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ABSTRACT We report mercury (Hg) mass dependent isotope fractionation (MDF) and non-mass dependent isotope fractionation (NMF) in hair samples of the Bolivian Esse Ejjas native people, and in several tropical fish species that constitute their daily diet. MDF with δ^{202}Hg ranging from –0.40 to –0.92 ‰ for fish and +1.04 to +1.42 ‰ for hair was observed. Hair samples of native people with a fish dominated diet are enriched by +2.0 ± 0.2 ‰ in δ^{202}Hg relative to the fish consumed. Both odd Hg isotopes, ^{199}Hg and ^{201}Hg, display NMF in fish (–0.14 to +0.38 ‰ for Δ^{201}Hg and –0.09 to +0.55 ‰ for Δ^{199}Hg) and in hair (+0.12 to +0.66 ‰ for Δ^{201}Hg and +0.14 to +0.81 ‰ for Δ^{199}Hg). No significant difference in NMF anomalies is observed between Hg in fish and in human hair, suggesting that the anomalies act as conservative source tracers between upper trophic levels of the tropical food chain.

Fish Hg NMF anomalies are ten-fold lower than those published for fish species from mid-latitude lakes. Grouping all Amazonian fish species per location shows that Δ^{199}Hg : Δ^{201}Hg regression slopes for the clear water Itenez river basin (0.95 ± 0.08) are significantly lower than those for the white water Beni river basin (1.28 ± 0.12). Assuming that the observed NMF originates from aquatic photoreactions, this suggests limited photodemethylation of monomethylmercury (MMHg) in the Beni river floodplains and insignificant photodemethylation in the Itenez river floodplains. This is possibly related to lower residence times of MMHg in Itenez compared to Beni river floodplains. Finally, significantly negative Δ^{201}Hg of –0.14 ‰ in Beni river fish suggests that the inorganic Hg precursor to the MMHg that bioaccumulates up the foodchain defines an ecosystem specific non-zero Δ^{201}Hg baseline. Calculation of photodemethylation intensities from Hg or MMHg NMF therefore requires a baseline correction.

INTRODUCTION

Mercury (Hg) is a globally distributed trace metal and its biogeochemical cycle and toxicity are largely controlled by its speciation. Excessive exposure to the neurotoxic organic form of Hg, monomethylmercury (MMHg) may cause problems such as trembling, eyesight problems, coordination disorders and ultimately death [1]. In the Bolivian Amazon MMHg levels ranging from 4300 to 19520 $ng.g^{-1}$ in hair of native people has been linked to elevated Hg in their fish diet [2, 3]. It is formed during inorganic Hg methylation by bacterial activities and abiotic reactions in aquatic systems [4]. In Bolivia, Hg is mainly released into the aquatic system by rock weathering and soil erosion, natural sources, but mercury input is increased by anthropogenic activities such as gold-mining, deforestation and agricultural slash and burn practices [5]. Riverine Hg transport mainly involves suspended particulate matter (SPM) [6] and it is during its transfer in floodplain lakes that Hg can be methylated and bioaccumulated in the foodchain [7, 8]. Comparative studies made in the Bolivian Andes [6] have suggested that Hg content in suspended sediments increased in human impacted areas compared to pristine valleys. However, there is a need to distinguish which of these Hg sources are responsible for high MMHg concentrations in the aquatic foodchain and consequently in human hair.

The natural fractionation of stable isotopes in the environment has already been studied for light elements, such as H, C, N, O and S. Recent analytical advances permit determination of the isotopic composition of heavier elements, such as Hg, using multi-collector ICP-MS [9, 10]. Hg has seven stable isotopes, ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg, which can be fractionated during physical, chemical and biological processes such as methylation, vaporization, oxidation or reduction. Thus, Hg isotopic variations may aid in the identification of sources and transformations of this element in the environment. Two types of Hg stable isotope fractionation have been documented: i. Mass dependent fractionation (MDF), which expresses the relative differences in isotope masses on kinetic and equilibrium processes of chemical reactions and phase transformations, and ii. Non-mass dependent fractionation (NMF) of the odd isotopes, ^{199}Hg and ^{201}Hg, possibly as a result of nuclear field shift [11] or magnetic isotope effects [12]. Observations of MDF in natural samples span a remarkably wide range of 7 ‰ on the $\delta^{202/198}Hg$ scale [13]. Large positive NMF anomalies, *i.e.* excess odd-isotope relative to MDF, have initially been observed in fresh water fish samples [14-16] and more recently in the marine fish certified reference material ERM-CE-464 tuna fish [17], and DORM-2 and DOLT-2 dogfish [15, 16]. Experimental photoreduction of Hg and MMHg [15] in the presence of fulvic acids has been shown to induce NMF and suggested to be possibly responsible for the Hg isotope patterns observed in fish. Bacterial methylation in sediments has been proposed as an alternative explanation for NMF in aquatic organisms, [16] yet recent bacterial methylation experiments show MDF only [18]. Negative NMF anomalies, i.e. odd-isotope deficits, have been documented indirectly in atmospheric Hg deposition, based on lichens [19], moss, peat and soil [20, 21]. Additional negative NMF signatures have been found in coal deposits [21] and sediments [22, 23], suggesting that a significant amount of atmospheric Hg has been incorporated in these geological reservoirs. Recently, liquid Hg evaporation has been shown to induce limited NMF [24]. However, the majority of biotic and abiotic reactions studied, such as microbial reduction and demethylation, abiotic atmospheric oxidation in a volcanic plume, hydrothermal HgS ore deposition, experimental abiotic reduction, derivatization, and Hg(0) volatilization do not induce NMF but only MDF [15, 17, 25-30].

