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ADVANCES IN CICHLID RESEARCH II

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. 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 species diversity. However, some zooplanktivorous and detritivorous haplochromine species recovered and shifted their diet towards macro invertebrates and fish. 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. Shifts in $\delta^{13}C$ signatures likely represented habitat differences and dietary changes. In addition, a shift to remarkably heavy $\delta^{13}C$ signatures in 2011 was found for all four species which might infer increased primary production and thus eutrophication although more research is needed to confirm this hypothesis. The observed temporal changes confirm previous findings that preserved specimens can be used to trace historical 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.

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Introduction

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 recommended to be a sensitive and accurate indicator of eutrophication (Paerl et al., 2003; Andersen et al., 2006) with some exception Garmendia et al. (2013) and Smith (2007). 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 thus might 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 food webs (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 ($\delta^{13}C < 1‰$) (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 lighter $\delta^{13}C$ signatures compared to heavier $\delta^{13}C$ signatures produced 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 have shown increased nitrogen and phosphorus loadings in the lake coinciding with decreased water transparency and oxygen levels (Mugidde, 1993; Hecky et al., 1994, 2010; Seehausen et al., 1997a; Verschuren et al., 2002). The eutrophication is thought to be caused by agricultural malpractices, urbanization and deforestation, although recent studies have suggested that climatic variability and wind stress played a crucial role as well (Kolding et al., 2008; Hecky et al., 2010; van Rijssel, 2014). 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 the ecosystem.

For the Mwanza Gulf, located in the southern part of the lake, even less data on productivity are available (Akiyama et al., 1977; Shayo et al., 2011; Cornelissen et al., 2014), although other environmental variables such as dissolved oxygen levels and Secchi depth data have been measured on a fairly regular basis in the last four decades (van Rijssel, 2014). 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, the introduced Nile perch, *Lates niloticus,* population boomed. The Nile perch is thought to have contributed to the eutrophication of Lake Victoria as well by accelerating and subsidizing productivity of the ecosystem through high turnover of fish biomass and consequently more rapid recycling of

nutrients (Kolding et al., 2008). Together with the eutrophication, the Nile perch boom caused a major decline of cichlid species (Witte et al., 1992; See-hausen et al., 1997a; Goudswaard et al., 2008). However, during the 1990s, some cichlid species, especially zooplanktivores and detritivores, recovered (Seehausen et al., 1997b; Witte et al., 2007; Kishe-Machumu et al., 2015) and shifted their diet towards macroinvertebrates and fish (Van Oijen & Witte, 1996 Katunzi et al., 2003; Kishe-Machumu et al., 2008; van Rijssel et al., 2015). With the use of formalin-preserved cichlid specimens collected over the past 35 years, we demonstrated that the recovered species showed morphological adaptive responses to the environmental changes (Witte et al., 2008; Van der Meer et al., 2012; Van Rijssel & Witte, 2013; van Rijssel et al., 2015).

Here, we use these unique cichlid museum specimens selected at triennial 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 on the research transect.

For this study, we used two closely related zooplanktivorous species (abbreviations of species in parentheses) *Haplochromis* (*Yssichromis*) *pyrrhocephalus* Witte & Witte-Maas 1987 (*pyr*), *H.*(*Y.*) *laparogramma* Greenwood & Gee 1969 (*lap*), the zooplankti/insectivorous species *H. tanaos* van Oijen 1996 (*tan*) and the mollusci/detritivorous species *Platytaeniodus degeni* Boulenger 1906 (*deg*). Dietary gut content analyses revealed that the species *pyr* and *lap* shifted their diet towards large macroinvertebrates such as aquatic insects, shrimps and molluscs as well as to fish during the 1990s, but reverted their diet (partly) back to zooplankton in the 2000s which is their original diet (Katunzi et al., 2003; Kishe-Machumu, 2012; van Rijssel et al., 2015). These two species also extended their habitat to shallower waters (Seehausen et al., 1997b; Kishe-Machumu et al., 2015). The species *tan* and *deg* both showed the most pronounced diet changes towards macroinvertebrates and fish during the 2000s and have extended their habitat to deeper waters (Van Oijen & Witte, 1996; Seehausen et al., 1997b; Kishe-Machumu et al., 2015; van Rijssel et al., 2015).

There are no substantial changes over time in the sedimentary δ^{15}N of the lake based on results from three sediment cores from various locations in northern Lake Victoria (R. E. Hecky, unpubl. data). Therefore, we expect the dietary changes to be reflected in the δ^{15}N signatures of the cichlids as was found by Kishe-Machumu et al. (this issue). However, the response of δ^{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 δ^{15}N.

The eutrophication of the lake coincided with increased primary productivity, altered species composition and higher 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., 2014). The shift from diatoms to cyanobacterial phytoplankton dominance was accompanied with an increase of 2‰ in the (Suess corrected) δ^{13}C of organic matter in sediment cores (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 δ^{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 the environment and in the fish's trophic level may be evident in the isotopic composition of the historical collection of haplochromine fishes from the Mwanza Gulf.

