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Mercury biomagnification in the food web of a neotropical stream

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ABSTRACT

Anthropogenic and natural mercury (Hg) contamination have been a major concern in South America since the early 1900s, but it remains unclear whether Hg levels pose a hazard to human health in regions that lack point sources. We studied Hg biomagnification patterns in the food web of Río Las Marías, an Andean piedmont stream in northern Venezuela, which supports a major subsistence fishery. Mercury concentrations and trophic positions in the food web (based on stable isotopes of nitrogen and carbon) were characterized for 24 fish species representing seven trophic guilds (piscivore, generalized carnivore, omnivore, invertivore, algivore, terrestrial herbivore, detritivore). Mercury showed significant biomagnification through the food web, but vertical trophic position explained little of the variation. Muscle Hg concentrations also increased with body mass across the food web. Trophic guild assignments offered a useful alternative to explicit analysis of vertical trophic position; piscivores showed the highest Hg concentrations and terrestrial herbivores had the lowest. There were no consistent seasonal differences in Hg concentrations within the 5 species sampled during both the wet and dry seasons, suggesting that bioavailability is unaffected by strong seasonal variation in rainfall. From a human health perspective, many medium- to large-bodied species that are commonly eaten had Hg concentrations that exceeded International Marketing Limit (IML) (0.5 μg/g) and World Health Organization (WHO) guidelines (0.2 μg/g) for consumption. We conclude that Hg concentrations may pose a health concern for local subsistence fishermen and their families. Our results suggest a need to perform risk assessment and better understand contaminant levels in subsistence and commercial fisheries even in areas that lack known Hg point sources.

1. Introduction

In South America, mercury (Hg) typically enters riverine food webs as a result of human activities such as gold mining and amalgamation, deforestation, and erosion from agriculture (Veiga, 1997). Small- and large-scale gold mining activities are particularly a concern in this region, accounting for the largest Hg emission source (>55%) in South America (Pacyna et al., 2010). Natural emissions of Hg, derived from forest fires, soil weathering, and volcanic emissions (Pacyna et al., 2010), are also gaining attention due to the high levels of Hg found in predatory fish located distant from known point sources (Belger and Forsberg, 2006; Fadini and Jardim, 2001). In the northern Amazon basin, the most important reported Hg source is mobilization of soil Hg following deforestation (Roulet et al., 1999). Mercury in aquatic systems can be methylated when terrestrial and aquatic vegetation decay (Roulet et al 2000, 2001a). Methylmercury (MeHg) bioaccumulates in aquatic food webs, thereby posing a health hazard to human populations consuming fish. Populations residing in the Amazonian low lands demonstrated high Hg levels in their blood and hair due to local fish consumption (Barbosa et al., 1995; Lebel et al., 1997; Malm et al., 1995).

MeHg is neurotoxic, and fish are an important source of MeHg to humans globally (Megler et al., 2007). The structure of the food web as well as the characteristics of the individual fish (e.g. body mass, age) influence the concentration of MeHg and total mercury (THg) in aquatic food webs (Campbell et al., 2008). With increasing recognition of the global extent of Hg contamination in fish targeted by subsistence and commercial fisheries, there is a pressing need to better characterize Hg levels in regions that are remote from known Hg sources. In addition, enhancing our understanding of controls on Hg concentrations within aquatic food webs would enable better prediction of which fish species are most likely to be health threats due to contamination.

Tropical rivers are typically characterized by seasonal rainfall that produces annual flood cycles (Lowe-McConnell, 1987). This flooding may increase the exposure of riverine species to Hg due to both release of Hg from soil and terrestrial material and methylation to MeHg in saturated soils and sediments. For instance, Roulet et al. (2001a) observed

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high MeHg concentration and anoxic conditions in Amazon rainforest soil experiencing seasonal inundation. The terrestrial detritus and aquatic vegetation that are flushed with flood pulses were identified as primary substrates for Hg methylation by sulfate-reducing bacteria (Guimaraes et al., 2000a) and the decomposition of these materials further accumulates MeHg in water (Roulet et al., 2000, 2001b). These sources subsequently contribute to increased bioavailability of MeHg to aquatic organisms (Roulet et al., 2001a). Such environmental factors emphasize the importance of investigating the potential seasonal differences in Hg bioavailability and bioaccumulation.

In the Andean piedmont of South America, river systems provide extensive fish migratory pathways and support fish from diverse trophic guilds including parasites, piscivores, omnivores, invertivores, algivores, and detritivores (Jepsen and Winemiller, 2007; Lowe-McConnell, 1987). These fish communities are often intensively harvested to support subsistence fisheries. Rivers in this region also differ in the extent of human disturbance of the riparian and channel habitats (Allan et al., 2006).

We studied Hg biomagnification patterns in a fourth-order perennial piedmont river in northern Venezuela that experiences relatively low watershed disturbance and anthropogenic pollution. Our study site lies in the Orinoco basin, originating from an elevation of 1200–1600 m and hosts a rich assemblage of approximately 75 fish species (McIntyre et al., 2008). Río Las Marías is a clear water system, surrounded by semi-deciduous forest with little deforestation but active cattle grazing (Allan et al., 2006). The river receives moderate sediment loading from natural and anthropogenic erosion. Epilithic algae are highly productive, and their growth is limited by nitrogen availability. The dominant substrates are cobbles and sand, with occasional patches of leaf litter and silt in pools. Due to the modest size of its catchment (331 km^2) in the Andean foothills, Río Las Marías experiences short periods of inundation and relatively low seasonal discharge that are more akin to flash flooding than the sustained floods typical of the large lowland river systems in South America. Nonetheless, such mid-sized tropical rivers are subject to substantial annual variation in water levels and discharge (0.1–25 m^3/s). Thus, extreme dry seasons can leave some areas of the river completely dry for several months, while storms during the wet season can produce severe and rapid flooding (Flecker and Feifarek, 1994).

