ATTACHMENT 10

Heidi M. Pickard et al., *PFAS and Precursor Bioaccumulation in Freshwater Recreational Fish: Implications for Fish Advisories*, 56 Env't Sci. & Tech. 15573 (2022) ("Pickard Study") and Supporting Information

Environmental Science & Technology

pubs.acs.org/est

Article

PFAS and Precursor Bioaccumulation in Freshwater Recreational Fish: Implications for Fish Advisories

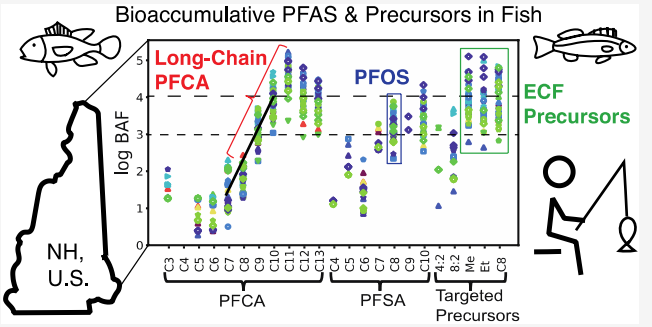
Heidi M. Pickard,* Bridger J. Ruyle, Colin P. Thackray, Adela Chovancova, Clifton Dassuncao, Jitka Becanova, Simon Vojta, Rainer Lohmann, and Elsie M. Sunderland

Cite This: *Environ. Sci. Technol.* 2022, 56, 15573−15583

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Per- and polyfluoroalkyl substances (PFAS) are a diverse class of fluorinated anthropogenic chemicals that include perfluoroalkyl acids (PFAA), which are widely used in modern commerce. Many products and environmental samples contain abundant precursors that can degrade into terminal PFAA associated with adverse health effects. Fish consumption is an important dietary exposure source for PFAS that bioaccumulate in food webs. However, little is known about bioaccumulation of PFAA precursors. Here, we identify and quantify PFAS in recreational fish species collected from surface waters across New Hampshire, US, using a toolbox of analytical methods. Targeted analysis of paired water and tissue samples suggests that many precursors below detection in water have a higher bioaccumulation potential than their terminal PFAA. Perfluorobutane sulfonamide (FBSA), a short-chain precursor produced by electrochemical fluorination, was detected in all fish samples analyzed for this compound. The total oxidizable precursor assay interpreted using Bayesian inference revealed fish muscle tissue contained additional, short-chain precursors in high concentration samples. Suspect screening analysis indicated these were perfluoroalkyl sulfonamide precursors with three and five perfluorinated carbons. Fish consumption advisories are primarily being developed for perfluorooctane sulfonate (PFOS), but this work reinforces the need for risk evaluations to consider additional bioaccumulative PFAS, including perfluoroalkyl sulfonamide precursors.



KEYWORDS: *PFAS precursors, targeted analysis, total oxidizable precursor (TOP) assay, Bayesian inference, suspect screening, bioaccumulation, consumption advisories, seafood*

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of anthropogenic chemicals with thousands of potential structures.[1,2] Human exposure to PFAS has been associated with many adverse health effects,[3] and seafood is known to be an important dietary PFAS source.[4,5] Many regions are developing fish consumption guidelines to reduce exposure risks for some of the most bioaccumulative legacy PFAS, predominantly perfluorooctane sulfonate (PFOS).[6,7] However, per- and polyfluoroalkyl precursors (hereon referred to as precursors) make up most of the PFAS mass in consumer products[8,9] and many contaminated aquatic ecosystems.[10] Prior work suggests some precursors have enhanced propensity for biological uptake relative to the terminal perfluoroalkyl acids (PFAA).[11]

PFAS precursors released to the environment may undergo abiotic or biotic transformation and eventually form PFAA as terminal products. Precursors that originate from the electrochemical fluorination (ECF) process have a fully fluorinated backbone in their chemical structure, while those manufactured by the fluorotelomerization (FT) process are not fully fluorinated.[12] Targeted mass spectrometry methods (LC-MS/MS) only capture a small fraction of the PFAS used in commerce and released to the environment.[13,14] It is challenging to detect most precursors using targeted methods because many analytical standards are not commercially available. High-resolution mass spectrometry (HRMS) can be used to confirm the presence of specific precursors and assign probable structures to unknown PFAS. However, these results are not quantitative and are difficult to interpret when diverse precursors are present at low concentrations, which is often the case with environmental samples. Semi-quantification of PFAS from HRMS measurements has been used to estimate the concentrations of analytes that lack matched analytical

Received: May 24, 2022
Revised: September 13, 2022
Accepted: September 14, 2022
Published: October 24, 2022

standards, but uncertainties are not quantifiable and could span an order of magnitude or more.[15,16]

To address some of the challenges associated with PFAS precursor detection, Ruyle et al.[17] developed a statistical method for interpreting results from the total oxidizable precursor (TOP) assay that groups precursors by their perfluorinated carbon chain length and manufacturing origin (ECF or FT) using Bayesian inference (hereon referred to as TOP + BI). The TOP assay transforms oxidizable precursors to perfluoroalkyl carboxylates (PFCA) with known perfluorinated carbon chain lengths that are detectable at trace levels using targeted LC-MS/MS analysis. The TOP + BI method is preferred over analytically detected changes in PFCA concentrations (only TOP) because it explicitly accounts for analytical uncertainties, incomplete recoveries, and variability in product yields following precursor degradation.

Many sites across the United States (US) have been contaminated by ECF- and FT-based aqueous film-forming foams (AFFF) that contain large quantities of precursors.[18,19] Some precursors, like per- and polyfluoroalkyl ether acids (PFEA), are known to be resistant to oxidation by the TOP assay.[20] Nonetheless, the TOP + BI method performs well at sites affected by AFFF chemistries that have many precursors present at low abundance.[10]

The main objective of this work was to better understand the bioaccumulation potential of PFAS and precursors present in inland surface waters. To do this, we used a toolbox of analytical and statistical methods to measure PFAS in muscle tissues from eight species of freshwater fish commonly caught by recreational fishers in New Hampshire (NH), US. Targeted analysis (LC-MS/MS) was used to detect a suite of up to 37 PFAS in paired surface water and fish tissue samples. Concentrations of PFAS precursors in fish grouped by perfluorinated carbon chain length (C_n where n = number of perfluorinated carbons) and manufacturing origin were interpreted using the TOP + BI method. Suspect screening was used to confirm the presence of additional precursors in fish muscle tissue. The combined data set provides insights into the accumulation of precursors in freshwater food webs. We discuss implications for developing and enhancing fish consumption advisories.

MATERIALS AND METHODS

Field Sample Collection. We collected paired water and fish samples from nine freshwater ecosystems in southern New Hampshire, US, in September−October 2017. Locations (denoted LOC) were selected based on proximity to suspected PFAS sources, including AFFF use (FF) (LOC 1, 3), waste disposal sites (WS) (LOC 2, 4), and plastics and textile manufacturing (MF) point sources (LOC 5−9, Figure S1). We were unable to differentiate PFAS profiles in fish based on potential sources due to limited sample sizes associated with each category.

A total of 23 surface water grab samples, 7 field duplicates, and 2 field blanks were collected in precleaned 1 L HDPE bottles and transported to Harvard University. Water samples were stored at 4 °C and analyzed within a month. Local recreational fishers and the NH Fish and Game Department assisted with fish harvesting. Fish (n = 62, 1−3 fish per species per location) were stored frozen at −20 °C and analyzed within a month, followed by reanalysis in 2021. Fish species included yellow perch: *Perca flavescens*; lake whitefish: *Coregonus clupeaformis*; bluegill: *Lepomis macrochirus*; pumpkinseed: *Lepomis gibbosus*; brown bullhead: *Ameiurus nebulosus*; chain pickerel: *Esox niger*; largemouth bass: *Micropterus salmoides*; and smallmouth bass: *Micropterus dolomieui*. The Supporting Information (SI) contains additional information on sampling (Sections 1.1−1.2, Table S1).

Chemicals and Reagents. Targeted analysis (LC-MS/MS) was used to detect up to 37 PFAS analytes, denoted by their perfluorinated carbon chain length (C_n where n = number of perfluorinated carbons). This list included eighteen PFAA [eleven PFCA (C3−C13) and seven perfluoroalkyl sulfonates (PFSA: C4−C10)] and up to nineteen targeted PFAS precursors [four fluorotelomer sulfonates (FTSA: 4:2, 6:2, 8:2, 10:2), six perfluoroalkyl sulfonamides (FASA: FBSA, FHxSA, FOSA, FDSA, N-MeFOSA, N-EtFOSA), two perfluoroalkyl sulfonamidoethanols (FASE: N-MeFOSE, N-EtFOSE), three perfluoroalkyl sulfonamidoacetic acids (FASAA: FOSAA, N-MeFOSAA, N-EtFOSAA), three fluorotelomer carboxylates (FTCA: 3:3, 5:3, 7:3), and one polyfluoroalkyl ether carboxylate (PFPE: ADONA)]. The SI Section 1.3 contains additional details on chemicals and materials used for analysis (Table S2).

Sample Extraction. Subsamples (500 mL) of 1 L water samples were obtained by sonicating and inverting the sample several times. Samples were extracted and analyzed for 25 targeted PFAS at Harvard University in 2017. Samples were spiked with 2 ng isotopically labeled internal standard followed by offline weak anion exchange (WAX) solid phase extraction (SPE), following established methods.[21] Fish muscle tissues were also extracted and analyzed for 25 targeted PFAS in 2017. In 2021, some fish tissue samples were re-extracted and analyzed for a larger suite of 37 targeted PFAS to compare with TOP assay results. For both fish extractions, 0.5 g of homogenized wet-weight muscle tissue fortified with internal standards was subjected to ion-pairing extraction, following established methods.[22] Bluegill muscle tissues were analyzed as composites (n = 3), and all other fish were analyzed individually. The SI Section 1.4 contains additional details on extraction methods.

Targeted Analysis. Water and fish muscle tissue extracts were analyzed for targeted PFAS using an Agilent (Santa Clara, CA) 6460 triple quadrupole liquid chromatograph-tandem mass spectrometer (LC-MS/MS) equipped with an Agilent 1290 Infinity Flex Cube online SPE, with slight modifications to previously published methods.[21] Each 100−300 μL extract was loaded onto an Agilent Zorbax SB-Aq (4.6 mm × 12.5 mm; 5 μm) online SPE cartridge with 0.85 mL of 0.1% aqueous formic acid at a flow rate of 1 mL min^{-1}. Analytes were eluted from the SPE cartridge and loaded onto an Agilent Poroshell 120 EC-C18 (3.0 mm × 50 mm; 2.7 μm) reversed-phase HPLC column using ammonium acetate (2 mM) in methanol and ammonium acetate (2 mM) in Milli-Q water at a flow rate of 0.5 mL min^{-1} and column temperature of 50 °C. Analytes were ionized with an electrospray ionization (ESI) source in negative ion mode and introduced to the tandem mass spectrometer at a temperature of 300 °C, gas flow rate of 13 L min^{-1}, and nebulizer pressure of 45 psi. Additional details are provided in SI Section 1.5.

