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Abstract

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

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

1. Introduction

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

The four largest freshwater lakes (Hourtin-Carcans, Lacanau, Cazaux-Sanguinet and Parentis-Biscarrosse) in southern France, are recognized as emblematic aquatic systems for both ecological and economic reasons. Recently, in the two more northern lakes (Hourtin-Carcans and Lacanau), high Hg concentrations exceeding the World Health Organization consumption recommendation (0.5 $\mu g.g^{-1}$ wet weight, equivalent to 2.5 $\mu g.g^{-1}$ dry weight (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 $\delta^{13}C$ 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 $\delta^{13}C$, nitrogen incorporation leads to an enrichment in $\delta^{15}N$ 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 $\delta^{15}N$ and $\delta^{13}C$ 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^2 (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^2), followed by L (1.19 km^2), HC (0.94 km^2) and CS (0.17 km^2; surface area of dense
126 stands > 50 $g.m^{-2}$ dw; Bertrin et al. 2017).

127 ### 2.2 Sampling and sample preparation

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

Asiatic clams, crayfish (*Procambarus clarkii*) and stems of aquatic macrophytes (*Lagarosiphon major, Egeria densa* and *Phragmites australis*) were collected by hand. Special attention was given to the macrophytes to preserve their epiphyton during collection. Epiphyton fixed on submerged reed stems (*Phragmites australis*, a common plant in the four lakes) was collected by scraping and washing with field water, maintained at 4°C for 24 h, then centrifuged for 3 min at 10 000 g. An extensive fishing sampling campaign (with both nets and lines) provided 490 individuals of various fish species. Five species common to the four lakes were targeted (n = 353) in order to compare the bioaccumulation and biomagnification of Hg between lakes: zander, northern pike, European perch (*Perca fluviatilis*), common bream (*Abramis brama)* and common roach (*Rutilus rutilus*). Based on the fact that size is correlated with the age of individuals, a size class (Table S2) was chosen in order to compare total Hg concentrations per lake for the five species (n = 128, Table 1). For Hg speciation and nitrogen and carbon stable isotope analyses, five individuals of each species and lake were selected. Extended fishing facilities at lake CS only (permitted by a collaboration with a fishing sampling campaign organized by the French Agency for 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 $\mu g.g^{-1}$ 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 (^{199}iHg and
177 ^{201}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 $ng.L^{-1}$ for IHg and 0.005
181 $ng.L^{-1}$ for MeHg. Hg concentrations are expressed in $ng.g^{-1}$ 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 $^{13}C/^{12}C$ (denoted $\delta^{13}C$) and $^{15}N/^{14}N$
188 (denoted $\delta^{15}N$) 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ 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 $\delta^{15}N$ values and
207 following the equation of Bergamino et al. (2011):

208 $$TP_i = [(\delta^{15}N_i - \delta^{15}N_{pc}) / 3.4] + 2 \quad \text{(Eq 1)}$$

209 where TP_i represents the average trophic position of species i; $\delta^{15}N_i$ the average $\delta^{15}N$ value of
210 species i; $\delta^{15}N_{pc}$ the average $\delta^{15}N$ value of primary consumers; 3.4 the mean $\delta^{15}N$ 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 $\delta^{15}N$ 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 ±0.32‰ for nitrogen and 1.30 ±0.30‰ 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 $$BMF_{PSC} = [Hg]_{predator} / \left(\sum_{i=1}^{n} ([Hg]_{prey\ i} \times f_{prey\ i}) \right) \text{ (Eq 2)}$$

236 where $[Hg]_{prey\ i}$ is the Hg concentration of prey i and $f_{prey\ i}$ 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 $$Log_{10}[Hg] = b\ (\delta^{15}N) + a \quad \text{(Eq 3)}$$

244 where a is the intercept. As for the TP calculation (Eq 1), we used $\delta^{15}N$ 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 $$FWMF = 10^{b} \text{ (Eq 4)}$$

