Per- and poly-fluoroalkyl compounds in freshwater fish from the Rhône River: Influence of fish size, diet, prey contamination and biotransformation

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Title:

Per- and poly-fluoroalkyl compounds in freshwater fish from the Rhône River: influence of fish size, diet, prey contamination and biotransformation

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Highlights

- Barbels, gudgeons, and roaches displayed different PFAS accumulation levels and profiles.
- These differences between species were partially explained by the diet and prey contamination.
- Small barbels displayed higher concentrations of most PFAS than did larger individuals.
- Concentration ratios suggest that biotransformation occurred in invertebrates and fish.
- C9-C14 compounds were biomagnified in gudgeon, as were C11-C14 compounds in barbel.

Abstract

Pools of aquatic plants and benthic invertebrates were collected along with 47 individuals from three cyprinid fish species (*Barbus barbus*, *Gobio gobio*, *Rutilus rutilus*) at a site in the Rhône River (France). Carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) and a wide range of per- and poly-fluorinated chemicals (PFASs) were analyzed in all samples. The sum of PFAS concentrations (ΣPFAS) increased from aquatic plants to fish dorsal muscles; molecular profiles were dominated by C9-C13 perfluorocarboxylic acids (PFCAs), while perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonamide (FOSA) were detected in all samples at lower concentrations. ΣPFAS and especially ΣPFCAs were higher in barbels (*B. barbus*) than in other species, while roaches (*R. rutilus*) were less contaminated by PFOS than barbels and gudgeons (*G. gobio*). Gudgeons accumulated significantly higher FOSA concentrations. Young (small) barbels displayed significantly higher PFOS, perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) concentrations than did large specimens; conversely, perfluorotetradecanoic acid (PFTeDA) concentrations were significantly higher in large barbels. Multiple linear regressions were performed on the whole set of fish samples with size, mass and isotopic ratios as explicative variables, and several single compounds as explained variables. Regardless of the compound, the regressions did not explain much of the contamination variability. However, adding species as a qualitative variable, i.e. performing analyses of covariance (ANCOVAs) improved the fit greatly, while adding sex did not. Diet (i.e. $\delta^{13}C$ and $\delta^{15}N$) was the main factor explaining interspecific differences. Biotransformation was assessed by comparing concentration ratios of PFOS or FOSA to their precursors in the food-web compartments. These ratios increased from invertebrates to fish, and differed among fish species, suggesting that biotransformation occurred but was species-specific. Biomagnification factor calculations showed that C11-C13 PFCAs, PFOS and FOSA were apparently biomagnified in barbels and gudgeons.

Keywords

Perfluoroalkyl chemical – bioaccumulation – fish – diet – body size – biotransformation

1. Introduction

Per- and poly-fluorinated substances (PFASs, named according to Buck et al. (2011), constitute a large class of chemicals that have been extensively studied in the environment since 2001, when the first review of the worldwide occurrence of perfluoro-octane sulfonate (PFOS) in wildlife was published (Giesy and Kannan, 2001). Since then, despite a number of studies conducted, the fate of these compounds, especially their accumulation in aquatic biota, remains incompletely understood (Ahrens, 2011; Ahrens and Bundschuh, 2014; Houde et al., 2011; Houde et al., 2006b). While size and age are rather well-acknowledged factors contributing to explaining the bioaccumulation of classic hydrophobic/lipophilic compounds such as polychlorobiphenyls in fish (e.g. Gewurtz et al., 2011; Parmanne et al., 2006; Paterson et al., 2006; Vives et al., 2005), very few studies have discussed this aspect for PFASs.

Shi et al. (2010) found no relationship between PFOS concentrations and age in their study of 59 fish samples (≥ six species) from six lakes and one adjacent river from the Tibetan plateau, a remote area subject to neither direct releases of this compound nor its precursors. Furthermore, a significant correlation between female age and log-transformed concentrations of C11-C16 perfluoro-carboxylic acids (PFCAs) was observed in Chinese sturgeons (*Acipenser sinensis*) eggs from the Yangtze River in China (Peng et al., 2010). There was not such a correlation for shorter-chain (C6-C10) PFCAs or PFOS. The concentrations of PFOS, perfluoroundecanoic acid (PFUnDA) and perfluorooctane sulfonamide (FOSA) in muscle and liver gradually increased with length in tilapia (N = 78) from 11 river locations throughout the Pearl River Delta area (Pan et al., 2014). Conversely, significant negative relationships between ΣPFASs, ΣPFCAs (C6-C13) or the sum of concentrations of C6 and C8 perfluoroalkyl sulfonates (PFSAs) and age were found in blue-spotted rays (*Neotrygon kuhlii*), a viviparous fish species, from the South-Eastern coast of Queensland, Australia (Baduel et al., 2014). Currently, there is no unifying theory explaining these different patterns.

The biomagnification of PFAS also remains controversial, because biomagnification factors (BMFs) and trophic magnification factors (TMFs) vary greatly among studies. This could be due in part to methodological issues (Franklin, 2016), but also to the yet unexplained differences in accumulation patterns among species and compounds. Methodological issues make the findings (i.e. biomagnification) uncertain, while unexplained differences among species or individuals rather relate to variability. This distinction is important, because uncertainty might be reduced by improving the study design, while variability cannot; nevertheless variability could be better understood (Linkov et al., 2001; Von Stackelberg et al., 2008). Biotransformation of some compounds such as FOSA, or for which observed concentrations might partially result from the biotransformation of so-called precursors, such as PFOS (Martin et al., 2010), might be another source of variability, beside

environmental or ecological factors. Frequently cited precursors include *N*-ethyl perfluorooctane sulfonamide (EtFOSA) and FOSA (Benskin et al., 2009; Martin et al., 2010; Tomy et al., 2004; Yeung et al., 2009). *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA) and *N*-methyl perfluorooctane sulfonamidoacetic acid (MeFOSAA) are also known FOSA (and thus PFOS) precursors (Buck et al., 2011; Olsen et al., 2005).

The Rhône River downstream from Lyon has been subject to a PFAS contamination, characterized by a peculiar molecular profile where long-chain PFASs (N carbon atoms ≥ 11) are predominant (Loos et al., 2009; Miège et al., 2012; Munoz et al., 2015). This provides a unique opportunity to investigate the influence of potential determinants of PFAS bioaccumulation in fish. Our first hypothesis was that fish size, diet, and prey contamination could explain the PFAS contamination patterns in various fish species. Secondly, we explored whether the most prominent PFASs present in this river (Miège et al., 2012; Munoz et al., 2015) were biomagnified, on the basis of predator-prey relationships. Finally, we examined whether the PFOS loads in fish tissues could result from the biotransformation of sulfonamides or sulfonamidoacetic precursors.

2. Materials & Methods

2.1. Study site

The Rhône River is the second largest river in France, with a mean (1966-2011) daily discharge downstream from the confluence with the Saône River of 1,040 m^3 sec^{-1}. The French course of the Rhône River begins at the outlet of Lake Geneva, and is divided into four successive geographic units: the “Haut-Rhône” (210 km, from Geneva Lake to the confluence with the Saône River), the “Rhône-moyen” (110 km, between the Saône and Isère river confluence), the “Rhône-inférieur” (160 km, from the confluence with the Isère River to the upstream end of the delta), and the delta. The Rhône River course from its outlet from Lake Geneva to the delta was greatly modified in the 19th and 20th centuries, with the construction of dikes and numerous dams. The study site (N 45°28’17.0”; E 4°46’43.4”) belongs to the “Rhône-moyen” and is located 4.5 km downstream of a power-supply dam (Vaugris), and 12 km upstream of another dam (St-Pierre-de-Boeuf). The site includes an oxbow connected to the river by its downstream end and the adjacent river stretch (Supplementary information [SI], Figure S1). This site is located 40 km downstream of a fluoropolymer manufacturing plant, where polyvinylidene fluoride (PVDF) and various fluorinated polymers have been synthesized since the 1980s (Dauchy et al., 2012).

