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# The cost of gold: Mercury contamination of fishes in a Neotropical river food web

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In South America, mercury contamination due to gold mining operations is a threat to both biodiversity and human health. We examined mercury (Hg) concentrations in fishes that constitute important subsistence fisheries from mined and non-mined tributaries in the middle Mazaruni River, Guyana. Mercury concentrations and trophic food web structure (based on carbon and nitrogen stable isotopes) were characterized for primary basal sources and 39 fish species representing seven trophic guilds. Fishes collected at mined sites had higher mercury concentrations; piscivores and carnivores had the highest Hg concentrations and exhibited significant Hg biomagnification. Our results showed that medium- to large-bodied fishes commonly eaten by local people contained Hg values that exceed the World Health Organization (WHO) criteria, and pose a health concern for riverine communities along the Mazaruni River that depend on fish as their main source of protein. Further research to determine the sources of Hg contamination and how it affects human health in this neotropical river must become a top priority. In addition, more research on how Hg contamination impacts the fishes themselves and overall aquatic biodiversity is also needed in the Mazaruni River which has both high fish endemism and diversity.

Keywords: Biomagnification, Gold mining, Guiana Shield, Stable isotopes, Trophic structure.

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Na América do Sul, a contaminação por mercúrio devido às operações de mineração de ouro é uma ameaça à biodiversidade e à saúde humana. Nós examinamos as concentrações de mercúrio (Hg) em peixes que constituem importantes pescarias de subsistência em afluentes minerados e não minerados no médio rio Mazaruni, Guiana. As concentrações de mercúrio e a estrutura trófica da teia alimentar (baseada em isótopos estáveis de carbono e nitrogênio) foram caracterizadas para fontes basais primárias e 39 espécies de peixes representando sete guildas tróficas. Os peixes coletados em locais minerados tiveram maiores concentrações de mercúrio; piscívoros e carnívoros tiveram as maiores concentrações de Hg e exibiram biomagnificação significativa de Hg. Nossos resultados mostraram que peixes de corpo médio a grande comumente consumidos pela população local continham valores de Hg que excedem os critérios da Organização Mundial de Saúde (OMS) e representam uma preocupação para a saúde das comunidades ribeirinhas ao longo do rio Mazaruni que dependem dos peixes como sua principal fonte de proteína. Outras pesquisas para determinar as fontes de contaminação por Hg e como isso afeta a saúde humana neste rio neotropical devem se tornar uma prioridade. Além disso, mais pesquisas sobre como a contaminação por Hg impacta os próprios peixes e a biodiversidade aquática em geral também são necessárias no rio Mazaruni, que tem alto endemismo e diversidade de peixes.

Palavras-chave: Biomagnificação, Mineração de ouro, Planalto das Guianas, Isótopos estáveis, Estrutura trófica.

## INTRODUCTION

Mercury (Hg) is a naturally occurring heavy metal that becomes highly toxic to living organisms when it transforms into the organic form of methylmercury (MeHg) (Sweet, Zelikoff, 2001). Mercury contamination is derived from both natural and anthropogenic sources and has been a major human-health concern in South America since the early 1900s (Veiga, 1997). Natural sources of mercury emissions include forest fires, weathering of soil, and volcanic emission (Pacyna *et al*., 2010), while the largest sources of anthropogenically-derived Hg in the environment include industrial emissions, deforestation, erosion from agriculture, and artisanal and small-scale gold mining (ASGM). ASGM, in particular, uses liquid elemental mercury ($Hg^0$) for gold amalgamation. After the amalgamation process, Hg is released into the atmosphere or deposited as mining waste directly into the soil and water (Telmer, Veiga, 2009). After Hg enters aquatic environments, it can persist there perpetually, and once methylated (MeHg), will bioaccumulate in aquatic food webs, thereby posing a health risk to human populations consuming fish. Controlling Hg contamination in the environment has been an issue of concern for local, national, and international health institutions because predatory fish that are consumed by riverside human populations in these developing countries contain high Hg levels (Veiga, 1997; UNEP, 2013).

The Guiana Shield ecoregion extends across the countries of Guyana, Suriname, French Guiana, Brazil, and Venezuela in northeastern South America and has considerable mineral resources such as gold, diamonds, iron, and bauxite (Hammond, 2005). This ecoregion houses the largest global repository of tropical forest vegetation on Precambrian terrain (Hammond, 2005; Dezécache *et al*., 2017) and the greatest concentration of global freshwater biodiversity with high levels of endemism (Reis *et al*., 2003; Alofs *et al*., 2014). However, this region is threatened by gold mining operations that have severely degraded aquatic habitats via siltation and river channel substrate alteration (Miller *et al*., 2003). In 2017, gold production in Guyana accounted for 10% of the total GDP (Hilson *et al*., 2019; Bank of Guyana, 2017), with ASGM accounting for 70% of the country's gold production (Watson *et al*., 2020). With the increased global demand for gold and the rising of gold price, mining operations (small-scale and medium-scale) have become more common in other countries within the Guiana Shield causing both environmental and social problems (Hammond *et al*., 2007; Dezécache *et al*., 2017; Martinez *et al*., 2018; Watson *et al*., 2020). Poorly managed mining operations intensify deforestation, increase sediment loads, and increase Hg accumulation in rivers, wildlife, and people (Cleary, 1990). High Hg concentrations derived from gold mining activities have been found in fluvial sediments of the Essequibo and Mazaruni Rivers in Guyana (Miller *et al*., 2003; Couture *et al*., 2005; Howard *et al*., 2011) and the Cuyuni River (an Essequibo tributary) in Venezuela (Nico, Taphorn 1994). Unsafe levels of Hg in carnivorous fish tissues have been reported from Suriname and French Guiana (Fréry *et al*., 2001; Ouboter *et al*., 2012) and in hair samples from indigenous communities in Guyana (Watson *et al*., 2020). Gold mining alters in-stream habitat structure (Lacerda *et al*., 2004; Mol, Ouboter, 2004), resulting in habitat transformation that shifts fish diversity and community composition (Miller *et al*., 2003; Mol, Ouboter, 2004; Barbieri, Gardon, 2009; Brosse *et al*., 2011). In the Upper Mazaruni River, gold mining is concentrated in the main channel, resulting in high turbidity and sediment accumulation (Alofs *et al*., 2014). This is particularly concerning because some species endemic to the Mazaruni drainage appear to be highly associated with main channel habitats (López-Fernández *et al*., 2012).

