Bioaccumulation and trophic transfer of total mercury in the subtropical Olifants River Basin, South Africa

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Vera Verhaert[1*], Johannes Teuchies[1], Wynand Vlok[2], Victor Wepener[2], Abraham Addo-Bediako[3], Antoinette Jooste[3], Ronny Blust[1], Lieven Bervoets[1]

[1] Systemic Physiological & Ecotoxicological Research, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

[2] Unit for Environmental Sciences and Management, Water Research Group, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

[3] Department of Biodiversity, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa

*Corresponding author: Laboratory of Systemic Physiological and Ecotoxicological Research, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium. Tel: +32(0)32653541, Fax: +32032653497, vera.verhaert@uantwerpen.be; Lieven.Bervoets@uantwerpen.be

Abstract

The present study describes total mercury (THg) levels in surface water, sediment and biota from the Olifants River Basin (ORB) (South Africa) and investigates the trophic transfer of THg by means of trophic magnification factors (TMFs) in the subtropical ORB food web.

Although levels in surface water, sediment and invertebrates were low, elevated levels of THg were measured in fish species of higher trophic levels (0.10 to 6.1 µg/g dw). This finding supports the biomagnificative character of mercury. THg concentrations in fish from the present study were find to be higher than most values reported in fish from other African aquatic ecosystems and comparable or lower compared to more industrialized regions. Fish length, trophic level, sediment THg levels and TOC in sediment were determining factors for THg levels in fish tissue. Concentrations were found to be higher in larger (and older) fish. Mercury has a high affinity for organic matter and will bind with the TOC in sediment, thus reducing the bioavailability of THg for aquatic biota which is reflected in the significant negative correlation between THg and TOC in sediment. A significant positive relationship between

relative trophic level and THg concentrations was observed and also TMFs indicate biomagnification in the ORB food web. However, the trend of lower TMFs in tropical areas compared to temperate and arctic regions was not supported by the results. The consumption of fish from higher trophic levels at the average South African consumption rate is expected to pose a significant health risk.

Keywords

Total Mercury; Bioaccumulation; Trophic Magnification Factors; Subtropical Olifants River Basin

Capsule

This paper contributes to the knowledge gap on the fate and transport of THg in (sub)tropical aquatic ecosystems, specifically in Africa.

1. Introduction

Mercury (Hg) is a trace metal which occurs naturally in the earth's crust. Natural but in particular human activities are responsible for elevated Hg concentrations in the environment. Mercury has no essential biological function but due to its unique physical properties, it is being used in many industrial (coal burning), mining (gold) and medical applications (Gochfeld, 2003). In addition to local inputs into lakes and rivers, Hg is a toxic metal of global concern because it is prone to atmospheric transport with depositions on soil and surface waters far from its source (Kidd et al., 2004). In aquatic ecosystems, the elemental and inorganic forms of Hg are predominant. This Hg settles on the sediment, where a part reacts with sulfide to form an insoluble mercuric sulfide precipitate, whereas a smaller percentage is biomethylated by bacteria to methylmercury (MeHg) (Shao et al., 2012). It is this MeHg which is readily bioavailable for uptake by aquatic biota and which biomagnifies in the aquatic food chain. Between 75 and 95% of the total mercury (THg) in fish is present in the MeHg form. This biomagnification results in THg concentrations in fish from higher trophic levels which can be a million-fold greater than surface water concentrations (Gochfeld et al., 2003). The elevated THg levels in fish are a potential risk for both fish-eating wildlife and humans. The consumption of fish and shellfish contaminated with MeHg is the main human exposure route of Hg (WHO/FAO, 2007). A broad range of adverse health effects are associated with Hg. It may have toxic effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes (WHO/FAO, 2007).

To minimize the risk of Hg pollution for aquatic ecosystems and human health, a good understanding of the fate and transport of Hg in aquatic ecosystems is essential. Levels of THg in aquatic biota are

61 determined by two main processes: (1) the bioavailability in surface water and sediment which has the
62 potential to bioaccumulate in organisms at the base of the food web and (2) the efficacy of the uptake
63 of THg at the base of the food web and the transfer to higher trophic levels (Hanna et al., 2015). These
64 two processes are represented in a frequently used risk assessment tool to investigate biomagnification
65 of THg in aquatic ecosystems: Trophic magnification factors (TMFs). TMFs represent the average food
66 web biomagnification and are calculated as the antilog of the slope of the regression between log-
67 transformed concentrations of a pollutant and the relative trophic level of the consumers of the food
68 web. If TMFs are above 1, biomagnification through the food web occurs. The bioavailability is defined
69 by the intercept of the regression model of trophic level and the Hg concentration in biota. Bioavailability
70 is mainly affected by physico-chemical characteristics of the ecosystem such as pH, dissolved organic
71 carbon, total organic carbon content of the sediment, Hg methylation rates, total phytoplankton
72 biomass/growth and nutrients (Borgå et al, 2012; Lavoie et al., 2013, Hanna et al., 2015). The second
73 process, the efficacy of uptake and transfer is depicted as the slope of the regression and thus the
74 average biomagnification and TMF (Borgå et al, 2012). This process is influenced by the properties of
75 the organisms (lipid content, length and weight, growth rate, metabolic rates), characteristics of the
76 ecosystem (productivity, species diversity, length of the food chain) and properties of the chemicals
77 (Borgå et al, 2012; Lavoie et al., 2013; Hanna et al., 2015). It can be expected that climate influences
78 both processes at different levels (Lavoie et al., 2013; Hanna et al., 2015). Lavoie et al. (2013) and
79 Borgå et al. (2012) predicted lower TMFs in (sub)tropical aquatic ecosystem due to higher biomass
80 dilution of contaminants, because temperature, primary productivity, growth rates and biomass- and
81 tissue turnover are higher and food webs are generally more complex. But research on this topic is
82 mainly conducted in temperate and arctic regions and (sub)tropical ecosystems are underrepresented.
83 In addition, previous work on Hg levels in aquatic biota in Africa report lower Hg concentrations than
84 expected. More research on biomagnification of THg in (sub)tropical aquatic ecosystems and more
85 specifically in Africa may help to shed light on global patterns of TMFs and the potential anomaly in Hg
86 accumulation across the African continent (Hanna et al., 2015; Black et al., 2011).
87 This study reports the THg pollution status of the Olifants River Basin (ORB) in South Africa and
88 investigates the trophic transfer and biomagnification of THg in the subtropical aquatic ecosystem by
89 means of TMFs. Sources of Hg pollution in South Africa are emissions from coal-fired power stations
90 and artisanal gold mining. The majority of these mining areas are in close proximity to South Africa's

water resources, yet the extent to which Hg impacts aquatic ecosystems is largely unknown. In a review on mercury pollution in South Africa (Walters et al., 2011), the highest Hg levels in sediment were measured in the ORB. In addition, the authors stress on the considerable knowledge gaps on the fate and transport of Hg and propose further environmental and human health studies. In Africa, fish from inland water bodies are considered to be an important food source. Consequently, the consumption of contaminated fish is an important route of human exposure to Hg (Hanna et al., 2015; Gerber et al., 2016).

Specific objectives are (1) to produce a baseline dataset on THg in surface water, sediment and biota from the Olifants River Basin; (2) to investigate trophic transfer of THg through a subtropical freshwater food based on trophic magnification factors (TMFs); and (3) to determine the potential human health risk by consumption of THg contaminated fish. In this way, the mentioned knowledge gap on the fate and transport of THg in (sub)tropical aquatic ecosystems and more specifically in Africa, is addressed.

2. Materials and methods

The samples from the present study were collected during the same field campaign as in Verhaert et al. (2017).

