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1 Is PCBs concentration variability between and within freshwater fish species explained by
2 their contamination pathways?
3

4 Lopes C. ^{a*}, Perga M.-E. ^b, Peretti A. ^a, Roger M.-C. ^a, Persat H. ^c, Babut M. ^a

5

6 ^a Cemagref, UR MALY, 3 bis Quai Chauveau – CP220, F-69336 LYON, France;

7 ^b INRA UMR CARTELE 75 avenue de Corzent, F-74203 Thonon les Bains, France

8 ^c UMR 5023 – Laboratoire d'Ecologie des Hydrosystèmes Fluviaux (LEHF), Université

9 Claude Bernard Lyon 1, 69221 Villeurbanne, France.

10

11 * To whom correspondence may be addressed:

12 Christelle Lopes

13 Cemagref, UR MALY, 3 bis Quai Chauveau – CP220, F-69336 LYON, France.

14 Tel: +33 (0)4-72-20-87-97 / Fax : +33 (0)4-78-47-78-75

15 E-mail: christelle.lopes@cemagref.fr

16 Abstract

17 Many chemical, physiological, and trophic factors are known to affect
18 bioaccumulation of polychlorinated biphenyls (PCBs) in biota. Understanding the primary
19 factors affecting fish contamination is critical for predicting and assessing risks to upper-
20 trophic level consumers, including humans. Here we identify PCB contamination pathways
21 that could explain within- and between-species variability in fish concentration levels. Three
22 freshwater river fish species (barbel, chub and bream) were sampled at three sites along the
23 Rhone River (France) where fish consumption is partially prohibited because of PCB levels
24 exceeding the European health-based benchmark. The trophic position was assessed using an
25 innovative approach based on stable isotope analyses and Bayesian inference, which takes
26 into account both isotope data variability and parameter uncertainty. The effect of foraging
27 habitat on fish contamination was addressed using stable isotope mixing models. The fish
28 trophic position and PCB concentrations were found to be unrelated while the exploitation of
29 sediment detrital carbon as a food source appeared to be a critical factor affecting fish
30 contamination. Fish length, PCB concentration of the sediment, and individual fish foraging
31 habitat (exploitation of detrital versus planktonic carbon sources) explained 80% of within-
32 and between-species variability observed in PCB concentrations. These results, obtained for
33 species that have overlapping TPs and exploit different carbon sources, reveal that the
34 important factor in fish PCB contamination is not only what fish consume, but also and
35 essentially the feeding location.

36 Keywords: freshwater river fish, PCB contamination, stable isotopes, mixing model,
37 Bayesian inference, predictive models

38 **1. Introduction**

39 The contamination of aquatic ecosystems by organic pollutants such as
40 polychlorinated biphenyls (PCBs) can result in consumption advisories and bans¹. In the
41 Rhone River near the city of Lyon, France, PCB levels above the European health-based
42 regulatory benchmark of 8 pg TEQ² / g (wet weight) were measured in fish, resulting in a
43 partial ban on fish consumption in 2007. Various studies have concluded that
44 bioconcentration (i.e., accumulation of a contaminant through direct uptake from water) was
45 the primary mechanism governing contamination of biota (Leblanc, 1995; Kucklick et al.,
46 1996; Campfens and Mackay, 1997; Zaranko et al., 1997; Burreau et al., 2004), while other
47 studies have suggested other mechanisms, such as biomagnification (i.e., an increase in
48 contaminant concentration in the food chain) (Zaranko et al., 1997; Burreau et al., 2004).
49 Nonetheless, it is commonly accepted that chemical concentrations in organisms tend to
50 increase with each step in the food chain, resulting in the concentration in organisms at the
51 top of food chains to be many times greater than those in organisms at the bottom (Gobas et
52 al., 1999; Walters et al., 2008).

53 Three types of factors are known to be important in the PCB bioaccumulation process
54 (Borgå et al., 2004): physico-chemical, physiological, and trophic. Physicochemical factors
55 include hydrophobicity, expressed as the K_{ow} value (octanol-water partition coefficient) of
56 each PCB congener, which accounts for their solubility in water as well as in lipids.
57 Physiological factors include lipid content (due to the PCB lipophilic property), body size
58 (related, via allometry, to the chemical elimination rate because of an altered surface-to-
59 volume ratio), and sex (Johnston et al., 2002). Many studies on PCB concentrations in aquatic
60 organisms consider the influence of lipids by lipid-normalizing the concentration, but this

¹ http://www.rhone-mediterranee.eaufrance.fr/usages-et-pressions/pollution_PCB/pcb-arretes-interdiction.php

² TEQ=toxic equivalent quantity for dioxin, furan and dioxin-like PCBs

61 practice is being debated (Hebert and Keenleyside, 1995). The effect of sex has been largely
62 debated, with the assumption that the depletion of lipids associated with female spawning
63 decreases accumulation of hydrophobic organic contaminants (Johnston et al., 2002; Borgå et
64 al., 2004; Debruyne et al., 2004). Finally, trophic factors include individual diet preferences,
65 habitat use (effects on bioaccumulation in terms of changes in exposure, both by water and
66 dietary uptake) (Guildford et al., 2008; Walters et al., 2008), the length of the food chain, and
67 thus trophic position (TP), as a consequence of the biomagnification processes at the
68 individual level, as explained above (Gobas et al., 1999). All of these factors are likely to
69 influence both between- and within-species variability in PCB concentrations. These factors
70 have generally been studied independently in statistical models of bioaccumulation, and their
71 relative contributions are less well known. Considering the ecological and economic impacts
72 of fish consumption bans and advisories in riverine ecosystems, understanding the relative
73 importance of the primary factors influencing bioaccumulation of PCBs in fish is critical for
74 predicting and assessing risks to upper-trophic-level consumers, including humans.

75 For many years, stable isotope analysis (SIA) has been widely used to relate fish
76 contamination and trophic factors, where TP is estimated deterministically from stable
77 nitrogen isotopes (Post, 2002) and ultimate carbon sources from stable carbon isotopes
78 (Vander Zanden and Rasmussen, 1996; Mazak et al., 1997; Kidd et al., 1998). Isotope mixing
79 models have also been developed to quantify the respective contribution of a few sources
80 (prey) to fish diet (Phillips, 2001; Phillips and Gregg, 2003). Studies combining PCB analysis
81 and SIA have long been used, principally in lakes (Vander Zanden et al., 2000; Tarvainen et
82 al., 2008). Such approaches have made it possible to identify the role played by TP in the
83 variability of contamination between species, but so far have failed to explain inter-individual
84 variability of contamination levels or the role played by habitat. Indeed, these approaches
85 generally suffer from potential sources of uncertainty around the mean estimates of sources,

86 such as in trophic fractionation factors, which were not adequately considered from a
87 technical point of view (Jardine et al., 2006). We assume that improving the SIA within such
88 ecotoxicological studies might help address the relationships between interindividual
89 variability in trophic behavior and in PCB contamination levels in fish.

90 The recent development of stable isotope mixing models based on Bayesian inference
91 (Jackson et al., 2009; Xue et al., 2009; Parnell et al., 2010) is a major advance in SIA since
92 these models take into account data variability and parameter uncertainty (Moore and
93 Semmens, 2008; Xue et al., 2009). The role of diet preferences and habitat partitioning in
94 PCB accumulation has not been adequately considered until now (Hebert and Haffner, 1991;
95 Paterson et al., 2006), although PCB levels differ between habitats because of the chemical
96 properties of the congeners. Indeed, PCB congeners are not all equally hydrophobic and
97 distribute differently among the various aquatic compartments. The use of these Bayesian
98 models in this context should therefore be very useful to determine the role played by
99 characteristic habitats on fish contamination pathways.

100 Within these models, however, no statistical method has been developed to include
101 data variability and parameter uncertainty in TP estimation. The TP within a single fish
102 species might be highly variable between individuals, inducing differences in PCB
103 bioaccumulation. Addressing the role of inter-individual variability in the TP estimated from
104 SIA on fish PCB contamination therefore requires the development of an adequate statistical
105 method. Using a Bayesian framework to estimate TP, the different biases related to the usual
106 deterministic estimation can be taken into account (Post, 2002).

107 Hence, the primary goal of this paper is to identify PCB contamination pathways that
108 explain between- and within-species variability in fish PCB concentrations observed in the
109 Rhone River, using an improved SIA approach. The potential role played by feeding habitats
110 was explored applying Bayesian isotope models, while a statistical method based on Bayesian

111 inference was developed to estimate the fish TP from stable isotope data. The results were
112 then integrated into a statistical predictive model that was developed to establish the
113 probability that the concentration of PCBs in a given fish species exceeds the health-based
114 fish consumption threshold.

