Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain)

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HIGHLIGHTS

- Seasonal variations of perfluoroalkyl substances in the Ebro Delta Area were studied.
- PFOA was the most frequently detected in water and sediments.
- Levels of PFASs in waters were characterised by a slight decrease during winter.
- Levels of PFASs in sediments showed a progressive decrease from autumn to summer.

GRAPHICAL ABSTRACT
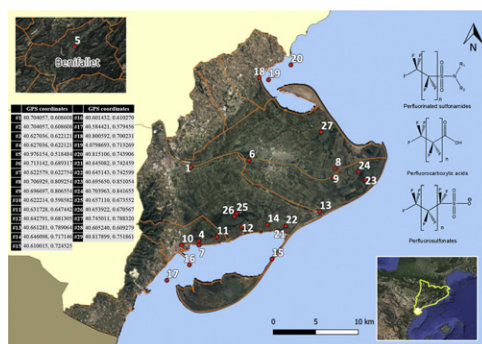
ABSTRACT

The main objective of this study was to assess the concentration levels and the seasonal variations of 13 poly- and perfluoroalkyl substances (PFASs) in different compartments (water, sediments and fish) of the Ebro Delta (NE Spain) and surrounding coastal areas. Perfluorooctanoic acid (PFOA) was the most frequently detected compound in waters and sediments. Perfluorocarboxylic acids (PFCAs) were the compounds found at the highest concentrations in water samples. On the other hand, sediments were more enriched in perfluorooctanesulfonate (PFOS) (range < 1.02–22.6 ng/g dw). Waters and sediments showed a different seasonal trend. While waters were characterised by a substantial constant level of PFASs over the year, sediments showed a progressive decrease from autumn to summer, revealing the great influence that environmental conditions exert on PFAS distribution in sediments. As regards fish samples, in spite of the ban of its production, PFOS was the most frequently detected compound in seawater fishes, in agreement with its high persistency, bioaccumulation and biomagnification. Moreover, PFASs showed to be more distributed in the skin rather than in muscle tissues. In addition, river fishes were characterised by very high PFAS levels ($\sum$ PFAS range from 63.8 ng/g ww to 938 ng/g ww), with perfluoroalkyl carboxylic acids being more concentrated than sulfonates. The PFASs concentrations in water, sediment, and biota revealed that one of the studied sites, Isla de Buda was the most contaminated site of the Ebro Delta. These results are consistent with its location at the final part of the estuary, where many irrigation channels are collected together.

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1. Introduction

Poly- and perfluoroalkyl substances (PFASs) are a wide group of synthetic substances with multiple industrial and domestic applications, such as stain repellents coatings for textiles and fire-fighting foams among many others (Arvaniti and Stasinakis, 2015; Zareitalabad et al., 2013). Because of the strong carbon-fluorine bond, these compounds are characterised by high thermal, chemical and biological stability. However, due to this high stability, they have been found to be persistent in the environment, with compounds such as the perfluorooctanesulfonate (PFOS) having a half-life of more than two months in waters and over six months in soils and sediments (Renzi et al., 2013). Moreover, PFASs show a tendency to bioaccumulate and biomagnify through the food chain (Ahrens et al., 2011; Naile et al., 2010), potentially causing adverse effects on organisms, such as hepatotoxicity reduction of the immune function among others (Lau et al., 2007; Zhang et al., 2013). Therefore, due to their persistence, accumulation in living organisms, the toxicity of some compounds and their wide distribution in the environment, the occurrence of PFASs is a cause for concern, and nowadays they are considered as emerging organic contaminants. For these reasons, the European Commission (EC) has set PFOS and its derivatives in the list of priority hazardous substances and has identified water and fish threshold concentrations for environmental quality assessment under the Water Framework Directive (WFD) (WFD, 2012). However, there is still a lack of legislation concerning most of these compounds in drinking water and food. Moreover, the Directive 2013/39/EC (EU Commission, 2013) laid down environmental quality standards (EQS) for priority substances in water and biota. The EQSs set for PFOS are 0.65 ng/l in inland surface waters (annual average concentration), 36 μg/l as maximum allowable concentration, and 9.1 μg/kg in biota. In the US Environmental Protection Agency (US EPA, 2016) has proposed a provisional threshold (between 0.01 and 0.09 μg/l) for drinking water with respect to only 7 compounds, including PFOS and perfluorooctanoic acid (PFOA).

Manufacturing facilities are considered to be one of the main sources of contamination by PFASs (Prevedouros et al., 2006; Pistocchi and Loos, 2009), along with wastewater treatment plants (WWTPs), which have been found to be inefficient in the removal of these compounds from wastewater influents (Ahrens et al., 2009; Boulanger et al., 2005; Schultz et al., 2006). Once released into the aquatic environment, they can easily be transferred into different environmental compartments, reaching groundwater (Houtz et al., 2013), soils (Houtz et al., 2013), sediments (Gao et al., 2015) and biota (Campo et al., 2016). Furthermore, these compounds have been found in remote environments, such as the Antarctica region (Llorca et al., 2012a). Once in the aquatic environment, PFASs are accumulated and biomagnified through the aquatic food chain whereby they reach human food (Pérez et al., 2014) and drinking water (Llorca et al., 2012b; Schwanz et al., 2016). The partitioning mechanism and their fate in the environment, though, are still not well-known (Ahrens, 2011). In addition, most studies have been mainly focused on more persistent and accumulative compounds such as PFOS and PFOA, while less information has been reported regarding the use of short-chain PFAS in the substitution of the 8-carbon chain compounds.

Different studies have already investigated the occurrence of PFASs in the aquatic environment, mainly focusing on their distribution in fresh waters, particularly rivers (Ahrens, 2011; Loos et al., 2013a; Munoz et al., 2015; Valsecchi et al., 2015; Lorenzo et al., 2016). But, up until now, scarce information is available about their seasonal fluctuation in coastal and highly productive areas, such as estuarine habitats. Those are fragile ecosystems that can be highly affected by human activities since they receive urban sewages and other by-products of human activities (Jiang et al., 2014).

Within this context, the main aim of this study was to assess the occurrence and environmental fate of 13 PFASs in the Ebro Delta (NE of Spain), as well as the surrounding coast: 8 perfluorocarboxylic acids, 4 perfluorosulfonates and 1 sulfonamide in a total number of 213 samples (87 waters, 71 sediments and 55 fishes). These compounds were analysed in the water, sediment and fish samples during three different seasons.

2. Materials and methods

2.1. Chemicals and reagents

Perfluoroalkyl compounds standards were provided by Wellington Laboratories Inc. (Canada) and were composed of: (i) a mixture of PFASs (PFAC-MXB, 2 μg/ml in methanol, purity >98%) containing perfluoropentanoic (PFPeA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), perfluoroundecanoic (PFUdA) and perfluorododecanoic (PFDoA) acids, and perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluorodecane sulfonate (PFDS); and (ii) the perfluoroctanesulfonamide (PFOSA). Surrogate internal standards used for quantification normalisation were supplied by Wellington Laboratories Inc. (Canada), and included: (i) a mixture of labelled PFASs (MPFAC-MXA, 2 μg/ml in methanol, purity > 98%), composed of $^{18}O_2$-perfluorohexanesulfonate (MPFHxS-18O2), $^{13}C_2$-perfluorohexanoic acid (MPFHxA 13C2), $^{13}C_4$-perlfuorooctanesulfonate (MPFOS-13C4), $^{13}C_4$-perfluorooctanoic acid (MPFOA-13C4), $^{13}C_5$-perfluorononanoic acid (MPFNA-13C5), $^{13}C_2$-perfluorodecanoic acid (MPFDA-13C2), $^{13}C_2$-perfluorododecanoic acid (MPFDoA-13C2); and (ii) $^{13}C_8$-perfluorooctanesulfonamide (M8FOSA, >99%).