Targeted PFAS were quantified using both isotopic dilution and extracted internal standard quantification with 7- to 11-point calibration curves. For PFAS without matched isotopically labeled standards, the internal standard closest in retention time and/or within the same functional group was used for quantification (Table S3). Milli-Q water was used for

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

procedural blanks, and two to three blanks were included with each water and fish tissue extraction (Table S4). Average (±standard deviation) spike recoveries using Milli-Q water as the spiking matrix were 105 ± 23% for the water extraction and 88 ± 10 and 104 ± 25% for the fish extractions. Average spike recoveries using fish muscle as the spiking matrix was 115 ± 33% for the fish extractions (Table S5). Sections 1.6−1.7 of the SI contain additional details on blanks, duplicates, spikes, and internal standard recoveries (Table S6).

Limits of detection (LODs) were calculated based on the average concentration at which the sample signal-to-noise ratio (S/N) was three. Method detection limits (MDLs) were determined based on sample dilution volumes or weight, and only values >MDL are reported here. MDLs for fish samples ranged between 0.001 and 1.27 ng g^{-1} (SI Section 1.8 and Table S7). Method trueness was assessed using NIST SRM 1947 (Lake Michigan fish tissue). Relative percent differences between NIST SRM 1947 analyzed in this study and the reference concentrations were within ±30% for all detectable PFAS, which compares favorably with other studies (SI Section 1.9 and Table S8).[23,24]

Total Oxidizable Precursor (TOP) Assay and Statistical Interpretation. The TOP assay uses hydroxyl radicals formed by heated persulfate under basic pH conditions to oxidize precursors into PFCA of the same or shorter perfluorinated carbon chain lengths that can be detected using targeted analysis.[25] The TOP assay was applied to one sample of fish muscle tissue from each location. We chose the fish species at each location that had the highest targeted PFAS concentrations (Table S9). The extract oxidation procedure was adapted from an aqueous oxidation procedure,[10] which itself is modified from the original method developed by Houtz and Sedlak.[25] Following ion-pairing extraction with ENVI-carb cleanup, extracts were transferred to 50 mL polypropylene tubes and evaporated to dryness. The tubes were vigorously shaken following addition of Milli-Q water (20 mL) and 0.12 M potassium persulfate and 0.25 M sodium hydroxide solution (20 mL). Samples were heated in an 85 °C water bath for ≥12 h and then neutralized to pH 7, as needed. Samples were processed similarly to water samples using SPE and prepped for targeted analysis (SI Section 1.10).

Precursor oxidation efficiency in the presence of fish tissue was evaluated with each sample batch by spiking fish muscle tissue with targeted precursors prior to extraction and oxidation. Complete oxidation of targeted precursor concentrations (concentrations < MDL) was verified after every batch of samples. Internal standards were added after the TOP assay to avoid oxidation of the isotopically labeled precursors. Molar yields of several targeted precursors oxidized in the presence of fish tissue to the corresponding PFCA were compared to literature data for other matrices (Table S10).[10,24−29] Targeted PFAS recovery spikes were included to assess the stability and recovery of PFAS after ion-pairing extraction and the TOP assay (Table S11). Low recoveries for the longer-chain PFCA (C > 8) indicated that PFAS loss occurred during the ion-pairing extraction and TOP assay, so they were omitted from further interpretation. The addition of internal standards after oxidation meant they could not be recovery-corrected. Instead, spike recoveries were used to correct oxidized C3−C8 PFCA concentrations with average recoveries ≥50%.[30] The change in C3−C8 PFCA after the TOP assay was calculated based on the recovery-corrected difference. Uncertainty in recoveries was accounted for in the Bayesian inference. We did not include modifications to the TOP assay to detect C < 3 PFCA[26] because the method used here is based on prior work[17] that did not require it to complete the PFAS mass balance, but this could be explored in future work. Additional details on the TOP assay validation are provided in SI Section 1.10.

Precursor concentrations (grouped by perfluorinated carbon chain length) were based on the recovery-corrected measured increases in C3−C8 PFCA (Table S9) produced by the TOP + BI method previously developed for aqueous samples.[10,17] Manufacturing origins [ECF vs FT] of precursors are identifiable based on their unique yields. FT precursors have n perfluorinated carbons followed by two or three aliphatic hydrocarbons (n:2, n = 4, 6, 8; n:3, n = 5, 7) and oxidize to form multiple PFCA analytes in the TOP assay (Table S10). ECF precursors include those with C_n (n = 4−8) perfluorinated carbons and generally oxidize to form one C_{n-1} PFCA with ∼100% yield (Table S10). Ten ECF and FT precursor groups with perfluorinated carbon chain lengths ranging from 4−8 were included in the statistical interpretation. Longer-chain precursor groups (C > 8) were not included due to reduced recovery of the longer-chain PFCA in the TOP assay. Inferred precursor classes based on this method incorporate those with analytical standards (i.e., targeted precursors) and others without that require suspect screening and/or nontargeted analysis to be identified. Precursors were inferred using their oxidation yields (Table S10) and measurements of their oxidation products by Markov chain Monte Carlo (MCMC) analysis implemented in Python 3.7.4 using *emcee* 3.0.2.[31] The likelihood of precursor concentrations, given the measurements, was determined by sampling the posterior distribution of precursor concentrations generated from the least-squares of the log difference between the model and measurements (yields of terminal PFCA generated by the TOP assay). We used a noninformative Jeffrey's prior because little is known about the presence of precursors in fish tissue and other biotic tissues (SI Section 1.11). Probability density functions were based on the nonparametric kernel density of oxidizable precursor concentrations. Here, we report the expected value (hereon referred to as the expected mean) and 95% confidence intervals (CI) of inferred precursor concentrations.

Bioaccumulation Factors (BAF). Field-measured BAF (μg PFAS kg^{-1} wet-weight fish tissue/μg PFAS L^{-1} water) were calculated for each sampling site. This calculation relies on detectable PFAS concentrations (>MDL) in both water and fish. Some longer-chain PFAS are frequently below detection in water but are known to be bioaccumulative and were detectable in fish muscle in this study. We therefore divided the measured tissue concentrations of PFAS detectable in fish by the MDL for each analyte in water to estimate the lower bound of their BAF (referred to as "potential BAF").

Suspect Screening and Nontargeted Analysis. Suspect screening and nontargeted analysis were performed on a subset of fish muscle tissue extracts at the University of Rhode Island using a SCIEX ExionLC AC UHPLC system coupled to a SCIEX X500R quadrupole time-of-flight tandem mass spectrometer (QTOF MS/MS). Each 20 μL extract was loaded onto a Phenomenex Gemini C18 analytical column (3 μm, 110 Å, 50 mm × 2 mm) preceded by a Phenomenex SecurityGuard cartridge at a flow rate of 0.3 mL min^{-1} and column temperature of 45 °C using ammonium acetate (10 mM) in methanol and ammonium acetate (10 mM) in Milli-Q water. An additional Phenomenex Gemini C18 column (5 μm,

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

110 Å, 50 mm × 4.6 mm) was used as the delay column for PFAS instrumental contribution. MS data were collected using both IDA and SWATH acquisitions in negative ESI mode at a temperature of 450 °C, curtain gas pressure of 30 psi, ion source gas 1 at 40 psi, and ion source gas 2 at 60 psi. Raw data were screened using the SCIEX Fluorochemical HRMS/MS Spectral Library 2.0. For quantitative comparison between targeted LC-MS/MS and suspect screening QTOF MS/MS results, a targeted HRMS/MS method was used, with the IS operated under the same conditions as for suspect screening. The SI Section 1.12 contains additional details on analyte parameters (Table S12) and suspect screening identification (Table S13).

Statistical Analyses. Statistical analyses were performed in R version 4.0.2 using NADA[32] and python version 3.7.4 using SciPy[33] and statsmodels.[34] We used hierarchical clustering (Figure S2) to group locations with similar PFAS profiles and then tested for statistically significant differences in fish PFAS concentrations among clusters using analysis of variance (ANOVA) and Tukey's HSD *post hoc* test (Table S14). Bluegill was the only fish species measured at every location. Samples with targeted PFAS measurements with ≤70% detection frequency were excluded from statistical summaries. For samples above this detection frequency that contained compounds < MDL, nondetects were imputed using robust regression on order statistics.[35] 7:3 FTCA was detected by LC-MS/MS in >90% of fish samples measured but was excluded from further evaluation due to the presence of a biological interference identified by HRMS (SI Section 1.13). Some biological molecules can interfere with quantification of certain PFAS if they have the same nominal mass in unit resolution.[36] Complementary measurements using HRMS are useful since interfering molecules in biological samples can be distinguished from PFAS using exact mass measurements.

RESULTS AND DISCUSSION

Concentrations of Targeted PFAS in Fish. Based on the reference dose value (RfD) derived by the state of New Hampshire for PFOS in 2019,[37] all but two fish samples analyzed in this study exceeded the daily consumption (8 oz meal) limit for adults (≤1.1 ng g^{-1}), and 21% of samples exceeded the weekly consumption limit (≤7.4 ng g^{-1}, Table S15). No samples exceeded the adult or child-based consumption limits for other PFAS (PFOA, PFNA, PFHxS) with available RfD values (Table S15). Although, New Hampshire already has a consumption limit of ≤4 meals/month of wild-caught fish based on mercury (SI Section 2.1). Linear PFOS was the predominant PFAS detected in all fish samples (0.21−52 ng g^{-1}, mean 5.1 ng g^{-1}, Table S16). Only 21% of samples had detectable branched PFOS isomers, and these were present at much lower concentrations (0.21−3.0 ng g^{-1}) than the linear isomer. This likely reflects preferential accumulation and retention of the linear isomer and/or reduced uptake and faster elimination of the branched isomers.[38,39]

The sum of targeted PFAS (∑PFAS) across all fish samples analyzed ranged from 0.95−60 ng g^{-1} (species averages: 1.1−11 ng g^{-1}). The C7−C13 PFCA (PFOA to PFTeDA), linear PFOS, PFDS, and three ECF precursors (FOSA, L-N-MeFOSAA, L-N-EtFOSAA) were detected in ≥80% of samples (Figure S3). After PFOS, the C10 PFCA (PFUnDA) was the PFAA with the highest average concentration (0.55 ± 0.43 ng g^{-1}), followed by the other long-chain PFCA (C9, C11−C12, Table S16). Limited sample sizes meant we were not able to assess statistically significant differences in PFAS concentrations among all locations. Instead, we grouped waterbodies with similar PFAS profiles using hierarchical clustering and tested differences among fish species within each cluster. Only a few statistically significant ($p < 0.05$) differences were observed among these clusters for individual PFAS (Table S14). LOC 4 (potential waste disposal site source) had higher ∑PFOS in bluegill, the only fish species measured at every location, compared to other locations (Table S16), but the difference was not statistically significant.

