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Biomagnification of DDT and its metabolites in four fish species of a tropical lake

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ABSTRACT

The concentrations and biomagnifications of dichlorodiphenyltrichloroethane (DDT) and its metabolites were examined in four fish species (*Clarias gariepinus, Oreochromis niloticus, Tilapia zillii,* and *Carassius auratus*) from Lake Ziway, Rift Valley, Ethiopia. Paired stomach content analysis, and stable isotope ratio of nitrogen (δ^{15}N, ‰) and carbon (δ^{13}C, ‰) were used to study the trophic position of the fish species in the lake. 4,4′-DDE, 4,4′-DDT and 4,4′-DDD were the main DDTs identified in the fish samples, with 4,4′-DDE as the most predominant metabolite, with mean concentration ranging from 1.4 to 17.8 ng g^{-1} wet weight (ww). The concentrations of DDTs found in fish from Lake Ziway were, in general lower than those found in most studies carried out in other African Lakes. However, the presence of DDT in all tissue samples collected from all fish species in the lake indicates the magnitude of the incidence. Moreover, the observed mean 4,4′-DDE to 4,4′-DDT ratio below 1 in *C. auratus* from Lake Ziway may suggest a recent exposure of these species to DDT, indicating that a contamination source is still present. 4,4′-DDE was found to biomagnify in the fish species of the lake, and increases with trophic level, however, the biomagnification rate was generally lower than what has been reported from other areas. Significantly higher concentrations of 4,4′-DDE were found in the top consumer fish in Lake Ziway, *C. gariepinus* than in *O. niloticus* ($t=2.6$, $P<0.01$), *T. zillii* ($t=2.5$, $P<0.02$) and *C. auratus* ($t=2.2$, $P<0.03$).

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1. Introduction

DDT is an organochlorine pesticide that has been used for vector and pest control since World War II (Foreman and Gates, 1997). This pesticide is grouped under the persistent organic pollutants (POPs) due to its toxic, lipophilic and persistent nature (Burreau et al., 2004; Holden, 1966; Jones and de Voogt, 1999). Coupled with its ability to bioaccumulate and magnify in the food chain (Alexander et al., 2007; Burreau et al., 2004; Mackay and Fraser, 2000; Rognerud et al., 2002), it has potential impact on top predator species, including humans. DDT and some of its metabolites are reported to affect the nervous and reproductive systems, and cause cancer (Beard, 2006; Kelce et al., 1995; McBlain, 1987). While the use of DDT has been limited internationally, some countries continued to use it in disease control programs as it was found a valuable short term line of attack for controlling malaria (Goldberg, 1991). In recognition of this pressing need, SC permits the production and use of DDT for disease vector control only by notifying the convention secretariats, provided that no safe, effective, and affordable alternatives are locally available (Stockholm Convention on Persistent Organic Pollutants, 2008).

Technical grade DDT is mainly a mixture of 4,4′-DDT (85 percent) and 2,4′-DDT (15 percent). Both DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis (4-chlorophenyl)ethane) exist as impurities in commercial DDT formulations. In the environment, DDT breaks down to form DDE or DDD (Foght et al., 2001; Pirnie et al., 2006; Sayles et al., 1997). DDT may enter rivers and lakes mainly through industrial release point sources, as runoff from agricultural fields, as well as from atmospheric deposition due to volatilization (Binelli and Provini, 2003; Schwarzbauer et al., 2001). DDT, DDD and DDE are strongly retained by soils, sediments, and biota lipids due to their low aqueous solubility and high octanol–water partitioning

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coefficients. 4,4′-DDE can often account for 70 percent of the total DDT in fish (Schmitt et al., 1990). The higher accumulation of 4,4′-DDE than the other metabolites is attributed to mixed-function oxidases that may have induced the dechlorination of 4,4′-DDT to 4,4′-DDE. The ratio of 4,4′-DDE to 4,4′-DDT is a helpful tool in revealing the significance of the degradation of DDT and to evaluate the current use of DDT in the given region (Strandberg and Hites, 2001). However, this method is limited in regions where dicofol is used. This is because dicofol contains high levels of DDT as an impurity (Qiu and Zhu, 2010).

There are two main routes by which fish can bioaccumulate chemicals in their natural aquatic habitat: (i) from water via body surfaces (e.g. gills) and (ii) through the diet (Burreau et al., 2004; Campbell et al., 2000; Holden, 1966). Stomach content analyses and stable nitrogen isotope ratio (δ^{15}N) provide complementary information which can be used in analysis of the trophic transfer and biomagnification rate of persistent contaminants, including DDTs (Rognerud et al., 2002; Sharma et al., 2009). It is well established that lipophilic compounds such as DDT preferentially accumulate in lipids of fishes and other animals (Mackay and Fraser, 2000), and the extent of accumulation is greater in fish that have high lipid content (Muir et al., 1990).

The Ethiopian Rift Valley Lakes (ERVLs) are the most northern part of the East African Rift Valley Lakes. In Central Ethiopia, the Great Rift Valley splits the Ethiopian highlands into northern and southern halves, and the ERVLs occupy the floor of the rift valley between the two highlands. Most lakes are highly productive and well known for their aquatic diversity and indigenous populations of edible fish species (Golubtsov et al., 2002). With changing environmental conditions under increasing anthropogenic influences, the nature of the Ethiopian Rift Valley Lakes is also changing.

Due to the intensive agricultural and deforestation activities in the catchments of the Ethiopian Rift Valley Lakes (Zinabu, 2002; Zinabu and Elias, 1989), there is a risk of chemical pollution from fertilizers and pesticides. Run off and erosion from the surrounding catchment could release pesticides (e.g. DDTs) sequestrated in the soil to the lake may enter the food chain, and reaching out to the fish. Hence, the abundance and quality of commercially important fish species, an important ecosystem service of the Rift Valley Lakes, may be at risk. Furthermore, consumption of contaminated fish is one of the main exposure routes to toxic organic chemicals like DDT for humans (e.g. Han et al., 2000; Svensson et al., 1995). However, the level and extent of DDTs contamination in fish species at various trophic levels, has not been studied. The objective of this study is therefore to determine the concentration levels of DDTs and their biomagnifications in relation to the trophic position of fish species from Lake Ziway, which is one of the important Ethiopian Rift Valley Lakes.