All solvents and reagents were of analytical grade. Water and methanol (CHROMASOLV® Plus), ammonium acetate (MW: 77.08, purity > 98%), and ammonium hydroxide (MW: 35.05, purity > 98%) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Area of study

The Ebro Delta is the third largest delta in the Mediterranean Sea. It is a wetland area of 320 km^2, highly relevant for conservation, which is included in the Ramsar Convention list. This estuarine habitat is characterised by a high biological productivity, thanks to the nutrients that are provided by the Ebro River (Lloret et al., 2004). The climate in the middle and lowland reaches of the River Ebro is typically Mediterranean, with rainfall concentrated in autumn and spring (200–300 mm) and intense summer drought (<50 mm). Flow regime is pluvio-nival because of the left-margin tributaries from the Pyrenees. The average annual temperature is between 10 and 15 °C. The lowest temperatures occur in winter (down to − 5 °C) and the highest in summer (>40 °C). Substratum in the area is mainly calcareous, with Cenozoic limestones, gypsum and alluvial sediments. Aquatic vegetation consists of macrophytes such as water crowfoot Ranunculus spp. and Scirpus spp. The land use is mainly for agriculture and cattle rearing approximately 13% of its total surface is composed of natural lagoons, bays and marshes, whereas the major part (77%) is dedicated mainly to agricultural activity such as rice and orchards. For this reason, since the 1960s, different dams and irrigation channels have been built in order to control Ebro River water and sediment inputs and to fulfil the surrounding water demand (Cardoch et al., 2002).

Amposta, Deltebre, Sant Jaume d'Enveja and Sant Carles de la Ràpita are the main towns that are located in this area, and they can potentially affect estuary environmental quality with the discharge of their treated sewages into the Ebro River. Chemical industries and a nuclear power plant on the northern side of the area (province of Tarragona) may be additional sources of contamination.

2.3. Sampling

Three sampling campaigns were carried out during October–November 2015 (autumn), February–April 2016 (winter), and June–July 2016 (spring-summer). A total number of 213 samples including 87 water, 71 sediment and 55 fish samples were collected. During the first campaign (i.e. autumn), only water and sediment were sampled, while fish samples were collected in the second (i.e. winter) and third (i.e. spring-summer) sampling campaigns, in addition to water and sediment. Detailed information about the locations of sampling sites and the samples are listed in Table S1 from Supporting information. In summary, water samples were collected from the Ebro River at 7 irrigation channels, from the emissary of the wastewater treatments plant (WWTP) that is located in Sant Carles de la Ràpita, at the influents and the effluents of 2 WWTPs (Sant Carles de la Rapita and Amposta); estuary water samples were collected in different lagoons (Illa de Buda, L'Encanyissada, La Tancada and Canal Vell) and seawater samples from the Fangar and Alfacs bays and at the open sea adjacent to these bays. Fish samples were collected both from seawater (during winter season) and from freshwater (during spring-summer period). Detailed information about fish communities is shown in Table 1. Regarding seawater fishes, a total of 15 specimens of different species were sampled from two sites of the Mediterranean Sea (Fangar bay, n = 4; Alfacs Bay, n = 5) and from one Ebro Delta site (Illa de Buda lagoon, n = 6). In particular, fish species were *Mugil cephalus*, *Squalius laietanus*, *Cyprinus carpio*, *Anguilla anguilla*, *Torpedo torpedo*, *Sarpa salpa*, *Trachurus mediterraneus*, *Boops boops*, *Diplodus annularis* and *Micropterus salmoides*. Fish were sampled by local fishers using nets. Individual fish samples were measured for fork/total length (FL/TL, ± 1 mm), weighed (wet body weight, ± 0.1 g), labelled, stored in ice and frozen (−20 °C) on the same date of collection.

Riverine fishes (n = 40) sampled during the spring-summer period were collected from two sites of the Ebro River upstream of the Delta: Xerta (n = 21) and Tortosa (n = 19). In particular, fish species were *Alburnus alburnus*, *Cyprinus carpio*, *Liza* sp., *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Silurus glanis*, and *Squalius laietanus*. To encompass the existing environmental variability, fish were collected from all meso-habitats present in the river (e.g. runs, riffles and pools), from the left and right margins along each sampling site (500 m river length). This allowed collecting a representative sample of fishes. Fishes were sampled by electrofishing from a boat (4.5-m aluminium hull) by using a 2000 W DC generator at 1000 V and 16 A (Model: 5.0-GPP

Smith-Root Inc., Vancouver, WA, USA), along with dip nets (2.5 m long pole, 50 cm diameter net, 10 mm mesh size). Two anodes were suspended from booms and mounted on the bow of the boat, and a cathode was mounted along each side of the hull. A single pass was made following a zigzagging and upstream direction without using block nets in every sampling site. After each survey was concluded, fish were identified until the species level and counted. Then, a fish sub-sample was immediately immersed in an overdose solution of anaesthetic (MS-222) for 15 min. Euthanized fish were measured for fork/total length (FL/TL, ± 1 mm), weighed (wet body weight, ± 0.1 g), labelled, stored in ice and frozen (−20 °C) on the same date of collection (<2 h since euthanasia) until laboratory processing. The remaining individuals of non-native species were euthanized according to the same procedure described above, while those of native fish species were kept in a tank with supplied oxygen (two battery operated aerators with portable pump) until fully recovered before being released. All field procedures complied with animal use and care regulations of Europe and Spain (specific licences were granted for Scientific Field Research in the River Ebro). Fish were collected by trained personnel (i.e. the holder of the licence, D. Almeida). Thus, no adverse effects were caused on the wildlife in the study habitats and all native fish fully recovered from the anaesthetic.

2.4. PFAS analysis

2.4.1. Analysis of water samples

Extraction and clean-up were carried out by using the method described by Llorca et al. (2012b). Briefly, 500 ml of seawater, 250 ml of river water and wastewater effluents and 150 ml of wastewater influents were spiked with 10 μl of a mixture of surrogate internal standards at 100 ng/ml. After 15 min, a time period that is necessary in order to reach the equilibrium, the samples were filtered and extracted by solid phase extraction (SPE) with Oasis WAX cartridges (30 cm³, 60 mg, 30 μm; Waters Corporation, MA, USA) that were previously conditioned with methanol and water. Cartridge elution was carried out with 4 ml of 10% NH_4OH in methanol. The extracts were evaporated under a gentle N_2 stream and reconstituted in 250 μl with a mixture of water and methanol (9:1). All the samples were processed in triplicates.

The extracts were analysed by ultra-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UPLC-QqQ-MS/MS). Chromatographic separation was achieved with an Acquity UPLC BEH C18 analytical column (2.1 × 50 mm, 1.7 μm particle size; Waters Corporation, USA) using the system Acquity UPLC H-Class (Waters Corporation). A pre-injection column PFASs isolator (Waters Corporation) was used, as well. Mobile phases consisted of 20 mM ammonium acetate in water (solvent A) and 20 mM ammonium acetate in methanol (solvent B) and injection was delivered at a flow rate of 0.4 ml/min. The elution programme was as follows: 20%B over a time period of 10 s, then linear gradient to 80%B over another time period of 4 min and 50 s, followed by a linear increase to 90%B during 2 min, followed by an isocratic hold at 90%B for a time period of 2 min and 50 s, and then an isocratic hold was implemented for 1 min more. At the minute 9:50, B was returned to 20% in 1 min. The total run time for each injection was 11 min and the injection volume was 10 μl.