In this study, we document Hg MDF and NMF in hair samples of the Bolivian Esse Ejjas native people, as well as in the tropical fish species that make up their daily diet. The specific objectives are to investigate Hg NMF in fish in relation to different physicochemical and hydrological properties of Amazonian rivers and floodplains. Secondly, we investigate the Hg isotopic variations between fish tissue and human hair as a first step in evaluating Hg isotopes as a metabolic process and/or Hg exposure tracer.

MATERIALS AND METHODS

Itenez basin fish samples

Fish samples were collected from several floodplain lakes adjacent to the San Martin river (13°18' S 63°36' W and 13°18' S 63°33' W) and the Blanco river (13°15' S 63°43' W) in the Itenez river basin (Figure 1), at the Bolivia-Brazil border. Two species of fish were analyzed: 6 *Pygocentrus naterreri,* also known as "pirañas", and 4 *Pellona castelnaeana*. *P. castelneana* is a strict piscivorous species that probably locally migrates between the river and the floodplain lake. *P. naterreri* is a sedentary species and considered as a voracious predator although various studied reported that it also consumed more than 30% of vegetal material [31].

Beni basin fish and hair samples

Beni basin fish samples were caught in the Beni river at Puerto Salinas (14°15' S 67°30' W) and 20 km downstream of the city of Rurrenabaque, in the Granja floodplain lake (14°16' S 67°28' W), regularly connected to the Beni river during periods of rising water and at the flood peak (Figure 1). Three fish species were analyzed: 9 *Pseudoplatystoma fasciatum*, locally named "surubi", was obtained from both locations, while 5 *P. naterreri* and 6 *Salminus brasiliensis*, both sedentary and carnivorous species, were collected from the Granja floodplain lake only. *P. fasciatum* is a strict piscivorous and migratory species, often exhibiting the highest concentrations in Hg_T [6] and one of the main fish species eaten by the local population. Hair of indigenous people was sampled in two different Esse Ejjas communities living along the banks of the Beni River: the community of Villa Copacabana (population A; 14°26' S 67°29' W) in 1998 (7 samples), and a family at Eyiyoquibo (population B; 14°25' S 67°33' W) in 2007 (7 samples) (Figure 1). Population A has an exclusive fish diet and leads a migratory existence along the Beni river limiting their contacts with the developed cities in the area, population B is permanently based in their village, practice limited agriculture, and are closer to the nearest town (Rurrenabaque: 30 min by direct road). Population B diet is consequently more diversified and besides fish includes fruits, rice, manioc, bread and meat.

Analytical methods

Total Hg concentrations (Hg_T) were measured by atomic absorption after combustion and gold-trapping with a Milestone DMA-80. Isotopic analyses were performed at the Laboratoire des Mécanismes et Transferts en Géologie (Toulouse; France) by cold vapor multi-collector inductively coupled plasma mass spectrometer MC-ICP-MS (Thermo-Fisher Neptune). See reference [32] for details. Mass-bias was corrected with the exponential law, using Tl as internal standard by bracketing samples with NIST 3133. Isotopic compositions are expressed in delta notation as follows

$$\delta^{x/198} = \left(\frac{{}^{x}Hg/{}^{198}Hg_{sample}}{({}^{x}Hg/{}^{198}Hg_{NIST\ 3133-1} + {}^{x}Hg/{}^{198}Hg_{NIST\ 3133-2})/2} - 1\right) * 1000$$

where x is the isotope number, and the standard 1 is analyzed before the sample and standard 2 after the sample. The reference material NIST 3133 used as a bracketing standard was at the same Hg_T and acid concentrations as samples. Blank signals were typically below 1 % of those of samples. Non-mass dependent fractionation is reported in "capital delta" notation as the difference between the measured $\delta^{x/198}$ and the theoretically predicted $\delta^{x/198}$ value using the relationship [33]:

$$\Delta^{x/198}Hg = \delta^{x/198}Hg - \beta * \delta^{202/198}Hg$$

where β is the equilibrium mass-dependent fractionation factor. Details on reference materials analyzed and analysis uncertainties by DMA-80 and MC-ICP-MS can be found in the Supplementary Information (SI).

