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1 **Specificity of the metabolic signatures of fish from cyanobacteria rich lakes**

2

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14

15

16

17 **Abstract**

18 With the increasing impact of the global warming, occurrences of cyanobacterial blooms in  
19 aquatic ecosystems are becoming a main worldwide ecological concern. Due to their capacity  
20 to produce potential toxic metabolites, interactions between the cyanobacteria, their  
21 cyanotoxins and the surrounding freshwater organisms have been investigated during the last  
22 past years. Non-targeted metabolomic analyses have the powerful capacity to study  
23 simultaneously a high number of metabolites and thus to investigate in depth the molecular  
24 signatures between various organisms encountering different environmental scenario, and  
25 potentially facing cyanobacterial blooms.

26 In this way, the liver metabolomes of two fish species (*Perca fluviatilis* and *Lepomis gibbosus*)  
27 colonizing various peri-urban lakes of the Île-de-France region displaying high biomass of  
28 cyanobacteria, or not, were investigated. The fish metabolome hydrophilic fraction was  
29 analyzed by <sup>1</sup>H-NMR analysis coupled with Batman peak treatment for the quantification and  
30 the annotation attempt of the metabolites. The results suggest that similar metabolome profiles  
31 occur in both fish species, for individuals collected from cyanobacterial blooming lakes  
32 compared to organism from non-cyanobacterial dominant environments. Overall, such  
33 environmental metabolomic pilot study provides new research perspectives in ecology and  
34 ecotoxicology fields, and may notably provide new information concerning the  
35 cyanobacteria/fish ecotoxicological interactions.

36

37 **Keywords:** fish; cyanobacteria; metabolomics; ecotoxicology; NMR

38

## 39 **1. Introduction**

40 Direct and indirect anthropogenic disturbances affect the global functioning and stability of  
41 freshwater ecosystems. Global warming, physical alteration, habitat loss, water withdrawal,  
42 pollutions, overexploitation and the introduction of non-native species constitute the main  
43 threats affecting freshwater ecosystems and their biocenoses (Dudgeon et al., 2006; Bunn 2016;  
44 Revenga et al., 2005). Over the past century, transformation of natural landscapes for industrial  
45 and urban needs have led to a general increase of chemical nutrients concentrations in  
46 freshwater ecosystems, and notably of phosphorus and nitrogen species that have caused the  
47 massive eutrophication of freshwater bodies (Schwarzenbach et al., 2010). One of the main  
48 consequences of this non-natural eutrophication process is a shift in the composition of the  
49 primary producers due to the appearance of noxious autotrophic bacteria, such as cyanobacteria  
50 (Chorus and Bartram 1999; O’Neil et al., 2012). In worldwide freshwater ecosystems,  
51 cyanobacteria are mainly present during summers, forming large surface scums generally  
52 accompanied by the presence of toxic compounds that have been pointed out to affect all trophic  
53 food web compartments (Codd et al., 2005; Ferrao-Filho et al., 2011; Sotton et al., 2015).  
54 *Microcystis*, *Aphanizomenon*, *Anabaena*, *Cylindrospermopsis* and *Planktothrix* are the main  
55 bloom-forming freshwater cyanobacterial genera found during summers in lentic waterbodies,  
56 and all have been often reported to produce potent toxic compounds, so-called cyanotoxins  
57 (Codd et al., 2017). Among them, the microcystins (MCs), a family of hepatotoxins consisting  
58 of more than 230 variants, are the most studied due to their high biological activity and their  
59 wide occurrence during freshwater cyanobacterial blooms (Catherine et al., 2017). For exposed  
60 macro-organisms, MCs may induce inhibition of the protein phosphatases 1 (PP-1) and 2A (PP-  
61 2A) as well as occurrence of a cellular oxidative stress *via* the formation of reactive oxygen  
62 species (ROS), with different physiological consequences depending on the organism and the  
63 species studied (Amado et al., 2010; Malbrouck and Kestemont 2006). The effects of freshwater  
64 cyanobacteria and their respective cyanotoxins, notably the MCs, have been widely studied on  
65 fish maintained in microcosm or mesocosm conditions, and fish have been proposed as valuable  
66 indicators of environmental disturbances associated to cyanobacteria proliferations (Malbrouck  
67 and Kestemont 2006; Bols et al., 2001; Le Manach et al., 2018). However, the actual knowledge  
68 on the genuine cyanobacteria bloom impacts for natural populations of fishes is mainly deduced  
69 from short-time experimentation generally performed in micro- or mesocosms with high  
70 concentrations of purified toxins. Furthermore, most of these studies were focused on the  
71 mechanisms involved in the dynamic of the MC accumulation-detoxification. It appears that

72 there is still a lack in our understanding of the real ecotoxicological effects of cyanobacterial  
73 biomass, particularly on the natural ichthyofauna population. Indeed, cyanobacterial blooms are  
74 producing at the same time a “cocktail” of potentially bioactive compounds, and are also  
75 potentially modifying other important ecological parameters of the water bodies.

76

77 The metabolome of an organism is constituted by the set of primary metabolites synthesized at  
78 a given time. It represents its metabolic chemical picture, which can potentially be altered when  
79 the ecological and environmental stress conditions change (Fiehn 2002; Bundy et al., 2008).  
80 The observed metabolome regulations represent the chemical phenotypic response of an  
81 organism that could potentially respond to counter-act the negative effects of stressors present  
82 in the ecosystems, and thus to adapt to the variable conditions (Hamilton et al., 2016). In this  
83 way, metabolomic studies have become a relevant approach to describe and analyze the  
84 integrated response of the organisms under specific environmental context and scenario  
85 (Franzosa et al., 2015; McLean 2013; Hultman et al., 2015). Then, the changes in the primary  
86 metabolite concentrations help to provide valuable and useful information concerning the  
87 physiological processes involved in the homeostatic or dys-regulated responses of the  
88 organisms encountering environmental stresses from potentially multiple origins. Nuclear  
89 magnetic resonance (NMR)-based metabolomics has been proved to be a powerful approach to  
90 address hypotheses relating to fish physiology or pollutant induced toxicity or diseases  
91 (Samuelsson and Larsson 2008; Viant 2008). However, despite its high potential for the  
92 understanding of the molecular mechanisms implicated in the ecotoxicological responses of  
93 organisms, the investigation of new qualitative and quantitative biomarkers characterizing the  
94 interactions between populations and their biotopes in various ecological context remains still  
95 rare and difficult to achieve (Sardans et al., 2013; Cappello et al., 2016; 2017; Qiao et al., 2016).

96

97 Thus, as toxic cyanobacterial blooms may represent important ecotoxicological and ecological  
98 constraints in freshwater environments, it can be supposed that organisms exposed to  
99 cyanobacterial blooms exhibit characteristic metabolome signatures, compared to others non-  
100 exposed to cyanobacterial dominant conditions. Their metabolism may respond and/or counter-  
101 act the potential negative effects of the cyanobacteria and thus adapt to the local environmental  
102 pressures. To date, no studies have been carried out using a global metabolomic approach in  
103 order to assess the specific metabolic changes that could be observed in fish exposed to  
104 cyanobacterial blooms in contrasted aquatic natural ecosystems. In this way, during the summer  
105 2015, young fish of two representative and common species of freshwater lakes from the

106 European temperate regions, the perch (*Perca fluviatilis*) and the pumpkinseed sunfish  
107 (*Lepomis gibbosus*), have been sampled in eight peri-urban lakes of the Île-de-France region  
108 contrasted by their phytoplanktonic community composition (“presence” or “absence” of  
109 cyanobacterial blooms). <sup>1</sup>H-NMR metabolomic analyses were performed on the fish liver in  
110 order to investigate the global metabolome local specificities of the two fish species collected  
111 from a gradient of distinct ecological contexts (comprising the cyanobacteria dominance) and  
112 to further identify the metabolic signatures related to these potential specific phenotypic  
113 responses.

