ASSESMENT OF POLYCHLORINATED BIPHENYLS (PCBs), POLYBROMINATED DIPHENYL ETHERS (PBDEs), PERFLUORINATED ALKYLATED SUBSTANCES (PFASs) IN EUROPEAN EELS (*ANGUILLA ANGUILLA*) FROM THE LOIRE ESTUARY (FRANCE)

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Introduction

During recent decades, the growing human activities have led to an increase of pollutants emissions into the environment leading to a degradation of the different media, notably the estuaries which represent the final receptacle of pollutants before the discharge in the sea. Mainly for these reasons, the estuaries and coastal waters are among the most modified and threatened aquatic environments. The Loire is the longest wild European river. Its basin (117,800 km^2) expands on more than 1/5 of the French territory and drains a lot of tributaries. Moreover, the Loire estuary runs through important urban sites with shipping, industrial and agricultural activities. It is the receptacle of diffusive pollutions including a mixture of contaminants such as heavy metals[1] and persistent organic pollutants[2] (POPs). POPs are particularly incriminated by the scientific community and are listed by authorities of different countries as priority pollutants to monitor in the environment. Some of the POPs present structural similarities displaying halogens in the molecule. These organohalogen compounds include well-known families of compounds such as polychlorinated biphenyls (PCBs), polybrominateddiphenyl ethers (PBDEs) and perfluorinatedalkylated substances(PFASs). All of these compounds are ubiquitous into the environment because of their properties such as low vapor pressures, low water solubility, high adsorption on particles and high lipophilicity implying their transport, resistance to biodegradation and bioaccumulation in sediments and biota. In the environment, the difficulty is the likely presence of a mixture of these compounds added to chronic exposure of the fauna. Due to its high lipid content and predatory feeding behavior, eel is highly prone to bioaccumulate lipophilic contaminants. Moreover, its sedentary way of life during the yellow eel phase reflects local pollution[3].Futhermore, *A. anguilla* is an economically important species in European coasts. For these reasons the eel is a highly suitable biomonitor for environmental contaminants[4]. This work was set out to reach two principal objectives. The first oneis to measure the contaminant impregnation in eels from the Loire estuary (France). Different POPs were investigated in muscle (PCBs, PBDEs, PFASs) to evaluate health risks for fish population and local consumers. A real lack of data on the POP contamination levels of eels exists in this estuary. Secondly, the sample collections were carried out at two different life stages (yellow and silver eels) and at three locations for yellow eels in order to evaluate variations according to the life stage and/or spatial locations in the contaminant levels and profiles.

Materials and methods

Sampling locations

The Loire is the longest river in France and its mouth is located on the North Atlantic coast. Three sampling locations were selected in this study near twocities.First, Varades(V) 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 two other sampling locations are upstream (UN) and downstream (DN) 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.

Fish sample collection

Eels (*Anguilla anguilla*) were fished by local fishermen according to the fishing authorizations, in the three sampling areas described above at two life stages (yellow (Y) and silver (S)) in November 2011 and June 2012, respectively. The aim is to assess the spatial and the life stage influence on chemical impregnation. A total of 45

females were randomly collected with fishing nets: 10yellow eels collected at Varades, 10 upstream and 10 downstream to Nantes, as well as 15 migrantsilver eels sampled during downstream migration.

Biological samples and biometric parameters

Once anesthetized, the body length (BL) and the body weight (BW) of each eels were recorded. The animals were then sacrificed and dissected at 4°C. Muscles were also removed whole and then stored at -20°C until chemical analysis. Biometric parameters were used to calculate the Fulton condition factor($K= (BW \times 10^5) / BL^3$, where BW and BL respectively expressed as g and mm.

