



Increasing mercury bioaccumulation and biomagnification rates of Nile perch (*Lates niloticus* L.) in Winam Gulf, Lake Victoria, Kenya

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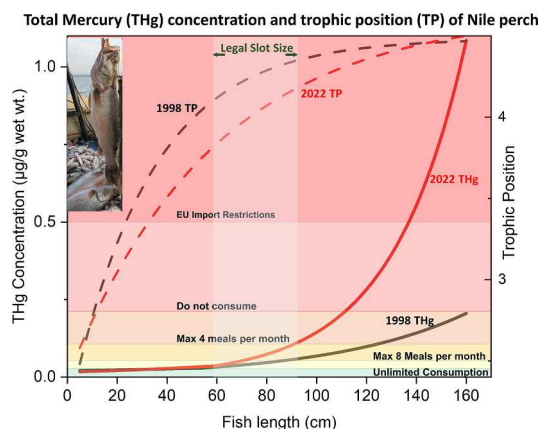
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HIGHLIGHTS

- Nile perch Hg bioaccumulation and biomagnification rates increased over time.
- Fish consumption advice for Nile perch became more restrictive over time.
- Increased bioaccumulation was due to reduced growth, not change prey Hg levels.

GRAPHICAL ABSTRACT



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ABSTRACT

The Nile perch (*Lates niloticus* L.) commercial fishery for Lake Victoria in East Africa is an important source of revenue and employment. We focused on shifts in food web structure and total mercury (THg) bioaccumulation and biomagnification in Nile perch, and lower food web items collected from Winam Gulf (Kenya) sampled 24 years apart (1998 and 2022). Stable isotope carbon ($\delta^{13}\text{C}$) values were higher in all species from 2022 compared

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to 1998. Stable nitrogen isotope ($\delta^{15}\text{N}$) values in baseline organisms were lower in 2022 compared to 1998. In Nile perch, $\delta^{15}\text{N}$ values were correlated with total length, but the $\delta^{15}\text{N}$ -length regressions were steeper in 1998 compared to 2022 except for one large (158 cm) Nile perch from 1998 with an uncharacteristically low $\delta^{15}\text{N}$ value. Total Hg concentrations were lower in lower trophic species from 2022 compared to 1998. However, the THg bioaccumulation rate (as a function of fish length) in Nile perch was greater in 2022 compared to 1998 resulting in 24.2 % to 42.4 % higher wet weight dorsal THg concentrations in 2022 Nile perch for market slot size (50 to 85 cm) fish. The contrasting observations of increased THg bioaccumulation with size in 2022 against decreases in the rate of trophic increase with size and lower THg concentrations of lower food web items imply reduced fish growth and potential bioenergetic stressors on Winam Gulf Nile perch. All samples except 1 large Nile perch (139 cm total length collected in 2022) had THg concentrations below the European Union trade limit (500 ng/g wet weight). However, for more vulnerable individuals (women, children and frequent fish eaters), we recommend a decrease in maximum monthly meal consumption for 55–75 cm Nile perch from 16 meals per month calculated for 1998 to a limit of 8 meals per month calculated for 2022.

1. Introduction

All global large lake ecosystems are now under threat from multiple stressors including watershed alteration, climate change, eutrophication, trophic disruption, and toxic contaminants, among other stressors (Jenny et al., 2020). At the same time, large lake ecosystems provision a disproportionate amount of global aquatic ecosystem services (Sterner et al., 2020). Multiple stressors can interact with one another in unanticipated ways, exacerbating the impacts of a given stressor (Gilbert et al., 2022; Navarro-Ortega et al., 2015). Mercury (Hg) is a global pollutant and environmental stressor that impacts many large lakes around the world (Driscoll et al., 2013; Mohapatra et al., 2007; Lavoie et al., 2013; Poste et al., 2015). As a stressor, Hg can compromise ecosystem integrity as well as food safety related to the consumption of contaminated fish (Driscoll et al., 2013; Eagles-Smith et al., 2018). Given that total Hg (THg) bioaccumulation is dependent on many ecological processes, its impacts are also subject to multiple stressor interactions that can enhance or reduce its local effects in a given environment (Eagles-Smith et al., 2018).

Mercury has a very high biomagnification potential comparable to the most persistent hydrophobic organic pollutants such as PCBs (Li et al., 2015; Mazzoni et al., 2020). In aquatic environments, Hg is readily biotransformed from less available inorganic species into highly bioavailable methyl-mercury that is efficiently assimilated from food by fish and slowly eliminated from fish tissues (Campbell et al., 2003a; Li et al., 2015). The bioaccumulation rate (increase in mercury concentration in organisms with age or size) and biomagnification rate (increase in mercury concentration with trophic position in food webs) depends on several physical, chemical and biological characteristics of a given ecosystem (Benoit et al., 2002; Campbell et al., 2003a; Karimi et al., 2007; Lavoie et al., 2013). Beyond change to mercury point sources and contaminant loadings, factors such as dissolved organic matter of water (Lavoie et al., 2013), mercury methylation rates (Benoit et al., 2002), number of trophic levels and trophic position of a given species (Kidd et al., 1995; Vander Zanden and Rasmussen, 1996; Hanna et al., 2015), variation and change in a species diet composition (Lepak et al., 2009; Hanna et al., 2016) and growth efficiencies (Cossa et al., 2012) of a given fish population influence the final THg concentrations achieved in fish tissues.

Co-occurring stressors such as eutrophication and trophic disruption alter many ecosystem processes that modulate THg behaviour in complex and sometimes unforeseen ways (Gilbert et al., 2022; Eagles-Smith et al., 2018). Changes to primary productivity due to eutrophication is most commonly associated with decreased THg bioaccumulation in fish related to biodilution processes operating at the ecosystem scale or by enhancing fish growth (Pickhardt et al., 2002; Chen and Folt, 2005; Karimi et al., 2007; Driscoll et al., 2012; Walters et al., 2015). However, the generality of this effect across study systems has been challenged leading Eagles-Smith et al. (2018) to conclude that increases in ecosystem primary production can lead to either reduced or enhanced THg bioaccumulation in fish. Trophic disruption, due to species

introductions or species loss, can have complex effects on fish trophic position, feeding ecology and growth that impact THg toxicokinetics and bioaccumulation potentials (Lavoie et al., 2013). While increase in a species trophic position is commonly associated with increased THg bioaccumulation (Vander Zanden and Rasmussen, 1996; Hanna et al., 2015), such responses can be confounded, or counteracted, by changes in fish growth (Swanson et al., 2006; Ward et al., 2010), spatial foraging or altered ecosystem methylation processes (Eagles-Smith et al., 2018). It is therefore important to characterize change in THg bioaccumulation dynamics in top predator fish from large-lake ecosystems and document how such patterns relate to multiple stressors and rapid ecological change occurring within these vulnerable yet ecologically and economically important study systems.

The objective of the present study was to evaluate change over time of THg bioaccumulation and biomagnification in a top predator fish, Nile perch (*Lates niloticus*), from Winam Gulf, Lake Victoria between 1998 and 2022. Winam Gulf is a large embayment of Lake Victoria that lies within Kenya's jurisdiction of this African Great Lake. Nile perch is one of three major fisheries present in both the gulf and Lake Victoria. The shallow waters of the gulf, coupled with slow water exchange and growing population pressure in its catchment have made it particularly vulnerable to multiple stressors (Hecky et al., 2010; Okely et al., 2010; Gikuma-Njuru et al., 2013). Major stressors of Winam Gulf include degraded water quality (Ongeri et al., 2012; Orata et al., 2011; Omwomo et al., 2015; Kundu et al., 2017; Outa et al., 2020b), frequent algal blooms (Sitoki et al., 2010; Nyamweya et al., 2020), habitat perturbations associated with invasive species (e.g. water hyacinth) (Wilson et al., 2007; Otieno et al., 2022), trophic perturbations due to non-native fish introductions and fish biodiversity loss (Kitchell et al., 1997), overfishing (Omwoma et al., 2014) and exponential expansion of aquaculture net-pen operations (Aura et al., 2018; Okechi et al., 2022; Mariott et al., 2023). These stressors have manifested themselves as declines in fish harvests from these traditionally highly productive waters, with total fish catch declining by 66 % between 1995 and 2009 (Witte et al., 2007; Kundu et al., 2017; Roegner et al., 2023; Omwomo et al., 2015).

Although there have been studies documenting THg bioaccumulation in Nile perch from Lake Victoria, including studies focused on Winam Gulf (Campbell et al., 2003b; Poste et al., 2012; Arinaitwe et al., 2020), there are no long-term studies documenting how THg bioaccumulation has changed through time while simultaneously accounting for change in baseline THg concentrations or fish trophic position. In the present study we examine THg bioaccumulation and biomagnification rates in Nile perch after accounting for baseline prey THg contamination and trophic shifts with size. We hypothesize that ecological stressors that have contributed to observed declines in fish production coincide with bioenergetic stressors that have offset declines in THg loads to Lake Victoria (Campbell et al., 2003c) and biodilution processes associated eutrophication of Winam Gulf waters.

