

Anomalous mercury isotopic compositions of fish and human hair in the Bolivian Amazon

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21 **ABSTRACT** We report mercury (Hg) mass dependent isotope fractionation (MDF) and non-mass
 22 dependent isotope fractionation (NMF) in hair samples of the Bolivian Esse Ejjas native people, and in
 23 several tropical fish species that constitute their daily diet. MDF with $\delta^{202}\text{Hg}$ ranging from -0.40 to $-$
 24 0.92 ‰ for fish and $+1.04$ to $+1.42$ ‰ for hair was observed. Hair samples of native people with a fish
 25 dominated diet are enriched by $+2.0 \pm 0.2$ ‰ in $\delta^{202}\text{Hg}$ relative to the fish consumed. Both odd Hg
 26 isotopes, ^{199}Hg and ^{201}Hg , display NMF in fish (-0.14 to $+0.38$ ‰ for $\Delta^{201}\text{Hg}$ and -0.09 to $+0.55$ ‰ for
 27 $\Delta^{199}\text{Hg}$) and in hair ($+0.12$ to $+0.66$ ‰ for $\Delta^{201}\text{Hg}$ and $+0.14$ to $+0.81$ ‰ for $\Delta^{199}\text{Hg}$). No significant
 28 difference in NMF anomalies is observed between Hg in fish and in human hair, suggesting that the
 29 anomalies act as conservative source tracers between upper trophic levels of the tropical food chain.

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

41

42 INTRODUCTION

43 Mercury (Hg) is a globally distributed trace metal and its biogeochemical cycle and toxicity are largely
 44 controlled by its speciation. Excessive exposure to the neurotoxic organic form of Hg,

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

57 The natural fractionation of stable isotopes in the environment has already been studied for light
58 elements, such as H, C, N, O and S. Recent analytical advances permit determination of the isotopic
59 composition of heavier elements, such as Hg, using multi-collector ICP-MS [9, 10]. Hg has seven stable
60 isotopes, ^{196}Hg , ^{198}Hg , ^{199}Hg , ^{200}Hg , ^{201}Hg , ^{202}Hg and ^{204}Hg , which can be fractionated during physical,
61 chemical and biological processes such as methylation, vaporization, oxidation or reduction. Thus, Hg
62 isotopic variations may aid in the identification of sources and transformations of this element in the
63 environment. Two types of Hg stable isotope fractionation have been documented: i. Mass dependent
64 fractionation (MDF), which expresses the relative differences in isotope masses on kinetic and
65 equilibrium processes of chemical reactions and phase transformations, and ii. Non-mass dependent
66 fractionation (NMF) of the odd isotopes, ^{199}Hg and ^{201}Hg , possibly as a result of nuclear field shift [11]
67 or magnetic isotope effects [12]. Observations of MDF in natural samples span a remarkably wide range
68 of 7 ‰ on the $\delta^{202/198}\text{Hg}$ scale [13]. Large positive NMF anomalies, *i.e.* excess odd-isotope relative to
69 MDF, have initially been observed in fresh water fish samples [14-16] and more recently in the marine
70 fish certified reference material ERM-CE-464 tuna fish [17], and DORM-2 and DOLT-2 dogfish [15,

71 16]. Experimental photoreduction of Hg and MMHg [15] in the presence of fulvic acids has been shown
72 to induce NMF and suggested to be possibly responsible for the Hg isotope patterns observed in fish.
73 Bacterial methylation in sediments has been proposed as an alternative explanation for NMF in aquatic
74 organisms, [16] yet recent bacterial methylation experiments show MDF only [18]. Negative NMF
75 anomalies, i.e. odd-isotope deficits, have been documented indirectly in atmospheric Hg deposition,
76 based on lichens [19], moss, peat and soil [20, 21]. Additional negative NMF signatures have been
77 found in coal deposits [21] and sediments [22, 23], suggesting that a significant amount of atmospheric
78 Hg has been incorporated in these geological reservoirs. Recently, liquid Hg evaporation has been
79 shown to induce limited NMF [24]. However, the majority of biotic and abiotic reactions studied, such
80 as microbial reduction and demethylation, abiotic atmospheric oxidation in a volcanic plume,
81 hydrothermal HgS ore deposition, experimental abiotic reduction, derivatization, and Hg(0)
82 volatilization do not induce NMF but only MDF [15, 17, 25-30].

