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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

I. Blanchet-Letrouvé[1]*, A. Zalouk-Vergnoux[1], A. Vénisseau[2], M. Couderc[1], B. Le Bizec[2], P. Elie[4], C. Herrenknecht[1], C. Mouneyrac[3], L. Poirier [1]

[1]Université de Nantes, MMS, EA 2160, 9 rue Bias, Nantes F-44322, France

[2]LUNAM Université, Oniris, Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France

[3]Université Catholique de l'Ouest, IBEA, CEREA, 44 rue Rabelais, Angers F-49008, France

[4]IRSTEA, 50 avenue de Verdun – Gazinet, Cestas F- 33612, France

* Corresponding author: +33685329898, isabelle.blanchet-letrouve@ac-nantes.fr

Abstract

To characterize the eel contamination by dioxin-like (dl) and non dioxin-like (ndl) PCBs and PCDD/Fs, 62 eels from the Loire estuary (France) were analyzed. PCB contamination significantly increased from glass eel stage (3.7 ±1.9 and 15.2±4.2 $ng.g^{-1}$ dw) to other life stages (for yellow eels: 62.8±34.4 and 381.8±181.8 $ng.g^{-1}$ dw; for silver eels: 93.7±56.3 and 463.2±244.6 $ng.g^{-1}$ dw respectively for dl and ndl PCB). An inter-site variability based on PCB levels and fingerprints was observed between the three studied sites. The glass eel pattern was mainly characterized by the less chlorinated PCBs contrarily to the other eels, underlying a different bioaccumulation pathway. Overall, eels from this estuary showed an intermediate contamination level compared to other international/national areas. However, more than 60% of studied silver eels displayed WHO_{2005} PCDD/F and dl-PCB TEQ values higher than the recommended level of 10 $pg.g^{-1}$ ww. This statement indicates a potential exposure to PCBs through eel consumption, especially with silver individuals, and could potentially lead to damages for the eel population.

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1. Introduction

Since the 1980s, monitoring studies in European countries have shown the decline of glass eels arriving in the coastal waters and estuaries (ICES, 2006). The disappearance of the prepubertal European eel (*Anguilla anguilla*) occurred as well a few decades earlier and stocks were estimated to be divided by ten (Dekker, 2003; Moriarty and Dekker, 1997). Several factors were brought forward to explain this decrease such as overfishing, obstacles to migration (Robinet and Feunteun, 2002), pathogens (Palstra et al., 2007b), climate change (Castonguay et al., 1994) and contaminants (Geeraerts et al., 2011; Palstra et al., 2007a; Roosens et al., 2010; van Ginneken et al., 2009)

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

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

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

2. Material and methods

2.1. Sampling sites

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

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

2.2. Sampled animals

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

2.3. Biometric parameters and life stages of the biological samples

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

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

The otoliths were utilized to determine the age of the organisms. The pair of otoliths named sagitta were removed from the eel's head. After extraction, otoliths were cleaned of all organic membranes in distilled water, dried with ethanol, and then stored in Eppendorf tubes. The otoliths were later embedded in synthetic resin (Synolithe), and then polished to the nucleus with a polishing wheel (Streuers Rotopol-35) using two different grits of sandpaper (1200 and 2400). Fine polishing was done by hand with alumina (1µm grain) on a polishing cloth. Etching was done using 10% EDTA. A drop of this solution was applied on the mold during fifteen minutes. Then, the otoliths were rinsed with distilled water and stored in dry condition. Yearly increments were revealed by staining with a drop of 5% Toluidine blue on the otolith and letting it dry. Growth rings were then counted under binocular magnifier. The age of each eel was determined by the number of increments starting from the nucleus, which was considered as year 1 of the eel's life. The otolithometry was realized in partnership with the IRSTEA (Cestas, France). Silver stage was determined by macroscopic characteristics such as the differentiated lateral line (presence of black corpuscules), a contrasting skin color (dark dorsal surface and a white ventral surface), the ocular diameter and the pectoral fin length.

