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Mercury in fish of the Madeira river (temporal and spatial assessment), Brazilian Amazon

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ABSTRACT

The Madeira River is the largest tributary of the Amazon River Basin and one of the most impacted by artisanal gold-mining activities, deforestation for agricultural projects, and recent hydroelectric reservoirs. Total Hg (and methylmercury-MeHg) concentrations was determined in 3182 fish samples of 84 species from different trophic levels as a function of standard size. Species at the top of the trophic level (Piscivorous, Carnivorous) showed the highest mean total Hg concentrations (51–1242 μg/kg), Planctivorous and Omnivorous species showed intermediate total Hg concentrations (26–494 μg/kg), while Detritivorous and Herbivorous species showed the lowest range of mean total Hg concentrations (9–275 μg/kg). Significant correlations between fish size (standard length) and total Hg concentrations were seen for Planctivorous ($r=0.474$, $p=0.0001$), Piscivorous ($r=0.459$, $p=0.0001$), Detritivorous ($r=0.227$, $p=0.0001$), Carnivorous ($r=0.212$, $p=0.0001$), and Herbivorous ($r=0.156$, $p=0.01$), but not for the Omnivorous species ($r=-0.064$, $p=0.0685$). Moreover, fish trophic levels influenced the ratio of MeHg to total Hg (ranged from 70% to 92%). When adjusted for standard body length, significant increases in Hg concentrations in the last 10 years were species specific. Spatial differences, albeit significant for some species, were not consistent with time trends for environmental contamination from past alluvial gold mining activities. Fish-Hg bioaccumulation is species specific but fish feeding strategies are the predominant influence in the fish-Hg bioaccumulation pattern.

1. Introduction

The Madeira River is the largest tributary of the Amazon River Basin and is characterized by high sediment yields and high suspended load. It contributes to 50% of the total suspended load transported by the Amazon River (Latrubesse et al., 2005) and 15% of the water that the Amazon River delivers to the Atlantic Ocean (see references in Bastos et al., 2007). Its catchment spreads over two countries (Bolivia and Brazil) in an area of 690×10^3 km^2 covering 14% of the Brazilian Amazon forest; geographically, 25% of its basin is in the Andes, 27% in the shield and 48% in the Amazon plain (Maurice-Bourgoin et al., 2000).

Due to the geographic vastness and environmental diversity of the Amazon it is challenging to gather a complete set of data to make an integrated interpretation of Hg bioaccumulation in this tropical rain-forest environment. Indeed, the fish Hg concentrations in Amazonian Rivers vary greatly and comparisons are frequently difficult due to fish habitat diversity, insufficient information of fish names (which vary from region to region), precise description of fish feeding hierarchy, and information related to fish size (length or weight) or age; these are important parameters in controlling the random nature of fish sampling (Barbosa et al., 2003). Therefore, information concerning mercury in fish from Amazonian Rivers is frequently unsatisfactory. As a result, when comparing rivers in the Amazon Basin, there were no salient features distinguishing the fish from rivers impacted by intense artisanal small scale gold-mining (ASGM) activities from the past, like the Madeira River, from the fish caught in ASGM non-impacted waters (Barbosa et al., 2003). Parameters related to physical-chemical characteristics of the aquatic (pH, DOC, sulfate), lotic and lenthic environments are likely to play a role in methylation potential.

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Mailman et al. (2006) reviewed strategies that might decrease the methylation potential of aquatic environments that included sediment removal. Extensive alluvial gold mining in the Madeira River disturbed, removed, and redistributed tons of sediments to extract gold during the hey-day of ASGM (Bastos et al., 2006). Lacerda et al. (1989) estimated that about 87 t of Hg (liquid and vapor phases) from ASGM were released in the Madeira River Basin between 1970 and 1990. After this period gold-extraction related emissions decreased drastically to a few kg per year, due to the exhaustion of gold deposits. However, on the Bolivian side of the watershed, including the Beni and the Madre de Dios Rivers, Hg emissions in the last 20 years before the year 2000 reached about 0.5 t yr^{-1} (Maurice-Bourgoin et al., 2000). Also, re-emission of deposited Hg has been reported from the higher basin of the Madeira River (Almeida et al., 2005). In the last 30 years, ASGM mining activities have declined (Bastos et al., 2006), and currently this form of gold mining is scarce and only encountered in remote parts of the river. Nevertheless, the amount of sediment removed during the gold rush did not result in lower fish-Hg concentrations when compared to results obtained in fish caught 20 years later (Bastos et al., 2006).

