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SPATIAL AND TEMPORAL TRENDS OF POLY- AND PERFLUOROALKYL SUBSTANCES IN FISH FILLETS AND WATER COLLECTED FROM POOL 2 OF THE UPPER MISSISSIPPI RIVER

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Abstract: In 2011, poly- and perfluoroalkyl substances (PFASs) were analyzed in surface water and fish fillet samples taken from Pool 2 of the Upper Mississippi River, a 33-mile stretch inclusive of the Minneapolis/St. Paul, Minnesota (USA) metropolitan area. Approximately 100 each of bluegill, freshwater drum, smallmouth bass, and white bass were sampled within the study area. Surface water samples were also collected from each of the 10 sampling reaches established for the study. Water and fillet samples were analyzed for perfluorinated carboxylic acids (C4–C12), perfluorinated sulfonic acids (C4, C6, and C8), and perfluorooctane sulfonamide. Perfluorooctane sulfonate (PFOS) was observed with the greatest frequency in fish fillets and ranged from 3.0 to 760 ng/g wet weight. Mean (geometric) PFOS concentrations in bluegill, freshwater drum, smallmouth bass, and white bass were 20, 28, 29, and 58 ng/g wet weight, respectively. When compared with fish data collected in 2009, a significant reduction ($p < 0.05$) in PFOS concentrations was noted. This finding was confirmed based on data from studies conducted in 2012 and 2013. Overall, between 2009 and 2013, PFOS concentrations decreased by 65, 76, and 50% for bluegill, freshwater drum, and white bass, respectively (44% decrease for smallmouth bass from 2009 to 2012). These declines in fish PFOS concentrations are consistent with ongoing efforts to effectively control sources of PFASs to the Mississippi River. *Environ Toxicol Chem* 2017;9999:1–10. © 2017 SETAC

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INTRODUCTION

The study of poly- and perfluoroalkyl substances (PFASs) in the environment has received considerable attention since the initial reports of their global distribution in human populations and wildlife [1,2]. Poly- and perfluoroalkyl substances have been measured in humans [3,4] as well as in freshwater and marine environments (water and animals) at concentration levels ranging from parts per billion down to parts per trillion [5–9]. Potential sources of human exposure to PFASs include inhalation of household dust, contact with consumer products, ingestion of drinking water, and consumption of food [10–12]. However, the importance of exposure sources in general human populations has not been well established and seems to vary depending on several factors. For instance, food consumption was estimated to contribute more than 90% of the total lifetime exposure of PFASs in some nonoccupationally exposed populations in North America and Europe [13]. This premise is supported by additional studies in Canada, Spain, Poland, China, and Sweden estimating that PFASs in freshwater fish and seafood can account for >50% of the exposure in human populations [12,14–17]. In contrast, studies in Norway and the United Kingdom found fish consumption to be a minor contributor to human exposure [18–20]. Differences in fish consumption by human populations can be attributed to a variety of socioeconomic factors such that it is necessary to consider region-specific dietary habits, food sources, and food PFAS concentrations to adequately evaluate the dietary exposure of human populations.

As a result of activities associated with the manufacture, use, and disposal of PFASs by 3M and other entities, studies have been conducted to evaluate the distribution and magnitude of these compounds in water and fish collected from the Upper Mississippi River Basin (MN, USA) with a focus on Pool 2 [21–23]. Prior to 2009, most of the studies conducted in Pool 2 represented limited efforts to collect fish from those areas adjacent to known point sources [23,24]. Of the PFASs monitored in these studies, perfluorooctane sulfonate (PFOS) was the greatest contributor to the total PFAS body burden in fish. However, because of relatively small sample sizes, sample locations that were local to known point sources, and fish species that varied between years, the true extent and magnitude of PFAS concentrations in fish throughout Pool 2 was uncertain. In 2009, the Minnesota Pollution Control Agency (MPCA) conducted the first comprehensive study of PFASs in fish in Pool 2 [25]. The study sampled bluegill (*Lepomis* spp.), smallmouth bass (*Micropterus dolomieu*), freshwater drum (*Aplodinotus grunniens*), white bass (*Morone chrysops*), and common carp (*Cyprinus carpio*) from 4 geographic sections that spanned the entirety of the pool. Species-specific differences were observed, with freshwater drum having the greatest arithmetic mean PFOS concentration of 229 ng/g (wet weight) and carp having the lowest arithmetic mean PFOS levels at 77 ng/g (wet weight).

In 2006, the Minnesota Department of Health (MDH) issued its first pool-wide fish consumption advisory for PFOS of one meal per week for bluegill based on a small number of fish collected near a known PFOS point source [25]. In 2007, the MDH broadened its PFOS fish consumption advisory in Pool 2 to include additional species, most of which had consumption

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frequency recommendations of one meal per month. However, this advice was also based on a very limited number of fish samples that did not represent the entirety of Pool 2 [26]. Following the 2009 MPCA study, the MDH issued a fish consumption advisory for freshwater drum of one meal per month on the basis that arithmetic mean PFOS concentrations in Pool 2 exceeded 200 ng/g wet weight [27]. Fish consumption advisories of one meal per week were also issued for bluegill, white bass, and carp based on arithmetic mean PFOS concentrations in these species exceeding 40 ng/g wet weight. No advice was warranted for smallmouth bass because it is subject to catch-and-release angling rules for this stretch of the river. Fish consumption advisories for mercury and polychlorinated biphenyls are also present in this section of the Mississippi River.

Over the past 10 to 15 yr, significant actions have been implemented to reduce the presence of PFASs in Pool 2 [25,27]. These have included the 3M manufacturing phase-out of perfluorooctanyl compounds and their precursors as announced in May 2000 as well as additional actions by 3M and other stakeholders to reduce the releases of PFASs to the river. To build on the 2009 Minnesota Pollution Control Agency fish sampling study, and because ongoing PFAS reduction efforts have been expected to reduce sources of these compounds to the river, the present study was conducted to provide additional site-specific information on PFASs in fish and water. The specific study objectives were to: 1) conduct a more detailed assessment of PFASs in Pool 2 surface water and fish, 2) evaluate spatial extent and species differences in PFAS concentrations, 3) characterize contributions of individual PFASs to total PFAS concentrations in water and fish, and 4) evaluate PFOS temporal trends in fish and water in Pool 2.