In aquatic ecosystems, food web structure and consumer traits (*e.g*., body size, trophic ecology) influence the concentration of Hg, in particular MeHg, assimilated and accumulated by organisms (Campbell *et al*., 2008) (Fig. 1). Primary producers (*e.g*., phytoplankton and periphyton) concentrate MeHg directly from water (Pickhardt, Fisher, 2007), whereas fish are exposed to MeHg via prey consumption (Driscoll *et al*., 2007). Globally, fish are recognized as important source of MeHg in humans and considerable efforts are underway to quantify Hg levels in species targeted for subsistence and commercial fisheries. Studies of Hg contamination in aquatic ecosystems in the Neotropics, and in particular, from the Guianas, are still lacking (Fréry *et al*., 2001; Kwon *et al*., 2012; Ouboter *et al*., 2012; Marshall *et al*., 2016). The seasonal hydrological cycle of tropical rivers is critical for Hg exposure of riverine species. For example, the extension of the streamflow events from river channels into floodplain during the seasonal inundation (Lowe-McConnel, 1987; Winemiller *et al*., 2014) have the potential to decrease oxygen availability for redox reactions, increasing the microbial conversion rate of Hg to MeHg (Gilmour *et al*., 1992; Singer *et al*., 2016). Guimarães *et al*. (2000) found that floating macrophytes and increased terrestrial detritus during the Amazon River's inundation pulse are important substrates for mercury methylation by sulfate

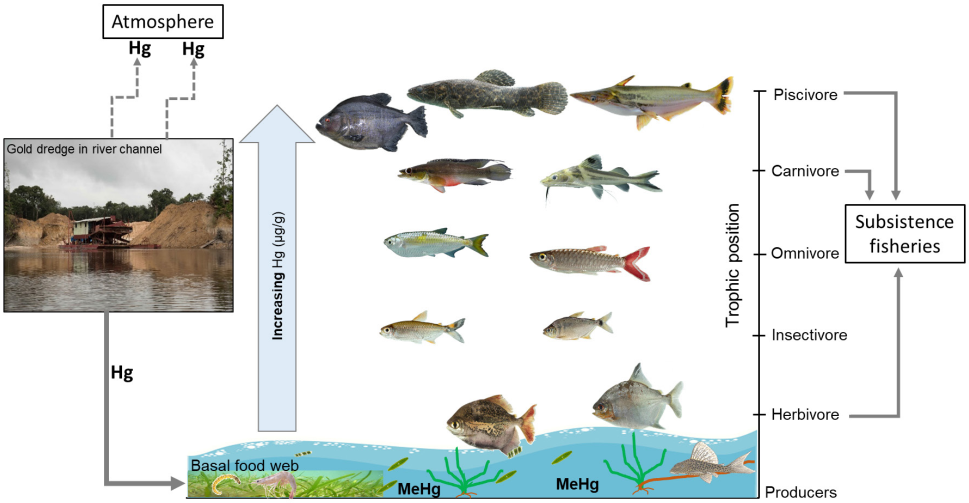


**FIGURE 1** | Conceptual model of how Hg enters riverine food webs of the Mazaruni River, Guyana. First, artisanal gold mining operations installed in the river use a suction dredge to reach gold contained in bottom sediments. These mining operations use mercury for gold amalgamation of which the majority is lost to the atmosphere or river. Once in the river, Hg can be methylated by microbes into the toxic Methylmercury (MeHg) and be assimilated rapidly by aquatic biota. Hg concentration bioaccumulates and biomagnifies in the trophic food chain. Fishes at higher trophic levels [piscivores and carnivores (*e.g*., piranhas, aimaras, and catfishes)] are important items in the diet of local communities and can become a direct source of Hg uptake via direct consumption.

reducing bacteria because as they decompose, they accumulate MeHg in the water and create direct and indirect pathways for MeHg to aquatic consumers (Roulet *et al*., 2001).

Tropical fish communities span a range of trophic guilds (*e.g*., herbivores, carnivores, piscivores) (Fig.1), and occupy virtually all ecological niches (*e.g*., benthic to pelagic species) (Lowe-McConnell, 1987; Winemiller, 1991; Montaña, Winemiller, 2013). In addition, many species are dependent on seasonal flooding pulses for extensive migrations and reproduction (Jepsen, Winemiller, 2007). Because these fish communities often comprise subsistence riverine fisheries, it is relevant to investigate the potential differences in Hg bioaccumulation of fishes in aquatic food webs. Mercury biomagnification in trophic networks (*i.e*., cumulative MeHg transfers between the successive consumer levels of the food chain) can lead to high Hg concentrations in piscivorous species (Lavoie *et al*., 2013; Pouilly *et al*., 2013) and extensive Hg burdens in the entire fish biomass (Watras *et al*., 1995). Biomagnification is positively correlated to food chain length (Cabana, Rasmussen, 1994; Chumchal *et al*., 2011), which can be derived from nitrogen stable isotopes. Nitrogen isotopes provide an estimate of consumer trophic position (Post, 2002). The slope of the relationship between the mean of Hg concentration and trophic position, referred as a food web magnification factor, can be used to compare the transfer efficiencies between MeHg and biomass in food webs (Cabana, Rasmussen, 1994; Jardine *et al*., 2006). A positive relationship between Hg concentrations and body size has been reported for several piscivorous fish species that are important fisheries across the Guianas such as *Hoplias malabaricus* (Bloch, 1794), *Serrasalmus rhombeus* (Linnaeus, 1766), and *Plagioscion squamosissimus* (Heckel, 1840) (Lacerda, Salomons, 1998; Mol *et al*., 2001; Maury-Brachet *et al*., 2020). Unfortunately, fish consumption is considered one of the main pathways of Hg intake by humans in South America (Pacyna *et al*., 2010). The World Health Organization (1990) established that the safe limit for fish consumption is 0.05 μg Hg $g^{-1}$ fish. Exposure of local people to Hg of concern because they consume large amounts of fish from local rivers that are contaminated with mercury. Several studies of fishes from neotropical rivers polluted by gold mining activities have already reported Hg concentrations that exceed these international guidelines (Fréry *et al*., 2001; Mol *et al*., 2001; Venturieri *et al*., 2017).

In this study, we quantified total Hg concentrations and biomagnification trends in 39 fish species from the middle Mazaruni River in Guyana to understand the extent of Hg contamination. We examined a range of trophic guilds and fish body sizes the collected in mined and non-mined sites and interpret these results in the context of both the ecosystem and human health. To evaluate how Hg bioaccumulates and biomagnifies through this river system, we used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to characterize the vertical trophic position and energy sources of consumers (Fry, 2006). To make inferences between Hg concentrations in fish and the potential risk to human health, we compared total Hg values to the standard values (0.5 μg Hg/g) established for human consumption (WHO, 1990). Our comparisons emphasized primarily fish species that are targeted for local subsistence fisheries (Tab. 1). Mercury concentrations were examined in fish assemblages from sites impacted by gold mining operations and from sites that were semi-natural, where nearby mining operations were not observed. We expected that differences in anthropogenic activities, primarily gold mining, are likely to affect the biological production, food web structure and consequently Hg bioaccumulation of riverine food webs.

## MATERIAL AND METHODS

**Study area.** The Mazaruni River in Guyana is a tributary of the Essequibo River in northern Guyana. The Essequibo River is the largest river in Guyana and known as the major fluvial system connecting to the Amazon River during the rainy season via the Rupununi wetlands of South-Central Guyana (Souza *et al*., 2020). The Mazaruni River, which is an important source of alluvial gold and diamonds, originates in the western forests of the Pakaraima Mountains and after it descends from the Guiana highlands, it curves northeastward near the town of Bartica, where it joins the Cuyuni River just upstream the confluence with the Essequibo River (Figs. 2A,B). From lowland to upstream reaches, the Mazaruni River changes in water properties, substrate composition and channel geomorphology (Miller *et al*., 2003). The lowland reaches of the Mazaruni River are characterized by a branching pattern with extensive bedrock exposure creating white water conditions, while the upper drainage reaches contain small pristine black water tributaries that are isolated from the lowland basin by rapids and waterfalls. Our survey sites were located in the middle Mazaruni River drainage (Figs. 2B,C). At this location, the main channel of the Mazaruni River and adjacent tributaries have been highly impacted by gold mining activities, consequently affecting the channel substrate, riparian soils and vegetation, and water turbidity. Our field observations suggest that sites impacted by gold mining activities contain homogeneous substrates (*e.g*., artificial tailing beaches) likely due to relocation of river substrate by gold dredges, but also high turbidity and high total suspended sediments caused by siltation from mining operations (Fig. 3).

