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1 **In situ Hg species photochemical transformations and associated isotopic**
2 **fractionation in the water column of high-altitude lakes from the Bolivian Altiplano**

3

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21

22

23 **ABSTRACT**

24 Photochemical reactions are major pathways for the removal of Hg species from aquatic ecosystems, lowering the
25 concentration of monomethylmercury (MMHg) and its bioaccumulation in foodwebs. Here we investigate the rates
26 and environmental drivers of MMHg photodegradation and inorganic Hg (IHg) photoreduction in waters of two high
27 altitude lakes from the Bolivian Altiplano representing meso- to eutrophic conditions. We incubated three contrasting
28 waters *in-situ* at two depths after adding Hg enriched isotopic species to derive rate constants. We found that
29 transformations mostly occurred in subsurface waters exposed to UV radiations and were mainly modulated by the
30 dissolved organic matter (DOM) level. In parallel, we incubated the same waters after the addition of low
31 concentrations of natural MMHg and followed the stable isotope composition of the remaining Hg species by
32 compound-specific isotope analysis allowing the determination of enrichment factors and MIF signatures during *in-*
33 *situ* MMHg photodegradation in natural waters. We obtained similar enrichment factors for the three waters
34 (average $\epsilon_{\delta^{202}} = -7.1 \pm 0.9 \text{ ‰}$ and $\epsilon_{\Delta^{199}} = -4.8 \pm 0.3 \text{ ‰}$, 1 SE) but the expression of the mass independent
35 fractionation ($\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ ratio) diverged depending on the DOM level. The $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ ratios ranged from
36 1.24 ± 0.03 to 1.34 ± 0.02 for the low and high DOM waters, respectively and matched very well the signatures of
37 MMHg recorded in fish (*Orestia* spp) collected in the same lake. The average extent of MMHg photodegradation
38 before its incorporation into the foodwebs from each lake ranged from 10 % in the eutrophic waters to 26 % in the
39 mesotrophic ones, which explain the higher Hg concentrations in biota observed in the eutrophic lake. Our results
40 call for more photodegradation experiments to be conducted under natural conditions in order to accurately use Hg
41 isotopes signatures recorded in biota to determine the extent of Hg species transformations and lifetime in aquatic
42 ecosystems.

43 Keywords: photodegradation; photoreduction; compound specific isotope analysis; enrichment factor; dissolved
44 organic matter; fish

45 Synopsis: MMHg photodegradation in lake waters was found to be modulated by DOM whereas the associated Hg
46 isotopes fractionation matched the Hg signatures recorded in fish.

47

48 INTRODUCTION

49 Hg concentrations have increased in all environmental compartments since the industrial revolution due to massive
50 release from anthropogenic activities.¹ In aquatic ecosystems, a fraction of the inorganic Hg (IHg) is transformed to
51 monomethylmercury (MMHg), which is the form that biomagnifies in food webs leading to human exposure.^{2,3} IHg
52 methylation is mostly carried out by bacterial strains possessing a specific genes cluster⁴ that is found in nearly all
53 anaerobic environments.⁵ Depending on the ecosystem, the major contributors to the MMHg pool include i) water
54 saturated systems (e.g., flooded soils and sediments)⁶, ii) the suboxic part of water column,^{7,8} and iii) anoxic
55 microniches found in oxic waters such as particulate flocs and biofilms.^{9,10} However, the Hg pools available to
56 aquatic organisms for subsequent methylation or trophic transfer result from the balance between multiple
57 reactions. Among these, IHg reduction and MMHg demethylation are considered important pathways reducing the
58 pool of IHg available for its methylation into MMHg and its further biomagnification.

59 In the aphotic zone, reduction and demethylation can be performed by various microorganisms carrying dedicated
60 genes or not.¹¹⁻¹⁴ The abiotic reduction of IHg at the surface of Fe(II)-bearing minerals is also well established as
61 a significant pathway^{15,16} while its reduction by OM¹⁷ is less certain, depending on the OM redox state, concentration
62 and composition.^{18,19} In the euphotic zone, photochemically induced reactions are considered a major sink of IHg
63 and MMHg in natural waters.^{20,21} These reactions are likely proceeding through multiple pathways depending on
64 the environmental conditions²² and several controlling parameters have been identified, among which light and
65 dissolved organic matter (DOM) are especially important. Regarding light, both the radiation intensity^{20,21,23} and
66 wavelength are influential, with the shortest wavelengths (i.e. UVB: 280 - 320 nm) being the most effective at
67 triggering the reactions.^{21,24,25} UV radiations therefore contribute the most to MMHg degradation and IHg reduction
68 near the surface but they are rapidly attenuated with depth and the photosynthetic active radiation (PAR) could
69 therefore be responsible for the largest share of the transformations over the whole water column.²²

70 On the other hand, DOM concentration and composition influence both reactions, e.g. by controlling the light
71 transmission, production/consumption of reactive oxygen species (ROS) and Hg complexation.²⁶⁻²⁹ Photochemical
72 reactions typically proceed more efficiently in the presence of DOM but reaction rates usually decrease at high
73 DOM concentrations.^{30,31} The breaking point depends on the DOM properties driving light attenuation and
74 quenching of radical species.^{27,32} While the direct photoreduction of IHg bound to DOM is possible under natural
75 light conditions,³³ the direct photolysis of MMHg is not,³¹ and MMHg complexation by thiol groups is a key

76 determinant of its photodegradation as it weakens the Hg-C bond.^{31,34} Laboratory studies further showed that the
77 reaction proceeds faster when MMHg is bound to the thiol group of a molecule also containing an aromatic
78 moiety.^{35,36} It suggests an intramolecular mechanism resulting from the direct energy transfer between the excited
79 triplet state of DOM (³DOM*) and the Hg-C bond, while indirect mechanisms involving several ROS were previously
80 suggested from field experiments.²²

