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## Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (*Anguilla anguilla*) from the Loire estuarine continuum: spatial and biological variabilities

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23  
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26 15 Abstract

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29 16 To characterize the eel contamination by dioxin-like (dl) and non dioxin-like (ndl) PCBs and  
30 17 PCDD/Fs, 62 eels from the Loire estuary (France) were analyzed. PCB contamination  
31 18 significantly increased from glass eel stage ( $3.7 \pm 1.9$  and  $15.2 \pm 4.2$  ng.g<sup>-1</sup> dw) to other life  
32 19 stages (for yellow eels:  $62.8 \pm 34.4$  and  $381.8 \pm 181.8$  ng.g<sup>-1</sup> dw; for silver eels:  $93.7 \pm 56.3$  and  
33 20  $463.2 \pm 244.6$  ng.g<sup>-1</sup> dw respectively for dl and ndl PCB). An inter-site variability based on  
34 21 PCB levels and fingerprints was observed between the three studied sites. The glass eel  
35 22 pattern was mainly characterized by the less chlorinated PCBs contrarily to the other eels,  
36 23 underlying a different bioaccumulation pathway. Overall, eels from this estuary showed an  
37 24 intermediate contamination level compared to other international/national areas. However,  
38 25 more than 60% of studied silver eels displayed WHO<sub>2005</sub> PCDD/F and dl-PCB TEQ values  
39 26 higher than the recommended level of 10 pg.g<sup>-1</sup> ww. This statement indicates a potential  
40 27 exposure to PCBs through eel consumption, especially with silver individuals, and could  
41 28 potentially lead to damages for the eel population.

## 30 1. Introduction

1  
2 31 Since the 1980s, monitoring studies in European countries have shown the decline of glass  
3  
4 32 eels arriving in the coastal waters and estuaries (ICES, 2006). The disappearance of the  
5  
6 33 prepubertal European eel (*Anguilla anguilla*) occurred as well a few decades earlier and  
7  
8 34 stocks were estimated to be divided by ten (Dekker, 2003; Moriarty and Dekker, 1997).  
9  
10 35 Several factors were brought forward to explain this decrease such as overfishing, obstacles to  
11  
12 36 migration (Robinet and Feunteun, 2002), pathogens (Palstra et al., 2007b), climate change  
13  
14 37 (Castonguay et al., 1994) and contaminants (Geeraerts et al., 2011; Palstra et al., 2007a;  
15  
16 38 Roosens et al., 2010; van Ginneken et al., 2009)

17  
18 39 Among these different causes, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-  
19  
20 40 dioxins and furans (PCDD/Fs) seem to be particularly suspected because of their potential as  
21  
22 41 estrogenic and anti-estrogenic disruptors (Canapa et al., 2002) and their neuroendocrine  
23  
24 42 effects (Kodavanti and Curras-Collazo, 2010), endangering several fish species and notably  
25  
26 43 the eel population (van Ginneken et al., 2009). PCBs represent a particularly persistent  
27  
28 44 chlorinated chemical group of 209 congeners, ubiquitous in the environment and from  
29  
30 45 anthropological origin exclusively. Two classes of PCBs were distinguished according to their  
31  
32 46 toxicological properties: the dioxin-like PCBs (dl-PCBs) which present analogous toxicity as  
33  
34 47 dioxin compounds and the non dioxin-like PCBs (ndl-PCBs) (European Union, 2011). These  
35  
36 48 classes were related to chemical structures such as the number and chlorine positions. Due to  
37  
38 49 their chemical stability, insulating and fire retardant properties, PCBs were used in the  
39  
40 50 manufacturing of electrical equipment, heat exchangers, hydraulic systems, and several other  
41  
42 51 specialized applications. In spite of the ban on their production during the eighties, the  
43  
44 52 accumulated production all over the world was estimated at 1,200,000 tons and approximately  
45  
46 53 30 % of this production is scattered in the environment, essentially in the oceanic environment  
47  
48 54 (Voltura and French, 2000). The contamination of aquatic organisms depends on the chemical  
49  
50 55 properties of each congener. The exposure level in the environment and various biotic factors  
51  
52 56 such as the metabolic capacity influence the bioaccumulation processes (Hubaux and  
53  
54 57 Perceval, 2011).

53  
54 58 Considered as a bottom dwelling fish, showing a high body lipid content, an important  
55  
56 59 longevity and a carnivorous status, the European eel is extremely exposed to lipophilic  
57  
58 60 persistent contaminants, such as PCBs, and represents a species sensitive to bioaccumulation  
59  
60 61 (Roche et al., 2000). Moreover, eels constitute an important economic value nearby estuaries

62 and rivers and a significant food resource (Perraudeau and Després, 2009). Significant levels  
63 of PCBs were detected in European eels from the Gironde and Adour estuary (France) (Tapie  
64 et al., 2011), in the Mondego estuary (Portugal) (Nunes et al., 2011), in the rivers of Italy  
65 (Mezzetta et al., 2011 ) and could be responsible for migration or reproduction impairments  
66 (van Ginneken et al., 2009). Assessing PCB contamination of the European eel is therefore of  
67 great interest since their level is threatening public health, beyond a maximal value (European  
68 Union, 2011) and is also a potential risk for its own health (for review, Geeraerts and  
69 Belpaire, 2010). The present study aims to assess PCB contamination of the European eel  
70 from the Loire estuary (France) which the basin (117,800 km<sup>2</sup>) drains a lot of tributaries.  
71 Moreover, the Loire estuary runs through important urban sites (Nantes, Saint-Nazaire) with  
72 shipping, industrial and agricultural activities. It displays a diffusive pollution including a  
73 mixture of contaminants such as heavy metals (Grobois et al., 2012), pesticides (Marchand et  
74 al., 2004), PAHs and PCBs (Hubaux and Perceval, 2011). For European eels, this estuary  
75 constitutes one of the most important continental migration path of glass eels. The  
76 preservation of its chemical quality is therefore essential for eel health. However, a real lack  
77 of data on the POPs contamination levels of European eels exists in this ecosystem. Only few  
78 individuals, sampling on the whole Loire river, have been analyzed in the French PCB  
79 framework (ONEMA, 2012). These results cannot be sufficiently representative of eels living  
80 in the estuary. In the present study, dl-PCB, ndl-PCB and PCDD/F levels were investigated in  
81 European eels fished in the Loire estuary. This work was set out to reach three objectives :  
82 i) to get a representative trend of PCB contaminants over life stages, from glass eels to silver  
83 eels; ii) to assess spatial PCB contamination variations on yellow individuals (similar size  
84 class distributions), along three different Loire estuary sites (Fig. 1), iii) to evaluate health  
85 risks for local consumers with PCDD/F and dl-PCB TEQs quantification according to WHO  
86 recommendations (van den Berg et al., 2006).

## 88 2. Material and methods

### 89 2.1. Sampling sites

90 As shown in Fig.1, three sampling sites were selected in this study. Varades is a small city  
91 (about 3550 locals), located upstream in the estuary at the limit of the salinity (100 km from  
92 the Loire mouth); it also presents few industrial activities and is particularly under agricultural  
93 pressure. The intermediate site is close to an important city, Nantes (about 600,000 locals)

94 located at 50 km from the mouth, characterized by an industrial harbor and an urban zone  
95 including two incineration factories. The third site, Cordemais, is downstream of Nantes with  
96 a strong influence of the North Atlantic Ocean and is well-known for its industrial activities,  
97 particularly the presence of a coal-fired power plant and closed to an industrial complex  
98 including oil refineries. These three sampling sites were chosen in order to represent the  
99 estuary displaying different kinds of human activities.