115 **2. Material and Methods**

116 **2.1. Study sites**

117 Fish, invertebrates, and sediments were collected at three sites in France along the
118 Rhone River: (1) Lône de la Morte (MTE), the relative reference site upstream from Lyon and
119 the first contaminated river reach; (2) Grand Large (GDL), a fluvial lake within the
120 contaminated area and close to the city of Lyon; and (3) Ile du Beurre (BRE), a site
121 downstream from Lyon. These sites were chosen for their expected sediment contamination
122 according to the potential contamination sources (a factory specialized in PCB incineration
123 between the two upstream sites and many industrial chemical sites between the two
124 downstream sites). Furthermore, dams border each site and thus limit fish migration to too
125 great a distance.

126 Sediment cores were collected at each site and radionuclide measurement was used to
127 age the successive layers in each core. The seven iPCB congeners were quantified by the
128 EUROFINS laboratory (Orléans, France) (see Supporting Information [SI] for greater detail).

129 **2.2. Fish and invertebrate sampling**

130 Fish and invertebrates were collected at each study site (Table 1) and the fauna were
131 inventoried by catching invertebrates with artificial substrates for an overview of the species
132 living therein.

133 Three large, long-lived freshwater cyprinid species were chosen because (i) they are
134 prone to PCB accumulation over a several-year period; (ii) although they exhibit a relatively
135 similar TP, they have a plastic trophic behavior maximizing trophic variability between
136 individuals (Philippart, 1977); and (iii) they have different diets and they exploit different
137 habitats: the barbel *Barbus barbus* (Linnaeus, 1758) is a bottom feeder and lives in running
138 rather than deep waters (Baras and Philippart, 1999), the European chub *Squalius cephalus*
139 (Linnaeus, 1758) is more often found in standing and running waters and feeds in all aquatic
140 compartments (top, middle and bottom waters) (Caffrey et al., 2008) and the bream *Abramis*
141 *brama* (Linnaeus, 1758) lives in standing waters and is a bottom and middle feeder (Persson
142 and Brönmark, 2002). Adult specimens were captured with nets or by electro-shocking (Table
143 1). To better control for within-species variability in PCB contamination and diets, only adults
144 were selected in order to limit the effects of age status. Length (cm) and weight (g) were
145 measured, sex was determined, and age was estimated by scalimetry (years). Stomach
146 contents were analyzed in the laboratory.

147 Large corbicula *Corbicula fluminea* (Müller, 1774) (>2 cm) and *Pisidium*
148 *tenuilineatum* (Stelfox, 1918) (Table 1), known to be preyed upon by these fish species, were
149 used as primary consumers for the isotopic baselines. They were chosen because they have a
150 known TP in the food web and they are characteristic of distinct carbon sources: large
151 *Corbicula* feed deeply in sediment (detrital carbon source) (Mouthon, 2003) and *Pisidium*
152 feed at the sediment surface (autochthonous carbon source) (Mouthon, 2008).

153 **2.3. Sample analysis**

154 Invertebrates were starved in the lab for 24 h to empty their digestive tract of residues
155 that could distort the PCB and isotopic analyses. Fish fillets and invertebrates were weighed,
156 frozen at -20°C , freeze-dried, weighed again, and finely ground.

157 Lipid content (%) and concentrations of the seven iPCB congeners (ng/g wet weight)
158 were measured by the CARSO-LSEHL group (Lyon, France), according to USEPA standard
159 1668. Uncertainty of concentrations was evaluated at 20%.

160 Stable isotope analyses (carbon and nitrogen, expressed as $\delta^{13}C$ and $\delta^{15}N$) were
161 performed using the IsoPrime spectrometer (*MicroMass*, Service Central d'Analyse, Solaize,
162 France) coupled to a EuroEA 3024 analyzer. The uncertainty was 0.3%. For invertebrate
163 baselines, measurements were taken in triplicate.

164 The protocol for stomach content analysis and the fauna inventory is explained in the
165 SI.

166 **2.4. Stable isotope data analysis**

167 **2.4.1. Stable isotope mixing models**

168 We used the SIAR package (Parnell et al., 2010), which incorporates temporal and
169 spatial variability in $\delta^{13}C$ and $\delta^{15}N$ in the stable isotope mixing model (SI, Eq. C1). Using a
170 Bayesian approach, SIAR determines the probability distribution of each respective source
171 contribution to the isotopic profiles of a consumer. For further detail, see Parnell et al.
172 (Parnell et al., 2010). From the two invertebrate baselines (*Pisidium* being used as a baseline
173 for autochthonous carbon and *Corbicula* for detrital carbon) (Mouthon, 2003, 2008), SIAR
174 was applied to each fish species and each site to determine the probability distribution of the
175 contribution of detrital carbon (*dC*) versus autochthonous carbon in supporting the food web.
176 At the individual level, SIAR could not be applied. Nevertheless, a simple stable isotope
177 mixing model with two stable isotopes and two carbon sources can be solved analytically (SI,
178 Eq. C2) in order to determine, for each individual, the percent contribution of detrital carbon
179 (*dC*) versus that of autochthonous carbon in supporting an individual fish's secondary
180 production.

181 **2.4.2. Trophic position estimation**

182 Post's equation (Post, 2002) with two baselines was used to estimate fish TP (SI, Eq.
183 C3). To take into account $\delta^{15}N$ and $\delta^{13}C$ data variability and parameter uncertainties, we used
184 Bayesian inference (prior information, software, number of iterations, etc. are presented in the
185 SI).

186 To estimate the individual TP, a bootstrapping method was used by drawing 10,000
187 parameter sets from the joint posterior distribution obtained for each species from each site.
188 The samples of the three species were pooled at each site and the mean value of each
189 parameter was used to report TP in the dual graph.

190 **2.5. Statistical tests and predictive models**

191 All statistical tests and models developed were performed using the R statistical
192 computing program at a confidence level of α equal to 0.05. The normality of each variable
193 was tested using a Shapiro test. Standard statistical tests were performed to analyze the data.

194 A log-linear regression model was developed to explain fish PCB concentration ($\sum_{i=1}^7 PCB$,
195 shortened PCB_{fish}) on the basis of all available explanatory variables: size, TP, $\delta^{13}C$ or the
196 percentage of detrital carbon exploited (dC), lipid content (LC), sex and site. The site effect
197 was represented by the maximum PCB concentration in the sediment to which fish were
198 exposed during their lifetime ($\sum_{i=1}^7 PCB$ in sediment, abbreviated to PCB_{sed}), estimated by
199 correlating fish age with sediment dating at each site. Beforehand, the correlation between
200 explanatory variables was tested with a Spearman rank test. Backward stepwise regression
201 was then carried out.

202 In the same way, a generalized linear model was developed to explain the probability
203 p that the PCB content of fish tissue exceeds the health-based benchmark using the same
204 explanatory variables.

205 **3. Results**

206 **3.1. Contamination data, biometric analysis, stomach content analysis** 207 **and fauna inventory**

208 In accordance with the study's objective, the results of sediment contamination are
209 only summarized here (see SI for further detail). The three sites appeared to be contaminated
210 differently, the site upstream from Lyon being far less contaminated than the other two
211 (confirming its suitability as a relative reference). During the past 10 years, the maximal PCB
212 concentration measured in the sediment has been 6.26, 69.7 and 55.1 ng/g dry weight at MTE,
213 GDL and BRE, respectively (historical trends presented in SI, Fig. A1).

214 Fish PCB contamination differed among species (ANOVA, $p < 0.0001$) and among
215 sites (ANOVA, $p < 0.0001$). The site upstream from Lyon was less contaminated than the two
216 downstream sites and chub was consistently the least contaminated fish species (SI, Fig. D1).
217 Fish contamination increased from upstream to downstream. This increase was expressed
218 both as mean PCB concentrations and also as the number of individuals exceeding the health-
219 risk-based threshold (SI, Fig. D1).

220 No correlation between PCB concentrations and sex, size, weight, lipid content, $\delta^{13}C$
221 and $\delta^{15}N$ was observed for any species at any site. The lack of a correlation between lipid and
222 PCB concentrations in fish indicates nonequilibrium conditions between individuals and their
223 environment. As a consequence, we did not lipid-normalize PCB concentrations in fish.

224 Stomach content analysis, expressed as prey occurrences (SI, Fig. B1) and mean diets
225 (SI, Fig. B2), showed differences among sites (suggesting a spatial heterogeneity concerning
226 prey availability) and between species (indicating specific preferences). The chub and barbel
227 diet spectra were broader than the bream spectrum, consistent with their opportunistic
228 behavior. Nevertheless, low individual variability was observed from stomach contents within
229 each species.

230 Finally, the inventory of the invertebrate fauna at MTE and GDL showed greater
231 diversity at GDL than at MTE (SI, Fig. B3), suggesting a different prey availability between
232 sites.

233 **3.2. Stable isotope data analysis**

234 **3.2.1. Stable isotope mixing models**

235 Using two end members, each representing a feeding habitat, the results of SIAR
236 showed only two patterns among the nine data sets tested (Fig. 1). The first pattern (Fig. 1A),
237 seen for bream and barbel at MTE and BRE, demonstrated that the two carbon sources were
238 exploited equally by the fish. The second pattern (Fig. 1B), seen for chub at all three sites and
239 bream and barbel at GDL, showed that autochthonous carbon sources were preferred to
240 detrital carbon sources.