2. Material and methods

2.1. Description of the study area

The study area, Lake Ziway, is located in the Rift Valley in the southeastern part of Ethiopia, at 1636 m a.s.l. (coordinates 7° 52′N and 38°4 5′E) (Fig. 1). It is part of the Ziway–Shala basin and has a catchment area of about 7000 km^2, and an average surface area of 490 km^2. The lake has an average volume of 1.8 km^3, and a maximum depth of 9 m (Vallet-Coulomb et al., 2001). There are two inflowing rivers, the Meki River from the north-west, and the Ketar River from the east. The lake drains towards the Lake Abiyata, through Bulbula River. The climate of the region is characterized by bimodal rainfall distribution with short-term, highly variable, relatively low rain fall from February to June, and higher rain fall from July to September (Zegeye et al., 2006). The mean annual air temperature is 25.5 °C, and the mean annual rainfall is about 702 mm (Zegeye et al., 2006). The pH of the lake ranges from 7 to 8, and the average conductivity is about 400 μS cm^{-1}, which is low compared to most Ethiopian Rift Valley Lakes (Erko et al., 2006; Zinabu et al., 2002).

The lake has an extended littoral zone, containing emergent and submerged macrophytes, which provide feeding, breeding, and nursery habitats for fish (Admassu and Ahlgren, 2000; Erko et al., 2006). The fish species of the lake include indigenous species like Nile tilapia (*Oreochromis niloticus*) and African big barb (*Barbus intermedius*), and introduced species such as African sharptooth catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), Golden carp (*Carassius auratus*) and *Tilapia zillii* (Negassa and Getahun, 2003). The lake has long been used as a resource of water supply for small scale irrigation, domestic water use, and fisheries. The recent expansion of floriculture industries around the lake may discharge untreated effluents directly into the lake, and as a result, excessive fertilizer and pesticide residues from the greenhouses may deteriorate the water quality as well as the aquatic life.

2.2. Sampling

Fish sampling was carried out between February and April 2008, partly by purchasing fish from the local fishermen upon landing, and partly by gillnetting, using experimental gill nets with mesh sizes from 5 to 45 mm (bar mesh). The total length (cm), weight (g) and sex of each fish were recorded (Table 1). The contents of the esophagus and stomach/first part of intestine were removed and preserved in 96 percent ethanol. Muscle samples were taken from each specimen, following the procedures in the EMERGE protocol, as described by Rosseland et al. (2001), and frozen. The frozen samples were transported to Norway for analysis of DDTs, stable isotopes of carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N). A total of 93 (40 females and 53 males) samples from four fish species (*C. auratus, C. gariepinus, O. niloticus,* and *T. zillii*) were examined and analyzed with respect to DDTs, and stable isotopes ratios of nitrogen and carbon (Table 1).

2.3. Stomach content analyses

The stomach content analyses were done at Hawassa University, Department of Applied Biology, Awassa, Ethiopia. A total of 143 stomach contents were examined for food composition. 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 percent composition by volume (percent) – volumetric analyses (Hyslop, 1980). The identification of the stomach contents was carried out using either a dissecting microscope for larger items, or by a compound light microscope for smaller items such as phytoplankton. The following food item categories were identified: phytoplankton (blue green algae, green algae and diatoms), macrophytes, detritus, zooplankton, aquatic insects, ostracods, gastropods and fish.

2.4. Stable isotope analyses

Stable isotope analyses were carried out at the Environmental Chemistry Section, Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (UMB). The stable isotopes of nitrogen (^{15}N and ^{14}N) and carbon (^{13}C and ^{12}C) were analyzed in homogenized and freeze-dried muscle samples subjected to combustion in a Flash Elemental Analyzer (EA) as described in Desta et al. (2007) and Sharma et al. (2009). The isotopic ratios (^{15}N/^{14}N, ^{13}C/^{12}C) were expressed in delta-values as follows:

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

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

2.5. Lipid content analyses

The relative lipid content (percent) in the fish muscle tissue was determined by a gravimetric method adopted from Lee et al. (1996). The method was tested using Standard Reference Material (SRM) 1946: Lake Superior fish tissue from USA National Institute Standard and Technology (NIST). The standard reference material was analyzed according to the proposed method and the result was within the certified range. About 5 g of fish tissue was defrosted, chopped into pieces, weighted and transferred into a 50 mL centrifuge tube. Each sample was completely homogenized using 25 mL of methyl tertbutyl ether (MTBE) and blended for 2 min with Polytron (Kinematica AG) at a moderate speed. To maintain a moderate and constant speed during blending is important because the high speed resulted in solvent vaporization and temperature rise. The extraction was repeated on the remaining, using 25 mL of MTBE to extract the fat completely. The extract was transferred to the same flask, and the volume was adjusted to 50 mL. Five milliliters of the extract (triplicate) was taken into a pre-weighted beaker. The sample was allowed to evaporate completely over night before the weight was determined, and finally the percentage of relative lipid content was calculated.