2. Methods

2.1. Study site, fish community and study species

Lake Victoria is the second largest freshwater lake in the world by surface area and largest lake in Africa (Hecky et al., 2010). The lake supports 73 million people with human population growth in the Lake Victoria basin being among the fastest in the continent (Nyamweya et al., 2016; Glaser et al., 2019; Roegner et al., 2020; Okechi et al., 2022; Simiyu et al., 2022). Kenya's jurisdiction of Lake Victoria covers 6 % of the lake's surface area (1400 km²) in the northeastern corner of the lake, most of which is encompassed by the relatively shallow (average depth of 6 m) embayment of Winam Gulf (Otieno et al., 2022). The City of Kisumu, located in the northeast corner of Winam Gulf is Kenya's third largest city supporting 1 million people and the largest urban contributor of industrial pollutants, whereas the gulf's catchment supports approximately 6.8 million people (Gikuma-Njuru et al., 2013; Alexander and Imberger, 2013; Simiyu et al., 2022). Winam Gulf connects with Lake Victoria via the relatively narrow Rusinga and Mbita Channels surrounding Rusinga Island (see Fig. 1). These constrictions limit water exchange between the gulf and lake, leading to a long water residence time of 3 years (Okely et al., 2010; Gikuma-Njuru et al., 2013).

The gulf is an important water source to urban and rural populations of the region and also supports the majority of Kenya's annual fish catch and fish export market (Outa et al., 2020a; Okechi et al., 2022). Despite multiple stressors, the productive waters of Winam Gulf continue to sustain higher fish biomass and species diversity compared to the open waters of Lake Victoria (Okechi et al., 2022). However, over the last three decades, there have been declines in fish production with total fish catch declining from 2.26×10^6 kg in 1995 to 7.6×10^5 kg in 2009 (Witte et al., 2007; Kundu et al., 2017; Roegner et al., 2023; Omwomo et al., 2015). The main commercial fish species from Winam Gulf include the endemic pelagic minnow Omena (*Rastrineobola argentea*) and introduced Nile perch, and to a decreasing extent, wild caught introduced Nile tilapia (*Oreochromis niloticus*) (Outa et al., 2020a).

The gulf's fish community, like that of Lake Victoria, has experienced dramatic changes over time and major losses of species diversity. In the 1970's a highly species rich community of native haplochromine cichlids dominated the fishery of the gulf (Okechi et al., 2022). However, many

of haplochromine species have been driven to extinction and today haplochromine biomass remains low although a few species appear to be recovering within the gulf's waters (Okechi et al., 2022). Nile perch, were introduced to Lake Victoria in the 1950's, and proliferated throughout the lake in the 1980's. This species has been cited as a main contributor to the onset of the haplochromine collapse (Kitchell et al., 1997; Abong'o et al., 2018; Mwamburi et al., 2020). However, other invasive species such as water hyacinth, which proliferated in the gulf after the 1980's, also contributed to declines in water quality and habitat alteration with associated trophic disrupting effects to the fish community (Otieno et al., 2022). Post haplochromine collapse, biomass of food web components in Winam Gulf became dominated by the freshwater shrimp (*Caradina nilotica*), Omena, Nile perch and Nile tilapia (Natugonza et al., 2016). Other common fish species in the gulf include the red-finned barb (*Enteromius apleurogramma*), Sadler's robber (*Brycinus sadleri*) and two species of catfish (*Synodontis victoriae* and *S. afrofischeri*) (Okechi et al., 2022). Introduced Nile tilapia populations have been declining in the gulf's waters by as much as 70 % in recent years leading to the rapid development tilapia net pen operations to augment the declining tilapia fishery (Njiru et al., 2021; Okechi et al., 2022).

Nile perch is the largest fish species and top predator in Lake Victoria (Natugonza et al., 2016). It can grow to a length of 2 m, weigh 200 kg and live up to 16 years (Ogutu-Ohwayo, 2004). Young of the year fish <5 cm feed primarily on zooplankton and quickly shift to larger macroinvertebrates dominated by *C. nilotica* (Cornelissen et al., 2018a). Fish become more prominent to the diet after 19 cm and can include haplochromines, Omena, juvenile Nile perch and juvenile Nile tilapia dependent on the abundances of prey encountered (Kitchell et al., 1997; Cornelissen et al., 2018a; Outa et al., 2020a). Fish 60–100 cm in size consume mainly juvenile Nile perch and other fish (Ogutu-Ohwayo, 1994) with cannibalism having become more common post haplochromine collapse (Campbell et al., 2003a).

2.2. Stable isotopes

Stable isotopes (SI) are used as ecological tracers of diet and energy flow in food webs (Paterson and Fry, 1987). Isotopic fraction of carbon occurs mainly during primary production and carbon isotopes are

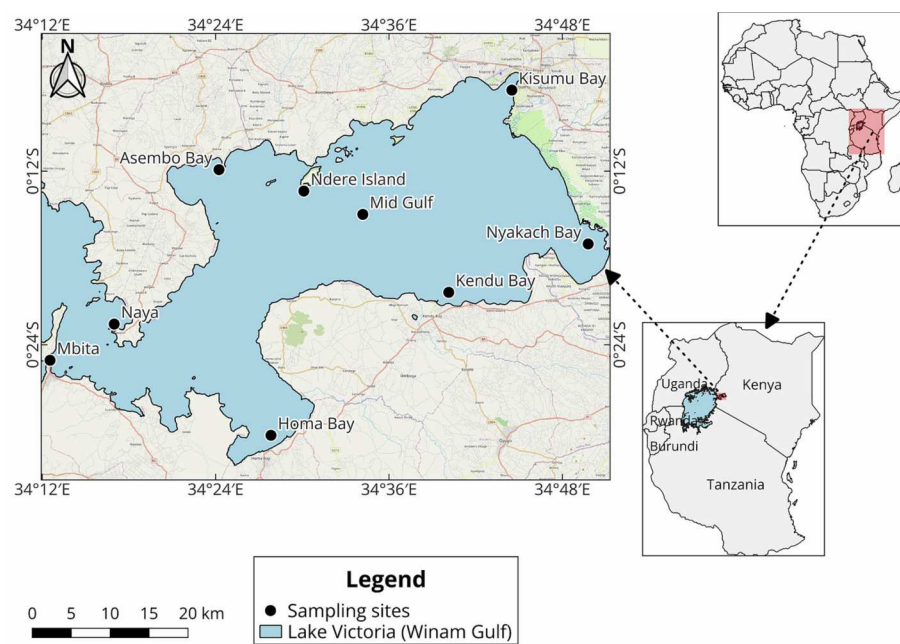


Fig. 1. 2022 trawl locations in Winam Gulf, Kenya.

commonly used as tracers of sources of carbon fixation incorporated in food web components (Hecky and Hesslein, 1995). Carbon isotopes have been used to distinguish allochthonous vs autochthonous carbon sources (Carpenter et al., 2005) or spatial patterns in autochthonous primary production (e.g. benthic vs pelagic primary production) (Hecky and Hesslein, 1995). However, carbon isotopes are also subject to change through time, with ^{13}C becoming depleted across decades due to anthropogenically altered atmospheric CO_2 isotope signatures (Van Rijssel et al., 2017) or enriched in response to increased eutrophication within a given system (Oczkowski et al., 2014). Given that eutrophication is most commonly associated with THg biodilution, enrichment of ^{13}C through time would be consistent with lower mercury bioaccumulation rates. Stable isotopes of nitrogen are commonly used to estimate organism trophic position after adjusting for nitrogen isotopes at the base of the food web (Post, 2002). As a biomagnifying contaminant, THg is positively correlated to organism trophic position (Vander Zanden and Rasmussen, 1996) and therefore change to organism trophic position is expected to result in comparable change in mercury bioaccumulation.

The common convention for reporting stable isotopes is delta notation (δ) in units of parts per thousand (‰) calculated as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \cdot 1000 \quad (1)$$

where $R = ^{13}\text{CO}_2/^{12}\text{CO}_2$ for $\delta^{13}\text{C}$ or $R = ^{15}\text{N}_2/^{14}\text{N}_2$ for $\delta^{15}\text{N}$. In each study year, the same reference (R_{standard}) for carbon or nitrogen was used and co-analyzed with food web samples during isotope analysis. For carbon, the reference was PeeDEE belemnite and for nitrogen it was nitrogen gas in ambient air. Trophic position (TP) of Nile perch was estimated based on a single point calibration relative to the mean $\delta^{15}\text{N}$ value (‰) measured in mussel ($\delta^{15}\text{N}_{\text{mussel}}$) samples collected in the same year as fish according to:

$$TP_{\text{fish}} = \frac{\delta^{15}\text{N}_{\text{N.perch}} - \delta^{15}\text{N}_{\text{mussel}}}{ER} + TP_{\text{Mussel}} \quad (2)$$

where $\delta^{15}\text{N}_{\text{N.perch}}$ is the mean $\delta^{15}\text{N}$ value (‰) measured in a given size class of fish, ER is the trophic enrichment factor set to a value of 2.4 (Kilham et al., 2009; Cornelissen et al., 2018a) and TP_{Mussel} is the trophic position of unionid mussels assumed to be 2 (Cabana and Rasmussen, 1996). The lower ER value applied in the present study compared to the more commonly used ER of 3.4 ‰ (Post, 2002) was based on the review of Kilham et al. (2009) who showed lower enrichment ratios in tropical systems. In addition, use of an ER of 2.4 ‰ provides compatibility with recent stable isotope food web studies conducted in Lake Victoria (Cornelissen et al., 2018a).

