High Rates of Mercury Biomagnification in Fish from Amazonian Floodplain-Lake Food Webs

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Abstract

Despite a global phase out of some point sources, mercury (Hg) remains elevated in aquatic food webs, posing health risks for fish-eating consumers. Many tropical regions have fast growing organisms, potentially short food chains, and few industrial point sources, suggesting low Hg baselines and low rates of trophic magnification with limited risk to people. Nevertheless, insufficient work on food-web Hg has been undertaken in the tropics and fish consumption is high in some regions. We studied Hg concentrations in fishes from floodplain lakes of the Juruá River, Amazonas, Brazil with three objectives: 1) determine rates of Hg trophic magnification, 2) assess whether Hg concentrations are high enough to impact humans eating fish, and 3) determine whether there are seasonal differences in fish Hg concentrations. A total of 380 fish-muscle samples were collected from 12 floodplain lakes during the low-water (September 2018) and falling-water (June 2019) seasons and analyzed for total Hg and stable nitrogen (N) isotopes. The average trophic magnification factor (increase per trophic level) was 10.1 in the low-water season and 5.4 in the falling-water season, both well above the global average for freshwaters. This high rate of trophic magnification, coupled with higher-than-expected Hg concentrations in herbivorous species, led to high concentrations (up to 17.6 mg/kg dry weight) in predatory pirarucu and piranha. Nearly 70% of all samples had Hg concentrations above the recommended human-consumption guidelines. Average concentrations were 42% higher in the dry season than the wet season, but differences varied by species. Since Hg concentrations are higher than expected and fish consumption in this region is high, future research should focus on Hg exposure for human populations here and in other tropical-rainforest regions, even in the absence of local point sources of Hg.

Key words: Trophic magnification, methylmercury, arapaima, subsistence fishing, low-water season, falling-water season

Introduction

Mercury (Hg) is released to the global environment from natural geological sources and anthropogenic activities (Pirrone et al. 2010). The latter includes coal burning, mining and smelting of metals, production of cement, artisanal gold mining, and industrial discharges, and emissions from these activities are declining with time in Europe and North America but increasing in Asia (Zhang et al. 2016, UN Environment 2019). As such, there remains a large global pool of Hg that can be transported long distances in the atmosphere to enter soil and water bodies through wet and dry deposition (Lyman et al. 2020), where it is methylated and bioaccumulates in food webs (Kidd et al. 2012). Wind patterns, temperature, landscape characteristics and flooding affect deposition and accumulation of Hg in aquatic environments (Obrist et al. 2018).

The fate of Hg in food webs is influenced by three main factors: Hg entering the base of the food web, length of the food chain and trophic magnification (Kidd et al. 2012), all of which differ across climate zones. Emission sources in the global south are dominated by artisanal gold mining (UN Environment 2019, Crespo-Lopez et al. 2021), though there are many tropical regions with no gold mining, intact forests and limited riparian disturbance. In those areas we could expect low or high baseline concentrations depending on naturally occurring Hg in soils (Fadini and Jardim 2001, Wasserman et al. 2003, Figueiredo et al. 2018). Mercury biomagnifies in food chains (Kidd et al. 2012), but is expected to do so at lower rates in tropical regions (Lavoie et al. 2013), because fish tend to grow quicker, possibly allowing for growth dilution of Hg in tissues (Chételat et al. 2020). Many tropical fish tend to have a short lifespan as well, preventing the bioaccumulation of Hg over a long period. Tropical regions are also expected to have shorter food chains than areas further from the Equator (Layman et al. 2005, Jardine 2016, Lacerot et al. 2021), owing to a high diversity of primary producers that create conditions favorable for herbivorous and omnivorous diets. Together, these features would suggest limited Hg risk for top predators in tropical regions compared with temperate and polar climates.

Mercury is a toxic metal, and its risk to human populations usually depends on a combination of concentrations in fish, and fish-consumption rates. Based on the consumption guidelines from many jurisdictions (e.g. United States Environmental Protection Agency, U.S. EPA), the maximum recommended concentration is 500 ng/g wet weight, with lower concentrations for regular consumers of fish. Recent studies in Amazonian rivers found that predators, such as barred catfish (caparari - *Pseudoplatystoma fasciatum*), wolf fish (trihara - *Hoplias malabaricus*), peacock bass (tucunaré - *Cichla ocellaris*) and pirarucu (*Arapaima* sp.), can have mean concentrations above the guideline, while herbivores and detritivores were always below this threshold (Albuquerque et al. 2020, Ferreira da Silva and de Oliveira Lima 2020). Typically, 20% to 50% of all fish samples will have Hg concentrations higher than the 500 ng/g guideline (Anjos et al. 2016, Lino et al. 2018). In the Amazon basin, inland fisheries produce 450,000 tonnes of fish annually due to the large protein demand by local populations (WWF 2020). Fish-protein per-capita consumption in Amazonian riverine communities is between 100 grams and 550 grams per day (Begossi et al. 2018), amongst the highest in the world. Mercury concentrations are therefore a serious concern for both the local communities in the Amazon River system (Passos and Mergler 2008), as well as for consumers of exported fish outside the Amazon (Meneses et al. 2022).

Flooding of rivers directly associated with the upper Amazon River occurs from about mid-December to mid-May while the low-water season usually occurs from June to December. These seasonal floods cause the re-suspension and distribution of particles in water, including Hg, that previously resided in the soils and sediment. Under anaerobic conditions caused by flooding and subsequent lake stratification (Brito et al. 2017), inorganic Hg can also be converted into bioavailable and toxic methyl Hg by sulphate reducing bacteria, leading to higher concentrations in the food web. The rate of methylation of Hg increases with increasing temperature, peaking during the hottest months, which could counteract some of the factors noted above that would keep Hg risk low for tropical top predators. However, in flooding seasons, there is an increase in interspecies interactions and forage ranges for fish, increasing nutrient availability and growth rates of plankton and fish, which can lead to growth dilution of Hg (Bastos et al. 2007, Brito et al. 2017). Based on these considerations, Hg concentrations could be either higher or lower in the high-water season compared to the low-water season.