Our surveys were conducted during the dry season of 2018 in mined (Puterang, Kurupung and Mazaruni River at Olive Creek) and non-mined (Eping Creek) river sites in the middle Mazaruni River drainage (Figs. 3A–H). Each site was sampled only once during this season. Eping Creek (06º07'29"N 60º04'08"W), is a blackwater tributary with low conductivity (range 4.0–13.0 µS/cm), low total suspended solids (TSS) in the water column (0.50 mg/L) and high water transparency (95–130 cm depth measured with Secchi disk). Eping Creek appeared not be mined at the time of our fish surveys. However, our field observation suggests that mining must have been occurred in the past because we observed abandoned mining equipment and a clear reconfiguration of the banks and riparian forest. In comparison with surveys conducted in the Eping Creek

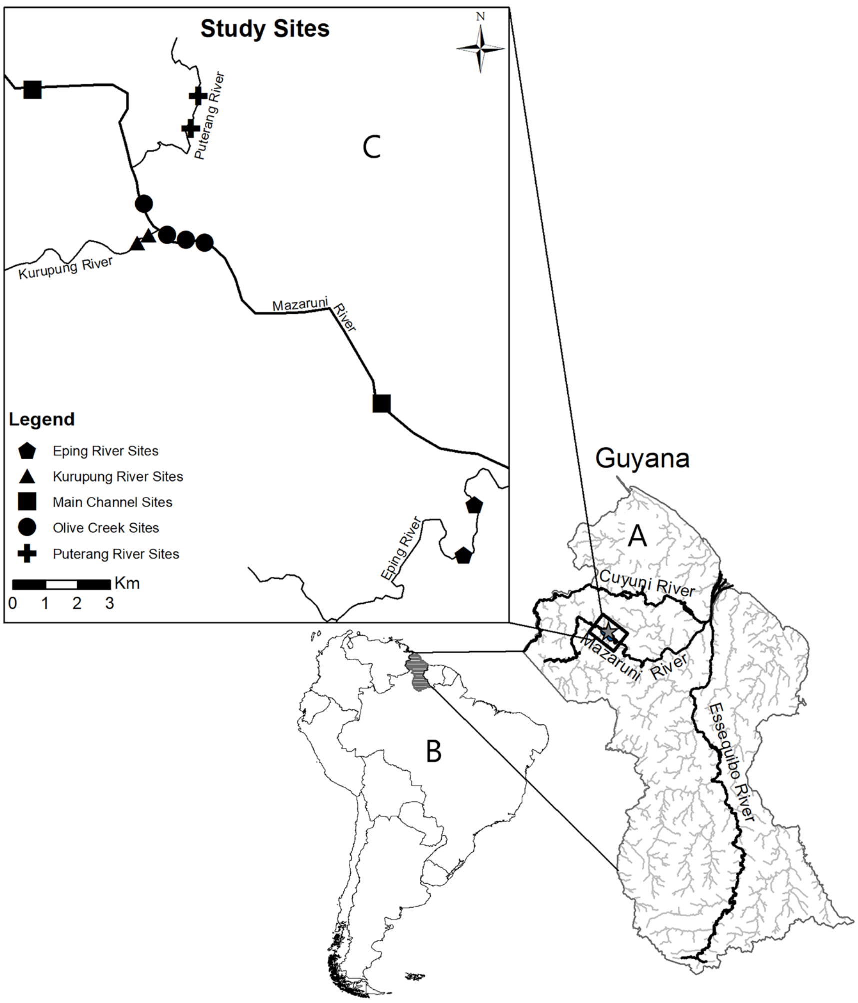


**FIGURE 2** | Locations of sampling sites in the middle Mazaruni River drainage in **A.** Guyana within **B.** South America. Sampling sites included location in the **C.** Main channel of the Mazaruni River (mined) and three main tributaries including Eping River (non-mined), Puterang and Kurupung rivers (mined).


**FIGURE 3 |** Sampling locations on the middle Mazaruni River: **A.** Main channel of the upper Eping River a small, black water tributary of the Mazaruni River. This river appeared more pristine and less impacted by gold mining activities; **B.** Sandy, shallow habitat sampled in middle channel of the upper Eping River; **C.** A gold dredge in the middle of the Kurupung River, on the right side is evidence of soil removal that resulted from mining activities; **D.** Deforestation (to establish mining stations) along the Kurupung River upstream, left bank from the confluence with the Mazaruni River; **E-F.** Main channel of the Mazaruni River at Olive Creek. At this location, the main channel of Mazaruni River is highly impacted by gold mining activities and gold dredges and 'tailing' beaches are commonly observed; **G-H**. The main channel of the lower Puterang River before the confluence with the Mazaruni River. At these locations, the Puterang River carries down heavily silted sediments that are spilled into the Mazaruni River.

in 2016 (Andrade *et al*., 2019), our observations of the landscape and water conditions did not report major changes in this creek. The riparian forest and its black waters (Figs. 3A,B) appeared less impacted as compared with other mined locations. The Puterang River (06º15'14"N 60º08'26"W), Kurupung River (06º12'36"N 60º12'59"W), and main channel of Mazaruni River (06º12'53"N 60º08'19"W) near Olive Creek were considered mined locations as we observed active mining activities along the main channels. In these three rivers, water conductivity was higher (range 14.2–39.3 µS/cm) while TSS (range 21.0–45.0 mg/L) and water transparency (10–45 cm depth) were lower than in Eping Creek.

**Sampling procedures and analysis.** Fishes were collected from multiple habitats within each site using multiple fishing gears including seines (6.0 m length x 1.8 m deep, 5-mm mesh), gillnets and hook-and-line. Shrimps were collected with seines. In the field, fishes were euthanized in clove oil, identified to lowest taxonomic levels and measured (standard length, SL in mm) before extracting muscle tissues for Hg and stable isotope analysis. Muscle tissues were taken from the dorsal flank of the fish near the dorsal fin using a sterilized stainless-steel scalpel. Tissue samples for total Hg analysis were placed in individual Ziploc bags and stored in ice until analysis. Tissues for stable isotope analysis were preserved in salt following the methods of Arrington, Winemiller (2002). The primary producers collected included aquatic macrophytes, benthic algae, and bryophytes (moss). Aquatic macrophytes and bryophytes were collected by hand, cut into small pieces, and placed into Ziploc bags for later processing. Sediment samples were collected from the surface of the bottom of the river about 5 cm depth using a Petri dish and spatula and were frozen until analysis. In the laboratory, all samples were soaked and rinsed with distilled water, placed in glass vials, and then dried in an oven at 60°C for 48 h. Once dried, samples were ground to a fine, homogenous powder using a mortar and pestle and then stored in clean glass vials 94°C.