81 Before entering the food-chain as MMHg, Hg species undergo a series of biogeochemical pathways affecting their
82 stable isotope composition, with both mass-dependent (MDF) and mass-independent fractionations (MIF) being
83 observed.³⁷ For instance, positive or negative MDF is observed for all kind of biotic and abiotic pathways, whereas
84 MIF of odd Hg isotopes is specifically observed for photochemical processes occurring in surface waters due to the
85 magnetic isotope effect (MIE).³⁸ Since its first demonstration in 2007,³⁹ several studies have examined Hg-MIF
86 during MMHg photodegradation and IHg photoreduction in synthetic waters of various composition but never in
87 natural waters due to analytical constraints. The MIF direction and signature ($\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$) have been shown to
88 vary depending on both the reaction involved, the light wavelength and the Hg bonding environment.⁴⁰⁻⁴⁴ MIF
89 enrichment factors determined in synthetic waters have been used to derive useful conclusions on Hg cycling in
90 aquatic ecosystems such as the extent of MMHg photodegradation before its uptake by biota. A paradox however
91 remains in that the MIF signatures observed in these laboratory experiments do not accurately match the one
92 recorded in biota.⁴³

93 In previous studies, we have examined several aspects of Hg cycling in lakes of the Bolivian Altiplano (3600-3800
94 m a.s.l.) which are highly reactive ecosystems where methylation can be stimulated under eutrophicated
95 conditions.⁴⁵ We provided a Hg contamination level and speciation inventory⁴⁶ and especially studied the Hg
96 accumulation and transformations in sediments, periphyton and benthic biofilms.⁴⁷⁻⁵⁰ We pointed out to sediments
97 and biofilms as compartments of MMHg production and bioaccumulation, but pathways leading to MMHg and Hg
98 removal were not addressed so far. Here, we specifically investigated Hg species transformations in the water
99 column of the same lakes, with a focus on demethylation and reduction as well as related isotope fractionation, to
100 get more insights into the fate of Hg before its incorporation in foodwebs. We carried out *in situ* incubation
101 experiments at two depths (with and without UV) using lake water from several sites showing contrasted DOC
102 levels and the addition of isotopically enriched tracers to determine transformations rate constants. In parallel
103 experiments, addition of low concentrations of natural MMHg allowed to determine the enrichment factors and MIF

104 signature during the photodegradation of MMHg in these waters. This information was then compared to the MMHg
105 isotopic composition recorded in fish from each lake to estimate the extent of MMHg photodegradation in their water
106 column and discuss the control exerted by DOM.

107 2. EXPERIMENTAL SECTION

108 2.1. Study sites.

109 Lake Titicaca (3809 m a.s.l.) comprises two connected basins, the Great Lake (7,131 km²; mean depth = 100 m)
110 and the Small Lake (1,428 km²; mean depth = 9 m). Each basin is fed by multiple streams that bring in various
111 pollutants according to their watershed characteristics (urban, industrial, mining or agricultural activities).⁴⁶ Lake
112 Uru Uru (3686 m a.s.l., 120 to 350 km² and 0.25 to 1 m depth) is a human-made reservoir located in the central
113 part of the Bolivian Altiplano, where numerous mining and smelting activities are concentrated. Sampling stations
114 were selected to represent various biogeochemical conditions of the hydrosystem in term of temperature, pH,
115 conductivity, dissolved oxygen, redox and DOC concentrations (Figure SI-1 and Table SI-1): CH is an oligotrophic
116 deep station (38 m) whereas HU presents an intermediate depths (5 m) and mesotrophic conditions. BC and UU
117 are both shallow (0.25 – 2 m) eutrophicated stations receiving mixed urban and mining effluents. A preliminary
118 campaign was conducted in November 2013 and two main campaigns were carried out in 2014, the first by the end
119 of the rainy season (April-May) and the second by the end of the dry one (October-November). Meteorological data
120 were recorded by a weather station (Campbell Scientific, Logan, UT, USA; equipped with a CR1000 datalogger)
121 installed on the shore of the Small Lake near the incubation sites whereas the spectral attenuation of light (UV-
122 PAR) through the water column was monitored with an underwater profiling C-OPS spectroradiometer (Biospherical
123 instrument Inc., San Diego, CA, USA).⁵¹

124

125 2.2. Sampling and incubations.

126 Lake water samples were collected at each station a few hours before incubations using a trace-metal clean 5-L
127 Teflon-lined General Oceanic (GO-FLO) bottle. Water was distributed in 125- or 250-mL Teflon® (Nalgene PFA)
128 bottles for incubations with enriched isotopic tracers or natural MMHg (Strem chemicals), respectively (Fig SI-2).
129 On one hand, Hg species transformations rate constants were determined using isotopically enriched tracers added
130 within ambient concentration ranges (0.1 to 0.2 ng.L⁻¹ for ²⁰¹MMHg and 0.8 to 1.6 ng.L⁻¹ for ¹⁹⁹IHg). Three controls
131 (*t*₀) per series were immediately acidified and stored in the dark at +4 °C, with half of the other bottles being mounted
132 on a homemade system (Figure SI-2) and incubated *in situ*. Incubations of samples from CH, HU and BC were all
133 conducted at the HU station with samples from HU and CH incubated at two different depths (0.5 and 3.5 m) to
134 discriminate the importance of specific light wavelengths. The remaining bottles were incubated in the dark in lake

135 surface water. Every incubation series were carried out in triplicates for the same period (4 to 8 hours) and
136 eventually stopped by acidification before being stored at +4 °C in the dark. On the other hand, Hg stable isotope
137 fractionation patterns during MMHg degradation were investigated by spiking lake water with natural MMHg (200
138 ng.L⁻¹) and a similar strategy as above but with longer incubations and a sacrificial sampling strategy where 3
139 bottles were sampled at 6, 12, 24 and 36 hours. One dark control incubation was also carried out for each site but
140 sampled only at 36 h, corresponding to the final time point of the diurnal incubation.