MATERIALS AND METHODS

Standards and reagents

In total, 13 PFASs, 3 perfluorosulfonic acids (PFSAs), 9 perfluorocarboxylic acids (PFCAs), and perfluorooctane sulfonamide (FOSA) were assessed (Supplemental Data, Table S2). Perfluoroheptanoic acid (PFHpA) was purchased from Sigma-Aldrich, and perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorododecanoic acid (PFDoA) were purchased from Oakwood Products. Predominantly linear isomeric standards of perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHS), PFOS, and the following stable isotope labeled reference standards were purchased from Wellington Laboratories: [1,2,3,4-$^{13}C_4$]perfluorobutanoic acid (PFBA), [1,2,3,4,5-$^{13}C_5$]perfluoropentanoic acid (PFPeA), [1,2-$^{13}C_2$]perfluoropentanoic acid (PFHxA), [1,2,3,4,5-$^{13}C_5$]perfluoroheptanoic acid (PFHpA), [1,2,3,4, 5, 6,7, 8-$^{13}C_8$]PFOA, [1,2,3,4,5,6,7,8,9-$^{13}C_9$]PFNA, [1,2,3,4,5-$^{13}C_6$]PFDA, [1,2,3,4,5,6,7-$^{13}C_7$]perfluoroundecanoic acid (PFUnA), [1,2-$^{13}C_2$]PFDoA, [1,2,3-$^{13}C_3$]PFHS, [1,2,3,4,5,6,7,8-$^{13}C_8$]PFOS, and [1,2,3,4,5,6,7,8-$^{13}C_8$]FOSA. The stable isotope labeled standard [$^{18}O_2$]perfluorobutane sulfonate (PFBS) was acquired from RTI International for use as an internal standard. The following isotopically labeled reference standards were purchased from Wellington Laboratories for use as surrogate recovery standards: [1,2,3,4-$^{13}C_4$] PFOA, [1,2,3,4-$^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA. The PFBS, FOSA, and standards containing both branched and linear isomers of PFOS (potassium salt, lot #217) and PFOA (ammonium salt, lot # 332) were obtained from 3M Company. All chemicals and reagents used in extraction procedures were from Sigma-Aldrich or VWR Scientific, and high-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were purchased from EM Science.

Study site

The upper and lower extent of Mississippi River Pool 2 is defined by 2 dams and locks (Figure 1). The upper extent is bounded by the Ford Dam (Lock & Dam #1) located between Minneapolis and Saint Paul, just north of the confluence of the Mississippi and Minnesota Rivers, while the lower extent is defined by the Hastings Dam (Lock & Dam #2) located just upstream of Hastings, Minnesota. Ten sampling reaches of approximately 3 miles in length were identified in Pool 2 with 100- to 200-m buffer zones between each of the reaches. These buffer zones were established to accomplish a distinct separation between the reaches and a higher degree of representativeness of the data generated. The present study

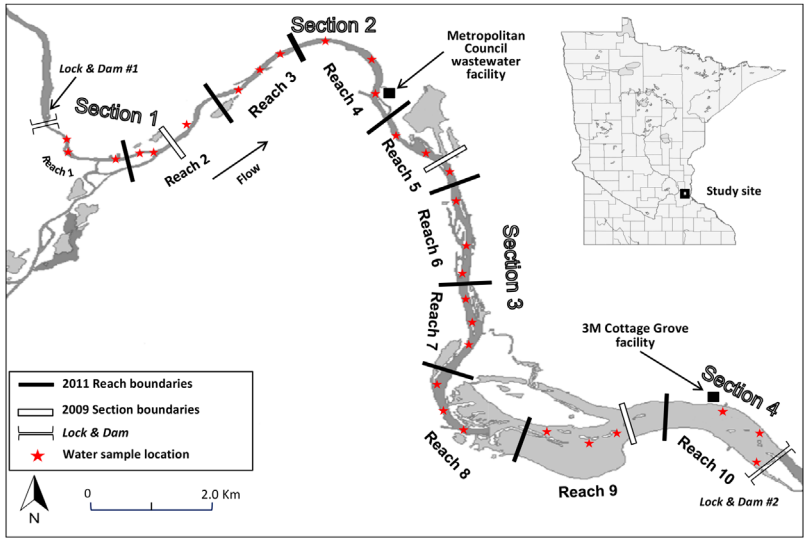

Figure 1. Areas of fish and water sample collection in the Upper Mississippi River, Pool 2 (MN, USA).

included a more geographically stratified sampling design of Pool 2 compared with the 2009 study. Rather than 4 sections of varying lengths sampled by the Minnesota Pollution Control Agency, the present study more finely divided the river into 10 reaches of approximately equal lengths (compare sections 1–4 versus reaches 1–10; Figure 1). Based on this approach, the data density for a given species (fish sampled per river mile) increased by 67%.

Water and fish collection

The fish collection focused on species that: 1) may be harvested by licensed anglers for consumption, 2) had been the focus of previous MPCA PFAS assessment studies, and/or 3) had demonstrated the highest PFAS levels in previous studies. The fish species collected included bluegill sunfish, freshwater drum, smallmouth bass, and white bass. The sampling objective was to collect 10 fish/species in each of the 10 sampling reaches, with each river bank being sampled equally to the extent possible. Electrofishing (Smith-Root) accounted for more than 95% of the fish collected but was supplemented with hook and line sampling when electrofishing was not effective. Because of precipitation events preceding the start of field efforts, water levels in Pool 2 were elevated and variable. As a result, electrofishing success was hampered, and 3 field collection rounds (round 1, 31 May–9 June 2011; round 2, 1–11 August 2011; round 3, 11–15 September 2011) were necessary to complete the fish collection and achieve the study objectives.