To examine the influence of energy sources and vertical trophic structure in mined and non-mined river sites, samples were analyzed for stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Samples of ground fish tissue were weighed (1.2–2.5 mg) into Ultrapure tin capsules, and sent to the Center of Applied Isotope Studies at University of Georgia (Athens, GA, USA) of for analysis using a Delta V mass spectrometer. Isotope ratios were reported in the standard of $\delta^{13}C$ and $\delta^{15}N$ notation (in parts per thousand ‰) relative to the international standards (atmospheric $N_2$ and PeeDee belemite) (Fry, 2006). Mercury analyses were conducted at the Texas Research Institute for Environmental Studies (TRIES) at Sam Houston State University (Huntsville, Texas, USA). Samples of fish muscle, primary producers and sediments were analyzed for Hg concentrations. Hg concentrations were measured in sediments, where methylation is supposed to occur and delivered to the basal food web (Singer *et al*., 2016).

All samples for Hg analysis were rinsed with distilled water, placed in glass vials, and then dried in an oven at 60°C for 48 h. Dried samples were homogenized into a fine powder sample and approximately 20 g of each dried sample were used for total Hg analysis. Mercury detection in the samples was determined with the method of cold vapor atomic absorption spectroscopy (CVAAS, Clesceri *et al*., 1998) using the Millennium Merlin Mercury Analyzer.

All fish species used for stable isotope and total Hg analyses were classified into major trophic guilds (Tab. 1) in the food web using the trophic designations of Richard *et al*. (2000), Hoeinghaus *et al*. (2003), and Montaña, Winemiller (2013). Trophic guilds include piscivore, carnivore, omnivore, invertivore, algivore, and algivore/detritivore guilds. In order to depict differences in the isotopic signatures and vertical trophic position of fish species between mined and non-mined sites, we constructed $\delta^{13}C$ and $\delta^{15}N$ biplots. Values of Hg concentration in sediment, primary producers, and fish (trophic guilds) were compared across surveyed sites and a polynomial regression was performed on total Hg concentration across producers and consumers in the riverine food web to depict overall patterns of Hg biomagnification. In addition, linear regression was performed on total Hg concentration and body size of target fish species known to be used for consumption by local riverine people in the middle Mazaruni River. Differences in $\delta^{15}N$ and total Hg among guilds were tested using a Kruskal-Wallis (K-W) test on untransformed data followed by a Mann-Whitney pairwise comparison test. For all tests, type I error was set to $\alpha = 0.05$.

## RESULTS

The values of total Hg concentrations and $\delta^{13}C$ and $\delta^{15}N$ in the fish assemblage from the dry season indicate Hg biomagnification is occurring in a wide range of trophic positions and basal energy sources in both mined and non-mined sites in this river food web (Figs. 4–5). Fish trophic levels spanned primary consumers (herbivores) to tertiary consumers (top predators: carnivores, piscivores) (Fig. 4). Most herbivore, algivore/detritivore and insectivore taxa in non-mined sites had mean $\delta^{13}C$ values similar to basal sources such as benthic algae and aquatic macrophytes and when compared to mined site, these values were lower on average (Fig. 4). At mined sites, the $\delta^{13}C$ of benthic algae became enriched on average. Thus, it appears that benthic algae could be the main production source supporting higher trophic consumers such as omnivores, carnivores and piscivores. The $\delta^{15}N$ values of basal sources also exhibited variation on average between study sites (Fig. 4). Shrimps became $\delta^{15}N$ enriched in mined sites when compared to non-mined and bryophyte and benthic algae were about two-fold higher in mined sites (Fig. 4). On average, values of $\delta^{15}N$ in consumer fishes were positively related to trophic guilds and $\delta^{15}N$ values were slightly more $\delta^{15}N$ enriched in carnivores and piscivores in mined sites. Overall, there was only a marginal variation (K-W: $H = 4.01$, $p < 0.05$) in the $\delta^{15}N$ values between mined and non-mined sites.

Mean Hg concentrations ranged from 0.02 to 5.92 μg/g among fish species (Tab. 1). Hg concentrations were significantly different among trophic groups between mined and non-mined sites (K-W test, $H = 12.7$, $p < 0.003$), with piscivores and carnivores having significantly greater Hg concentrations than the lower trophic guilds. Fishes from mined sites, and in particular, carnivores and piscivores had higher Hg concentration on average (Tab. 1). Regressing Hg concentrations against $\delta^{15}N$ values (7.50 to 12.74‰) of all surveyed samples indicated substantial biomagnification of Hg in the Mazaruni River food web ($F = 4.40$, $p < 0.004$; Fig. 5). The linear regression was $\log Hg = -1.19 + 0.16 (\delta^{15}N)$ with a weak strength of the relationship ($r^2 = 0.13$). The Hg concentrations for carnivores and piscivores were higher than any other trophic group, and in many

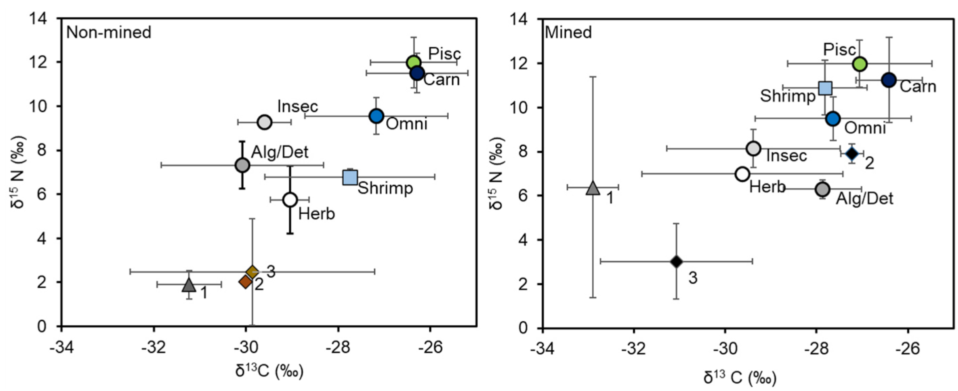


**FIGURE 4** | Mean nitrogen (δ15N) and carbon (δ13C) isotope ratios of fish species collected from mined (right panel) and non-mined (left panel) sites in the Mazaruni River, Guyana. Species were grouped together based on their trophic guild. Each symbol represents the average of all species within each trophic guild. Numbers represent the basal resources collected at surveyed sites (1 = bryophyte, 2 = benthic algae, 3 = aquatic macrophytes). The abbreviations for the fish trophic guilds: Algivore/Detritivore (Alg/Det), Insectivore (Insec), Herbivore (Herb), Omnivore (Omni), Carnivore (Carn), Piscivore (Pisc), as well as one shrimp (*Macrobrachium*).

cases, some piscivores showed Hg concentrations that were above the WHO criteria (0.5 µg Hg/g) for fish tissue (Fig. 5). Piscivores such as *S. rhombeus* and *H. malabaricus*, which were collected in both mined and non-mined sites had Hg concentrations above 0.5 µgHg/g (Hg ranges between 0.51 – 5.92 µgHg/g). These Hg levels are of special concern because both Piranhas (*Serrasalmus*) and Houri (*Hoplias*) along with some catfishes (*e.g.*, Dwalla - *Ageneiosus* spp.) and Tiger catfish (*Pseudoplatystoma fasciatum* (Linnaeus, 1766)) and Lukanani (*e.g.*, *Cichla* spp.) (Tab. 1) are commonly consumed by local communities along the Mazaruni River.