In the Amazon, fish is a key source of protein and essential nutrients that complements a cassava-based diet of riverine/traditional populations (Dórea, 2004). A fish-based diet is recognized to play an important nutritional and health role in the diet of rich countries (see references in Burger et al., 2014) in subsistence populations of the Amazon Basin, fish-based diets are also important to infant nutrition (Marques et al., 2013) and cardiovascular health (Dórea et al., 2005a). However, fish is also the major source of environmental Hg exposure of these subsistence populations (Barbosa et al., 2003; Dórea et al., 2005b; Oliveira et al., 2010). As a result, fish-Hg exposure is a concern to health of Amazonian population because of adverse effects on neurodevelopment. Additionally, there are also risks related to immunological and cardiovascular effects. Therefore, the objective of this work is to gain insights to the patterns of total Hg and meHg concentrations in fish from the Madeira River.

2. Materials and methods

This study represents fish collected in specified sites on the Madeira River over a period of four years (from October 2009 to August 2013) that included *igarapés*, tributary rivers and lakes (see map in Fig. 1). During the 1970s and 1980s, Madeira River upstream of Porto Velho city was intensely dredged for alluvial gold.

The fish were caught with nets (30–200 mm) set for a period of 24 h with 4 h intervals for fish collection, depending on the abundance of catches. After collection, fish were properly identified, and muscle samples were taken for Hg determination. Fish identification was based on feeding habits according to taxonomy keys as described elsewhere (Santos and Jegu, 1996; Britski et al., 2007; Buckup et al., 2007; Santos and Zuanon 2008). Then, fish were measured and weighed, and standard length (SL) (the distance from the tip of the nose until the last vertebra in the caudal rays) in mm was measured with an ichtyometer (model Piscis100, Krauss & Henre, Lima, Peru). After these procedures, approximately 20 g (wet weight) of muscle samples were immediately cut, frozen and transported to the biogeochemistry laboratory at the Federal University of Rondônia for analysis.

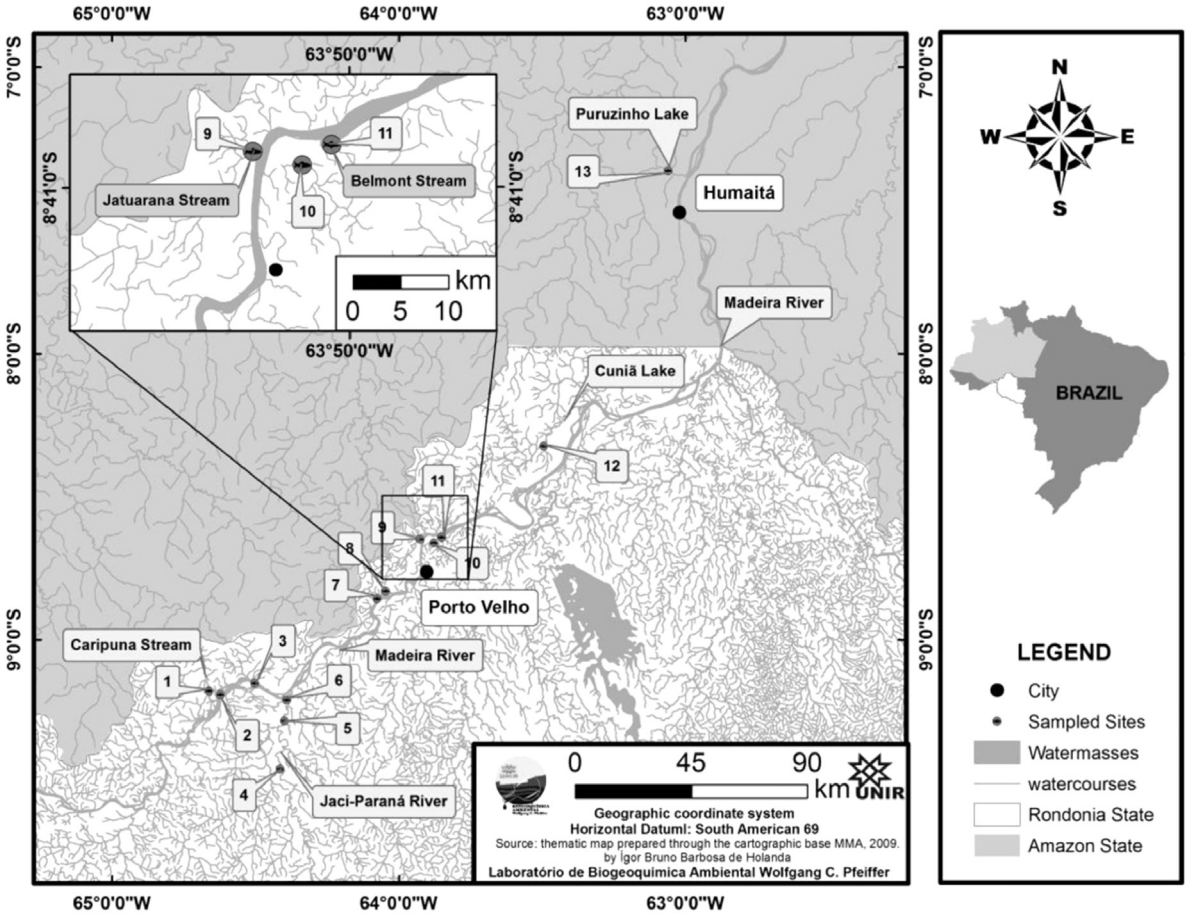

Fig. 1. Map of the fish sampling area marking sites of fishing. Porto Velho city is the capital of Rondônia State.

