Bioaccumulation of Mercury in Fish Species from the Ethiopian Rift Valley Lakes

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***Abstract*- The concentration and accumulation of total mercury (THg) in relation to size, diet and trophic position of the fish have been investigated in three fish species, i.e., *Barbus intermedius, Oreochromis niloticus* and *Clarias gariepinus*, from Lake Koka and Lake Ziway, Ethiopia. Stomach content analysis and stable isotope ratios of nitrogen (δ^{15}N, ‰) and carbon (δ^{13}C, ‰) were used to determine the diet and the trophic position of the fish. The fish species studied represented different trophic positions in the food chain of the lakes. The THg concentrations in each of the three fish species, *B. intermedius, C. gariepinus,* and *O. niloticus,* ranged between non-detectable (ND) and 0.9 mg kg^{-1} (ww) in Lake Koka, and between ND and 0.5 mg kg^{-1} (ww) in Lake Ziway, respectively. Significantly a higher concentration of THg was found in *B. intermedius* than in *C. gariepinus* and *O. niloticus,* in Lake Koka, whereas in Lake Ziway, *B. intermedius* had significantly higher THg concentrations than *O. niloticus* only. Species variation in total THg accumulation is attributed to size, diet and trophic position of the fish.**

Keywords*- *Fish Species; Rift Valley Lakes; Trophic Position; Hg

I. INTRODUCTION

Mercury (Hg) exists in the environment in different chemical forms: elemental (metallic), inorganic, and organic forms. Inorganic Hg is converted to MeHg, presumably by sulfate reducing bacteria which usually live in sediments [1, 2]. The ecological and toxicological effects of mercury depend on the chemical species present [3]. Generally, organic mercury forms are more toxic to aquatic organisms and birds than the inorganic forms [4]. It is also documented that virtually all 50–98% of the total Hg (THg) in the edible tissue of fish is in the form of MeHg [5, 6]. The key factor dictating total concentration of Hg in the biota (e.g. fish) is the MeHg concentration in water [7], and the important sources are precipitation, runoff from wetlands and in-lake methylation [8, 9]. Hg can accumulate to elevated levels in the biota even in remote areas which are devoid of local industrial and natural sources; due to long-range atmospheric transport followed by deposition [10]. Local factors such as the presence of hot springs around lakes is also thought to account for high Hg concentrations in the waters of Ethiopian Rift Valley soda lakes [11]. It is also important to take into account biological factors such as the food web structure, and age of the fish to explain the behaviour of Hg, including bioaccumulation and biomagnification. The concentration of Hg biomagnifies along the food chain i.e. it increases with trophic position in the food chain [12, 13, 14]. The trophic position of aquatic organisms in a food chain or food web may be assessed using the δ^{15}N value, which usually correlates with the Hg concentrations [15, 16]. The objective of the present study was therefore to determine the level of THg and to assess the influence of size, diet and trophic position of the fish on key fish species: Big barb (*Barbus intermedius*), African sharp tooth (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) from Lake Koka and Lake Ziway.

II. MATERIALS AND METHODS

A. Study Area

Lake Koka and Lake Ziway are part of the Ethiopian Rift Valley Lakes (ERVLs) (Figure 1). Lake Koka, situated at 8^{o}24’12.66’’N; 39^{o}05’31.50’’E, is a dammed hydroelectric reservoir made within the River Awash, and now receiving its inflows from River Awash and River Mojo. Lake Ziway, situated at 7°58'18.37’’N and 38°50'29.88’’E, is a shallow lake in the Ethiopian Rift Valley, located approximately 40 km and 120 km south of Lake Koka and Addis Ababa, respectively. The main inflows to this lake are River Meki and River Katar (Figure 1). General water quality characteristics of the lakes have been described in [17]. Lake Koka and Lake Ziway are both located in an area characterized by a semi-arid to sub-humid climate. The annual precipitation and temperature of the area range, respectively, from 600 mm and 25^{o}C in the semi-arid parts to 1200

mm and 15°C in the humid areas. Accordingly, the period from October to February falls in the dry season, and July to September is the main rainy season, as it is in most parts of Ethiopia. The fish species sampled in the present study include Nile tilapia (*O. niloticus*), African big barb (*B. intermedius*), and African sharp tooth catfish (*C. gariepinus*) from each lake (Lake Koka and Lake Ziway).

