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**Drivers of variability in mercury and methylmercury bioaccumulation and biomagnification in temperate freshwater lakes**

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26 **Abstract**

27

28         The four largest freshwater lakes in southern France are of both ecological and  
29 economic importance. However, some of them are subjected to mercury (Hg) contamination,  
30 resulting in the ban of human consumption of piscivorous fish. Moreover, beyond predatory  
31 fish, little information exist regarding Hg levels in other species of these ecosystems. In this  
32 context, we used a food web analytical approach to investigate Hg bioaccumulation and  
33 biomagnification in relation to the trophic structure of these four lakes. More specifically,  
34 various organisms (macrophytes, epiphyton, invertebrates and fish) were collected at the four  
35 lakes and analysed for carbon and nitrogen stable isotopes as well as for total Hg (THg) and  
36 methylmercury (MeHg). A spatial variability of bioaccumulation in organisms was observed,  
37 particularly in carnivorous fish, with higher Hg levels being found in the two more northern  
38 lakes (median±SE: 3491±474 and 1113±209 ng THg.g<sup>-1</sup> dw in lakes HC and L, respectively)  
39 than in the southern pair (600±117 and 911±117 ng THg.g<sup>-1</sup> dw in lakes CS and PB,  
40 respectively). Methylmercury biomagnification was observed through the food webs of all  
41 four lakes, with different trophic magnification slopes (HC=0.16; L=0.33; CS=0.27;  
42 PB=0.27), even though the length of the food chains was similar between the lakes. Our  
43 results suggest that rather than the food web structure, anthropogenic inputs (sulfate in  
44 northern lakes and phosphorus inputs in southern ones) may have a strong impact, more or  
45 less directly, on Hg methylation in freshwater environments, and lead to concentrations  
46 exceeding environmental recommendations despite low mercury backgrounds in sediment and  
47 water.

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50 **Keywords:** methylmercury, fish, invertebrates, epiphyton, stable isotope, lakes.

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## 53 **1. Introduction**

54

55 Mercury (Hg) is a toxic trace metal found naturally in the environment, and whose  
56 contemporary releases mainly come from anthropogenic activities (Driscoll et al. 2013;  
57 Lindberg et al. 2007). Methylmercury (MeHg) is the most toxic Hg species, mostly produced  
58 within aquatic ecosystems by prokaryotes (e.g. Bridou et al. 2011; Hamelin et al. 2011;  
59 Gilmour et al. 2013), bioaccumulated in biota mainly by trophic pathway, and biomagnified  
60 through aquatic food webs (Clarkson et al. 2006; Watras et al. 1998). Nonetheless, wide  
61 disparities that exist within and between ecosystems in measured Hg concentrations point to  
62 the complexity of the Hg biogeochemical cycle. Indeed, MeHg formation and transfer in food  
63 webs are influenced by a multitude of factors, such as levels of environmental inorganic Hg,  
64 the activity of prokaryotes carrying out most of the methylation processes, the bioavailability  
65 of inorganic Hg to these prokaryotes, the bioavailability of MeHg at the base of the food web,  
66 as well as the ecological mechanisms affecting the efficiency of biomagnification among  
67 which are primary productivity, habitat use, bioenergetics and food web structure (Arcagni et  
68 al. 2018; Braaten et al. 2020; Clayden et al. 2013; Eagles-Smith et al. 2018; Lavoie et al.  
69 2013; Ward et al. 2010; Wyn et al. 2009). Thus, identifying environmental factors influencing  
70 Hg bioaccumulation and biomagnification within specific aquatic ecosystems is essential for  
71 predicting where risks may be high for humans and wildlife, and to find remedial solutions.

72 The four largest freshwater lakes (Hourtin-Carcans, Lacanau, Cazaux-Sanguinet and  
73 Parentis-Biscarrosse) in southern France, are recognized as emblematic aquatic systems for  
74 both ecological and economic reasons. Recently, in the two more northern lakes (Hourtin-  
75 Carcans and Lacanau), high Hg concentrations exceeding the World Health Organization  
76 consumption recommendation ( $0.5 \mu\text{g}\cdot\text{g}^{-1}$  wet weight, equivalent to  $2.5 \mu\text{g}\cdot\text{g}^{-1}$  dry weight  
77 (dw) WHO, 1990) were detected in the carnivorous fish zander (*Sander lucioperca*) and

78 northern pike (*Esox lucius*) (ANSES, 2003). Significantly in this context, no local Hg  
79 emission sources have been identified in Southwestern France, nor is the region a localized  
80 'hot spot' for Hg atmospheric emissions (Colette et al. 2016) with Hg deposition  
81 measurements revealing normal Hg fluxes (Roustan et al. 2006 and data not shown).  
82 Understanding these spatial differences is therefore important for the conservation of these  
83 ecosystems. On this basis, the first objective of the present study was to determine and  
84 contrast the concentrations of Hg and MeHg in primary producers, invertebrates and fishes  
85 across the four lakes. The second objective was to assess the food web structure in each lake  
86 using stable C and N isotopes. The third objective was to determine and contrast Hg  
87 biomagnification across these four lakes and investigate underlying factors to the higher Hg  
88 concentrations measured in northern lakes.

89 To this end, we used a food web analytical approach combining carbon and nitrogen  
90 stable isotopes, which are useful tools to reveal feeding relationships among consumers. This  
91 approach is also used to understand Hg bioaccumulation in ecosystems because of the  
92 importance of diet as a route of exposure for Hg (Hall et al. 1997). Indeed, carbon and  
93 nitrogen stable isotopes can explain variability in Hg concentrations of different animal  
94 populations (Jardine et al. 2006). The relatively low enrichment of  $\delta^{13}\text{C}$  along food chains  
95 (1‰ between two trophic levels) enables discriminating the different sources of organic  
96 carbon (Peterson, 1999; Hobson et al. 2002). Thereby, it may be possible to link the various  
97 consumers to the various primary producers located at the base of food chains and to locate  
98 the entry point of matter and xenobiotics into food webs (Cabana & Rasmussen, 1994; Vizzini  
99 et al. 2002). In contrast to  $\delta^{13}\text{C}$ , nitrogen incorporation leads to an enrichment in  $\delta^{15}\text{N}$  of  
100 approximately 3 to 5‰ between each trophic level (Peterson, 1999; Cabana & Rasmussen,  
101 1994), allowing the trophic position of species to be inferred. Thus, nitrogen stable isotope  
102 allow assessing simultaneously the importance of food chain length and dietary pathway in  
103 Hg biomagnification processes (Jardine et al. 2006). In addition, the use of isotopic mixing

104 models, based on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in prey and predators, complements these analyses by  
105 enabling the feeding relationships between organisms to be more precisely estimated (Phillips  
106 et al. 2005).

## 107 **2. Methods**

108

### 109 2.1 Study area

110 The four lakes under study are located in the Nouvelle Aquitaine region (Southwestern  
111 France) (Figure 1). Lake Hourtin-Carcan (hereafter termed 'HC'; 45°06'00.4"N 1°07'05.1"W)  
112 flows into lake Lacanau ('L'; 44°58'26.6"N 1°07'18.7"W), whereas lake Cazaux-Sanguinet  
113 ('CS'; 44°29'22.1"N 1°09'35.6"W) flows into lake Parentis-Biscarosse ('PB'; 44°21'05.3"N  
114 1°09'58.6"W). The waterway connection between each pair is via a canal with locks that  
115 control lake water level. The watershed surfaces of the lakes, which belong to a common type  
116 of ecoregion (Carvalho et al. 2008), are between 200 to 360 km<sup>2</sup> (Table S1). Thermal  
117 stratification has already been observed in the two most southern lakes, but this is not  
118 systematic and the waters do not reach complete anoxia. Cyanobacteria blooms appear  
119 frequently in the eutrophic PB lake due to high amounts of sedimentary phosphorus  
120 (Cellamare et al. 2012). The lakes have been subjected to increasing anthropogenic pressure  
121 for several years, mainly due to recreational activities, especially angling and boating. They  
122 have also been exposed to the development of invasive aquatic macrophytes such as  
123 *Lagarosiphon major* and *Egeria densa*, particularly for the L and PB lakes (Bertrin et al.  
124 2017). The PB lake presents the highest colonized surface of invasive macrophytes (4.17  
125 km<sup>2</sup>), followed by L (1.19 km<sup>2</sup>), HC (0.94 km<sup>2</sup>) and CS (0.17 km<sup>2</sup>; surface area of dense  
126 stands > 50 g.m<sup>-2</sup> dw; Bertrin et al. 2017).

### 127 2.2 Sampling and sample preparation

128 Organisms, macrophytes and periphyton associated with macrophytes (epiphyton)  
129 were mainly collected during the autumn of 2015. Macrophytes and Asiatic clams (*Corbicula*  
130 *fluminea*) were also collected during the fall of 2017 and 2018 (Asiatic clams: 2017 in lake L,  
131 2018 in lake PB; *Lagarosiphon*: 2016 in lake CS, 2017 in lakes PB and HC; *Egeria*: 2017 in  
132 lake PB). Isotopic and Hg data (see below) were pooled in assuming similar food web  
133 structures and baselines in both sampling years. As part of this study, sediment sampling in  
134 the four lakes has been previously conducted and reported (collected in April 2014 and in  
135 January 2015 for the two northern lakes, and in spring and summer 2016 for the two southern  
136 lakes; Canredon et al. 2019).