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

89

90 **MATERIALS AND METHODS**

91 **Itenez basin fish samples**

92 Fish samples were collected from several floodplain lakes adjacent to the San Martin river (13°18' S
93 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
94 (Figure 1), at the Bolivia-Brazil border. Two species of fish were analyzed: 6 *Pygocentrus nattereri*,
95 also known as “pirañas”, and 4 *Pellona castelnaeana*. *P. castelneana* is a strict piscivorous species that
96 probably locally migrates between the river and the floodplain lake. *P. nattereri* is a sedentary species

97 and considered as a voracious predator although various studies reported that it also consumed more
98 than 30% of vegetal material [31].

99 **Beni basin fish and hair samples**

100 Beni basin fish samples were caught in the Beni river at Puerto Salinas (14°15' S 67°30' W) and
101 20 km downstream of the city of Rurrenabaque, in the Granja floodplain lake (14°16' S 67°28' W),
102 regularly connected to the Beni river during periods of rising water and at the flood peak (Figure 1).
103 Three fish species were analyzed: 9 *Pseudoplatystoma fasciatum*, locally named “surubi”, was obtained
104 from both locations, while 5 *P. nattereri* and 6 *Salminus brasiliensis*, both sedentary and carnivorous
105 species, were collected from the Granja floodplain lake only. *P. fasciatum* is a strict piscivorous and
106 migratory species, often exhibiting the highest concentrations in Hg_T [6] and one of the main fish
107 species eaten by the local population. Hair of indigenous people was sampled in two different Esse Ejjas
108 communities living along the banks of the Beni River: the community of Villa Copacabana (population
109 A; 14°26' S 67°29' W) in 1998 (7 samples), and a family at Eyiyuquibo (population B; 14°25' S 67°33'
110 W) in 2007 (7 samples) (Figure 1). Population A has an exclusive fish diet and leads a migratory
111 existence along the Beni river limiting their contacts with the developed cities in the area, population B
112 is permanently based in their village, practice limited agriculture, and are closer to the nearest town
113 (Rurrenabaque: 30 min by direct road). Population B diet is consequently more diversified and besides
114 fish includes fruits, rice, manioc, bread and meat.

115 **Analytical methods**

116 Total Hg concentrations (Hg_T) were measured by atomic absorption after combustion and gold-trapping
117 with a Milestone DMA-80. Isotopic analyses were performed at the Laboratoire des Mécanismes et
118 Transferts en Géologie (Toulouse; France) by cold vapor multi-collector inductively coupled plasma
119 mass spectrometer MC-ICP-MS (Thermo-Fisher Neptune). See reference [32] for details. Mass-bias
120 was corrected with the exponential law, using Tl as internal standard by bracketing samples with NIST
121 3133. Isotopic compositions are expressed in delta notation as follows

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

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]:

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

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 $\delta^{202}\text{Hg}$ and $\delta^{200}\text{Hg}$, with $\delta^{202}\text{Hg}$ 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 $\Delta^{199}\text{Hg}$ of +0.04 ‰ to +0.35 ‰ and $\Delta^{201}\text{Hg}$ 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,

146 respectively (Table S1-S1). Even for carnivorous or piscivorous species feeding at the upper trophic
147 levels of the aquatic foodchain, these Hg_T levels are extremely elevated and easily exceed the EPA
148 consumption limit [34] of $100 \text{ ng.g}^{-1}.\text{d}^{-1}$ wet mass, corresponding to $333 \text{ ng.g}^{-1}.\text{d}^{-1}$ dry mass for the
149 muscles of consumed predator fish.