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

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

3. Results

3.1 Mercury concentrations in biota

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

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

3.2 Food web structure and feeding behavior of top predators

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

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

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

3.3 Mercury biomagnification factors

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

4. Discussion

4.1 Mercury concentrations in biota

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

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

4.2 Food web structure

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

Methyl Hg trophic transfer efficiency in carnivorous fishes is influenced by the composition of food web and feeding relationships (Vander Zanden and Rasmussen, 1996; Cabana and Rasmussen, 1994). Here, it is thus unlikely that food web structure was the main factor explaining the observed differences in Hg bioaccumulation in top predatory fishes. On the other hand, processes at the base of food webs that influence MeHg availability are generally the principal contributors to bioaccumulation and biomagnification of Hg in ecosystems (Gorski et al. 2003; Chasar et al. 2009; Molina et al. 2010). Significant differences observed in $\delta^{13}C$ values between invasive macrophytes and other organisms indicated that macrophytes do not represent a major feeding resource for grazing fish (common roach) or detritivorous (crayfish) in the four lakes. Conversely, their epiphyton (epiE, epiphyton *Egeria*) and epiphyton associated with endemic plants (epiR, epiphyton reed) appears to be a food source for these organisms. Previous studies have highlighted the relative importance of epiphytic algae compared to macrophytes as a carbon source in benthic food webs (France, 1995a). In addition to allochtonous inputs, therefore, other autochthonous 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 µg $THg.g^{-1}$ dw). However, the MeHg proportion in organic sediment was higher in
403 HC than the other three lakes (2.5± 1% of THg for lake HC, 1.7± 0.7% for L, 0.5± 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 µM, CS = 126 µM, L = 276 µM and HC = 392 µM),
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 $\delta^{15}N$ 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

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

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LEGENDS TO FIGURES

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

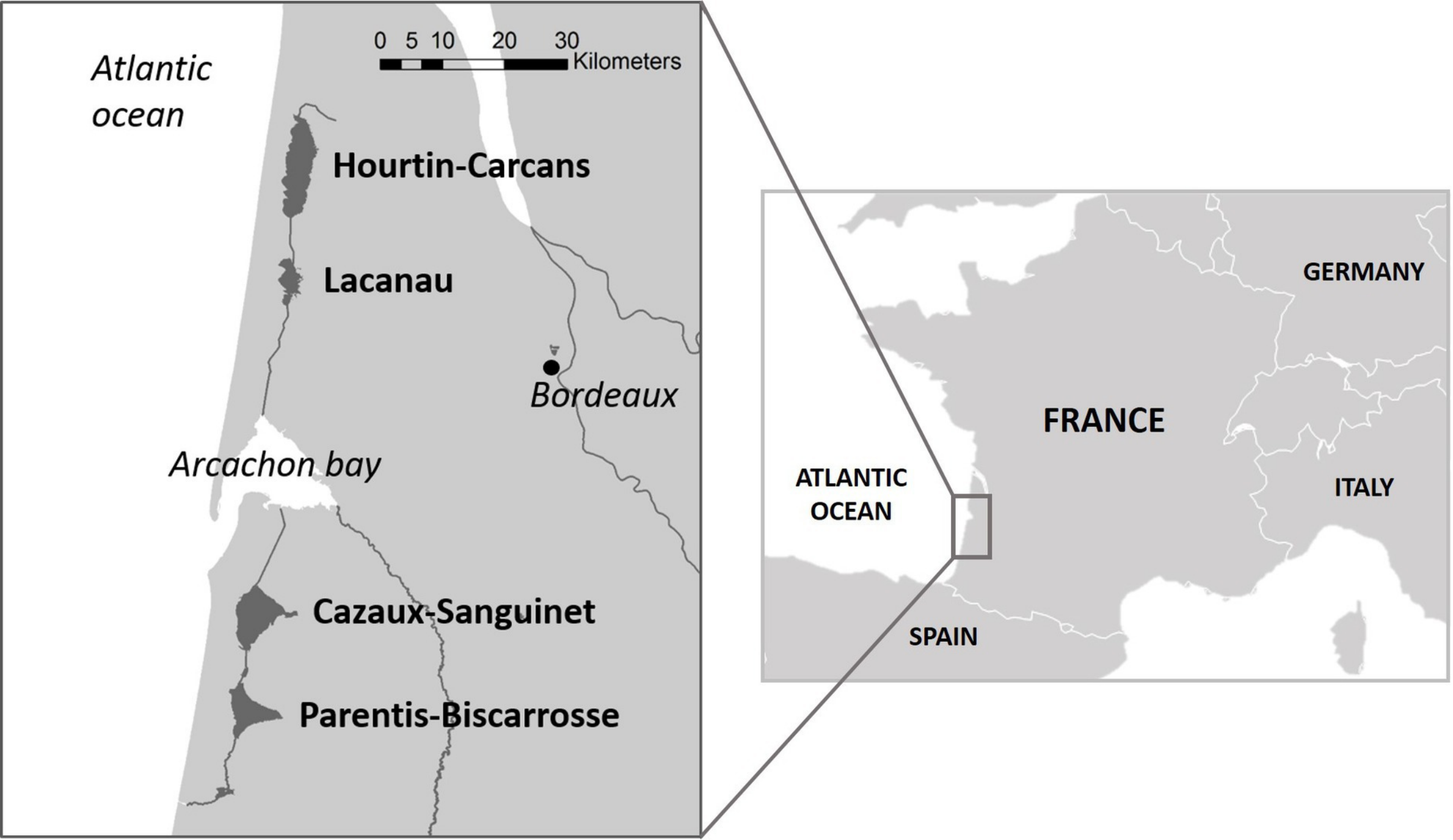
Figure 2. Boxplots of total mercury (left) and methylmercury (right) concentrations in muscle tissue of carnivorous (zander, northern pike and European perch) and omnivorous fish species (common roach and common bream) in lakes Hourtin-Carcans (HC), Lacanau (L), Cazaux-Sanguinet (CS) and Parentis-Biscarrosse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

