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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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HIGHLIGHTS

- PCBs and PCDD/Fs in eels from a moderately polluted estuary (France) were determined.
- Variability according to the site and life stage was observed.
- PCB pattern of glass eels underlined a different bioaccumulation pathway.
- Overall, eels from this estuary showed an intermediate contamination level.
- More than 60% of silver eels displayed values higher than the EU permissible level.

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ABSTRACT

To characterize the eel contamination by dioxin-like (dl) and non dioxin-like (ndl) polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), sixty-two eels from the Loire estuary (France) were analyzed. PCB contamination significantly increased from glass eel stage (3.71 ± 1.85 and 15.2 ± 4.2 ng g^{-1} dw) to other life stages (for yellow eels: 62.8 ± 34.4 and 382 ± 182 ng g^{-1} dw; for silver eels: 93.7 ± 56.3 and 463 ± 245 ng g^{-1} dw respectively for dl and ndl − PCBs). An inter-site variability based on PCB levels and profiles was observed among the three studied sites. For glass eels, the profile was mainly characterized by less chlorinated PCBs contrary to the other eels, displaying a different bioaccumulation pathway. Overall, the contamination level in the eels from this estuary was shown to be low for PCDD/Fs and intermediate for dl and ndl-PCBs, compared to other international/national areas. However, more than 60% of the studied silver eels displayed higher values for PCDD/F and dl-PCB WHO_{2005} TEQ than the EU permissible level of 10 pg g^{-1} ww. This statement suggests a potential exposure to PCBs through eel consumption, especially with silver eels, and also points out apparent contamination that could eventually affect the reproductive success of the species.

1. Introduction

Since the 1980s, monitoring studies in European countries have shown the decline of glass eels arriving in coastal waters and estuaries (ICES, 2006). Similar steep declines of the prepubertal European eel (*Anguilla anguilla*) were also reported a few decades earlier and stocks were now 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 factors, polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) seem to be particularly incriminated because of their potentials as estrogenic and anti-estrogenic disruptors (Canapa et al., 2002) and their neuroendocrine effects (Kodavanti and Curras-Collazo, 2010). They endanger several fish species including the eel population (Van Ginneken et al., 2009). PCBs represent a particularly persistent chlorinated chemical group of 209 congeners, which is ubiquitous in the environment and from an 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

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compounds and the non dioxin-like PCBs (ndl-PCBs) (European Union, 2011). These classes were related to chemical structure features such as the number and positions of the chlorine atoms. 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 1980s, the accumulated production all over the world was estimated at 1,200,000 t and approximately 30% of this production is scattered in the environment, with essentially all 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 high body lipid content, a significant longevity and a carnivorous status, the European eel is extremely exposed to lipophilic persistent contaminants, such as PCBs, and represents a species sensitive to their bioaccumulation (Roche et al., 2000). Moreover, eels constitute an important economic value for nearby estuaries and rivers and an essential food resource (Després, 2009). Significant levels of PCBs have already been reported in European eels from the Gironde and Adour estuaries (France) (Tapie et al., 2011), in the Mondego estuary (Portugal) (Nunes et al., 2011), in the rivers of Italy (Mezzetta et al., 2011) and were suggested to 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 maximum value (European Union, 2011) and is also a potential risk for eel health itself (for review, Geeraerts and Belpaire, 2010). The Loire estuary's basin (117,800 km^2) drains tributaries and 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 (Grosbois 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 paths of glass eels. The preservation of its chemical quality is therefore essential for eel health. However, a significant lack of data on the POPs contamination levels of European eels exists in this ecosystem, as only few individuals, sampled on the entire 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. The present study was set out to reach three objectives: i) to get a representative assessment of PCB and PCDD/F contamination of European eel in the Loire estuary, according to the life stage; ii) to assess spatial PCB contamination variations with yellow individuals (similar size class distributions), along three different Loire estuary sites (Fig. 1), and iii) to evaluate health risks for local consumers 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 at the upstream boundary of the estuary (100 km from the Loire mouth); it also presents few industrial activities and is under particular agricultural pressure. The intermediate site is close to an important city, Nantes (about 600,000 locals) 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. It is strongly influenced by the North Atlantic Ocean and is well-known for its industrial activities, particularly the presence of a coal-fired power plant and its close proximity to an industrial complex including oil refineries. These three sampling sites were chosen to display different kinds of human activities on the estuary.

2.2. Sampled animals

Eels were sub-sampled from a previous ecotoxicology study (Blanchet-Letrouvé et al., 2013). The number of samples was in agreement with similar studies involving eel contamination (Daverat et al., 2011; Ferrante et al., 2010; Quadroni et al., 2013). During a year and a half, i.e. from May 2009 to January 2011, European eels were captured by local fishermen according to the fishing regulations, in the three sampling sites described above. Sixty-two yellow and silver eels were collected with fyke and stow nets respectively. Sixteen 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 per site. To evaluate the trend in contaminant level over life stage, glass eels (two pools of 40 individuals) 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 for a few hours until dissection under a natural photoperiod (L15/D9) and at a temperature around 12 ± 2 °C, which is 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

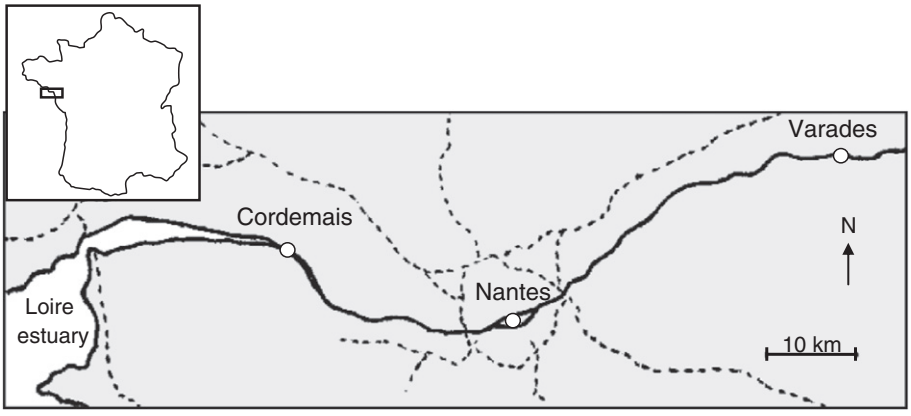

Fig. 1. Studied area: the Loire estuary (France). Three sampling locations (Cordemais; Nantes and Varades).