After collection, fish were transferred to clean coolers containing bagged ice and transported to a laboratory for processing. Fish were examined for general health and overt abnormalities, measured, weighed, and photographed. Otoliths were removed for age determinations. Fish were filleted based on United States Environmental Protection Agency guidelines [28]. The fillet type for all fish species was scaled and skin-on (major bones removed), which is consistent for data used by the MDH to establish fish consumption advisories [25]. Fillets were weighed and homogenized in a chemically cleaned stainless food processor (Robo-Coupe) using a freeze-fracture method. Homogenized fillet tissue was then put into clean polyethylene bags and kept frozen until analysis. In total, 396 fish were collected; sample size and physical parameters are summarized in the Supplemental Data, Table S1.

Surface water was collected in the first 2 rounds of the fish collection. In round 1, abbreviated because of high river water levels, water samples were collected from only 14 locations; in round 2, a complete set was collected, with 3 water samples being obtained from each reach (total of 30 locations sampled). Samples were taken from the water column approximately 30 to 60 cm below the surface and from the main river channel proximal to where fish were collected. All samples were collected as grab samples using large polyethylene bottles and were distributed to 250-mL high-density polyethylene (HDPE) sample bottles that contained internal standards and surrogate recovery standards.

Fish tissue analysis

Analytical methods for fish tissues as are given in Malinsky et al. [29]. Briefly, fillet samples were frozen with dry ice and homogenized to achieve a fine powder consistency. After homogenization, the tissue was transferred to a polyethylene bag and stored unsealed at –20 °C to allow the residual dry ice to sublimate. Approximately 0.5 g of each sample homogenate was transferred to centrifuge tubes, and each sample aliquot received a fixed quantity of stable isotope-labeled internal standards and surrogate recovery standards for use in quantitation, and for evaluation of analyte recovery, respectively. Extraction of each sample was performed by homogenization in 2.5 mL of acetonitrile, followed by centrifugation and analysis by HPLC–tandem mass spectrometry (MS/MS).

Samples were analyzed using an Agilent 1100 HPLC system with a binary pump interfaced with an AB Sciex API 5000 mass spectrometer equipped with an electrospray interface and operated in negative-ion mode (Supplemental Data, Table S2). The PFASs were separated from sample extracts chromatographically on an Oasis HLB online column (3×20 mm; 25-μm particle size) at 30 °C using 2 mM ammonium acetate in water and acetonitrile mobile phase system at a flow rate of 400 μL/min and an injection volume of 40 μL.

Water analysis

The analytical methods for water samples are as described by Wolf et al. [30]. Briefly, all samples, calibration standards, and associated quality control samples were extracted using either a preconditioned Waters C18 solid phase extraction (SPE) cartridge (PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHS, PFOS, and FOSA) or a preconditioned Oasis HLB SPE cartridge (PFBA, PFPeA, PFHxA, PFHpA, and PFOA). Target analytes were extracted with methanol into polypropylene vials with a 25× concentration factor. Samples were then analyzed on an Agilent 1200 series HPLC system interfaced with an AB Sciex API 5500 mass spectrometer equipped with an electrospray interface and operated in negative-ion MS/MS mode (Supplemental Data, Table S2). Analysis of the target analytes was performed on a Betasil™ C18 analytical column (2.1×100 mm; 5-μm particle size) held at 30 °C with 2 mM ammonium acetate in water and methanol mobile phase system. The flow rate was 300 μL/min and the injection volume was either 5 or 10 μL.

Quality assurance/quality control

For surface water samples, mean field matrix spike recoveries of C4–C12 PFCAs; C4, C6, and C8 PFSAs; FOSA; and [1,2,3,4- $^{13}C_4$]PFOA, [1,2,3,4- $^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA ranged from 87.5 to 124%. Water laboratory control sample recoveries ranged from 91% for PFHxA up to 106% for mixed PFOA (Supplemental Data, Table S3). All fish samples were analyzed in duplicate, and every 10th sample included 3rd and 4th sample aliquots that were fortified with the target PFAS as low and high laboratory matrix spike samples. Mean surrogate recoveries and their relative standard deviations based on all samples analyzed were $107 \pm 13\%$ ($n = 974$), $103 \pm 10\%$ ($n = 974$), and $106 \pm 11\%$ ($n = 971$) for [1,2,3,4- $^{13}C_4$]PFOA, [1,2,3,4- $^{13}C_4$]PFOS, and [1,2-$^{13}C_2$]PFUnA, respectively. Mean laboratory matrix spike sample recoveries and relative standard deviations ($n = 47$) of all the analytes and surrogate recovery standards were 91.7 to 113% and 8 to 20%, respectively (Supplemental Data, Table S4). Mean laboratory control sample ($n = 224$–234) recoveries and relative standard deviations for the linear isomers were $113 \pm 21\%$ for PFBA, $106 \pm 24\%$ for PFPeA, $102 \pm 13\%$ for PFHxA, $93.6 \pm 10\%$ for PFHpA, $96.1 \pm 15\%$ for PFOA (linear), $99.2 \pm 11\%$ for PFNA, $102 \pm 11\%$ for PFDA, $96.7 \pm 9.7\%$ for PFUnA, $98.9 \pm 9.8\%$ for PFDoA, $98.8 \pm 13\%$ for PFBS, $97.0 \pm 9.8\%$ for PFHS, $96.4 \pm 8.9\%$ for PFOS (linear), $95.8 \pm 7.0\%$ for FOSA,

109% ± 12% for $^{13}C_4$-PFOA, 105 ± 8.6% for $^{13}C_2$-PFUnA, and 105 ± 8.5% for $^{13}C_4$-PFOS (Supplemental Data, Table S4). The mean laboratory control sample ($n = 225$–231) values for technical mixtures (mixed linear and branched isomers) of PFOA, PFHS, and PFOS were 87.8 ± 13%, 110 ± 8.6%, and 116 ± 8.1%, respectively. These results met the quality assurance/quality control objectives for the present study (Supplemental Data, Table S4).

