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Bioaccumulation of methylmercury in fish tissue from the Roosevelt River, Southwestern Amazon basin

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ABSTRACT

Mercury is a major pollutant in the Amazon River system, and its levels in fish and human hair are usually above the limit recommended by health agencies. The objective of this study was to analyze the methylmercury (MeHg) concentration in fish tissue from the Roosevelt River. The river's water velocity, depth, pH, temperature, electrical conductivity, dissolved oxygen and substrate type were measured, and fifty specimens distributed in 14 fish species were collected. A total of 64.3% of the sampled species were of the order Characiform and 71.4% of the species were carnivores. Fifty percent of the species had MeHg concentrations above threshold limit (Hg-T 0.5 mg kg^{-1}) established for food by the World Health Organization. *Cichla monoculus* had the highest value of MeHg (2.45 mg kg^{-1}). The MeHg concentration in fish varied according to dietary habits. The study also found bioaccumulation of MeHg in fish tissue in the following descending order: carnivorous > detritivorous > frugivore. Low significant correlations were found between fish weight or length and MeHg. Further studies on MeHg contamination are recommended in tissues of fish consumed in human riverine communities in the Roosevelt River Basin.

Keywords: biomagnification, chemical contamination, water pollution.

Bioacumulação de metilmercúrio em tecidos de peixes no rio Roosevelt, Sudoeste da Bacia Amazônica

RESUMO

O mercúrio tem sido um dos principais poluentes no sistema do rio Amazonas, cujos níveis em peixes e cabelo humano são geralmente acima dos limites recomendados pelos órgãos de saúde. O objetivo deste estudo foi analisar a concentração de metilmercúrio (MeHg) em tecidos de peixes do rio Roosevelt. Os atributos medidos do rio foram a velocidade da água, profundidade, pH, temperatura, condutividade elétrica, oxigênio dissolvido, e profundidade. Cinquenta espécimes distribuídas em 14 espécies de peixes foram coletadas. A ordem Characiformes representou 64,3% das espécies amostradas e os carnívoros representaram 71,4%. Cinquenta por cento das espécies apresentaram concentrações de MeHg acima do limite (Hg-T 0,5 mg kg^{-1}) estabelecido para os alimentos pela Organização Mundial de Saúde. *Cichla monoculus* apresentou o maior valor de MeHg (2,45 mg kg^{-1}). A concentração de MeHg variou por hábitos alimentares. Este estudo demonstrou bioacumulação de MeHg em tecidos de peixes, como segue: carnívoros > detritívoros > frugívoros. Baixas correlações entre peso ou cumprimento de peixe e MeHg foram encontradas. Recomenda-se um estudo mais aprofundado de contaminação do MeHg em tecidos de peixes consumidos nas comunidades ribeirinhas na bacia do rio Roosevelt.

Palavras-chave: biomagnificação, contaminação química, poluição aquática.

1. INTRODUCTION

Mercury (Hg) is one of the most hazardous environmental pollutants, with a large number of physical and chemical forms (Nevado et al., 2010). Although Hg is a naturally occurring element, anthropogenic activities may also release it into the environment (Cristol et al., 2008), and there has been an increase in the amount currently cycled in the biosphere (Hugget et al., 2001). Complex chemical transformations cause the cycling of Hg in the environment, although methylmercury (MeHg) formation is the predominant reason for Hg bioaccumulation in the aquatic food chain (Dorea et al., 2006).

Research has shown that about 60 to 95% of the total mercury in muscle tissue occurs in the form of MeHg, which is one of the most toxic forms for humans (WHO, 1991). Methylmercury causes brain damage, impaired motor coordination coupled to impaired speech and gait, paresthesia, neurasthenia, tremors, lack of balance, weakness, fatigue, difficulty in concentration, decreased visual field and hearing, and other effects, such as teratogenicity, and can lead to death (Goyer and Clarkson, 2001).