Relatively few studies have assessed Hg contamination and biomagnification trends in regions of South America that lack point sources. In the current study, we characterized THg concentrations and biomagnification trends in a large suite of fish species from Río Las Marías to understand the extent of Hg contamination. Our goal was to capture the full range trophic strategies and body sizes present in this system, thereby enabling rigorous evaluation of the relative influence of vertical trophic position in the food web, energy sources, body size, and trophic guild on Hg bioaccumulation. To test for seasonal dynamics of Hg concentrations in fish, we also compared THg concentrations between the wet and dry seasons for common species targeted by fisherman.

We interpreted the Hg survey results in both food web and human health contexts. To understand how Hg bioaccumulates through the food web in this ecosystem, we used stable isotopes of nitrogen and carbon to characterize relative vertical trophic position and energy sources of each species (Caut et al., 2009; Fry, 2006). Stable nitrogen isotope values, expressed as $\delta^{15}N$, are widely used to measure vertical trophic position of animals within food webs. Consumers typically have 2–4‰ higher $\delta^{15}N$ values than their prey (Caut et al., 2009; Post, 2002). Stable carbon isotopes ($\delta^{13}C$) provide insight into the energy sources supporting a consumer (Fry, 2006). In rivers, $\delta^{13}C$ values of fishes indicate relative contributions of allochthonous (e.g., terrestrial detritus) and autochthonous energy sources (e.g., benthic algae) (Fry, 2006). We tested whether vertical trophic position or energy sources were a better predictor of muscle THg concentrations in fish. To place our results in a human health context, we compared muscle THg to established standards for human consumption. We paid special attention to species that are targeted in the local subsistence fishery (McIntyre et al., 2007), and therefore could pose the greatest threat to human health. Though quantitative fish consumption data are not available, our extensive creel surveys and frequent interactions with fishermen indicate that many subsistence fishermen and their families consume fish from the study site at least once per week.

2. Materials and methods

Fish were collected using nets, hook-and-line and an electroshocker from a 2-km reach of the main channel of Río Las Marías, Venezuela (9°10′ N, 69°44′ W). This forested reach is representative of piedmont rivers in the region, and has been the focus of our long-term research on aquatic ecosystem dynamics. Details of the watershed, hydrology, and chemistry of Río Las Marías have been summarized by Allan et al. (2006). A total of 64 individual fishes representing 24 species were analyzed (Table 1). All the fish were collected from the same area during both the wet and dry seasons. All but one species were collected in the dry season (January–March 2004), when fishing pressure is most intense in Río Las Marías. Wet-season sampling (July and October 2003) focused on five common species that are often targeted by fishermen *(Salminus hilarii, Astyanax integer, Parodon apolinari, Brycon whitei, Prochilodus mariae)*, and included one large species not captured during the dry season (*Hemisorubim platyrynchus*). Species sampled during the dry season included many of the most abundant species in the community, and represented all seven of the major trophic guilds in the food web (piscivore, generalized carnivore, omnivore, invertivore, algivore, terrestrial herbivores and detritivore; McIntyre et al., 2008) (Table 1). Trophic guild designations follow McIntyre et al. (2008), and were based on stomach content analysis and extensive field observations spanning from 1997 to 2004. After measuring total body length (cm), standard length (cm) and wet mass (g), we dissected dorsal muscle tissue free of skin, scales, or bones using a stainless steel scalpel. All the tools were wiped thoroughly between each sample to avoid cross-contamination. Muscle samples of each fish were placed in glass vials, oven-dried at 60 °C for 48 h, and stored frozen until analysis. Dried fish samples were ground to a fine, homogenous powder inside the original glass vial using a clean stainless steel spatula.

Samples were analyzed for stable isotope ratios of carbon and nitrogen to test the influence of energy sources and vertical trophic position in the food web upon Hg bioaccumulation. Homogenized fish muscle powder was weighed into tin capsules (1.2 mg), and analyzed using a Finnigan MAT Delta Plus Isotope Ratio Mass Spectrometer at the Cornell University Stable Isotope Laboratory, Ithaca, New York, USA. Isotope values were expressed in the standard $\delta^{15}N$ and $\delta^{13}C$ notation (in parts per thousand ‰) relative to international standards (atmospheric N_2 and PeeDee belemnite) (Fry, 2006). Analytical precision was quantified using in-house standard materials (trout tissue, methionine) interspersed throughout each sample set every 8 samples; all SD were less than 0.2‰.

Mercury analyses were carried out in a Queen's University ultra-trace clean room laboratory outfitted with HEPA filters and engineered for positive pressure relative to outside the room. Teflon vials were placed in 10% trace metal grade nitric acid (HNO_3) and bromine monochloride (BrCl) bath for 24 h and washed with deionized water prior to the analysis. Approximately 20-mg of dried, homogenized muscle was transferred to pre-cleaned Teflon vials. In cases where the available mass of sample material was below the minimum needed for analysis, we combined material in equal portions from multiple conspecific individuals that were similar in body size and stable isotope values (Table 1). Samples were digested with 5 mL trace metal grade HNO_3 in the Microwave Accelerated Reaction System (200 °C for 15 min, 30 min cool down), following the USGS Standard Operating Procedure No. HC520B (USGS, 1996). The

Table 1
List of species analyzed for this study, along with the number of samples analyzed for THg concentrations and stable isotopes, and the mean ± 1 SD for stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), body mass (g), and muscle THg concentration (μg/g wet weight). Species are organized by trophic guild following McIntyre et al. (2008). N_a indicates number of samples analyzed for THg concentration (parentheses representing the wet season samples). N_b represents total number of individuals pooled based on the stable isotope values. Species for which individual samples were pooled for THg analysis are denoted with an asterisk.