2.3. Sample collections

A total of five 30-min trawls were performed between Jun 23–29, 2022 in the Kendu Bay, Homa Bay, Naya Bay and in waters off Mbita of Winam Gulf (Fig. 1) via KMFRI's research vessel the RV Uvumbuzi. From the above trawls, 7 live unionid mussels (species not identified) were obtained, shucked and analyzed together as a single pool. Fish collections included 5 Nile tilapia (20.0 to 28.6 cm) and 35 Nile perch (8.3 cm to 139.5 cm). Ethical review of animal collections, handling and fish euthanasia for 2022 collections were approved under the University of Windsor's Animal Care Committee under an Animal User Procedure and Protocol (AUPP-23-05) lead by K. Drouillard.

Raw data from 1998 were obtained from Campbell et al. (2003b). Data were truncated to a common set of species and sizes collected in both 1998 and 2022 that included unionid mussels (unidentified species), Nile tilapia (*Oreochromis niloticus* L.) and Nile perch (*Lates niloticus* L.). Samples from 1998 were collected in December by trawl net or gill net sets in conjunction with the Kenya Institute for Marine Fisheries Research (KMFRI), from Kisumu to the Rusinga Channel or purchased

directly from local fishers who verified the location of collection as being from Winam Gulf, Kenya. The 1998 collections had a wide range of fish and invertebrate species, so for this study, we included data for two samples of unionid mussels, 4 Nile tilapia (25.5 to 29.5 cm) and 28 Nile perch (4.9 to 158 cm).

2.4. Analytical chemistry

Samples from 1998 were shipped frozen to Waterloo, Ontario, Canada and subsequently freeze-dried at the University of Waterloo. Total Hg analysis was performed by atomic fluorescence spectroscopy (AFS) equipped with a purge-and-trap inlet operated at the Ontario Ministry of Environment laboratory in Dorset, Ontario, Canada. Stable isotopes were analyzed at the University of Waterloo Environmental Isotope Laboratory using a Micromass VG-Isochrome Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS). Isotope analysis was performed without lipid extraction given the low lipid contents (<1 %). Details of analytical protocols and quality control measures for THg and stable isotopes are provided by Campbell et al. (2003b, 2003d).

Samples from 2022 were oven dried (60 °C, overnight) in Kisumu, Kenya to determine sample moisture contents and shipped dry to the University of Windsor, Ontario, Canada for analytical measurements. Between 0.02 and 0.10 g of dry sample were analyzed for THg concentration by Direct Mercury Analyzer (DMA-80) as per EPA Method 7473. Total Hg concentrations were calculated based on the signal response relative to a 10 point calibration curve generated from a certified liquid Hg standard (High-Purity Standards, Charleston, SC, USA). Sample replicates ($n = 4$ duplicates across the run) and certified reference tissues (CRMs; National Research Council of Canada – DORM-3 and DOLT-4) were run with every 10th sample. The mean $\pm$ standard deviation (STD) Hg signal response for blanks was 0.21 ± 0.19 ng ($n = 5$) and the detection limit adjusted for average sample size was 13.0 ng/g dry weight. Mean $\pm$ STD % recoveries of THg in CRMs was 92.3 ± 0.04 %. Replicate measurements of 4 randomized samples had a precision, expressed as the mean $\pm$ STD of the % deviation from a given duplicate average of 4.1 ± 3.6 %.

Stable isotopes were performed on samples after lipid extraction using a 2:1 chloroform:methanol mixture followed by analysis on a Delta V Plus Thermoscientific CF-IRMS with a 4010 Elemental Combustion System. Stable isotope ratios in the sample were calculated using the same reference standards used in 1998. CRMs co-analyzed for SI included National Institute of Standards and Technology (NIST) and U.S. Geological Survey (USGS) reference materials: NIST 8573, 8542, 8574 and USGS 40 for $\delta^{13}\text{C}$ and NIST 8573, 8574, 8547 and USGS 40 for $\delta^{15}\text{N}$ co-analyzed with every 10th sample. Mean $\pm$ STD CRM percent recoveries of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were 99.8 ± 0.9 % and 113.2 ± 25.3 %, respectively. Sample precision of replicated SI measures among 4 duplicate samples was 0.13 ± 0.10 % and 0.80 ± 0.38 % of the reported paired mean ‰ values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Given that 1998 samples were not lipid extracted, a subset of 10 samples from the 2022 collections were repeated for analysis without lipid-extraction. Linear regression on matched 2022 samples demonstrated no-significant differences ($R^2 = 0.95$; $p > 0.7$ for $\delta^{13}\text{C}$ and $R^2 = 0.96$, $p > 0.5$ for $\delta^{15}\text{N}$) in measured isotope values for lipid versus non-lipid extracted samples (Fig. S1).

2.5. Human fish consumption recommendations

While THg concentration for all biota from both 1998 was reported on a dry-weight (dw) basis, most regulatory and human-health advisories are based on wet-weight (ww) values. We obtained moisture correction values for the 2022 samples by recording wet and dry weights for each sample. The mean 2022 moisture content (77.8 %) determined for Nile perch was similar to those reported by Outa et al. (2020a) at 80 % and Machiwa et al. (2003) at 79 %. For the 1998 samples, we applied the mean 2022 wet-dry difference (0.222) for converting the 1998 THg

values originally expressed in dry weight units to wet-weight equivalents.

The wet-weight THg concentrations for individual Nile perch were first evaluated against the EU Trade limit of 500 ng/g ww THg for imported food to determine the fraction of fish samples and fraction of fish in the legal slot size that exceed this regulatory limit (European Union, 2022). We subsequently adapted the Ontario Ministry of Environment, Conservation and Parks (OMEC) consumption advisory methodology for generating maximum consumption frequency recommendations issued to the public who regularly consume wild caught fish. The OMEC methodology computes separate advisories that are issued to the general public and to vulnerable groups (women of childbearing age and children under fifteen) across different size bins of fish in 5 cm intervals across the legal slot sizes of fish (Gandhi et al., 2017). For the present study, we focused our calculations using the methodology applied to fish consumption advice issued to vulnerable groups. The OMEC approach estimates the maximum monthly meals of a given species and size class that can be safely consumed by an individual that would generate Hg exposures protective of tolerable daily intake (TDI) limits and associated toxicological health risks. The OMEC guidelines were adapted for Kenya by assuming a similar meal portion size (MS) of 226 g of skinless dorsal muscle (Gandhi et al., 2017) but adjusting for the average Kenyan body weight (BW) of 60.7 kg, based on continental African average (Walpole et al., 2012).

Tolerable daily intakes or tolerable weekly intakes (TWI) for methyl-mercury (MeHg) for the protection of the women and children were reviewed across jurisdictions and are 200 ng/kg BW/d for Canada (McLean Pirkle et al., 2016), 190 ng/kg BW/d for the European Union (EFSA, 2012) and 100 ng/kg BW/d for the United States (US FDA/EPA, 2022). The lowest TDI above was adopted and converted into tolerable monthly intake (TMI) for total mercury (3158 ng/kg BW/month) assuming 95 % of THg in fish is in the form of MeHg (Arinaitwe et al., 2020). The above information was used to calculate benchmark THg concentrations in fish muscle ($C_{f,max}$; ng/g ww) that are protective of the TMI following consumption of 0, 4, 8, 16 or 32 fish meals per month according to:

$$C_{f,max} = \frac{TMI \cdot BW}{MS \cdot \#Meals}$$

The THg concentration benchmarks are provided in Table S1. Next, the best predictive statistical model of geometric mean dry weight mercury concentrations in Nile perch as a function of size was used to estimate the dorsal muscle mercury concentration for the largest fish in each size bin. The dry weight estimate was converted to a wet weight concentration by dividing by 0.222 and compared against monthly meal frequency category benchmarks in S1 to arrive at monthly consumption advice for each size bin.

2.6. Statistical analysis

Multiple regression models were used to explore within species relationships between carbon or nitrogen isotopes or THg concentration as a function of fish total length, collection year and the total length x year interaction. Year was coded as a dummy variable set equal to 0 for 1998 and 1 for 2022 to allow testing the year x group interaction. Where either year or the year x group interaction was found to be significant, linear regressions were then performed on SI's or THg separately for each year of data to provide within year predictive relationships for each parameter as a function of fish size or trophic position.