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

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

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

Table 2. Trophic Magnification Slope (TMS) and Food Web Magnification factor (FWMF) calculated for THg and MeHg in each lake.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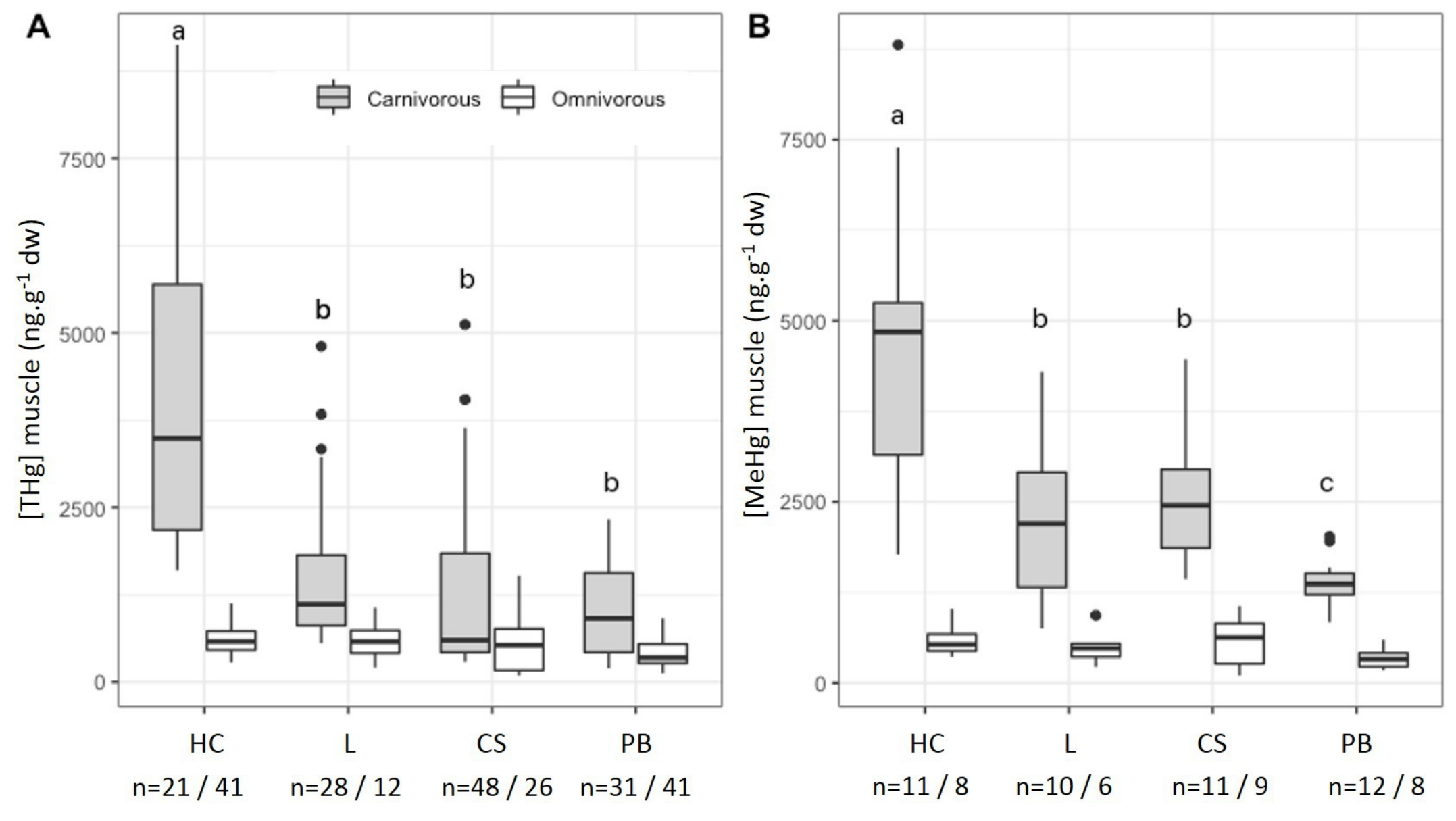
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