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# Journal Pre-proof

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1 **CRedit Author Statements**

2 Bastien Duval, Alberto de Diego and David Amouroux devised the research.

3 Bastien Duval wrote the manuscript.

4 Bastien Duval and Emmanuel Tessier performed all mercury measurements.

5 Acquisition of the financial support for the project leading to this publication by David Amouroux and

6 Alberto de Diego.

7 All co-authors helped in the data interpretation and manuscript writing.

Journal Pre-proof

1 **Dynamics, distribution, and transformations of mercury**  
2 **species from Pyrenean high-altitude lakes**

3

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## 28 **Abstract**

29           While mercury (Hg) is a major concern in all aquatic environments because of its  
30 methylation and biomagnification pathways, very few studies consider Hg cycling in remote alpine  
31 lakes which are sensitive ecosystems. Nineteen high-altitude pristine lakes from Western / Central  
32 Pyrenees were investigated on both northern (France) and southern (Spain) slopes (1620 to 2600  
33 m asl.). Subsurface water samples were collected in June 2017/2018/2019 and October  
34 2017/2018 for Hg speciation analysis of inorganic mercury (iHg(II)), monomethylmercury (MMHg),  
35 and dissolved gaseous mercury (DGM) to investigate spatial and seasonal variations. In June  
36 2018/2019 and October 2018, more comprehensive studies were performed in four lakes by  
37 taking water column depth profiles. Besides, in-situ incubation experiments using isotopically  
38 enriched Hg species (<sup>199</sup>iHg(II), <sup>201</sup>MMHg) were conducted to investigate Hg transformation  
39 mechanisms in the water column. While iHg(II) (0.08 to 1.10 ng L<sup>-1</sup> in filtered samples; 0.11 to  
40 1.19 ng L<sup>-1</sup> in unfiltered samples) did not show significant seasonal variations in the subsurface  
41 water samples, MMHg (<0.03 to 0.035 ng L<sup>-1</sup> in filtered samples; <0.03 to 0.062 ng L<sup>-1</sup> in unfiltered  
42 samples) was significantly higher in October 2018, mainly because of in-situ methylation. DGM  
43 (0.02 to 0.68 ng L<sup>-1</sup>) varies strongly and can exhibit higher levels in comparison with other pristine  
44 areas. Depth profiles and incubation experiments highlighted the importance of in-situ biotic  
45 methylation triggered by anoxic conditions in bottom waters. In-situ incubations confirm that  
46 significant methylation, demethylation and photoreduction extents are taking place in the water  
47 columns. Overall, drastic environmental changes occurring daily and seasonally in alpine lakes  
48 are providing conditions that can both promote Hg methylation (stratified anoxic waters) and  
49 MMHg photodemethylation (intense UV light). In addition, light induced photoreduction is a major  
50 pathway controlling significant gaseous Hg evasion. Global warming and potential eutrophication  
51 may thus have direct implications on Hg turnover and MMHg burden in those remote ecosystems.

52

53 *Keywords:*

54 Mercury; Biogeochemistry; Alpine lakes; Methylation; Demethylation; Photoreduction

## 55           **1. Introduction**

56           Natural sources of mercury (Hg) (volcanic activities and degassing, evasion from aquatic  
57 and terrestrial surfaces) and increasing anthropogenic sources (e.g. fossil fuel combustion and  
58 gold mining), combined to a high volatility, contribute to the global pool of Hg in the atmosphere  
59 and lead to long-range dispersion and deposition away from point sources<sup>1-3</sup>. Hg has also created  
60 a scenario of global health concerns due to its conversion into an organometallic compound of  
61 elevated neurotoxicity, namely monomethylmercury (MMHg).

62           Hg is a biogeochemically driven contaminant because its most harmful organometallic  
63 species (MMHg) is naturally produced in aquatic systems thanks to a complex interplay between  
64 microbial and chemical processes. MMHg is a contaminant that biomagnifies from one trophic  
65 level to another, leading to elevated concentration on top of food chains. The European Water  
66 Framework Directive (WFD-2000/60/EG) classifies Hg as one of the 30<sup>th</sup> most “precarious  
67 dangerous pollutants”. Human exposure to MMHg is well defined, and mainly associated with fish  
68 consumption<sup>4</sup>. Yet, most risk assessment studies lack fundamental information on what exactly  
69 controls Hg levels in fish. Without detailed knowledge of the Hg biogeochemical cycle and, in  
70 particular, the fundamental processes controlling MMHg production<sup>5-7</sup>, it is difficult to precisely  
71 estimate Hg’s health impacts and socio-economical costs<sup>8</sup>.

72           The net production of MMHg in aquatic ecosystems is closely related to physical  
73 conditions and ecological characteristics, such as temperature, pH, organic matter, redox  
74 conditions, ionic strength and either solar radiation or the occurrence of microorganisms, that are  
75 able to transform various Hg compounds. Consequently, all these environmental settings have a  
76 direct influence on the Hg compounds reactivity or bioavailability, and regulate the extent of  
77 methylation, demethylation and reduction pathways<sup>9</sup>. MMHg bioaccumulation in aquatic food  
78 chains is largely dependent on these biotic and abiotic pathways that control its production and  
79 degradation or the removal of Hg from the ecosystem (e.g., volatilization, sedimentation). In most  
80 surface aquatic systems, a large array of conditions can control these various pathways according  
81 to geological, hydroecological or climatic conditions. However, the methylation of inorganic Hg  
82 (iHg(II)) in aquatic ecosystems is more specifically due to anaerobic microorganisms such as  
83 sulphate-reducing or iron-reducing bacteria, among other groups<sup>10,11</sup>.

84            Among the aquatic compartments, lowland lakes have been widely studied regarding Hg  
85    pollution, speciation and species transformations <sup>12-15</sup>. Several studies highlighted a link between  
86    organic matter and Hg in lake ecosystems <sup>13,16</sup>, with evidence of the catchment influence on the  
87    Hg levels. Significant photo-reduction and -demethylation were observed in lakes <sup>17,18</sup>, exceeding  
88    the external inputs of MMHg (rain, snow, runoff) and therefore suggesting MMHg sources from  
89    bottom sediments. Nevertheless, the biogeochemical processes that control the speciation and  
90    fate of Hg, and especially MMHg levels, remain poorly established <sup>12</sup>. Alpine lakes are better  
91    witnesses of Hg past and present contamination in comparison with lowland lakes. They are  
92    usually characterized by a low catchment-to-lake-surface-area ratio, as the catchment surface is  
93    commonly smaller than lowland lakes. Therefore, atmospheric deposition and catchment  
94    weathering are important processes influencing greatly the lake water geochemistry <sup>19</sup>. Thus,  
95    mountain lake catchments are viewed and used as excellent proxies of background diffuse  
96    contamination <sup>3,20</sup>. While alpine lakes, through sediment cores analysis, have been successfully  
97    used to reconstruct temporal trends in atmospheric Hg deposition <sup>2,3,21</sup>, Hg behaviour in the  
98    aquatic compartment of those pristine areas has been barely investigated. For instance, *Guédrón*  
99    *et al.* <sup>22,23</sup> and *Alanoca et al.* <sup>24</sup> have reported Hg cycling features in high-altitude Bolivian lakes.  
100    Despite anthropogenic influences in these urban and mining areas, Hg species cycling is highly  
101    dynamic and MMHg production was driven by eutrophication in these lakes, while intense UV  
102    light promoted significant demethylation and reduction pathways. Regarding pristine high-altitude  
103    lakes, Hg species distribution has been only investigated in one study on four lakes from the  
104    French Alps <sup>25</sup>. Thus, while Hg transformations in the aquatic compartment have been well  
105    described in lower altitude or more accessible aquatic systems <sup>15,24,26-33</sup>, there is a lack of studies  
106    in more pristine areas such as alpine lakes, particularly sensitive to global changes and long  
107    range transport of pollutants <sup>20,34</sup>.

108            In that sense, *Chételat et al.* <sup>12</sup> suggested that profound environmental changes may be  
109    impacting the cycling and bioaccumulation of Hg. The responsibility of Humans on the dispersion  
110    of aerosols and Hg is undeniable <sup>35,36</sup>, yet it is important to take into account the intensification of  
111    natural processes occurring through Global Change in the biogeochemical cycle of Hg species.  
112    In particular, Climate Change also accelerates many processes having an impact on alpine lakes  
113    dynamics: droughts and losses of snow cover that enhance dust production and soil runoff,

114 potential decreases of cloud density and ice cover period in lakes, surface waters temperature  
115 increase and water column stratification <sup>20</sup>. All these processes will have a direct effect on the  
116 aquatic cycling of Hg through enhance microbial or photochemical transformations of Hg or drastic  
117 changes in the transport of Hg via inputs to lake waters or sediments and atmospheric exchanges.

118 To better understand the potential role of global change of the Hg cycling in high altitude  
119 lakes, we report here for the first time a complete inventory of Hg levels and speciation (iHg(II),  
120 MMHg, DGM) as well as in situ measurements of Hg compounds transformation rates in the water  
121 column of alpine lakes from the Central - Western Pyrenees. The main objectives of this study  
122 were i) to assess the Hg species levels and variability in the aquatic compartment of these  
123 ecosystems, and better understand how seasonal or geographical conditions and lake trophic  
124 status may control such variability ii) to evaluate the importance of abiotic and biotic pathways  
125 affecting the fate of Hg in mountain lakes, and further estimate how selected lakes can be  
126 considered as sources of methylmercury and either enhance Hg burial or reemission iii) to  
127 highlight potential effects of climatic and biogeochemical changes on these identified pathways  
128 and their consequence on the future Hg cycling in remote and alpine aquatic environments.

## 129 2. Material and methods

### 130 2.1. Site description

131 The 19 studied high-altitude lakes (elevation: 1640 – 2600 m asl) are located in three  
132 different valleys of the Central-Western Pyrenees (France/Spain): Caunterets (n=7), Panticosa  
133 (n=9) and Ayous (n=3) areas (**see Figure 1, Figure A1 and Table A1**). These small lakes show  
134 similar physical properties (lake size: 0.37 – 12.82 ha; catchment size: 15 – 3229 ha; maximum  
135 depth: 3 – 35 m) but differ from their catchment characteristics and geological background, i.e.  
136 mainly granite core (pDe-GR) versus sedimentary rocks (Devonian, De-SR; Permo-Triassic, PT-  
137 SR; Cretaceous Cr-SR) <sup>37,38</sup>. Bioclimatic conditions are also substantially different with a decrease  
138 in temperature (2 – 19 °C), an increase in precipitation and an increase in the duration of the snow  
139 cover (50 – 246 days per year; determined according to *Gascoin et al.* <sup>39</sup> using data from Theia  
140 Snow collection (<https://www.theia-land.fr/product/neige/>) and published elsewhere (*Duval, PhD*  
141 *Thesis* <sup>40</sup>) at higher altitude. It is worth noting that the winter 2017 – 2018 was colder and/or with



142 much higher wet deposition and snow accumulation than the winters 2016 – 2017 and 2018 –  
 143 2019.

144 The water geochemistry of these lakes has been described elsewhere (*Duval, PhD*  
 145 *Thesis*<sup>40</sup>) and the raw data are gathered in **Table A2**. Basically, despite the increase of the  
 146 primary productivity during the summer (increasing Total Organic Carbon (TOC) associated to  
 147 decreasing nitrate ( $\text{NO}_3^-$ )) and the influence of agropastoralism on lakes, all the studied lakes are  
 148 classified as oligotrophic. The results, in accordance with previous Pyrenean lakes studies<sup>19,41–</sup>  
 149<sup>43</sup>, highlighted the importance of the geological background, the atmospheric inputs, and, although  
 150 very limited, the Human impact on the aquatic geochemistry of these high-altitude lakes.

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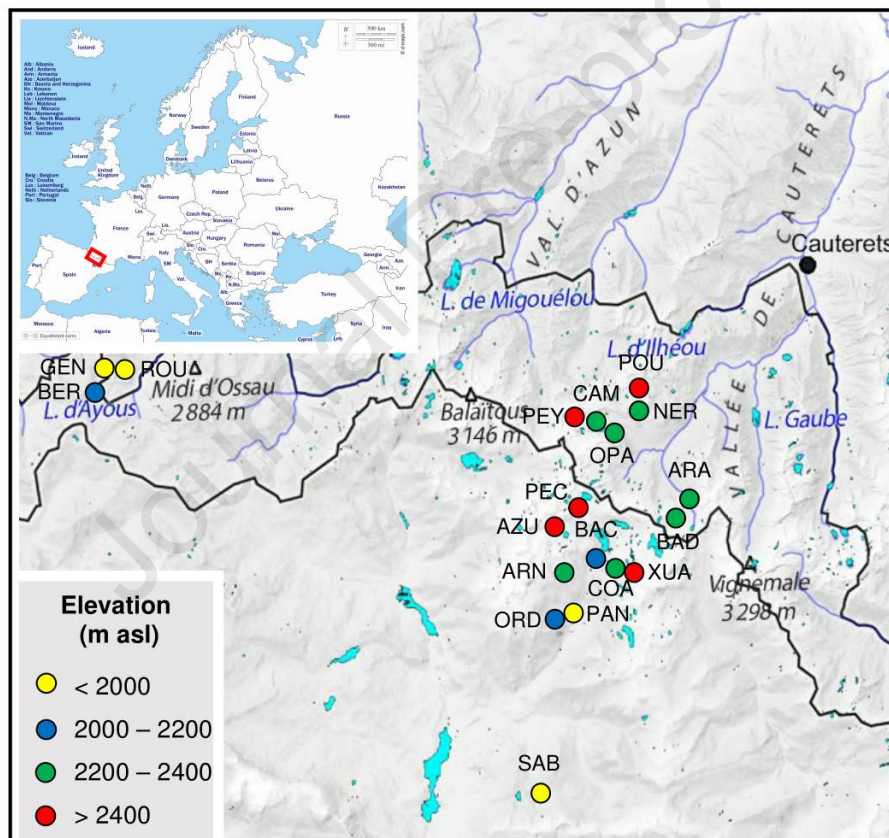
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**Figure 1** : Studied area; circles show position of the lakes, and colors indicate the elevation of the corresponding lakes. Lake acronyms are detailed in **Table A1**. See **Figure A1** for the whole map, including details about the geology of the lakes' catchment.

## 172 2.2. Water samples collection and processing

173 Subsurface (~0.5 m depth) water samples were collected during all the sampling  
174 campaigns (June 2017 [15 lakes, n=81]; October 2017 [10 lakes, n=58]; June 2018 [16 lakes,  
175 n=50]; October 2018 [16 lakes, n=40]; June 2019 [4 lakes, n=18]) with triplicate for the first two  
176 sampling campaigns to check for the spatial variability in the studied lakes. Additionally, water  
177 column profiles were investigated in Lac Gentau (GEN, 5 depths), Lac d'Arratille (ARA, 3 depths),  
178 Ibón de Sabocos (SAB, 6 depths) and Ibón Azul Alto (AZU, 3 depths), by sampling at different  
179 depths along the day. This study provides for the first time a unique Hg species data set with a  
180 total of 319 water samples (**Table A3 and Table A4**).

181 The particles in the water have a key role in the transport and fate of Hg species<sup>16</sup>. Thus,  
182 to evaluate their influence on the distribution of Hg species, two kinds of samples were collected  
183 and further analysed: filtered (F, dissolved fraction) and unfiltered (UF, total fraction). It is worth  
184 noting that considering the general oligotrophic status and elevation of the studied lakes, the  
185 particles were scarce and thus it was impossible to collect and analyse them.

186 Briefly, all the material needed up to the lake was transported by hiking. An inflatable  
187 rubber boat was used to reach the sampling locations. To collect the subsurface water samples,  
188 a manually operated ultra-clean sampler (*5L Teflon Coated Go-Flo Water Sampler, General  
189 Oceanic*) was deployed using powder-free gloves and avoiding the surface microlayer. For depth  
190 sampling, the Go-Flo sampler was deployed using a Kevlar cable and operated at the required  
191 depth with a plastic-coated messenger. Back ashore, the water sample was distributed with a  
192 clean silicone tubing in dedicated flasks according to the parameter to be further analysed.

193 First, an appropriate subsampling procedure was applied for dissolved gaseous Hg  
194 species (DGM). A 250 mL Teflon container was filled overflow without headspace and somehow  
195 following the protocol for the Winkler method (i.e. dissolved oxygen). Then, aliquots for unfiltered  
196 and filtered (Sterivex Filter unit, PVDF, 0.22 µm) non-gaseous Hg species (iHg(II), MMHg) were  
197 collected in 125 mL Teflon containers and acidified to 0.5 % v/v CH<sub>3</sub>COOH 99% (Trace metal  
198 grade)<sup>33</sup>. The Teflon containers were closed tightly and stored in double PE Zip-lock bags in a  
199 portable cooler (5 – 10 °C), protected from light.

200 Other methods used for the measurement of ancillary parameters (conductivity, pH,  
201 temperature, dissolved oxygen saturation, chlorophyll-a, total organic carbon TOC, dissolved

202 inorganic carbon DIC, total alkalinity TA, silicate, sulphate  $\text{SO}_4^{2-}$ , chloride  $\text{Cl}^-$ , nitrate  $\text{NO}_3^-$ ) are  
203 described elsewhere (*Duval, PhD Thesis*<sup>40</sup>).

### 204 **2.3. Non-gaseous Hg species (iHg(II), MMHg) analysis**

205 The quantification of non-gaseous Hg species (i.e. iHg(II) and MMHg) was carried out by  
206 double species-specific isotopic dilution method (D-SS-IDA) and analysis by capillary GC-ICP-  
207 MS<sup>33,44</sup>. Note that this analytical methodology allows to quantify the iHg(II) and the methylated  
208 species (MeHg, i.e., the sum of MMHg and DMHg,). Nevertheless, the purge sample experiment  
209 (**see Appendix 1 and Figure A2**) highlights the fact that the measured MeHg are mainly in the  
210 form of MMHg. LoD ranged from 9 to 34  $\text{pg L}^{-1}$  for iHg(II) and from 3 to 4  $\text{pg L}^{-1}$  for MMHg, in good  
211 agreement with recent publications<sup>33,44</sup>.

212 Repeatability of the analytical measurement (n=3) was 2% for both iHg(II) and MMHg.  
213 The relative standard deviations (RSD) associated to the samples collected at three different  
214 locations of each lake (intra-lake variability, two first sampling campaigns) are 15 and 20%  
215 respectively for unfiltered and filtered MMHg, and 17 and 20% respectively for unfiltered and  
216 filtered iHg(II) and will be used to describe uncertainties in this paper.

### 217 **2.4. Gaseous Hg species (DGM) analysis**

218 The samples for gaseous Hg species (DGM) were processed close to the sampling site  
219 within 1 to 4 hours after sampling by stripping out and trapping the volatile Hg species from the  
220 water sample into a gold-coated sand trap<sup>45,46</sup>. For that purpose, the water sample was gently  
221 transferred into a Teflon purge vessel and subsequently purged under an argon flow ( $500 \text{ mL min}^{-1}$ ,  
222 20 min). The moisture in the gas stream was eliminated by a cold trap ( $-20^\circ\text{C}$ ) before gaseous  
223 Hg collection in the gold-based trap. A second gold trap was always positioned after the sampling  
224 trap to prevent atmospheric contamination. Gold traps were sealed with polypropylene Teflon-  
225 lined caps and stored in double PE Zip-lock bags, in the dark ( $< 4^\circ\text{C}$ ) until analysis. All this  
226 procedure was carried out in an on-field laboratory installed in a mountain hut close to the  
227 sampling site.

228 The analysis of gaseous Hg species was done by double amalgamation on gold-AFS  
229 (DA/Au-AFS)<sup>46</sup>. Thermodesorption efficiency was controlled by carrying out two consecutive

230 analyses of the gold-coated sand trap. Gaseous Hg quantification was done by external  
231 calibration of the DA/Au-AFS device using a controlled Hg(0) source. Finally, the analysis of purge  
232 blanks allowed us estimating the efficiency of the sample treatment procedure. Considering the  
233 five sampling campaigns, the purge efficiency was assessed to reach  $(95\pm 3)\%$  ( $n=12$ ). Note that  
234 this analytical methodology allows the measurement of the sum of Hg(0) and volatile  
235 dimethylmercury (DMHg). Nevertheless, regarding the very small levels of methylated species  
236 (MeHg), i.e. the sum of MMHg and DMHg, in comparison with DGM, we can assume that DGM  
237 is mainly elemental mercury Hg(0).

238 LoD were quite constant over the sampling campaigns, ranging from 0.1 to 0.5  $\text{pg L}^{-1}$ ,  
239 which is consistent with the LoD of 0.4  $\text{pg L}^{-1}$  reported by *Bouchet et al.* <sup>46</sup>.

## 240 **2.5. Hg species transformation assessments**

241 In-situ water incubation experiments over 7h (usually from 9 a.m. to 4 p.m. UTC+2) using  
242 isotopically enriched Hg species ( $^{199}\text{iHg(II)}$ ,  $^{201}\text{MMHg}$ ) were conducted in the June 2018, October  
243 2018 and June 2019 campaigns at lakes Gentau, Arratille, and Sabocos to investigate Hg species  
244 transformation mechanisms in the water column (methylation, demethylation, reduction)  
245 according to the procedure published elsewhere <sup>47-49</sup>.

246 Unfiltered water samples were collected to perform the incubation experiments. Different  
247 conditions of incubation were tested depending on sampling depth (subsurface, middle depth,  
248 and bottom) or light exposure (Diurnal vs Dark) (**Table A5**).

249 After collecting a water sample using Go-Flo sampler, a first set of samples was prepared.  
250 Three 125 mL Teflon container (triplicate) per condition were directly filled with the water sample  
251 up to the top. Isotopically enriched spikes were added to each of the replicate to obtain  
252 concentrations in  $^{199}\text{iHg(II)}$  and  $^{201}\text{MMHg}$  of respectively 2 and 0.2  $\text{ng L}^{-1}$ . It corresponds to about  
253 10 times the natural concentrations observed in the studied ecosystems: high enough to overlap  
254 the natural concentrations and low enough to avoid any biotic stress. Using a simple mooring line  
255 at the sampling point, incubation bottles, either protected from the light or not, were placed at the  
256 corresponding depth. After 7 hours, the Teflon containers were collected, and the incubation  
257 processes were stopped by adding high-purity HCl (1 % v/v). Teflon containers were closed tightly  
258 and stored in double PE Zip-lock bags in a portable cooler (5-10 °C), protected from light, and

259 further transported and stored in the laboratory (5-10 °C). This first set of samples allows the  
 260 determination of methylation, demethylation and MMHg loss rates.

261 A second set of samples was prepared to determine Hg reduction rates. After collection  
 262 of water using the Go-Flo sampler, two 250 mL Teflon containers (duplicate) per condition were  
 263 filled overflow without headspace and somehow following the protocol for the Winkler method and  
 264 spiked with 2 and 0.2 ng L<sup>-1</sup> isotopically enriched <sup>199</sup>iHg(II) and <sup>201</sup>MMHg, respectively, like in the  
 265 previous case. They were incubated together with the samples of the first set. The difference in  
 266 this case is that, at the end of the 7 hours, the elemental Hg (Hg(0)) was immediately recovered  
 267 in gold-coated sand traps by purging the water samples.