2.6. DDTs analyses

The DDTs (2,4′-DDE, 4,4′-DDE, 2,4′-DDD, 4,4′-DDD, 2,4′-DDT and 4,4′-DDT) were analyzed at the laboratory of Norwegian Institute for Agricultural and

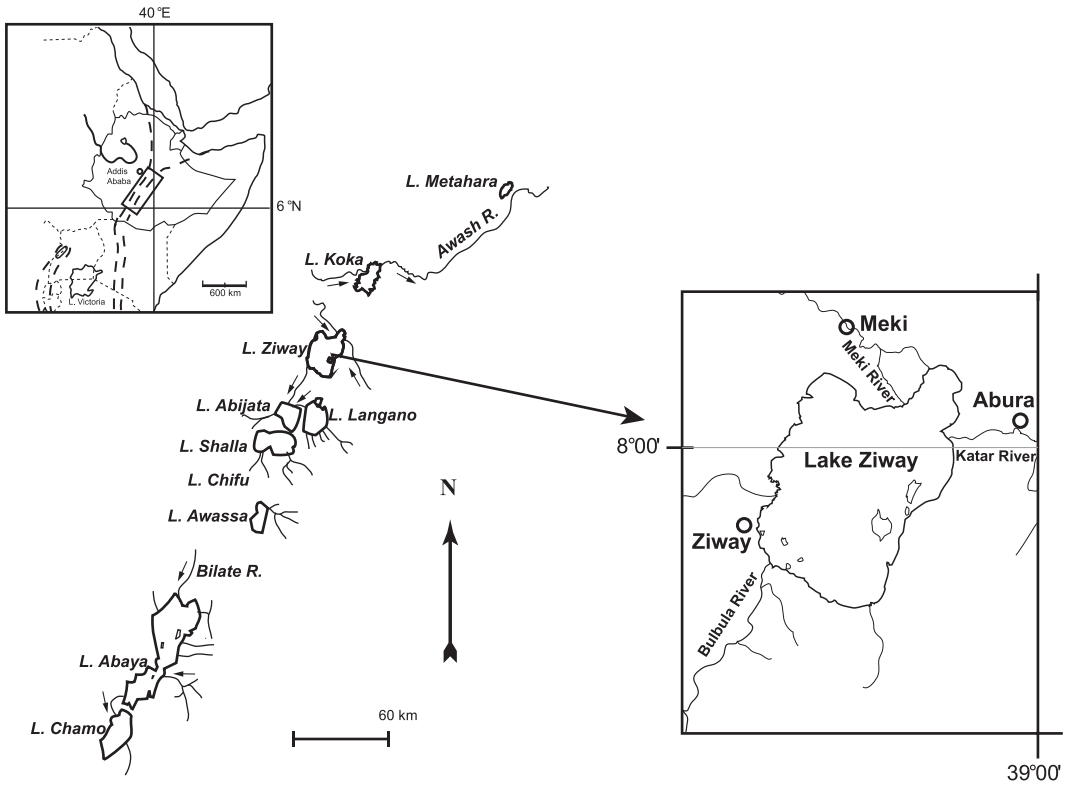

Fig. 1. Map of Lake Ziway.

Table 1
Mean ± standard deviation, minimum and maximum values of total length (T_L, cm), and weight (W, g), number of samples (N) and sex (female (♀) and male (♂)) of the examined fish species (*C. auratus, C. gariepinus*, *O. niloticus,* and *T. zillii*) from Lake Ziway, Ethiopia, sampled in 2008.

Fish Spp	*N* (Sex: ♀/ ♂)	T_L (cm)			*W* (g)		
		Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
C. auratus	22 (♀=9, ♂=13)	27.5 ± 5.8	18.6	39	291.6 ± 199.4	76	810
C. gariepinus	27(♀=13, ♂=14)	50.3 ± 20.5	22.1	87	1045 ± 1015	63	3350
O. niloticus	24(♀=10, ♂=14)	26.5 ± 4.4	14.5	31	309.5 ± 141.1	38	480
T. zillii	20(♀=8, ♂=12)	21.0 ± 2.9	15.1	24.8	195.4 ± 76.2	63	335

Environmental Research (Bioforsk): Chemistry and Pesticide Section, Norway. The fish tissue samples were extracted with acetonitrile as described in Norli et al. (2011). Control spiking experiments in *O. niloticus* at 5 and 50 ng g^{-1} ww extracted using this method showed recovery for all DDTs ranged from 86 to 115 percent. To further test the method using acetonitrile as extraction solvent, we analyzed the standard reference material SRM 1946®, Lake Superior Fish tissue homogenate. This is a certified trout material containing known residues of pesticides, PCBs, heavy metals and other compounds in a broad concentration range. The results from certified trout material were all within the certified range. These samples were analyzed and the LOQ values were estimated for DDT and its metabolites using the the root mean square signal-to-noise ratio (RMS *S/N*) parameter in the Chem Station software.

The measurements were carried out on an Agilent 6890 gas chromatograph connected to an Agilent 5973 mass spectrometer with an inert ion source using ChemStation Software. The gas chromatograph was equipped with a Gerstel (Mühlheim Ruhr, Germany) programmable temperature vaporizing (PTV) injector with a sintered liner. The separation column was a fused silica J&W Scientific HP-5MSI 30 m with 0.25 mm internal diameter and 0.25 mm film thickness. A 2.5 m methyl deactivated pre column (Varian Inc. Lake Forest, CA, USA) of same internal diameter was connected to the analytical column. The columns were connected by a press fit connector (BGB Analytic, Switzerland). The temperature program was as follows: 70 °C held for 3 min, 25 °C/min to 150 °C, held for 0 min, 3 °C/min to 200 °C, held for 0 min, 20 °C/min to 280 °C, held for 6 min, 50 °C/min to 325 °C, held for 1 min, total runtime 34.77 min. The PTV program was as follows: injection volume 15 μL with a slow plunger speed. The solvent vent temperature was kept at 50 °C in 1.89 min with a solvent vent flow at 200 mL/min. After 1.80 min the split valve was closed and the injector temperature raised by 720 °C/min to 280 °C and kept for 1.2 min. Pressurized air was used as a coolant for the PTV injector. The mass spectrometer was operated in selected ion monitoring mode with target and qualifier ions. Transfer line temperature was set at 280 °C, ion source temperature at 230 °C and quadrupole temperature at 150 °C.