114

## 115 **2. Experimental section**

### 116 *2.1. Lakes and physico-chemical parameters measurements*

117 *In-situ* sampling campaigns were performed during summer 2015 in eight lakes of the Île-de-  
118 France region (France), chosen for their different dominant phytoplankton communities already  
119 described in previous studies and notably the presence or the absence of recurrent  
120 cyanobacterial blooms (Catherine et al., 2008; Maloufi et al., 2016). Cergy “les Galets” lake  
121 (C), Champs-sur-Marne “la Sablière” lake (CM), Maurepas “la Courance” lake (M), Rueil  
122 “Saint Cucufa” lake (R), Verneuil “la grosse Pierre” lake (V), Varenne-sur-Seine “Le grand  
123 Marais” lake (VS), Fontenay-sur-loing “le grand Fontenay” lake (F) and Triel lake (T)  
124 (Supplementary figure S1) were thus studied and sampled with electric fishing device for  
125 capturing fish alive. Previous investigation of the fish guild populating every one of these lakes  
126 (using both electric device and fish net sampling) had indicated that only the perch (*Perca*  
127 *fluviatilis*) and pumpkinseed sunfish (*Lepomis gibbosus*) were presents in all or almost all the  
128 lakes and were further selected as bioindicative species for further metabolomic analyses.

129 In all lakes, dissolved oxygen (O<sub>2</sub>) concentrations, pH, temperatures and conductivity were  
130 measured in the water column using a multiparameter probe (YSI EXO2) (Supplementary table  
131 S1).

132

### 133 *2.2. Phytoplankton sampling*

134 In every lake, sub-surface chlorophyll-*a* equivalent concentrations attributed to the four-main  
135 phytoplankton groups (Chlorophyta, Diatoms, Cyanobacteria, Cryptophyta) were measured  
136 with an *in-situ* fluorometer (Fluoroprobe II, Bbe-Moldenke, Germany) and samples of water  
137 were filtered through 1.2 µm GF/C filters (Nucleopore, Whatman) and stored at -80°C until  
138 total chlorophyll-*a* concentrations analyses using the ethanolic extraction as described by

139 Yepremian and co-workers (2017). Samples of water were fixed in Lugol iodine solution and  
140 kept at 4°C until the identification. The estimation (%) of the abundance of the different  
141 cyanobacterial genera was performed using an Utermohl's counting chamber and an inverted  
142 microscope as described by Catherine et al. (2016). In parallel, phytoplankton biomass was  
143 collected using an Apstein's type phytoplankton net (20- $\mu$ m mesh size) and kept at -80°C until  
144 metabolite characterization by mass spectrometry (MS) and MCs content analyses.

145

### 146 *2.3. Metabolite characterization of phytoplanktonic biomass by mass spectrometry*

147 The collected biomass from the sampled lakes were freeze-dried and then sonicated in 75%  
148 methanol, centrifuged at 4°C (15,000 g, 10 min). This step was performed twice, and the pooled  
149 supernatants were collected with a Pasteur pipet and kept at -80°C prior to analysis. The  
150 supernatant was transferred and acidified with formic acid and 5  $\mu$ L were analyzed in triplicats  
151 on an HPLC (Ultimate 3000, ThermoFisher Scientific) coupled to an electrospray ionization  
152 and quadrupole time-of-flight hybrid mass spectrometer (ESI-QqTOF, QStar® Pulsar i,  
153 Applied Biosystems®, France).

154 High-performance liquid chromatography (HPLC) of 5  $\mu$ L of each of the metabolite extracts  
155 was performed on a C<sub>18</sub> column (Discovery® Bio wide pore, 15cm x 1mm, 5  $\mu$ m, Sigma) at a  
156 50  $\mu$ L.min<sup>-1</sup> flow rate with a gradient of acetonitrile in 0.1% formic acid (10 to 80% for 60 min).  
157 The metabolite contents were then analyzed on positive mode using information dependent  
158 acquisition (IDA), which allows switching between mass spectrometry (MS) and tandem mass  
159 spectrometry (MS/MS) experiments, as previously described (Marie et al., 2012). The data was  
160 acquired and analyzed with the Analyst QS software (Version 1.1). Peak lists were generated  
161 from MS spectra acquired between 10 and 55 min, filtering noise threshold at 2% maximal  
162 intensity and combining various charge states and related isotopic pattern. Metabolite  
163 annotation was attempted according to the accurate mass of the molecules, then to their  
164 respective MS/MS fragmentation pattern with regard to an in-house database of more than 700  
165 cyanobacterial metabolites.

166 A molecular network was created using the online workflow at GNPS (Wang et al., 2016). The  
167 data was filtered by removing all MS/MS peaks within +/- 17 Da of the precursor m/z. The data  
168 was then clustered with MS-Cluster with a parent mass tolerance of 1.0 Da and a MS/MS  
169 fragment ion tolerance of 0.5 Da to create consensus spectra. Further, consensus spectra that  
170 contained less than 2 spectra were discarded. A network was then created where edges were  
171 filtered to have a cosine score above 0.6 and more than 5 matched peaks. Further edges between  
172 two nodes were kept in the network if and only if each of the nodes appeared in each other's

173 respective top 10 most similar nodes. The spectra in the network were then searched against  
174 GNPS' spectral libraries. The library spectra were filtered in the same manner as the input data.  
175 All matches kept between network spectra and library spectra were required to have a score  
176 above 0.6 and at least 5 matched peaks. All GNPS results are available at  
177 <http://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=a158701fa7894ab08c0df4c60994ccf1>

178

#### 179 *2.4. Cyanotoxin quantification*

180 The 75% methanol extracts of the phytoplankton biomass were further analyzed by ELISA  
181 (enzyme-linked immunosorbent assay), with the microcystins (Adda-specific) Kit (AD4G2  
182 antibody, Abraxis), in order to globally measure the total MC content, and integrating the signal  
183 obtained for all MC variants in presence. Prior to analysis, samples were dissolved with the  
184 ELISA sample diluent to reach a methanol concentration below 5% to avoid any interactive  
185 effect and to stay in the detection range of the kit (0.1-5  $\mu\text{g}\cdot\text{L}^{-1}$ ) for all samples. The results  
186 were expressed in microcystin-LR equivalents ( $\mu\text{g eq. MC-LR}\cdot\text{mg}^{-1}$  dry weight).

187 The free and bound  $\beta$ -Methylamino-L-alanine (BMAA) were also measured in the  
188 phytoplankton biomass according to the hydrophobic interaction liquid chromatography  
189 (HILIC)/MS-MS based methods described previously (Combes et al., 2014; Faassen et al.,  
190 2016). This method used solid-phase extraction based on mixed mode sorbent to concentrate  
191 and clean up the phytoplankton extract that contained free BMAA. After the acidic lysis of the  
192 phytoplankton biomass, the bound fraction of BMAA was analyzed by LC/MS-MS. This  
193 quantitative method has proved to be specific and reliable in a range of concentration level from  
194 0.25 to 1.6  $\text{ng}\cdot\text{mg}^{-1}$  dry weight.

195

#### 196 *2.5. Fish sampling and tissue extraction procedure for metabolomic analysis*

197 Immature individuals of perch (*Perca fluviatilis*) and pumpkinseed sunfish (*Lepomis gibbosus*),  
198 two representative fish species of European freshwater lakes (that were selected according to  
199 their presence in almost, if not, all investigated lakes), were targeted by electric fishing (FEG  
200 8000, EFKO, Leutkirch, Germany) performed in the riparian area of every lake. Alive caught  
201 fish ( $n < 6-10$  per lake and per species) were directly measured, weighed (Table S2), briefly  
202 euthanized by neck dislocation and then liver of each individual was shortly sampled, deep-  
203 frozen in liquid nitrogen and kept at  $-80^{\circ}\text{C}$  until metabolomics analyses, in accordance with  
204 animal ethical concerns and regulations.

205 Individual liver metabolome extraction was carried out using the methanol/chloroform/water  
206 (ratio 2/2/1.8) method, on the basis of existing literature (Lin et al., 2007; Wu et al., 2008).



