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Caroline Simmonet-Laprade[a], Hélène Budzinski[b] Marc Babut[c], Karyn Le Menach[b], Gabriel Munoz[a], Mathilde Lauzent[a], Benoît JD Ferrari[d], Pierre Labadie[b*]

[a] Univ. Bordeaux, EPOC, UMR 5805, LPTC research group, F-33400 Talence, France

[b] CNRS, EPOC, UMR 5805, LPTC research group, F-33400 Talence, France

[c] Irstea, Irstea, RIVERLY Research Unit, Lyon-Villeurbanne Center, 5 rue de la Doua CS 20244, F-69625 Villeurbanne, France

[d] Swiss Centre for Applied Ecotoxicology, EPFL ENAC IIE-GE, Station 2, CH-1015 Lausanne, Switzerland

*Corresponding author. Contact: pierre.labadie@u-bordeaux.fr

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Highlights:

- The trophic magnification of 17 PFASs was investigated in five riverine food webs
- ∑PFASs was in the range 0.9-213 ng g^{-1} in invertebrates and 6.9-1811 ng g^{-1} in fish
- TMFs were determined using a Kendall regression and a GLMM model
- Long-chain PFCAs and PFOS were generally biomagnified (TMF > 1)
- Low to moderate TMF variability was observed for almost all biomagnified PFASs

Abstract:

The occurrence at different trophic levels of 17 poly- and perfluoroalkyl substances (PFASs), including perfluoroalkyl acids (PFAAs) and some of their precursors (e.g., perfluoroalkane sulfonamides, 6:2 fluorotelomer sulfonate (6:2 FTSA)), was investigated in riverine freshwater food webs in Southeastern France. Two fish species (*Barbus barbus* and *Squalius cephalus*) and various invertebrate taxa were collected in five rivers to assess the spatial variability of trophic magnification factors (TMFs). Particular attention was devoted to sample and data processing to minimize potential biases associated with the TMF determination. Fish were significantly more contaminated than invertebrates (ΣPFAS = 7–1811 *vs.* 0.9–213 ng g^{-1} wet weight (ww)). Those from the Rhône River presented significantly higher levels due to high concentrations of perfluoroundecanoic acid (406 ng g^{-1} ww) and perfluorotridecanoic acid (566 ng g^{-1} ww) ascribed to an industrial point source. Perfluorooctane sulfonate (PFOS) was dominant at the other sites (concentration range = 3.6–134 ng g^{-1} ww). Two linear regression models were compared (i.e., Kendall regression *vs.* Generalized Linear Mixed-Effect Model, GLMM). Results showed that TMFs calculated using the non-weighted Kendall regression were higher than those obtained using the GLMM approach. GLMM-based TMFs were consistently > 1 for C_9–C_{14} perfluorocarboxylic acids (PFCAs), PFOS and perfluorodecane sulfonate (PFDS), indicating their apparent biomagnification in the investigated food webs. Comparatively, 6:2 FTSA and N-ethylperfluorooctane sulfonamidoacetic acid (N-EtFOSAA) were less often detected and were not significantly biomagnified, probably because of metabolization. TMF estimates were generally consistent across sites although some PFASs (in particular C_9, C_{10} and C_{13} PFCAs) displayed higher variability, due to a unique extreme value that may have resulted from the contribution of unattributed precursor biotransformation.

Keywords:

PFASs; River; Trophic Magnification Factors; Biotransformation; Spatial variability

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) have been synthesized since the 1960s for a wide range of industrial and commercial applications (Kissa, 2001), generating numerous direct or indirect inputs into the environment (Prevedouros et al., 2006). The worldwide presence of perfluoroalkyl carboxylates (PFCAs) and perfluoroalkane sulfonates (PFSAs) in aquatic environments has been abundantly demonstrated (e.g. Giesy and Kannan, 2001; Ahrens, 2011; Gewurtz et al., 2013). In particular, high concentrations of perfluorooctane sulfonate (PFOS) and several PFCAs were reported in aquatic biota (Kannan et al., 2001; Houde et al., 2006a, 2011), indicating a high bioaccumulative potential. Exposure to such substances can induce adverse effects on cellular functions (Hu et al., 2003) and endocrine-disrupting effects in fish (Jarque and Piña, 2014; Liu et al., 2011). The United States Environmental Protection Agency also set up a stewardship agreement with chemical industries to reduce the production and uses of long-chain PFCAs ($\geq C_8$) (US-EPA, 2009). PFOS was also added to annex B of the list of Persistent Organic Pollutants (POP) of the Stockholm Convention (UNEP, 2009) and included in the list of priority substances of the European Union Water Framework Directive (WFD). The WFD set two environmental quality standards (EQS) for PFOS in fish, with different protection goals, i.e., "Human health via consumption of fishery products" ($EQS_{biota\ PFOS}$ = 9.1 µg kg^{-1} wet weight) (EU, 2013) and "secondary poisoning of top predators" ($EQS_{biota,secpois\ PFOS}$ = 33 µg kg^{-1} wet weight) (EU, 2014).

The bioaccumulative (B) character of a contaminant is often predicted on the basis of its octanol-water partition coefficient (K_{ow}). Owing to their hydrophobic and oleophobic properties, this approach cannot be applied to PFCAs and PFSAs (Cahill et al., 2003). Common metrics used to assess the bioaccumulative potential of chemicals include the bioconcentration factor (BCF) and the bioaccumulation factor (BAF), as well as the biomagnification factor (BMF) or the trophic magnification factor (TMF) derived from field-based data (Gobas et al., 2009). The latter accounts for trophic exposure pathways and thereby provides a more holistic assessment of the B property. In a

regulatory perspective, TMFs (or BMFs) greater than 1 are indicative of biomagnification and may be used as lines of evidence for bioaccumulation assessment (Mackay et al., 2016). The published TMFs for PFOS and a few PFCAs include, however, values both below and above this threshold (Franklin, 2016). Such a variability might be due to food web properties, different environmental conditions, or differences in study design and data processing (Borgå et al., 2012; Burkhard et al., 2013). For instance, the use of concentrations determined in whole organisms or in specific tissues (e.g., liver, muscle or plasma) can directly impact the calculated TMF values (Houde et al., 2006b). In addition, some studies suggested that the biotransformation of perfluoroalkyl acid (PFAA) precursors could bias, either positively or negatively, the assessment of PFAA biomagnification (Franklin, 2016; Gebbink et al., 2016).