137 Asiatic clams, crayfish (*Procambarus clarkii*) and stems of aquatic macrophytes  
138 (*Lagarosiphon major*, *Egeria densa* and *Phragmites australis*) were collected by hand.  
139 Special attention was given to the macrophytes to preserve their epiphyton during collection.  
140 Epiphyton fixed on submerged reed stems (*Phragmites australis*, a common plant in the four  
141 lakes) was collected by scraping and washing with field water, maintained at 4°C for 24 h,  
142 then centrifuged for 3 min at 10 000 g. An extensive fishing sampling campaign (with both  
143 nets and lines) provided 490 individuals of various fish species. Five species common to the  
144 four lakes were targeted (n = 353) in order to compare the bioaccumulation and  
145 biomagnification of Hg between lakes: zander, northern pike, European perch (*Perca*  
146 *fluviatilis*), common bream (*Abramis brama*) and common roach (*Rutilus rutilus*). Based on  
147 the fact that size is correlated with the age of individuals, a size class (Table S2) was chosen  
148 in order to compare total Hg concentrations per lake for the five species (n = 128, Table 1).  
149 For Hg speciation and nitrogen and carbon stable isotope analyses, five individuals of each  
150 species and lake were selected. Extended fishing facilities at lake CS only (permitted by a  
151 collaboration with a fishing sampling campaign organized by the French Agency for  
152 Biodiversity) resulted in the capture of additional fish species, namely bleak (*Alburnus*

153 *alburnus*), white bream (*Blicca bjoerkna*), gudgeon (*Gobio gobio*), ruffe (*Gymnocephalus*  
154 *cernua*) and black bullhead (*Ameiurus melas*) (see Table 1 for details).

155 Dorsal muscle tissues free of skin were dissected from fish, while muscle free of shell  
156 and soft tissues were dissected from crayfish and molluscs, respectively. A single tissue  
157 sample from molluscs comprised 10 pooled individuals. Samples were freeze-dried and  
158 homogenised by automatic grinding using Teflon® balls and mortars, washed with 3% HCl  
159 and rinsed with ultrapure water between each sample. The ground material obtained was  
160 stored in ultraclean amber glass tubes at 4°C.

161

### 162 2.3 Total mercury analysis

163 Total Hg (THg) concentrations in samples were determined by flameless atomic  
164 absorption spectrometry (Altec AMA 254). The detection limit of this method is 0.01 ng and  
165 the limit for quantification is 0.010 µg.g<sup>-1</sup> dw. Reference material IAEA 436 (International  
166 Atomic Energy Agency, Tuna Fish Flesh Homogenate) was used every ten samples to control  
167 analytical accuracy, which averaged 101.5%.

168

### 169 2.4 Mercury speciation analysis

170 Plants and epiphyton were digested with 6N nitric acid, whereas fish and crayfish  
171 muscle and mollusc soft tissues were digested with TMAH (Tetramethylammonium  
172 hydroxide), under microwave radiation. All samples were analysed by GC-ICP-MS (gas  
173 chromatography-inductively coupled plasma-mass spectrometry; Focus GC and ICPMS X2  
174 series, Thermo Electron) as described elsewhere (Rodriguez Martin-Doimeadios et al. 2002;  
175 Clémens et al. 2011). Quantification of Hg species was performed by species-specific isotope  
176 dilution, by adding the appropriate amount of isotopically enriched Hg standards (<sup>199</sup>iHg and  
177 <sup>201</sup>MeHg) (Rodriguez Martin-Doimeadios et al. 2002). Each assay was analyzed three times,  
178 with the measurement error for MeHg and iHg being <2%. Data quality was checked by



179 blanks and by IAEA 407 reference material (Fish Homogenate) with a recovery rate of 93.4%  
180 for MeHg and 106.6% for IHg. The limits of quantification are 0.02 ng.L<sup>-1</sup> for IHg and 0.005  
181 ng.L<sup>-1</sup> for MeHg. Hg concentrations are expressed in ng.g<sup>-1</sup> on a dry weight basis.

182

## 183 2.5 Stable isotope analysis

184 Homogenized powder samples were weighed into tin capsules using a microbalance  
185 (XPE26, Mettler Toledo®). Isotopic analyses were performed by the platform ‘Spectrométrie  
186 Isotopique’ (LIENSs laboratory, La Rochelle) using a Thermo Scientific Delta V Advantage  
187 isotope ratio mass spectrometer (Chartier et al. 2014). The <sup>13</sup>C/<sup>12</sup>C (denoted δ<sup>13</sup>C) and <sup>15</sup>N/<sup>14</sup>N  
188 (denoted δ<sup>15</sup>N) ratios, expressed in ‰, were calculated as the relative differences between the  
189 sample and the conventional standard following Peterson and Fry (1987). Standards were run  
190 in duplicate every twenty measurements. The analytical precision for δ<sup>13</sup>C and δ<sup>15</sup>N were  
191 0.2‰ and 0.3‰, respectively. According to Post et al. (2007), organisms with a C/N ratio  
192 of > 4 (solely molluscs and bleak) were systematically lipid extracted. Lipids were removed  
193 by successive cyclohexane washing (addition of 4 ml cyclohexane to 15 mg of homogenized  
194 powder samples, placed 5 min in an ultrasonic bath, 10 min vortex, and centrifugation 5 min  
195 at 4500 rpm). The supernatant containing lipids was removed and cyclohexane washings were  
196 performed on the pellet until the supernatant was clear, then the pellet was dried at 45°C.  
197 Carbonate extraction was also tested for macrophytes and epiphyton from the four lakes.  
198 Carbonates were removed prior to elemental analyses by adding HCl 0.5 N on samples (until  
199 cessation of bubbling) and placed for 3 min in an ultrasonic bath. Subsequently, samples were  
200 dried at 50 °C, homogenized using an ultrasonic bath after addition of milliQ water, and  
201 freeze-dried before ground again. No between lake differences were observed, except for reed  
202 epiphyton from HC where the δ<sup>13</sup>C value after carbonate extraction was kept.

203

## 204 2.6 Data analyses

205 2.6.1 Trophic position calculation

206 The trophic position (TP) of each organism was calculated based on  $\delta^{15}\text{N}$  values and  
207 following the equation of Bergamino et al. (2011):

208 
$$\text{TP}_i = [(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{pc}}) / 3.4] + 2 \text{ (Eq 1)}$$

209 where  $\text{TP}_i$  represents the average trophic position of species  $i$ ;  $\delta^{15}\text{N}_i$  the average  $\delta^{15}\text{N}$  value of  
210 species  $i$ ;  $\delta^{15}\text{N}_{\text{pc}}$  the average  $\delta^{15}\text{N}$  value of primary consumers; 3.4 the mean  $\delta^{15}\text{N}$  trophic  
211 enrichment occurring per trophic level (Post, 2002); and 2, the trophic position of the baseline  
212 organism (filter feeders in our study). Because of our inability to find a filter feeder in lake  
213 HC and since HC waters flow into lake L, calculations of an organism's trophic position in  
214 the former lake were based on the  $\delta^{15}\text{N}$  value for filter feeders from the latter.

215

216 2.6.2 Isotopic mixing model

217 The relative isotopic contributions of the different food sources to the three  
218 carnivorous fish species studied were estimated by incorporating C and N isotope data into a  
219 Bayesian stable isotope mixing model (SIMMR package in R software, Parnell et al. 2013).  
220 Potential prey for each of these species were chosen according to the literature (Bruslé et al.  
221 2001; Keith et al. 2011; Schlumberger and Elie, 2008) and validated with stomach content  
222 observations: crayfish, common roach and common bream were identified as common food  
223 sources for European perch, northern pike and zander; juvenile and small European perch  
224 were considered as additional prey for northern pike and zander. We used trophic enrichment  
225 factors of  $2.90 \pm 0.32\text{‰}$  for nitrogen and  $1.30 \pm 0.30\text{‰}$  for carbon between prey and predator  
226 muscles, as previously advised in the study of Mc Cutchan et al. (2003). The degree of  
227 uncertainty associated with the mixing-model outputs was usually close to 10%.

228

229 2.6.3 Mercury biomagnification factors

230 2.6.3.1 Biomagnification factor (BMF)

231 Biomagnification factor (BMF) is a magnification factor between a predator and its  
232 main preys. To quantify the proportion of sources (PS) consumed by a predator (consumer,  
233 C), we used our results obtained from the stable isotope mixing models in the following  
234 equation modified according to Lavoie et al. (2010):

$$235 \quad \text{BMF}_{\text{PSC}} = [\text{Hg}]_{\text{predator}} / \left( \sum_{i=1}^n ([\text{Hg}]_{\text{prey } i} \times f_{\text{prey } i}) \right) \text{ (Eq 2)}$$

236 where  $[\text{Hg}]_{\text{prey } i}$  is the Hg concentration of prey  $i$  and  $f_{\text{prey } i}$  is the proportion of prey  $i$  in the diet  
237 of its predator. Proportions of sources are provided in Table S3.