In addition to linear regressions, alternative models were explored for predicting isotopes or THg as a function of fish length or trophic position where non-linear patterns were evident in the data. Alternative models included piecewise regressions or asymptote non-linear regressions. Piecewise regression examines for breakpoints in the slope across different data regions, and where they are observed, generate different regression coefficients (i.e. separate linear regressions) for each

data region. Where the piecewise regression showed improvement of fit ($R^2 > 0.03$) over a single linear regression, the piecewise regression was selected as the better model. Asymptote non-linear regressions were examined in cases where data as a function of fish size suggested an asymptotic trend at the upper end of the size range. Asymptote non-linear regressions were fit according to:

$$\delta^{15}N = [A - (A - B)] \times e^{(-k \cdot TL)}$$

where fitted coefficient A is the maximum, or asymptote value of the isotope ratio, B is initial isotope ratio for the smallest size class, k relates the change in isotope ratio as a function of length and TL is the total length (cm) measured for a given fish. As with piecewise regressions, where the non-linear regression had an $R^2 > 0.03$ above the linear regression, it was considered a better predictor of trends in the data. Non-linear regressions were initialized with different starting coefficient estimates and re-ran multiple times until an optimal solution was obtained.

SYSTAT Version 12 statistical software was used to perform group contrasts, multiple-regressions linear regression and asymptote non-linear regression analysis. Piecewise regressions were performed using R-studio using the segmented library. Prior to multiple or linear regression, data were tested for normality by Lilefor's test. Where required, data transformation by $\log_{10}$ was performed to conform to linear regression or group contrast assumptions. Where test assumptions could not be met by data transformation, non-parametric Kruskal-Wallis test with Conover-Iman's pairwise comparisons was used to contrast parameters between groups.

3. Results

3.1. Stable isotopes

A biplot of $\delta^{15}N$ vs $\delta^{13}C$ for Nile perch, Nile tilapia and mussels show consistently lower $\delta^{13}C$ values in samples from 1998 (−18 to −22 ‰) compared to those from 2022 (−14 to −20 ‰) (Fig. 2). Nile perch had highest $\delta^{15}N$ values in both 1998 (11.5 ‰) and 2022 (10.3 ‰) (Table 1, Fig. 2), consistent with known trophic structure in the Lake Victoria food web (Campbell et al., 2003b, 2003d; Cornelissen et al., 2018a, 2018b). Mussels from 1998 had lower $\delta^{15}N$ values (7.4 ‰) relative to Nile tilapia (8.30 ‰) collected in the same year, whereas mussels from 2022 had similar $\delta^{15}N$ values (6.9 ‰) as 2022 Nile tilapia (6.9 ‰) (Table 1, Fig. 2). On a within species basis, Nile perch had significantly lower $\delta^{13}C$ values (−22 ‰) in 1998 compared to 2022 (−18.7 ‰, $p < 0.01$; Kruskal-Wallis test) by an average of 3.3 ‰. Nile tilapia $\delta^{13}C$ values (Table 1, Fig. 2) were not significantly different ($p > 0.3$; Kruskal-Wallis test) between years but still exhibited a similar difference in mean $\delta^{13}C$ values of 3.2 ‰ between years. Similarly, mussels from 1998 were depleted in $\delta^{13}C$ by 3.4 ‰ compared to the 2022 mussel pool (Table 1, Fig. 2).

A multiple regression model for Nile perch $\delta^{13}C$ values demonstrated significant effects by year ($p < 0.001$; t-test); total length ($p < 0.001$; t-test) and a significant length x year interaction ($p < 0.001$). When examined separately by year, the 1998 Nile perch $\delta^{13}C$ values were significantly dependent on total length ($p < 0.001$; t-test; Table 2) as reported by Campbell et al. (2003b, 2003d). However, there was no relationship ($p > 0.9$; t-test) between $\delta^{13}C$ values and total length for 2022 Nile perch.

The multiple regression model for Nile perch $\delta^{15}N$ values indicated a significant effect by year ($p < 0.05$; t-test) and total length ($p < 0.001$; t-test) but non-significant ($p > 0.7$; t-test) year x total length interaction. Based on the negative coefficient with year, average $\delta^{15}N$ values were lower in 2022 Nile perch compared to 1998 whereas total length was strongly positively related to $\delta^{15}N$ in both years (Fig. 3). However, given that mussels also showed a 0.53 ‰ decrease in $\delta^{15}N$ between years, at least a portion of the observed decrease in 2022 Nile perch $\delta^{15}N$ values could be attributed to changes in baseline nitrogen signatures of the gulf

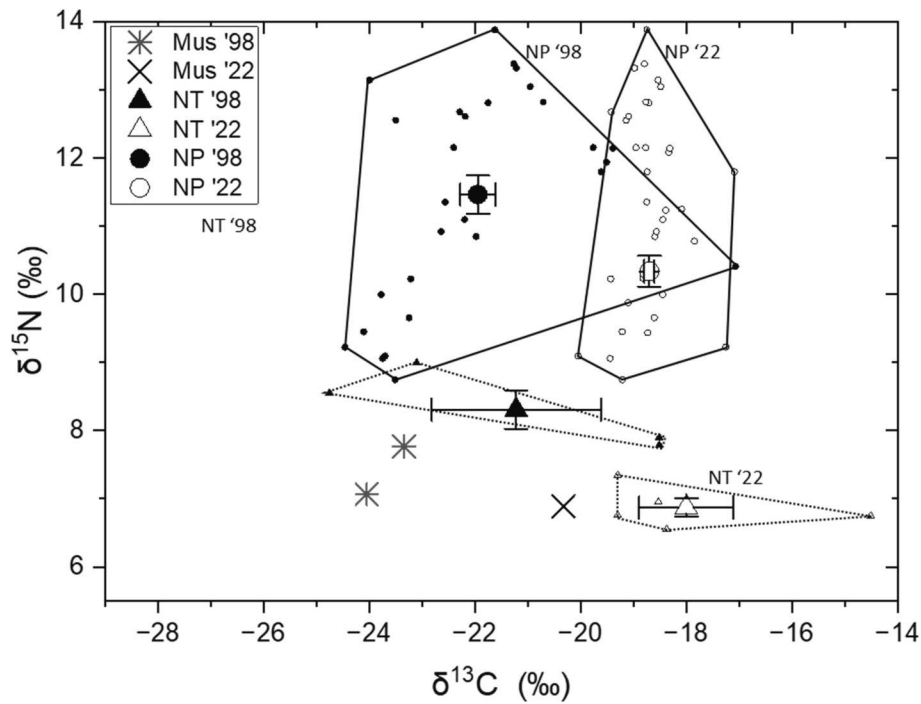


Fig. 2. Stable isotope values in common species sampled from Winam Gulf in 1998 and 2022. Small symbols present raw data for fish, large symbols are mean $\pm$ standard error for fish or individual measures for mussels. Solid lines represent convex hulls around Nile Perch, dashed lines are convex hulls around Nile Tilapia.

Table 1

Mean values $\pm$ standard error (range and n) for total mercury (THg, ng/g dw), stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, ‰) and total length (TL, cm) for freshwater mussels, Nile tilapia and Nile perch from Winam Gulf, Lake Victoria.

Taxon, year	TL	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	[THg]
Mussel, 1998	NA, n = 2	-23.70 (-23.35 to -24.05, 2)	7.42 (7.07–7.77, 2)	53.9 (63.2–80.6, 2)
Mussel, 2022	7 $\pm$ 1.8, n = 1	-20.33 (NA, 1)	6.89 (NA, 1)	25.8 (NA, 1)
Nile tilapia, 1998	27.6 $\pm$ 0.8 (25.5–29.5, 4)	-21.22 $\pm$ 1.60 (-18.51 to -24.76, 4)	8.30 $\pm$ 0.28 (7.79–8.99, 4)	41.5 $\pm$ 5.7 (40.1–42.2, 3)
Nile tilapia, 2022	23.6 $\pm$ 1.6 (20–28.6, 5)	-18.00 $\pm$ 0.89 (-19.30 to -14.51, 5)	6.87 $\pm$ 0.13 (6.55–7.35, 5)	25.5 $\pm$ 3.4 (15.4–34.0, 5)
Nile Perch, 1998	50.4 $\pm$ 8.0 (4.9–158, 20)	-22.02 $\pm$ 0.33 (-24.45 to -17.07, 28)	11.45 $\pm$ 0.29 (8.75–13.88, 28)	181.9 $\pm$ 44.1 (14.3–885.7, 19)
Nile Perch, 2022	43.7 $\pm$ 5.8 (8.3–139.5, 35)	-18.70 $\pm$ 0.10 (-20.05 to -17.09, 35)	10.34 $\pm$ 0.23 (8.55–13.00, 35)	310.0 $\pm$ 108.2 (63.0–3622.9, 35)

^a Mussels from 2022 $n = 7$ individuals were pooled together and analyzed as a single sample. All other replicates presented in Table 1 refer to individual samples.

through time. Therefore, the multiple regression model was repeated after adjusting for the difference in mussel $\delta^{15}\text{N}$ between years (i.e. the $\delta^{15}\text{N}$ in each 2022 Nile perch was increased by 0.53 ‰). Multiple regression on the adjusted data demonstrated a non-significant (but approaching significance; $p = 0.06$; t-test) difference by year and highly significant difference ($p < 0.001$) by length following removal of the non-significant interaction term from the model. Close inspection of the data (Fig. 3) further revealed that the $\delta^{15}\text{N}$ versus length relationship

Table 2

Selected models predicting stable isotopes (‰) or THg concentration (ng/g dw) in Nile Perch for 1998 and 2022.