2.1 Study area

The Olifants River basin (ORB) is a mining, agricultural and urban region situated in the north-east of South-Africa (Figure 1). The basin has been described as one of the most threatened in southern Africa (De Villiers and Mkwelo, 2009), although it has a key role in nature conservation, since the ORB is one of the main water sources for the Kruger National Park (KNP) and delivers important goods and services to the residing communities. Samples were collected from four locations: the Flag Boshielo Dam (FBD), the Phalaborwa Barrage (PB), Mamba Weir (MW) and the Olifants Gorge (OG). The locations were selected upon their position in the ORB: two upstream locations inside the mining, agricultural and urban region and two locations downstream, in the KNP. The Flag Boshielo Dam (FBD) is situated in the middle basin and is fed by the Olifants and Elands River (Figure 1). The dam was constructed to provide water for mining, urban, agriculture and recreation (IWMI, 2008). This dam area is characterized by large scale agricultural activities causing poor to unacceptable ecological status of the Elands River (De Villiers and Mkwelo, 2009). The other three sampling points are situated downstream in the lower basin. The Phalaborwa Barrage (PB) is built to provide drinking water as well as water for mining, urban and

industrial use (Buermann et al., 1995). PB is located next to a largescale copper and phosphorus mine (De Villiers and Mkwelo, 2009). Sluice gates of the barrage open at the bottom allowing deposited silt to be washed through, resulting in a high sediment flux into the KNP (Buermann et al., 1995). The two river sample points Mamba Weir (MW) and Olifants Gorge (OG) are both situated in the lower ORB within the KNP, where no discharge into the river system is allowed. MW is situated on the western boundary of the KNP, while OG is further east into the park, close to the border with Mozambique. Major crocodile and fish mortalities at OG demonstrated that the ecosystem functions of the river are disturbed, even within the KNP (Ashton, 2010; Van Vuuren, 2009). The ORB is characterized by a humid subtropical climate with high flow rates during hot and wet summers and low flow rates during mild to cool and dry winters. The average annual temperature is 22.4°C and the average annual rainfall is 561mm. More detailed information on geological, hydrogeological and climate regimes in the ORB is reported by eWISA (2010) and IWMI (2008).

2.2 Sample Collection

At each location, samples of surface water, sediment, invertebrates and fish were collected during the summer, high flow (April 2012) and the winter, low flow season (September 2012). Physio-chemical water quality variables including temperature (°C), pH, oxygen saturation (%), dissolved oxygen (mg/L) and conductivity (µS/cm^2) were measured in situ via a handheld WTW 340i multimeter at each location preceding sampling (Table S1).

For THg analysis in surface water, 3 replicates per sampling location were collected and directly filtered over a 0.20 µm cellulose acetate filter and stored at -20 °C until analysis.

At FBD and PB, sediment samples were taken with a Van Veen Grab from a boat. At MW and OG sediment was collected with a 50mL vial from the shallow river banks due to boat and wildlife constraints. At each location, three sediment samples were collected per location, immediately frozen and brought to the laboratory for analysis of total mercury and total organic carbon content (TOC).

Concerning invertebrates, dragonfly larvae (*Gomphidae*, Odonata) and the snail species *Tarebia granifera* (Thiaridae, Gastropoda) were collected with a sweep net (mesh size: 0.5mm) and kept frozen at -20°C until total mercury and stable isotope analysis.

All fish from FBD and PB were collected with gill nets (70 to 120 mm stretched mesh size). In MW and OG, *Clarias gariepinus* (Sharptooth catfish) and *Hydrocynus vittatus* (Tiger fish) were collected with

artificial lures and bait. All other fish species in these sites were collected by electrofishing. The following species were selected for analysis, based on their distribution throughout the study area: *Labeo rosae* (Rednose labeo), *Labeo congoro* (Purple labeo), *Synodontis zambesensis* (Plain squeaker), *Schilbe intermedius* (Silver catfish), *Labeobarbus marequensis* (Largescale yellowfish), *H.vittatus* (Tiger fish), *C. gariepinus* (Sharptooth catfish) and *Oreochromis mossambicus* (Mozambique tilapia). Fish length was measured (0.1cm) and weight (0.001g). The caudal muscle tissue and liver tissue were collected for THg and stable isotope analysis were performed on caudal muscle tissue. All fish samples were stored at -20°C prior to extraction.

2.3. Total Organic Carbon

For TOC, 3 sediment samples per location were analyzed. TOC was determined through Loss on Ignition (LOI). To this, the sediment samples (5-10g dw) were incinerated at 550 °C for 4 h, weight loss was determined and LOI was calculated (Heiri et al., 2001):

$LOI\ (\%) = (m_b – m_c / m_b - m_a) * 100$

with m_a = weight empty crucible (g), m_b = weight crucible + sediment sample before heating in muffle furnace(g), m_c = weight crucible + sediment sample after heating in muffle furnace

To calculate the total amount of organic carbon a conversion factor of 1.724 is used, assuming that organic carbon makes up 58% of the total organic matter content (Nelson and Sommers, 1996).

2.4. Total mercury (THg)

2.4.1. Sample preparation

In the laboratory, filtered surface water samples (10 mL) were acidified with 150 µL of pure HNO_3 (69%, Merck, Damstadt Germany) and stored at −20 °C until trace element analysis. For sediment and biota (fish muscle and liver and invertebrates), 0.5 g ww was accurately weighed on a Mettler AT261 DeltaRange® sensitive balance (accuracy of 0.01 mg). After freeze drying, the dry weight (dw) was determined. Samples were digested as described in Mataba et al. (2016). A volume of 500 µL of concentrated HNO_3 (69%), 1500 µL of HCl (37%), 200 µL of H_2O_2 and a magnetic stirrer were added to each freeze dried sample. Sample digestion (SP-Discover Microwave (CEM, USA)) was executed in two steps: (1) 120 °C, ramp time of 5 min, hold time of 5 min, maximum pressure of 34 bars at 300 W

and low stirring; (2) 160 °C, while the other parameters remained unchanged. Samples were diluted upon 5–6% acid for THg analysis.

2.4.2. Total mercury analysis

Analysis of THg in all samples was performed in cold plasma mode by a High Resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS) (Thermo scientific Finnigan element 2, Waltham, MA, USA). The method quantification limit (MQL) was 0.01 µg/L. For sediment, the limit of detection (LOD) is 0.0002 µg/g dw and for biota, the LOD is 0.001µg/g dw.

2.4.3. Quality Assurance and Quality Control (QA/QC)

For quality control, blanks and certified reference materials (CRM) were added every 20 samples and prepared in the same way as the samples. Reference materials were channel sediment (BCR 320R) and lyophilized Cod Muscle (BCR 422) from the Institute for Reference Materials and Measurements (IRMM, Geel Belgium) and freeze dried mussel tissue (no. 2976) from NIST (National Institute of Standards and Technology–USA). The measured concentrations agreed with the certified concentrations in the reference materials, ranging from 99 to 117%. All blanks were below the instrumental detection limit (IDL).

2.5. Stable Isotope analysis

Biota samples were dried at 60 °C, homogenized with a mortar and pestle into fine powder, weighed to the nearest 0.001 mg and encapsulated in pre-weighed 5 × 8 mm Sn capsules to determine $\delta^{13}C$ and $\delta^{15}N$. For the dragonfly larvae HCl was added to remove traces of non-dietary carbonates due to the presence of an exoskeleton (for this Ag cups were used instead) (Verhaert et al. 2013). Stable isotope measurements were performed using a Thermo Flash HT/EA coupled to a Thermo DeltaV Advantage IRMS with a Conflo IV interface at the Department of Earth and Environmental Sciences, KULeuven (Belgium). Stable isotope results are expressed using following formula:

$\delta^{13}C$; $\delta^{15}N = [(R_{sample}/R_{reference}) - 1] \times 1000$

with R = ratio $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen.