2.4. PCB and PCDD/F analysis

Eel filets and pools of glass eels were analyzed for 18 PCBs (n= 62 and 2 pools of glass eels). Among them, 12 are dl-PCBs (#77; 81; 105; 114; 118; 123; 126; 156; 157; 167; 169; 189) and 6 are ndl-PCBs (#28; 52; 101; 138; 153; 180). Ndl-PCB and dl-PCB levels in eel filets and pools of glass eels were expressed as a sum of all congeners. In order to assess a potential health risk, PCDD/F analyses were achieved on 11 out of 62 eels (5 yellow and 6 silver individuals) and on the 2 pools of glass eels. The PCDD/Fs analyzed were the 17 congeners regulated by the European Union (EC/1259/2011). PCB and PCDD/F levels were expressed by congeners or as a sum of all congeners in $ng.g^{-1}$ dry, lipid or wet weight (dw, lw or ww).

2.4.1 Reagents and Chemicals

All organic solvents (Promochem) were Picograde® quality. Silica (Fluka), sodium sulfate (Merck), and sulfuric acid (SDS) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from Cambridge Isotope Laboratories (CIL) and Wellington

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

2.4.2 Sample preparation procedure

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

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

2.4.3 GC-HRMS measurement

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

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

2.4.4 Toxic equivalency calculation

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

2.4.5. Quality assurance/quality control

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

2.5. Statistical analysis

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

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3. Results and discussion

3.1 Biometric parameters

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

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

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

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

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

Regarding the silver eels, dl-PCB levels, expressed as $ng.g^{-1}$ dw, were significantly higher than results for yellow eels from Varades and Cordemais. Considering the same unit, ndl-PCB levels for silver eels were significantly higher than levels for yellow eels from Varades only. Considering dl- and ndl-PCB levels expressed as $ng.g^{-1}$ lw, the results tend to decrease but are only significantly different to those for yellow eels from Nantes, and whatever all the size classes. This results can be explained by the highly lipid content in silver eels leading to a dilution of the contaminants.

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

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

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

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

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

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

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

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Varades are the lowest contaminated by dl and ndl-PCBs closely related with lower LW. The eels from Nantes and some of those from Cordemais are more contaminated, showing a higher LW. However, eels from Nantes present a higher heterogeneity. The inter-site differences observed could be also related to differences of biometric parameters such as the BL and the age, characterizing a different exposition time (9.8 ± 1.9 years for eels from Nantes compared to 4.4 ± 1.4 years and 5.9 ± 1.9 years for eels from Cordemais and Varades, respectively).

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

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

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

The result of the PCA correlation loading is shown in Fig.3A. The first two principal components of the PCA (respectively PC1 and PC2) describe 85.74% of the total variability among eels. PC1 and PC2 respectively represent 68.85 and 16.89%. This figure highlights that the first principal component is positively correlated to all the individual PCB levels. The second one is negatively correlated to low chlorinated PCBs and positively correlated to highly chlorinated ones.

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

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

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

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

Considering the sampling site influence, an inter-site variability is observed, particularly for eels from Cordemais which display patterns with significantly lower relative proportion of PCBs #28 and 118 whereas the relative proportion of #180 is significantly higher than those of the other sites. About eels from Nantes, the relative proportion of the PCB #101 is significantly higher to the detriment of the #180 with a relative proportion significantly lower. Finally, the PCB #180 is the only one able to discriminate the sampling sites. Its proportion is higher in Cordemais eels, lower in Nantes eels and intermediate in Varades eels. For the other marker-PCBs (#153, 138 and 52), no significant specific trend is observed according to the sampling site.