Variable predicted	Model type	Equation	R ² , df
$\delta^{15}\text{N}_{\text{(Nile Perch, 1998)}}$	Linear regression	$0.031 \pm 0.008^{**} \cdot \text{TL} + 10.03 \pm 0.49$	0.42, 18
	Non-linear regression	$13.38 - [(13.38 - 7.76) \cdot \exp(-0.031 \cdot \text{TL})]$	0.80, 17
$\delta^{15}\text{N}_{\text{(Nile Perch, 2022)}}$	Linear regression ^a	$0.033 \pm 0.004^{***} \cdot \text{TL} + 8.87 \pm 0.20$	0.72, 33
	Non-linear regression ^a	$13.25 - [(13.78 - 8.37) \cdot \exp(-0.017 \cdot \text{TL})]$	0.80, 32
$\text{Log}_{10}\text{THg}_{\text{(Nile Perch, 1998)}}$	Linear regression ^b	$0.007 \pm 0.001^{***} \cdot \text{TL} + 1.82 \pm 0.08$	0.64, 12
	Piecewise regression ^b	$\text{TL} \leq 53.5; 0.003 \cdot \text{TL} + 1.96$ $\text{TL} > 53.5; 0.008 \cdot (\text{TL} - 53.5) + 2.115$	0.62, 12
$\text{Log}_{10}\text{THg}_{\text{(Nile Perch, 2022)}}$	Linear regression	$0.011 \pm 0.001^{***} \cdot \text{TL} + 1.72 \pm 0.04$	0.86, 32
	Piecewise regression	$\text{TL} \leq 58; 0.0058 \cdot \text{TL} + 1.87$ $\text{TL} > 58; 0.0146 \cdot (\text{TL} - 58) + 2.20$	0.90, 32
$\text{Log}_{10}\text{THg}_{\text{(All Species, 1998)}}$	Linear regression ^b	$0.098 \pm 0.023^{**} \cdot \delta^{15}\text{N} + 0.97 \pm 0.24$	0.39, 25
$\text{Log}_{10}\text{THg}_{\text{(All Species, 2022)}}$	Linear regression	$0.221 \pm 0.024^{***} \cdot \text{adjusted } \delta^{15}\text{N} - 0.22 \pm 0.24$	0.69, 39

^a $\delta^{15}\text{N}_{\text{(Nile Perch, 2022)}}$ linear and non-linear regression models generated after adjusting raw 2022 $\delta^{15}\text{N}$ Nile perch values for the difference in mean mussel $\delta^{15}\text{N}$ values between 2022 and 1998.

^b One fish TL = 73.4 cm with THg = 14.32 ng/g dry weight removed from model calibration as a low outlier.

^{**} Linear regression slope was significantly different from zero ($p < 0.01$).

^{***} Linear regression slope was significantly different from zero ($p < 0.001$).

was not linear but asymptotic for the largest fish (Fig. 3). Non-linear asymptote regressions were fitted to Nile perch $\delta^{15}\text{N}$ data (after adjusting 2022 data for between year differences in mussel $\delta^{15}\text{N}$ data) for each year separately. Both models generated similar $\delta^{15}\text{N}$ estimates at the smallest and largest size range of fish. For example, 8 cm fish were

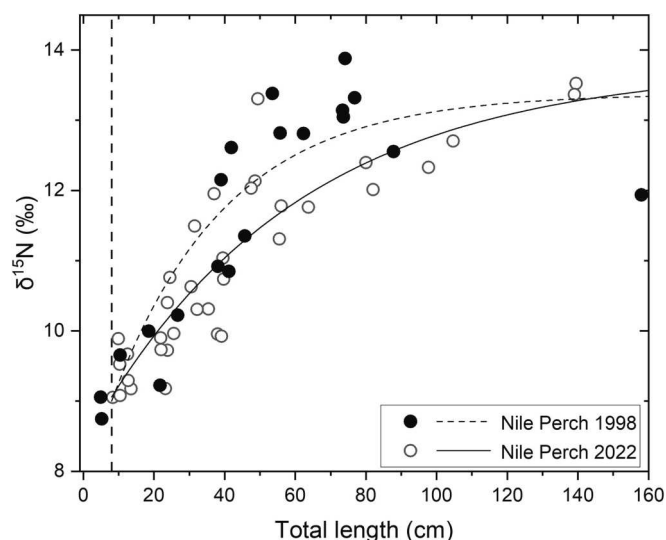


Fig. 3. $\delta^{15}\text{N}$ values in Nile Perch as a function of total length (cm) for data from 1998 (solid circles) and 2022 (open circles). Dashed curve is the asymptotic regression fit to the 1998 data, solid line is the fit to 2022 data. Vertical line denotes the 8 cm size cut off from which asymptotic regression fits were restricted to.

predicted by each model to have a $\delta^{15}\text{N}$ of 9.0 and 9.1 ‰ for 1998 and 2022 fish, respectively. The asymptote $\delta^{15}\text{N}$ for 1998 and 2022 non-linear models were 13.4 and 13.8 ‰, respectively. However, the two curves deviate from one another in $\delta^{15}\text{N}$ values by an average of 0.3 ‰ for fish across the 16–104 cm size range such that 1998 fish achieved higher relative trophic position at lower sizes compared to 2022 fish (Fig. 3). The largest deviation between predicted $\delta^{15}\text{N}$ values across models was for the 46 cm sized fish which differed by 0.73 ‰ (the deviation for non-mussel adjusted 2022 $\delta^{15}\text{N}$ values would have been 1.25 ‰).

3.2. THg bioaccumulation and biomagnification

Mercury concentrations across species and years are summarized in Table 1. Combined 1998 and 2022 Nile perch had significantly elevated THg concentration compared to Nile tilapia ($p < 0.001$; Kruskal-Wallis test). Combined 1998 and 2022 mussels had generally similar THg concentrations to combined 1998 and 2022 Nile tilapia (Table 1, not statistically tested). Nile tilapia THg concentrations were significantly lower in 2022 than in 1998 ($p < 0.05$; Kruskal-Wallis test), with an apparent decline of 38.6 % across years. Similarly, mussel THg concentrations were approximately 52 % lower in 2022 compared to 1998.

A multiple regression model for Nile perch THg concentration as a function of size, year and size $\times$ year interaction was then performed. However, it was noticed that one 1998 Nile perch (TL = 73.4 cm) had an uncharacteristically low THg concentration of 14.32 ng/g dw relative to other samples from this species and year (shown in Fig. 4). This outlier was omitted from the 1998 THg regression against fish length (or isotopes described below). The multiple regression indicated a highly significant effect of total length ($p < 0.01$; t-test) and a significant total length $\times$ year interaction ($p < 0.05$; t-test), but non-significant effect by year ($p > 0.5$; t-test). Individual linear regression models were established for Nile perch THg concentration as a function of total length for each year (Table 2) showing a higher mercury bioaccumulation slope for 2022 compared to 1998. In addition, piecewise regressions were explored for each year's data separately. For the 1998 data, piecewise regression generated a lower R^2 (0.62) compared to linear regression ($R^2 = 0.64$) with a weak inflection point at 53.5-cm sized Nile perch (Fig. 4, Table 2). Thus, a single linear regression was considered a slightly stronger predictor of 1998 THg in Nile perch compared to the piecewise regression model. For the 2022 data, there was a strong breakpoint observed after fish achieved sizes >58 cm total length. The piecewise regression generated an improved fit ($R^2 = 0.90$) over the linear regression model ($R^2 = 0.86$) and therefore the piecewise regression was considered a better model for estimating THg concentration for the 2022 data compared to the single regression eq.