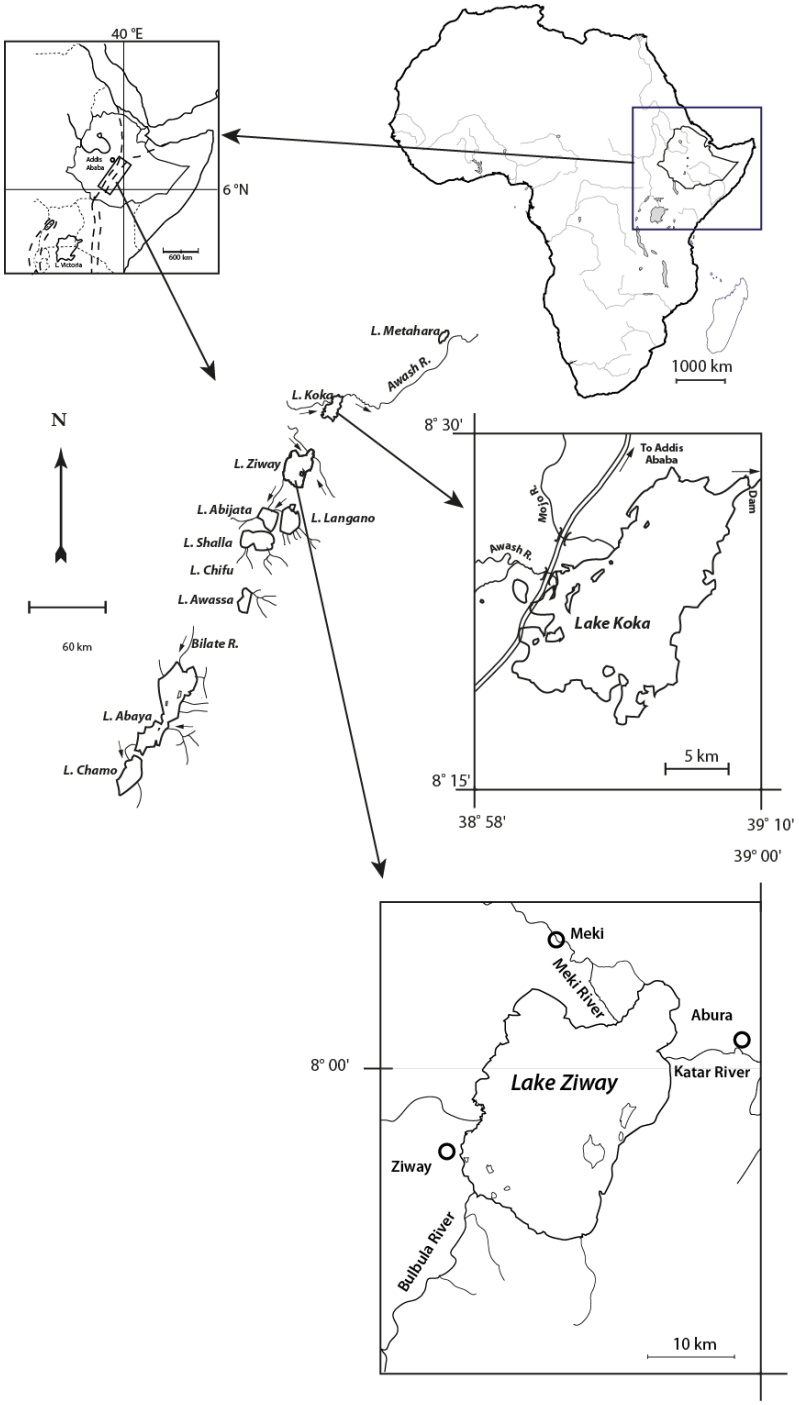

Fig. 1 Map of Lake Koka and Lake Ziway

B. Fish Sampling and Handling

A total of 141 fish were sampled and analyzed for THg and stable isotope analyses. Fish sampling was carried out from February–April 2008. In addition, *B. intermedius* were sampled in Lake Ziway during July to October 2009 (Table 1). Fish were partly purchased from the local fish market in each lake and partly using our experimental gillnets. Total length (LT) and weight (WT) were measured to the nearest centimeter and gram, respectively. Fish dissection of muscle samples and handling were carried out following the EMERGE protocol [18]. Fish muscle tissue for total mercury (THg) and stable isotope analyses were wrapped in aluminum foil, kept in a plastic bag with zipper lock and stored in an ice box until transferred to a deep freezer. Stomach contents were stored in small plastic vials, and preserved, using 90% ethanol.