The concentrations of mercury in Amazonian fishes have been a matter of concern for human health and wildlife for over 25 years (Kehrig et al., 2008). Fish constitute an important source of protein for Amazonian riverine population (Dorea et al., 2006) since its excellent, low-fat protein source provides several benefits (Patterson, 2002). High concentrations of mercury found in the Amazonian ecosystem have been usually attributed to gold mining (Nriagu et al., 1992), soils with relatively high concentrations of natural mercury (Lechler et al., 2000), and the atmospheric transport and deposition of mercury from anthropogenic activities (Lacerda, 1995).

While several studies have been conducted in the Amazon involving the contamination of fish by mercury, the Roosevelt River Basin (southwestern Amazon Basin), a potential mercury hotspot, has not yet been investigated for methylmercury. Therefore, the objective of this study was to analyze methylmercury (MeHg) concentration in fish tissue from the Roosevelt River.

2. MATERIAL AND METHODS

2.1. Study area

The area under analysis lies on the right bank of the Madeira River, Brazil (Figure 1). The Roosevelt River is a clear water tributary on the right bank of the Aripuanã River, one of the most important tributaries on the east side of the Madeira River Basin. The regional climate according to Köppen classification is Af, which represents a tropical rainforest climate with annual rainfall between 2,300 and 2,750 mm, with January - April as the wettest season (Brasil, 1978). The sampling points are located between parallel 8° S, and meridians 60° and 61° W (Figure 1; Table 1).

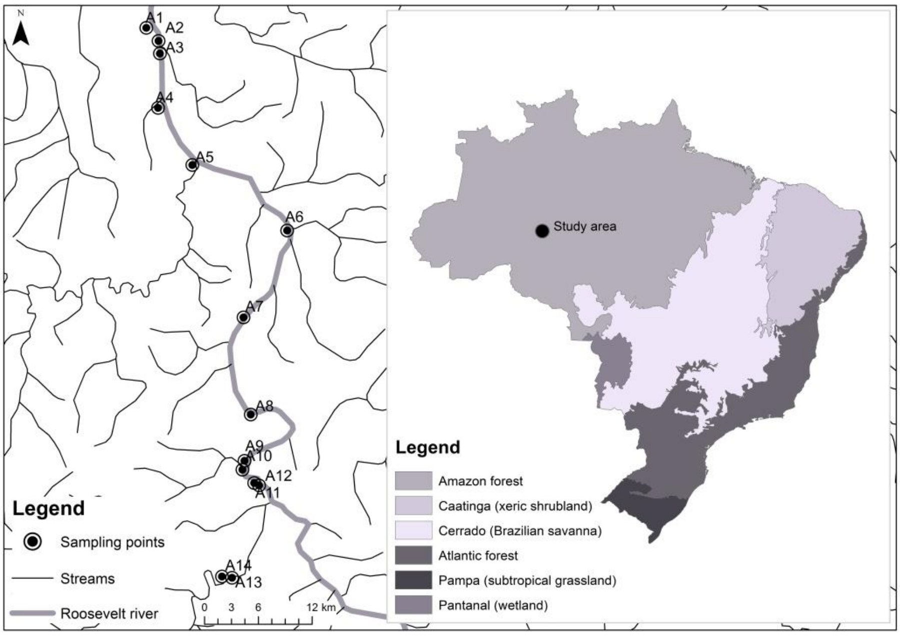

Figure 1. Sampling points at Roosevelt River in the southwestern Amazon Basin, Brazil.

Table 1. Geographical coordinates of sampling points at Roosevelt River in Southwestern Amazon basin, Brazil.