238

### 239 2.6.3.2 Trophic Magnification Slope (TMS)

240 The biomagnification potential throughout the entire food web for each lake was  
241 assessed using the slope (b), termed Trophic Magnification Slope (TMS), of the simple linear  
242 regression that included all organisms (Eq 3):

$$243 \quad \text{Log}_{10}[\text{Hg}] = b (\delta^{15}\text{N}) + a \text{ (Eq 3)}$$

244 where  $a$  is the intercept. As for the TP calculation (Eq 1), we used  $\delta^{15}\text{N}$  values from filter  
245 feeders taken from lake L to calculate the TMS for HC.

246

### 247 2.6.3.3 Food web magnification factor (FWMF)

248 In order to compare different ecosystems, the biomagnification potential through each  
249 lake's entire food web was corrected by taking into account the different trophic enrichment  
250 factors and the baseline differences and was calculated as follow (Fisk et al. 2001):

$$251 \quad \text{FWMF} = 10^b \text{ (Eq 4)}$$

252 where  $b$  is the TMS from Eq (3) using TL instead of  $\delta^{15}\text{N}$ .

253

### 254 2.6.4 Statistical analyses

255 Factorial ANOVAs were used to study differences in species THg concentrations for  
256 each site. Assumptions of normality and homoscedasticity of the error term were tested. If the

257 assumption was satisfied, the parametric post hoc LSD Fisher test was applied; if not, the non-  
258 parametric Kruskal-Wallis test was used. Comparisons of means within the same lake for two  
259 different groups of individuals (*e.g.* carnivorous versus omnivorous) were performed using  
260 Student's t-test when the assumption was met or the non-parametric Wilcoxon test when it  
261 was not met. An ANCOVA followed by a post hoc Tukey's test was run to check if the  
262 difference in TMS differed significantly between lakes. In each test,  $p < 0.05$  was considered  
263 significant. All statistical analyses were performed using *STATISTICA* version 6.1 software  
264 (Statsoft, USA).

265

### 266 3. Results

#### 267 3.1 Mercury concentrations in biota

268 Total Hg concentrations measured in macrophytes, epiphyton, invertebrates and low-trophic  
269 level fish (both herbivorous and omnivorous) in all four lakes ranged from 5.5 ng. g<sup>-1</sup> (plant  
270 *Egeria densa*, PB) to 982 ± 325 ng. g<sup>-1</sup> (common roach, CS, Table 1). Carnivorous fish  
271 (European perch, northern pike, zander) expressed the highest THg concentrations and MeHg  
272 percentages in all lakes, particularly in HC (maximum value: 7384 ± 1536 ng. g<sup>-1</sup>, 94% MeHg  
273 in zander, Table 1). Total Hg and MeHg concentrations were also averaged for omnivorous  
274 and carnivorous fish to compare Hg levels between lakes (Figure 2). In omnivorous species, a  
275 significant lake effect on THg concentrations was observed (ANOVA,  $F_{3,89}=3.2$ ,  $p=0.03$ ).  
276 Post hoc LSD Fisher's multiple comparison test indicated that THg concentrations were  
277 significantly lower in PB than in the other three lakes (all  $p<0.05$ ). However, no significant  
278 effect of lake with respect to MeHg concentrations was observed ( $F_{3,27}=1.4$ ,  $p=0.26$ ). In  
279 carnivorous species, a significant lake effect was observed for both THg ( $F_{3,124}=25.04$ ,  
280  $p<0.001$ ) and MeHg ( $F_{3,40}=14.1$ ,  $p<0.001$ ) concentrations. Both THg and MeHg  
281 concentrations were significantly higher in HC than in the other lakes (all  $p<0.001$ ).

282 Moreover, MeHg concentrations were also significantly higher at L and CS than at PB (both  
283  $p < 0.05$ ).

284

### 285 3.2 Food web structure and feeding behavior of top predators

286 Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes mean values for the different species  
287 analysed are summarised in Table 1 and plotted for each lake in Figure 3 in order to visualize  
288 the food web relationships among the species. For three of the four lakes (L, CS and PB), the  
289  $\delta^{13}\text{C}$  values were significantly different between species (Kruskal-Wallis test, L:  $H = 19.00$ ;  
290 CS:  $H = 36.22$ ; PB:  $H = 30.20$ , all  $p < 0.01$ ) except between the Asiatic clams and European  
291 perch in lake L, between gudgeon and ruffe and gudgeon and Asiatic clams in lake CS, and  
292 between the Asiatic clams and northern pike in PB (all  $p < 0.05$ ). Carbon values were not  
293 significantly different between species for HC (ANOVA,  $F_{8,22} = 0.91$ ,  $p = 0.53$ ). Carbon  
294 isotopic ratios in carnivorous fish were significantly different between lakes (ANOVA,  
295  $F_{3,51} = 0.91$ ,  $p = 0.15$ ) with higher values occurring in HC than in the other three lakes (Post  
296 hoc LSD Fisher test,  $p < 0.001$ ).

297 Few significant differences in  $\delta^{15}\text{N}$  values were found between species within each  
298 lake (Kruskal-Wallis test, HC:  $H = 24.76$ ; L:  $H = 23.11$ ; CS:  $H = 47.00$ ; PB:  $H = 40.56$ , all  
299  $p < 0.01$ ), and when present, were mainly between species of low- and high-trophic levels (HC:  
300 zander vs common roach / zander vs crayfish; L: clam vs northern pike; CS: zander vs clam /  
301 zander vs gudgeon; PB: zander vs clam, all  $p < 0.05$ ). Nitrogen isotopic ratios in carnivorous  
302 fish were significantly different between lakes (Kruskal-Wallis test,  $H = 47.28$ ,  $p < 0.001$ )  
303 with higher values in PB than in the other lakes  $p < 0.05$ ). Trophic positions (TP, Table 1)  
304 ranged from  $1.4 \pm 0.1$  for crayfish (HC) to  $4.1 \pm 0.1$  for zander (PB). Trophic positions of  
305 zanders (considered as the top predator) were not statistically different between lakes  
306 (Kruskal-Wallis test,  $H = 9.98$ ,  $p = 0.018$ ). The diets of zander, northern pike and European  
307 perch are presented in Table S3. Overall, these species had a similar diet, both within and

308 between lakes. However, some variability was observed: at HC, L and CS: northern pike and  
309 European perch had higher isotopic contribution from benthic prey in their diet (for northern  
310 pike, 23 to 51% crayfish and 18 to 53% common bream; for European perch, 44 to 50%  
311 crayfish and 24 to 25% common bream) than of pelagic prey (for northern pike, 8 to 18%  
312 common roach and 13 to 15% European perch; for European perch, 15 to 26% common  
313 roach). Conversely, zander had approximately equal isotopic contribution of the different prey  
314 species (see Table S3 for details).

315

### 316 3.3 Mercury biomagnification factors

317 The significant and positive linear relationships between  $\log_{10}[\text{MeHg}]$  and  $\delta^{15}\text{N}$  values  
318 (Figure 4) indicated that MeHg biomagnified along the food web of each lake (HC:  $F_{1,23}=7.5$ ,  
319  $R^2=0.21$ ,  $p=0.01$ ; L:  $F_{1,24}=39.2$ ,  $R^2=0.60$ ,  $p<0.01$ ; CS:  $F_{1,45}=184$ ,  $R^2=0.80$ ,  $p<0.001$ ; PB:  
320  $F_{1,30}=136.4$ ,  $R^2=0.81$ ,  $p<0.001$ ). Trophic Magnification Slopes (TMS) and Food Web  
321 Magnification Factors (FWMF) were calculated based on MeHg and THg concentrations  
322 measured in organisms from each lake (Table 2). The TMS for THg and MeHg ranged from  
323 0.13 (PB) to 0.23 (L) and from 0.16 (HC) to 0.33 (PB), respectively. The TMS for MeHg was  
324 significantly steeper than for the THg of each lake (L:  $F_{52,53}=4.13$ ;  $p<0.05$ ; CS:  $F_{100,101}=11.13$ ;  
325  $p<0.001$ ; PB:  $F_{68,69}=20.86$ ;  $p<0.001$ ), except for HC (HC:  $F_{51,52}=0.03$ ;  $p=0.86$ ). A significant  
326 lake effect on the TMS for MeHg was observed (ANCOVA,  $F_{3,122} = 2.8$ ,  $p = 0.045$ ), with  
327 lower TMS values measured at HC than the three other lakes (Post hoc Tukey's test, all  
328  $p<0.01$ ). Food web magnification factors ranged from 2.96 (PB) to 5.35 (L) for THg and from  
329 3.48 (HC) to 12.87 (L) for MeHg. For carnivorous fish, the highest  $\text{BMF}_{\text{psc}}$  was observed for  
330 European perch from HC for both THg and MeHg (9.5), whereas the lowest  $\text{BMF}_{\text{psc}}$  was  
331 observed for zander from L for THg (2.0) and for northern pikes from L and CS for MeHg  
332 (2.9) (Table 1). For lakes L, CS and PB, the  $\text{BMF}_{\text{psc}}$  for carnivorous fish were systematically  
333 higher than the FWMF for MeHg, which was not the case for lake HC (Tables 1 and 2).