241 The application of these mixing models at the individual level showed that the PCB
242 concentration in fish tissue increased with the proportion of carbon from a detrital source (SI,
243 Fig. C1), especially at GDL.

244 **3.2.2. TP estimation**

245 Bayesian inference was performed on $\delta^{15}N$ and $\delta^{13}C$ data for each species at each site.
246 Thin posterior distributions for all parameters were obtained (SI, Fig. C2), meaning that each

247 data set was sufficiently informative to obtain a good estimation of each parameter. For all
248 species, the most plausible value of the enrichment in $\delta^{15}N$ per trophic level, ΔN , was around
249 10% higher at all sites than the mean of 3.4‰ currently used (Post, 2002), except for the
250 bream at BRE. The inferred TP showed similar values between species and between sites.
251 Chub had the lowest mean TP at MTE, barbell had the lowest mean TP at GDL, and bream
252 had the lowest mean TP at BRE. The empirical joint posterior distribution showed that TP
253 was negatively correlated with ΔN , as expected (SI, Fig. C3).

254 The results of SIA (SI, Fig. C4 and C5) and their relation to PCB concentrations (SI,
255 Fig. C1 and C6), are summarized in Fig. 2, illustrating differences not only in trophic levels
256 (along the y-axis), but also in detrital carbon source exploitation (along the x-axis). Overall, it
257 appeared that (i) the exploitation of detrital carbon increased with a decrease in TP; (ii) the
258 difference in TP between species is not highly relevant, while the individual variability in TP
259 could be strong at the same site (up to one trophic level), as in the exploitation of detrital
260 carbon; and (iii) a contamination gradient was particularly marked at GDL according to the x-
261 axis, the most contaminated fish being those exploiting the detrital carbon sources the most
262 (SI, Fig. C1). On the other hand, no contamination gradient was observed according to TP (SI,
263 Fig. C6).

264 **3.3. Predictive statistical models**

265 There was no significant difference in fish age between sites and the mean age was
266 estimated at 7.3 years. The maximum PCB concentrations in the sediment to which fish were
267 exposed during their life were 6.26, 69.7 and 55.1 ng/g dry weight at MTE, GDL and BRE,
268 respectively (SI, Fig. A1). No correlation was found between all the explanatory variables.

269 The backward stepwise log-linear regressions carried out on PCB concentration levels,
270 for all fish species and sites combined, showed no significant effect of sex, lipid content, or

271 TP. The best model was obtained using only three significant explanatory variables — fish
272 size, percentage of detrital carbon exploited and maximal PCB concentration in the sediment
273 — which together explained 78% of the total variability:

$$\log_{10}(PCB_{fish}) = -0.569(\pm 0.167) + 0.036(\pm 0.003) * size + 0.779(\pm 0.188) * dC + 0.591(\pm 0.065) * \log_{10}(PCB_{sed}) \quad (1)$$

275 Figure 3 presents the observed versus predicted contamination data obtained from Eq.
276 1. This representation is useful to evaluate the calibration of the predictive model to the data
277 set and its utility from a risk assessment perspective, even though an independent data set is
278 needed to validate the model's predictive capability. Only a few individuals fall within the
279 quadrant representing an overestimation of the PCB contamination risk (the predicted value
280 exceeds the measured value and exceeds the health-based benchmark). Similarly, only a few
281 samples fall within the quadrant representing an underestimation of risk, i.e., the predicted
282 concentrations are less than the health-based benchmark and the measured concentrations
283 exceed it. The latter case is of greater concern from a risk assessment perspective.
284 Nevertheless, for all species and all sites, only a few points are concerned (3/113), and for two
285 of the three, the measurement uncertainty interval (PCB concentration uncertainty estimated
286 at 20%) overlaps the regulatory threshold.
287 The best generalized linear model obtained is presented in the SI.

288 **4. Discussion**

289 Many studies have highlighted the importance of chemical and biological factors in
290 PCB accumulation by aquatic biota (Borgå et al., 2004). The factors leading to differential
291 accumulation among species have become a major focus of ecotoxicology and environmental
292 chemistry studies in the past few years (Borgå et al., 2004; Guildford et al., 2008; Walters et
293 al., 2008; Gewurtz et al., 2009). There are a number of interacting factors determining within-
294 and between-species variations in PCB concentrations and the importance of these factors

295 varies both temporally and spatially. Many studies have shown that size, sex, TP and lipid
296 content are important predictors of PCB concentrations in aquatic organisms (Kidd et al.,
297 1998; Burreau et al., 2004; Missildine et al., 2005).

298 The use of stable isotope mixing models to describe diet behaviors showed that chub is
299 the only species to predominantly feed on autochthonous carbon sources at the three sites,
300 while bream and barbel showed a similar behavior only at GDL where invertebrate fauna is
301 plentiful and widely available (as observed with the fauna inventory). Combined with
302 observations from gut contents, these results are consistent with the opportunistic behavior of
303 the chub. Moreover, we identified PCB contamination pathways that could explain the
304 individual and between-species variability in PCB contamination levels. High within-species
305 variability was observed for TP and habitat exploitation and PCB contamination levels,
306 whereas gut contents were relatively similar. One reason for these observations might be that
307 only adults, whose diet is assumed to be fixed, were sampled. The variability observed for
308 isotope data and PCB concentrations could stem from different foraging habitats and/or
309 individual life history. Moreover, we observed that sediments are historically more
310 contaminated at GDL than at BRE, while fishes are more contaminated at BRE than at GDL
311 and they specifically exploit more detrital carbon sources at BRE than at GDL. Furthermore,
312 we showed that PCB concentration increases with the exploitation of detrital carbon sources,
313 confirming the results obtained by Berglund et al. (Berglund et al., 2005), who showed that
314 individuals associated with the detrital pathway have higher PCB concentrations than those
315 associated with the algae pathway. All these results confirmed that deposited sediment plays a
316 central role in food-web contamination (Gewurtz et al., 2009), not only its contamination
317 level, but also the ultimate carbon sources supporting the food web. The important factor in
318 fish PCB contamination in the study sites examined was therefore not only what fish
319 consume, but also and particularly the degree of contamination of the food consumed,

320 suggesting that spatial gradients of contamination were more important than the type of food
321 consumed and its trophic status.

322 The present study shows that combining isotopic and contaminant determination
323 provides an efficient tool to assess the trophic transfer of pollutants such as PCBs within food
324 webs and to analyze bioaccumulation processes. Nevertheless, this combined use implies that
325 they have a similar rate of turnover in fish tissues. As mentioned by Perga and Gerdeaux
326 (Perga and Gerdeaux, 2005), large consumers such as fish have tissue turnover rates ranging
327 from months to years and their isotopic signature therefore represents their diet over a long
328 period of time. The turnover of PCBs in tissues is different for each congener, given their
329 different transformation rates. The congener profiles obtained here were always similar, with
330 a predominance of congener 153, known to accumulate at the highest concentrations
331 (Paterson et al., 2007). As a consequence, it is reasonable to think that for the temporal scale
332 considered here, the values of stable isotopes and PCB concentrations used here can be
333 compared.

334 Lau et al. (Lau et al., 2009) showed that the isotopic signature of consumers and their
335 foods varied with the season and shade conditions in streams, thus affecting the food-web
336 baselines. Nevertheless, employing isotopic values for a single taxon as the baseline seems
337 preferable to using the mean value of primary consumers, because it reduces the high degree
338 of isotopic variability among taxa (Lau et al., 2009). The problem of seasonal and individual
339 variability is considered in the Bayesian framework here by defining uncertainty around
340 baseline isotope values, nitrogen enrichment per trophic level (commonly set at $\Delta N=3.4\%$)
341 and TP, and by defining the variability of isotopic values. This new approach demonstrated
342 that: (i) the variability of the $\delta^{15}N$ values is higher than that of the $\delta^{13}C$ values, (ii) the chub
343 has the highest $\delta^{15}N$ variability and the lowest TP, and (iii) ΔN was estimated to be higher

344 than 3.4%. As a consequence, neglecting such sources of variability and uncertainties would
345 result in an overall estimation of fish TP for the data studied.