Code	Site	Latitude (S)	Longitude (W)
A1	Estação 35 Igarapé da Praia	8°02'22.1"	61°04'36.8"
A2	Estação 36 Igarapé da Camponesa	8°03'09.6"	61°03'52.8"
A3	Estação 37 Igarapé do Pavão	8°03'55.1"	61°03'48.0"
A4	Estação 38 Igarapé da Capela	8°07'11.4"	61°03'54.6"
A5	Estação 39 Machadinho	8°10'38.0"	61°01'50.9"
A6	Estação 41 Igarapé do Alfinete	8°14'35.0"	60°56'08.9"
A7	Estação 43 Poço da Morcega	8°19'49.0"	60°58'46.5"
A8	Estação 47 Poço do Diogo	8°25'40.2"	60°58'20.4"
A9	Estação 51 Cachoeira da Glória	8°28'27.5"	60°58'42.9"
A10	Estação 52 Poço do Esperança	8°28'59.7"	60°58'50.4"
A11	Estação 53 Poço da Pirapitinga	8°29'47.7"	60°58'07.9"
A12	Estação 54 Poço do Santa Rita	8°29'56.6"	60°57'50.3"
A13	Estação 55 Poço da Pirarara	8°35'30.4"	60°59'27.7"
A14	Estação 56 Lago do Tucunaré	8°35'25.8"	61°00'04.1"

2.2. Data collection

The analyzed attributes of sampling areas were water velocity (m s^{-1}), depth (m), pH, temperature (ºC), electric conductivity (µS cm^{-1}), dissolved oxygen (mg L^{-1}) and substrate type (>75% of a substrate type on the channel by visual observation). The fish capture effort was standardized in 500 m^2 for 24 hours at each sample point with fish harvested every six hours, using 14 gillnets (10x1.5 m or 10x1.5 m with mesh sizes between adjacent knots: 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180 and 200 mm). Longlines were also set and exposed at strategic locations near the mouth of each tributary during the collections. Collected fish were fixed in 10% formaldehyde and conserved in 70% alcohol, except those used in the MeHg analysis. Standard length (cm) and total body weight (g) were recorded in the laboratory. Voucher specimens were deposited at the Laboratório de Ictiologia e Ordenamento Pesqueiro do Vale do Rio Madeira – LIOP/UFAM.

2.3. MeHg analysis

After collection, fish were properly identified, measured and weighed. 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.

The method described by EPA-Method 1630 (USEPA, 2001) and Liang et al. (1994) was used for the MeHg determination in the fish muscle. A known amount (200 mg) of muscle tissue (wet weight) was weighed in a PTFE tube and 5.0 mL of 25% (w/v) KOH methanolic solution was used to extract the MeHg in an oven with the temperature controlled 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 the MeHg. Subsequently, the ethylation process was done with 300 µL of 272 g L^{-1} 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 MeHg determinations were ensured by the use of certified material (Dogfish Muscle, DORM-2, National Research Council of Canada, Ottawa) which was run with each batch of samples with a mean recovery of 108% MeHg.

2.4. Statistical analysis

We assumed the WHO threshold limit of 0.50 mg kg^{-1} for Hg (WHO, 1991) as the threshold limit for MeHg. Estimates of MeHg averages (±95% confidence interval) were calculated for species and trophic composition measurements by bootstrapping the resampled time series over 1,000 interactions (Efron and Tibshirani, 1993). The confidence interval is a range of values calculated by statistical methods which includes the desired true parameter, the mean in this case. Confidence interval is an alternative to null hypothesis significance testing because it provides information about the probability of the sign of an effect (Natrella, 1960; Berry, 1986; Gardner & Altman, 1986; Brandstätter & Linz, 1999). The confidence intervals were calculated by the ‘boot’ package in R program (R Core Team, 2015).

3. RESULTS AND DISCUSSION

Mean water velocity was 7.0 m s^{-1}, electric conductivity was 6.8 µS cm^{-1}, mean depth was 4.3 m, pH ranged from 5.6 to 8.0, mean temperature was 29.3°C, and mean dissolved oxygen was 9.4 mg L^{-1} (Table 2). The substrate type of the river channel was mainly composed of coarse litter and bedrock.