On average, mercury concentrations in sediments (all samples, 0.03 µg/g[SD±0.05]) and basal primary producers (all samples, 0.04 µg/g[±0.11]) were significantly lower when compared to consumers (all samples, 1.87µg/g[±1.20]). When examined by site, there was a trend for increasing Hg concentrations in sediments and benthic algae (mean: 0.07µg/g[±0.01] and 0.12 µg/g[±0.01], respectively) at mined sites (Fig. 6). On average, Hg concentrations appeared to bioaccumulate at the top of the food web with piscivorous fishes containing the highest Hg values in mined sites. The polynomial trendline ($y = 0.05x^2 - 0.22 + 0.28$) also suggested a strong strength of the relationship ($R^2 = 0.98$) (Fig. 6). Among the 39 species examined in this study, several species including piscivores, carnivores and herbivores (*i.e.*, frugivores) are targeted by local fishermen for food consumption. Overall, Hg concentrations did not appear to correlate with body size in herbivorous fish (*e.g.*, *Myloplus asterias*) ($F = 2.16$, $p = 0.14$, $r^2 = 0.03$; Fig. 7). However, in carnivores and piscivores, we found a significant ($F = 38.8$, $p < 0.003$, $r^2 = 0.40$; Fig. 7), positive relationship between increasing consumer body size and Hg concentration in fish tissue.

**TABLE 1** | Fish species and their mean mercury concentrations (µg Hg/g, dry weight) from mined or non-mined sites. Species were classified to trophic guilds based on published literature. N indicates total number of samples analyzed.

| *Scientific name* | Trophic guild | Use for consumption | N | Mined (mean THg) | Non-mined (mean THg) |
|---|---|---|---|---|---|
| *Acestrorhynchus microlepis* | Piscivore | N | 2 | 2.37 | |
| *Ageneiosus inermis* | Piscivore | Y | 5 | 3.24 | |
| *Ageneiosus ucayalensis* | Piscivore | Y | 1 | 5.92 | |
| *Auchenipterus nuchalis* | Piscivore | Y | 1 | 3.01 | |
| *Boulengerella cuvieri* | Piscivore | Y | 3 | 4.29 | |
| *Cynodon septenarius* | Piscivore | Y | 5 | 4.41 | 0.51 |
| *Cichla ocellaris* | Piscivore | Y | 2 | | 0.85 |
| *Hoplias malabaricus* | Piscivore | Y | 3 | 2.68 | 0.77 |
| *Hydrolycus armatus* | Piscivore | Y | 4 | 2.15 | 0.55 |
| *Serrasalmus rhombeus* | Piscivore | Y | 59 | 1.52 | 0.32 |
| *Serrasalmus eigenmanni* | Piscivore | Y | 14 | 1.01 | 0.15 |
| *Trachycorystes trachycorystes* | Piscivore | N | 4 | 0.51 | 0.4 |
| *Pseudoplatystoma fasciatus* | Piscivore | Y | 1 | 2.78 | |
| *Charax gibbosus* | Carnivore | N | 6 | 4.21 | 0.42 |
| *Crenicichla lugubris* | Carnivore | N | 3 | 2.22 | 0.58 |
| *Pimelodella* sp. | Carnivore | N | 3 | 1.05 | 0.46 |
| *Pimelodella gergi* | Carnivore | N | 1 | 1.01 | |
| *Hemisorubim platyrhynchos* | Carnivore | Y | 2 | 1.7 | 0.45 |
| *Pachypops fourcroi* | Carnivore | Y | 1 | 0.4 | |
| *Pseudopimelodus bufonius* | Carnivore | Y | 3 | 1.33 | 0.54 |
| *Anostomus anostomus* | Omnivore | N | 5 | 1.08 | 0.31 |
| *Brycon pesu* | Omnivore | N | 6 | 2.68 | 0.32 |
| *Bryconops melanurus* | Omnivore | N | 6 | 1.21 | 0.59 |
| *Caenotropus labyrinthicus* | Omnivore | N | 2 | 0.75 | |
| *Chalceus macrolepidotus* | Omnivore | N | 3 | 2.6 | 0.59 |
| *Cichlasoma bimaculatum* | Omnivore | Y | 3 | 2.56 | 0.24 |
| *Doras carinatus* | Omnivore | N | 3 | 0.47 | |
| *Leporinus agassizi* | Omnivore | N | 2 | | 0.31 |
| *Triportheus albus* | Omnivore | Y | 8 | 2.32 | 0.37 |
| *Biotodoma cupido* | Insectivore | N | 3 | | 2.01 |
| *Geophagus* n. sp. | Insectivore | N | 2 | 0.23 | |
| *Jupiaba polylepis* | Insectivore | N | 9 | 1.12 | 0.25 |
| *Brycon amazonicus* | Herbivore | Y | 1 | | 0.02 |
| *Myloplus asterias* | Herbivore | Y | 6 | 0.19 | 0.06 |
| *Myloplus rubripinnis* | Herbivore | Y | 2 | 0.15 | |
| *Curimata ocellata* | Algivore/Detritivore | N | 4 | 0.085 | 0.035 |
| *Hypostomus* sp. | Algivore/Detritivore | N | 6 | 0.51 | 0.34 |
| *Parodon* sp. | Algivore/Detritivore | N | 5 | | 0.06 |
| *Platydoras hancocki* | Algivore/Detritivore | N | 4 | 0.31 | |