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 killed, skinned and dissected in order to collect filets and otoliths. Biometric parameters were recorded to evaluate the Fulton's condition factor ($K = (BW \times 10^5)/BL^3$ with BW and BL respectively expressed as g and mm) (Fulton, 1904).

The otoliths were used 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 and dried with ethanol. 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 (1 μm grain) on a polishing cloth. Etching was done using 10% EDTA. A drop of this solution was applied on the mold for fifteen minutes. Then, the otoliths were rinsed with distilled water, stored in dry condition and stained with a drop of 5% Toluidine blue following grinding of the convex side (ICES, 2006). After drying, growth rings were counted using a light stereomicroscope. Individual age of eel was determined from reading annual otolith rings starting from the nucleus, considered as year 1 of the eel's life. The otolithometry was realized in partnership with the IRSTEA (Cestas, France). The life stage of silver eel 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). 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). Dl, ndl and marker PCBs in eel filets and pools of glass eels were expressed as a sum of all congeners or by congeners in ng g^{-1} wet, dry or lipid weight (ww, dw or lw respectively). Moreover, to estimate health risks from consumption and effects on eel, Toxic Equivalent (TEQ) values were calculated according to the World Health Organization Toxic Equivalency Factors (WHO TEFs) for human. To easily compare results from our study with literature, WHO_{1998} TEFs and WHO_{2005} TEFs were used (Van den Berg et al., 1998; Van den Berg et al., 2006). Results of dl-PCBs and PCDD/Fs were thus expressed in pg g^{-1}, in pg WHO_{1998} TEQ g^{-1} and in pg WHO_{2005} TEQ g^{-1} on a fresh weight basis.

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 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 (dw) of filets and pools of glass eels were cut, dehydrated, and milled using a turbo-mixer with glass bowl. 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 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 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 PCDD/F fraction was purified on a Carbopack C/Celite 545 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, and $^{13}C_{12}$-1,2,3,4-TCDD for PCDD/Fs), 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 magnetic and electric sector high resolution mass spectrometer (Jeol, Tokyo, Japan). A DB5MS (30 m × 0.25 mm × 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 (7 min), for PCDD/Fs the GC program was 120 °C (3 min), 20 °C/min to 170 °C (0 min), 3 °C/min to 260 °C (0 min) and finally 25 °C/min to 300 °C (5 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 a 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. Quality assurance/quality control

All these procedures integrated quality control parameters to fulfill the requirements of the Commission Directive 2012/252/EC and 2012/278/EC, 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 feeding stuffs 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 test (Kruskal–Wallis followed by Conover–Iman post-hoc test) was used to compare 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) was performed. PCDD/F and dl-PCB WHO_{2005} TEQ values were compared according to life stages using Mann–Whitney test at a significant level of 5%. All statistical treatments were performed with XLstat software.

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. These parameters allow characterizing of the local status of sampled individuals. The increase of BW was positively correlated with the increase of BL whatever the life stage (yellow or silver). The linear regression equations were BL = 128.92 lnBW-169.82 $R^2 = 0.97$ (n = 49) for yellow eels and BL = 220.94 lnBW-685.9 $R^2 = 0.98$ (n = 13) for silver eels. For yellow eels, no significant difference in the regression was shown according to the different sampling sites. These high correlations are in agreement with those found in other different European sites (Adam et al., 2010) and permit to divide the sampled fraction of the population according to a coherent set of biological characteristics. 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 other sites and for silver eels. Regarding the Fulton's condition factor values (K), no significant difference was found between eels from the different sampling site with values ranging from 0.13 to 0.17. As well, no difference was observed between silver and yellow eels, except with those from Varades (p-value <0.003). According to Feunteun (2002), these values are representative of good eel health in the Loire estuary. Such values are similar to Fulton's condition factor values found in other studies about European areas (Gravato et al., 2010; Palstra et al., 2007b; Tapie et al., 2011). Nevertheless, better eel conditions were reported for eels in some other studied sites like the River Rhine Watershed and Lake Ijsselmeer despite higher PCB contamination levels (Haenen et al., 2010; Santillo et al., 2005). Eels from less impacted Italian water bodies showed a low growth rate and condition status (Quadroni et al., 2013). No relationship between the Fulton's condition factor and levels of contaminants can be noticed since this heterogeneous factor characterizes also the habitat context and trophic conditions (Melià et al., 2006).

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 LOQ ranged from 0.16 (PCB #169) to 1.72 (PCB #156) pg g^{-1} ww for dl-PCBs and from 0.67 (PCB #101) to 13.4 (PCB #28) pg g^{-1} ww for ndl-ones). The sums of dl and ndl-PCBs are 3.71 ± 1.85 ng g^{-1} dw and 15.2 ± 4.2 ng g^{-1} dw respectively. These low but significant levels could be the result of a contamination via the food web during the leptocephali stage and of a direct exposure from the aquatic compartment. Indeed, at the end of the reproduction period in the Sargasso Sea, eel eggs hatch and free leptocephali larvae drift back towards the Atlantic coasts (Tesch and White, 2003). During their 6000 km migration from important pollution sources, these larvae eat essentially oceanic plankton and are mainly contaminated by feeding uptake. When they approach the continental shelf claim, the metamorphosis in glass eels occurs and is associated with a feeding interruption (Elie et al., 1982). During this period, glass eels are exposed to contaminants only by direct exposure from the abiotic compartment (dissolved phase, particles and sediments) (Tapie et al., 2011). Another explanation could be an intergenerational transfer of contaminants during oogenesis