Until now, studies on PFAS trophic magnification have been essentially carried out in lakes or marine areas (Xu et al., 2014; Franklin, 2016), whereas more dynamic riverine ecosystems have not been investigated. Recently, the use of TMFs has been suggested in the context of freshwater monitoring (EU, 2014). The approach requires a preliminary assessment of the variability of this metrics, including spatial variations, to design robust monitoring procedures (Irvine, 2004). This also has important implications if the field-generated TMFs are, in the future, used in predictive models to assess the compliance of contamination levels in freshwater fish with EQS_{biota} (Kidd et al., 2019). To the authors' best knowledge, the variability of TMFs has seldom been addressed in the PFAS literature within a single study (Houde et al., 2006b). While meta-analyses of literature are useful, it is also critical to assess the spatial variability of the metrics using common sampling, analytical, and statistical treatment procedures across sites to neglect inter-laboratory biases in quantitation or data handling.

In the present study, we investigated for the first time the spatial variability of the biomagnification potential of 17 selected PFASs in riverine trophic webs. The targeted analytes included PFCAs, PFSAs, and some of their precursors. Biota samples (n = 106), including two omnivorous cyprinid fish species and a wide range of benthic invertebrates, were collected at five different locations from rivers in

Southeastern France with different anthropogenic pressures. TMFs were subsequently determined at each study site, with particular attention dedicated to data acquisition, handling and processing, and the spatial variability of TMFs for a given chemical was discussed.

2. Material and methods

2.1. Study sites

The five sampling sites were selected within four basins corresponding to the Rhône, the Bourbre, the Furan and the Luynes rivers, which are characterized by different hydrological features and located in southeastern France (Figure S1 and Table S1 in the supplementary information, SI). Site 1 (Rhône River) is located about 50 km downstream from the city of Lyon, a major urban area, and about 40 km downstream from a fluoropolymer manufacturing plant (Dauchy et al., 2012). The other sites are located downstream from areas hosting a variety of small or medium size industrial areas.

2.2. Sample collection

Sampling was carried out between 2013 and 2014. At each site, benthic invertebrates (crustaceans, bivalve mollusks, gastropods, insect larvae, and worms) were collected with a surber net and pooled by family or genus. Fish were caught by electrofishing, targeting two bentho-pelagic species, the common barbel (*Barbus barbus*) and the chub (*Squalius cephalus*). Fish size and mass were measured on site, and sex was recorded in the laboratory when possible. Larger fish (ca. ≥ 20 cm) were dissected in three fractions, namely dorsal muscle (fillets), liver, and the remaining tissues (viscera, skin, head and bones). At sites 1 and 2, only fillets were available for PFAS analysis. All samples were frozen, freeze-dried, and finely ground prior to analysis. More details are provided in section 1.2 of the SI.

2.3. Standards and reagents

The complete list of analyzed PFASs, their acronyms, molecular formulas and associated internal standard (ISs) are provided in Table S4. PFAS native standards, including PFCAs (C_8–C_{14} PFCAs), C_6–C_8 and C_{10} PFSAs, perfluorooctane sulfonamide (FOSA) and related compounds (N-MeFOSA, N-EtFOSA,

N-MeFOSAA and N-EtFOSAA), and 6:2 fluorotelomer sulfonate (6:2 FTSA) were all acquired from Wellington Laboratories at a purity of 98 % or higher, while isotope-labeled ISs were also purchased from Wellington Laboratories. Details on other chemicals, standards and reagents are provided in the SI (section SI 2.1).

2.4. PFAS analysis

The sample preparation was based on a previously validated method with few modifications (Munoz et al. 2017). Between 50 and 250 mg dry weight (dw) of each sample was extracted, depending on the available sample amount. Briefly, PFASs were extracted under a microwave field with 12 mL of methanol (MeOH) and extracts were subsequently purified on Strata X-AW (200 mg, Phenomenex) and graphitized carbon black cartridges Supelclean^{TM} ENVI^{TM}–Carb SPE Tubes (250 mg, Supelco).

Analyses were carried out using a 1200 LC system coupled with a 6490 triple quadrupole mass spectrometer from Agilent technologies (Massy, France) (Munoz et al., 2017). Details on the detection method are presented in the SI (Table S5). PFAS identification and quantification were carried out by isotopic dilution using either linear or quadratic calibration curves (0.5–50 ng g^{-1} in MeOH, IS concentrations set at 10 ng g^{-1}).

2.5. Stable isotope analysis

Stable isotope analysis (N and C) was performed on tissues of invertebrates, fish fillets, and whole fish previously freeze-dried, finely ground into homogeneous powder (using a micro ball mill), and defatted. Because lipids are depleted in ^{13}C relative to proteins and carbohydrates and because the lipid content varies among organisms or tissue types, lipid extraction is necessary to avoid a bias in C stable isotope analyses (Post et al., 2007). The extraction of lipids was carried out by accelerated

solvent extractor ASE350 (Dionex) with dichloromethane (100°C, 100 bar, one static cycle of 13 min) as described by Bodin et al. (2009). The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios were determined using a Thermo Scientific Delta V Plus Isotope Ratio MS connected to a Thermo Scientific Flash 2000 Organic Elemental Analyzer (EA-IRMS). Carbon and nitrogen isotope compositions were expressed as per mil (‰) in the δ notation relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2, respectively.

2.6. Quality assurance / quality control

Quality assurance and quality control samples were incorporated in each batch of samples, and included procedural blanks, accuracy tests (spiked sole *Solea Solea* tissues from the Gironde Estuary, native PFAS and IS addition before extraction), recovery tests (spiked sole tissues from the Gironde Estuary, native PFAS addition before extraction and IS addition before LC-MS/MS analysis), and analysis of an in-house control matrix (spotted seabass *Dicentrarchus punctatus* from the Gironde Estuary). Whole-method trueness was also controlled, using NIST SRM 1947 reference material (Lake Michigan trout).