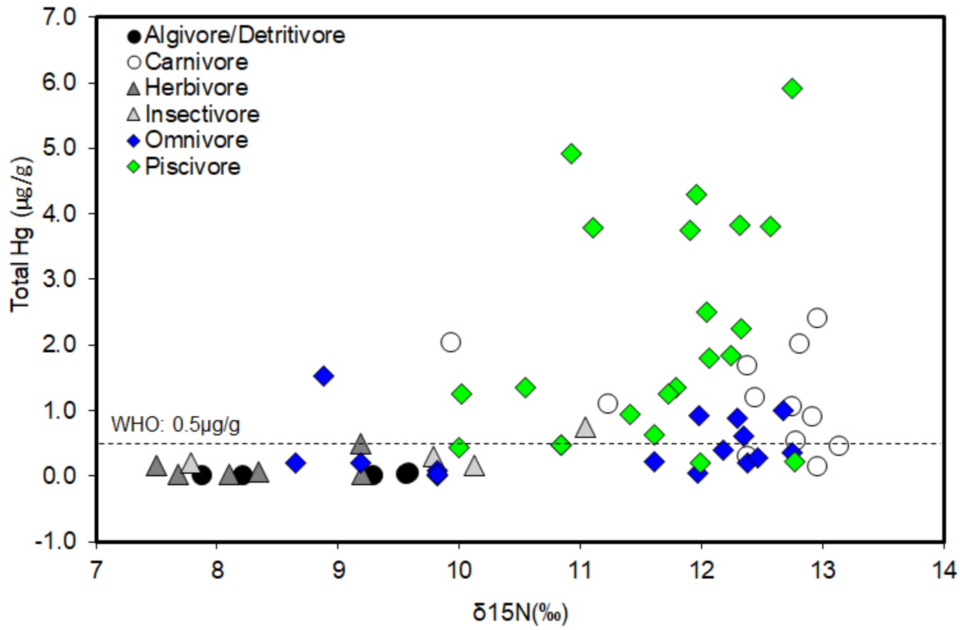


**FIGURE 5** | Biomagnification of Hg in the Mazaruni River, Guyana, represented as the log total Hg concentrations plotted against nitrogen (δ15N) isotope ratios. Species were grouped together based on their trophic guild. Each symbol represents the average of all species within each trophic guild. The dashed horizontal line indicates the reference limit of 0.5 µg/g by WHO guideline for Hg in fish consumed by humans.

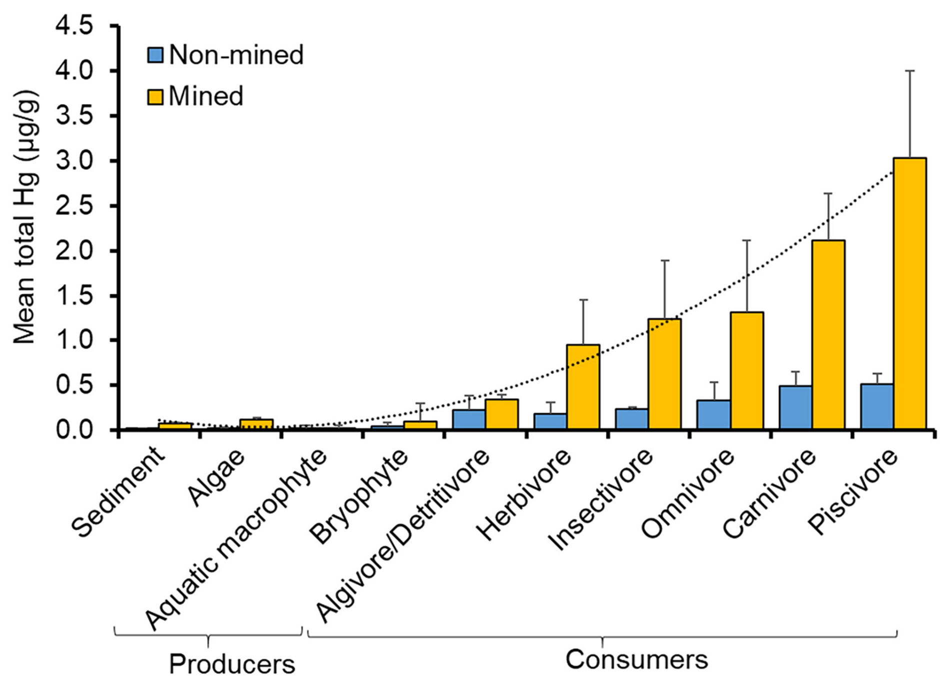


**FIGURE 6** | Mean Hg concentrations (dry weight) in sediments and instream biota separated by trophic groups (producers and consumers) between mined and non-mined sites in the Mazaruni River, Guyana. Species were grouped together based on their trophic guild. Polynomial trendline: y = 0.05x2 - 0.22x + 0.28, coefficient of determination $R^2$ = 0.98.

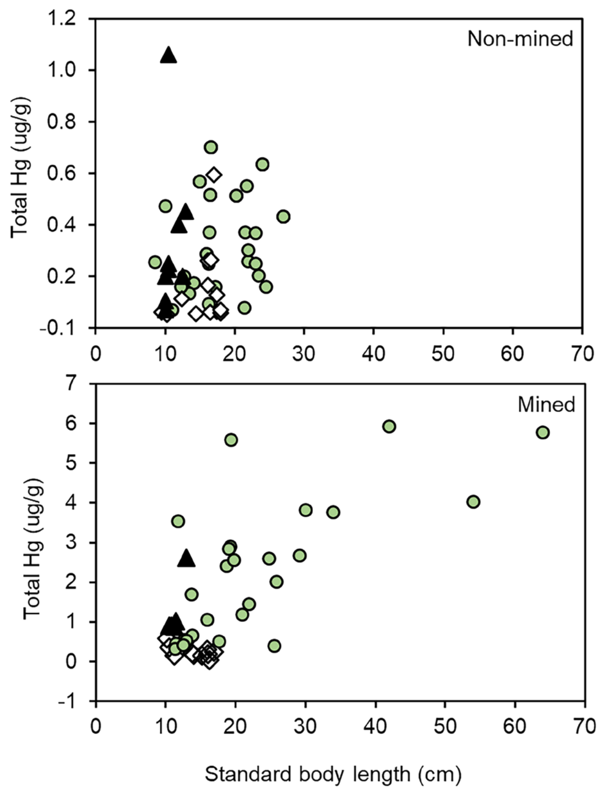


**FIGURE 7** | Relationships between body size (standard length, mm) and total Hg of fishes commonly used for consumption. Top panel (non-mined) contains three species that were common at mined sites (bottom panel). Relationships were estimated for five species that are commonly found and consumed, and for what a class structure was observed. Symbols represent individual fish. Clear diamonds = frugivores [1 sp.: *Myloplus asterias* (n = 19)], dark triangles = carnivores [1 sp.: *Serrasalmus eigenmanni* (n = 14)], green circles = piscivores [(3 spp.: *S. rhombeus* (n = 24), *Ageneiosus ucayalensis* (n = 2), *Hoplias malabaricus* (n = 1)].

## DISCUSSION

Our findings confirm widespread Hg contamination in aquatic food webs of the middle Mazaruni River due to increased ASGM activities. In this specific region, a large number of gold dredges have been installed in the main channel of the Mazaruni River with observable consequences in the river water quality such as high turbidity, high concentration of suspended sediments in the water column, and presence of "tailing" beaches which have resulted from mining operations. The positive relationship between the vertical food web structure (*i.e.*, estimated by $\delta^{15}N$ of fishes) and the concentrations of Hg found in fish tissues suggest that mercury biomagnification is occurring in the aquatic food web of the Mazaruni River. Fish Hg contamination appears linked to biomagnification enhanced by benthic algae and primary consumers (*e.g.*, shrimps, the main invertebrate analyzed) contribution to the food web and probably in combination with the altered river conditions that perhaps facilitate methylation in this floodplain river.