346 Using all available explanatory variables available, we developed a predictive
347 statistical model to explain PCB levels in freshwater river fishes and the probability of those
348 levels exceeding the health-based benchmark. Fish body length, maximum concentration of
349 PCBs in the sediment to which fish were exposed during their lifetime, and fish foraging
350 behavior (and the associated diet) had significant effects, whereas sex, lipid content and TP
351 were not significant predictors for these three fish species that have overlapping TPs and
352 different ultimate carbon sources. It is clear that there are many interacting factors
353 determining within-species variation in PCB concentrations and that the importance of each
354 factor probably varies both temporally and spatially. Nevertheless, the role of sediment
355 contamination in controlling fish contamination has been underlined and most mechanistic
356 food-web models explicitly consider this compartment as a major exposure route (Morrison et
357 al., 1997; Gewurtz et al., 2009). The variability in TP estimated from the present data set is
358 too low to observe a significant relation between TP and PCB concentrations, probably
359 because no piscivorous fish species were considered. The results obtained here are limited to
360 the species considered and cannot be extrapolated to species at a higher trophic level. As a
361 consequence, because of the small differences in TP between the three species studied here
362 and the substantial differences in their feeding areas, the effect of biomagnification processes
363 cannot be entirely excluded. Nevertheless, a correlation between TP and PCB concentrations
364 has rarely been observed between individuals of the same species (Rasmussen et al., 1990).
365 Until now, the fact that uncertainty was neglected in the deterministic estimation of TP could
366 explain why no correlation was found. The Bayesian approach proposed here provides a more
367 robust estimation and thus contributes support to TP's small effect on PCB levels.

368 The log-linear model developed here to predict PCB concentration in fish (Eq. 1) can
369 be used in a risk assessment perspective for fish consumption in the sense that there was an
370 underestimation of risk in only 3% of cases. Nevertheless, it is clear that the amount of
371 detrital carbon in the fish diet is not a convenient variable for environmental managers and
372 that an independent data set is needed to validate the model's predictive capability.

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384 comments.

385 **References**

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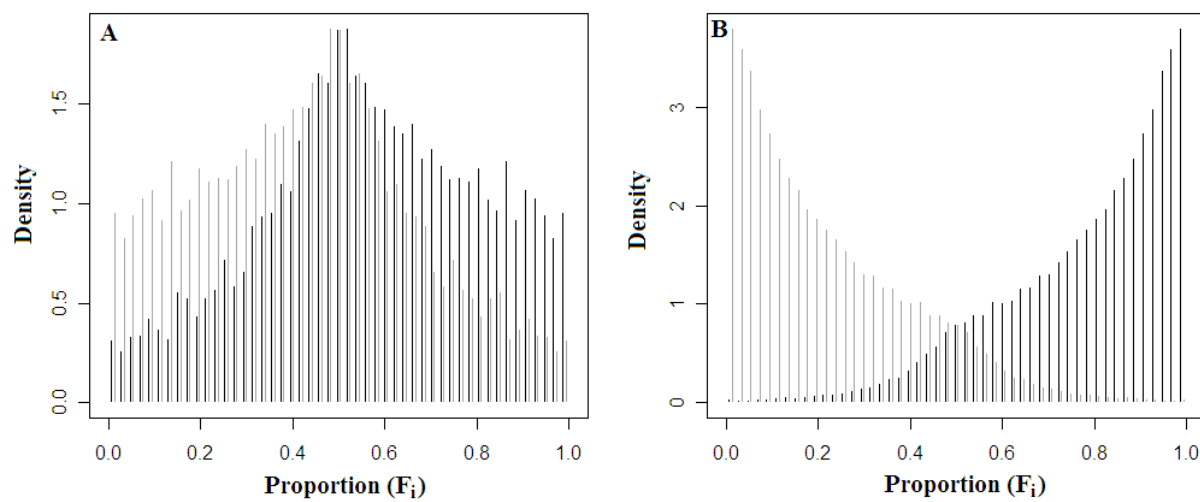
505 **Figure legends**

506 Fig. 1: Proportion distribution of autochthonous carbon (in black) and detrital carbon (in grey)
507 in fish isotopic profiles determined by stable isotope mixing models. (A) Profile obtained for
508 bream and barbel at the MTE and BRE sites. (B) Profile obtained for chub at the three sites
509 and bream and barbel at site GDL.

510 Fig. 2: Fish trophic position according to the percentage of detrital carbon exploited, with
511 their level of PCB contamination. PCB concentrations are indicated relative to the health-risk
512 benchmark (equal to $\sum_{i=1}^7 \text{PCB} \approx 153 \text{ ng/g ww}$, (Babut et al., 2009)) and three times this
513 threshold.

514 Fig. 3: Relationship between predicted (Eq. 1) and observed PCB concentrations (in a \log_{10}
515 basis) in breams (\square), barbels (\circ) and chubs (Δ) at MTE (white), GDL (grey) and BRE (black).
516 Dotted lines correspond to the health-risk benchmark of 153 ng/g wet weight for the sum of
517 the seven indicator PCBs.

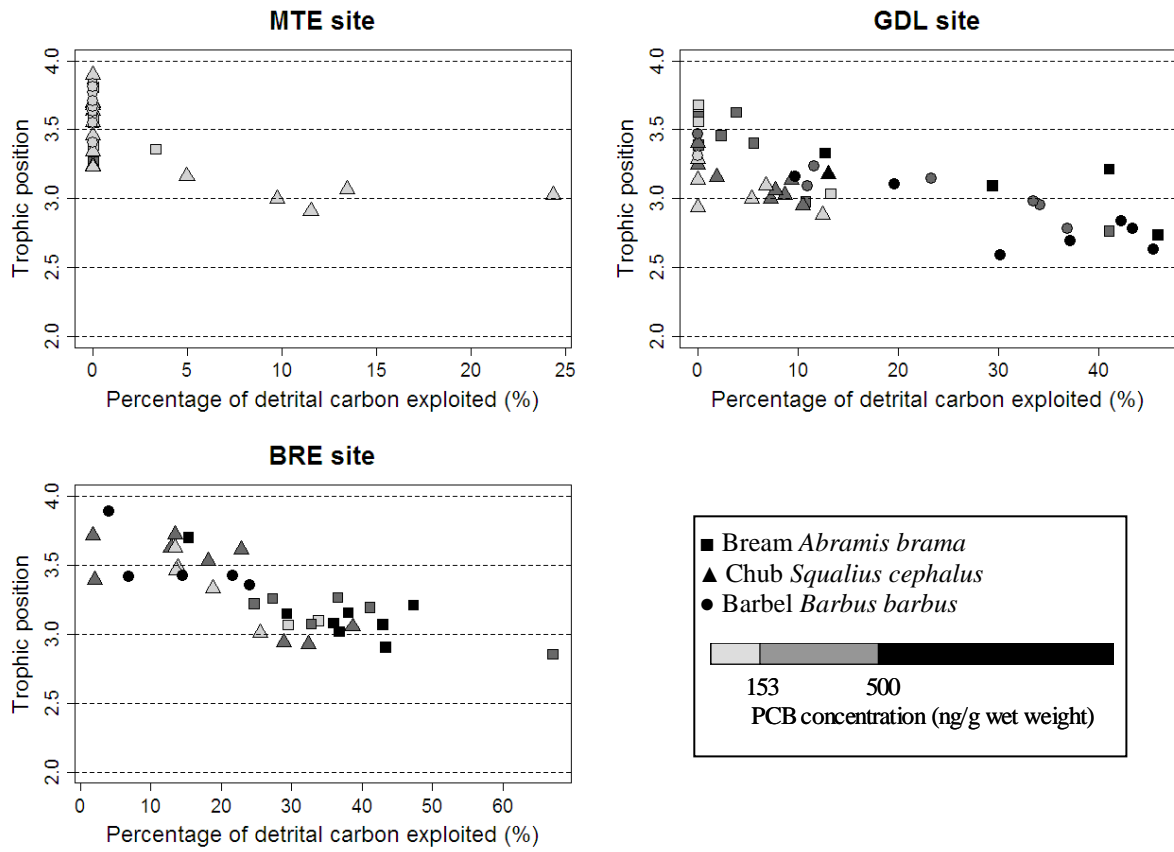
518 **Figures**



519

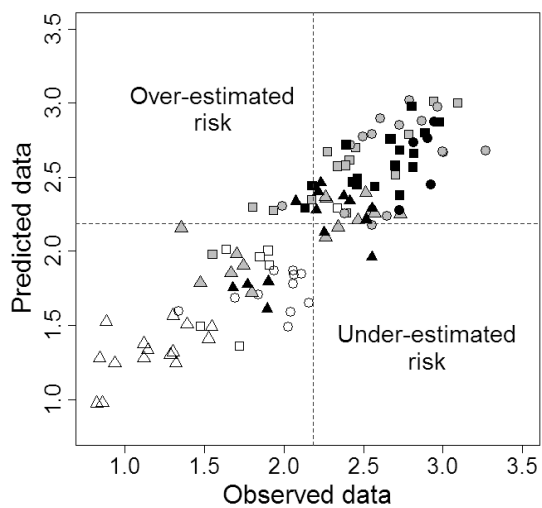
520

Fig. 1



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522

Fig. 2



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Fig. 3

525 **Tables**

526 Table 1: Number of fishes and invertebrates collected on each site between August 2008 and
 527 January 2009 (N), with the distinction between females (♀) and males (♂) for fishes; mean
 528 size (\pm standard deviation); mean weight (\pm standard deviation); mean age (\pm standard
 529 deviation) and mean lipid content (\pm standard deviation).