Total Hg determination was done in the samples after treatment with H_2SO_4:HNO_3 (1:1) solution and $KMnO_4$ (5% w/v) for oxidation. For 500 mg of sample (wet weight), 5.0 mL of acid mixture was added and digested in a digestion block at 60 °C for 1 h (Tecnal-Mod.007 A, Piracicaba, São Paulo, Brazil). After digestion, 4.0 mL of $KMnO_4$ solution (5% w/v) was added to the sample, leaving it for 30 min more at the digestion block. After cooling until the room temperature, drops of hydroxylamine hydrochloride solution at 12% (w/v) was added and the volume was made up to 10 mL with ultra-pure water (Milli-Q Plus, Millipore, Bedford, MA, USA). All glassware was washed clean in 10% (w/v) HNO_3 and rinsed with ultra-pure H_2O. Total Hg measurements were carried out by cold vapor atomic absorption spectrophotometry (Flow Injection Mercury System-FIMS-400-Perkin Elmer, Überlingen, Germany) (Malm et al., 1989; Bastos et al., 1998).

For the MeHg determination in fish muscle, we used the method described by EPA-Method 1630 (2001) and Liang et al. (1994). A known amount (200 mg) of muscle tissue (wet weight) was weighed in PTFE tube and 5.0 mL of 25% (w/v) KOH methanolic solution was used to extract MeHg in an oven with controlled temperature at 70 °C (Nova Instruments, Model NI 1512, São Paulo, Brazil) for 6 h with gentle stirring every hour; the samples were then kept in the dark to avoid possible degradation of MeHg. Subsequently, the ethylation process was done with 300 μL of 272 g/L sodium acetate buffer (pH 4.5) followed by the addition of 30 μL of sample and 50 μL of tetra ethyl sodium borate solution (1% w/v) according to Taylor et al. (2011). The final volume was brought to 40.0 mL with ultra-pure water (milli-Q, Millipore, Cambridge, MA, USA) and analyzed on a MERX-TM automated MeHg system from Brooks Rand Labs (Seattle, USA) equipped with an auto-sampler, a purge and trap unit, a packed column GC/pyrolysis unit, and a Model III atomic fluorescence spectrophotometer. Method accuracy of Hg determinations were ensured by the use of certified material (Tuna Fish, BCR-463 – THg= 2850 ± 160 μg/kg and MeHg= 3040 ± 160 μg/kg) which was run with each batch of samples; mean recovery of 96% (2750 ± 50 mg/kg) and 97% (2960 ± 50 mg/kg) for total Hg and MeHg, respectively. All analysis of samples and certified reference materials were run in triplicate. Limit of detection for THg was 7.0 μg/kg (FIMS) e for MeHg was 8.1 μg/kg (MERX).

Data normality was assessed by a Shapiro–Wilk test and analyzed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). Differences in Hg concentrations between sampling times were assessed by Mann–Whitney *U*-Test, and between specific sites by Kruskal–Wallis test and Bonferroni post-hoc test. Correlations among variables of interest were assessed with Spearman (non-parametric) correlation matrix. Statistically significant differences signify that $p < 0.05$.

3. Results

In total, the overall fishing expeditions resulted in 3182 fish samples of 84 species separated in 6 different conventional alimentary habits: 13 Detritivorous, 6 Herbivorous, 7 Planctivorous, 25 Omnivorous, 17 Carnivorous and 16 Piscivorous with 68% as being Characiforme and 23% Siluriforme fish. The mean total Hg concentrations and respective standard length (used as an