Data were calibrated using a combination of IAEA-C6, IAEA-N1, and acetanilide, which had been calibrated in house for $\delta^{13}C$ and $\delta^{15}N$. Estimated precision is generally better than 0.15‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Relative trophic levels were derived from animal $\delta^{15}N$ values using the following equation (Post, 2002):

$$TL_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/\Delta\ \delta^{15}N]+2$$

where $TL_{consumer}$ is the trophic level of the organism, $\delta^{15}N_{consumer}$ is $\delta^{15}N$ of the organism, $\delta^{15}N_{primary\ consumer}$ is the mean $\delta^{15}N$ of a local long-lived primary consumer, 2 is the trophic level of the primary consumer and $\Delta\ \delta^{15}N$ is the trophic enrichment factor, or the shift in $\delta^{15}N$ between two consecutive trophic levels (Post, 2002). In the present study, the primary consumer used as a baseline was the snail species *T. granifera.* A $\Delta\delta^{15}N$ trophic fractionation of 3‰ was used, as this is the most adequate estimate for non-acid treated muscle tissue (McCutchan et al., 2003; Vanderklift and Ponsard 2003). TMFs were based on THg concentrations and relative trophic levels, and were calculated from the slope of the regression of the log-transformed concentrations of pollutants versus trophic level calculated based on $\delta^{15}N$ (Borgå et al., 2012).

$$\text{Log [contaminant]} = a + b\ TL + \varepsilon \qquad TMF = 10^b$$

2.6. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, Inc) and the SPSS 15.0 statistical package. The level of statistical significance was defined at $p < 0.05$. For THg concentrations <IDL, the value of IDL/2 was incorporated. Prior to analyses, data was tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's tests) after which log transformation was applied to data sets where necessary. Differences in TOC levels, sediment THg levels and contamination levels per biota species for locations and seasons were tested with two-way ANOVA, followed by Post-Hoc Tukey test. One-way ANOVA was used for the comparison of THg contamination between species. A paired sampled T-test was performed for the identification of any significant differences in means between corresponding liver and muscle samples. Pearson's correlation coefficients were calculated for (1) TOC content in sediment and sediment contamination levels; (2) the relation between fish biological characteristics (lipid content, length, weight) and THg levels in biota tissue; (3) the relation between THg levels in sediment and biota tissue THg levels and (4) the relation between latitude and TMF. A multiple regression was conducted to investigate which independent

variables (length, weight, lipid content, trophic level, species, invertebrate THg levels, THg sediment concentrations and TOC content) are most determining for the fish THg levels. The relative importance of the different environmental factors was determined by a forward selection procedure. This was done by starting with a single factor, i.e., the THg concentration in the sediment, and stepwise adding the other terms and evaluating their contribution to the observed variation.

2.7. Human health risk assessment

The ATSDR (2016), USEPA (UNEP, 2008) and WHO/FAO (2007) defined respectively a Minimum Risk Level (MRL), Reference Dose (RfD), and Provisional tolerable daily intake (PTDI) for methylmercury. For fish, it is assumed that 80-100% of THg is methylmercury and a conversion factor of 1.0 has been suggested based on the MeHg/THg proportion (Power, 2002; EFSA, 2012). With these standards, the maximum amount of fish (g) which can be consumed by a person of 60 kg without potential human health risks is calculated based on the 50^{th} and 95^{th} percentile of measured THg concentration for all fish species sampled in the ORB. The following formula is used:

$Y = W \times M$; $Q = Y / C$; $Q = W \times M / C$

with:

Y (µg/day) = maximum amount of THg a 60 kg person can consume per day without posing health risk; M (µg/kg body weight/day) = Minimum Risk Level (MRL) for oral intake of THg; W (kg) = weight of an average person of 60 kg; Q (g) = maximum amount of contaminated fish muscle a 60 kg person can consume per day without posing health risks; C (µg/g ww) = 50th and 95th percentiles of the observed THg concentration in the fish muscle.

3. Results

3.1. Surface water and sediment

3.1.1. THg

Dissolved THg concentrations in surface water were all < MQL (0.01µg/L) in the present study. The THg levels in sediment samples ranged from 0.001 to 0.078 µg/g dw (Table 1). No significant interaction between seasons and locations was observed. But significant differences between locations were determined with higher THg levels in sediment from FBD and PB compared to MW and OG ($F_{3,13}$=9.1; p=0.0017) (Figure 2). No significant differences between seasons were found.

3.1.2. TOC

The total organic carbon content (TOC in %) in the sediment samples from the different sites ranged from 0.57% to 10% in the high flow season and 0.28 to 3.2% in winter low flow (Table 1). A significant interaction between seasons and locations was observed ($F_{2,8}$=42, p<0.0001). In the summer high flow season, TOC values in sediment from the dams FBD and PB were significantly higher than TOC in sediment from the river points MW and OG. In addition, TOC was higher in FBD than in PB (FBD > PB > MW = OG). In the winter low flow season, TOC levels in sediment from FBD were significantly higher than TOC in sediment from MW and OG. Per location, a significant difference between summer and winter was only observed for FBD with S>W.

A significant positive correlation between TOC (%) and the mercury concentrations in the sediment was observed (r(15)=0.86, r^2=0.74, p<0.0001, N=17) (Figure S1).

3.2. Invertebrates

The lipid content varied between 0.85% and 1.6% for *Gomphidae* larvae and between 0.82% and 1.3% for the snail *T. granifera,* with no significant difference among locations or between seasons (Table 2). THg levels in the snail species *T. granifera* ranged from 0.061 to 0.29 µg/g dw (0.014-0.086 µg/g ww). In *Gomphidae* larvae, levels ranged from 0.088 to 0.69 µg/g dw (0.014-0.14 µg/g ww) (Table 2, Figure 2). No significant differences between species per location were observed.

A significant interaction between seasons and locations was observed for *T. granifera* (F1,23=14; p=0.0011). In the summer high flow season, THg levels in *T. granifera* from FBD were significantly lower than in snails from MW and OG (F2,18=13; p=0.0003). In the winter low flow season, higher levels of

THg in *T. granifera* from OG compared to snails from MW were observed (t(11)=2.4; p=0.035). For MW and OG, significant differences between seasons of THg levels in *T. granifera* were tested for MW and OG. For MW, significant higher THg levels were observed in the summer high flow season (t(11)=2.9, p=0.014). In OG however, significant higher levels were determined in the winter low flow season (t(12)=2.6, p=0.024). For *Gomphidae* larvae, no significant differences in THg levels between seasons and locations were determined.

3.3. Fish

The lipid content percentage in fish muscle varied from 0.09% for *L. congoro* to 4.6% for *S. zambezensis* (Table 3). No significant difference in lipid content for the same species at different locations or seasons were identified.

Mercury levels in fish muscle tissue ranged from 0.10 to 6.1 µg/g dw (0.021-1.1 µg/g ww) (Table 3, Figure 2). At FBD, mercury levels in *S. intermedius* were significantly higher than in muscle tissue for *L. rosae, L. marequensis, S. zambesensis* and *O. mossambicus* ($F_{5,23}$=7; p=0.001). At MW, THg levels in *C. gariepinus* and *H. vittatus* were significantly higher than THg levels in *L. congoro* and *L. marequensis* ($F_{3,21}$=11; p<0.001). At OG, mercury levels in *C. gariepinus* and *H. vittatus* were significantly higher than levels in *L. congoro* ($F_{4,27}$=5; p=0.006). No clear trend in variation among locations and between seasons was observed for THg levels in fish.

The THg levels in liver tissue ranged from 0.081 to 3.5 µg/g dw (0.033 – 1.2 µg/g ww) (Table S2). A significant correlation between THg muscle tissue levels and liver tissue levels was found for *C. gariepinus* (r(15)=0.89, r^2=0.79, p<0.001, N=17), *L. marequensis* (r(8)=0.65, r^2=0.42, p=0.04, N=10), *S. zambesensis* (r(3)=0.92, r^2=0.85, p=0.03, N=5) and *H. vittatus* (r(8)=0.83, r^2=0.69, p=0.0028, N=10). Overall, no difference between THg levels in liver and muscle tissue was observed but per species, significant differences were found. For *C. gariepinus* and *H. vittatus*, the mercury levels in muscle tissue were higher than in liver tissue (t(16)=4, p=0.001 and t(9)=7, p<0.001, respectively). However, for *L. rosae* and *S. intermedius*, mercury concentrations in liver were significantly higher than in muscle tissue (t(5)=-3, p=0.026 and t(5)=-5, p=0.004).