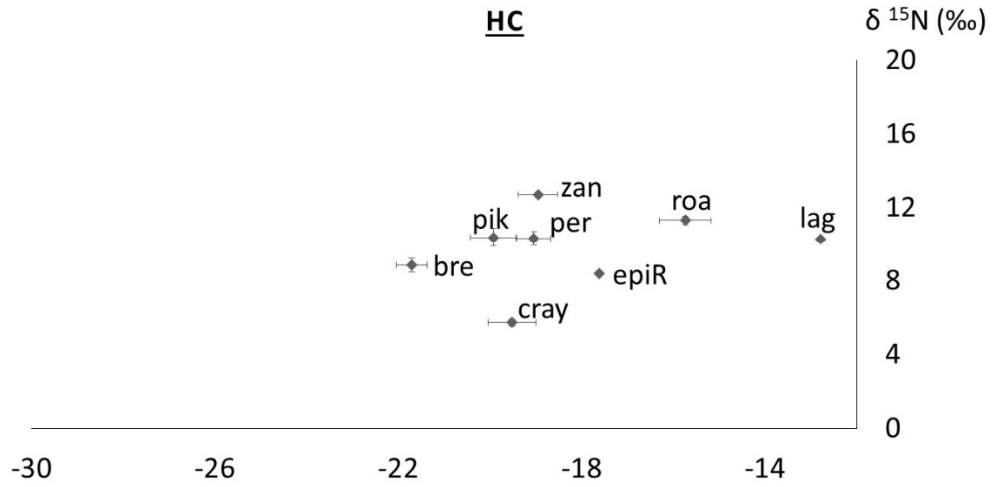
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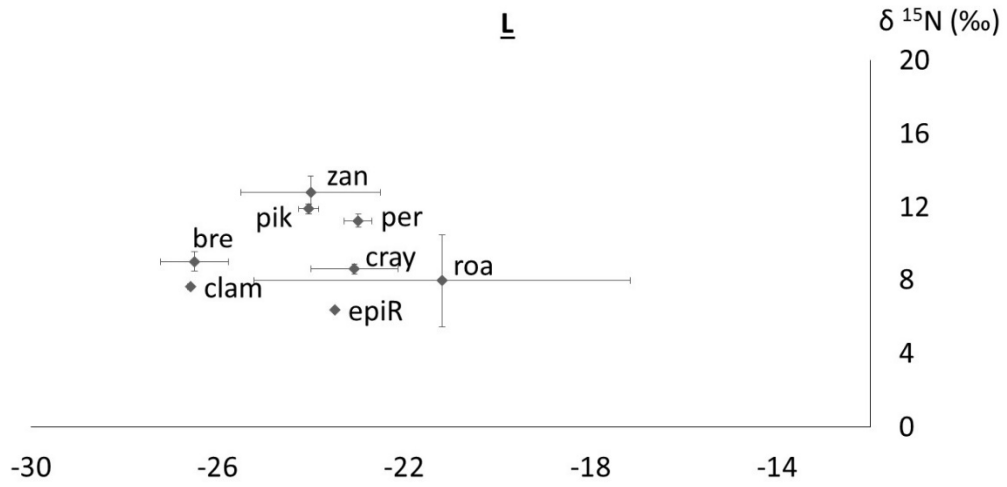
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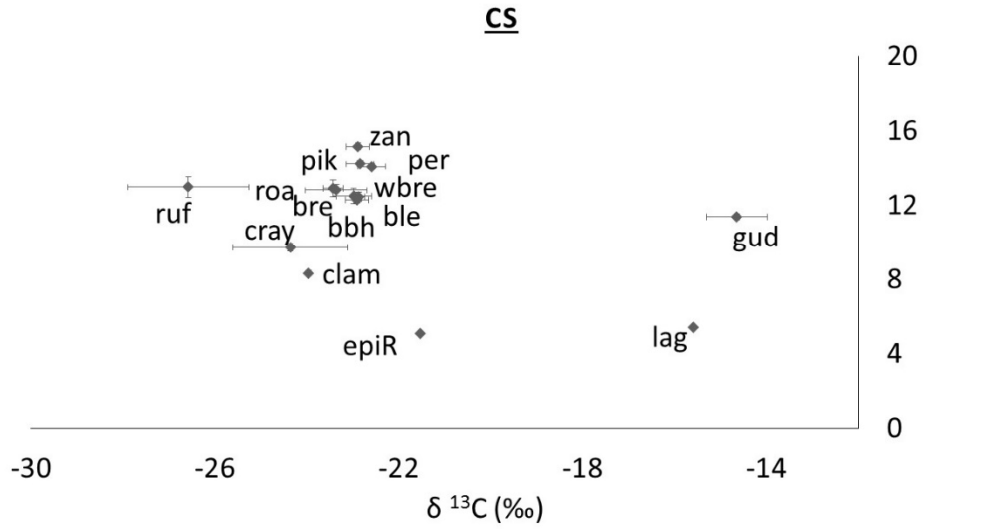