## 100 2.2. Sampled animals

101 During one year and a half, *i.e.* from May 2009 to January 2011, European eels were  
102 captured by local fishermen according to the fishing authorizations, in the three sampling sites  
103 described above. Using specific methods, 62 yellow and silver eels were collected with fyke  
104 and stow nets respectively. The aim of the sampling procedure was to evaluate the potential  
105 spatial variability of contaminant levels in eels on these 3 different sites, to upstream from  
106 downstream. Consequently, 16 yellow eels were captured in Varades, 16 in Nantes and 17 in  
107 Cordemais. The captured eels were preferentially selected in order to obtain a similar size  
108 class distribution, *i.e.* about 4 to 5 eels per size class and par site. To evaluate the trend in  
109 contaminant level over life stage, glass eels and 13 silver eels were also captured. Individuals  
110 were transported to the laboratory in aerated 200 L tanks filled with water from the sampling  
111 site. They were maintained in the laboratory few hours until dissection under a natural  
112 photoperiod (L15/D9) and at a temperature around  $12 \pm 2$  °C, equivalent to the fishing site  
113 conditions. Glass eels were collected with a specific fishing net (authorized mesh size) in  
114 January 2011 in the estuary entry, near Cordemais. These glass eels had no pigment and  
115 corresponded to a stage before the onset of the feeding (Elie et al., 1982). They were directly  
116 frozen at -20°C in aluminum foil after fishing and later divided into two different pools.

## 117 2.3. Biometric parameters and life stages of the biological samples

118 Eels were anesthetized in a water bath of 10 L added with 1.5 to 2 mL of clove oil solution  
119 dissolved in ethanol (70%), according to the weight of eels (Palstra et al., 2007a). Once  
120 anesthetized, the body length (BL in mm) and the body weight (BW in g) of each European  
121 eel were measured. The animals were then sacrificed, skinned and dissected in order to collect  
122 filets and otoliths. Biometric parameters were recorded to evaluate the Fulton's condition

124 factor ( $K = (BW \times 10^5) / BL^3$  with BW and BL respectively expressed as g and mm) (Fulton,  
1 125 1904).

126 The otoliths were utilized to determine the age of the organisms. The pair of otoliths named  
2 127 sagitta were removed from the eel's head. After extraction, otoliths were cleaned of all organic  
3  
4 128 membranes in distilled water, dried with ethanol, and then stored in Eppendorf tubes. The  
5  
6 129 otoliths were later embedded in synthetic resin (Synolithe), and then polished to the nucleus  
7  
8 130 with a polishing wheel (Streuers Rotopol-35) using two different grits of sandpaper (1200 and  
9  
10 131 2400). Fine polishing was done by hand with alumina (1 $\mu$ m grain) on a polishing cloth.  
11  
12 132 Etching was done using 10% EDTA. A drop of this solution was applied on the mold during  
13  
14 133 fifteen minutes. Then, the otoliths were rinsed with distilled water and stored in dry condition.  
15  
16 134 Yearly increments were revealed by staining with a drop of 5% Toluidine blue on the otolith  
17  
18 135 and letting it dry. Growth rings were then counted under binocular magnifier. The age of each  
19  
20 136 eel was determined by the number of increments starting from the nucleus, which was  
21  
22 137 considered as year 1 of the eel's life. The otolithometry was realized in partnership with the  
23  
24 138 IRSTEA (Cestas, France). Silver stage was determined by macroscopic characteristics such as  
25  
26 139 the differentiated lateral line (presence of black corpuscles), a contrasting skin color (dark  
27  
28 140 dorsal surface and a white ventral surface), the ocular diameter and the pectoral fin length.  
29  
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#### 33 142 2.4. PCB and PCDD/F analysis

34  
35 143 Eel filets and pools of glass eels were analyzed for 18 PCBs (n= 62 and 2 pools of glass eels).  
36  
37 144 Among them, 12 are dl-PCBs (#77; 81; 105; 114; 118; 123; 126; 156; 157; 167; 169; 189) and  
38  
39 145 6 are ndl-PCBs (#28; 52; 101; 138; 153; 180). Ndl-PCB and dl-PCB levels in eel filets and  
40  
41 146 pools of glass eels were expressed as a sum of all congeners. In order to assess a potential  
42  
43 147 health risk, PCDD/F analyses were achieved on 11 out of 62 eels (5 yellow and 6 silver  
44  
45 148 individuals) and on the 2 pools of glass eels. The PCDD/Fs analyzed were the 17 congeners  
46  
47 149 regulated by the European Union (EC/1259/2011). PCB and PCDD/F levels were expressed  
48  
49 150 by congeners or as a sum of all congeners in ng.g<sup>-1</sup> dry, lipid or wet weight (dw, lw or ww).  
50  
51

#### 52 151 2.4.1 Reagents and Chemicals

53  
54 152 All organic solvents (Promochem) were Picograde<sup>®</sup> quality. Silica (Fluka), sodium sulfate  
55  
56 153 (Merck), and sulfuric acid (SDS) were of superior analytical quality. Native and <sup>13</sup>C-labeled  
57  
58 154 standards were purchased from Cambridge Isotope Laboratories (CIL) and Wellington  
59  
60

155 Laboratory. Standard solutions were prepared in toluene. All reference solutions were stored  
156 in darkness at a temperature < 6°C.

#### 157 2.4.2 Sample preparation procedure

158 Eel filets and pools of glass eels were homogenized, weighed and freeze-dried. Five grams of  
159 filets and pools of glass eels were cut, dehydrated, and milled using a turbo-mixer with glass  
160 bowl. Each experiment was realized with disposable material. Then, samples were powdered  
161 and transferred into cells in order to be extracted by Accelerated Solvent Extraction (ASE)  
162 using a Dionex ASE 300. Before extraction, eighteen <sup>13</sup>C-labelled PCB congeners were added  
163 to the samples for internal standard calibration and quantification by the isotope dilution  
164 method. Pressure and temperature were set to 100 bars and 120°C respectively. The extraction  
165 solvent was a mixture of toluene/acetone 70:30 (v/v), and three successive extraction cycles  
166 (5 min each) were performed. The extract was evaporated to dryness by rotary evaporation  
167 (40°C), allowing the gravimetric determination of the fat content, in order to assess the filet  
168 lipid weight (LW in % of wet weight). The extracts were dissolved in 25 mL of hexane for  
169 sample clean-up.

170 Three purification steps were then performed, using successively acid silica, Florisil<sup>®</sup> and  
171 celite/carbon columns. After removal of fat on the first silica gel column activated with  
172 sulfuric acid, PCBs were separated from PCDDs/PCDFs on the second Florisil<sup>®</sup> column. The  
173 separation of coplanar (non-ortho) PCBs from non coplanar PCBs was achieved on an  
174 activated mixture of Florisil<sup>®</sup>/ Carbopack C/Celite 545 (overnight at 130°C). After the  
175 addition of external standards for the recovery calculation (<sup>13</sup>C<sub>12</sub>-PCB #111 for PCBs), final  
176 sample extracts were evaporated under a nitrogen stream to dryness and reconstituted in 20  
177 μL, 50 μL and 10 μL of toluene for coplanar PCBs, non coplanar PCBs and PCDD/Fs  
178 respectively.