Traces of C_8–C_{14} PFCAs, PFOS, 6:2 FTSA and FOSA were detected in procedural blanks (Table S6); blank correction was therefore applied to the corresponding samples. Mean recoveries ranged between 34 % (N-EtFOSA) and 74 % (6:2 FTSA). The method accuracy was satisfactory, with values between 80–113 % except for PFHpS (66 %) (Table S7). The results for in-house control matrix and SRM 1947 agreed with those previously obtained in our laboratory (Munoz et al. 2017) and those reported by Reiner et al. (2012) (Table S8). Variation coefficients (relative standard deviation < 30 %) indicated suitable whole-method precision as per international guidelines (Shoemaker et al., 2009).

For analytes detected in blanks, the Limit of Detection (LOD) was determined as the standard deviation of the blanks multiplied by the $t_{n-1,95}$ Student coefficient. For other analytes, LODs were determined as the concentration yielding a signal to noise ratio of 3

Overall, LODs varied from 0.001 ng g^{-1} wet weight (ww) to 0.72 ng g^{-1} ww depending on the compound, the tissue and the sample batch (Table S9). LOQs were set at 10/3 x LODs and ranged between 0.01 and 2.3 ng g^{-1} ww.

Every 15 samples, the accuracy of stable isotope analyses was controlled using certified matrices, i.e., USG24 (graphite) and IAEA-N2 (ammonium sulfate) for $\delta^{13}C$ and $\delta^{15}N$, respectively (Table S10). When sample amounts were sufficient, isotopic analyses were performed in triplicate (analyzed in different batches) and the variation coefficient rarely exceeded 10 %, indicative of suitable analytical reproducibility and tissue homogenization.

2.7. Determination of trophic levels and Trophic Magnification Factors

Trophic levels (TLs) were determined from $\delta^{15}N$ measurements according to Eq. 1 (Post, 2002):

$$TL = TL_{baseline} + (\delta 15N_{consumer} - \delta 15N_{baseline})/\Delta N \qquad \text{(Eq. 1)}$$

Where $TL_{baseline}$ corresponds to the TL of an organism at the base of the food web, $\delta^{15}N_{consumer}$ (‰) and $\delta^{15}N_{base}$ (‰) are the respective N isotopic ratios of the consumer and the baseline species, and ΔN is the mean trophic enrichment. $TL_{baseline}$ was fixed at 2, corresponding to primary consumers, and ΔN at 3.4 ‰ (Post, 2002).

TMFs were derived from the regression of the logarithm-transformed PFAS concentrations (ng g^{-1} ww) versus TL (Eq.2 and 3, from Borgå et al., 2012):

$$Log[contaminant] = a \times TL + b \qquad \text{(Eq.2)}$$

$$TMF = 10^{a} \qquad \text{(Eq.3)}$$

Two regression approaches were considered: a Kendall regression and a generalized linear mixed-effect model (GLMM) (Munoz et al. 2017). While both approaches take into account non-detect data, the GLMM model also takes into consideration the interspecific variability (random effect) as well as the different number of samples per taxon. Note that concentrations used in the TMF determination process referred to either whole body (WB) in the case of individual fish, or to pool homogenates in the case of invertebrates. Concentrations were systematically expressed on a ww basis.

2.8. Statistical treatment

Data analysis was conducted using R statistical software (R version 3.2.1, R Core Team, 2015) and XLstat 2015. The significance of differences between groups was determined with non-parametric tests, i.e., Mann-Whitney (k = 2) and Kruskal-Wallis (k > 2). Analysis of covariance (ANCOVA) was applied to determine the parameters that could influence PFAS levels and molecular patterns. For all tests, the significance threshold was set at 0.05.

The Kendall regression and GLMM approach were implemented with R statistical software using the *cenken* function (Kendall regression, within the NADA R package) (Helsel, 2012) and the *lmec* (Linear Mixed-Effects Models with Censored Responses) function (LMEC R package) (Vaida and Liu, 2009), respectively. For a given sampling site, the TMF calculation was performed only when the detection frequency of an analyte was higher than 40 %.

3. Results and discussion

3.1. Isotopic ratios and food-web characterization

Mean $\delta^{13}C$ values at each site ranged from -28.2 ‰ to -25.6 ‰, while average $\delta^{15}N$ ranged from 10.4 ‰ to 15.1 ‰ (Table S11). $\delta^{13}C$ were significantly higher at sites 3 and 4 than at sites 2 and 5. $\delta^{15}N$ values were also higher at sites 3 and 4 than at sites 1, 2 and 5. *Oligocheta* ($\delta^{15}N$: 7.9–13.2 ‰), *Gammaridae* ($\delta^{15}N$: 8.2–13.6 ‰) and *Chironomidae* ($\delta^{15}N$: 7 – 13 ‰) were positioned at the lower end of the $\delta^{15}N$ values distribution (Figure S2), consistent with their detritus-feeding behavior (Tachet et al., 2010). Conversely, *Anisopterae* and *Zygopterae* (*Odonates)* larvae displayed $\delta^{15}N$ values close to those of fish (10.3–15.9 ‰ and 10.0–17.2 ‰ respectively), as expected for these predators (Tachet et al., 2010). Fish displayed consistently higher $\delta^{15}N$ values than most invertebrates (barbel: 12.2–14.5 ‰, chub: 10.1–17.1 ‰). At sites 2 and 5, where both fish species were present, barbel had significant higher $\delta^{15}N$ (p-value < 0.014) than chub, and more negative $\delta^{13}C$ values only at site 5 (p-value = 0.004), thus showing distinct feeding regimes. The respective $\delta^{15}N$ distributions in fillets and WB of fish were similar (Kolmogorov Smirnov test, p-value = 0.08), thereby allowing the use of $\delta^{15}N$ measured in fillets to estimate TLs.

As recommended by previous studies (Post, 2002; Vander Zanden and Rasmussen, 2001; Anderson and Cabana, 2007), the selection of baseline organisms targeted primarily long-lived primary consumers, such as *Corbicula fluminea* (bivalve) at site 1, *Simuliidae* (insect) at site 5, *Hydropsychidae* (*Trichoptera*) at site 4 and *Heteroptera* at site 3, with a TL set at 2. Because none of these taxa were available at site 2, *Oligochaetes* were used as a surrogate baseline organism, with a TL also set at 2. Calculated TLs thus varied from 1.8 to 4.9 (Table S12) and the difference between the highest and the lowest trophic levels ranged between 1.8 (site 4) and 3.6 (site 3).