A short-chain perfluorobutane sulfonamide ECF precursor (FBSA) was detected using targeted analysis in every fish sample analyzed for this analyte. The average measured concentration of FBSA was greater than any other targeted precursor (1.1 ± 1.8 ng g^{-1}). FBSA is a degradation product and major metabolite of other precursors in some AFFF formulations and surface treatment products.[40,41] Detection of FBSA in environmental samples has only recently been reported.[42,43] Concentrations similar to those measured in this study were detected in freshwater fish from different waterbodies across Canada and the Great Lakes region,[44] suggesting widespread presence of FBSA in the environment.

Differences between Surface Water and Fish PFAS Composition. PFAS frequently detected in water (i.e., ∑PFOS, PFOA, and a few other short-chain PFAA) are the focus of current regulatory efforts across the US.[45] Figure 1 contrasts the PFAS composition between paired water and fish tissue samples. In New Hampshire surface waters, the shorter-chain PFCA (C3, C5−C7) are most abundant in water, whereas fish muscle tissue predominantly contains ∑PFOS (sum of linear and branched isomers) and longer-chain PFCA (C7, C9−C13) (Tables S16 and S17). The ECF precursors (FOSA, L-N-MeFOSAA, and L-N-EtFOSAA) were only above detection limits in one water sample but were detected in ≥84% of fish samples. This means relative PFAS abundance in water is not a good proxy for those detected in fish. Long-chain PFCA, in particular, are close to or below limits of detection in water but bioaccumulate in fish to levels that may be considered a human exposure risk.[46,47] Presently, fish consumption advisories are focused mainly on PFOS as the predominant analyte detected in fish and overlook many of the other frequently detected compounds.[6,7,48]

Field-Measured Bioaccumulation Factors (BAF) for Fish. Many studies report liver tissue or whole-body fish PFAS concentrations to estimate risks to wildlife,[50] but concentrations in fish muscle tissue are most relevant for human consumption. Figure 2 shows field-measured bioaccumulation factors (BAF) for muscle tissue for the eight freshwater fish species from this study (Table S18). BAF calculated for the C7−C10 PFCA and C8 PFSA (∑PFOS) were based on detectable concentrations in both water and muscle tissue for ≥70% of samples and are indicated by solid markers (Figure 2). A statistically significant ($p < 0.05$) linear relationship (R^2 = 0.81) between measured BAF and PFCA chain length is evident for the C7−C10 PFCA, with an average increase in BAF by 0.78 ± 0.14 log units per perfluorinated carbon (Figures 2 and S4). A similar relationship could not be constructed for the PFSA because most were below detection other than ∑PFOS. The log BAF for the C7−C10 PFCA ranged from 0.9−4.3 and 2.5−3.9 (mean of 3.2) for the C8 PFSA (∑PFOS). Log BAF between 3 and 4 indicate

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

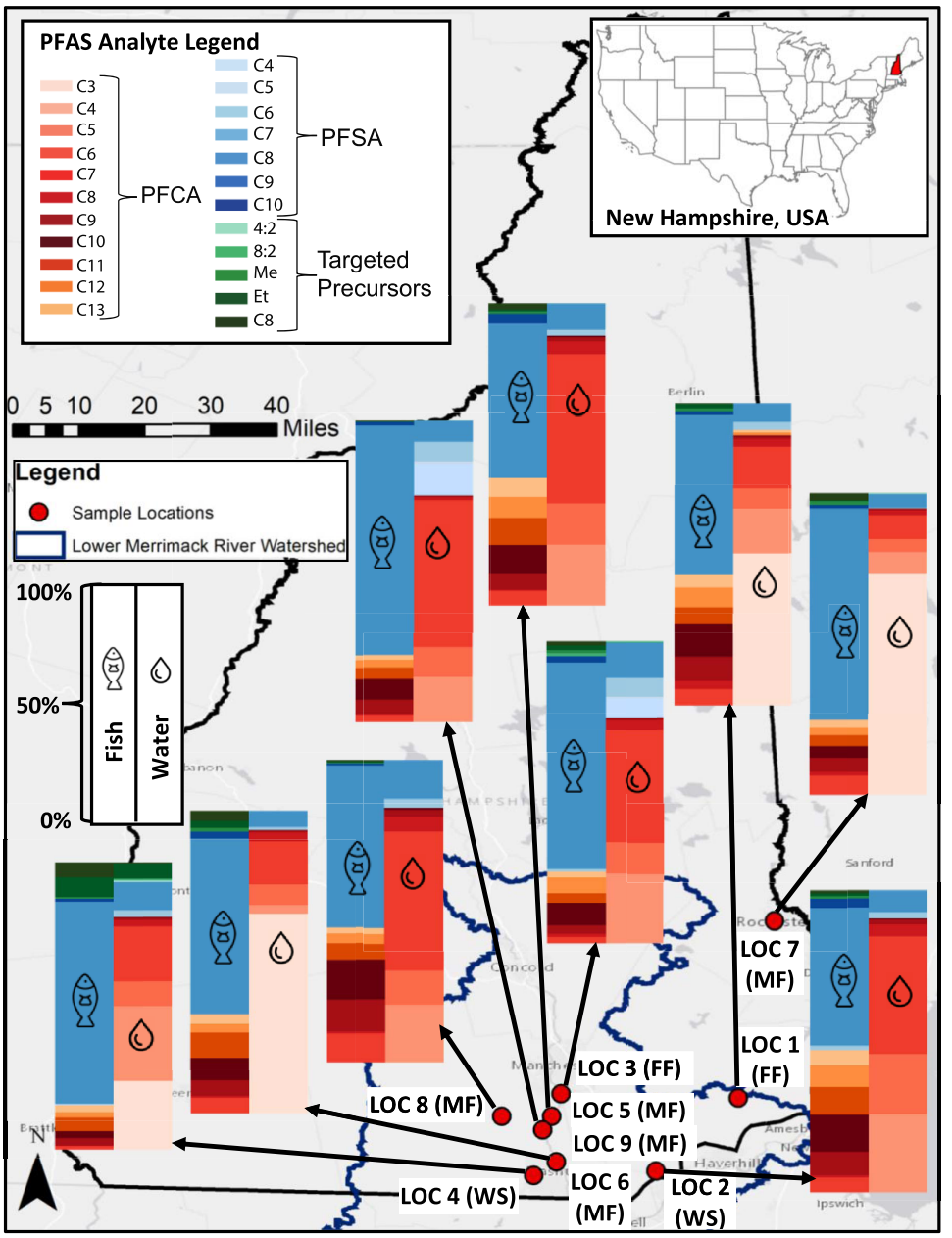


Figure 1. Composition of targeted PFAS measured in water and fish collected from the lower Merrimack River Watershed in New Hampshire, US, in 2017. PFAS are labeled by perfluorinated carbon (C_n) chain length: perfluoroalkyl carboxylates (PFCA) range from C3−C13, perfluoroalkyl sulfonates (PFSA) from C4−C10, and targeted precursors include 4:2 and 8:2 fluorotelomer sulfonates, perfluorooctane sulfonamide (C8), and *N*-methyl (Me) and *N*-ethyl (Et) perfluorooctane sulfonamidoacetic acids. Sample locations are denoted by LOC + site number with potential source types in brackets: FF = aqueous film-forming foam, WS = waste disposal site, and MF = plastics or textile manufacturing. The map was created using ArcGIS software by Esri.[49] Sources: Esri, HERE, Garmin, OpenStreetMap contributors, and the GIS user community.

substances with a tendency to bioaccumulate, while those with log BAF ≥ 4 are considered very bioaccumulative.[51]

Potential log BAF represent the lower limit of bioaccumulation potential for analytes that were below detection in water (by substituting the MDL for the concentration in water, Figure 2). Potential log BAF ranged from 3.1−5.2 for C11−C13 PFCA, 2.7−4.3 for C10 PFSA (PFDS), and 2.6−5.1 for the ECF precursors (FOSA, L-N-MeFOSAA, and L-N-EtFOSAA), indicating they are all very bioaccumulative. High BAF have previously been reported for ECF and FT precursors, including FOSA, N-EtFOSA, 6:2 fluorotelomer phosphate diester (6:2 diPAP), and select PFECA.[4,52,53] We did not measure FBSA in water samples and therefore could not estimate a potential BAF in this study. Another study recently reported FBSA has a log BAF of 2.0−3.2,[54] indicating a tendency to bioaccumulate.

A limitation of potential BAF calculations is that they reflect both the inherent properties of the chemicals to accumulate in fish and analytical detection limits. While a higher potential BAF for the C11 PFCA is consistent with the log linear increase observed for C7−C10 PFCA (Figure 2), it could also reflect the lower analytical MDL for water compared to the C12 and C13 PFCA (Table S7). Irrespective of detection limits, lower bioaccumulative potential for PFCA > C11 has been observed in other studies,[53,55−57] supporting the trends indicated by potential BAF in this study (Figure 2). This may

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

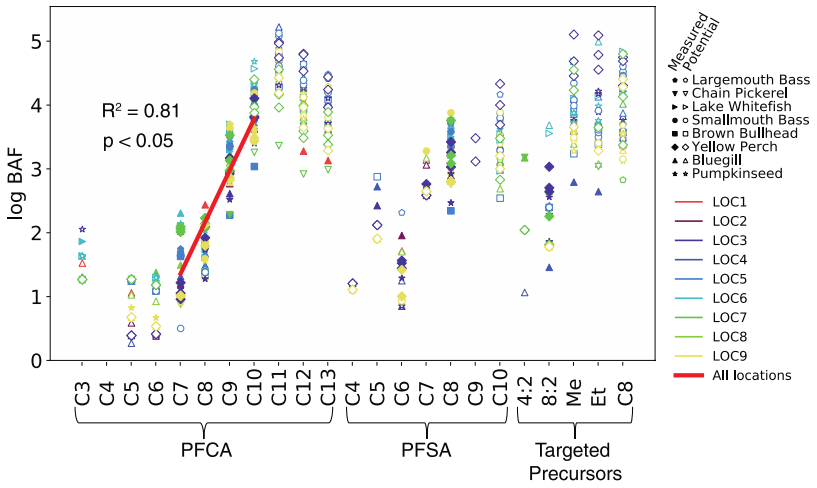


Figure 2. Empirically derived bioaccumulation factors (BAF, L kg^{-1}) for different PFAS, fish species, and sampling locations in New Hampshire, US. Each marker indicates an individual measurement, and each marker type denotes the fish species. Solid markers show measured BAF based on detectable water and fish concentrations, while open markers show potential BAF calculated from method detection limits for water and measured fish concentrations. Abbreviations for precursors are: perfluorooctane sulfonamide (C8), *N*-methyl (Me), and *N*-ethyl (Et) perfluorooctane sulfonamidoacetic acids, and fluorotelomer sulfonates indicated by carbon number (*n*:2). The red line and R^2 value are based on linear regression of the measured BAF data for the C7−C10 PFCA. Location-specific regressions are shown in Figure S4.

reflect reduced bioavailability of larger molecules due to a steric hindrance to uptake past a certain chain length.