3.4. Determining factors for THg levels in fish

Per species, the relationships between THg levels in fish and length, weight, lipid content, THg invertebrate levels, THg sediment levels and TOC content were determined. An overview of the significant correlations is given in Table S3. A significant positive correlation was found between length (cm) and THg (µg/g ww) for *C. gariepinus, S. intermedius* and *H. vittatus* (Figure S2). In addition, for *C. gariepinus* a significant positive correlation was observed for weight (g) and THg level (µg/g ww). A negative correlation was observed for lipid content (%) and THg (µg/g ww) for *L. marequensis.* In addition, a significant correlation was observed between THg levels in *L. marequensis* (µg/g ww) and mean THg levels in invertebrates (µg/g ww). Significant negative correlations were determined between THg levels in *C. gariepinus* and *L. marequensis* (µg/g ww) and sediment THg (µg/g dw) and TOC (%) levels (Figure S2).

Multiple regressions were run to investigate if THg levels in fish could be predicted from THg sediment concentrations or other biological and environmental characteristics (weight, length, lipid content, TL, species, THg in invertebrates and TOC content). THg levels (µg/g ww) could be predicted from a linear combination of sediment concentrations (µg/g dw), fish length (cm), TL and TOC content (%) (log-transformed data – $F_{4,67}$=12, p<0.001, R^2=0.41): $[THg_{fish}] = -2.2 + (0.12 * [THg_{sediment}]) + (0.35 * \text{fish length}) + (0.33 * TL) - (0.62 * TOC)$.

3.5. Trophic transfer of mercury through a subtropical food web

3.5.1. Trophic level

Ranges and median levels of nitrogen stable isotopes are presented in table 2 and 3. Trophic levels (TL) ranged from 2.0±0.1 for *T. granifera* to 4.0±0.6 for *S. intermedius*. On average, TL increased from detrivores to omnivores to piscivores (Figure 3).

3.5.2. Trophic transfer and trophic magnification factors

TMFs are based on the relation between the TL and the log contaminant concentration. Log THg concentrations expressed in both ww and dw were tested (ANCOVA, no significant differences in slopes between ww and dw values, not tested for THg levels in lipid weight (lw) since the majority of the literature reports in dw and ww, not in lw. In this way, a comparative study is possible). Results are presented in dw. When THg could be detected in both invertebrates and fish, the relationship between TL and log THg concentrations were tested (the case for FBD, MW and OG). At FBD, invertebrates could not be collected in the winter low flow season, so trophic transfer and TMFs are not calculated for

this season. In the summer high flow season at FBD, a significant relation between TL and THg was observed. At MW and OG, THg, was significantly related to TL in both seasons (Figure 4). Based on the slopes of these relationships, TMFs were calculated (Table 4). TMFs ranged from 1.9 to 4.2 and biomagnification is significantly higher in the winter low flow period than in summer high flow (MW $F_{1,41}$=9.0; p=0.0045 and OG $F_{1,53}$=5.9; p=0.018) (Figure 4).

The trophic magnification slopes (slope from the linear regression between log THg levels and $\delta^{15}N$ = TMS) of different studies worldwide reported in Lavoie et al. (2013) were transformed to TMFs with the formula $TMF = 10^{TMS*3‰}$ (3‰ is the enrichment factor between 2 trophic levels) (Figure 5). Only values from freshwater river ecosystems were used (Table S4). The relationship between latitude (absolute degrees) and TMFs of freshwater river ecosystems based on data of Lavoie et al. (2013) and present study was not significant ($F_{1,70}$=0.074; p=0.79).

3.6. Minimum Risk Levels for Human Health

Table 5 presents the maximum amount of fish (g) which can be consumed by a person of 60 kg without potential human health risks calculated with the 50th and 95th percentile of the measured concentrations of THg for all fish species sampled in the ORB. A person of 60 kg can consume only 6.4 g of *C. gariepinus*, 7.0 g of *S. zambesensis* and *H. vittatus* and 16 g of *S. intermedius*, without posing a potential human health risk following the USEPA RfD (Table 5).

4. Discussion

4.1. Surface water and sediment

4.1.1. THg

Dissolved surface water THg levels were < LOQ (0.01 µg/L). This is in contrast to levels up to 0.43 µg/L reported from a wetland adjacent to a petrochemical industry in central South Africa (De Klerk et al., 2013). Regarding sediment, a review on mercury pollution in South Africa reports that the Upper Olifants River had amongst the highest measured sediment THg values of South Africa with values exceeding 0.05 µg/g dw (Walters et al. 2011). The present study shows that THg levels in sediment from the dams, located in the populated area with agriculture and industry, (FBD and PB) are significantly higher than in the remote sampling points located downstream, in the Kruger National Park (MW and OG). Although levels in FBD and PB are elevated, with values up to 0.078 µg/g, these levels are still lower than THg

concentrations in sediment from African rivers close to gold mining areas (Taylor et al., 2005 (0.66 µg/g dw); Mataba et al., 2016 (0.34 µg/g dw)) and petrochemical industries (De Klerk et al., 2013 (3.8 µg/g dw)). In general, THg levels from the present study were observed to be in the same range as found in other African aquatic ecosystems (Mataba et al., 2016; Ouédraogo et al., 2015) but lower than concentrations from Europe and US (Gati et al., 2016; Domagalski, 2001; Van Ael et al., 2014).

4.1.2. TOC

Sediment characteristics, such as total organic carbon (TOC) are imperative in the fate and retention of THg in the sediment with expected higher sediment THg levels when TOC content increases due to the availability of more strong binding sites (Miglioranza et al., 2002; Munn and Gruber, 1997; Chakraborty et al. 2015; Wu et al. 2013). In the present study a positive relationship between TOC and THg was found in the sites PB and FBD.

4.2. Invertebrates

To our knowledge, research on mercury levels in African invertebrates is limited which makes a comparison difficult. However, our results were in the same range as found in gastropods of different reservoirs in Burkina Faso (Ouédraogo et al.,2015) and gastropods from the Nikonga River, far downstream of a gold mining area in Tanzania (Taylor et al., 2005). No clear trends in differences between locations and seasons were observed for THg levels in invertebrates.

4.3. Fish

Previous studies on the fate and transport of mercury in African aquatic ecosystems reported unexpected low concentrations which is referred to as the tropical African mercury anomaly (Black et al. 2011; Hanna et al., 2015; Poste et al., 2015). The processes and patterns behind this African mercury anomaly are still unclear which underlines the importance of mercury research in (sub)tropical aquatic ecosystems. THg concentrations in fish from the present study are generally higher than in fish from other African aquatic ecosystems. Table S5 shows mean THg levels in fish with similar TLs from different African freshwater ecosystems. In a review of Walters et al. (2011) on mercury pollution in South Africa, fish MeHg levels ranged from 0.00040 to 0.27µg/g ww, which is a factor 4 lower than the THg levels from present study, taking into account that the proportion of MeHg in total Hg is usually within the range