RESULTS AND DISCUSSION

Hg analyses in fish

The Hg_T concentrations of freeze-dried *P. naterreri* and *P. castelnaena* samples from the Itenez river basin range from 467-1140 $ng.g^{-1}$ and 706-1085 $ng.g^{-1}$ dry mass (d.m.), respectively (Table S1-S1). The Hg_T concentrations of the corresponding fresh-frozen samples range from 95-249 $ng.g^{-1}$ and 163-232 $ng.g^{-1}$ wet mass (w.m.) respectively, and are linearly correlated (slope = 3.78, r^2 = 0.99, n = 24) with freeze-dried Hg_T. Mass loss of the fish samples upon freeze-drying was 70 %, which is consistent with the wet and dry mass Hg_T concentration ratios. Variations in $\delta^{202}Hg$ and $\delta^{200}Hg$, with $\delta^{202}Hg$ ranging from –0.70 to –0.40 ‰ are consistent with MDF (Figure S1-S1, Table S1-S1). Significant NMF was observed for both odd isotopes, with $\Delta^{199}Hg$ of +0.04 ‰ to +0.35 ‰ and $\Delta^{201}Hg$ of +0.15 ‰ to +0.35 ‰ (Figure S1-S1).

The Hg_T concentrations of *P. fasciatum*, *P. naterreri* and *S. brasiliensis* fish samples from the Beni river range from 1597 to 10315 $ng.g^{-1}$, from 2376 to 9584 $ng.g^{-1}$ and from 1668 to 6458 $ng.g^{-1}$ dry mass, respectively (Table S1-S1). Even for carnivorous or piscivorous species feeding at the upper trophic levels of the aquatic foodchain, these Hg_T levels are extremely elevated and easily exceed the EPA consumption limit [34] of 100 $ng.g^{-1}.d^{-1}$ wet mass, corresponding to 333 $ng.g^{-1}.d^{-1}$ dry mass for the muscles of consumed predator fish.

P. fasciatum, *P. naterreri* and *S. brasiliensis* of the Granja floodplain lake (Beni river) are characterized by δ^{202}Hg values ranging from –0.92 to –0.61 ‰, from –0.61 to –0.40 ‰ and from –0.63 to –0.45 ‰ respectively (Figure 2). In addition, significant odd isotope NMF is observed with Δ^{199}Hg and Δ^{201}Hg values ranging from +0.36 to +0.55 ‰ and +0.24 to +0.38 ‰, respectively. The δ^{202}Hg values of *P. fasciatum* of the Beni river at Puerto Salinas range from –0.79 to –0.59 ‰, the Δ^{199}Hg values from –0.09 to +0.27 ‰ and the Δ^{201}Hg values from –0.14 to +0.19 ‰. δ^{202}Hg values for the pelagic *P. naterreri* ($P = 0.74$) species are not significantly different between the Itenez and Beni river basins. In the Granja floodplain lake, the δ^{202}Hg values between *P. naterreri* and *P. fasciatum* as well as between *S. brasiliensis* and *P. fasciatum* are significantly different ($P = 0.003$ and $P = 0.01$ respectively) but not between *P. naterreri* and *S. brasiliensis* ($P = 0.54$). This may relate to different ecological factors such as foraging behavior (*P. naterreri* is pelagic while *P. fasciatum* and *S. brasiliensis* is benthopelagic species) and to trophic levels. The Δ^{201}Hg values are not significantly different among the three Granja floodplain lake species. However, the δ^{202}Hg and Δ^{201}Hg for *P. fasciatum* from the Beni river at Puerto Salinas are significantly lower than those from the Granja floodplain lake ($P = 0.03$ and $P = 0.013$ respectively).

Hg NMF in fish

Anomalies of the odd Hg isotopes have been related to both nuclear field shift (NFS) and magnetic isotope effects (MIE) [12, 35]. NFS induced anomalies depend on nuclear volume and shape properties, parameterized by the nuclear charge radius of individual isotopes. NFS isotopic fractionation has been estimated to induce a Δ^{199}Hg : Δ^{201}Hg ratio between 2 and 3 depending on the choice of experimentally determined nuclear charge radii [20, 24, 36]. Experimental Δ^{199}Hg : Δ^{201}Hg linear regression slopes of

1.00 for photoreduction of Hg(II) and of 1.36 for photodemethylation of MMHg, both in the presence of fulvic acids, have been reported and have been suggested to result from MIE effects [15]. Δ^{199}Hg : Δ^{201}Hg ratios may therefore be a powerful means to distinguish NFS from MIE, as well as to identify different MIE inducing reactions. Bergquist and Blum [15] have shown that temperate lake fish samples containing predominantly MMHg have Δ^{199}Hg and Δ^{201}Hg up to +4.97 ‰ with a Δ^{199}Hg : Δ^{201}Hg regression slope of 1.28 ± 0.03 (2 SE standard error). The similarity between MMHg photoreduction and fish MMHg Δ^{199}Hg : Δ^{201}Hg ratios have led them to suggest that photochemical demethylation is the cause of Hg NMF observed in fish. Jackson *et al.* [16] have also observed positive Hg NMF anomalies (Δ^{199}Hg up to +5.2 ‰) in crustaceans and fish from three Canadian lakes (Ontario, Shipiskan and Cli) and have suggested bacterial methylation in sediments to be the main NMF inducing process. Recent bacterial methylation experiments however have only shown MDF and no NMF [18]. In addition, experimental works including biochemical reactions such as bacterial Hg(II) reduction and MMHg demethylation have shown complete absence of NMF [26, 27, 37] and theoretical considerations on biochemical radical chemistry have also indicated the unlikelyness of biochemical NMF [37, 38]. Therefore, we interpret our observed anomalies in Amazonian fish in the context of photochemical Hg and MMHg reduction, the only relevant process that has been shown to induce substantial NMF. Our observations (Figure 3) on tropical freshwater fish show similarities as well as differences with Bergquist and Blum's and Jackson et al's., temperate and boreal lake studies: i) Δ^{201}Hg values of tropical fish are lower (maximum of +0.38 ‰) than those observed in temperate freshwater (+3.89 ‰, [15]) and marine fish (+2.18 ‰, [17]), ii) several tropical fish carry limited, but significantly, negative Δ^{201}Hg values down to -0.14 ‰, and iii) the ensemble of Beni river and floodplain lake fish species exhibits a Δ^{199}Hg : Δ^{201}Hg slope of 1.28 ± 0.12 (2 SD) (Figure 3, $r^2 = 0.99$, n = 20), similar to the mid-latitude lakes study [15].