In this study, we analyzed Hg concentrations in fish from the Juruá River, Amazonas, Brazil. The Brazilian Amazon has been changing rapidly due to migration and regional development (Bastos et al. 2007), but more isolated areas, such as the middle Juruá, still have intact forests and protected reserves. There is no artisanal gold mining in the Juruá catchment, and forestry activities are limited, though some atmospheric transport of Hg from mining and fires could lead to Hg deposition in the headwaters. Together with expected food-web properties, low Hg concentrations were anticipated at the base of the food chain, limited Hg trophic magnification through the food web, and short food chains that would suggest a small risk of Hg to local people despite their high fish-consumption rates. Based on this information, we had three objectives: 1) calculate Hg trophic magnification factors in the fish food web, 2) compare fish Hg concentrations to guidelines for consumption, and 3) determine whether there are seasonal differences in Hg concentrations in fish that could point to underlying factors responsible for Hg sources and its fate in food webs.

Methods

The Secretaria de Estado do Meio Ambiente Amazonas (SEMA-DEMUC; Ref. 41/2018), Instituto Chico Mendes de Conservacão da Biodiversidade (ICMBio; Ref. SISBIO 62427-1), and the Ethic Committee of the Instituto Nacional de Pesquisas da Amazônia (INPA; Ref. 040/2018) authorized the research. Samples were collected from 12 floodplain lakes along the Juruá River in September 2018 during the low-water season and during the falling-water season in June 2019 (Hawes & Peres 2016). The Juruá's floodplain lakes are important sites for inland fisheries that support the livelihoods and basic diets for people living in remote rural settlements and larger market towns within the region (Newton et al. 2012, Endo et al. 2016). These lakes are dominated by sand and mud substrate with limited aquatic vegetation. They fill with sediment-laden water from the main river during the high-water season, but, unlike in other Amazonian lakes (Gomes et al. 2020), pH remains high in the low-water season when the lakes become disconnected. During the low-water sampling, lakes were generally circumneutral, with low conductivity and a range of trophic status from oligotrophic to hypereutrophic (Table S1), consistent with earlier work on a broader range of lakes within the region (Campos-Silva et al. 2021).

Approximately 60 species of fish were collected using gill nets. Due to the limited taxonomic knowledge of some groups, especially for juveniles, some sibling species may have been grouped, but they would have similar trophic relationships. All specimens were weighed and measured in situ for standard and total length, and a small skinless, dorsal muscle-tissue sample was collected from each fish, yielding a total of 377 samples (Table S2). These were immediately stored in a freezer (-20°C) for <30 days until arrival at INPA, where tissues were freeze-dried and ground. Samples of 20.0 ± 5.0 mg were placed in nickel boats for analysis of total Hg (ng/g dry weight) on a Direct Mercury Analyzer (Milestone DMA-80) calibrated with a certified reference material (TORT-3, lobster hepatopancreas). Recovery of a second certified reference material (DORM-4, fish protein) was 93 ± 6% S.D. (n = 35). Blanks were consistently less than half of the detection limit, so samples were not blank-corrected.

Many studies assume that methyl Hg is the dominant form in fish tissue (Bloom, 1992) so total Hg can serve only as a proxy for methyl Hg. However, % methyl Hg can be variable in fish tissues, especially small-bodied and omnivorous species (Lescord et al. 2018), so % methyl Hg was calculated for a subset of the fish samples (n = 24) using the methods given in Barst et al. (2013) (Supplementary Information). Samples tested for % methyl Hg covered a range of species and trophic levels/feeding guilds.

To assess trophic magnification, samples were analysed for stable N isotopes. Samples were packed into tin capsules at 1.0 ± 0.2 mg, combusted in an elemental analyser (Costech

ECS4010), and delivered via continuous flow to an isotope ratio mass spectrometer (Thermo Scientific Delta V). Data are reported in delta notation according to $\delta^{15}N$ = $(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{AIR})$ - 1 x 1000, where AIR is atmospheric nitrogen, the standard for $\delta^{15}N$ measurements. Instrumental precision, measured as the standard deviation of repeated in-house standards, was 0.15‰ (n = 70).

Total Hg concentrations were $\log_{10}$-transformed for all statistical comparisons. Concentrations were regressed against $\delta^{15}N$, which is a proxy for trophic position (Jacobi et al. 2020). The slope of this linear relationship is known as the Trophic Magnification Slope (TMS) which averages 0.16 ± 0.11 for total Hg worldwide (Lavoie et al. 2013). A TMS greater than zero indicates there is biomagnification in the food web, whereas a negative slope indicates biodilution. The Trophic Magnification Factor (TMF) can be calculated from the TMS and represents the increase in Hg concentration per trophic level, as calculated from: $TMF = 10^{(TMS\ x\ 3.4)}$, where 3.4 is an average increase in $\delta^{15}N$ with each trophic level, known as the trophic enrichment factor (TEF, Post 2002). A TMF greater than 1 indicates biomagnification and a TMF less than one indicates biodilution. The average TMF globally for total Hg is 3.5 (Lavoie et al. 2013), indicating a 3.5-fold increase in total Hg with each step in the food chain.

The six species of fish with the largest sample sizes: aruanã (silver arowana, *Osteoglossum bicirrhosum*), bodó (Amazon sailfin catfish, *Liposarcus pardalis*), curimatã (black prochilodus, *Prochilodus nigricans*), piranha (red-bellied piranha, *Pygocentrus nattereri*), pirarucu (*Arapaima* sp.) and tucunaré (peacock bass, *Cichla* sp.), were compared and analysed for Hg concentrations, N-isotope ratios and trophic position for the wet and dry season. Trophic position (TP) was calculated as TP = 2 + $(\delta^{15}N_{fish} - \delta^{15}N_{baseline})/3.4$ (Post 2002) where $\delta^{15}N_{baseline}$ was the $\delta^{15}N$ value of primary consumers (zooplankton, insects, snails) that occupy trophic position 2 (Jacobi et al. 2020) and 3.4 is the TEF. While a TEF of 3.4‰ is likely high for these species (Jacobi et al. 2020), it was applied to maintain consistency with the broader trophic magnification literature (Lavoie et al. 2013). We also include a calculation of alternative TMF values when applying lower TEFs (Supplementary Information, Table S3). All baseline invertebrate samples were collected from the same lakes at the same time as the fish samples.

All Hg concentrations were compared with the U.S. EPA Guidelines for Hg exposure (500 ng/g wet weight or 2000 ng/g dry weight assuming 75% moisture). Values from each trip were also compared separately to determine if there were any seasonal changes in Hg concentrations. Data were tested for homogeneity of variance using Levene's Test. A general linear model was then constructed for all lakes and both seasons, with season and species as fixed factors and lake as a random factor. The model included all pairwise interactions. Species-specific models were then analysed to determine which species showed seasonal differences, including total length and trophic position as covariates and lake as a random factor. Statistical significance was set at alpha = 0.05. All analyses were conducted in R (1.2.5001).