268 Quantification was carried out by isotope pattern deconvolution (IPD) isotope dilution  
 269 analysis (IDA) and analysis by capillary GC-ICP-MS for the water samples following the same  
 270 protocol used for the determination of organomercury species <sup>32</sup>. The particularity is that the  
 271 samples were spiked with other enriched isotope solution (<sup>198</sup>iHg(II) and <sup>202</sup>MMHg).

272 The gaseous species trapped in the gold-coated sand traps were quantified by IPD and  
 273 analysed by thermal desorption cryogenic trapping (CT) followed by GC-ICP-MS. Recoveries and  
 274 mass balance of the added Hg spikes during the experiment were systematically established in  
 275 order to provide accurate transformation yield as previously described <sup>18,32,48</sup>.

276 The Hg species incubation experiment allowed us to calculate methylation (M) (iHg(II)  
 277 into MMHg), demethylation (D) (MMHg into iHg(II)), MMHg loss (L) (MMHg into iHg(II) and Hg(0))  
 278 and reduction (R) (iHg(II) into Hg(0)) potentials (in % day<sup>-1</sup>) (**Figure A3**) according to *Rodriguez-*  
 279 *Gonzales et al.* <sup>48</sup> with the following formula:

$$280 \quad \text{Methylation (M)} = \frac{0^{199}\text{MMHg}_t - 0^{199}\text{MMHg}_{t0}}{0^{199}\text{iHg(II)}_{t0}} \times \frac{1440}{t} \times 100$$

$$281 \quad \text{Demethylation (D)} = \frac{0^{201}\text{iHg(II)}_t - 0^{201}\text{iHg(II)}_{t0}}{0^{201}\text{MMHg}_{t0}} \times \frac{1440}{t} \times 100$$

$$282 \quad \text{MMHg Loss (L)} = \frac{0^{201}\text{MMHg}_{t0} - 0^{201}\text{MMHg}_t}{0^{201}\text{MMHg}_{t0}} \times \frac{1440}{t} \times 100$$

$$283 \quad \text{Reduction (R)} = \frac{0^{199}\text{Hg(0)}_t - 0^{199}\text{Hg(0)}_{t0}}{0^{199}\text{iHg(II)}_{t0}} \times \frac{1440}{t} \times 100$$

284 where  $t$  is the time of incubation (min),  $^{xxx}iHg(II)_{t_0}$ ,  $^{xxx}MMHg_{t_0}$  and  $^{xxx}Hg(0)_{t_0}$  are the  
 285 concentrations measured at the beginning of the incubation experiment,  $^{xxx}iHg(II)_t$ ,  $^{xxx}MMHg_t$  and  
 286  $^{xxx}Hg(0)_t$  are the concentrations measured at the end of the incubation experiment.

287 To predict the variations of MMHg concentrations in the lake, the potential net Hg  
 288 methylation ( $ng\ L^{-1}\ day^{-1}$ ) was calculated using the methylation and demethylation rates (% day<sup>-1</sup>)  
 289 according to the following formula:

$$290 \quad Net\ Methylation = \frac{M}{100} \times [iHg(II)]_{ambient} - \frac{D}{100} \times [MMHg]_{ambient}$$

291 where  $M$  is the methylation potential (% day<sup>-1</sup>),  $[iHg(II)]_{ambient}$  is the ambient  $iHg(II)$  concentration  
 292 ( $ng\ L^{-1}$ ),  $D$  is the demethylation potential (% day<sup>-1</sup>),  $[MMHg]_{ambient}$  is the ambient MMHg  
 293 concentration ( $ng\ L^{-1}$ ).

294 It is worth noting that we chose to use demethylation rates instead of MMHg loss rates to  
 295 avoid an underestimation of the net methylation since we have only determined MMHg production  
 296 from  $iHg(II)$ . Thus, in that case, we specifically consider the most significant reversible  
 297 methylation/demethylation pathways between  $iHg(II)$  and MMHg forms.

## 298 **2.6. Hg gaseous fluxes at the air-water interface**

299 According to *Sharif et al.*<sup>32</sup>, gaseous Hg fluxes at the air-water interface (volatilization flux  
 300 densities [FD]) ( $ng\ m^{-2}\ h^{-1}$ ) were calculated using the following equation:

$$301 \quad Volatilization\ FD = k_w \times \left( [DGM] - \frac{[TGM]}{H} \right)$$

302 where DGM is the concentration of DGM (i.e.  $Hg(0)_{aq}$ ) measured in the subsurface water ( $ng\ m^{-3}$ )  
 303 <sup>3</sup>, TGM is the concentration of TGM (i.e.  $Hg(0)_g$ ) measured in the atmosphere. As a reference  
 304 value for atmospheric concentration of TGM we used the measurement performed at the Pic du  
 305 Midi (2860 m asl.) between February 2012 and January 2013<sup>50</sup> ( $1.8\ ng\ m^{-3}$ ).  $H$  is the  
 306 dimensionless Henry's Law constant corrected for water temperature and salinity<sup>51</sup> and  $k_w$  is the  
 307  $Hg(0)$  gas transfer velocity ( $m\ h^{-1}$ ) at the air-water interface.  $k_w$  was calculated, according to *Sharif*



308 *et al.*<sup>32</sup> and using a specific model developed for CO<sub>2</sub> exchanges in sheltered lakes<sup>52</sup>, as a  
309 function of wind speed.

310 The wind speed has been measured during daytime in Lake Gentau ( $w = (3.2 \pm 1.4) \text{ m s}^{-1}$  in June  
311 2018;  $w = (1.4 \pm 0.8) \text{ m s}^{-1}$  in October 2018;  $w = (2.6 \pm 2.0) \text{ m s}^{-1}$  in June 2019), Lake Sabocos  
312 ( $w = (1.5 \pm 1.2) \text{ m s}^{-1}$  in June 2018;  $w = (2.8 \pm 2.1) \text{ m s}^{-1}$  in October 2018;  $w = (2.0 \pm 2.0) \text{ m s}^{-1}$  in  
313 June 2019), Lake Arratille ( $w = (1.5 \pm 0.7) \text{ m s}^{-1}$  in June 2018) and Lake Azul ( $w = (0.8 \pm 0.8) \text{ m}$   
314  $\text{s}^{-1}$  in June 2018). Thus, to estimate the FD in all the sampled lakes, two wind speed ranges will  
315 be considered: smooth breeze at  $1 \text{ m s}^{-1}$  to stronger wind at  $3 \text{ m s}^{-1}$ , which corresponds well to  
316 sheltered lakes conditions as proposed by *Cole and Caraco*<sup>52</sup>.

### 317 3. Results and discussion

#### 318 3.1. Total Hg (Hg<sub>TOT</sub>) in all lake waters

319 The concentrations of iHg(II) and MMHg, both in filtered (F) and unfiltered (UF) samples,  
320 and DGM are presented in **Table A3** and their variability is illustrated in **Figure 2**. Total Hg (Hg<sub>TOT</sub>)  
321 is defined here as the sum of concentrations of iHg(II), MMHg and DGM in unfiltered samples  
322 (DGM only measured in unfiltered samples).

323 In the following discussions, to establish a proper global assessment of the total Hg  
324 (Hg<sub>TOT</sub>) in the subsurface water of the high-altitude lakes, seven outliers are discarded from the  
325 whole database based on to the fact that the concentrations observed were too high compared  
326 to the normal distribution of our data and that no valid explanation could be given for such higher  
327 values (**see Appendix 2 for more details**). Hg<sub>TOT</sub> is quite homogeneous along the five sampling  
328 campaigns, ranging from 0.17 to 1.37 ng L<sup>-1</sup> with a median value of 0.48 ng L<sup>-1</sup> and an average  
329 of  $(0.54 \pm 0.26) \text{ ng L}^{-1}$  (n=66). The homogeneity in the Hg<sub>TOT</sub> concentrations in the Pyrenean lakes  
330 suggests that local inputs through geogenic paths (erosion, lixiviation) are not significant and does  
331 not play a key role in the biogeochemistry of Hg as it was found for other trace elements<sup>38,43,53</sup>.  
332 This also confirms the fact that Hg inputs in alpine environments like the Pyrenees are mainly due  
333 to wet and dry atmospheric deposition. Only two previous studies showed that unfiltered Hg<sub>TOT</sub>  
334 varies from 2 to 14 ng L<sup>-1</sup> in the precipitation from the Pyrenees (880 m asl) in 2014<sup>54</sup> and from  
335 2 to 170 ng L<sup>-1</sup> in the surface snow from the Alps (2448 m asl) in 2008 – 2009<sup>55</sup>. In our study, Hg

336 species in meltwater and ice were also measured in Lake Cambalès in June 2018. The  $Hg_{TOT}$   
337 concentrations were much higher in ice ( $Hg_{TOT} = 19.41 \text{ ng L}^{-1}$ ) than in meltwater ( $Hg_{TOT} = 1.05 \text{ ng}$   
338  $\text{L}^{-1}$ ) and subsurface water ( $Hg_{TOT} = 0.45 \text{ ng L}^{-1}$ ). These reported concentrations in atmospheric  
339 depositions are much higher than the levels observed in the subsurface water from the high-  
340 altitude lakes from this study, suggesting a simple dilution effect or more complex exchanges in  
341 lake waters as discussed thereafter.

## 342 3.2. Mercury compounds distribution in water samples

### 343 3.2.1. Variability in subsurface waters of all lakes

#### 344 Inorganic mercury (iHg(II)) (Figure 2)

345 Filtered and unfiltered iHg(II) levels ( $iHg(II)_F$  and  $iHg(II)_{UF}$ ) in subsurface water from the  
346 19 sampled lakes over the five sampling campaigns were slightly lower than other worldwide  
347 lakes<sup>15,22,24,25,28-31</sup> and freshwaters from the same area<sup>32,33</sup> (**Table 1**).  $iHg(II)_F$  ranges from 0.08  
348 to  $1.10 \text{ ng L}^{-1}$  with a median value of  $0.23 \text{ ng L}^{-1}$  and  $iHg(II)_{UF}$  ranges from 0.11 to  $1.19 \text{ ng L}^{-1}$  with  
349 a median value of  $0.38 \text{ ng L}^{-1}$ , similar to concentration levels found in marine waters<sup>56,57</sup> and  
350 typical from a pristine environment.

351 The filtered fraction of Hg ( $iHg(II)_F$ ) represents about  $68 \pm 16 \%$  (range from 19 to 100 %)   
352 of the unfiltered fraction ( $iHg(II)_{UF}$ ), suggesting that most of the mercury in the Pyrenean lakes is  
353 found in the dissolved fraction. However, subsurface water samples from Azul in Spring 2017 (39  
354 %), Peyregnets (47 %) and Gentau (19 % to 44 %) in Spring 2018 and Cambalès (41%),  
355 Peyregnets (49 %) and Gentau (41 %) in Autumn 2018 are exhibiting higher particulate Hg  
356 fraction.

357 On **Figure 2**, the homogeneity observed in the iHg(II) concentrations, either filtered or  
358 unfiltered, along the five different seasons demonstrates a rather steady state in the Hg mass  
359 balance between the investigated periods. Another study reports seasonal variations of iHg(II) in  
360 the high altitude subtropical Lake Uru Uru (3686 m asl)<sup>24</sup>. In that case, concentrations were  
361 significantly higher during the dry season over the wet season because of the enhanced  
362 evaporation occurring at the end of the dry season. In pristine Pyrenean lakes, the concentration



363 of iHg(II) in Autumn (2017 and 2018) is not significantly higher than in early Spring (2017, 2018  
364 and 2019) (Kruskal-Wallis test, p-value = 0.79 and 0.54 respectively for iHg(II)<sub>UF</sub> and iHg(II)<sub>F</sub>).

### 365 **Monomethylmercury (MMHg) (Figure 2)**

366 Filtered and unfiltered MMHg levels (MMHg<sub>F</sub> and MMHg<sub>UF</sub>) in subsurface water from the  
367 19 sampled lakes over the five sampling campaigns vary respectively from <0.003 to 0.035 ng L<sup>-1</sup>  
368 <sup>1</sup> and from <0.003 to 0.062 ng L<sup>-1</sup> (**Table 1**). The median values, 0.008 for MMHg<sub>F</sub> and 0.011 for  
369 MMHg<sub>UF</sub>, are typical from pristine aquatic environments, in the range of what has been found in  
370 Lake Superior (MMHg<sub>F</sub> = 0.005 ± 0.001 ng L<sup>-1</sup> in April 2000; MMHg<sub>F</sub> = 0.008 ± 0.002 ng L<sup>-1</sup> in  
371 August 2000) <sup>15</sup> and in some high-altitude lakes in the Alps (0.002 – 0.005 ng L<sup>-1</sup>) <sup>25</sup>. MMHg  
372 represents (4±3) % of the filtered non-gaseous Hg and (4±2) % of the unfiltered non-gaseous Hg.

373 From Hg speciation analyses in surface snow from the French Alps (2448 m asl)  
374 *Maruszczak et al.* <sup>55</sup> concluded that biotic production of MMHg in the snowpack is unlikely,  
375 considering the constant ratio MMHg/THg measured throughout the season in their study. The  
376 main contributor to Hg in the high-altitude lakes being atmospheric deposition, the higher  
377 proportion of MMHg observed in surface waters from high-altitude lakes in the Alps (4 ± 3 % for  
378 MMHg<sub>F</sub> and 3 ± 2 % for MMHg<sub>UF</sub>) in comparison to surface snow (MMHg vary from non-  
379 determined to 1.21 % of the total Hg) might be due to in-situ aquatic methylation.

380 Regarding the MMHg levels in surface waters of the investigated Pyrenean lakes, the  
381 Autumn 2018 sampling campaign stands out from the other ones (**Figure 2**). Median values for  
382 MMHg<sub>UF</sub> in Spring 2017, Autumn 2017, Spring 2018, and Spring 2019 were respectively 0.010,  
383 0.006, 0.011 and 0.013 ng L<sup>-1</sup> while the median value in Autumn 2018 was 0.025 ng L<sup>-1</sup>. A  
384 comparison of MMHg<sub>UF</sub> levels in Spring 2018 and Autumn 2018 reveals substantial increases in  
385 lakes Arratille (+248 %), Gentau (+360 %), Roumassot (+235 %), Bachimaña (+211 %), Coanga  
386 (+231 %), Panticosa (+583 %) and Sabocos (+216 %) and more moderate increases in most of  
387 the other studied lakes. Spring - summer algal bloom are controlling the whole biological turnover  
388 in those high-altitude and oligotrophic lakes <sup>58</sup>, and intense bloom events might be responsible  
389 for such increase by promoting methylation processes in the sediments <sup>29,59</sup>. Arratille, Gentau,  
390 Roumassot, Coanga and Sabocos showed the lowest nitrate (NO<sub>3</sub><sup>-</sup>) concentrations (from below  
391 LOD to 0.171 mg L<sup>-1</sup>) in Autumn 2018, which can be an indicator of its removal by higher biological  
392 productivity <sup>19,20,58,60</sup>. In-situ methylation rates in lakes have already been linked to the trophic

393 status, with increasing eutrophication leading to increasing organic matter loading and  
394 subsequent Hg methylation <sup>22</sup>.

#### 395 **Dissolved Gaseous Mercury (DGM) (Figure 2)**

396 Dissolved Gaseous Mercury (DGM) varied strongly, from 0.02 to 0.68 ng L<sup>-1</sup> (outliers  
397 excluded), and, overall, was higher than the measured DGM in other pristine areas (**Table 1**).  
398 Indeed, DGM in Bolivian lakes vary from 0.001 to 0.017 ng L<sup>-1</sup> in Lake Titicaca <sup>22</sup> and from 0.003  
399 to 0.125 ng L<sup>-1</sup> in Lake Uru Uru <sup>24</sup>. In the Lake Superior, in Canada, DGM measured in august  
400 2000 was 0.020 ± 0.003 ng L<sup>-1</sup> <sup>15</sup>. Even in the Adour Estuary, downstream to the Pyrenees, DGM  
401 content was lower than in the Pyrenean Lakes with values ranging from 0.024 to 0.056 ng L<sup>-1</sup> <sup>32</sup>.  
402 Finally, the DGM measured in the present study, with a median value of 0.11 ng L<sup>-1</sup>, was in the  
403 range of the open ocean waters <sup>57</sup>. DGM accounted for 25 ± 11 % of the Hg<sub>TOT</sub> and sometimes  
404 unexpectedly represents up to 55% of the total Hg.

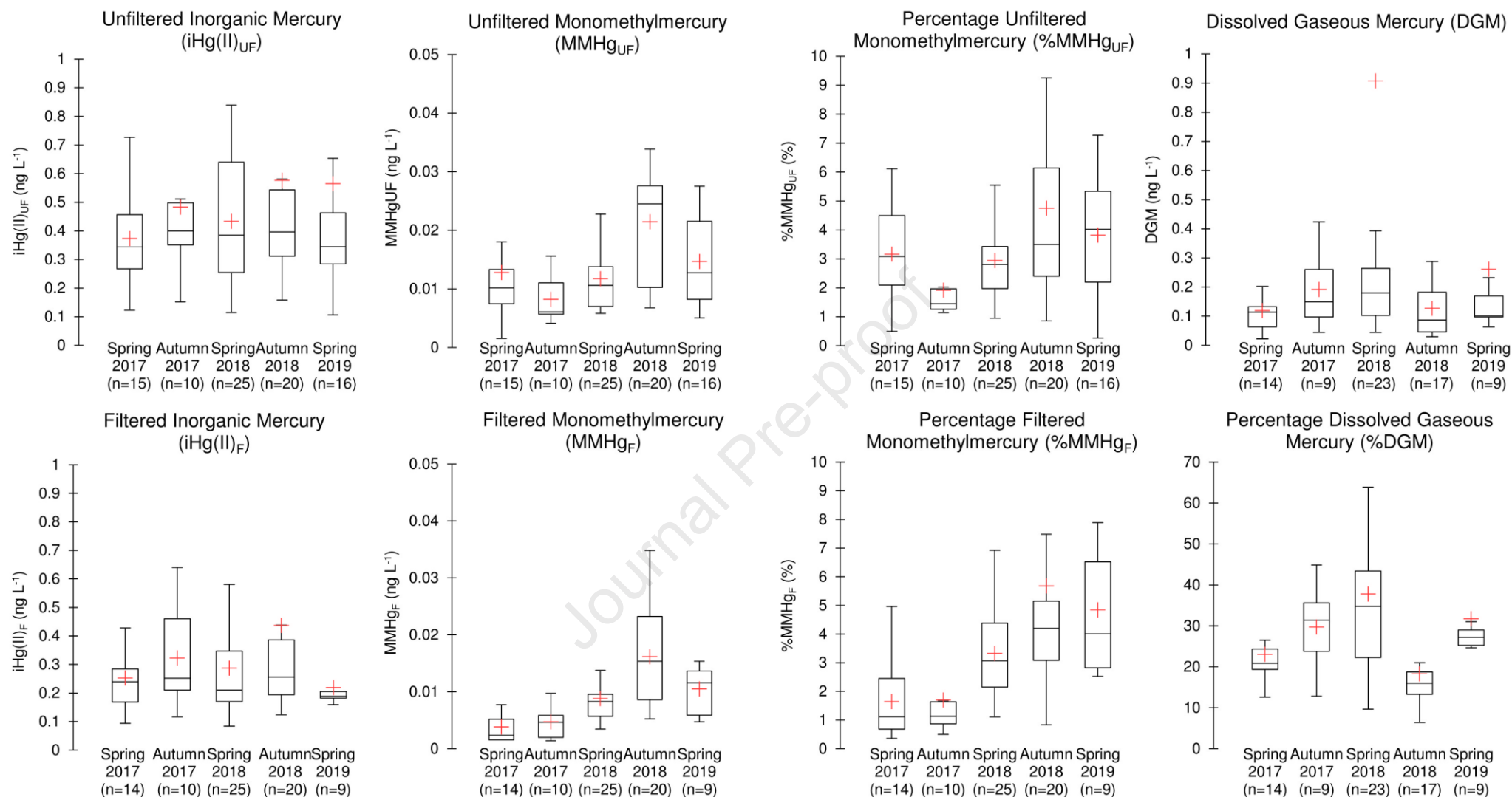
405 The homogeneous iHg(II) concentrations contrasts with the high DGM levels measured  
406 in the Pyreneans lakes of our study. Photoreduction is probably important in high-altitude lakes  
407 from the Pyrenees. This photoreduction might be triggered by important solar radiation, and the  
408 general oligotrophic state of the Pyrenean lakes, in comparison with Bolivian <sup>22,24</sup> or Canadian  
409 lakes <sup>28</sup>, might play a key role in this process. Indeed, organic matter is believed to have an impact  
410 on Hg photoreduction, and it is worth noting that DOC concentrations exhibit low to moderate  
411 values in the Pyrenean lakes (from 0.62 to 4.63 mg L<sup>-1</sup>). Higher Hg photoreduction to elemental  
412 Hg(0) therefore likely takes place in open water in which low to moderate DOC content has been  
413 observed <sup>57</sup>.