Confirmatory analyses of 41 fish representing approximately 10% of the total number of fish sampled and all round 2 surface water samples were conducted by a second analytical testing laboratory (AXYS Analytical Services; summary in Supplemental Data, Table S5). Except for FOSA, average relative percent differences between the 2 laboratories for the PFASs in fish were 22% or less. The result for FOSA, average of 31%, was based on only 4 matched samples whose concentrations were all less than 15 ng/g. On average, PFOS fish concentrations reported by AXYS were approximately 18% greater than those reported by 3M. Overall, the deviations between the results reported by both laboratories are comparable to those observed in other interlaboratory comparisons [31,32].

A poststudy QC interlaboratory comparison was conducted that included blind sample splits of archived fish samples and National Institute of Standards and Technology standard reference material (NIST SRM) for lake trout from Lake Superior (SRM 1946) and Lake Michigan (SRM 1947; Supplemental Data, Table S6). Compared with the original results, the average relative percent difference for PFOS for archived fish from both laboratories was 4.7%. For the standard reference materials, average PFOS concentrations reported by 3M were within the acceptable range reported by NIST for both SRM 1946 and SRM 1947 [33].

Interlaboratory comparisons of PFAS concentrations for the split water samples were limited because of the large number of nondetects reported by both laboratories. Using data from samples with measured concentrations above the limit of quantitation (LOQ), relative percent differences ranged from 1.3 to 69%, with an overall arithmetic mean of 33%. For PFOS, the relative percent difference ranged from 17 to 56%, with an arithmetic mean of 35% (Supplemental Data, Table S5). Overall, the deviations between the results reported by both laboratories are comparable to those observed in other interlaboratory comparisons [31,32].

Data analysis

Statistical analyses were performed with SAS, Ver 9.3. Nondetect data in both surface water and fish tissue were set to one-half of the LOQ prior to use in statistical analysis. A Kolmogorov–Smirnov test and probability plots were used to evaluate normality, and, if necessary, data were log-transformed to approximate normality. Variance homogeneity was evaluated with Levene's test. Species and location PFAS concentration differences were evaluated with mixed models (PROC MIXED) and least square means to account for unequal sample sizes. Tukey's test with the Kramer approximation was used to evaluate differences between treatments (species and/or location). To evaluate differences between groups, *t* tests with the Satterthwaite approximation were used, while correlations were evaluated with Spearman's rank test. Species and location differences in PFAS composition were examined by principal component analysis (PROC Factor) where analytes with ≥80% detects (PFOS, FOSA, PFDA, PFUnA, and PFDoA) were used in the analyses. Percent contributions of the sum of the 5 PFASs were arcsine-transformed to normalize the data for concentration differences. Principal component analysis was performed using a correlation matrix, pairwise deletion of missing data, and a Promax rotation. Differences with $p < 0.05$ are significant unless noted otherwise. Unless specifically noted, all surface water and fish fillet PFAS concentrations are reported as geometric means ± standard errors.

RESULTS

Surface water PFAS concentrations

Except for PFBA, PFOA, and PFOS, PFAS concentrations in surface water samples collected from reaches 1 through 9 (stations 1–27) were dominated by values below the LOQ (Figure 2; Supplemental Data, Table S7). In 9 of the 10 reaches, PFBA was measured, with detectable concentrations ranging from 11 ng/L (reach 1) to 648 ng/L (reach 10). All 10 reaches had at least one sample with measurable levels of PFOA that ranged from 2.3 ng/L (reaches 2, 4, and 6) to 219 ng/L (reach 10). In 9 of 30 water samples, PFOS was measured, with measurable values being first observed at station 15 (reach 5). The PFOS concentrations ranged from 2.15 (reach 8) to 136 ng/L (reach 10). Although concentrations of PFASs varied over the entirety of Pool 2, the greatest concentrations of all 13 targeted PFASs were in one samples collected in reach 10 (station 28). This station was located immediately downriver from the 3M Cottage Grove facility outfall, identified as a source of PFASs to the river [25].

Fish tissue concentrations

Although measurable PFAS concentrations were reported in all fish species, reported values for PFBA, PFPeA, PFHxA,

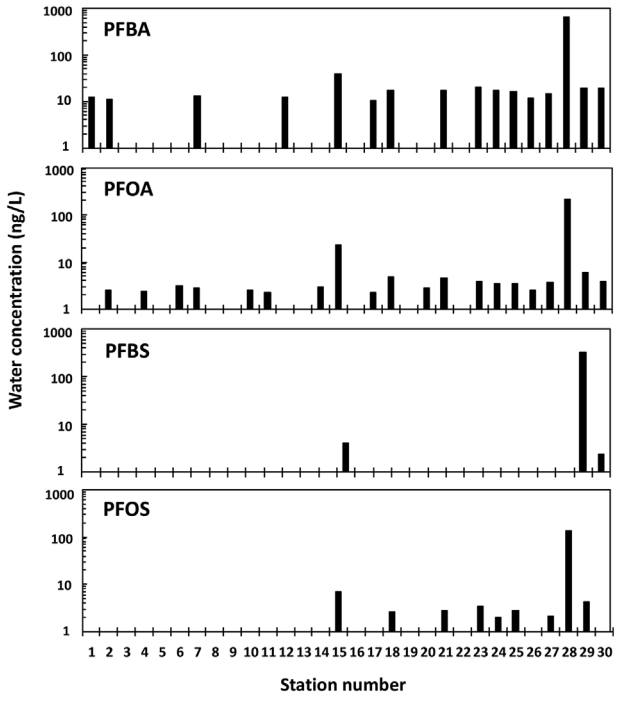

Figure 2. Poly- and perfluoroalkyl substance (PFAS) surface water concentrations in samples collected from Pool 2 of the Upper Mississippi River (MN, USA). Sample stations and reaches are numbered from upstream to downstream. Only measurable values are presented. LOQ values were 10 ng/L for PFBA, and 2.0 ng/L for the other PFAS. PFBA = perfluorobutanoic acid; PFOA = perfluorooctanoic acid; PFBS = perfluorobutane sulfonate; PFOS = perfluorooctane sulfonate.