Methods

Fish collection

Most fish were collected from a research transect in the northern part of the Mwanza Gulf (6–14 m) on the southern coast of Lake Victoria. Fish were caught with a bottom trawler during the period 1978-2011. The species *pyr* and *lap* and were mainly caught above mud at station G (12–14 m) of the transect. Selected *pyr* specimens from 1987 were from Luanso Bay (Goldschmidt et al., 1993), a shallow bay (3–4 m) 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 (Fig. 1). Fish were preserved in 4% formaldehyde (buffered with borax) and after

shipment to Leiden, The Netherlands transferred to 70% ethanol and stored at theNaturalis Biodiversity Center. Species determination and distinction occurred for each individual in the field. F. Witte was responsible for the species re-determination for
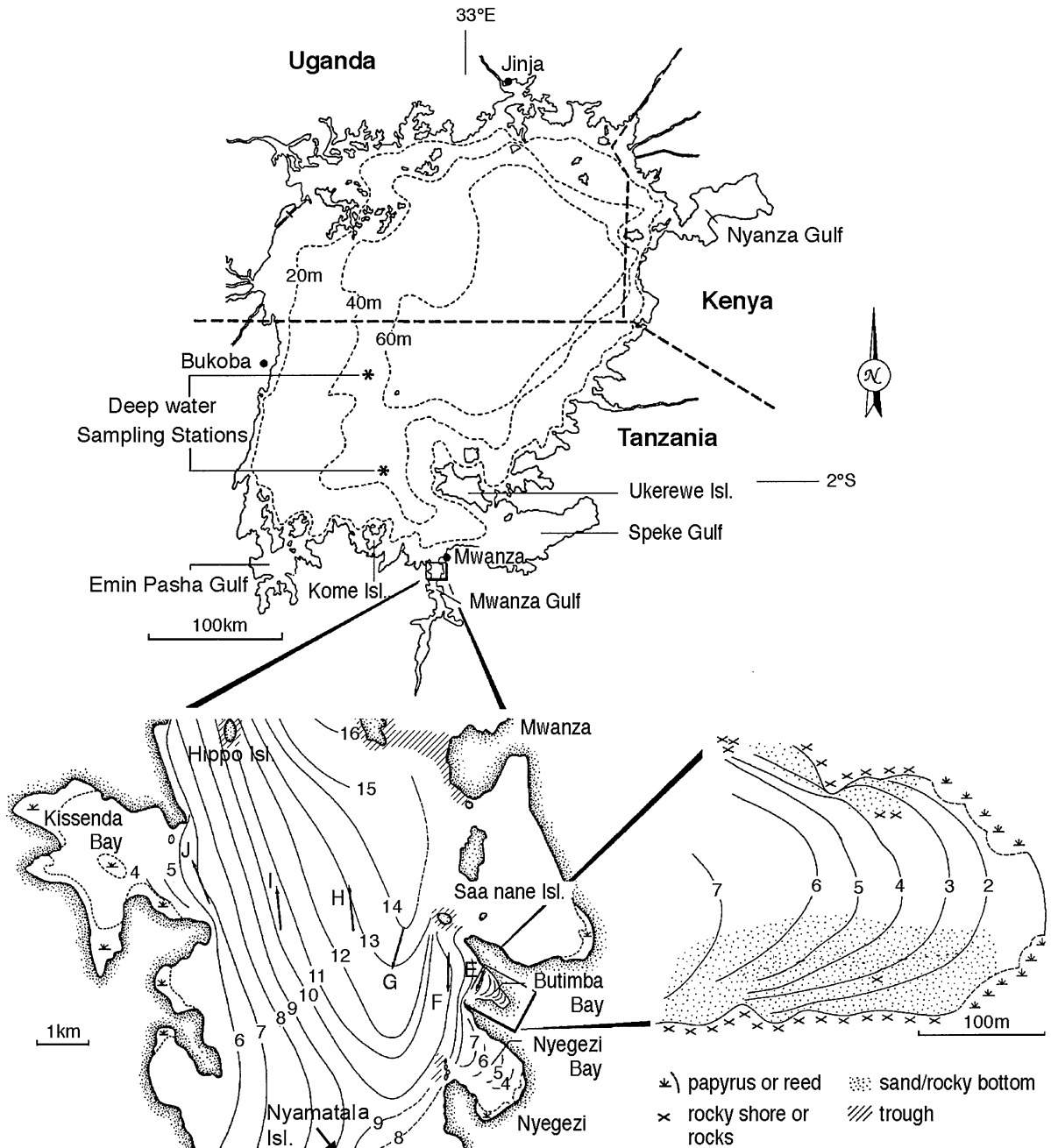

Fig. 1 Map of Lake Victoria with the sampled research transect in the Mwanza Gulf. Sampling stations are indicated with *diagonal lines*, *contour lines* are isobaths indicating depth in metres

every preserved individual. A total of 273 male specimens (eight fish per year per species on average) was 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) and van Rijssel et al. (2015) (Table S1 in Electronic Supplementary Material).