414 **Table 1:** Comparison of filtered and unfiltered inorganic mercury (iHg(II)), monomethylmercury (MMHg) and Dissolved Gaseous Mercury (DGM) concentrations in the  
 415 subsurface water samples of the 19 studied lakes with literature data for worldwide pristine areas (oceans, boreal lakes, high altitude lakes) and local areas (freshwaters and  
 416 estuary). %MMHg is calculated as the ratio between MMHg and non-gaseous Hg (MMHg + iHg(II)). %DGM is calculated as the ratio between DGM and total Hg ( $Hg_{TOT} =$   
 417  $MMHg + iHg(II) + DGM$ ). \*THg, \*\*Reactive Hg and \*\*\*Surface and Depth samples.

| Reference  | Location  | Elevation (m asl) | Sampling period           | iHg(II) <sub>F</sub><br>ng L <sup>-1</sup> | iHg(II) <sub>UF</sub><br>ng L <sup>-1</sup> | MMHg <sub>F</sub><br>(% MMHg <sub>F</sub> )<br>ng L <sup>-1</sup> | MMHg <sub>UF</sub><br>(% MMHg <sub>UF</sub> )<br>ng L <sup>-1</sup> | DGM<br>(% DGM)<br>ng L <sup>-1</sup>            |
|--|---|-------------------|---------------------------|--|---|---|---|---|
| This Work  | Central Pyrenees<br>(France/Spain)  | 1640 - 2600       | 2017-2019                 | 0.08 - 1.10<br>median = 0.23               | 0.11 - 1.19<br>median = 0.38                | <0.003 - 0.035<br>(4 ± 3%)<br>median = 0.008                      | <0.003 - 0.062<br>(4 ± 2%)<br>median = 0.011                        | 0.02 - 10.79<br>(29 ± 18%)<br>median = 0.12     |
| Fitzgerald et al. (2007) <sup>57</sup><br>(and references therein) | Equatorial Pacific Ocean<br>North Pacific Ocean<br>North Atlantic Ocean<br>South Atlantic Ocean |                   |                           | 0.03 - 0.39*                               | 0.08 - 1.38**                               | 0.209 ± 0.217   | <0.010 - 0.116  | 0.003 - 0.138<br>0.096 ± 0.062<br>0.241 ± 0.160 |
| Cavalheiro et al. (2016) <sup>33</sup>                             | Freshwaters (France)  |                   | 2012                      | <0.14 - 2.10                               |   | <0.04 - 0.14  |   |   |
| Sharif et al. (2014) <sup>32</sup>                                 | Adour Estuary (France)  |                   | 2007 & 2010               | 0.28 - 0.59                                | 0.40 - 2.66                                 | 0.014 - 0.054   | 0.018 - 0.124   | 0.024 - 0.056                                   |
| Emmerton et al. (2018) <sup>28</sup>                               | Boreal Lakes (Canada, n=50)   |                   | 2012 - 2016               |  | 0.36 - 5.33*                                |   | <0.01 - 0.344   |   |
| Bravo et al. (2017) <sup>29</sup>                                  | Boreal Lakes (Sweden, n=10)***  | 3 - 229           | 2012 & 2013               |  | 0.9 - 7.3                                   |   | 0.2 - 2.9   |   |
| Rolfhus et al. (2003) <sup>15</sup>                                | Lake Superior (Canada/USA)  | 183               | April 2000<br>August 2000 |  | 0.57 ± 0.07*<br>0.47 ± 0.03*                |   | 0.005 ± 0.001<br>0.008 ± 0.002                                      | 0.020 ± 0.003                                   |
| Meuleman et al. (1995) <sup>30</sup>                               | Lake Baïkal (Russia)***   | 456               | 1992 & 1993               | 0.14 - 0.77*                               |   | 0.002 - 0.038   |   |   |
| Malczyk and Branfireun (2015) <sup>31</sup>                        | Lake Zapotlán (Mexico)  | 1497              | 2007 & 2008               | 0.5 - 2.4*                                 | 0.9 - 10.7*                                 | 0.006 - 0.119   |   |   |
| Maruszczak et al. (2011) <sup>55</sup>                             | Lake (Alps, n=4)  | 1648 - 2448       | 2008                      |  | <0.1 - 3.12*                                |   | 0.002 - 0.005   |   |
| Alanoca et al. (2016) <sup>24</sup>                                | Uru Uru Lake (Bolivia)  | 3686              | 2010 & 2011               | 0.7 - 6.3                                  | 0.2 - 2.5                                   | 0.2 - 3.8   | 0.2 - 4.5   | 0.003 - 0.125                                   |
| Guédron et al. (2017) <sup>22</sup>                                | Lake Titicaca   | 3809              | 2013 & 2015               | 0.10 - 0.82*                               | 0.08 - 1.81*                                | 0.003 - 0.243   | 0.013 - 0.306   | 0.001 - 0.017                                   |

418

419



**Figure 2:** Boxplot representations of unfiltered and filtered iHg(II), MMHg and percentage of MMHg (calculated as the ratio between MMHg and non-gaseous Hg (MMHg + iHg(II))), and DGM and percentage of DGM (calculated as the ratio between DGM and total Hg ( $Hg_{TOT} = MMHg + iHg(II) + DGM$ )) in subsurface water samples of the 19 studied lakes. Bars indicate 10<sup>th</sup> and 90<sup>th</sup> percentile, boxes indicate 25<sup>th</sup> and 75<sup>th</sup>, marks within each box are medians, and red crosses are mean. Outliers' values especially measured for DGM are included in the graphic presentations (see Appendix 2).

### 3.2.2. Mercury compounds distribution in the water column of selected alpine lakes

**Figure 3** (Lake Gentau) and **Figure 4** (Lake Sabocos) display some physico-chemical parameters, including temperature, dissolved oxygen saturation (not corrected from altitude), chlorophyll-a, TOC, Silicate,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$  and  $\text{NO}_3^-$  that might be useful to understand the distribution of mercury species (iHg(II), MMHg and DGM) in the water column of these two different lakes intensively monitored during June 2018, October 2018 and June 2019. The results are also summarised in **Table A4**. Non stratified and shallow Lakes Arratille and Azul depth profiles (respectively **Figure A5** and **Figure A6**) are described in **Appendix 3**.

#### Lake Gentau (Figure 3)

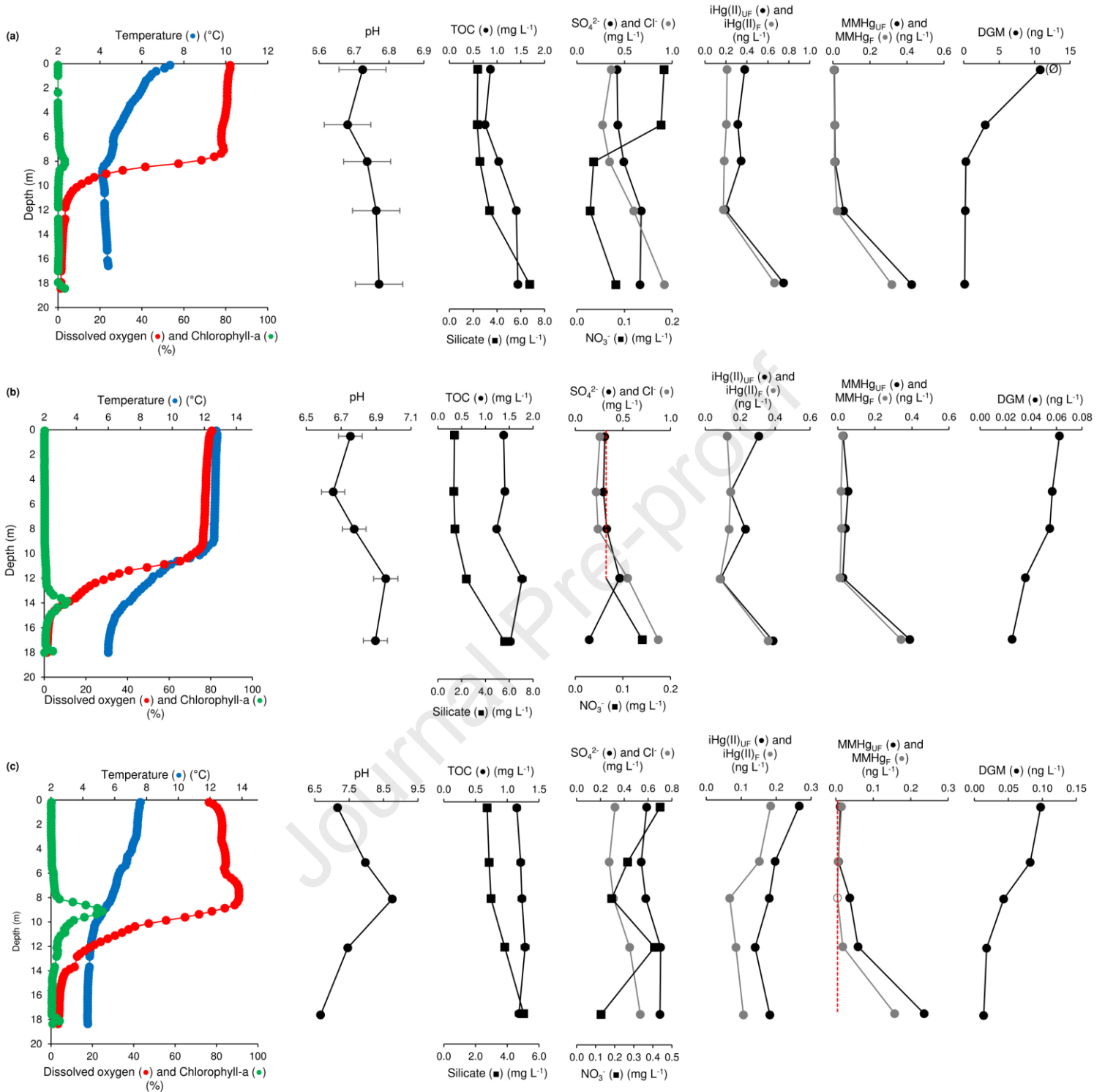
The water column of Lake Gentau was stratified in the three sampling campaigns (June 2018, October 2018, and June 2019). Indeed, this lake is divided in three various sections: epilimnion, metalimnion, and hypolimnion, all along the year except during the short overturn spring and autumn periods<sup>61</sup>. In addition to the oxygen chemocline (located around 7m depth in June 2018, 10m depth in October 2018 and 8m in June 2019), Gentau also presented a thermocline in the June 2018 sampling campaign (at 10m depth), which was here not matching the depth of the chemocline.

The highest iHg(II) concentrations, either unfiltered or filtered, were detected at the deepest sampling point of the lake in June (0.75 and 0.66 ng L<sup>-1</sup> in unfiltered and filtered samples, respectively) and October 2018 (0.39 and 0.36 ng L<sup>-1</sup> in unfiltered and filtered samples, respectively). In June 2019, the surface water samples were characterized by the highest iHg(II) concentrations (0.27 and 0.18 ng L<sup>-1</sup> in unfiltered and filtered samples, respectively). *Bravo et al.*<sup>29</sup> have shown that the highest total mercury concentrations in river stream systems were associated with important terrestrial DOM (Dissolved Organic Matter) and low nutrient content. In our study, TOC was low and relatively constant all along the water column during the three sampling campaigns (1.1 ± 0.3 mg L<sup>-1</sup> in June 2018, 1.5 ± 0.2 mg L<sup>-1</sup> in October 2018, and 1.2 ± 0.1 mg L<sup>-1</sup> in June 2019). In addition, nitrate ( $\text{NO}_3^-$ ), indicator of the biological productivity, decreased with depth in June 2018 (0.18 to 0.08 mg L<sup>-1</sup>) while it increased with depth in October

450 2018 ( $<0.06$  to  $0.14 \text{ mg L}^{-1}$ ), exhibiting the seasonal changes in primary productivity and water  
451 column remineralisation of nitrogen. Also, the important increase observed for  $\text{iHg(II)}$  in the  
452 deepest point of the lake in June and October 2018 is probably due to some inputs of  $\text{iHg(II)}$  at  
453 the water-sediment interface.

454 MMHg concentrations were significantly higher at the deepest point of the lake in  
455 comparison with the other four sampled points. Indeed, while unfiltered and filtered MMHg in the  
456 water column in June 2018 varied from  $0.008$  to  $0.057 \text{ ng L}^{-1}$  and  $0.006$  to  $0.023 \text{ ng L}^{-1}$   
457 respectively, MMHg unfiltered and filtered levels were respectively  $0.426$  and  $0.318 \text{ ng L}^{-1}$  at  $18$   
458 m depth. In October 2019, the same trend was observed for MMHg: unfiltered and filtered MMHg  
459 varied from  $0.027$  to  $0.055 \text{ ng L}^{-1}$  and  $0.011$  to  $0.026 \text{ ng L}^{-1}$ , respectively, while the deepest  
460 sampled point ( $17\text{m}$  depth) exhibited MMHg levels as high as  $0.388$  and  $0.341 \text{ ng L}^{-1}$  for the  
461 unfiltered and filtered samples. In June 2019, unfiltered and filtered MMHg concentrations varied  
462 from  $0.007$  to  $0.059 \text{ ng L}^{-1}$  and  $<0.004$  to  $0.019 \text{ ng L}^{-1}$  respectively, while it reached  $0.236$  and  
463  $0.157 \text{ ng L}^{-1}$  at the deepest sampling point ( $17.5\text{m}$  depth). Moreover, MMHg represents a higher  
464 fraction of the non-gaseous Hg species (MMHg and  $\text{iHg(II)}$ ) at the deepest sampled point, from  
465  $36$  to  $56\%$  for unfiltered MMHg and from  $32$  to  $60\%$  for filtered MMHg. Anoxic conditions observed  
466 in the deepest point of Lake Gentau can host anaerobic microbial activities responsible of in-situ  
467 Hg biotic methylation in both water and surface sediments<sup>49</sup>. Such anoxic conditions may not be  
468 permanent in those lakes but last for several months and affect MMHg burden in lake waters.  
469 Oxygen complete consumption in bottom waters is related to both dark respiration during the ice  
470 cover in winter/spring time and also to the "Bowl-shape" - basins (Gentau, Sabocos) in which the  
471 organic deposits from the whole lake will rapidly consume bottom waters oxygen between two  
472 vertical mixing events.

473 Finally, vertical distribution of DGM in Gentau was quite simple with a constant and  
474 progressive decrease from surface waters ( $10.79 \text{ ng L}^{-1}$  in June 2018;  $0.06 \text{ ng L}^{-1}$  in October  
475 2018;  $0.10 \text{ ng L}^{-1}$  in June 2019) to the deepest sampled point ( $0.14 \text{ ng L}^{-1}$  in June 2018 at  $18\text{m}$   
476 depth;  $0.03 \text{ ng L}^{-1}$  in October 2018 at  $17\text{m}$  depth;  $0.01 \text{ ng L}^{-1}$  in June 2019 at  $17.5\text{m}$  depth). DGM  
477 concentrations were also lower in October 2018 than in June 2018 and 2019 all along the water  
478 column. This supports the previous assumption regarding the importance of photoreduction  
479 processes in those high-altitude lakes, induced by significant solar radiation on surface waters.



505

**Figure 3:** Depth profiles of temperature, percentage of dissolved oxygen saturation, chlorophyll-a (RFU) and some other chemical parameters including mercury speciation obtained in (a) June 2018, (b) October 2018 and (c) June 2019 in Lake Gentau. Red dot points correspond to the LoD.  $\odot$  correspond to outlier data for DGM in surface water (Grubbs's test; see Table A3).

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509



**Lake Sabocos (Figure 4)**

Lake Sabocos is less stratified than Lake Gentau, but oxygen and temperature varied strongly along the water column with depletion of both physico-chemical parameters with depth, leading to almost anoxic conditions in the bottom part of this lake. Indeed, dissolved oxygen varied from 96 to 1% in June 2018, from 79 to 2% in October 2018 and from 84 to 5% in June 2019, and the chemocline, where dissolved oxygen starts decreasing, was located at 4, 10 and 5m in June 2018, October 2018, and June 2019, respectively. The thermocline was located at the same depths than chemocline and temperature varied from 17 to 5°C in June 2018, 11 to 6°C in October 2018 and 12 to 5°C in June 2019. This lake has also one specificity: it is the only one classified as an alkaline lake with pH values ranging from 7.47 to 7.71 in the surface water (June and October 2018).

iHg(II), either unfiltered or filtered, strongly varied all along the water column in the three sampling campaigns (respectively from 0.39 to 1.29 ng L<sup>-1</sup> and from 0.29 to 0.53 ng L<sup>-1</sup> in June 2018; from 0.53 to 0.90 ng L<sup>-1</sup> and from 0.17 to 0.68 ng L<sup>-1</sup> in October 2018; from 0.08 to 0.34 ng L<sup>-1</sup> and from 0.02 to 0.21 ng L<sup>-1</sup> in June 2019). There is no evidence for any specific trend in the iHg(II) distribution in the Lake Sabocos but the strong variations suggest that important Hg species transformations may occur.

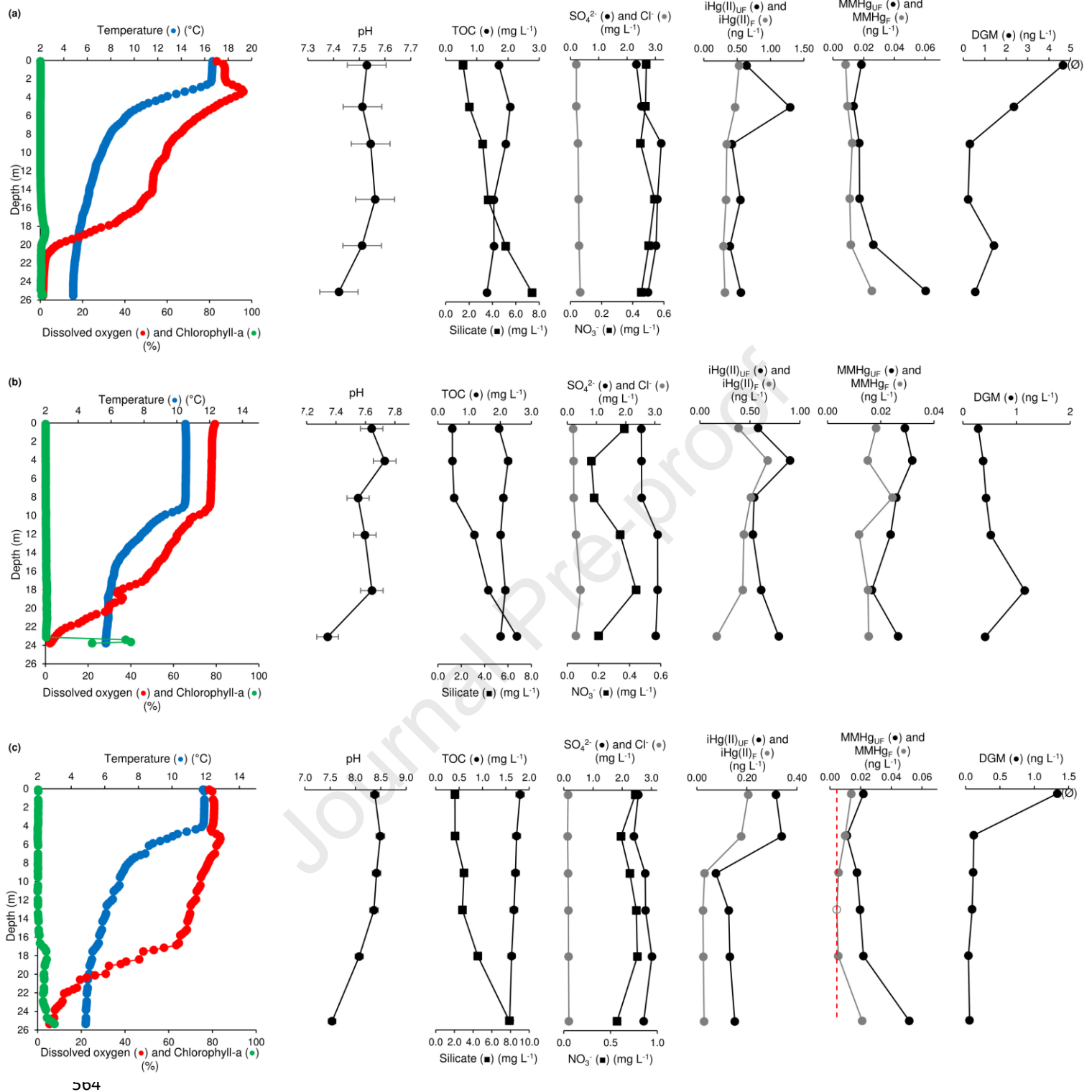
MMHg depth profiles in Lake Sabocos indicate potential in-situ methylation with the highest MMHg levels measured in the deepest part of the lake where the oxygen level was the lowest. MMHg concentrations in unfiltered et filtered waters were respectively 0.060 and 0.025 ng L<sup>-1</sup> (10 and 7% of the non-gaseous Hg), and 0.052 and 0.021 ng L<sup>-1</sup> (25 and 42% of the non-gaseous Hg) in June 2018 and 2019. Increase of MMHg levels was not observed in October 2018. During this sampling campaign, no maximum of chlorophyll was detected in the water column, probably indicating a lower biological production.

Regarding the DGM concentrations, the high levels measured in surface waters in both June 2018 and 2019 (4.65 and 1.34 ng L<sup>-1</sup> respectively), in comparison with deepest samples (0.56 and 0.06 ng L<sup>-1</sup> respectively), is consistent with intense photoreduction enhanced during spring conditions.

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539





**Figure 4:** Depth profiles of temperature, percentage of dissolved oxygen saturation, chlorophyll-a (RFU) and some other chemical parameters including mercury speciation obtained in (a) June 2018, (b) October 2018 and (c) June 2019 in Lake Sabocos. Red dot points correspond to the LoD. ∅ correspond to outlier data for DGM in surface water (Grubbs's test; see Table A3).

### 3.3. Mercury species transformations and volatilization in the water column of selected alpine lakes

Hg species incubation experiments were conducted in lakes Gentau, Sabocos and Arratille as described in **Section 2.5**, to quantify the importance of the Hg species transformations occurring in these high-altitude lakes. The obtained results are summarized in **Table 2**, together with transformation rates obtained in previous studies.

#### 3.3.1. Methylation and Demethylation pathways

##### Inorganic mercury Methylation

Methylation potentials under dark and diurnal conditions obtained in unfiltered water samples ranged between  $<0.03$  and  $6.97 \text{ \% day}^{-1}$  in Lake Gentau, between  $<0.03$  and  $0.95 \text{ \% day}^{-1}$  in Lake Sabocos, and between  $<0.03$  and  $0.44 \text{ \% day}^{-1}$  in Lake Arratille (**Table 2**). These methylation potentials are in agreement with data obtained for water samples in Canadian lakes<sup>26</sup>, high-altitude Bolivian lakes<sup>24</sup> and for marine and coastal waters<sup>32,47</sup> using similar experimental methods. The highest methylation potentials have been measured under dark conditions in the bottom anoxic zone for Lake Gentau which exhibit  $4.34 \pm 0.40 \text{ \% day}^{-1}$  in June 2018,  $6.97 \pm 0.44 \text{ \% day}^{-1}$  in October 2018 and  $1.63 \pm 0.11 \text{ \% day}^{-1}$  in June 2019. For Lake Sabocos methylation is  $0.81 \pm 0.33 \text{ \% day}^{-1}$  in June 2018,  $0.95 \pm 0.38 \text{ \% day}^{-1}$  in October 2018 and  $0.63 \pm 0.28 \text{ \% day}^{-1}$  in June 2019. No significant difference was observed for methylation potentials for incubation performed under dark or diurnal conditions, confirming that light induced methylation is not significant in those high-altitude pristine lakes. These results also support depth profile measurements and demonstrate that reducing conditions, especially in stratified anoxic waters, promote Hg methylation due to methylating anaerobic bacteria.

Lower but measurable methylation extent were determined in the oxic subsurface waters of Lake Gentau with  $0.42 \pm 0.12 \text{ \% day}^{-1}$  under diurnal conditions and  $0.40 \pm 0.17 \text{ \% day}^{-1}$  under dark conditions in June 2018, and  $0.21 \pm 0.17 \text{ \% day}^{-1}$  under diurnal conditions in June 2019. In Lake Sabocos, surface water methylation was  $0.09 \pm 0.05 \text{ \% day}^{-1}$  under diurnal conditions and  $0.26 \pm 0.02 \text{ \% day}^{-1}$  under dark conditions in June 2018, and  $0.34 \pm 0.25 \text{ \% day}^{-1}$  under diurnal conditions and  $0.17 \pm 0.07 \text{ \% day}^{-1}$  under dark conditions in October 2018. Lake Arratille exhibit

596 methylation potential of  $0.44 \pm 0.04 \text{ \% day}^{-1}$  under diurnal conditions in June 2018. The formation  
597 of MMHg in the oxic freshwater column is not fully understood yet. A recent work conducted in  
598 Lake Geneva highlights that particles sinking through oxygenated water column can be  
599 responsible for the production of MMHg<sup>27</sup>. In our study, although the methylation rates measured  
600 remain low, it is measurable and suggests that reducing conditions occurring in particles  
601 microenvironment within the oxic layer could also play a role.