Table 1. Mean concentrations of PFAS compounds in fish from Pool 2 of the Upper Mississippi River (MN, USA)[a]

	Bluegill FD[b]		Freshwater drum FD		Smallmouth bass FD		White bass FD	
PFAS	%	Mean	%	Mean	%	Mean	%	Mean
PFBA	9	0.16 ± 0.015 A	16	0.22 ± 0.020 A	4	0.17 ± 0.011 A	8	0.17 ± 0.015 A
PFPeA	7	0.11 ± 0.011 A	6	0.12 0.011 A	22	0.15 ± 0.012 B	3	0.092 ± 0.009 A
PFHxA	12	0.13 ± 0.011 A	15	0.18 ± 0.013 B	10	0.12 ± 0.009 A	9	0.15 ± 0.012 B
PFHpA	10	0.031 ± 0.002 A	14	0.027 ± 0.002 A	3	0.032 ± 0.002 A	6	0.026 ± 0.002 A
PFOA	11	0.064 ± 0.004 A	25	0.079 ± 0.006 A	1	0.067 ± 0.003 A	9	0.066 0.004 A
PFNA	41	0.078 ± 0.005 A	86	0.23 ± 0.015 B	7	0.043 ± 0.003 C	88	0.42 0.031 D
PFDA	100	1.0 ± 0.070 A	100	1.5 ± 0.077 B	100	1.6 ± 0.066 C	100	3.4 ± 0.012 D
PFDoA	100	0.47 ± 0.029 A	100	0.88 ± 0.053 B	100	1.1 ± 0.042 C	100	1.4 ± 0.055 D
PFUnA	100	0.65 ± 0.040 A	100	0.94 ± 0.047 B	100	1.1 ± 0.041C	100	1.7 ± 0.58 D
PFBS	7	0.042 ± 0.003 A	8	0.058 ± 0.005 A	1	0.047 ± 0.003 A	2	0.049 ± 0.004 A
PFHS	38	0.069 ± 0.008 B	31	0.044 ± 0.004 B	7	0.032 ± 0.002 A	55	0.062 ± 0.006 B
PFOS	100	20 ± 1.9 A	100	28 ± 2.5 B	100	29 ± 1.8 B	100	58 ± 2.4 C
FOSA	93	0.068 ± 0.004 A	100	0.21 ± 0.017 B	100	0.45 ± 0.038 C	100	1.1 ± 0.064 D

[a]Fillet concentrations in ng/g wet weight. Geometric means and standard error. Nondetects reported as one-half of the reporting limit. Different capital letters (A, B, C, D) indicate significant ($p < 0.05$) differences between species.
[b]Frequency of detection (FD): Samples sizes for bluegill (100), freshwater drum (100), smallmouth bass (100), and white bass (96).
PFAS = poly- and perfluoroalkyl substances; PFBA = perfluorobutanoic acid; PFPeA = perfluoropentanoic acid; PFHxA = perfluorohexanoic acid; PFHpA = perfluoroheptanoic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFDoA = perfluorododecanoic acid; PFUnA = perfluoroundecanoic acid; PFBS = perfluorobutane sulfonate; PFHS = perfluorohexane sulfonate; PFOS = perfluorooctane sulfonate; FOSA = perfluorooctane sulfonamide.

PFHpA, PFOA, PFBS, and PFHS were dominated by values below their LOQ, where their total frequency of measurable values was 9.3, 9.6, 12, 8.3, 12, 4.5, and 33%, respectively (Table 1). The frequencies of measurable values for PFNA and FOSA were 55 and 99%, respectively, whereas they were 100% for PFDA, PFUnA, PFDoA, and PFOS. Except for PFOS, tissue concentrations of the other target PFASs generally did not exceed 5.0 ng/g wet weight. The pool-wide mean PFOS concentration was 31 ng/g wet weight for all fish, whereas mean concentrations in bluegill, freshwater drum, smallmouth bass, and white bass were 20, 28, 29, and 58 ng/g wet weight, respectively (Table 1). In Pool 2, species-specific differences in PFOS concentrations were observed, with bluegill concentrations being statistically ($p < 0.05$) less than the other 3 species; in contrast, concentrations in white bass were greater than in the other 3 species. The PFOS concentrations in freshwater drum and smallmouth bass were not statistically different. Weak ($r \leq 0.30$), but statistically significant correlations ($p \leq 0.05$) were observed between PFOS concentrations and fish mass, length, and age, with no single association accounting for greater than 30% of the total variation (Supplemental Data, Table S8). In addition, while many of the associations identified were positive (i.e., increases in concentration with mass, length, or age), some were negative. For example, there was a positive correlation between mass and PFOS concentrations in bluegill ($r = 0.22$, $p = 0.03$), whereas it was negative for freshwater drum ($r = -0.30$, $p = 0.02$). These results (i.e., no clear and consistent relationship between PFOS concentration and mass, length, or age of fish) are in alignment with other study findings [21,25].

Except for PFOS and FOSA, analyses of the spatial distribution of PFASs did not indicate any clear concentration gradient within Pool 2 (Supplemental Data, Table S9). For PFOS, the greatest concentrations were observed in fish collected from reach 10 and an area located between the lower portion of reach 5 and the upper portion of reach 6 (Figure 3). Both areas are downriver of known PFAS point sources to Pool 2. The highest FOSA concentrations in all 4 species were observed in reach 10, ranging from maximums of 0.18 ng/g wet weight for bluegill to 6.0 ng/g wet weight for smallmouth bass.