After separation, the detection was carried out using a triple quadrupole analyser Xevo TQ-MS (Waters Co.) with an electrospray ionisation (ESI) source operating in negative conditions.

2.4.2. Analysis of solid samples

Sediment sample analyses were carried out by the method that was previously developed and validated by our group (Llorca et al., 2012b). For the extraction of sediment samples, 1 g dried sediment was spiked with 20 μl of a mixture of internal standards (100 ng/ml) and left to reach equilibrium for 20 min. After this period, 10 ml of pure methanol was added, and the sediments were extracted by ultrasonic assisted extraction (UAE) for 1 h. The extracts were then centrifuged for 20 min at

Table 1
Fish communities.

Habitat	Scientific name	Common name	Taxonomic family	Origin
Sea	*Torpedo torpedo*	Common torpedo	Torpedinidae	Native
Sea	*Trachurus mediterraneus*	Jack mackerel	Carangidae	Native
Sea	*Boops boops*	Bogue	Sparidae	Native
Sea	*Diplodus annularis*	Annular sea bream	Sparidae	Native
Sea	*Sarpa salpa*	Salema	Sparidae	Native
Delta	*Anguilla anguilla*	European eel	Anguillidae	Native
Delta	*Micropterus salmoides*	Largemouth bass	Centrarchidae	Non-native
Delta	*Mugil cephalus*	Flathead grey mullet	Mugilidae	Native
Delta/river	*Squalius laietanus*	Ebro chub	Cyprinidae	Native
Delta/river	*Cyprinus carpio*	Common carp	Cyprinidae	Non-native
River	*Alburnus alburnus*	Bleak	Cyprinidae	Non-native
River	*Rutilus rutilus*	Roach	Cyprinidae	Non-native
River	*Scardinius erythrophthalmus*	Rudd	Cyprinidae	Non-native
River	*Silurus glanis*	European catfish	Siluridae	Non-native
River	*Liza* sp.	Mullet sp.	Mugilidae	Native

4000 rpm at 17 °C. After centrifugation, 4 ml of the supernatant was dried with a gentle stream of N_2, reconstituted in 100 μl of a mixture water:methanol (9:1) and directly injected in an on-line clean-up system. Extracts and the posterior analysis were performed in triplicates.

For the analysis of PFASs in fish, skin and muscle were processed separately, according to the validated procedure described by Llorca et al. (2012a). Briefly, 1 g of wet sample was spiked with 20 μl of a mixture of internal standards (100 ng/ml) and left at equilibrium for 20 min. The extraction procedure was based on alkaline extraction, mixing the sample with a solution of 10 ml of methanol with 10 mM sodium hydroxide and digesting for 2 h in an orbital shaker. After digestion, the mixture was centrifuged at 4000 rpm and 17 °C for 20 min. Then, 4 ml of the supernatant was processed as described above for the analysis of PFASs in sediment samples (dried and reconstituted before on-line clean-up process). Due to the differences in weight and size of the selected species, the smallest fish samples were processed and analysed as a pool of individuals, whereas the biggest fish samples were treated as individuals. Whenever possible, the guts were removed from the fish body and only muscle and skin tissues were analysed, but whenever this was impossible, the whole fish body was extracted and analysed.

Extracts of sediments and fish were purified in an on-line clean-up system (Thermo Fisher EQuan™) based on turbulent flow chromatography (TFC). For the purification, two columns were used, Cyclone (50 mm × 0.5 mm, 60 μm particle size, 60 Å pore size) and C18 XL (50 mm × 0.5 mm, 60 μm particle size, 60 Å pore size), connected in tandem. Loading and eluting solvents are summarised in Table S2. After purification, the extracts were directly pumped to the analytical column Hypersil GOLD PFP (3 × 50 mm, 3 μm particle size; Thermo Fisher Scientific). Sample injection volume was set at 20 μl. Detection was carried out using a triple quadrupole analyser TSQ Quantiva (Thermo Fischer Scientific) equipped with an electrospray ionisation (ESI) source operating in negative conditions.

2.5. Quality assurance and quality control

In order to rule out any system contamination, instrumental blanks made of methanol:water (1:9) were run every three sample injections, while different points of the calibration curve were analysed before, during and after samples in order to check sensibility drifts. For water analyses, procedural blanks were prepared in parallel to samples in order to discard any contamination step during sample treatment.

In Tables S3 and S4, the method limits of detection and quantification, and the recoveries for different matrices, respectively, are presented.

2.6. Data analysis

Statistical analyses were performed with R software. All the values that were below the method limit of quantification (mLOQ) were substituted with half the limit of quantification. Variables with <10% of detections were removed from the dataset for statistical analyses. Correlation between variables in the different matrices was investigated using Spearman's rank-order correlation. Differences among the seasonal periods were studied through the non-parametric Kruskal-Wallis test. Reported values are means ± SD. The significance level was set at p-value $(\alpha) < 0.05$.

3. Results and discussion

In water samples, among the 13 selected PFASs, only 5 compounds were detected in all of the sampling campaigns (autumn 2015; winter and spring-summer 2016), with perfluorocarboxylic acids (PFCAs) being the most abundant group. Table S5 summarises the main physicochemical parameters of water samples, while Table S6 provides a comparison between campaigns, along with summary statistics of the analysed PFASs. PFOA was the carboxylic acid detected at the highest

frequency (67% in autumn 2015, 42%, in winter and 76% in spring), followed by PFPeA (30%, 17% and 66%, respectively) and PFNA (22%, 21% and 31%, respectively). Among perfluorinated sulfonates, PFOS was the most abundant compound with frequencies of 22% in autumn, 4% in winter and 86% during spring. Additionally, PFOS was almost the unique sulfonate compound detected, with the exception of PFHxS, found only in one sample during the autumn period, and PFDS, detected only during the spring period. PFOSA was not detected in any of the analysed samples. PFOA and PFOS were the most commonly compounds found in river water, which is in agreement with previous studies (Campo et al., 2015; Llorca et al., 2012a; Valsecchi et al., 2015). Though, it is worth to be noted that PFOS annual average concentrations (0.52 ng/l in freshwater and 0.26 ng/l in seawater) were below the EQS set by EU Directive 2013/39/EU. Moreover, PFUdA, PFDA and PFDS were the only longer-chain compound detected, at low frequencies. In detail, PFUdA was present in only two samples from the winter campaign, whereas PFDA and PFDS were recorded only in spring season. Lower distribution of longer-chain compounds in water samples is not surprising, firstly because of their lower solubility, and secondly because of their current replacement in human production with shorter chain compounds, which have lower bioaccumulation potential (Onghena et al., 2012).

Table S7 reports concentrations of PFASs in surface waters of different published studies. Globally, the most abundant compound was once again PFOA, confirming the results showed in this work. Shorter chain compounds, especially PFHxA were also quite abundant, as in Yangtze River (China) or in Ebro River (Spain), confirming their high solubility and their increase in use and production in spite of the longer chain PFASs. Sulfonates, and PFOS above all, showed a similar span of values, comparable to PFOA (for comparison of range values, see Table S7).