Studies in other temperate and tropical aquatic systems suggest that Hg biomagnification trends based on fish-only food webs normally fall between 0.2 and 0.3 (Campbell *et al*., 2003, 2005; Pouilly *et al*., 2013). Our estimated slope of 0.22 suggests that Hg bioaccumulation rates in the Mazaruni River food webs are as high as those observed in other Neotropical rivers (Pouilly *et al*., 2013). Although studies in freshwater ecosystems have demonstrated a positive relationship between Hg biomagnification rates and food chain length (Cabana, Rasmussen, 1994; Molina *et al*., 2010), the strength of trophic biomagnification appears to be affected by the productivity of the river system (Walters *et al*., 2015). For instance, in productive systems with abundant algae, Hg bioaccumulation appears to decline via the mechanism of bloom dilution (MeHg burden per cell decreases in algal bloom; Tsui *et al*., 2010; Walters *et al*., 2015). The high Hg bioaccumulation that we may be observing could be as a result that high turbidity levels and high amounts of suspended sediments in the water column, due to increased ASGM activities along the Mazaruni River, have consequently reduced the accumulation and distribution of algae in this system due to light limitation. It is important to highlight that in tropical systems, $\delta^{15}N$ fractionation is low in trophic transfers compared to other ecosystems (Kilham *et al*., 2009) perhaps due to the rapid turnover rate of the tissues (McIntyre, Flecker, 2006), therefore, in this context of Hg biomagnification, one should expect increase of Hg per unit change of $\delta^{15}N$. When examining the middle Mazaruni River food web, our results appear to support this prediction, as Hg concentrations in consumer tissues were positively correlated with their trophic position in the food web.

Fish trophic position (measured by enrichment of $\delta^{15}N$ ratios) is an important variable to consider in Hg studies because it influences dietary exposure to MeHg. In the Mazaruni River section we studied, carnivores and piscivores exhibited the highest Hg concentrations. Even fishes from these two trophic groups that were collected from non-mined sites showed Hg values above the WHO criteria. As Hg bioaccumulates, it is possible that some fish from non-mined sites still retain high Hg concentrations in their tissues from past mining activities. Although little information is known about mercury deposition, bioaccumulation, and speciation in sediments of the Mazaruni River, one would expect that mining in any particular area of the Mazaruni could contaminate the entire river drainage. For instance, Hg can get buried in sediments for long periods of time. Also, sediments can spread downstream, carrying Hg with them, to all main channel substrate downstream. Migratory species or those that undergo long-distance movements amongst different sites could influence these observed results as well. For instance, some of the large piscivorous catfishes and predatory species like *Hoplias*, *Hydrolycus*, and *Serrasalmus* (Fig. 1) are believed to have seasonal migrations associated with reproduction events (Goulding *et al*., 2003; Van der Sleen, Albert, 2018). During these migrations, fishes could assimilate Hg from more contaminated locations along the Mazaruni River and tributaries and then migrate into our study sites. Because of widespread of ASGM activities in the Mazaruni River and its tributaries and placer mine introduction of inorganic Hg into these aquatic ecosystems, there is high potential for inorganic Hg in sediments to be transformed by methylation microbes (*e.g*., sulfate-and iron-reducing bacteria) into bioaccumulative MeHg (Donovan *et al*., 2016; Singer *et al*., 2016), consequently increasing the likelihood of MeHg entering the aquatic food webs. Our results of total Hg concentrations in sediments from mined sites roughly yielded similar concentrations of Hg (0.077 µgHg/g) as documented by Miller *et al*. (2003) in a reach of 350 km along the Mazaruni River. Likewise, in their extensive study of Hg contamination of alluvial sediments within the Essequibo (160 km river reach) and Mazaruni River basins, Miller *et al*. (2003) showed that high Hg accumulation in alluvial deposits was related to anthropogenic activities such as gold mining.

Although many factors may play a role in Hg transformation into MeHg in riverine sediments, clearly tropical floodplain rivers can represent ideal systems for Hg methylation. This is due to their seasonal connectivity between the main channel and floodplains (*i.e*., flood pulse; Junk *et al*., 1989), which can alter the redox conditions and thereby facilitate the microbial conversion of inorganic Hg to the organic form-MeHg (Gilmour *et al*., 1992; Benoit *et al*., 1999). However, Hg can enter the river systems via atmospheric deposition as approximately 55% of the mercury used in gold mining operations is assumed to be lost to the atmosphere (Pfeiffer, Lacerda, 1988). Although unlikely, this should be interpreted with caution, high Hg levels in fishes from non-mined sites in Mazaruni River could be attributed to atmospheric transportation and deposition in sediments. While working on pristine and gold mined impacted streams in Suriname, a country that borders Guyana to the east, Ouboter *et al*. (2012) found that sediment samples and fishes from pristine areas had high levels of Hg concentrations comparable to mined areas. Their main conclusion is explained by the fact that atmospheric transportation of Hg from adjacent mining areas is deposited in pristine areas where Hg can be freely available for methylation.

We expected that differences in water quality due to mining impacts would affect trophic structure since these patterns have been observed in rivers in Suriname and Bolivia (Ouboter *et al*., 2012; Pouilly *et al*., 2013). In regards to $\delta^{15}$C, values of basal resources such as bryophytes, benthic algae, and aquatic macrophytes were more $\delta^{13}$C depleted on average, with the exception of benthic algae at mined sites that showed enriched $\delta^{13}$C values. Lower $\delta^{15}$C values in tropical (Sanseverino *et al*., 2012) and temperate (Bastviken *et al*., 2003) freshwater ecosystems have been associated with methane production from anoxic sediments during flooding events. Wantzen *et al*. (2002) suggested that the seasonal flood pulse in the Brazilian Pantanal affect variations of the methane production resulting in low $\delta^{15}$C values, which was also reflected in herbivore fishes. We did not eliminate the possibility of methane production in the Mazaruni River due to increased sedimentation attributed to gold mining activities and deforestation (Miller *et al*., 2003; Alofs *et al*., 2014), although our lower $\delta^{13}$C values could tentatively be interpreted as an effect of high methane production by methanotrophic bacteria further research will be necessary. The $\delta^{13}$C values for trophic consumers also appeared to vary between sites. For example, in mined sites, more consumers appear aligned with $\delta^{13}$C originating from benthic algae sources that may explain a consistent relationship between high Hg values for benthic algae and primary consumers at mined sites. Benthic algae along with aquatic macrophytes, and riparian trees are dominant energy sources in tropical food webs source (Roach, 2013; Roach *et al*., 2014; Montaña *et al*., 2020). Therefore, the link between $\delta^{13}$ C and Hg concentrations in fishes at mined sites could reflect that methylation is occurring at the bottom of the food web, where perhaps sediment accumulation and low productivity reinforce Hg methylation and transportation to higher trophic levels. Unfortunately, information about bioavailable Hg in tropical rivers is still lacking. Roulet *et al*. (2001) suggested that in large floodplain rivers, MeHg is associated with allochthonous sources and therefore fishes feeding in allochthonous materials may enhance Hg assimilation. Although we did not measure either Hg or stable isotopes in allochthonous sources (*e.g*., riparian plants), we are aware that aquatic macrophytes may contribute to the energy source of herbivorous fishes (Roach *et al*., 2014). There was not a consistent relationship with Hg concentration, as herbivores were lower in the food web.