2.7. Statistical analyses

Comparisons of the concentrations of DDTs in different fish species were performed by analysis of variance (ANOVA), using MINITAB 16. Log transformed values of each DDTs concentrations in ng g^{-1} ww were regressed against total length (T_L) in cm for each fish species to find the relationship between fish size and DDTs concentrations. Total length was used instead of weight to avoid bias due to the difference in weight caused by the variation in gut fullness. Biomagnification rate of DDTs was determined by regressing the log transformed DDTs concentrations against δ^{15}N values and against percentage of lipids. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Fish diets

Mean volume (percent) of food items for *C. gariepinus*, *C. auratus*, *T. zillii, and O. niloticus* are presented in Fig. 2. Their diet was composed of plants, invertebrates, fish and detritus, with variations among the species. Consumed plants consisted of both phytoplankton (blue green algae, green algae and diatoms) and macrophytes.

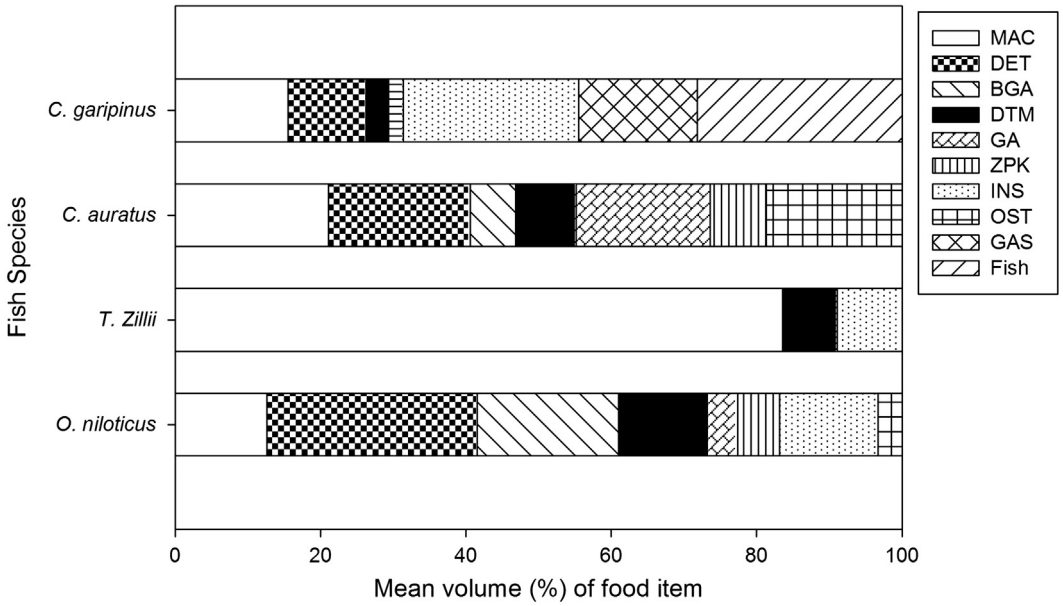

Fig. 2. Mean volume (percent) of food items in four fish species sampled in Lake Ziway, during February–April, 2008 and July–October 2010 (MAC, macrophyte; DET, detritus; BGA, blue green algae; DTM, diatoms; GA, green algae; ZPK, zooplankton; INS, insect; OST, ostracods; GAS, gastropods).

Algae were the most important food group of *O. niloticus* in Lake Ziway, and formed 34.2 percent (by volume) of the total stomach content, of which blue green algae, diatoms and green algae accounted for 19.4 percent, 10.6 percent and 4.2 percent, respectively. Detritus and macrophytes contributed with 29.0 percent and 12.6 percent of the mean volume, respectively. Regardless of fish size, macrophytes were found in the stomachs of all *T. Zillii* in Lake Ziway, and were the most important item in the diet, forming 82.5 percent (by volume) of the total stomach content. Aquatic insects and diatoms contributed with 8.9 percent and 7.5 percent of the mean volume, respectively. The stomach contents of *C. auratus* were diverse, with macrophytes, detritus, algae and ostracods all important, by volume. The diet of *C. gariepinus* included many food items from detritus to fish, but fish, aquatic insects, and gastropods, made up 28.2 percent, 24.1 percent, and 16.3 percent of the stomach contents by volume, respectively. The fish recorded as food items of *C. gariepinus* was mainly Nile tilapia.

Table 2
Mean ± standard deviation (SD), with minimum and maximum values of stable isotope ratios of nitrogen (δ^{15}N, ‰), stable isotope ratios of carbon (δ^{13}C, ‰), and lipid content (percent) for the examined fish species from Lake Ziway, Ethiopia, sampled in February-April 2008.

Variable	Spp	No. of samples (*N*)	Mean	SD	Min.	Max.
δ^{15}N (‰)	*C. auratus*	22	12.32	0.85	9.61	13.73
	C. gariepinus	27	12.38	1.12	8.6	14.71
	O. niloticus	24	9.98	0.89	8.67	11.56
	T. zillii	20	11.46	0.75	10.19	12.93
δ^{13}C (‰)	*C. auratus*	22	−22.54	1.81	−24.26	−18.02
	C. gariepinus	27	−24.56	3.19	−34.62	−20.92
	O. niloticus	24	−23.56	1.47	−26.64	−19.93
	T. zillii	20	−19.59	2.12	−23.94	−16.98
Lipid (percent)	*C. auratus*	22	0.31	0.03	0.28	0.34
	C. gariepinus	27	0.61	0.06	0.54	0.69
	O. niloticus	24	0.13	0	0.12	0.13
	T. zillii	20	0.49	0.06	0.42	0.57

3.2. Stable isotopes

The δ^{15}N values of the four fish species ranged from 8.6‰ to 14.7‰ (Table 2). The mean values of the δ^{15}N were larger for *C. gariepinus* and *C. auratus* than for *T. zillii* and *O. niloticus,* indicating the higher trophic level of the two species, *C. gariepinus* and *C. auratus* (Fig. 3). The δ^{13}C values, ranging from −34.6 to −17.0‰ (Table 2), show the fish species of the lake utilized carbon sources with both littoral and pelagic origin (Fig. 3).