Table 1
Means and standard deviations of PCB levels (dl, ndl and seven markers) 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	Σ dl-PCBs		Σ ndl-PCBs			Σ 7 PCBs	
									ng g^{-1} ww	ng g^{-1} dw	ng g^{-1} ww	ng g^{-1} dw	ng g^{-1} lw	ng g^{-1} ww	ng g^{-1} lw
Glass eels		<200	2 pools	≤90	62 ± 12	<1	4.0 ± 0.8	n.d.	0.78 ± 0.48	3.71 ± 1.85	18.6 ± 8.3	3.03 ± 0.43	15.2 ± 4.2	78.1 ± 26.3	3.49 ± 0.16	89.2 ± 21.8
Yellow eels	Varades	200–300	5	279 ± 14	30 ± 3	5.2 ± 0.8	4.9 ± 2.3	0.14 ± 0.02	13.0 ± 4.6	41.4 ± 12.4	286 ± 69	79.7 ± 28.6	254 ± 78	1764 ± 458	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	12.1 ± 4.2	37.6 ± 9.3	349 ± 372	72.7 ± 22.2	228 ± 47	1183 ± 510	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	14.0 ± 2.2	48.2 ± 6.5	334 ± 198	76.7 ± 14.2	261 ± 12	1770 ± 1006	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	10.1 ± 7.5	29.0 ± 13.8	169 ± 121	69.1 ± 58.8	195 ± 118	1041 ± 619	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	23.3 ± 8.9	71.8 ± 15.7	266 ± 131	144 ± 49.1	449 ± 78.5	1657 ± 722	158 ± 54.7	1810 ± 799
		400–500	4	452 ± 33	129 ± 30	9.5 ± 0.5	10.2 ± 3.2	0.14 ± 0.01	26.0 ± 3.0	82.2 ± 10.3	278 ± 103	152 ± 16.4	482 ± 74.4	1669 ± 797	167 ± 17.3	1827 ± 852
		500–600	4	546 ± 24	259 ± 49	10.5 ± 1.2	11.2 ± 6.7	0.16 ± 0.01	32.5 ± 13.5	97.8 ± 31.0	344 ± 135	181 ± 77.2	543 ± 171.3	1909 ± 767	199 ± 84.5	2100 ± 828
		>600	3	678 ± 63	551 ± 183	11.0 ± 3.9	11.6 ± 8.9	0.17 ± 0.03	46.6 ± 37.7	135 ± 83	488 ± 244	252 ± 188	735 ± 402	2706 ± 1365	279 ± 210.0	2986 ± 1508
	Cordemais	200–300	5	272 ± 10	26 ± 2	3.1 ± 0.7	6.6 ± 2.8	0.13 ± 0.01	13.4 ± 3.6	45.6 ± 11.4	238 ± 126	95.8 ± 23.5	327 ± 78.8	1291 ± 490	102 ± 25.1	1910 ± 1258
		300–400	5	342 ± 36	61 ± 19	3.8 ± 0.6	12.0 ± 3.6	0.15 ± 0.02	20.8 ± 9.0	61.8 ± 22.7	172 ± 45.5	136 ± 63.1	404 ± 166	1130 ± 378	146 ± 67.7	1217 ± 401
		400–500	4	455 ± 17	147 ± 12	5.5 ± 0.4	7.7 ± 3.3	0.16 ± 0.01	13.2 ± 2.9	46.1 ± 6.7	191 ± 68.8	88.1 ± 21.0	307 ± 42.6	1275 ± 476	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	26.2 ± 7.2	76.1 ± 17.2	185 ± 54.4	165 ± 37.0	479 ± 68.9	1170 ± 285	178 ± 40.9	1263 ± 312
Silver eels		>500	13	659 ± 124	517 ± 344	12.4 ± 3.8	25.6 ± 3.5	0.16 ± 0.01	41.5 ± 26.5	93.7 ± 56.3	161 ± 96	205 ± 113	463 ± 245	800 ± 425	229 ± 130	895 ± 485

n.d.: non determined.

and vitellogenesis (Van Ginneken et al., 2009). It has been shown that PCBs and other hydrophobic pollutants can be transferred from females to eggs and thus impairing larval survival and development (Gutleb et al., 1999, 2007). This has been demonstrated in different Teleost fish (for instance, the European hake *Merluccius merluccius* and the zebrafish *Danio rerio*) for congener #153 (Bodiguel et al., 2009; Daouk et al., 2011).

Concerning yellow eels, the PCB contamination was significantly higher than that of glass eels whatever the sampling site and the size class considered. Regarding each site, no significant difference in ndl or dl-PCB levels expressed as ng g^{-1} dw or lw was found between the different size classes (Kruskal–Wallis, p-value >0.1). This observation could be attributed to the low sample number per size class. For eels from Nantes, ndl and dl-PCB levels expressed on dw basis increase with increasing length, weight and age (Pearson correlations with p-values <0.05 for correlations between ndl and dl-PCB levels (dw basis) and BW; and p-values <0.01 for correlations between ndl and dl-PCB levels (dw basis) and BL or age). These correlations were not observed for Cordemais and Varades eels. Eels from Nantes were relatively older (9.8 ± 1.9 years compared to 4.4 ± 1.4 and 5.9 ± 1.9 years for eels from Cordemais and Varades, respectively; p-values <0.006). Moreover, they presented a Fulton's condition factor equivalent to silver eels. Comparatively, in Cordemais and Varades, individuals were globally smaller and tend to present a lower Fulton's condition factor. This statement could indicate that yellow eels sampled in Nantes present some physiological specificity suggesting for instance a reversal silver process or sedentism (Adam et al., 2010).

Regarding the silver eels, dl-PCB levels (dw basis) were significantly higher than dl-PCB levels for yellow eels from Varades and Cordemais. Considering the same unit, ndl-PCB levels of silver eels were significantly higher than levels depicted for yellow eels from Varades only. Considering dl- and ndl-PCB levels expressed on lw basis, the results of silver eels tend to be lower than those of the yellow ones. This statement was however only significant when comparing to yellow eels from Nantes regardless of the size class. These differences could be linked to physiological modifications occurring with silvering process. On the one hand, silver eels accumulated energy reserves in order to ensure their sexual maturation and their swimming towards spawning areas (Durif et al., 2005). This accumulation is gradual during the silvering. In this study, significant high lipid content was noticed for silver eels compared to yellow eels. Several authors showed an accumulation of pollutants linked to the increase of eel lipid content (Durif et al., 2005; Robinet and Feunteun, 2002). This could partially explain the higher dw basis PCB concentrations in silver eels. On the other hand, silver eels are migrants and could come from less contaminated upstream fresh water areas. In these areas, pollutants could be less accumulated which could explain the lower lw basis concentrations in silver eels. Finally, when eels start their downstream migration, they begun their starvation and must have started to mobilize their energy supply from liver and fat (Durif et al., 2005). This process induces a release of lipophilic compounds such as PCBs into the blood (Geeraerts and Belpaire, 2010). This hypothesis was not validated in our work and requires to be tested by specific PCB measures on lipids using a sufficient number of larger yellow and silver eels growing towards their sexual maturity.