334

## 335 4. Discussion

336

### 337 4.1 Mercury concentrations in biota

338 Total Hg concentrations in epiphyton and macrophytes (Table 1) were relatively  
339 similar to those previously measured (Gentès et al. 2013a) in all four lakes, except PB where  
340 Hg levels were six times higher in epiphyton associated with *Egeria densa* than in *Egeria*  
341 *densa* itself. This could be explained by the fact that epiphyton is known to be a trap for  
342 pollutants such as Hg, due to their rich organic matter composition (Coelho-Souza et al. 2011;  
343 Gentès et al. 2013b; Klaus et al. 2016). Total Hg concentrations in macroinvertebrates  
344 (crayfish) and omnivorous species (common bream and common roach) were below the  
345 WHO guideline threshold and were relatively similar to other European aquatic systems,  
346 including streams (Babut et al. 2011; Noël et al. 2013) and lakes (Łuczynska et al. 2018;  
347 Orтели et al. 2009). However, THg levels in the carnivorous fish were close to, or exceeded,  
348 the WHO guideline, especially in the two northern lakes (HC and L), where THg levels were  
349 two to six times higher (in fish of similar standard length) than in fish from similar  
350 ecosystems (Babut et al. 2011; Luczynska et al. 2018), but were close to THg levels found in  
351 disturbed temperate freshwater lakes (Gorski et al. 2003; Chasar et al. 2009) or tropical  
352 ecosystems (Berzas-Nevado et al. 2010).

353 Moreover, a spatial variability in MeHg concentrations between lakes was observed in the  
354 three carnivorous fish, highlighting a South-North positive gradient for MeHg, although this  
355 was less evident than for THg. A similar gradient for MeHg has been previously observed in  
356 the organic sediment of these lakes (Canredon et al. 2019), and also in epiphyton and crayfish,  
357 although statistical testing was lacking due to a paucity of sampled organisms.

358

### 359 4.2 Food web structure

360 In our study, carbon and nitrogen isotopic data suggest that the food web structures  
361 were not significantly different between the four studied lakes (despite that less organisms  
362 were sampled in lakes HC and L). Indeed, we found few differences between trophic levels of  
363 organisms (i.e., the same trophic guilds), and equivalent food web lengths (even if food web  
364 length of the two most southern lakes were slightly longer than the two northern ones).  
365 However, significantly higher nitrogen ratios found in carnivorous fish of lake PB than at  
366 three other lakes show higher enrichment in nitrogen, but could be explained by the presence  
367 of high biomass of invasive macrophyte species (Bertrin et al., 2017) and regular  
368 cyanobacteria bloom (Cellamare et al., 2012) in this eutrophic system, leading to a higher  
369 incorporation of atmospheric nitrogen.

370 Methyl Hg trophic transfer efficiency in carnivorous fishes is influenced by the  
371 composition of food web and feeding relationships (Vander Zanden and Rasmussen, 1996;  
372 Cabana and Rasmussen, 1994). Here, it is thus unlikely that food web structure was the main  
373 factor explaining the observed differences in Hg bioaccumulation in top predatory fishes. On  
374 the other hand, processes at the base of food webs that influence MeHg availability are  
375 generally the principal contributors to bioaccumulation and biomagnification of Hg in  
376 ecosystems (Gorski et al. 2003; Chasar et al. 2009; Molina et al. 2010). Significant  
377 differences observed in  $\delta^{13}\text{C}$  values between invasive macrophytes and other organisms  
378 indicated that macrophytes do not represent a major feeding resource for grazing fish  
379 (common roach) or detritivorous (crayfish) in the four lakes. Conversely, their epiphyton  
380 (epiE, epiphyton *Egeria*) and epiphyton associated with endemic plants (epiR, epiphyton  
381 reed) appears to be a food source for these organisms. Previous studies have highlighted the  
382 relative importance of epiphytic algae compared to macrophytes as a carbon source in benthic  
383 food webs (France, 1995a). In addition to allochthonous inputs, therefore, other autochthonous  
384 sources of carbon need to be investigated within the trophic chain, such as phytoplankton,



385 other macrophyte species and their associated epiphyton, as well as particulate organic matter,  
386 in order to assess more precisely food web functioning (France, 1996).

387

#### 388 4.3 Methyl Hg biomagnification

389 A significant MeHg biomagnification was observed along the food webs of all lakes  
390 (Figure 4) with TMS values similar to those observed in other temperate freshwater lakes  
391 (Lavoie et al. 2013). Food Web Magnification Factor (FWMF) were similar to equivalent data  
392 from other studies (Lavoie et al., 2010; Fisk et al. 2001) and were higher for MeHg than for  
393 THg in all the lakes, but this was not systematically the case for the  $BMF_{psc}$  of carnivorous  
394 fish, which showed the greatest bioavailability and the largest proportion of MeHg in  
395 organisms. The elevated  $BMF_{psc}$  calculated for European perch from HC could be explained  
396 by the greater weight of individuals sampled from this lake.

397 The trophic transfer efficiency of Hg depends on local geochemical and biological  
398 factors (Luoma and Rainbow, 2005; Veltman et al. 2008). In a related aspect of the current  
399 project, an extensive mapping of the sediment characteristics and their Hg rates was  
400 conducted in the four lakes (Canredon et al. 2019), which revealed no sediment contamination  
401 and similar THg concentrations (averaged concentration in organic sediment for the four  
402 lakes:  $213 \mu\text{g THg}\cdot\text{g}^{-1} \text{ dw}$ ). However, the MeHg proportion in organic sediment was higher in  
403 HC than the other three lakes ( $2.5 \pm 1\%$  of THg for lake HC,  $1.7 \pm 0.7\%$  for L,  $0.5 \pm 0.1\%$  for  
404 CS and PB; Canredon et al. 2019), following the same South-North positive gradient as that  
405 observed in the present study for carnivorous fish and crayfish. Since the origin of carbon in  
406 HC seems to be more benthic than in the other lakes, HC's organic sediment and the epifauna  
407 living on this substrate (such as crayfish) could be the main entry point of MeHg into the food  
408 web. The MeHg trophic transfer efficiency associated with benthos is generally considered to  
409 be less than that associated with the pelagic compartment (Cossa and Gobeil, 2000), thus

410 explaining the lowest TMS observed for HC. Here, the greater input of MeHg in sediment  
411 would offset the latter's lower trophic transfer efficiency, thereby providing an explanation  
412 for the highest biota contamination in this lake compared to the others.

413 In their study, Canredon et al. (2019) also highlighted a South-North positive gradient  
414 of sulfate in the water column (PB = 114  $\mu$ M, CS = 126  $\mu$ M, L = 276  $\mu$ M and HC = 392  $\mu$ M),  
415 with optimal concentrations for the development of sulfate reducing microorganisms (SRM)  
416 occurring in the HC water column. Sulfate reducers are considered as the main source of  
417 MeHg production in the environment (King et al. 2001; Bridou et al. 2011). Moreover, a  
418 laboratory incubation with sulfate-enrichment of epiphyton on floating macrophyte roots  
419 (*Ludwigia* sp.) from CS and two other ecosystems of the region showed that Hg  
420 transformation processes were mainly due to SRM activity (Gentès et al. 2013b).  
421 Consequently, in these lakes, the sulfate concentration gradient in water could entail a biotic  
422 MeHg production gradient in epiphyton as well as in organic sediment. A natural origin of  
423 sulfate is unlikely since (i) the geological formation of these lakes and their respective  
424 watersheds are similar, and (ii) the distances between them are relatively small. Agricultural  
425 activity has been identified as a source of sulfate for these lakes, especially HC, due to the  
426 addition of lime to soils to control crop pH (Canredon et al. 2019). A survey on the use of  
427 sulfate by local farmers should therefore be conducted to further examine this hypothesis. If  
428 confirmed, solutions could be proposed to reduce these inputs and thus lower Hg  
429 concentrations in the biota of the more northern lakes (Braaten et al. 2020).

430 Parentis-Biscarrosse is the lake with the lowest THg and MeHg concentrations measured in  
431 biota in parallel with the lowest sulfate concentrations measured in its water (Canredon et al.  
432 2019). However, in addition to the presence of invasive macrophytes (Bertrin et al. 2017),  
433 cyanobacterial blooms occur regularly in PB (Cellamare et al. 2012). Another explanation for  
434 such low Hg levels in PB biota could be the presence of multiple carbon sources that in turn  
435 enhance the dilution factor for Hg bioavailability at the base of the food chain (Chen and Folt

436 2005). As a consequence, biodilution would be an indirect beneficial effect of the  
437 eutrophication on Hg dynamics in this lake, reducing bioaccumulation and the  
438 biomagnification factor in top predators.

439 Seasonal variation can affect TMS, for example through changes in  $\delta^{15}\text{N}$  values in lower  
440 TP organisms, with a resultant influence on the entire food web (Borgå et al. 2011). To assess  
441 Hg biomagnification variability during the course of a year, different seasonal sampling of  
442 biota should be considered for these lakes, and particularly PB.