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 freeze dried for 72 h and ground into fine powder. A subsample of 1.25 mg was placed into tin cups and shipped to the UC Davis Stable Isotope Facility for analysis. Stable isotope analysis of ^{13}C and ^{15}N was carried out with a PDZ Europa 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 Pee Dee Belemnite) and air, respectively. The difference (δ) in isotopic ratio between the sample and standards was calculated as follows:

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

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 NIST Standard Reference Materials such as IAEA-N1, IAEA-N2, IAEA-N3, USGS-40 and USGS-41.

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 is most severe as the present day is approached (Verburg, 2007). As atmospheric and aquatic CO_2 equilibrate rapidly in the upper mixed layer of lakes and oceans, 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:

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

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‰).

Kishe-Machumu et al. (this issue) showed that formalin/ethanol preservation had a small but consistent effect on the stable isotopes of Lake Victoria cichlids. The preservation depleted $\delta^{13}C$ with 0.66‰ and $\delta^{15}N$ values increased on average with 0.34‰, which is consistent with directional shifts induced by formalin and ethanol preservation of fish reported in previous studies (Arrington & Winemiller, 2002; Sarakinos et al., 2002; Kelly et al., 2006; Gonzalez-Bergonzoni et al., 2015). So far, studies on long-term preservation effects of fish stable isotopes showed that these are independent of time (Kaehler & Pakhomov, 2001; Ogawa et al., 2001; Ponsard & Amlou, 1999; Sarakinos et al., 2002; Sweeting et al., 2004) which we assume is also the case for the cichlids used in this study. Although all fish of our study have been preserved, the same way over time and preservation effects are expected not to influence our results, we corrected the $\delta^{13}C$ and $\delta^{15}N$ values for preservation effects with +0.66‰ and −0.34‰, respectively (as reported by Kishe-Machumu et al., this issue) to approach more accurate stable isotope signatures.

Lipids are also known to influence $\delta^{13}C$ analyses by fractionation which results in differences between $\delta^{13}C$ values of lipids and other tissue like protein in a single organism (Deniro & Epstein, 1977; Mcconnaughey & Mcroy, 1979; Sweeting et al., 2006). Lipid content of tissue can be estimated by its C:N ratio (Mcconnaughey & Mcroy, 1979) after which a mathematical normalization technique can be applied to correct $\delta^{13}C$ values for lipid content. By plotting $\delta^{13}C$ values of each sample against its C:N ratio, we found a minor (slope = −0.02), but significant ($P < 0.001$) effect of lipids in our dataset with lower lipid contents in more recent years (Fig. S1). We

corrected the dataset for lipid content using the formula:

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times \text{ C} : \text{N}$$

which is proposed by Post et al. (2007) to use for aquatic organisms where $\delta^{13}C_{normalized}$ is the $\delta^{13}C$ value normalized for lipid content and is comparable with $\delta^{13}C$ after chemical lipid extraction. Fish samples without any lipids have a C:N ratio close to 3.0 (Kiljunen et al., 2006; Post et al., 2007). The average lipid content (C:N ratio) per sample in our study was fairly low (3.4, which is equal to 4.3% lipid), and it would therefore not be necessary to account for lipids (Post et al., 2007). Nonetheless, we corrected the samples for lipid content in order to adjust for the minor but significant trend found between $\delta^{13}C$ and the lipid content.

In addition to generating stable isotope values from preserved cichlid specimens, we used stable isotope values of particulate organic matter (POM) to test how intra-lake variation in POM would influence our results. These POM stable isotope data were collected on a transect from Mwanza to Port Bell, Lake Victoria (see Fig. 2 in Hecky et al., 2010 for sampling transect). Every 20 km, surface water samples were taken from the trans-lake ferry MV Bukoba in October 1995. Additionally, water samples were taken from location V96-5MC in the middle of the lake, from Bugaia Island in the northern part of the lake in July 1995 and April 1996 (Campbell et al., 2003a; Hecky et al., 2010)

and from offshore sites XL1, XL4, XL6-9 in May 1995 (Mugidde et al., 2003). Stable isotope signatures were derived from these samples using the methods of Hecky & Hesslein (1995) at the Freshwater Institute Laboratory (Winnipeg, Canada) and were also Suess corrected (Verburg, 2007).

Statistical analysis

We divided stable isotopes signatures into three different time periods according to van Rijssel et al. (2015). The pristine period (1978–1984), which is considered as the period before severe environmental changes; the perturbed period (1987–2002), which is considered as the period of severe environmental changes and changes in diet of the haplochromine cichlids; the recovery period (2006–2011), in which environmental changes seem less severe and some haplochromine species (partially) returned to their original diet.