602

### 603 **MMHg Demethylation**

604 Significant demethylation potentials were measured in the subsurface waters of Lake  
605 Gentau ( $23.8 \pm 4.4 \text{ \% day}^{-1}$  in June 2018;  $23.9 \pm 2.9 \text{ \% day}^{-1}$  in October 2018 and  $35.6 \pm 2.2 \text{ \%}$   
606  $\text{day}^{-1}$  in June 2019) and Lake Sabocos ( $35.2 \pm 9.7 \text{ \% day}^{-1}$  in June 2018;  $9.0 \pm 0.5 \text{ \% day}^{-1}$  in  
607 October 2018 and  $12.4 \pm 4.2 \text{ \% day}^{-1}$  in June 2019) only at daylight conditions. These important  
608 demethylation potentials have been observed in previous studies and were associated to both  
609 abiotic and biotic processes<sup>32,49</sup>. In our study, demethylation under dark conditions was detected  
610 only in Lake Sabocos within the oxicleine in June 2019 ( $6.8 \pm 4.7 \text{ \% day}^{-1}$ ), while in other lakes and  
611 season dark demethylation was always below  $4 \text{ \% day}^{-1}$ . Therefore, our results suggest that in  
612 high-altitude pristine lakes, direct light-induced photochemical demethylation in UV exposed  
613 surface waters is the most significant pathway that contributes to control the extent of MMHg in  
614 the water column<sup>23</sup>.

615 Lake Sabocos exhibited an interesting result regarding the middle depth (9m), only  
616 studied in June 2019. While in Lake Gentau significant demethylation was measured at the middle  
617 depth of the lake (8m) in June 2018 ( $13.6 \pm 1.2 \text{ \% day}^{-1}$ ) and June 2019 ( $13.6 \pm 0.4 \text{ \% day}^{-1}$ ) only  
618 under diurnal conditions and in lower extent than for the subsurface, Lake Sabocos exhibited  
619 significant demethylation at both diurnal and dark conditions in June 2019 ( $8.2 \pm 3.9 \text{ \% day}^{-1}$  and  
620  $6.8 \pm 4.7 \text{ \% day}^{-1}$  respectively). Thus, in both stratified lakes, demethylation occurred within the  
621 oxicleine and cannot be related to UV photochemical reactions (i.e., no UV light). These results  
622 suggest that specific microbial activity are involved due to heterotrophic (Gentau, Sabocos) and/or  
623 phototrophic (Sabocos) bacteria specifically developing at the oxycline (middle depth).

624

625

**626 Demethylation vs total MMHg loss**

627 As mentioned previously, demethylation, especially in subsurface waters, is important in  
628 Lake Gentau and Lake Sabocos. As a reminder, demethylation potentials calculated here  
629 correspond to the transformation of MMHg into iHg(II). In **Table 2**, in addition to the demethylation  
630 potentials, total loss of MMHg potential from the experimental water solution has also been  
631 determined. Total loss of MMHg correspond to the decrease of MMHg during the incubation and  
632 could be connected to both the transformation of MMHg into iHg(II), and the transformation of  
633 MMHg into Hg(0) or other precipitated insoluble forms. The linear regression between both MMHg  
634 degradation processes (excluding middle depth dark conditions of Lake Sabocos, June 2019)  
635 exhibits a very good coefficient of determination ( $R^2 = 0.97$ ; linear trend MMHg Loss =  $(1.7 \pm 0.1)$   
636  $\times$  Demethylation) (**Figure A7**). The covariation of both calculated potentials (demethylation and  
637 loss of MMHg) suggests that similar pathways occur during the different incubation experiments.  
638 These results demonstrate that a significant fraction (always below 50%) of the degraded MMHg,  
639 not recovered as soluble iHg(II), is converted to Hg(0) (reductive (photo)demethylation) or to  
640 insoluble Hg forms as final products.

641

**642 Hg Net methylation assessment**

643 In anoxic waters, the net methylation assessment (**Table 2**) allows to exhibit a potential  
644 significant production of MMHg in the bottom part of Lake Gentau with  $3.25 \pm 0.30 \text{ ng L}^{-1} \text{ day}^{-1}$  in  
645 June 2018,  $2.71 \pm 0.17 \text{ ng L}^{-1} \text{ day}^{-1}$  in October 2018, and  $0.29 \pm 0.02 \text{ ng L}^{-1} \text{ day}^{-1}$  in June 2019.  
646 While lower ones are determined in Lake Sabocos with  $0.45 \pm 0.19 \text{ ng L}^{-1} \text{ day}^{-1}$  in June 2018,  
647  $0.75 \pm 0.30 \text{ ng L}^{-1} \text{ day}^{-1}$  in October 2018, and  $0.09 \pm 0.04 \text{ ng L}^{-1} \text{ day}^{-1}$  in June 2019. On the one  
648 hand, at the subsurface, low potential production of MMHg was observed in Lake Arratille with  
649 net methylation of  $0.07 \pm 0.01 \text{ ng L}^{-1} \text{ day}^{-1}$  (diurnal condition) and  $0.07 \pm 0.04 \text{ ng L}^{-1} \text{ day}^{-1}$  (dark  
650 condition) in June 2018. On the other hand, under diurnal conditions, the subsurface waters of  
651 Lake Gentau and Lake Sabocos behave as a sink of MMHg, as net methylation remains negative  
652 and varies from  $-0.01 \pm 0.09$  to  $-0.64 \pm 0.08 \text{ ng L}^{-1} \text{ day}^{-1}$ .

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### 3.3.2. Reduction and volatilization from alpine lakes

#### 657 **Reduction and Volatilization Flux Density in selected alpine lakes (Arratille, Gentau and** 658 **Sabocos)**

659 High iHg(II) reduction potentials (**Table 2**) were measured under diurnal conditions in  
660 subsurface waters of Lake Gentau ( $81.2 \pm 0.8 \text{ \% day}^{-1}$  in June 2018;  $20.8 \pm 0.1 \text{ \% day}^{-1}$  in October  
661 2018 and  $16.9 \pm 0.1 \text{ \% day}^{-1}$  in June 2019) and Lake Sabocos ( $13.2 \text{ \% day}^{-1}$  in June 2019), and  
662 were less significant in subsurface waters of Lake Arratille ( $17.3 \pm 2.9 \text{ \% day}^{-1}$  in June 2018;  $2.8$   
663  $\pm 0.2 \text{ \% day}^{-1}$  in October 2018), but still remained among the highest in comparison to previous  
664 studies<sup>24,32,47</sup>. These important reduction potentials are consistent with the high DGM  
665 concentrations measured, especially in Lakes Gentau and Sabocos. Regarding the dark  
666 conditions, reduction potentials at subsurface waters were detected at lower extents only in June  
667 2018 ( $9.1 \pm 2.6 \text{ \% day}^{-1}$ ) and October 2018 ( $1.7 \pm 0.1 \text{ \% day}^{-1}$ ) for Lake Gentau, June 2019 ( $3.5$   
668  $\pm 0.1 \text{ \% day}^{-1}$ ) for Lake Sabocos. Therefore, intense UV light occurring in surface waters of high-  
669 altitude lakes promotes Hg reduction.

670 Using the reduction potentials and the non-gaseous Hg measured at the subsurface  
671 waters, we can estimate the amount of Hg reduced per day within the first meter depth under  
672 diurnal conditions and compare it with the Volatilization Flux Density (FD) calculated as described  
673 in **Section 2.6**. All the fluxes calculated are gathered in **Table A3**.

674 In Lake Gentau, the amount of Hg reduced per day was  $473 \pm 5 \text{ ng m}^{-2} \text{ day}^{-1}$  in June  
675 2018,  $54 \pm 1 \text{ ng m}^{-2} \text{ day}^{-1}$  in October 2018 and  $47 \pm 1 \text{ ng m}^{-2} \text{ day}^{-1}$  in June 2019. Median values  
676 of Volatilization FD in Lake Gentau were in the same order of magnitude with  $48 \text{ ng m}^{-2} \text{ day}^{-1}$  for  
677  $1 \text{ m s}^{-1}$  wind speed (from 12 to  $143 \text{ ng m}^{-2} \text{ day}^{-1}$ ) and  $72 \text{ ng m}^{-2} \text{ day}^{-1}$  for  $3 \text{ m s}^{-1}$  wind speed (from  
678 18 to  $216 \text{ ng m}^{-2} \text{ day}^{-1}$ ) (n=10). For Lake Sabocos, the quantity of Hg reduced per day was in the  
679 same range than Lake Gentau with  $46 \pm 1 \text{ ng m}^{-2} \text{ day}^{-1}$  in June 2019, and in the same order of  
680 magnitude than the Volatilization FD median values,  $121 \text{ ng m}^{-2} \text{ day}^{-1}$  for  $1 \text{ m s}^{-1}$  wind speed (from  
681 31 to  $288 \text{ ng m}^{-2} \text{ day}^{-1}$ ) and  $184 \text{ ng m}^{-2} \text{ day}^{-1}$  for  $3 \text{ m s}^{-1}$  wind speed (from 46 to  $436 \text{ ng m}^{-2} \text{ day}^{-1}$ )  
682 (n=4). In these two lakes, even if the Volatilization FD median values are not significantly different  
683 from the extent of Hg reduced per day, the overall range remains important. The amount of Hg  
684 reduced in Lake Arratille was lower than the other two lakes with  $31 \pm 5 \text{ ng m}^{-2} \text{ day}^{-1}$  in June 2018  
685 and  $14 \pm 1 \text{ ng m}^{-2} \text{ day}^{-1}$  in October 2018, which is also similar to the Volatilization FD calculated

686 (from 20 to 178 ng m<sup>-2</sup> day<sup>-1</sup> for 1 m s<sup>-1</sup> wind speed and from 30 to 270 ng m<sup>-2</sup> day<sup>-1</sup> for 3 m s<sup>-1</sup>  
687 wind speed (n=7)). Overall, the gaseous Hg evasion estimated from the first meter of the water  
688 column (subsurface) represents a similar extent than the reduction potential obtained from our  
689 incubation experiments. Our results strongly support that UV light induced in-situ reduction is a  
690 major pathway controlling significant volatilization of Hg from these alpine lakes.

691

#### 692 **Volatilization Flux Density (FD) in all the studied lakes and comparison with other fluxes**

693 Median values of Volatilization FD in all the lakes (except Arratille, Sabocos and Gentau  
694 discussed below) were 60 ng m<sup>-2</sup> day<sup>-1</sup> for 1 m s<sup>-1</sup> wind speed (from 7 to 375 ng m<sup>-2</sup> day<sup>-1</sup>) and 92  
695 ng m<sup>-2</sup> day<sup>-1</sup> for 3 m s<sup>-1</sup> wind speed (from 11 to 568 ng m<sup>-2</sup> day<sup>-1</sup>). Volatilization FD did not vary  
696 strongly depending on the sampling campaign with median values from 45 to 111 ng m<sup>-2</sup> day<sup>-1</sup> (1  
697 m s<sup>-1</sup>) and from 68 to 168 ng m<sup>-2</sup> day<sup>-1</sup> (3 m s<sup>-1</sup>).

698 It is interesting to compare these estimated Volatilization FD with wet Hg deposition fluxes  
699 (direct input and snow-ice melt), and Hg accumulation rates in sediments. The latter is commonly  
700 used as a probe for past Hg contamination in alpine lakes and atmospheric burden.

701 Hg concentration in wet deposition at the peatland of Pinet (Central Pyrenees, 880m asl)  
702 was recently measured at 8.0 ± 4.6 ng L<sup>-1</sup> <sup>54</sup>. Considering that annual precipitation is 1161 mm at  
703 Pinet peat (average for the period 2010-2013) <sup>54</sup> and 2000 mm at lake Marboré (Central Pyrenees,  
704 2612 m asl) <sup>3</sup>, the studied lakes in this work (from 1620 m asl to 2600 m asl) are subjected to 25  
705 to 44 ng m<sup>-2</sup> day<sup>-1</sup> Hg inputs from wet deposition. This is well in accordance with model results of  
706 Hg deposition in the Pyrenees Region from European Monitoring and Evaluation Programme  
707 (EMEP), ranging from 33 to 49 ng m<sup>-2</sup> day<sup>-1</sup> (Website. <http://www.emep.int/>). The median  
708 Volatilization FD calculated in this work are 60 to 92 ng m<sup>-2</sup> day<sup>-1</sup> (1 and 3 m s<sup>-1</sup> wind speed,  
709 respectively) which is slightly above the maximum wet deposition. Nevertheless, considering the  
710 surface of the studied lakes the median Volatilization Flux Density can be estimated to be 2 to 3  
711 mg day<sup>-1</sup>, while considering the surface of the catchment the wet deposition inputs to Pyrenean  
712 high-altitude lakes can be estimated to be 45 to 79 mg day<sup>-1</sup>. We conclude that Hg evasion does  
713 not constitute a major Hg loss at the catchment scale, but it will drastically affect its water column  
714 biogeochemistry.

715 Lake Marboré has been deeply studied<sup>3,62,63</sup>, and one recent publication is also dedicated  
716 to the Hg in sediment cores from this lake (*Duval, PhD Thesis*<sup>40</sup>). In this study, the most recent  
717 sample (2004 AD) exhibits a Hg accumulation rate of  $40 \mu\text{g m}^{-2} \text{year}^{-1}$ , which corresponds to an  
718 average daily Hg fluxes of  $110 \text{ ng m}^{-2} \text{day}^{-1}$ . This flux is in the same range as the median  
719 Volatilization Flux Density calculated with the gas exchange model.

720 Overall, the evasion fluxes calculated for the alpine lakes are very significant, and due to  
721 the high DGM levels measured in these lakes. It is worth noting that these estimations are made  
722 using an empirical water-air gas exchange model providing a rough estimate, and that the  
723 calculated Volatilization Flux Density corresponds to the free-ice period (negligible during ice  
724 cover). However, it is clear that the Hg deposited in bottom lake sediments will be drastically  
725 affected by DGM evasion and direct input from snow melt and runoff.

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**Table 2:** Methylation (M), Demethylation (D), MMHg Loss (L), Net Methylation (NM) and Reduction (R) potentials (mean  $\pm$  SD, n=3 for M, D, L and NM, n=2 for R) in unfiltered waters performed under varying light and dark conditions at different depths for Lakes Gentau, Sabocos and Arratille and for sampling campaigns June and October 2018 and June 2019, together with data from the literature. Detection limits are 0.03, 4, 4 and 1 % day<sup>-1</sup> for Methylation, Demethylation, MMHg Loss and Reduction yields, respectively. n.d. is not determined.

| Location   | Sampling Period | Sampling Type     | Incubation Time (h) | iHg(II) Methylation (%day <sup>-1</sup> ) |               | MMHg Demethylation (%day <sup>-1</sup> ) |               | MMHg Loss (%day <sup>-1</sup> ) |                | Hg Reduction (%day <sup>-1</sup> ) |               | Net Methylation (ng L <sup>-1</sup> day <sup>-1</sup> ) |                  |
|--|-----------------|-------------------|---------------------|---|---------------|--|---------------|---------------------------------|----------------|------------------------------------|---------------|---|------------------|
|  |                 |                   |                     | Diurnal                                   | Dark          | Diurnal                                  | Dark          | Diurnal                         | Dark           | Diurnal                            | Dark          | Diurnal   | Dark             |
| <b>Lake Gentau</b><br><b>(Central Pyrenees)</b>  | June 2018       | Subsurface (0.5m) | 7.4                 | 0.4 $\pm$ 0.1                             | 0.4 $\pm$ 0.2 | 23.8 $\pm$ 4.4                           | <LOD          | 44.5 $\pm$ 4.4                  | 7.8 $\pm$ 1.9  | 81.2 $\pm$ 0.8                     | 9.1 $\pm$ 2.6 | -0.01 $\pm$ 0.09  | 0.23 $\pm$ 0.10  |
|  |                 | Middle Depth (8m) | 8.5                 | <LOD                                      | <LOD          | 13.6 $\pm$ 1.2                           | <LOD          | 23.3 $\pm$ 0.3                  | 8.7 $\pm$ 3.6  | 19.5 $\pm$ 0.3                     | 5.2 $\pm$ 0.6 | -0.17 $\pm$ 0.01  | -0.01 $\pm$ 0.09 |
|  |                 | Bottom (17m)      | 8.7                 | n.d.                                      | 4.3 $\pm$ 0.4 | n.d.                                     | <LOD          | n.d.                            | 7.0 $\pm$ 3.3  | n.d.                               | <LOD          | n.d.  | 3.25 $\pm$ 0.30  |
|  | October 2018    | Subsurface (0.5m) | 7.2                 | <LOD                                      | <LOD          | 23.9 $\pm$ 2.9                           | <LOD          | 41.7 $\pm$ 7.8                  | 23.2 $\pm$ 2.8 | 20.8 $\pm$ 0.1                     | 1.7 $\pm$ 0.1 | -0.64 $\pm$ 0.08  | <LOD             |
|  |                 | Middle Depth (8m) | 6.0                 | 0.4 $\pm$ 0.2                             | 0.5 $\pm$ 0.2 | <LOD                                     | <LOD          | 9.1 $\pm$ 7.4                   | <LOD           | <LOD                               | <LOD          | 0.08 $\pm$ 0.05   | 0.11 $\pm$ 0.06  |
|  |                 | Bottom (17m)      | 7.2                 | n.d.                                      | 7.0 $\pm$ 0.4 | n.d.                                     | <LOD          | n.d.                            | 28.9 $\pm$ 8.6 | n.d.                               | <LOD          | n.d.  | 2.71 $\pm$ 0.17  |
|  | June 2019       | Subsurface (0.5m) | 8.7                 | 0.2 $\pm$ 0.2                             | <LOD          | 35.6 $\pm$ 2.2                           | <LOD          | 61.5 $\pm$ 7.0                  | <LOD           | 16.9 $\pm$ 0.1                     | <LOD          | -0.43 $\pm$ 0.07  | <LOD             |
|  |                 | Middle Depth (8m) | 8.5                 | <LOD                                      | <LOD          | 13.6 $\pm$ 0.4                           | <LOD          | 31.5 $\pm$ 8.3                  | <LOD           | 3.7 $\pm$ 1.4                      | <LOD          | -0.51 $\pm$ 0.01  | <LOD             |
|  |                 | Bottom (17m)      | 8.5                 | n.d.                                      | 1.6 $\pm$ 0.1 | n.d.                                     | <LOD          | n.d.                            | <LOD           | n.d.                               | <LOD          | n.d.  | 0.29 $\pm$ 0.02  |
| <b>Lake Sabocos</b><br><b>(Central Pyrenees)</b> | June 2018       | Subsurface (0.5m) | 6.3                 | 0.1 $\pm$ 0.0                             | 0.3 $\pm$ 0.0 | 35.2 $\pm$ 9.7                           | <LOD          | 52.8 $\pm$ 15.4                 | <LOD           | n.d.                               | n.d.          | -0.52 $\pm$ 0.18  | 0.18 $\pm$ 0.01  |
|  |                 | Bottom (27m)      | 6.3                 | n.d.                                      | 0.8 $\pm$ 0.3 | n.d.                                     | <LOD          | n.d.                            | <LOD           | n.d.                               | n.d.          | n.d.  | 0.45 $\pm$ 0.19  |
|  | October 2018    | Subsurface (0.5m) | 6.3                 | 0.3 $\pm$ 0.2                             | 0.2 $\pm$ 0.1 | 9.0 $\pm$ 0.5                            | <LOD          | 12.2 $\pm$ 3.7                  | 4.3 $\pm$ 1.7  | n.d.                               | n.d.          | -0.06 $\pm$ 0.13  | 0.10 $\pm$ 0.04  |
|  |                 | Bottom (23m)      | 6.3                 | n.d.                                      | 1.0 $\pm$ 0.4 | n.d.                                     | <LOD          | n.d.                            | <LOD           | n.d.                               | n.d.          | n.d.  | 0.75 $\pm$ 0.30  |
|  | June 2019       | Subsurface (0.5m) | 6.8                 | <LOD                                      | <LOD          | 12.4 $\pm$ 4.2                           | <LOD          | 14.8 $\pm$ 7.5                  | <LOD           | 13.2                               | 3.5 $\pm$ 0.1 | -0.19 $\pm$ 0.06  | <LOD             |
|  |                 | Middle Depth (9m) | 6.8                 | 0.2 $\pm$ 0.2                             | 0.1 $\pm$ 0.0 | 8.2 $\pm$ 3.9                            | 6.8 $\pm$ 4.7 | 22.5 $\pm$ 4.6                  | 24.7 $\pm$ 2.3 | 3.7 $\pm$ 0.4                      | 2.4 $\pm$ 0.2 | -0.14 $\pm$ 0.07  | -0.11 $\pm$ 0.08 |
|  |                 | Bottom (25m)      | 6.8                 | n.d.                                      | 0.6 $\pm$ 0.3 | n.d.                                     | <LOD          | n.d.                            | <LOD           | n.d.                               | <LOD          | n.d.  | 0.09 $\pm$ 0.04  |



Table 2 (continued)  
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|   |              |  |     |             |             |              |              |             |           |            |            |                           |             |  |
|---|--------------|--|-----|-------------|-------------|--------------|--------------|-------------|-----------|------------|------------|---------------------------|-------------|--|
| <b>Lake Arratille<br/>(Central Pyrenees)</b>          | June 2018    | Subsurface (0.5m)                            | 6.0 | 0.4 ± 0.0   | n.d.        | <LOD         | n.d.         | 22.6 ± 15.7 | n.d.      | 17.3 ± 2.9 | 13.6 ± 0.8 | 0.07 ± 0.01               | 0.07 ± 0.04 |  |
|   |              | Middle Depth (6m)                            | 5.3 | 0.4 ± 0.2   | <LOD        | <LOD         | <LOD         | 16.4 ± 1.4  | 7.8 ± 0.6 | n.d.       | 18.7 ± 5.4 | n.d.                      | <LOD        |  |
|   | October 2018 | Subsurface (0.5m)                            | 5.3 | n.d.        | n.d.        | n.d.         | n.d.         | n.d.        | n.d.      | 2.8 ± 0.2  | <LOD       | n.d.                      | n.d.        |  |
|   |              | Bottom (12m)                                 | 6.7 | n.d.        | n.d.        | n.d.         | n.d.         | n.d.        | n.d.      | n.d.       | <LOD       | n.d.                      | n.d.        |  |
| <b>Lake (Canada)</b> <sup>26</sup>                    | 2002         | Oxycline                                     | 24  | 0.6 - 14.8  |             |              |              |             |           | <12        |            |                           |             |  |
| <b>Mediterranean Sea</b><br><sub>47</sub>             | 2003 & 2004  | Surface Water                                | 24  | <0.02 - 6.3 | <0.02 - 3.8 | 3.3 - 24.5   | <1.5 - 10.9  |             |           | 1.1 - 16.9 | 1.0 - 12.3 |                           |             |  |
| <b>Lake Moreno<br/>(Argentina)</b> <sup>64</sup>      | April 2007   | Upper limit of<br>metalimnion (30m<br>depth) | 72  | 27.3 - 50.8 | 15.4 - 23.5 |              |              |             |           |            |            |                           |             |  |
| <b>Arcachon Bay<br/>(France)</b> <sup>49</sup>        | 2006 & 2007  | Water column                                 | 24  | <0.02 - 0.8 | <0.02 - 1.1 | 1.8 - 11.9   | 1.3 - 9.0    |             |           |            |            |                           |             |  |
| <b>Adour river estuary<br/>(France)</b> <sup>32</sup> | 2007 & 2010  | Surface Water                                | 24  | <0.01 - 0.4 | <0.01 - 0.1 | 6.6 - 55.3   | <2.0 - 22.1  |             |           | 4.3 - 43.5 | 0.3 - 14.7 | -0.02 - 0.00              |             |  |
| <b>Lake Uru Uru<br/>(Bolivia)</b> <sup>24</sup>       | 2010 & 2011  | Surface Water                                | 24  | <0.02 - 4.9 | <0.02 - 7.7 | <0.02 - 21.0 | <0.02 - 20.5 |             |           | 0.1 - 1.0  |            | -0.11 - 0.20 -0.06 - 0.45 |             |  |