2.2. Invertebrate and aquatic plant sampling

Composite samples of aquatic plants were gathered by hand at the oxbow downstream end in October 2012. Composite benthic invertebrate samples were also collected at the oxbow

downstream end with a surber net in October 2012, June and October 2013, and sorted out on site on a 500 µm mesh sieve, at the family or order level, so as to collect approximately 1 g of fresh material in each category. The sorting out was refined in the laboratory, where invertebrate samples were weighed, and then freeze-dried.

2.3. Fish sampling

Three bentho-pelagic species were targeted, based on a previous study at a large spatial scale showing that these species tend to accumulate higher PFAS concentrations (Babut et al., 2011) , while representing different feeding behaviours: the barbel (*Barbus barbus*), which feeds mainly on benthic invertebrates, such as small crustaceans, insect larvae, molluscs, mayfly, and midge larvae; the gudgeon (*Gobio gobio*) which feeds on insect larvae, molluscs, and crustaceans; and the roach (*Rutilus rutilus*), whose diet is composed of benthic invertebrates, zooplankton, plant material, and detritus (Fishbase, http://www.fishbase.org/search.php , accessed May 13th, 2016). Fish were caught along the right bank (±500 m up- and downstream from the oxbow downstream end) by electro-fishing in October 2011; large barbels were collected with nets set overnight mainly along the left bank opposite the oxbow mouth in November 2011. Fish were euthanized and kept on ice until arrival at the laboratory, where size and mass were measured, and sex was determined whenever possible. Individual fish were dissected, in order to separate fillets and livers and collect the stomach contents. Fillets and livers were freeze-dried and ground, prior to analysis. Stomach contents were preserved in 5 % formalin before their examination under a microscope. Diet residues were identified and assigned to eight categories (namely aquatic insects, terrestrial insects, crustaceans, molluscs, fish, plants, detritus, and unidentified-empty). Prey occurrence (the percentage of fish eating the aforementioned categories) and individual variability of the diet (number of each prey versus the number of items in each stomach) were determined.

2.4. PFAS analysis

Chemicals were purchased from Wellington Laboratories (via BCP Instruments, Irigny, France) and Sigma-Aldrich (St Quentin Fallavier, France).

Full details on the chemicals and materials, as well as on the sample preparation procedure and LC-MS/MS operating conditions, are provided in companion papers (Bertin et al., 2014; Bertin et al., 2016). Briefly, upon surrogate standard addition (2 ng each), PFASs were extracted by sonication using methanol (MeOH), and the extracts subsequently concentrated under a nitrogen stream. Extracts were diluted in ultra-pure water and loaded through Strata X-AW cartridges. Analytes were recovered with 2 x 4 mL of basic methanol (MeOH/NH4OH 0.2 %), the extracts being passed through ENVI-Carb graphite cartridges directly connected under the Strata X-AW cartridges (tandem clean-

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up). The resulting eluates were concentrated to 400 µl under a nitrogen stream and transferred into injection vials. PFASs (see the detailed list of compounds in table S1 of the SI) were analysed using an Agilent 1200 Infinity high performance liquid chromatography chain interfaced with an Agilent 6490 triple quadrupole mass spectrometer, both from Agilent Technologies (Massy, France)

Fortified samples were run along with the samples to control accuracy rates and recovery rates of the whole method (Bertin et al., 2014; Labadie and Chevreuil, 2011). Recovery rates were generally in the range of 80–120%, with the exception of PFTeDA and MeFOSA (50–60%). The relative standard deviations were lower than 20%. The addition of suitable internal standards ensured adequate accuracy rates for the full list of targeted analytes. Method trueness was evaluated via the analysis of reference samples (NIST SRM 1947 Lake Michigan Fish Tissue); the determined concentration was within ± 20 % of the target value.

Replicate procedural blanks were also included in each sample batch. When applicable, PFAS concentrations were therefore systematically blank-corrected. For compounds present in procedural blanks, the limits of detection (LoDs) were defined as three times the standard deviation of the blank, and the limits of quantification (LoQs) were set at three times the LoDs (Muir and Sverko, 2006). For analytes not detected in blanks, LoDs and LoQs were determined as the concentration with a signal-to-noise ratio of 3 and 9, respectively. Alternatively, for analytes detected in neither blanks nor field samples, LoDs and LoQs were derived from the signal-to-noise ratio observed in low-level spiked samples.

2.5. Isotopic ratios determination and trophic level determination

C and N isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$ respectively) were determined according to Bodin et al. (2009). In brief, freeze-dried subsamples (fish muscle or invertebrates) were ultra-finely ground and precisely (± 0.01 mg) weighed, prior to lipid removal by accelerated solvent extraction with hexane and dichloromethane. C and N isotope measurements were determined using a Thermo Finnigan Delta V EA-IRMS (Elemental Analyzer – Isotope Ratio Mass Spectrometry) with a Conflo IV interface. All 10–15 analyses, IAEA-N2 ($\delta^{15}N$ = 20.3 ‰) and USG-24 ($\delta^{13}C$ = -16.1 ± 0.2 ‰) reference materials were included to ensure the validity of the results.

Determination of the trophic level (TL) was based on Eq. 1 (Post, 2002)

$$TL_{cons} = 2 + \frac{(\delta^{15}N_{cons} - \delta^{15}N_{base})}{\Delta N} \qquad \text{Eq. 1}$$

with TL_{cons} the trophic level of a consumer (predator) species, $\delta^{15}N_{cons}$ and $\delta^{15}N_{base}$ the respective N isotopic ratios of the predator and the baseline species, and ΔN the mean trophic enrichment. In this equation the baseline species is assigned a TL of 2, corresponding to a primary consumer. We used

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Corbicula fluminea, a long-living filter-feeding mollusc living in the sediment depth as the baseline. ΔN was set at 3.4 ‰ (Post, 2002).

2.6. Biomagnification factors

Biomagnification factors (BMFs) can be determined on the basis of fish specific tissue (e.g. Kannan et al., 2005) or whole-body measurements (Houde et al., 2006a). As whole-body concentration estimates were not available for all fish species, both types of BMFs are presented.

Biomagnification factors adjusted to trophic levels (BMF_{TL}) were determined for fish individuals on the basis of Eq. 2, according to Fisk et al. (2001):

$$BMF_{TL} = \frac{[predator]/[prey]}{TL_{predator}/TL_{prey}} \qquad \text{Eq. 2}$$

with [*predator*] and [*prey*] the respective concentrations in predator (fish tissue) and prey tissues, and *TL* determined following Eq. 1. Although this type of adjustment leads to lower values than adjusting on respective TLs by a simple subtraction, both approaches are conceptually similar. When based on whole-body concentrations, BMFs adjusted to TL are noted $BMF_{TL\text{-}WB}$; BMF_{TL} thus correspond to factors based on concentrations in fish dorsal muscle.