150 *P. fasciatum*, *P. naterreri* and *S. brasiliensis* of the Granja floodplain lake (Beni river) are
151 characterized by $\delta^{202}\text{Hg}$ values ranging from -0.92 to -0.61 ‰, from -0.61 to -0.40 ‰ and from -0.63
152 to -0.45 ‰ respectively (Figure 2). In addition, significant odd isotope NMF is observed with $\Delta^{199}\text{Hg}$
153 and $\Delta^{201}\text{Hg}$ values ranging from $+0.36$ to $+0.55$ ‰ and $+0.24$ to $+0.38$ ‰, respectively. The $\delta^{202}\text{Hg}$
154 values of *P. fasciatum* of the Beni river at Puerto Salinas range from -0.79 to -0.59 ‰, the $\Delta^{199}\text{Hg}$
155 values from -0.09 to $+0.27$ ‰ and the $\Delta^{201}\text{Hg}$ values from -0.14 to $+0.19$ ‰. $\delta^{202}\text{Hg}$ values for the
156 pelagic *P. naterreri* ($P = 0.74$) species are not significantly different between the Itenez and Beni river
157 basins. In the Granja floodplain lake, the $\delta^{202}\text{Hg}$ values between *P. naterreri* and *P. fasciatum* as well as
158 between *S. brasiliensis* and *P. fasciatum* are significantly different ($P = 0.003$ and $P = 0.01$ respectively)
159 but not between *P. naterreri* and *S. brasiliensis* ($P = 0.54$). This may relate to different ecological
160 factors such as foraging behavior (*P. naterreri* is pelagic while *P. fasciatum* and *S. brasiliensis* is
161 benthopelagic species) and to trophic levels. The $\Delta^{201}\text{Hg}$ values are not significantly different among the
162 three Granja floodplain lake species. However, the $\delta^{202}\text{Hg}$ and $\Delta^{201}\text{Hg}$ for *P. fasciatum* from the Beni
163 river at Puerto Salinas are significantly lower than those from the Granja floodplain lake ($P = 0.03$ and P
164 $= 0.013$ respectively).

165 **Hg NMF in fish**

166 Anomalies of the odd Hg isotopes have been related to both nuclear field shift (NFS) and magnetic
167 isotope effects (MIE) [12, 35]. NFS induced anomalies depend on nuclear volume and shape properties,
168 parameterized by the nuclear charge radius of individual isotopes. NFS isotopic fractionation has been
169 estimated to induce a $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ ratio between 2 and 3 depending on the choice of experimentally
170 determined nuclear charge radii [20, 24, 36]. Experimental $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ 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]. $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ 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 $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ up to +4.97 ‰ with a $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ regression slope of 1.28 ± 0.03 (2 SE standard error). The similarity between MMHg photoreduction and fish MMHg $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ 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 ($\Delta^{199}\text{Hg}$ up to +5.2 ‰) in crustaceans and fish from three Canadian lakes (Ontario, Shipikan 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 unlikeliness 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) $\Delta^{201}\text{Hg}$ 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 $\Delta^{201}\text{Hg}$ values down to -0.14 ‰, and iii) the ensemble of Beni river and floodplain lake fish species exhibits a $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ slope of 1.28 ± 0.12 (2 SD) (Figure 3, $r^2 = 0.99$, $n = 20$), similar to the mid-latitude lakes study [15].

194 Ecosystem specific Hg NMF baselines

195 Itenez basin fish species show a lower $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ slope of 0.95 ± 0.08 (2 SD) ($r^2 = 0.99$, $n = 10$)
 196 that is similar to the slope of 1.0 accompanying inorganic Hg photoreduction [15]. The negative $\Delta^{201}\text{Hg}$
 197 values in fish suggest however that newly methylated Hg may have inherited anomalies i.e. the
 198 inorganic Hg precursor to MMHg already possessed a small but significant $\Delta^{201}\text{Hg}$ anomaly ≤ -0.14 ‰.
 199 Supporting evidence for such an ecosystem specific negative baseline is provided by overbank sediment
 200 $\Delta^{201}\text{Hg}$ signatures of -0.15 ± 0.10 ‰ (2 SD) from the adjacent Mamore river basin which also drains the
 201 Andean cordillera [22]. It has been suggested that Hg NMF in fish can be used to calculate net
 202 photochemical demethylation extents. If we assume (as in ref. [15]) that i) the $\Delta^{201}\text{Hg}$ baseline is 0 ‰,
 203 ii) that photodemethylation only takes place in water, and iii) that a $\Delta^{201}\text{Hg}$ fractionation factor
 204 $10^3 \ln \alpha_{\text{Hg(II)}-\text{Hg(0)}}^{\Delta^{201}\text{Hg}}$ of 1.0057 corresponding to an experimental fulvic acid concentration of 10 mg C/L
 205 applies (supporting online information of ref. [15]), then the maximum $\Delta^{201}\text{Hg}$ of +0.38 ‰ in Granja
 206 floodplain lake fish indicates MMHg losses via photoreduction of 5 ‰. On the contrary, if we assume
 207 that the Beni river $\Delta^{201}\text{Hg}$ baseline is at least -0.14 ‰, the calculated MMHg loss increases to 8 ‰.
 208 Despite the importance of a baseline correction, the largest uncertainty in the photodemethylation
 209 calculation remains the uncertainty of the NMF fractionation factor. Overall, photodemethylation in
 210 tropical floodplain lakes appears to be one order of magnitude less intense than in the cited mid-latitude
 211 lakes.