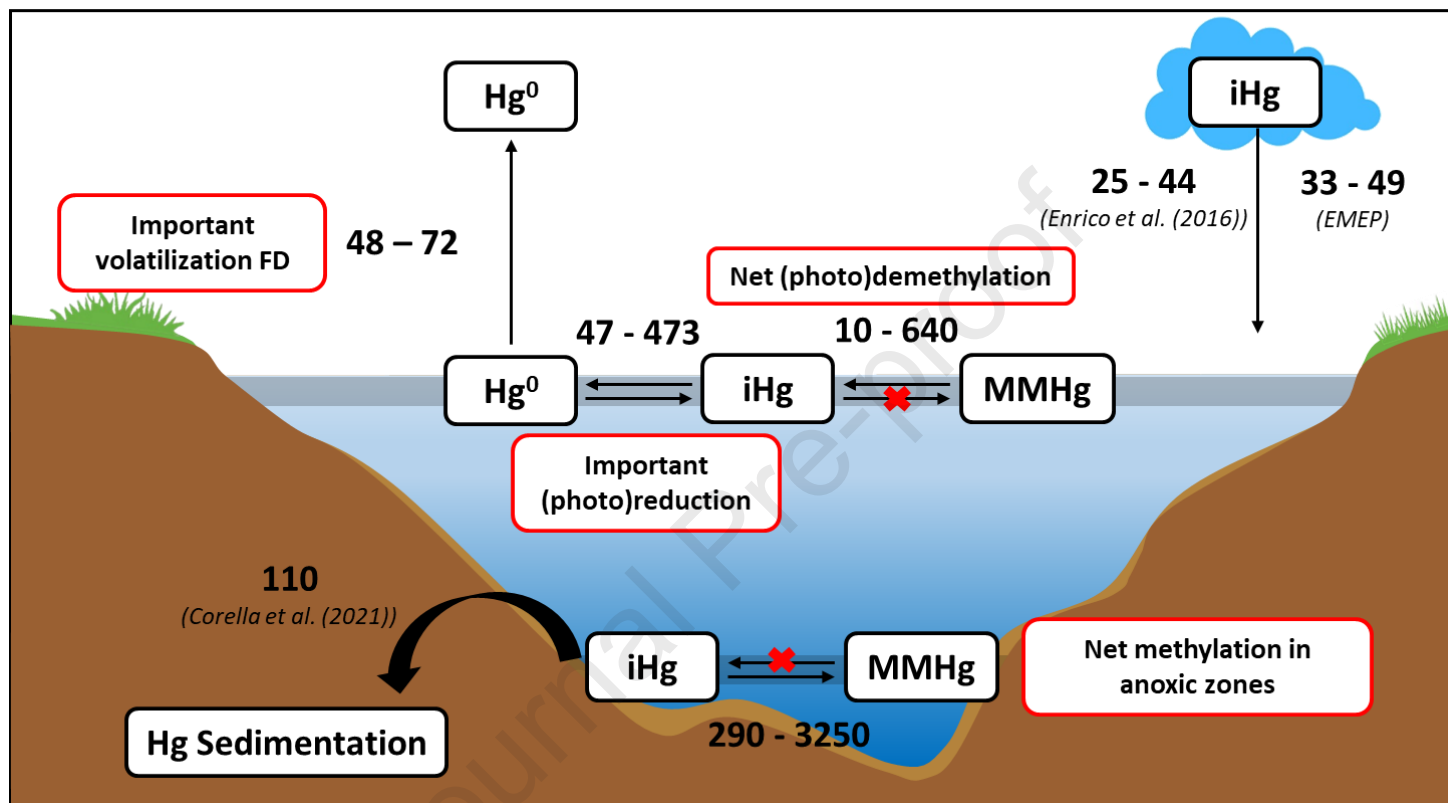
**758 Implication for Hg cycling in alpine lakes and climate driven perspectives**

759 Overall, this work focusing on the water column provides a first global picture of the Hg  
760 cycling in the high-altitude lake's ecosystems including various transformations rates measured  
761 and estimated volatilization fluxes for the first time. As an example, results obtained from Lake  
762 Gentau (**Figure 5**) allow us to estimate the fate of Hg in such stratified alpine lakes and to predict  
763 the influence of changes in the hydrological cycle, the water column stratification, and the  
764 biological productivity due to anthropogenic pressure and/or climate conditions. Indeed, important  
765 methylation ( $290$  to  $3250$   $\text{ng m}^{-3} \text{day}^{-1}$ ) occurs in the deepest and anoxic zone of this lake with  
766 possible harmful impacts on the biota, while important (photo)demethylation ( $10$  to  $640$   $\text{ng m}^{-3}$   
767  $\text{day}^{-1}$ ) and (photo)reduction ( $47$  to  $473$   $\text{ng m}^{-3} \text{day}^{-1}$ ) leading to Hg evasion ( $50$  to  $75$   $\text{ng m}^{-2} \text{day}^{-1}$ )  
768 take place in the surface of the lake. Future in-depth studies must be conducted on these sentinel  
769 ecosystems to better constrain fluxes and transformations and obtain a more accurate mass  
770 balance of Hg species. Consequently, further experimental studies are clearly required to assess  
771 the effect of lake waters stratification, to quantify atmospheric deposition via direct input and  
772 snow/ice melt and to evaluate the eutrophication status and major biogeochemical changes.

773 Direct changes in the alpine ecosystem due to human activities (e.g. tourism,  
774 agropastoralism) or more global forcing due to climate change, have a potential effect on major  
775 drivers such as water temperature or light incidence. These effects will thus have drastic  
776 consequences on the Hg cycle at high altitude lakes that can be anticipated as follow: first, warmer  
777 conditions might increase lake water stratification and anoxic conditions in bottom waters that can  
778 promote Hg methylation by microorganisms. Such conditions can also increase both Hg  
779 (photo)reduction and evasion from surface waters to the atmosphere as we observed during this  
780 study. However large uncertainties remain in those evaluations considering that major changes  
781 are also expected with atmospheric deposition at local scale and that specific evolution of the  
782 lakes depends on their geographic and topographic situations. In this work, we addressed for the  
783 first time the importance of photoreduction on the fate of Hg through gaseous Hg evasion flux  
784 densities from alpine lakes. In addition, methylation and mainly photodemethylation extents were  
785 simultaneously measured in this work, allowing to calculate the net methylation rate to assess the  
786 net MMHg balance of these competitive pathways under different seasonal and annual  
787 conditions. These preliminary assessments provide here some first conclusions on the potential

788 effect of global or climate changing drivers on the Hg cycling and impact in those “sentinel”  
 789 ecosystems. However more complex features were not taken into account in this work and might  
 790 be involved especially during winter ice cover period, very warm summer conditions or water  
 791 column mixing events.

792



**Figure 5:** Hg transformations (ng  $\text{m}^{-3} \text{day}^{-1}$ ) and fluxes (ng  $\text{m}^{-2} \text{day}^{-1}$ ) in Lake Gentau. Red crosses are pathways not measured in this study.

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803 **References**

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Journal Pre-proof



1 **Appendices: Dynamics, distribution, and transformations of**  
2 **mercury species from Pyrenean high-altitude lakes**

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## 27 **Appendix 1: Recovery of gaseous and non-volatile Hg species during sample processing**

28 One concern drove our attention regarding the results for Hg species. Indeed, the unfiltered non-  
29 gaseous mercury, calculated as the sum of iHg(II) and MMHg, ranges from 0.11 to 3.13 ng L<sup>-1</sup> in the  
30 subsurface water samples with a median value of 0.39 ng L<sup>-1</sup>, while DGM can be very significant and ranges  
31 from 0.02 to 10.79 ng L<sup>-1</sup> with a median value of 0.11 ng L<sup>-1</sup>. As a consequence, the recovery of DGM,  
32 mainly as Hg(0) (and potentially as DMHg), in collected water samples filtration, acidification and storage is  
33 always questionable. To answer properly to that question, in the last sampling campaigns June 2019, we  
34 collected in 125 mL Teflon container the water sample that has been previously purged to collect DGM  
35 species on gold-coated sand trap. These “purged samples” were acidified adding high-purity HCl (1% v/v)  
36 (Trace metal grade) and analysed by GC-ICP-MS following the protocol described in **2. Material and**  
37 **Methods**. The **Figure A2** shows a comparison between results obtained in purged and unpurged unfiltered  
38 samples for both methylated (MMHg) and inorganic (iHg(II)) Hg. On the one hand, with good coefficient of  
39 determination ( $R^2 = 0.91$ ) and a slope not significantly different from 1 ( $0.94 \pm 0.07$ ), the results for iHg(II)  
40 analysis confirm the fact that gaseous Hg (DGM) is not a significant contributor to Hg(II) measured in  
41 unpurged samples. On the other hand, the slope obtained for the MMHg analysis is lower than 1 ( $R^2 = 0.98$ ,  
42 slope =  $0.75 \pm 0.02$ ), thus suggesting that in unpurged samples some DMHg was also occurring. This was  
43 further confirmed by the detection of DMHg ( $< 0.2$  pg L<sup>-1</sup>) in carbotrap columns, used in a parallel study to  
44 quantify the volatile selenium species<sup>1,2</sup>. Indeed, in the last depth of Lake Gentau (17.5m, anoxic zone) and  
45 in the last two depths of Lake Sabocos (18 and 25m, anoxic zone) and the middle depths of Lake Sabocos  
46 (9 and 13m, maximum chlorophyll-a, witness of algae production), DMHg was detected in the carbotrap  
47 columns. Regarding the methylated species quantified by GC-ICPMS, removing the samples exhibiting  
48 suboxic to anoxic conditions (three last depths from Gentau, 8, 12 and 17.5m; last depth from Sabocos  
49 25m), the slope becomes not significantly different from 1 ( $R^2 = 0.91$ , slope =  $0.92 \pm 0.09$ ). Overall, in most  
50 of the cases, especially regarding subsurface samples, DMHg is negligible in comparison with MMHg, and  
51 the measured methylated species are mainly in the form of MMHg.

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## 55 Appendix 2: Hg measurement outliers for Total Hg ( $Hg_{TOT}$ ) and DGM

56 Regarding total Hg ( $Hg_{TOT}$ ), defined here as the sum of concentrations of  $iHg(II)$ , MMHg and DGM,  
57 in unfiltered samples (DGM only measured in unfiltered samples), seven specific samples stood out as  
58 outliers (Grubbs's tests,  $p$ -value < 0.05 then  $p$ -value = 0.063 > 0.05).

59 Major five outliers concern two specific and particular lakes : Lake Gentau (June 2018, 10:50 a.m.)  
60 and Lake Sabocos (June 2018, 09:45 a.m., 00:35 p.m. and 3:35 p.m.; June 2019, 00:30 p.m.). This is mainly  
61 because Lakes Gentau and Sabocos exhibited important DGM with extremely high values around midday  
62 (DGM = 10.79  $ng\ L^{-1}$  in June 2018 for Lake Gentau; DGM = 4.65  $ng\ L^{-1}$  in June 2018 and DGM = 1.34  $ng\ L^{-1}$   
63  $L^{-1}$  in June 2019 for Lake Sabocos) when the light incidence was the highest (**Figure A4**). The explanation  
64 is not clear, but this important DGM might be due to i) accumulation of DGM under the ice cover during the  
65 winter or ii) important  $iHg(II)$  provided by the snow melt then converted into  $Hg(0)$  by reduction pathways. In  
66 both cases, it implies that the sampling has been done right after the spring period, where the lakes are  
67 frozen and the catchment is covered by snow, which is the case in June 2018. Indeed, a few days before  
68 the sampling, more than 90 % of the studied lakes were completely frozen. However, it remains difficult to  
69 explain such exceptionally high levels of DGM measured only in these two lakes. Further studies, with  
70 intensive monitoring and sampling during several days at the snowmelt period are needed to fully  
71 understand the mechanisms occurring in those Pyrenean Lakes. To avoid bias interpretation, these five  
72 samples are discarded for the discussion on DGM levels and volatilization in high-altitude lakes.

73 In Lake Coanga in October 2018 at 8:50 a.m., very high levels of  $iHg(II)$  were measured in both  
74 filtered ( $iHg(II)_F = 2.68\ ng\ L^{-1}$ ) and unfiltered ( $iHg(II)_{UF} = 2.88\ ng\ L^{-1}$ ) samples. In this sample, filtered and  
75 unfiltered MMHg and DGM does not differ strongly from the other samples suggesting that this high  $iHg(II)$ ,  
76 either filtered or unfiltered, might be due punctual  $iHg(II)$  inputs or simply to contamination during the sample  
77 collection: this sample will be discarded from the discussion on  $iHg(II)$ , and consequently from  $Hg_{TOT}$ . In  
78 Lake Panticosa in October 2017 at 00:30 p.m., the relatively high levels of both  $iHg(II)$  ( $iHg(II)_F = 0.64\ ng\ L^{-1}$ ;  
79  $iHg(II)_{UF} = 0.68\ ng\ L^{-1}$ ) and DGM (DGM = 1.21  $ng\ L^{-1}$ ) suggested that this specific sample was enriched  
80 in Hg, probably coming either from the catchment (the biggest one, 3229 Ha) or from punctual high Hg  
81 inputs.

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83 **Appendix 3: Mercury species distribution in the water column of Lake Arratille (Figure A5) and Lake**  
84 **Azul (Figure A6)**

85 In the shallow alpine Lake Arratille (2256 m asl.), physico-chemical parameters measured in-situ  
86 with the multiparameter probe (temperature, oxygen and chlorophyll) did not vary strongly within the water  
87 column. Indeed, the average temperature was  $5.0 \pm 0.4$  °C in June 2018 and  $8.0 \pm 0.9$  °C in October 2018,  
88 and the average dissolved oxygen was  $65 \pm 23$  % in June 2018 and  $71 \pm 10$  % in October 2018.  
89 Nevertheless, it is worth noting that a short decrease in both temperature and oxygen is observed in the last  
90 2 meters of the lake in both June and October 2018. Concerning the mercury species distribution, no  
91 significant difference was observed for unfiltered and filtered iHg(II) ( $0.26 \pm 0.10$  and  $0.18 \pm 0.05$  ng L<sup>-1</sup>,  
92 respectively), unfiltered and filtered MMHg ( $0.010 \pm 0.04$  and  $0.008 \pm 0.03$  ng L<sup>-1</sup>, respectively) and DGM  
93 ( $0.13 \pm 0.06$  ng L<sup>-1</sup>) along the water column, suggesting a well-mixed lake with clear water.

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95 In the shallower and more elevated Lake Azul (2420 m asl.), dissolved oxygen ( $76 \pm 1$  % in June  
96 2018 and  $72 \pm 2$  % in October 2018) and temperature ( $4.8 \pm 0.1$  °C in June 2018 and  $5.1 \pm 0.2$  °C in October  
97 2018) did not vary at all along the water column. Regarding Hg species, on the one hand, no specific trend  
98 was observed in June 2018 for unfiltered and filtered iHg(II) ( $0.36 \pm 0.05$  and  $0.30 \pm 0.02$  ng L<sup>-1</sup>, respectively)  
99 unfiltered and filtered MMHg ( $0.006 \pm 0.001$  and  $0.004 \pm 0.001$  ng L<sup>-1</sup>, respectively) and DGM ( $0.12 \pm 0.04$  ng  
100 L<sup>-1</sup>). On the other hand, a significant increase was observed with depth in October 2018 for all Hg  
101 compounds, probably as a result of sediment punctual remobilisation of Hg.

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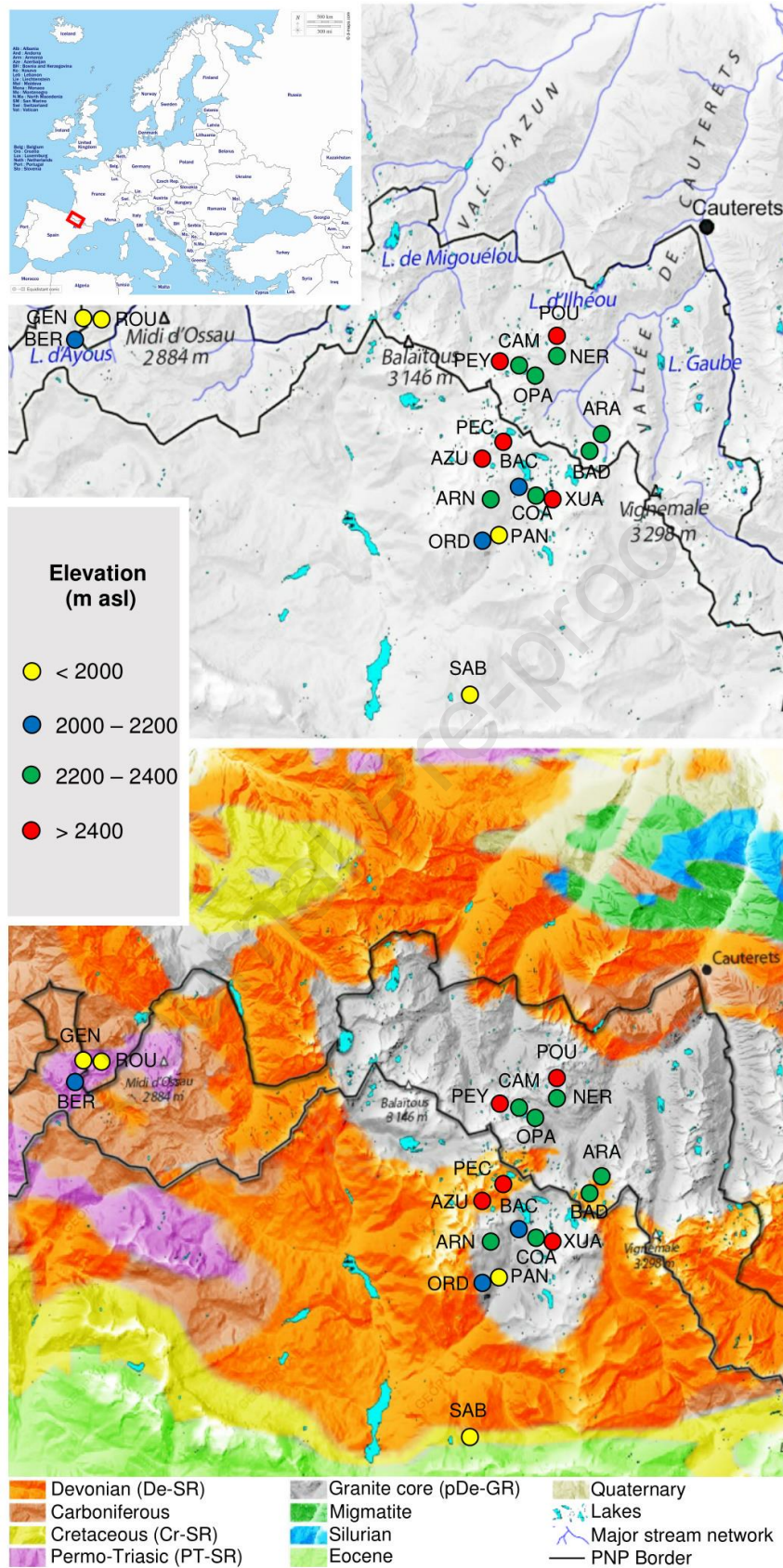
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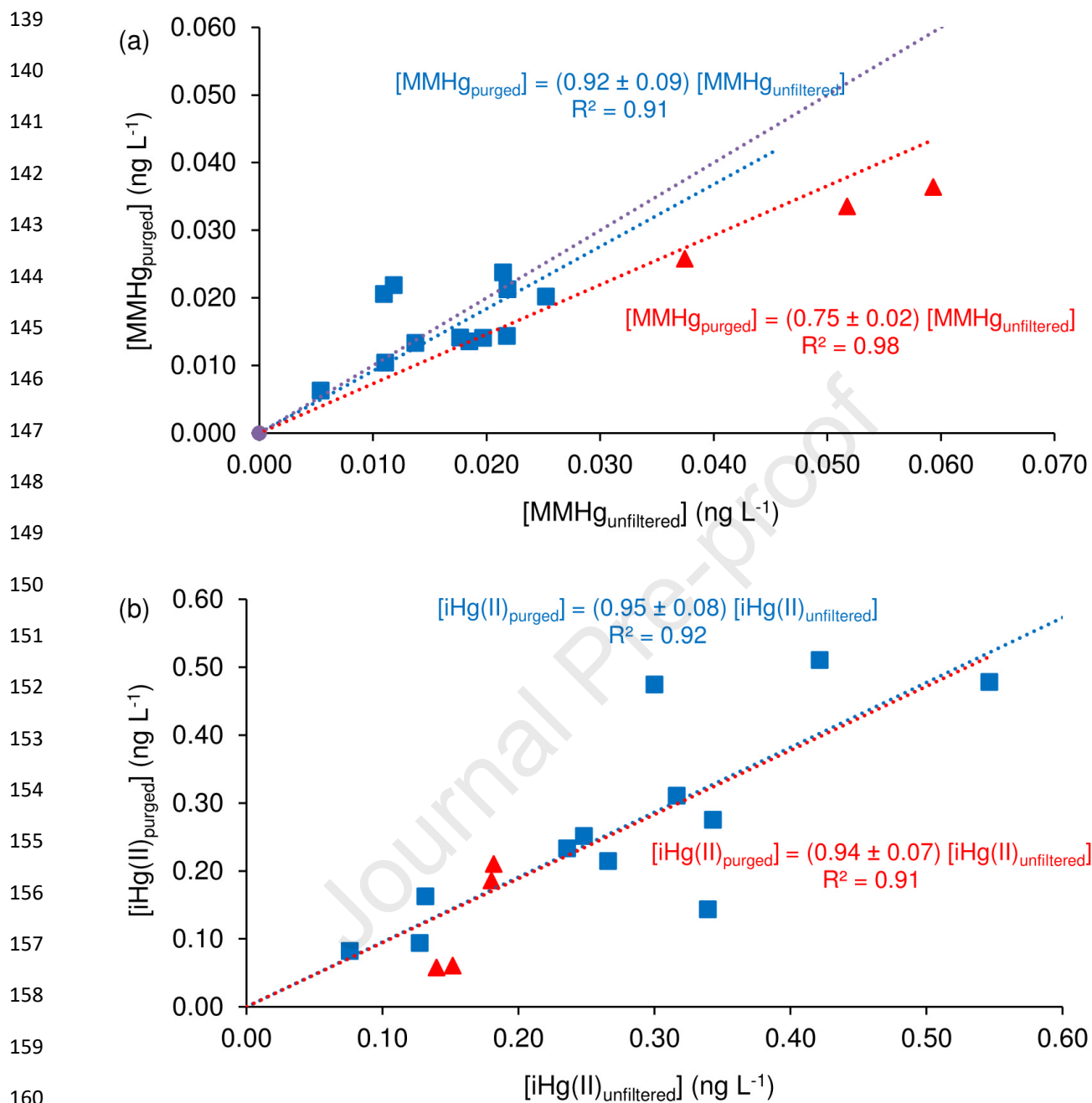
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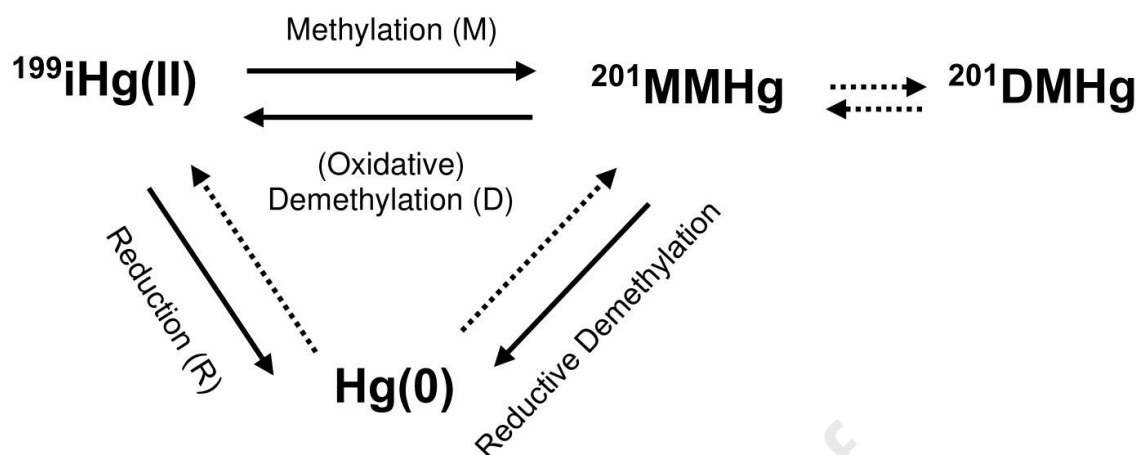


**Figure A1:** Studied lakes together with geology of their catchments (adapted with permission from *Zaharescu et al.*<sup>3</sup>); circles show position of the lakes, and colors indicate the elevation of the corresponding lakes. Lake acronyms are detailed in **Table A1**.