443

## 444 **5. Conclusion**

445 In this study, THg and MeHg concentrations were measured in the biota of the four largest  
446 lakes of southwestern France, in relation to their trophic structure. High Hg concentrations in  
447 piscivorous fish from the two northern lakes were confirmed, whereas fish from the two more  
448 southern lakes were below the WHO consumption recommendation. Methyl Hg  
449 biomagnification was observed in all four lakes, and surprisingly, the most contaminated lake  
450 displayed the lowest TMS. Food web structure alone could not explain this difference,  
451 although sulfate concentrations in the water column seems to be the main driver. In addition,  
452 the lake with the lowest Hg concentrations in biota was eutrophic as a consequence of  
453 preceding phosphorus inputs, and was subjected to regular algal blooms. Eutrophication could  
454 therefore have an indirect beneficial (reducing) effect on Hg bioaccumulation. This study  
455 therefore highlights the view that even in environments with very low mercury backgrounds  
456 in sediment and water, and beyond the immediate interest of controlling mercury release,  
457 other anthropogenic inputs (here, sulfate and phosphorus) may have a strong impact, more or  
458 less directly, on Hg methylation in freshwater environments.

459 Future studies of these ecosystems will aim to assess the methylation and demethylation  
460 potentials in different compartments at the base of the food web (organic sediment, epiphyton  
461 associated with invasive and endemic plants) using enriched stable isotopes of Hg to identify

462 the role played by each compartment in Hg transformations. In addition, microorganism  
463 diversity associated with each compartment will be studied, as well as sulfate reduction rates,  
464 since MeHg production in these aquatic compartments seems to be linked to the activity of  
465 sulfate reducers. Additionally, important information would derive from characterizing the Hg  
466 sources in these lacustrine ecosystems using Hg stable isotopes (isotopic fractionation).

467

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683

### 684 LEGENDS TO FIGURES

685

686 Figure 1. Location of the four sampled freshwater lakes in southwestern France.

687

688 Figure 2. Boxplots of total mercury (left) and methylmercury (right) concentrations in muscle  
689 tissue of carnivorous (zander, northern pike and European perch) and omnivorous fish species  
690 (common roach and common bream) in lakes Hourtin-Carcans (HC), Lacanau (L), Cazaux-  
691 Sanguinet (CS) and Parentis-Biscarrosse (PB). n: number of samples. Letters indicate  
692 statistical differences ( $p < 0.05$ ).

693

694 Figure 3. Relationships between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  mean values (‰) of species in the four lakes.  
695 Bars represent standard error. lag: *Lagarosiphon*, ege: *Egeria*, epiR: epiphyton reed, epiE:  
696 epiphyton *Egeria*, epiL: epiphyton *Ludwigia*, cray: crayfish, roa: common roach, bre:  
697 common bream, per: European perch, pik: northern pike, zan: zander, clam: Asiatic clams,  
698 ble: bleak, wbre:white bream, gud:gudgeon, ruf: ruffe, bbh: black bullhead.

699

700 Figure 4. Averages of  $\log_{10}$  MeHg concentrations versus trophic levels ( $\delta^{15}\text{N}$ ) measured in  
701 biota of the four lakes. Bars represent standard errors for  $\delta^{15}\text{N}$  and [MeHg]. The linear  
702 regression and its correlation coefficient ( $R^2$ ) were calculated for all individual species. lag:  
703 *Lagarosiphon*, ege: *Egeria*, epiR: epiphyton reed, epiE: epiphyton *Egeria*, epiL: epiphyton  
704 *Ludwigia*, cray: crayfish, roa: common roach, bre: common bream, per: European perch, pik:  
705 northern pike, zan: zander, clam: Asiatic clams, ble: bleak, wbre:white bream, gud:gudgeon,  
706 ruf: ruffe, bbh: black bullhead.

707

708 Table 1. Average mercury concentrations (Mean THg,  $\text{ng}\cdot\text{g}^{-1}$  dry weight), percentages of  
709 MeHg (%), stable isotope ratios ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , ‰) and trophic positions (TP) of organisms  
710 sampled from the four lakes. Biomagnification Factor ( $\text{BMF}_{\text{PSC}}$ ) were calculated in zander,  
711 northern pike and European perch for MeHg and THg for each lake. Biometric characteristics  
712 of organisms are also indicated: total weight (g) and standard length (cm). Values are means  $\pm$   
713 standard error; N: number of samples for  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and THg analysis, n: number of samples  
714 for MeHg analysis.

715

716 Table 2. Trophic Magnification Slope (TMS) and Food Web Magnification factor (FWMF)  
717 calculated for THg and MeHg in each lake.