Results

Log (Hg concentration) vs δ^{15}N regressions strongly fit the data for each lake in both seasons (Table 1, Figure S1). In the low-water season, r^2 values were between 0.50 and 0.95 and all p-values were < 0.01. In the falling-water season, the r^2 values ranged from 0.26 to 0.95, and all regressions were significant except at Sacado do Jiburi where the δ^{15}N range was limited (<3‰). The average TMS determined for all floodplain lakes was 0.282 in the low-water season, corresponding to an average TMF of 10.1, and 0.208 in the falling-water season, corresponding to a TMF of 5.4 (Table 1). Five of the 12 lakes had significantly lower slopes in the falling-water season, indicated by a significant interaction between season and lake (Figure S1). Trophic magnification slopes were all positive and TMFs were all greater than 1, regardless of lake or season (Table 1). The highest TMF was at Marari Grande in the low-water season (23.5) and the lowest was at Sacado do Jiburi in the falling-water season (2.7).

Concentrations of total Hg in many of the fish species were much higher than the recommended guidelines of 2000 ng/g dry weight (d.w.). The arithmetic-mean concentration for all the samples was 1638 ng/g (d.w.) and the geometric mean was 968 ng/g (d.w.). The lowest individual value was 70 ng/g (d.w.) in a cará (Family Cichlidae), with a trophic position of 2.34 and the highest individual concentration was 17610 ng/g (d.w.) in a pirarucu with a trophic position of 3.58. High concentrations in piranha, despite their small body size, illustrate the importance of trophic position in driving Hg accumulation. Four of the six most common species collected had an average Hg concentration higher than guidelines (Table 2). The majority of the Hg was methyl Hg, with % methyl Hg between 80 and 90% in 20 of the 22 samples tested (Figure S2). While detritivorous species appeared to have lower % methyl Hg (Figure S2), the samples from bodó and curimatã, which are among the most commonly caught and consumed species for local subsistence, still had values >80%.

Mercury concentrations were generally higher in the low-water season than in the falling-water season. Five of the six common fish species we captured had higher values in the low-water season than the falling-water season, and the differences were strongest in the top predators (Figure 1). There were differences among species, seasons and lakes, and all two-way interactions were significant ($p < 0.05$), with the interactions driven by bodó that had a higher concentration in the falling-water season. Species-specific models indicated that the carnivorous aruanã, tucunaré, pirarucu and piranha all had significantly higher Hg concentrations during the low-water season ($p < 0.05$), while the detritivorous bodó had higher concentrations during the falling-water season ($p < 0.05$), and concentrations in the detritivorous curimatã were not significantly different between seasons. The effects of length and trophic position depended on the species. Length was significant for the predators tucunaré, pirarucu, piranha and aruanã, while trophic position was significant for pirarucu, piranha, bodó and aruanã.

Discussion

Mercury concentrations increased strongly with trophic level in Amazon floodplain-lake food webs, resulting in TMFs that are well into the upper end of the range observed worldwide and running counter to earlier predictions about lower trophic magnification in tropical food webs (Lavoie et al. 2013). Concentrations of Hg reached values that are a concern for the health of human consumers, and these high concentrations were apparent in both low- and falling-water seasons when fish are consumed at high rates. Future studies should assess Hg exposure in local human populations and fish-eating wildlife to determine whether there are adverse effects due to Hg toxicity, and consider the potential for co-occurring elements such as selenium in ameliorating Hg toxicity risk (Rocha et al. 2014, Ralston et al. 2019).

Trophic magnification of Hg in Juruá floodplain lakes was higher than averages found in previous studies. Globally, TMFs for total Hg are 3.2 and 4.8 for freshwater and marine sites, respectively (Lavoie et al. 2013). The mean TMF values in this study were above these values in both the low- and falling-water season, and well above a previously reported mean value for tropical freshwaters (2.6, Lavoie et al. 2013) that included other locations in the Amazon (TMF = 4.5 to 5.2, Azevedo-Silva et al. 2016). Tropical freshwaters outside of South America have even lower TMF values (Jardine et al. 2012, Ouedraogo et al. 2015), and Hg concentrations therefore were expected to be lower in tropical predatory fish for a given food-chain position. However, concentrations in this study exceeded those even for typical cold-water fish at northern latitudes (Depew et al. 2013), opposite to expectations. This occurred despite relatively low trophic positions of predators such as pirarucu, aruanã, tucunaré, and piranha (Table 2), which were comparable to those from tropical African reservoirs (Ouedraogo et al. 2015). Application of lower $\delta^{15}N$ TEFs resulted in TMFs that were more in line with global averages (Table S3), but applying such low TEFs would also increase mean trophic positions beyond expected values based on known diets of these species. For example, applying a TEF of 2.0‰ results in a mean trophic position for pirarucu of 4.2, much higher than the mean value calculated from stomach contents (3.6, Jacobi et al. 2021).

Though high TMFs were unexpected, TMFs were estimated from tabulated data in da Silva et al. (2005) for another Amazonian system, the Tapajós River, and calculated values ranged from 7.1 to 14.3, suggesting that high Hg trophic magnification may be more widespread than expected. Rapid growth rates in tropical regions were expected to lead to growth dilution of Hg in fish (Chételat et al. 2020), but the high concentrations observed in pirarucu that is amongst the fastest growing fishes in the world, do not accord with that hypothesis. This highlights the complexities around food consumption rates, assimilation efficiencies, and elimination rates that are the subject of Hg mass-balance models (Trudel and Rasmussen 2006, Madenjian et al. 2021).

High TMFs should be associated with low baseline concentrations (Lavoie et al. 2013), yet these Juruá floodplain lakes had high TMFs and high baselines, the latter indicated by high concentrations in species occupying low food-chain positions. In other tropical freshwaters, large-bodied herbivores and detritivores had lower concentrations than those observed here. For example, concentrations in Nile tilapia (*Oreochromus niloticus*) ranged from 0.003 to 0.014 µg/g wet weight, equivalent to ~12 to 56 ng/g dry weight (Ouedraogo et al. 2015), roughly one quarter of the concentrations we observed in large-bodied herbivore/detritivores, while bony bream (*Nematalosa erebi*) in northern Australia had concentrations under 100 ng/g dry weight (Jardine et al. 2012), less than half of that in the Juruá. This suggests a much higher Hg baseline concentration in the Juruá likely owing to high natural background in soils (Wasserman et al. 2003) that are seasonally flooded. High baseline concentrations, evidenced by high concentrations in non-piscivorous fishes (~150 to 400 ng/g dry weight, da Silva et al. 2005), occur in the Tapajós River, which has intensive artisanal gold mining, suggesting that these Amazonian tributaries have high Hg levels whether or not they are exposed to local point sources.