In a previous work (Tapie et al., 2011), a literature review about marker PCB levels in *A. anguilla* filets was achieved. The seven marker PCB congeners are #28, 52, 101, 118, 138, 153 and 180 and correspond to about half of the amount of total PCBs. This sum is considered as an appropriate marker for occurrence and human exposure of PCBs (European Union, 2011).the dl-PCB congener #118 was usually used as a marker PCB until the current regulation (European Union, 2011) which defined now 6 PCBs markers on the basis of the ndl-ones exclusively. To compare PCB levels of eels from the Loire estuary to biomonitoring results from other countries, the values obtained in this study for the seven marker congeners were summed, and expressed as ng g^{-1} ww and lw (Σ 7 PCBs; Table 1).

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, where Σ 7 PCBs ranged from 14 to 756 ng g^{-1} lw (Bordajandi et al., 2003; Corsi et al., 2005; McHugh et al., 2010; Santillo et al., 2005). However, higher levels were observed for the River Elbe in Czech Republic, the Tevere river in Italy, Flanders in Belgium, different lakes in Finland and some estuaries in The Netherlands, where Σ 7 PCBs ranged from 2176 to 9947 ng g^{-1} lw (Belpaire et al., 2011; De Boer et al., 2010; Maes et al., 2008; Santillo et al., 2005; Tulonen and Vuorinen, 1996). Throughout France, eels from the Loire estuary are slightly more contaminated than those from the Vacares lagoon (Σ 7 PCBs = 176 ng g^{-1} ww) and about two times more than those from the Thau pound (Σ 7 PCBs = 1036 ng g^{-1} lw) (Oliveira Ribeiro et al., 2008; Santillo et al., 2005), whereas they are less contaminated than eels from the Rhone River (Σ 7PCBs ranging from 4117 to 12759 ng g^{-1} lw) and the Gironde estuary (Σ 7PCBs ranging from 2569 to 4410 ng g^{-1} lw) (Tapie et al., 2011).

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 on dw basis. Since silver eels are not strictly territorial, due to their downstream migration, they could have originated from other sites than the 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 Fig. 2 (respectively Fig. 2A and 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 is consistent with the results of other authors and was to be expected according to the lipophilic properties of PCBs and the increase of lipid content during the last years of the yellow phase (Van der Oost et al., 1996; Durif et al., 2005). This positive relationship could explain the increase of PCB levels with growth depicted for yellow eels from Nantes.

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 B underlines that eels from Varades are the lowest contaminated by dl and ndl-PCBs (dw basis). This result was however closely related with lower LW (Mann–Whitney *U* test, p-value = 0.03). Lipid normalized PCB concentrations in eels from Varades, were actually comparable with those from Cordemais and Nantes (Mann–Whitney U tests, p-values for dl and ndl-PCBs respectively: 0.11 and 0.22 comparing to Cordemais; 0.33 and 0.47 comparing to Nantes). Considering this lw basis, the contamination level in Varades was then similar with Cordemais and Nantes. The eels from Nantes tend to be the most contaminated, with a high heterogeneity. A significant difference of lipid normalized PCB levels was found between eels from Nantes and Cordemais (Mann–Whitney U tests, p-values = 0.004 and 0.030 for dl and ndl-PCBs respectively). Varades, which is located upstream in the estuary, is a small and quite preserved city with about 3550 locals and with few industrial activities. It is relatively unexpected that the contamination level of this site was similar with Cordemais and Nantes. Nantes is indeed an important city with about 600,000 locals and Cordemais is downstream of Nantes and well-known for its industrial activities. Therefore, the living area of eels seems therefore to affect the contamination level as it was already shown in the Gironde estuary (Tapie et al., 2011). These differences were also closely related to variations of biometric parameters such as BL and age, both characterizing for

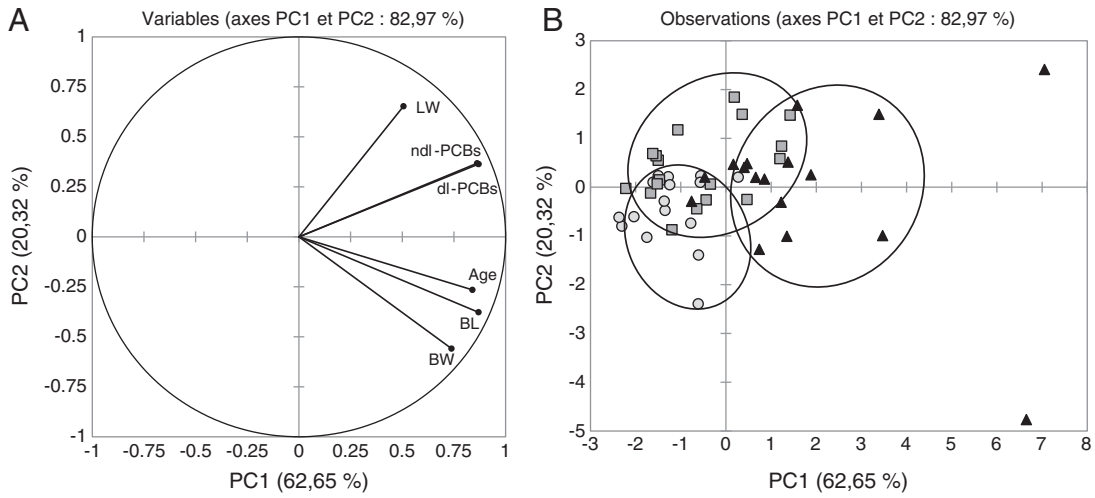

Fig. 2. Principal Component Analysis of biometric parameters and dl- and ndl-PCB levels expressed in ng g^{-1} dw in muscles of yellow eels from 3 sampling sites (n = 49): Varades, Nantes and Cordemais. A: correlation loadings (BW: body weight; BL: body length; LW: lipid weight); B: sample representation (circles = eels from Varades; triangles = eels from Nantes; squares = eels from Cordemais).

instance a higher exposure time for yellow eels from Nantes, such as previously described. These inter-site differences are highlighted in the next section dealing with each PCB profile.