C. Stomach Content Analysis

Stomach content analysis was carried out at the Department of Applied Biology laboratory, University of Hawassa. Large items were identified visually, and smaller items were identified under a dissecting microscope. For microscopic food item like algae, the stomach contents from each fish were first diluted with water to a known volume, mixed thoroughly, a drop of it was placed on a microscopic slide and identified. The relative importance and contribution of each food item to the diet of each fish species was determined using the frequency of occurrence method and the relative composition by volume (%) − volumetric analysis [19].

D. Mercury Analysis

Tissue concentration of THg in all fish samples was analyzed at the Environmental Chemistry Section of the Department of Plant and Environmental Sciences (IPM), Norwegian University of Life Sciences (UMB). The wet muscle tissues (200 mg) were first weighed, and then digested, using an Anton Paar microwave oven. THg concentrations were analyzed using the Perkin-Elmer model FIMS 400 flow injection Hg system. The equipment was calibrated by plotting calibration curves using the measurement values of four different synthetic standards. The curves were linear, and calibration was rechecked after every five samples. The concentrations of synthetic standards varied depending on the concentrations of Hg in the samples. The accuracy of the method was controlled against DORM-2 (piked dogfish *Squalus acanthias* L.), certified reference material, National Research Council of Canada, Ottawa. The reference material was run once after every 15 samples and was measured five times during the routine of measuring THg including muscle samples of other fish species. The mean ± SD of the certified reference samples was 4.598 ± 0.093 $mgkg^{-1}$, and it is within the range of the certified reference value (4.64 ± 0.26 $mgkg^{-1}$, Recovery = 110–125%). Blanks were used and values were <0.001 $mgkg^{-1}$. Sample values were corrected against blank values.

E. Stable Isotope Analyses

The stable isotope analyses were carried out at the Environmental Chemistry Section, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (UMB) as described in [20]. Fish filets of approximately 10–100 mg, were homogenized with a blender, after adding about 50 mL of distilled water. Homogenized and freeze-dried muscle tissue samples were weighed (0.8–1.2 mg) in tin capsules which were sealed to avoid sample loss. The samples in tin capsule were subjected to combustion in a Flash Elemental Analyzer (EA), and stable isotopes of nitrogen (15N and 14N) and carbon (13C and 12C) were determined by a Continuous Flow-Infrared Mass Spectrometer (CF-IRMS) as also described in [20, 21]. The isotopic ratios (15N/14N, 13C/12C) were expressed in delta-values as follows:

$$\delta^{15}N \text{ and } \delta^{13}C\ (‰) = [(RSample/RStandard) - 1] * 1000$$

where, $R = {}^{15}N/{}^{14}N$ for $\delta^{15}N$ or $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$.

The spatial variation of the stable isotopes of nitrogen between the two lakes was corrected by normalizing the mean value of $\delta^{15}N$ in the main primary consumer, *O. niloticus* in the two lakes [22].

F. Statistical Analysis

Comparisons of the THg concentrations among different fish species for each lake and comparisons of the THg concentrations for each fish species between the two lakes were performed by analyses of variance (ANOVA), using MINITAB 16. Biomagnification rate of Hg was determined by regressing the log transformed THg concentrations against $\delta^{15}N$ values. Log transformed Hg concentrations were regressed against LT to see the relationship between THg concentration and age (assume large fish tend to be older). Differences in THg mean values were considered as statistically significant when $p < 0.05$.

III. RESULTS

A. Diet and Trophic Position

Regardless of fish size, algae were the most important diet of *O. niloticus* in Lake Koka, besides zooplankton. The diet of *O. niloticus* from Lake Ziway included detritus, macrophytes and aquatic insects in its diet, in addition to algae and some zooplankton (Figure 2). The diet of *C. gariepinus* included food items extending from detritus to fish, but fish and aquatic invertebrates were the major food items of this species in both lakes. The stomach contents of *B. intermedius* in Lake Koka included aquatic insects, fish, detritus and macrophytes. Aquatic insects were the most dominant food item of this species (Fig. 2). Since the stomach of most individuals of *B. intermedius* was empty, the stomach content was not analyzed for this species.