	N	Size (cm)	Weight (g)	Age (year)	Lipid (%)
Abramis brama					
La Morte	7 (3♀ + 4♂)	53.11 \pm 8.18	1636 \pm 775	6.43 \pm 1.51	9.24 \pm 4.76
Grand-Large	15 (9♀ + 6♂)	54.14 \pm 5.78	2025 \pm 695	8.67 \pm 3.96	22.85 \pm 13.93
Ile du Beurre	17 (10♀ + 7♂)	52.45 \pm 4.69	2009 \pm 607	5.94 \pm 2.34	28.34 \pm 11.22
Squalius cephalus					
La Morte	20 (13♀ + 7♂)	40.02 \pm 8.76	861 \pm 500	6.3 \pm 1.78	3.2 \pm 1.15
Grand-Large	15 (6♀ + 9♂)	44.0 \pm 5.80	1075 \pm 457	5.27 \pm 1.98	7.77 \pm 3.89
Ile du Beurre	17 (12♀ + 5♂)	42.58 \pm 7.67	1061 \pm 533	5.82 \pm 2.40	7.62 \pm 3.91
Barbus barbus					
La Morte	11 (11♀ + 0♂)	52.1 \pm 2.92	1215 \pm 117	10 \pm 1	5.85 \pm 2.94
Grand-Large	15 (8♀ + 7♂)	54.7 \pm 6.36	1710 \pm 767	9.4 \pm 2.32	13.62 \pm 6.14
Ile du Beurre	5 (3♀ + 2♂)	56.6 \pm 5.45	1853 \pm 575	9.8 \pm 3.56	17.65 \pm 4.14
Invertebrates					
	<i>Pisidium</i>	<i>Corbicula</i>			
La Morte	140	15			
Grand-Large	160	13			
Ile du Beurre	130	60			

530

531 *Supporting Information*

532

533 Lopes C. ^{a*}, Perga M.-E. ^b, Peretti A. ^a, Roger M.-C. ^a, Persat H. ^c, Babut M. ^a

534

535 ^a Cemagref, UR MALY, 3 bis Quai Chauveau – CP220, F-69336 LYON, France;

536 ^b INRA UMR CARTELE 75 avenue de Corzent, F-74203 Thonon les Bains, France

537 ^c UMR 5023 – Laboratoire d’Ecologie des Hydrosystèmes Fluviaux (LEHF), Université

538 Claude Bernard Lyon 1, 69221 Villeurbanne, France.

539

540 The supporting Information is divided in five Appendices :

541 • Appendix A : Age sediment dating and contamination

542 • Appendix B : Gut Content Analysis

543 • Appendix C : Stable isotope analysis

544 • Appendix D : Fish contamination

545 • Appendix E : Statistical predictive models

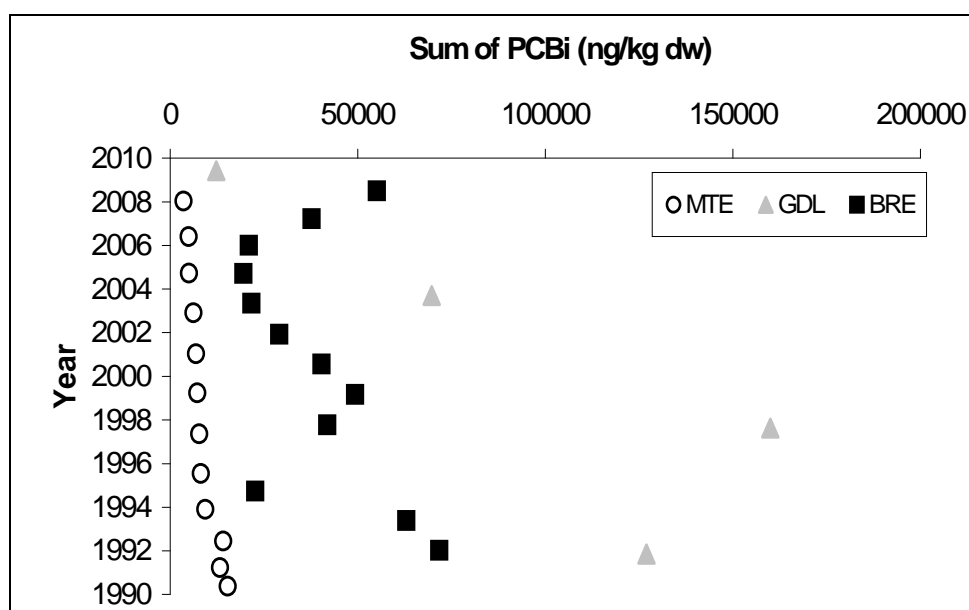
546 **Appendix A: Age Sediment age dating and contamination**

547 The results presented here are the subject of a paper in preparation. Only those
548 relevant to this study are summarised here.

549 Sediment cores were collected at each site with a gravity corer fitted with a 1.2-m
550 liner. Radionuclide (^{210}Pb , ^{137}Cs) measurement was used to age the successive layers in each
551 core. PCB analysis was performed using high-resolution mass spectrometry (HRGC-HRMS)
552 by a contract laboratory (EUROFINS in Orléans, France).

553 Measurements of ^{210}Pb and ^{137}Cs and correlation with documented hydrosedimentary
554 events were used to estimate a mass accumulation rate (MAR; $\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1}$) for each interval in
555 each core. At MTE, the estimated sediment MAR is 1.25 cm per year. At GDL the MAR is
556 estimated at 1.73 cm per year. At BRE, the MAR is estimated at 2.5 cm per year.

557 From this age sediment dating, PCB levels measured in the cores are reported in the
558 respective time scale. The concentrations observed during these last 20 years in each site are
559 presented in Fig. A.1.



560

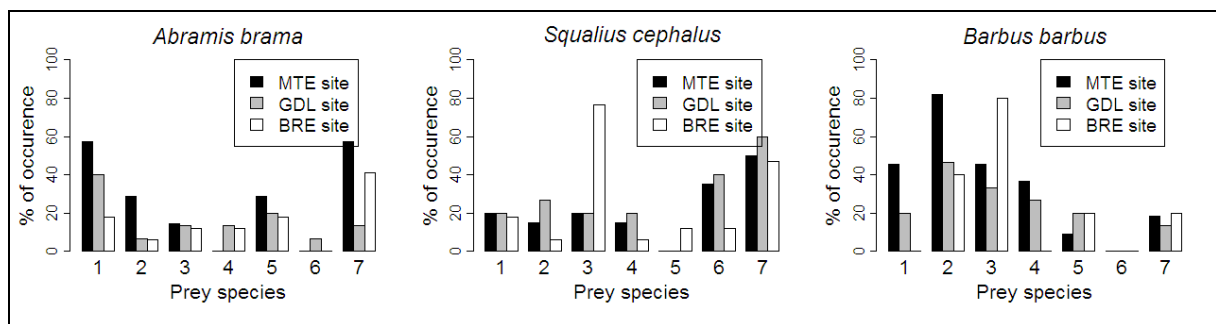
561 Fig. A1: Historical PCB contamination of sediment in the three study sites.

562 Appendix B: Gut Content Analysis and fauna inventory

563 Stomach content analysis

564 Stomach contents were preserved in 5% formalin before analysis. Prey species were
565 identified and numbered. Fish tissues for PCB analysis were sampled according to the
566 European regulation No. 1883/2006 of the Commission³. All species found in the stomach
567 were listed and grouped in seven groups according to their family: (1) dipterans, (2) other
568 aquatic insects, (3) crustaceans, (4) gastropods, (5) bivalves, (6) macrophytes and (7) others.

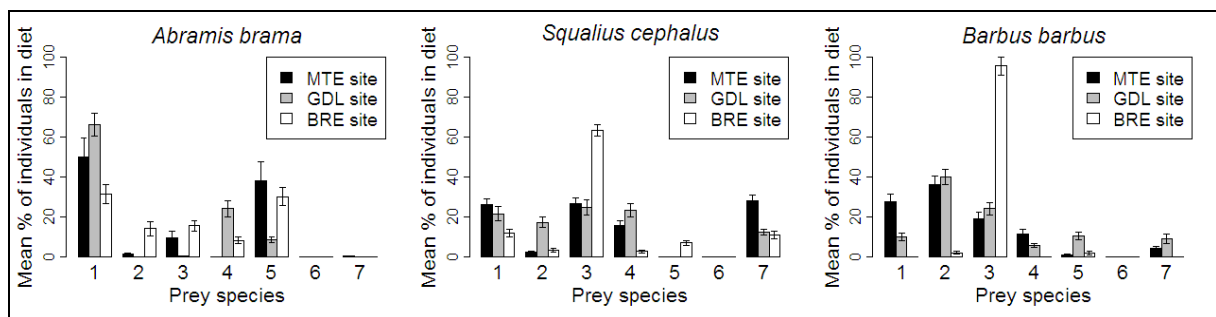
569 Prey occurrence (expressed as percentage of fish that ate each prey category) were
570 calculated for each fish species for each site, and individual variability and average diet (in
571 number of individuals) were determined. The results for prey occurrence and mean percentage
572 of individuals of each prey class in fish diet are presented in Fig. B1 and B2 respectively.