To test our hypotheses on heavier $\delta^{13}C$ and $\delta^{15}N$ signatures over time, we applied circular statistics using Oriana 4.0 to quantify directional food web changes following Schmidt et al. (2007). We calculated the direction (angle of change) and length (magnitude of change) of both $\delta^{13}C$ and $\delta^{15}N$ combined per species over time (both for the three periods as for individual years). The directional change was calculated by considering the $\delta^{13}C$ and $\delta^{15}N$ values as x, y coordinates where delta y (^{15}N) was divided by delta x (^{13}C). These values were then converted into

Fig. 2 Arrow histograms of the angle of isotopic change between **a** the pristine and perturbed period, **b** the perturbed and recovery period for all four cichlid species. *Arrows* represent the direction and magnitude of isotopic change. The *straight dashed line* represents the mean vector of change; the *curved dashed line* on the rim represents the 95% confidence interval around the mean vector of change
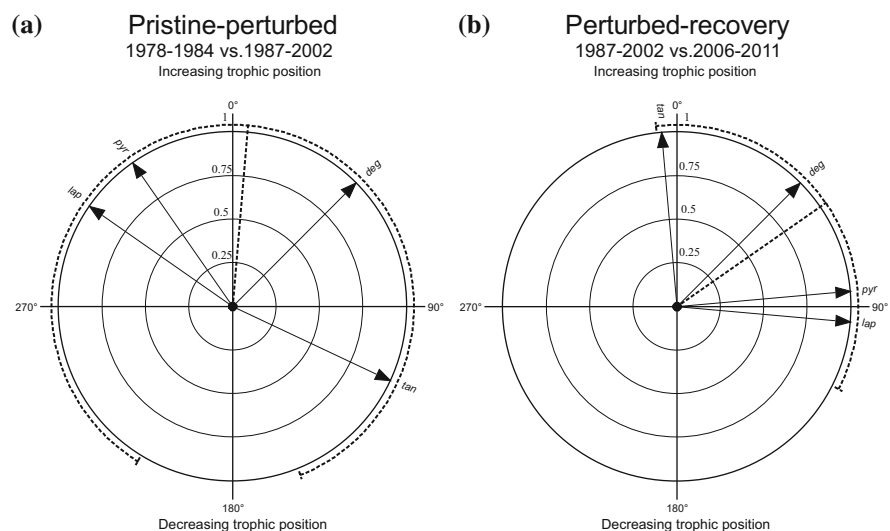
angles using the ATAN function in Excel. The direction (mean angle, μ) and the length (r) of the mean vector of all the species of the same period were calculated where (r) provides a unit of concentration of turning angles, r values close to 0 indicate that the directions are uniformly distributed, r values close to 1 indicate all angles are concentrated in the same direction. Angular standard deviation is given as measure of angular variance. We constructed arrow diagrams to visualize the direction and magnitude of each food web component. We used both Rayleigh's test as Rao's spacing test to assess whether the direction of the angles was uniformly distributed (null hypothesis).

Differences in stable isotopes per year were also tested with a One-way ANOVA. 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–Wilks test. In three out of four species (*lap*, *pyr* & *tan*), only five significant correlations between $\delta^{13}C$ and SL were found within years. 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 (Table S2).

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 (SD) of the stable isotopes per year (Table S3). We applied the same method to test for seasonal effects by correlating the number of catch dates with the SD of the stable isotopes.

Slope differences between stable isotope data of POM and preserved cichlid species were tested with an ANCOVA. All these statistical tests were performed with SPSS 20.

Results

Common isotopic responses per period

The direction of change in stable isotope signatures between the pristine and perturbed period differed between the four species (Fig. 2a; Table 1). The $\delta^{13}C$ signatures of the two zooplanktivores *pyr* and *lap* changed unexpectedly towards lighter values while $\delta^{15}N$ signatures increased as expected. The $\delta^{13}C$

signatures of species *tan* and *deg* increased to heavier values and where $\delta^{15}N$ signatures increased for *deg*, they unexpectedly decreased for *tan* (Fig. 2a). Arrow diagrams show that in the recovery period, stable isotope signatures of 3 out of 4 species (*pyr*, *lap* and *deg*) changed into the expected similar direction (Fig. 2b). This is supported by Raleigh's and Rao's spacing test which both indicate almost significant patterns of consistent change ($0.10 > P > 0.05$; Table 1) and a relatively high r (0.78). The $\delta^{13}C$ signatures of *pyr*, *lap* and *deg* increased while those of *tan* remained similar. The $\delta^{15}N$ signatures remained similar for *pyr* and *lap* while they increased for *tan* and *deg* (Fig. 2b).