A second multiple regression model, which included data for all

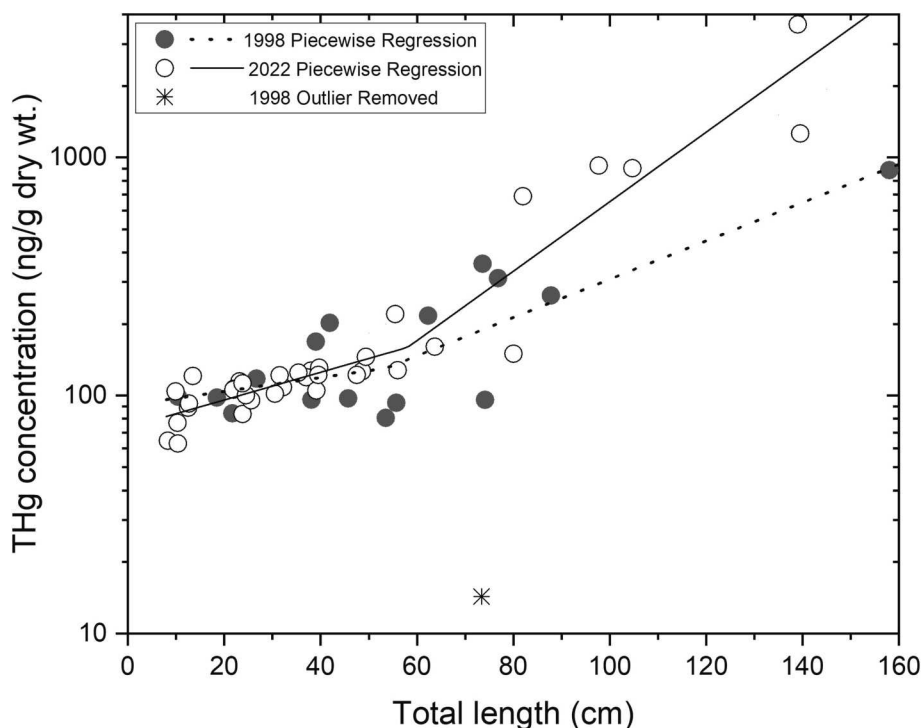


Fig. 4. Total mercury concentration in Nile Perch as a function of size for data from 1998 (solid circles) and 2022 (open circles). Dashed line is the piecewise regression fit to the 1998 data, solid line is the piecewise regression fit to 2022 data.

three species, was fit to predict THg concentration based on $\delta^{15}\text{N}$ (or mussel-adjusted $\delta^{15}\text{N}$ for 2022 data), year and the $\delta^{15}\text{N} \times \text{year}$ interaction (Fig. 5). Although the data were non-normal ($p < 0.05$), the interaction term from the multiple regression model was highly significant ($p < 0.001$; ANCOVA) (Table 2). The 2022 biomagnification slope was 2.3-fold higher compared to the 1998 slope (Fig. 5). The 1998 linear regression generated an elevated intercept relative to 2022 such that the two regression lines intersected at $\delta^{15}\text{N}$ of 9.6 ‰ corresponding to the isotope value for smaller Nile perch (8–12 cm). The combined observations imply declines in THg concentrations in lower food web items over time yet increased mercury bioaccumulation and biomagnification in Nile perch.

3.3. Fish consumption advice

One Nile perch collected in 2022 (139 cm TL, THg concentration of 822 ng/g ww) exceeded the European Union trade limit of 500 ng/g ww, and would generate a “Do Not Consume” advisory for the vulnerable population. However, this individual fish was outside the legal slot size (50–85 cm total length) imposed for harvests of Nile perch in Kenyan waters. All fish from the legal slot size were under the European Union food trade limit. All fish from the legal slot size were under the European Union food trade limit. Piecewise regressions from 1998 and 2022 (Table 3) were applied to predict THg concentrations in Nile perch across 5-cm intervals over the legal slot size range of 50–85 cm (Table 3). In 1998, Nile perch in the legal slot sizes had THg concentrations commensurate with maximum monthly meal recommendations of between 8 meals (80–85 cm) to 16 meals per month (55–75 cm fish). In 2022, maximum monthly meal recommendations remained at 8 meals for the largest slot size of fish (80–85 cm). However, 2022 fish sized between 60 and 75 cm had more stringent consumption frequency recommendations of 8 meals/month down from 16 meals per month recommended for the same sizes in 1998. The 2022 piecewise regression also indicate that Nile perch >137 cm are likely to exceed the European

Table 3

Maximum monthly meals advice associated with mercury contamination of Winam Gulf Nile Perch issued to women and children and the general population based on Ontario Ministry of Environment Climate Change (OMECC) fish consumption advice protocols.

Nile Perch size interval (cm)	1998		2022	
	Mercury concentration estimate ^a (ng/g wet weight)	Advice for women and children (max. meals per month)	Mercury concentration estimate ^a (ng/g wet weight)	Advice for women and children (max. meals per month)
50–55	35.4 ^a	16	46.7 ^a	16
55–60	38.4 ^a	16	53.0 ^a	8
60–65	41.6 ^a	16	60.2 ^a	8
65–70	45.1 ^a	16	68.3 ^a	8
70–75	48.9 ^a	16	77.5 ^a	8
75–80	53.0 ^a	8	88.0 ^a	8
80–85	57.5 ^a	8	99.9 ^a	8

^a Estimated THg concentration generated for the largest size (e.g. 54.9 for 50–55 cm size bin) based on year specific piecewise regression equation reported in Table 2 and converting dry weight to wet weight concentration equivalents by multiplying by 0.77.

Union trade limit of 500 ng/g ww. The smallest size class of Nile perch that would warrant a “Do Not Consume” recommendation for the vulnerable population in 2022 was 115 cm.

4. Discussion

4.1. Spatial and temporal trends of THg in fish from Lake Victoria

Hanna et al. (2015) provided a review of mercury in freshwater fish from Continental Africa and Campbell et al. (2003a) reviewed mercury in abiotic and biotic samples from Lake Victoria. While THg concentrations in fish tend to be anomalously low in Continental Africa

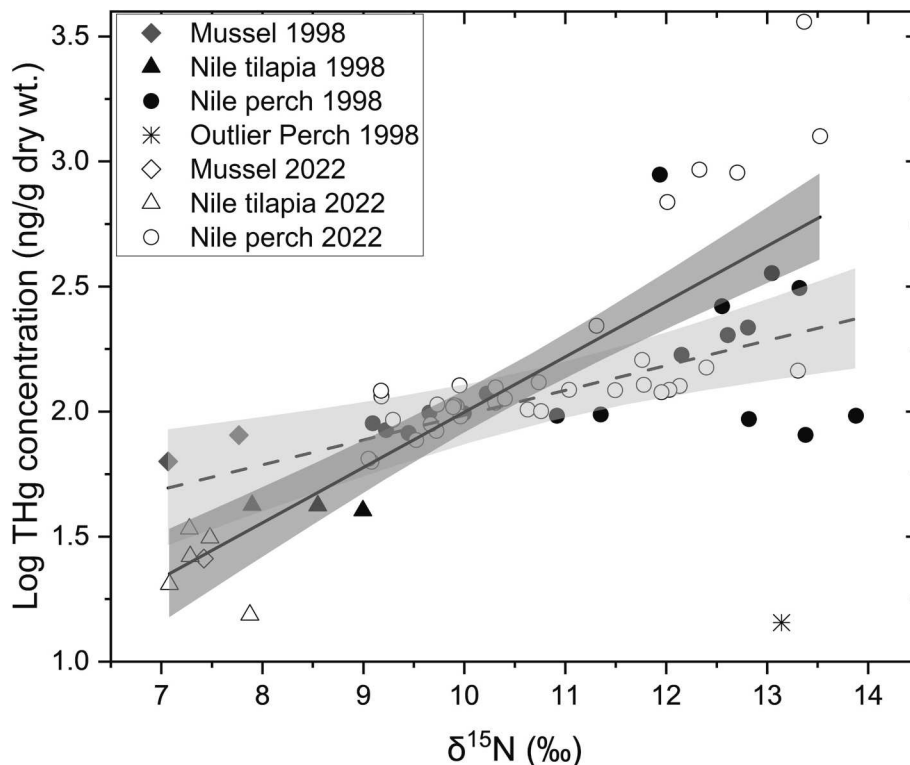


Fig. 5. Total mercury in biota as function of $\delta^{15}\text{N}$ during 1998 and 2022. The 2022 $\delta^{15}\text{N}$ values were adjusted according to the difference in mussel $\delta^{15}\text{N}$ baselines between years. Shaded areas present 95 % confidence intervals for linear regression fits.

compared to other regions around the world, Lake Victoria and the associated goldfields within its basin have received considerable attention (Hanna et al., 2015; Campbell et al., 2003a). More recent studies have since reported on THg concentrations in Lake Victoria Nile perch from different gulfs, embayment's and open waters of the lake (Campbell et al., 2004; Machiwa, 2005; Poste et al., 2012; Kundu et al., 2017; Arinaitwe et al., 2020). The highest THg concentration from the present study (822 ng/g ww, 139-cm Nile perch) exceeded the maximum THg concentration reported for this species in the most recent Lake Victoria fish THg studies (Poste et al., 2012; Kundu et al., 2017; Arinaitwe et al., 2020), but was lower than the maximum THg concentration of 1231 ng ww reported for Nile perch from Mwanza Gulf in the early 1990's (Migiro, 1996). However, most 2022 Nile perch in the 22–105 cm size range had a THg concentration between 17 and 217 ng/g ww which is generally comparable to the recently published literature for Lake Victoria. Fifty Nile Perch collected between 2010 and 2016 from Winam Gulf had mean reported mercury concentrations between 150 and 410 ng/g ww similar to data presented in the present study (Kundu et al., 2017). Alternatively, THg concentration in fish collected in proximity to Lake Victoria goldfields exceed those found in fish collected from the open waters of Lake Victoria and Winam Gulf (Hanna et al., 2015). Goldfields in the Rawamagasa area of Tanzania and Migori-Transmara region of Kenya have some of the highest THg concentration reported in continental Africa (Taylor et al., 2005; Ikingura et al., 2006; Ngure et al., 2014; Odumo et al., 2014; Hanna et al., 2015; Tampushi et al., 2022; Kola et al., 2019; Ondo et al., 2023). Kola et al. (2019) reported elevated THg concentration in pond-reared Nile tilapia (180–920 ng/g ww or 815–4167 ng/g dw) from 10 ponds across the Migori county Goldfield area that exceeded the highest concentrations reported here. The above studies point to the need to better characterize mercury loadings associated with goldfield area river outlets draining into Lake Victoria.