141 Fish samples analyzed in this study consist of muscles from several individuals of *Orestias* Spp ($n_{\text{total}} = 43$), an
142 endemic fish species of the Bolivian Altiplano region.⁵² Most of them were collected by local fishermen using gillnets
143 during dedicated scientific surveys performed in Lake Titicaca (n=15) and lake Uru Uru (n=28) in October 2010 (9
144 additional samples from 2005 and 2014 were added to the database) and were preserved at – 20°C before being
145 freeze-dried and homogenized. MMHg compound specific stable isotope analysis (CSIA) was carried out for all fish
146 samples using the methodology detailed previously.⁵³

147

148 **2.3. Laboratory analyses.**

149 *Ancillary data.*

150 Dissolved organic carbon (DOC) concentrations were determined using a Non Dispersive Infra-Red (NDIR) CO₂
151 Shimadzu® (Model VCSN) spectrometer after wet oxidation in a sodium persulfate solution at 100 °C. Specific
152 ultraviolet absorbance at 254 nm (SUVA₂₅₄) was obtained by dividing the UV absorbance measured with an UV-
153 VIS (Perkin Elmer Lambda 35) by the DOC concentration of the same water sample. A submersible multiparameter
154 probe (HANNA HI-9828, Hanna Instruments) was used to characterize the basic physicochemical conditions (pH,
155 conductivity, dissolved oxygen concentration, oxygen saturation, and temperature).

156

157 *Hg species concentrations and calculation of transformation rates.*

158 Directly after incubations, Hg⁰ was purged from the samples onto gold-coated sand traps and analyzed according
159 to protocols previously described.⁵⁴ Teflon® purging vessels (0.5 L) were bubbled for 30 min with a Hg-free nitrogen
160 flow (c.a. 400 mL min⁻¹) and the gas stream was dried through a moisture trap before species trapping. Gold traps
161 were analyzed on-site (within a few hours) by double amalgamation on gold (method detection limit about 2 fM).
162 IHg and MMHg concentrations were determined back in the laboratory according to Rodriguez-Gonzalez et al.⁵⁵

163 Briefly, Hg species were analyzed by species-specific isotope dilution using a complementary pair of isotopes
164 (^{198}IHg and $^{202}\text{MMHg}$), derivatization with sodium tetrapropylborate and liquid-liquid extraction into isooctane. After
165 a vigorous shake, the organic phase was recovered and injected in triplicate into the gas chromatograph (GC)
166 hyphenated to an inductively coupled plasma mass spectrometer (ICP-MS, Thermo-Electron Series XII). The
167 concentrations of the added and formed Hg species deriving from the enriched isotopes 199 and 201 were
168 calculated by isotopic pattern deconvolution methodology and the demethylation rate was calculated based on the
169 loss of $^{201}\text{MMHg}$. The determination of IHg and MMHg concentrations in the incubations with added natural MMHg
170 was carried out according to Monperrus et al.,⁵⁶ using ^{199}IHg and $^{201}\text{MMHg}$, on a small subsample (1.5 mL).

171

172 *Hg compounds specific stable isotope analysis (CSIA) for water samples.*

173 IHg and MMHg CSIA were determined by on-line preconcentration, separation by gas chromatography and
174 detection by MC-ICP-MS as described in details in Bouchet et al.⁵⁷ Briefly, 100 mL of the incubated water were
175 derivatized with sodium tetrapropylborate and extracted into hexane, of which 25 μL were injected into the GC
176 using a programmed temperature vaporization injector. A straight liner (2 mm ID, 2.75 mm OD, 120 mm length,
177 ThermoFisher Scientific, France) packed with a styrene-divinylbenzene polymeric resin (Bondesil-ENV, 125 μm ,
178 Agilent Technologies) was used to preconcentrate the Hg species on-line before their separation and detection by
179 GC/MC-ICP-MS. A Trace Ultra GC (ThermoFisher Scientific, France) was fitted with a MXT-1 capillary column
180 (0.53 mm ID, 1 μm thick coating, 30 m length, Restek, France) and interfaced to a Nu Plasma HR (Nu instruments,
181 UK) through a commercial heated interface and dual inlet glass torch allowing the simultaneous introduction of an
182 isotopically certified TI solution (200 $\mu\text{g}\cdot\text{L}^{-1}$ in 2% HNO_3) to correct for instrumental mass bias. Analysis followed a
183 Sample Standard Bracketing (SSB) sequence where the SRM NIST SRM-3133 (IHg) and STREM (MMHg) were
184 used as primary standards and matched to the sample concentrations within 25%. Isotopic ratios were calculated
185 using the Linear Regression Slope (LRS) method and Hg isotopic compositions are commonly reported as delta
186 values relative to the NIST SRM-3133 IHg standard.⁵⁸ For IHg, delta values were calculated as follow:

187

$$\delta^{xxx}\text{IHg}(\text{‰}) = \left[\frac{({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{sample}}}{({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{NIST 3133}}} - 1 \right] \times 1000$$

188 Where xxx can be 204, 202, 201, 200 or 199 and $(^{xxx}\text{Hg}/^{198}\text{Hg})_{\text{NIST 3133}}$ is the averaged isotopic ratio of the two
 189 bracketing standards. For MMHg, the delta values were first calculated against the STREM MMHg standard and
 190 then converted relatively to the NIST SRM-3133 according to:

$$191 \quad \delta^{xxx}\text{MMHg}(\text{‰}) = \left[\left(\frac{\delta^{xxx}(\text{STREM vs NIST 3133})_{\text{CCVG}}}{1000} + 1 \right) \times \left(\frac{\delta^{xxx}(\text{sample vs STREM})}{1000} + 1 \right) - 1 \right] \times 1000$$