While Neotropical freshwater fishes are among the most diverse taxa on the planet, they have been increasingly impacted by humans due to the reliance of societies on freshwater ecosystem services and the lack of sustainable practices and conservation policies (Pelicice *et al*., 2017). For decades, most countries within the Guiana Shield including Guyana, Suriname and French Guiana, have economically relied on artisanal and small-scale gold mining (ASGM) activities. The widespread use of Hg in gold mining activities has driven contamination of Guianan river ecosystems including fishes and humans, threatened ecosystems and biodiversity, human health and livelihoods of local riverine communities (Hammond *et al*., 2007; Ouboter *et al*., 2012; Maury-Brachet *et al*., 2020; Watson *et al*., 2020). Unsafe Hg concentrations have been reported in carnivorous fishes and human hair samples from the lower Mazaruni River (Singh *et al*., 2001; Watson *et al*., 2020). Watson *et al*. (2020) also reported high mercury levels in hair samples for residents living close to ASGM activities in the southern Rupununi region in Guyana. From our findings, it is noticeable that fish trophic guild exhibited a clear relationship with Hg levels, which helps to provide information about the trophic groups that can be safety for human consumption. Most of the piscivorous species analyzed in this study including *Ageneiosus* spp., *H. malabaricus*, *S. rhombeus*, *Cichla ocellaris* Bloch & Schneider, 1801, and *Hydrolycus armatus* (Jardine, 1841), are commonly used by indigenous people (*i.e*., Amerindians) in the Mazaruni for food protein. Exceptionally high Hg concentrations were observed in these species, and these patterns are consistent with studies in other rivers within the Guianas (for example: Mol *et al*., 2001 (Suriname); Richard *et al*., 2000; Fréry *et al*., 2001; Ouboter *et al*., 2012; and Maury-Brachet *et al*., 2020 (French Guiana) and Amazon basin (Roach *et al*., 2013; Venturieri *et al*., 2017).

Our findings have important implications for fisheries conservation and human health in the Mazaruni Region. First, the Mazaruni River drainage is recognized for having high fish diversity and endemism (Albert, Reis, 2011; Reis, 2013), yet this ichthyological biodiversity is poorly studied and threatened by gold mining operations. Second, fishing provides a major source of protein for indigenous communities in Guyana (Couture *et al*., 2005; Watson *et al*., 2020). Watson *et al*. (2020) reported that more than 50% of all participants from four communities in South Rupununi, Guyana, consumed fish daily and that indigenous people living close to the ASGM activities and consuming fish daily showed the highest concentrations of mercury in their hair samples. From our personal observations during field surveys along the Mazaruni, there are certainly many families that consume fish, more than once per week. This situation may pose a health risk to people because many of the fish species analyzed from Mazaruni River had high concentrations of mercury that exceeds the WHO recommended criteria. Thus, assuming that 95% of the total Hg present in fish tissues was MeHg (Bloom, 1992), MeHg concentrations reported for most piscivores, carnivores and some omnivores were above the WHO tissue criteria for consumption. Among the nineteen species commonly reported for consumption by local people (Tab. 1), a positive relationship between body size and Hg bioaccumulation was observed for piscivorous species with a large size structure (*e.g*., *S. rhombeus*) indicating the certain trophic guilds (*e.g*., piscivores) and long-lived fish can have elevated Hg concentrations that can be passed on to humans via consumption. As expected, our results found support for patterns between trophic guilds and Hg concentrations reported for many tropical and temperate aquatic systems, with larger piscivores typically having higher Hg concentrations due to biomagnification through the food web. Our results are critical for informing how trophic position and fish size are factors that can be easily identified by riverine people in Guyana who consume fish regularly and could be used to help them to minimize the intake of fishes that are likely to contain high Hg concentrations.

Although our results are from a single season, they suggest that mercury contamination due to gold mining activities may be responsible for the loss of Neotropical freshwater fish diversity. Hg contaminated habitats were also highly turbid resulting in lower quality habitats that reduced fish diversity and shifted in community structure. Hg exposure can have inhibitory effects on fish reproduction such as spawning behavior, fertilization success, and fecundity (Depew *et al*., 2012). This is particularly concerning for fish species that are very sensitive to changes in habitat and water quality. In the Mazaruni basin, for example, some species of catfish (*Rhamdia*) and undescribed hypopomid knifefish appear primarily associated with undisturbed river habitats (Alofs *et al*., 2014). Likewise, high concentrations of Hg bioaccumulation can alter the immune system and disrupt the metabolic processes in fishes, particularly those species feeding at higher trophic levels (Morcillo *et al*., 2017). Loss or reduction of certain functional groups (*e.g*., carnivores and piscivores) following high Hg toxicity not only impact the ecological functions of this aquatic ecosystem, but the fisheries (*e.g*., food security, market value, yields) and other ecosystem services.

In light of the importance of subsistence fisheries in the Mazaruni River, high Hg concentration in fishes and biomagnification rate, we issue a warning to local inhabitants about the health risk of regularly eating large amounts of fish captured from this region. Also, our results agree with other studies conducted in other rivers within the Guiana Shield, which have demonstrated alarming concentrations of mercury accumulation in fish. Accumulation of Hg levels in Indigenous Guyanese populations needs more comprehensive assessments of Hg sources to better inform the potential health effects, both short- and long-term effects, from fish consumption.

While the importance of Hg contamination through fish uptake in humans is well established. The impacts of Hg contamination in Neotropical fish and overall aquatic biodiversity needs further investigations. In Guyana, the ASGM operations have increased in recent decades due to the rising gold price, and along with these operations, greater deforestation and river dredging have been documented (Hammond *et al*., 2007; Alofs *et al*., 2014). Experimental studies have shown that high levels of mercury cause behavioral, hormonal, and reproductive changes in birds and mammals (Scheuhammer *et al*., 2007), but very little is known about the adverse impacts of Hg in fish behavior, gonadal development, reproduction, or even their biomass and diversity. Water turbidity and accumulation of sediments on the main channel of the Mazaruni River are noticeable, which could create hostile living habitats for fishes and affect their biomass as well. Of particular concern about mercury contamination in the Mazaruni

River and other rivers in Guyana (*e.g*., Rupununi and Essequibo) is that the health of these ecosystems is vital for the persistence of biological and cultural communities. High fish endemism has been reported for Mazaruni drainage, but very little is known about how mercury derived from gold mining is impacting these species that appear to be primarily habitat specialists. Although work in other rivers of the Guiana Shield reported effects of ASGM operations on fish communities (Brosse *et al*., 2011), we recommend more detailed medium-long term studies for monitoring the fish communities in Mazaruni River; and in particular, studies related to changes in fish biomass, diversity, and fish health and reproduction are needed to evaluate the effect of mercury contamination in freshwater biota.

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## AUTHORS' CONTRIBUTION

**Carmen G. Montaña:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing-original draft, Writing-review and editing.

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**Donald C. Taphorn:** Investigation, Writing-review and editing.

**Christopher M. Schalk:** Conceptualization, Formal analysis, Investigation, Writing-review and editing.

## ETHICAL STATEMENT

The Staff of the Centre for the Study of Biological Diversity (CSBD) at the University of Guyana for the verification process of the fish and EPA-Guyana for processing the research and export permits. This study was conducted under EPA-Guyana permit No. 031516 BR003 to EL, CGM and DCT.

## COMPETING INTERESTS

The authors declare no competing interests.

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