The $\delta^{15}N$ values of the three fish species ranged from 13.2‰ to 19.0‰, and 6.8‰ to 16.5‰ in Lake Koka and Lake Ziway, respectively (Table 1). The mean values of the $\delta^{15}N$ in Lake Koka were relatively larger for *C. gariepinus* and *B. intermedius* than for *O. Niloticus* while the mean value of $\delta^{15}N$ of *C. gariepnus* in Lake Ziway was larger than *B. intermedius* and *O. niloticus* (Fig. 3). However, a much larger variation of $\delta^{15}N$ values was obtained among individuals of *B. intermedius* from Lake Ziway (Fig. 3). The $\delta^{13}C$ values ranged from -26.0 to -16.3‰ in Lake Koka, and from -34.6 to -19.9‰ in Lake Ziway (Table 1). The fish species from the two studied lakes utilized carbon sources both from littoral and pelagic origin (Fig. 3).

However, *B. intermedius* in Lake Koka exhibited relatively more enriched $\delta^{13}C$ than the other fish species. The differences in levels of the corrected $\delta^{15}N$ values for each species between the two study lakes were insignificant (Fig. 3).

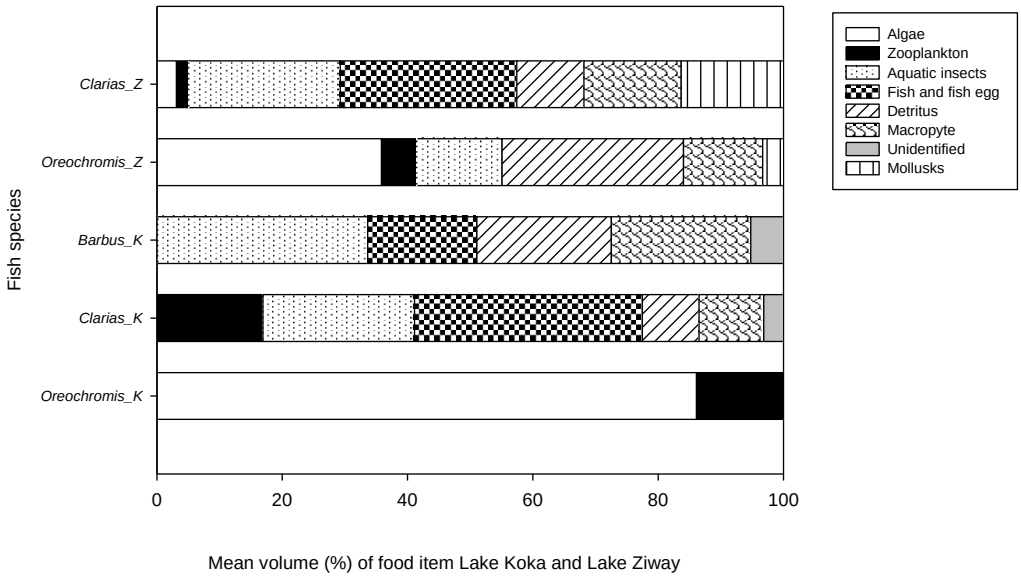

Fig. 2 Mean volume (%) of food items in the fish species *B. intermedius*, *C. gariepinus* and *O. niloticus* sampled from Lake Koka (K) and Lake Ziway (Z), during February-April, 2008 and July-October 2010 (K = Koka, Z = Ziway)