192
 193 where $\delta^{xxx}(\text{STREM vs NIST SRM-3133})_{\text{CCVG}}$ is the previously reported isotopic composition of the STREM MMHg
 194 standard relative to the NIST SRM-3133 measured by CCVG / MC-ICP-MS⁵⁹ and $\delta^{xxx}(\text{sample vs STREM})$ is the
 195 isotopic composition of the sample MMHg versus the STREM MMHg standard calculated as follows:

$$196 \quad \delta^{xxx}\text{Sample vs STREM}(\text{‰}) = \left[\frac{(^{xxx}\text{Hg}/^{198}\text{Hg})_{\text{sample}}}{(^{xxx}\text{Hg}/^{198}\text{Hg})_{\text{STREM}}} - 1 \right] \times 1000$$

197
 198 The Δ notation is used to express the mass independent fractionation (MIF), calculated as $\Delta^{xxx}\text{Hg} = \delta^{xxx}\text{Hg} -$
 199 $\beta_{\text{kin}} \times \delta^{202}\text{Hg}$ where $\beta_{\text{kin}} = \ln(m_{198}/m_{xxx})/\ln(m_{198}/m_{202})$.⁵⁸ Enrichment factors were calculated as the slope of the best
 200 linear fit of relation between $\ln(\Delta^{199}\text{Hg})$ or $\ln(\delta^{202}\text{Hg})$ and $\ln(f)$.

201

202 RESULTS AND DISCUSSION

203 Characteristics of the water column at the investigated stations.

204 Both lakes are influenced by a high-altitude tropical climate (rainy season between December and March) and their
205 hydrological regime is dominated by evaporation. This results in high pH values (Table SI-1), usually ranging from
206 7.4 to 9.5 although lower values are found at the BC station (6.6 – 7.1) due to severe anthropogenic inputs and OM
207 mineralization.⁶⁰ Conductivity ($\sim 1100 - 6200 \mu\text{S}\cdot\text{cm}^{-1}$) and DOC concentrations ($2.5 - 29 \text{ mg L}^{-1}$) overall reflect the
208 eutrophication status of each station with a gradual increase from CH to UU while the oxygen saturation ($15 - 183$
209 %) shows an inverse trend. SUVA values in Lake Titicaca increase with the DOC values but remain overall low
210 ($0.004 - 0.07 \text{ L mg}^{-1} \text{ m}^{-1}$) due to limited terrestrial inputs from the watershed and thus the prevalence of
211 autochthonous OM in the lake. Typical incident PAR intensities along the day and specific wavelength penetration
212 depths in the water column are presented in Figure SI-3. The daily and seasonal variations of light attenuation were
213 limited ($< 10 \%$, data not shown). At the CH and HU station where incubations were carried out and representative
214 for lake water DOC concentrations of $2 - 3 \text{ mg L}^{-1}$, PAR radiations could reach down to about 8 m while UV-A are
215 attenuated between 3 and 8 m depending on the wavelength and UV-B are not transmitted at all below 3m.

216

217 Hg species transformations in the water column.

218 For the various conditions investigated, the demethylation rate constants stretched over an order of magnitude but
219 were relatively constant for each station independent of the season (Figure 1) and fell in two groups, which clearly
220 differ in their DOC concentrations. The demethylation at CH and HU ($0.003 - 0.018 \text{ h}^{-1}$, DOC $2.6 - 4.5 \text{ mg L}^{-1}$) were
221 indeed consistently lower than at BC and UU ($0.036 - 0.05 \text{ h}^{-1}$) where the DOC concentration is usually 2 – 3 times
222 higher ($6.1 - 9.9 \text{ mg L}^{-1}$, Table S1). The demethylation rates were on average 25 % lower in bulk than in filtered
223 waters exposed to light. It implies that the photodegradation reaction is mainly induced by DOM and that the
224 presence of particles (including bacteria and algae) hamper it through both attenuated light transmission (absorption
225 & scattering) and radical scavenging. The demethylation rates largely decreased (3 to 8 times) for the incubations
226 conducted at depth (3.5 m) compared to the subsurface ones (0.5 m) reflecting the sharp attenuation of (UV) light
227 and especially UV-B with depth (Figure SI-3). In the dark controls, rates were always below (or very close) to
228 detection limits (DLs). The relationship between MMHg photodegradation and DOC concentration follows a bell
229 curve with a maximum around 6 mg L^{-1} (Figure SI-4). This is consistent with previous studies demonstrating that a

230 minimal amount of DOM is required to complex MMHg and trigger the photodegradation process^{31,34,61} but that
231 photodegradation rates decrease with an increase in DOM due to light attenuation and radical scavenging.^{32,62,63}
232 Interestingly, Girard et al.⁶⁴ found a very similar relationship in Arctic lakes spanning DOC contents from about 1 to
233 11 mg L⁻¹, with a breaking point around 7 mg L⁻¹.
234 Similarly to MMHg demethylation, the IHg reduction rates were also relatively constant within the stations and
235 independent of the season. They fell in the same two groups (Figure 1) but exhibited an opposite trend with respect
236 to demethylation, i.e. higher at CH and HU (0.009 – 0.017 h⁻¹) than at BC and UU (0.003 – 0.004 h⁻¹). There is
237 overall a negative trend with DOC (Figure SI-4) suggesting that the optimal DOC concentration for IHg reduction
238 lies in the lower range examined here (< 5 mg L⁻¹) and higher concentrations only decrease the reaction rate. The
239 reduction rates dropped by 2 to 7 times for the incubations conducted at depth (CH and HU) compared to the
240 subsurface samples, reflecting again the UV attenuation. The reduction rates in the dark were negligible (< 0.001
241 h⁻¹) demonstrating that the dark biotic or abiotic contribution to reduction was limited within the experiment
242 timeframe, except for HU during the dry season (0.002 – 0.003 h⁻¹).
243 No methylation was detected in waters during the two seasons investigated. However, our incubations were all
244 carried out during daytime while most methylating bacteria are (facultative) anaerobic and could thus be more active
245 at night when the oxygen level decreases. Moreover, the high ambient MMHg concentrations, especially in BC and
246 UU (73 – 1030 pg L⁻¹, Table SI-1), make it difficult to detect the formation of new MMHg from the low concentrations
247 of added IHg (1 - 1.5 ng L⁻¹). Therefore, methylation in the water columns of these lakes cannot be ruled out from
248 these experiments with enriched tracers. Methylation was however detected in waters from UU incubated with
249 stable isotopes during the dry season (see below), consistent with previous observations in this shallow lake where
250 strong redox shift and high concentrations of suspended particles favor the establishment of reducing microniches
251 and restrict photodemethylation in the water column.^{47,65} Regarding Lake Titicaca, several compartments can
252 contribute to the pool of MMHg present in the water column as methylation was previously clearly demonstrated in
253 benthic biofilms, green-algae periphyton and carbonate-rich sediments^{49,50} but also in the water column itself under
254 anoxic conditions that developed after a severe algal bloom.⁴⁵