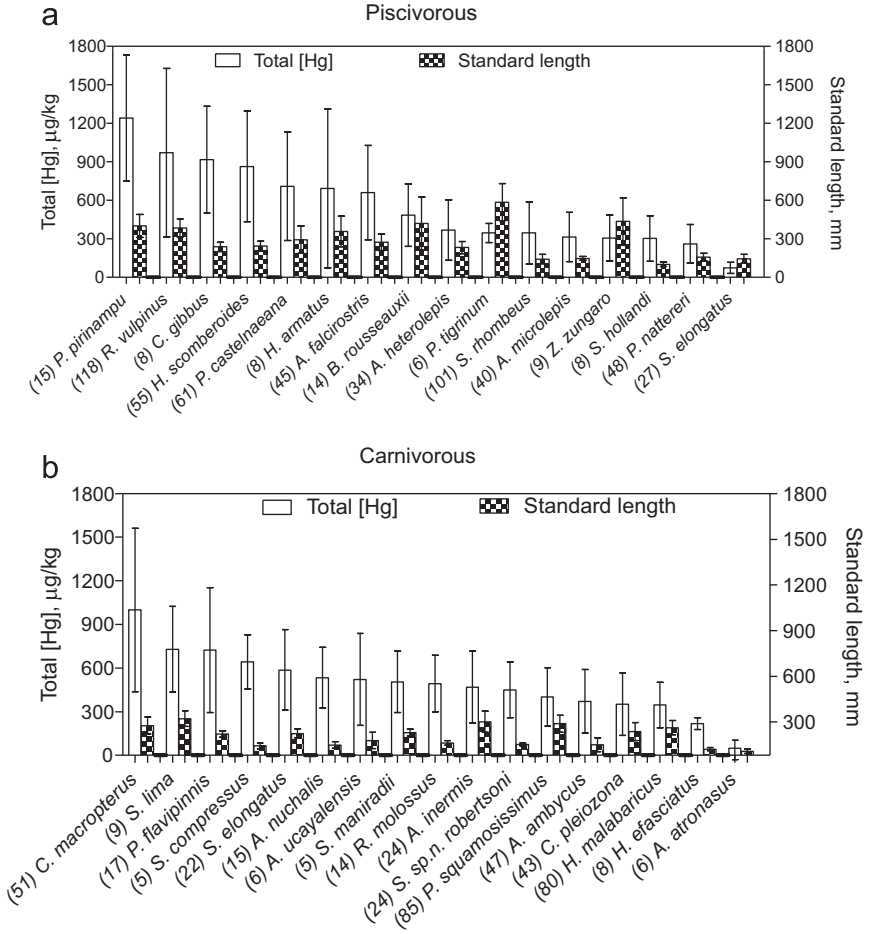

Fig. 2. Bar graph representing mean (SD) total Hg concentrations and standard length as a function of sampled species according to feeding habit classification; (a) Piscivorous (597)=number of samples; (b) Carnivorous (461)=number of samples.

ecological parameter for Hg biomagnification) for each species are represented in Figs. 2 and 3 according to trophic levels. In Fig. 2, species at the top of the trophic level (Piscivorous, Carnivorous) showed the highest mean levels from 51 to 1242 μg/kg. Among these species, Siluriformes and the Characiformes orders showed the highest Hg concentrations; maximum concentrations reached 3334 μg/kg in *Calophysus macropterus* (piracatinga) and 6065 μg/kg for *Rhaphiodon vulpinus* (barba-chata), respectively. In fact, 7.7% of the specimens of these two species showed Hg concentrations higher than 1000 μg/kg; most of them (98%) were from the Pimelodidae and the Cynodontidae families.

Low and intermediate fish-Hg concentrations are shown in Fig. 3. Planctivorous and Omnivorus species showed intermediate total Hg mean concentrations from 26 to 494 μg/kg; it should be noted that in these categories *Anodus elongatus* (orana) and the omnivorous *Triportheus albus* (sardinha) presented total Hg maximum concentrations of 1184 and 1041 μg/kg, respectively, similar to the piscivorous with maximum concentrations. Detritivorous and Herbivorous species showed the lowest range of mean total Hg concentrations from 9 to 275 μg/kg; however, in two species, *Psectrogaster rutiloides* (branquinha) and *Schizodon fasciatus* (piau), individual maximum total Hg concentrations reached 1041 and 1855 μg/kg, respectively.

Considering variability in fish size and Hg concentrations after pooling data from the species from all the sites, significant correlations between size (standard length) and total Hg concentrations were seen for Planctivorous ($r=0.474$, $p=0.0001$), Piscivorous ($r=0.459$, $p=0.0001$), Detritivorous ($r=0.227$, $p=0.0001$), Carnivorous ($r=0.220$, $p=0.00001$) and Herbivorous ($r=0.156$, $p=0.011$), but not for the Omnivorous species ($r=-0.064$, $p=0.069$).