207 Briefly, fresh frozen livers were weighed, homogenized in ice-cold methanol (8 mL per gram  
208 of tissue, AnalaR Normapur, min. 99.8 %, VWR, Pennsylvania, USA) and ice cold milliQ water  
209 (2.5 mL.g<sup>-1</sup>), and then vortexed for 1 min. Subsequently, ice cold chloroform (4 mL.g<sup>-1</sup>,  
210 Normapur, 99.3 %, VWR, Pennsylvania, USA) and milliQ water (4 mL.g<sup>-1</sup>) were added to  
211 extract the hydrophobic metabolites. Then, the mixture was vortexed for 1 min and incubated  
212 on ice for 10 min to obtain a complete solvent partition. The resulting supernatant was then  
213 centrifuged at 4°C for 10 min at 2,000 g, resulting in a biphasic solution. The upper polar and  
214 lower non-polar layers were carefully removed. The upper polar fraction was then transferred  
215 to 2-mL Eppendorf tubes, dried under Speed-vac device (Speed-vac Plus SC110A, Savant) and  
216 then kept at -80°C until NMR analysis. Prior to <sup>1</sup>H-NMR measurement, the polar tissue extracts  
217 were dissolved in 550 μL of 0.1 M sodium phosphate buffer (pH 7.0) prepared in D<sub>2</sub>O (10%  
218 v/v) containing 0.25 mM sodium-3-tri-methylsilylpropionate (TMSP) as an internal standard.  
219 Finally, the resulting samples were transferred to 5-mm NMR tubes (Norell, France) and  
220 immediately analyzed by <sup>1</sup>H-NMR.

221

## 222 *2.6. <sup>1</sup>H-NMR spectroscopy and metabolite quantification.*

223 All NMR data were recorded at 298 K on a 600 MHz Bruker AVANCE III HD spectrometer  
224 equipped with a 5-mm TCI CryoProbe (<sup>1</sup>H-<sup>13</sup>C-<sup>15</sup>N) with Z-gradient. One-dimensional <sup>1</sup>H-  
225 NMR spectra were acquired using a standard Bruker noesygppr1d pulse sequence to suppress  
226 water resonance. Each spectrum consisted of 256 scans of 32,768 data points with a spectral  
227 width of 7.2 kHz, a relaxation delay of 2.5 s and an acquisition time of 2.3 s.

228 The relative metabolite quantification was performed using the BATMAN (an acronym for  
229 Bayesian AuTomed Metabolite Analyser for NMR spectra) R-package (Hoa et al., 2014),  
230 which deconvolutes peaks from 1-dimensional <sup>1</sup>H-NMR spectra to automatically assign  
231 chemical shifts to specific metabolites from a target list and then estimate their respective  
232 concentrations. This can be achieved thanks to an implementation of a Bayesian-based  
233 modelling procedure. BATMAN uses, in a two-component joint model, resonances of every  
234 assigned proton from a list of catalogued metabolites (this library is provided with the  
235 BATMAN R-package, and includes the chemical shift information of various targeted  
236 metabolites measured at pH 7.0) and noisy information to finally reconstruct the empirical  
237 NMR spectrum. But, in absence of confirmatory analytical methods, they were further  
238 mentioned as candidate metabolic biomarkers sharing the same <sup>1</sup>H-NMR parameters with the  
239 catalogued metabolites. Therefore, 222 potential metabolites were quantified from Bruker  
240 spectra files using the following parameters: i) the chemical shift regions belonging to the two

241 following regions: 0.5 to 4.60 ppm and 5.40 to 10.0 ppm, ii) 400 burn-in iterations, iii) 200  
242 post-burn-in iterations and iv) 5000 iterations for batman rerun. Calculations were performed  
243 on a HP Z820 workstation using two 3.30 GHz Intel Xeon® CPU E5 processors and 64 Go  
244 RAM by activating 12-parallel threads processing.

245

### 246 2.7. Statistical exploration of data

247 The mixOmics library (Rohart et al., 2017) was used to carry out the multivariate analyses  
248 (Tenenhaus et al., 2014). Regularized canonical correlation analysis (rCCA) is a multivariate  
249 statistical method used to assess correlations between two multivariate datasets acquired on the  
250 same individuals. Here, it was used as a factorial discriminant analysis that modeled the  
251 relationships between the species (*Perca* and *Lepomis*), the quantitative chemical data measured  
252 in the different lakes (total Chl-*a* concentrations, Chl-*a* estimated concentrations related to  
253 cyanobacterial biomass (BBE<sub>cya</sub>), MCs concentrations, O<sub>2</sub> concentrations, pH and  
254 conductivity), together with the lake of sampling, constituting the Y matrix, and the semi-  
255 quantitative levels of metabolites determined by the BATMAN algorithm with no  
256 normalization of the data (X matrix) (supplementary data 1). The *rcc()* function was used to  
257 define the canonical correlations and the canonical variates, the *network()* function was used to  
258 produce the network of interactions, using default parameters. Cross-validation of MANOVA  
259 results was obtained thanks to a bootstrap-based procedure with corrected *p*-value ( $P < 0.05$ ) by  
260 applying a random assignment of any statistical individual to a given group of treatments  
261 (Krishnamoorthy and Lu, 2010). In order to evaluate the effects of the experimental factors  
262 (species, chemical data and their interaction) on the relative concentrations of metabolites  
263 highlighted by multivariate analyses and the subsequent relevant networks, simple two-way  
264 ANOVAs followed by a Student-Newman-Keuls post-hoc test were performed.

265

## 266 3. Results

### 267 3.1. Phytoplankton, microcystins and chemical conditions of the studied lakes

268 During 2015 summer sampling campaign (Supplementary fig. S1), distinct phytoplanktonic  
269 compositions and concentrations were observed in the eight lakes targeted (Fig. 1). Among  
270 them, F, T, V and VS, exhibited remarkable high and/or dominance of cyanobacterial-specific  
271 chlorophyll-*a* concentrations reaching 106, 14.8, 135 and 48.3  $\mu\text{g eq. Chl } a \text{ L}^{-1}$ , respectively  
272 (Fig. 1A). In the other lakes, chlorophyll-*a* concentrations of the total phytoplanktonic  
273 community ranged between 7.2 and 241.5  $\mu\text{g. Chl } a \text{ L}^{-1}$  (supplementary table S1) but were

274 mainly dominated by chlorophytes, diatoms and/or cryptophytes (Fig. 1A), major  
275 phytoplanktonic phyla, that are so far not known to produce any toxins nor other ecological  
276 concerns as cyanobacteria does. In these lakes, only low concentrations of cyanobacterial-  
277 specific chlorophyll-*a*, ranging from 2.9 to 10.9  $\mu\text{g eq. Chl } a \text{ L}^{-1}$ , were measured (Fig. 1A).  
278 Furthermore, in the cyanobacteria-dominated lakes, different cyanobacterial genera were  
279 observed during samplings (Fig. 1B). F and T were dominated by *Planktothrix* (94% of the  
280 cyanobacteria present in the sample) and *Pseudanabaena* (71%), respectively, whereas V and  
281 VS, were both dominated by *Microcystis* (Fig 1B). Furthermore, we notice that the modeste  
282 blue green signal observed in C corresponds to 100% *Spirulina* sp., a cyanobacteria genus that  
283 is so far known not to produce any known toxic compounds, as it was further confirmed.  
284 Microcystin concentration was investigated in order to estimate the amount of the  
285 cyanobacterial bioactive compounds present in the various lake water (Fig. 2; supplementary  
286 table S2). MC-LR equivalent concentrations were measured at concentrations ranging from  
287 above the quantification limit ( $> 1 \mu\text{g.g}^{-1} \text{ DW}$ ) to 3367  $\mu\text{g.g}^{-1} \text{ DW eq. MC-LR}$ . The highest MCs  
288 concentrations were found in lakes dominated by cyanobacteria and particularly in F, V and VS  
289 where 2067  $\mu\text{g.g}^{-1}$ , 166  $\mu\text{g.g}^{-1}$  and 3367  $\mu\text{g.g}^{-1} \text{ MC-LR eq.}$  were measured, respectively.  
290 Interestingly, V that exhibited the highest cyanobacterial concentrations (dominated by  
291 *Microcystis*) measured in this study, did not exhibit the highest MCs concentrations (3367  $\mu\text{g.g}^{-1}$   
292  $\text{MC-LR eq.}$  in VS) illustrating the fact that MCs concentrations are not linearly linked to the  
293 cyanobacterial biomass measured in the lakes, as it is also dependent on the production of  
294 metabolite by the dominant cyanobacterial clones, which may or not be producing MCs. On  
295 one side, none of these samples presented detectable amount of the potential neurotoxin BMAA  
296 (neither in free or bound form), saxitoxins nor anatoxins. On the other side, various classes of  
297 cyanobacterial secondary metabolites comprising different variants of hepatotoxic  
298 microcystins, together with various other potentially bioactive metabolite families, such as  
299 anabaenopeptins, aeruginosins, microginins and cyanopeptolins, were detected in the  
300 phytoplanktonic biomass of collected from the lakes where cyanotoxin-producing  
301 cyanobacteria reached higher concentrations (Fig. 2; supplementary figure S2-S3).