411 of 80-99% for fish muscle tissue (Power er al., 2002). When compared to THg fish levels from studies
412 in Europe and the sub-Arctic, levels of the present study were lower or in the same range (Carrasco et
413 al., 2011; Power et al, 2002; Nguetseng et al. 2015). The Environmental Quality Standard (EQS) for
414 THg in biota from the European Commission is 0.02 µg/g ww. This biota EQS is set to protect top
415 predators (birds and mammals including humans) against secondary poisoning through consumption of
416 contaminated aquatic biota (EC, 2014). Up to 94% of the fish from present study has THg levels which
417 exceeds this biota THg EQS. However, Chvojka (1990) divided fish THg concentrations in 5 categories,
418 i.e. 50-150 ng/g ww (very low), 150-250 ng/g ww (low), 250-350 ng/g ww (medium), 350-450 ng/g ww
419 (high) and >450 ng/g ww (very high). Applying this classification, in the present study, 61% of the fish
420 has very low concentrations, 17% low, 5% is categorized as medium, 7% is high and 10% of the fish
421 have very high THg concentrations. The fish species from the category 'very high' had the highest trophic
422 levels (*C. gariepinus, H. vittatus and S. zambezensis*) indicating that mercury is biomagnificative.
423 Significant differences in THg levels among species were found. Species of higher trophic levels (*S.*
424 *intermedius, C. gariepinus* and *H. vittatus)* contained significantly higher THg levels than fish from lower
425 trophic levels (*L. rosae, L. marequensis, S. zambesensis* and *L. congoro)*. This finding also supports the
426 biomagnificative character of mercury.
427 THg levels in liver were not always higher than in muscle tissue, but differences were species-specific.
428 Higher levels of THg in muscle of *L. victorianus* compared to liver was also observed by Mataba et al.
429 (2016). It was previously reported that feeding habits have an effect on the THg organ distribution with
430 piscivorous fish having higher liver THg levels than non-piscivores (Havelková et al., 2008). However,
431 in present study, the only piscivore fish, *H. vittatus,* was found to have higher concentrations in muscles
432 compared to liver.
433
434 **4.4. Determining factors for THg levels in fish**
435 Biological characteristics and environmental characteristics, including fish length, fish weight, lipid
436 content, THg invertebrate levels, THg sediment levels and TOC content in sediment are previously
437 reported as determining factors for THg bioaccumulation (Kidd et al., 2003). Compared to Campbell et
438 al. (2004) and Kidd et al.(2003) increasing THg levels with increasing fish size was found in species of
439 higher trophic levels (*C. gariepinus, S. intermedius* and *H. vittatus)*. Mercury has a high affinity for
440 organic matter and will bind with TOC in sediment, reducing the bioavailability of THg for aquatic biota

(Shao et al., 2012; Taylor et al., 2012). This is reflected in the significant negative relationships between THg levels and TOC in sediment and THg levels in *C. gariepinus* and *L. marequensis*.

The contribution of biological and environmental factors on the variation in THg concentration in the ORB fish was tested with a multiple regression model. Weight, length, lipid content, TL, species, THg in invertebrates and TOC content were considered. The variation in fish THg levels was explained for 41% by sediment THg levels, fish length, TL and TOC content. Significant single correlations between sediment THg levels, length and TOC content and fish THg levels per species were already observed. So the multiple regression confirms the importance of these factors to the THg fish tissue levels. The relation between THg fish levels and trophic level was observed to be important. This will be discussed in 4.5.

Although THg levels in surface water and sediment were low, relatively high bioaccumulation of THg in fish was observed. Knowing that the proportion of MeHg in THg is within 80-99% in fish (Power er al., 2002), the high bioaccumulation could be due to favorable conditions in our study area for the methylation of Hg. Biomagnification results in THg concentrations in fish from higher trophic levels which can be a million-fold greater than surface water concentrations (Gochfeld et al., 2003).

When elemental and inorganic Hg enter the aquatic ecosystem, it often accumulates in the sediment, where it can react with sulfides to form an insoluble HgS precipitate, whereas a smaller percentage is biomethylated by bacteria (Shao et al., 2012). It is this MeHg which is rapidly bioavailable for aquatic organisms and biomagnifies in the food chain. The biotic methylation process is controlled by different factors including temperature, pH, redoxpotential, presence of complexing agents, organic matter, sulfur, oxygen availability, … (Paranjape and Hall, 2017). In this light, it is interesting to note that the presence of dams on the Olifants River may stimulate the methylation of Hg. Dams can seasonally become stratified. This creates anoxic environments which are especially favorable for methylation and bioaccumulation of Hg. Recent studies have shown that river impoundment can often have greater effects on the downstream river ecosystem than on the reservoir itself (Kasper et al., 2014).

4.5. Trophic transfer of mercury through a subtropical food web

4.5.1. Trophic level

The ratio of ^{15}N to ^{14}N ($\delta^{15}N$) is a powerful tool to estimate the trophic position of organisms since stable nitrogen isotope ratios of consumers are typically enriched by 2-4‰ relative to their diet (Layman et al.,

2012). The trophic levels from present study correspond well with the levels reported on Fishbase (www.fishbase.org) for *L. marequensis, C. gariepinus, S intermedius and H vittatus*. According to Fishbase, *S. zambesensis* and *L. rosae* have a trophic level of 2.7±0.34 and 3.5±0.29 respectively, which is slightly lower than trophic levels determined in the present study. But on average, trophic levels increased from detrivores to omnivores to piscivores.

4.5.2. Trophic transfer and trophic magnification factors

The calculated TMFs > 1 indicate biomagnification of THg in the food web of the Olifants river. TMFs for THg exceeding 1 are found in most studies worldwide and our values are consistent with other values reported in (sub)tropical African aquatic ecosystems (Poste et al., 2008; Campbell et al., 2008; Campbell et al., 2004; Campbell et al., 2003; Kidd et al., 2003; Kidd et al., 2004; Tadiso et al., 2011; Ouédraogo et al., 2015).

Previous research predicted that ecosystems in tropical regions have lower TMFs compared to those in temperate and Arctic regions due to differences in temperature (Borgå et al., 2012; Lavoie et al., 2013; Poste et al., 2015). Lavoie et al. (2013) described 3 mechanisms that could explain this temperature effect: (1) Growth biodilution is higher in tropical regions because of higher growth rates, (2) warmer temperatures result in higher excretion rates of Hg and (3) more complex food webs in tropical regions (high species diversity) could result in lower Hg biomagnification compared to basic food webs in arctic aquatic ecosystems. However, large variation in TMFs are existing. Type of ecosystem (lake – river – marine), seasonal differences or physicochemical site characteristics are expected to have an influence on TMFs. In the present study, TMFs in the cooler winter were found to be significantly higher compared to summer values which is possibly related to lower biomagnification and higher biodilution and excretion rates in summer. Yet, on a worldwide scale, no significant relationship (was found between the latitude (absolute degrees) and TMF of freshwater river ecosystems, based on data of Lavoie et al. (2013) and present study (Figure 5).

4.6. Minimum Risk Levels for Human Health

Fish is an essential food source for rural communities inhabiting the OR catchment (Gerber et al., 2016). Since THg is accumulated and biomagnified in the food web of the Olifants River, also human

consumers can be at risk (Du Preez et al., 2003; Afful et al., 2010). The consumption of fish and shellfish, contaminated with methylmercury is the main human exposure route to mercury (WHO/FAO, 2007). A broad range of adverse health effects are associated with mercury, including effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin and eyes (WHO/FAO, 2007).

The FAO (2010) estimated the average fish consumption rate of the South African population on 21 g per day. Given the THg concentrations of the present study, a potential risk resulting from consumption of fish originating from the OR is present. For fish belonging to higher trophic levels (*C. gariepinus, S. zambesensis, S. intermedius,* and *H. vittatus*), less than the average consumption rate of the South African population (21 g per day) can be consumed. Using the most stringent minimum risk levels to prevent human health from the USEPA, a maximum of 6.4 g of *C. gariepinus* could be consumed on a daily basis. Furthermore, it can be expected that subsistence fishermen and people living along the shores of the river and dams consume more fish than the average South African fish consumption set by the FAO. Additionally, larger fish are expected to be selected for consumption while THg levels were found to increase with length which might result in higher uptake rates. Fish length and species can be easily identified by the subsistence fishermen and could be used to help individuals minimize Hg intake by selecting fish for consumption by species (lower TL) and size (smaller fish) (Hanna et al., 2015).

5. Conclusions

THg levels in sediment from the upstream sites, located in the mining, agricultural and urban region were higher than in the sites located in the Kruger National Park. The overall THg levels in sediment however, were low and comparable to other African aquatic ecosystems.

The present study confirms the previously reported biomagnificative character of Hg. Although THg levels in surface water, sediment and invertebrates are low, THg levels in fish from higher trophic levels were significantly higher than in species from lower levels of the food web.