573

574

Fig. B1 : Occurrence of each prey category for each fish species at each site.



575

576

Fig. B2 : Mean percentage of individuals in the diet of each fish species at each site.

³ E.C. (2006). Commission Regulation (EC) no. 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs, Official Journal of the European Union. 1883/2006: L 364/32 - 364/43

577 **Fauna inventory**

578 A fauna inventory was made by catching invertebrates with artificial substrates.

579 Taxonomic determination, abundance and weight were determined in the laboratory. The

580 fauna present in artificial substrates was listed and grouped in 6 groups, the same as the ones

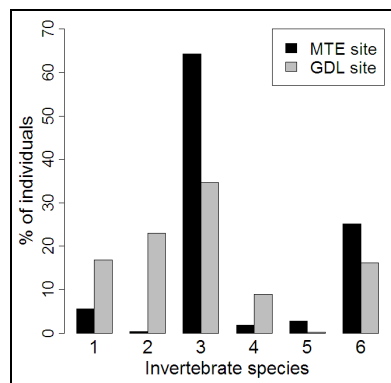
581 mentioned above for gut contents except that macrophytes are grouped with the last group.

582 A problem occurred with the substrat at BRE (site downstream Lyon), so as no result

583 for this site is available. The inventory of the invertebrate fauna at MTE (upstream Lyon) and

584 GDL (site closed to Lyon) shows a higher diversity at GDL than at MTE (Figure B3),

585 suggesting a different prey availability between sites.



586

587 **Figure B3: Distribution of invertebrates at MTE and GDL. (1) Dipterans; (2) Other aquatic**

588 **insects; (3) Crustaceans; (4) Gastropoda; (5) Bivalves; (6) Others.**

589 Appendix C: Stable isotope analysis

590 Stable isotope mixing models

591 The stable isotope mixing model used in SIAR is expressed as follows:

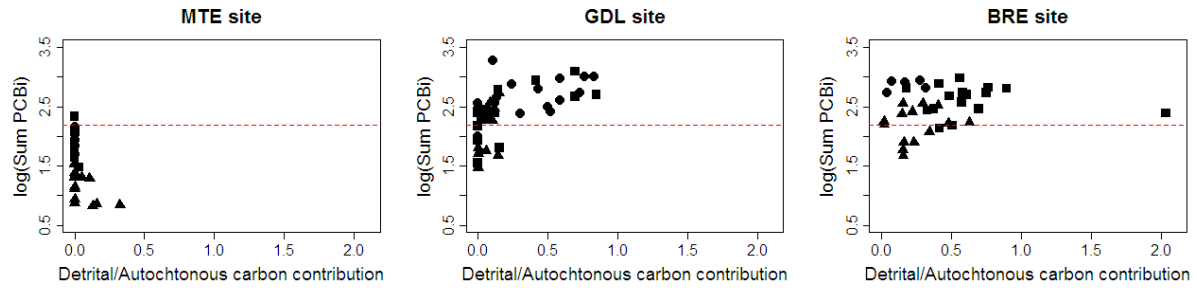
$$592 \quad X_{c,j} = \sum_i F_i (X_{i,j} + C_{i,j}) + \varepsilon_{c,j} \quad (\text{Eq. C1})$$

593 where $X_{c,j}$ is the observed isotope value j of the fish species c , $X_{i,j}$ is the source value i on
 594 isotope j (normally distributed), F_i is the contribution of the source i (estimated by the model),
 595 $C_{i,j}$ is the trophic enrichment factor for isotope j on source i (normally distributed) and $\varepsilon_{c,j}$ is
 596 the residual error, describing additional inter-observation variance not described by the model
 597 (distributed according to a centered normal distribution for which the standard deviation is
 598 estimated by the model).

599 At the individual level, a simple stable isotope mixing model with two stable isotopes and two
 600 carbon sources can be analytically solved :

$$601 \quad \begin{cases} \delta^{13}C_p = \sum_{i=1}^2 F_i * \delta^{13}C_i \\ \delta^{15}N_p = \sum_{i=1}^2 F_i * \delta^{15}N_i \\ \sum_{i=1}^2 F_i = 1 \end{cases} \Leftrightarrow \begin{cases} F_1 = \frac{\delta^{13}C_p + \delta^{15}N_p - \delta^{13}C_2 - \delta^{15}N_2}{\delta^{13}C_1 + \delta^{15}N_1 - \delta^{13}C_2 - \delta^{15}N_2} \\ F_2 = \frac{\delta^{13}C_1 + \delta^{15}N_1 - \delta^{13}C_p - \delta^{15}N_p}{\delta^{13}C_1 + \delta^{15}N_1 - \delta^{13}C_2 - \delta^{15}N_2} \end{cases} \quad (\text{Eq. C2})$$

602 The relation between individual PCB contamination of individuals and the ratio between
 603 detrital and autochthonous carbon contributions calculated above are presented Fig. C1:



604

605 Fig. C1: Relation between individual PCB contamination of individuals and the ratio between
 606 detrital and autochthonous carbon contributions in fish isotopic profiles obtained with stable
 607 isotope mixing models applied to each individual (Eq. C2) for bream (■), barbel (●) and chub
 608 (▲). The dotted line represents the health-risk benchmark.

609 **TP estimation**

610 Post's equation

611 Post's equation with two baselines was used to estimate fish TP:

612
$$TP = \lambda + \frac{\delta^{15}N_p - (\alpha \delta^{15}N_{base1} + (1-\alpha) \delta^{15}N_{base2})}{\Delta N} \quad \text{with } \alpha = \frac{\delta^{13}C_p - \delta^{13}C_{base2}}{\delta^{13}C_{base1} - \delta^{13}C_{base2}} \quad (\text{Eq. C3})$$

613 where λ is the TP of the baselines used (primary consumers, $\lambda = 2$); $\delta^{15}N_p$, $\delta^{13}C_p$, $\delta^{15}N_{base1}$,
 614 $\delta^{13}C_{base1}$, $\delta^{15}N_{base2}$ and $\delta^{13}C_{base2}$ are the values of $\delta^{15}N$ and $\delta^{13}C$ measured in the consumers
 615 and in the two baselines, respectively; α is the proportion of nitrogen in the consumer
 616 ultimately derived from the base of the first food web and ΔN is the enrichment in $\delta^{15}N$ per
 617 trophic level (estimated at a mean 3.4‰) (Post, 2002).

618 Bayesian Inference procedure

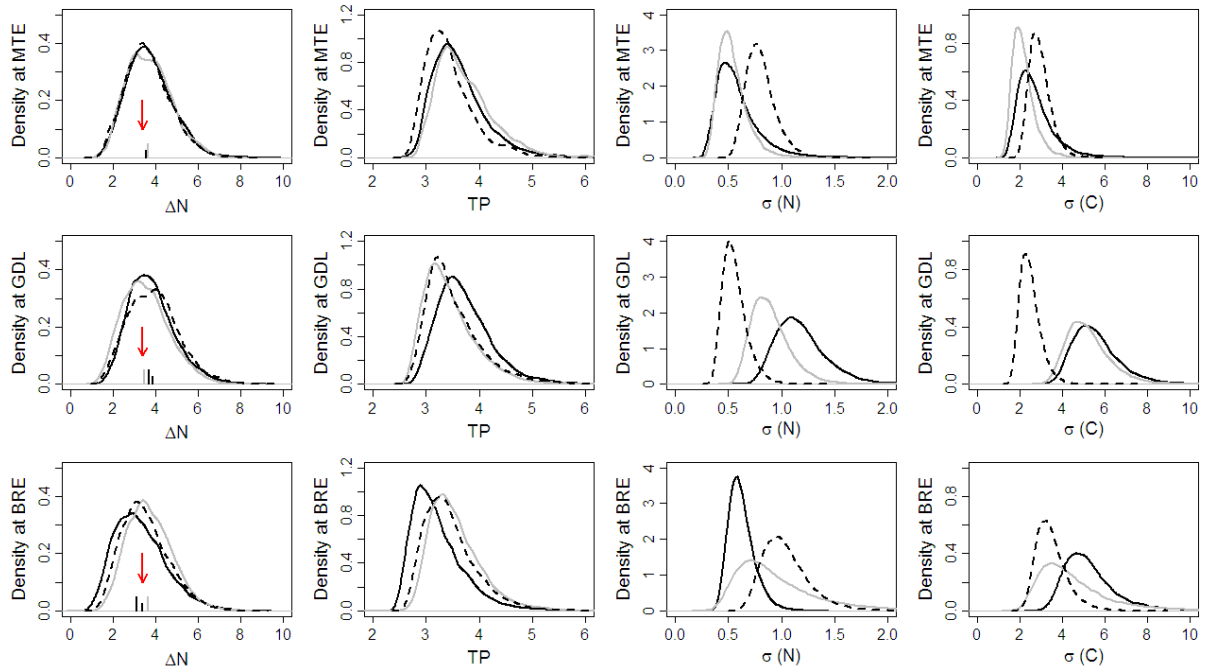
619 Based on prior distributions assigned to variables and parameters, multiplied by the
 620 likelihood of the data, the bayesian approach yields not only the marginal posterior
 621 distribution of each parameter, but also an unnormalised joint posterior distribution of the

622 parameters conditional upon the data (allowing potential structural correlations between the
623 parameters to be controlled).