Fish PFAS composition

Five PFASs accounted for approximately 97% of the total body burden in fish and included PFOS (84%), PFDA (5.2%), PFUnA (3.2%), PFDoA (2.9%), and FOSA (1.2%). Based on these 5 PFASs, principal component analysis identified 3 factors that accounted for approximately 99% of the total variation, with factors 1, 2, and 3 accounting for 48.2, 27.1, and 23.3% of the total variation, respectively. Significant loadings ($r > 0.7$) on factor 1 were associated with long-chain PFCAs (i.e., PFUnA, PFDoA, and PFDA), whereas factors 2 and 3 were associated with PFOS and FOSA, respectively. A plot of factors scores showed very little overlap in the distribution of samples

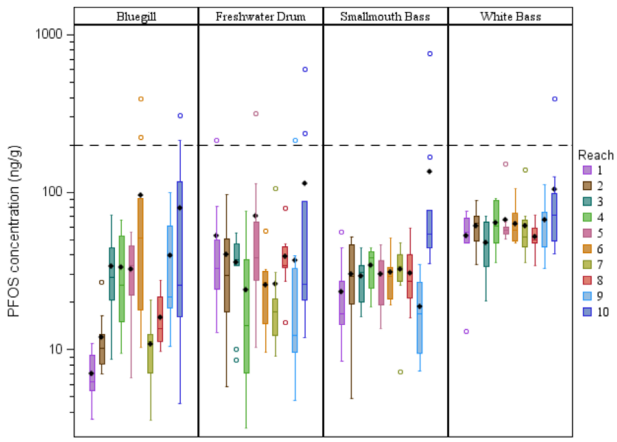

Figure 3. Spatial distribution of perfluorooctane sulfonate (PFOS) in fish collected from Pool 2 of the Upper Mississippi River (MN, USA) in 2011. The marker inside the box is the median, the line represents the line is the group mean, the box represents the interquartile range (IQR), the whiskers represent values that are 1.5 × IQR or less, and the values outside the whiskers are outliers. The dashed line is the one meal per month fish consumption value for Minnesota.

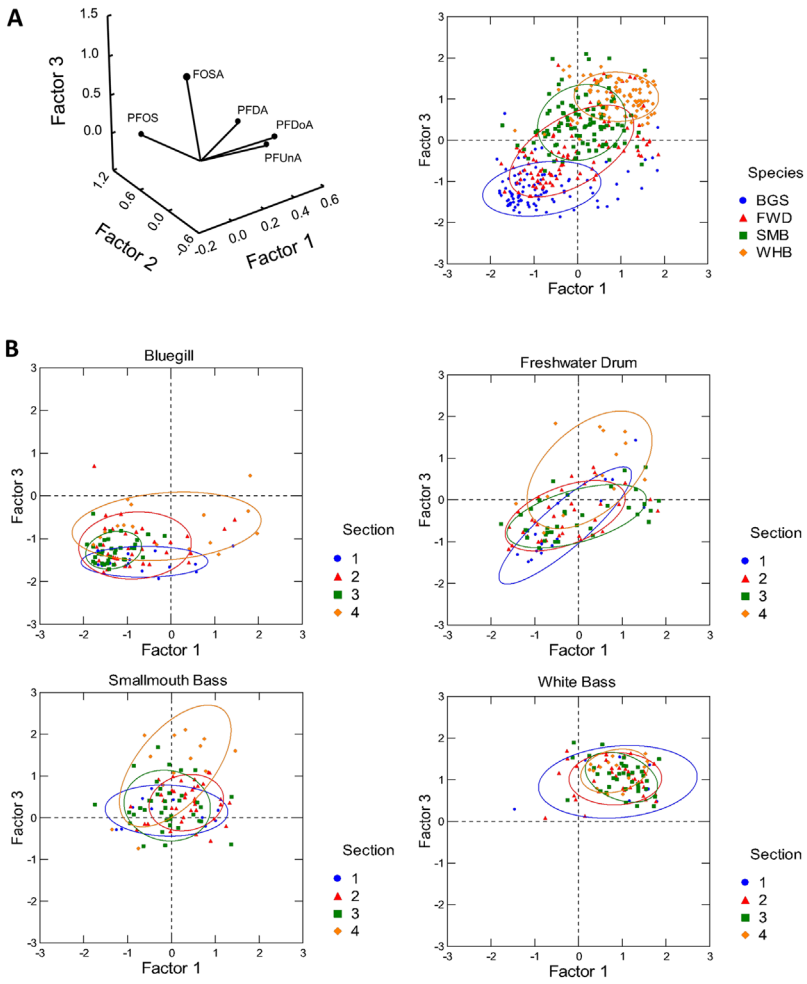

Figure 4. Principal component analysis (PCA) of poly- and perfluoroalkyl substances (PFASs) in fish collected from Pool 2 in the Upper Mississippi River (MN, USA) in 2011. (**A**) PCA results for all fish species collected in Pool 2. (**B**) Species-specific PCA results based on their spatial distribution in Pool 2. Sections based on the Minnesota Pollution Control Agency designations [25]. BGS = bluegill; FWD = freshwater drum; SMB = smallmouth bass; WHB = white bass.

associated with bluegill and white bass, indicating that these 2 species have distinct patterns of PFAS accumulation in Pool 2 (Figure 4A). In contrast, there was a significant overlap in the distribution of samples associated with freshwater drum and smallmouth bass, indicating a similar pattern of accumulation. When evaluated river reach/section, principal component analysis results indicated little to no differentiation in PFAS patterns within Pool 2 regardless of the proximity of the sample location to known point sources to the river (Figure 4B). The only exception was observed with bluegill, where the distribution in sample for fish collected from the most upstream section differed from that observed for fish collected from the most downstream section.

DISCUSSION

After the present study was completed, 2 additional and comparable studies were performed. In 2012, the MPCA conducted essentially a repeat study of its 2009 work [27]. Then in fall 2013, another study collected bluegill sunfish, common carp, freshwater drum, and white bass in Pool 2 based on river sections that were defined in the 2009 MPCA effort [34]. The collective data from these 4 major studies, including surface water results generated from each effort, will be considered in the remaining sections.

Water concentrations

Water PFAS concentrations in samples collected in the 2009 to 2013 studies had similar spatial patterns in terms of measurable values in Pool 2. The spatial distributions for PFDA, PFUnA, PFDoA, and FOSA were characterized by a preponderance of samples with values less than their LOQ throughout Pool 2. In contrast, PFBA and, to a lesser extent, PFOA were detected throughout Pool 2, indicating that both point and nonpoint sources were likely contributors. For PFOS, the uppermost portion of Pool 2 was also dominated by samples with concentrations less than the LOQ; however, there was an increase in the incidence of measurable PFOS levels starting in mid-Pool 2 that continued downstream to Dam 2 at Hastings. The locations, patterns, and magnitude of measurable PFAS constituents, especially when encountered midpool and downstream in an urban setting generally are indicative of the presence of point source contributions.