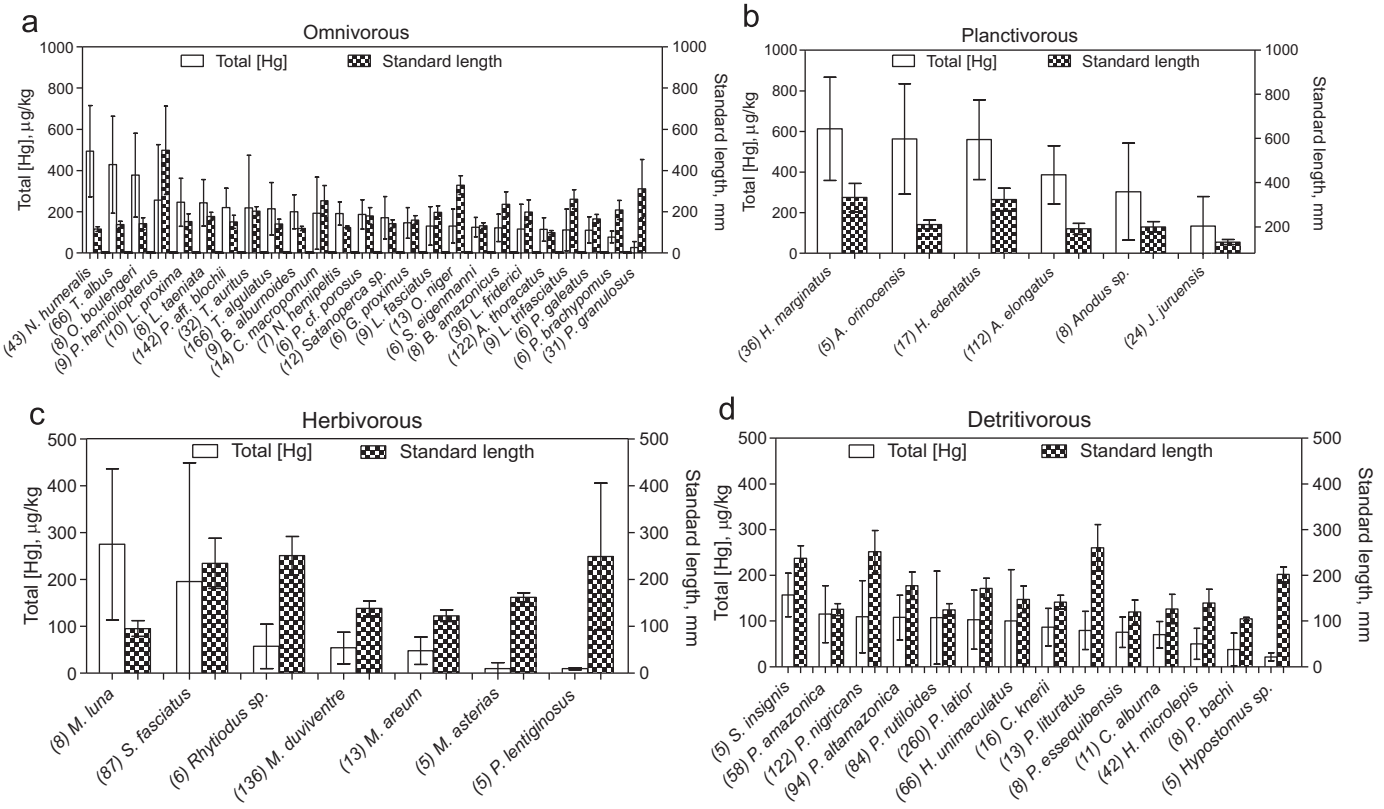

Fig. 3. Bar graph representing mean (SD) total Hg concentrations and standard length as a function of sampled species according to feeding habit classification; (a) Omnivorous (784)=number of samples; (b) Planctivorous (202)=number of samples; (c) Herbivorous (260)=number of samples; (d) Detritivorous (834)=number of samples.

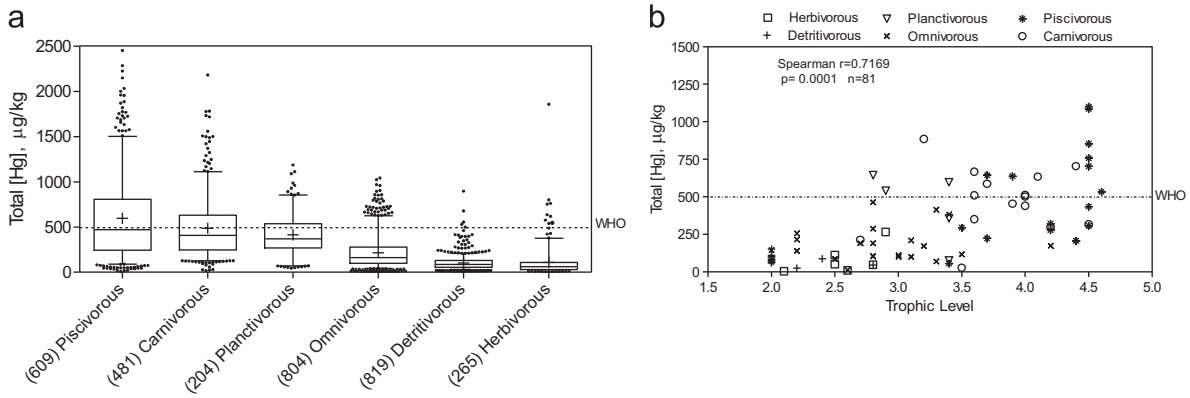

Fig. 4. Fish mercury concentration *versus* trophic level represented as (a) box-plot of conventional classification of fish-feeding strategies, and as (b) scatter plot of fish Hg concentration and trophic level according to 'fishbase' (Fishbase, 2014). Dotted line represents WHO (1990) action level of fish-Hg concentrations; (3182)= number of samples.