Overall, these results suggest many precursors have enhanced propensity for bioaccumulation compared to their terminal degradation products. Potential BAF for precursors may be underestimated if any were biotransformed *in vivo* into intermediate and terminal PFAA. Conversely, BAF calculations may overestimate the accumulation potential of terminal PFAA if precursor biotransformation has contributed to observed tissue burdens.[58]

Oxidizable Precursors in Fish Muscle Tissue. Following the TOP assay, analytically detectable increases in concentrations of the C3−C8 PFCA of greater than 1 nmol L^{-1} (nM) were measured in 35% of tissue samples analyzed (Figure S5). TOP + BI results indicated ECF rather than FT precursors were the predominant class present in these samples (Figures 3 and S6). Similar distributions of inferred precursors were observed in multiple fish species from the same locations. This likely means that any cross-species differences in uptake were less important than aqueous exposures for observed tissue concentrations of precursors.

Results from the TOP + BI analysis for LOC 3 samples (Figure 3A,B) showed C4 ECF precursors had higher concentrations (*x*-axis) and probabilities of occurrence (indicated by higher, narrower peaks, *y*-axis) than the other C3−C8 precursors. The highest expected mean concentrations from the TOP + BI analysis for LOC 3 samples ranged from 10−13 nM for the C4 ECF precursors, followed by 1.9−3.7 nM for the C6 ECF precursors (Figure 3 and Table S19). Relatively small uncertainties (narrow probability distributions) in the concentration ranges of C4 ECF precursors were enabled by high measured concentrations of FBSA (a C4 ECF precursor) in the targeted analysis (9.5−11 nM, Table S20), which was used to constrain the inference. The expected mean concentrations of C4 ECF precursors from the TOP + BI analysis were within 18% of the targeted FBSA concentrations, suggesting FBSA was likely the only C4 ECF precursor present in fish muscle tissue from LOC 3. In the LOC 4 sample, the C8 ECF precursors had the highest probabilities of occurrence and were well-constrained by a narrow probability density function (Figure 3C).

Greater uncertainty in the TOP + BI results was apparent for other locations with lower concentrations of targeted and inferred (≤2 nM) precursors (e.g., LOC 5 and LOC 9) (Figure S6 and Tables S19 and S20). Fish tissue from these locations showed suggestive evidence of C4 ECF precursors (expected means of 2.2−4.0 nM, Table S19) but had lower targeted FBSA concentrations (≤1.7 nM, Table S20), which provides a measurement constraint for the inference. For these locations, the probability density functions for inferred C4 ECF precursors were shallow and broader (Figure S6), indicating greater uncertainty (Figure S7). Inferred C4 ECF precursor expected mean concentrations were 2−12 times higher than targeted concentrations of FBSA (Table S20). Given the uncertainty in the posteriors for the LOC 5 and LOC 9 samples, we do not consider this robust evidence for additional C4 ECF precursors. Detection of other C4 ECF precursors from HRMS would be needed to confirm such a finding.

Uncertainty in the TOP + BI results for the C4 ECF precursors highlights some of the limitations of standard analytical techniques. The TOP assay oxidizes FBSA to the C3 PFCA (PFBA), which can be challenging to measure at low concentrations in biological tissues. Short-chain PFAS coelute with many biological molecules in LC-MS/MS analysis due to their small size.[36] This coelution with matrix interferences affects ionization efficiency and increases the background, leading to reduced and variable recovery and higher detection limits. Measurement uncertainties are considered in the TOP + BI method and propagate to uncertainty in the posterior probability distribution of inferred concentrations (Figures 3 and S6−S8). These results highlight some of the challenges associated with measuring low concentrations of PFAS and precursors in biological tissues.

In summary, we find the TOP + BI method is most informative when total precursor concentrations in samples exceed 9 nM (concentration ranges for LOC 3 and LOC 4 samples). For samples with lower total precursor concen-

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

trations (e.g., <2 nM for LOC 5 and LOC 9), large uncertainties in the inferred concentrations (broad posterior probability density functions) make results less informative (Table S20). In general, targeted precursor measurements are useful for constraining uncertainty in the statistical inference, emphasizing the need for additional commercially available standards.

Evaluation of Consistency in Precursor Detection across Analytical Methods. We compared the expected mean concentrations of all C4−C8 precursors from the TOP + BI analysis to the summed concentrations of targeted precursors of each chain length. Targeted analysis accounted for 75−92% of the expected mean concentration of precursors from the TOP + BI analysis in LOC 3 samples, 46% in LOC 4, and 8−22% in LOC 5 and LOC 9 (Table S20). The differences between targeted and mean inferred precursor concentrations were greatest for LOC 5 and LOC 9 samples that had relatively low concentrations of precursors compared to other sites but were in better agreement (3−69% difference) with the lower 95% CI of inferred concentrations (Table S20).

Results from suspect screening analysis for the same fish tissue samples subjected to the TOP + BI analysis confirmed the detection of a C3 ECF precursor, perfluoropropane sulfonamide (FPrSA), and a C5 ECF precursor, perfluoropentane sulfonamide (FPeSA) at a high confidence level (2a[59]) and high detection frequency (Table S13). High abundance of these precursors was determined based on peak area. In the LOC 3 samples, peak areas for FPeSA were almost double those of FBSA (Table S21). Concentrations of FPeSA were semi-quantitatively estimated to be 2× greater than FBSA in LOC 3 YP1 (20 nM FPeSA vs 8.7 nM FBSA) and 3× greater in LOC 3 PS2 (32 nM FPeSA vs 11 nM FBSA) (Figure 4 and Table S22). Standards for the even-chain PFSA were used for semi-quantification because no matched analytical standards are available for these compounds. Uncertainty in such measurements cannot be quantified but could be large. Concurrence or discrepancies with the TOP + BI results are therefore useful for establishing additional lines of evidence or uncertainty for the abundance of a particular nontargeted and semi-quantified compound.

No additional PFAS analytes were identified using the nontargeted analysis. Fewer precursors in biological tissues were identified by suspect screening in this work compared to more contaminated locations such as those directly impacted by AFFF.[60] This study is more representative of background levels of PFAS, likely from multiple environmental sources.

Results from targeted analysis, suspect screening analysis, and TOP + BI measurements showed reasonable agreement for the C4, C6, and C8 ECF precursors (Figure 4 and Table S20). In contrast, semi-quantified concentrations of FPeSA from the suspect screening analysis greatly exceeded even the upper 95% CI of inferred concentrations of C5 ECF precursors from the TOP + BI analysis (Figure 4 and Tables S19 and S21). For example, the semi-quantified concentrations of FPeSA (C5 ECF) in samples from LOC 3 exceeded 20 nM compared to the upper 95% CI TOP + BI concentration of 3.4 nM, which accounts for analytical uncertainty and variable recoveries (Figure 4). The maximum analytically measured increase in the C4 PFCA (PFPeA) following TOP (the oxidation product of FPeSA) was 3.1 nM. The TOP + BI comparison thus suggests that semi-quantified concentrations are overestimated.

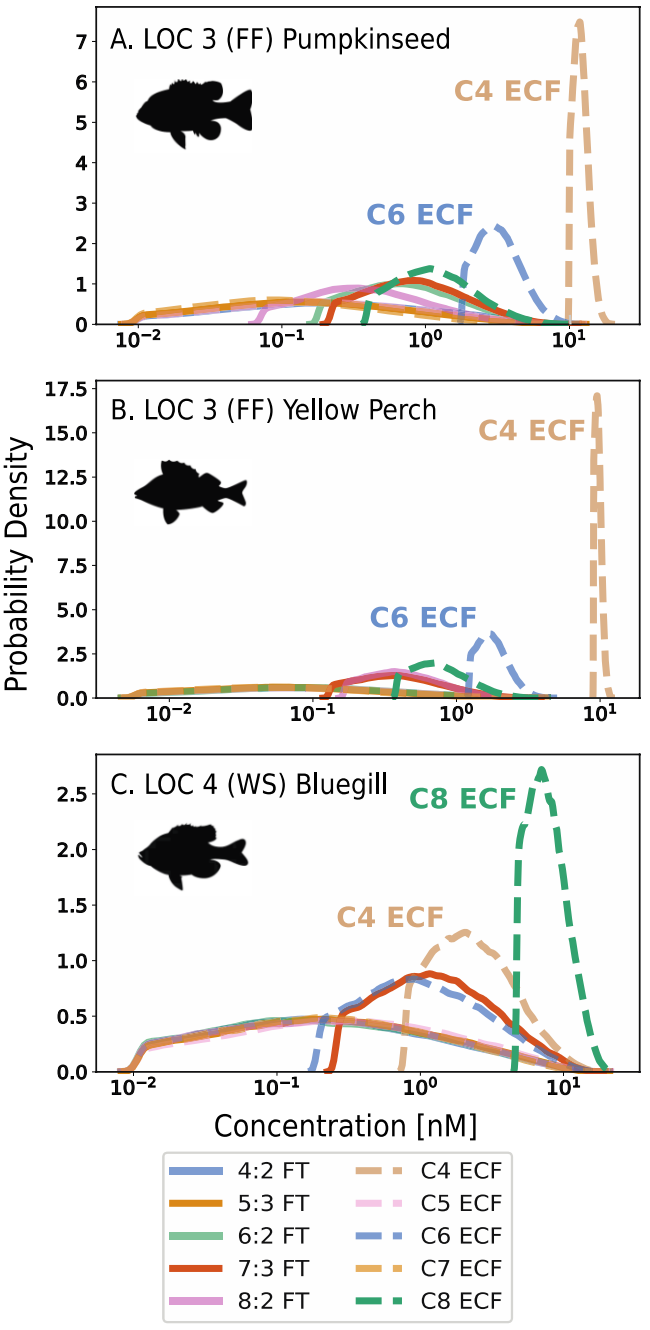


Figure 3. Inferred concentrations (nM) of oxidizable precursors and their perfluorinated carbon chain length based on total oxidizable precursor (TOP) assay results interpreted using Bayesian inference (TOP + BI). Panels (A) and (B) show probability density functions for the concentrations of oxidizable precursors for two species (pumpkinseed (PS2) and yellow perch (YP1)) from location 3 (LOC 3) where AFFF (FF) is a potential source. Panel (C) shows bluegill (BGcomp) from location 4 (LOC 4) where waste disposal (WS) is a potential source. Higher peaks with narrower ranges indicate greater probability (less uncertainty) in inferred concentrations. Results for samples from LOC 5 and 9 are provided in the SI (Figure S6).