2.7. Statistics

We used Pro-UCL 5.0 software (https://www.epa.gov/land-research/proucl-software) to determine compound distributions accounting for left-censored results. Mann-Whitney (pair comparison) or Kruskal-Wallis (comparison of several samples) tests were applied for comparing contamination levels between groups with XLStat (2013.1) software. When the Kruskal-Wallis test yielded significant differences, the Dunn post-hoc procedure (comparison of rank means, assuming an asymptotic Gaussian distribution) was applied in order to specify which group(s) differ from the others. Multiple linear regressions were applied for exploring the relationships between contamination levels (dependent variable) and fish size, mass and isotopic ratios (explanatory variables). Model performance was assessed by analyzing the variance in two ways: Type I sum of squares analysis allowed the most significant variables to be identified, while the type III sum of squares informed about the effect of removing a variable on model fit. An analysis of covariance (ANCOVA) was applied in the same perspective as multiple linear regression, with species or sex as qualitative variables. The significance threshold was set at 0.05 in all analyses.

3. Results

3.1. Fish size, mass and diet

The data set included 20 barbels (101-620 mm, 12-3033g), 15 gudgeons (100-113 mm, 12.2-16.5 g)

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and 12 roaches (104-148 mm, 17.5-54 g). While gudgeon and roach sizes were quite homogenous, precluding the possibility of investigating the relationships between size and contamination for these species, barbels were distributed in two distinct groups: 11 young specimens, whose size ranged from 101 to 142 mm and mass from 12 to 40.1 g, and nine large individuals, whose size ranged from 450 to 620 mm and mass from 1,077 to 3,033 g.

Stomach contents were assigned to eight categories: aquatic insects, terrestrial insects, crustaceans, molluscs, fish, plants, detritus, and unidentified. This allowed us to determine the proportion of each prey category in individual diets from each fish species (Table 1).

Species (N: number of examined stomach contents)	aquatic insects	terrestrial insects	crustaceans	molluscs	fish	plants	detritus	unidentified or empty
B. barbus (17)	58.8%	0.0%	88.2%	47.1%	0.0%	11.8%	11.8%	0.0%
R. rutilus (12)	8.3%	0.0%	16.7%	50.0%	8.3%	83.3%	0.0%	8.3%
G. gobio (15)	73.3%	13.3%	20.0%	20.0%	0.0%	0.0%	20.0%	13.3%

Table 1 – Occurrences of prey categories in selected fish species at one sampling site from the Rhône River

As expected, roach stomach content included mainly aquatic plants, while those of barbels or gudgeons were more varied. Chironomids were systematically identified when the stomach contained aquatic insects, whereas Trichoptera occurred in only 9% of these samples. Gammarids were identified in 90% of the contents that included crustaceans; mollusc species could not be identified in several instances (one out of 16 in barbel stomach contents, four out of five for roach). Young barbels ate both chironomids larvae and gammarids, while large individuals tended to abandon chironomids. The distribution of some types of prey among individuals was based on identifiable residues (insects, crustaceans, molluscs), expressed as percentages of the number of residues in the bolus. In the case of gudgeons, the crustaceans' third quartile equalled 1%, while it was 85% in barbels. Conversely, the first quartile of insects equalled 99% for gudgeons, but 0% for barbels. With a third quartile equalling 9%, the consumption of molluscs by barbels was certainly underestimated, since only shell shreds were retrieved, prohibiting an accurate count of the number of ingested molluscs.

$\delta^{13}C$ values ranged from -14.41‰ and -29.23‰ in plants and invertebrates (Figure S2-A in SI), and from -21.81‰ to -25.65‰ in fish (Figure S2-B in SI). Most invertebrates displayed $\delta^{13}C$ values between -23.38‰ and -29.23‰; only plants and some molluscs (Gastropods) had less negative $\delta^{13}C$

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values, consistent with the macrophyte photosynthetic activity, and the plant consumption by Gastropods (Tachet et al., 2010), while other benthic invertebrates relied on sediment carbon sources from terrestrial origins (Dubois, 2012). Gudgeon $\delta^{13}C$ values (median -24.06‰, standard deviation (SD) 0.32‰) were less variable than those of barbel (median -23.91‰ SD 1.16‰) and roach (median -23.64‰, SD 1.116‰), suggesting a less diverse diet in terms of carbon sources. Furthermore, $\delta^{13}C$ was significantly less negative in young barbels than in large/older ones (Mann-Whitney test, *p*-value=0.001). These values are consistent with the fish respective stomach contents, while providing a wider understanding of the exploited carbon sources: roaches feed on macrophytes and pelagic invertebrates, in variable proportions among individuals. Gudgeon $\delta^{13}C$ values were slightly less negative than chironomid ones, suggesting that chironomids indeed contributed most to gudgeons diet, along with a minor other carbon source (yet unidentified). Barbel diet shifted toward benthic preys when getting older and larger.

On the basis of Eq.1, calculated TLs ranged between 1.77 and 2.89 in invertebrates, and between 3.12 and 3.65 in fish; there was no difference among the three fish species (Table S2 in SI). When $\delta^{15}N$ values of young and large barbels were compared (Mann-Whitney test), the *p*-value (0.058) was slightly above the 0.05 threshold.

3.2. PFAS occurrence and concentration levels

PFAS concentrations (ΣPFAS, i.e. the sum of the concentrations of the measured compounds) increased from aquatic plants to fish muscle (Figure 1).

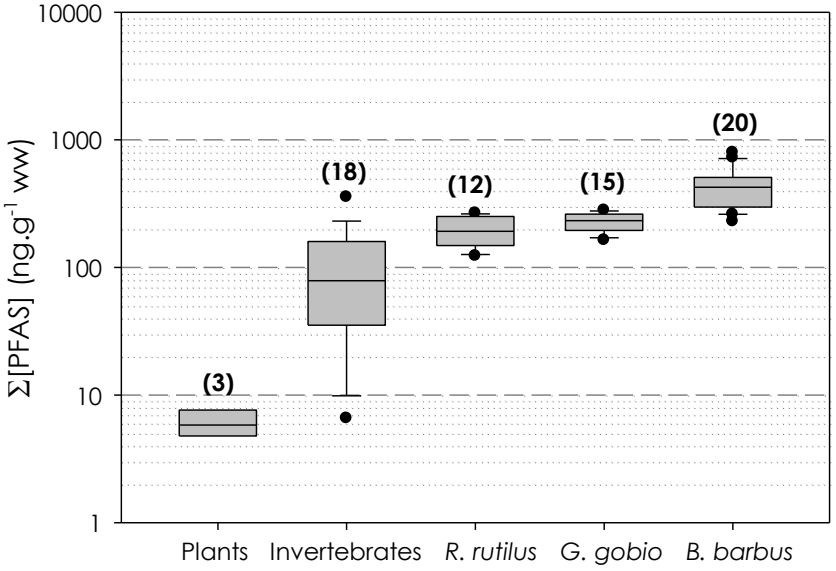

Figure 1 – Box-plots of ΣPFAS concentrations (ng.g^{-1} ww) in plants, invertebrates and three fish species. Respective sample sizes are reported above each box.