Fig. 4a illustrates the representation of fish-Hg concentrations per conventional classification (Fig. 4a) and as trophic level classification proposed by Fishbase (Boettiger et al., 2012; Fishbase, 2014). Fig. 4b shows Detritivorous between 2.0 and 2.4, Herbivorous between 2.1 and 2.9, while Omnivorous shows the widest range (2.1–4.2); Planctivorous (range 2.3–3.4) are mostly higher than Detritivorous/Herbivorous. Curiously, Carnivorous have a range (2.2–4.4) wider than Piscivorous (3.4–4.6) but lower, as expected.

A sub-sample of each guild ($n=264$) was analyzed for MeHg and results are shown Fig. 5. MeHg concentrations showed a wide range of values, varying from 12 to 1981 μg/kg, and were higher in the piscivorous fish (Fig. 5). However, some Planctivorous fish also presented high methyl-Hg concentrations reaching 988 μg/kg in *Hypophthalmus marginatus* (mapará). The percentage of methyl-Hg relative to the total Hg content varied from 70% to 92%. However, feeding habits did not directly determine methyl-Hg percentage (Fig. 5).

In Table 1 we compared time tendencies (1990/2002 and 2009/2013) of total Hg concentrations in fish. There was a significant increase in fish-Hg concentrations for *Pterygoplicthys* sp. ($p=0.0089$; Detritivorous), *H. edentates* ($p=0.0004$), *H. marginatus* ($p=0.0029$; Planctivorous), *T. albus* ($p=0.0012$; Omnivorous), and *P. pirinampu* ($p=0.0381$; piscivorous); however, *Serrassalmus rhombeus* ($p=0.0355$) showed a significant decrease in Hg concentrations. Indeed not only there was variation between species in relation to total Hg concentrations but there were also differences between ratios of MeHg to total Hg concentration.

Fig. 6 shows a summary of total Hg per sampling site. Overall, total Hg concentrations showed significant differences. However, sampling sites where dredging gold mining activities took place more than 30 years ago did not show a pattern consistent with a legacy of Hg used in gold extraction.

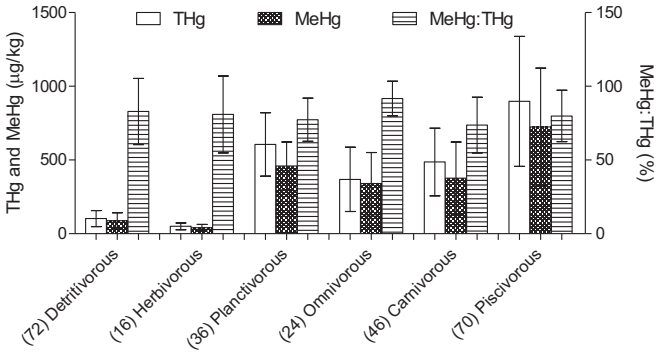

Fig. 5. Bar graph showing mean and (SD) of Total mercury (THg), methylmercury (MeHg), and MeHg:THg ratios in a subsample (n) of fish according to trophic level.

4. Discussion

The fresh-water fish of the Madeira River showed a wide range of mercury concentrations between and within trophic levels. It seems that fish feeding strategies of trophic generalists favored Hg bioaccumulation over specialists. Globally, however, when we take into account fish age/size, Omnivorous species were the only ones to show a negative and non-statistically significant correlation between size and Hg concentrations; also, trophic level was significantly correlated to fish-Hg concentrations.

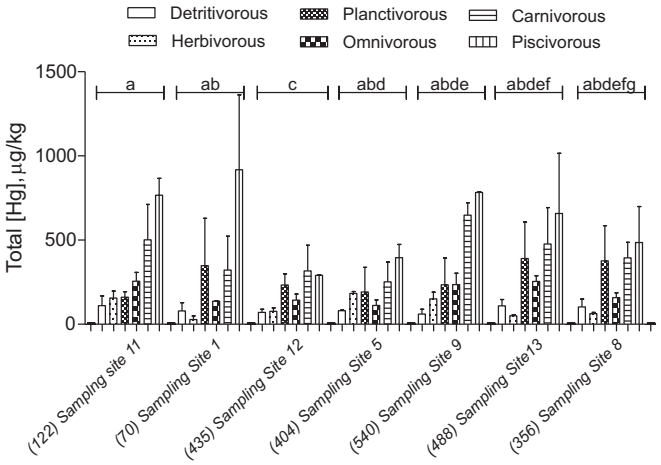

Fig. 6. Bar graph showing mean and (SD) and comparing overall fish-Hg concentrations among sampling sites with representative fish of all conventional classification of feeding strategies and samples ($n>5$) for all groups. Sampling sites with different letters (Bonferroni post-hoc test) show statistically significant differences ($p=0.05$); (2415)=number of samples.