The PCB contamination variability between **t**he three sampled sites could be partially explained by the anthropogenic activities existing in the area. Indeed, Varades is a relatively small city, marked by several agricultural activities where PCB sources are probably less important than in Nantes or Cordemais. On the contrary, Nantes is an important urban and industrial city, with an important economic and demographic development, where two incinerator factories and various maritime and industrial activities exist. A lot of domestic, industrial and agricultural effluents are discharged into the Loire estuary more or less previously depolluted in sewage treatment plants. Cordemais presents another profile because it is a very small rural city but dominated by its economic and industrial activities, directly

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

The PCB sources are therefore multiple along the estuary and the PCB contamination of this ecosystem could be done following atmospheric or aquatic routes. This complexity prevents from establishing easy correlations between the congener profiles and the sources. Motelay-Massei et al. (2004) showed, in the Seine river basin, that less chlorinated PCB congeners are transported over longer distances from the source sites because of their longer residence time in the atmosphere, whereas the heaviest PCBs tend to be adsorbed on particles and to settle near production sources. Therefore, our results could suggest that eels from Cordemais were living closer to a PCB source than eels from the other sites.

Moreover, the Loire estuary presents strong hydrodynamic, sedimentary and abiotic parameters (Dauvin, 2008). Today, the effects of the tide can be observed within 97 km from the estuary mouth and the salinity moves upstream. This modifies also the temperature from the mouth of the estuary to the upstream front of the salinity which varies of 5°C from downstream to upstream. The Loire estuary is also characterized by a fluid mud which extends over 20 km; it is an important factor in the rapid sedimentation of the estuary, so the turbidity varies from 2 $g.L^{-1}$ at the surface to 20 $g.L^{-1}$ near the bottom. All these parameters (salinity, turbidity, temperature and tidal amplitude) could affect the exposition level of PCBs potentially present in the estuary, and their chemical bioavailability for eels. Moreover, the biological variability of eel PCB levels could also be explained by differences of diets, ecology, physiology or metabolism capacities in relation to polyhaline, mesohaline and oligohaline ecozones.

Regarding the difference of the relative proportions found for silver eels compared to yellow eels, the only significant one is for PCB #28 which is higher in silver eels compared to yellow eels whatever the sampling site considered. The patterns of silver eels are closer to those of eels from Nantes, then Varades, whereas they display many significant differences comparatively to those of eels from Cordemais: all the relative proportions are significantly different except the one of PCB #138.

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

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

3.5 PCDD/F, PCB levels and public health

Mean PCDD/F and dl-PCB levels expressed according to the 2005 WHO recommendations were 5.21 ± 1.78 and 9.88 ± 4.14 $pg.g^{-1}$ WHO_{2005} PCDD/F and dl-PCB TEQ (toxic equivalents) ww for yellow (n=5) and silver individuals (n=6) respectively. Glass eels depicted a mean level significantly lower (0.27 ± 0.03 $pg.g^{-1}$ WHO_{2005} PCDD/F and dl-PCB TEQ ww). The maximum level established for the level of PCDD/Fs in eel filets is currently 3.5 $pg.g^{-1}$ WHO_{2005} PCDD/F TEQ ww and 10 $pg.g^{-1}$ WHO_{2005} PCDD/F and dl-PCB TEQ wet weight (European Union, 2011). These values were not reached regarding the yellow eels. Nevertheless, in the case of silver ones, biological variability was high and 4 out of 6 studied eels displayed WHO_{2005} PCDD/F and dl-PCB TEQ values higher than 10 pg WHO_{2005} PCDD/F and dl-PCB TEQ per gram of wet filet.

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

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

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

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

Conclusion

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

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

Apart from an eventual sanitary problem, the contamination of eels could lead to damages for the eel population by affecting their reproduction and by a transfer of pollutants to eggs. Indeed, since these compounds are lipophilic, the results showed that the PCB levels are correlated to the lipid content in the filets. Lipids are essential compounds for the migration reproduction (van Ginneken et al., 2009) and for both fat deposition in the oocytes and later incorporation of vitellogenic stores in eggs. Moreover, acclimation of eels to seawater, silvering process and reproduction migration are under different endocrine controls and fuel consuming. This energetic cost is described to increase significantly when lipid filets of swimming eels are charged in PCB mixture (after intraperitoneally injection of 5000 ng g^{-1} PCB # 153, 7 ng g^{-1} PCB # 126 and 50 ng g^{-1} PCB # 77) (Thillart et al., 2009). PCB levels determined in eels from the Loire estuary could thus potentially have an impact on the reproduction success of European eels.