Aquatic plants, identified as *Myriophyllum spicatum*, *Ceratophyllum demersum* and *Valisneria spiralis*, displayed low PFAS concentrations, with ΣPFAS ranging from 4.78 to 7.63 $ng.g^{-1}$ expressed on a wet weight (ww) basis. The highest measured concentrations in these samples were for PFNA (1.02–1.68 $ng.g^{-1}$ ww), PFUnDA (1.28–2.31 $ng.g^{-1}$ ww) and PFTrDA (1.99 – 2.91 $ng.g^{-1}$ ww). In invertebrates, ΣPFAS ranged from 6.56 $ng.g^{-1}$ ww in a chironomid sample to 355.9 $ng.g^{-1}$ ww in a gammarid sample (details presented in Table S3 in SI). PFUnDA, PFTrDA, PFOS (linear isomer, L-PFFOS), FOSA and 6:2 FTSA were detected in 100% of these samples; PFNA, PFDA, PFDoDA, PFTeDA and PFHxS were also frequently detected (80–90%), while PFOA, PFDS and MeFOSAA occurrence frequencies ranged between 60% and 70%. PFHpS and MeFOSA occurrence frequencies were 39% and 5.6% respectively. PFTrDA and PFUnDA displayed concentrations well above those of the other targeted PFASs, followed by PFNA and PFOS. EtFOSAA was less frequently detected and at lower concentrations than FOSA, while MeFOSAA was measured in 13 out of 17 samples, at concentrations about five times lower than FOSA. Detection rates in fish dorsal muscles (fillets) varied greatly, from 0% for MeFOSA, EtFOSA and EtFOSAA, to 100% for C9-C14 PFCAs, PFOS (branched and linear isomers), MeFOSAA and FOSA. PFHxS, PFDS and PFOA were detected in 93.6%, 42.6% and 29.8% of fish fillet samples respectively. The molecular profiles of these samples looked similar to those observed in the other compartments (Figure S3 in SI), though significant differences could be noted for some groups/compounds, e.g. between barbels and gudgeons for PFNA or between invertebrates and barbels for PFTrDA (Kruskal-Wallis / Dunn tests). Contamination levels were higher in barbels than in other species for ΣPFAS as well as individual PFCAs, in particular PFUnDA and PFTrDA (Kruskal-Wallis test, *p*-value < 0.0001). Gudgeons and barbels also displayed significantly higher PFNA and PFOS concentrations than roaches (*p*-value < 0.0001). In the case of PFOS, the *p*-value of the Dunn test comparing gudgeons and barbels equaled 0.051, slightly above the 0.05 threshold. Gudgeons accumulated significantly higher FOSA concentrations than the two other species (*p*-value < 0.0001), which displayed similar concentrations of this compound. Gudgeons also displayed higher occurrences of PFOA, PFDS and 6:2 FTSA compared with the other two species, but the respective concentrations remained low (90^{th} percentiles 0.05 – 0.11 $ng.g^{-1}$ ww; see table S4 in SI).

In all, 19 liver samples were analysed, i.e. for ten roaches and nine barbels. Both ΣPFAS and individual PFAS concentrations were much higher in the liver samples than in the corresponding fillets (**Erreur ! Source du renvoi introuvable.**), as expected from the literature (e.g. Hoff et al., 2003; Kannan et al., 2002; Labadie and Chevreuil, 2011; Martin et al., 2003a). The variability of PFAS concentrations in roach livers was higher than in fillets, and no pattern was apparent. With the exception of one outlier, barbel ΣPFAS liver and fillet concentrations were correlated (R^2=0.83, *p*-value=0.002, *N*=8). Contrary to roaches, for which liver mass varied greatly while the size range

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remained limited, barbel liver and fillet masses increased as fish size increased.

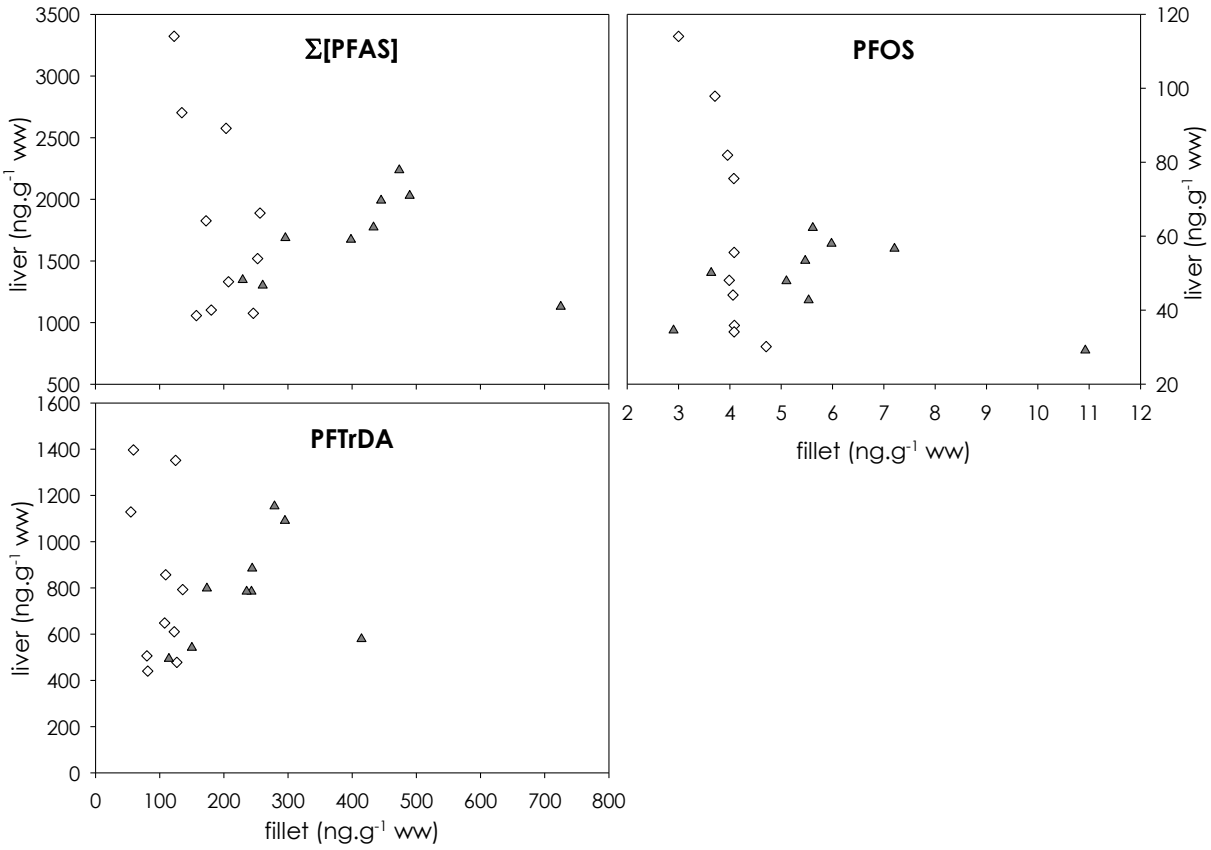

Figure 2 – Comparison of PFAS concentrations (ng.g^{-1} ww) in dorsal muscles (x axis) and livers (y axis) for 10 roaches (open diamonds) and eight barbels (gray triangles)

3.3. Influence of size on barbels' contamination

Young barbels displayed significantly higher PFOS, PFNA and PFDA fillet concentrations than large specimens. PFUnDA, PFDoDA, PFTrDA and ΣPFAS concentrations were not significantly different between young and large barbels. Only PFTeDA had significantly higher levels in large barbels compared with young ones (*p*-value=0.0003). PFDS was undetected in ten out of 11 young barbels, and two out of nine large ones; when >LoD, the measured concentrations remained low, ranging from 0.045 to 0.073 ng.g^{-1} ww.