3.3. PCB profiles

To evaluate the influence of the sampling site on PCB profiles in yellow eels, a second PCA was performed using each individual PCB level expressed as ng g^{-1} dw. The result of the PCA correlation loading is shown in Fig. 3A. The first two principal components (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 individual PCB levels. The second one is negatively correlated to low chlorinated PCBs and positively to highly chlorinated congeners.

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 on the right bottom of the correlation circle. The higher chlorinated congeners with several Cl atoms in meta and para-positions are located in the right top of the circle. Ndl-PCBs are considered as particularly persistent and ubiquitous contaminants 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).

3.3.1. Influence of the life stage

Overall, the ndl-PCBs contributed mainly to total PCB levels compared to dl-PCBs. Their shares were on average 79.4% for glass eels, 86.0% for yellow eels and 83.0% for silver eels. The accumulation profiles of the seven marker-PCBs in glass, silver and yellow eels from the three sampling sites were presented in Fig. 3B. Whatever the life stage, the main congener was PCB #153. Interestingly, this PCB is considered as a non metabolizable congener, a tracer of bioaccumulation process. Variation in its proportion suggests a difference of contamination profiles related to a difference of diet, ecology, physiology or metabolization capacities (Tapie et al., 2011). Its share was the lowest for glass eels with a mean value of 24.7%. For silver eels, this congener contributed to the marker-PCB profile with a mean of 39.3%. A higher value (mean 41.5%) was found for yellow eels from Varades. The highest contributions (mean 42.3 and 42.8% respectively) were depicted for individuals

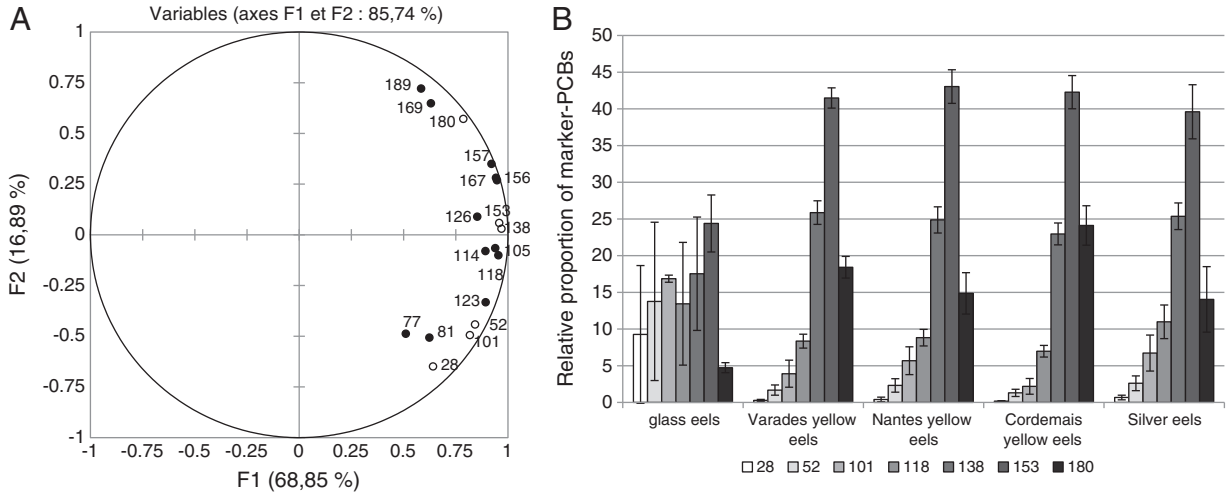

Fig. 3. Representation of PCB patterns. A: correlation loadings of Principal Component Analysis of dl and ndl-PCB muscle levels expressed in ng g^{-1} dw in yellow eels from 3 sampling sites (n = 49): Varades, Nantes and Cordemais (dl-PCBs: black circles; ndl-PCBs: white circles); B: relative proportion (in %) of marker-PCBs in eel filets according to the life stage and the sampling site.

from Cordemais and Nantes. These values were significantly higher than values depicted for glass and silver eels (Kruskal–Wallis test, p-values <0.003 and 0.002 respectively). Overall, PCB profiles of silver eels were closer to those depicted by yellow eels from Nantes as only PCBs #28 and #153 were different, with the percentage of PCBs #28 being significantly higher in silver eels (p-value <0.0001). The eels from Cordemais displayed the most significant difference because all the PCB relative percentages are significantly different from those of silver eels (p-values ranging from 0.0001 to 0.002). The singular profile of silver eels could be the result of several factors. Silver eels fished during the downstream run could have come from upstream, from less contaminated fresh water areas far from PCB sources. In these areas, the less chlorinated PCBs are more present as these light compounds are transported over longer distances because of their longer residence time in the atmosphere (Motelay-Massei et al., 2004). On the other hand, we could suppose that the mobilization of lipids during silvering process lead to differences of PCB profiles according to the life stage.

Concerning the glass eels, a contrasting profile was noticed, mainly characterized by an important proportion of less chlorinated PCBs to the detriment of the heaviest PCBs, which implied a different bioaccumulation process. This was already shown in a previous study (Tapie et al., 2011) in which congeners 28, 50, 52, 101, and 118 represented 51% of accumulation profile for glass eels sampled in the Gironde estuary. Comparatively, the relative proportion of these congeners (without the PCB #50 not analyzed in this study) represents 53% of glass eels in our study. On one hand, according to Tapie et al. (2011), this singular profile could confirm a contamination mainly by direct exposure during the period of metamorphosis into glass eels. In the water column, the less-chlorinated compounds, more polar, shared a significant percentage of the dissolved phase and particulate matter (Cailleaud et al., 2007). On the other hand, the transfer of low chlorinated PCBs during the oogenesis, more efficient than those of the heavy chlorinated PCBs, could be linked to this singular profile (Bargar et al., 2001; Verreault et al., 2006).