#### 179 2.4.3 GC-HRMS measurement

180 PCB and PCDD/F measurements were performed by gas chromatography coupled to high  
181 resolution mass spectrometry (GC-HRMS) using an 7890A gas chromatograph (Agilent)  
182 coupled to a JMS 700D or a JMS 800D double electromagnetic sector high resolution mass  
183 spectrometer (Jeol, Tokyo, Japan). A DB5MS (30 m x 0.25 mm x 0.25 μm) capillary column  
184 (J&W) was used in the splitless mode. The GC program for PCBs was 120°C (3 min),  
185 20°C/min to 170°C (0 min), 3°C/min to 245 °C (0 min) and finally 20°C/min to 275°C

186 (7 min). Ionization was achieved in the electron ionization mode (42 eV electron energy). The  
187 spectrometric resolution was set at 10,000 (10% valley), and the signal acquisition was  
188 performed in the Single Ion Monitoring (SIM) mode focusing on the two most abundant  
189 signals from each target molecular ion ( $^{35}\text{Cl}$  and  $^{37}\text{Cl}$  isotopic contributions). Signals were  
190 integrated by JEOL Diok software (v.4). The detection and quantification limits (LOD and  
191 LOQ respectively) are calculated by JEOL Diok software according to the regulation for  
192 dioxin compounds analysis (LOD=LOQ at Signal/Noise=3). A LOD is calculated for each  
193 congener and each sample (according to the sample mass).

#### 194 2.4.4 Toxic equivalency calculation

195 Toxic Equivalent Quotient values (TEQ) were calculated according to the 2005 World Health  
196 Organization Toxic Equivalency Factors (van den Berg et al., 2006) and basically expressed  
197 on a fresh weight basis.

#### 198 2.4.5. Quality assurance/quality control

199 All these procedures integrated quality control parameters to fulfill the requirements of the  
200 Commission Directive 2002/69/EC and 2002/70/EC of July 2002, laying down the sampling  
201 methods and the methods of analysis for the official control of dioxins and the determination  
202 of dl-PCBs in foodstuffs and feedingstuffs respectively. Moreover, all analyses were  
203 performed upon a double quality management system associated with an accreditation system  
204 according to the ISO 17025:2005 standard for analytical measurements.

#### 205 2.5. Statistical analysis

206 The Shapiro-Wilk and the Kolmogorov-Smirnov tests were employed to determine the  
207 normality of the results. Consequently to these tests, non-parametric tests (Kruskal-Wallis and  
208 pair-wise comparison tests) were used in order to highlight significant differences of PCB  
209 levels and fingerprints in filets of eels with different life stages and from different sites. The  
210 significant level of each test was determined according to Bonferroni correction (corrected  
211 significant level of 0.005). To compare PCB levels in eel filets from different sites and  
212 facilitate their discrimination, Principal Component Analysis (PCA) were performed.  
213 WHO<sub>2005</sub> PCDD/F and dl-PCB TEQ values were compared according to life stages using  
214 Mann-Whitney test at a significant level of 5%. All statistical treatments were realized with  
215 XLstat software.

216

### 217 3. Results and discussion

#### 218 3.1 Biometric parameters

219 Table 1 shows biometric parameters of the European eels collected in the Loire estuary  
220 according to life stage, sampling site and size class. The increase of BW is positively  
221 correlated with the increase of BL whatever the life stage (yellow or silver), and the sampling  
222 site. The linear regression equations for yellow eels are: Varades  $BL=126.18 \ln BW-154.9$   
223  $R^2=0.97$  (n=16); Nantes  $BL=152.8 \ln BW-292.5$   $R^2=0.97$  (n=16); Cordemais  $BL=113.14$   
224  $\ln BW-105.2$   $R^2=0.98$  (n=17) and those of silver eels was  $BL=220.94 \ln BW-685.9$   $R^2=0.98$   
225 (n=13). The age of eels is associated to BL and BW, only for yellow individuals from  
226 Cordemais ( $BL=60.9 \text{ Age}+109.2$   $R^2=0.77$  (n=17)). No significant correlation was observed  
227 for yellow individuals from others sites and for silver eels. Fulton's condition factor values  
228 (K) are roughly similar in the range of the different size classes studied as well as according to  
229 the sampling site and the life stage, with values ranging from 0.13 to 0.17. According to  
230 (Feunteun, 2002), these values are representative of eel good health of in the Loire estuary.  
231 Such values are similar to the Fulton's condition factor found in other studies about European  
232 areas (Gravato et al., 2010; Palstra et al., 2007b; Tapie et al., 2011). Nevertheless, better eel  
233 conditions were calculated in some other studied sites like the River Rhine watershed and  
234 Lake Ijsselmeer (Haenen et al., 2010).

#### 235 3.2 Influence of life stage, sampling site and size class on dl and ndl-PCB levels

236 Table 1 shows the PCB levels (dl and ndl-PCBs) according to the life stage, the sampling site  
237 and the size class. As it was already reported in a previous work (Tapie et al., 2011), PCB  
238 levels determined for glass eels were higher than the limit of quantification of the analytical  
239 methods. The sums of dl and ndl-PCBs are  $3.7 \pm 1.9 \text{ ng.g}^{-1} \text{ dw}$  and  $15.2 \pm 4.2 \text{ ng.g}^{-1} \text{ dw}$   
240 respectively. These levels could be the result of a contamination via the food web during the  
241 leptocephali stage (plankton) and to a direct exposure from the aquatic compartment. Another  
242 hypothesis could be an intergenerational transfer of contaminants (Palstra and van den  
243 Thillart, 2011).

244 Regarding yellow eels, the PCB contamination increases and becomes significantly higher  
245 compared to glass eels whatever the sampling site and the size class considered. Regarding  
246 each site, the trends of ndl and dl-PCB levels expressed as  $\text{ng.g}^{-1} \text{ dw}$  or  $\text{lw}$  are similar and

247 showed no significant difference according to the size classes. This observation could be  
1 248 attributed to the low sample number per size class. Nevertheless, it is possible to conclude that  
2 249 PCB levels were not correlated to BL and BW, except for eels from Nantes (Table 1).  
3 250 Considering the results depicted for eels from Nantes and the eel ecology (bottom dwelling  
4 251 fish), the increasing contamination with BL and BW could be attributed to the continental  
5 252 phase longevity and consequently to the time spent in the estuary environment, in close  
6 253 contact with potentially contaminated sediments. It could be also related to the trophic chain  
7 254 based on a more or less contaminated food.

15 255 Regarding the silver eels, dl-PCB levels, expressed as  $\text{ng.g}^{-1}$  dw, were significantly higher  
16 256 than results for yellow eels from Varades and Cordemais. Considering the same unit, ndl-PCB  
17 257 levels for silver eels were significantly higher than levels for yellow eels from Varades only.  
18 258 Considering dl- and ndl-PCB levels expressed as  $\text{ng.g}^{-1}$  lw, the results tend to decrease but are  
19 259 only significantly different to those for yellow eels from Nantes, and whatever all the size  
20 260 classes. This results can be explained by the highly lipid content in silver eels leading to a  
21 261 dilution of the contaminants.

29 262 In a previous work (Tapie et al., 2011), a review about marker PCB levels in *Anguilla*  
30 263 *anguilla* filets was achieved from the literature. Marker PCB congeners are #28, 52, 101, 118,  
31 264 138, 153 and 180. To compare with this synthetic review, the values obtained in this study  
32 265 for the last congeners were summed, and expressed as  $\text{ng.g}^{-1}$  ww and lw (Table 1). The PCB  
33 266 congener #118 is usually used as a marker PCB until now (ANSES, 2011). The percentage of  
34 267 this congener was relatively constant and represented an average of  $9.39 \pm 2.7$  % of total PCB  
35 268 marker level.