Common isotopic responses per sampling year

All four species showed significant changes through time in $\delta^{13}C$ (ANOVA, $P < 0.001$) and $\delta^{15}N$ (ANOVA, $P < 0.01$; Figs. 3, 4; see Table S4 for F-statistics, degrees of freedom and P values). From 1978–1981, the three zooplanktivorous species shifted towards lighter $\delta^{13}C$ values and heavier $\delta^{15}N$ values while the species *deg* shifted in the opposite direction (Figs. 3a, 4a, b, c). From 1981 to 1984, *pyr*, *lap* and *deg* all shifted back towards heavier isotopic $\delta^{13}C$ signatures, while $\delta^{15}N$ values remained similar ($\mu = 0.86$; Rayleigh's test $P = 0.10$; Table 2, Figs. 3b, 4a, b, d). From 1984 to 1987, these three species all showed a striking drop in $\delta^{15}N$ signatures while $\delta^{13}C$ values of *lap* and *deg* remained similar and those of *pyr* shifted towards heavier values ($\mu = 0.82$; Rayleigh's test $P = 0.13$; Table 2; Figs. 3c, 4a, b, d). It must be noted that specimens of *pyr* caught in 1987 came from the Luanso Bay, a shallow bay (3–4 m) 10 kilometres south of the transect which might be the cause of the relatively heavy $\delta^{13}C$ values. In the periods 1987–1991, 1991–1993, 1993–1999 stable isotope signature of the two zooplanktivores *pyr* and *lap* both shifted towards lighter $\delta^{13}C$ values and heavier $\delta^{15}N$ values (Table 2; Figs. 3d, e, f, 4a, b) while from 1999 to 2002 a major shift towards heavier $\delta^{13}C$ and lighter $\delta^{15}N$ values occurred ($\mu = 1.0$; Rayleigh's test $P = 0.14$; Table 2; Figs. 3g, 4a, b). While the isotopic signatures of these two zooplanktivores continued to shift towards heavier $\delta^{13}C$ values from 2002 to 2006, $\delta^{15}N$ values of *tan* increased and $\delta^{13}C$ values of *deg* shifted towards lighter values (Figs. 3h, 4). From 2006 to 2011, the isotopic signatures of all four species made a major shift towards remarkably heavy $\delta^{13}C$

Table 1 Circular statistics on changes in stable isotopes between the pristine (1978–1984), perturbed (1987–2002) and recovery period (2006–2011)

Period	N	Mean vector		Circular SD	Raleigh's test		Rao's spacing test	
		Direction (μ)	Length (r)		Z	P	U	P
1978–1984 to 1987–2002	4	4.9	0.41	76.8	0.66	0.55	98.3	0.50–0.90
1987–2002 to 2006–2011	4	55.2	0.78	40.2	2.4	0.08	170.7	0.05–0.10

Direction length values (r) close to 1 indicate a common isotopic change in direction

values, while $\delta^{15}N$ values decreased for *pyr*, *lap* and *tan* during this period ($\mu = 0.95$; Rayleigh's test $P = 0.02$; Table 2, Figs. 3h, 4).

Effect of catch location

Fish from multiple catch locations showed a higher catch within year variation in $\delta^{13}C$ compared to 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 SD 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 SD of $\delta^{13}C$ (Table 3).

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 (Table 3).

The $\delta^{13}C$ and $\delta^{15}N$ values of POM exhibit an inverse relationship (Fig. 5, Pearson correlation, $r = -0.50$, $P = 0.002$) indicating intra-lake variation. 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, -0.21 and -0.30, respectively (Figs. 4a–c). An ANCOVA on the slopes of these species and that of POM showed that the slope of *lap* differed significantly ($P = 0.004$) from POM but both slopes of *pyr* and *tan* did not (Table S5). The species *deg* showed a positive slope (0.41, Fig. 4d) which differed

significantly from the slope of POM ($P < 0.001$; Table S5).

Effect of catch date

There was a significant positive correlation between the number of catch dates and the SD 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 SD of $\delta^{15}N$ and the number of catch dates per year (Table 3).

Discussion

Stable isotope changes through time

This study shows how dietary shifts are reflected in the stable isotopes of formalin-preserved 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. However, the hypothesized increase of $\delta^{13}C$ did not occur until the 2000s, while we expected it to increase at the onset of eutrophication in 1987, the start of the perturbed period.