In water samples we found that the concentrations of PFASs were minor during the winter period (Fig. 1), coinciding with a higher dilution after the rainy season, as it can be inferred from the mean flow rate of Ebro River, which was c.a. 160 m^3/s during autumn and spring, and c.a. 450 m^3/s in winter (Table S5). However, the concentrations can be considered fairly constant along the course of the year. As expected, the major concentrations were reported in wastewater, with PFPeA being the compound reaching the highest concentrations, with 2329, 2775 and 345 ng/l in autumn, winter and spring, respectively, in agreement with its use as replacement compound (Wang et al., 2013). Nevertheless, effluents collected after the WWTPs showed a great efficiency in the removal of PFPeA from contaminated waters. In contrast, the two WWTPs revealed that they were inefficient in the removal of the other PFASs. For example, in the samples collected during autumn 2015, in spite of the significant removal of PFASs during the wastewater treatment, the final effluents of both WWTPs, Amposta and Sant Carles de la Ràpita, still showed notable concentrations of PFOA. The influents of Amposta and Sant Carles were 6.8 and 8.7 ng/l of PFOA while the final effluents were 3.5 and 6.0 ng/l of PFOA, respectively, indicating that >50% of this compound remains in the effluent. Higher concentrations of PFASs in effluents than in influents of WWTPs have already been reported by different studies as a result of the incomplete degradation of their precursors (such as polyfluoroalkyl phosphates and fluorotelomer alcohols) during water treatment processes with activated sludges (Guo et al., 2010; Lee et al., 2010; Loos et al., 2013b; Wang et al., 2005). Detection of PFHpA only at the effluents of the Sant Carles de la Ràpita WWTP is a further evidence of other related PFASs (e.g. fluorotelomers) partial degradation into shorter-chain PFASs. In river waters, the concentrations were below 6 ng/l of PFOA as the most recalcitrant, followed by PFHxA and PFNA. It is noteworthy that the control site "before Amposta", located in the Ebro River far from the estuary area and selected as reference site, reported a slight contamination by PFASs, especially for PFCAs. This fact suggests that contamination of the estuary environment is not only due to the different surrounding human activities, which may impact on water quality, but reflects a contamination which originates at a far distance from the estuary. As it can be
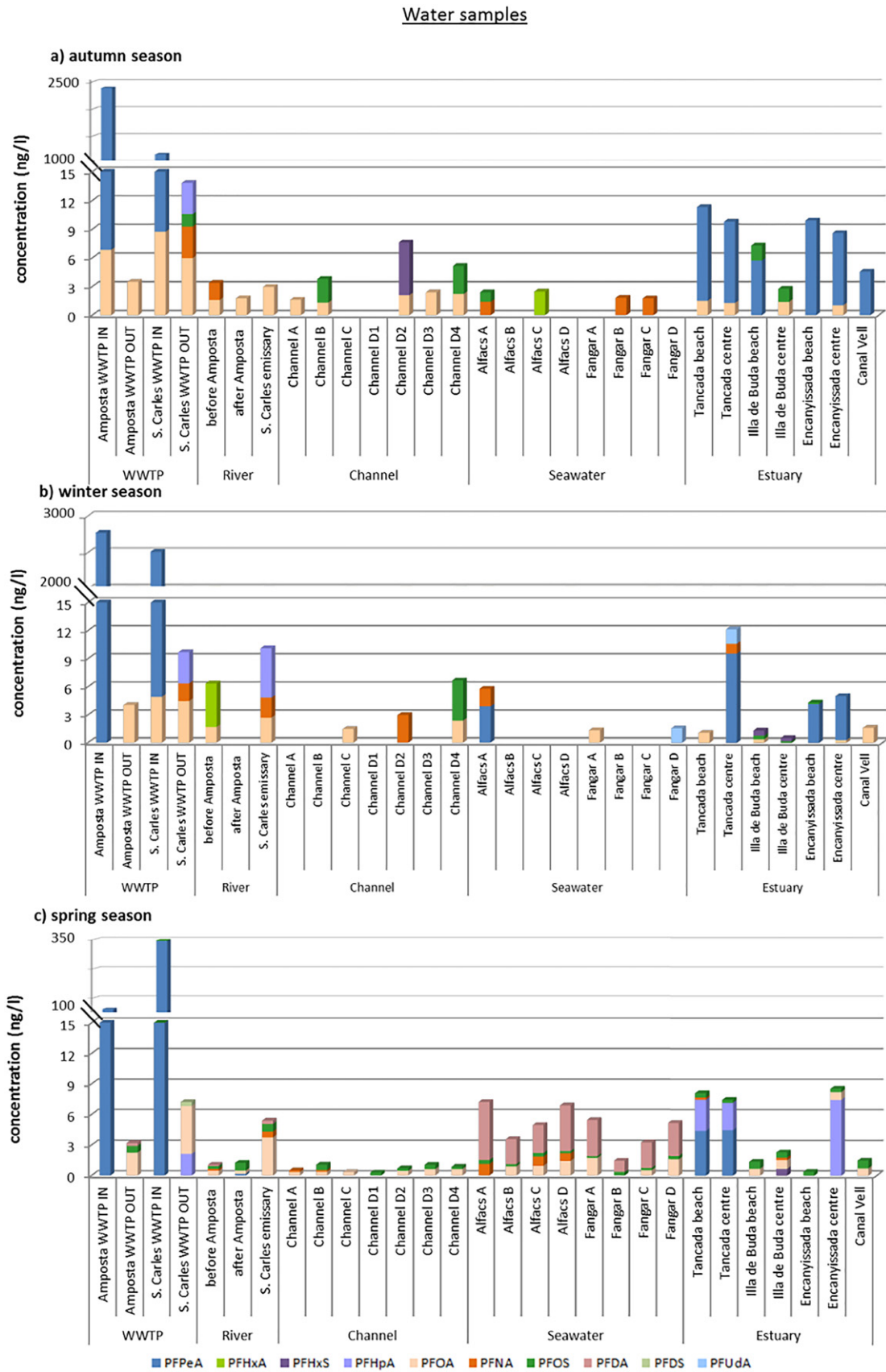
Water samples

a) autumn season

b) winter season

c) spring season

Legend: PFPeA, PFHxA, PFHxS, PFHpA, PFOA, PFNA, PFOS, PFDA, PFDS, PFUdA

Fig. 1. PFAS concentrations (ng/l) detected in water samples during the autumn season (a), winter season (b) and spring-summer season (c).

appreciated in Fig. 1, the final part of the Ebro Delta (La Tancada, L'Encanyissada and Illa de Buda) was more affected by PFASs contamination, since it collects all waters from the surrounding irrigation channels. Comparing river water and seawater concentration patterns over the year, it is noteworthy that samples taken during the winter period showed lower concentrations than those samples collected during the autumn and spring-summer periods. Although PFOA still remained the most frequent compound among all PFASs, its frequency of detection decreased during the second sampling campaign, but was comparable between the first and third sampling campaigns (52% in autumn, 35% in winter, 55% in spring, excluding WWTPs data). In agreement with these data, the average concentrations for PFOA were 1.6, 0.97 and 0.87 ng/l in freshwaters (without WWTPs) for autumn, winter and spring, respectively (excluding WWTPs data). The dilution factor due to annual fluctuation of the Ebro River should be taken into account for freshwater samples: the lower PFAS concentrations detected in the wet winter season were due to the higher river flow rate of Ebro River (as explained before), while lower river flow rates lead to a higher concentration of contaminants in water during the dry season. In addition to the fluctuations of the Ebro River flow rates, the increment of population during the summer-autumn period, and specific weather conditions during the different sampling campaigns (i.e. temperature and evaporation rates) may influence PFASs occurrence in the aquatic compartment.