Ecosystem specific Hg NMF baselines

Itenez basin fish species show a lower Δ^{199}Hg : Δ^{201}Hg slope of 0.95 ± 0.08 (2 SD) (r^2 = 0.99, n = 10) that is similar to the slope of 1.0 accompanying inorganic Hg photoreduction [15]. The negative Δ^{201}Hg values in fish suggest however that newly methylated Hg may have inherited anomalies i.e. the inorganic Hg precursor to MMHg already possessed a small but significant Δ^{201}Hg anomaly ≤ –0.14 ‰. Supporting evidence for such an ecosystem specific negative baseline is provided by overbank sediment Δ^{201}Hg signatures of –0.15 ± 0.10 ‰ (2 SD) from the adjacent Mamore river basin which also drains the Andean cordillera [22]. It has been suggested that Hg NMF in fish can be used to calculate net photochemical demethylation extents. If we assume (as in ref. [15]) that i) the Δ^{201}Hg baseline is 0 ‰, ii) that photodemethylation only takes place in water, and iii) that a Δ^{201}Hg fractionation factor $10^3 \ln \alpha^{\Delta^{201}Hg}_{Hg(II)-Hg(0)}$ of 1.0057 corresponding to an experimental fulvic acid concentration of 10 mg C/L applies (supporting online information of ref. [15]), then the maximum Δ^{201}Hg of +0.38 ‰ in Granja floodplain lake fish indicates MMHg losses via photoreduction of 5 %. On the contrary, if we assume that the Beni river Δ^{201}Hg baseline is at least –0.14 ‰, the calculated MMHg loss increases to 8 %. Despite the importance of a baseline correction, the largest uncertainty in the photodemethylation calculation remains the uncertainty of the NMF fractionation factor. Overall, photodemethylation in tropical floodplain lakes appears to be one order of magnitude less intense than in the cited mid-latitude lakes.

The negative sign of the Beni/Mamore Andean Δ^{201}Hg baseline may suggest that a fraction of inorganic Hg, before being methylated, has already cycled through the atmosphere during a previous photoreductive process. It is well known that soils act as net sinks for atmospheric Hg deposition [39], Recent studies on the Hg isotopic composition of lichens, moss and peat suggest that atmospheric Hg deposition carries large negative Δ^{201}Hg, down to –1.0 ‰ [19-21]. Our suggestion then implies that Andean soils have acquired a small Δ^{201}Hg of –0.14 ‰ by mixing of non-anomalous bedrock Hg with negative anomalous atmospheric deposition. This soil Hg pool has subsequently been mobilized by weathering processes and deposited in the Amazon floodplains, sites of Hg methylation.

Aquatic photoreduction of inorganic Hg and MMHg

The $\Delta^{199}Hg$: $\Delta^{201}Hg$ slope of 0.95 for the clear water Itenez basin suggest that the MMHg carrying these NMF anomalies did not undergo significant photochemical demethylation. In addition, it may be possible that the inorganic Hg source for the Itenez fish MMHg did undergo photochemical reduction to acquire the range of positive $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values on the $\Delta^{199}Hg$: $\Delta^{201}Hg$ slope of 0.95. The variation in Itenez fish $\Delta^{199}Hg$ and $\Delta^{201}Hg$ along the slope 0.95 line then reflects either i) a constant inorganic mercury $\Delta^{199}Hg = \Delta^{201}Hg$ baseline, modified by local variations in inorganic Hg photoreduction, ii) mixing of MMHg produced from inorganic Hg reservoirs with variable $\Delta^{199}Hg = \Delta^{201}Hg$ baselines between 0 and +0.5 ‰, or iii) different photodemethylation processes in tropical ecosystem resulting in different $\Delta^{199}Hg$: $\Delta^{201}Hg$ relationships than have been thus far observed. Variable positive baselines may have been induced over geological times, based on recent evidence of NMF signatures in geological reservoirs. Hydrothermal deposit $\Delta^{199}Hg$ of up to +0.27 ‰ were observed in the Yellowstone hydrothermal field [40]. Of ultimate interest here is an explanation as to why photochemical demethylation result in different $\Delta^{199}Hg$: $\Delta^{201}Hg$ slopes in the Beni and the Itenez basin. In addition to this, within the Beni basin, fish NMF anomalies in the Granja floodplain lake are higher (+0.24 to +0.38 ‰ for $\Delta^{201}Hg$) than in the Beni mainstream (–0.14 to +0.19 ‰ for $\Delta^{201}Hg$) suggesting that MMHg photodemethylation mostly takes place in the floodplain lake. A qualitative explanation for these two observations may relate to the influence of regional geology and associated river water chemistry, and the hydrodynamics of the river-floodplain systems.