Table 2. Environmental attributes of sampling points at Roosevelt River in the southwestern Amazon Basin, Brazil. DO = dissolved oxygen.

Code	Water velocity ($m.s^{-1}$)	Depth (m)	Conductivity ($\mu S.cm^{-1}$)	pH	Temperature (°C)	DO ($mg.L^{-1}$)	Substrate type (75%)
A1	5.80	4.30	7.25	6.7	29.7	7.7	Sand
A2	5.30	2.80	7.75	6.6	28.5	7.3	Coarse litter
A3	7.30	2.77	6.50	5.6	27.4	10.0	Coarse litter
A4	5.20	3.40	6.00	6.8	29.0	8.9	Coarse litter
A5	10.70	2.21	6.75	5.9	29.1	-	Sand
A6	3.55	3.20	5.00	6.7	31.0	-	Bedrock
A7	7.20	3.70	7.75	6.7	30.2	12.8	Bedrock
A8	7.30	5.00	5.75	6.6	29.2	8.3	Bedrock
A9	7.30	9.00	7.75	6.6	28.9	11.1	Bedrock
A10	6.00	7.50	6.75	6.7	29.6	10.0	Sand
A11	15.00	3.90	6.50	6.7	29.0	10.1	Bedrock
A12	5.60	2.25	8.00	6.6	29.1	11.0	Bedrock
A13	6.90	4.30	6.00	6.7	30.0	8.9	Bedrock
A14	4.82	5.45	8.00	6.2	30.1	7.0	Coarse litter
Mean	**7,0**	**4,3**	**6,8**	**6,5**	**29,3**	**9,4**	

A total of 50 individuals from 14 fish species were collected and analyzed in the Madeira River Basin (Table 3). A total of 64.3% of the sampled species were of the order Characiform, followed by the Perciform (21.4%) and Siluriform (14.3%) orders. Fish were grouped according to preferential feeding habits. Carnivores represented 71.4% of the sampled species, while frugivores represented 14.3% and detritivores and omnivores were 7.1%. The standard length of the fish ranged between 31 and 77.7 cm and body weight varied from 577.3 to 9,267.3 g. The most abundant species were *Prochilodus nigricans*, *Hydrolycus scomberoides* and *Boulengerella cuvieri*, in that order.

Seven species exceeded the WHO threshold limit, such as *Cichla monoculus*, *Phractocephalus hemioliopterus* and *Hydrolycus scomberoides* (Figure 2). On the other hand, *Hydrolycus tatauaia* was at the WHO threshold limit and six species showed concentrations below the threshold limit. Carnivorous fish presented a mean total MeHg value above the WHO threshold limit (Figure 3), showing the importance of the MeHg bio magnification. No detritivore, omnivore and frugivore fish (4 spp.) showed concentrations above the threshold limit.

The low positive correlation was significant (p-value > 0.05) between length (0.39) or weight (0.49) and MeHg for fish in the study area. The strong positive correlation was significant between weight and length (0.86).

Table 3. Fish species, number of individuals (N), trophic level, standard length (cm), total body weight (g) in Roosevelt River in the southwestern Amazon basin, Brazil. Values in parentheses mean confidence interval.