Mercury concentrations in fish were expected to be higher in the flooding season than the dry season. Flooding and filling of floodplain lakes causes resuspension and redistribution of Hg from soils and sediments, and breakdown of organic matter leads to anoxia and Hg methylation by sulfur-reducing bacteria (Acha et al. 2011, Pestana et al. 2019). The higher concentrations in detritivorous bodó in the falling-water season suggests a detrital entry point for the Hg (Fostier et al. 2015) that is highest during and after flooding, with Hg transmitted to top predators later in the low-water season. This temporal disconnect also contributed to the observed differences in TMFs between seasons, but only half of the lakes showed this pattern. Differences in concentrations between the seasons have also been hypothesized as the result of greater prey density in the dry season compared to the wet, or may be due to physio-chemical differences (pH and electrical conductivity, Gomes et al. 2020). Overall, while there are significant seasonal changes in concentrations and TMFs, both seasons have high enough concentrations to warrant concern for fish-eating consumers.

The oral reference dose for methyl Hg is 0.1 ng/g body weight per day for non-carcinogenic endpoints (Rice et al. 2000). This value is the concentration that can be consumed daily over time without causing any detectable adverse effects such as neurotoxicity in children, adults and fetuses (Rice et al. 2000). If people in the region randomly consumed the species tested, they would be exposed to far greater concentrations than the reference dose, considering the average weight of a human is 70 kg, average daily fish consumption in the Juruá is 100 to 550 grams and the average Hg concentration is 1638 ng/g dry weight (~410 ng/g wet weight). This would result in a daily exposure between 0.6 and 3.2 ng/g body weight per day, greatly exceeding the reference dose. Although the reference dose is conservative (Clarkson 2002), most of the species tested in our study also exceeded the national consumption guideline in Brazil (500 ng/g wet weight). Based on the fish consumption rates by people in the Juruá, these standards are not sufficient and human populations are likely at risk of Hg toxicity (Passos and Mergler 2008, Crespo-Lopez et al. 2021). However, detritivores, herbivores and omnivores represent the majority of the subsistence catch in the Juruá (Instituto Juruá, unpublished data), and since these species had much lower concentrations than the carnivorous species such as tucunaré, piranha, aruanã, and the iconic pirarucu, Hg exposure risk will be reduced. Also, the lower molar ratios of Hg:Se in herbivorous species (Lima et al. 2005, Lino et al. 2020) and other foods (Rocha et al. 2014, da Silva Junior et al. 2022) could protect consumers in rural communities against the high concentrations of Hg in the carnivores. Therefore, future studies should incorporate the consumption rates of fish species from different trophic guilds and plant foods in Hg risk assessments to provide a clearer picture regarding the exposure of such communities.

Humans are not the only top predators that may be affected by high Hg concentrations. Caimans are amphibious carnivores, and the black caiman (*Melanosuchus niger*) is among the largest predators in the Amazonian ecosystem. This species includes fish in its diet (Laverty & Dobson 2013), and average Hg concentrations of 1100 ng/g have been measured in their claws (Gomes et al. 2020). River dolphins (*Inia* spp. and *Sotalia fluviatilis*) are also likely to be affected, with their diet including piranhas, shrimp, crabs and turtles (Mosquera-Guerra et al. 2019). Mercury concentrations ranged from 870 ng/g to 3990 ng/g in muscle tissues of these dolphin species (Mosquera-Guerra et al. 2019), consistent with values for high-trophic-level consumers in our study, and likely exceed expected toxicity thresholds (Kershaw and Hall 2019). Mercury exposure and effects in these and other fish-eating predators, such as giant otters (*Pteronura brasiliensis*) would warrant further investigation.

With these high observed Hg concentrations, we are left with little anthropogenic explanation for their origin. The main anthropogenic sources of Hg in the Amazon are from artisanal small-scale gold mining, deforestation and biomass burning, and hydroelectric dams (Crespo-Lopez et al. 2021). Though artisanal gold mining is prominent elsewhere in the Amazon, including the Tapajós, we are unaware of any local activity in the Juruá catchment. Tree leaves are known to accumulate atmospheric Hg (Ericksen et al. 2003), but Hg loading to forest canopies is low beyond approximately 50 km from these emission sources (Gerson et al. 2022), much farther than the nearest likely Hg emission source from gold mining. Forestry causes disturbances and erosion of soils and sediments, allowing Hg to enter waterbodies, including Hg added through gold mining (Telmer et al. 2006). However, there is little evidence for significant deforestation upstream of our sites or on its tributaries. Furthermore, while the construction of hydroelectric dams remobilizes and resuspends Hg from sediments and soils (Arrifano et al. 2018), there are no dams upstream of the study sites. As such, while there are many sources present in the Amazon, there are no obvious local sources to which we could attribute such high concentrations in the Juruá River.

Future studies in this region should closely examine the biogeochemistry of Hg by testing seasonal patterns in methylation potential, influences of water quality (e.g. dissolved oxygen depletion) and other factors expected to influence availability of Hg at the base of the food web. In parallel, studies should aim to link species-specific consumption patterns with any adverse effects on communities surrounding these waterbodies by assessing endpoints of Hg exposure and potential Hg-toxicity symptoms. Those studies, along with our current data, could lead to recommendations on what fish species can be eaten safely, to provide a critical source of protein, vitamins and essential fatty acids for people in these riverine communities while minimizing risks from Hg exposure.

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Table 1. Slopes of $\log_{10}$ (Hg concentration) vs. $\delta^{15}N$ relationships and corresponding Trophic Magnification Factors (TMFs) for 12 floodplain lakes along the Juruá River, Amazonas, Brazil, during the low-water season (September 2018) and the falling-water season (June 2019). Interaction p values indicate whether slopes were equivalent within lakes between the low- and falling-water seasons, while season p values indicate whether intercepts were equivalent between the low- and falling-water seasons.