In addition, trophic transfer of THg was observed and TMFs were > 1, so biomagnification occurred in the food web of the ORB. THg TMFs were consistent with other observations in (sub)tropical African aquatic ecosystems. Due to this biomagnification, a potential risk of THg contamination resulting from consumption of fish originating from the ORB is present.

6. Acknowledgements

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539 Tables

540

541 Table 1: The number of samples analyzed (N), ranges and median levels of Total Organic Carbon
542 content (TOC%) and THg (µg/g dw) in sediment from the Olifants River Basin, South Africa.

Location	N	TOC %		N	THg	
		S	W		S	W
FBD	3	8.2-10	3.0-3.2	3	0.007-0.046	0.006-0.048
		8.3	3.0		0.043	0.028
PB	3	4.8-5.1	-	3	0.033-0.078	-
		5.0			0.037	
MW	3	0.57-0.65	0.42-0.47	2	0.001-0.004	0.002-0.003
		0.61	0.42		0.002	0.002
OG	3	1.1-1.3	0.28-0.34	2	0.009-0.016	0.001-0.002
		1.2	0.31		0.012	0.001

543 <LOQ: below limit of quantification; - No sample collected; S: Summer High Flow Season; W: Winter Low Flow Season

544

545

546 Table 2: The number of samples analyzed (N), lipid levels (%) and ranges and median levels of $\delta^{15}N$
547 (‰) and THg (µg/g dw) of invertebrates from the Olifants River Basin, South Africa. Also the dw/ww
548 ratio is presented.

Species per Location	N	Lipid content		N		δ15N		dw/ww		THg	
		S	W	S	W	S	W	S	W	S	W
Gomphidae											
FBD	1*	0.85	-	4	-	9-11	-	6.1	-	0.088-0.69	-
						10		6.1		0.21	
PB	1*	0.82	-	6	-	11	-	3.1-6.8	-	0.27-0.55	-
						11		4.5		0.36	
MW	1*	1.4	1.6	4	7	10-12	12-13	7.6	6.0-9.0	0.12-0.33	0.13-0.22
						11	12	7.6	7.6	0.25	0.16
OG	1*	1.5	1.1	8	7	11-12	10-12	5.2-7.6	4.9-5.9	0.15-0.35	0.15-0.22
						11	12	6.2	5.3	0.21	0.17
Tarebia granifera											
FBD	1*	1.10	-	7	-	8.0-8.8	-	4.1-4.6	-	0.061-0.15	-
						8.4		4.3		0.085	
MW	1*	1.2	1.3	4	3	10-11	12	3.2-6.2	3.2-3.9	0.14-0.26	0.11-0.17
						10	12	5.1	3.5	0.20	0.14
OG	1*	1.3	1.05	7	7	10-11	12	5.2-6.5	3.2-3.7	0.099-0.19	0.11-0.29
						11	12	6.1	3.4	0.13	0.22

549 <LOQ: below limit of quantification; - No sample collected; S: Summer High Flow Season; W: Winter Low Flow Season; *N=1
550 samples are pooled for invertebrates;

551

552

553

554 Table 3: The number of samples analyzed (N), ranges and median lipid levels (%), $\delta^{15}N$ (‰) and THg
555 (µg/g dw) of muscle tissue of fish from the Olifants River Basin, South Africa. Also the dw/ww ratio is
556 presented.

	N		Lipid%		$\delta^{15}N$		N		dw/ww		THg	
	S	W	S	W	S	W	S	W	S	W	S	W
Clarias gariepinus												
FBD	3	-	0.23-0.32	-	13-16	-	2	-	5.6	-	0.23-0.91	-
			0.26		15				5.6		0.57	
PB	3	-	0.25-0.47	-	14-16	-	3	-	5.6-6.5	-	0.39-1.4	-
			0.27		14				5.9		0.55	
MW	3	3	0.40-0.54	0.20-0.40	15-16	15-16	3	3	4.4-5.4	5.4-5.8	0.63-1.1	0.55-2.1
			0.54	0.36	15	16			5.1	5.4	0.93	1.1
OG	3	3	0.22-0.30	0.17-0.31	11-14	17	3	3	5.2-5.6	4.8-5.3	0.54-1.8	3.84-5.0
			0.27	0.28	12	17			5.5	5.1	1.3	4.5
Labeo rosae												
FBD	3	3	0.30-0.38	0.12-0.54	12-13	12-14	3	3	5.4-6.11	5.2-5.6	0.24-0.32	0.28-0.39
			0.31	0.22	13	14			5.4	5.3	0.31	0.33
Labeo congoro												
MW	3	4	0.09-0.54	0.12-0.65	11-14	12-14	3	3	4.7-4.8	4.8-5.0	0.10-0.14	0.32-0.53
			0.22	0.33	14	13			4.7	5.0	0.13	0.37
OG	3	3	0.16-0.35	0.39-0.75	11-18	13-14	3	3	4.8-5.3	4.6-5.4	0.12-0.29	0.33-0.39
			0.27	0.71	14	13			5.0	4.7	0.15	0.36
Labeobarbus marequensis												
FBD	-	3	-	1.2-2.2	-	13-17	-	3	-	5.1-5.6	-	0.23-0.34
				1.5		16				5.2		0.32
MW	3	2	1.1-2.1	0.72-1.8	12-14	14-15	3	3	3.2-5.3	4.2-4.8	0.14-0.37	0.26-0.81
			1.1	1.3	13	15			4.3	4.7	0.32	0.37
OG	3	-	0.53-1.5	-	12-13	14-15	2	3	4.3-4.4	4.7-5.1	0.36-0.47	0.78-1.13
			0.92		13	15			4.4	4.9	0.41	0.88
Synodontis zambezensis												
FBD	3	-	1.4-4.6	-	12-14	-	3	-	4.0-5.5	-	0.33-0.61	-
			1.7		14				4.7		0.37	
OG	2	2	0.51-0.92	0.60-1.6	14	15	2	3	4.5-5.3	5.3-5.4	0.84-1.19	0.55-6.1
			0.72	1.1	14	15			4.9	5.3	1.0	0.81
Schilbe intermedius												
FBD	3	3	0.44-1.7	0.09-0.28	15-17	12-14	3	3	5.3-5.8	5.3-6.2	1.2-2.2	0.75-0.99
			0.86	0.19	16	13			5.7	5.9	1.4	0.77
Hydrocynus vittatus												
MW	1	3	0.87	1.4-20	17	17-18	1	3	4.7	3.6-4.7	1.2	1.2-1.7
			0.87	1.4	17	18			4.7	4.2	1.2	1.2
OG	3	3	0.31-0.68	0.32-1.3	13-18	18-19	3	3	4.2-4.5	4.2-4.6	2.0-3.5	2.5-4.0
			0.66	0.75	13	18			4.3	4.6	2.7	3.5
Oreochromis mossambicus												
FBD	3	-	0.49-1.6	-	-	-	4	-	4.9-5.5	-	0.11-0.16	-
			0.88						5.0		0.13	

557 < LOQ: below limit of quantification; - No sample collected; S: Summer High Flow; W: Winter Low Flow Season
558

Table 4: Slope, r^2, and p-value of the regression analysis between TL and the logarithm of the contaminant concentration and TMFs per location.

	Summer High Flow				Winter Low Flow			
	Slope	**r^2**	**p**	**TMF**	**Slope**	**r^2**	**p**	**TMF**
FBD	0.34	0.53	<0.0001	2.2	No data			
MW	0.29	0.43	0.0017	1.9	0.57	0.81	<0.0001	3.7
OG	0.39	0.47	<0.0001	2.5	0.62	0.85	<0.0001	4.2

Table 5: Maximum amounts of fish (kg) which can be consumed by a person of 60 kg without potential human health risks based on MRL (ATSDR, 2010), Rfd (UNEP, 2008) and PTDI (WHO/FAO, 2007) and taken into account the 50th and 95th percentile of observed concentration of THg (µg/g ww) for all fish species sampled in Olifants River, South Africa. Maximum edible amounts which are less than the average fish consumption rate of the South African population (0.021 kg) are indicated in bold.