718

*Atlantic  
ocean*

0 5 10 20 30  
Kilometers

**Hourtin-Carcans**

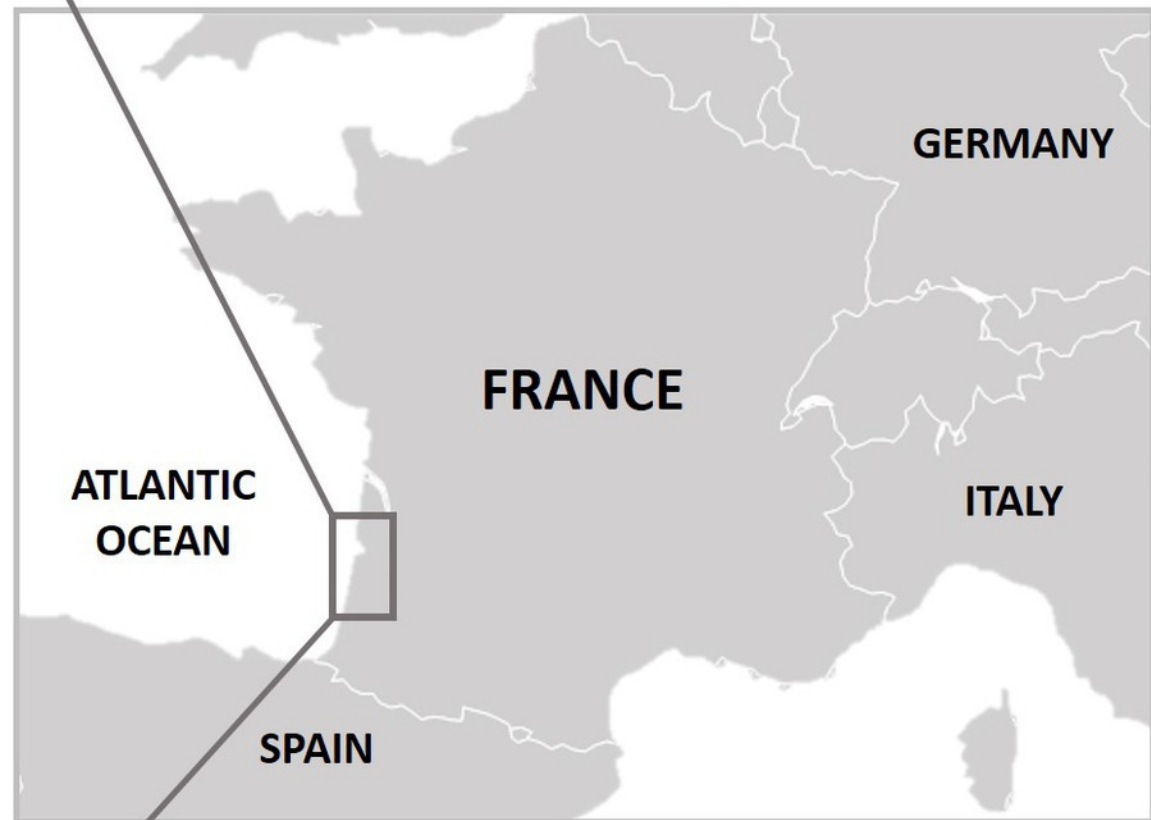
**Lacanau**

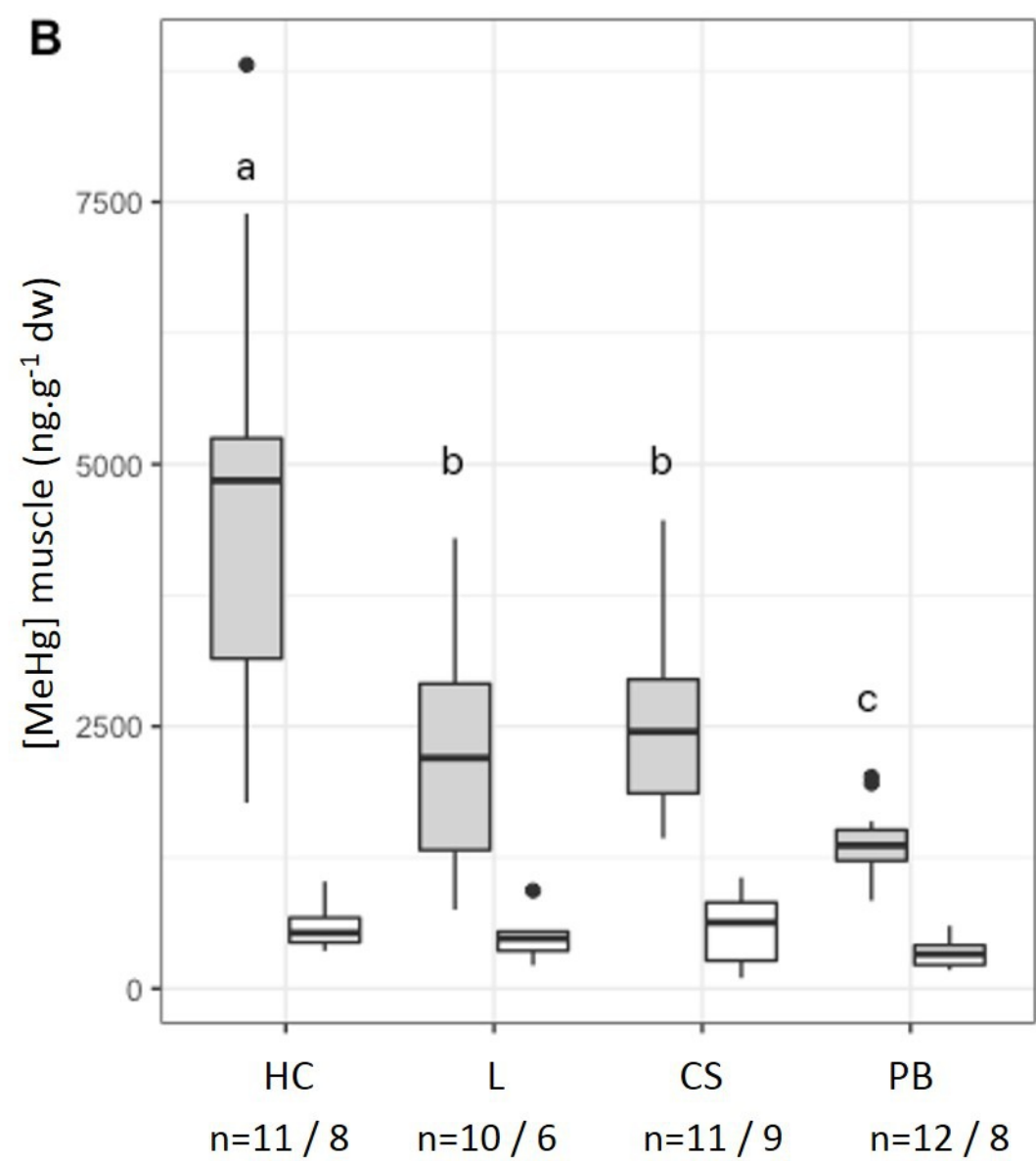
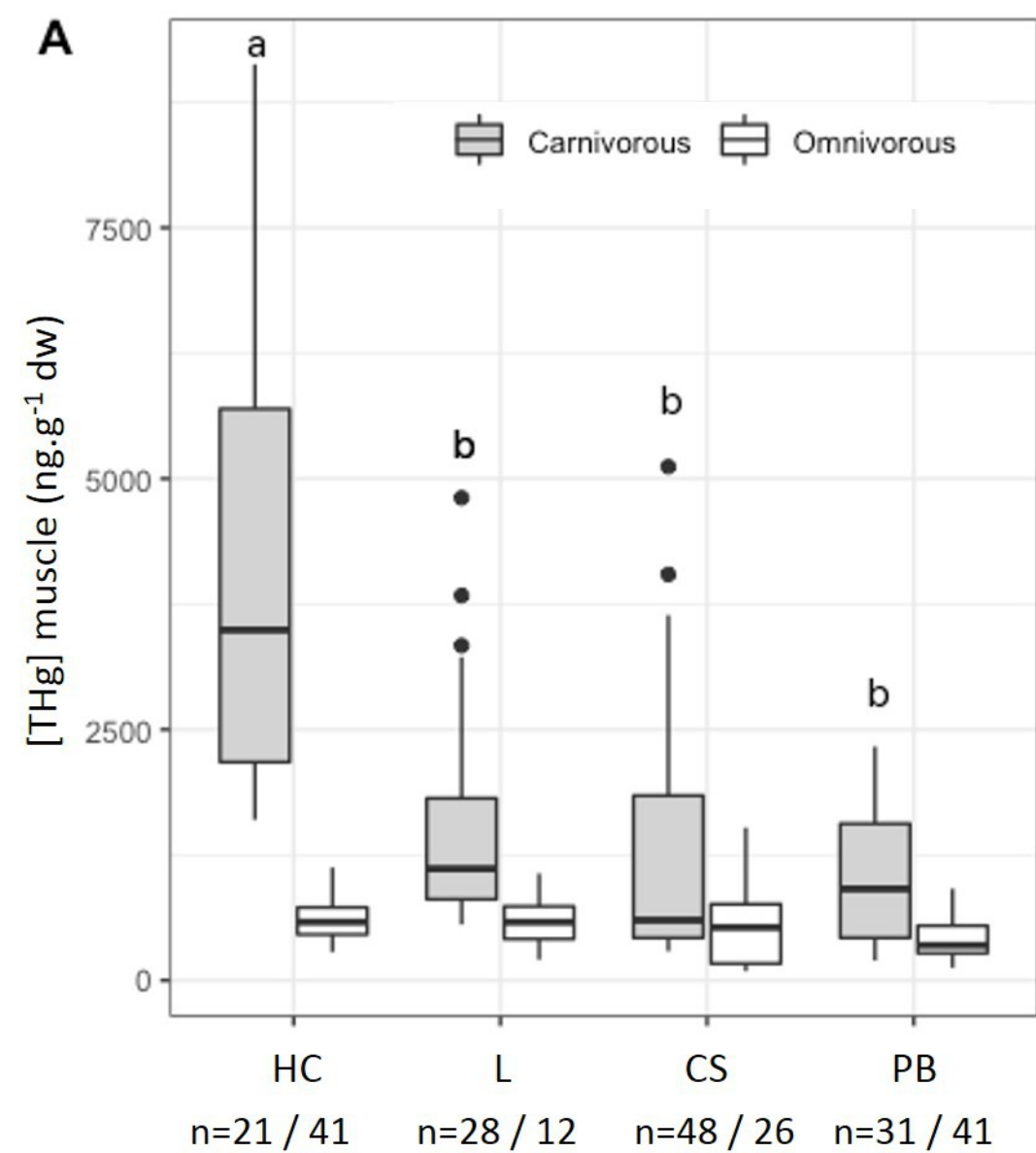
*Bordeaux*