624 Data variability was incorporated by considering that $\delta^{15}N$ and $\delta^{13}C$ data followed
625 normal distributions with a mean equal to the mean of the observed values and a standard
626 deviation σ_N and σ_C estimated by the model. Parameter uncertainties were taken into account
627 by specifying priors: (i) the enrichment in nitrogen per trophic level, ΔN , followed a normal
628 distribution with 3.4‰ as the mean and 1.5‰ as the standard deviation (Post, 2002); (ii)
629 isotope data for the baselines followed a normal distribution; and (iii) TP followed a uniform
630 distribution between 2 and 5. The TP of the baselines was set at 2 (primary consumers).
631 Bayesian inference was applied via a Monte-Carlo Markov-Chain (MCMC), computed using
632 the WinBugs software (Lunn et al., 2000). **The implementation of the procedure needs to**
633 **define a data file per fish species and per site (with $\delta^{15}N$ and $\delta^{13}C$ values for each fish species**
634 **and each invertebrate baselines), and a general model data file specifying the parameters to**
635 **estimate and the priors.** For each data set, the inference was made on 6×10^3 iterations
636 following 10^4 adaptive iterations, on three independent MCMCs (with three different initial
637 parameter values), resulting in a total of 18×10^3 parameter sets.

638 Parameter estimates

639 The posterior distribution obtained by Bayesian inference to estimate each parameter is
640 presented in Fig. C2, for each site.



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645

Fig. C2: Posterior distributions obtained for each parameter by Bayesian inference from the bearm (—), barbel (---) and chub (- -) data sets at MTE, GDL and BRE. For ΔN , vertical lines represent medians obtained for each species and the arrow corresponds to the commonly used value of 3.4‰. $\sigma(N)$ and $\sigma(C)$ represent variability around $\delta^{15}N$ and $\delta^{13}C$ data, respectively.

646

Joint posterior distributions

647

The joint posterior distribution obtained by Bayesian inference to estimate TP is

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presented in Fig. C3, for each species at each site.

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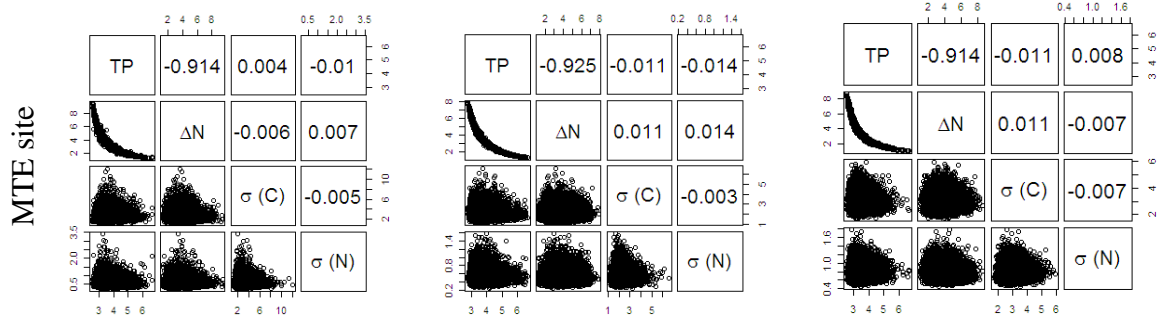
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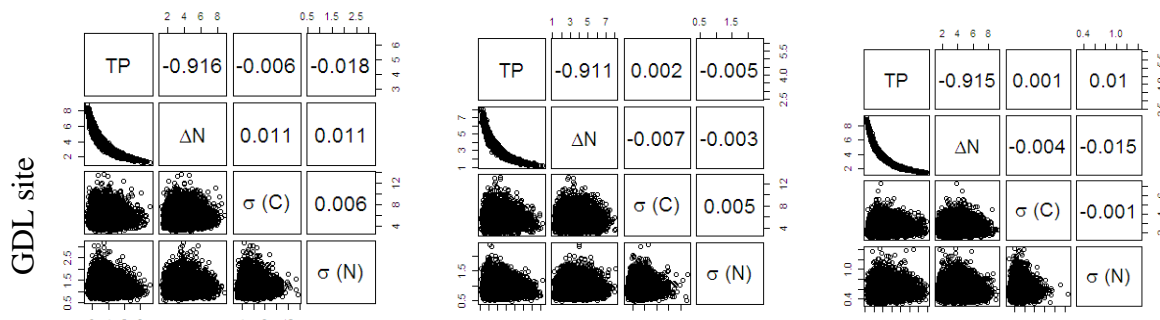
Abramis brama

Barbus barbuis

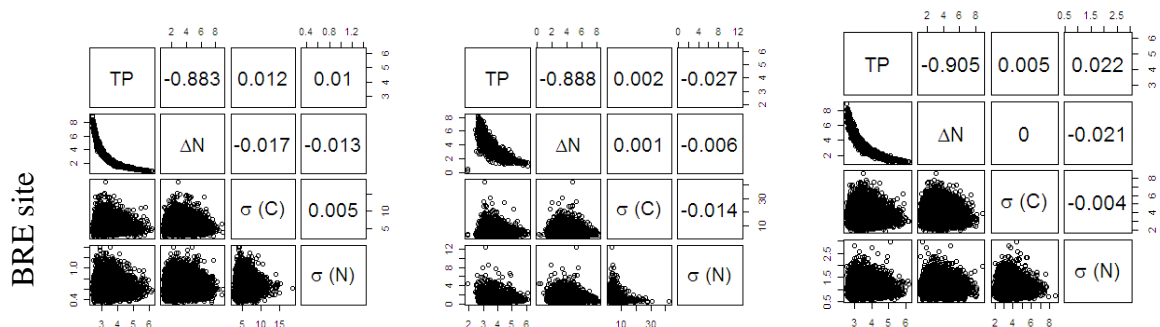
Squalius cephalus



656



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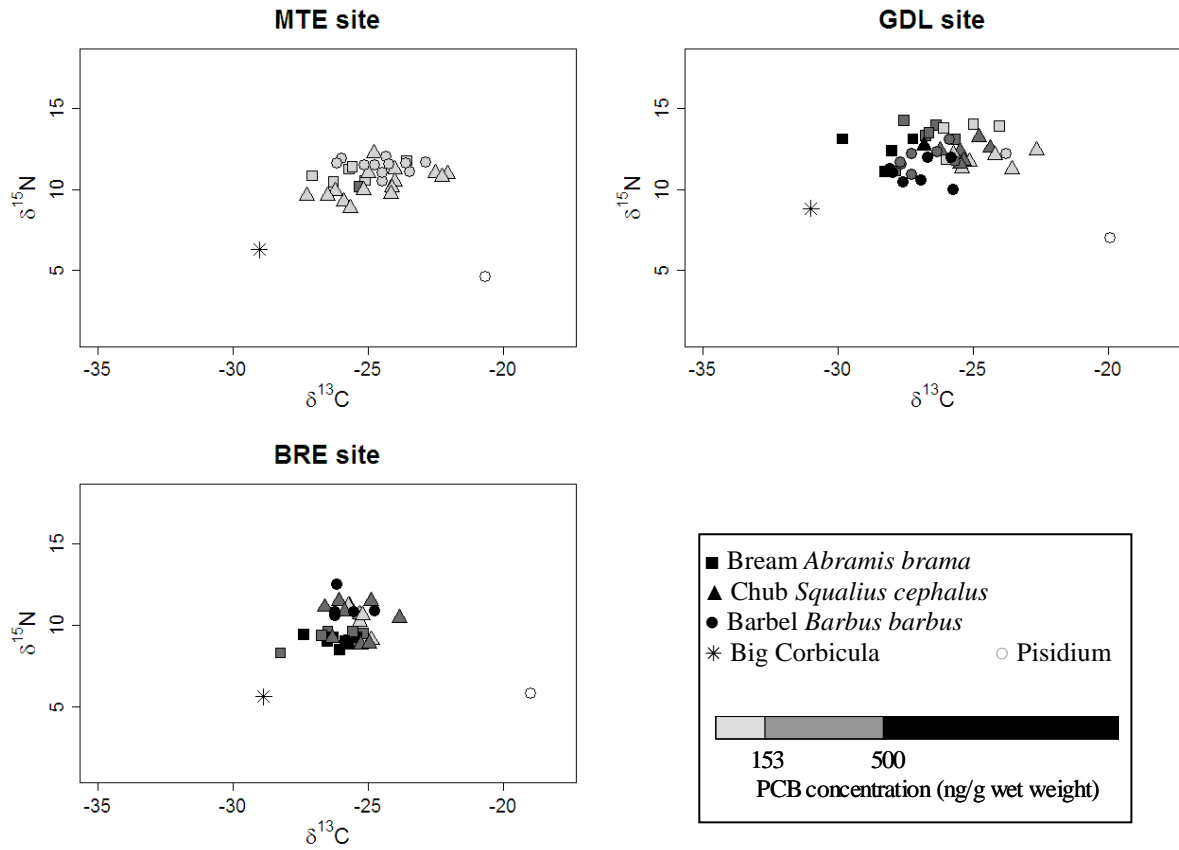


658

659 Fig. C3: joint posterior probability distribution of the four parameters obtained for each
 660 species data set at each site. The projection of MCMC chains in the plane of each pair of
 661 parameters are represented in the bottom left of each figure, correlation coefficients between
 662 each pair of parameters are represented in the top right of each figure.