To further address the issue of location, a comparison of the surface water data from 2009 and 2012 MPCA was conducted because both studies sampled the same locations in Pool 2. Using only samples with measurable PFAS concentrations, there was at least a 1.6-fold increase in PFBA and PFOA concentrations in surface water concentrations regardless of sampling location within Pool 2. For PFOS, concentration

increases ranged from 1.6- to 2.3-fold between 2009 and 2012 for sites with measurable values in both years. Temporal comparisons over a longer period can also be made on a more location-specific manner. In this analysis, several proximate sampling locations were examined across the 4 studies. For example, station 12 in the 2009 studies, station 515 in the 2011 study, and the station at RM814.3 in the 2013 study represent proximate locations of waters leaving Pool 2 that can be used to evaluate surface water concentrations over a 4-yr period (Supplemental Data, Table S10). For PFBA, average water concentrations were 44, 19, 190, 190, and 41 ng/L in 2009, 2011, 2012, 2013 (October), and 2013 (September), respectively. Average PFOA levels were 17, 6.1, 37, 12, and 8.6 ng/L, while for PFOS they were 15, 4.2, 25, 6.7, and 4.0 ng/L, in 2009, 2011, 2012, 2013 (October), and 2013 (September), respectively. Similar observations can be noted for other portions of Pool 2. Thus, based on this somewhat limited dataset, it is apparent that no clear and consistent trend in water concentrations can be observed in Pool 2. While a more comprehensive evaluation is necessary to evaluate potential temporal differences, the high frequency of samples ≤LOQ, differences in sample locations, and differences in analytical methodologies and their attendant LOQs between studies obscure any indication of temporal changes in Pool 2.

The question of hydrological status during the time of collection is of some importance given the differences in river stage height observed among the 4 studies. However, this factor can be largely isolated by shifting from considerations of measured concentrations to mass loadings. To assess this issue further, concentration data for the most upstream and most downstream sampling locations have been combined with available flow rate information to calculate Pool 2 inlet and outlet mass flux rates for PFBA, which has a ubiquitous distribution within Pool 2, and PFOS, which is more closely associated with point sources in Pool 2 (Supplemental Data, Table S11). In the upper section of Pool 2, the only consistently quantifiable parameter was PFBA, and its flux rate generally ranged from 0.05 to 0.08 kg/d, with a maximum value of 0.51 kg/d that was observed in the 2011 study. At the most downriver locations in Pool 2, no clear pattern was evident for PFBA, with mass flux rates varying from 1.0 to 2.0 kg/d. At the outlet of Pool 2, the mass flux rates for PFOS in 2009 and 2011 were 0.36 and 0.33 kg/d, respectively, but then decreased by more than 75% in surface water collected in 2013. Similar patterns were also observed for PFOA and PFHxS. Thus, while changes in stage height and water flow in rivers has been shown to affect the concentration of chemicals in surface waters [35], this analysis based on mass loadings essentially isolates this phenomenon and reveals a downward trend at the outlet to Pool 2 for the higher homologue constituents.

Fish PFAS concentrations

In fish sampled in the 2011 study, pool-wide fish PFOS concentrations were statistically less than those observed in 2009, with reductions of 60, 59, 42, and 30% for bluegill, freshwater drum, smallmouth bass, and white bass, respectively [25]. The downward trend in PFOS concentrations was also observed in the subsequent 2012 and 2013 studies [27,40]. From 2009 to 2013, PFOS concentrations in bluegill, freshwater drum, white bass, and carp decreased by approximately 65, 76, 50, and 63%, respectively. For smallmouth bass, PFOS concentrations decreased by 44% between 2009 and 2012. (Smallmouth bass were not included in the 2013 study).

To evaluate the change in fish PFOS concentrations in a more spatially explicit manner, fish collected in the 2011 study were assigned to river sections 1 through 4 established in the 2009 MPCA study using GPS coordinates associated with electrofishing runs (Figure 1). Results from this analysis indicate that the magnitude of changes in PFOS concentrations was not only species-specific, but was also influenced by location within Pool 2. When evaluated spatially, the greatest changes in mean PFOS concentrations that occurred between 2009 and 2011 were observed in fish collected within section 4, where significant decreases of approximately 64, 83, 75, and 40% were observed in bluegill, freshwater drum, smallmouth bass, and white bass, respectively (Figure 5). The least amount of change was observed in section 2, where PFOS mean concentrations declined by approximately 17, 36, and 21% in white bass, bluegill, and freshwater drum, respectively. Interestingly, PFOS concentration in smallmouth bass from section 2 increased approximately 30% over this time period. The greatest variation in species-specific reductions in PFOS concentrations between river sections was observed in bluegill, while the least variation was observed in white bass. The difference in section-related PFOS concentrations for both species was most likely because of differences in the migratory behavior and home ranges of both species [36–38]. The steady and less variable reductions in PFOS concentrations noted for white bass most likely were because of the greater home range and movement of white bass within Pool 2 compared with the other species. Thus changes in white bass PFOS concentrations are most likely the best indicator of overall reductions of PFOS exposure in Pool 2. In contrast, bluegill have much smaller home ranges that

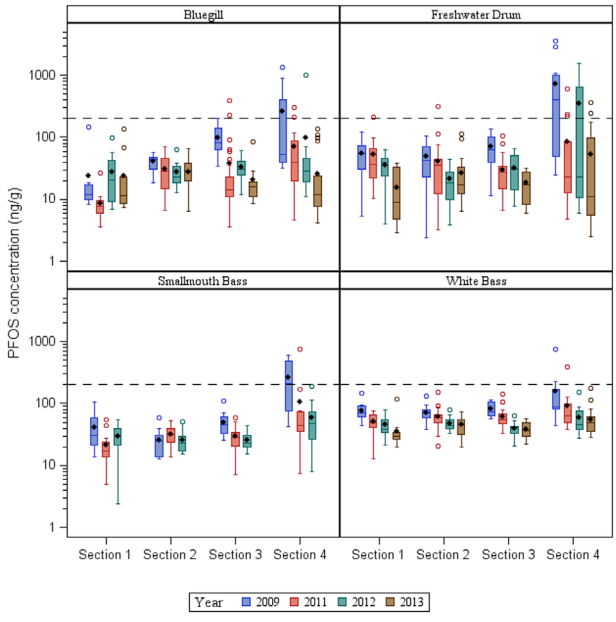

Figure 5. Comparison of perfluorooctane sulfonate (PFOS) fish tissue concentrations in 2009 and 2013 by species in Pool 2 of the Mississippi River (MN, USA). The marker inside the box is the median, the line represents the group mean, the box represents the interquartile range (IQR), the whiskers represent values that are 1.5 × IQR or less, and the values outside the whiskers are outliers. The dashed line is the one meal per month fish consumption value for Minnesota.

would more closely reflect local changes and variability in environmental PFOS concentrations.