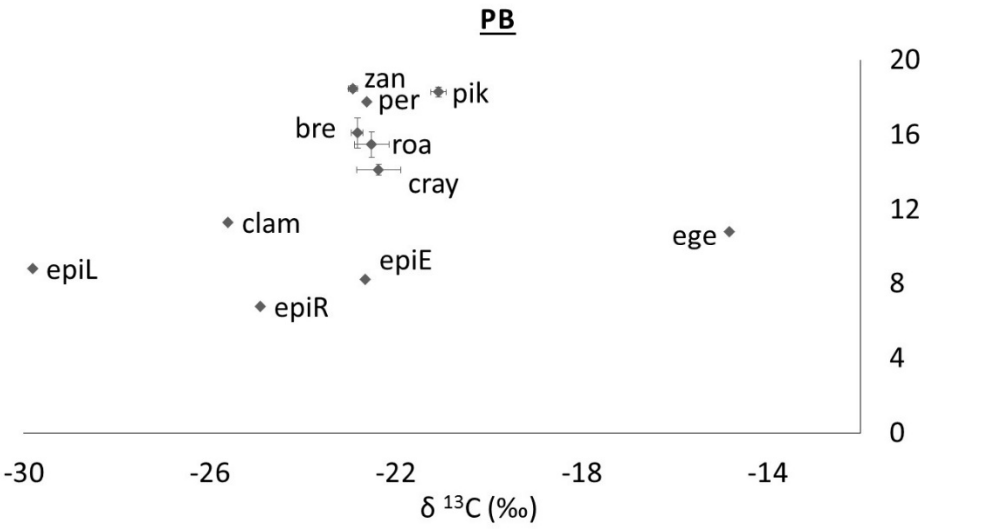
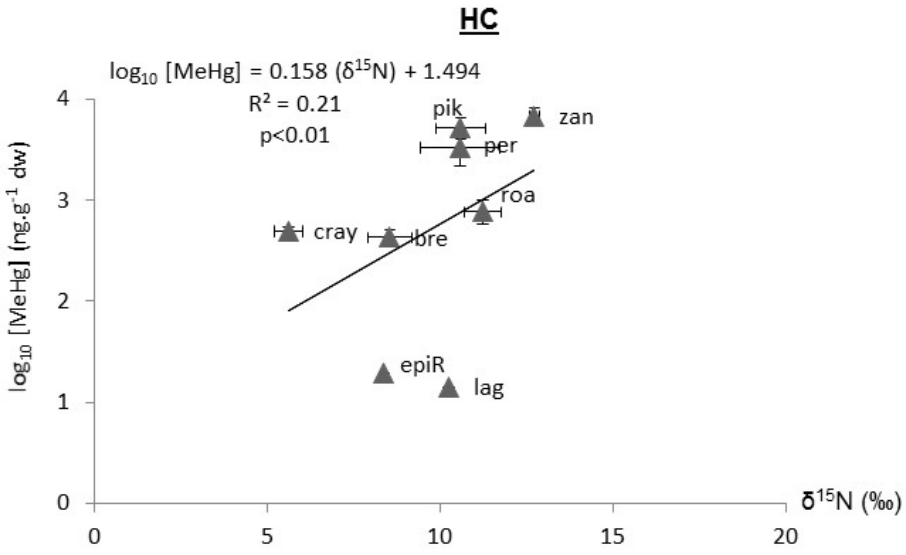
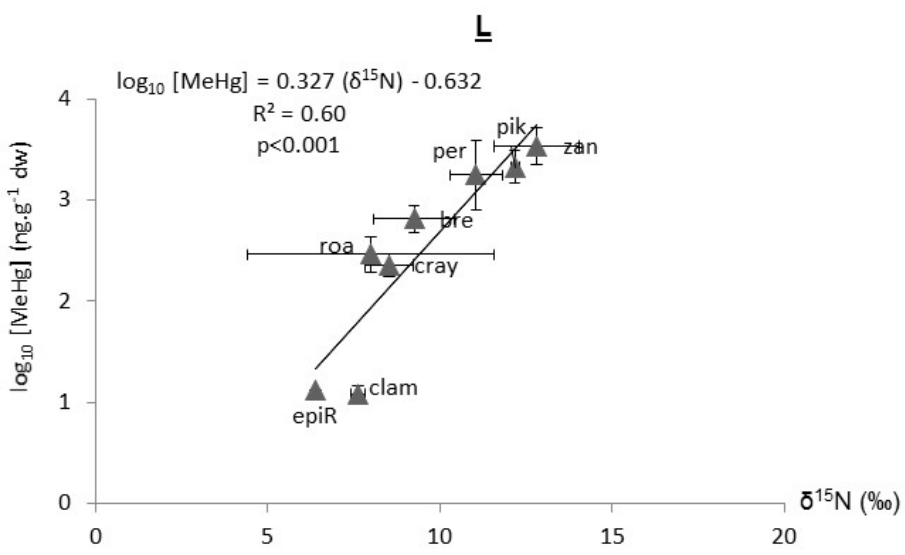
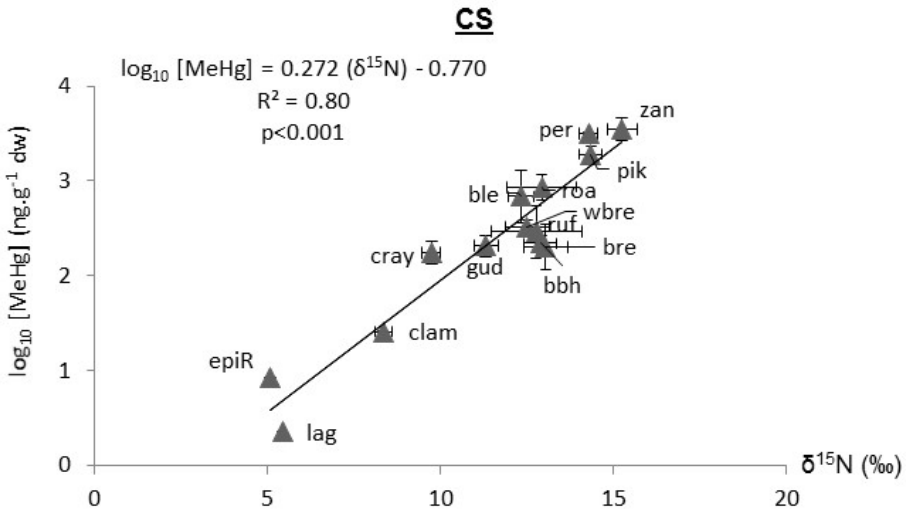
clam
epiR
-30
-26
-22
-18
-14