663 **Dual graphs**

664 Raw isotopic data ($\delta^{15}N$ and $\delta^{13}C$) are presented in Fig. C4 for each site and its associated
 665 dual graph with TP in Fig. C5, in link with individual PCB contamination levels. The figure
 666 C6 represents the relation between PCB concentration of individuals and their TP.



667

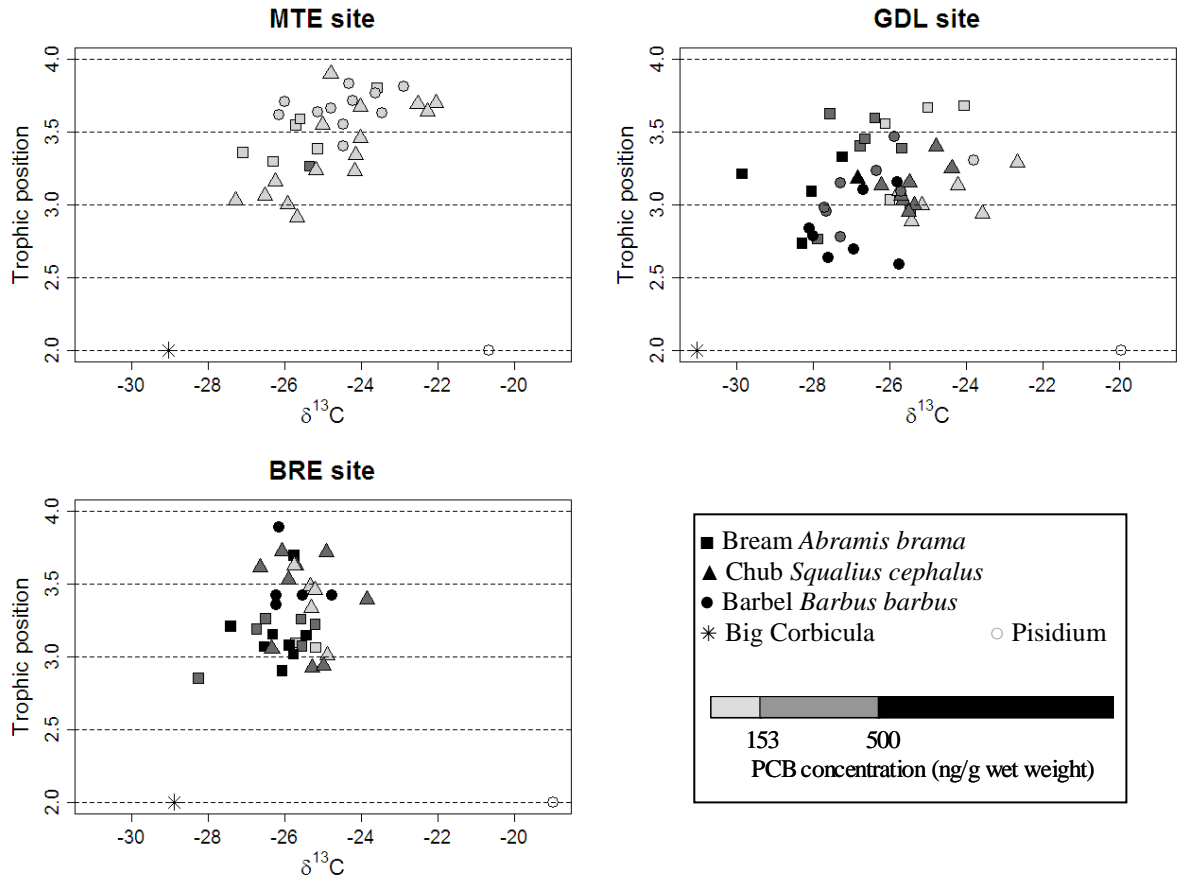
668

669

670

Fig. C4: Dual isotope plots in each site, with their level of PCB contamination. PCB concentrations are expressed in relation to the sanitary threshold (corresponding to

$$\sum_{i=1}^7 i \text{PCB} \approx 153 \text{ ng/g ww, (Babut et al., 2009)) and three times this threshold.}$$



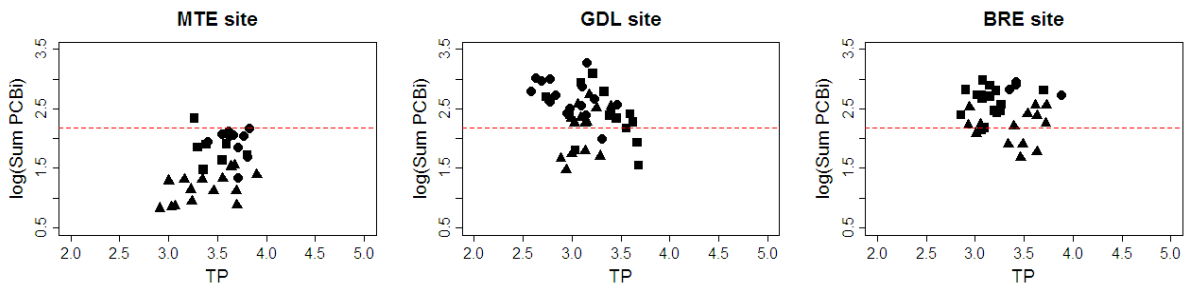
671

672 Fig. C5: TP versus isotope carbon values in each site, with their level of PCB contamination.

673 PCB concentrations are expressed in relation to the sanitary threshold (corresponding to

674
$$\sum_{i=1}^7 i \text{PCB} \approx 153 \text{ ng/g ww, (Babut et al., 2009)} \text{ and three times this threshold.}$$

675



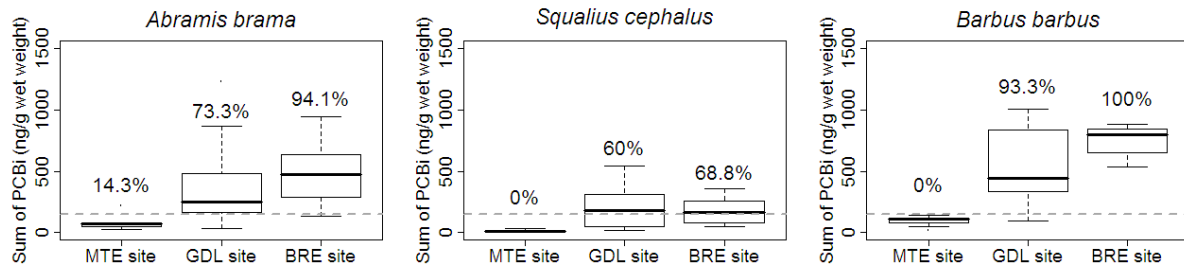
676

677 Fig. C6: Relation between individual PCB contamination of individuals and their TP for

678 bream (■), barbell (●) and chub (▲). The dotted line represents the health-risk benchmark.

679 **Appendix D: Fish contamination**

680 The results for PCB concentrations in fish tissue are presented in Fig. D1.



681
682 Fig. D1: Boxplots of PCB contamination for fish. The dotted line denotes the European
683 health-risk benchmark level of 8 pg TEQ (dioxin, furan and dioxin-like PCBs) / g of fish wet
684 weight, corresponding to $\sum_{i=1}^7 PCB_i \approx 153$ ng/g ww. The percentages correspond to the
685 proportion of individuals above this threshold.

686 **Appendix E: Statistical predictive models**

687 The best generalised linear model was obtained with the same explanatory variables as
688 before, explaining 70% of the variation in the probability of exceeding the health-risk
689 benchmark:

690 $\text{logit}(p) = -20.8(\pm 4.65) + 0.253(\pm 0.067) * \text{size} + 6.80(\pm 3.21) * dC + 5.01(\pm 1.28) * \log_{10}(PCB_{sed})$ (Eq. E1)

691