The spatial and temporal trends observed for the other PFASs were similar to those observed for PFOS, but because of the large number of nondetects, the magnitude of these trends is difficult to quantify. In general, the frequency of detection in fish tissue was very low ($\leq$10%) in all studies for PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS, and PFHS. For the remaining constituents (PFNA, PFDA, PFUnA, PFDoA, and FOSA), the fish tissue concentrations were low, with maximum levels for all species in all studies generally at or below 40 ng/g. In part because of these relatively low concentrations, PFAS composition did not vary greatly between species, nor did it vary greatly between reaches. As a result, it is difficult to associate the presence of PFASs in fish with a specific source, especially for those species that move freely throughout Pool 2. This conclusion is also supported by the principal component analysis, which demonstrated little difference in PFAS body burden patterns when evaluated on a species-specific or spatially explicit manner. Overall, the concentration data for these analytes suggest that their concentrations are decreasing from 2009 levels in a manner similar to that observed for PFOS.

To provide additional context to PFOS concentrations measured in 2009, a retrospective analysis was conducted with fish samples prior to this date. The validity of such an analysis will depend on the extent to which the following assumptions are true: 1) the same species have been collected over the time period under consideration, 2) the sampling locations have not significantly changed over the time period in question, 3) differences in analytical methodologies do not influence the observed trends, 4) the data are of similar quality, and 5) sample size between years is sufficient to detect changes in concentrations. When the entirety of Pool 2 is considered, few of these assumptions are met, given the sporadic and limited nature of previous fish collections [23–25]. However, somewhat comparable data are available if the analysis is restricted to only the tail waters of Pool 2, an area encompassed by section 4 in the 2009 MPCA study [25] and reach 10 of the 2011 study. Graphically, PFOS concentrations in bluegill, smallmouth bass, and white bass collected between 2005 and 2008 as part of very localized range-finding work appear to be similar in magnitude to those observed in the fish collected in 2009 (Table 2). Regression analyses of PFOS concentration data in bluegill, smallmouth bass, and white bass for samples collected from 2005 to 2013 determined half-lives of 2.7, 2.8, and 2.5 yr, respectively. While the temporal span encompassed by these data is short, the consistent downward trend for all species is readily apparent. The log transformation of the data somewhat masks the fact that species-specific PFOS concentrations have declined significantly, especially between 2009 and 2011.

The general temporal trend in Pool 2 fish PFOS concentrations was consistent with that observed in other fauna that have been studied in the Upper Mississippi River basin. Route et al. [39] observed approximately a 2-fold decrease in PFOS concentrations in bald eagle blood collected from 2006 to 2011 from an area that included Pool 2. In contrast, the decrease in PFOS concentrations in eagle blood collected from locations distant from known point sources associated with the Minneapolis/St. Paul area exhibited only slight decreases in PFOS concentrations (i.e., no greater than 16%) over the same time period [38]. Reductions in great blue

Table 2. Temporal trends in perfluorooctane sulfonate concentrations in fish collected from the tail waters (section 4) of Pool 2 in the Upper Mississippi River (MN, USA)[a]

	2005		2008		2009		2011		2012		2013					
Species	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean	Model[b]	r-square	*p* value	Half-life (yr)
Bluegill	6	130 ± 65	5	160 ± 27	15	110 ± 35	11	40 ±11	15	36 ± 9.4	15	2.5 ± 2.5	Y = 8.512–0.3538(X)	0.3538	<0.0001	1.28
Smallmouth bass	17	213 ± 67	5	130 ± 38	15	180 ± 42	13	50 ± 15	15	44 ±9.2	ND	ND	Y = 8.384 – 0.4.018(X)	0.4018	<0.0001	1.73
White bass	5	286 ± 150	ND	ND	15	118 ± 21	14	72 ± 11	13	52 ± 6.9	26	49 ± 4.2	Y = 8.337 – 0.3524(X)	0.3524	<0.0001	1.96

[a]Concentration data presented as geomeans and standard errors. All data in ng/g wet weight.
[b]Regression model includes perfluorooctane sulfonate concentration (ng/g) and year (X).
ND = no data.

heron egg PFOS concentrations have also been observed in colonies from Pig's Eye Lake, which is located in Pool 2 in close proximity to the Metro Wastewater Treatment Plant [39]. Geometric mean (95% confidence limit) PFOS concentrations in eggs collected from this location in 1993, 2010, and 2011 were 940 (608–1455), 288 (171–483), and 396 (203–773) ng/g wet weight, respectively. This represents a reduction of 69% in 2010 and 57% in 2011 when compared with PFOS concentrations measured in 1993.

Overall, the measured declines in fish PFOS concentrations that were documented in the present study, as well as in other studies [39,40], coincide with the >10-yr cessation of manufacturing of products based on perfluorooctanyl chemistry and with ongoing efforts in Minnesota and other areas to effectively control sources of PFASs to the environment. These efforts include the reduction of PFAS releases from known point sources such as the Metropolitan Council Metro wastewater treatment plant and the 3M Cottage Grove plant. However, the association between changes in biota PFAS concentrations and changes in surface water concentrations cannot be definitively determined because of the lack of measurable PFAS surface water concentrations that could be used to track similar changes over the same time period.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3891.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (John.Newsted@obg.com).

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