Lake	Low-water Slope ± SE (TMF)	r^2, p (n)	Falling-water Slope ± SE (TMF)	r^2, p (n)	Interaction p	Season p
Bomfim	0.296 ± 0.033 (10.1)	0.85, <0.001 (16)	0.256 ± 0.026 (7.4)	0.87, <0.001 (16)	0.357	0.040
Damião	0.192 ± 0.034 (4.5)	0.80, <0.001 (10)	0.246 ± 0.085 (6.9)	0.48, 0.018 (12)	0.557	0.011
Mandioca	0.308 ± 0.025 (11.1)	0.80, <0.001 (51)	0.199 ± 0.033 (4.7)	0.82, <0.001 (10)	0.038	NA
Marari Grande	0.403 ± 0.128 (23.5)	0.50, 0.010 (17)	0.176 ± 0.027 (4.0)	0.75, <0.001 (16)	0.040	NA
Puca	0.282 ± 0.018 (9.1)	0.95, <0.001 (15)	0.178 ± 0.044 (4.0)	0.58, 0.002 (15)	0.026	NA
Pupunha de Baixo	0.267 ± 0.033 (8.1)	0.78, <0.001 (20)	0.293 ± 0.028 (9.9)	0.95, <0.001 (10)	0.540	<0.001
Resaca do Xibauá	0.240 ± 0.039 (6.5)	0.65, <0.001 (22)	0.172 ± 0.048 (3.8)	0.62, 0.007 (15)	0.326	0.021
Sacado do Jiburi	0.291 ± 0.037 (9.8)	0.85, <0.001 (13)	0.128 ± 0.073 (2.7)	0.26, 0.113 (12)	0.046	NA
Samaúma	0.366 ± 0.030 (17.6)	0.83, <0.001 (38)	0.221 ± 0.026 (5.6)	0.87, <0.001 (15)	0.013	NA
Santa Clara	0.226 ± 0.037 (5.9)	0.79, <0.001 (12)	0.216 ± 0.026 (5.4)	0.83, <0.001 (16)	0.831	0.611
São Sebastião	0.235 ± 0.021 (6.3)	0.92, <0.001 (13)	0.222 ± 0.036 (5.7)	0.80, <0.001 (13)	0.771	0.844
Veado	0.280 ± 0.042 (9.0)	0.79, <0.001 (29)	0.189 ± 0.040 (4.4)	0.76, 0.002 (9)	0.160	0.208

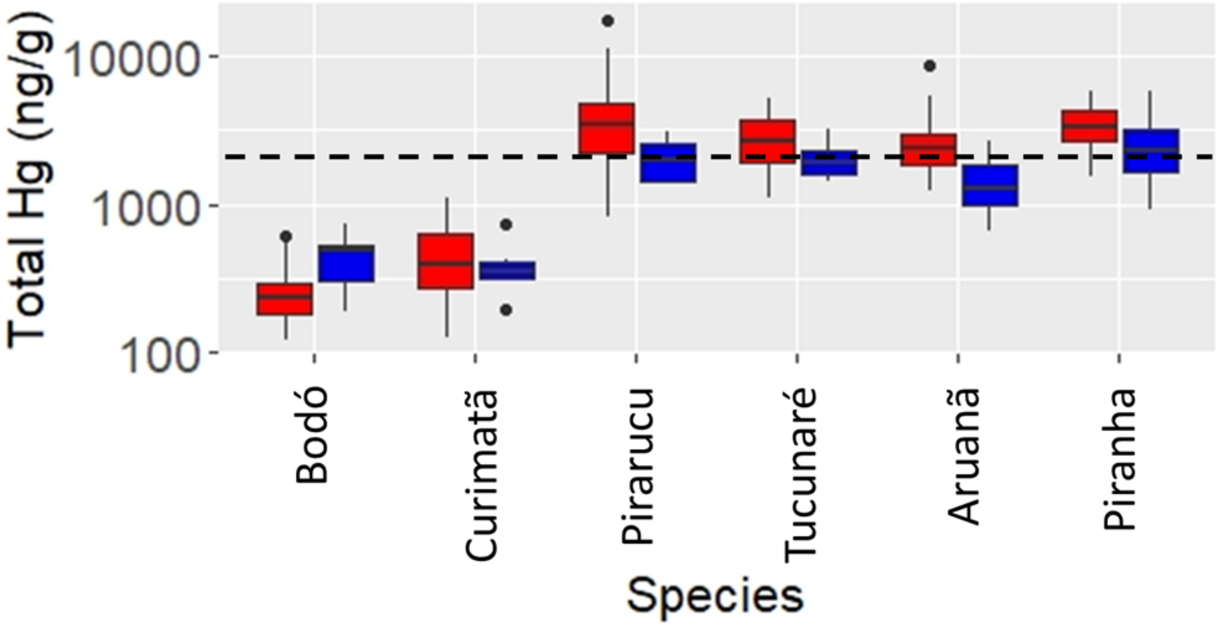
Table 2. Mean (± 1 S.D.) body sizes, stable-nitrogen-isotope ($\delta^{15}N$) ratios, trophic positions and feeding guilds for six common fish species found in floodplain lakes along the Juruá River.

Species	Season	Body length (cm)	$\delta^{15}N$	Trophic position	Feeding guild
Bodó	Low (24)	36.2 ± 5.8	7.3 ± 1.2	2.4 ± 0.2	Detritivore
	Falling (10)	40.6 ± 3.5	7.9 ± 1.0	2.6 ± 0.2	
Curimatã	Low (32)	32.7 ± 6.1	8.0 ± 0.9	2.6 ± 0.3	Detritivore
	Falling (6)	22.8 ± 2.1	8.7 ± 0.4	2.6 ± 0.3	
Pirarucu	Low (34)	185.7 ± 31.4	9.9 ± 0.8	3.2 ± 0.3	Carnivore
	Falling (5)	142.7 ± 32.8	9.8 ± 0.5	3.2 ± 0.4	
Tucunaré	Low (15)	38.4 ± 3.6	10.8 ± 0.3	3.4 ± 0.4	Carnivore
	Falling (16)	31.6 ± 7.6	10.6 ± 0.3	3.4 ± 0.4	
Aruanã	Low (20)	63.4 ± 11.3	11.1 ± 0.5	3.7 ± 0.4	Carnivore
	Falling (15)	59.2 ± 11.9	10.5 ± 0.4	3.4 ± 0.4	
Piranha	Low (9)	19.3 ± 2.3	11.2 ± 0.3	3.8 ± 0.4	Carnivore
	Falling (28)	19.2 ± 3.2	11.0 ± 0.6	3.5 ± 0.4	

Figure captions

Figure 1. Median log total Hg concentrations (ng/g) in muscle tissue of six common fish species found in floodplain lakes along the Juruá River in the low-water (red) and falling-water (blue) seasons. Boxes represent the interquartile range, lines show minima and maxima, and points are outliers. The horizontal dashed line indicates the Hg consumption guideline (~2000 ng/g assuming 75% moisture).