3.2. PFAS concentrations and molecular patterns

3.2.1. Occurrence

Among the 17 investigated PFASs, only the linear isomer of PFOS was systematically detected in all samples of each site (Tables S13–S14). Other compounds were also detected at 100 % depending on the site: PFUnDA, PFTrDA and FOSA at site 1, FOSA at site 2, C_{10}-C_{14} PFCAs, Br-PFOS, PFDS, 6:2 FTSA and FOSA at site 3, C_{12}–C_{13} PFCAs at site 4 and C_{12}–C_{14} PFCAs at site 5. Overall, long-chain PFCAs (C_9–C_{14}), PFOS isomers, FOSA and PFDS were found in more than 80 % of samples. The detection frequency of PFOA, PFHxS, PFHpS, 6:2 FTSA, N-MeFOSAA and N-EtFOSAA was lower (33–76 % on average). PFOA and 6:2 FTSA were more frequently found in invertebrates (71–76 % in average) than in fish (31–38 % in average). At site 2, all precursors except FOSA had low detection rates (< 50 %). N-MeFOSA and N-EtFOSA were never detected.

3.2.2. PFAS concentrations and compliance to EQS

The total PFAS concentrations (∑PFASs) in invertebrates and whole fish are shown in Figure-1A and 1B respectively (descriptive statistics are given in Tables S13 and S14). For invertebrates, ∑PFASs ranged from 0.87 ng g^{-1} ww to 213 ng g^{-1} ww depending on site and species. According to the median ∑PFASs, site 1 appeared significantly more contaminated than sites 4, 3, and 2 in decreasing order (*p*-value < 0.0001). However, site 1 was not significantly more contaminated than site 5 due to large interspecific variability. Indeed, taxa with similar TLs displayed different contamination levels: for instance, in *Oligochaeta*, ∑PFASs ranged from 3.4 to 199 ng g^{-1} ww *vs.* 1.4 to 5.3 ng g^{-1} ww in *Chironomidae.* Similarly, *Anisoptera and Zygoptera* displayed relatively low concentrations (1.9–26.2 ng g^{-1} ww) as compared to *Hydropsychidae* (30–45 ng g^{-1} ww), gammarids (0.8–212 ng g^{-1} ww) or planars (99.1 ng g^{-1} ww). Such differences might be explained by feeding behaviors and physiology, including metabolic

capacities (Bertin et al., 2016; Prosser et al., 2016). PFAS concentrations in invertebrates have not often been reported, in particular in riverine systems (Houde et al., 2011; Ahrens and Bundschuh, 2014). Overall, concentrations measured in this study were higher than those reported in invertebrate marine shellfish along French coasts (Munschy et al., 2013), which is consistent with a general decreasing gradient of water contamination observed from rivers to open-ocean, and may be explained by the lower dilution rate of urban and industrial effluents in rivers (Ahrens, 2011).

PFASs were analyzed in dissected and whole fish from sites 3, 4 and 5, and only in the fillet of fish from sites 1 and 2. For the latter, PFAS concentrations in whole fish ($PFAS_{WB}$) were estimated from concentrations in fillet multiplied by a fillet-to-whole-body conversion factor as suggested by Kidd et al. (2019) and the European Commission (EU, 2014) (procedure detailed in SI 3.2.2). Briefly, these factors were directly determined for each compound from the slope of the linear regression of whole-body concentrations *vs.* fillet concentrations of dissected fish from sites 3, 4 and 5 (using a mass balance approach). For all PFASs except PFOA, PFHpS and N-EtFOSAA, a slope significantly different from zero was found (p-values < 0.004) and conversion factors ranged between 1.56 and 4.09 (see Table S16).

Fish were systematically more contaminated than invertebrates, in agreement with previous findings (Ahrens and Bundschuh, 2014). $\sum PFAS_{WB}$ ranged from 6.9 to 1811 ng g^{-1} ww, and the average at site 1 (1043 ng g^{-1} ww) appeared significantly higher (10–71 times) than at all other sites (< 97.3 ng g^{-1} ww). The contamination levels at site 1 are mainly explained by the high concentrations of two long-chain carboxylates of odd carbon numbers, PFUnDA (C_{11}) and PFTrDA (C_{13}), which reached respectively 691 and 983 ng g^{-1} ww (< 39 ng g^{-1} ww at the other sites). This specific pattern is attributed to the presence of a fluorochemical industrial discharge located about 40 km upstream of site 1 (Dauchy et al., 2012). At the other sites, PFOS presented the highest concentrations, and fish from sites 3 and 5 were the most contaminated ($\sum PFOS$ = 45.5 ± 22.3 and 46.9 ± 27.9 ng g^{-1} ww respectively). These levels are higher than those reported for multiple fish species in the Gironde Estuary (France) (Munoz et al.,

2017). They are similar to concentrations observed in muscle of chub from the Orge River (France) (Labadie and Chevreuil, 2011) and to those reported for other freshwater fish species worldwide (Houde et al., 2006a; Naile et al., 2010; Houde et al., 2011; Thompson et al., 2011; Pan et al., 2014).

In the present study, the mean concentration of ΣPFOS in fish exceeded the EU EQS_{biota} at all sites, as regularly observed for numerous fish species from other European rivers (Svihlikova et al., 2015; Fliedner et al., 2016; Pico et al., 2019). At sites 3 and 5, individual PFOS concentrations were systematically above the EU EQS_{biota}. These results demonstrate that, despite PFOS phase-out in Europe, the aquatic contamination by PFOS is a long-term environmental issue due to its persistence and the slow environmental degradation of some of its precursors. Additionally, PFOS production phase-out does not mean that PFOS-based products are no longer used and introduced in the environment (Kotthoff et al., 2015).