255

256 **MMHg photodegradation and associated isotopic fractionation in the water column.**

257 *Hg species concentrations along incubation experiments.*

258 No significant loss of MMHg (or increase in IHg) was observed in the dark controls incubated for 36 h in the dark
259 for each site or in the incubation performed with HU water at 3.5 m depth, where mostly PAR with little UV-A (<
260 10% of the incident surface radiation) but no UV-B radiations are transmitted (Figure SI-3). The results of these
261 experiments conducted over a longer duration and with higher MMHg concentrations compared to the incubations
262 with enriched isotopes (200 vs 0.2 ng.L⁻¹) confirmed that demethylation in the dark or at 3.5 m, below the UV-B
263 penetration depth, was not significant. In the three sets of incubations carried out at 0.5 m, the MMHg concentrations
264 decreased from an initial value close to 200 ng L⁻¹ to a final value ranging from 140 to 170 ng L⁻¹ (Figure 2). A first
265 decrease was observed between 0 and 6 h for all but then the time-courses diverged during the night period (10 –
266 22 h): the MMHg concentrations were either still decreasing (BC, high demethylation rate), stable in HU (moderate
267 demethylation rate) or slightly increasing in UU, which indicates a re-methylation of the produced IHg. A decrease
268 was finally observed for all incubations during the second day (24 - 36 h). At 6h, the average demethylation rate
269 constants obtained from triplicate experiments were thus 0.011 ± 0.003 , 0.036 ± 0.006 and 0.036 ± 0.002 h⁻¹ for
270 HU, BC and UU, respectively. These values are consistent with those previously obtained from the enriched isotope
271 incubations, i.e. 0.010, 0.030 and 0.046 for HU, UU and BC, respectively (see above). It demonstrates a similar
272 behavior for the photodegradation of the added MMHg, despite the concentrations being more than 1000 times
273 higher, extending previous observations made on added versus ambient MMHg.²⁵

274 On the other hand, the concentrations of IHg increased from 6 ± 2 , 9 ± 3 and 10 ± 1 ng L⁻¹ in BC, UU and HU
275 respectively to 30 ± 5 , 21 ± 1 and 23 ± 5 ng L⁻¹ after 36 h (Figure 2). The IHg concentrations showed the inverse
276 trend relative to the MMHg losses, i.e. a first increase followed by a plateau during the night period and a second
277 increase during the next day. On average, only 30 % of the lost MMHg was recovered as IHg in the solution at the
278 end of the experiment (Figure SI-6) but the recovery was higher for HU and BC than UU (45, 37 and 20 %,
279 respectively at 36 h). These losses cannot be solely explained by the production and evasion of Hg⁰ as the
280 reduction rates are lower than the demethylation ones and exhibit an inverse trend (HU >> UU ≥ BC). Altogether,
281 this suggests that the missing IHg fraction also comprises a refractory phase that escapes the derivatization
282 (propylation) reaction needed prior to GC-ICP-MS analysis. We hypothesize that IHg was gradually transformed
283 into HgS nanoparticles along the incubations, most likely by direct precipitation with sulfides produced through
284 sulfate reduction occurring in low oxygen microniches.⁴⁷ However, HgS was also observed to form within a few
285 hours upon UV exposure of IHg complexed with thioglycolic acid⁶⁶ and in the dark when IHg is left aging with OM

286 for a long period.⁶⁷ The occurrence of sulfate reduction in microniches of UU waters is very plausible given: (i) the
287 high DOM, SPM and sulfate that favor the development of aggregates,⁴⁷ and (ii) the observed re-methylation of the
288 IHg issued from the demethylation.

289

290 *Hg species isotopic fractionation along the incubation experiments.*

291 No significant MMHg MIF ($\Delta^{199}\text{Hg}_{\text{MMHg}}$) could be measured in any of the dark controls or the incubation carried out
292 at 3.5 m depth at HU, for which the MMHg degradation was never significant. In the subsurface incubations, the
293 increase in $\Delta^{199}\text{Hg}_{\text{MMHg}}$ mirrored the decrease in MMHg concentrations as expected and went from 0 to 2.80 ± 0.26
294 ‰, 5.50 ± 0.67 ‰ and 6.06 ± 0.25 ‰ after 36 h for HU, UU and BC, respectively (Figure 2). The corresponding
295 MDF ($\delta^{202}\text{Hg}_{\text{MMHg}}$) increased to 0.2 ± 0.1 ‰, 0.7 ± 0.2 ‰ and 0.4 ± 0.1 ‰, leading to an average $\Delta^{199}\text{Hg}/\delta^{202}\text{Hg}$ of
296 7.5 ± 0.7 (1 SE, York regression, Figure SI-5), which is higher than the previous reported slope (2.4 ± 0.1 , 1 SE).^{37,39}
297 However, the variability in the data is large and may originate from other reactions that MMHg undergo besides
298 photodegradation but also from analytical uncertainties.⁵⁷ For example, the $\delta^{202}\text{Hg}_{\text{MMHg}}$ decreased following the re-
299 methylation of the produced IHg in UU waters, consistent with previous observations that lighter Hg isotopes are
300 preferentially taken up and methylated.^{68,69}