43 269 At the international scale, eels from the Loire estuary appear to be more contaminated than  
44 270 those from some other sites in Poland, Ireland, Spain, Italy and the UK (Bordajandi et al.,  
45 271 2003; Corsi et al., 2005; McHugh et al., 2010; Santillo et al., 2005). However, other sites are  
46 272 more contaminated than the Loire estuary (twice to 10 times higher), i.e. the River Elbe in  
47 273 Czech Republic and Germany, the Tevere and Gagliarino rivers in Italy, Flanders in Belgium  
48 274 and different lakes in Finland (Belpaire et al., 2011; Maes et al., 2008; Tulonen and Vuorinen,  
49 275 1996; van der Oost et al., 1996). Throughout France, eels from the Loire estuary are slightly  
50 276 more contaminated than those from the Vacares lagoon and about three times more than those  
51 277 from the Thau pound (Oliveira Ribeiro et al., 2008; Santillo et al., 2005), whereas they are

278 less contaminated than eels from the Rhone River (about ten times less) and the Gironde  
279 estuary (about two times less, whatever the life stage and the size class) (Tapie et al., 2011).

280 In the study of Tapie et al. (2011), a significant decrease of marker-PCB levels expressed as  
281  $\text{ng.g}^{-1}$  dw was observed for eels exceeding 600 mm. These authors hypothesized that this  
282 decrease could be induced by two parameters regarding the sexual maturity of the individuals  
283 of this size class. On the one hand, eels could be at the onset of the silvering and coming from  
284 upstream areas, less contaminated. On the other hand, silver eels could be already in  
285 starvation and start to mobilize lipid stores as fuel energy to ensure the sexual maturation and  
286 swimming towards spawning areas. This mobilization of lipids was already proposed to  
287 explain a decrease in lipid contents observed in filets of eels larger than 800 mm (Durif et al.,  
288 2005). In this present work, no decrease is observed for eels with length superior than 600  
289 mm whatever the unit expression. .

290  
291 In order to evaluate the correlations between biometric parameters and PCB levels as well as  
292 the sampling site effect, a principal component analysis (PCA) was performed by using  
293 biometric parameters (age, BW, BL and LW) and dl and ndl-PCB levels expressed as  $\text{ng.g}^{-1}$   
294 dw. Since silver eels are not strictly territorial, due to their downstream migration, they could  
295 be originated from other sites than sampling ones. For that reason, the PCA was performed  
296 with yellow eels only. As it was shown in the Table 1, the size class distributions between the  
297 3 studied sites are comparable. Consequently, it is possible to study and discuss the presence  
298 of an eventual sampling site effect on yellow eel impregnation.

299 The correlation loading and sample representation are shown on figure 2 (respectively Fig.2 A  
300 and Fig.2 B). The first two principal components (respectively PC1 and PC2) describe  
301 82.97% of the total variability among eels. PC1 and PC2 represent respectively 62.65 and  
302 20.32%.

303 The correlation loading (Fig.2A) highlights that biometric parameters (BW, BL and age) are  
304 correlated to each other as it was depicted in Table 1. Concerning LW, it appears to be quite  
305 correlated to both levels of dl- and ndl-PCBs. This observation was expected and already  
306 well-known according to the lipophilic properties of PCBs (van der Oost et al., 1996).  
307 Regarding the sample presentation in Fig.2B, the eels are relatively clustered according to the  
308 three different sampling sites. The comparison of Fig.2A and Fig.2B underlines that eels from

309 Varades are the lowest contaminated by dl and ndl-PCBs closely related with lower LW. The  
1 310 eels from Nantes and some of those from Cordemais are more contaminated, showing a  
2 311 higher LW. However, eels from Nantes present a higher heterogeneity. The inter-site  
3 312 differences observed could be also related to differences of biometric parameters such as the  
4 313 BL and the age, characterizing a different exposition time ( $9.8 \pm 1.9$  years for eels from  
5 314 Nantes compared to  $4.4 \pm 1.4$  years and  $5.9 \pm 1.9$  years for eels from Cordemais and Varades,  
6 315 respectively).

13 316 Moreover, Varades is a small city (about 3550 locals), relatively preserved, located upstream  
14 317 in the estuary, and with few industrial activities. It is probably for these reasons that the eels  
15 318 from this sampling site are less contaminated than the others. Nantes is indeed an important  
16 319 city (about 600000 locals) and Cordemais is downstream of Nantes and well-known for its  
17 320 industrial activities. In this study, the living area of eels seems to affect their contamination  
18 321 level as it was already shown in the Gironde estuary (Tapie et al., 2011). These inter-site  
19 322 differences would be highlighted in the next section dealing with eels PCB fingerprints.  
20 323

### 27 324 3.3 PCB fingerprints in eels according to the sampling site and life stage

28 325 A sampling site effect has been previously demonstrated (Fig.2). A second PCA was then  
29 326 performed using individual PCB levels expressed as  $\text{ng.g}^{-1}$  dw. For the same reason that 3.2  
30 327 paragraph, this second PCA was realized with yellow eels only. Consequently, this PCA was  
31 328 useful in order to evaluate the influence of the sampling site on PCB fingerprints in yellow  
32 329 eels.  
33 330

34 331 The result of the PCA correlation loading is shown in Fig.3A. The first two principal  
35 332 components of the PCA (respectively PC1 and PC2) describe 85.74% of the total variability  
36 333 among eels. PC1 and PC2 respectively represent 68.85 and 16.89%. This figure highlights  
37 334 that the first principal component is positively correlated to all the individual PCB levels. The  
38 335 second one is negatively correlated to low chlorinated PCBs and positively correlated to  
39 336 highly chlorinated ones.  
40 337

41 338 Each PCB congener is represented around the right part of the correlation circle. Nevertheless,  
42 339 the repartition of the different PCBs seems to be due to their chemical structure, i.e. the  
43 340 number and the position of Cl atoms. Low chlorinated PCBs with few Cl atoms in meta and  
44 341 para-positions are in the right bottom of the circle. The more the number of total Cl atoms and  
45 342

340 of Cl atoms in meta- and para- positions are important, the upper their localization is. Ndl-  
1 341 PCBs are considered as particularly persistent and present in the environment, representing  
2 342 about 50% of all of the PCB congeners found in food from animal origin (AFSSA, 2006).  
3 343 According to Fig.3A, they were well distributed around the right part of the correlation circle  
4 344 and among all the other PCBs, emphasizing their qualitative representativeness of all the PCB  
5 345 congeners (Cariou et al., 2010).

11 346 Fig.3B enhances this result showing the accumulation patterns of marker-PCBs in yellow eels  
12 347 from the three sampling sites comparatively to those in the glass and silver eels.

16 348 Concerning the main congener #153, it contributes to an average of 42% of the contamination  
17 349 of the yellow eels whatever the sampling site. This PCB is interesting because it is non  
18 350 metabolizable and a tracer of bioaccumulation process. The percentages of this congener are  
19 351 not significantly different for glass and silver eels compared to yellow eels from Varades but  
20 352 they are lower than those found for yellow eels from Cordemais and Nantes.

26 353 Considering the sampling site influence, an inter-site variability is observed, particularly for  
27 354 eels from Cordemais which display patterns with significantly lower relative proportion of  
28 355 PCBs #28 and 118 whereas the relative proportion of #180 is significantly higher than those  
29 356 of the other sites. About eels from Nantes, the relative proportion of the PCB #101 is  
30 357 significantly higher to the detriment of the #180 with a relative proportion significantly lower.  
31 358 Finally, the PCB #180 is the only one able to discriminate the sampling sites. Its proportion is  
32 359 higher in Cordemais eels, lower in Nantes eels and intermediate in Varades eels. For the other  
33 360 marker-PCBs (#153, 138 and 52), no significant specific trend is observed according to the  
34 361 sampling site.