A high bias in semi-quantified concentrations for some precursors in this study is consistent with past work that has shown semi-quantification using surrogate reference standards produces results that can be biased due to ionization or fragmentation differences.[61] Past work suggests semi-quantified concentrations may be overestimated by up to four times the

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

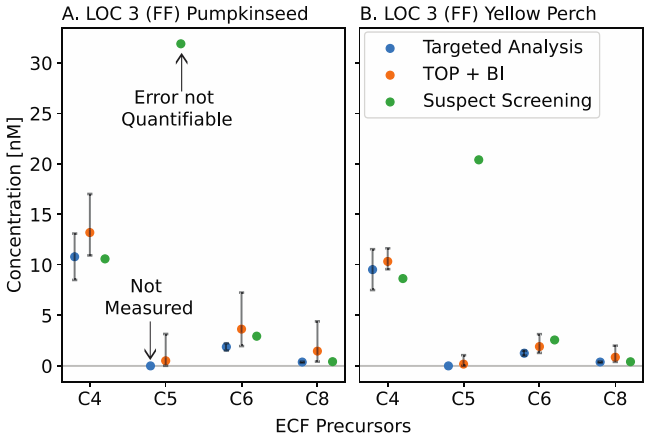


Figure 4. Comparison of measured, inferred, and semi-quantified concentrations of precursors. The C4, C5, C6, and C8 ECF precursors for LOC 3 samples are shown where the potential source is AFFF (FF). Panel (A) shows LOC 3 pumpkinseed (PS2), and panel (B) shows LOC 3 Yellow Perch (YP1). The blue circles show targeted analysis results for ECF precursors (FBSA (C4), FHxSA (C6), and FOSA/L-N-MeFOSAA (C8)) with concentrations quantified using analytical standards. C5 was not measured by targeted analysis since no C5 ECF precursor standards were available. Error bars for targeted analysis are based on the average relative percent difference between sample and spike duplicates (n = 2−6). The orange circles show expected mean molar concentrations of ECF precursor classes from the TOP+BI analysis and 95% confidence intervals of the inference. The green circles show results from suspect screening. The C5 ECF precursor (FPeSA) is semi-quantified (no C5 analytical standard). The suspect screening results do not have error bars as the error cannot be quantified for these measurements.

TOP assay results due to lower accuracy and limited analytical standards.[15,62] Alternatively, the same studies have suggested TOP assay results can underestimate true concentrations due to incomplete oxidation and low recoveries.[62] However, we have accounted for these factors in the Bayesian inference interpretation of the TOP assay (TOP + BI). HRMS/MS is not subject to the same interferences that can be problematic for LC-MS/MS due to exact mass measurements, so this is not expected to be a factor in the high bias in concentration. Instead, we attribute the variability to the lack of commercial analytical standards to quantify concentrations associated with instrumental results from the HRMS/MS analysis. These results emphasize the benefits of using a toolbox of methods to better understand the robustness of any given measurement, especially for compounds lacking commercially available standards.

In summary, both the TOP + BI results and suspect screening analysis indicate the presence of short-chain ECF precursors (perfluoroalkyl sulfonamides) in fish muscle samples that were not detected by targeted analysis. Analytical standards for additional short-chain sulfonamide compounds (e.g., C3: FPrSA and C5: FPeSA) are needed to quantify concentrations of these bioaccumulative precursors more accurately.

IMPLICATIONS

Results of this study reinforce the high bioaccumulation propensity of several long-chain PFAA[63−65] that are frequently detected in human serum[46] and breastmilk.[47] Exposure to long-chain PFAA has been associated with adverse toxicological outcomes and is correlated with reported fish consumption, highlighting the importance of seafood as an exposure source.[66,67] Results of this study also emphasize the bioaccumulative potential for ECF precursors, specifically short-chain perfluoroalkyl sulfonamides (C3−C5) such as FBSA (C4). The C1−C8 sulfonamide congeners have pKa values of 5.86−9.72 compared to <4 for PFAA,[68] indicating the presence of more neutral species in solution that will have a greater propensity to partition into cells due to hydrophobic interactions.[69] These precursors were detected in multiple species of recreational fish across New Hampshire, US. The widespread detection of perfluoroalkyl sulfonamide precursors in biota indicates that additional exposure and risk evaluations are needed for some understudied PFAS.

Federal and state regulatory efforts are presently focused on legacy PFAS predominantly detected in water and do not consider the full range of highly bioaccumulative terminal PFAA and precursors discussed in this work. Metabolism of precursors that exhibit a higher bioaccumulation potential than their terminal degradation products will enhance exposures to terminal PFAA of concern.[11,70] Some studies have suggested sulfonamide precursors have greater bioactivity than PFAA of similar perfluorinated carbon chain length due to their higher pKa, greater fraction of neutral species at similar pH, and interactions with lipids and membranes facilitated by the sulfonamide head group.[69,71−73] Additional physicochemical and toxicological data on diverse precursors, particularly the sulfonamides, are needed to better understand their bioaccumulation potential and toxicity. Our work suggests that more comprehensive fish advisories are needed to account for potential human exposures to the full suite of highly bioaccumulative longer-chain PFAA and ECF precursors.

We found reasonable agreement among analytical methods for measuring PFAS in biota (targeted analysis, semi-quantification, and TOP + BI) in samples that had relatively higher PFAS concentrations (total precursor concentrations > 9 nM). More uncertainty among analytical methods was apparent for samples with total precursor concentrations < 2 nM, in part reflecting challenges associated with detection and recoveries in a more complex tissue matrix at low PFAS concentrations. Matrix interferences that affect accurate quantification are a challenge for new measurement techniques that aim to characterize unknown PFAS present in environmental samples at low levels. The toolbox of analytical methods used in this study allowed us to identify additional precursors and quantitatively estimate the lower and upper bounds of their concentrations in these fish samples. However, without individual PFAS analytical standards, accurate quantification of the short-chain perfluoroalkyl sulfonamide precursors in biota will remain a challenge. Thus, additional commercially available standards for potentially bioaccumulative PFAS precursors are essential for more comprehensively characterizing PFAS exposures for all fish consumers.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c03734.

Sampling information; supporting descriptions of extraction methods, analyses, and quality assurance/quality control; tables of concentration and BAF data; details of the Bayesian inference; and precursor evaluations across methods (PDF)

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

Fish concentrations (Table S16) (XLSX)
Water concentrations (Table S17) (XLSX)

AUTHOR INFORMATION

Corresponding Author

Heidi M. Pickard − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States;* orcid.org/0000-0001-8312-7522; Email: hpickard@fas.harvard.edu

Authors

Bridger J. Ruyle − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States;* orcid.org/0000-0003-1941-4732

Colin P. Thackray − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States;* orcid.org/0000-0003-0439-3270

Adela Chovancova − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States*

Clifton Dassuncao − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States; Eastern Research Group, Inc., Arlington, Virginia 22201, United States;* orcid.org/0000-0001-7140-1344

Jitka Becanova − *Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882, United States;* orcid.org/0000-0002-3091-1054

Simon Vojta − *Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882, United States*

Rainer Lohmann − *Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882, United States;* orcid.org/0000-0001-8796-3229

Elsie M. Sunderland − *Harvard John A. Paulson School of Engineering and Applied Sciences, Cambridge, Massachusetts 02138, United States; Department of Environmental Health, Harvard T. H. Chan School of Public Health, Harvard University, Boston, Massachusetts 02115, United States;* orcid.org/0000-0003-0386-9548

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.2c03734

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the National Institute of Environmental Health Sciences Superfund Research Program (P42ES027706). H.M.P. was supported by a fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC). The authors thank NH Fish and Game guides (Hope Eagleson, Bill Horgan, Mike Ivone, Gardener Murphy, and Larry Murphy) for their assistance with field sampling, and staff at the NH DES for input on the data.

REFERENCES

(1) Organisation for Economic Co-operation and Development (OECD). *Toward a New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs): Summary Report on Updating the OECD 2007 List of per- and Polyfluoroalkyl Substances (PFASs); ENV/JM/MONO(2018)7*; 2018.

(2) Wang, Z.; DeWitt, J. C.; Higgins, C. P.; Cousins, I. T. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environ. Sci. Technol.* **2017**, *51*, 2508−2518.

(3) Agency for Toxic Substances and Disease Registry. *Toxicological Profile for Perfluoroalkyls*; Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2021, DOI: 10.15620/cdc:59198.

(4) Miranda, D. A.; Benskin, J. P.; Awad, R.; Lepoint, G.; Leonel, J.; Hatje, V. Bioaccumulation of Per- and Polyfluoroalkyl Substances (PFASs) in a Tropical Estuarine Food Web. *Sci. Total Environ.* **2021**, *754*, No. 142146.

(5) Augustsson, A.; Lennqvist, T.; Osbeck, C. M. G.; Tibblin, P.; Glynn, A.; Nguyen, M. A.; Westberg, E.; Vestergren, R. Consumption of Freshwater Fish: A Variable but Significant Risk Factor for PFOS Exposure. *Environ. Res.* **2021**, *192*, No. 110284.

(6) Massachusetts Department of Public Health. Freshwater Fish Consumption Advisory List (2021). https://www.mass.gov/doc/public-health-freshwater-fish-consumption-advisories-2021/download (accessed August 8, 2022).

(7) Minnesota Department of Health. Great Lakes Consortium for Fish Consumption Advisories: Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines (2019). https://www.health.state.mn.us/communities/environment/fish/docs/consortium/bestpracticepfos.pdf (accessed September 13, 2022).

(8) Tokranov, A. K.; Nishizawa, N.; Amadei, C. A.; Zenobio, J. E.; Pickard, H. M.; Allen, J. G.; Vecitis, C. D.; Sunderland, E. M. How Do We Measure Poly- and Perfluoroalkyl Substances (PFASs) at the Surface of Consumer Products? *Environ. Sci. Technol. Lett.* **2019**, *6*, 38−43.

(9) Schaider, L. A.; Balan, S. A.; Blum, A.; Andrews, D. Q.; Strynar, M. J.; Dickinson, M. E.; Lunderberg, D. M.; Lang, J. R.; Peaslee, G. F. Fluorinated Compounds in U.S. Fast Food Packaging. *Environ. Sci. Technol. Lett.* **2017**, *4*, 105−111.

(10) Ruyle, B. J.; Pickard, H. M.; LeBlanc, D. R.; Tokranov, A. K.; Thackray, C. P.; Hu, X. C.; Vecitis, C. D.; Sunderland, E. M. Isolating the AFFF Signature in Coastal Watersheds Using Oxidizable PFAS Precursors and Unexplained Organofluorine. *Environ. Sci. Technol.* **2021**, *55*, 3686−3695.