The comparison of eel biomonitoring studies highlighted heterogeneity in sampled individuals. To better correlate studies at the international level, it appears necessary to standardize parameters such as age, length, sex and sexual maturation stage. To preserve this endangered species and such as recommended by scientists (van Ginneken et al., 2009), the environmental quality of its habitats should be restored and protected. Considering our results, the European eels from the Loire estuary appeared moderately contaminated compared to eels from other major international estuaries, suggesting a moderate PCB contamination of the Loire estuarine system. These conditions could contribute therefore to preserve genitors.

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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 ($ng.g^{-1}$ dw)	ndl-PCB levels ($ng.g^{-1}$ dw)	dl-PCB levels ($ng.g^{-1}$ lw)	ndl-PCB levels ($ng.g^{-1}$ lw)	ndl-PCB levels ($ng.g^{-1}$ ww)	Marker-PCB levels ($ng.g^{-1}$ ww)	Marker-PCB levels ($ng.g^{-1}$ 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

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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 $ng.g^{-1}$ 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 $ng.g^{-1}$ 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.

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10.1016/j.scitotenv.2013.11.037

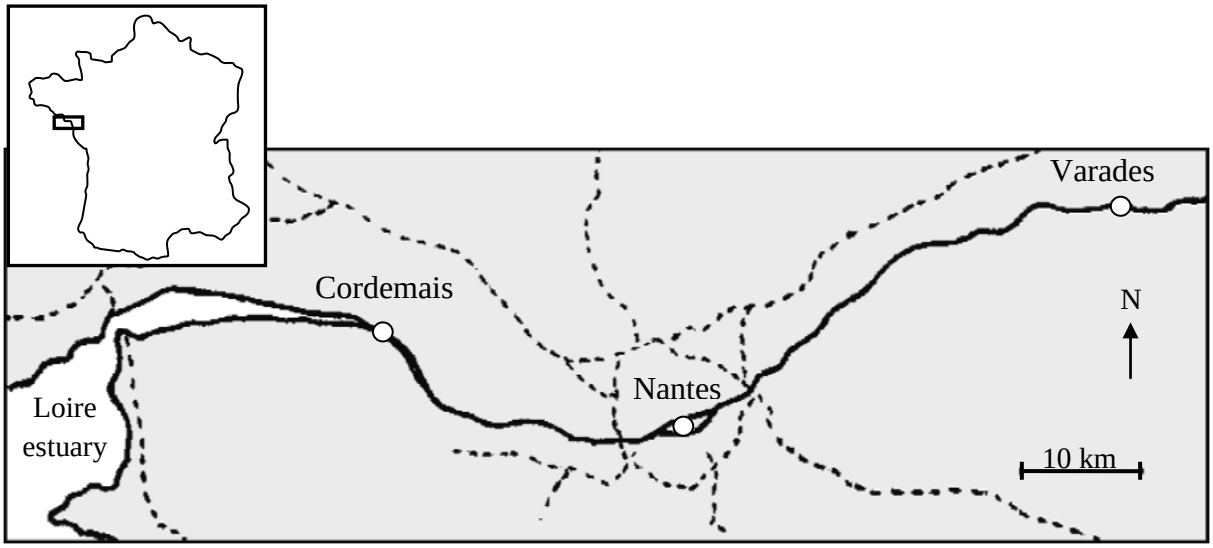

Figure 1

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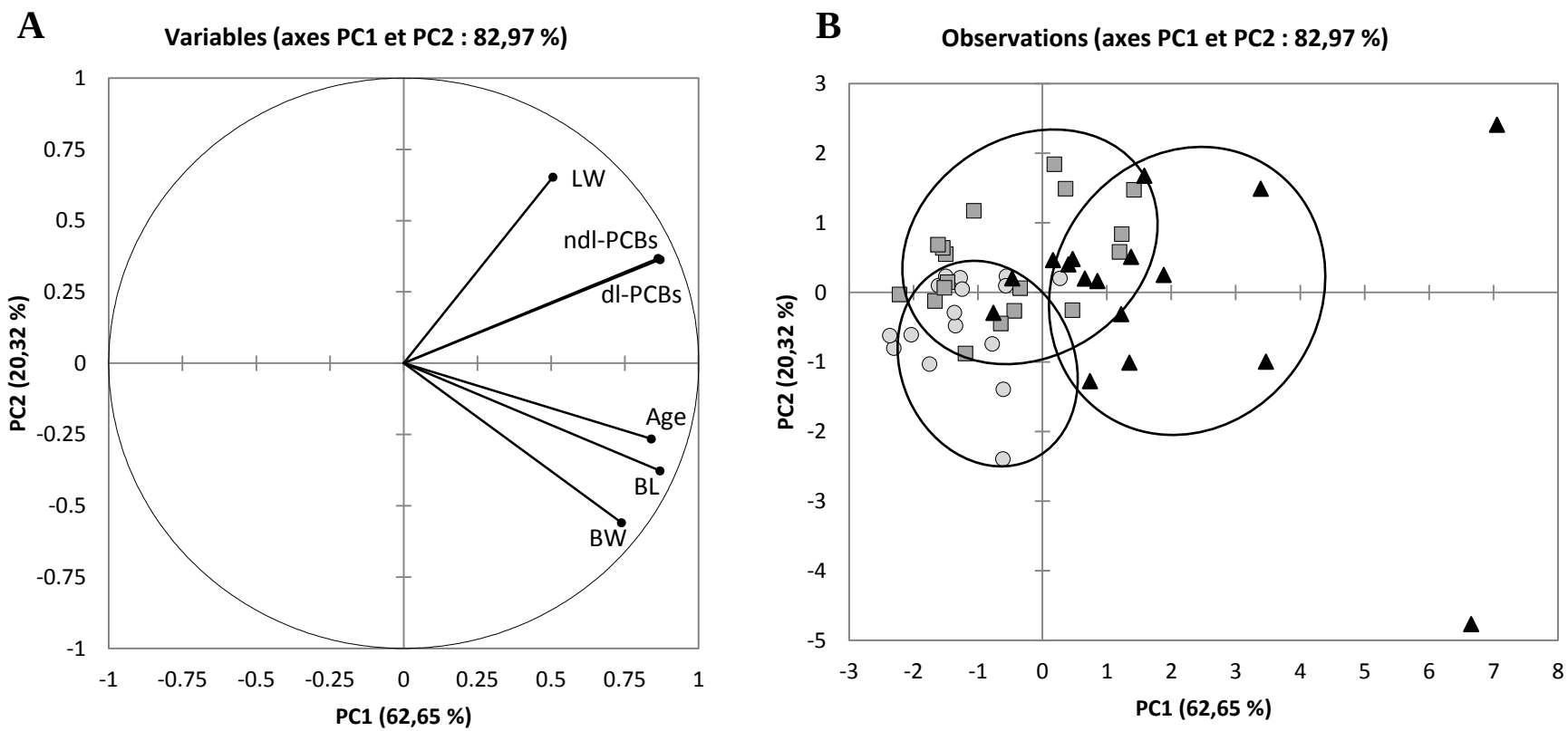