3.2.3. PFAS Molecular pattern

The mean molecular pattern at each site is illustrated in Figure 2. For both invertebrates and fish, site 1 differed from the others due to the large dominance of PFUnDA and PFTrDA, explaining together 71 % and 83 % of ∑PFAS in average for invertebrates and fish, respectively, in good agreement with Miège et al. (2012). A similar profile has been observed in fish from Lake Kejimkujik (Nova Scotia, Canada) and was also related to industrial releases (Gewurtz et al., 2013). At the other sites, ΣPFOS was dominant and explained 32 to 52 % of ∑PFASs, as usually reported in biota worldwide (Labadie and Chevreuil, 2011; Houde et al., 2006b; Naile et al., 2010). The linear PFOS isomer alone explained 83–95 % of ∑PFOS, which is consistent with previous findings (Houde et al., 2008; Munoz et al., 2017). The other most abundant PFASs were PFDoDA and PFDA at sites 3 and 4 and PFUnDA and PFDA at sites 2 and 5. However, the order of importance was reversed in some taxa such as *Gammaridae*, which displayed a pattern systematically dominated by PFCAs (76 % of ∑PFASs on average) (Figure S3).

Significant differences were observed between fish and invertebrates. The relative abundance of PFOA, PFNA and the sum of PFAA-precursors were systematically lower in fish. Furthermore, PFHxS and PFHpS were more abundant, relatively to ΣPFASs, in invertebrates than in fish from sites 2 and 4. Similar patterns have been observed, for instance when comparing fish and zooplankton from Taihu Lake (Xu et al., 2014), fish and oysters from Sydney Harbor (Thompson et al., 2011) or fish, crustaceans, gastropods, and bivalves from coastal areas in Korea (Naile et al., 2010). This further supports the conclusions that fish are more effective in eliminating the shortest-chain PFAAs and have better abilities to metabolize PFAA precursors. Previous reports indicated that some fish species could metabolize perfluorooctane sulfonamide derivatives into PFOS, with FOSA being an intermediate biotransformation product (Tomy et al., 2004; Benskin et al., 2013).

3.2.4. Biotransformation of PFAA-precursors

In a companion paper that focused on the food-web from site 1 and that was here extended to a larger number of taxa, it was shown that the biotransformation of FOSA to PFOS and N-MeFOSAA to FOSA in fish was species-specific (Babut et al., 2017). PFOS/FOSA and FOSA/N-MeFOSAA concentration ratios were indeed higher in fish than in invertebrates, suggesting that biotransformation likely occurred in this food web.

In the present work, we extended the approach to four additional study sites. PFOS/FOSA and FOSA/OSD (OSD standing for “other sulfonamide derivatives”, i.e., the sum of N-MeFOSAA and N-EtFOSAA in the present case) concentration ratios were calculated for all invertebrates and whole fish samples, provided that both values were above the LOD (25/106 samples) (Table 1). An ANCOVA was performed on the whole data set with site, sample type (i.e., invertebrate *vs.* fish), $\delta^{15}N$ and $\delta^{13}C$ as explicative variables of PFOS/FOSA or FOSA/OSD ratio. The PFOS/FOSA ratio was weakly ($R^2 = 0.33$)

but significantly related to the site (p-value = 0.001) and $\delta^{15}N$ (p-value = 0.004) according to the analysis of Type III Sum of Squares. Positive correlations between the PFOS/FOSA ratio and $\delta^{15}N$ were found at sites 2, 3 and 5. In addition, the PFOS/FOSA ratio was significantly higher in fish than in invertebrates at all sites, except site 4. Thus, these results suggest that the biotransformation of FOSA to PFOS occurred at most sites. Contrasted conclusions have been drawn in previous studies, probably due to differences in site contamination patterns and target species (Asher et al., 2012; Gebbink et al., 2016; Munoz et al., 2017). The FOSA/OSD ratio was not related to $\delta^{15}N$ at any site; however, significant differences were observed between invertebrates and fish (p-value < 0.0001). This ratio was always higher in fish than in invertebrates, suggesting the metabolization of N-MeFOSAA and N-EtFOSAA in fish.

3.3. Trophic Magnification Factors

The TMF is usually proposed as a relevant indicator of the bioaccumulation potential of contaminants, considering the complex mechanisms of their transfer along trophic webs (Conder et al., 2011). Borgå et al. (2012) listed some methodological recommendations to limit biases related to the concentration expression, the calculation method and the amplitude of TL differences between the base and the top of the considered trophic web, the choice of the baseline organism, or the consideration of non-detect data. In this study, TMFs were estimated from the slope of the linear regression between log C (concentration in whole body of fish and pool of whole invertebrates, expressed in ng g^{-1} ww) and TL. For this purpose, two regression approaches were considered (i.e., Kendall regression *vs.* GLMM). These approaches may help limit the bias generated by the substitution of non-detect data by an arbitrary value such as LOD/2 (Helsel, 2012), limit the impact of outliers on the regression (rank-order correlations), and correct for unbalanced sample size among taxa (GLMM).

The field-derived TMF values and associated regression parameters are compiled in the SI (section 3.3). Depending on the compound, the comparison between the Kendall regression and GLMM approaches revealed some differences. Probably because of the high dispersion of contamination levels, and especially for sites where the TL amplitude was low (inferior to the recommended ΔTL of 2), the Kendall regression indicated no significant correlation between log C and TL (*p*-value < 0.05) for PFNA, C_6–C_{10} PFSAs and FOSA at site 1, PFDA, PFDoDA, PFTrDA and FOSA at site 2, PFOA, PFHxS, PFHpS, 6:2 FTSA, FOSA, N-MeFOSAAA and N-EtFOSAA at site 3, C_9–C_{14} PFCAs, C_6–C_{10} PFSAs at site 4 and PFNA, PFTeDA and PFHxS at site 5. In contrast, the GLMM almost systematically provided slopes significantly different from 0, allowing the calculation of TMFs. TMFs computed using the Kendall regression were almost systematically superior to the upper bound of the confidence interval returned by the GLMM, especially at site 1 (TMF = 5.6–11.7 *vs.* 1.9–5.7 for PFAA) and site 3 (TMF = 2.0–7.0 *vs.* 1.8–3.3 for PFAA). This may be the consequence of high differences between invertebrate and fish contamination levels and it illustrates the consequence of imbalanced study design, which may result in regressions substantially weighted with samples from higher TLs (Borgå et al., 2012). The GLMM approach was therefore preferred for the discussion of the TMFs computed for the five trophic webs considered in the present study (Table 2).