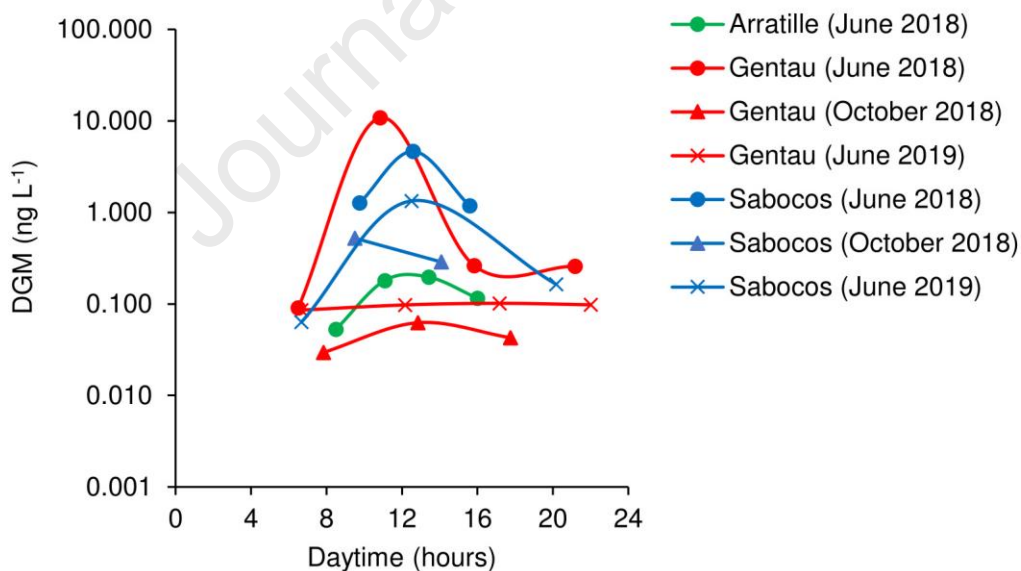




**Figure A2:** Comparison of (a) MMHg concentrations ( $\text{ng L}^{-1}$ ) obtained in purged and unfiltered samples (last Lake Gentau depth not shown) and (b) iHg(II) concentrations ( $\text{ng L}^{-1}$ ) obtained in purged and unfiltered samples for June 2019 sampling campaign. Square blue points correspond to samples collected in well oxygenated depths (oxic water) while triangle red points correspond to samples collected in not well oxygenated depths (anoxic water). Red dashed line is the linear regression using all the samples while blue dashed line included only samples from oxic water.



176 **Figure A3:** Reactivity model of Hg compounds. Solid arrows correspond to  
 177 the reaction pathways that can be calculated with the incubation  
 178 experiments, and dotted arrows the pathways that cannot be quantified.  
 179 MMHg Loss is calculated as the sum of Oxidative and Reductive  
 180 Demethylation.

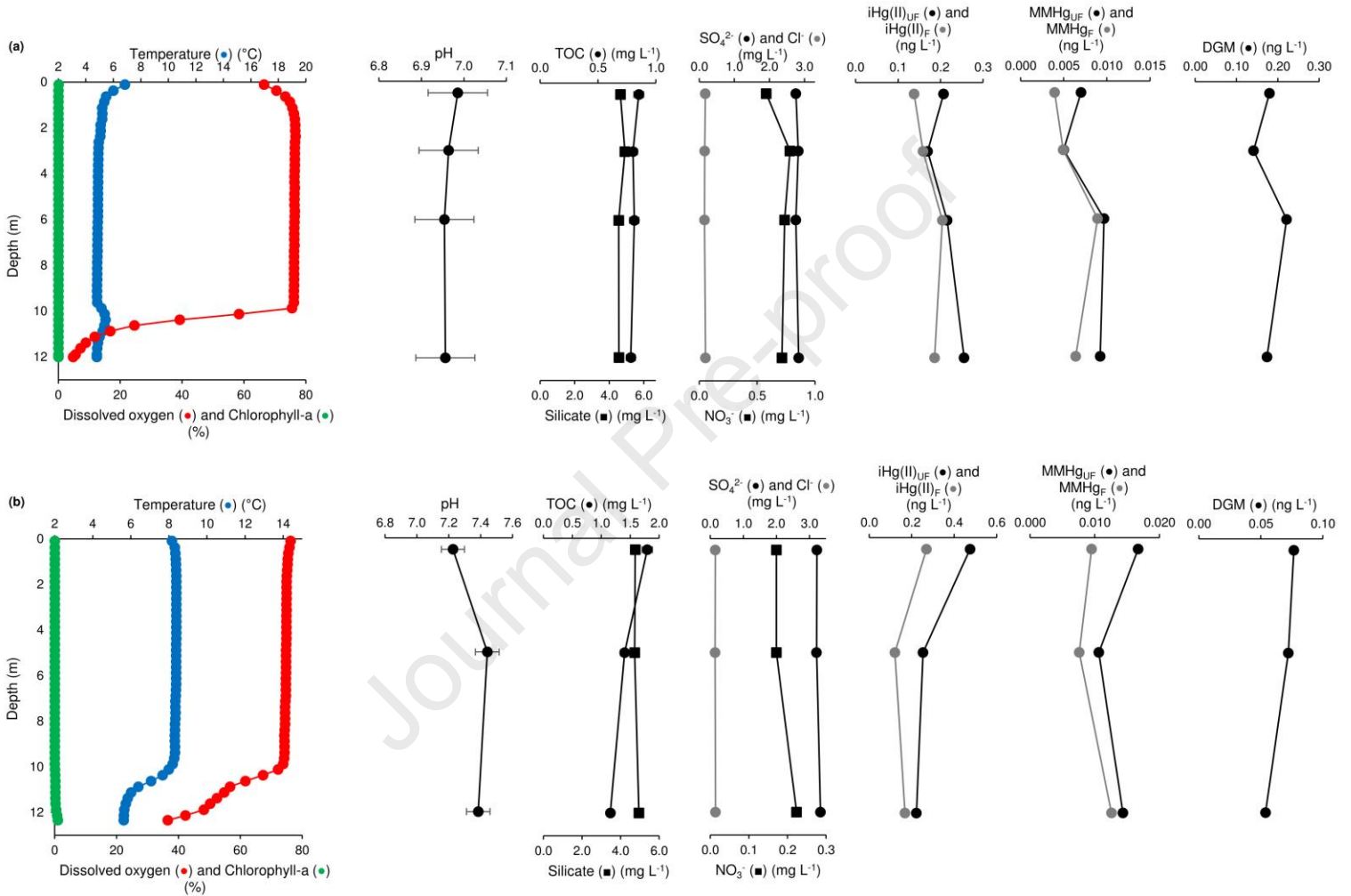


192 **Figure A4:** Daily variation of DGM in Lakes Arratille, Gentau and Sabocos.

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**Figure A5:** Depth profiles of temperature, percentage of dissolved oxygen saturation, chlorophyll-a (RFU) and some other chemical parameters including mercury speciation obtained in (a) June 2018 and (b) October 2018 in Lake Arratille.

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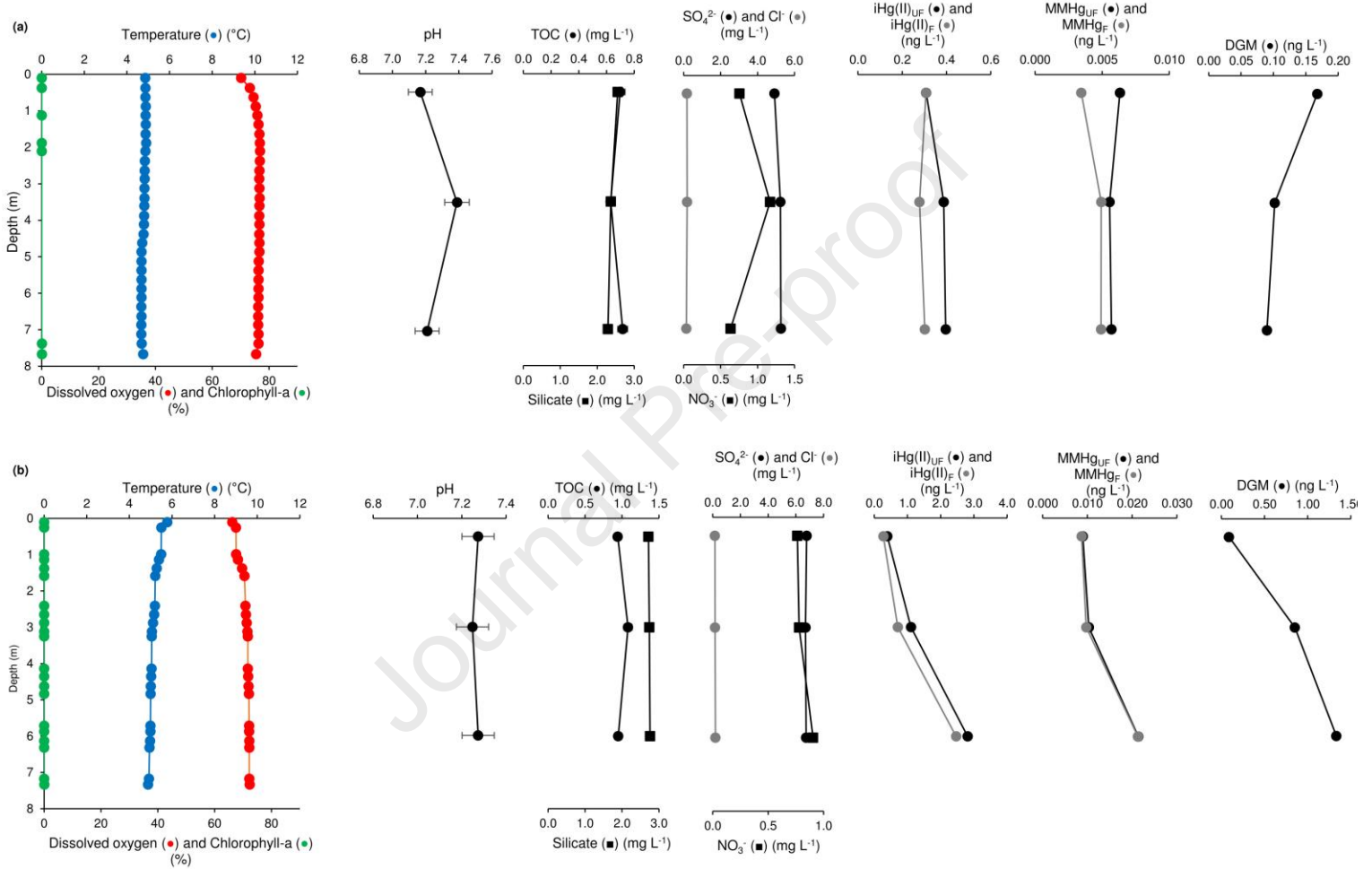
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**Figure A6:** Depth profiles of temperature, percentage of dissolved oxygen saturation, chlorophyll-a (RFU) and some other chemical parameters including mercury speciation obtained in (a) June 2018 and (b) October 2018 in Lake Azul.

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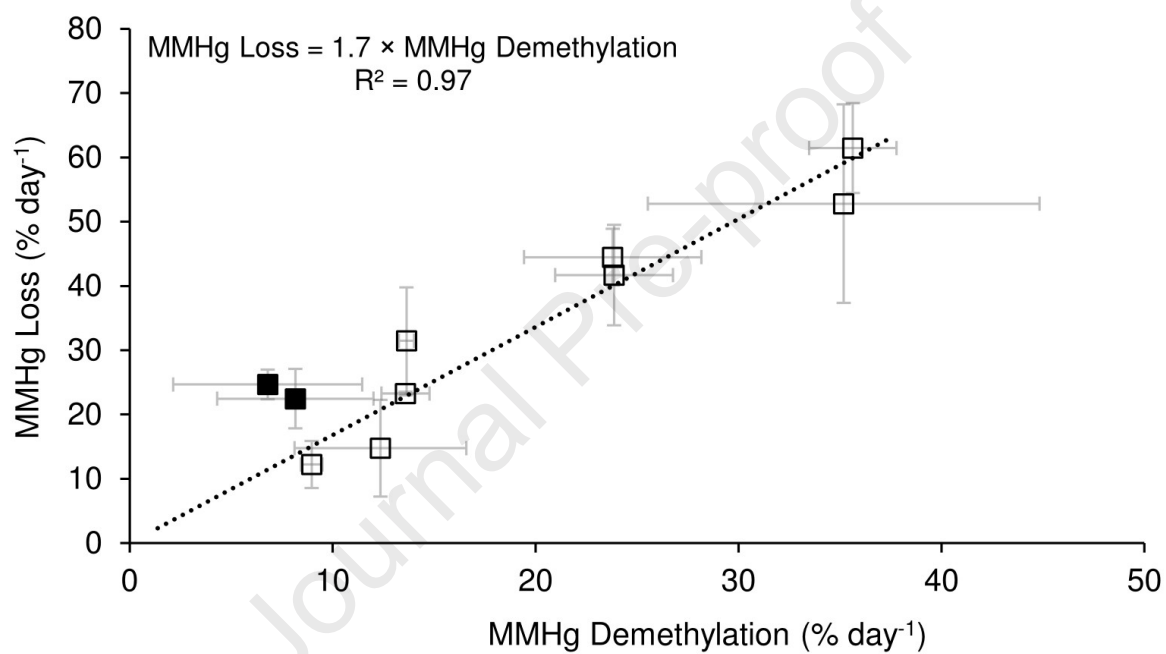
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**Figure A7:** Linear relationship between MMHg Demethylation and MMHg Loss. The two black squares, out of the trend, correspond to MMHg demethylation / MMHg loss under dark conditions in the middle depth of Lake Sabocos (June 2019).

**Table A1:** Some physical characteristics of the sampled lakes. Note that max depth was either measured or estimated from size using allometric relation, and volume of the lakes was also estimated from max depth using a geometric approximation (error can be large). Pre-Devonian Granitic rocks (pDe-GR), Devonian sedimentary rocks (De-SR) include limestone, sandstone and shale. Permo-Triassic sedimentary rocks (PT-SR) include conglomerate, sandstone, lutite, and andesite. Cretaceous sedimentary rocks (Cr-SR) are mainly composed by carbonate rocks.

| Lake name                     | ID Lake | Latitude | Longitude | Elevation<br>(m asl) | Size<br>(Ha) | Catchment<br>(Ha) | Max depth<br>(m) (*calculated) | Volume<br>(m <sup>3</sup> ) (calculated) | Prevailing<br>bedrock |
|-------------------------------|---------|----------|-----------|----------------------|--------------|-------------------|--------------------------------|--|-----------------------|
| <b>Cauterets area</b>         |         |          |           |                      |              |                   |                                |  |                       |
| Lac d'Arratille               | ARA     | 42.8009  | -0.1748   | 2256                 | 5.87         | 296.4             | 12                             | 264307                                   | De-SR                 |
| Lac de la Badète              | BAD     | 42.7938  | -0.1820   | 2341                 | 6.97         | 79.9              | 7                              | 243990                                   | De-SR                 |
| Grand lac de Cambalès         | CAM     | 42.8297  | -0.2251   | 2344                 | 3.46         | 179.7             | 15                             | 208745                                   | pDe-GR                |
| Lac de Peyregnets de Cambalès | PEY     | 42.8324  | -0.2379   | 2493                 | 1.17         | 15.2              | 9                              | 52987                                    | pDe-GR                |
| Lac de Petite Opale           | OPA     | 42.8284  | -0.2177   | 2290                 | 0.64         | 129.3             | 6*                             | 25788                                    | pDe-GR                |
| Lac Nère                      | NER     | 42.8350  | -0.2029   | 2304                 | 2.91         | 94.8              | 12                             | 178522                                   | pDe-GR                |
| Lac de Pourtet                | POU     | 42.8432  | -0.2031   | 2403                 | 5.95         | 48.7              | 13                             | 387705                                   | pDe-GR                |
| <b>Ayous Area</b>             |         |          |           |                      |              |                   |                                |  |                       |
| Lac Gentau                    | GEN     | 42.8482  | -0.4874   | 1942                 | 8.62         | 186.2             | 20                             | 993736                                   | PT-SR                 |
| Lac Roumassot                 | ROU     | 42.8480  | -0.4793   | 1843                 | 5.15         | 268.2             | 16                             | 424200                                   | PT-SR                 |
| Lac Bersau                    | BER     | 42.8392  | -0.4952   | 2080                 | 12.82        | 61.4              | 35                             | 2266475                                  | PT-SR                 |

Table A1 (continued)

| Panticosa Area                        |            |         |         |      |      |        |     |         |        |
|---------------------------------------|------------|---------|---------|------|------|--------|-----|---------|--------|
| <b>Ibón de Arnales</b>                | <b>ARN</b> | 42.7738 | -0.2435 | 2320 | 2.60 | 93.5   | 9   | 84579   | pDe-GR |
| <b>Ibón de Ordicuso Inferior</b>      | <b>ORD</b> | 42.7571 | -0.2478 | 2100 | 0.37 | 14.9   | 3   | 14171   | De-SR  |
| <b>Ibón de los Baños de Panticosa</b> | <b>PAN</b> | 42.7589 | -0.2370 | 1640 | 5.50 | 3229   | 15  | 470072  | pDe-GR |
| <b>Ibón Azul Alto</b>                 | <b>AZU</b> | 42.7898 | -0.2461 | 2420 | 3.89 | 151.4  | 8   | 273527  | De-SR  |
| <b>Ibón de Pecico de la Canal</b>     | <b>PEC</b> | 42.7992 | -0.2251 | 2460 | 0.91 | 167.5  | 9*  | 38954   | De-SR  |
| <b>Ibón de Xuans</b>                  | <b>XUA</b> | 42.7773 | -0.2090 | 2600 | 2.97 | 41.3   | 15  | 183875  | pDe-GR |
| <b>Ibón de Coanga</b>                 | <b>COA</b> | 42.7774 | -0.2199 | 2304 | 0.58 | 27.9   | 5   | 23208   | pDe-GR |
| <b>Ibón de Bachimaña Bajo</b>         | <b>BAC</b> | 42.7813 | -0.2266 | 2178 | 3.08 | 1470.1 | 13* | 193335  | pDe-GR |
| <b>Ibón de Sabocos</b>                | <b>SAB</b> | 42.6926 | -0.2574 | 1900 | 9.56 | 231.7  | 25  | 1183798 | Cr-SR  |

**Table A2:** Main chemical parameters of the studied lakes measured by the multiparametric probe (temperature, conductivity, redox potential), the TOC analyser (TOC as NPOC), the VINDTA 3C Instrument (DIC) and the ionic chromatograph (Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and SO<sub>4</sub><sup>2-</sup>). pH was calculated according to Kortazar *et al.*<sup>4</sup>. Associated error corresponds to standard deviation of three replicated (n=3) points sampled per lake (June 2017 and October 2017).