Fish sepcies	Concentration Fish (µg/g ww)		Maximum edible amount (kg/day/60 kg person)					
			ATSDR (2016)		USEPA		WHO/FAO (2007)	
	50th	95th	50th	95th	50th	95th	50th	95th
Clarias gariepinus	0.21	0.94	0.086	**0.019**	0.029	**0.0064**	0.066	**0.015**
Labeo rosae	0.055	0.07	0.33	0.26	0.11	0.086	0.25	0.2
Labeo congoro	0.058	0.093	0.31	0.19	0.1	0.065	0.24	0.15
Labeobarbus marequensis	0.083	0.21	0.22	0.088	0.072	0.029	0.17	0.067
Synodontis zambesensis	0.13	0.82	0.14	**0.022**	0.045	**0.007**	0.1	**0.017**
Schilbe intermedius	0.19	0.38	0.095	0.047	0.032	**0.016**	0.073	0.036
Hydrocynus vittatus	0.51	0.85	0.035	**0.021**	**0.012**	**0.007**	0.027	**0.016**
Oreochromis mossambicus	0.024	0.03	0.75	0.6	0.25	0.2	0.57	0.46

Figures

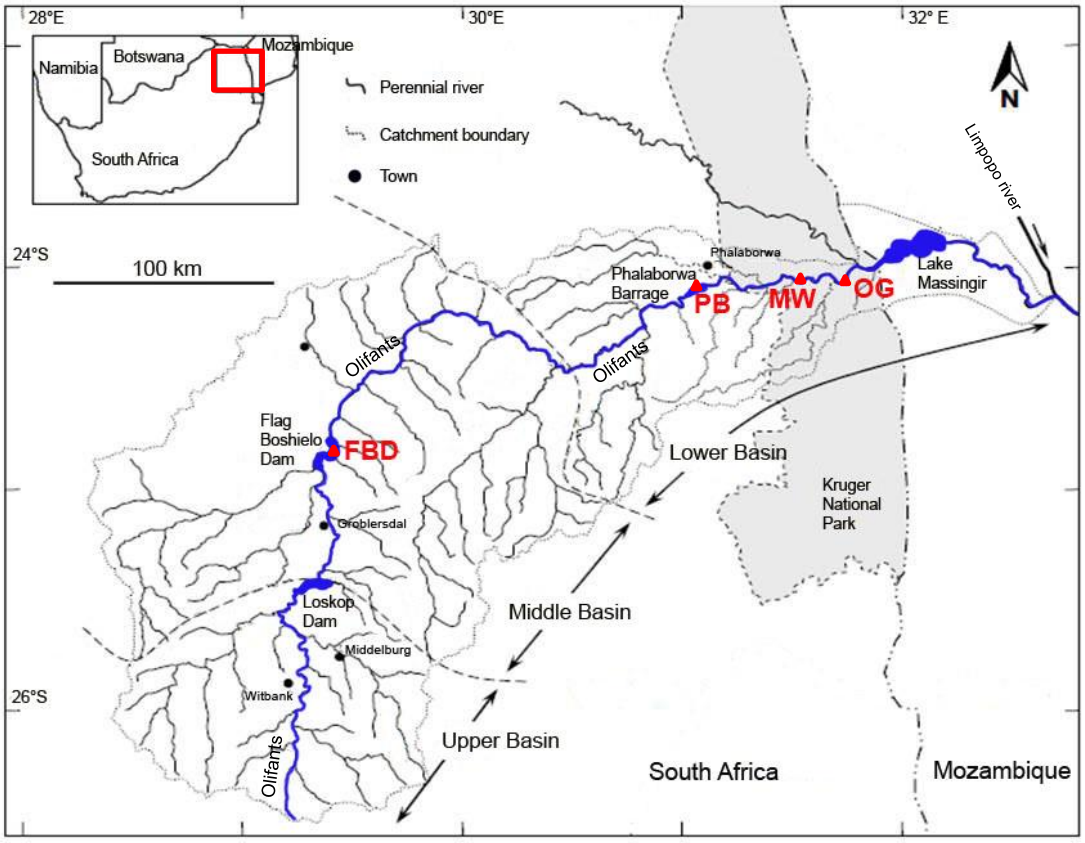


Figure 1: Adapted from Ashton (2010) illustrating the Upper, Middle and Lower Sub Basins of the Olifants River Catchment, South Africa. Sampling locations are indicated in bold: Δ FBD: Flag Boshielo Dam, Δ PB: Phalaborwa Barrage, Δ MW: Mamba Weir, Δ OG: Olifants Gorge.

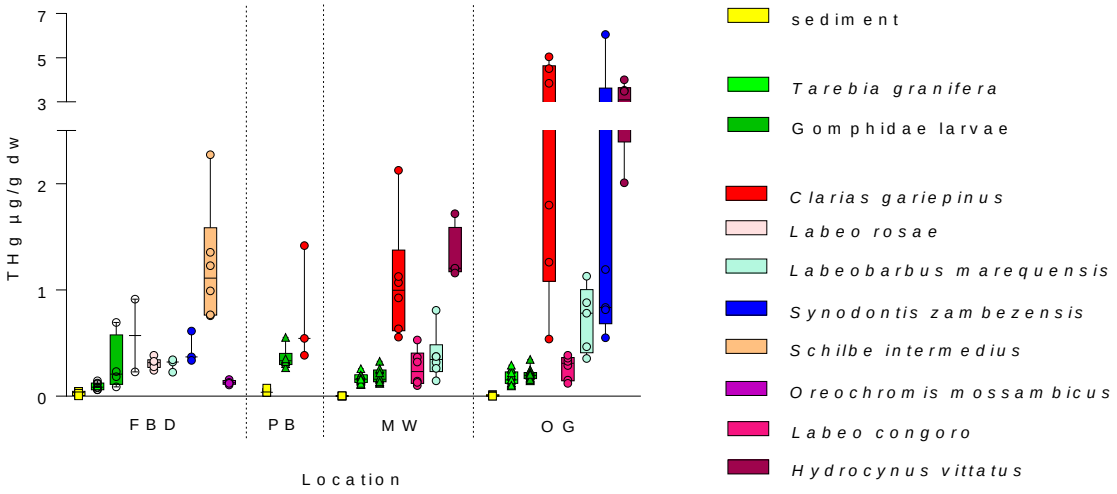
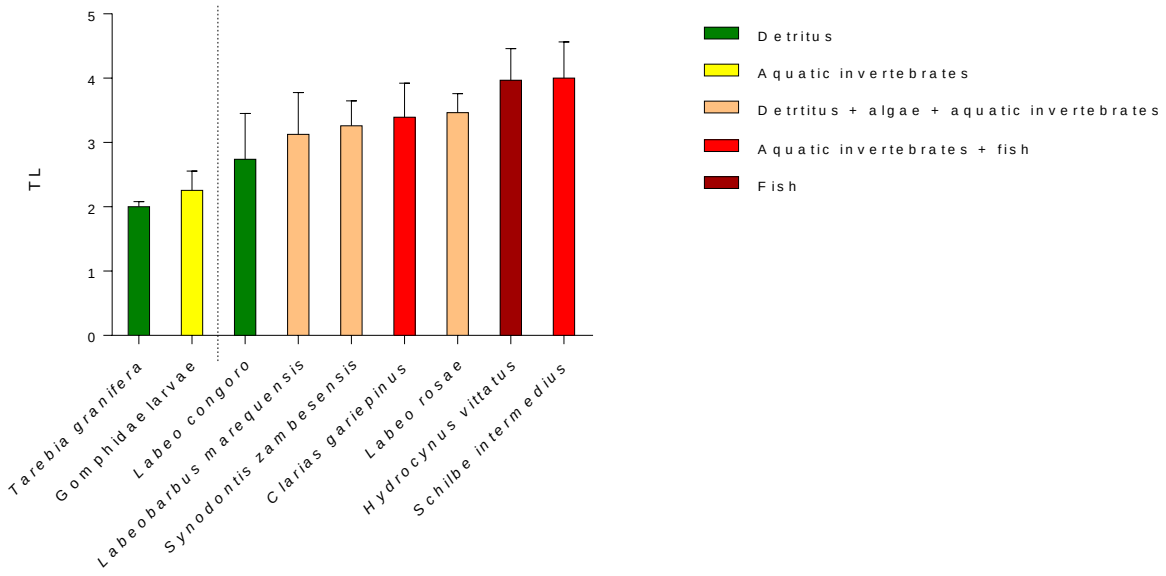