For PFOA, PFHxS, PFHpS, 6:2 FTSA, N-MeFOSAA and N-EtFOSAA, TMFs were not systematically calculated because of low detection frequencies at some sites (i.e., < 40 % or when the compound was detected neither in fish nor in invertebrates). For these compounds, the slope of the regression was not significantly different from zero, or TMF was below 1, thereby suggesting the absence of relation between log C and TL and, thus, the absence of biomagnification. Due to the poor bioaccumulation of shorter-chain PFAAs and the potential metabolization of perfluorooctane sulfonamide derivatives and 6:2 FTSA (Hoke et al., 2015), it is not surprising to observe steady or decreasing concentration of these chemicals with TL. Some exceptions (TMFs > 1) were, however, observed for PFHxS, PFHpS and N-MeFOSAA at site 3, and PFHxS at site 1. The concentrations of the latter compounds were often close to their respective LOQs, which might increase analytical uncertainties not accounted for in the

regression model; therefore, caution should be exercised when interpreting such results. As discussed previously, N-alkyl perfluorooctane sulfonamide derivatives may undergo biotransformation in a species-dependent manner. This might induce variable apparent biomagnification, depending on the trophic web. In another study, PFOS/FOSA ratios varying up to 4 orders of magnitude were noted between marine mammal top predators (Galatius et al., 2013). This may be partly related to different metabolic capacities between species, but also to specific exposures in certain regions of the world (Dassuncao et al., 2017). Thus, the use of TMF as a predictive model for these chemicals that may be biotransformed seems questionable in a regulatory context such as the EU WFD.

As regards PFDA and PFUnDA, TMFs were significantly >1. L-PFOS, Br-PFOS, ∑PFOS and PFTrDA showed TMFs > 1 at sites 2–5 and PFDoDA and PFDS at sites 3, 4 and 5. The amplitude of TMF between-site variability differed according to the compounds. Limited spatial variability was observed for PFUnDA (2.4–4.2), PFDoDA (1.8–2.7), PFTeDA (1.9–2.8), ∑PFOS (2.4–4.1) and PFDS (2.1–3.0), suggesting that TMF could be used as a reasonable predictive model for these chemicals. TMFs were more variable for PFTrDA (1.8–14.9), PFNA (0.6–9.9), PFDA (2.6–10.9) and the branched isomer of PFOS (1.4–8.3) but this was, however, mainly due to one extreme value (Table 2). The acquisition of this dataset represented an ambitious effort in terms of both sampling and analysis, and provided useful results for a sound preliminary assessment of the PFAS TMF variability in riverine ecosystems. However, it proved insufficient to identify the factors influencing the TMF of these compounds (e.g., sampled invertebrate species, baseline contamination level, etc.), through comprehensive descriptive bivariate or multivariate analyses (e.g. correlation test, ANCOVA, Principal Component Analysis, data not shown). A plausible hypothesis would be that the occurrence of unidentified precursors and their enhanced biotransformation in fish compared to invertebrates is a confounding factor leading to the overestimation of TMFs at some sites. Other possible explanations include site differences in rates of biotransformation and growth, sediment-water concentration ratios, extent of food web omnivory or spatial concentration gradients (Mackay et al., 2016).

TMFs for the most studied PFASs (PFOS, C_8–C_{14} PFCAs) were previously reported, especially for lacustrine, marine and estuarine food webs, where the span in trophic levels might be longer and trophic web less variable than those in riverine systems (Martin et al., 2004; Houde et al., 2006b; Kelly et al., 2009; Tomy et al., 2009; Loi et al., 2011; Xu et al., 2014; Munoz et al., 2017). Rivers are also subject to short-term changes in pollution discharge and exhibit lower dilution capability. These variable emissions can influence PFAS contamination levels and patterns as well as the isotopic signatures of aquatic biota, which might raise concerns about the reliability of TMF calculation for such ecosystems (Borgå et al., 2012). However, our results were consistent with previously reported TMFs. The lowest $TMF_{\sum PFOS}$ value found in this study (2.4 at site 1) is consistent with those from a fish trophic web from the Lake Taihu in China (Xu et al., 2014) whereas the maximum value (4.1 at site 4) was close to the value determined for a marine food web from Charleston Harbor, South Carolina, U.S.A. (TMF_{PFOS} = 4.9) (Houde et al., 2006b). Likewise, the TMFs of PFUnDA and PFDoDA were in the range of values reported for the Gironde Estuary and the Charleston Harbor food webs (Houde et al., 2006b; Munoz et al., 2017). This is also the case for most TMFs calculated for PFNA, PFDA, PFTrDA and PFTeDA. Further studies are, however, needed to get further insight into the variability of TMFs. Additional data on factors that can impact PFAS concentrations in biota and thus TMFs would be needed. These include, but are not limited to, spatial and seasonal variability of the contamination in both abiotic compartments and trophic web baseline, contamination levels and half-lives values of PFAA precursors in key species, and biological or ecological variables (e.g., age, sex, life traits, change in the feeding ecology, etc.) (Franklin, 2016).

4. Conclusion

This study showed the widespread occurrence of 15 PFASs including C_8–C_{14}PFCAs, C_7–C_{10}PFSAs and some precursors (6:2 FTSA, FOSA, MeFOSAA and EtFOSAA) in invertebrates and fish from 5 rivers in

France. Organisms collected downstream of a fluorochemical industrial effluent discharge presented the highest PFAS levels and a peculiar contamination profile largely dominated by two long-chain PFCAs (namely, PFTrDA and PFUnDA). Mean concentrations of PFOS in fish systematically exceeded the EU EQS_{biota}. This work provides, for the first time, TMFs for PFASs in freshwater riverine ecosystems. TMFs varied from 0.14 to 14.9 depending on the compound and the site. Here, we provide further evidence for the apparent biomagnification of C_{10}–C_{14} PFCAs, PFOS, and PFDS. Comparatively, PFOA, N-EtFOSAA and 6:2 FTSA were not biomagnified and more variable conclusions were drawn for PFHxS, PFHpS, PFNA and some precursors. Low to moderate spatial variability of TMF was observed for almost all biomagnified PFASs but extreme values at some sites could not be explained. Further work addressing the reduction of both the variability and the uncertainties related to the computation of TMFs would be useful, especially if this metrics is to be applied in a regulatory context such as the EU WFD.