3.3. Lipid content

The lipid content of the fish species from Lake Ziway was within the range 0.12–0.69 percent (Table 2). The highest mean lipid content was recorded in *C. gariepinus*, followed by *T. zillii, C. auratus,* and *O. niloticus.*

3.4. DDT residues

The DDTs frequently detected in the fish tissue samples were 4,4′-DDT, 4,4′-DDE and 4,4′-DDD, and these accounted for 85.8 percent of all the DDTs (Fig. 4). The 4,4′-DDE was the predominant group, and made up 61.8 percent, 38.74 percent, 78.0 percent and 30.0 percent of the mean DDTs concentration in *C. gariepinus*, *C. auratus*, *T. zillii* and *O. niloticus*, respectively.

The mean concentrations of DDTs found in *C. gariepinus*, *C. auratus*, *T. zillii, and O. niloticus* are presented in Table 3. 4,4′-DDE was detected in all fish tissue samples analyzed with concentrations in the four fish species ranging between 4.7 ng $g^{-1}$and 17.8 ng g^{-1} ww. Significantly higher concentrations of 4,4′-DDE were found in *C. gariepinus* than in *O. niloticus* ($t=2.6$, $P<0.01$), *T. zillii* ($t=2.5$, $P<0.02$) and *C. auratus* ($t=2.1$, $P<0.03$). The ratios of 4,4′-DDE to 4,4′-DDT in *C. gariepinus*, *O. niloticus* and *C. auratus* were 16.7, 2.8 and 0.89, respectively (Fig. 4). 4,4′-DDT was below the detection limit in *T. zillii*, as well as in 36.4 percent of *C. auratus.*

3.4.1. Concentrations of DDTs and fish size

Concentration of DDTs shows a positive correlation with fish size in all species (Fig. 5). The concentration of log transformed

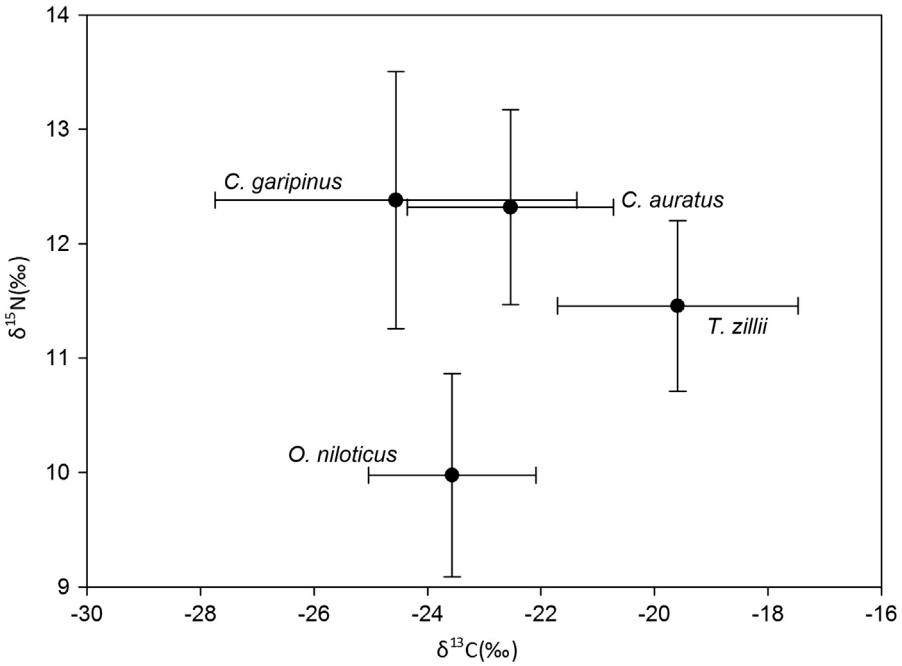

Fig. 3. Relative trophic position of four fish species sampled in February–April 2008 in Lake Ziway, based on the mean and ± SD of stable isotope ratios of nitrogen (δ^{15}N, ‰) and carbon (δ^{13}C, ‰).

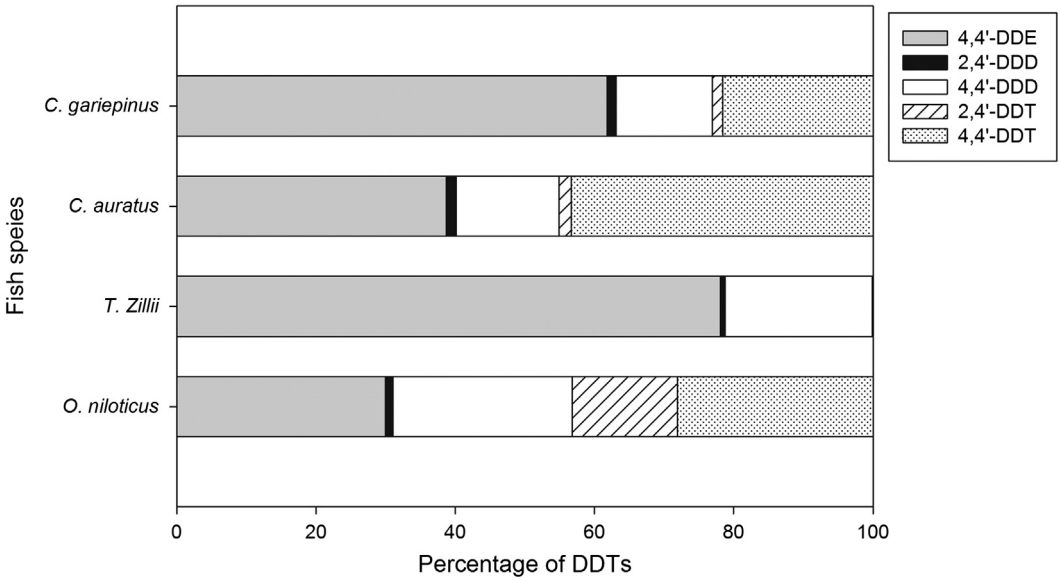

Fig. 4. The mean percentage of DDTs in the fish species *O. niloticus*, *C. gariepinus*, *T. zillii* and *C. auratus* from Lake Ziway sampled in February–April 2008.