302

### 303 3.2. Metabolomic analysis of sampled fish

304  $^1\text{H-NMR}$  raw files (supplementary fig. S4) were preprocessed thanks to the R BATMAN library  
305 (Hao et al., 2014) to get a relative quantification of a preselected set of metabolites replacing  
306 an analytical assignment based on different and complementary analyses. This approach allows  
307 for each individual spectrum to determine a metabolite quantification, even for very low

308 concentrated metabolites when compared to the main ones, as the differences in their relative  
309 concentrations could have been validated by both uni- and multivariate statistical tests (Hao et  
310 al., 2014). A multivariate analysis was performed by using a rCCA analysis between the matrix  
311 of the relative concentrations of the 222 metabolites (X) and the dummy matrix (Y)  
312 corresponding to the following variables (supplementary data S1): *i.e.* fish species,  
313 cyanobacterial-specific Chl-*a* and MCs concentrations, O<sub>2</sub>, pH, conductivity and the total  
314 Chlorophyll-*a* concentrations and their interaction using the mixOmics package in R and a  
315 MANOVA bootstrap procedure applied to the dataset in order to highlight the significant effects  
316 of the environmental variables on the observed metabotypes (*i.e.* the specific metabolic profiles  
317 according to the environmental factors).

318 MANOVA bootstraps reveal significant effects of species ( $p = 0$ ), cyanobacterial-specific chl-  
319 *a* (BBE<sub>cy</sub>) concentrations ( $p < 10^{-43}$ ), MC concentrations ( $p < 10^{-11}$ ), pH ( $p < 10^{-28}$ ), O<sub>2</sub> ( $p <$   
320  $10^{-28}$ ), conductivity ( $p < 10^{-47}$ ), total chlorophyll-*a* concentrations ( $p < 0.011$ ) from the rCCA  
321 model. However, a clear species effect is observable through the dimension 1 ( $p < 10^{-16}$ ) with  
322 *Perca* and *Lepomis* clearly separated by the first dimension whatever the lake considered (fig.  
323 2A). On the dimension 2, a clear effect correlated with cyanobacterial biomass is observable ( $F$   
324  $= 1931$ ,  $p < 10^{-16}$ ), as fish coming from cyanobacterial dominated lakes (in green) are clearly  
325 separated by this dimension whatever the fish species considered (Fig. 3A). In addition, pH, O<sub>2</sub>  
326 concentrations and conductivity variables ( $F = 599$  and  $p < 10^{-16}$ ,  $F = 867$  and  $p < 10^{-16}$ , and  $F$   
327  $= 419$  and  $p < 10^{-16}$ , respectively) are also significantly correlated to the different metabotypes  
328 observed on this dimension but to a much lesser extent compared to cyanobacterial biomass  
329 contents (Fig. 3A). In addition, no significant interactive effects between species and the  
330 environmental factors could have been observed in this analysis suggesting that the  
331 metabotypes of the two species seem to be similarly driven by the different environmental  
332 factors.

333 The relevance network based on the second rCCA dimension specifically highlights metabolites  
334 discriminating the fish from dominated and non-dominated cyanobacteria lakes and linking to  
335 the correlated environmental factors (mainly BBE<sub>cy</sub> concentrations but also pH) (Fig. 3B).  
336 Overall, it appears that all these metabolites shown by this network exhibit negative correlation  
337 with both cyanobacterial concentrations and pH values. In addition, two-way ANOVA (Table  
338 1) were performed on all putative metabolites highlighted in the figure 3B. A significant effect  
339 of the BBE<sub>cy</sub> and pH is shown for putative metabolites annotated as 16 $\alpha$ -hydroxyestrone,  
340 acetylcholine, androsterone, glycerophosphocholine, isovalerylcarnitine, L-glutamine and L-  
341 palmitoylcarnitine whereas for glycerol, homo-L-arginine and L-arginine, a significant effect

342 of the BBECya concentrations, only, have been confirmed by two-way ANOVA analyses (Table  
343 1). Furthermore, no interaction effect between the species and the BBECya concentrations or  
344 pH has been observed for any metabolites suggesting the occurrence, for both fish species and  
345 for these two discriminating environmental factors, the occurrence of a similar metabolic  
346 responses. Negative correlations between relative concentrations of certain metabolites and the  
347 BBECya concentrations and the pH conditions are corresponding to lowest relative  
348 concentrations of these metabolites in the liver of fish captured in cyanobacterial dominated  
349 lakes (Table 1; supplementary figure S5-S6). Globally, it appears that liver of fish sampled in  
350 T, F and V lakes, and in VS lake in a lesser extent, exhibits the most significant differences in  
351 the relative concentrations of the highlighted metabolites compared to fish coming from other  
352 investigated lakes, where cyanobacteria are notably in low or not dominant proportions.

353

## 354 **Discussion**

### 355 *4.1. Diversity of ecosystems: environmental, chemical and biological conditions*

356 Cyanobacterial blooms are frequent phenoma in peri-urban lakes from the Île-de-France region  
357 due to the eutrophic and hyper-eutrophic states of these environments. Interestingly, these  
358 freshwater aquatic ecosystems are characterized by a wide range of environmental conditions  
359 and anthropogenic pressures that influence the phytoplankton biomass and compositions  
360 present in these ecosystems (Maloufi et al., 2016; Catherine et al., 2010; 2016). During summer  
361 2015 sampling, in the lakes from Rueil (R), Cergy (C), Maurepas (M) and Champs-sur-Marne  
362 (CM), diatoms or chlorophyta were dominant and cyanobacteria were present with chl-*a*  
363 concentrations never exceeding 5 µg eq. chl-*a* L<sup>-1</sup> (supplementary table S1). In the other lakes,  
364 the *Microcystis* genera appeared largely dominant, such as in Varenne-sur-Seine (VS) and in  
365 Verneuil (V) lakes with cyanobacterial-specific chl-*a* (BBECya) concentrations reaching 135  
366 and 48 µg eq. chl-*a* L<sup>-1</sup>, respectively. In the Fontenay (F) lake, *Planktothrix* genera was found  
367 to be dominant with BBECya concentration reaching 106 µg eq. chl-*a* L<sup>-1</sup>. Interestingly, in Triel  
368 (T) lake, the cyanobacteria belonging to the *Pseudanabaena* and *Anabaena* genera were present  
369 at relatively lower but even substantial BBECya concentrations (around 15 µg eq. chl-*a* L<sup>-1</sup>).  
370 However, even though cyanobacterial amount in T appears maybe not as high as in other  
371 cyanobacterial-dominated lakes of this study (F, V and VS), the presence in this T lake of  
372 noticeable and recurrent amounts of other contaminants, such as heavy metals, polychlorinated  
373 biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) was measured in both water  
374 and fish samples (Teil et al., 2014; Gaspéri et al., 2010; Azimi and Rocher 2016), and would

375 indicate why the fish present in the T lake exhibit an even more divergent metabolic profile,  
376 when compared to those of the non-cyanobacteria-dominated (R, C, M and CM) and the other  
377 cyanobacteria-dominated (V, VS and C) lakes.