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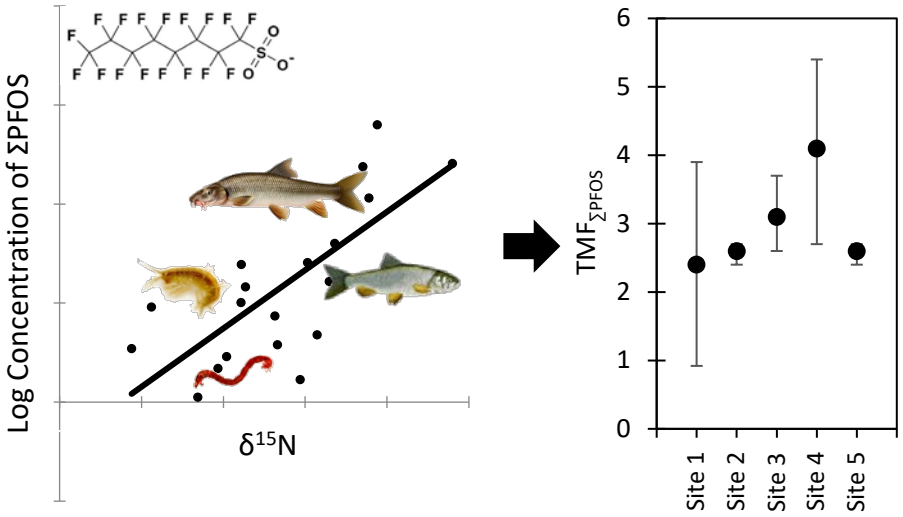
Graphical Abstract:



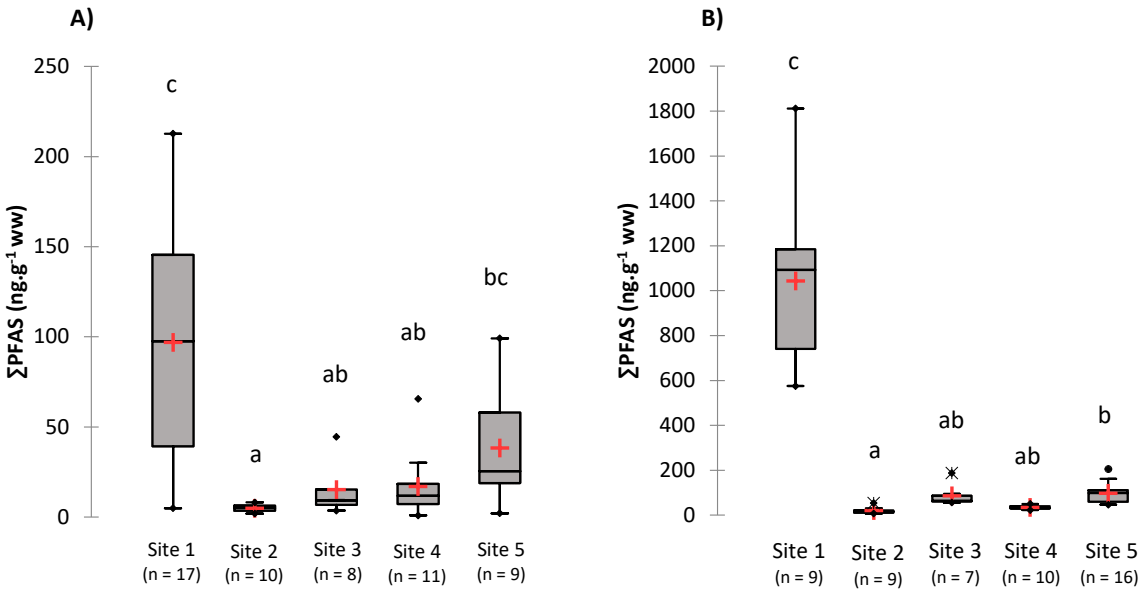


Figure 1: ∑PFASs (ng g^{-1} ww) in invertebrates (A) and whole fish (B). Similar lowercase letters indicate the absence of significant difference. Red crosses correspond to the mean value; lower, middle and upper box lines correspond to the first quartile, the median and the third quartile, respectively.

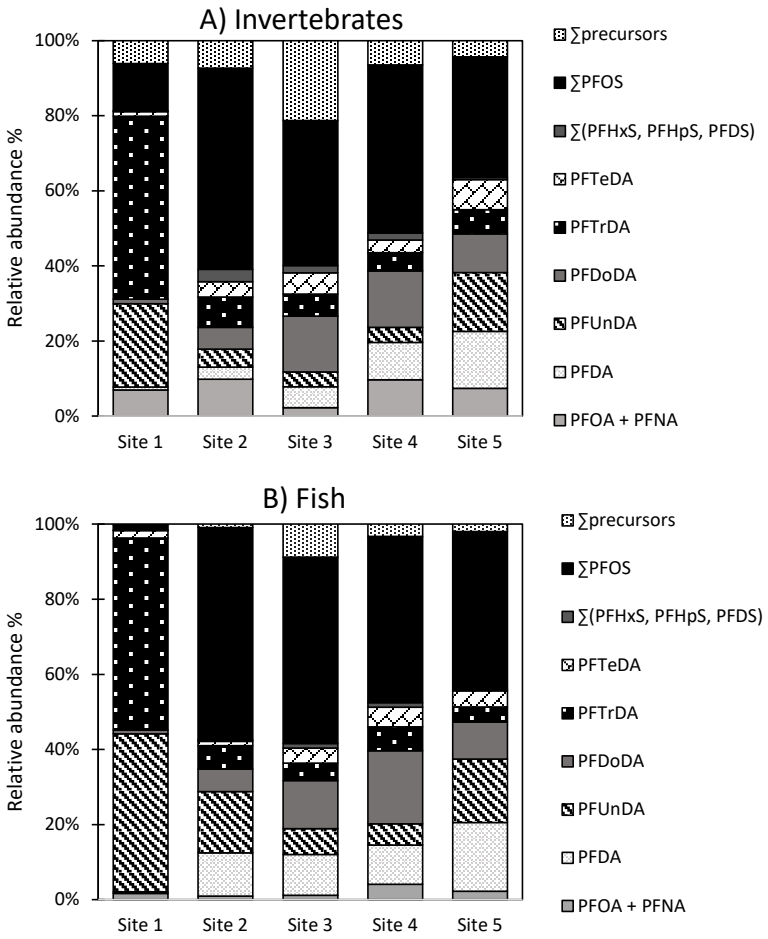


Figure 2: Mean PFAS molecular pattern in invertebrates (A) and fish (B) at each study site.