43 362 The PCB contamination variability between the three sampled sites could be partially  
44 363 explained by the anthropogenic activities existing in the area. Indeed, Varades is a relatively  
45 364 small city, marked by several agricultural activities where PCB sources are probably less  
46 365 important than in Nantes or Cordemais. On the contrary, Nantes is an important urban and  
47 366 industrial city, with an important economic and demographic development, where two  
48 367 incinerator factories and various maritime and industrial activities exist. A lot of domestic,  
49 368 industrial and agricultural effluents are discharged into the Loire estuary more or less  
50 369 previously depolluted in sewage treatment plants. Cordemais presents another profile because  
51 370 it is a very small rural city but dominated by its economic and industrial activities, directly

371 based on the presence of the Loire estuary such as a coal-fired power plant and close to an  
1 372 industrial complex which includes oil refineries.  
2

3  
4 373 The PCB sources are therefore multiple along the estuary and the PCB contamination of this  
5  
6 374 ecosystem could be done following atmospheric or aquatic routes. This complexity prevents  
7  
8 375 from establishing easy correlations between the congener profiles and the sources. Motelay-  
9  
10 376 Massei et al. (2004) showed, in the Seine river basin, that less chlorinated PCB congeners are  
11  
12 377 transported over longer distances from the source sites because of their longer residence time  
13  
14 378 in the atmosphere, whereas the heaviest PCBs tend to be adsorbed on particles and to settle  
15  
16 379 near production sources. Therefore, our results could suggest that eels from Cordemais were  
17 380 living closer to a PCB source than eels from the other sites.  
18

19  
20 381 Moreover, the Loire estuary presents strong hydrodynamic, sedimentary and abiotic  
21  
22 382 parameters (Dauvin, 2008). Today, the effects of the tide can be observed within 97 km from  
23  
24 383 the estuary mouth and the salinity moves upstream. This modifies also the temperature from  
25  
26 384 the mouth of the estuary to the upstream front of the salinity which varies of 5°C from  
27  
28 385 downstream to upstream. The Loire estuary is also characterized by a fluid mud which  
29  
30 386 extends over 20 km; it is an important factor in the rapid sedimentation of the estuary, so the  
31  
32 387 turbidity varies from 2 g.L<sup>-1</sup> at the surface to 20 g.L<sup>-1</sup> near the bottom. All these parameters  
33  
34 388 (salinity, turbidity, temperature and tidal amplitude) could affect the exposition level of PCBs  
35  
36 389 potentially present in the estuary, and their chemical bioavailability for eels. Moreover, the  
37  
38 390 biological variability of eel PCB levels could also be explained by differences of diets,  
39  
40 391 ecology, physiology or metabolism capacities in relation to polyhaline, mesohaline and  
41  
42 392 oligohaline ecozones.  
43

44 393 Regarding the difference of the relative proportions found for silver eels compared to yellow  
45  
46 394 eels, the only significant one is for PCB #28 which is higher in silver eels compared to yellow  
47  
48 395 eels whatever the sampling site considered. The patterns of silver eels are closer to those of  
49  
50 396 eels from Nantes, then Varades, whereas they display many significant differences  
51  
52 397 comparatively to those of eels from Cordemais: all the relative proportions are significantly  
53  
54 398 different except the one of PCB #138.  
55

56 399 Finally, concerning the glass eels, a contrasting pattern is noticed, underlying a different  
57  
58 400 bioaccumulation phenomenon, characterized mainly by an important proportion of less  
59  
60 401 chlorinated PCBs to the detriment to the heaviest PCBs. This was already shown in a previous  
61  
62 402 study (Tapie et al., 2011) in which congeners 28, 50, 52, 101, 118 represented 51% of  
63

403 accumulation pattern for glass eels sampled in the Gironde estuary. Comparatively, the  
1 404 relative proportion of these congeners (without the PCB #50 not analyzed in this study)  
2 405 represent 53% for glass eels in our study. The less chlorinated PCBs are transported over  
3 406 longer distances from the PCB sources sites because of their longer residence time in the  
4 407 atmosphere (Motelay-Massei et al., 2004). Moreover, in aquatic environments, these PCBs  
5 408 that are more polar, are found to be dominant in dissolved phase and particulate organic  
6 409 matter (Cailleaud et al., 2007). Glass eels come from the oceanic platform after the  
7 410 metamorphosis of larvae leptocephali stage. These transparent larvae move with currents  
8 411 (pelagic compartment) for months, or years, in seawaters far from important pollution  
9 412 sources. During their travel, the larvae were mainly contaminated by feeding uptake. When  
10 413 they approach the continental shelf claim, the metamorphosis in glass eels occurs. They stop  
11 414 then to feed and the contamination is then by direct exposure which leads to a pattern similar  
12 415 to that of water column dominated by less chlorinated compounds. It is likely that the specific  
13 416 PCB pattern of glass eels could be the result of these two phenomena. Another hypothesis is  
14 417 the transfer of PCBs from adult eels to eggs and, consequently, to glass eels, low chlorinated  
15 418 PCBs being more efficiently transferred than the heavy chlorinated ones (Bargar et al., 2001;  
16 419 Verreault, 2006).

### 31 32 420 3.5 PCDD/F, PCB levels and public health

33  
34 421 Mean PCDD/F and dl-PCB levels expressed according to the 2005 WHO recommendations  
35 422 were  $5.21 \pm 1.78$  and  $9.88 \pm 4.14$   $\text{pg}\cdot\text{g}^{-1}$  WHO<sub>2005</sub> PCDD/F and dl-PCB TEQ (toxic  
36 423 equivalents) ww for yellow (n=5) and silver individuals (n=6) respectively. Glass eels  
37 424 depicted a mean level significantly lower ( $0.27 \pm 0.03$   $\text{pg}\cdot\text{g}^{-1}$  WHO<sub>2005</sub> PCDD/F and dl-PCB  
38 425 TEQ ww). The maximum level established for the level of PCDD/Fs in eel filets is currently  
39 426  $3.5$   $\text{pg}\cdot\text{g}^{-1}$  WHO<sub>2005</sub> PCDD/F TEQ ww and  $10$   $\text{pg}\cdot\text{g}^{-1}$  WHO<sub>2005</sub> PCDD/F and dl-PCB TEQ wet  
40 427 weight (European Union, 2011). These values were not reached regarding the yellow eels.  
41 428 Nevertheless, in the case of silver ones, biological variability was high and 4 out of 6 studied  
42 429 eels displayed WHO<sub>2005</sub> PCDD/F and dl-PCB TEQ values higher than  $10$   $\text{pg}$  WHO<sub>2005</sub>  
43 430 PCDD/F and dl-PCB TEQ per gram of wet filet.

44 431 Regarding the congeners # 28, 52, 101, 138, 153 and 180 (ndl-PCB), sampled eels did not  
45 432 present levels superior than the 2005 WHO recommendation of  $300$   $\text{ng}\cdot\text{g}^{-1}$  ww. Silver eels and  
46 433 yellow individuals from Nantes depicted the highest levels (mean of  $204.6 \pm 113.3$  and  $175.7$   
47 434  $\pm 90.7$   $\text{ng}\cdot\text{g}^{-1}$  ww, respectively), but few individuals (3/29) presented concentrations higher

435 than the recommended level (Table 1). Yellow individuals from Cordemais presented  
436 intermediate levels (mean of  $117.9 \pm 47.7 \text{ ng.g}^{-1} \text{ ww}$ ) and those from Varades the lowest ones  
437 (mean of  $75.5 \pm 25.2 \text{ ng.g}^{-1} \text{ ww}$ ).