Diet-related stable isotope changes

Although the species shifted their diet already in 1987 (van Rijssel et al., 2015), 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 (van Rijssel et al., 2015), which explains the low $\delta^{15}N$ values. Campbell et al. (2003b) reported that *Caridina* (shrimps) had substantially
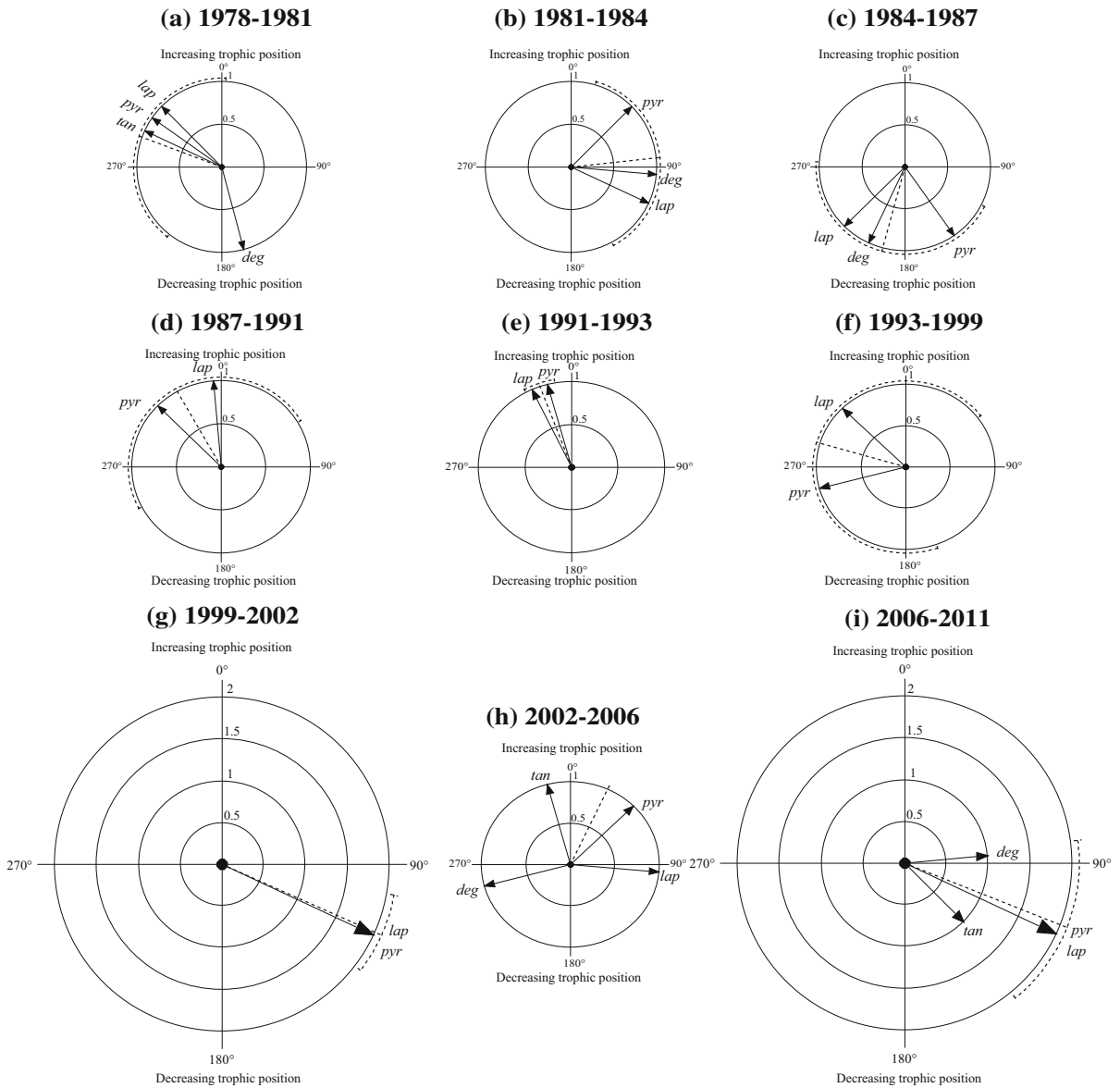
Fig. 3 Arrow histograms of the angle of isotopic change between individual years for all four cichlid fish species. *Arrows* represent the direction and magnitude of isotopic change. The *straight dashed line* represents the mean vector of change, the *curved dashed line* on the rim represents the 95% confidence interval around the mean vector of change. Note that only in **a**, **h**, **i** all four species could be included in the histogram and that axes for magnitude differ for **g** and **i**

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 that year, 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; van Rijssel et al., 2015), it is likely that

this species had shifted to a similar diet 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 (Van Oijen & Witte, 1996). Although higher $\delta^{15}N$ values might be expected with a shift to larger prey, *tan* already