301 On the contrary, the MIF of the produced/residual IHg ($\Delta^{199}\text{Hg}_{\text{IHg}}$) decreased from 0 to -9.9 ± 0.2 ‰, -5.7 ± 0.6 ‰
302 and -8.5 ± 0.6 ‰ after 36 h for HU, UU and BC, respectively with a plateau phase or a slight decrease during the
303 night period for HU and BC but a slight increase for UU. These negative MIF values mirror the positive MMHg MIF
304 values in BC and UU but not in HU where the photoreduction of IHg is the strongest. The photoreduction of IHg
305 has been found to produce a positive MIF on the remaining IHg^{39,44} while UV-B photolysis experiments of Hg^0 in
306 the presence of halogens generated a negative MIF in the produced IHg.⁷⁰ We thus suggest that a large fraction of
307 the measured IHg in the HU incubations originates from the re-oxidation of Hg^0 , the only mechanism shown to
308 produce negative MIF until now and which is likely favored by our closed incubation bottles. The corresponding
309 MDF ($\delta^{202}\text{Hg}_{\text{IHg}}$) exhibited two different patterns depending on the stations, an increase to 0.8 ± 0.5 ‰ for UU but
310 a decrease for both HU and BC to -0.7 ± 0.1 ‰ and -0.86 ± 0.1 ‰, respectively. The increase in $\delta^{202}\text{Hg}_{\text{IHg}}$ observed
311 in UU can be explained by the precipitation of HgS as a positive Hg-MDF was evidenced during this reaction⁷¹ and

312 this is also consistent with the fact that only a small fraction of the degraded MMHg is recovered as IHg in UU as
313 discussed above (Figure SI-6).

314

315 *MIF signatures and enrichment factors.*

316 The average $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ slope of the residual MMHg was 1.31 ± 0.01 (1 SE, York regression, $r^2 = 0.999$, Figure
317 3) when considering all data. However, the value for the HU waters (1.24 ± 0.03 with 3 mg L^{-1} DOC, 1 SE, York
318 regression) was significantly lower than the ones for BC and UU (1.33 ± 0.02 and 1.34 ± 0.02 with 6 and 16 mg L^{-1}
319 DOC, respectively, both 1 SE and York regression). Overall, our $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}_{\text{MMHg}}$ ratios are lower than previous
320 values obtained through lab experiments that used “open” systems where the Hg° produced is continuously
321 removed and matrix consisting in purified DOM extracts. Indeed, Bergquist and Blum⁵⁸ originally reported a ratio of
322 1.36 ± 0.02 (2 SE) while Chandan et al.⁴³ later found an average ratio of 1.38 ± 0.02 (2 SE) with experiments
323 conducted with three contrasted DOM extracts and low ratios between MMHg and strong OM-thiol ligands
324 (MMHg: $S_{\text{red}}\text{-DOM}$ ratios of 10^{-2} to 10^{-1}). Our values are actually closer to the range obtained for higher MMHg: $S_{\text{red}}\text{-}$
325 DOM ratios (0.2 - 2) by Chandan et al.⁴³ while our estimated MMHg: $S_{\text{red}}\text{-DOM}$ ratios range from 10^{-4} to $6 \cdot 10^{-3}$ (Table
326 SI-2). While we cannot explain such difference, our experiments with natural waters also indicate that the Hg-MIF
327 signature imprinted by MMHg photodegradation decreases when the MMHg: $S_{\text{red}}\text{-DOM}$ increases and point to a
328 control by the MMHg bonding environment, i.e. its distribution between strong OM-thiol ligands ($S_{\text{red}}\text{-DOM}$) and
329 weaker inorganic ones such as N and O ligands associated to DOM or dissolved Cl⁻.⁴³ On the other hand, the slope
330 for the measured IHg was 1.30 ± 0.03 (1 SE, York regression, $r^2 = 0.985$), similar to the residual MMHg. The other
331 reactions affecting IHg such as photo-reduction/oxidation therefore did not significantly change its MIF signature in
332 our experiments.

333 Regarding enrichment factors, all experimental data points fell on the same line (except one in HU) and the average
334 MIF enrichment factor ($\epsilon_{\Delta^{199}}$) for MMHg was $-4.8 \pm 0.3 \text{ ‰}$ (1 SE, $r^2 = 0.95$, York regression, Figure 4) while the
335 average MDF enrichment factor ($\epsilon_{\delta^{202}}$) was $-7.1 \pm 0.9 \text{ ‰}$ (1 SE, $r^2 = 0.7$, York regression). The larger variability
336 associated to the MDF enrichment factor may originate from other reactions that MMHg undergo besides
337 photodegradation but also from analytical uncertainties.⁵⁷ The individual MIF enrichment factors thus did not differ
338 among the three stations that presented contrasted DOC concentrations (3 to 16 mg L^{-1}) but consistent low
339 MMHg: $S_{\text{red}}\text{-DOM}$ ratio. Our average MIF enrichment factor is slightly higher but comparable to the one originally

340 determined by Bergquist and Blum³⁹ (-3.33 ‰ for 1 mg L^{-1} DOC) under natural light and about 3 times lower than
341 the average determined by Chandan et al.⁴³ for low MMHg:S_{red}-DOM ratios (-14.9 ± 1.2 ‰, 2 SE) but artificial light
342 conditions.

