish (I. J. M. Cornelissen, unpubl. data), which supports the above mentioned hypothesis. Zooplankton grazing experiments on phytoplankton in the Mwanza Gulf would be needed to draw definitive conclusions.

Conclusions

Using a unique long term sampling data set, our study shows that stable isotope changes are reflecting dietary and habitat changes of four formalin fixed haplochromine species. In contrast, there does not seem to be a seasonal effect on the stable isotopes. Besides ecological changes, we suggest that the stable isotopes of these fishes might be reflecting variation in primary production and varying degrees of eutrophication over the last several decades. This would imply that these haplochromines could serve as indicators of eutrophication and could be used for eutrophication assessment methods. Our results also suggest that not only cichlids, but also Nile perch and dagaa seem to be quite stenotopic and might show less horizontal dispersal as previously thought.

The temporal variability of stable isotopes in these fishes shows that museum specimens can be used to trace historic changes in fish ecology and the aquatic environment. The reconstruction of the ecology and environment by stable isotope analysis might be applicable to other aquatic organisms as well. This highlights the need for continued sampling of fish and as well as other aquatic organisms important to fish feeding to reconstruct and predict environmental changes in aquatic ecosystems.

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Appendix Table 6.1 Pearson correlations per species and year between $\delta^{13}C$, $\delta^{15}N$ and SL. Significant values are depicted in bold.

			$\delta^{13}C$		$\delta^{15}N$	
Species	**Year**	**N**	**r**	***P***	**r**	***P***
H. laparogramma	1978	5	-0.702	0.187	0.569	0.317
	1981	5	-0.420	0.482	0.848	0.069
	1984	10	**-0.672**	**0.033**	0.444	0.198
	1987	10	**-0.690**	**0.027**	-0.017	0.963
	1991	10	-0.570	0.085	-0.203	0.574
	1993	10	0.551	0.099	-0.448	0.194
	1999	6	-0.612	0.197	0.049	0.926
	2001	10	-0.139	0.702	0.275	0.442
	2006	10	0.019	0.959	-0.066	0.856
	2011	10	0.228	0.527	0.115	0.752
H. pyrrhocephalus	1978	5	-0.489	0.403	0.511	0.379
	1981	5	-0.323	0.596	-0.063	0.920
	1984	10	0.111	0.761	0.056	0.878
	1987	10	-0.273	0.446	-0.285	0.425
	1991	10	-0.511	0.131	-0.575	0.082
	1993	10	0.060	0.869	0.187	0.605
	1999	10	-0.427	0.219	-0.515	0.128
	2001	10	**-0.729**	**0.017**	-0.489	0.151
	2006	10	-0.125	0.731	0.018	0.960
	2011	10	0.371	0.291	-0.073	0.841
H. tanaos	1978	5	-0.607	0.278	0.663	0.222
	1981	5	0.707	0.182	0.350	0.564
	1993	4	-0.668	0.332	-0.729	0.271
	2001	10	0.345	0.329	0.546	0.103
	2006	10	**0.812**	**0.004**	0.336	0.342
	2011	10	**-0.668**	**0.035**	0.221	0.539
P. degeni	1978	5	0.251	0.684	-0.093	0.882
	1981	5	0.242	0.695	-0.361	0.550
	1984	10	0.267	0.456	0.202	0.576
	1987	4	-0.634	0.366	0.218	0.782
	2001	10	0.005	0.989	-0.299	0.402
	2006	10	0.303	0.394	0.202	0.575
	2011	10	-0.281	0.463	0.346	0.361


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