4,4′-DDE ($P<0.01$) is significantly related to total length for all species except *T. zillii*. A significant relationship was also found between the concentration of log transformed 4,4′-DDD and total length in *C. gariepinus* and *O. niloticus*. However, the concentration of log transformed 4,4′-DDT ($P<0.01$) was not significant at all for all fish species.

3.4.2. Concentrations of DDTs and trophic position

When all fish species were pooled together, the relationship between log transformed 4,4′-DDE and δ^{15}N was significant ($F=10.0$, $df=1$, $P=0.002$, $N=93$). The concentrations of 4,4′-DDT also showed a positive correlation with δ^{15}N, however, the relationship between the concentrations of the other DDT metabolites (4,4′-DDT and 4,4′-DDD) and δ^{15}N was not significant.

3.4.3. Concentrations of DDTs and lipid content in fish

The relationships between log transformed 4,4′-DDE and percentage of lipids were significant ($F=11.1$, $df=1$, $P=0.001$, $N=93$), when all the data were pooled. However, the relationship between concentrations of the other DDT metabolites (4,4′-DDT and 4,4′-DDD) and percentage of lipids were not significant. The highest concentration of 4,4′-DDE, 4,4′-DDD and ∑DDT was found in *C. garipienus,* the species with the highest mean percentage (Table 3).

Table 3
Mean values ± standard deviation of concentration of DDTs in ng g^{-1} ww, with min–max values and frequency of detection in four fish species from Lake Ziway, sampled in February–April 2008.

DDTs	Spp	No. of samples (*N*)	Frequency	Mean	StDev	Min.	Max.
4,4′-DDT (ng g^{-1})	*C. auratus*	22	63.64	12.49 (b)	13.96	4.94	56.49
	C. gariepinus	27	100	6.22(a)	2.82	3.28	17.37
	O. niloticus	24	100	5.31(a)	1.51	2.86	8.95
	T. zillii	20	ND	-	–	–	–
4,4′-DDE (ng g^{-1})	*C. auratus*	22	100	7.10(a)	4.57	1.53	20.23
	C. gariepinus	27	100	17.81 (b)	22.61	3.8	122.77
	O. niloticus	24	100	5.67(a)	4.53	1.00	19.31
	T. zillii	20	100	4.71(a)	8.57	0.89	40.73
4,4′-DDD (ng g^{-1})	*C. auratus*	22	95.45	2.84(a)	3.38	0.35	14.12
	C. gariepinus	27	100	3.98(a)	5.73	0.37	30.6
	O. niloticus	24	100	4.87(a)	11.24	0.77	56.87
	T. zillii	20	90	1.42(a)	2.84	0.33	12.73
∑DDT (ng g^{-1})	*C. auratus*	22	100	18.33 (a)	15.49	3.65	76.17
	C. gariepinus	27	100	28.78 (a)	30.92	7.8	171.96
	O. niloticus	24	100	18.93 (a)	26.33	4.78	139.01
	T. zillii	20	100	6.04(b)	11.28	0.89	53.47

Means with different letters within a column are significantly different ($P < 0.05$).

4. Discussion

The concentrations of DDTs found in fish from Lake Ziway (Table 3) are generally lower than those found in Lake Nubia, between Egypt and Sudan (Elzorgani et al., 1979) with a mean concentration of 184 ng g^{-1} ww and fish from Lake Victoria, Kenya with a wide range of concentrations, from 3–460 ng g^{-1} ww (Mitema and Gitau, 1990). However, they are comparable with fish from the Densu River Basin, Ghana, which had a mean concentration of 8.0 and 4.0 ng g^{-1} ww for 4,4′-DDE and 4,4′-DDT, respectively (Fianko et al., 2011) and higher than those found in fish from lake Bosomtwi, Ghana, which had a mean concentration of 5.2 and 3.7 ng g^{-1} ww for 4,4′-DDE and 4,4′-DDT, respectively (Darko et al., 2008), and from lake Koka, Ethiopia which had mean concentration of ∑DDT between 4.5 and 15.6 ng g^{-1} ww (Deribe et al., 2011). Fish species, trophic position and the organ(s) analyzed etc. varied in these studies, and may make direct comparisons difficult.

The detection of at least one DDT metabolite in all tissue samples indicates that the consumption of fish from Lake Ziway cannot be considered to have no risks. Moreover, the observed mean 4,4′-DDE to 4,4′-DDT ratio below 1 in *C. auratus* (0.89) may suggest a recent exposure of this species to DDT, indicating that a contamination source is still present. According to Qiu and Zhu (2010), this ratio can be affected in regions where dicofol is being used. This is because dicofol contains high levels of DDT as an impurity. To our knowledge, the use of dicofol is limited in the study area and therefore the presence of dicofol is unlikely to affect this ratio. DDT is probably still used in vector control in the region, but contamination from obsolete pesticides may also contribute. Runoff and erosion from the surrounding catchment could potentially release pesticides (e.g. DDTs) sequestrated in the soil to the lake and reaching out to the fish. However, a time sequence study on the percentage of 4,4′-DDE in comparison with the 4,4′-DDT concentration is required in order to get a better understanding of the current use of DDT in this area of the Ethiopian rift valley.

The progressive increase in concentrations of 4,4′-DDE and 4,4′-DDD in *C. gariepinus* and *O. niloticus,* as well as the increase of 4,4′-DDE in *C. auratus* with increasing total length of the fish is most probably a result of bioaccumulation of DDTs with age. As pointed out by Rognerud et al. (2002) and Vives et al. (2005) the length and weight of fish are important variables for explaining the concentration of DDTs. The length distribution of analyzed *T. zillii* was very narrow and represents mainly small individuals, and may explain why the concentrations of 4,4′-DDE and 4,4′-DDD in this species did not increase significantly with increasing length. The concentration of 4,4′-DDT in *C. gariepinus*, *O. niloticus,* and *C. auratus* also showed a positive correlation with size, however, the relationship was not significant. This is probably due to larger individuals of these species accumulating a more degraded form of DDT, as DDT is transformed to DDE and DDD over time.