Table 1
THg concentration (median, min-max) in fish species with conventional trophic level classification between 2 periods 1999–2002 (P1) and 2009–2013 (P2) standardized by standard length (total $n=520$).

Scientific name	Feeding behavior	Trophic level****	*n*	SL* (mm) Range	P1** THg (μg/kg)	P2*** THg (μg/kg)	Mann–Whitney, *U*-Test (p)
Colossoma macropomum	Omnivorous	2.0 ± 0.10	28	150–430	63.50 (12.00–251.00)	145.37 (21.93–551.90)	0.1900
Pterygoplichthys spp	Detritivorous	2.0 ± 0.00	26	180–303	27.10 (10.00–106.00)	63.02 (29.06–110.56)	0.0089
Prochilodus nigricans	Detritivorous	2.4 ± 0.18	42	210–311	105.00 (10.00–195.00)	74.69 (30.66–187.69)	0.5628
Schizodon fasciatus	Herbivorous	2.5 ± 0.20	30	230–281	57.00 (20.00–149.00)	57.45 (7.74–87.14)	0.2539
Mylossoma aureum	Herbivorous	2.5 ± 0.23	26	102–180	37.00 (10.00–46.00)	49.50 (10.82–109.35)	0.1735
Hypophthalmus edentatus	Planctivorous	2.9 ± 0.30	10	220–308	28.90 (22.10–77.00)	468.24 (346.22–790.55)	0.0004
Pimelodus spp	Omnivorous	3.1 ± 0.43	20	130–190	132.50 (10.00–322.00)	150.88 (74.74–275.32)	0.7337
Triportheus albus	Omnivorous	3.4 ± 0.38	80	95–290	146.00 (12.10–1331.00)	257.07 (64.82–459.45)	0.0012
Serrasalmus rhombeus	Piscivorous	4.2 ± 0.70	74	104–387	239.00 (39.00–1759.00)	212.97 (20.63–1263.99)	0.0355
Plagioscion squamosissimus	Carnivorous	4.4 ± 0.80	72	180–430	241.50 (12.00–1320.00)	204.43 (89.45–346.48)	0.5888
Hoplias malabaricus	Carnivorous	4.5 ± 0.80	68	229–380	298.50 (13.00–1054.00)	240.50 (150.74–469.11)	0.1602
Hydrolycus scomberoides	Piscivorous	4.5 ± 0.80	14	159–550	535.00 (90.00–1068.00)	554.51 (280.34–879.22)	0.7015
Pinirampus pirinampu	Piscivorous	4.5 ± 0.80	30	244–538	385.00 (20.00–1696.90)	1101.22 (605.00–2030.00)	0.0381

* SL=Standard length.
** P1=Period of 1999–2002.
*** P2=Period of 2009–2013.
**** www.fishbase.org. Only three species showed normal distribution (mean and SD) and group differences were analyzed by t-test as follows: *Schizodon fasciatus (P1: 67.6 ± 38.6, P=51.3 ± 28.4); Pimelodus* spp (*P1: 144.1 ± 83.1, P=161.4 ± 71.3*); *Hydrolycus scomberoides (P1: 517.7 ± 361.6, P=548.4 ± 206.4).*

Nevertheless, in the tropical-forest environment of the Madeira River, fish food resources are abundant but there are cycles with annual flooding, resulting in changes in fish diet. Not only aquatic but terrestrial plants, fruits and seeds are food sources for fish (Goulding et al., 1988; Araújo-Lima et al., 1995 Agostinho and Zalewski, 1995; Albrecht and Caramaschi, 2003; Alvim and Peret, 2004) along with terrestrial invertebrates (Lowe-McConnel, 1987; Goulding et al., 1988; Fugi et al., 2005) that fall on to the water during flight. Furthermore, ants are abundant in the aquatic environment during the flooding season (Goulding et al., 1988). Also, during seasonal flooding of the tropical forest, the inundated land is incorporated into the aquatic environment; then, terrestrial invertebrates that are not usually available become temporarily important dietary items for many fish species. Creatures of cryptic habits, such as earthworms (Oligochaeta) and termites (Isoptera), are added to available food sources for fish (Durães et al., 2001; Balassa et al., 2004). However, trophic position, age or fish body size, and feeding range can modify the patterns of mercury bioaccumulation (Barbosa et al., 2003; Mason et al., 2006; Gammons et al., 2006).