Table 1: PFOS/FOSA and FOSA/Other sulfonamide derivatives (OSD) ratios in invertebrates and fish, arranged per sampling site. *nc: not calculated.

		PFOS/FOSA Ratio					**FOSA/OSD Ratio**	
		Mean ± std deviation	Invertebrates *vs.* Fish (Mann-Whitney)	Correlation with TL (Pearson)			Mean ± std deviation	Invertebrates *vs.* Fish (Mann-Whitney)
			p-value	*p*-value	R^2	equation		*p*-value
Site 1	Invertebrates	12 ± 11.5	0.041	0.155	nc*	nc	8.2 ± 13.0	0.002
	Fish	19.6 ± 5.6					19.8 ± 6.7	
Site 2	Invertebrates	12.7 ± 4.2	<0.0001	0.0003	0.554	y = 96.3x -223	2.9 ± 0.9	nc
	Fish	96.0 ± 71.4					nc	
Site 3	Invertebrates	19.2 ± 28.9	0.018	0.012	0.398	y = 21.1x - 59.8	2.3 ± 1.7	0.001
	Fish	35.5 ± 21.6					12.7 ± 6.3	
Site 4	Invertebrates	33.4 ± 32.5	0.079	0.058	nc	nc	2.3 ± 1.0	0.0003
	Fish	36.2 ± 14.2					15.9 ± 9.2	
Site 5	Invertebrates	25.6 ± 27.3	0.013	0.001	0.618	y = 25.3x - 47.5	2.3 ± 1.8	0.002
	Fish	50.1 ± 22.3					10.4 ± 8.6	

Table 2: PFAS Trophic Magnification Factors calculated with a generalized linear mixed-effect model GLMM (*lmec* function). DF = detection frequency, IC 95% = 95 % confidence interval. Values indicated in bold black font indicate TMFs significantly higher than 1, while values indicated in grey font indicate TMFs not significantly different from 1. *nc: not calculated (detection frequency < 40 %)

	Site 1 (n = 26)		Site 2 (n = 19)		Site 3 (n = 15)		Site 4 (n = 21)		Site 5 (n = 25)	
	DF %	TMF (IC 95%)	DF %	TMF (IC 95%)	DF %	TMF (IC 95%)	DF %	TMF (IC 95%)	DF %	TMF (IC 95%)
PFOA	42	nc*	32	nc	67	0.50 [0.40; 0.60]	76	0.57 [-0.10; 1.2]	60	0.39 [0.02; 0.77]
PFNA	92	**9.9 [5.5; 14.3]**	58	1.4 [-0.9; 3.7]	87	**3.1 [2.8; 3.5]**	90	**4.5 [2.4; 6.6]**	100	0.61 [0.57; 0.65]
PFDA	92	**5.7 [4.2; 7.2]**	79	**10.9 [3.6; 32.8]**	100	**2.6 [2.3; 2.9]**	95	**3.3 [2.4; 4.3]**	100	**3.2 [3.0; 3.4]**
PFUnDA	100	**2.6 [2.3; 2.9]**	95	**4.2 [2.7; 6.6]**	100	**2.4 [2.0; 2.7]**	95	**2.5 [1.7; 3.3]**	100	**3.4 [3.0; 3.7]**
PFDoDA	96	1.4 [0.8;2.0]	95	2.1 [0.88; 3.3]	100	**1.8 [1.5; 2.1]**	100	**2.1 [1.4; 2.7]**	100	**2.7 [2.3; 3.2]**
PFTrDA	100	0.9 [0.8;1.0]	84	**1.8 [1.1; 2.6]**	100	**1.9 [1.6; 2.3]**	100	**2.5 [1.5; 3.5]**	96	**14.9 [11.1; 18.6]**
PFTeDA	92	**1.9 [1.5; 2.3]**	84	0.7 [-0.5; 1.8]	100	**2.1 [1.8; 2.4]**	95	**2.8 [1.7; 4.0]**	100	**1.4 [1.2; 1.7]**
PFHxS	92	**1.5 [1.3; 1.7]**	42	nc	80	**3.7 [2.4; 4.9]**	76	0.36 [0.11; 0.62]	88	0.76 [0.59; 0.92]
PFHpS	61	0.65 [0.60; 0.69]	5	nc	67	**8.3 [5.4; 11.3]**	43	nc	67	1.20 [0.95; 1.5]
L-PFOS	100	0.81 [0.72; 0.90]	100	**2.4 [2.3; 2.6]**	100	**3.3 [2.6; 3.9]**	100	**4.5 [3.0-5.9]**	100	**2.6 [2.4; 2.7]**
Br-PFOS	80	1.4 [0.93; 1.9]	95	**8.3 [6.7; 9.8]**	100	**1.9 [1.5; 2.4]**	95	**2.8 [1.7; 3.8]**	100	**3.0 [2.9; 3.0]**
∑PFOS	100	2.4 [0.92; 3.9]	100	**2.6 [2.4; 2.7]**	100	**3.1 [2.5; 3.7]**	100	**4.1 [2.7; 5.4]**	100	**2.6 [2.5; 2.7]**
PFDS	69	0.73 [0.41;1.06]	68	17.9 [-10.6; 46.4]	100	**2.1 [1.8; 2.4]**	76	**2.1 (1.9-2.3)**	96	**3.0 [2.4; 3.6]**
6:2-FTSA	65	0.14 [0.08; 0.19]	16	nc	100	0.55 [0.47; 0.63]	81	1.79 [-0.22; 3.8]	21	nc
FOSA	100	**1.3 [1.15; 1.5]**	100	0.56 [0.52; 0.59]	100	0.69 [0.67; 0.72]	90	**5.9 [5.0; 6.8]**	100	1.1 [0.98; 1.2]
N-MeFOSAA	85	1.2 [0.85;1.5]	32	nc	93	**2.3 [1.9; 2.7]**	67	1.8 [-0.12; 3.7]	96	0.54 [0.46; 0.61]
N-EtFOSAA	0	nc	0	nc	73	0.43 [0.36; 0.50]	33	nc	63	0.64 0.15; 1.13]