3.3.2. Influence of the sampling site and environmental parameters

The sampling site influence has been explored regarding the non-migrant yellow eels. A variability of PCB profiles was observed, particularly for eels from Cordemais which displayed profiles with significantly lower proportions of PCBs #28 (p-values <0.004) and 118 (p-values <0.0002), and higher proportion of #180 (p-values <0.0001) compared to eels from other sites. PCB #180 appeared to be the only congener able to discriminate amongst the sampling sites. Its proportion was shown to be high in Cordemais eels, low in Nantes eels and intermediate in Varades eels. For the marker-PCB#153, no significant difference was observed according to the sampling site (p-values >0.047).

In estuaries, the PCB sources are multiple and the contamination could be done following atmospheric or aquatic routes. PCBs are mainly sorbed to suspended particulate matter and sediment, but they may also be free in the dissolved phase, bound to dissolved organic matter (Cailleaud et al., 2009). Hydrophobic organic contaminant exchanges between the suspended particulate matter or sediments and the water column have been shown by resuspension of in-place sediments during tidal and wind events (Eggleton and Thomas, 2004). In several estuaries, inputs resulting from PCB resuspension processes were shown to be the dominant source compared to fluvial inputs or atmospheric depositions (Cailleaud et al., 2009). Moreover, the Loire estuary is clearly tidally dominated, leading to strong hydrodynamic, sedimentary and abiotic parameters (Dauvin, 2008). The marine tidal effects can be observed up to 97 km away from the estuary mouth. This dynamic modifies the temperature from the mouth of the estuary to the upstream boundary leading to variations of 5 °C from downstream to upstream. The Loire estuary is also characterized by a fluid mud which extends over 20 km in the downstream part (Cordemais site) and the magnitude is influenced by tidal currents; so the turbidity varies from 2 $g \cdot L^{-1}$ at the surface to 30 $g \cdot L^{-1}$ near the bottom (GIP, 2010). All these parameters (salinity, turbidity, temperature and tidal cycles) were known to affect the bioavailability of PCBs (Cailleaud et al., 2009; Turner, 2003). For instance, in the Seine estuary, highest PCB levels were observed in surface and bottom waters when suspended particulate matter remobilizations were maximum, in relation to higher speed currents. In the Loire estuary, geochemical processes and transport of organochlorinated contaminants during tidal cycles are lacking and must be investigated to assess the water quality of this estuary.

On the other hand, the three sampled sites showed different anthropogenic pressures. 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 a significant economic and demographic development, where various maritime and industrial activities exist. High amounts of domestic, industrial and agricultural effluents are discharged into the Loire estuary, with somewhat efficient preliminary depollution in sewage treatment plants. The eels from Cordemais presented a unique PCB profile, which could be explained by the presence of PCB sources around Cordemais. Indeed, this area is dominated by substantial industrial activities such as a coal-fired power plant, an incinerator factory, an industrial complex including oil refineries and an old industrial waste dump (Boudet, 2003).

3.4. Dioxins, furans and dl-PCBWHO TEQ values

Table 2 presented the mean concentrations of PCDD/Fs and dl-PCBs in eels, according to the life stage. Mean concentration of PCDD/Fs (Σ PCDD/Fs) was 1.42 pg g^{-1} ww for glass eels. A higher value (2.94 pg g^{-1} ww) was noticed for yellow eels and a maximum level of 3.61 pg g^{-1} ww has been reached for silver eels. According to WHO_{1998} TEFs, mean values (Σ PCDD/F TEQ 98) of 0.12, 0.68 and 1.00 pg WHO_{1998} TEQ g^{-1} ww were respectively obtained. The WHO_{2005} concentrations (Σ PCDD/F TEQ 05) were about 18% less on average. These levels are similar to concentrations measured in eel from Polish lagoons (0.30–1.88 pg WHO_{1998} TEQ g^{-1} ww; Szlinder-Richert et al., 2010). Lower concentrations were measured in eels from the River Turia in Spain (0.369–0.580 with mean of 0.2 pg WHO_{1998} TEQ g^{-1} ww; Bordajandi et al., 2003). Higher concentrations have

Table 2
Concentrations of PCDD/Fs and dl-PCBs in the European eels from the Loire estuary. For each life stage and contaminant class, the number of eel analyzed (n), the mean concentrations expressed as $pg \cdot g^{-1}$ wet weight (ΣPCDD/Fs or Σdl-PCBs), pg WHO_{1998} TEQ g^{-1} (Σ TEQ 98) and pg WHO_{2005} TEQ g^{-1} (Σ TEQ 05) are given.

		Glass eels	Yellow eels	Silver eels
PCDD/Fs	n	2 pools	5	6
	Σ PCDD/Fs	1.42 ± 0.11 (1.35–1.50)	2.94 ± 1.77 (1.54–5.99)	3.61 ± 1.78 (2.25–6.80)
	Σ PCDD/F TEQ 98	0.12 ± 0.01 (0.11–0.13)	0.68 ± 0.39 (0.40–1.37)	1.00 ± 0.59 (0.47–2.00)
	Σ PCDD/F TEQ 05	0.09 ± 0.01 (0.09–0.10)	0.56 ± 0.32 (0.33–1.11)	0.82 ± 0.50 (0.42–1.70)
dl-PCBs	n	2 pools	49	13
	Σ dl-PCBs	780 ± 480 (440–1120)	20283 ± 13555 (4753–89218)	41517 ± 26496 (6939–115786)
	Σ dl-PCB TEQ 98	0.24 ± 0.07 (0.19–0.30)	6.18 ± 3.61 (1.39–20.5)	13.5 ± 5.69 (2.84–24.8)
	Σ dl-PCB TEQ 05	0.17 ± 0.02 (0.16–0.19)	3.82 ± 2.26 (0.80–10.5)	8.91 ± 3.34 (2.14–13.8)

Data are expressed as mean ± standard deviation (minimum–maximum).

been noted in several European sites in the last decade: in pools of yellow eels (1.2 pg WHO_{1998} TEQ g^{-1} ww; Geeraerts et al., 2011) and in silver eels (2.92 pg WHO_{1998} TEQ g^{-1} ww; Byer et al., 2013) from Belgium, in the Baltic sea (0.4–5.9 pg WHO_{1998} TEQ g^{-1} ww; Karl et al., 2010), in Irish waters (0.2–4.4 pg WHO_{1998} TEQ g^{-1} ww; McHugh et al., 2010), in southern Norway (5–23 pg WHO_{1998} TEQ g^{-1} ww; Knutzen et al., 2003) and in the Elbe river and its tributaries (0.5 to 12 pg WHO_{1998} TEQ g^{-1} ww; Stachel et al., 2007).