*Arcachon bay*

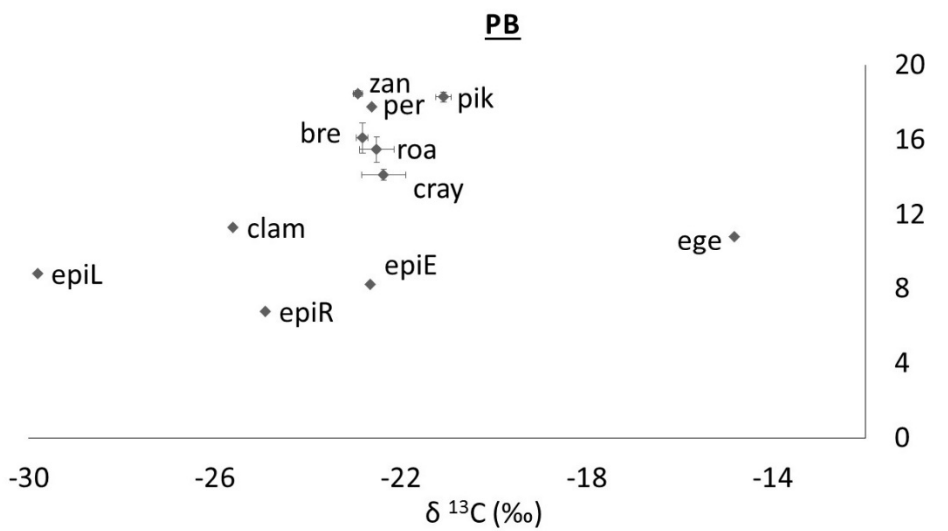
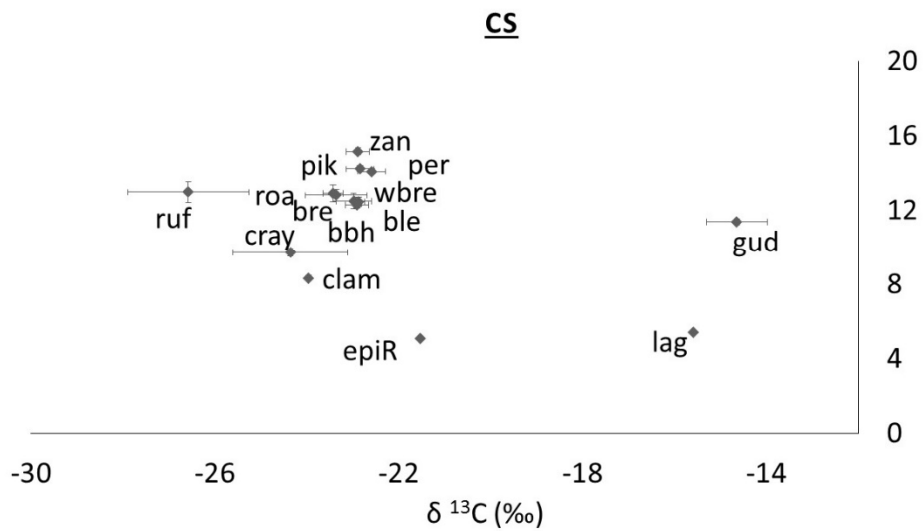
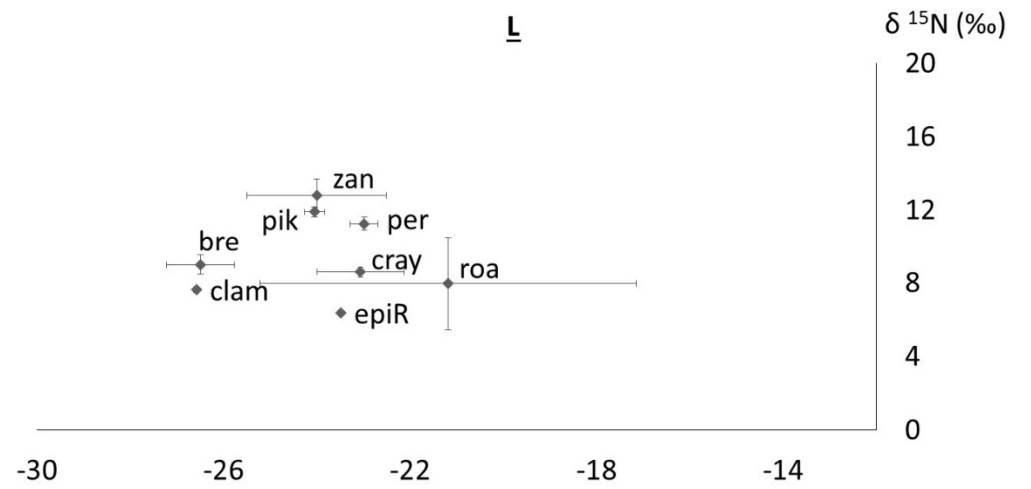
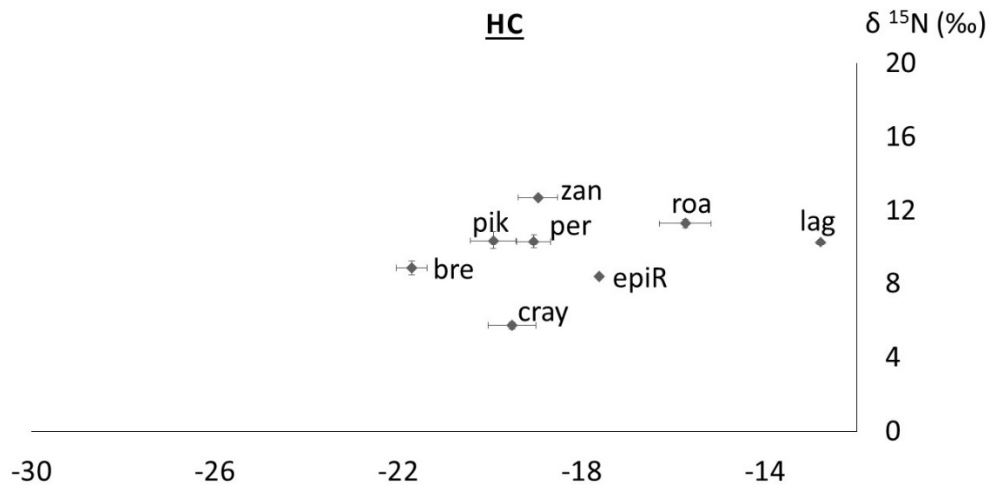
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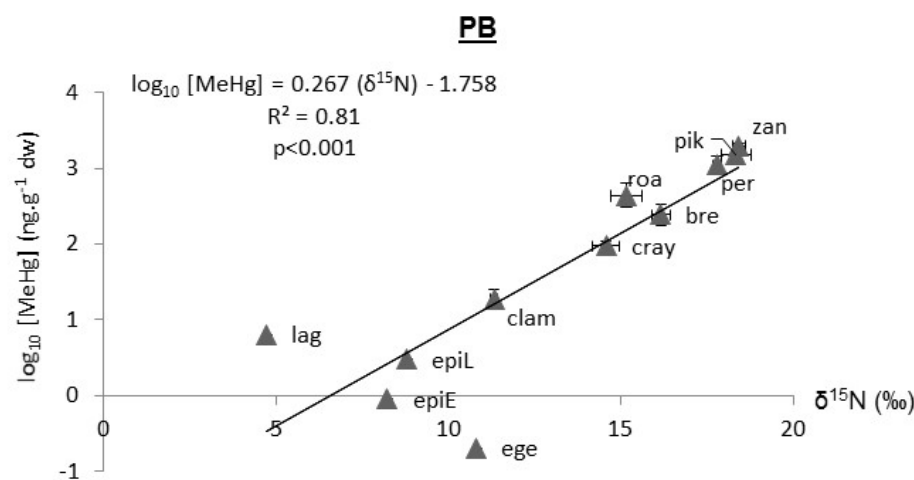
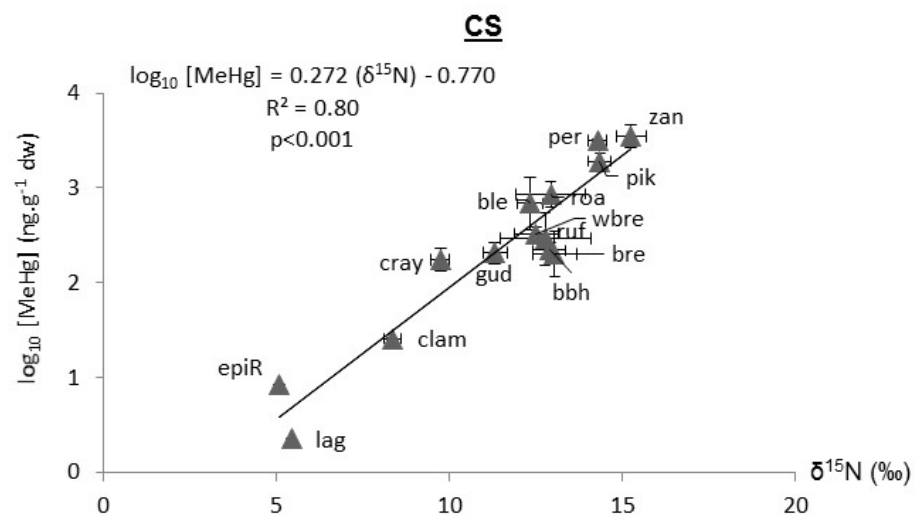
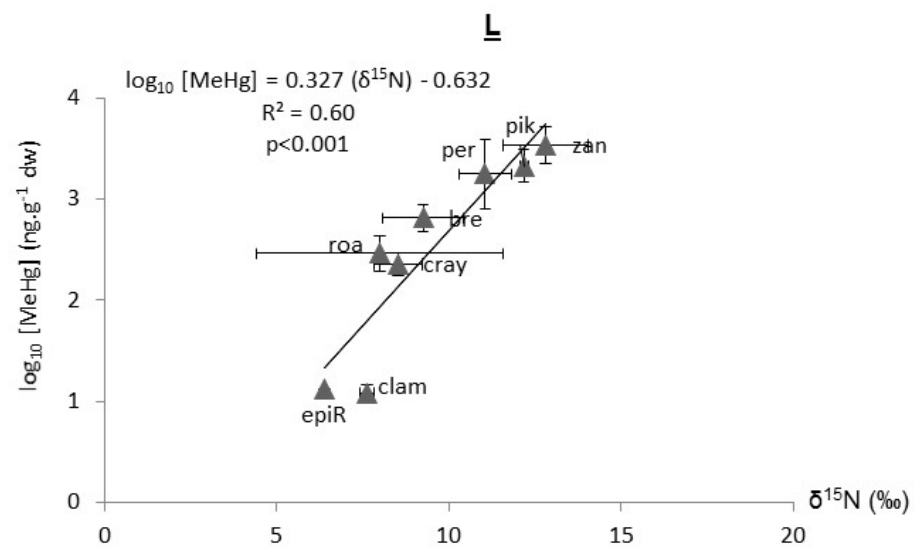
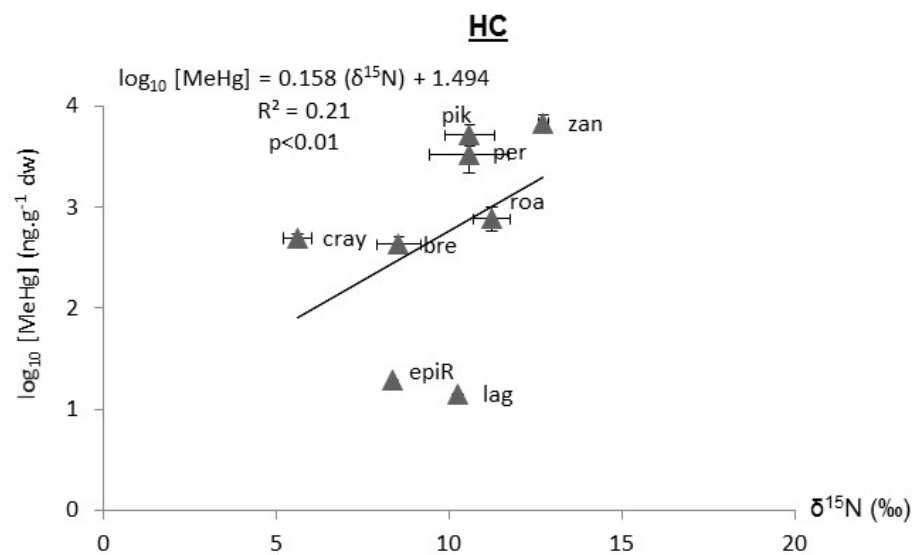
**Parentis-Biscarrosse**