Figure 1.



Supporting Information for:

High Rates of Mercury Biomagnification in Fish from Amazonian Floodplain-Lake Food Webs

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SI 1. Limnological data

We measured several basic water quality parameters to characterize the floodplain lakes (Table S1). Temperature, pH and conductivity were measured with a handheld tester (Hanna), and a Secchi depth reading was taken from a canoe at the perceived deepest part of each lake. Total nitrogen (TN) and total phosphorus (TP) were measured by spectrophotometry after acid digestion. Chlorophyll *a* concentrations were determined by spectrophotometry according to Golterman et al. (1978). Extraction was performed with 90% acetone in a low-light environment and samples were read at a wavelength of 663nm. Correction for probable interference from other colored compounds and turbidity was made by reading at a wavelength of 750 nm, in which absorption by these pigments is minimal.

SI 2. Methods for estimating % methyl Hg by difference.

To estimate % methyl Hg in the fish samples, we followed methods outlined in Barst et al. (2013). We first analysed samples for total Hg using a Direct Mercury Analyser (DMA-80, Milestone, Inc.). Next, we weighed ~100 mg of the sample into a centrifuge tube and added 1 mL of high strength HCl (12 M). We microwaved the sample at 10% power for 30 seconds and let it cool for five minutes. Any higher power caused the centrifuge tube to burst open. Five mL of toluene were then added and the tube was shaken for 20 minutes with a wrist-action shaker. The sample was then centrifuged for 15 minutes at 966 g. The supernatant was transferred into a 5 mL centrifuge tube and diluted with DI water to minimize the chance of corrosion in the DMA. Liquid samples were then pipetted into quartz boats and analysed on the DMA for inorganic Hg concentrations. The inorganic Hg concentration was then subtracted from the total Hg concentration to estimate the methyl Hg concentration.

SI 3. Quality Assurance/Quality Control for the inorganic Hg determination

We subjected three different certified reference materials (CRMs) to the extraction procedure and compared results to expected values for inorganic Hg (Table S2). Fish protein (DORM-4, certified value = 56 µg/kg), lobster hepatopancreas (TORT-3, certified value = 155 µg/kg) and dogfish liver (DOLT-5, certified value = 1780 µg/kg) were the CRMs that were closest in composition to the fish matrix we were testing in our samples. The CRM with the lowest expected inorganic Hg concentration, DORM-4, yielded higher than expected inorganic Hg (104 +/- 23 µg/kg; 185.7 ± 40.5% recovery), but the other two CRMs were within specifications (TORT-3 = 150 +/- 19 µg/kg, 97.0 ± 12.2% recovery; DOLT-5 = 2082 +/- 134 µg/kg, 116.9 ± 7.5% recovery).

SI 4. % methyl Hg in Juruá River fish samples

Percent methyl Hg values were typically between 80 and 90% (Figure S2). Two samples had unusually low % methyl Hg (55% and 71%) but we do not know if this was an error in the extraction procedure or if it is real. With those two outliers included, the % methyl Hg vs trophic position regression is not significant ($r^2 = 0.02$, $p = 0.496$), while after their removal it was significant ($r^2 = 0.33$, $p = 0.005$).

SI 5. Estimating trophic magnification factors with alternative δ^{15}N trophic enrichment factors

Our earlier work in these Juruá floodplain-lake food webs suggested a lower-than-expected trophic enrichment factor (TEF) for δ^{15}N. Based on stomach content analyses of pirarucu (*Arapaima* sp.) paired with δ^{15}N values, Jacobi et al. (2020) reported a TEF of only 1.0. However, stomach contents of the other species were not investigated. Since it has been suggested that tropical organisms may have lower TEFs (Kilham et al. 2009), here we show how TMFs would differ when applying lower TEFs (Table S2).

Table S1. Locations, sampling dates, and water-quality measurements for 12 sampled floodplain lakes along the Juruá River, Amazonas, Brazil.

Lake	Latitude	Longitude	Season	Date	TN (mg/L)	TP (mg/L)	Chl a (µg/L)	Temp (°C)	pH	Cond.	Secchi (cm)
Bomfim	-6.0055	-67.876	Low	05/09/18	0.40	0.038	11.83	29.5	7.07	55	50
			Falling	12/06/19	1.61	0.263	-	30.4	-	80	-
Damião	-5.6867	-67.769	Low	08/09/18	0.47	0.022	0.68	29.4	6.76	54	90
			Falling	17/06/19	0.49	0.045	-	28.7	6.57	97	-
Mandioca	-5.8769	-67.829	Low	01/09/18	0.89	0.107	316.72	34.0	7.53	100	150
			Falling	14/06/19	0.58	0.049	-	29.1	6.93	104	-
Marari Grande	-5.941	-67.766	Low	03/09/18	0.41	0.056	12.56	29.0	6.46	36	70
			Falling	13/06/19	0.53	0.029	-	29.5	6.37	42	-
Puca	-5.5923	-67.561	Low	10/09/18	2.42	0.116	56.04	32.3	9.07	65	45
			Falling	20/06/19	0.60	0.043	-	28.4	6.83	95	-
Pupunha de Baixo	-5.5936	-67.764	Low	09/09/18	0.53	0.069	15.01	31.1	7.15	21	20
			Falling	18/06/19	0.52	0.025	-	31.6	6.83	54	-
Ressaca do Xibauá	-5.9052	-67.8653	Low	02/09/18	0.58	0.184	43.69	31.6	7.55	24	8
			Falling	15/06/19	0.77	0.053	-	27.1	6.65	96	-
Sacado do Jiburi	-5.1469	-67.225	Low	12/09/18	0.34	0.026	3.64	33.8	7.10	73	87
			Falling	21/06/19	0.62	0.016	-	28.9	6.78	49	-
Samaúma	-5.5276	-67.634	Low	11/09/18	0.63	0.029	0.91	34.9	7.09	73	75
			Falling	19/06/19	0.56	0.053	-	29.4	6.99	93	-
Santa Clara	-5.9846	-67.8033	Low	04/09/18	0.45	0.034	6.14	29.1	6.69	56	75
			Falling	11/06/19	0.45	0.027	-	28.5	6.91	78	-
São Sebastião	-6.0592	-67.878	Low	06/09/18	0.69	0.043	33.67	31.1	7.68	56	80
			Falling	10/06/19	1.01	0.064	-	30.3	6.93	37	-
Veado	-5.8269	-67.799	Low	07/09/18	1.11	0.100	9.56	34.5	9.68	60	25
			Falling	16/06/19	0.59	0.031	-	28.7	6.65	73	-