PCB, PBDE and PFASanalysis in fish muscles

All of compounds were analyzed using methodsvalidated and accredited according to the ISO 17025 standard and described elsewhere[5,6]. Targeted substances were 18 PCBs, among them 12 dioxin-like PCBs (dl-PCB #77; 81; 105; 114; 118; 123; 126; 156; 157; 167; 169; 189) and 6 non dioxin-like PCBs (ndl-PCB: #28; 52; 101; 138; 153; 180), 7 PBDEs (#28, 47, 99, 100, 153, 154, 183) and a total of 17 PFAS: 5 PerfluoroalkylSulfonates (PFBS, PFHxS, PFHpS, PFOS, PFDS) and 11 Perfluoroalkyl Carboxylic Acids (PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA). Briefly, for PCBs and PBDEs analysis, muscles were cut, freeze-dried, milled and extracted by Accelerated Solvent Extraction. The gravimetric determination of the fat content was calculated after evaporation to dryness by rotary evaporation (40°C) in order to assess the muscle lipid weight (LW in % of wet weight). Three purification steps were then performed, using successively acid silica, Florisil® and celite/carbon columns.PCBs and PBDEs measurements were performed by gas chromatography coupled a double sector high resolution mass spectrometer(GC-HRMS) set at a resolution of 10 000.Concerning PFASs, compounds were extracted from muscles using a liquid solid extraction (LSE) overnight. The supernatant was purified using two solid phase extraction (SPE) constituted of Oasis Wax and SupelcleanEnvicarb stationary phases.The extracts were analyzed by LC-MS/MS (qQq) in the SRM acquisition mode.

Results and discussion

Biometric parameters

Table 1 shows the biometric parameters of the eels according to the life stage and the sample location. BL and BW present similar variations, *i.e.* yellow eels taller and bigger in the locations near Nantes (UN and DN) compared to Varades. Considering silver eels, they are intermediate. About LW values, they are lower for eels from V and DN than those of silver eels, UN values being intermediate. Fulton's condition factor values (K) are not significantly different, except those calculated for UN, with values ranging from 0.14 to 0.18. According to Feunteun[7], these values are representative of good health conditions of eels in the Loire estuary. Such values are similar to the Fulton's condition factor found in other studies about European areas[8].

Table 1: Means and standard deviations of biometric parameters (Body Length BL, Body Weight BW, Lipid Weight LW) and Fulton's condition factor (K).

Life stage	Sampling site	n	BL (mm)	BW (g)	LW (%)	K
Yellow eels	Varades(V)	10	504±57	192±96	6.2±5.0	0.14±0.02
	Upstream Nantes (UN)	10	717±113	727±409	17.7±11.1	0.18±0.03
	Downstream Nantes (DN)	10	522±70	222±94	13.3±12.5	0.15±0.02
Silver eels		15	618±100	384±248	26.9±4.4	0.15±0.01

PCB levels in eels muscles

Fig.1 shows eel impregnation of total PCBs. The trends observed for dl- and ndl-PCBs are similar. The yellow eels from Varades are significantly less contaminated than yellow eels from the others sites as well as the silver eels. Two hypotheses could be proposed including the influence of the sampling site and the LW of eels. Assuming that PCBs are lipophilic compounds, their levels are correlated to LW. Consequently, eels from Varades displaying lower LW show smaller PCB contamination. On the other hand, eels from Varades are far away from industrial and urban influences explaining the differences with eels from the other sites. Fig.2

corresponds to patterns of ndl-PCBs. The major congener present in the muscles is #153. The patternsare different but no trends depending on the sampling location orthe life stage are found.