Fig. 4 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 for comparison with particulate organic matter (POM) data, see discussion
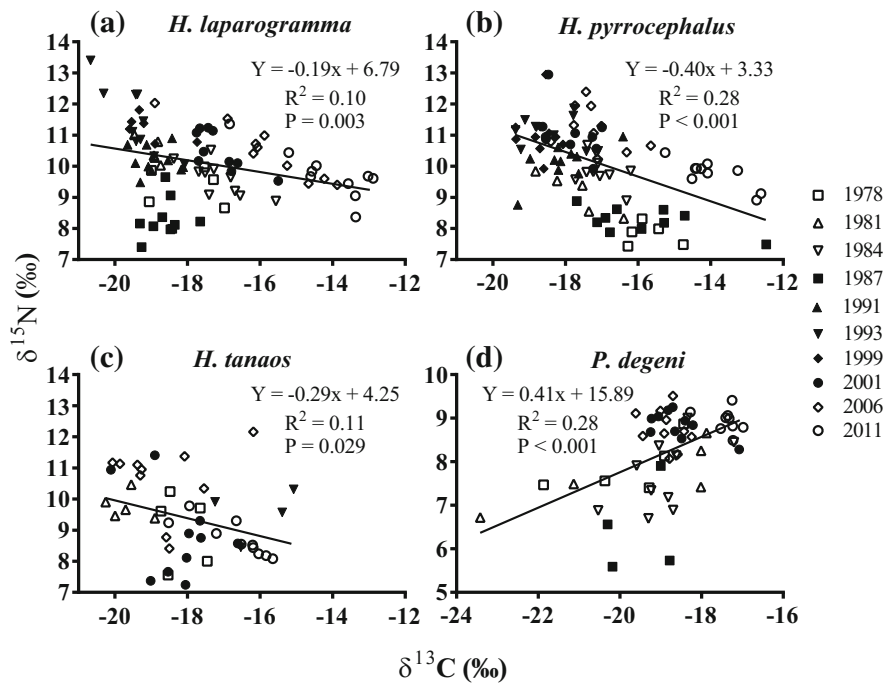

Table 2 Circular statistics on changes in stable isotopes between the individual years

Period	N	Mean vector		Circular SD	Raleigh's test		Rao's spacing test	
		Direction (μ)	Length (r)		Z	P	U	P
1978–1981	4	290.5	0.58	60.3	1.32	0.29	158.1	0.10–0.50
1981–1984	3	83.9	0.86	31.0	2.24	0.10	n.a.	n.a.
1984–1987	3	195.2	0.82	36.0	2.02	0.13	n.a.	n.a.
1987–1991	2	330.6	0.94	20.1	1.77	0.19	n.a.	n.a.
1991–1993	2	340.3	1.00	2.1	2.00	0.14	n.a.	n.a.
1993–1999	2	286.8	0.88	29.0	1.55	0.24	n.a.	n.a.
1999–2002	2	113.9	1.00	3.1	1.99	0.14	n.a.	n.a.
2002–2006	4	24.7	0.35	83.1	0.49	0.64	75.5	0.50–0.90
2006–2011	4	111.6	0.95	18.7	3.60	**0.02**	218.1	**<0.05**

Direction length values (r) close to 1 indicate a common isotopic change in direction

Significant values are depicted in bold

n.a. Indicates that a result could not be calculated due to low sample size

Table 3 Pearson correlations per species between the number of catch locations, catch dates per year and the SD of $\delta^{13}C$, $\delta^{15}N$

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 correlation

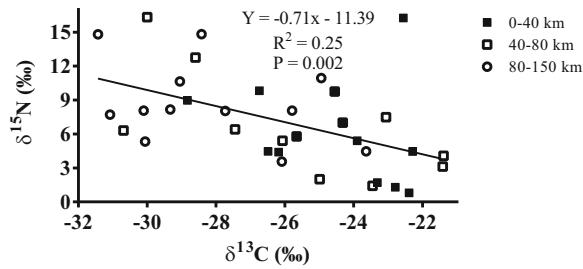Significant values are depicted in bold

Fig. 5 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, from Bugaia Island in the northern part of the lake and from several offshore sites in 1995/1996 (see Campbell et al., 2003a; Mugidde et al., 2003; Hecky et al., 2010)

included quite a high percentage of insects in their diet (8% chironomids, 5% *Chaoborus* larvae and 24% other 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., 2003b; Ojwang et al., 2004), which might explain the lack of $\delta^{15}N$ increase in 1993 (Fig. 4; Table S4). In 2006, *tan* included even more fish in their diet than in 1993 (van Rijssel et al., 2015) which is reflected in the increase of $\delta^{15}N$ values as well (Figs. 3, 4; Table S4). 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; van Rijssel et al., 2015) 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 (data from van Rijssel et al., 2015). 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 $\delta^{15}N$ in the fish through time (Table S6). The lack of correlation can be caused by three factors: (1) these fish 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 (van Rijssel et al., 2015). These lower food web organisms show a high variability in their stable isotope signatures (Campbell et al., 2003b) which might be reflected in the stable isotopes of the fish; (2) the stomach and gut contents only reflect what the fish has been eating that day (or night) and do not always have to reflect fish's diet on the long term; (3) climatic variability seems to be affecting the mixing depths of the Mwanza Gulf (van Rijssel, 2014) 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 higher number of catch locations resulted in 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 versus lighter $\delta^{13}C$ and heavier $\delta^{15}N$ offshore) as has been 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 5 km. Our results show that the slopes between $\delta^{13}C$ and $\delta^{15}N$ in three out of four species have a similar direction compared to the slope of POM (with the slopes of *pyr* and *tan* showing no significant difference with that of POM). However, the slopes of these three species are less steep than that of POM, and so geographical 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 positive slope of *deg* is in the opposite direction of the slope of POM so variation in *deg* isotopes can only be explained by a shift to isotopically heavier prey as both $\delta13C$ and $\delta15$ N increase together from earlier to later years. The species *pyr* and *lap* did not extend their habitat to deeper water (as would be expected from the POM data and from the lighter $\delta^{13}C$ signatures in these species through the 1980s and 1990s) but rather they occupied shallower