TN = total nitrogen; TP = total phosphorus; Chl a = chlorophyll a; Temp = temperature; Cond. = conductivity; Secchi = Secchi depth

Table S2. Sample sizes for different fish taxa collected from floodplain lakes of the Juruá River in the low-water and falling-water seasons.

Common name	**Family**	**Scientific name**	**Low**	**Falling**
piau	Anostomidae	*Leporinus* spp.		3
matrinxã	Bryconidae	*Brycon cephalus*		1
arari	Chalceidae	*Chalceus macrolepidotus*		2
palometa	Characidae	*Stethaprion erythrops*	1	
piaba	Characidae	*Ctenobrycon spilurus*	2	
piaba, lambari	Characidae	*Moenkhausia dichroura*	3	
rabo de fogo	Characidae	*Moenkhausia lepidura*	1	
sardinha	Characidae	*Triportheus* spp.	3	1
acará	Cichlidae	*Aequidens tetramerus*	1	
acará	Cichlidae	*Apistogramma agassizii*		1
acará	Cichlidae	*Chaetobranchus semifasciatus*	1	
acará	Cichlidae	*Cichlasoma amazonarum*	1	
acará	Cichlidae	*Heros efasciatus*	1	
acará	Cichlidae	*Heros* spp.	1	
acará bandeira	Cichlidae	*Pterophyllum scalare*	1	
acará disco	Cichlidae	*Symphysodon spp.*		1
acará festivo	Cichlidae	*Mesonauta festivus*		1
acará/cará	Cichlidae	Cichlidae	8	6
acará-açu	Cichlidae	*Astronotus crassipinnis*	6	1
acará-prata	Cichlidae	*Chaetobranchus flavescens*	1	
jurupari/papa-terra	Cichlidae	*Satanoperca jurupari*	1	
tucunaré	Cichlidae	*Cichla* spp.	15	16
branquinha	Curimatidae	*Curimata roseni*	1	
branquinha cascuda	Curimatidae	*Psectrogaster amazonica*	1	
branquinha cascuda	Curimatidae	*Psectrogaster rutiloides*	1	
curimbatá	Curimatidae	*Curimatella alburna*	1	
mocinha	Curimatidae	*Potamorhina altamazonica*	1	2
sardinha	Curimatidae	*Cyphocharax spiluropsis*	1	
small fishes	Curimatidae	*Potamorhina latior*		1
tapioca	Curimatidae	*Potamorhina pristigaster*	5	2
bagre	Doradidae	*Nemadoras humeralis*	1	
bagre	Doradidae	Doradidae		1
cuiú-cuiú	Doradidae	*Pterodoras granulosus*		1
quiri-quiri	Doradidae	*Amblydoras affinis*		1
traira	Erythrinidae	*Hoplias malabaricus*	6	1
peitudo	Gasteropelecidae	*Gasteropelecus* spp.	1	
charuto	Hemiodontidae	*Hemiodus immaculatus*	1	

tuvira	Hypopomidae	*Brachyhypopomus spp.*		1
ancistrus	Loricaridae	*Ancistrus* spp.		3
bodó	Loricaridae	*Liposarcus pardalis*	24	10
cascudo	Loricaridae	*Ancistrus dolichopterus*		1
aruanã	Osteoglossidae	*Osteoglossum bicirrhosum*	20	15
pirarucu	Osteoglossidae	*Arapaima* spp.	34	5
bico de pato	Pimelodidae	*Sorubim lima*	1	
mandi	Pimelodidae	*Pimelodus blochii*	4	
sorubim	Pimelodidae	*Pseudoplatystoma corruscans*		2
sardinhão	Pristigasteridae	*Pellona castelnaeana*		3
curimatã	Prochilodontidae	*Prochilodus nigricans*	32	6
jaraqui	Prochilodontidae	*Semaprochilodus insignis*	1	1
pescada	Sciaenidae	*Plagioscion squaumosissimus*	1	
pacu	Serrasalmidae	*Mylossoma aureum*	10	2
pacu	Serrasalmidae	Serrasalmidae		7
piranha	Serrasalmidae	Serrasalmidae	6	
piranha branca	Serrasalmidae	*Pristobrycon striolatus*	4	15
piranha caju/vermelha	Serrasalmidae	*Pygocentrus nattereri*	9	28
piranha preta	Serrasalmidae	*Serrasalmus rhombeus*		3
pirapitinga	Serrasalmidae	*Piaractus brachypomus*		1
tambaqui	Serrasalmidae	*Colossoma macropomum*	2	14
sardinha	Triportheidae	*Triportheus angulatus*	1	
sardinha	Triportheidae	*Triportheus elongatus*	2	

Table S3. Estimated Mercury Trophic Magnification Factors (TMFs) for floodplain lakes of the Juruá River when using different trophic enrichment factors (TEFs) for δ^{15}N. Values in parentheses indicate TEFs reported in different meta-analyses (3.4‰, Post 2002; 2.8‰, Caut et al. 2009; 2.0‰, McCutchan et al. 2003) that were applied to the data.