The observation of intensified MMHg photodemethylation in the floodplain lake correlates qualitatively with increased DOC levels (Beni river, 1-5 $mg.L^{-1}$; Granja floodplain lake, 1-20 $mg.L^{-1}$), increased water residence times and higher SPM (Granja floodplain lake, 8 – 1050 $mg.L^{-1}$ SPM with an average value of 100 $mg.L^{-1}$; Beni river, 40 - 6300 $mg.L^{-1}$ SPM with an average value of 1400 $mg.L^{-1}$). This is consistant with experimental studies that have showed that both $\Delta^{199}Hg$ and $\Delta^{201}Hg$ NMF anomalies increase with DOC [15], and with the general notion that photoreduction rates are first order in light intensity [15, 41].

The inter-basin differences in floodplain lake MMHg photodemethylation involve differences in water chemistry. The Itenez river, similar to the Brazilian Tapajós river, drains the Pre-Cambrian shield with typically low SPM (1-227 $mg.L^{-1}$). The Beni river, which drains the younger Andean cordillera, is a white water river with higher SPM (40 - 6300 $mg.L^{-1}$) and dissolved solids. No difference was observed in net Hg methylation in periphyton between clear and white water floodplain lakes of the Amazon basin [42]. Moreover, DOC levels are not significantly different between the Granja floodplain lake (4.5 $mg.L^{-1}$) and the studied Itenez floodplain lakes (7.5 $mg.L^{-1}$). The Granja floodplain lake area (0.90 km^2) is approximately one order of magnitude larger than the Itenez floodplains (0.05 to 0.14 km^2), for the same distance between floodplain lake and river, and the associated residence time of aqueous MMHg is probably higher in the Granja floodplain lake than in the Itenez floodplains. Given the difficulty in explaining all river and floodplain observations based on variations in SPM and DOC, we propose here that the aquatic MMHg residence time in a given water body is perhaps one of the key parameters in determining the evolution of Hg and MMHg NMF. MMHg residence times in the smaller Itenez floodplains are then insufficient to produce significant anomalies *via* photochemical demethylation. It is highly likely that the large MMHg NMF anomalies of +4.97 ‰ observed in Lake Michigan are due to the long MMHg residence time of approximately 72 years [43]. Finally, it should be noted that Hg(II) photoreduction by formic acid, a simple carboxylic acid, led to MDF but not NMF [29]. Clearly, our mechanistic understanding of photochemical NMF by MIE is insufficient, and more work is needed to map out the role of Hg complexation sites and chromophoric properties of organic ligands on Hg NMF, and in particular on the possible range $\Delta^{199}Hg$: $\Delta^{201}Hg$ ratios.

Hg MDF and NMF in hair samples

Hg_T concentrations in hair of indigenous people, who have a daily fish diet, are elevated: from 6300 to 23701 $ng.g^{-1}$ (Table S1-S1). The hair $\delta^{202}Hg$ of native people living in Villa Copacabana (population A) averages +1.15 ± 0.16 ‰ (2 SD, n = 6) and odd isotopes of Hg present positive anomalies of +0.19 ± 0.06 ‰ for Δ^{199}Hg and +0.12 ± 0.08 ‰ for Δ^{201}Hg (2 SD, n = 6). The hair δ^{202}Hg of native people living in Eyiyoquibo (population B, all belonging to the same family), ranges from +1.04 to +1.42 ‰ and the odd isotope anomalies range from +0.25 to +0.81 ‰ for Δ^{199}Hg and from +0.15 to +0.66 ‰ for Δ^{201}Hg. Both MDF and NMF signatures of hair of population A are remarkably homogeneous and reflect identical diet, mobility, and/or consanguinity. Whereas population A has an exclusive fish diet, population B diet is more diversified due to their proximity to the city of Rurrenabaque. Δ^{201}Hg changes with age in population B (Figure 4), with the youngest people having the highest anomalies. In population A, this relationship was not observed, but Hg_T concentrations are also much higher than Hg_T concentrations of population B, presumably due to exclusive fish diet. At present it is not possible to offer a conclusive interpretation for this age vs. Δ^{201}Hg trend in the population B. Biochemical Hg NMF, in the form of bacterial methylation, has previously been suggested to contribute to Hg NMF observed in aquatic organisms [16]. Without well-constrained experimental evidence on non-photochemical, i.e. biochemical Hg NMF, we suggest that the observed Δ^{201}Hg variation in population B is more likely due to i) dietary diversification, i.e. children of population B eat different food with different Δ^{201}Hg than adults; in particular recent food aid programs to the Eyiyoquibo community in the form of conserved marine fish (sardines, tuna), which is known to have high Δ^{201}Hg [15-17], may have shifted children's Δ^{201}Hg to higher values than adults; and ii) a potential contamination of children from recent government vaccination programs. Some vaccines are known to be still stabilized with ethylmercury and only delivered to babies and young children [44]. The exact nature of a high Δ^{201}Hg food source requires further investigation.