3.4. Regression approaches

Multiple linear regressions were performed on the whole set of fish fillet samples (*N* = 47) with size, mass and isotopic ratios as explicative variables, and various single compounds (PFOS, FOSA, PFNA and PFTrDA) as explained variables. These compounds were selected because (i) their accumulation patterns differed among the three species (Figure S4 in SI) and (ii) they were quantified in all samples. Weak but significant correlations (Pearson) were noted for PFOS and FOSA (*p*-value <

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0.0001, R^2 = 0.33), PFOS and PFNA (*p*-value < 0.0001, R^2 = 0.31), FOSA and PFNA (*p*-value = 0.02, R^2 = 0.12), and PFNA and PFTrDA (*p*-value = 0.0001, R^2 = 0.28). Overall, regardless of the tested compound, the multiple linear regressions did not explain much of the contamination variability. In the case of PFOS, the *p*-value of the model including all explaining variables was slightly above the threshold (0.059; Table 2-A). Removing the variable "mass" from the dataset, as suggested by the results of the type III sum of squares analysis, yielded a significant relationship (*p*-value = 0.027), but the explained variance remained low. Size, $\delta^{13}C$ and $\delta^{15}N$ were the most significant variables (*p*-values < 0.0001, 0.016 and 0.005, respectively, in the type I sum of squares analysis) explaining FOSA concentrations. However, the type III analysis suggested removing first $\delta^{13}C$ (*p*-value = 0.588), which did not improve the fit. Size, $\delta^{13}C$ and $\delta^{15}N$ were also the most significant variables in the PFNA models. For PFTrDA, the regression using all variables was not significant (*p*-value = 0.162, Table 2-A); removing $\delta^{15}N$, as suggested by the type III sum of squares analysis, could make sense because trophic levels of all individuals from the three species were in the same range, and not determined by species. Nonetheless, it did not lead to a significant relationship (*p*-value = 0.09).

A- Multiple linear regression

Compound	Variables	Adjusted R^2	*p*	Contributing quantitative variables (*p*)
PFOS	size, mass, $\delta^{13}C$, $\delta^{15}N$	0.11	0.059	mass, $\delta^{15}N$ (0.057); $\delta^{13}C$ (0.150); size (0.828)
	size, $\delta^{13}C$, $\delta^{15}N$	0.13	0.027	$\delta^{15}N$ (0.054); size (0.057); $\delta^{13}C$ (0.133)
	size, $\delta^{15}N$	0.15	0.010	$\delta^{15}N$ (0.015); size (0.054)
FOSA	size, mass, $\delta^{13}C$, $\delta^{15}N$	0.42	< 0.0001	size (<0.0001); $\delta^{15}N$ (0.005); $\delta^{13}C$ (0.016); mass (0.216)
	size, mass, $\delta^{15}N$	0.43	< 0.0001	size (<0.0001); $\delta^{15}N$ (0.0003); mass (0.212)
PFNA	size, mass, $\delta^{13}C$, $\delta^{15}N$	0.33	0.0003	size (< 0.0001); $\delta^{13}C$ (0.085); $\delta^{15}N$ (0.290); mass (0.725)
	size, $\delta^{13}C$, $\delta^{15}N$	0.34	< 0.0001	size (< 0.0001); $\delta^{13}C$ (0.072); $\delta^{15}N$ (0.305)
PFTrDA	size, mass, $\delta^{13}C$, $\delta^{15}N$	0.06	0.162	size (0.035); $\delta^{13}C$ (0.164); $\delta^{15}N$ (0.718); mass (0.857)

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B- ANCOVA

Compound	Variables	Adjusted R^2	*p*	Contributing quantitative variables (*p*)	Qualitative variables (*p*)
PFOS	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species	0.55	< 0.0001	mass, $\delta^{15}N$ (0.009); $\delta^{13}C$ (0.046); size (0.761)	< 0.0001
	mass, $\delta^{13}C$, $\delta^{15}N$ / species	0.56	< 0.0001	$\delta^{15}N$ (0.007); mass (0.008); $\delta^{13}C$ (0.089)	< 0.0001
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / sex	0.23	0.011	$\delta^{15}N$ (0.042); size (0.045); $\delta^{13}C$ (0.124); mass (0.690)	0.025
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species + sex	0.53	< 0.0001	$\delta^{15}N$ (0.011); size (0.012); $\delta^{13}C$ (0.051); mass (0.610)	< 0.0001 (spp.); 0.003 (sex)
FOSA	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species	0.71	< 0.0001	size, $\delta^{15}N$ (< 0.0001); mass (0.085); $\delta^{13}C$ (0.447)	< 0.0001
	size, mass, $\delta^{13}C$, $\delta^{15}N$ /sex	0.43	< 0.0001	mass (0.0003), $\delta^{15}N$ (0.0004), size (0.014), $\delta^{13}C$ (0.585)	0.267
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species + sex	0.70	< 0.001	mass, $\delta^{15}N$ (<0.0001), size (0.001), $\delta^{13}C$ (0.449)	< 0.0001 (spp.); 0.085 (sex)
PFNA	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species	0.74	< 0.0001	mass (< 0.0001); $\delta^{13}C$ (0.007); size (0.008); $\delta^{15}N$ (0.094)	< 0.0001
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / sex	0.74	< 0.0001	mass (< 0.0001); $\delta^{13}C$ (0.007); size (0.008); $\delta^{15}N$ (0.094)	0.010
PFTrDA	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species	0.80	< 0.0001	size (< 0.0001); d13C (0.004); $\delta^{15}N$ (0.437); mass (0.697)	< 0.0001
	size, $\delta^{13}C$, $\delta^{15}N$ / species	0.75	< 0.0001	size (0.118); $\delta^{13}C$ (0.141); $\delta^{15}N$ (0.343)	< 0.0001
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / sex	0.29	0.003	mass (0.025); $\delta^{13}C$ (0.112); size (0.351); $\delta^{15}N$ (0.679)	0.002
	size, mass, $\delta^{13}C$, $\delta^{15}N$ / species + sex	0.80	< 0.0001	mass (< 0.0001); $\delta^{13}C$ (0.004); size (0.085); $\delta^{15}N$ (0.440)	< 0.0001 (spp.); 0.463 (sex)

Table 2 – Regression models for PFOS, FOSA, PFNA and PFTrDA

The inclusion of species as a qualitative variable in the regression improved the fit for all compounds (Table 2-B). The best results were obtained for PFTrDA (R^2 = 0.80; *p*-value < 0.0001) and FOSA (R^2 = 0.71; *p*-value < 0.0001) with all explaining variables included. Again, removing the less contributing variables, such as mass for PFTrDA or size for PFOS, only slightly increased the explained variances. Furthermore, including sex alone as a qualitative variable led to significant relationships with *p*-value ranging from < 0.0001 for PFNA to 0.01 for PFOS, but low R^2, except for FOSA, which had a high *p*-value for sex. Combining species and sex in the same ANCOVA yielded fits similar to species alone, since sex was not significant (*p*-value 0.463), contrary to species (Table 2, part B). As hypothesized, PFAS bioaccumulation in fish was thus influenced by several factors, namely fish size and fish diet, to extents varying according to the compound.

3.5. Biomagnification

BMFs adjusted to TL (BMF_{TL}, Eq. 2) were calculated based on barbel or gudgeon fillet concentrations

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and contamination of their main prey, namely gammarids for barbels and chironomids for gudgeons. Because invertebrates were analysed as pools, only two prey data were available, while fish were analysed as individuals. All the possible predator-prey combinations were tested, yielding a range of BMF_{TL}, values for each fish species. BMF_{TL}s were nevertheless considered as undetermined when PFAS were <LoD in fish or their prey, or both. BMF_{TL}s could be determined for C9-C14 PFCAs, C6-C8 PFSAs and FOSA in barbels (Figure S5 A in SI), and C8-C13 PFCAs, PFHxS, PFOS and FOSA in gudgeons (Figure S5 B in SI). BMF_{TL}s could also be calculated for PFDS in large barbels. The respective values are reported in Table 3.