343

344 **Hg species isotopic composition in fish from Lakes Titicaca and Uru Uru: implications for MMHg**
345 **photodegradation and aquatic cycling.**

346 Fish collected from Lake Titicaca and Uru Uru display a similar weight (44 ± 11 and 49 ± 13 g, respectively, $n = 15$
347 and 28, Table SI-3) but different HgT concentrations with higher values in Uru Uru compared to Titicaca (642 ± 392
348 vs $354 \pm 156 \text{ ng g}^{-1}$ d.w., respectively) and MMHg accounting overall for 89 ± 10 % of HgT. The $\delta^{202}\text{Hg}_{\text{MMHg}}$ values
349 ranged from 0.05 to 1.11 ‰ among the two lakes and were slightly higher in Lake Titicaca (0.82 ± 0.06 ‰, 1 SE)
350 than Uru Uru (0.59 ± 0.04 ‰, 1 SE). The $\Delta^{199}\text{Hg}_{\text{MMHg}}$ values ranged from 1.1 to 5.9 ‰ and were also significantly
351 higher in Lake Titicaca (4.3 ± 0.4 ‰, 1 SE) compared to Uru Uru (1.7 ± 0.1 ‰, 1 SE). Based on these values and
352 our MIF enrichment factor, we calculated that the extent of MMHg photodegradation before its incorporation into
353 biota ranges from 2 to 31 % and averages 26 ± 2 and 10 ± 1 % in Lake Titicaca and Uru Uru, respectively. These
354 estimates of MMHg photodegradation would be about 30 % higher if the “classical” MIF enrichment factor (-3.33
355 ‰)³⁹ was used. These averages correspond to respectively 18 and 3 h of exposition to sunlight before MMHg is
356 either incorporated or escapes the photic zone, and thus over 30 h of residence time for MMHg in Lake Titicaca
357 when assuming 11 h light per day. Overall, the higher concentrations of MMHg found in both water and biota from
358 Lake Uru Uru compared to Titicaca⁴⁶, and exemplified here with fish, can be explained by both a higher methylation
359 activity in this lake^{47,49} and a lower photodegradation within its water column. This latter can be ascribed to a
360 reduced depth penetration of UV light owing to high DOC and SPM concentrations and also probably to a more
361 efficient scavenging of MMHg by SPM resulting in its downward export.

362 The $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}_{\text{MMHg}}$ ratio ranged from 1.14 to 1.47 and averaged 1.31 ± 0.01 ($n = 43$, 1 SE), typical of freshwater
363 fish,^{37,43} but the average ratio was lower in Lake Titicaca than Uru Uru, 1.24 ± 0.01 vs 1.35 ± 0.01 (1 SE),
364 respectively. These ratios are strikingly similar to the ones determined experimentally for the HU and UU waters
365 indicating that experiments conducted with natural waters and low MMHg concentrations lead to similar MIF
366 expression than the ones recorded in fish from each lake. Assuming that the MIF expression of MMHg during
367 photodegradation is controlled by the MMHg:S_{red} ratio, it implies that the distribution of MMHg among ligands in

368 incubations is similar to the ambient one, even if the concentrations of added MMHg are higher than the ambient
369 ones. The lower $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}_{\text{MMHg}}$ ratio measured in fish from the oligo-mesotrophic Lake Titicaca thus seems to
370 reflect an overall higher MMHg: S_{red} -DOM ratio in the upper part of its water column compared to the eutrophic Lake
371 Uru Uru. Considering that their demethylation rate constants were similar to the ones obtained from the incubations
372 with enriched isotopes at close to ambient concentrations, it can be eventually concluded that these incubations
373 mimic MMHg *in situ* photodegradation better than laboratory experiments performed with purified DOM extracts as
374 the sole matrix, which relevance was previously questioned.⁴³

375 Finally, our results confirm that the MIF extent and signature of MMHg in fish record both the extent of demethylation
376 and the MMHg bonding environment during its photodegradation.⁴³ However, given the importance of enrichment
377 factors to infer the extent of MMHg photodegradation in surface waters and considering the remaining uncertainties
378 regarding the influence of water chemistry on MMHg photodegradation and associated isotopic fractionation, there
379 is still clearly a need to conduct a systematic assessment of enrichment factors with contrasted natural waters.
380 More (in situ) incubations should be performed at low MMHg: S_{red} -DOM ratios to obtain representative MIF
381 enrichment factor(s) and decipher the combined effects of DOM as well as inorganic ions concentrations and
382 composition on the expression of MIF. This will further improve our ability to reconstruct the MMHg cycling and
383 controlling factors using biota archives.

384

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389

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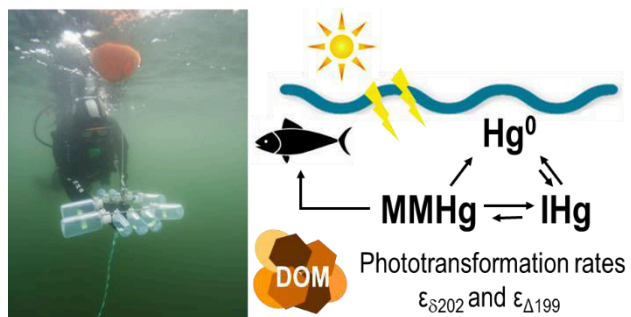
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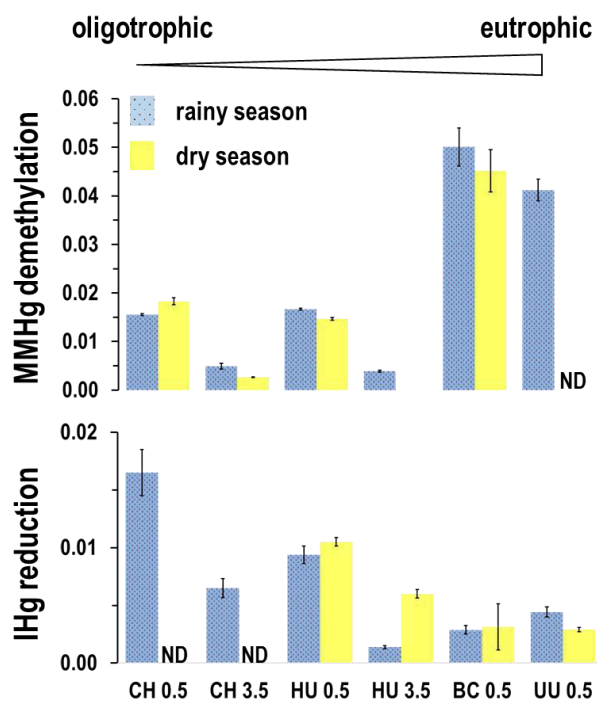


Figure 1: MMHg demethylation and IHg reduction rate constants (in h⁻¹, error bars represent 1 sd of triplicate incubations) determined with isotopically enriched tracers over the 2 seasons and for the various stations and depths (0.5 and/or 3.5 m) investigated.