A student t-test on Δ^{199}Hg and Δ^{201}Hg shows that the average anomalies of all analyzed Esse Ejjas hair are not significantly different from those of their main fish diet represented by *P. fasciatum* (respectively P = 0.525 and P = 0.349). In addition, the slope of Δ^{199}Hg : Δ^{201}Hg for hair is 1.16 ± 0.04 (2 SD) (Figure 3, excluding one outlier) which is not significantly different from the slope defined by

Beni basin fish (slope of 1.28 ± 0.12): P = 0.11 (ANCOVA test). NMF anomalies therefore appear to act as conservative source tracers for dietary MMHg exposure. In contrast, δ^{202}Hg of Esse Ejjas hair are enriched in heavy isotopes by +2.0 ± 0.2 ‰ relative to *P. fasciatum*, suggesting that substantial MDF takes place during MMHg human metabolism. Excretion in faeces of light isotopes of Hg is one possible way, yet other metabolic reactions such as demethylation or blood-hair transfer should not be excluded. The direction of fractionation is similar to that for the lighter elements C, and N, which are typically enriched by several ‰ per trophic level increase. As humans and *P. fasciatum* define approximately one trophic level difference, MMHg metabolism is potentially accompanied by ~ +2 ‰ per trophic level enrichment of heavier Hg isotopes in δ^{202}Hg. The striking similarity in population B δ^{202}Hg (and Δ^{201}Hg) compositions, despite a much larger variation in Beni basin *P. fasciatum* δ^{202}Hg (and Δ^{201}Hg) suggests that the metabolic process responsible for the large MDF does not vary much from one individual to another. If this is valid for MMHg metabolism in humans in general, then a +2.0 ± 0.2 ‰ correction may be applied to human hair δ^{202}Hg to find the average dietary MMHg δ^{202}Hg signature. Such an approach may be of use in future human MMHg exposure studies.

ACKNOWLEDGMENTS

We would like to thank the INSU-CNRS and the WWF (IRD KN10 grant) for funding. LL acknowledges a PhD grant from the Ministère de l'Enseignement et de la Recherche in France. HH thanks the Midi-Pyrenees Observatory for a sabbatical grant. J. Chincheros, G. Crespo and J-L. Duprey are thanked for technical assistance at the LCA laboratory (UMSA, La Paz). C. Boucayrand, M. Carayon and C. Causserand at the LMTG are thanked for help during sample preparation, R. Freydier for assistance on the Neptune, B. Guerrero for help with Fig.1 and D. Point for fruitful discussions. We thank the reviewers for their helpful comments in improving this paper.

Supporting Information available

Detailed study area and detailed experimental including sample treatment prior to analysis, analytical methods and delta values.

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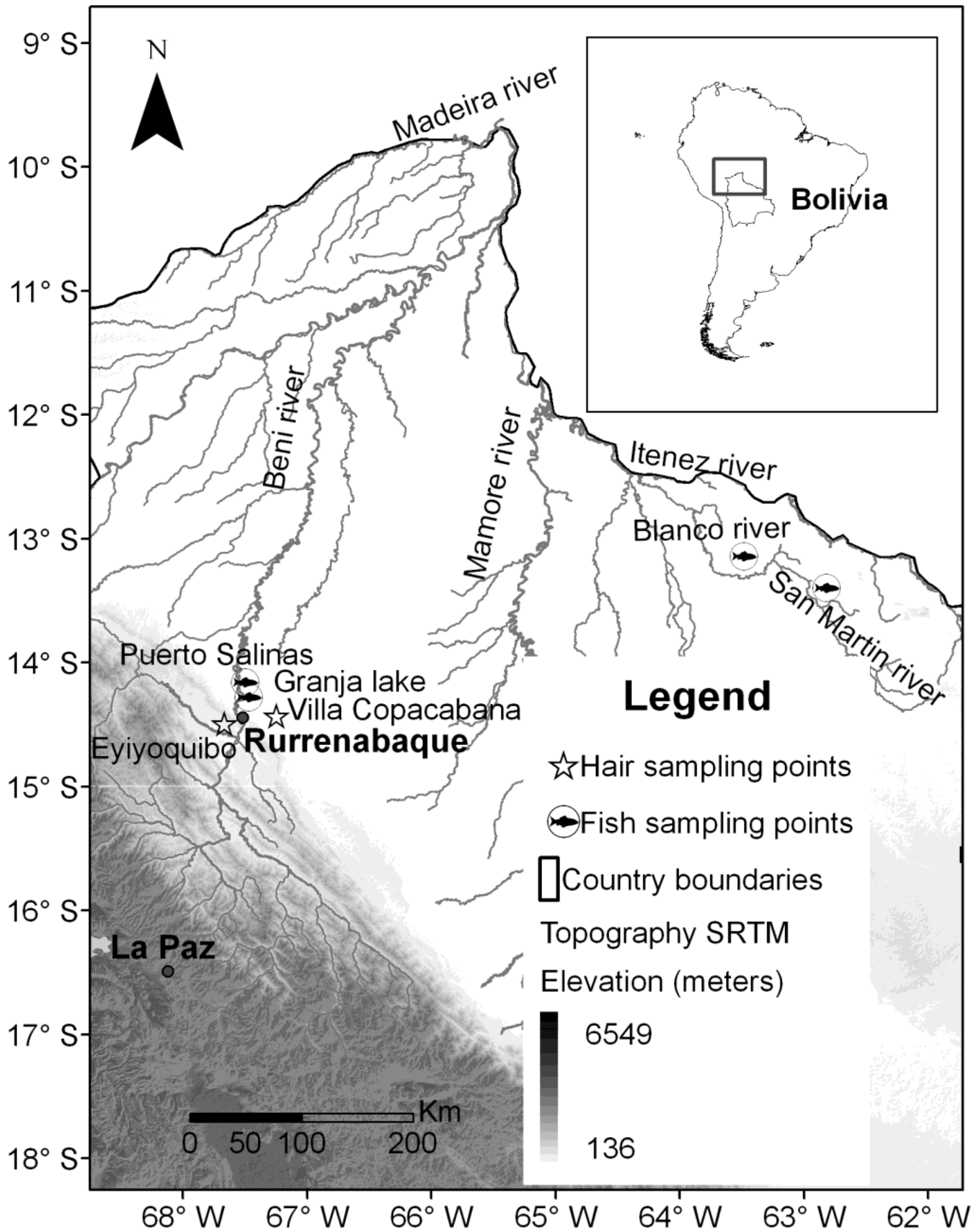
Figures