Scientific name	Trophic guild	Code	N_a	N_b	Mean $\delta^{13}C$	Mean $\delta^{15}N$	Mean mass	Mean THg
Ochmacanthus alterus	Piscivore	Om	1	4	−23.43	11.85	0.39	175.00
Hoplias malabaricus	Piscivore	Hm	2	2	−21.75 ± 1.48	9.79 ± 1.13	310.80 ± 238.30	1.13 ± 0.18
*Salminus hilarii**	Piscivore	Sh	3(2)	12	−22.04 ± 1.01	10.87 ± 0.36	141.63 ± 48.92	1.60 ± 0.96
Serrasalmus rhombeus	Piscivore	Sr	3	3	−21.90 ± 0.61	11.23 ± 0.56	130.31 ± 31.51	0.59 ± 0.35
Hemisorubim platyrynchus	Piscivore	He	(1)	1	−20.59	10.17	400.00	0.70
Caquetaia kraussi	Carnivore	Ck	2	2	−21.55 ± 0.64	10.34 ± 0.17	116.5 ± 18.38	1.02 ± 0.59
Crenicichla geayi	Carnivore	Cg	2	2	−22.60 ± 1.56	9.01 ± 0.07	95.04 ± 74.23	0.35 ± 0.02
*Pimelodus blochii**	Carnivore	Pb	2	3	−21.68 ± 0.46	8.92 ± 0.88	44.87 ± 4.59	0.19 ± 0.10
Potamotrygon orbignyi	Carnivore	Po	2	2	−21.05 ± 1.20	10.54 ± 0.01	482.95 ± 524.74	0.42 ± 0.42
*Rhamdia quelen**	Carnivore	Rq	2	3	−25.58 ± 2.51	7.22 ± 0.43	109.38 ± 127.60	0.37 ± 0.21
Leporellus vittatus	Omnivore	Lv	2	2	−22.70 ± 0.85	9.29 ± 0.04	35.05 ± 16.36	0.23 ± 0.21
*Leporinus striatus**	Omnivore	Ls	2	3	−22.40 ± 1.84	10.77 ± 0.03	24.35 ± 0.82	0.20 ± 0.19
Aequidens pulcher	Invertivore	Ap	2	2	−24.75 ± 2.33	8.30 ± 0.66	26.78 ± 17.71	0.63 ± 0.50
*Apteronotus albifrons**	Invertivore	At	2	5	−22.81 ± 0.20	7.98 ± 0.74	4.38 ± 1.23	0.34 ± 0.06
*Astyanax integer**	Invertivore	Ai	4(2)	15	−23.53 ± 0.46	9.38 ± 0.95	16.34 ± 1.25	0.37 ± 0.22
*Cetopsorhamdia insidiosa**	Invertivore	Ci	2	4	−22.50 ± 1.20	9.89 ± 0.37	7.54 ± 2.64	0.26 ± 0.14
*Lebiasina erythrinoides**	Invertivore	Le	2	4	−25.14 ± 0.05	7.37 ± 0.13	9.17 ± 3.77	0.07 ± 0.03
*Rhamdella sp.**	Invertivore	Rh	2	6	−23.33 ± 0.42	9.49 ± 0.19	2.57 ± 0.33	0.13 ± 0.06
*Parodon apolinari**	Algivore	Pa	3(2)	14	−18.04 ± 0.59	9.09 ± 0.59	13.84 ± 4.78	0.20 ± 0.07
*Chaetostoma milesi**	Algivore	Cm	2	4	−23.28 ± 3.22	8.87 ± 0.25	28.83 ± 32.37	0.17 ± 0.05
*Brycon whitei**	Terr. herbivore	Bw	2(2)	9	−22.82 ± 0.54	8.08 ± 0.32	144.68 ± 21.00	0.16 ± 0.06
Schizodon isognathus	Terr. herbivore	Si	3	3	−24.13 ± 0.49	7.69 ± 0.94	127.60 ± 5.02	0.12 ± 0.06
*Prochilodus mariae**	Detritivore	Pm	4(2)	15	−27.30 ± 1.37	7.50 ± 1.10	112.62 ± 32.98	0.33 ± 0.11
*Steindachnerina argentea**	Detritivore	Sa	2	6	−30.22 ± 0.54	7.04 ± 0.42	6.70 ± 0.85	0.22 ± 0.14

digested samples were diluted using ultra purified trace-metal quality water and 200 μL of BrCl was added to prevent the volatilization of Hg.

Digested samples were analyzed using a Tekran Model 2600 cold vapor atomic fluorescence spectrometer following EPA method 1631 in a clean room laboratory. The method detection limit was 0.1 pg/g THg. Analytical precision was measured using 4 blanks (n = 16, <0.5 pg/g), 2 spiked samples (n = 8, recovery 110–118%), and 3–4 National Research Council (Canada) certified reference materials (DORM-2, n = 5, 4.64 ± 0.26 mg THg/kg, recovery 95–99%; DORM-3, n = 2, 0.382 ± 0.06 mg THg/kg, recovery 91–97%; DOLT-3, n = 7, 3.37 ± 0.14 mg THg/kg, recovery 99–126%) for each run.