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

221 **Aquatic photoreduction of inorganic Hg and MMHg**

222 The $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ slope of 0.95 for the clear water Itenez basin suggest that the MMHg carrying
 223 these NMF anomalies did not undergo significant photochemical demethylation. In addition, it may be
 224 possible that the inorganic Hg source for the Itenez fish MMHg did undergo photochemical reduction to
 225 acquire the range of positive $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ values on the $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ slope of 0.95. The
 226 variation in Itenez fish $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ along the slope 0.95 line then reflects either i) a constant
 227 inorganic mercury $\Delta^{199}\text{Hg} = \Delta^{201}\text{Hg}$ baseline, modified by local variations in inorganic Hg
 228 photoreduction, ii) mixing of MMHg produced from inorganic Hg reservoirs with variable $\Delta^{199}\text{Hg} =$
 229 $\Delta^{201}\text{Hg}$ baselines between 0 and +0.5 ‰, or iii) different photodemethylation processes in tropical
 230 ecosystem resulting in different $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ relationships than have been thus far observed.
 231 Variable positive baselines may have been induced over geological times, based on recent evidence of
 232 NMF signatures in geological reservoirs. Hydrothermal deposit $\Delta^{199}\text{Hg}$ of up to +0.27 ‰ were observed
 233 in the Yellowstone hydrothermal field [40]. Of ultimate interest here is an explanation as to why
 234 photochemical demethylation result in different $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ slopes in the Beni and the Itenez basin.
 235 In addition to this, within the Beni basin, fish NMF anomalies in the Granja floodplain lake are higher
 236 (+0.24 to +0.38 ‰ for $\Delta^{201}\text{Hg}$) than in the Beni mainstream (-0.14 to +0.19 ‰ for $\Delta^{201}\text{Hg}$) suggesting
 237 that MMHg photodemethylation mostly takes place in the floodplain lake. A qualitative explanation for
 238 these two observations may relate to the influence of regional geology and associated river water
 239 chemistry, and the hydrodynamics of the river-floodplain systems.

240 The observation of intensified MMHg photodemethylation in the floodplain lake correlates
 241 qualitatively with increased DOC levels (Beni river, 1-5 mg.L⁻¹; Granja floodplain lake, 1-20 mg.L⁻¹),
 242 increased water residence times and higher SPM (Granja floodplain lake, 8 – 1050 mg.L⁻¹ SPM with an
 243 average value of 100 mg.L⁻¹; Beni river, 40 - 6300 mg.L⁻¹ SPM with an average value of 1400 mg.L⁻¹).
 244 This is consistent with experimental studies that have showed that both $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ NMF

245 anomalies increase with DOC [15], and with the general notion that photoreduction rates are first order
246 in light intensity [15, 41].

247 The inter-basin differences in floodplain lake MMHg photodemethylation involve differences in water
248 chemistry. The Itenez river, similar to the Brazilian Tapajós river, drains the Pre-Cambrian shield with
249 typically low SPM (1-227 mg.L⁻¹). The Beni river, which drains the younger Andean cordillera, is a
250 white water river with higher SPM (40 - 6300 mg.L⁻¹) and dissolved solids. No difference was observed
251 in net Hg methylation in periphyton between clear and white water floodplain lakes of the Amazon
252 basin [42]. Moreover, DOC levels are not significantly different between the Granja floodplain lake (4.5
253 mg.L⁻¹) and the studied Itenez floodplain lakes (7.5 mg.L⁻¹). The Granja floodplain lake area (0.90 km²)
254 is approximately one order of magnitude larger than the Itenez floodplains (0.05 to 0.14 km²), for the
255 same distance between floodplain lake and river, and the associated residence time of aqueous MMHg is
256 probably higher in the Granja floodplain lake than in the Itenez floodplains. Given the difficulty in
257 explaining all river and floodplain observations based on variations in SPM and DOC, we propose here
258 that the aquatic MMHg residence time in a given water body is perhaps one of the key parameters in
259 determining the evolution of Hg and MMHg NMF. MMHg residence times in the smaller Itenez
260 floodplains are then insufficient to produce significant anomalies *via* photochemical demethylation. It is
261 highly likely that the large MMHg NMF anomalies of +4.97 ‰ observed in Lake Michigan are due to
262 the long MMHg residence time of approximately 72 years [43]. Finally, it should be noted that Hg(II)
263 photoreduction by formic acid, a simple carboxylic acid, led to MDF but not NMF [29]. Clearly, our
264 mechanistic understanding of photochemical NMF by MIE is insufficient, and more work is needed to
265 map out the role of Hg complexation sites and chromophoric properties of organic ligands on Hg NMF,
266 and in particular on the possible range $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ ratios.