Figure 1. Study area and location of hair and fish sampling points.

Figure 2. NMF anomaly Δ^{201}Hg plotted as a function of δ^{202}Hg in fish and hair samples collected in the Beni river basin: *Pseudoplastyma fasciatum, Pygocentrus naterreri* and S*alminus brasiliensis* from Puerto Salinas (A) and Granja floodplain lake (B), native people, Esse Ejjas, hair from Villa Copacabana and Eyiyoquibo, and in fish sampled in the Itenez river basin: *Pygocentrus naterreri* (C) and *Pellona castelnaeana* (C). Error bars represent external reproducibility (2 SD). Human hair δ^{202}Hg is enriched in heavy Hg isotopes by +2.0 ± 0.2 ‰ relative to the *P. fasciatum,* the dominant fish species in the diet of the Copacabana population. Conversely, hair Δ^{201}Hg is not significantly different from fish Δ^{201}Hg.

Figure 3. Linear correlations between Δ^{199}Hg and Δ^{201}Hg (‰) for: (A) fish samples from two basins: Beni river, and Beni floodplain (Granja floodplain lake), slope = 1.28 ± 0.12 (2 SD, n = 20, r^2 = 0.99) and floodplains of the Itenez river, slope = 0.95 ± 0.08 (2 SD, n = 10, r^2 = 0.99). Both slopes are significantly different P = 0.002 (ANCOVA test); (B) hair samples of Esse Ejjas communities from Villa Copacabana (population A) and Eyiyoquibo (population B) from Beni river basin, slope = 1.16 ± 0.04 (2 SD, n = 13, r^2 = 0.99). Error bars represent external reproducibility (2 SD).

Figure 4. Δ^{201}Hg as a function of age in Esse Ejjas native people hair. Δ^{201}Hg changes with age in population B: showing highest anomalies for the youngest people. No trends are observed for Esse Ejjas from Villa Copacabana (population A). Higher Δ^{201}Hg in Eyiyoquibo (population B) children most likely reflect different dietary sources compared to adults.



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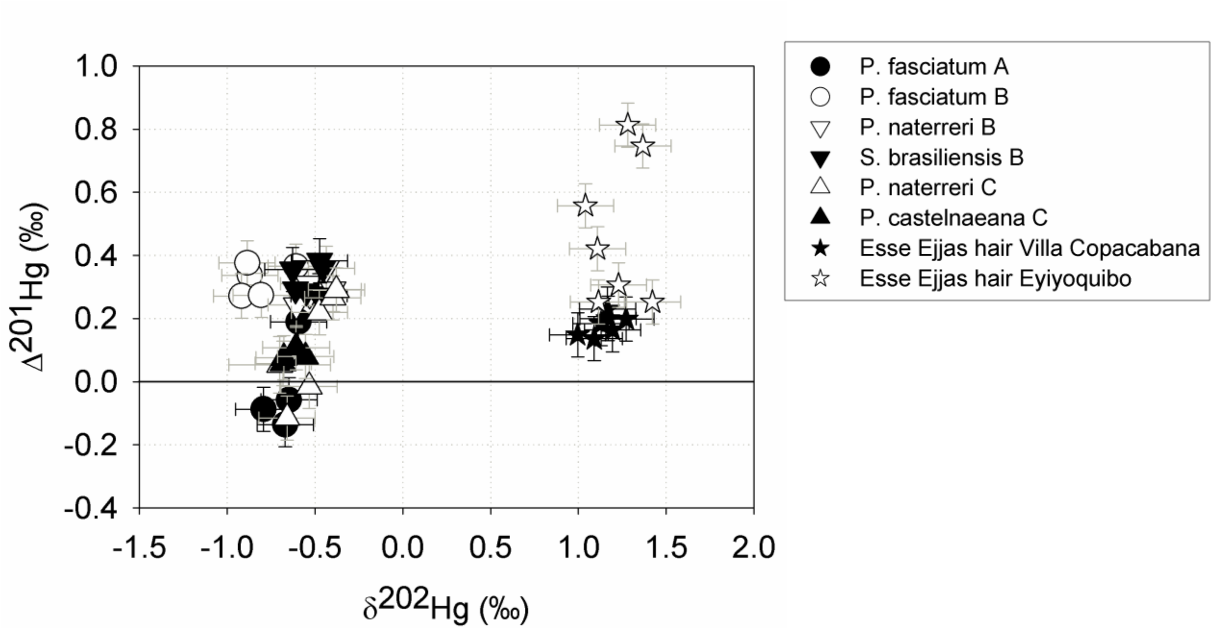