(11) Zhang, X.; Lohmann, R.; Sunderland, E. M. Poly- and Perfluoroalkyl Substances in Seawater and Plankton from the Northwestern Atlantic Margin. *Environ. Sci. Technol.* **2019**, *53*, 12348−12356.

(12) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integr. Environ. Assess. Manage.* **2011**, *7*, 513−541.

(13) Yeung, L. W. Y.; Mabury, S. A. Are Humans Exposed to Increasing Amounts of Unidentified Organofluorine? *Environ. Chem.* **2016**, *13*, 102−110.

(14) Koch, A.; Kärrman, A.; Yeung, L. W. Y.; Jonsson, M.; Ahrens, L.; Wang, T. Point Source Characterization of Per- and Polyfluoroalkyl Substances (PFASs) and Extractable Organofluorine (EOF) in Freshwater and Aquatic Invertebrates. *Environ. Sci.: Processes Impacts* **2019**, *21*, 1887−1898.

(15) Nickerson, A.; Rodowa, A. E.; Adamson, D. T.; Field, J. A.; Kulkarni, P. R.; Kornuc, J. J.; Higgins, C. P. Spatial Trends of Anionic, Zwitterionic, and Cationic PFASs at an AFFF-Impacted Site. *Environ. Sci. Technol.* **2021**, *55*, 313−323.

(16) Charbonnet, J. A.; Rodowa, A. E.; Joseph, N. T.; Guelfo, J. L.; Field, J. A.; Jones, G. D.; Higgins, C. P.; Helbling, D. E.; Houtz, E. F. Environmental Source Tracking of Per- and Polyfluoroalkyl Substances within a Forensic Context: Current and Future Techniques. *Environ. Sci. Technol.* **2021**, *55*, 7237−7245.

(17) Ruyle, B. J.; Thackray, C. P.; McCord, J. P.; Strynar, M. J.; Mauge-Lewis, K. A.; Fenton, S. E.; Sunderland, E. M. Reconstructing the Composition of Per- and Polyfluoroalkyl Substances in Contemporary Aqueous Film-Forming Foams. *Environ. Sci. Technol. Lett.* **2021**, *8*, 59−65.

(18) Andrews, D. Q.; Hayes, J.; Stoiber, T.; Brewer, B.; Campbell, C.; Naidenko, O. V. Identification of Point Source Dischargers of Per- and Polyfluoroalkyl Substances in the United States. *AWWA Water Sci.* **2021**, *3*, No. e1252.
(19) Leeson, A.; Thompson, T.; Stroo, H. F.; Anderson, R. H.; Speicher, J.; Mills, M. A.; Willey, J.; Coyle, C.; Ghosh, R.; Lebrón, C.; Patton, C. Identifying and Managing Aqueous Film-Forming Foam-Derived Per- and Polyfluoroalkyl Substances in the Environment. *Environ. Toxicol. Chem.* **2021**, *40*, 24−36.
(20) Zhang, C.; Hopkins, Z. R.; McCord, J.; Strynar, M. J.; Knappe, D. R. U. Fate of Per- and Polyfluoroalkyl Ether Acids in the Total Oxidizable Precursor Assay and Implications for the Analysis of Impacted Water. *Environ. Sci. Technol. Lett.* **2019**, *6*, 662−668.
(21) Weber, A. K.; Barber, L. B.; LeBlanc, D. R.; Sunderland, E. M.; Vecitis, C. D. Geochemical and Hydrologic Factors Controlling Subsurface Transport of Poly- and Perfluoroalkyl Substances, Cape Cod, Massachusetts. *Environ. Sci. Technol.* **2017**, *51*, 4269−4279.
(22) Dassuncao, C.; Pickard, H.; Pfohl, M.; Tokranov, A. K.; Li, M.; Mikkelsen, B.; Slitt, A.; Sunderland, E. M. Phospholipid Levels Predict the Tissue Distribution of Poly- and Perfluoroalkyl Substances in a Marine Mammal. *Environ. Sci. Technol. Lett.* **2019**, *6*, 119−125.
(23) Reiner, J. L.; O'Connell, S. G.; Butt, C. M.; Mabury, S. A.; Small, J. M.; De Silva, A. O.; Muir, D. C. G.; Delinsky, A. D.; Strynar, M. J.; Lindstrom, A. B.; Reagen, W. K.; Malinsky, M.; Schäfer, S.; Kwadijk, C. J. A. F.; Schantz, M. M.; Keller, J. M. Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials. *Anal. Bioanal. Chem.* **2012**, *404*, 2683−2692.
(24) Simonnet-Laprade, C.; Budzinski, H.; Maciejewski, K.; Le Menach, K.; Santos, R.; Alliot, F.; Goutte, A.; Labadie, P. Biomagnification of Perfluoroalkyl Acids (PFAAs) in the Food Web of an Urban River: Assessment of the Trophic Transfer of Targeted and Unknown Precursors and Implications. *Environ. Sci.: Processes Impacts* **2019**, *21*, 1864−1874.
(25) Houtz, E. F.; Sedlak, D. L. Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff. *Environ. Sci. Technol.* **2012**, *46*, 9342−9349.
(26) Janda, J.; Nödler, K.; Scheurer, M.; Happel, O.; Nürenberg, G.; Zwiener, C.; Lange, F. T. Closing the Gap − Inclusion of Ultrashort-Chain Perfluoroalkyl Carboxylic Acids in the Total Oxidizable Precursor (TOP) Assay Protocol. *Environ. Sci.: Processes Impacts* **2019**, *21*, 1926−1935.
(27) Göckener, B.; Eichhorn, M.; Lämmer, R.; Kotthoff, M.; Kowalczyk, J.; Numata, J.; Schafft, H.; Lahrssen-Wiederholt, M.; Bücking, M. Transfer of Per- and Polyfluoroalkyl Substances (PFAS) from Feed into the Eggs of Laying Hens. Part 1: Analytical Results Including a Modified Total Oxidizable Precursor Assay. *J. Agric. Food Chem.* **2020**, *68*, 12527−12538.
(28) Martin, D.; Munoz, G.; Mejia-Avendaño, S.; Duy, S. V.; Yao, Y.; Volchek, K.; Brown, C. E.; Liu, J.; Sauvé, S. Zwitterionic, Cationic, and Anionic Perfluoroalkyl and Polyfluoroalkyl Substances Integrated into Total Oxidizable Precursor Assay of Contaminated Groundwater. *Talanta* **2019**, *195*, 533−542.
(29) Wang, B.; Yao, Y.; Wang, Y.; Chen, H.; Sun, H. Per- and Polyfluoroalkyl Substances in Outdoor and Indoor Dust from Mainland China: Contributions of Unknown Precursors and Implications for Human Exposure. *Environ. Sci. Technol.* **2022**, *56*, 6036−6045.
(30) Shoemaker, J. A.; Tettenhorst, D. R. *Method 537.1. Determination of Selected Per- and Polyflourinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS); EPA/600/R-18/352*; Cincinnati, OH, 2018; Vol. *1*.
(31) Foreman-Mackey, D.; Hogg, D. W.; Lang, D.; Goodman, J. Emcee: The MCMC Hammer. *Publ. Astron. Soc. Pac.* **2013**, *125*, 306−312.
(32) Lee, L. NADA: Nondetects and DataAnalysis for Environmental Data. https://cran.r-project.org/web/packages/NADA/index.html (accessed June 03, 2020).
(33) Virtanen, P.; Gommers, R.; Oliphant, T. E.; Haberland, M.; Reddy, T.; Cournapeau, D.; Burovski, E.; Peterson, P.; Weckesser, W.; Bright, J.; van der Walt, S. J.; Brett, M.; Wilson, J.; Millman, K. J.; Mayorov, N.; Nelson, A. R. J.; Jones, E.; Kern, R.; Larson, E.; Carey, C. J.; Polat, İ.; Feng, Y.; Moore, E. W.; VanderPlas, J.; Laxalde, D.; Perktold, J.; Cimrman, R.; Henriksen, I.; Quintero, E. A.; Harris, C. R.; Archibald, A. M.; Ribeiro, A. H.; Pedregosa, F.; van Mulbregt, P.; et al. SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python. *Nat. Methods* **2020**, *17*, 261−272.
(34) Seabold, S.; Perktold, J. *Statsmodels: Econometric and Statistical Modeling with Python*, American University, 2010; pp 92−96 DOI: 10.25080/Majora-92bf1922-011.
(35) Helsel, D. R. *Statistics for Censored Environmental Data Using Minitab and R*; Proceedings of the Python in Science Conference, 2nd ed.; Wiley: Denver, Colorado, 2012.
(36) Bangma, J. T.; Reiner, J.; Fry, R. C.; Manuck, T.; McCord, J.; Strynar, M. J. Identification of an Analytical Method Interference for Perfluorobutanoic Acid in Biological Samples. *Environ. Sci. Technol. Lett.* **2021**, *8*, 1085−1090.
(37) New Hampshire Department of Environmental Services (NHDES). Technical Background Report for Proposed Maximum Contaminant Levels (MCLs) and Ambient Groundwater Quality Standards (AGQSs) for Perfluorooctane Sulfonic Acid (PFOS), Perfluorooctanoic (PFOA), Perfluorononanoic Acid (PFNA), and Perfluorohexane Sulfonic Acid (PFHxS), 2019, https://www.des.nh.gov/sites/g/files/ehbemt341/files/documents/r-wd-19-29.pdf (accessed August 08, 2022).
(38) Houde, M.; Czub, G.; Small, J. M.; Backus, S.; Wang, X.; Alaee, M.; Muir, D. C. G. Fractionation and Bioaccumulation of Perfluorooctane Sulfonate (PFOS) Isomers in a Lake Ontario Food Web. *Environ. Sci. Technol.* **2008**, *42*, 9397−9403.
(39) Zhong, W.; Zhang, L.; Cui, Y.; Chen, M.; Zhu, L. Probing Mechanisms for Bioaccumulation of Perfluoroalkyl Acids in Carp (Cyprinus Carpio): Impacts of Protein Binding Affinities and Elimination Pathways. *Sci. Total Environ.* **2019**, *647*, 992−999.
(40) Barzen-Hanson, K. A.; Roberts, S. C.; Choyke, S.; Oetjen, K.; McAlees, A.; Riddell, N.; McCrindle, R.; Ferguson, P. L.; Higgins, C. P.; Field, J. A. Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environ. Sci. Technol.* **2017**, *51*, 2047−2057.
(41) Chu, S.; Letcher, R. J. In Vitro Metabolic Formation of Perfluoroalkyl Sulfonamides from Copolymer Surfactants of Pre- and Post-2002 Scotchgard Fabric Protector Products. *Environ. Sci. Technol.* **2014**, *48*, 6184−6191.
(42) Kaboré, H. A.; Goeury, K.; Desrosiers, M.; Vo Duy, S.; Liu, J.; Cabana, G.; Munoz, G.; Sauvé, S. Novel and Legacy Per- and Polyfluoroalkyl Substances (PFAS) in Freshwater Sporting Fish from Background and Firefighting Foam Impacted Ecosystems in Eastern Canada. *Sci. Total Environ.* **2022**, *816*, No. 151563.
(43) Kaboré, H. A.; Goeury, K.; Desrosiers, M.; Vo Duy, S.; Liu, J.; Cabana, G.; Munoz, G.; Sauvé, S. Fish Exhibit Distinct Fluorochemical and Δ15N Isotopic Signatures in the St. Lawrence River Impacted by Municipal Wastewater Effluents. *Front. Environ. Sci.* **2022**, *10*, 1−14.
(44) Chu, S.; Letcher, R. J.; McGoldrick, D. J.; Backus, S. M. A New Fluorinated Surfactant Contaminant in Biota: Perfluorobutane Sulfonamide in Several Fish Species. *Environ. Sci. Technol.* **2016**, *50*, 669−675.
(45) Post, G. B. Recent US State and Federal Drinking Water Guidelines for Per- and Polyfluoroalkyl Substances. *Environ. Toxicol. Chem.* **2021**, *40*, 550−563.
(46) Hu, X. C.; Dassuncao, C.; Zhang, X.; Grandjean, P.; Weihe, P.; Webster, G. M.; Nielsen, F.; Sunderland, E. M. Can Profiles of Poly- and Perfluoroalkyl Substances (PFASs) in Human Serum Provide Information on Major Exposure Sources? *Environ. Health* **2018**, *17*, No. 11.
(47) Zheng, G.; Schreder, E.; Dempsey, J. C.; Uding, N.; Chu, V.; Andres, G.; Sathyanarayana, S.; Salamova, A. Per- and Polyfluoroalkyl

Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS. *Environ. Sci. Technol.* **2021**, *55*, 7510−7520.
(48) ECOS (Environmental Council of the States). *State PFOS Fish Tissue Advisory Values, Compiled June 2020*. https://www.ecos.org/wp-content/uploads/2020/09/PFOS-thresholds-in-state-fish-advisories-9_14_20B-ECOS.pdf.
(49) Esri. "State Map" [basemap]. Scale Not Given. "World Light Gray Base". June, 2021, https://services.arcgisonline.com/ArcGIS/rest/services/Canvas/World_Light_Gray_Base/MapServer (accessed September 13, 2022).
(50) Burkhard, L. P. Evaluation of Published Bioconcentration Factor (BCF) and Bioaccumulation Factor (BAF) Data for Per- and Polyfluoroalkyl Substances Across Aquatic Species. *Environ. Toxicol. Chem.* **2021**, *40*, 1530−1543.
(51) United States Environmental Protection Agency (USEPA) *EPA's PBT Chemical Substances Initiative*, 1998; Vol. *63*.
(52) Chen, M.; Zhu, L.; Wang, Q.; Shan, G. Tissue Distribution and Bioaccumulation of Legacy and Emerging Per-and Polyfluoroalkyl Substances (PFASs) in Edible Fishes from Taihu Lake, China. *Environ. Pollut.* **2021**, *268*, No. 115887.
(53) Li, Y.; Yao, J.; Zhang, J.; Pan, Y.; Dai, J.; Ji, C.; Tang, J. First Report on the Bioaccumulation and Trophic Transfer of Perfluoroalkyl Ether Carboxylic Acids in Estuarine Food Web. *Environ. Sci. Technol.* **2022**, *56*, 6046−6055.
(54) Munoz, G.; Mercier, L.; Duy, S. V.; Liu, J.; Sauvé, S.; Houde, M. Bioaccumulation and Trophic Magnification of Emerging and Legacy Per- and Polyfluoroalkyl Substances (PFAS) in a St. Lawrence River Food Web. *Environ. Pollut.* **2022**, *309*, No. 119739.
(55) Ng, C. A.; Hungerbühler, K. Bioaccumulation of Perfluorinated Alkyl Acids: Observations and Models. *Environ. Sci. Technol.* **2014**, *48*, 4637−4648.
(56) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Bioconcentration and Tissue Distribution of Perfluorinated Acids in Rainbow Trout (Oncorhynchus Mykiss). *Environ. Toxicol. Chem.* **2003**, *22*, 196−204.
(57) Inoue, Y.; Hashizume, N.; Yakata, N.; Murakami, H.; Suzuki, Y.; Kikushima, E.; Otsuka, M. Unique Physicochemical Properties of Perfluorinated Compounds and Their Bioconcentration in Common Carp Cyprinus Carpio L. *Arch. Environ. Contam. Toxicol.* **2012**, *62*, 672−680.
(58) Lewis, A. J.; Yun, X.; Spooner, D. E.; Kurz, M. J.; McKenzie, E. R.; Sales, C. M. Exposure Pathways and Bioaccumulation of Per- and Polyfluoroalkyl Substances in Freshwater Aquatic Ecosystems: Key Considerations. *Sci. Total Environ.* **2022**, *822*, No. 153561.
(59) Charbonnet, J. A.; McDonough, C. A.; Xiao, F.; Schwichtenberg, T.; Cao, D.; Kaserzon, S.; Thomas, K. V.; Dewapriya, P.; Place, B. J.; Schymanski, E. L.; Field, J. A.; Helbling, D. E.; Higgins, C. P. Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry. *Environ. Sci. Technol. Lett.* **2022**, *9*, 473−481.
(60) Koch, A.; Yukioka, S.; Tanaka, S.; Yeung, L. W. Y.; Kärrman, A.; Wang, T. Characterization of an AFFF Impacted Freshwater Environment Using Total Fluorine, Extractable Organofluorine and Suspect per- and Polyfluoroalkyl Substance Screening Analysis. *Chemosphere* **2021**, *276*, No. 130179.
(61) Dubocq, F.; Wang, T.; Yeung, L. W. Y.; Sjöberg, V.; Kärrman, A. Characterization of the Chemical Contents of Fluorinated and Fluorine-Free Firefighting Foams Using a Novel Workflow Combining Nontarget Screening and Total Fluorine Analysis. *Environ. Sci. Technol.* **2020**, *54*, 245−254.
(62) Nickerson, A.; Maizel, A. C.; Kulkarni, P. R.; Adamson, D. T.; Kornuc, J. J.; Higgins, C. P. Enhanced Extraction of AFFF-Associated PFASs from Source Zone Soils. *Environ. Sci. Technol.* **2020**, *54*, 4952−4962.
(63) Dassuncao, C.; Hu, X. C.; Nielsen, F.; Weihe, P.; Grandjean, P.; Sunderland, E. M. Shifting Global Exposures to Poly- and Perfluoroalkyl Substances (PFASs) Evident in Longitudinal Birth Cohorts from a Seafood-Consuming Population. *Environ. Sci. Technol.* **2018**, *52*, 3738−3747.
(64) Conder, J. M.; Hoke, R. A.; Wolf, W.; de Russell, M. H.; Buck, R. C. Are PFCAs Bioaccumulative? A Critical Review and Comparison with Regulatory Criteria and Persistent Lipophilic Compounds. *Environ. Sci. Technol.* **2008**, *42*, 995−1003.
(65) Muir, D.; Bossi, R.; Carlsson, P.; Evans, M.; De Silva, A.; Halsall, C.; Rauert, C.; Herzke, D.; Hung, H.; Letcher, R.; Rigét, F.; Roos, A. Levels and Trends of Poly- and Perfluoroalkyl Substances in the Arctic Environment − An Update. *Emerging Contam.* **2019**, *5*, 240−271.
(66) Skogheim, T. S.; Villanger, G. D.; Weyde, K. V. F.; Engel, S. M.; Surén, P.; Øie, M. G.; Skogan, A. H.; Biele, G.; Zeiner, P.; Øvergaard, K. R.; Haug, L. S.; Sabaredzovic, A.; Aase, H. Prenatal Exposure to Perfluoroalkyl Substances and Associations with Symptoms of Attention-Deficit/Hyperactivity Disorder and Cognitive Functions in Preschool Children. *Int. J. Hyg. Environ. Health* **2020**, *223*, 80−92.
(67) Kawabata, K.; Matsuzaki, H.; Nukui, S.; Okazaki, M.; Sakai, A.; Kawashima, Y.; Kudo, N. Perfluorododecanoic Acid Induces Cognitive Deficit in Adult Rats. *Toxicol. Sci.* **2017**, *157*, 421−428.
(68) Rayne, S.; Forest, K. A New Class of Perfluorinated Acid Contaminants: Primary and Secondary Substituted Perfluoroalkyl Sulfonamides Are Acidic at Environmentally and Toxicologically Relevant PH Values. *J. Environ. Sci. Health, Part A* **2009**, *44*, 1388−1399.
(69) Nouhi, S.; Ahrens, L.; Campos Pereira, H.; Hughes, A. V.; Campana, M.; Gutfreund, P.; Palsson, G. K.; Vorobiev, A.; Hellsing, M. S. Interactions of Perfluoroalkyl Substances with a Phospholipid Bilayer Studied by Neutron Reflectometry. *J. Colloid Interface Sci.* **2018**, *511*, 474−481.
(70) Dassuncao, C.; Hu, X. C.; Zhang, X.; Bossi, R.; Dam, M.; Mikkelsen, B.; Sunderland, E. M. Temporal Shifts in Poly- and Perfluoroalkyl Substances (PFASs) in North Atlantic Pilot Whales Indicate Large Contribution of Atmospheric Precursors. *Environ. Sci. Technol.* **2017**, *51*, 4512−4521.
(71) Slotkin, T. A.; MacKillop, E. A.; Meinick, R. L.; Thayer, K. A.; Seidler, F. J. Developmental Neurotoxicity of Perfluorinated Chemicals Modeled in Vitro. *Environ. Health Perspect.* **2008**, *116*, 716−722.
(72) Han, J.; Gu, W.; Barrett, H.; Yang, D.; Tang, S.; Sun, J.; Liu, J.; Krause, H. M.; Houck, K. A.; Peng, H. A Roadmap to the Structure-Related Metabolism Pathways of Per- and Polyfluoroalkyl Substances in the Early Life Stages of Zebrafish (Danio Rerio). *Environ. Health Perspect.* **2021**, *129*, No. 077004.
(73) Rericha, Y.; Cao, D.; Truong, L.; Simonich, M. T.; Field, J. A.; Tanguay, R. L. Sulfonamide Functional Head on Short-Chain Perfluorinated Substance Drives Developmental Toxicity. *iScience* **2022**, *25*, No. 103789.

https://doi.org/10.1021/acs.est.2c03734
Environ. Sci. Technol. 2022, 56, 15573−15583

Supporting Information

PFAS and Precursor Bioaccumulation in Freshwater Recreational Fish: Implications for Fish Advisories

Heidi M. Pickard*, Bridger J. Ruyle, Colin Thackray, Adela Chovancova, Clifton Dassuncao, Jitka Becanova, Simon Vojta, Rainer Lohmann, Elsie M. Sunderland

Number of pages: 34 pages

Number of figures: 8 SI figures: Figure S1-S8

Number of tables: 22 SI tables: Table S1-S22

Table of Contents

1. Methods & QA/QC

1.1. Surface Water Sampling. Water samples were collected in high-density polyethylene (HDPE) bottles with polypropylene caps. Bottles (1L) were pre-cleaned with LC-MS grade methanol and Milli-Q (MQ) water and rinsed 3 times with sample water before filling. All PFAS water samples were unfiltered, stored on ice in the field, and then stored at 4°C until analysis. Surface water samples were taken at nine locations to assess the PFAS composition at selected riverine and lacustrine waterbodies (Figure S1). Two to three samples were collected at each sampling site. The NH Fish and Game Department's bathymetry maps were used to determine the sampling areas and specific points at target locations.