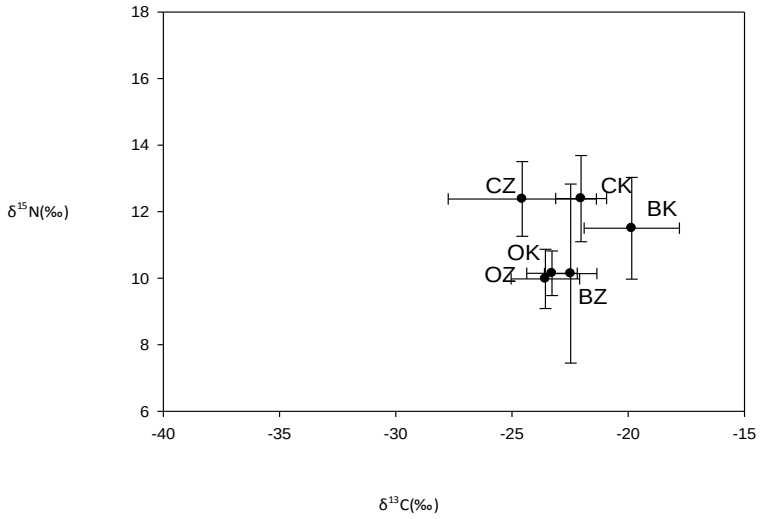

Fig. 3 Corrected relative trophic positions for the fish species *B. intermedius* (B), *C. gariepinus* (C) and *O. niloticus* (O) sampled between February–April 2008 from Lake Koka (K) and Lake Ziway (Z), based on the mean and ± SD of stable isotope ratios of nitrogen ($\delta^{15}N$, ‰) and carbon ($\delta^{13}C$, ‰)

TABLE 1 MEAN VALUES ±STANDARD DEVIATION (SD) OF THG CONCENTRATIONS (IN MG KG^{-1} WW), RATIO OF STABLE ISOTOPE VALUES OF NITROGEN, RATIO OF STABLE ISOTOPE VALUES OF CARBON, WITH MINIMUM AND MAXIMUM VALUES AND FREQUENCY OF DETECTION (%) IN THE FISH SPECIES *B. INTERMEDIUS*, *C. GARIEPINUS*, AND *O. NILOTICUS* FROM LAKE KOKA (A) AND ZIWAY (B), SAMPLED IN 2008 AND 2009

a)

		Sample Size	Total Length (cm)	$\delta^{15}N$	$\delta^{13}C$	THg	
Lake	**Code**	**N**	**Mean ± SD (Min -Max)**	**Mean ± SD (Min -Max)**	**Mean ± SD (Min -Max)**	**%**	**Mean ± SD (Min -Max)**
Koka	*B. intermedius*	15	37.2 ±5.0	15.9 ±1.5	-19.8 ±2.0	100	0.39 ±0.3[a]
			(25.2–44)	(13.2–18.1)	-(23.6–16.3)	-	(0.03–0.9)
	C. gariepinus	24	57.5 ±25.4	16.7 ±1.3	-22.4 ±1.5	100	0.14 ±0.1[b]
			(30–126)	(13.2–19.0)	-(26.0–20.2)	-	(0.05–0.64)
	O. niloticus	31	28.794 ±3.4	14.5 ±0.7	-23.3 ±1.0	64.5	0.01 ±0.0[b]
			(21.2–34.2)	(13.4–16.3)	-(24.9–20.7)	-	(ND–0.02)

Means of THg that do not share a letter are significantly different ($p < 0.05$) among fish species. ND means not detected.

b)

Lake	Code	Sample Size N	Total Length (cm) Mean ± SD (Min -Max)	$\delta^{15}N$ Mean ± SD (Min -Max)	$\delta^{13}C$ Mean ± SD (Min -Max)	THg %	THg Mean ± SD (Min -Max)
Ziway	*B. intermedius*	11	33.3 ± 5.0	10.1 ± 2.7	-22.5 ± 1.1	100	0.13 ± 0.2[a]
			(28.1–44.4)	(6.8–16.5)	-(24.5–20.0)	-	(0.01–0.5)
	C. gariepinus	34	46.5 ± 20.2	12.9 ± 1.4	-24.3 ± 3.0	30	0.06 ± 0.04[ab]
			(20.7–87)	(8.6–14.0)	-(34.6–20.9)	-	(ND–0.16)
	O. niloticus	26	25.2 ± 6.1	10.1 ± 1.0	-23.6 ± 1.4	70	0.01 ± 0.0[b]
			(5.8–31)	(8.7–11.7)	-(26.6–19.9)	-	(ND – 0.04)

Means of THg that do not share a letter are significantly different ($p < 0.05$) among fish species. ND means not detected.

B. Mercury Concentrations and Biomagnifications

The THg concentrations in each of the three fish species, *B. intermedius*, *C. gariepinus*, and *O. niloticus*, ranged between ND and 0.9 mg kg^{-1} (ww) in Lake Koka, and between ND and 0.5 mg kg^{-1} (ww) in Lake Ziway, respectively (Table 1). Significantly a higher concentration of THg was found in *B. intermedius* than in *C. gariepinus* and *O. niloticus*, in Lake Koka, whereas in Lake Ziway, *B. intermedius* had significantly higher THg concentrations than *O. niloticus* only (Table 1). The THg concentrations of *B. intermedius* in Lake Koka ($P < 0.05$) were significantly higher than *B. intermedius* in Lake Ziway, similarly the THg concentrations of *C. gariepinus* in Lake Koka ($P < 0.05$) were significantly higher than *C. gariepinus* in Lake Ziway.