Figure 2

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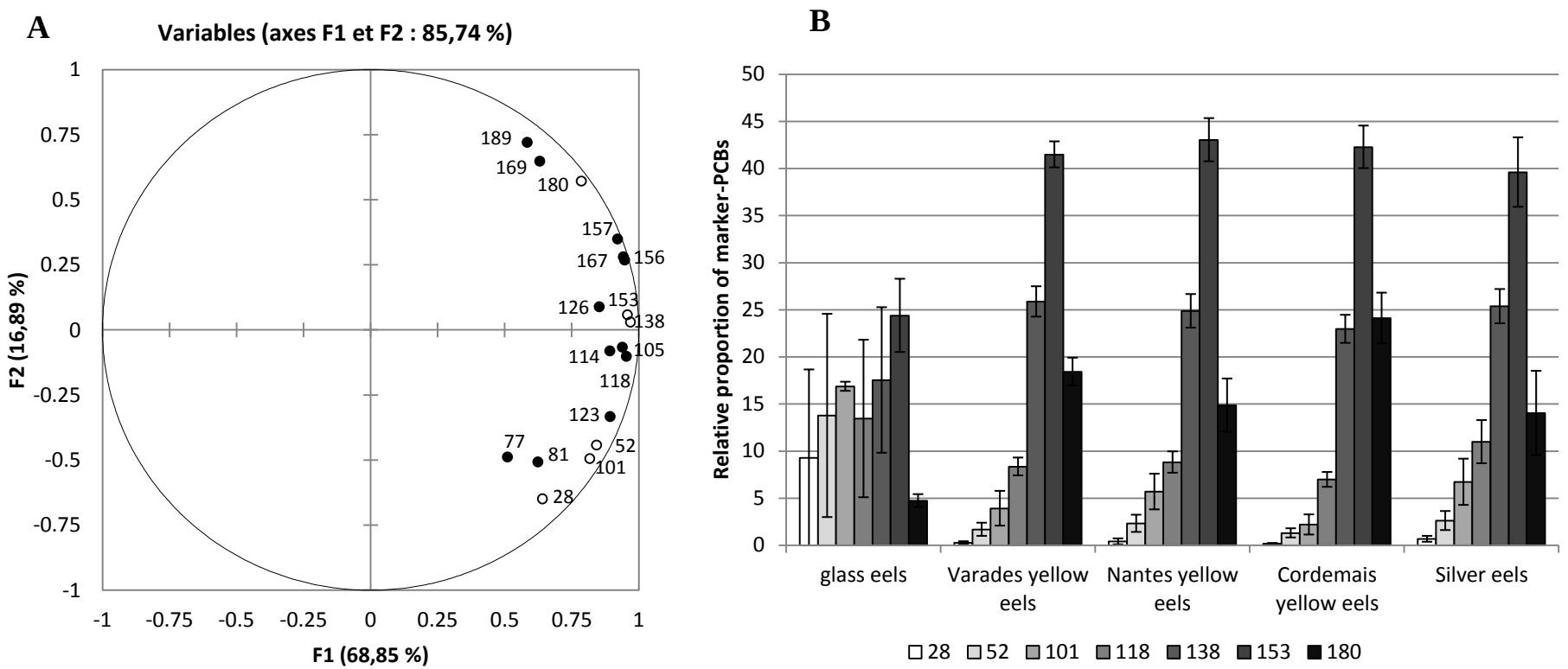

Figure 3

*Conflict of interest

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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.

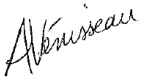
We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (**isabelle.blanchet-letrouve@ac-nantes.fr**).

Signed by all authors as follows:

Author name	Date	Signature
I. Blanchet-Letrouvé	27/06/2013	
A. Zalouk-Vergnoux	09/07/2013	
A. Vénisseau	11/07/2013	
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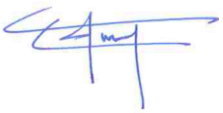
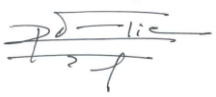
B. Le Bizec	1st July 2013	
P. Elie	27/06/2013	
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