BMF_{TL}	*G. gobio* / chironomids			*B. barbus* (small) / gammarids			*B. barbus* (large) / gammarids		
	Min	Median	Max	Min	Median	Max	Min	Median	Max
PFOA	0.28	0.44	0.72						
PFNA	2.81	14.68	64.25	1.53	2.42	3.40	0.15	0.22	0.87
PFDA	11.45	15.97	19.99	0.45	0.88	1.42	0.34	0.48	0.94
PFUnDA	14.26	35.08	78.97	1.29	3.32	6.18	1.79	2.56	4.61
PFDoDA	139.56	176.68	231.49	0.47	0.97	2.51	0.54	1.02	1.89
PFTrDA	11.70	28.23	67.76	1.00	1.46	2.83	0.88	1.90	3.19
PFTeDA	nd	nd	nd	0.42	0.94	3.00	1.73	6.07	11.64
PFHxS	1.43	2.57	4.70	0.18	0.28	0.51	0.10	0.14	0.30
PFHpS	nd	nd	nd	0.27	0.37	0.57	0.09	0.15	0.50
PFOS	1.98	3.93	7.98	0.52	0.85	1.10	0.32	0.59	1.13
PFDS	nd	nd	nd	nd	nd	nd	1.24	1.52	2.19
FOSA	5.67	13.66	39.16	0.63	0.78	1.14	0.20	0.30	0.41

Table 3 - BMF_{TL} distributions (based on PFAS measurements in fillets); nd: not determined

PFDoDA BMFs calculated for gudgeon-chironomid pairs seem unreliable, because the concentrations in chironomids are too close to the LoD. Excluding PFDoDA, median BMF_{TL} for gudgeon-chironomid pairs were correlated to the number of perfluorinated carbon atoms (Pearson test, $N = 8$, $R^2 = 0.73$ p-value 0.007). BMF_{TL}s for PFNA, PFDA, PFTeDA, PFHxS, PFHpS, PFOS and FOSA of small/young barbels were higher than those of large barbels. Conversely, BMF_{TL}s for PFTrDA and PFTeDA were higher for large barbels than for small ones, while PFUnDA and PFDoDA were similar in the two groups. The pattern relating the number of perfluorinated carbon atoms and BMFs was quite complex: BMF_{TL}s of small barbels first increased with the number on perfluorinated carbon atoms, till N=10, i.e. PFUnDA, and then decreased. BMF_{TL}s of PFCAs based on large barbels increased linearly as according to the number of perfluorinated carbon atoms (N=6, R^2=0.625, p-value =0.03) BMF_{TL}s of sulfonates were higher than those of PFCAs having the same number of perfluorinated carbon atoms.

BMFs based on whole-body concentrations ($BMF_{TL\text{-}WB}$) or estimates thereof would be more robust than BMFs based on measurements in fractions of the predator such as fillet or liver (Franklin, 2016). Accordingly we attempted to estimate whole-body concentrations for the barbel, based on regressions developed elsewhere for this species (as summarized in the SI, Tables S5 and S6). Median

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$BMF_{TL\text{-}WB}$ values for barbels ranged from 0.36 for PFNA to 21.97 for FOSA (Figure 3; Table S7 in SI). BMF ranges for PFNA and PFDA were in part below 1, this benchmark falling between the median and the third quartile for the former, and between the first quartile and the median for the latter. In the case of PFUnDA, with a minimum BMF at 0.91, almost all of the range was above 1. $BMF_{TL\text{-}WB}$ ranges for PFDoDA, PFTrDA, PFOS and FOSA were completely above 1 (Figure 3). Despite a rather high R^2 value (0.75), the correlation of PFCA $BMF_{TL\text{-}WB}$ with the number of perfluorinated carbon atoms was not significant (*p*-value 0.06).

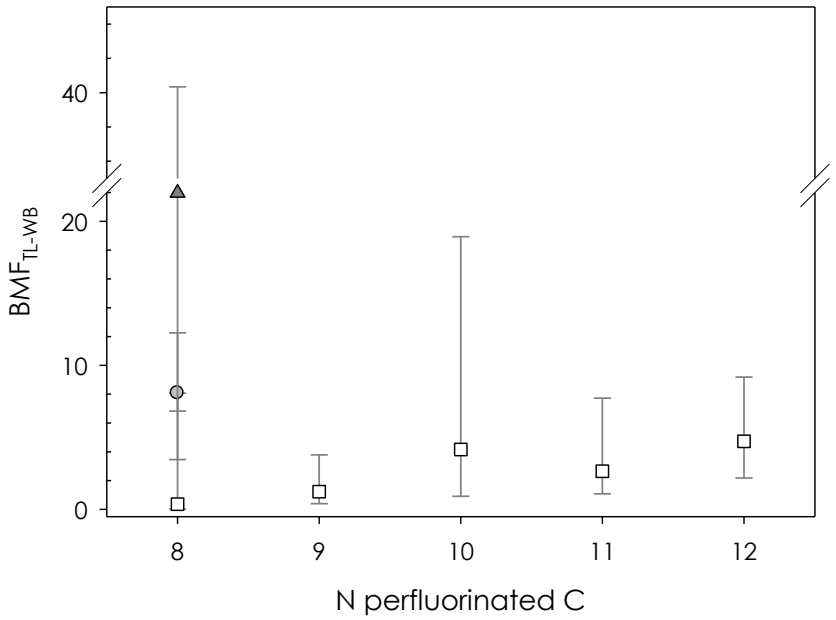

Figure 3 - Median $BMF_{TL\text{-}WB}$ for barbels for selected PFCAs (open squares), PFOS (gray dot) and FOSA (dark gray triangle); error bars correspond to minimum and maximum values.

As noted recently by Franklin (2016), it is somewhat tricky to compare BMFs from published studies; this remark stems from methodological differences, such as the predator fraction used in the calculation, or adjustment to trophic level. Other sources of uncertainty in such comparisons include the species or the ecosystems studied. For these reasons we only compared our results to BMFs involving fish as predators. PFNA, PFFHxS and PFOS BMFs based on concentrations in fillet of barbels were similar to those of rainbow trout (0.23 – 0.42) from a feeding experiment (Goeritz et al., 2013). As noted above, BMFs obtained for gudgeon were consistently above 1 for the same compounds. BMFs based on whole-body measurements in barbels (our study, Table S7 in SI) were in the same range than in lake trout (Martin et al., 2004), higher for FOSA and PFOS (2.9 and 1.4 respectively), and PFTrDA (2.5) to a lesser extent, while it was the opposite for PFNA (2.3) and PFDA (2.7). These $BMF_{TL\text{-}WB}$ were overall consistent with other field studies dealing with PFAS biomagnification in freshwater food-webs (e.g. Fang et al., 2014; Xu et al., 2014) who found trophic magnification factors

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> 1 for long-chain PFCAs and PFOS. The main difference is for FOSA, which displayed high BMFs in this study while it was not in previous field studies (Fang et al., 2014), or to a much lower extent (Martin et al., 2004).