438 Our results indicate a potential exposure to PCBs through eel consumption in this estuary, and  
439 especially with silver ones. The French Food Safety Agency proposed a tolerable daily intake  
440 (TDI) of 10 ng/ kg body weight/day (for the 6 ndl-PCB congeners), which represents 700  
441 ng/day for a 70 kg person or 150 ng/day for a child of 15 kg (under 3 years) (French Food  
442 Safety Agency, 2010). We could thus recommend to limit the consumption of eel from the  
443 Loire estuary to one portion (150 g) per month for the general population, which represent an  
444 average dietary daily intake of 694 ng/day. This is more restricted than the French Food  
445 Safety Agency recommendations which limit the consumption of PCB bioaccumulating fish  
446 to two portions per month for the general population. Specific recommendations (a portion of  
447 60 g every two months) exist for the most sensitive populations (pregnant and breastfeeding  
448 women, young and adolescent girls, women of childbearing age, and children under 3) and are  
449 in agreement with our results, representing an average dietary daily intake of 139 ng/day.

450 A national study assessing the PCB impregnation of freshwater fish consumers performed on  
451 six investigation sites including the Loire (French Food Safety Agency, 2011), revealed that  
452 only 13% of participants (on a total of 606 amateur anglers and members of their households  
453 and 16 professional anglers) are strong PCB-bioaccumulator freshwater fish consumers, with  
454 a moderate consumption frequency of 1 time per month. Among the strong PCB-  
455 bioaccumulator freshwater fish species, eel is consumed with a mean annual frequency of 2.6  
456 times per year. Considering these local practices and our results, a dietary daily intake of ndl-  
457 PCBs varying from 22 to 504 ng/day with a mean of 150 ng/day could be estimated. The TDI  
458 value is not exceeded and the risk seems then to be moderate for an adult consumer but really  
459 present for the most sensitive populations.

## 461 Conclusion

462 This study gives a first assessment of the PCB contamination of a European eel population  
463 fraction from the Loire estuary, along a hundred-km long portion of ecosystem. The  
464 quantitative and qualitative contents of PCBs in eel filets are different depending on their life  
465 stage and the sampling sites. The eels sampled in the site next to Varades (small city under

466 agricultural pressure) appeared less contaminated than the two other sites, *i.e.* Nantes (an  
1 467 important city) and Cordemais (a town hosting a coal-fired power station). Regarding the  
2  
3 468 PCB patterns, the sampled sites of Varades and Nantes could be associated to urban  
4  
5 469 influences whereas the one of Cordemais, more impacted by heavy chlorinated PCBs, would  
6  
7 470 be nearer from a PCB industrial source. Compared to other international or national areas, the  
8  
9 471 ndl-PCB levels in eels from Loire estuary show an intermediate contamination. Our results  
10  
11 472 indicate a potential exposure to PCBs through eel consumption, and especially with silver  
12  
13 473 ones. According to TDI value, the consumption must be limited to once per month for the  
14  
15 474 general population and to once every two month for the most sensitive ones.

16  
17 475 Apart from an eventual sanitary problem, the contamination of eels could lead to damages for  
18  
19 476 the eel population by affecting their reproduction and by a transfer of pollutants to eggs.  
20  
21 477 Indeed, since these compounds are lipophilic, the results showed that the PCB levels are  
22  
23 478 correlated to the lipid content in the filets. Lipids are essential compounds for the migration  
24  
25 479 reproduction (van Ginneken et al., 2009) and for both fat deposition in the oocytes and later  
26  
27 480 incorporation of vitellogenic stores in eggs. Moreover, acclimation of eels to seawater,  
28  
29 481 silvering process and reproduction migration are under different endocrine controls and fuel  
30  
31 482 consuming. This energetic cost is described to increase significantly when lipid filets of  
32  
33 483 swimming eels are charged in PCB mixture (after intraperitoneally injection of 5000 ng g<sup>-1</sup>  
34  
35 484 PCB # 153, 7 ng g<sup>-1</sup> PCB # 126 and 50 ng g<sup>-1</sup> PCB # 77) (Thillart et al., 2009). PCB levels  
36  
37 485 determined in eels from the Loire estuary could thus potentially have an impact on the  
38  
39 486 reproduction success of European eels.

40 487 The comparison of eel biomonitoring studies highlighted heterogeneity in sampled  
41  
42 488 individuals. To better correlate studies at the international level, it appears necessary to  
43  
44 489 standardize parameters such as age, length, sex and sexual maturation stage. To preserve this  
45  
46 490 endangered species and such as recommended by scientists (van Ginneken et al., 2009), the  
47  
48 491 environmental quality of its habitats should be restored and protected. Considering our results,  
49  
50 492 the European eels from the Loire estuary appeared moderately contaminated compared to eels  
51  
52 493 from other major international estuaries, suggesting a moderate PCB contamination of the  
53  
54 494 Loire estuarine system. These conditions could contribute therefore to preserve genitors.

55 495  
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57  
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60  
61

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**Table 1:** Means and standard deviations of PCB levels (dl, ndl and marker) and biometric parameters (Body Length BL, Body Weight BW, Lipid Weight LW), Fulton's condition factor (K) and age of sampled European eels (n = 62) from the three studied sites in the Loire estuary according to life stage and size classes.