In spite of that, no significant differences ($p > 0.05$) were found regarding the total PFAS concentrations over the year, stating their persistence in the water compartment. In addition, the specific features of non-polar compounds must be noted. For example, it is known that PFOS is partitioned into sediments (see next section) and that the concentrations detected in water are related to suspended organic material. In this case, the concentration of non-polar compounds can be influenced by heavy rain periods that redilute these compounds and increment their concentration in water, with a resulting constant trend of concentrations throughout the year.

The concentration data regarding sediment samples are summarised in Fig. 2 and Table S8. As in waters, for the first sampling campaign the most common compound was PFOA, detected in almost all analysed samples (96% frequency), along with PFHxA and PFHpA, recorded in 41% and 36% of samples, respectively. Among sulfonates, PFOS was predominant, with a maximum of 22.6 ng/g dw and mean value of 2.7 ± 5.6 ng/g dw in autumn. It is noteworthy that during the winter period the only PFASs detected were PFOA and PFOS, and they were at lower concentrations compared to autumn sampling (mean value in winter of 0.84 ± 1.10 ng/g dw for PFOA; 0.61 ± 0.50 ng/g dw for PFOS) and lower frequencies (25% for PFOA, 15% for PFOS). PFDoA was also detected in both sampling periods, but at a higher frequency in winter (15%) than in autumn (5%), even if at low concentrations (mean value 0.91 ± 0.29 ng/g dw). Samples collected during the spring period did not show any concentration of PFASs above the mLOQ.

Globally, sediment mean concentrations found in this work were in agreement with those ones recorded in the upstream section of Ebro River recorded by Lorenzo et al. (2016) and with other rivers of the Spanish peninsula (Campo et al., 2016). Though, they were much higher than concentrations detected in Yangtze River sediments (Pan et al., 2014a, 2014b) and in Chinese river sediments (Pan et al., 2014a), due to different sources of introduction and different environmental characteristics of rivers. For a better comparison of the ranges detected in those works, please refer to Table S7.

Fig. 2 reports the distribution of PFAS concentrations during autumn and winter periods. The most contaminated sites were the two lagoons of Illa de Buda and L'Encanyissada, along with Canal Vell, where PFOA and PFOS concentrations were accompanied by the detection of some of their replacement products in the autumn season (PFHxA, PFHpA, PFHxS, PFBS). Winter season displayed an evident decrease in PFASs detection, especially for samples taken from the open sea, where no compound was recorded; the estuarine environment showed a similar

trend, with only some positive measurements in Illa de Buda and Canal Vell. Samples collected in the freshwater system, on the other hand, showed higher similarities between the two seasons. The Kruskal-Wallis test that was run on the samples of the first and second campaigns confirmed the different biogeochemical features between the two sampling periods, showing statistically significant differences for the occurrence of PFHxA, PFHpA, PFOA and PFNA ($p < 0.05$), which are reported in Table S9. This seasonal pattern suggests that PFAS concentrations in sediments strongly depend not only on water-sediment interactions, but also on the surrounding environmental conditions, such as temperature, precipitations and water currents that may occur consequently to the higher rainfall rates. In this context, the decrease in concentrations of PFASs in sediments could be due to a resuspension of sediments in coincidence with the rainy season, leading to an increase of PFASs in the dissolved particulate matter and a correspondent decrease in sediments.

Only a few works focusing on PFAS seasonal trend in water and sediment have been conducted so far. Pan et al. (2014a) found no substantial variation of PFASs in sediments of Yangtze River in summer and winter seasons, even though the detected concentrations were very low ($\sum$ PFASs range of 0.05–1.44 ng/g dw), and most PFASs were not detected in the majority of the sampling sites. Pan et al. (2014b) in rivers of the Pearl River Delta region (South China) also showed comparable concentrations of PFASs in winter and summer, in contrast with the results obtained in this study. However, it should be highlighted that both studies were focused on river basins. The estuarine system of a delta environment is more complex since it is subjected to the influence of both inland waters and open seawaters. The anomalous behaviour of concentrations in sediments of Ebro delta could be explained by sediment resuspension that is produced consequently to heavy rainfalls during winter and spring, which leads initially to a depletion of the shorter chain compounds, less hydrophobic than the longer chain ones, as is actually registered for PFAS concentrations in winter season. Moreover, tidal events and strong water currents occurring during winter period in the Mediterranean Sea could cause a mobilization of superficial sediments, resulting in the removal and transport of sediments towards far distant areas along the coastline, as an effect of coastal erosion.