| Lake                                 | Cl <sup>-</sup> | NO <sub>3</sub> <sup>-</sup><br>mg L <sup>-1</sup> | SO <sub>4</sub> <sup>2-</sup> | T<br>°C | Cond.<br>µS cm <sup>-1</sup> | pH           | E<br>mV | NPOC<br>mg C L <sup>-1</sup> | DIC<br>µmol kg <sup>-1</sup> |
|--------------------------------------|-----------------|--|-------------------------------|---------|------------------------------|--------------|---------|------------------------------|------------------------------|
| <b>Cauterets Area – June 2017</b>    |                 |  |                               |         |                              |              |         |                              |                              |
| ARA                                  | 0.20 ± 0.03*    | 0.64 ± 0.03*                                       | 2.90 ± 0.03*                  | 9.53    | 46                           | 7.35 ± 0.02* | 271     | 1.57 ± 0.19*                 | 680 ± 3*                     |
| BAD                                  | 0.20 ± 0.04*    | 0.77 ± 0.01*                                       | 3.59 ± 0.06*                  | n.d.    | n.d.                         | 7.46         | n.d.    | 2.00 ± 0.17*                 | 787 ± 3*                     |
| CAM                                  | 0.17 ± 0.01*    | 0.40 ± 0.06*                                       | 0.39 ± 0.01*                  | 8.71    | 8                            | 6.42 ± 0.02* | 160     | 0.68 ± 0.05*                 | 124 ± 2*                     |
| PEY                                  | 0.16 ± 0.01*    | 0.41 ± 0.06*                                       | 0.28 ± 0.03*                  | 5.45    | 17                           | 6.01 ± 0.01* | 208     | 0.63 ± 0.01*                 | 92 ± 3*                      |
| OPA                                  | 0.16            | 0.65 ± 0.08*                                       | 0.51 ± 0.01*                  | n.d.    | n.d.                         | 6.92 ± 0.01* | n.d.    | 1.06 ± 0.07*                 | 308 ± 4*                     |
| NER                                  | 0.16 ± 0.01*    | 0.30 ± 0.08*                                       | 0.38 ± 0.01*                  | 11.13   | 7                            | 6.27 ± 0.04* | 119     | 0.78 ± 0.01*                 | 109 ± 2*                     |
| POU                                  | 0.23 ± 0.03*    | 0.40 ± 0.03*                                       | 0.43 ± 0.01*                  | 9.46    | 12                           | 6.75 ± 0.05* | 202     | 1.02 ± 0.17*                 | 190 ± 1*                     |
| <b>Cauterets Area – October 2017</b> |                 |  |                               |         |                              |              |         |                              |                              |
| ARA                                  | 0.15 ± 0.01*    | 0.21 ± 0.02*                                       | 3.74 ± 0.04*                  | 9.85    | 65                           | 7.91 ± 0.03* | 80      | 2.80 ± 0.35*                 | 822 ± 3*                     |
| BAD                                  | 0.20 ± 0.05*    | 0.41 ± 0.08*                                       | 3.72 ± 0.11*                  | 9.72    | 67                           | 7.59 ± 0.04* | 46      | 3.05 ± 0.59*                 | 858 ± 14*                    |
| CAM                                  | 0.19 ± 0.01*    | 0.21 ± 0.01*                                       | 0.63 ± 0.02*                  | 9.71    | 14                           | 6.65 ± 0.03* | 121     | 1.27 ± 0.22*                 | 172 ± 2*                     |
| PEY                                  | 0.17 ± 0.01*    | <LoD*  | 0.35 ± 0.03*                  | 9.57    | 7                            | 6.00         | 108     | 1.40                         | 87                           |
| OPA                                  | 0.16 ± 0.01*    | 0.58 ± 0.04*                                       | 0.71 ± 0.02*                  | 9.89    | 25                           | 6.93 ± 0.04* | 141     | 1.32 ± 0.06*                 | 322 ± 3*                     |
| <b>Cauterets Area – June 2018</b>    |                 |  |                               |         |                              |              |         |                              |                              |
| ARA-t1                               | 0.27            | 0.85   | 2.64                          | 5.86    | 38                           | 7.09         | 176     | 0.88                         | 597                          |
| ARA-t2                               | 0.17            | 0.58   | 2.75                          | 5.70    | 38                           | 6.99         | 84      | 0.85                         | 597                          |
| ARA-t3                               | 0.18            | 0.71   | 2.65                          | 5.72    | 35                           | 6.94         | 317     | 0.79                         | 590                          |
| ARA-t4                               | 0.19            | 0.61   | 2.80                          | 6.64    | 36                           | 6.88         | 310     | 0.82                         | 580                          |
| BAD                                  | 0.19            | 1.13   | 5.43                          | 5.89    | 34                           | 7.21         | 207     | 0.89                         | 915                          |
| CAM                                  | 0.13            | 0.34   | 0.35                          | 2.43    | 5                            | 5.80         | 172     | 0.85                         | 108                          |
| CAM-meltwater                        | n.d.            | n.d.   | n.d.                          | n.d.    | n.d.                         | n.d.         | n.d.    | 1.03                         | 315                          |
| CAM-ice                              | n.d.            | n.d.   | n.d.                          | n.d.    | n.d.                         | n.d.         | n.d.    | 3.34                         | 52                           |
| PEY                                  | 0.07            | 0.10   | <LoD                          | 6.41    | 16                           | 4.87         | 36      | 0.84                         | 33                           |
| OPA                                  | 0.17            | 0.56   | 0.49                          | 3.29    | 15                           | 6.48         | 91      | 0.71                         | 270                          |

Table A2 (continued)

| Cauterets Area – October 2018 |      |      |      |       |      |      |      |      |      |
|-------------------------------|------|------|------|-------|------|------|------|------|------|
| <b>ARA</b>                    | 0.15 | 0.17 | 3.23 | 8.32  | 50   | 7.23 | 170  | 1.79 | 777  |
| <b>BAD</b>                    | 0.15 | 0.29 | 2.96 | 5.33  | 45   | 7.37 | 136  | 1.26 | 778  |
| <b>CAM</b>                    | 0.18 | 0.21 | 0.40 | 8.47  | 10   | 6.36 | 222  | 1.08 | 162  |
| <b>PEY</b>                    | 0.14 | <LoD | <LoD | 6.81  | 5    | 5.94 | 151  | 1.64 | 79   |
| <b>OPA</b>                    | 0.17 | 0.51 | 0.48 | 5.90  | 17   | 6.74 | 127  | 1.13 | 307  |
| Ayous Area – June 2018        |      |      |      |       |      |      |      |      |      |
| <b>GEN-t1</b>                 | 0.25 | 0.10 | 0.49 | 6.89  | 20   | 6.70 | 269  | 0.85 | 341  |
| <b>GEN-t2</b>                 | 0.36 | 0.18 | 0.42 | 6.87  | 20   | 6.72 | 202  | 0.86 | 346  |
| <b>GEN-t3</b>                 | 0.26 | 0.21 | 0.49 | 6.87  | 20   | 6.90 | 202  | 0.62 | 339  |
| <b>GEN-t4</b>                 | 0.38 | 0.16 | 0.47 | 7.21  | 20   | 6.64 | 75   | 1.09 | 340  |
| <b>ROU</b>                    | 0.30 | 0.14 | 0.50 | n.d.  | n.d. | n.d. | n.d. | 0.87 | 345  |
| <b>BER</b>                    | 0.23 | 0.37 | 0.32 | n.d.  | n.d. | n.d. | n.d. | 0.77 | 168  |
| Ayous Area – October 2018     |      |      |      |       |      |      |      |      |      |
| <b>GEN-t1</b>                 | 0.30 | <LoD | <LoD | 12.63 | 24   | 6.74 | 166  | 1.30 | 368  |
| <b>GEN-t2</b>                 | 0.26 | <LoD | 0.31 | 12.80 | 24   | 6.75 | 96   | 1.38 | 358  |
| <b>GEN-t3</b>                 | 0.37 | <LoD | 0.38 | 12.81 | 24   | 6.71 | 137  | 1.25 | 359  |
| <b>ROU</b>                    | 0.29 | <LoD | 0.47 | 13.38 | 25   | 6.85 | 36   | 1.86 | 359  |
| <b>BER</b>                    | 0.31 | <LoD | <LoD | 12.09 | 15   | 7.43 | 38   | 1.58 | 237  |
| Ayous Area – June 2019        |      |      |      |       |      |      |      |      |      |
| <b>GEN-t1</b>                 | 0.27 | 0.33 | 0.55 | 7.17  | 30   | n.d. | 234  | 1.50 | n.d. |
| <b>GEN-t2</b>                 | 0.32 | 0.44 | 0.59 | 7.57  | 30   | n.d. | 285  | 1.15 | n.d. |
| <b>GEN-t3</b>                 | 0.29 | 0.29 | 0.49 | 7.57  | 30   | n.d. | 285  | 1.16 | n.d. |
| <b>GEN-t4</b>                 | 0.27 | 0.33 | 0.51 | 8.53  | 32   | n.d. | 240  | 1.15 | n.d. |
| <b>ROU</b>                    | 0.26 | 0.30 | 0.58 | 12.69 | 34   | n.d. | 234  | 1.45 | n.d. |
| <b>BER</b>                    | 0.24 | 0.22 | <LoD | 2.14  | 9    | n.d. | 206  | 1.12 | n.d. |

Table A2 (continued)

| Panticosa Area – June 2017    |              |              |              |       |     |              |     |              |           |
|-------------------------------|--------------|--------------|--------------|-------|-----|--------------|-----|--------------|-----------|
| <b>ARN</b>                    | 0.17 ± 0.01* | 0.66 ± 0.01* | 1.40 ± 0.02* | 8.43  | 18  | 6.83 ± 0.02* | 122 | 1.03 ± 0.13* | 225 ± 1*  |
| <b>ORD</b>                    | 0.18 ± 0.01* | 0.12 ± 0.03* | 1.86 ± 0.09* | 18.29 | 61  | 7.34 ± 0.03* | 157 | 2.74 ± 0.39* | 695 ± 32* |
| <b>PAN</b>                    | 0.33 ± 0.09* | 0.73 ± 0.13* | 2.29 ± 0.14* | 10.29 | 27  | 7.02         | 195 | 1.54 ± 0.17* | 402       |
| <b>AZU</b>                    | 0.20 ± 0.02* | 0.89 ± 0.16* | 6.51 ± 0.04* | 8.22  | 58  | 7.47         | 131 | 2.14 ± 0.22* | 797       |
| <b>PEC</b>                    | 0.18 ± 0.01* | 0.79 ± 0.06* | 2.61 ± 0.04* | 8.84  | 36  | 7.13 ± 0.02* | 198 | 1.51 ± 0.02* | 457 ± 2*  |
| <b>XUA</b>                    | 0.20 ± 0.04* | 0.90 ± 0.06* | 0.61 ± 0.03* | 4.63  | 12  | 6.68 ± 0.02* | 110 | 0.85 ± 0.05* | 202 ± 5*  |
| <b>COA</b>                    | 0.15 ± 0.01* | 0.21 ± 0.02* | 0.47 ± 0.02* | 7.11  | 23  | 6.42         | 97  | 1.02 ± 0.05* | 134       |
| <b>BAC</b>                    | 0.23 ± 0.01* | 0.69 ± 0.15* | 1.71 ± 0.04* | -     | -   | 6.94 ± 0.02* | -   | 1.23 ± 0.09* | 310 ± 3*  |
| Panticosa Area – October 2017 |              |              |              |       |     |              |     |              |           |
| <b>ARN</b>                    | 0.19 ± 0.01* | 0.66 ± 0.02* | 2.72 ± 0.07* | 10.19 | 31  | 7.07 ± 0.03* | 117 | 1.61 ± 0.35* | 356 ± 3*  |
| <b>PAN</b>                    | 0.33 ± 0.11* | 0.43 ± 0.01* | 2.57 ± 0.25* | 11.12 | 51  | 7.02 ± 0.07* | 146 | 2.06 ± 0.26* | 430 ± 5*  |
| <b>AZU</b>                    | 0.17 ± 0.01* | 0.68 ± 0.03* | 7.56 ± 0.05* | 8.77  | 72  | 7.60 ± 0.05* | 102 | 2.50 ± 0.10* | 882 ± 6*  |
| <b>COA</b>                    | 0.20 ± 0.01* | <LoD*        | 0.57 ± 0.03* | 11.08 | 15  | 6.67 ± 0.05* | 81  | 1.90 ± 0.11* | 190 ± 5*  |
| <b>BAC</b>                    | 0.18 ± 0.01* | 0.37 ± 0.02* | 2.35 ± 0.05* | 10.71 | 36  | 7.10 ± 0.05* | 114 | 1.80 ± 0.28* | 413 ± 12* |
| Panticosa Area – June 2018    |              |              |              |       |     |              |     |              |           |
| <b>ARN</b>                    | 0.19         | 0.62         | 1.13         | 7.45  | 13  | 6.33         | 200 | 0.77         | 184       |
| <b>ORD</b>                    | 0.20         | 0.20         | 1.86         | 17.50 | 57  | 7.02         | 97  | 2.26         | 722       |
| <b>PAN</b>                    | 0.27         | 0.71         | 1.88         | 11.18 | 28  | 6.67         | 176 | 1.01         | 359       |
| <b>AZU-t1</b>                 | 0.18         | 0.76         | 4.91         | 4.87  | 46  | 7.17         | 149 | 0.70         | 675       |
| <b>AZU-t2</b>                 | 0.17         | 0.69         | 4.76         | 5.03  | 43  | n.d.         | 39  | 0.65         | 661       |
| <b>PEC</b>                    | 0.17         | 1.03         | 1.68         | 4.33  | 21  | 6.64         | 149 | 0.65         | 314       |
| <b>COA</b>                    | 0.24         | 0.41         | 0.35         | 10.09 | 7   | 5.85         | 169 | 1.03         | 108       |
| <b>BAC</b>                    | 0.16         | 0.57         | 1.68         | 12.45 | 25  | 6.55         | 129 | 0.94         | 290       |
| <b>SAB</b>                    | 0.21         | 0.49         | 2.33         | 17.25 | 115 | 7.53         | 207 | 1.71         | 1723      |



Table A2 (continued)

| <b>Panticosa Area – October 2018</b> |      |      |      |       |     |      |     |      |      |
|--------------------------------------|------|------|------|-------|-----|------|-----|------|------|
| <b>ARN</b>                           | 0.21 | 0.81 | 2.19 | 7.41  | 20  | 6.79 | 221 | -    | 309  |
| <b>ORD</b>                           | 0.39 | 0.14 | 1.50 | 9.32  | 48  | 6.90 | 225 | 4.63 | 573  |
| <b>PAN</b>                           | 0.68 | 0.63 | 2.20 | 9.87  | 25  | 6.64 | 193 | 2.03 | 372  |
| <b>AZU</b>                           | 0.16 | 0.76 | 6.78 | 5.39  | 50  | 7.27 | 110 | 0.94 | 803  |
| <b>PEC</b>                           | 0.19 | 0.69 | 2.39 | 8.13  | 35  | 6.79 | 216 | 1.08 | 444  |
| <b>COA</b>                           | 0.25 | 0.09 | <LoD | 6.18  | 7   | 6.02 | 163 | 1.87 | 125  |
| <b>BAC</b>                           | 0.22 | 0.82 | 1.89 | 8.97  | 32  | 6.75 | 207 | 1.05 | 358  |
| <b>SAB-t1</b>                        | 0.23 | 0.13 | 2.48 | 10.42 | 100 | 7.71 | 188 | 3.02 | 1691 |
| <b>SAB-t2</b>                        | 0.20 | 0.37 | 2.54 | 10.70 | 101 | 7.64 | 245 | 1.96 | 1694 |
| <b>SAB-t3</b>                        | 0.22 | <LoD | 2.56 | 10.80 | 101 | 7.67 | 262 | 2.62 | 1692 |
| <b>Panticosa Area – June 2019</b>    |      |      |      |       |     |      |     |      |      |
| <b>SAB-t1</b>                        | 0.15 | 0.73 | 2.62 | 12.47 | 141 | n.d. | 212 | 1.86 | n.d. |
| <b>SAB-t2</b>                        | 0.15 | 0.66 | 2.49 | 11.87 | 130 | n.d. | 138 | 1.79 | n.d. |
| <b>SAB-t3</b>                        | 0.15 | 0.76 | 2.55 | 11.87 | 130 | n.d. | 138 | 1.80 | n.d. |

**Table A3:** Filtered and unfiltered inorganic mercury (iHg(II)), monomethylmercury (MMHg) and Dissolved Gaseous Mercury (DGM) concentrations measured in the subsurface water samples of the 19 studied lakes. volatilization flux densities (FD) are calculated according to the methodology detailed in *Sharif et al.*<sup>5</sup> for DGM fluxes and using the specific gas exchange model for sheltered lakes developed by *Cole and Caraco*<sup>6</sup>. Associated error corresponds to standard deviation of three replicated (n=3) points sampled per lake (June 2017 and October 2017). <sup>o</sup> correspond to outlier (Grubbs's test).

| Lake                                 | iHg(II) <sub>F</sub> | iHg(II) <sub>UF</sub> | MMHg <sub>F</sub>  | MMHg <sub>UF</sub> | DGM  | FD (1m s <sup>-1</sup> )             | FD (3m s <sup>-1</sup> )             |
|--------------------------------------|----------------------|-----------------------|--------------------|--------------------|------|--------------------------------------|--------------------------------------|
|                                      |                      |                       | ng L <sup>-1</sup> |                    |      | ng m <sup>-2</sup> day <sup>-1</sup> | ng m <sup>-2</sup> day <sup>-1</sup> |
| <b>Cauterets Area – June 2017</b>    |                      |                       |                    |                    |      |                                      |                                      |
| <b>ARA</b>                           | 0.15                 | 0.26 ± 0.01*          | 0.011 ± 0.001*     | 0.012 ± 0.001*     | 0.33 | 178                                  | 270                                  |
| <b>BAD</b>                           | 0.16 ± 0.03*         | 0.27 ± 0.03*          | <LoD               | 0.009 ± 0.001*     | 0.02 | 7                                    | 11                                   |
| <b>CAM</b>                           | 0.26 ± 0.02*         | 0.38 ± 0.03*          | <LoD               | 0.018 ± 0.002*     | 0.12 | 61                                   | 93                                   |
| <b>PEY</b>                           | 0.19 ± 0.02*         | 0.31 ± 0.09*          | 0.012              | 0.014 ± 0.003*     | 0.12 | 60                                   | 92                                   |
| <b>OPA</b>                           | 0.11 ± 0.01*         | 0.12 ± 0.01*          | 0.007              | 0.007 ± 0.002*     | 0.07 | 34                                   | 52                                   |
| <b>NER</b>                           | 0.41 ± 0.05*         | 0.47 ± 0.02*          | 0.008              | 0.010 ± 0.002*     | 0.13 | 68                                   | 103                                  |
| <b>POU</b>                           | 0.26 ± 0.03*         | 0.44 ± 0.07*          | <LoD               | 0.010 ± 0.002*     | 0.11 | 57                                   | 86                                   |
| <b>Cauterets Area – October 2017</b> |                      |                       |                    |                    |      |                                      |                                      |
| <b>ARA</b>                           | 0.12 ± 0.02*         | 0.15 ± 0.02*          | 0.005 ± 0.001*     | 0.006 ± 0.001*     | 0.04 | 20                                   | 30                                   |
| <b>BAD</b>                           | 0.34 ± 0.27*         | 0.46 ± 0.12*          | 0.004 ± 0.001*     | 0.006 ± 0.001*     | 0.26 | 141                                  | 214                                  |
| <b>CAM</b>                           | 0.50 ± 0.05*         | 0.51 ± 0.06*          | 0.006 ± 0.002*     | 0.009 ± 0.002*     | 0.42 | 233                                  | 352                                  |
| <b>PEY</b>                           | 0.17 ± 0.05*         | 0.29 ± 0.02*          | <LoD               | 0.006 ± 0.002*     | 0.12 | 65                                   | 98                                   |
| <b>OPA</b>                           | 0.22 ± 0.03*         | 0.42 ± 0.11*          | 0.004 ± 0.001*     | 0.006 ± 0.001*     | 0.10 | 50                                   | 75                                   |
| <b>Cauterets Area – June 2018</b>    |                      |                       |                    |                    |      |                                      |                                      |
| <b>ARA-t1</b>                        | 0.08                 | 0.11                  | 0.005              | 0.007              | 0.05 | 24                                   | 37                                   |
| <b>ARA-t2</b>                        | 0.14                 | 0.21                  | 0.004              | 0.007              | 0.18 | 96                                   | 146                                  |
| <b>ARA-t3</b>                        | 0.17                 | 0.17                  | 0.007              | 0.007              | 0.20 | 106                                  | 160                                  |
| <b>ARA-t4</b>                        | 0.15                 | 0.20                  | 0.004              | 0.010              | 0.12 | 60                                   | 91                                   |
| <b>BAD</b>                           | 0.16                 | 0.18                  | 0.005              | 0.006              | 0.12 | 62                                   | 95                                   |
| <b>CAM</b>                           | 0.19                 | 0.26                  | 0.009              | 0.012              | 0.19 | 100                                  | 152                                  |
| <b>CAM-meltwater</b>                 | 0.53                 | 1.01                  | 0.010              | 0.035              | n.d. | n.d.                                 | n.d.                                 |
| <b>CAM-ice</b>                       | 1.03                 | 18.80                 | 0.054              | 0.612              | n.d. | n.d.                                 | n.d.                                 |
| <b>PEY</b>                           | 0.31                 | 0.67                  | 0.015              | 0.023              | 0.39 | 217                                  | 328                                  |
| <b>OPA</b>                           | 0.19                 | 0.24                  | 0.007              | 0.007              | 0.04 | 19                                   | 29                                   |

Table A3 (continued)  
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| Cauterets Area – October 2018 |      |      |       |       |      |    |    |
|-------------------------------|------|------|-------|-------|------|----|----|
| <b>ARA</b>                    | 0.27 | 0.47 | 0.010 | 0.017 | 0.08 | 38 | 58 |
| <b>BAD</b>                    | 0.23 | 0.28 | 0.010 | 0.010 | 0.04 | 19 | 29 |
| <b>CAM</b>                    | 0.19 | 0.45 | 0.008 | 0.011 | 0.09 | 44 | 67 |
| <b>PEY</b>                    | 0.20 | 0.40 | 0.009 | 0.010 | 0.09 | 48 | 72 |
| <b>OPA</b>                    | 0.20 | 0.31 | 0.008 | 0.009 | 0.06 | 27 | 41 |

| Ayous Area – June 2018 |      |      |       |       |                    |      |      |
|------------------------|------|------|-------|-------|--------------------|------|------|
| <b>GEN-t1</b>          | 0.16 | 0.84 | 0.007 | 0.008 | 0.09               | 46   | 69   |
| <b>GEN-t2</b>          | 0.21 | 0.38 | 0.006 | 0.008 | 10.79 <sup>ø</sup> | 6090 | 9226 |
| <b>GEN-t3</b>          | 0.19 | 0.68 | 0.009 | 0.013 | 0.26               | 143  | 216  |
| <b>GEN-t4</b>          | 0.17 | 0.39 | 0.009 | 0.014 | 0.26               | 140  | 213  |
| <b>ROU</b>             | 0.18 | 0.30 | 0.014 | 0.014 | 0.13               | n.d. | n.d. |
| <b>BER</b>             | 0.35 | 0.70 | 0.016 | 0.018 | 0.27               | n.d. | n.d. |

| Ayous Area – October 2018 |      |      |       |       |      |    |    |
|---------------------------|------|------|-------|-------|------|----|----|
| <b>GEN-t1</b>             | 0.12 | 0.24 | 0.024 | 0.024 | 0.03 | 12 | 18 |
| <b>GEN-t2</b>             | 0.13 | 0.31 | 0.026 | 0.029 | 0.06 | 30 | 46 |
| <b>GEN-t3</b>             | 0.14 | 0.16 | 0.026 | 0.027 | 0.04 | 19 | 29 |
| <b>ROU</b>                | 0.21 | 0.38 | 0.017 | 0.034 | 0.03 | 14 | 22 |
| <b>BER</b>                | 0.17 | 0.21 | 0.005 | 0.007 | 0.05 | 21 | 32 |

| Ayous Area – June 2019 |      |      |       |       |      |     |     |
|------------------------|------|------|-------|-------|------|-----|-----|
| <b>GEN-t1</b>          | 0.17 | 0.24 | 0.012 | 0.018 | 0.09 | 43  | 65  |
| <b>GEN-t2</b>          | 0.18 | 0.27 | 0.015 | 0.012 | 0.10 | 50  | 75  |
| <b>GEN-t3</b>          | 0.16 | 0.30 | 0.014 | 0.011 | 0.10 | 52  | 79  |
| <b>GEN-t4</b>          | 0.19 | 0.25 | 0.006 | 0.014 | 0.10 | 50  | 76  |
| <b>ROU</b>             | 0.29 | 0.42 | 0.012 | 0.025 | 0.17 | 90  | 136 |
| <b>BER</b>             | 0.41 | 0.55 | 0.012 | 0.021 | 0.23 | 126 | 191 |