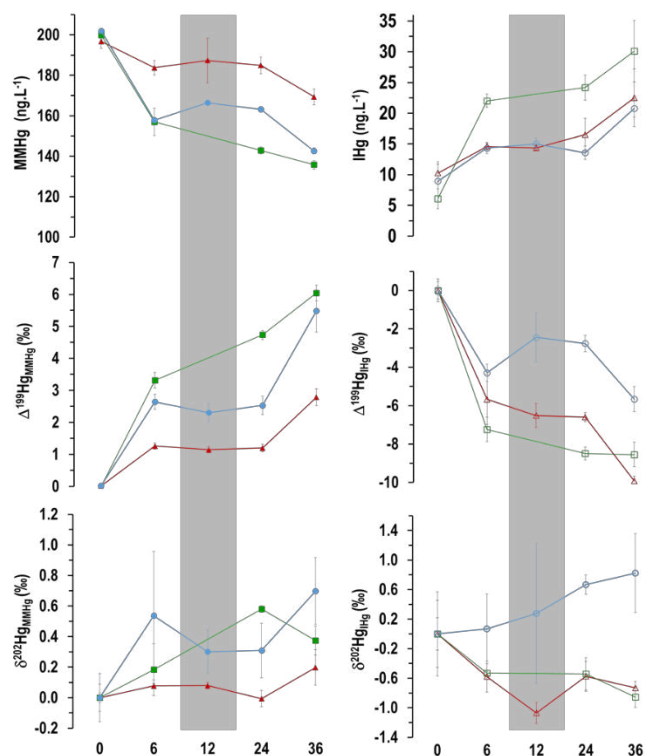


Figure 2: Average concentrations (upper panels), Hg mass independent (middle panels) and mass dependent fractionation (lower panels) of MMHg and IHg along time (x axis in h) in the stable isotope incubations experiments (error bars represent 1 sd of triplicate incubations and shaded areas the night period, red triangles: HU, green squares: BC and blue circles: UU). All isotopic composition data were normalized to t_0 in order to correct for the fractionation that occurred upon addition of MMHg.

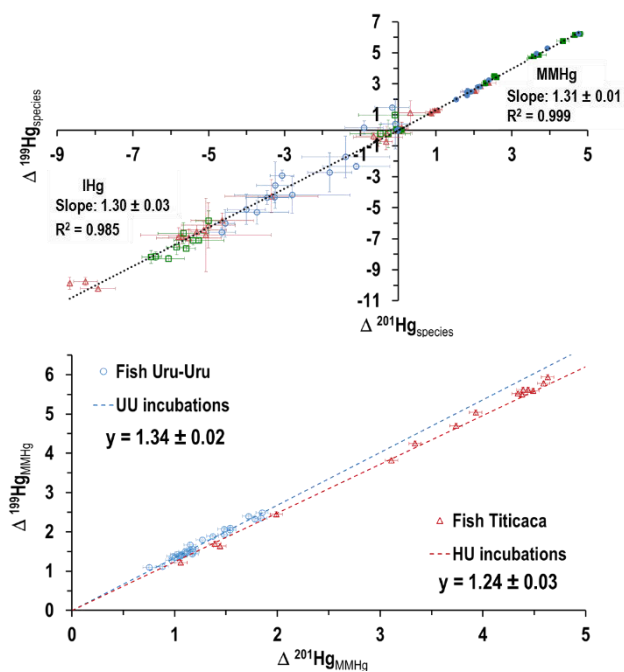


Figure 3: Upper panel: $\Delta^{199}\text{Hg}$ vs $\Delta^{201}\text{Hg}$ for MMHg (right part) and IHg (left part) in the incubation experiments (red triangles: HU, green squares: BC and blue circles: UU, each point represents an individual experiment and error bars 1 sd of triplicate measurements). Lower panel: $\Delta^{199}\text{Hg}$ vs $\Delta^{201}\text{Hg}$ for MMHg recorded in fish from each lakes superimposed on the regression lines obtained from the incubation experiments.

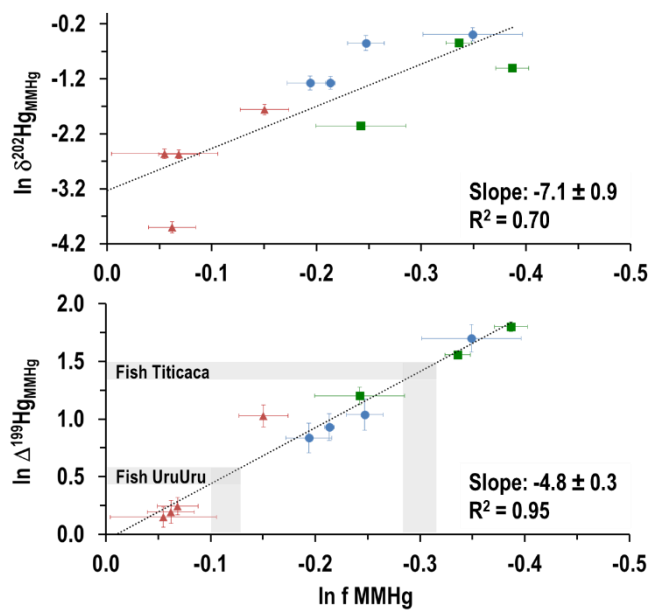


Figure 4: Hg_{MMHg} mass dependent (upper panel) and independent (lower panel) fractionation as a function of demethylation yield along the incubations (error bars represent 1 sd of triplicate incubations, red triangles: HU, green squares: BC and blue circles: UU). Grey bands indicate the $\Delta^{199}\text{Hg}_{\text{MMHg}}$ values (Compound Specific Isotopic Analysis) recorded in fish from each lake (mean \pm 1 SE).