Regarding the analysis of biota, the data which report PFASs accumulation in fish from Ebro River near the municipalities of Xerta and Tortosa are listed in Table S10, while the data of fishes that were collected in the Ebro Delta (estuarine and seawaters) are reported in Table S11. Notwithstanding the differences in sample preparation, PFOA was the most abundant compound, being detected both in the whole fish body and in muscle and skin tissues, and confirming its bioaccumulation potential (Llorca, 2012). As could be expected, pool samples showed higher PFOA concentrations (from 94.2 to 330 ng/g ww) compared to samples for which only data on muscle and skin were available, since PFASs have been proved to bioaccumulate preferentially in liver and kidney, rather than in muscles or fat matter (Llorca, 2012). On the other hand, PFDA and PFOSA showed the highest frequencies of detection, being detected in all samples, with relatively high mean concentration for PFDA (141 ± 187 ng/g ww) and lower values for PFOSA (7.4 ± 6.0 ng/g ww). In particular, PFDA reached 459 ng/g ww in European catfish (*Silurus glanis*), which is at the top of the aquatic food-chain, and 454 and 455 ng/g ww in mullet fish (*Liza* sp.) and bleak (*Alburnus alburnus*), respectively, which both feed on small molluscs, insect larvae, worms and small fishes. PFOS (range of values from <mLOQ to 154 ng/g ww) and the short chain PFHxA (range < mLOQ–122 ng/g ww) were also found at remarkable concentrations. Focusing, in more detail, on the individual contribution of PFASs in freshwater biota (Fig. 3), concentrations of whole-body revealed a similar pattern in PFAS bioaccumulation, both for pool samples (Fig. 3a) and for individual samples (Fig. 3b), showing the predominance of PFOA among all the PFASs. It is well-known that the longer chain compounds exhibit more bioaccumulative potential than the shorter chain compounds. In this
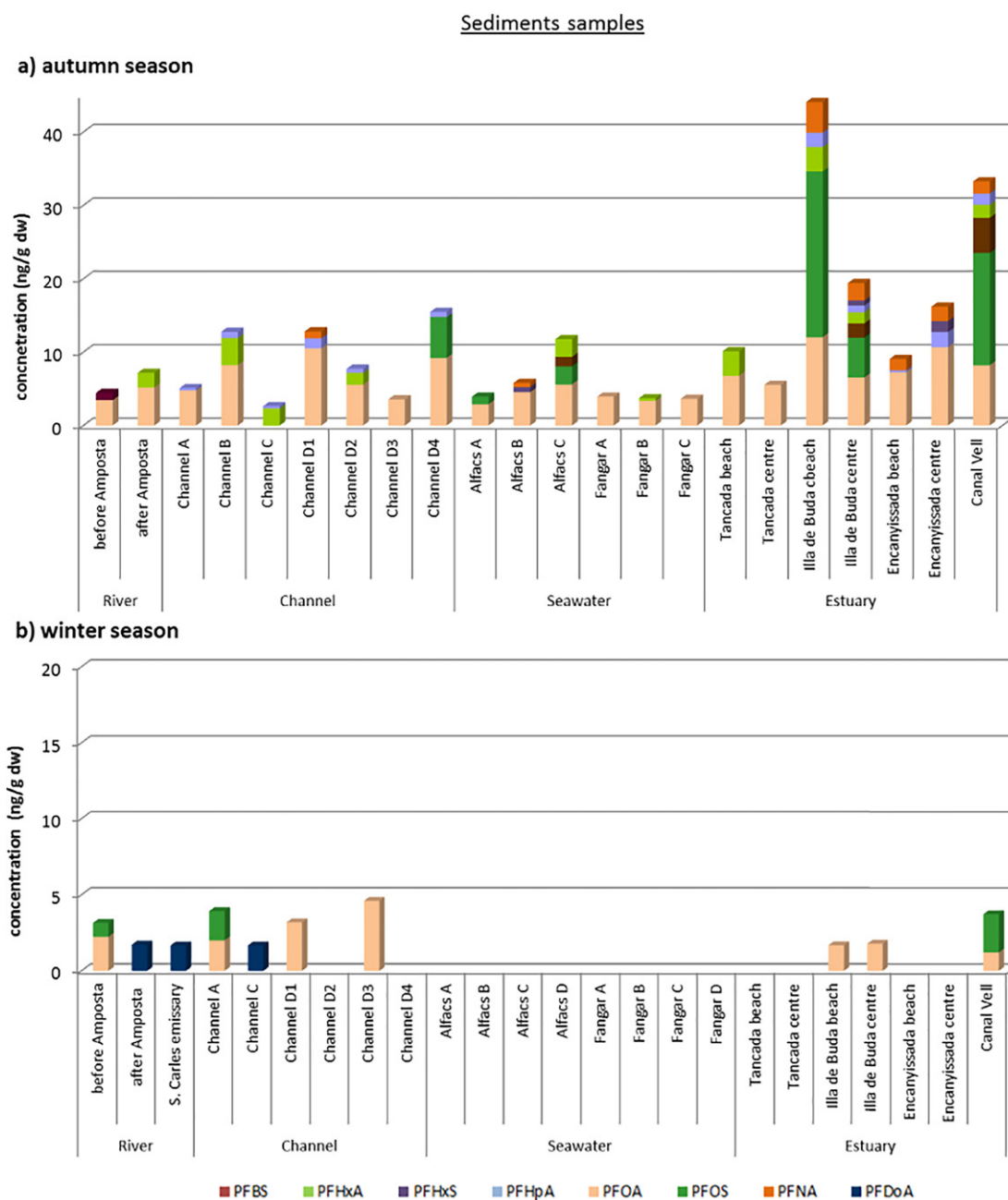
Sediments samples

a) autumn season

b) winter season

Fig. 2. PFAS concentrations (ng/g dw) detected in sediment samples during the autumn season (a) and the winter season (b).

study PFDA was the most frequently long-chain detected compound, contributing to the total PFAS amount of 9% on average, and comparable to the 6-carbon chain PFHxA (5% of total contribution). This suggests that, due to their higher use and replacement of 8-chain PFASs, short-chain compounds could lead over time to similar hazardous effects on organisms as those normally associated with long-chain compounds. PFOSA was detected in species such as *Cyprinus carpio* and *Silurus glanis*, even though no concentrations above the mLOQ were registered in waters or sediments. These fishes are long-lived species, reaching large sizes, and moreover, *Silurus glanis* is a common predator at the top of the aquatic food chain; PFOSA detection only in these species could be clear evidence of the biomagnification process throughout the trophic chain. In addition, detected concentrations of PFOSA can originate from amination of PFOS (Dimitrov et al., 2004) or from the metabolisation of the *N*-ethyl-perfluorooctane-sulfonamido-ethanol (N-Et-FOSE) (Frömel Tobias, 2010). Lower detection of PFOA in muscle and skin tissue (Fig. 3c), in comparison to the concentration registered

in the whole body (Fig. 3a and b), reinforce its preferential partition in liver, whereas PFDA showed a higher detection frequency, reaching almost 60% of PFAS contribution in fish muscle-skin tissues.

PFASs accumulation in fish organisms has already been studied by several authors, although the high differences in fish preparation, as well as the wide variety of analysed species, make it difficult to compare the results. Nevertheless, a rough comparison of the results obtained in the study can be done with values taken from literature and reported in Table S7. As it can be seen, data regarding fish biota can be very different from one work to another, and are mostly dependent on the species selected and their habits. However, as a general pattern displayed in the majority of works, PFOS shows to be the most bioaccumulative compound, while among PFCAs, the longer chain compounds (>C8) are the most abundant and the most frequently detected ones.

Data of freshwater biota were particularly compared to the results reported by Lorenzo et al. (2016) in the Ebro River, in order to compare the results obtained in different time periods but in the same river, even

Freshwater fishes
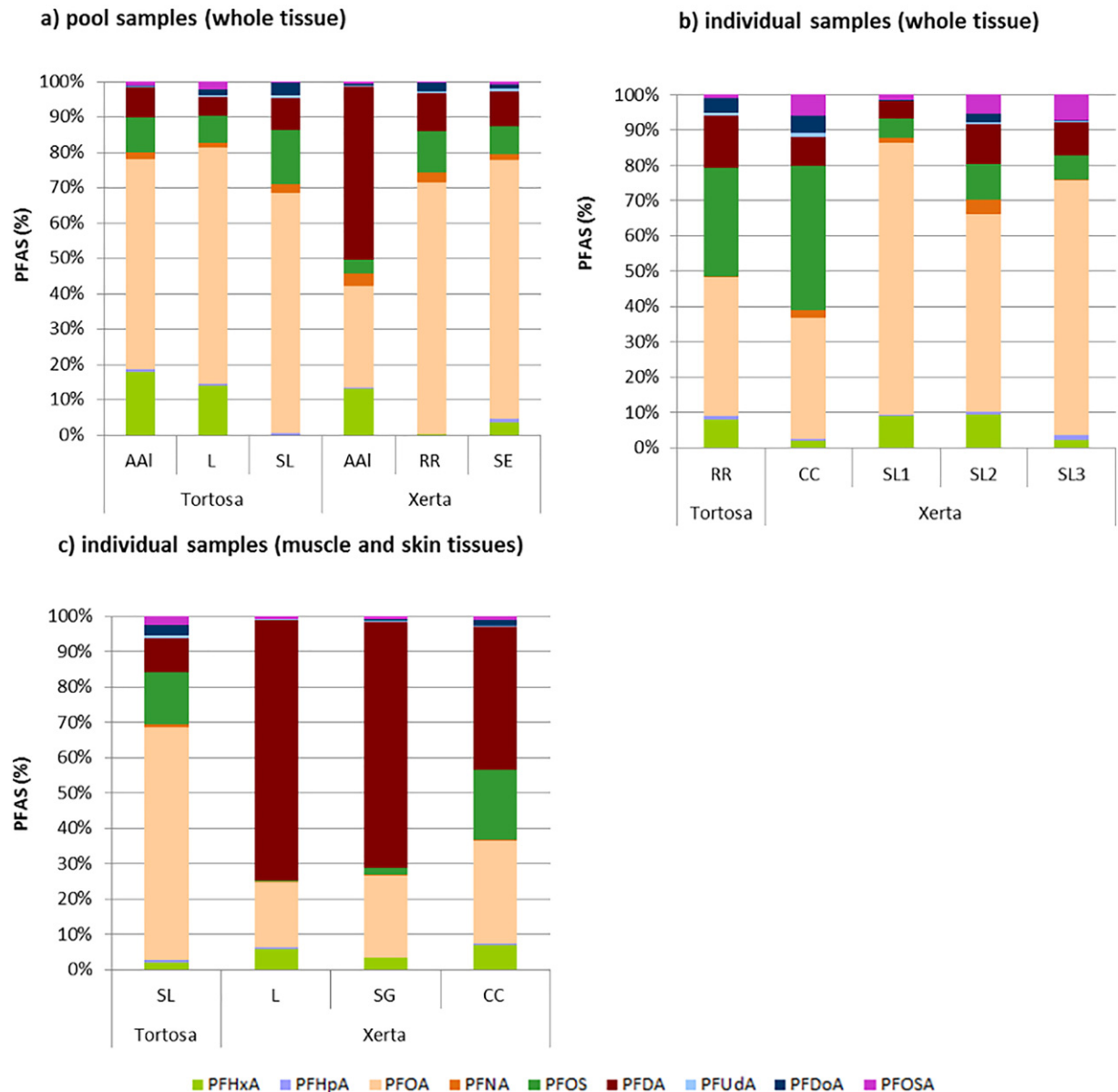
Fig. 3. PFAS concentration contributions (%) in freshwater fish samples. Data refer to the whole fish body concentration calculated from a pool of fishes (a) and from individuals (b), and muscle and tissue concentrations of individuals (c). The selected species are as follows: AAl: *Alburnus alburnus*; L: *Liza* sp.; SL: *Squalius laietanus*; RR: *Rutilus rutilus*; SE: *Scardinius erythrophthalmus*; CC: *Cyprinus carpio*; SG: *Silurus glanis*.