378

#### 379 *4.2. Which cyanobacterial-related parameters may influence the fish metabolome?*

380 Cyanobacteria are well known to produce a wide variety of potentially toxic secondary  
381 metabolites including MCs, that have been detected in remarkable amount especially in the VS  
382 and F lakes, together with various other cyanobacterial bioactive compounds, such as those that  
383 have been highlighted by the global network analysis, including cyanopeptolins, aeruginosins,  
384 microginins and anabaenopeptins. Previous experimental studies have been able to show that  
385 cyanobacterial exposure could induce significant physiological impairs in fish exhibiting  
386 different metabolomic dysregulation, in relation with the various secondary metabolite content  
387 of the investigated strains (Sotton et al., 2017a; 2017b). Nevertheless, the genuine ecological  
388 consequences of the production of this chemical bioactive (including potent toxic) metabolites  
389 by cyanobacteria remain largely uncovered.

390 Indeed, in addition to the production of such potentially toxic secondary metabolites, changes  
391 in physico-chemical parameters of the water-bodies have also been reported during  
392 cyanobacterial bloom episodes. Massive decrease of dissolved O<sub>2</sub> concentrations may occur  
393 during important bloom senescence events, due to the bacterial degradation of cyanobacterial  
394 cell biomass, inducing sometimes spectacular massive death of various fish species, also  
395 described as fish-kill phenomenon (Pearl and Paul 2011). During the present study, no  
396 noticeable depletion of the dissolved O<sub>2</sub> concentrations of neither surface nor bottom water or  
397 any fish-kill have been observed, indicating that the high cyanobacterial biomass (observed in  
398 V, VS, T and F lakes) were not in senescence. Furthermore, O<sub>2</sub> concentrations does not appear  
399 to significantly drive the liver metabolic differences observed in the fish species studied,  
400 suggesting that no metabolic disturbances linked to O<sub>2</sub> concentration variation were observed  
401 here. Among the other environmental factors measured on the studied lakes that are noticeably  
402 correlated with metabolic differences in fish livers based on rCCA analyses, the pH exhibits  
403 large variations between sampled aquatic ecosystems. Indeed, it appears that lakes dominated  
404 by cyanobacteria show higher pH values (above 9), excepted in Fontenay (F) lake where the  
405 value is more similar (between 7.1 and 8.3) to those found in lakes where cyanobacteria are in  
406 less abundance (supplementary table S1). Such high pH value phenomenon has been already  
407 reported to be directly related with cyanobacteria photosynthesis process that removes carbon  
408 dioxide from the water and increases hydroxide ion concentration (Lopez-Archilla et al., 2004).

409 However, in the present study, it is not possible to directly conclude whether the elevated pH  
410 observed in cyanobacterial dominated lakes is a consequence of the photosynthetic activity of  
411 cyanobacteria or is due to local geochemical conditions of the respective lake. To better  
412 characterize the causality of these elevated pH in further investigation, it should be suitable to  
413 monitor pH value before, during and at the end of the bloom. Further studies need now to be  
414 performed in order to disentangle the specific and/or synergic effects of the various bioactive  
415 metabolite production together with those of other physicochemical parameters associated with  
416 cyanobacterial blooms.

417

#### 418 *4.3. Metabolic changes correlated to perturbed lakes: cyanobacterial concentrations and pH* 419 *as environmental drivers of fish liver metabolome?*

420 Thanks to NMR and multivariate analyses, the present results show that comparable metabolic  
421 changes are observable in both fish species exposed to higher cyanobacterial biomass (F, VS,  
422 T and V lakes). In these analyses the most constraining factors correlated to the metabolic  
423 changes in fish were the cyanobacterial concentrations and the pH values that exhibit the  
424 strongest correlations with the changes of relative concentrations of various metabolites in the  
425 two fish species sampled. In this study, it is also very likely that the combination of both high  
426 pH values and high cyanobacterial biomass could lead to the more contrasted metabolome  
427 changes in fish. This would be in agreement with the fact that fish from F lake, presenting high  
428 cyanobacterial and MCs concentrations but moderate pH value around 7.5, exhibit intermediate  
429 metabolomes compared to fish captured in T, V and VS (high cyanobacterial and MCs  
430 concentrations and high pH) and fish captured in other lakes (C, CM, M, R), not dominated by  
431 cyanobacteria.

432 The present results indicate that MCs may not be in this case the most constraining factor for  
433 fish from those natural ecosystems. However, in addition to MC cyanobacteria produce a large  
434 set of bioactive compounds that could also influence the fish health and the complexity of such  
435 secondary metabolite mixtures aims at being more characterized in an ecotoxicological point  
436 of view. One should take also into account that fish may not necessarily be exposed by direct  
437 ingestion of cyanobacterial cells, and that trophic transfer may be another source of  
438 cyanobacterial bioactive (including toxic) metabolites (Sotton et al., 2014). It would be now  
439 interesting to consider other fish species feeding on phytoplankton in order to test whether they  
440 are indeed exposed to higher cyanotoxin contents, by direct ingestion of cyanobacterial cells,  
441 and whether they exhibit similar or even more drastic metabolome alterations than such  
442 carnivorous ones.

443 Interestingly, the fish collected from the T lake exhibits one of the most divergent global  
444 metabolotypes, suggesting that it could potentially be influenced by the occurrence of multi-stress  
445 conditions, comprising cyanobacteria proliferations together with the other heavy metal, PCB  
446 and PAH contaminants, that have been observed in this specific environments (Catherine et al.,  
447 2016). Indeed, T lake was not characterized by the highest concentration of cyanobacteria  
448 observed during this study. However, due to the presence of other pollutants already monitored  
449 in past studies in this pound and not in the other one (Teil et al., 2014; Gaspéri et al., 2010;  
450 Azimi and Rocher 2016), this observation suggests that additive and/or synergistic effects of  
451 multi-pollutants together with cyanobacterial bloom seem to be involved in similar metabolic  
452 variations than those of fish from pounds which are the most stressed by cyanobacterial blooms.  
453 But such assumption now need further investigations before been fully validated. On order to  
454 fully validate potential metabolite biomarkers of cyanobacterial exposure pressure on fish liver  
455 metabolome, further investigation would now aim at investigating a larger set of lake from a  
456 wider geographic area, and also to firmly confirm the metabolite identification by orthogonal  
457 approaches such as 2-D NMR, spiking of standard, and or to develop in parallel mass  
458 spectrometry based metabolomic investigations.

459

## 460 **5. Conclusion**

461 The present study demonstrates that cyanobacterial blooms occurrence can locally be correlated  
462 with metabolic divergences in relation to the fish response to environmental stress. Such  
463 metabolomic analyses support experimentally observed processes but also give us innovative  
464 perspectives for the characterization of the environmental and genuine interactions between fish  
465 and cyanobacteria proliferations.

466 In this way, further studies should be performed in order to investigate longitudinal variations  
467 along seasonal variations, fish life history, populational genetic distance and the specific  
468 changes of fish metabolomes during bloom episodes, which could help us to better understand  
469 and characterize the main environmental factors potentially disturbing fish physiology. Also,  
470 the understanding of the ecosystem functioning under ecotoxicological pressure induced by  
471 cyanobacterial blooms would gain at investigating other fish species feeding directly on  
472 phytoplankton organisms. It should highlight whether higher metabolome and toxicological  
473 changes would be driven by directly feeding on cyanobacterial cells and higher contact with  
474 with the various potentially toxic secondary metabolites, or by other ecological parameters  
475 impacted by cyanobacterial proliferations. Furthermore, complementary omic analyses, such as



476 proteomics and transcriptomics, would provide additional support to generate system biology  
477 understanding of the global organism response to environmental stress, and help at  
478 characterizing potential biomarkers useable at the fish population levels in various aquatic  
479 environments.