Mean TEQ concentrations for dl-PCBs (Σ dl-PCB TEQ 98) ranged from 0.24 for glass eels to 13.5 pg WHO_{1998} TEQ g^{-1} ww for silver ones. These values were about 2 times higher than Σ PCDD/F TEQ 98 for glass eels and from 12 to 16 times higher for yellow and silver individuals. As for PCDD/F TEQ concentrations, eels from the Loire estuary are less polluted with dl-PCBs than eels from Belgium (1–139 pg WHO_{1998} TEQ g^{-1} ww; Geeraerts et al., 2011) and from Germany (8.5–47 pg WHO_{1998} TEQ g^{-1} ww; Stachel et al., 2007). However, these levels were similar to those measured in eels from the Baltic sea (0.93–15.3 pg WHO_{1998} TEQ g^{-1} ww; Karl et al., 2010) and higher than those noted in eels from Irish waters (0.17–1.24 pg WHO_{1998} TEQ g^{-1} ww; McHugh et al., 2010), the southern Norway (1.4–3.9 pg WHO_{1998} TEQ g^{-1} ww; Knutzen et al., 2003), Spain (0.09 pg WHO_{1998} TEQ g^{-1} ww; Bordajandi et al., 2003), Italian bodies of water (0.35–4.34 pg WHO_{2005} TEQ g^{-1} ww; Quadroni et al., 2013) and Poland (1.55–7.11 pg WHO_{1998} TEQ g^{-1} ww; Szlinder–Richert et al., 2010).

Dl-PCBs accounted for the highest percentage (mean 91.6%, range 82.1–96.8%) to the total-TEQ value (Σ PCDD/F TEQ 98 + Σ dl-PCB TEQ 98) for yellow and silver eels. These results were in agreement with data on Belgium and German eels (Stachel et al., 2007; Geeraerts et al., 2011), but this percentage is high compared to other European areas which showed dl-PCB levels ranging from 17% to 70% of the total-TEQ value (Knutzen et al., 2003; Karl et al., 2010; McHugh et al., 2010). A lower percentage was calculated for glass eels (67.0 ± 4.2%) emphasizing a different bioaccumulation process.

The contribution of PCDDs, PCDFs, dl-PCBs to the PCDD/F TEQ and dl-PCB TEQ values were respectively shown in Fig. 4A and B. Whatever the life stage, the relative congener profiles of PCDD/Fs were dominated by 2,3,4,7,8-PeCDF which shared 44.1% to 50.2% on average of the PCDD/F TEQ. Similar results were denoted by Szlinder-Richert et al. (2010) and Geeraerts et al. (2011) in eels from Poland and Belgium respectively. For dioxins, the congener 1,2,3,7,8-PeCDD predominated, its shares in the PCDD/F TEQ was 20.5% for glass eels and on average 32.4% (range 23.1–43.2%) for yellow and silver eels. The congener 2,3,7,8-TeCDD, known as the most toxic dioxin, contributed on average 5.65% for glass eels and 8.55% for yellow and silver eels.

Among the dl-PCBs, the four non-ortho substituted PCBs (no-PCBs) and the eight mono-ortho substituted PCBs (mo-PCBs) contributed differently regarding the life stage to the dl-PCB TEQ values. The no-PCBs accounted for about 49.0% in yellow eels, 57.4% in silver eels and 62.7% in glass eels, so the mo-PCBs contributed 51.0%, 42.6% and 37.3%, respectively. Among the no-PCBs, PCB #126, the most toxic congener, predominated and contributed most to the no-PCB TEQ (mean of 98.0%), regardless of the life stage. Eel from Belgium also showed a dominant contribution of PCB #126 to the dl-PCB TEQ with 52% on average, but without information on the individual life stage (Geeraerts et al., 2011). An Italian study (Quadroni et al., 2013) showed a similar percentage (according to WHO_{1998} TEFs, mean of 57.1%) for silver individuals living in the heavily urbanized zone (Tevere river). On the other hand, in the less anthropized areas (Caproce Lake and Lesina Lagoon), different shares were reported ranging from 83.5% to 98.0% (Quadroni et al., 2013). In Poland, Szlinder-Richert et al. (2010) reported the contribution of no-PCBs in the total TEQ as the highest with a predominance of PCB #126 (70 to 80% of the no-PCB TEQ). On the contrary, in eel from the Camargue (France), this congener was not detected (Oliveira Ribeiro et al., 2008).

As concerns the mo-PCBs, PCB #118 was the most dominant congener and its share in the dl-PCB TEQ was about 17.7% and consistent over the life stage. Notable contributions of PCB #156 could also be noticed showing significant variations between glass eels (7.09%), yellow eels (19.4%) and silver eels (13.6%). Again these observations were in agreement with other results reported on Belgium eels (PCB #118: mean 16% and PCB #156: mean 18%) (Geeraerts et al., 2011).

PCDD/F, dl and ndl-PCB contaminants could lead to damages for the eel population by affecting their reproduction and by a transfer of pollutants to eggs. Palstra et al. (2006) observed disrupting effects of dl-PCBs on development and survival of eel embryos at levels below 4 pg WHO_{1998} TEQ g^{-1} in gonad. Negative consequences on reproduction should be expected for the most polluted silver eels of the Loire estuary, as dl-PCBs accumulated in muscle fats are metabolized together with the lipid metabolization occurring during migration and sexual maturation. On the other hand, acclimation of eels to seawater, silvering process and reproduction migration are under different endocrine controls and fuel consuming. This energetic cost has been reported to increase significantly when lipid filets of swimming eels are charged with PCB mixture (after intraperitoneal injection of 5000 ng g^{-1} of PCB #153, 7 ng g^{-1} of PCB #126 and 50 ng g^{-1} of PCB #77) (Thillart et al., 2009).