Lakes	Species	Common name (code)	Trophic guild	Body weight	Standard length	N/n	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	TP	Mean [THg]	% MeHg	BMF <sub>PSC</sub> THg	BMF <sub>PSC</sub> MeHg
<b>Hourtin-Carcans (HC)</b>	<i>Lagarosiphon major</i>	Lagarosiphon (lag)	PP			1/1	-12.8	10.3		187.1	7.5		
	Epiphyton <i>Phragmites australis</i>	(epiR)				1/1	-17.6	8.4		35.8	53.7		
	<i>Procambarus clarkii</i>	Crayfish (cray)	Det			5/4	-19.5±1.2	5.7±0.4	1.4±0.1	523.9±79.6	90.7±3.2		
	<i>Rutilus rutilus</i>	Common roach (roa)	Omni-Herbi	281±135	22±4	5/4	-15.7±1.3	11.3±0.5	3.1±0.1	803±218.4	97.2±3.2		
	<i>Abramis brama</i>	Common bream (bre)	Omni-Benth	427±387	25±8	5/4	-21.7±0.8	8.8±0.9	2.4±0.3	440.1±110.6	90.4±1.5		
	<i>Perca fluviatilis</i>	European perch (per)	Carni-Pisci	1660±416	36±3	5/4	-19±0.8	10.3±0.9	2.8±0.3	5463.9±1023.3	96.3±0.6	9.5	9.5
	<i>Esox spp.</i>	Northern pike (pik)	Carni	2030±416	56±3	5/4	-19.9±1.1	10.4±1.1	2.8±0.3	3518.5±1317.5	96.9±1.5	3.1	3.2
	<i>Sander lucioperca</i>	Zander (zan)	Carni-Pisci	2914±429	60±5	3/3	-18.9±0.7	12.7±0.1	3.5±0	7383.8±1536.1	93.9±2.7	3.6	4.3
<b>Lacanaul (L)</b>	Epiphyton <i>Phragmites australis</i>	(epiR)	PP			1/1	-23.5	6.4		30.8	43		
	<i>Corbicula fluminea</i>	Asiatic Clam (clam)	FF	0.95±0.05	32±3	5/5	-26.6±0.1	7.6±0.2	2	138.1±13.9	8.8±1.3		
	<i>Procambarus clarkii</i>	Crayfish (cray)	Det			5/4	-23.1±2.1	8.6±0.6	2.3±0.2	260.1±64.9	87.4±2.5		
	<i>Rutilus rutilus</i>	Common roach (roa)	Omni-Herbi	89-421	15-25	2/2	-25.2; -17.1	10.5-5.5	2.1±1.1	414.3-206.7	93.1-100.0		
	<i>Abramis brama</i>	Common bream (bre)	Omni-Benth	1454±186	40±2	5/4	-26.5±1.6	9±1.2	2.4±0.3	753.4±216.6	93±1.3		
	<i>Perca fluviatilis</i>	European perch (per)	Carni-Pisci	910±421	33±5	5/4	-23±0.7	11.3±0.8	3.1±0.2	1961.2±1500.7	96.2±1.1	4.2	5.0
	<i>Esox spp.</i>	Northern pike (pik)	Carni	2415±452	62±4	5/4	-24±0.5	11.9±0.6	3.3±0.2	2160.5±834.9	94.5±1.1	2.8	2.9
	<i>Sander lucioperca</i>	Zander (zan)	Carni-Pisci	2400-5100	56-71	2/2	-25.5; -22.5	11.9-13.7	3.5±0.4	4809.3-2642.9	96.4-96.3	2.0	4.9
<b>Cazaux-Sanguinet (CS)</b>	<i>Lagarosiphon major</i>	Lagarosiphon (lag)	PP			1/1	-15.6	5.4		41.3	5.5		
	Epiphyton <i>Phragmites australis</i>	(epiR)				1/1	-21.5	5.1		23.3	35.8		
	<i>Corbicula fluminea</i>	Asiatic Clam (clam)	FF	0.5±0.1	20±2	3/3	-24±0.1	8.3±0.2	2	295.8±18.2	8.8±0.6		
	<i>Procambarus clarkii</i>	Crayfish (cray)	Det			2/2	-23.1; -25.6	9.9-9.5	2.4±0.1	181.8-240.4	79.0-89.6		
	<i>Rutilus rutilus</i>	Common roach (roa)	Omni-Herbi	270±146	23±4	5/5	-23.4±0.5	12.9±1	3.3±0.3	982.5±325.5	91.5±3		
	<i>Abramis brama</i>	Common bream (bre)	Omni-Benth	1146±496	37±5	5/4	-23.4±1.5	12.8±0.7	3.3±0.2	234.7±110.4	83.9±7.1		
	<i>Perca fluviatilis</i>	European perch (per)	Carni-Pisci	950±294	34±3	5/3	-22.6±0.7	14.1±0.3	3.7±0.1	2582.7±1210.3	92.8±2.3	6.2	5.2
	<i>Esox spp.</i>	Northern pike (pik)	Carni	2220±396	59±4	5/4	-22.8±0.7	14.2±0.4	3.7±0.1	1920.1±476.9	95.6±0.5	2.9	2.9
	<i>Sander lucioperca</i>	Zander (zan)	Carni-Pisci	3443±527	63±3	5/4	-22.9±0.6	15.2±0.4	4±0.1	3533.6±1044	96.3±1.8	4.0	4.2
	<i>Alburnus alburnus</i>	Bleak (ble)	Omni	39±8	14±1	5/4	-22.9±0.6	12.3±0.3	3.2±0.1	747.3±466	90.9±1.3		
	<i>Blicca bjoerkna</i>	White bream (wbre)		183±41	20±2	5/4	-22.9±0.3	12.5±0.6	3.2±0.2	336.2±82.3	91.1±2.9		
	<i>Gobio gobio</i>	Gudgeon (gud)		7±1	9±1	5/4	-14.6±1.5	11.4±0.4	2.9±0.1	261.7±56	83.2±6.7		
	<i>Gymnocephalus cernua</i>	Ruffe (ruf)		18±3	10±1	5/4	-26.6±2.9	13±1.3	3.2±0.3	377.1±232.2	82.1±5.8		
	<i>Ameiurus melas</i>	Black bullhead (bbh)		123±49	18±3	5/4	-23±0.9	12.5±0.9	3.4±0.4	239.4±67.7	85.2±4.7		
<b>Parentis-Biscarrosse (PB)</b>	<i>Lagarosiphon major</i>	Lagarosiphon (lag)	PP			1/1	-11.3	4.7		149.9	4.2		
	<i>Egeria densa</i>	Egeria (ege)				1/1	-14.8	10.8		5.5	3.7		
	Epiphyton <i>Egeria densa</i>	(epiE)				1/1	-22.6	8.2		33	2.7		
	Epiphyton <i>Ludwigia</i> sp.	(epiL)				1/1	-29.8	8.8		43	7		
	Epiphyton <i>Phragmites australis</i>	(epiR)				1	-24.9	6.8		20.7			
	<i>Corbicula fluminea</i>	Asiatic Clam (clam)	FF	0.8±0.1	20.9±0.8	5/5	-25.6±0.2	11.3±0.1	2	199.7±9.9	9.8±2.7		
	<i>Procambarus clarkii</i>	Crayfish (cray)	Det			5/4	-22.4±0.7	14.1±1.1	2.8±0.3	116.7±20.7	75.6±13.4		
	<i>Rutilus rutilus</i>	Common roach (roa)	Omni-Herbi	311±197	25±4	5/4	-22.5±1.5	15.5±0.8	3.2±0.2	531.7±151.7	90.6±6		
	<i>Abramis brama</i>	Common bream (bre)	Omni-Benth	395±46	27±2	5/4	-22.8±1.8	16.1±0.3	3.4±0.1	275.2±91.1	86.4±4.7		
	<i>Perca fluviatilis</i>	European perch (per)	Carni-Pisci	826±224	32±2	5/4	-22.6±0.2	17.8±0.1	3.9±0	1156.9±317.9	93.8±1.5	3.9	4.5
	<i>Esox spp.</i>	Northern pike (pik)	Carni	2460±451	65±3	5/4	-21.1±0.6	18.3±0.4	4±0.1	1459.4±332.5	95±2.3	3.4	3.9
	<i>Sander lucioperca</i>	Zander (zan)	Carni-Pisci	1890±284	55±3	5/4	-22.9±0.4	18.5±0.2	4.1±0.1	2065.7±258.1	92.5±1.4	4.8	5.1

PP: Primary producers; FF: Filter-feeders; Det: detritivorous; Omni: Omnivorous; Herbi: Herbivorous; Benthophagous: Benth; Carni: Carnivorous; Pisci: Piscivorous

lakes	THg		MeHg	
	TMS	FWMF	TMS	FWMF
HC	0.16	3.06	0.16*	3.48
L	0.23	5.35	0.33	12.87
CS	0.18	4.06	0.27	8.46
PB	0.13	2.96	0.27	8.13

\*: ANCOVA,  $p < 0.05$