We summarized the overall pattern of Hg biomagnification from the species collected during the dry season and by regressing individual log THg concentrations against $\delta^{15}N$ using a linear regression model. To determine the best predictors of THg, a multiple regression model was used with $\delta^{15}N$, $\delta^{13}C$, and log-transformed wet mass from the dry season species as predictors. We chose body mass rather than length to characterize fish size because metabolism and consumption rates scale more closely with fish mass. There was weak colinearity between $\delta^{15}N$ and $\delta^{13}C$, but none between isotope data and body mass. We used analysis of covariance (ANCOVA) to test the predictive value of trophic guild categories, using $\delta^{15}N$, $\delta^{13}C$, and log-transformed mass from the dry season species as covariates. Pairwise differences in log THg among guilds were tested using Tukey's HSD. We also used ANCOVA to test for seasonal differences in THg concentrations within species sampled during both the wet and dry seasons, using log-transformed mass as a covariate. All statistical tests were performed using SYSTAT. THg concentrations and fish wet mass were always log-transformed for statistical analyses. Assumptions of normality, homoscedasticity, and homogeneity-of-slopes were met for the relevant statistical tests.

3. Results

Although our data do not encompass the entire Río Las Marías food web, the $\delta^{15}N$ and $\delta^{13}C$ values of the fish species sampled during wet and dry seasons indicate a wide range of energy sources and vertical trophic positions (Fig. 1). THg concentrations ranged from 0.05 to 3.22 μg/g among species. Regressing THg against $\delta^{15}N$ values (5.62 to 11.66‰) indicated substantial biomagnification in the dry season of Río Las Marías food web ($F_{1,50} = 7.54$, $p = 0.008$; Fig. 2). The linear regression equation was log THg = −1.51 + 0.10 ($\delta^{15}N$), but the strength of relationship was weak ($r^2 = 0.13$). The seasonal data indicated equivalent THg concentrations between wet and dry seasons (ANCOVA; $F_{1,19} = 0.11$, $p = 0.747$; Table 2, Fig. 3). $\delta^{15}N$ and $\delta^{13}C$ values indicated that trophic roles were also similar in the wet and dry seasons.

The multiple regression model indicated significant, independent effects of both $\delta^{15}N$ and log body mass on log THg concentrations across all fish species during the dry season ($\delta^{15}N$; $t_{48} = 2.54$, $p = 0.015$; log body mass; $t_{48} = 2.67$, $p = 0.010$), but no significant

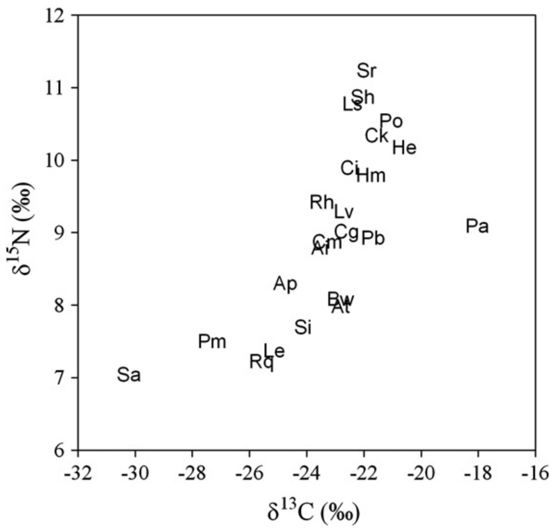

Fig. 1. Mean stable nitrogen ($\delta^{15}N$) and carbon isotope ($\delta^{13}C$) ratios of the fish species collected during dry and wet seasons in Río Las Marías, Venezuela. Letters indicate species names following Table 1. $\delta^{15}N$ indicates vertical trophic position in the food web, while $\delta^{13}C$ indicates energy sources from terrestrial inputs (more negative) to benthic algal productivity (less negative).

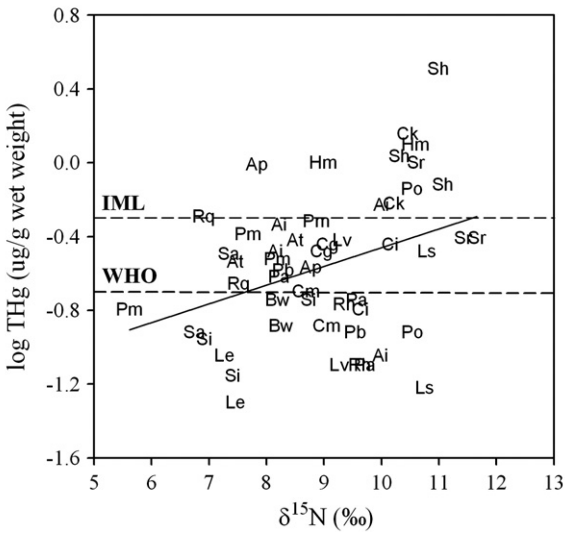

Fig. 2. Biomagnification of Hg in the dry season of Río Las Marías, represented as the log total mercury concentrations (log THg) plotted against stable nitrogen isotope ($\delta^{15}N$) ratios. Letters indicate species names following Table 1. Dash horizontal lines indicate the International Marketing Limit (IML; 0.5 μg/g wet weight) and WHO guideline (WHO; 0.2 μg/g wet weight) for Hg in fish consumed by humans and the solid line indicates the biomagnification trend of THg.