267

268 **Hg MDF and NMF in hair samples**

269 Hg_T concentrations in hair of indigenous people, who have a daily fish diet, are elevated: from 6300 to
270 23701 ng.g⁻¹ (Table S1-S1). The hair $\delta^{202}\text{Hg}$ of native people living in Villa Copacabana (population A)

271 averages $+1.15 \pm 0.16$ ‰ (2 SD, $n = 6$) and odd isotopes of Hg present positive anomalies of $+0.19 \pm$
272 0.06 ‰ for $\Delta^{199}\text{Hg}$ and $+0.12 \pm 0.08$ ‰ for $\Delta^{201}\text{Hg}$ (2 SD, $n = 6$). The hair $\delta^{202}\text{Hg}$ of native people
273 living in Eyiyoquibo (population B, all belonging to the same family), ranges from $+1.04$ to $+1.42$ ‰
274 and the odd isotope anomalies range from $+0.25$ to $+0.81$ ‰ for $\Delta^{199}\text{Hg}$ and from $+0.15$ to $+0.66$ ‰ for
275 $\Delta^{201}\text{Hg}$. Both MDF and NMF signatures of hair of population A are remarkably homogeneous and
276 reflect identical diet, mobility, and/or consanguinity. Whereas population A has an exclusive fish diet,
277 population B diet is more diversified due to their proximity to the city of Rurrenabaque. $\Delta^{201}\text{Hg}$ changes
278 with age in population B (Figure 4), with the youngest people having the highest anomalies. In
279 population A, this relationship was not observed, but Hg_T concentrations are also much higher than Hg_T
280 concentrations of population B, presumably due to exclusive fish diet. At present it is not possible to
281 offer a conclusive interpretation for this age vs. $\Delta^{201}\text{Hg}$ trend in the population B. Biochemical Hg
282 NMF, in the form of bacterial methylation, has previously been suggested to contribute to Hg NMF
283 observed in aquatic organisms [16]. Without well-constrained experimental evidence on non-
284 photochemical, i.e. biochemical Hg NMF, we suggest that the observed $\Delta^{201}\text{Hg}$ variation in population
285 B is more likely due to i) dietary diversification, i.e. children of population B eat different food with
286 different $\Delta^{201}\text{Hg}$ than adults; in particular recent food aid programs to the Eyiyoquibo community in the
287 form of conserved marine fish (sardines, tuna), which is known to have high $\Delta^{201}\text{Hg}$ [15-17], may have
288 shifted children's $\Delta^{201}\text{Hg}$ to higher values than adults; and ii) a potential contamination of children from
289 recent government vaccination programs. Some vaccines are known to be still stabilized with
290 ethylmercury and only delivered to babies and young children [44]. The exact nature of a high $\Delta^{201}\text{Hg}$
291 food source requires further investigation.

292 A student t-test on $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ shows that the average anomalies of all analyzed Esse Ejjas
293 hair are not significantly different from those of their main fish diet represented by *P. fasciatum*
294 (respectively $P = 0.525$ and $P = 0.349$). In addition, the slope of $\Delta^{199}\text{Hg} : \Delta^{201}\text{Hg}$ for hair is 1.16 ± 0.04
295 (2 SD) (Figure 3, excluding one outlier) which is not significantly different from the slope defined by