480

#### 481 **Conflicts of interest**

482 None

483

#### 484 **Declaration of interest**

485 None

486

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497

#### 498 **References**

- 499 Amado, L., Monserrat, J., 2010. Oxidative stress generation by microcystins in aquatic  
500 animals: Why and how. *Environment International* 36, 226–235.
- 501 Azimi, S., Rocher, V., 2016 Influence of the water quality improvement on fish population in  
502 the Seine River (Paris , France) over the 1990 – 2013 period. *Science of the Total*  
503 *Environment* 542, 955–964.
- 504 Bols, N. C., Brubacher, J. L., Ganassin, R. C., Lee, L. E. J., 2001. Ecotoxicology and innate  
505 immunity in fish. *Developmental and Comparative Immunology* 25(8–9), 853–873.
- 506 Bundy, J. G., Davey, M. P., Viant, M. R., 2008. Environmental metabolomics: a critical  
507 review and future perspectives. *Metabolomics* 5(1), 3–21.
- 508 Bunn, S. E., 2016. Grand challenge for the future of freshwater ecosystems. *Frontiers in*

509 *Environmental Science* 4, 1–4.

510 Cappello, T., Fernandes, D., Maisano, M., Casano, A., Bonastre, M., Bebianno, M. J., ... &  
511 Porte, C., 2017. Sex steroids and metabolic responses in mussels *Mytilus*  
512 *galloprovincialis* exposed to drospirenone. *Ecotoxicology and environmental safety*  
513 143, 166-172

514 Cappello, T., Brandão, F., Guilherme, S., Santos, M. A., Maisano, M., Mauceri, A., Canário,  
515 J., Pacheco, M., Pereira, P., 2016. Insights into the mechanisms underlying mercury-  
516 induced oxidative stress in gills of wild fish (*Liza aurata*) combining 1H NMR  
517 metabolomics and conventional biochemical assays. *Science of the Total Environment*  
518 548–549, 13–24.

519 Catherine, A., Troussellier, M., Bernard, C., 2008. Design and application of a stratified  
520 sampling strategy to study the regional distribution of cyanobacteria (Ile-de-France,  
521 France). *Water research* 42(20), 4989–5001.

522 Catherine, A., Mouillot, D., Escoffier, N., Bernard, C., Troussellier, M., 2010. Cost effective  
523 prediction of the eutrophication status of lakes and reservoirs. *Freshwater Biology*  
524 55(11), 2425–2435.

525 Catherine, A., Selma, M., Mouillot, D., Troussellier, M., Bernard, C., 2016. Patterns and  
526 multi-scale drivers of phytoplankton species richness in temperate peri-urban lakes.  
527 *Science of The Total Environment* 559, 74–83.

528 Catherine, A., Bernard, C., Spooof, L., Bruno, M., 2017. Microcystins and Nodularins. In  
529 *Handbook on Cyanobacterial Monitoring and Cyanotoxin Analysis*, Meriluoto, J.,  
530 Spooof, L., Codd, G. A., Eds., John Wiley and Sons, p 576.

531 Chorus, I., Bartram, J. *Toxic cyanobacteria in water: a guide to their public health*  
532 *consequences, monitoring and management.*, E & FN Spon, 1999.

533 Codd, G. A., Morrison, L. F., Metcalf, J. S. 2005. Cyanobacterial toxins: risk management for  
534 health protection. *Toxicol. Appl. Pharm.* 203(3), 264–272.

535 Codd, G. A., Meriluoto, J., Metcalf, J. S., 2017. Introduction: Cyanobacteria, Cyanotoxins,  
536 Their Human Impact, and Risk Management. In *Handbook of Cyanobacterial*  
537 *Monitoring and Cyanotoxin Analysis*, Meriluoto, J., Spooof, L., Codd, G. A., Eds., John  
538 Wiley and Sons, p 548.

539 Combes, A., El Abdellaoui, S., Vial, J., Lagrange, E., Pichon, V., 2014. Development of an  
540 analytical procedure for quantifying the underivatized neurotoxin  $\beta$ -N-methylamino-l-  
541 alanine in brain tissues. *Analytical and Bioanalytical Chemistry* 406(19), 4627–4636.

542 Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque,

543 C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., et al., 2006.  
544 Freshwater biodiversity: importance, threats, status and conservation challenges.  
545 *Biological Reviews of the Cambridge Philosophical Society* 81 (2), 163–182.

546 Faassen, E. J., Antoniou, M. G., Beekman-Lukassen, W., Blahova, L., Chernova, E.,  
547 Christophoridis, C., Combes, A., Edwards, C., Fastner, J., Harmsen, J., et al., 2016. A  
548 collaborative evaluation of LC-MS/MS based methods for BMAA analysis: Soluble  
549 bound BMAA found to be an important fraction. *Marine Drugs* 14(3).

550 Ferrão-Filho, A. da S., Kozłowsky-Suzuki, B., 2011. Cyanotoxins: bioaccumulation and  
551 effects on aquatic animals. *Marine drugs* 9(12), 2729–2772.

552 Fiehn, O., 2002. Metabolomics - the link between genotypes and phenotypes. *Plant*  
553 *Molecular Biology* 48, 155–171.

554 Franzosa, E. A., Hsu, T., Sirota-Madi, A., Shafquat, A., Abu-Ali, G., Morgan, X. C.,  
555 Huttenhower, C., 2015. Sequencing and beyond: integrating molecular “omics” for  
556 microbial community profiling. *Nature reviews. Microbiology* 13(6), 360–372.

557 Gaspéri, J., Moreau-Guigon, E., Labadie, P., Blanchard, M., Teil, M.-J., Tlili, K., Dinh, T. .,  
558 Tran, C., Alliot, F., Desportes, A., et al., 2010 Contamination de la Seine par les  
559 micropolluants organiques : Effet des conditions hydriques et de l'urbanisation.  
560 <https://hal-enpc.archives-ouvertes.fr/hal-00766537>

561 Hamilton, P. B., Cowx, I. G., Oleksiak, M. F., Griffiths, A. M., Grahn, M., Stevens, J. R.,  
562 Carvalho, G. R., Nicol, E., Tyler, C. R., 2016. Population-level consequences for wild  
563 fish exposed to sublethal concentrations of chemicals ??? a critical review. *Fish and*  
564 *Fisheries* 17(3), 545–566.

565 Hao, J., Liebeke, M., Astle, W., De Iorio, M., Bundy, J. G., Ebbels, T. M. D., 2014. Bayesian  
566 deconvolution and quantification of metabolites in complex 1D NMR spectra using  
567 BATMAN. *Nature protocols* 9(6), 1416–1427.

568 Hultman, J., Waldrop, M. P., Mackelprang, R., David, M. M., McFarland, J., Blazewicz, S. J.,  
569 Harden, J., Turetsky, M. R., McGuire, A. D., Shah, M. B., et al., 2015. Multi-  
570 omics of permafrost, active layer and thermokarst bog soil microbiomes.  
571 *Nature* 521, 208–212.

572 Krishnamoorthy, K., & Lu, F., 2010. A parametric bootstrap solution to the MANOVA under  
573 heteroscedasticity. *Journal of Statistical Computation and Simulation* 80(8), 873-887.

574 Le Manach, S., Sotton, B., Huet, H., Duval, C., Paris, A., Marie, A., ... & Marie, B. (2018).  
575 Physiological effects caused by microcystin-producing and non-microcystin producing  
576 *Microcystis aeruginosa* on medaka fish: A proteomic and metabolomic study on liver.

577 *Environmental pollution*, 234, 523-537.

578 Lin, C. Y., Wu, H., Tjeerdema, R. S., Viant, M. R., 2007. Evaluation of metabolite extraction  
579 strategies from tissue samples using NMR metabolomics. *Metabolomics* 3(1),  
580 55–67.

581 Lopez-Archilla, A., Moreira, D., Lopez-Garcia, P., Guerrero, C., 2004. Phytoplankton  
582 diversity and cyanobacterial dominance in a hypereutrophic shallow lake with  
583 biologically produced alkaline pH. *Extremophiles* 8, 109–115.