Table A3 (continued)  
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| Panticosa Area – June 2017    |                           |                           |                |                |                   |      |      |
|-------------------------------|---------------------------|---------------------------|----------------|----------------|-------------------|------|------|
| <b>ARN</b>                    | 0.18 ± 0.02*              | 0.31 ± 0.02*              | <LoD           | <LoD           | 0.06              | 26   | 39   |
| <b>ORD</b>                    | 0.52 ± 0.04*              | 0.73 ± 0.13*              | 0.014 ± 0.005* | 0.047 ± 0.003* | 0.18              | 98   | 148  |
| <b>PAN</b>                    | 0.24 ± 0.03*              | 0.34 ± 0.05*              | 0.006          | 0.012 ± 0.002* | 0.10              | 54   | 82   |
| <b>AZU</b>                    | 0.09 ± 0.02*              | 0.24 ± 0.13*              | <LoD           | <LoD           | 0.06              | 29   | 44   |
| <b>PEC</b>                    | 0.24 ± 0.01*              | 0.27 ± 0.03*              | <LoD           | 0.007 ± 0.002* | 0.04              | 17   | 26   |
| <b>XUA</b>                    | 0.29 ± 0.04*              | 0.49 ± 0.12*              | 0.008 ± 0.001* | 0.024 ± 0.002* | 0.13              | 70   | 106  |
| <b>COA</b>                    | 0.43 ± 0.17*              | 0.60 ± 0.07*              | <LoD           | 0.011 ± 0.001* | 0.20              | 109  | 165  |
| <b>BAC</b>                    | n.d.                      | 0.36 ± 0.07*              | n.d.           | 0.008 ± 0.001* | n.d.              | n.d. | n.d. |
| Panticosa Area – October 2017 |                           |                           |                |                |                   |      |      |
| <b>ARN</b>                    | 0.23 ± 0.11*              | 0.36 ± 0.13*              | <LoD           | 0.004 ± 0.001* | 0.15              | 78   | 119  |
| <b>PAN</b>                    | 0.64 ± 0.15* <sup>Ø</sup> | 1.19 ± 0.17* <sup>Ø</sup> | 0.010 ± 0.003* | 0.016 ± 0.002* | 0.68 <sup>Ø</sup> | 375  | 568  |
| <b>AZU</b>                    | 0.28 ± 0.01*              | 0.38 ± 0.06*              | <LoD           | 0.005 ± 0.001* | 0.21              | 111  | 168  |
| <b>COA</b>                    | 0.53 ± 0.05*              | 0.72 ± 0.03*              | 0.006 ± 0.001* | 0.012 ± 0.002* | 0.37              | 200  | 303  |
| <b>BAC</b>                    | 0.23 ± 0.03*              | 0.35 ± 0.09*              | 0.009 ± 0.001* | 0.013 ± 0.004* | 0.05              | 25   | 38   |
| Panticosa Area – June 2018    |                           |                           |                |                |                   |      |      |
| <b>ARN</b>                    | 0.31                      | 0.38                      | 0.006          | 0.007          | 0.08              | 39   | 59   |
| <b>ORD</b>                    | 0.54                      | 0.55                      | 0.027          | 0.030          | n.d.              | n.d. | n.d. |
| <b>PAN</b>                    | 0.36                      | 0.53                      | 0.009          | 0.011          | n.d.              | n.d. | n.d. |
| <b>AZU-t1</b>                 | 0.34                      | 0.31                      | 0.003          | 0.006          | 0.17              | 89   | 135  |
| <b>AZU-t2</b>                 | 0.31                      | 0.38                      | 0.005          | 0.006          | 0.09              | 48   | 73   |
| <b>PEC</b>                    | 0.20                      | 0.25                      | 0.006          | 0.007          | 0.05              | 23   | 34   |
| <b>COA</b>                    | 0.42                      | 0.60                      | 0.009          | 0.011          | 0.20              | 108  | 164  |
| <b>BAC</b>                    | 0.30                      | 0.44                      | 0.010          | 0.012          | 0.11              | 57   | 87   |
| <b>SAB-t1</b>                 | 0.58                      | 0.70                      | 0.010          | 0.012          | 1.27 <sup>Ø</sup> | 693  | 1050 |
| <b>SAB-t2</b>                 | 0.53                      | 0.64                      | 0.008          | 0.019          | 4.65 <sup>Ø</sup> | 2553 | 3867 |
| <b>SAB-t3</b>                 | 0.67                      | 0.73                      | 0.010          | 0.018          | 1.18 <sup>Ø</sup> | 645  | 977  |

Table A3 (continued)  
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| Panticosa Area – October 2018 |                   |                   |       |       |                   |      |      |
|-------------------------------|-------------------|-------------------|-------|-------|-------------------|------|------|
| <b>ARN</b>                    | 0.37              | 0.52              | 0.008 | 0.011 | 0.20              | 110  | 167  |
| <b>ORD</b>                    | 1.10              | 1.16              | 0.023 | 0.025 | 0.18              | 97   | 147  |
| <b>PAN</b>                    | 0.68              | 0.96              | 0.035 | 0.062 | n.d.              | n.d. | n.d. |
| <b>AZU</b>                    | 0.27              | 0.39              | 0.009 | 0.009 | 0.09              | 45   | 68   |
| <b>PEC</b>                    | 0.24              | 0.34              | 0.005 | 0.009 | n.d.              | n.d. | n.d. |
| <b>COA</b>                    | 2.68 <sup>ø</sup> | 2.88 <sup>ø</sup> | 0.022 | 0.025 | 0.20              | 107  | 162  |
| <b>BAC</b>                    | 0.33              | 0.38              | 0.014 | 0.026 | 0.11              | 56   | 85   |
| <b>SAB-t1</b>                 | 0.39              | 0.58              | 0.025 | 0.027 | 0.52              | 288  | 436  |
| <b>SAB-t2</b>                 | 0.39              | 0.58              | 0.018 | 0.029 | 0.29              | 156  | 237  |
| <b>SAB-t3</b>                 | 0.44              | 0.53              | 0.022 | 0.029 | n.d.              | n.d. | n.d. |
| Panticosa Area – June 2019    |                   |                   |       |       |                   |      |      |
| <b>SAB-t1</b>                 | 0.19              | 0.34              | 0.005 | 0.005 | 0.06              | 31   | 46   |
| <b>SAB-t2</b>                 | 0.21              | 0.32              | 0.014 | 0.022 | 1.34 <sup>ø</sup> | 741  | 1122 |
| <b>SAB-t3</b>                 | 0.18              | 0.35              | 0.005 | 0.018 | 0.16              | 87   | 132  |

**Table A4:** Filtered and unfiltered inorganic mercury (iHg(II)), monomethylmercury (MMHg) and Dissolved Gaseous Mercury (DGM) concentrations measured in the depth water samples of the 4 intensively studied lakes (Gentau, Sabocos, Aritama and Azul Superior) together with some physicochemical parameters.

| Depth<br>m                        | iHg(II) <sub>F</sub> | iHg(II) <sub>UF</sub> | MMHg <sub>F</sub><br>ng L <sup>-1</sup> | MMHg <sub>UF</sub> | DGM   | Cl <sup>-</sup> | NO <sub>3</sub> <sup>-</sup><br>mg L <sup>-1</sup> | SO <sub>4</sub> <sup>2-</sup> | T<br>(°C) | DO<br>% | pH   | NPOC<br>mg C L <sup>-1</sup> |
|-----------------------------------|----------------------|-----------------------|---|--------------------|-------|-----------------|--|-------------------------------|-----------|---------|------|------------------------------|
| <b>Lake Gentau – June 2018</b>    |                      |                       |   |                    |       |                 |  |                               |           |         |      |                              |
| <b>0.5</b>                        | 0.21                 | 0.38                  | 0.006                                   | 0.008              | 10.79 | 0.36            | 0.18   | 0.42                          | 6.87      | 82      | 6.72 | 0.86                         |
| <b>5</b>                          | 0.20                 | 0.31                  | 0.009                                   | 0.010              | 3.04  | 0.27            | 0.18   | 0.43                          | 4.97      | 78      | 6.68 | 0.75                         |
| <b>8</b>                          | 0.18                 | 0.34                  | 0.009                                   | 0.013              | 0.31  | 0.34            | 0.04   | 0.49                          | 4.37      | 27      | 6.74 | 1.03                         |
| <b>12</b>                         | 0.18                 | 0.19                  | 0.023                                   | 0.057              | 0.23  | 0.60            | 0.03   | 0.68                          | 4.23      | 3       | 6.76 | 1.41                         |
| <b>18</b>                         | 0.66                 | 0.75                  | 0.318                                   | 0.426              | 0.14  | 0.92            | 0.08   | 0.66                          | 4.40      | 1       | 6.77 | 1.44                         |
| <b>Lake Gentau – October 2018</b> |                      |                       |   |                    |       |                 |  |                               |           |         |      |                              |
| 0.5                               | 0.13                 | 0.31                  | 0.026                                   | 0.029              | 0.06  | 0.26            | <LoD   | 0.31                          | 12.80     | 79      | 6.75 | 1.38                         |
| 5                                 | 0.14                 | 0.15                  | 0.017                                   | 0.055              | 0.06  | 0.22            | <LoD   | 0.30                          | 12.68     | 78      | 6.65 | 1.34                         |
| 8                                 | 0.14                 | 0.23                  | 0.020                                   | 0.041              | 0.05  | 0.24            | <LoD   | 0.33                          | 12.62     | 77      | 6.77 | 1.43                         |
| 12                                | 0.08                 | 0.09                  | 0.011                                   | 0.027              | 0.04  | 0.55            | <LoD   | 0.47                          | 8.52      | 33      | 6.96 | 2.39                         |
| <b>17</b>                         | 0.36                 | 0.39                  | 0.341                                   | 0.388              | 0.03  | 0.87            | 0.14   | <LoD                          | 6.05      | 2       | 6.90 | 5.60                         |
| <b>Lake Gentau – June 2019</b>    |                      |                       |   |                    |       |                 |  |                               |           |         |      |                              |
| <b>0.5</b>                        | 0.18                 | 0.27                  | 0.015                                   | 0.012              | 0.10  | 0.32            | 0.44   | 0.59                          | 7.57      | 79      | n.d. | 1.15                         |
| <b>5</b>                          | 0.15                 | 0.20                  | 0.006                                   | 0.007              | 0.08  | 0.27            | 0.27   | 0.54                          | 6.76      | 84      | n.d. | 1.21                         |
| <b>8</b>                          | 0.07                 | 0.18                  | 0.004                                   | 0.037              | 0.04  | 0.31            | 0.18   | 0.58                          | 5.82      | 91      | n.d. | 1.23                         |
| <b>12</b>                         | 0.08                 | 0.14                  | 0.019                                   | 0.059              | 0.02  | 0.45            | 0.41   | 0.71                          | 4.51      | 20      | n.d. | 1.28                         |
| <b>17.5</b>                       | 0.11                 | 0.18                  | 0.157                                   | 0.236              | 0.01  | 0.53            | 0.13   | 0.70                          | 4.30      | 4       | n.d. | 1.18                         |

Table A4 (continued)  
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| Lake Sabocos – June 2018    |      |      |       |       |      |      |      |      |       |     |      |      |
|-----------------------------|------|------|-------|-------|------|------|------|------|-------|-----|------|------|
| <b>0.5</b>                  | 0.53 | 0.64 | 0.008 | 0.019 | 4.65 | 0.21 | 0.49 | 2.33 | 17.25 | 102 | 6.79 | 1.71 |
| <b>5</b>                    | 0.47 | 1.29 | 0.009 | 0.013 | 2.36 | 0.21 | 0.48 | 2.51 | 10.70 | 82  | 6.78 | 2.07 |
| <b>9</b>                    | 0.35 | 0.42 | 0.012 | 0.017 | 0.31 | 0.27 | 0.45 | 3.21 | 7.57  | 61  | 6.79 | 1.93 |
| <b>15</b>                   | 0.33 | 0.55 | 0.011 | 0.017 | 0.22 | 0.29 | 0.54 | 3.07 | 6.01  | 49  | 6.95 | 1.54 |
| <b>20</b>                   | 0.29 | 0.39 | 0.012 | 0.026 | 1.43 | 0.31 | 0.50 | 3.02 | 5.13  | 8   | 6.78 | 1.55 |
| <b>25</b>                   | 0.32 | 0.56 | 0.025 | 0.060 | 0.56 | 0.35 | 0.46 | 2.74 | 4.80  | 1   | 6.78 | 1.32 |
| Lake Sabocos – October 2018 |      |      |       |       |      |      |      |      |       |     |      |      |
| <b>0.5</b>                  | 0.39 | 0.58 | 0.018 | 0.029 | 0.29 | 0.20 | 0.37 | 2.54 | 10.70 | 78  | 7.64 | 1.96 |
| <b>4</b>                    | 0.68 | 0.90 | 0.015 | 0.032 | 0.38 | 0.22 | 0.15 | 2.54 | 10.56 | 78  | 7.73 | 2.25 |
| <b>8</b>                    | 0.51 | 0.54 | 0.024 | 0.026 | 0.43 | 0.22 | 0.17 | 2.55 | 10.53 | 77  | 7.55 | 2.10 |
| <b>12</b>                   | 0.44 | 0.53 | 0.012 | 0.024 | 0.52 | 0.30 | 0.34 | 3.09 | 7.87  | 62  | 7.60 | 2.01 |
| <b>18</b>                   | 0.43 | 0.61 | 0.015 | 0.017 | 1.15 | 0.46 | 0.44 | 3.10 | 5.97  | 35  | 7.64 | 2.16 |
| <b>23</b>                   | 0.17 | 0.79 | 0.015 | 0.026 | 0.41 | 0.30 | 0.20 | 3.03 | 5.70  | 5   | 7.34 | 2.01 |
| Lake Sabocos – June 2019    |      |      |       |       |      |      |      |      |       |     |      |      |
| <b>0.5</b>                  | 0.21 | 0.32 | 0.014 | 0.022 | 1.34 | 0.15 | 0.76 | 2.55 | 11.87 | 79  | n.d. | 1.80 |
| <b>5</b>                    | 0.18 | 0.34 | 0.010 | 0.011 | 0.12 | 0.13 | 0.61 | 2.40 | 10.18 | 82  | n.d. | 1.73 |
| <b>9</b>                    | 0.03 | 0.08 | 0.006 | 0.018 | 0.11 | 0.14 | 0.71 | 2.79 | 7.07  | 76  | n.d. | 1.71 |
| <b>13</b>                   | 0.02 | 0.13 | 0.004 | 0.020 | 0.09 | 0.16 | 0.78 | 2.80 | 6.09  | 70  | n.d. | 1.67 |
| <b>18</b>                   | 0.03 | 0.13 | 0.005 | 0.022 | 0.04 | 0.16 | 0.79 | 3.02 | 5.26  | 48  | n.d. | 1.62 |
| <b>25</b>                   | 0.03 | 0.15 | 0.021 | 0.052 | 0.06 | 0.17 | 0.57 | 2.73 | 4.87  | 7   | n.d. | 1.58 |



Table A4 (continued)  
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| Lake Arratille – June 2018    |      |      |       |       |      |      |      |      |      |    |      |      |
|-------------------------------|------|------|-------|-------|------|------|------|------|------|----|------|------|
| <b>0.5</b>                    | 0.14 | 0.21 | 0.004 | 0.007 | 0.18 | 0.17 | 0.58 | 2.75 | 5.70 | 72 | 6.99 | 0.85 |
| <b>3</b>                      | 0.16 | 0.17 | 0.005 | 0.005 | 0.14 | 0.15 | 0.78 | 2.82 | 4.91 | 76 | 6.96 | 0.80 |
| <b>6</b>                      | 0.20 | 0.22 | 0.009 | 0.010 | 0.22 | 0.15 | 0.74 | 2.75 | 4.87 | 76 | 6.95 | 0.81 |
| <b>12</b>                     | 0.19 | 0.26 | 0.006 | 0.009 | 0.17 | 0.18 | 0.71 | 2.83 | 4.79 | 5  | 6.96 | 0.78 |
| Lake Arratille – October 2018 |      |      |       |       |      |      |      |      |      |    |      |      |
| <b>0.5</b>                    | 0.27 | 0.47 | 0.010 | 0.017 | 0.08 | 0.15 | 0.17 | 3.23 | 8.32 | 76 | 7.23 | 1.79 |
| <b>5</b>                      | 0.12 | 0.25 | 0.008 | 0.011 | 0.07 | 0.14 | 0.17 | 3.22 | 8.38 | 75 | 7.44 | 1.40 |
| <b>12</b>                     | 0.17 | 0.22 | 0.013 | 0.014 | 0.05 | 0.16 | 0.22 | 3.33 | 5.64 | 45 | 7.39 | 1.16 |
| Lake Azul – June 2018         |      |      |       |       |      |      |      |      |      |    |      |      |
| <b>0.5</b>                    | 0.31 | 0.31 | 0.003 | 0.006 | 0.17 | 0.18 | 0.76 | 4.91 | 4.87 | 74 | 7.17 | 0.70 |
| <b>3.5</b>                    | 0.28 | 0.39 | 0.005 | 0.006 | 0.10 | 0.19 | 1.17 | 5.23 | 4.82 | 77 | 7.39 | 0.63 |
| <b>7</b>                      | 0.30 | 0.40 | 0.005 | 0.006 | 0.09 | 0.15 | 0.64 | 5.26 | 4.67 | 76 | 7.21 | 0.72 |
| Lake Azul – October 2018      |      |      |       |       |      |      |      |      |      |    |      |      |
| <b>0.5</b>                    | 0.27 | 0.39 | 0.009 | 0.009 | 0.09 | 0.16 | 0.76 | 6.78 | 5.39 | 68 | 7.27 | 0.94 |
| <b>3</b>                      | 0.71 | 1.11 | 0.010 | 0.010 | 0.85 | 0.17 | 0.78 | 6.70 | 5.08 | 71 | 7.25 | 1.08 |
| <b>6</b>                      | 2.46 | 2.81 | 0.021 | 0.021 | 1.33 | 0.20 | 0.90 | 6.73 | 4.98 | 72 | 7.27 | 0.95 |

**Table A5:** Operating conditions for the incubation experiments. Note that PFA Teflon bottles (Nalgene) were used to allow in-situ transmission of both UV A and B during incubation (of subsurface waters) <sup>5</sup>.

| Lake name   | Sampling Depth    | Light exposure   | Water temperature (°C) | Incubation time (h) |
|---|-------------------|------------------|------------------------|---------------------|
| <b>Lac Gentau, June 2018</b><br>• Good weather<br>• Analytes: iHg(II), MMHg and DGM   | Subsurface (0.5m) | Diurnal and Dark | 6.87                   | 7.4                 |
|   | Middle depth (8m) | Diurnal and Dark | 4.37                   | 8.5                 |
|   | Bottom (17m)      | Dark             | 4.40                   | 8.7                 |
| <b>Lac Gentau, October 2018</b><br>• Cold, Rainy morning, Sunny spell midday, Cloudy afternoon<br>• Analytes: iHg(II), MMHg and DGM | Subsurface (0.5m) | Diurnal and Dark | 12.80                  | 7.2                 |
|   | Middle depth (8m) | Diurnal and Dark | 12.62                  | 6.0                 |
|   | Bottom (17m)      | Dark             | 6.05                   | 7.2                 |
| <b>Lac Gentau, June 2019</b><br>• Cold, Windy, Sunny spell morning<br>• Analytes: iHg(II), MMHg and DGM                             | Subsurface (0.5m) | Diurnal and Dark | 7.57                   | 8.7                 |
|   | Middle depth (8m) | Diurnal and Dark | 5.82                   | 8.5                 |
|   | Bottom (17m)      | Dark             | 4.30                   | 8.5                 |
| <b>Ibón de Sabocos, June 2018</b><br>• Good weather<br>• Analytes: iHg(II), MMHg  | Subsurface (0.5m) | Diurnal and Dark | 17.25                  | 6.3                 |
|   | Bottom (27m)      | Dark             | 4.80                   | 6.3                 |
| <b>Ibón de Sabocos, October 2018</b><br>• Cold, Cloudy afternoon<br>• Analytes: iHg(II), MMHg                                       | Subsurface (0.5m) | Diurnal and Dark | 10.70                  | 6.3                 |
|   | Bottom (23m)      | Dark             | 5.70                   | 6.3                 |
| <b>Ibón de Sabocos, June 2019</b><br>• Windy, Cloudy<br>• Analytes: iHg(II), MMHg and DGM   | Subsurface (0.5m) | Diurnal and Dark | 11.87                  | 6.8                 |
|   | Middle depth (9m) | Diurnal and Dark | 7.07                   | 6.8                 |
|   | Bottom (25m)      | Dark             | 4.87                   | 6.8                 |
| <b>Lac d'Arratille, June 2018</b><br>• Good weather<br>• Analytes: iHg(II), MMHg and DGM  | Subsurface (0.5m) | Diurnal and Dark | 5.70                   | 6.0                 |
|   | Middle depth (6m) | Dark             | 4.87                   | 5.3                 |
| <b>Lac d'Arratille, October 2018</b><br>• Rain, Wind, Bad weather<br>• Analytes: iHg(II), MMHg and DGM                              | Subsurface (0.5m) | Diurnal and Dark | 8.32                   | 5.3                 |
|   | Bottom (12m)      | Dark             | 5.64                   | 6.7                 |

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## 1 **Highlights**

- 2 • Hg speciation and cycling was studied in waters of Pyrenean alpine lakes
- 3 • Spatial and seasonal variations of Hg levels and speciation remained low
- 4 • Hg species transformation rates were measured by in-situ experiments
- 5 • Significant Hg methylation was measured in stratified anoxic waters
- 6 • Large photochemical reactions and Hg(0) evasion occurred in surface waters
- 7 • Climate change and eutrophication can be important drivers of those pathways

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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