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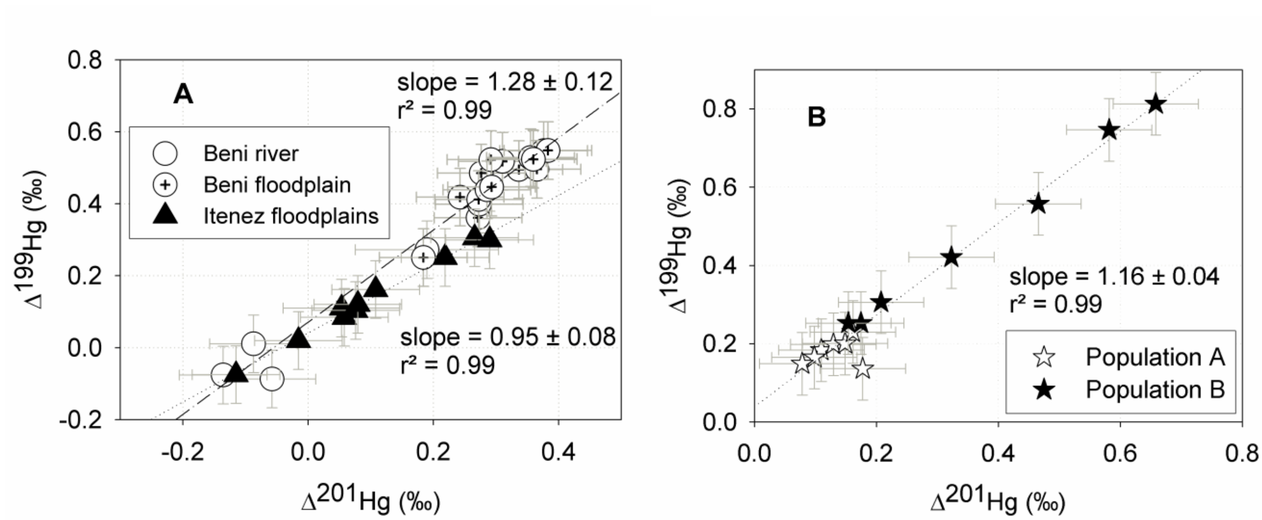

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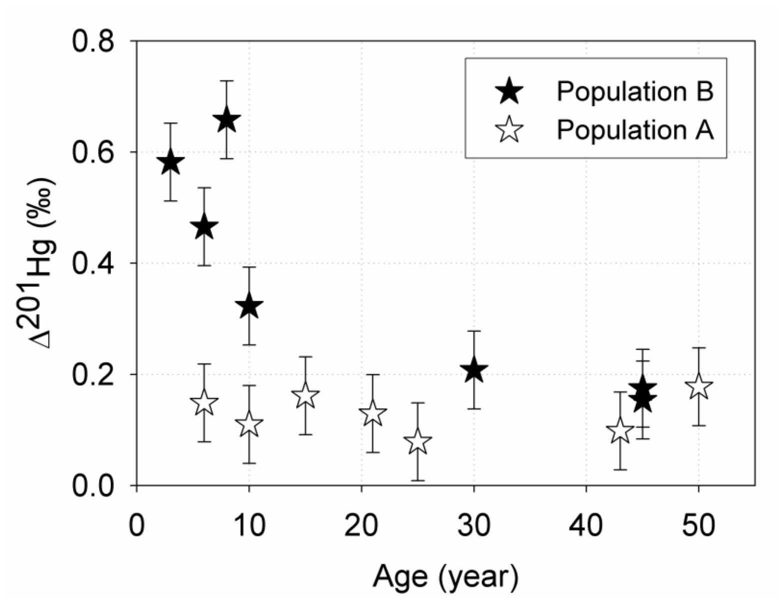

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Table of Contents Brief: Hg non-mass dependent fractionation evidence in hair samples of the Bolivian Esse Ejjas native people and in tropical fish species that constitute their daily diet.