A few studies reported on spatial contrasts of THg concentration in Nile perch from Lake Victoria after accounting for between site differences in fish size and/or trophic position. Campbell et al. (2003b) demonstrated Napoleon Gulf Nile perch and Nile tilapia to be 2.4-fold higher in THg concentration compared to Winam Gulf fish after standardizing for size and stable isotopes in baseline organisms. The authors concluded that Napoleon Gulf had higher bioavailable Hg at the base of the food web but similar trophic transfer (or biomagnification rates) of mercury at both sites. These trends were not consistent with the lack of difference in THg concentrations in water samples between sites (Campbell et al., 2003b) implying other drivers of mercury bioavailability differences between the embayments. Poste et al. (2012) identified a marginal but significant increase in THg of Nile perch from Murchison Bay (42.0 ± 22.0 ng/g ww for 19–96 cm fish) compared to fish from Napoleon Gulf (34.8 ± 13 ng/g ww for 9.7–63.5 cm fish) but did not size-correct their data. Differences were also noted between THg vs $\delta^{15}\text{N}$ relationships across sites, with Napoleon Gulf having a higher biomagnification rate compared Murchison Bay that was not changed after baseline correction of isotope data. Arinaitwe et al. (2020) reported 2 to 3-fold higher THg concentrations in Nile perch from Ugandan open-water sites compared to nearshore sites (Bunjako and Napoleon Bays) but the authors did not size-adjust their data. The open-water site $\delta^{15}\text{N}$ values were also elevated in Nile perch compared to the Napoleon Gulf site by 3–4 ‰ whereas differences in $\delta^{15}\text{N}$ between Bunjako Bay and open water were less clear. Given a lack of difference in Nile tilapia THg concentration values between Napoleon Bay and open-water sites coupled with differences in $\delta^{15}\text{N}$ values of Nile perch from these locations, the authors interpreted the higher open-water mercury content of Nile perch to be due to the higher trophic status of open water fish.

Temporal trends of mercury in abiotic samples and fish samples from Winam Gulf and Lake Victoria are limited. An examination of mercury in sediment cores from Itome Bay of Napoleon Gulf and central Lake Victoria collected in 1995 and 1996 indicated that sedimentary mercury began increasing in the lake after 1960 and peaked around 1980

followed by declines thereafter (Campbell et al., 2003c). The post-1980 declines were interpreted to reflect either reduced loadings to the lake or increased eutrophication causing increased sediment burial rates due to higher phytoplankton productivity through time (Campbell et al., 2003c). Kundu et al. (2017) reported THg concentrations in sediments from Winam Gulf collected in 2015 being lower than those from 2012 and 2013. However, major changes in mercury sediment contamination over a time frame as short as 3 years are more likely a result of spatial differences in sampling, or potential shifts in contaminated-sediment zones rather than system-wide changes in the THg sediment mass balance (Drouillard et al., 2020). Poste et al. (2012) contrasted their fish THg concentration in Napoleon Gulf fish with those of Campbell et al. (2003b) and suggested that THg was declining in fish from Napoleon Gulf through time. Likewise, Arinaitwe et al. (2020) noted that Nile perch THg concentration from Ugandan nearshore areas inclusive of Napoleon Bay were lower than previously reported by Campbell et al. (2003b). Given the consistency of declining THg concentration trends across studies, there is general support for overall declining Hg loads to Lake Victoria abiotic and biotic compartments through time that corroborates with the decline in THg concentrations observed for mussels and Nile tilapia reported here. However, Nile perch from the present research showed an opposite pattern of increasing THg bioaccumulation and biomagnification rates through time and this is the first study that we are aware of from the contemporary literature on Lake Victoria to show such a temporal increase. Based on the piecewise regression relationships generated for 1998 and 2022, 2022 perch in the 50 to 85 cm slot size had between 1.3 and 1.7-fold increased THg concentration compared to 1998 Nile perch. Increased THg bioaccumulation was also more strongly observed for Nile perch >60 cm in length but less apparent for smaller fish.

4.2. Multiple stressor impacts on THg bioaccumulation and biomagnification

Lake Victoria and its associated embayment's are subject to a variety of multiple stressors that have strong implications for the sustainability of its fisheries (Hecky et al., 2010; Outa et al., 2020; Roegner et al., 2023). In the present research, stable isotopes of carbon and nitrogen in baseline food web items and Nile perch were used to characterize and contrast different multiple stressor – food web interactions occurring in Winam Gulf through time. Stable isotopes of carbon were used to tease out climate change (e.g. altered $\delta^{13}\text{C}$ values due to accumulation of fossil fuel emission in the atmospheric) (Van Rijssel et al., 2017) from eutrophication signals apparent in the gulf. Values of $\delta^{13}\text{C}$ in 2022 fish and mussels were enriched by 3.2 to 3.4 ‰, respectively, compared to the same species from 1998 inconsistent with the declines in atmospheric carbon $\delta^{13}\text{C}$ values through time due to accumulation of atmospheric fossil fuel emissions (Van Rijssel et al., 2017; Dombrosky, 2020). Applying similar Suess corrections of Dombrosky (2020) to adjust 2022 data for declines in atmospheric $\delta^{13}\text{C}$ values would indicate even greater enrichment of 2022 $\delta^{13}\text{C}$ values in food web samples on the order of 3.8 to 4.2 ‰. Alternatively, the temporal increase in $\delta^{13}\text{C}$ values of food web items are consistent with expected trends of increased primary productivity related to increased eutrophication of the gulf through time (Hecky, 1993; Hecky et al., 2010; Oczkowski et al., 2014). Increasing eutrophication of Winam Gulf has previously been described (Gikuma-Njuru et al., 2005; Hecky et al., 2010) while associated increases in key limiting nutrients such as total phosphorus have nearly doubled between 2000 and 2008 (Roegner et al., 2023). Elsewhere in Lake Victoria, Van Rijssel et al. (2017) observed $\delta^{13}\text{C}$ values to increase by a similar 4–5 ‰ between 1998 and 2011 in archived cichlid samples (*Haplochromis laparogramma* and *H. pyrrhocephalus*) from Mwanza Gulf and attributed these increases to eutrophication. Overall, carbon isotopes in the present study are consistent with an increased influence of eutrophication stress through time. In turn, increased eutrophication is predicted to enhance biodilution processes and reduce bioavailable mercury (Pickhardt et al.,

2002; Chen and Folt, 2005; Karimi et al., 2007; Driscoll et al., 2012). Indeed, evidence from the lower food web items (mussels and Nile tilapia) supports an ecosystem biodilution prediction, with both lower food web items showing between 38.6 % and 52 % declines in their THg concentrations across years. However, the biodilution effect was not apparent for Nile perch, which showed little to no change in THg concentration at the smallest size range and increasing THg concentrations in larger fish. The lack of support for eutrophication-driven biodilution in a top predator fish from this system is not unprecedented compared to other studied systems experiencing eutrophication (Eagles-Smith et al., 2018) and implies that other stressors are counteracting the biodilution effect in this species even though the effect was apparent in lower food web items.

Trophic disruption was another major stressor identified for Winam Gulf resulting from species introductions, species extinctions, temporal fluctuations in fish biomass and overall food web simplification (Hecky et al., 2010; Gikuma-Njuru et al., 2013; Otieno et al., 2022; Kitchell et al., 1997). Stable isotopes of nitrogen were used as a tracer of changes in trophic position of Nile perch and baseline organisms across study years. Baseline $\delta^{15}\text{N}$ values in mussels were lower in 2022 by 0.53 ‰ and in Nile tilapia by 1.43 ‰ compared to 1998 samples. Mussels are considered ideal baseline organisms for evaluating lower food web isotopes given that they consistently occupy trophic level 2, are sessile and long lived, have a consistent filter-feeding diet (Cabana and Rasmussen, 1996; McKinney et al., 1999). Decreases in baseline organism $\delta^{15}\text{N}$ can occur due to change in nitrogen sources. For example, increase in synthetic fertilizers over organic fertilizers can lower $\delta^{15}\text{N}$ values by 9 to 18 ‰ (Kendall et al., 2008; Cormier et al., 2021). Alternatively, increased proportions of microbially fixed nitrogen also depletes seston $\delta^{15}\text{N}$ values by 2.2 to 3.7 ‰ (Unkovich, 2013). Similar to mussels, lower 2022 $\delta^{15}\text{N}$ values were observed for Nile tilapia. Nile tilapia in the 20–30 cm sizes consume a mixture of algae, plants, detritus, invertebrates and fish (Njiru et al., 2004). The larger decline in $\delta^{15}\text{N}$ values of Nile tilapia between 1998 and 2002 compared to mussels could be attributed to a combination of change in nitrogen source as described for mussels coupled with a greater incorporation of plant material to the diet in later years. Given the apparent change in $\delta^{15}\text{N}$ of lower food web items, nitrogen isotopes in 2022 Nile perch were baseline adjusted.