| Life stage  | Sampling site | Size class (mm) | n       | BL (mm) | BW (g)  | Age (year) | LW (%)    | K         | dl-PCB levels (ng.g <sup>-1</sup> dw) | ndl-PCB levels (ng.g <sup>-1</sup> dw) | dl-PCB levels (ng.g <sup>-1</sup> lw) | ndl-PCB levels (ng.g <sup>-1</sup> lw) | ndl-PCB levels (ng.g <sup>-1</sup> ww) | Marker-PCB levels (ng.g <sup>-1</sup> ww) | Marker-PCB levels (ng.g <sup>-1</sup> lw) |
|-------------|---------------|-----------------|---------|---------|---------|------------|-----------|-----------|---------------------------------------|--|---------------------------------------|--|--|---|---|
| Glass eels  |               | < 200           | 2 pools | ≤ 90    | 62±12   | < 1        | 4.0 ± 0.8 | n.d.      | 3.7±1.9                               | 15.2±4.2                               | 18.6±8.3                              | 78±26                                  | 3.0±0.4                                | 3.5±0.2                                   | 89±21                                     |
| Yellow eels | Varades       | 200-300         | 5       | 279±14  | 30±3    | 5.2±0.8    | 4.9±2.3   | 0.14±0.02 | 41.4±12.4                             | 253.7±78.1                             | 286.9±69.2                            | 1764±458                               | 79.7±28.6                              | 86.8±31.3                                 | 1918±491                                  |
|             |               | 300-400         | 5       | 349±31  | 59±17   | 5.7±1.8    | 6.4±4.9   | 0.14±0.01 | 37.6±9.3                              | 228.0±46.9                             | 349.3±372.9                           | 1183±510                               | 72.7±22.2                              | 79.3±24.7                                 | 1284±546                                  |
|             |               | 400-500         | 4       | 433±26  | 111±21  | 5.6±1.8    | 6.0±4.4   | 0.14±0.01 | 48.2±6.5                              | 261.0±12.0                             | 334.5±198.8                           | 1770±1006                              | 76.7±14.2                              | 84.5±15.2                                 | 1953±1111                                 |
|             |               | 500-600         | 2       | 533±31  | 207±2   | 9.0±2.1    | 10.1±11.7 | 0.14±0.02 | 29.0±13.8                             | 195.1±118.1                            | 169.7±121.4                           | 1041±619                               | 69.1±58.8                              | 74.6±63.0                                 | 1132±682                                  |
|             | Nantes        | 300-400         | 5       | 366±37  | 81±28   | 8.6±0.7    | 10.4±5.7  | 0.16±0.02 | 71.8±15.7                             | 448.5±78.5                             | 266.8±131.2                           | 1657±722                               | 144.8±49.1                             | 158.3±54.7                                | 1810±799                                  |
|             |               | 400-500         | 4       | 452±33  | 129±30  | 9.5±0.5    | 10.2±3.2  | 0.14±0.01 | 82.2±10.3                             | 482.2±74.4                             | 278.5±103.6                           | 1669±797                               | 151.9±16.4                             | 166.6±17.3                                | 1827±852                                  |
|             |               | 500-600         | 4       | 546±24  | 259±49  | 10.5±1.2   | 11.2±6.7  | 0.16±0.01 | 97.8±31.0                             | 542.8±171.3                            | 344.4±135.5                           | 1909±767                               | 180.8±77.2                             | 199.0±84.5                                | 2100±828                                  |
|             |               | >600            | 3       | 678±63  | 551±183 | 11.0±3.9   | 11.6±8.9  | 0.17±0.03 | 134.9±82.9                            | 734.6±401.9                            | 488.0±244.5                           | 2706±1365                              | 252.0±187.9                            | 278.6±210.0                               | 2986±1508                                 |
|             | Cordemais     | 200-300         | 5       | 272±10  | 26±2    | 3.1±0.7    | 6.6±2.8   | 0.13±0.01 | 45.6±11.4                             | 326.7±78.8                             | 238.7±126.2                           | 1291±490                               | 95.8±23.5                              | 102.3±25.1                                | 1910±1258                                 |
|             |               | 300-400         | 5       | 342±36  | 61±19   | 3.8±0.6    | 12.0±3.6  | 0.15±0.02 | 61.8±22.7                             | 403.8±166.4                            | 172.5±45.5                            | 1130±378                               | 135.7±63.1                             | 146.2±67.7                                | 1217±401                                  |
|             |               | 400-500         | 4       | 455±17  | 147±12  | 5.5±0.4    | 7.7±3.3   | 0.16±0.01 | 46.1±6.7                              | 307.3±42.6                             | 191.1±68.8                            | 1275±476                               | 88.1±21.0                              | 94.9±22.4                                 | 1372±508                                  |
|             |               | 500-600         | 3       | 522±11  | 227±22  | 6.3±1.0    | 15.0±6.8  | 0.16±0.00 | 76.1±17.2                             | 479.3±68.9                             | 185.1±54.4                            | 1170±285                               | 164.8±37.0                             | 177.9±40.9                                | 1263±312                                  |
| Silver eels |               | >500            | 13      | 659±124 | 517±344 | 12.4±3.8   | 25.6±3.5  | 0.16±0.01 | 93.7±56.3                             | 463.2±244.6                            | 161.6±96.1                            | 800±425                                | 204.6±113.3                            | 229.0±130.3                               | 895±485                                   |

n.d.: non determined

Figure 1: Studied area: the Loire estuary (France). Three sampling locations (Cordemais; Nantes and Varades)

Figure 2: Principal Component Analysis of biometric parameters and dl- and ndl-PCB levels expressed in  $\text{ng.g}^{-1}$  dw in muscles of yellow eels from 3 sampling sites ( $n = 49$ ): Varades, Nantes and Cordemais.

A: correlation loadings (BW: body weight; BL: body length; LW: lipid weight);

B: sample representation (circles = eels from Varades; triangles = eels from Nantes; squares = eels from Cordemais).

Figure 3: Representation of PCB patterns.

A: correlation loadings of Principal Component Analysis of dl and ndl-PCB muscle levels expressed in  $\text{ng.g}^{-1}$  dw in yellow eels from 3 sampling sites ( $n = 49$ ): Varades, Nantes and Cordemais (dl-PCBs: black circles; ndl-PCBs: white circles);

B: Relative proportion (in %) of marker-PCBs in eel filets according to the life stage and the sampling site.