Most neotropical fish have great adaptability to changes in diet and, depending on feed availability, many species are able to take advantage of prevailing opportunity (Gerking, 1994). This trophic adaptability or adaptive flexibility (Dill, 1983) allows them to change ecological niche, reflecting on feed availability and attendant Hg bioaccumulation. It seemed, however, that Omnivorous species responded to prey preference and foraging ecology, leading to a different pattern in mercury bioaccumulation; no linear and significant correlation was seen between fish size and Hg concentrations. However, in most species a temporal increase in Hg concentration was observed.

Although patterns of mercury bioaccumulation have been studied in fish species from the Amazon Basin (Dórea et al., 2006; Bastos et al., 2007; Bastos et al., 2008; Rabitto et al., 2011), interpretation and or reporting of results are not always satisfactory in explaining diversity of findings. The situation in the Madeira River typifies the environmental perturbation brought by anthropogenic activities due to ASGM, hydroelectric damming, and deforestation for agricultural projects. Although ASGM has almost ceased for the last 30 years, in this area, the other high-impact human activities have increased.

In the vast and complex Amazon ecosystem, even in a defined region with a history of ASGM in its recent past, it is challenging to understand the dynamics of MeHg mobilization. Certainly, anthropogenic activities related to ASGM have not left a discernible impact on fish Hg concentrations. In most species, significant temporal increases in Hg concentrations were observed (Table 1). Amazon studies that compared fish-Hg accumulation over time reported a decline or an increase due to river damming (Fearnside, 2005; Kasper et al., 2012); however, in these studies there were no correction for fish size. Boudou et al. (2005) reported both increase and decrease in fish-Hg from Amazon waters in French Guyana; Hg from ASGM activities did not account for high fish Hg concentrations. Indeed, in Canada, Hodson et al. (2014) reported that fish-Hg concentrations were unrelated to mercury legacy in the Saint Lawrence River sediments. In none of these studies there were corrections for fish size in relation to Hg concentrations. Nevertheless a review of different aquatic systems that were previously impacted by Hg input showed recoveries regarding fish-Hg concentrations after reduction of environmental Hg loading (Munthe et al., 2007). However, anthropic environmental disturbances can affect fish habitat (Petrere Junior et al., 2004); machines used to extract alluvial gold in Amazonian rivers interfered with upriver spawning migration of catfishes (Petrere Junior et al., 2004). Therefore, not only direct contamination by Hg but also other type of anthropic activities can have consequences on fish foraging and Hg uptake.

With the complexity of the Amazonian ecosystem a simple comparison between single environmental variables may not explain most of the fluctuation in fish tissue Hg concentrations. Therefore, a chosen environmental stressor is insufficient to understand the variability in fish-Hg concentrations when correction for fish size and season are not taken into account (Dórea et al., 2006). Thus empirical linkages between fish-Hg and specific environmental Hg responses may not be sufficient to justify environmental policies.

For frequent fish consumers, making informed risk decisions about species to be consumed from the Madeira River is not site-specific; levels of fish-Hg concentrations are indeed species-specific. Fish-Hg concentrations in the Amazon Basin, besides constitutional characteristics (life span, size, and trophic level), depend also on high or low waters (Dórea et al., 2006; Bastos et al., 2007). In the present case, most species showed Hg concentrations above the recommended limits of 500 μg/kg (wet weight) (WHO, 1990). Trade in fish from the Madeira River has been estimated to reach 1000 t/yr (Doria et al., 2012); therefore, a conservative estimate of 0.5 kg Hg (or 0.4 kg MeHg) is transferred to human consumption, mainly in Porto Velho. Araújo-Lima et al. (1986) report that more than 30% of the fish consumed in the Amazon basin are Detritivorous of order Characiformes (families Prochilodontidae and Curimatidae) and that their food chain originates from phytoplankton.

There are few studies in the Amazon ecosystem that have measured season differences in the same water body. In our case, the sampling sites coincided with past ASGM, and the trend for an increase in total Hg concentration was statistically significant for most of the tested species (Table 1); only one species of 'piranha' (*S. rhombeus*) showed a decrease with time. In our case, we corrected for variability in fish age/size by comparing only time differences within the same range of standard length. Given the volume of water transported by the Madeira River, the legacy of ASGM, if any, cannot be detected after 30 years of peak activity. Indeed, sampling site locations did not show a statistical significance (Fig. 5).

A potential confounding factor reported in many studies is comparison of fish-Hg concentrations without correction for fish size; in our temporal comparison we could achieve this degree of strength by comparing fish within an established standard length. Another robust feature of our study was the breadth of species and the number of samples; by far the most comprehensive inventory of fish-Hg concentration in a single Amazon Basin river.

5. Conclusions

Spatial differences, albeit significant for some species, were not consistent with time trends for environmental contamination from past alluvial gold-mining activities. Fish-Hg bioaccumulation is species specific but fish feeding strategies are the predominant influence on the fish-Hg bioaccumulation pattern.

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