Based on the stomach content analyses, the diet composition of all fish species except *C. auratus* from Lake Ziway, agreed with the trophic levels identified by δ^{15}N signatures. The low δ^{15}N of *O. niloticus* corresponds well with the diet being predominantly algae and detritus. This species has a similar diet in other Ethiopian Rift Valley Lakes such as Lake Awassa (Desta et al., 2007; Getachew, 1987, 1989; Getachew and Fernando, 1989), Lake Koka (Deribe et al., 2011), and Lake Chamo (Getachew, 1993), as well as in other East African lakes (Lowe-McConnell, 1958).

Macrophytes were the most important component in the diet of *T. zillii*, this has also been found by Negassa and Getahun (2003) in an earlier study in Lake Ziway. The diet composition of *T. zillii* along with the δ^{15}N indicates that *T. zillii* is occupying the lowermost trophic level, together with *O. niloticus*. Tadiso et al. (2011) found that *T. zillii* had a lower mean δ^{15}N signal (8.4) compared to the signals found in this study, mean δ^{15}N signal (11.5). Nitrogen is not only an important nutrient but it is also abundant in sewage and chemical fertilizers such as ammonium sulfate. Intense anthropogenic activities may lead to rapid loading of nitrate which may have resulted in high δ^{15}N in the lake biota including fish. Such an increase of δ^{15}N is observed in *Chaenogobius isaza* a fish species collected from the north basin of Lake Biwa Isaza, Japan, and is associated with an increase in nitrate loading due to eutrophication (Ogawa et al., 2001). Since all species had higher δ^{15}N in 2008 (our study) compared to fish sampled in 2006 (Tadiso et al., 2011), and all samples were analyzed at the same laboratory, a possible explanation may be that the nitrogen levels have risen through increased use of fertilizers. The shrinkage of littoral macprophyte areas (personal observations), which play an important role in water purifications in Lake Ziway is also likely to be associated with an increased loading of nitrate.

Although fish were not found as part of the diet of *C. auratus*, this species has a δ^{15}N signature almost the same level as the partly piscivorous *C. gariepinus*, suggesting that *C. auratus* may include fish and carnivorous invertebrates in its diet at other times of the year. Stable isotope analyses provide a relatively longer-term and time integrated measurement compared to stomach content analysis which can be regarded as a snapshot in time of an organism's diet. As demonstrated in other studies (Creach et al., 1997), discrepancies between stomach content and stable isotope analysis is not uncommon, but may provide a better understanding of the trophic position of fish than either method can deliver in isolation.

The top consumer in Lake Ziway, in both the present study and according to Tadiso et al. (2011), is *C. gariepinus*. This species is not only entirely piscivorous but also includes both plants and invertebrates in its diet, as described by Bruton (1979) from Lake Sibaya. Larger individuals of *C. gariepinus* are more typically piscivores in Lake Ziway as well as in other localities (Bruton, 1978; Dadebo, 2000; Desta et al., 2007), and may explain the increase in δ^{15}N signatures with increasing size of *C. gariepinus. C.*

gariepinus from Lake Ziway had larger mean value of δ^{15}N than those found in Lake Awassa. This difference may be due to differences in the mean value of δ^{15}N in primary consumers at the base of the food web in the two lakes.

The δ^{13}C signatures of the studied fish species from Lake Ziway, in general indicate a wide range of carbon sources, from pelagic to littoral areas. However, *T. zillii* was found to inhabit the littoral area of the lake, apparently an ideal foraging ground for the fish to exploit the macrophytic vegetation. This is in line with the mean δ^{13}C values obtained in the study by Tadiso et al. (2011), which showed the preference of adult *T. zillii* to a littoral habitat. Moreover, the littoral vegetation presumably provides protection for juvenile fish from predation. Although there is no documented evidence about the predation risk of *T. zillii* in Lake Ziway, the presence of unidentified fish prey in the gut of *C. gariepinus* in our study suggest that *T. zillii* could have been one of the fish prey of *C. gariepinus*. A switch in the main food item is not unusual in *C. gariepinus,* the cichlid *O. niloticus* was the main fish prey of *C. gariepinus* in Lake Awassa in the years 1987–1988 (Dadebo, 2000). Desta et al. (2007) showed that the major prey fish utilized was the small barb *B. paludinosus* in the same lake in the years 2004–2005. The switch in prey fish species might be a response to a decline in the population density of *O. niloticus* due to their exploitation by the commercial fishery in Lake Awassa. This might also be the case in our study in Lake Ziway. *C. gariepinus* may have switched to feed on *T. zillii* in response to a decline in the population density of *O. niloticus* due to their exploitation by the commercial fishery in the lake. As *O. niloticus* and *C. auratus* presented similar mean carbon isotope ratios this indicates that these species foraged in the same area during a given season. This is also supported by the diet overlap of the two species, which shows both species include macrophytes, algae and detritus in their food.