Several studies have reported increases in Nile perch $\delta^{15}\text{N}$ values with body size (Campbell et al., 2003b, 2004; Poste et al., 2012; Cornelissen et al., 2018a) consistent with the known greater incorporation of higher-trophic fish to the diet of larger Nile perch, along with extensive cannibalism on its young (Goudswaard et al., 2006; Kische-Machumu et al., 2012; Outa et al., 2017). In the present research, subtle differences in $\delta^{15}\text{N}$ accumulation with size and associated trophic position were noted between the two study years validating that trophic disruption has occurred in Winam Gulf between 1998 and 2022. The asymptote regression models predicting $\delta^{15}\text{N}$ values in Nile perch with size for 1998 and 2022 predicted similar trophic positions for the smallest and largest fish with trophic level estimates of 2.7 for 8-cm size classes and trophic levels between 4.3 and 4.4 for 120-cm size classes (Eq. (2)), respectively. However, the two models deviated in the rate at which trophic position increased with size for 16–104 cm fish. For these intermediate sizes, the 1998 fish had higher trophic positions compared to equivalent sized 2022 fish with the maximum deviation occurring for 46 cm fish where the 1998 and 2022 trophic positions were 3.92 and 3.62, respectively. These between year differences could be explained by later year fish incorporating larger proportions of lower trophic organisms such as *Caridina* shrimp in their diet (Goudswaard et al., 2006). Cornelissen et al. (2018a) indicated that Nile perch between 1.5 and 65 cm show the largest degree of ontogenetic diet shifts, transitioning from invertebrates to mostly fish. The 1998 $\delta^{15}\text{N}$ asymptote model fit is consistent with the above, i.e., fish 65 cm in length were at 94 % of their asymptotic $\delta^{15}\text{N}$ value and approached their maximum trophic position. However, 2022 Nile perch did not reach their 94 % asymptotic $\delta^{15}\text{N}$ value until 115 cm and therefore were delayed in the time required to

achieve maximum trophic position. Cornelissen et al. (2018a) indicated fish up to 70 cm still incorporate *Caridina* into their diets especially those in deeper waters and during the rainy season. The present work suggests that 2022 Nile perch from Winam Gulf continue to incorporate invertebrates to their diets beyond 100 cm. Delays in the transitioning from invertebrates to predominately fish diets would be expected to lead to reduced growth given higher foraging costs, and lower net energy gains per meal for fish consuming smaller prey (Sherwood et al., 2002).

From the perspective of THg biomagnification, the decrease in Nile perch trophic position of 2022 fish, particularly in the 16–104 cm sizes, would be predicted to lower THg exposures that should carry forward into reduced THg concentrations at the largest size classes (Vander Zanden and Rasmussen, 1996). However, the opposite trend was observed and both the bioaccumulation rate and biomagnification rate of THg increased over time as did THg concentrations in dorsal muscle of the largest size classes. This implies that the observed shift in fish trophic position was not responsible for heightened mercury exposures of this species. Fish trophic position, average prey THg concentration and fish growth each affect mercury exposures, sometimes in counteracting ways (Swanson et al., 2006). Trophic position is typically related to average prey THg concentrations but can also be positively related to fish growth (Chételat et al., 2021) given that trophic position often scales with prey size (Kopf et al., 2021). Chételat et al. (2021) concluded that trophic position and associated prey THg concentration generated stronger effects on mercury bioaccumulation in two arctic salmonid species compared to the counteracting effect of increased growth. However, in the present work, mercury in baseline organisms declined as did fish trophic position leaving only reduction in growth as an alternative explanation for the enhanced mercury accumulation. Poste et al. (2012) came to a similar conclusion for Nile perch in Ugandan waters of Lake Victoria, with the higher THg biomagnification rate seen for Murchison Bay compared to those from Napoleon Gulf being attributed to higher growth dilution in Napoleon Gulf fish.

4.3. Fish consumption advice

Nile perch in the 50–85 cm slot size remained below the European Union import limits of 500 ng/g ww for mercury in fish and therefore THg concentrations in this species does not currently pose a threat to the export market. However, our calculations suggest that recommended maximum monthly meal frequencies for 60–75 cm Nile perch have dropped between 1998 to 2022 from 16 to 8 meals per month. The 8 meal/month limit corresponds to a recommendation of consuming <21.7 kg of Nile perch per year. This level of consumption is likely above the expected consumption rates of this species by Kenyan people living in proximity to Winam Gulf. Outa et al. (2020a) reported an average Kenyan per capita fish consumption of 4.5 kg per person/year which is nearly five-fold lower than the maximum recommended Nile perch consumption rate. Furthermore, Nile perch in the larger slot sizes of the legal range are less likely to contribute substantially to the total annual fish consumption given the decreasing abundances of fish in this size and higher market value, since such specimens are more likely diverted towards international markets. In the Rwamagasa artisanal gold mining area of northwest Tanzania, Taylor et al. (2005) estimated maximum weekly consumption of 250 g Nile perch (13 kg/year) which is also under the limit implied by our calculations. While assessing pond-raised Nile tilapia near artisanal gold mining sites in the Migori County (southern Kenya), Kola et al. (2019) adopted U.S. EPA fish consumption frequency calculations to compute weekly meal allowances which ranged from 4 to 16 meals per month dependent on the pond in which fish were collected and were somewhat more restrictive than advice generated here. It should be noted that the $C_{f,max}$ benchmarks computed for this study were 2.3-fold lower compared to Ontario (OMECP) benchmarks owing to the use of the lower US FDA/EPA TDI and lower Kenyan body weight compared to those used in OMECP calculation protocols. Finally, the calculated meal advisory recommendations for

this study do not factor in other contaminants such as organochlorines and assume that most of the mercury exposure to the local Winam Gulf population occurs from the consumption of fish (Campbell et al., 2003a). Overall, the local population from around Winam Gulf is currently unlikely to be exposed to excess Hg due to consumption of Nile perch from Winam Gulf. However, the results from the present research indicate a disconcerting trend of increasing mercury bioaccumulation with size for this species, a multiple-stressor outcome attributed changes in the growth rather than change in mercury loadings, baseline prey contamination or trophic position. Given the observed increases in THg bioaccumulation in Nile perch with time, and associated change in risk to human consumers of these fish, THg concentrations in Nile perch from Winam Gulf should continue to be monitored regularly.

5. Concluding remarks

The present research highlights shifts in carbon and nitrogen isotope composition and THg bioaccumulation in Nile perch and lower food web items from Winam Gulf, Lake Victoria between 1998 and 2022. After adjusting for between-year difference in baseline mussel $\delta^{15}\text{N}$ values, non-linear asymptote regressions indicated that 2022 Nile perch exhibited slower increases in their trophic position with body length compared to 1998. In contrast, THg bioaccumulation and biomagnification rates increased with body length in 2022 compared to 1998 even though declines in THg concentrations of lower food web items were observed. These contrasting observations imply cumulative multiple stressor impacts have lowered growth efficiencies of Nile perch leading to elevated THg concentration in dorsal muscle tissues of the larger size classes of this species. The increased THg concentration of 2022 Nile perch led to more stringent recommended fish consumption limits for vulnerable populations in 2022 compared to 1998, from 16 to 8 meals per month for fish in the 55–75 cm size range. Given the increasing rate of THg bioaccumulation over time, it is recommended that Nile perch continued to be monitored for contaminants, isotope signatures and growth rates given that continued increases of THg concentrations may eventually jeopardize the export market for this species and further increase local health risks of Hg exposures to the local population.

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CRediT authorship contribution statement

Ken G. Drouillard: Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Linda Campbell:** Writing – original draft, Investigation, Formal analysis, Data curation. **Dennis Otieno:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **James Achiya:** Investigation. **Albert Getabu:** Writing – review & editing, Conceptualization. **Lewis Sitoki:** Writing – review & editing, Investigation. **Reuben Omondi:** Writing – review & editing, Writing – original draft. **Anakalo Shitandi:** Writing – review & editing. **Bethwell Owuor:** Writing – review & editing. **James Njiru:** Resources, Project administration. **George Bullerjahn:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **R. Michael Mckay:** Investigation, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Kefa M. Otiso:** Supervision, Project administration, Funding acquisition. **Emma Tebbs:** Writing – review & editing, Supervision. NSF-IRES Lake Victoria Research Consortium. Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.170059>.

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