Species	N	Trophic level	Length (cm)	Weight (g)
Boulengerella cuvieri	6	Carnivore	55.7 (5.7)	1791.6 (558.3)
Cichla monoculus	1	Carnivore	38.0 (0.0)	1500.0 (0.0)
Cichla orinocensis	1	Carnivore	44.0 (0.0)	1380.0 (0.0)
Hydrolycus scomberoides	7	Carnivore	52.5 (13.2)	2932.0 (1950.0)
Hydrolycus tatauaia	4	Carnivore	37.8 (2.9)	577.3 (150.0)
Leiarius marmoratus	1	Carnivore	70.0 (0.0)	8600.0 (0.0)
Leporinus friderici	1	Omnivore	32.0 (0.0)	840.0 (0.0)
Myleus pacu	3	Frugivore	35.3 (4.5)	1271.3 (225.0)
Myleus torquatus	3	Frugivore	33.3 (2.0)	1263.4 (225.0)
Phractocephalus hemioliopterus	2	Carnivore	77.7 (10.0)	9267.3 (4350.0)
Plagioscion squamosissimus	2	Carnivore	48.5 (1.5)	2322.1 (325.0)
Prochilodus nigricans	11	Detritivore	37.9 (3.7)	1148.9 (308.7)
Serrasalmus rhombeus	4	Carnivore	31.0 (4.0)	761.8 (560.0)
Serrasalmus spilopleura	4	Carnivore	35.8 (4.2)	1311.7 (528.7)

Only carnivores exhibited methylmercury (MeHg) concentrations above the WHO threshold limit in food for human consumption. The results highlight carnivorous fish as good indicators of contamination by MeHg and indicate the occurrence of MeHg bio magnification in food webs for fish in the southwestern Amazon Basin. Several studies indicated Madeira River and its tributaries, such as the Roosevelt River, as the principal route in Hg exportation to their associated aquatic systems due to soil erosion caused by forest burning, extensive cattle ranching, soybean plantations and cassiterite mining (Lechler et al., 2000).

As expected for neotropical rivers, fish of the Characiform order were the most abundant sampled species in the Roosevelt River (Lowe-McConnel, 1999). In fact, they include the most important commercial and subsistence fish of South American inland waters (Ardura et al., 2010). Some carnivore fish species consumed by the population have considerable potential for bioaccumulation due to their position at the top of the aquatic food chain (Dorea et al., 2006). These include *Cichla monoculus* (tucunaré-amarelo) and *Phractocephalus hemioliopterus* (pirarara), which are inappropriate for human consumption (Bastos et al., 2008). In the case of Cichla, its feeding habits evolve from planktivore in the early to piscivorous in the mature stage (Kehrig et al., 2008). On the other hand, herbivorous fish species consumed by the population, such as *Myleus torquatus* (pacú-branco) were not inappropriate for human consumption, even though the herbivore pacú-branco may have higher Hg concentrations during the rainy season (Dorea et al., 2006).