water (Seehausen et al., 1997b; Kishe-Machumu et al., 2015). 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; van Rijssel et al., 2015), 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. It is still unclear why some species have shifted from shallow to deeper waters while other species showed shifts in the opposite direction. Several causes of these habitat shifts have been suggested and most probably, a combination of these causalities has resulted in the habitat shifts. (1) A shift to the littoral habitat could be a response to heavy predation by Nile perch in the sublittoral zone; (2) the same shift could be a response to lower oxygen levels and reduced water transparency caused by increased eutrophication; (3) the shift from the littoral to the sublittoral habitat might be explained by competitive release and the opportunity to invade previously unoccupied spatial niches and; 4) ecological and morphological adaptive responses might have facilitated the habitat extension to deeper waters (Seehausen et al., 1997b; van der Meer et al., 2012; van Rijssel, 2014; Kishe-Machumu et al., 2015).

Although our dataset does not allow us to make within year comparisons, we suggest that geographical variation in δ^{13}C isotopes might be present on a small scale like our research transect in cichlids. Specimens from the two closely related zooplanktivorous species *pyr* and *lap* from 1978 were caught all along the transect while fish of these species from 1981 were caught only at the deepest station of the transect, G (Table S1). Stomach and gut content analysis revealed these fish mainly fed on zooplankton and that there was no within species difference in volume percentages of this prey type before 1987 (van Rijssel et al., 2015).This is why we consider the shift towards lighter δ^{13}C values of the two zooplanktivorous species in 1981 compared to 1978 (Figs. 3a, 4a, b) more likely to be the result of geographical variation than a change in diet over time. The observed trend for lighter δ^{13}C values in deeper offshore water has been reported on a larger scale by Hecky et al. (2010). They attributed these lighter offshore δ^{13}C values to a lower offshore algal (cyanobacteria) productivity and biomass compared to inshore. Although our research transect is only 5 km wide, the stable isotope data suggest that a

similar 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. (this issue) who found heavier δ^{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, depths or parts of the water column but a virtual lack of horizontal migration that would be required to explain our data have 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 et al., 2015).

Seasonal variation

Primary producers are known to have within year temporal variation in both δ^{13}C and δ^{15}N stable isotopes (Cabana & Rasmussen, 1996; Post, 2002). Enriched (heavy) δ^{13}C and decreased δ^{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 small 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 museum material. The heavy δ^{13}C and light δ^{15}N values of 2011 found for *lap*, *pyr* and *tan* could, in theory, 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 fish caught

during the wet season in the year 1999 shows that the latter actually had lighter δ^{13}C and higher δ^{15}N values, opposite from what one would expect if there is a strong seasonal effect. This leads us to believe that, based on our data, stable isotope signatures are a reflection of the fish's diet and habitat rather than any possible seasonal effect. In addition, so far, no seasonal variation in the diet of Lake Victoria cichlids has been reported (Katunzi et al., 2003; Van Oijen & Witte, 1996; Kishe-Machumu, 2012; Kishe-Machumu et al., 2008; van Rijssel et al., 2015). Studies on seasonal variation of stable isotope signatures in Lake Victoria cichlids would provide definitive conclusions on this matter.

Signs of increased primary productivity?

Unexpectedly, the δ^{13}C values in the studied zooplanktivorous species shifted to lighter values during the 1990s (the perturbed period) 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 δ^{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. (2014) found that phytoplankton productivity 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 δ^{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, van Rijssel et al., 2015), the preyed upon zooplankton (mainly copepods) which 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., 2014).

However, grazing experiments indicated that Lake Victoria's crustacean zooplankton (mainly cyclopoid copepods) do not control the cyanobacteria-dominated phytoplankton biomass (Branstrator et al., 1998; Lehman & Branstrator, 1993). In addition, other studies found cyanobacteria (*Microcystis*) to be toxic, nutritionally inadequate and able to suppress feeding in copepods (Demott & Moxter, 1991; Demott et al., 1991; Fulton & Paerl, 1987). On the other hand, there is a growing amount of evidence 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 δ^{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 δ^{13}C stable isotope values as for our fish (Cornelissen 2015), which supports the above-mentioned theory. Zooplankton grazing experiments on phytoplankton in the Mwanza Gulf would be needed to draw definitive conclusions.

Conclusion

With the use of a unique long-term formalin preserved sampling dataset, our study shows that stable isotope changes are reflecting dietary and habitat changes of four 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 fish 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.

The temporal variability of stable isotopes in these fishes confirms previous findings that museum specimens can be used to trace historical changes in fish ecology and the aquatic environment. 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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