though the fishes that were analysed by the authors belong to the river upstream section, while fishes analysed in this work are closer to the Delta River mouth. Overall, both studies reported the occurrence of PFCAs such as PFOA and PFHxA as the main PFASs accumulated in biota. This is in contrast with the majority of other studies (Labadie and Chevreuil, 2011; Houde et al., 2011; Xu et al., 2014; Ye et al., 2008) that assessed perfluorinated sulfonates, and PFOS among all of them, as the most bioaccumulative of the perfluoroalkyl group. The major content of PFCAs in fish could be due to the higher occurrence of perfluorocarboxylic acids than sulfonates found in waters. However, concentrations of almost all PFASs detected in this study were much higher than those obtained by Lorenzo et al. (2016). Moreover, these very high values seemed not to be related to water data, which in turn showed much lower concentrations ($\sum$ PFASs of Ebro River near Xerta: 3.4 ng/l in autumn, 6.4 ng/l in winter and 1.0 ng/l in spring-summer). Bioaccumulation is the result of long time interactions between organisms and the contaminated environment, and can be thus

considered the evidence of water contamination events occurred in the past. Furthermore, fishes can move freely along the river and may have been affected by PFASs in different river transects far from the sampling area. Size and age of species, as well as gender, additionally influence the contaminants bioaccumulation and biotransformation process (Houde et al., 2011). All these aspects can thus explain such high PFASs concentrations detected in riverine fishes.

For each of the fish species collected in Xerta, a PFAS experimental bioaccumulation factor was calculated according to the formula BAF = C_b / C_w, and expressed as L/kg. C_b is the PFAS concentrations in fish and C_w its concentration in water, considering a mean value of water concentrations detected in Xerta throughout the year. BAF values were calculated only for PFHxA, PFOA, PFNA and PFOS because detections in water above the mLOQ were only available for these compounds in at least two sampling campaigns. LogBAF values are reported in Table S12. Among the four variables, PFNA showed the lowest logBAF values, while PFHxA, PFOA and PFOS showed comparable

values (mean value of 4.2 for PFHxA, 5.1 for PFOA and 5.0 for PFOS), suggesting a higher bioavailability and uptake of these compounds in comparison to PFNA.

Contamination by PFASs was also found in fish collected from the estuarine and coastal areas (Fig. 4). For these species, it was possible to separate skin from muscle, therefore, results for these two tissues are displayed separately. In this case, PFOS was the predominant compound, with the highest concentrations detected in Illa de Buda lagoon: the common carp (*Cyprinus carpio*, 14.5 ng/g ww) and the European eel (*Anguilla anguilla*, 21.6 ng/g ww). PFUdA and shorter-chain PFCAs (PFNA, PFOA, PFHpA, PFHxA) were also detected, but at lower concentrations (<5 ng/g ww). On the other hand, the flathead grey mullet (*Mugil cephalus*) and the largemouth bass (*Micropterus salmoides*) showed lower concentrations (max value of 4.2 ng/g ww for PFOS). Among the marine fishes that were collected in the open sea (Alfacs Bay and Fangar Bay), only the salema (*Sarpa salpa*) did not present contamination by PFASs. This is consistent with its feed which is based on algae. Fishes taken at Alfacs Bay showed slightly higher concentrations of PFASs with respect to Fangar Bay, especially regarding muscle tissue. The fish species common torpedo (*Torpedo torpedo*) and bogue (*Boops boops*) were found to be very similar in their PFAS accumulation level, even though they are characterised by different behavioural habits (*Torpedo torpedo* is a benthic predator, while *Boops boops* is an omnivorous semi-pelagic organism). Anyway, the very low range (<2 ng/g ww) at which PFAS concentrations were detected did not allow to distinguish any possible difference in the uptake of PFASs between the two species. No differences were found for PFAS distribution in muscle and

skin tissues, except for PFHxA (*p*-value of Kruskal-Wallis test = 0.02), as can be seen in the graphs, where PFHxA is preferentially distributed in fish skin, rather than in muscle. It is well-known that PFASs tend to bind preferentially to protein and accumulate in liver and blood (Kannan et al., 2005); the higher concentrations found in the skin for PFHxA are thus not likely to be related to a bioaccumulation process of the organisms, but rather to skin contact with contaminated lagoonal sediments.