296 Beni basin fish (slope of 1.28 ± 0.12): $P = 0.11$ (ANCOVA test). NMF anomalies therefore appear to act
297 as conservative source tracers for dietary MMHg exposure. In contrast, $\delta^{202}\text{Hg}$ of Esse Ejjas hair are
298 enriched in heavy isotopes by $+2.0 \pm 0.2$ ‰ relative to *P. fasciatum*, suggesting that substantial MDF
299 takes place during MMHg human metabolism. Excretion in faeces of light isotopes of Hg is one
300 possible way, yet other metabolic reactions such as demethylation or blood-hair transfer should not be
301 excluded. The direction of fractionation is similar to that for the lighter elements C, and N, which are
302 typically enriched by several ‰ per trophic level increase. As humans and *P. fasciatum* define
303 approximately one trophic level difference, MMHg metabolism is potentially accompanied by $\sim +2$ ‰
304 per trophic level enrichment of heavier Hg isotopes in $\delta^{202}\text{Hg}$. The striking similarity in population B
305 $\delta^{202}\text{Hg}$ (and $\Delta^{201}\text{Hg}$) compositions, despite a much larger variation in Beni basin *P. fasciatum* $\delta^{202}\text{Hg}$
306 (and $\Delta^{201}\text{Hg}$) suggests that the metabolic process responsible for the large MDF does not vary much
307 from one individual to another. If this is valid for MMHg metabolism in humans in general, then a $+2.0$
308 ± 0.2 ‰ correction may be applied to human hair $\delta^{202}\text{Hg}$ to find the average dietary MMHg $\delta^{202}\text{Hg}$
309 signature. Such an approach may be of use in future human MMHg exposure studies.

310

311 ACKNOWLEDGMENTS

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

319

Supporting Information available

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

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458 **Figures**

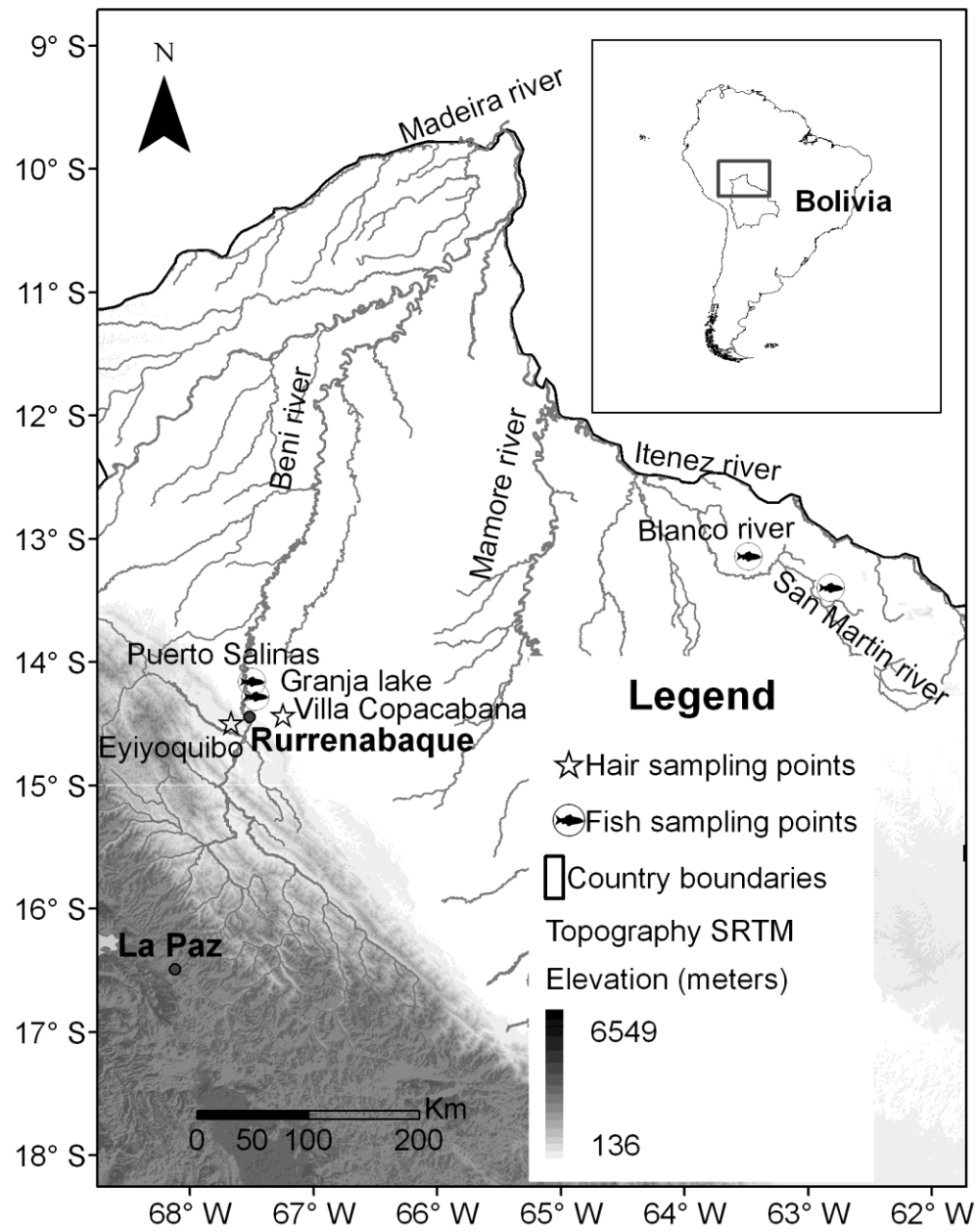
459 **Figure 1.** Study area and location of hair and fish sampling points.