584 Malbrouck, C., Kestemont, P., 2006. Effects of microcystins on fish. *Environ. Toxicol. Chem.*  
585 25(1), 72–86.

586 Maloufi, S., Catherine, A., Mouillot, D., Louvard, C., Couté, A., Bernard, C., Troussellier,  
587 M., 2016. Environmental heterogeneity among lakes promotes hyper  $\beta$ -diversity across  
588 phytoplankton communities. *Freshwater Biology* 61(5), 633–645.

589 Marie, B., Huet, H., Marie, A., Djediat, C., Puiseux-Dao, S., Catherine, A., Trinchet, I.,  
590 Ederly, M., 2012. Effects of a toxic cyanobacterial bloom (*Planktothrix agardhii*) on  
591 fish: Insights from histopathological and quantitative proteomic assessments following  
592 the oral exposure of medaka fish (*Oryzias latipes*). *Aquatic toxicology (Amsterdam,*  
593 *Netherlands)* 114–115C, 39–48.

594 McLean, T. I., 2013. “Eco-omics”: A Review of the Application of Genomics,  
595 Transcriptomics, and Proteomics for the Study of the Ecology of Harmful Algae.  
596 *Microbial Ecology* 65(4), 901–915.

597 Paerl, H. W., Paul, V. J., 2011. Climate Change: Links to Global Expansion of Harmful  
598 Cyanobacteria. *Water Research* 46(5), 1363–1349.

599 O’Neil, J. M., Davis, T. W., Burford, M. A., Gobler, C. J., 2012. The rise of harmful  
600 cyanobacteria blooms: The potential roles of eutrophication and climate change.  
601 *Harmful Algae* 14, 313–334.

602 Qiao, Q., Le Manach, S., Huet, H., Duvernois-Berthet, E., Chaouch, S., Duval, C., Sotton, B.,  
603 Ponger, L., Marie, A., Mathéron, L., et al., 2016. An integrated omic analysis of  
604 hepatic alteration in medaka fish chronically exposed to cyanotoxins with possible  
605 mechanisms of reproductive toxicity. *Environ. pollut.* 219, 119–131.

606 Revenga, C., Campbell, I., Abell, R., de Villiers, P., Bryer, M., 2005. Prospects for  
607 monitoring freshwater ecosystems towards the 2010 targets. *Philosophical*  
608 *transactions of the Royal Society of London. Series B, Biological sciences* 360(1454),  
609 397–413.

610 Rohart, F., Gautier, B., Singh, A., & Le Cao, K. A., 2017. mixOmics: An R package for

611 'omics feature selection and multiple data integration. *PLoS computational biology*  
612 13(11), e1005752.

613 Samuelsson, L. M., Larsson, D. G. J., 2008. Contributions from metabolomics to fish  
614 research. *Molecular bioSystems* 4(10), 974–979.

615 Sardans, J., Peguelas, J., Rivas-Ubach, A., 2011 Ecological metabolomics: Overview of  
616 current developments and future challenges. *Chemoecology* 21(4), 191–225.

617 Schwarzenbach, R. P., Egli, T., Hofstetter, T. B., von Gunten, U., Wehrli, B., 2010. Global  
618 Water Pollution and Human Health. *Annual Review of Environment and Resources*  
619 35(1), 109–136.

620 Sotton, B., Guillard, J., Anneville, O., Maréchal, M., Savichtcheva, O., Domaizon, I., 2014.  
621 Trophic transfer of microcystins through the lake pelagic food web: Evidence for the  
622 role of zooplankton as a vector in fish contamination. *Science of The Total*  
623 *Environment* 466–467, 152–163.

624 Sotton, B., Domaizon, I., Anneville, O., Cattaneo, F., Guillard, J., 2015. Nodularin and  
625 cylindrospermopsin: a review of their effects on fish. *Reviews in Fish Biology and*  
626 *Fisheries* 25, 1–19.

627 Sotton, B., Paris, A., Le Manach, S., Blond, A., Lacroix, G., Millot, A., ... & Marie, B.,  
628 2017a. Global metabolome changes induced by cyanobacterial blooms in three  
629 representative fish species. *Science of the Total Environment* 590, 333-342.

630 Sotton, B., Paris, A., Manach, S. Le, Blond, A., Lacroix, G., Duval, C., Huet, H., Qiao, Q.,  
631 Labrut, S., Chiapetta, G., et al., 2017b. Metabolic changes in Medaka fish induced by  
632 cyanobacterial exposures in mesocosms : an integrative approach combining  
633 proteomic and metabolomic analyses. *Scientific reports* 7, 1–13.

634 Teil, M.-J., Tlili, K., Blanchard, M., Labadie, P., Alliot, F., Chevreuil, M., 2014.  
635 Polychlorinated Biphenyls, Polybrominated Diphenyl Ethers, and Phthalates in Roach  
636 from the Seine River Basin (France): Impact of Densely Urbanized Areas. *Archives of*  
637 *environmental contamination and toxicology* 66, 41–57.

638 Tenenhaus, A., Philippe, C., Guillemot, V., Le Cao, K. A., Grill, J., & Frouin, V., 2014.  
639 Variable selection for generalized canonical correlation analysis. *Biostatistics* 15(3),  
640 569-583.

641 Wang, Mingxun, et al., 2016. Sharing and community curation of mass spectrometry data with  
642 Global Natural Products Social Molecular Networking. *Nature Biotechnology* 34/8,  
643 828-837.

644 Wu, H., Southam, A. D., Hines, A., Viant, M. R., 2008. High-throughput tissue extraction

645 protocol for NMR- and MS-based metabolomics. *Analytical biochemistry* 372 (2),  
646 204–212.

647 Yepremian, C., Catherine, A., Bernard, C., 2017. SOP202: Chlorophyll a extraction. In  
648 *Handbook on Cyanobacterial Monitoring and Cyanotoxin Analysis*, Meriluoto, J.,  
649 Spoof, L., Codd, G. ., Eds., p 458.

650

651 **Table**

652

Putative metabolites	BBECya		pH	
	<i>F-value</i>	<i>p-value</i>	<i>F-value</i>	<i>p-value</i>
16a-Hydroxyestrone	26.604	<b>1.02e<sup>-06</sup></b>	8.295	<b>0.00472</b>
Acetylcholine	43.903	<b>1.08e<sup>-09</sup></b>	5.892	<b>0.01672</b>
Androsterone	19.932	<b>1.85e<sup>-05</sup></b>	10.776	<b>0.00135</b>
Glycerophosphocholine	38.040	<b>1e<sup>-08</sup></b>	8.892	<b>0.00348</b>
Glycerol	29.455	<b>3.1e<sup>-07</sup></b>	3.908	0.0504
Homo-L-arginine	32.501	<b>8.95e<sup>-08</sup></b>	1.795	0.18287
Isovalerylcarnitine	32.028	<b>1.08e<sup>-07</sup></b>	9.392	<b>0.0027</b>
L-Arginine	30.269	<b>2.22e<sup>-07</sup></b>	2.228	0.13820
L-Glutamine	30.004	<b>2.47e<sup>-07</sup></b>	14.025	<b>0.000280</b>
L-Palmitoylcarnitine	24.965	<b>2.05e<sup>-06</sup></b>	12.888	<b>0.000483</b>

653

654 **Table 1:** Effects of significantly-related environmental factors on the putative metabolite  
655 expressions revealed by two-ways ANOVA analyses performed on the environmental  
656 parameter and metabolite matrix for all individuals (supplementary data 1). Bold p-values  
657 correspond to p-values < 0.05.

658

659

660 **Legends of figures**

661 **Figure 1. Phytoplanktonic chlorophyll-a concentrations ( $\mu\text{g.L}^{-1}$  eq. Chl-a) in the sampled**  
662 **lakes (A) and the cyanobacterial genus composition observed in the cyanobacteria-**  
663 **dominated lakes (B).** ND = non-detected. Cergy (C), Champs-sur-Marne (CM), Maurepas lake  
664 (M), Rueil lake (R), Verneuil lake (V), Varenne-sur-Seine lake (VS), Fontenay lake (F) and  
665 Triel lake (T).