Overall, the estuarine and marine biota analysed in this study showed an accumulation of perfluoroalkyl substances that are not found in waters or sediments, such as the short-chain PFHxA and PFHpA (see Figs. 1 and 2 for comparison). This is consistent with the fact that these compounds in waters and sediments are influenced by a high variability, due to the continuous changes of environmental conditions (e.g. temperature, pH, precipitation rates, water currents), while bioaccumulation through the aquatic food chain is the product of a longer time period exposure of organisms to contaminants. Seawater fishes showed lower concentrations compared to freshwater fishes, in contrast to what would be expected from water and sediments results, which highlighted a greater contamination of Illa de Buda lagoon, located in the final stretch of the Ebro Delta, with lower salinity than seawater sampling points, but much higher salinity than freshwater zones (Table S5). PFAS biota concentrations that are higher in freshwater than in seawater ecosystems have already been reported, and a possible explanation can be related to the lower solubility, and thus lower bioavailability, of PFAS in marine water (Zhao et al., 2011). It is noteworthy, however, that the majority of the species that were captured in the
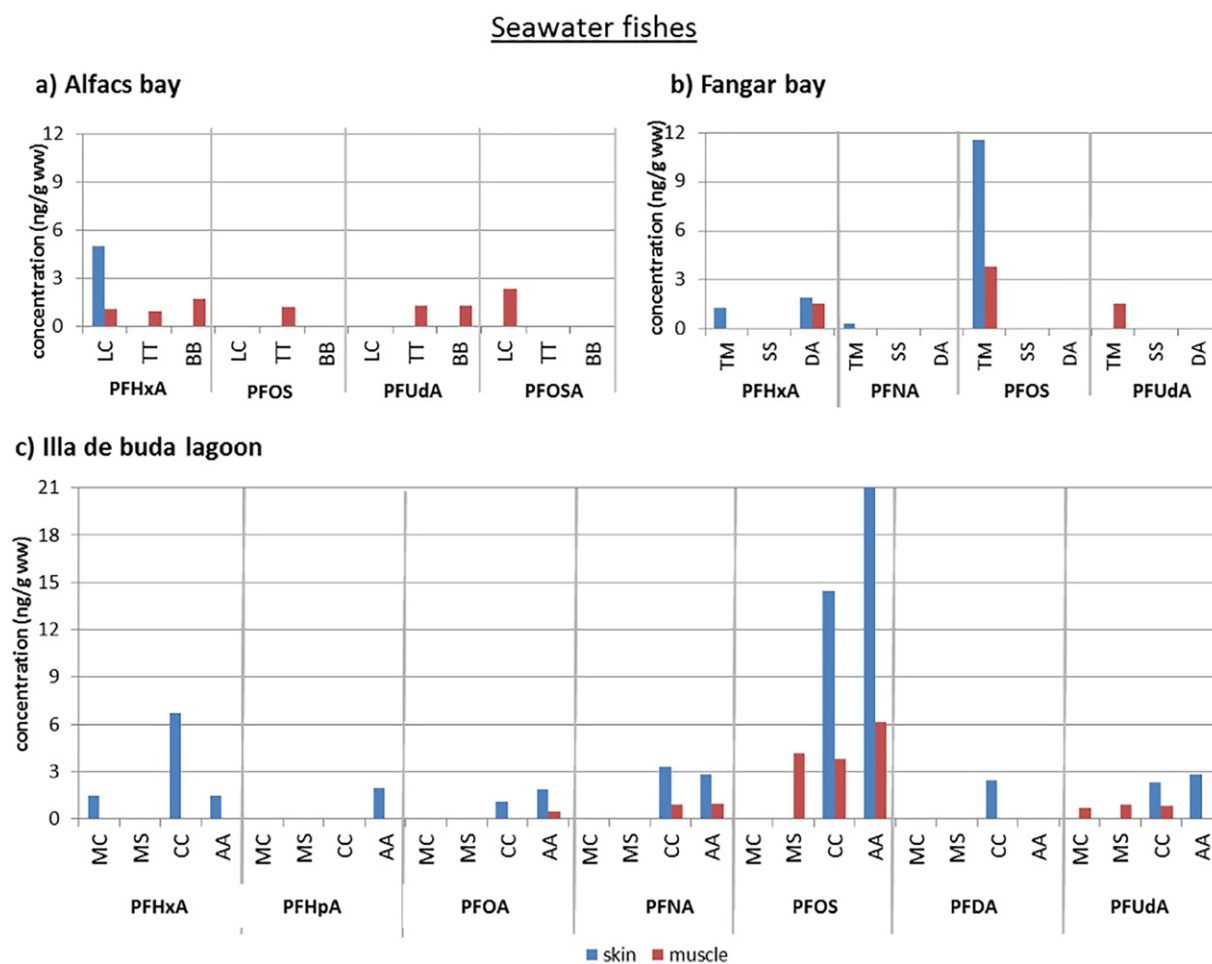
Fig. 4. PFAS concentrations (ng/g ww) in skin and muscles of coastal fishes collected in Alfacs Bay (a), Fangar Bay (b) and Illa de Buda lagoon (c). Fishes species are as follows: LC (*Mugil cephalus*), TT (*Torpedo torpedo*), BB (*Boops boops*), TM (*Trachurus mediterraneus*), SS (*Sarpa salpa*), DA (*Diplodus annularis*), MC (*Mugil cephalus*), MS (*Micropterus salmoides*), CC (*Cyprinus carpio*), AA (*Anguilla anguilla*).

estuarine area and in river were different. Among the common species that were captured in both environments, such as the common carp, those from Illa de Buda were younger. Caution should thus be exercised in comparing these results.

Although, in this study, the common carp (*Cyprinus carpio*) was the most affected species with respect to PFAS accumulation in tissues, both in river and estuarine environments. Bioaccumulation of contaminants is strictly species-specific, being mostly dependent on the metabolism of the selected organism. The results of this study point out that the common carp is a good marker of PFAS contamination in biota, as it is also well known for the many POPs and metal pollutants. These results should be taken into consideration when addressing future research on PFAS bioaccumulation.

PFASs accumulation in fish is an issue of great concern, since it can be related to human exposure to PFASs through intake of contaminated fish. In this context, EU Directive 2013/39/EU set an Environmental Quality Standard (EQS) for PFOS in fish biota of 9.1 μg/kg, in order to safeguard ecosystem and human health; no EQS for the other PFASs have been set yet, as a consequence of the lack of information about their real toxicity levels. Concentrations detected in this study in the edible muscle tissue of seawater fishes were acceptable according to the EU Directive; on the contrary, almost all freshwater fishes exceeded the EU threshold for PFOS (range < mLOQ–154 μg/kg dw; mean value 49.7 ± 50.7 μg/kg dw), thus revealing a very anomalous situation in Ebro River. To conclude, PFASs accumulation in freshwater fishes should thus be better analysed, considering that PFOA and PFDA showed even higher concentrations than PFOS, in order to understand if PFAS levels detected in fish of Ebro River can pose a risk for human beings or the ecosystem.

4. Conclusions

This study focused on the occurrence and biogeochemical features of PFASs in waters, sediments and fishes of the Ebro Delta region (Catalonia, Spain). Sampling campaigns in different time periods were carried out in order to investigate seasonal trends of PFASs. The study revealed a difference in the sampling campaigns that is likely due to the different environmental conditions, with the main influencing factors being temperature and rainfall regimes. PFOA was confirmed to be the predominant compound among all the perfluoroalkyl substances, both in water and in sediment. With respect to waters, PFPeA was the most abundant compound, reaching very high concentrations, especially in the WWTPs, as a consequence of its widespread use as an alternative to PFOA and PFOS. Concerning sediments, PFOS was found to be the most abundant perfluorinated compound, being detected at higher concentrations than those found in waters, and revealing its preferential behaviour to be adsorbed on sediment particles rather than staying in a water-dissolved phase. Sediments registered a very different pattern of PFASs than did water, consisting of a progressive decrease in the occurrence of PFASs throughout the year. This decrease reflects a very high influence of the environmental conditions on PFAS distribution in sediments and suggests water-sediment partitioning is happening over a long-term time-scale. Seawater fishes showed PFAS concentrations higher in their skin than in their muscle tissues; PFOS was once again the most abundant and the most detected compound. Results on waters, sediments and biota confirmed Illa de Buda to be the most contaminated site of the Ebro Delta. On the other hand, freshwater fishes showed very high concentrations of both sulfonates and carboxylic acids, in contrast with those ones registered for seawater fishes. Such high differences in concentrations could be due to a different uptake mechanism between freshwater and seawater fishes and to the different behavioural habitats of the two fish types.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.07.025.

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