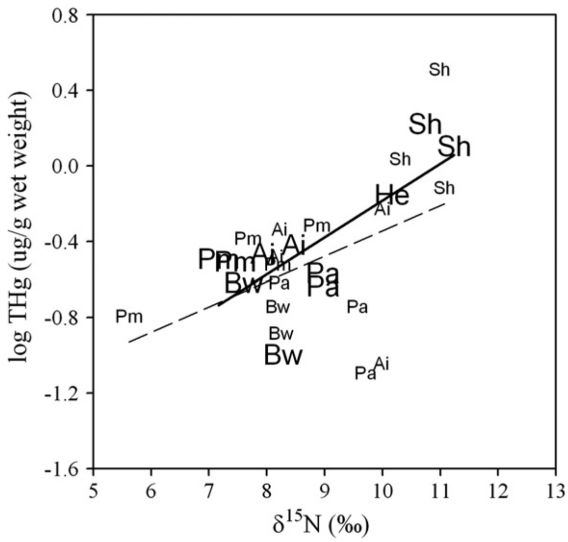

Fig. 3. Biomagnification of THg during the wet (large type with solid line) and dry (small type with dash line) season in Río Las Marías. Letters indicate species names following Table 1.

effect of $\delta^{13}C$ (Table 3). When trophic guild was included in an ANCOVA along with $\delta^{15}N$, $\delta^{13}C$ and log body mass, only the trophic guild effect was significant ($F_{6,42}=3.91$, $p=0.003$; Table 4). Pairwise comparisons (Tukey's HSD) among trophic guilds indicated that piscivores were significantly higher in THg than terrestrial herbivores ($p=0.001$) and omnivores ($p=0.017$; Table 5). Terrestrial herbivores had the lowest mean THg concentrations, but were not significantly lower than most other guilds.

A total of 6 species (*Hoplias malabaricus, S. hilarii, Serrasalmus rhombeus, H. platyrynchus, Caquetaia kraussi, Aequden pulcher)* had average THg concentrations that exceeded the International Marketing Level (IML) (0.5 μg/g wet weight), and an additional 10 species (*Crenicichla geayi, Potamotrygon orbignyi, Rhamdia quelen, Leporellus vittatus, Apteronotus albifrons, A. integer, P. apolinari, Cetopsorhamdia insidiosa, P. mariae, Steindachnerina argentea*) had average THg concentrations above the World Health Organization (WHO) guidelines (0.2 μg/g wet weight). Among these high-Hg fishes, four species (*P. mariae, S. hilarii, A. integer, C. geayi*) are frequently targeted by fishermen (McIntyre et al., 2007). Note that the *Ochmacanthus alternus* has been excluded from our main results and statistical analysis because the THg estimate was 62% higher than our highest calibration standard, and additional material from this species was not available for re-analysis (see Table 1).

4. Discussion

The significant positive relationship between vertical trophic position, as estimated by $\delta^{15}N$ of fishes in the food web, and the concentration of THg shows that biomagnification occurs in Río Las Marías. However, unlike many other aquatic ecosystems observed in arctic (Campbell et al., 2005; Power et al., 2002), temperate (Kidd et al., 1995), and tropical (Campbell et al., 2003, 2008; Kidd et al., 2003) areas, this relationship was weak ($r^2=0.13$). Campbell et al. (2005) noted that the slope of Hg biomagnification trends normally falls between 0.2 and 0.3 based on fish-only food webs. Thus, our estimated slope of 0.10 suggests relatively low bioaccumulation rates in this food web.

Several factors could affect the slope of the $\delta^{15}N$–THg relationship in Río Las Marías. First, tropical streams show relatively low fractionation of $\delta^{15}N$ during trophic transfers compared to other ecosystem types (Kilham et al., 2009), perhaps due to rapid turnover of tissue $\delta^{15}N$ in tropical aquatic organisms (McIntyre and Flecker, 2006) or temperature-dependent fractionation of $\delta^{15}N$ (Sweeting et al., 2007). In the context of Hg biomagnification, low fractionation of $\delta^{15}N$ in tropical steams (Kilham et al., 2009) should increase the rate of Hg per unit change of $\delta^{15}N$. Our results do not match this prediction, suggesting that geographic biases in trophic fractionation of $\delta^{15}N$ are not responsible for low apparent biomagnification rates of THg in Río Las Marías.

Second, the high proportion of inorganic Hg in the primary consumers (algivores and detritivores) could also affect the strength of Hg biomagnification. Tropical aquatic systems such as Río Las Marías demonstrate rich abundance and wide distribution of algal- and detrital-feeding fish that are analogous to benthic invertebrates in temperate systems in that they utilize periphyton, algae and sediments as food sources (Flecker, 1992). Direct consumption of these basal resources could be a major uptake route of inorganic Hg. With much lower assimilation efficiency and trophic transfer of inorganic Hg compared to the MeHg (Mason et al., 1995), we may be observing relatively weak biomagnification trend of THg because primary consumers have high inorganic Hg that is not transferred up the food chain.

Table 2
Results of analysis of covariance (ANCOVA) with season (wet vs. dry), species, and log-transformed body mass (log (mass)) as predictors of log THg concentrations ($r^2=0.69$).

Factor	df	F	p
Species	4	8.30	<0.001
Season	1	0.11	0.747
Log (mass)	1	0.17	0.684

Table 3
Statistical results from multiple regression model with $\delta^{15}N$, $\delta^{13}C$, and log transformed body mass (log (mass)) as predictors of log THg concentrations. ($r^2=0.26$, adjusted $r^2=0.22$, $F=5.75$, df = 3, 48, $p=0.002$).