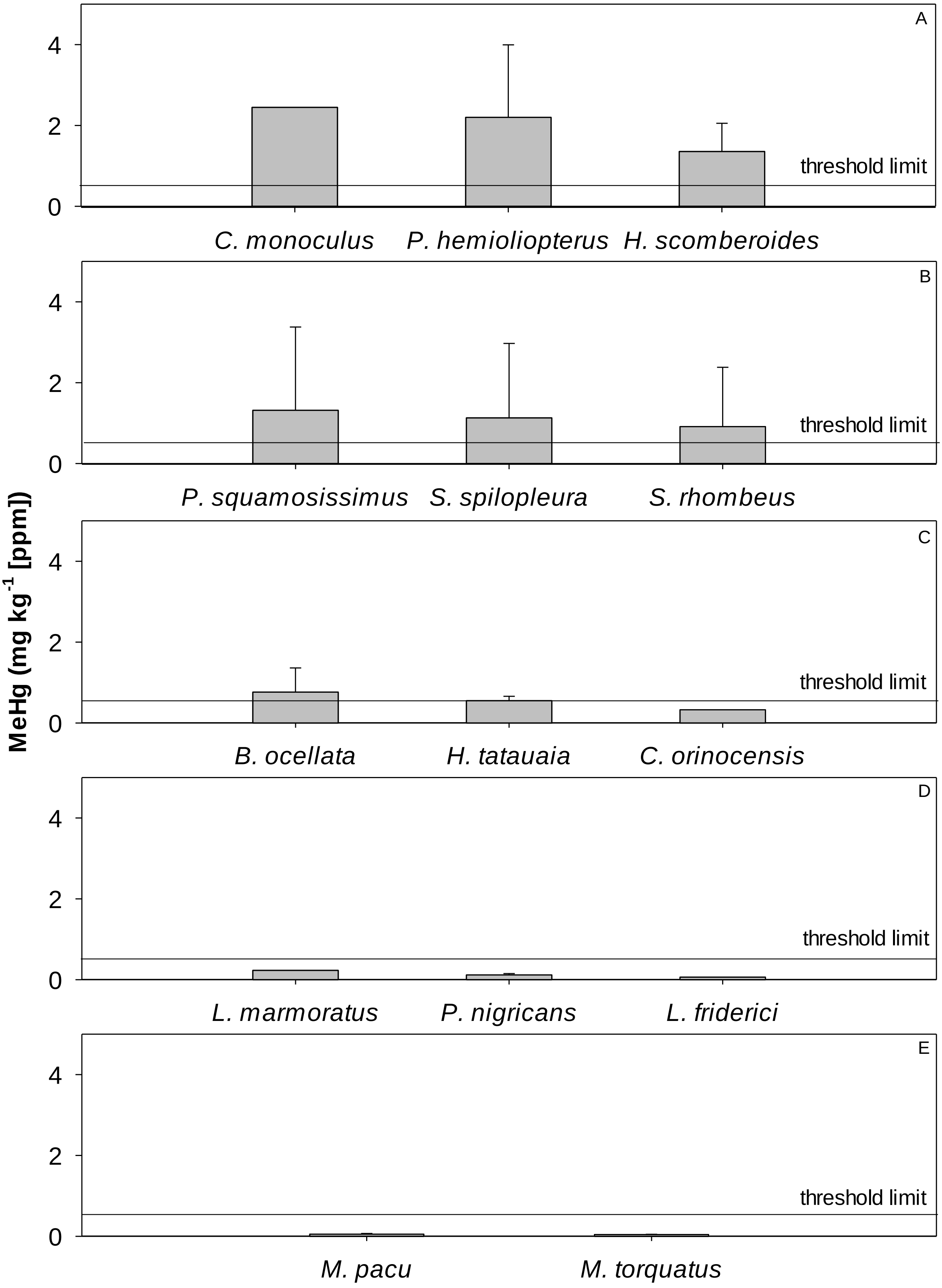

Figure 2. Concentration of MeHg in fish tissue of sampled species at Roosevelt River in the southwestern Amazon Basin, Brazil. Bars represent ±95% confidence interval.