3.6. Biotransformation

Biotransformation was assessed by comparing ratios PFOS or FOSA concentrations with those of their precursors (MeFOSAA, EtFOSAA, MeFOSA, EtFOSA, and FOSA) in the food-web compartments. Concentrations of these compounds were also available for surface sediments from the same site at the same period (Bertin et al., 2014; Bertin et al., 2016); Table S8 in SI). EtFOSAA was the most significant precursor measured in these sediment samples, with concentrations ranging from <LoD to 0.48 ng.g^{-1} dw, a range comparable to that of PFOS. FOSA concentrations in this compartment were an order of magnitude lower, and the other precursors remained undetected in most instances. In sediment, [FOSA]/[EtFOSAA] ratios could be determined in three instances, and ranged between 0.16 and 0.18. This ratio was undetermined in several invertebrate samples (*N*=9 out of 17) when EtFOSAA concentrations were <LoD; when EtFOSAA was measurable (*N*=8), the ratio was greater than in sediment (median value 2.19). As EtFOSAA was not detected in fish, this ratio was not calculated for this compartment. Both [PFOS]/[FOSA] and [FOSA]/[MeFOSAA] ratios increased from sediment to barbel samples, but not roach nor gudgeon (Figure 4).

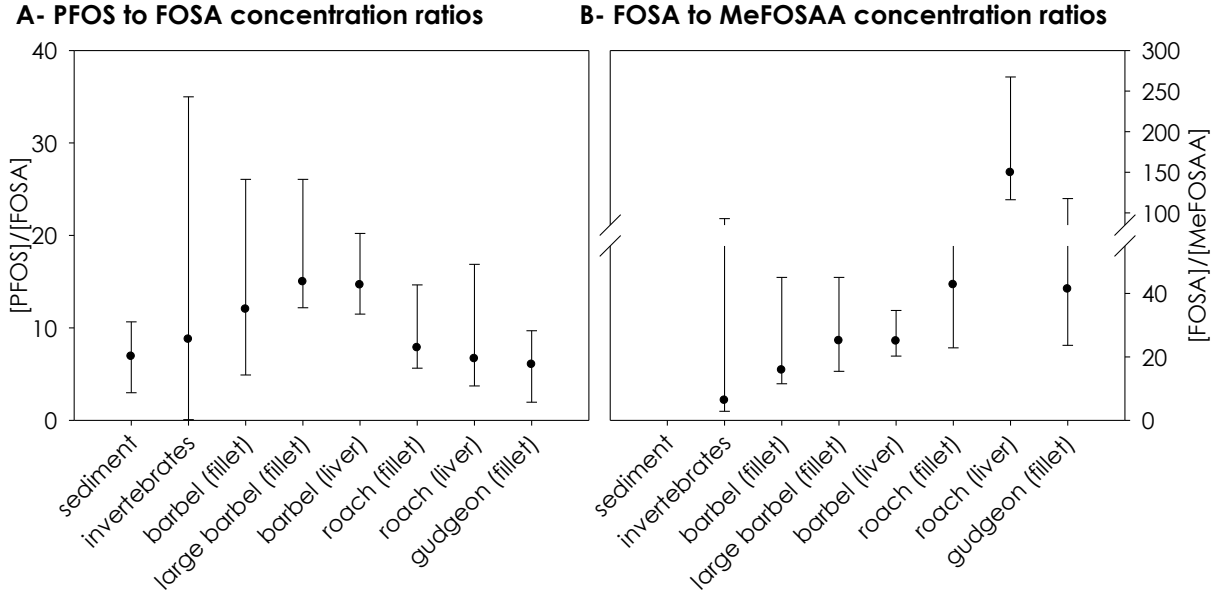

Figure 4 – Median and min/max values of PFOS precursor concentration ratios in the studied food webs: PFOS versus FOSA (A) and FOSA versus MeFOSAA (B)

[PFOS]/[FOSA] ratios were significantly lower in gudgeon (median value, 6.03) than in barbel fillets

(median value 12.01), while roach ratios (median value 7.84) were not significantly different from those of either barbel or gudgeon. Both ratios were significantly higher in large barbels compared with small ones. [FOSA]/[MeFOSAA] ratios were higher than [PFOS]/[FOSA] ratios, and significantly lower in barbel fillets (median value 15.81) than in roach or gudgeon (respective median values 42.72 and 41.35). When calculated on the basis of concentrations in barbel or roach livers, these ratios displayed contrasted patterns (**Erreur ! Source du renvoi introuvable.**): while [PFOS]/[FOSA] ratios were similar in muscle and liver, as well as barbel [FOSA]/[MeFOSAA] ratios, the latter were much higher in roach livers compared with fillets.

When accounting for all samples (invertebrates pools and fish individuals), the [FOSA]/[MeFOSAA] ratio tended to increase with increasing δ^{15}N values (Mann-Kendall, *p*-value 0.01), but (i) the range of δ^{15}N values was limited (7.58 ‰ – 13.97‰), and (ii) the respective sample sizes of invertebrates and fish individuals were clearly unbalanced. This trend should accordingly be taken with caution. Conversely, there was no evidence of such a trend for the [PFOS]/[FOSA] ratio.

4. Discussion

4.1. Factors driving PFAS accumulation in fish

Contamination levels were higher in barbels than in other species for ΣPFAS as well as PFCAs, especially PFUnDA and PFTrDA. For PFOS and FOSA, gudgeons were more contaminated than the other species. Fish diet appears to be an important explanation factor, supported by stomach content distribution and prey contamination, as well as δ^{15}N or δ^{13}C in multiple linear regressions (Table 2). Indeed, these parameters were among the most contributing variables in all regressions, i.e., δ^{15}N for PFOS and FOSA, and δ^{13}C for PFNA and PFTrDA. More negative δ^{13}C values correspond to detrital organic matter, in particular from terrestrial plants (Dubois, 2012), thus a carbon source related to sediment particles, exemplified by the δ^{13}C signature of *Corbicula fluminea* (Fig. S2 in SI), which is a filter-feeder bivalve living in the sediment (Tachet et al., 2010). Furthermore, longer-chain PFASs are readily sorbed to sediment particles (Higgins and Luthy, 2006; Higgins and Luthy, 2007), while poorly soluble in water (Munoz et al., 2015). This suggests a stronger link to sediment as a source for these two PFCAs; absorption from water would be very limited compared to absorption from food brought with sediment particles. This connection to sediment was shown previously in a field study focusing on a Lake Ontario food web, although in this case there was not much difference among the compounds (Martin et al., 2004). We suggest that habitat use and feeding behavior, which are important factors for bioaccumulation of persistent chemicals (Borgå et al., 2004), as well as the predominance of long-chain PFCAs in the molecular profile in the Rhône (Munoz et al., 2015), may explain why the sediment connection appeared more important for long-chain PFCAs than in the Lake Ontario study.

According to the regression analyses (Table 2), size was another important factor explaining the contamination patterns. Nevertheless, this finding is only due to barbels, since for the two other species size did not vary much. The influence of size was somewhat counterintuitive, in the sense that for PFOS and PFNA large individuals displayed lower concentrations than young ones, while the opposite was true for PFTeDA. Compounds with perfluoroalkyl chain lengths lying between PFNA and PFTeDA were similar in both groups. Baduel et al. (2014) observed higher ΣPFAS concentrations in young blue-spotted stingray livers. Moreover, they found a negative relationship between PFOS or PFNA concentrations with age or size, and concluded that this pattern might be explained by differing uptake kinetics in response to e.g. a flood event. In another study, it was hypothesized that juvenile rainbow trout might not have an active mode of elimination for perfluoroalkyl acids (Martin et al., 2003b), while the enterohepatic recirculation would increase the assimilation efficiency of PFASs. Our results support the latter hypothesis for shorter-chain compounds, i.e., PFOS, PFNA and PFDA. Compounds with longer perfluorinated chains could be less easily filtered in the kidney, because of their molecular size or shape, similar to other vertebrates (Atherton, 2012; Elger et al., 1987; Ohlson et al., 2001). Accordingly, their concentration levels would increase as a function of fish age (or size, which is related to the former variable).