	Coefficient	Standard error	Standard coefficient	t	p
Log (mass)	0.221	0.083	0.339	2.67	0.010
$\delta^{15}N$	0.116	0.046	0.405	2.54	0.015
$\delta^{13}C$	−0.029	0.023	−0.194	−1.24	0.223

Table 4
Results of analysis of covariance (ANCOVA) with $\delta^{15}N$, $\delta^{13}C$, log-transformed body mass (log (mass)), and trophic guild as predictors of THg concentrations ($r^2 = 0.53$).

Factor	df	F	p
Log (mass)	1	3.19	0.081
$\delta^{15}N$	1	0.03	0.855
$\delta^{13}C$	1	0.22	0.638
Trophic guild	6	3.91	0.003

Third, we consider the most likely explanation for weak biomagnification to be the unusual size structure of the food web in Río Las Marías. Various primary consumers in this system achieve body sizes comparable to top predators (Layman et al., 2005; Winemiller, 1990), such that vertical trophic position (estimated by $\delta^{15}N$) is not predicted by body size in Río Las Marías and other Orinoco tributaries (Layman et al., 2005). Given that these species are likely to grow slowly on their low energy diet (Hood et al., 2005), large-bodied detritivores and grazers may have been accumulating contaminants for years longer than smaller species that occupy higher vertical trophic positions. Indeed, body mass proved to be a significant predictor of fish THg concentrations when considered alongside $\delta^{15}N$, reflecting THg in large-bodied species occupying low vertical trophic position (e.g., *P. mariae*) that is comparable to that of smaller fishes at higher vertical trophic positions in the food web. Thus, the presence of large-bodied primary consumers in this and many other tropical river food webs may reduce the apparent rate of Hg biomagnification. Further work will be required to resolve the mechanisms that are responsible for low biomagnification rate in this food web.

In addition to analyzing biomagnification using $\delta^{15}N$, the large number of species and diverse diets of fishes in this study enabled us to test for differences among trophic guilds and effects of energetic pathways on Hg levels. Unlike stable isotope data, information on trophic guild membership is readily available for most freshwater fishes from FishBase (Froese and Pauly, 2011). We found that including trophic guild in the statistical model obviated body mass and $\delta^{15}N$ as predictors of THg (Table 4). Though guild differences were generally strong at the ends of the trophic spectrum – fish-eating species showing the highest levels and terrestrial herbivores the lowest – it was nonetheless striking that individual vertical trophic position based on $\delta^{15}N$ offered no significant additional predictive value after accounting for guild affiliations. This result indicates that relative levels of Hg, and thus safety for human consumption, may be predicted for some species based on trophic guild alone in the absence of stable isotope data or direct analyses of Hg. However, the fact that detritivores were considerably higher in THg than generalized herbivores, as also observed in the Río Negro (Barbosa et al., 2003), underscores the complexity of making robust predictions.

In addition, we recorded an exceptionally high THg concentration (175 μg/g) and vertical trophic position ($\delta^{15}N = 11.85‰$) in an *O. alternus*, a parasitic species that consumes the mucus coating of other fish (Winemiller, 1990) (Table 1). This anomalous datum was excluded from our main quantitative results because it exceeded the highest value in our analytical calibration, however we are confident that the THg concentration was indeed very high. Unfortunately, no additional tissue was available from this species for further analyses. Nonetheless, this provisional result is intriguing because it suggests that the unique parasitic feeding strategies of tropical fishes may sometimes yield dramatically elevated tissue Hg.

Sources of bioavailable Hg are poorly understood in tropical rivers, and data on Hg concentrations in soil, sediments, and water are scarce. Large floodplain rivers receive methylated Hg associated with terrestrial organic matter during high water (Roulet et al., 2000), suggesting that feeding on allochthonous resources might enhance Hg in fishes. We found no support for that hypothesis; fish $\delta^{13}C$ indicates the relative contributions of autochthonous and allochthonous energy sources, but showed no consistent relationship with muscle THg concentrations (Table 3). Moreover, our terrestrial herbivores that specialize on allochthonous resources (e.g. fruits, seeds, flowers) had the lowest THg concentrations. Unambiguous interpretation of $\delta^{13}C$ in tropical rivers is sometime difficult due to the multitude of potential energy sources (e.g. grasses, trees, phytoplankton, macrophytes, periphyton, sediment). However, our previous experience in sampling of basal resources, invertebrates, and fishes at the study site suggests that riparian trees, and benthic algae, which are easily distinguishable using $\delta^{13}C$, are dominant energy sources for the aquatic food web (McIntyre and Flecker, unpublished data). This lack of significant effect of $\delta^{13}C$ on THg concentrations in our fish could reflect that riparian soils near Río Las Marías do not experience long-term seasonal inundation throughout the wet season, which underlies the high levels of methylated Hg in soils in the Amazon floodplain (Roulet et al., 2001a). Instead, our study site experiences strong seasonality of rainfall but only occasional, temporary flooding beyond the river channel (Flecker and Feifarek, 1994). Thus, there may be less seasonal change in methylation and transport of terrestrial Hg than in floodplain systems, as suggested by both the lack of differential biomagnification based on energy sources and the similarity of THg levels in five common fish species sampled during both the wet and dry seasons (Table 2, Fig. 3). This conclusion also accords with previous evidence that seasonal variation in fish Hg is less important than habitat influences even in large floodplain rivers (Dorea et al., 2006; Guimaraes et al., 2000b).