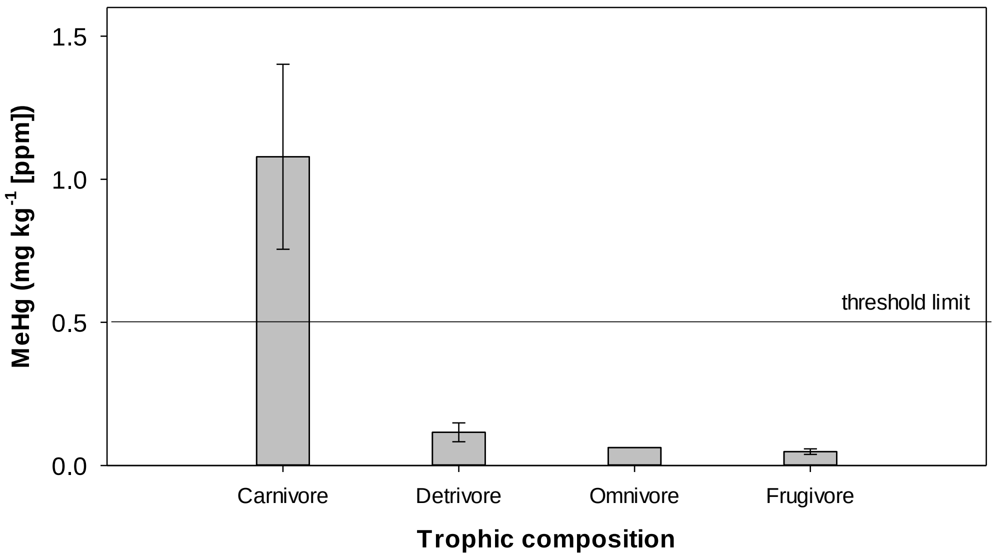

Figure 3. Concentrations of MeHg in fish tissues by fish trophic composition at Roosevelt River in the southwestern Amazon Basin, Brazil. Bars represent ±95% confidence interval.

Amaro et al. (2014) recorded a seasonal variation in Hg level with higher rates at the end of the dry season, in contrast to results by Coelho et al. (2007) in which rainfall has a strong influence in Hg levels in aquatic environments. This is due to leaching, which increases the availability of organic matter and enhances the bio-magnification process in the river sediment. Some water bodies have favorable characteristics for the mobilization of mercury and organification, such as subanoxia, anoxia, low pH, and high dissolved organic matter (Lacerda and Malm, 2008).

Regular annual flooding alters the aquatic environment and affects fish feeding strategies (Dorea et al., 2006). They reported fish, debris and insects as the most important food resources throughout the year, while the percentages of fruit, invertebrates and fish were reduced during the low-water period. Regardless of hydrological period effects, ranges of total Hg concentrations studied by Dorea et al. (2006) were higher for carnivores than for omnivore, detritivore and herbivore species.

In the case of human populations, one possible exposure route is through the ingestion of contaminated food. This is highly important for riverine communities, especially for indigenous peoples for whom fish constitutes their daily protein diet (Boischio and Cernichiari, 1998). Malm et al. (1997) showed some areas with both high Hg levels in fish and in samples of human hair in the Madeira and Tapajós Basins in Brazil. In fact, concentrations were high enough to cause negative health effects.

The frequency of consumption and daily intake are essential components in the evaluation of health risks even if the fish consumed contains concentrations of mercury below rates established by sanitary legislation (Brabo et al., 1999). The frequency of consumption constitutes a most important factor in assessing the risk of mercury contamination in communities that do not have alternative food sources (Brabo et al., 1999). Indications of neurotoxic effects related to MeHg exposure due to fish consumption have been reported in at least one riverine community of the Tapajos River Basin (Grandjean et al., 1999).

Although in current analysis there was a low significant correlation between fish length or weight and MeHg level in the sampled fish tissue, information on the species weight, size and feeding habits is important in evaluating MeHg bioaccumulation processes (Brabo et al. 1999). According to Roulet and Maury-Brachet (2001), it is rare to observe significant correlations in Amazonian fish. Other authors did not find significant correlation between fish length or weight and MeHg level (Brabo et al., 1999; Amaro et al., 2014; Bastos et al., 2008). The current authors underscore that MeHg contamination may be associated not only to the variability in the diet of each fish species and migration capacity, but also to other variables of aquatic environment, such as flow dynamics, water depth, levels of mercury on the bottom and suspended sediments, and in planktonic and benthic communities (Reuther, 1994).

4. CONCLUSIONS

Fifty specimens from 14 fish species were collected and analyzed for contamination by methylmercury (MeHg) in the Madeira River Basin. More than 60% of the sampled species were of the Characiform order and over 70% were carnivores. They include the most important commercial and subsistence fish of South American inland waters whose contamination poses a risk to human consumption. Seven species exceeded the WHO threshold limit, such as *Phractocephalus hemioliopterus* and *Hydrolycus scomberoides*. Only carnivores exhibited MeHg concentrations above the WHO threshold limit in food for human consumption which means that they are good indicators of contamination by MeHg. Therefore, MeHg concentrations in fish varied due to dietary habits. The study also demonstrated that MeHg bioaccumulation in fish tissue occurs in the following descending order: carnivore > detritivore > frugivore.

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