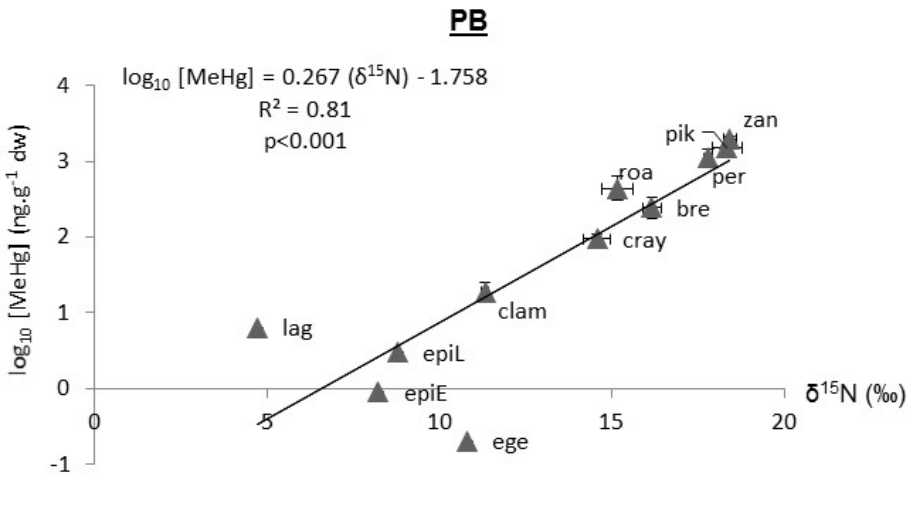

CS
20
16
12
8
4
0
zan
pik
per
wbre
roa
bre
ble
bbh
ruf
cray
clam
gud
epiR
lag
-30
-26
-22
-18
-14
δ 13C (‰)