Our results have important implications for human health in the study region, where subsistence fishing provides a major source of protein for the rural population. For instance, Barbosa et al. (2001) surveyed populations along the Río Negro in Brazil, where there is no recent history of gold mining and human population depend heavily on river fisheries for nutrition. They found that the majority of respondents (78.6%) eat fish at least twice a day, and estimated per capita intake of 200 g wet mass per day (Barbosa et al., 1995). Though our casual observations of local fishermen during the dry season since the mid-1980s suggest lower consumption rates at Río Las Marías, there are certainly many families that consume fish for more than one meal per week. Even weekly dining on fish may pose a substantial risk to subsistence fishermen and their families because many of the fish species we analyzed from Río Las Marías had THg concentrations that exceeded the International Marketing Limit (0.5 μg/g) and WHO (0.2 μg/g) recommended guidelines established for vulnerable populations such as children, pregnant women and regular consumers (Fig. 2). Of the eleven fish species caught

Table 5
Pairwise comparisons between log THG concentrations of 7 trophic guilds. Entries represent probability values from Tukey's HSD multiple comparisons.

	Algivore	Carnivore	Detritivore	Invertivore	Omnivore	Piscivore	Terrestrial herbivore
Algivore	1.000	–	–	–	–	–	–
Carnivore	0.993	1.000	–	–	–	–	–
Detritivore	1.000	0.997	1.000	–	–	–	–
Invertivore	0.995	1.000	0.999	1.000	–	–	–
Omnivore	1.000	0.996	1.000	0.997	1.000	–	–
Piscivore	0.120	0.624	0.254	0.061	0.017	1.000	–
Terrestrial herbivore	0.859	0.460	0.814	0.279	0.506	0.001	1.000

regularly by fishermen at our study site (McIntyre et al., 2007), four species had average THg exceeding WHO consumption guidelines. Most important among these species is *P. mariae*, which makes up roughly half of total fish biomass in Río Las Marías (McIntyre et al., 2008) and other neotropical rivers (Silva and Uieda, 2007). This and closely related species are caught in large numbers during dry-season migrations, supporting important fisheries in the Andean piedmont and the llanos floodplain regions (Duque et al., 2008; McIntyre et al., 2008). Average mercury concentration for *P. mariae* was 0.33 μg/g, and some individuals almost exceeded the IML consumption guideline of 0.5 μg/g (Table 1). Many other genera that are heavily fished throughout the study region and elsewhere in South America also had high THg, including *Salminus, Hoplias, Serrasalmus, Caquetaia,* and *Crenicichla*. These results are troubling in that they indicate that major fisheries target species are likely to pose considerable health risk to human consumers who have few other options.

5. Conclusions

Despite low biomagnification rates through the food web, several fish species had sufficiently elevated THg concentrations warranting human consumption advisories in the region. While the source of Hg is not yet confirmed, high-trophic piscivores, certain trophic guilds and large long-lived fish species had consistently elevated THg concentrations. Further investigation is required to understand the role of allochthonous and autochthonous resources as sources of bioavailable Hg in tropical rivers. In light of the importance of subsistence fisheries throughout our study region, there is a need for more comprehensive assessments of Hg sources and bioaccumulation. Public education efforts are also needed to inform rural populations about the potential health effects of fish consumption. Such outreach efforts could use fish trophic guilds as an easily understood predictor of likely Hg contamination.

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References

Allan JD, Flecker AS, Segnini S, Taphorn DC, Sokol E, Kling GW. Limnology of Andean piedmont rivers of Venezuela. J N Am Benthol Soc 2006;25:66–81.

Barbosa AC, Boischio AA, East GA, Ferrari I, Goncalves A, Silva PRM, et al. Mercury contamination in the Brazilian Amazon. Environmental and occupational aspects. Water Air Soil Pollut 1995;80:109–21.

Barbosa AC, Jardin W, Dorea JG, Fosberg B, Souza J. Hair mercury speciation as a function of gender, age, and body mass index in inhabitants of the Negro River basin, Amazon, Brazil. Arch Environ Contam Toxicol 2001;40:439–44.

Barbosa AC, de Souza J, Dorea JG, Jardim WF, Fadini PS. Mercury biomagnification in a tropical black water, Río Negro, Brazil. Arch Environ Contam Toxicol 2003;45:235–46.

Belger L, Forsberg BR. Controlling Hg levels in two predatory fish species in the Negro river basin, Brazilian Amazon. Sci Total Environ 2006;367:451–9.

Campbell LM, Hecky RE, Nyaundi K, Muggide R, Dixon DG. Distribution and food web transfer of mercury in Napoleon and Winam Gulfs, Lake Victoria, East Africa. J Great Lakes Res 2003;29(2):267–82.

Campbell LM, Norstrom RJ, Hobson KA, Muir DCG, Backus S, Fisk AT. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Sci Total Environ 2005;351–352:247–63.

Campbell L, Verburg P, Dixon DG, Hecky RE. Mercury biomagnification in the food web of Lake Tanganyika (Tanzania, East Africa). Sci Total Environ 2008;402:184–91.

Caut S, Angulo E, Courchamp F. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotope values and applications for diet reconstruction. J Appl Ecol 2009;46:443–53.

Dorea JG, Barbosa AC, Silva GS. Fish mercury bioaccumulation as a function of feeding behavior and hydrological cycles of the Río Negro, Amazon. Comp Biochem Physiol 2006;142:275–83.

Duque AB, Taphorn DC, Winemiller KO. Ecology of the coporo, *Prochilodus mariae* (Characiformes, Prochilodontidae), and status of annual migrations in western Venezuela. Environ Biol Fishes 2008;53:33–46.

Fadini PS, Jardim WF. Is the Negro River basin (Amazon) impacted by naturally occurring mercury? Sci Total Environ 2001;275:71–82.