	Low-water			Falling-water		
Lake	TMF (3.4‰)	TMF (2.8‰)	TMF (2.0‰)	TMF (3.4‰)	TMF (2.8‰)	TMF (2.0‰)
Bomfim	10.1	6.7	3.9	7.4	5.2	3.2
Damião	4.5	3.5	2.4	6.9	4.9	3.1
Mandioca	11.1	7.3	4.1	4.7	3.6	2.5
Marari Grande	23.5	13.5	6.4	4.0	3.1	2.3
Puca	9.1	6.2	3.7	4.0	3.1	2.3
Pupunha de Baixo	8.1	5.6	3.4	9.9	6.6	3.9
Resaca do Xibauá	6.5	4.7	3.0	3.8	3.0	2.2
Sacado do Jiburi	9.8	6.6	3.8	2.7	2.3	1.8
Samaúma	17.6	10.6	5.4	5.6	4.1	2.8
Santa Clara	5.9	4.8	2.8	5.4	4.0	2.7
São Sebastião	6.3	4.6	3.0	5.7	4.2	2.8
Veado	9.0	6.1	3.6	4.4	3.4	2.4
Mean ± S.D.	10.1 ± 5.4	6.6 ± 2.8	3.8 ± 1.1	5.4 ± 2.0	4.0 ± 1.2	2.7 ± 0.6

Figure S1. Log Hg concentration (µg/kg) vs. δ^{15}N (‰) regressions for the 12 floodplain lakes in the low-water (blue triangles) and falling-water (red circles) seasons. Shading indicates standard errors around slope estimates, and asterisks indicate differences in slopes between seasons.

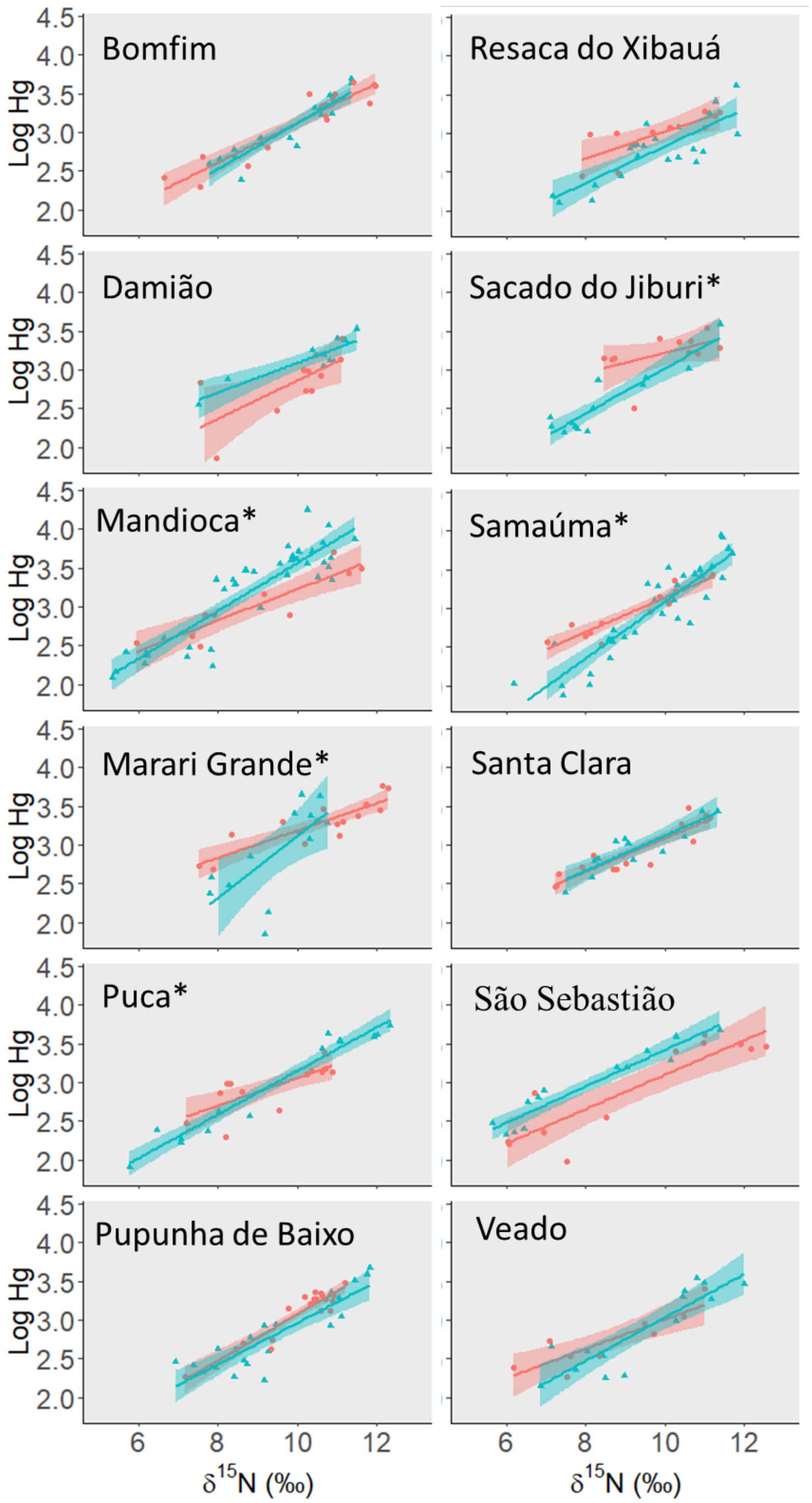
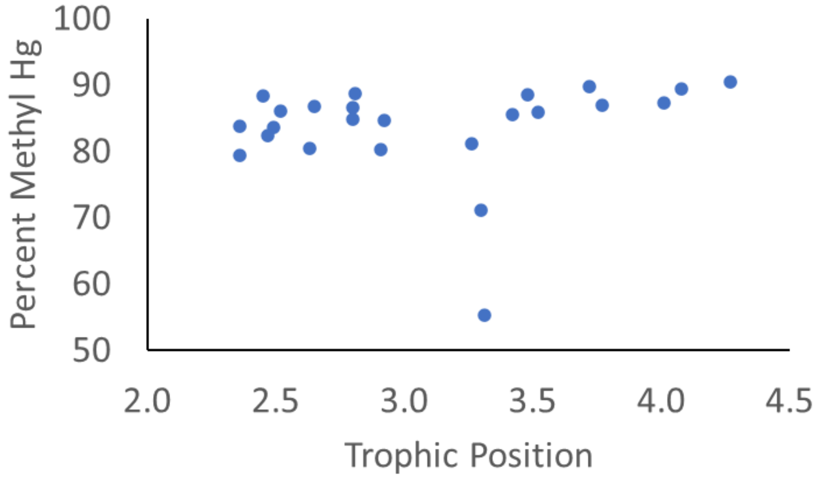


Figure S2. Percent methyl mercury (MeHg) in a subset of fish samples (n = 24) where inorganic mercury was measured using extraction methods and a Direct Mercury Analyser as outlined in Barst et al. (2013), relative to trophic position as estimated from δ^{15}N.



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