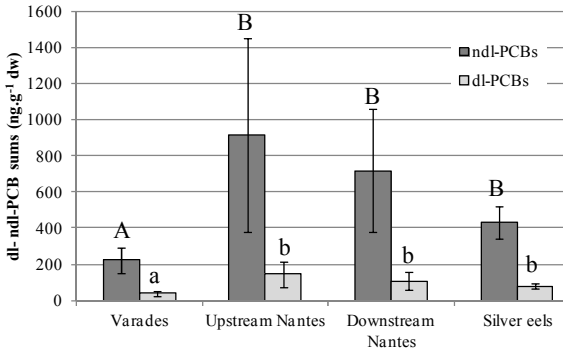

Fig.1: dl- and ndl-PCB sums

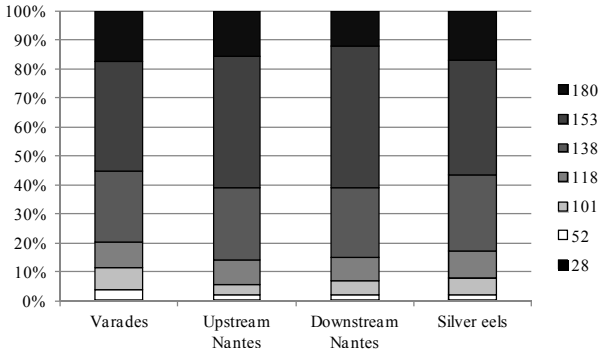

Fig.2: Marker-PCB patterns

PBDE levels in eel muscle

Fig.3 shows the impregnation of the fish in PBDEs. According the sexual stage, silver eels are significantly more contaminated by PBDE compounds than the yellow eels, except those from UN. Concerning the location influence on yellow eels, PBDE levels are significantly higher in fish from UN than V, DN being intermediate. The observed trend for PBDE levels is similar to LW trend. As for PCBs, the correlation between PBDEs contamination and LW exists, explaining high PBDE levels depicted in silver eels. Regarding yellow individuals, eels from UN and DN (urbanized and industrialized sites) seem to be more exposed to PBDEs than those from V (under agricultural pressure). Yellow individuals from UN present singular physiological characteristics close to the silver ones.The PBDE patterns are presented in Fig.4.Whatever the sexual stage and location, PBDEs #47, 100 and 154 are the most abundant congeners in eel muscles, respectively.The patternsare different but no trends depending on the sampling location orthe life stage are found.

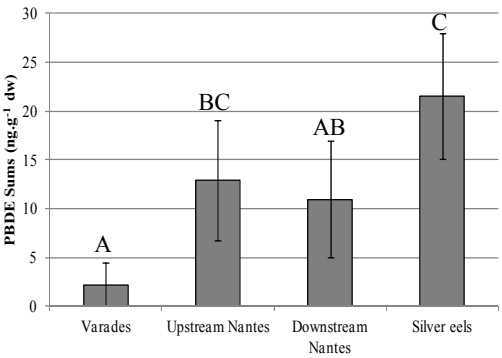

Fig.3: PBDE sums in sampled eels

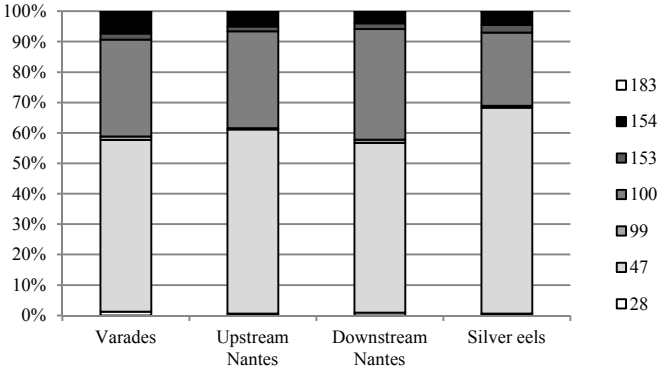

Fig.4: PBDE patterns

PFASlevels in eel muscles

No significant difference between the PFASsums is observed in Fig.5.The impregnations of eels in PFASs are independent to the sampling sites, life stages, and indirectly biometric parameters.Fig.6 about the PFAS patterns shows that PFOS (perfluorooctanesulfonate) is the major PFASdetected. Its contribution to the sum is higher than 80%. As for PCBs and PBDEs, the patterns seem to be similar whatever the sampling location and the life stage, except for yellows individuals from DN which present a significant higher percentage of PFHxS.