PB
20
16
12
8
4
0
zan
per
pik
bre
roa
cray
clam
ege
epiE
epiL
epiR
-30
-26
-22
-18
-14
δ 13C (‰)


HC
log10 [MeHg] = 0.158 (δ15N) + 1.494
R² = 0.21
p<0.01
log10 [MeHg] (ng.g-1 dw)
δ15N (‰)
pik
zan
per
roa
cray
bre
epiR
lag


L
log10 [MeHg] = 0.327 (δ15N) - 0.632
R² = 0.60
p<0.001
log10 [MeHg] (ng.g-1 dw)
δ15N (‰)
pik
zan
per
bre
roa
cray
clam
epiR


CS
log10 [MeHg] = 0.272 (δ15N) - 0.770
R² = 0.80
p<0.001
log10 [MeHg] (ng.g-1 dw)
δ15N (‰)
zan
per
pik
ble
roa
wbre
ruf
bre
cray
gud
bbh
clam
epiR
lag


PB
log10 [MeHg] = 0.267 (δ15N) - 1.758
R² = 0.81
p<0.001
log10 [MeHg] (ng.g-1 dw)
δ15N (‰)
pik
zan
roa
per
bre
cray
clam
lag
epiL
epiE
ege

Lakes	Species	Common name (code)	Trophic guild	Body weight	Standard length	N/n	Mean $\delta^{13}C$	Mean $\delta^{15}N$	TP	Mean [THg]	% MeHg	BMF_{PSC} THg	BMF_{PSC} MeHg
Hourtin-Carcans (HC)	*Lagarosiphon major*	Lagarosiphon (lag)	PP			1/1	-12.8	10.3		187.1	7.5		
	Epiphyton *Phragmites australis*	(epiR)				1/1	-17.6	8.4		35.8	53.7		
	Procambarus clarkii	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		
	Rutilus rutilus	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		
	Abramis brama	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		
	Perca fluviatilis	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
	Esox spp.	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
	Sander lucioperca	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
Lacanau (L)	Epiphyton *Phragmites australis*	(epiR)	PP			1/1	-23.5	6.4		30.8	43		
	Corbicula fluminea	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		
	Procambarus clarkii	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		
	Rutilus rutilus	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		
	Abramis brama	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		
	Perca fluviatilis	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
	Esox spp.	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
	Sander lucioperca	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
Cazaux-Sanguinet (CS)	*Lagarosiphon major*	Lagarosiphon (lag)	PP			1/1	-15.6	5.4		41.3	5.5		
	Epiphyton *Phragmites australis*	(epiR)				1/1	-21.5	5.1		23.3	35.8		
	Corbicula fluminea	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		
	Procambarus clarkii	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		
	Rutilus rutilus	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		
	Abramis brama	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		
	Perca fluviatilis	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
	Esox spp.	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
	Sander lucioperca	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
	Alburnus alburnus	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		
	Blicca bjoerkna	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		
	Gobio gobio	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		
	Gymnocephalus cernua	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		
	Ameiurus melas	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		
Parentis-Biscarrosse (PB)	*Lagarosiphon major*	Lagarosiphon (lag)	PP			1/1	-11.3	4.7		149.9	4.2		
	Egeria densa	Egeria (ege)				1/1	-14.8	10.8		5.5	3.7		
	Epiphyton *Egeria densa*	(epiE)				1/1	-22.6	8.2		33	2.7		
	Epiphyton Ludwigia sp.	(epiL)				1/1	-29.8	8.8		43	7		
	Epiphyton *Phragmites australis*	(epiR)				1	-24.9	6.8		20.7			
	Corbicula fluminea	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		
	Procambarus clarkii	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		
	Rutilus rutilus	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		
	Abramis brama	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		
	Perca fluviatilis	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
	Esox spp.	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
	Sander lucioperca	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

	THg		MeHg	
lakes	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, $p < 0.05$