The significant positive relationship was found between the concentration of log transformed THg and total length for *C. gariepinus* in Lake Koka only ($p < 0.05$ $R^2 = 0.44$) (Fig. 4).

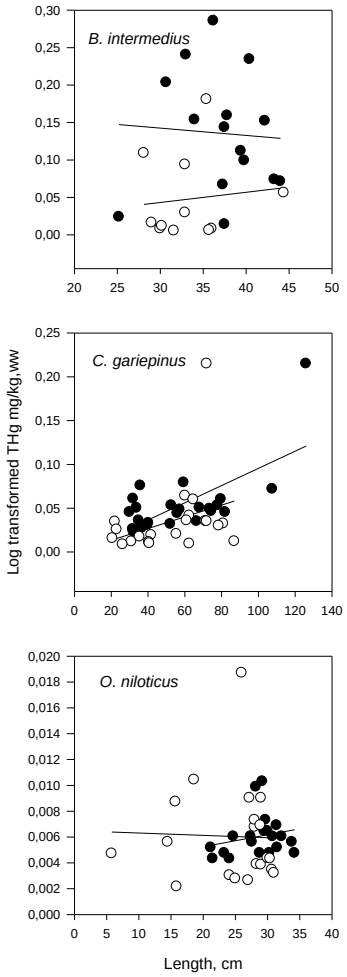

Fig. 4 The relationship between the log transformed concentration of THg (mg kg-1ww) and total length (cm) for the fish species *B. intermedius*, *C. gariepinus*, and *O. niloticus*, from Lake Koka (•) and Lake Ziway (o), sampled in 2008 and 2009

For all fish species and individuals pooled, a significant positive relationship was found between log transformed THg and $\delta^{15}N$ both in Lake Koka ($p < 0.05$, $R^2 = 0.35$) and Lake Ziway ($p < 0.05$, $R^2 = 0.26$) (Fig. 5).

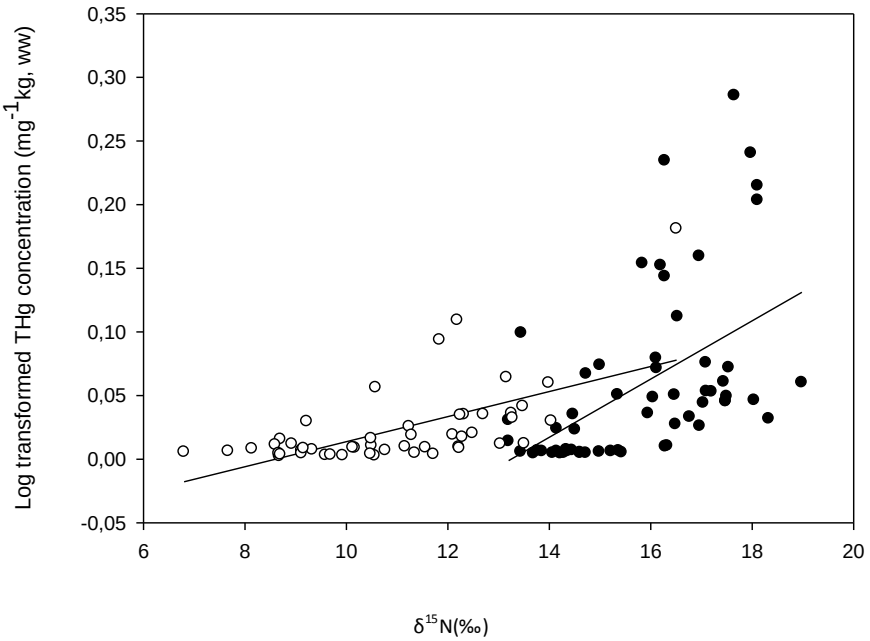

Fig. 5 The relationship between log transformed THg (mg.kg-1 ww) and $\delta^{15}N$ signatures for all fish species (*B. intermedius*, *C. gariepinus* and *O. niloticus*) pooled, from Lake Koka (•) and Lake Ziway (o) during 2008 and 2009