Figure 2: Boxplot of THg in sediment, invertebrate and fish species (µg/g dw) per location (Box: 25-75 percentiles, line: median, whiskers: minimum and maximum, each individual value was plotted)

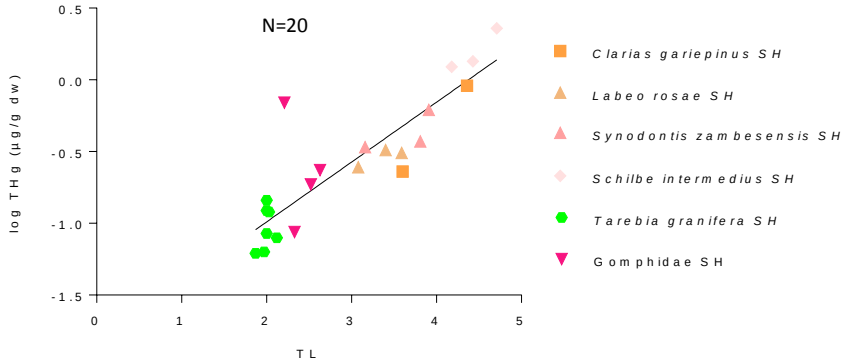


615

616 Figure 3: Mean trophic level per species for all locations and seasons together. Different colours
617 represent feeding mode (Error bars represent SD). Dotted line separates invertebrates from fish species.

618

A.



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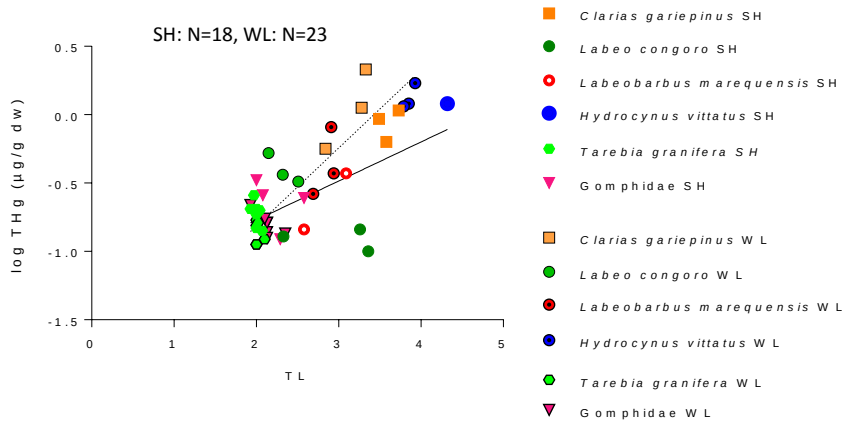

C.

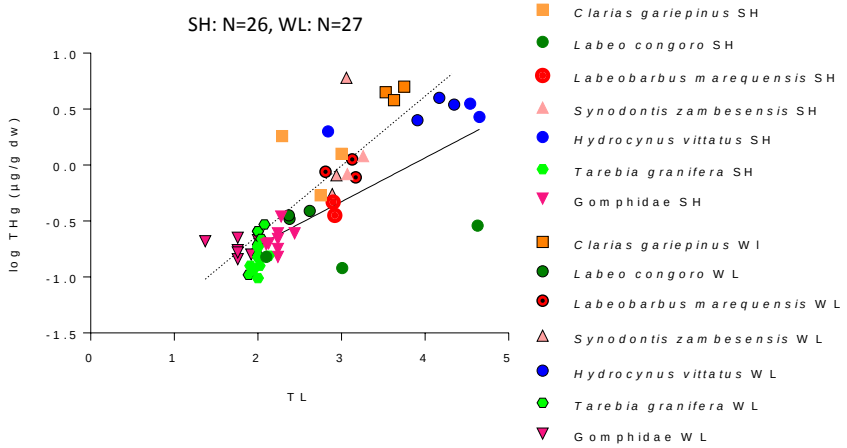

Figure 4: Relationship of TL and log concentrations of THg of different food webs in the summer high flow (———) and winter low flow season (- - - -) at FBD (A), MW (B) and OG (C).

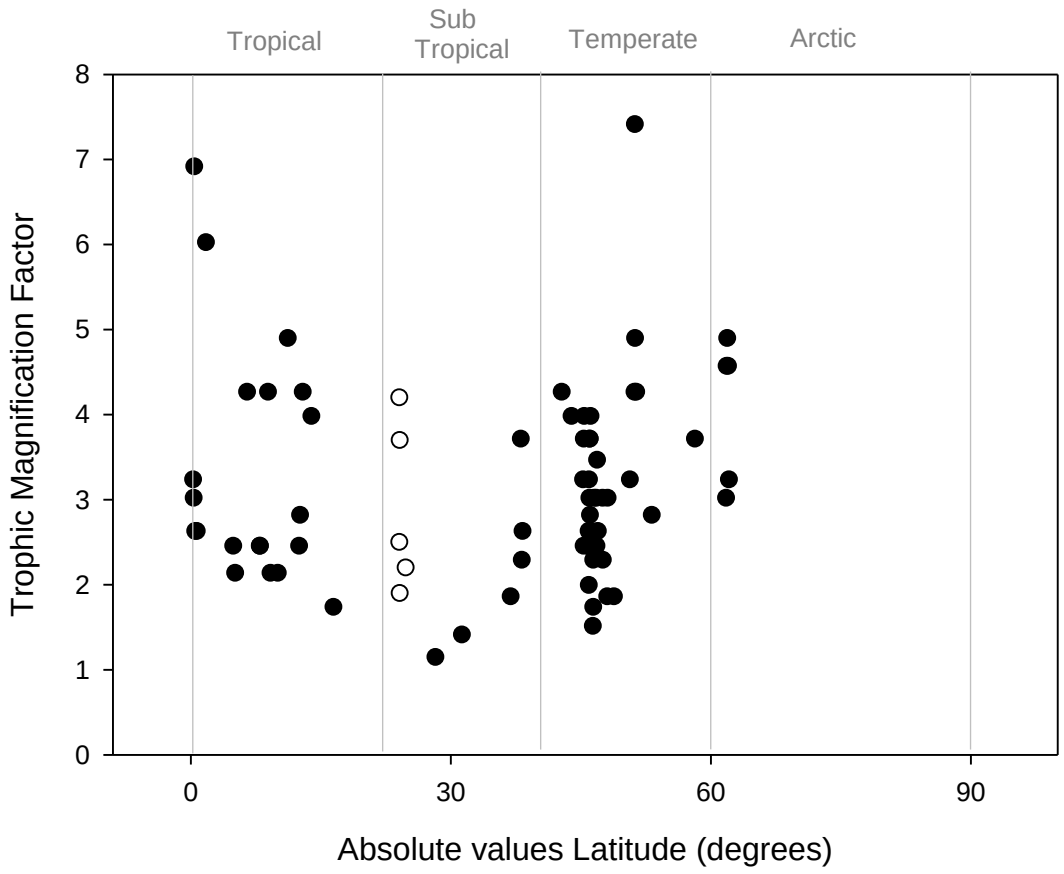

Figure 5: Relationship between THg TMFs against latitude for freshwater ecosystems of different climates. Data from present study (○) and data obtained from Lavoie et al. (2013) (●), transformed to TMF and Latitudes changed to absolute values. Climate regions were divided according to latitudes (absolute values) with 0 to 23°30' tropical, 23°30' to 40° subtropical, 40° to 60° temperate and 60° to 90° Arctic climate.

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