Flecker AS. Fish trophic guilds and the structure of a tropical stream: weak direct vs. strong indirect effects. Ecology 1992;73:927–40.

Flecker AS, Feifarek B. Disturbance and the temporal variability of invertebrate assemblages in two Andean streams. Freshw Biol 1994;31:131–42.

FishBase. Froese R, Pauly D. version 11/2010. World Wide Web electronic publication. www.fishbase.org.

Fry B. Stable isotope ecology. Baton Rouge (LA): Louisiana State University; 2006.

Guimaraes JRD, Meili M, Hylander LD, Silva EC, Roulet M, Mauro JBN, et al. Mercury net methylation in five tropical flood plain regions of Brazil: high in the root zone of floating macrophyte mats but low in surface sediments and flooded soils. Sci Total Environ 2000a;261:99-107.

Guimaraes JRD, Roulet M, Lucotte M, Mergler D. Mercury methylation along a lake-forest transect in the Tapajos river floodplain, Brazilian Amazon: seasonal and vertical variations. Sci Total Environ 2000b;261:91–8.

Hood JM, Vanni MJ, Flecker AS. Nutrient recycling by two phosphorus-rich grazing catfish: the potential for phosphorus-limitation of fish growth. Oecologia 2005;146: 247–57.

Jepsen DB, Winemiller KO. Basin geochemistry isotopic ratios of fishes and basal production sources in four neotropical rivers. Ecol Freshw Fish 2007;16:267–81.

Kidd KA, Hesslein RH, Fudge RJ, Hallard KA. The influence of trophic level as measured by δ^{15}N on mercury concentrations in freshwater organisms. Water Air Soil Pollut 1995;80:1011–5.

Kidd KA, Bootsma HA, Hesslein RH, Lockhart WL, Heckly RE. Mercury concentrations in the food web of Lake Malawi, East Africa. J Great Lakes Res 2003;29(2):258–66.

Kilham SS, Hunte-Brown M, Verburg P, Pringle CM, Whiles MR, Lips KR, et al. Challenges for interpreting stable isotope fractionation of carbon and nitrogen in tropical aquatic ecosystems. Verh Internat Verein Limnol 2009;30:749–53.

Layman CA, Winemiller KO, Arrington DA, Jepsen DB. Body size and trophic position in a diverse tropical food web. Ecology 2005;86:2530–5.

Lebel J, Roulet M, Mergler D, Lucotte M, Larribe F. Fish diet and mercury exposure in a riparian Amazonian population. Water Air Soil Pollut 1997;97:31–47.

Lowe-McConnell RH. Ecological studies in tropical fish communities. Britain: Cambridge; 1987.

Malm O, Branches FJP, Akagi H, Castro MB, Pfeiffer WC, Harada M, et al. Mercury and methylmercury in fish and human hair from the Tapagos River basin, Brazil. Sci Total Environ 1995;175:141–50.

Mason RP, Reinfelder JR, Morel FMM. Bioaccumulation of mercury and methylmercury. Water Air Soil Pollut 1995;80:915–21.

McIntyre PB, Flecker AS. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia 2006;148:12–21.

McIntyre PB, Jones LE, Flecker AS, Vanni MJ. Fish extinctions alter nutrient recycling in tropical freshwaters. PNAS 2007;104:4461–6.

McIntyre PB, Flecker AS, Vanni MJ, Hood JM, Taylor BW, Thomas SA. Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? Ecology 2008;89:2335–46.

Megler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, et al. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio 2007;36:3-11.

Pacyna EG, Pacyna JM, Sundseth K, Munthe J, Kindbom K, Wilson S, et al. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmos Environ 2010;44:2487–99.

Post DM. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 2002;83:703–18.

Power M, Klein GM, Guiguer KRRA, Kwan MKH. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. J Appl Ecol 2002;39:819–30.

Roulet M, Lucotte M, Farella N, Serique G, Coelho H, Sousa Passos CJ, et al. Effects of recent human colonization on the presence of mercury in Amazonian ecosystems. Water Air Soil Pollut 1999;112:297–313.

Roulet M, Lucotte M, Guimaraes IR. Methylmercury in water, seston, and epiphyton of an Amazonian river and its floodplain, Tapajos River, Brazil. Sci Total Environ 2000;261:43–59.

Roulet M, Goch F, Peleja JRP. Spatio-temporal geochemistry of mercury in waters of the Tapajos and Amazon rivers, Brazil. Limnol Oceanogr 2001a;46:1141–57.

Roulet M, Guimaraes JRD, Lucotte M. Methylmercury production and accumulation in sediments and soils of an Amazonian floodplain-effect of seasonal inundation. Water Air Soil Pollut 2001b;128:41–60.

Silva TB, Uieda VS. Preliminary data on the feeding habits of the freshwater stingrays *Potamotrygon falkneri* and *Potamotrygon motoro* (Potamotrygonidae) from the Upper Parana River basin, Brazil. Biota Neotrop 2007;7:221–6.

Sweeting CJ, Barry J, Barnes C, Polunin NVC, Jennings S. Effects of body size and environment on diet-tissue δ^{15}N fractionation in fishes. J Exp Mar Biol Ecol 2007;340:1-10.

U.S. Geological Survey. Analysis of fish for total mercury. Standard operating procedure SOP no. HC520B. SOP (version 1.0). Ann Arbor: Michigan; 1996.

Veiga MM. Mercury in Artisanal gold mining in Latin America: facts, fantasies and solutions. UNIDO 1997:1-23.

Winemiller KO. Spatial and temporal variation in tropical fish trophic network. Ecol Monogr 1990;60:331–67.