IV. DISCUSSION

An assessment of the dietary intake by fish can be achieved by using a combination of conventional stomach content analysis and stable isotope analysis as it has been done in the present study. The diets of the fish species *B. intermedius*, *C. gariepinus*, and *O. niloticus*, from Lake Koka and Lake Ziway were comparable and in good accordance with studies from other lakes (Fig. 2). *O. niloticus* in both lakes mainly fed on algae, macrophytes and detritus, and is thus a typical herbivore. This species is also herbivorous in earlier study in these lakes [22, 23, 24] and in other Ethiopian rift-valley lakes, such as Lake Awassa [20, 25, 26, 27]. Both *B. intermedius* and *C. gariepinus* in Lake Koka, and *C. gariepinus* in Lake Ziway are omnivorous according to their diet preferences, but the large individuals of both species include fish in their diet. Similar findings were also reported for *C. gariepinus* in Lake Awassa [23, 28], and for *B. intermedius* in Lake Victoria, Uganda [29]. The diet compositions of the fish species from Lake Koka and Lake Ziway were further substantiated by the $\delta^{15}N$ signals. The two species *B. intermedius* and *C. gariepinus* were occupying a higher trophic level than *O. niloticus* in Lake Koka, however, the mean $\delta^{15}N$ value for *B. intermedius* in Lake Ziway indicated that this species was occupying the same trophic level as *O. niloticus* in Lake Ziway. The stomach contents from *B. intermedius* in Lake Ziway were not available, but the large SD of the mean $\delta^{15}N$ value indicates a large variation between individuals as regards food items as it has been also exhibited in Lake Koka.

The $\delta^{15}N$ values of the three fish species ranged from 13.2‰ to 19.0‰, and 6.8‰ to 16.5‰ in Lake Koka and Lake Ziway, respectively (Table 1). The range in $\delta^{15}N$ signatures, being larger than 7‰ in the studied lakes, indicates that there are at least 2 trophic levels in the fish community of the two lakes, following a mean enrichment value of $\delta^{15}N$ at 3–4‰ per successive trophic level [30], indicating that the fraction could be much lower than 3–4‰ in tropical ecosystems. This is perhaps due to rapid turnover of tissue $\delta^{15}N$ in tropical aquatic organisms [31] or temperature dependent fractionation of $\delta^{15}N$ [32]. Therefore, there may be more trophic levels between the most herbivorous and the most piscivorous fish species in our study.

The $\delta^{13}C$ signatures in general indicated that the studied fish species from the two lakes have a wide range of carbon sources in their diets that extend from pelagic to littoral resources. Some individuals had very "light'' (more negative) $\delta^{13}C$ signature, indicating a pelagic energy pathway, in contrast to those with "heavier'' (less negative) $\delta^{13}C$ signatures which are likely due to benthic carbon sources [33, 34]. However, *B. intermedius* in Lake Koka and Lake Ziway exhibited relatively more enriched $\delta^{13}C$ than other studied fish species, suggesting that this species obtained a large proportion of its prey from benthic sources.

As pointed out by [35], the length and weight (size) of fish are important variables for explaining the concentration of THg. The significant positive relationship between the concentrations of THg and total length was found only in *C. garipienus* in Lake Koka which was mainly due to the considerable influence of one large individual of exceptionally high Hg concentration (Fig. 4). However, the fish from Lake Koka and Lake Ziway might have followed the phenomenon of biodilution of THg, and

such biodilution of contaminants in fish because of fast growth (growth dilution) has also been observed in other studies in the same region [20, 22]. For all fish species and individuals pooled, a significant positive relationship was found between log transformed THg and $\delta^{15}N$ both in Lake Koka ($P < 0.05$, $R^2 = 0.35$) and Lake Ziway ($P < 0.05$, $R^2 = 0.26$) (Fig. 5). Therefore, the present study demonstrates a bioaccumulation of Hg in the fish species of Lake Koka and Lake Ziway, and thus increases as the trophic level increases.

V. CONCLUSION

The present study demonstrates the accumulation of heavy metals (e.g, Hg) in ERVLs, and species variation in total THg accumulation is attributed to size, diet and trophic position of the fish.

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