666

667 **Figure 2. Diversity of metabolites observed in the lakes, dominated or not by**  
668 **cyanobacterial proliferations revealed by molecular networking based on MS/MS**  
669 **fragmentation data.** When various phospholipids are observed in all lakes, potentially bio-  
670 active metabolites known to be produced by certain cyanobacteria, such as microcystins  
671 (hepatotoxin), aeruginosins, cyanopeptolins, anabaenopeptins, microginins, are only observed  
672 in cyanobacterial dominated lakes.

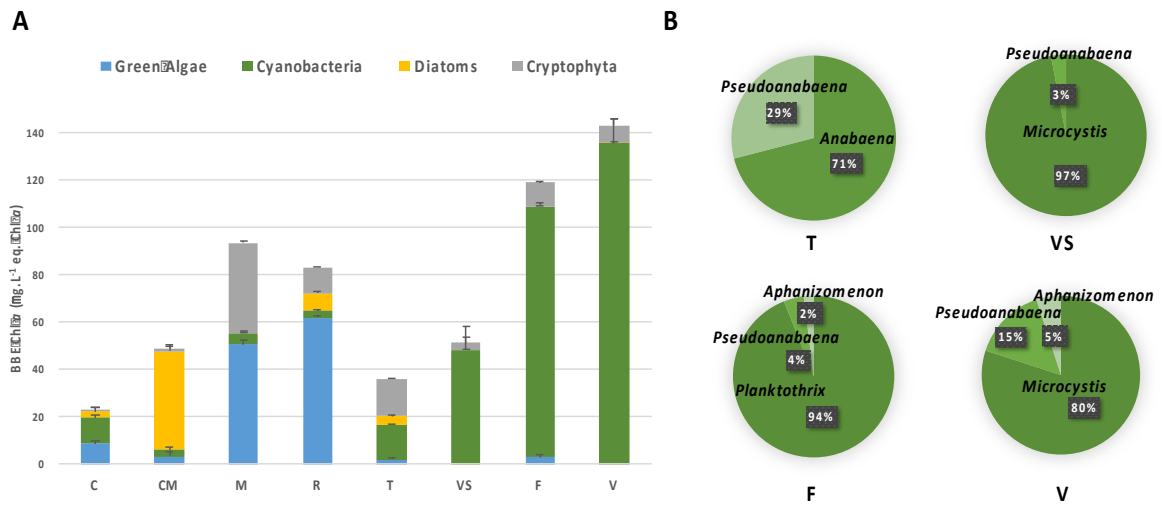
673

674 **Figure 3.  $^1\text{H-NMR}$  liver metabolomes and relevance network of fish sampled in the different**  
675 **lakes.** The individual plots of regularized canonical correlations analysis (rCCA) for  
676 dimensions 1–2 (A). Perch individuals are on the left side and pumpkinseed individuals on the  
677 right side of the graphic. Lakes are represented by their respective letters that in blue correspond  
678 to control lakes and in green to perturbed lakes. Cergy (C), Champs-sur-Marne (CM),  
679 Maurepas lake (M), Rueil lake (R), Verneuil lake (V), Varenne-sur-Seine lake (VS), Fontenay  
680 lake (F) and Triel lake (T). Relevance network providing from rCCA analysis on the dimension  
681 2 (B). Putative metabolites above a correlation threshold of  $|0.3|$  were kept, revealing that the 2  
682 most correlated variables are pH and BBECya (cyanobacterial specific content of chlorophyll a  
683 measured by BBE fluorescence probe). Green edges correspond to negative correlations with  
684 the discriminant ecological factors.

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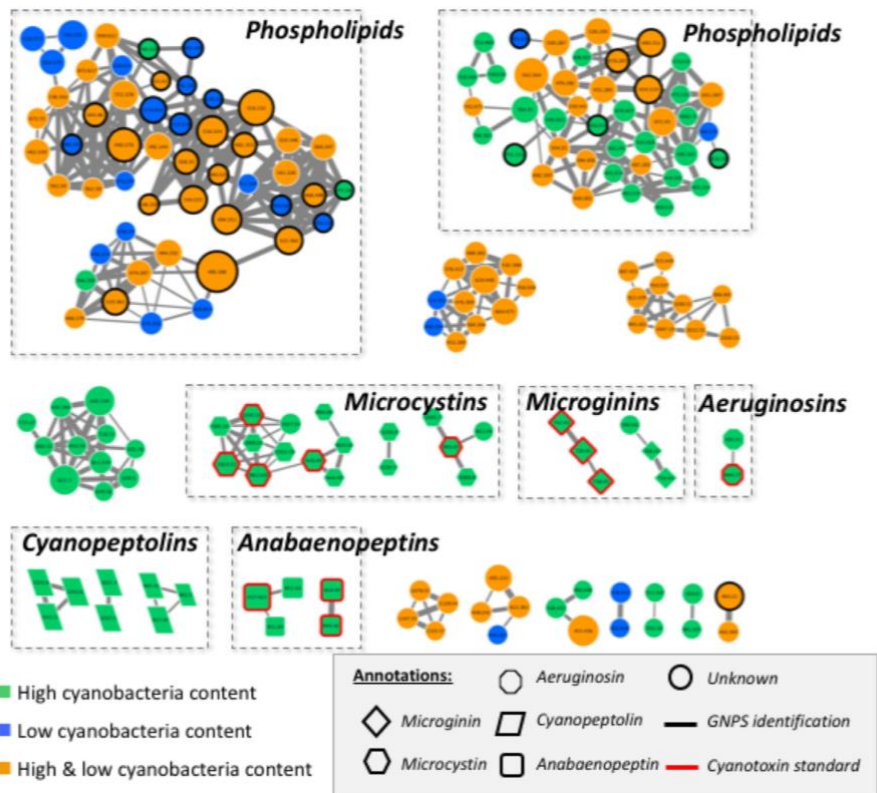


686 **Fig. 1.**



687

688 Fig. 2.

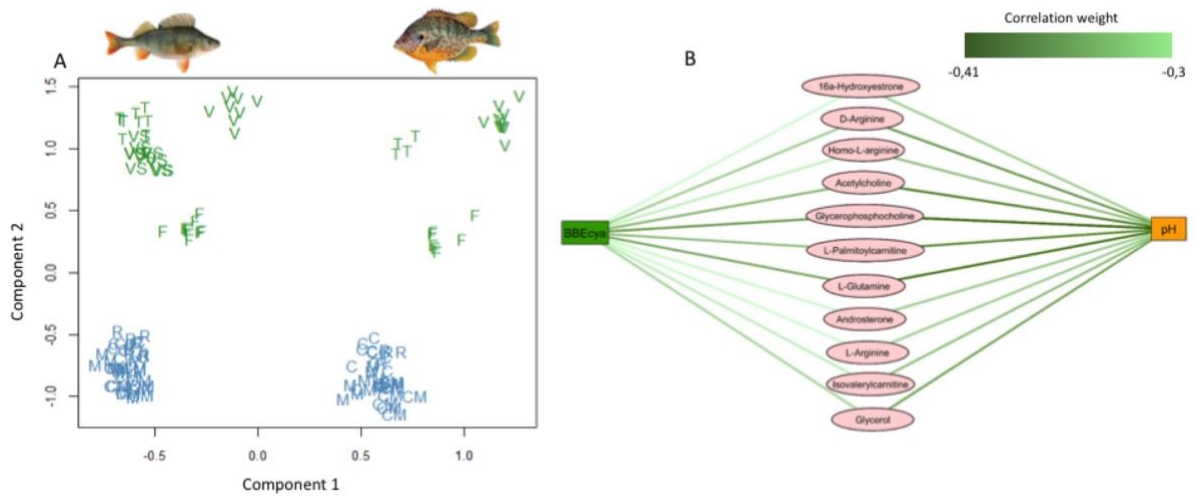


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692 **Fig. 3.**



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