Fig.7 shows results of the PCA performed with PCB, PBDE, PFAS levels in muscles and biometric parameters of the eels from Loire estuary. The contaminants are clustered according to the chemical family as it is shown on the correlation loadings. PBDEs are particularly correlated to biometric parameters, *i.e.* BW, BL and LW.

Regarding sample representation, eels from V are the less contaminated and well differentiated from silver eels. Eels from UN or DN are more or less clustered, with eels from UN close to silver eels.

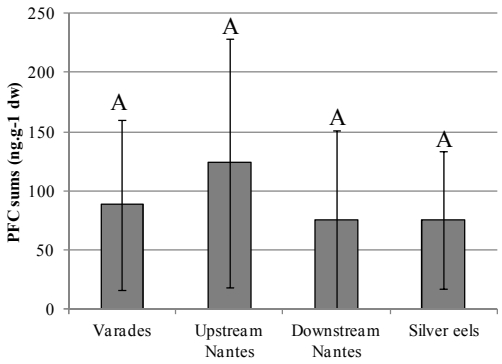

Fig.5:PFASsums in eel

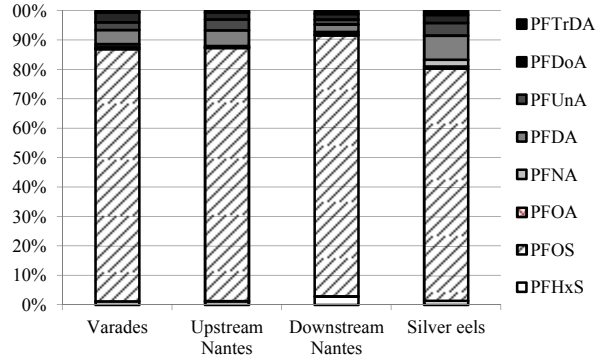

Fig.6:PFAS patterns

Principal Component Analysis (PCA)

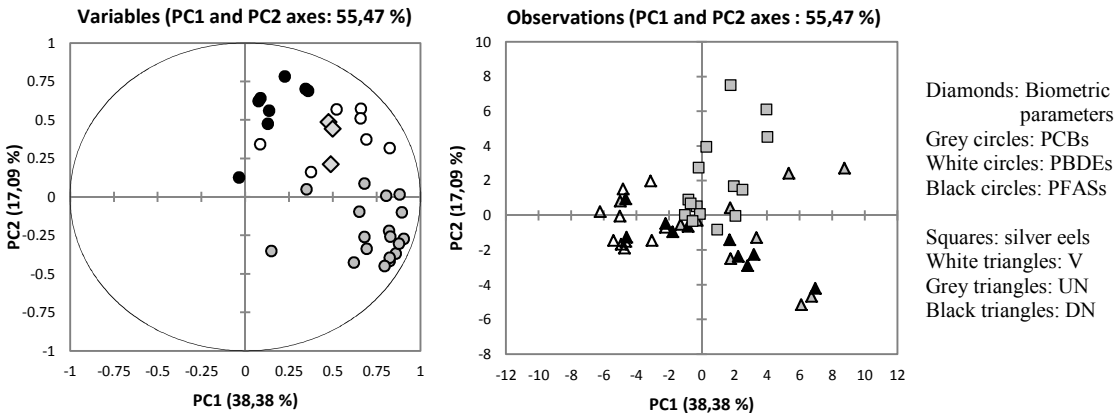

Fig.7:PCA: correlation loadings and sample representation.

Conclusions

The high PBDE levels depicted in silver eels could be explained by their high lipid content. Regarding yellow individuals, eels from UN and DN (urbanized and industrialized sites) seemed to be more exposed to PBDE than those from Varades (under agricultural pressure). The PCB levels seem to be more influenced by the sampling site. PFAS contaminants seem to be less influenced by the sexual stage and the sampling location.

Acknowledgments

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