4.2. Influence of biotransformation on PFOS and FOSA accumulation

PFOS in aquatic biota is thought to originate not only from direct releases, but also from the degradation of precursors (Benskin et al., 2009; Martin et al., 2010; Wang et al., 2009). FOSA could be either a PFOS precursor or an end-product of the transformation process (Buck et al., 2011). Degradation processes may be abiotic, occurring in the atmosphere (Wallington et al., 2006), or biotic, especially involving hepatic microsomes in vertebrates. Such a process was described in a fish cell line (Tomy et al., 2004) and various mammal cell lines (Benskin et al., 2009; Letcher et al., 2014; Wang et al., 2009). This mechanism could contribute toward explaining interspecific differences of PFAS accumulation (Galatius et al., 2013). Higher biotransformation rates at higher TLs than at lower TLs might yield high BMFs in the field (Franklin, 2016). If this were the case, the relative proportions of PFOS or FOSA compared with those of their respective precursors should change from sediment to biota and from lower to higher TLs. Among the precursors analyzed in this study, only MeFOSAA, EtFOSAA and FOSA could be reliably measured in several compartments. EtFOSAA was detected in sediment and invertebrates, but not in fish. MeFOSAA was >LoQ in 76% invertebrate and in 100% fish samples, while FOSA was systematically > LoQ in both compartments. Nevertheless for both MeFOSAA and FOSA, concentration levels in fish were lower than in invertebrates. As hypothesized, [PFOS]/[FOSA] and [FOSA]/[MeFOSAA] concentration ratios increased from sediment or invertebrates to fish. [FOSA]/[MeFOSAA] ratios as high as 10-93 were found in molluscs

(*Potamopyrgus antipodarum*, *Corbicula fluminea*) or oligochaetes. Similarly, higher [PFOS]/[FOSA] ratios (i.e. 10 – 35) were observed for oligochaetes, chironomids, or Zygoptera. These findings consistently support the hypothesis of biotransformation, and suggest that it occurs not only in vertebrates but also in some benthic invertebrates. Moreover, these findings indicate that the biotransformation capacity is species-specific: roach and gudgeon especially displayed much higher [FOSA]/[MeFOSAA] ratios than barbels, suggesting that they metabolized MeFOSAA more efficiently. This might also partly explain the higher accumulation of FOSA in gudgeons. Thus, the contrasted biotransformation capacities could contribute to explaining the different accumulation patterns among species. For barbels, the different ratio values between small and large individuals also point to evolving biotransformation capacity from juveniles to adult fish.

4.3. Uncertainties in BMF estimation

Among published field-based BMFs, the sources of variation pointed by Franklin (2016) were related to methodological aspects, such as input concentrations referring to a specific organ instead of whole-body, or to inherently variable factors, such as feeding ecology, body size and so forth. Franklin's review referred to these items alternatively as sources of uncertainty or sources of variability. These concepts are nevertheless not equivalent: while uncertainty can be reduced for instance by a better study design, variability can only be better understood (Von Stackelberg et al., 2008). Most factors listed above as variable, such as body size or feeding ecology, exhibit also some uncertainty due to the respective investigation or measurement methods. This uncertainty is nevertheless expected to be low compared to the actual variability (Linkov et al., 2001). It is thus normal to observe variable BMFs in the field, even in a single ecosystem; the main issue accordingly pertains to reducing the uncertainty.

We calculated BMFs adjusted to the respective trophic levels of barbels, gudgeons, and their main prey in order to determine whether biomagnification occurred and for which compounds. The BMF model assumes that the system is at (or close to) equilibrium (Franklin, 2016; Gobas et al., 2009), a condition difficult to achieve in dynamic systems such as rivers. Nevertheless, such systems might present conditions favouring steady state in areas where the flow velocity slows down (e.g., fluvial annexes), or when the fish contamination pathway is related to sediments, or if the system is continuously exposed to relatively constant contaminant releases. On the other hand the composition of the fish "food basket" is obviously variable over a year. Invertebrates such as chironomid larvae are mainly found in spring and summer, while other taxa such as gammarids are more or less present in all seasons. Moreover, fish feeding rates also vary over the year, with typically lower rates observed in winter. In our case large barbels fed mainly on benthic invertebrates, especially from the fluvial annex, which displays a substantial sediment deposit (Figure

S1 in SI). Small barbels and gudgeons were collected along the river bank, where sediment settles down because the flow velocity decreases. Overall, we assumed that the conditions allowed for the calculation of tentative BMFs.

For all tested compounds, multiple linear regressions generally pointed to $\delta^{13}C$ and $\delta^{15}N$, and thus food as a factor explaining fish contamination. Predator-prey relationships were identified on the basis of stomach contents. Carbon isotopic ratios values confirmed the relative importance of gammarids in the large barbels' diet, while the small/young ones had more diverse food sources. With a slightly more negative $\delta^{13}C$ signature than gudgeons, chironomids constituted the major carbon source exploited by this species. These findings support the selection of two predator-prey pairs, i.e. barbel-gammarids and gudgeon-chironomids, for BMF calculations.

Barbel median $BMF_{TL\text{-}WB}$ values exceeded 1 for C10-C13 PFCAs (PFDA to PFTrDA), PFOS, and FOSA. The whole range of values was above 1 for PFDoDA, PFTrDA, PFOS and FOSA. For PFUnDA this threshold lay between the minimum (0.91) and the first quartile (2.19), while for PFDA it was above the third quartile. The BMFs determined for gudgeons were based on PFAS concentrations in fillets, and were therefore likely underestimated, as they did not account for accumulation in organs such as the liver or the blood. We conclude that C11-C13 PFCAs (PFUnDA to PFTrDA), PFOS, and FOSA were biomagnified in both species at this site, while PFNA and PFDA were biomagnified only in gudgeons.

5. Conclusions

Based on the stomach contents and isotopic ratios, diet was found to be an important explanatory factor to PFAS accumulation in the three cyprinid fish species studied, namely the barbel (*Barbus barbus*), the gudgeon (*Gobio gobio*), and the roach (*Rutilus rutilus*). Small/young barbels displayed significantly higher levels of several PFASs than did large/older individuals; increasing perfluoroalkyl backbone length shifted concentrations to the opposite direction, suggesting that the elimination efficiency depends on both the perfluoroalkyl carbon chain length and fish age. MeFOSAA and EtFOSAA were biotransformed along the food webs. End-product to precursors to concentration ratios were substantially variable among fish species, suggesting different abilities to metabolize the precursor. Stomach contents and isotopic ratios allowed us to identify relevant predator-prey pairs, namely barbel-gammarid and gudgeon-chironomid; the estimation of biomagnification factors adjusted to the respective trophic levels confirmed that long-chain perfluoro-alkyl compounds (>C11) were biomagnified in this system, while C8-C9 compounds were biomagnified only in gudgeons. Nevertheless, this assessment relied only on a part of the fish diet and was performed at a single site, with a rather specific contamination profile. Performing additional studies on a larger spatial scale would help gain further insights into the mechanisms involved in the trophic magnification of PFAS.

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