460 **Figure 2.** NMF anomaly $\Delta^{201}\text{Hg}$ plotted as a function of $\delta^{202}\text{Hg}$ in fish and hair samples collected in the
461 Beni river basin: *Pseudoplatystoma fasciatum*, *Pygocentrus nattereri* and *Salminus brasiliensis* from
462 Puerto Salinas (A) and Granja floodplain lake (B), native people, Esse Ejjas, hair from Villa
463 Copacabana and Eyiyoquibo, and in fish sampled in the Itenez river basin: *Pygocentrus nattereri* (C)
464 and *Pellona castelnaeana* (C). Error bars represent external reproducibility (2 SD). Human hair $\delta^{202}\text{Hg}$
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466 in the diet of the Copacabana population. Conversely, hair $\Delta^{201}\text{Hg}$ is not significantly different from fish
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468 **Figure 3.** Linear correlations between $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ (‰) for: (A) fish samples from two basins:
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470 and floodplains of the Itenez river, slope = 0.95 ± 0.08 (2 SD, $n = 10$, $r^2 = 0.99$). Both slopes are
471 significantly different $P = 0.002$ (ANCOVA test); (B) hair samples of Esse Ejjas communities from
472 Villa Copacabana (population A) and Eyiyoquibo (population B) from Beni river basin, slope = $1.16 \pm$
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474 **Figure 4.** $\Delta^{201}\text{Hg}$ as a function of age in Esse Ejjas native people hair. $\Delta^{201}\text{Hg}$ changes with age in
475 population B: showing highest anomalies for the youngest people. No trends are observed for Esse Ejjas
476 from Villa Copacabana (population A). Higher $\Delta^{201}\text{Hg}$ in Eyiyoquibo (population B) children most
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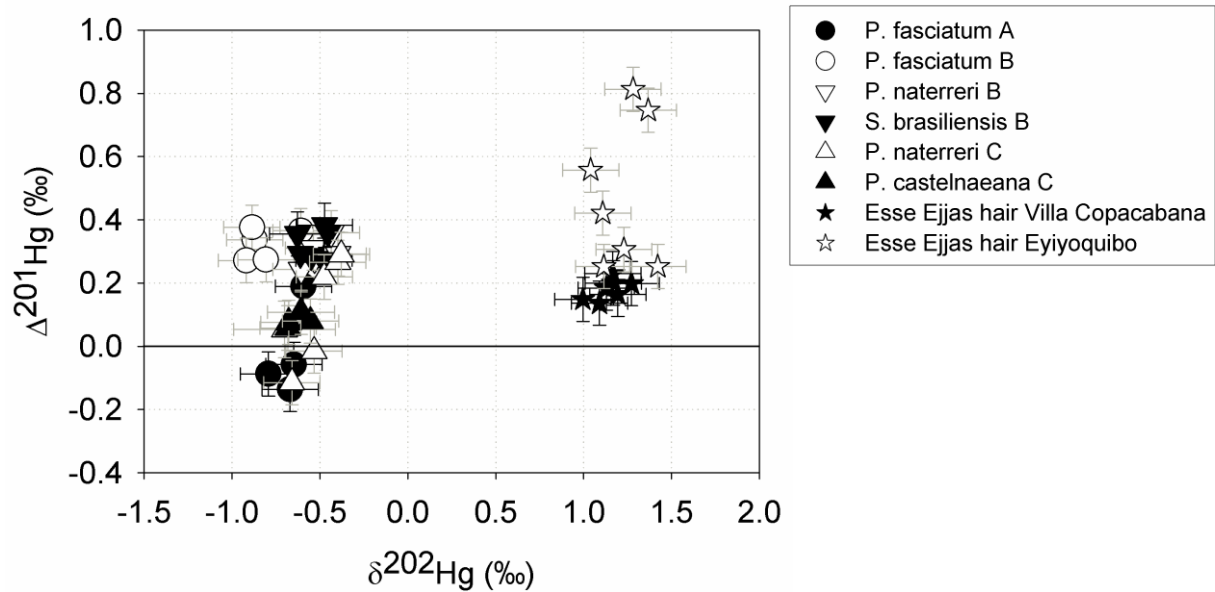
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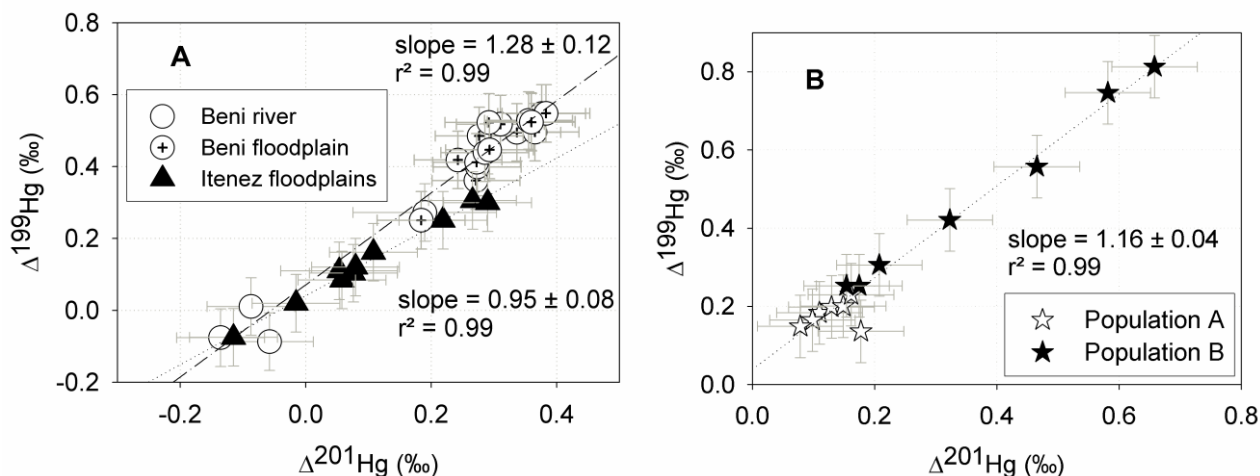
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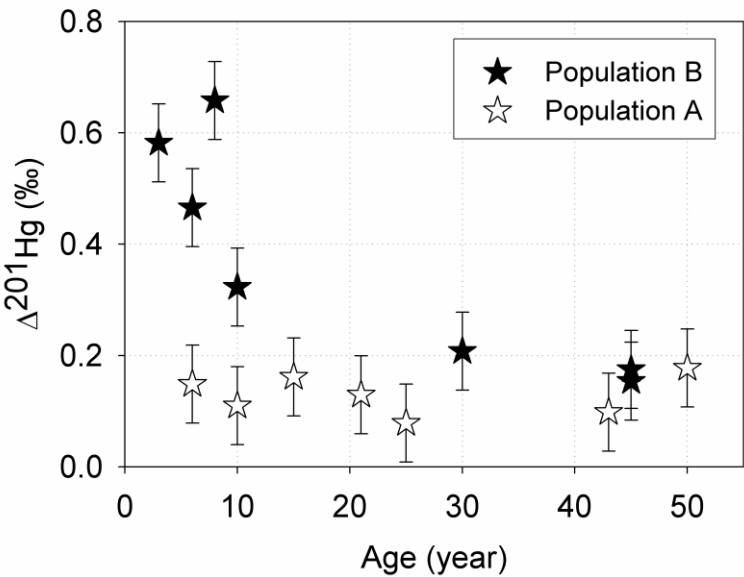


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537 **Table of Contents Brief:** Hg non-mass dependent fractionation evidence in hair samples of the
538 Bolivian Esse Ejjas native people and in tropical fish species that constitute their daily diet.
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