3.5. Public health

The maximum levels set for PCDD/Fs and dl-PCBs for eel filets are currently 3.5 pg WHO_{2005} TEQ g^{-1} ww for the PCDD/F TEQ and 10 pg WHO_{2005} TEQ g^{-1} ww for the PCDD/F TEQ + dl-PCB TEQ (European Union, 2011). These values were not reached regarding the yellow eels. However, in the case of the silver ones, biological variability was high and 4 out of 6 studied eels displayed PCDD/F TEQ + dl-PCB TEQ values higher than permissible limits (Table 2).

Regarding congeners #28, 52, 101, 138, 153 and 180 (ndl-PCBs), sampled eels did not present levels superior than the maximum level of 300 ng g^{-1} ww (European Union, 2011). Silver and yellow eels from Nantes depicted the highest levels (mean of 205 ± 113 and 176 ± 91 ng g^{-1} ww, respectively), but few individuals (3/29) presented concentrations higher than the maximum level (Table 1). Yellow individuals from Cordemais presented intermediate levels (mean of 118 ± 48 ng g^{-1} ww) whereas those from Varades are the least contaminated (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 eels. 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). It could then be recommended to limit the consumption of eel from the Loire estuary to one portion (150 g) per month for the general population, which represents an average dietary daily intake of 694 ng/day. This is more restrictive 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 revealed that only 13% of participants are strong PCB-bioaccumulator freshwater fish consumers, with a moderate consumption frequency of 1 time per month (French Food Safety Agency, 2011). 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 the results of this study, a dietary daily intake of ndl-PCBs varying from 22 to 504 ng/day with a mean of 150 ng/day could be estimated. According to the TDI value noted above, the risk seems to be moderate for an adult consumer but exists for the most sensitive populations.

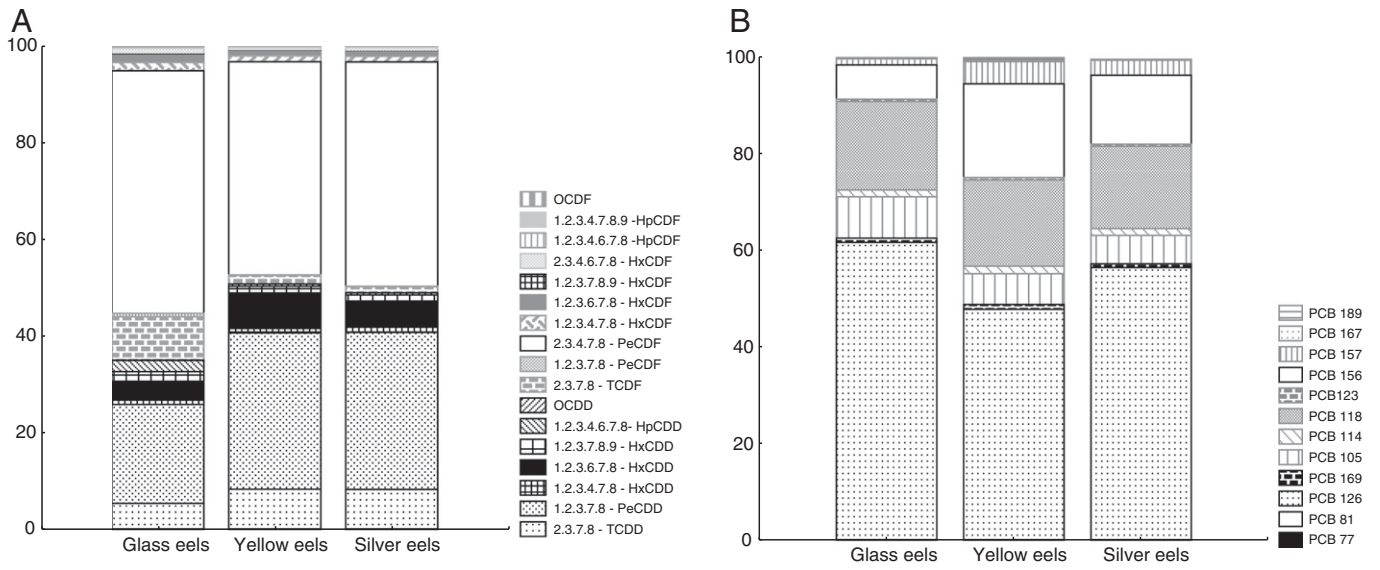

Fig. 4. Distributions of the PCDD/F (A) and dl-PCB congeners (B) (pg WHO_{1998} TEQ g^{-1} ww), according to the life stage.

4. Conclusion

This study gives a first assessment of the PCB and PCDD/F contamination of a European eel population fraction from the Loire estuary, along a hundred-km long portion of this ecosystem. The quantitative and qualitative contents of PCBs and PCDD/Fs in eel filets are different depending on their life stage and the sampling sites. The eels sampled in the site next to Nantes (the most important city of the estuary) appeared more contaminated than the two other sites, i.e. Varades (small city under agricultural pressure) and Cordemais (a town hosting a coal-fired power station). Regarding the PCB profiles, the sampled sites of Varades and Nantes could be associated to urban influences whereas the presence of PCB sources could be noticed around Cordemais site. Compared to other international or national areas, the contamination level of eels from the Loire estuary was low regarding PCDD/Fs but intermediate for dl and ndl-PCBs. A potential exposure to PCBs through eel consumption, and especially with silver eels, was noticed. According to the French TDI value (French Food Safety Agency, 2010), the consumption should be limited to once per month for the general population and once every two months for the most sensitive people.

The comparison of eel biomonitoring studies highlighted heterogeneity in sampled individuals. In order to better correlate all studies at the international level, it appears to be necessary to standardize parameters such as age, length, sex and sexual maturation stage. To preserve this endangered species and 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 and PCDD/F contamination of the Loire estuarine system. This state of moderate contamination of the Loire estuary should be maintained in order to preserve genitors of the eel's population.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.11.037.

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