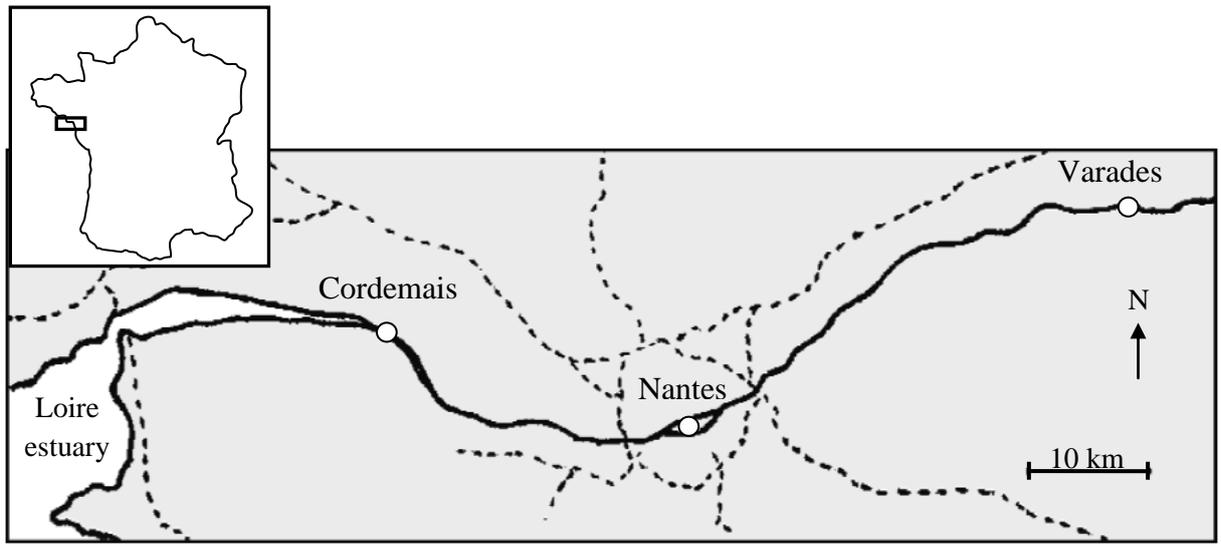


Figure 1

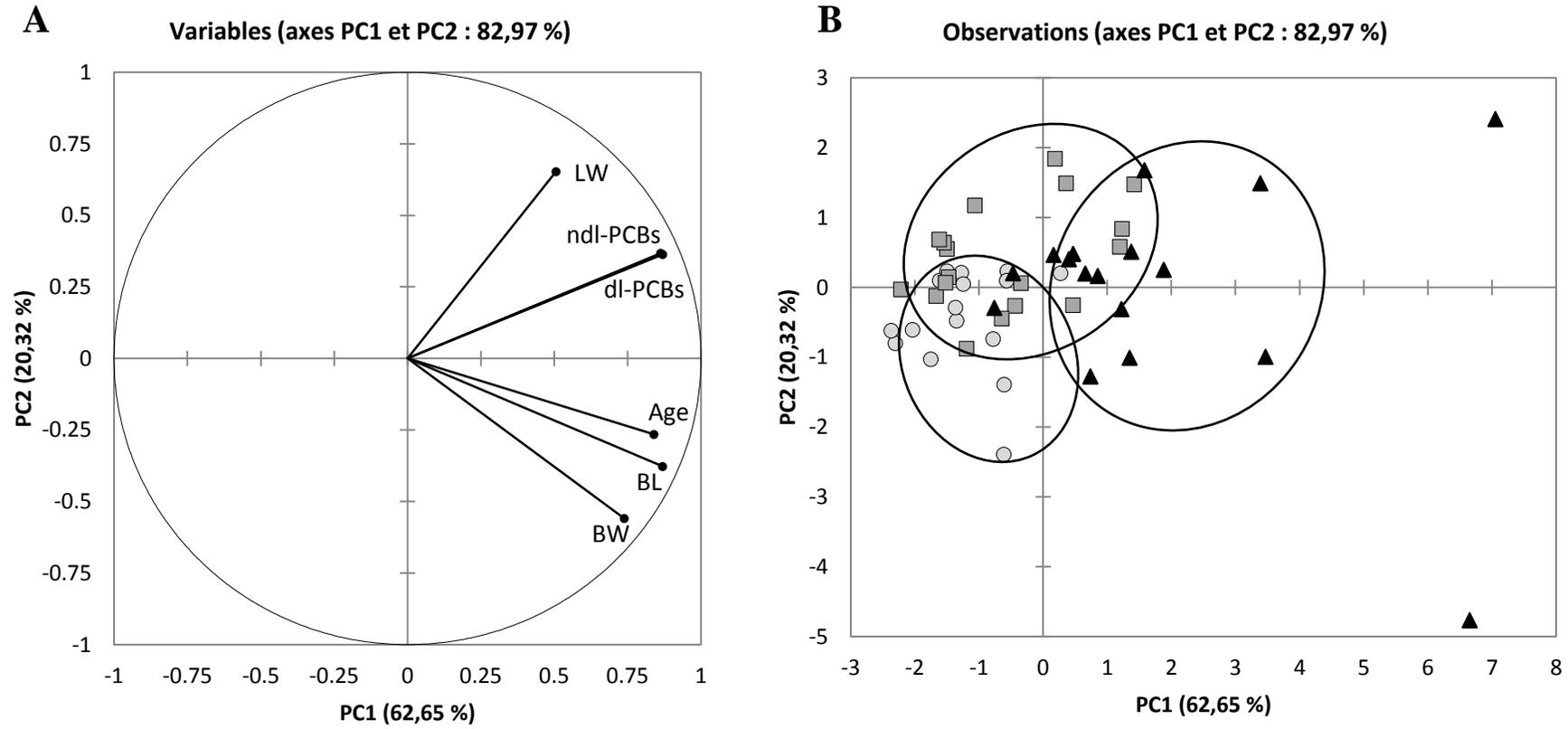


Figure 2



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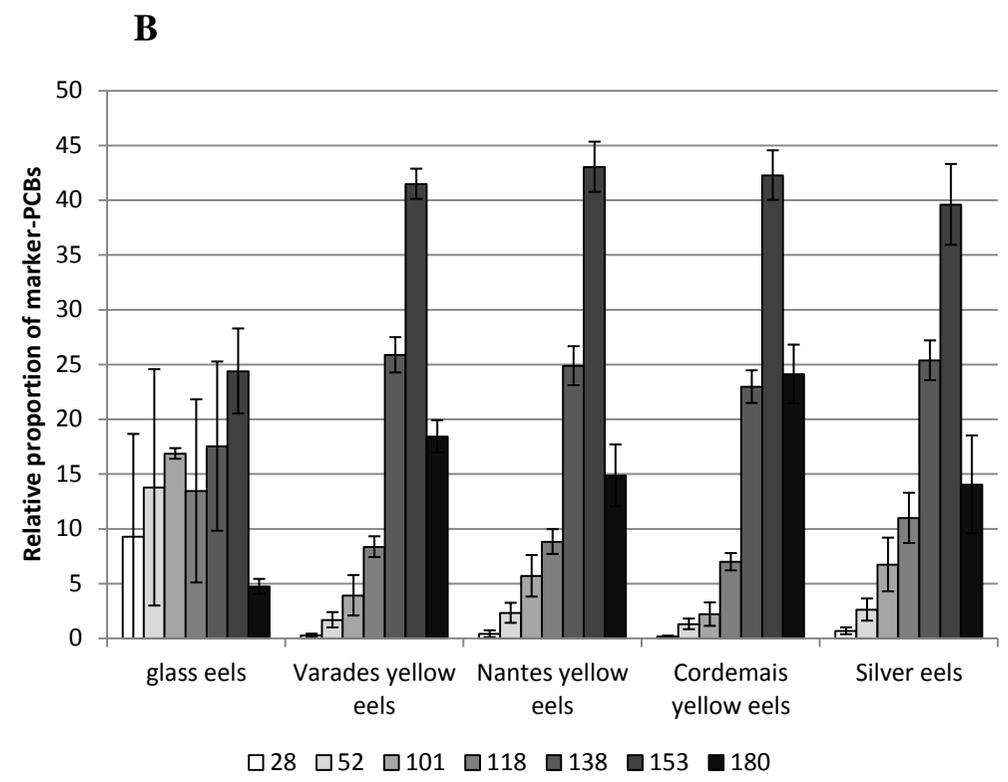
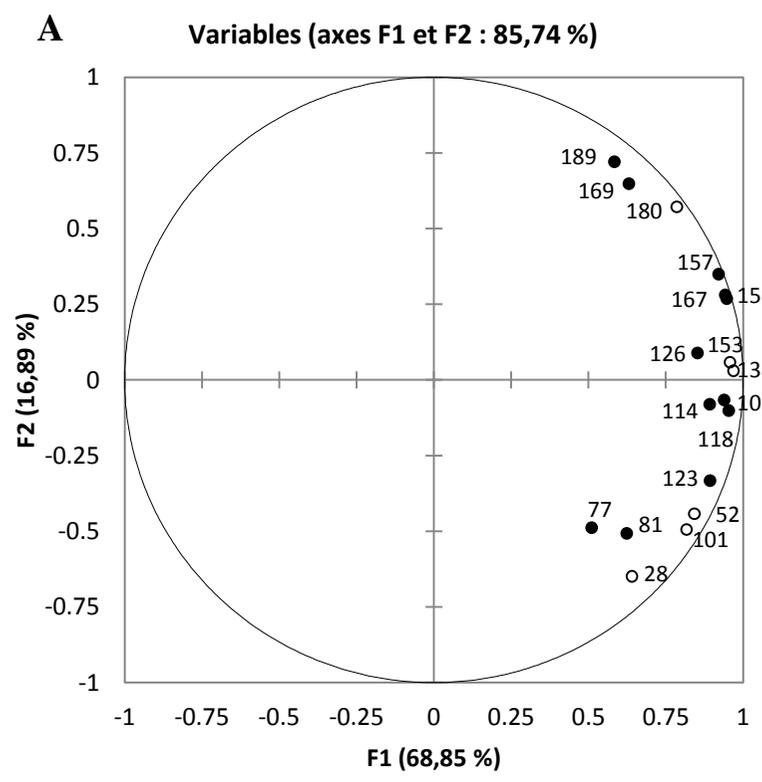


Figure 3

## AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication entitled " Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (*Anguilla anguilla*) from the Loire estuarine continuum: spatial and biological variabilities" and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

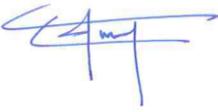
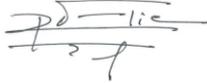
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