4,4′-DDE is biomagnified in the food web of the lake, and thus concentrations increase as the trophic level increases. However, the biomagnification rate of 4,4′-DDE in the food web of Lake Ziway is lower than what has been reported from other areas (Table 4). This is presumably because tropical food webs are more

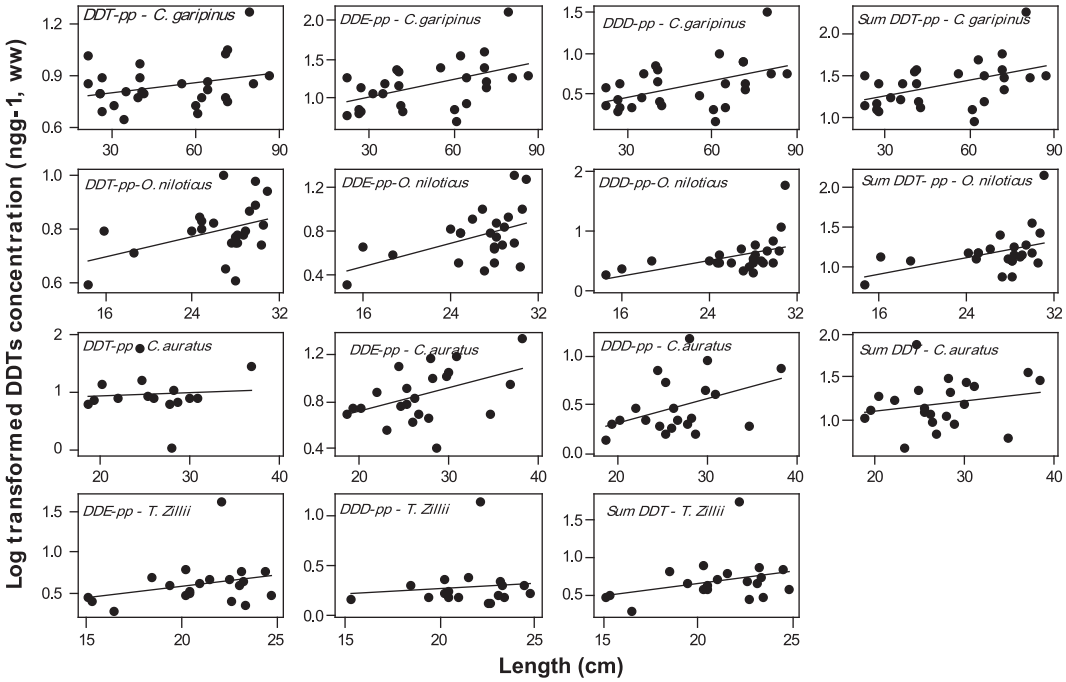

Fig. 5. The relationship between DDTs (4,4′-DDT, 4,4′-DDE, 4,4′-DDD and sum of DDT) concentrations in ng g^{-1}ww and length in cm of individual fish from Lake Ziway: (O, *O. niloticus;* Cl, *C. gariepinus;* Ca, *C. auratus;* B, *B. intermedius*).

Table 4
The slope and r^2 of the linear regression equation between log transformed DDTs concentration and stable isotope ratio of nitrogen (δ^{15}N, ‰) showing the biomagnification rate of DDTs in different regions.

Authors	Study Area	DDTs	Slope	r^2
Our study	Lake Ziway, Ethiopia	4,4′-DDE	0.077	0.099
Ikemoto et al. (2008)	Mekong Delta, South Vietnam	∑DDT	0.152	0.33
		4,4′-DDT	0.177	0.53
		4,4′-DDD	0.174	0.32
		4,4′-DDE	0.16	0.3
Hoekstra et al. (2003)	Southern Beaufort-Chukchi Seas, in the Arctic	∑DDT	2.95	0.31
		4,4′-DDE	5.35	0.48
		4,4′-DDT	1.53	0.05
Ruus et al. (2002)	Marine food web from southeastern Norway	∑DDT	0.95	0.72
Kidd et al. (2000)	Lake Malawi, East African tropical region	∑DDT	0.2	0.32
		4,4′-DDE	0.26	0.43
Kidd et al. (1998)	Sub arctic lakes in Yukon Territory	∑DDT	0.21–1.53	0.28–0.82

complex than temperate systems because of higher biodiversity, which likely promotes greater diversity of diets in the species. In addition, higher biomass or tissue turnover in tropics may decrease trophic magnification factors (TMFs) due to higher biomass dilution of contaminants. The results from the present study, albeit limited, call for a further study on the biomagnifications rate of organic contaminants in the tropics.

The lipid content seems to be an important variable for explaining the concentrations of 4,4′-DDE in the fish species of Lake Ziway. This was also found by Campbell et al. (2000) for concentrations of organochlorine in the food web of the subalpine Bow Lake, Banff National Park. Likewise, Kidd et al. (2000) found that lipid content was a better predictor of DDTs than δ^{15}N in fish from Lake Malawi. However, the concentration 4,4′-DDD in the Lake Ziway fish was not related to lipid content, probably due to the less lipophilic nature of 4,4′-DDD compared to 4,4′-DDE (the octanol-water partition coefficient of 4,4′-DDD and 4,4′-DDE is logK_{ow}=5.69 and logK_{ow}=6. 02, respectively). The lack of a significant relationship between the concentration of 4,4′-DDT and lipid content is probably due to the fact that 4,4′-DDT was below the detection limit in *T. zillii*, as well as in 36.4 percent of *C. auratus*, although 4,4′-DDT has the largest octanol–water partition coefficient (logK_{ow}=6.36).

4,4′-DDE was found to biomagnify and hence reached the highest concentrations in the piscivorous *C. gariepinus*. Moreover, the mean ratio of 4,4′-DDE to 4,4′-DDT was found to be larger in this species than in the other species, probably due to the higher lipid content of this species. Similar results were also obtained by Zhou et al. (2007) in fish species from Qiantang River, East China, probably as a result of more efficient transfer of DDT to phytoplankton and macrophyte consuming fish, while the piscivorous fish species feeding on these species accumulate a more DDE, a degradation product of DDT.

5. Conclusion

Although the low concentrations of DDT found in the fish species from Lake Ziway may indicate that the fish is safe for human consumption, the presence of at least one metabolite of DDT in all fish sampled from the lake, and the recent exposure of some of the fish indicate the current use of DDT in the study area. Paired stomach content and stable isotope analysis provides a better understanding of the trophic positions of the fish species studied from Lake Ziway that neither method can deliver in isolation. The present study demonstrates a biomagnification of persistent contaminants (e.g. DDTs) in tropical lakes, although this is lower than other areas. Although there are a number of potential reasons for the lower biomagnification rate, the main reason here is likely to be the complex food web, the species diversity and species abundance in tropical lakes result in different food resources with variable DDT burdens.

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