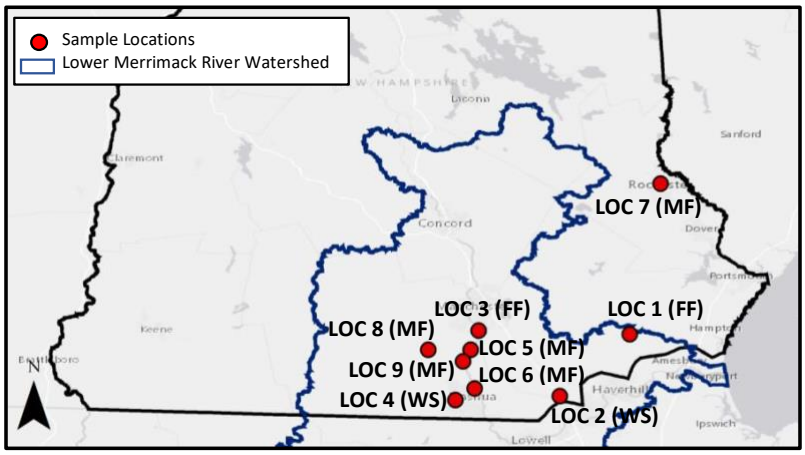


Figure S1. Map of sampling locations in Southern New Hampshire, U.S. Red dots indicate paired water and fish sampling locations. Code abbreviations in brackets are for potential point sources to each location: FF = AFFF, WS = waste disposal sites, and MF = plastics and textile manufacturing point sources. This map was created using ArcGIS® software by Esri.[1] Sources: Esri, HERE, Garmin, © OpenStreetMap contributors, and the GIS user community.

1.2. Fish Sampling. Fish species were selected based on information about which fish species occupied polluted areas, following consultation with local fishermen and the NH Fish and Game Department. Samples (between 1-3 fish per species per location) were collected in conjunction with the surface water samples. Table S1 summarizes the number of fish species collected from each location along with their length and weight. All species except Lake Whitefish, which is a coldwater species, are popular warmwater species in New Hampshire.

Table S1. Locations and species of freshwater fish collected

Site	Sample Site	Subwatershed Name	Fish Sampled	# Fish Sampled	Sample ID	Fish Length (cm)	Fish Weight (g)
1	Great Pond (Kingston, NH)	Powwow River 010700061403	Bluegill	3	LOC1BGcomp*	19	125
2	Hedgehog Pond (Salem, NH)	Spicket River 010700061102	Bluegill	3	LOC2BGcomp	21	181
3	Pine Island Pond (Manchester, NH)	Cohas Brook 010700060703	Bluegill	3	LOC3BGcomp	14	49
			Pumpkinseed	3	LOC3PS1	14	54
					LOC3PS2	14	56
					LOC3PS3	11	25
			Yellow Perch	3	LOC3YP1	17	52
					LOC3YP2	17	54
					LOC3YP3	15	33

4	Nashua River (Nashua, NH)	Unkety Brook – Nashua River 010700040402	Bluegill	3	LOC4BGcomp	17	102
5	Merrimack River (Merrimack, NH)	Little Cohas Brook – Merrimack River 010700060804	Smallmouth Bass	3	LOC5SM1	26	238
					LOC5SM2	26	265
					LOC5SM3	24	174
			Largemouth Bass	2	LOC5LM1	27	385
					LOC5LM2	23	141
			Brown Bullhead Juv	1	LOC5CF1	18	80
			Bluegill	3	LOC5BGcomp	19	148
			Bluegill Juv	3	LOC5BGcompJ	14	49
6	Merrimack River (Nashua, NH)	Nesenkeag Brook – Merrimack River 010700061002	Bluegill	3	LOC6BGcomp	18	142
			Pumpkinseed	3	LOC6PS1	19	166
					LOC6PS2	19	166
					LOC6PS3	18	121
			Lake Whitefish	3	LOC6DA1	26	216
					LOC6DA2	26	187
					LOC6DA3	22	97
7	Cocheco River (Rochester, NH)	Middle Cocheco River 010600030603	Chain Pickerel	1	LOC7PICK	36	319
			Largemouth Bass	2	LOC7LM1	29	305
					LOC7LM2	29	311
			Bluegill	3	LOC7BGcomp	17	95
			Yellow Perch	3	LOC7YP1	24	181
					LOC7YP2	21	122
					LOC7YP3	18	62
8	Baboosic Lake (Amherst, NH)	Baboosic Brook 010700060905	Bluegill	2	LOC8BGcomp	16	85
9	Horseshoe Pond (Merrimack, NH)	Nesenkeag Brook – Merrimack River 010700061002	Largemouth Bass	3	LOC9LM1	28	344
					LOC9LM2	24	137
					LOC9LM3	29	336
			Bluegill	3	LOC9BGcomp	18	106
			Smallmouth Bass	1	LOC9SM1	19	63
			Yellow Perch	2	LOC9YP1	22	124
					LOC9YP2	26	212
			Pumpkinseed	3	LOC9PS1	14	49
					LOC9PS2	14	45
					LOC9PS3	13	41

*Bluegill samples (n=3) from each location were composited for extraction and analysis. All other fish species were extracted and analyzed individually.

1.3. Chemicals and Materials. MQ water with a resistivity of >18 MΩ cm^{-1} was obtained from a GenPure™ xCAD Plus UV-TOC system (Thermo Scientific™ Barnstead™, Lake Balboa, CA). LC-MS grade methanol (J.T. Baker, Center Valley, PA), HPLC grade methyl tert-butyl ether (J.T. Baker, Center Valley, PA), HPLC grade (98%) tetrabutylammonium hydrogen sulfate (J.T. Baker, Center Valley, PA), ACS grade ammonium hydroxide, and MACRON ACS AR sodium hydroxide pellets were purchased from VWR (Radnor, PA). Reagent grade formic acid, BioUltra ammonium acetate, ACS grade acetic acid, BioXtra sodium bicarbonate, BioXtra sodium carbonate, Honeywell ACS reagent potassium persulfate, and Supelclean ENVI-Carb (120-400 mesh, 100 m^2 g^{-1} surface area) were obtained from Sigma Aldrich (St. Louis, MO). Oasis WAX cartridges (6 mL, 150 mg, 30 µm particle size) were obtained from Waters (Milford, MA). PFAS standards were purchased from Wellington Laboratories (Guelph, Canada). A list of PFAS compounds analyzed are in Table S2.

Table S2. Name, acronym, perfluorinated chain-length and molecular weight of targeted PFAS analytes

Compound Name	Acronym	Number of perfluorinated carbons	Molecular weight (g/mol)
Perfluoroalkyl Carboxylates			
Perfluorobutanoate	PFBA	3	213
Perfluoropentanoate	PFPeA	4	263
Perfluorohexanoate	PFHxA	5	313
Perfluoroheptanoate	PFHpA	6	363
Perfluorooctanoate	PFOA	7	413
Perfluorononanoate	PFNA	8	463
Perfluorodecanoate	PFDA	9	513
Perfluoroundecanoate	PFUnDA	10	563
Perfluorododecanoate	PFDoDA	11	613
Perfluorotridecanoate	PFTrDA	12	663
Perfluorotetradecanoate	PFTeDA	13	713
Perfluoroalkyl Sulfonates			
Perfluorobutane sulfonate	PFBS	4	299
Perfluoropentane sulfonate	PFPeS	5	349
Perfluorohexane sulfonate	PFHxS*	6	399
Perfluoroheptane sulfonate	PFHpS	7	449
Perfluorooctane sulfonate	PFOS*	8	499
Perfluorononane sulfonate	PFNS	9	549
Perfluorodecane sulfonate	PFDS	10	599
Perfluoroalkyl Sulfonamides			
Perfluorobutane sulfonamide	FBSA	4	298
Perfluorohexane sulfonamide	FHxSA	6	398
Perfluorooctane sulfonamide	FOSA	8	498
Perfluorodecane sulfonamide	FDSA	10	598
N-methyl perfluorooctanesulfonamide	N-MeFOSA	8	512
N-ethyl perfluorooctanesulfonamide	N-EtFOSA	8	526
Fluorotelomer Sulfonates			
4:2 fluorotelomer sulfonate	4:2 FTSA	4	327
6:2 fluorotelomer sulfonate	6:2 FTSA	6	427
8:2 fluorotelomer sulfonate	8:2 FTSA	8	527
10:2 fluorotelomer sulfonate	10:2 FTSA	10	627
Perfluoroalkyl Sulfonamidoethanols			
N-methyl perfluorooctane sulfonamidoethanol	N-MeFOSE	8	556
N-ethyl perfluorooctane sulfonamidoethanol	N-EtFOSE	8	570
Perfluoroalkyl Sulfonamidoacetic Acids			
N-methyl perfluorooctane sulfonamidoacetic acid	N-MeFOSAA*	8	570
N-ethyl perfluorooctane sulfonamidoacetic acid	N-EtFOSAA*	8	584
Perfluorooctanesulfonamidoacetic acid	FOSAA	8	556
Polyfluoroalkyl Ether Carboxylates			
Dodecafluoro-3H-4,8-dioxanonanoate	ADONA	5	377
Fluorotelomer Carboxylates			
3:3 fluorotelomer carboxylate	3:3 FTCA	3	241
5:3 fluorotelomer carboxylate	5:3 FTCA	5	341
7:3 fluorotelomer carboxylate	7:3 FTCA	7	441

*Branched and linear isomers were available for these standards and were integrated separately

1.4. Sample Extraction. Water samples were initially extracted and analyzed in 2017 as part of batch 1 for targeted PFAS analysis (25 compounds). Water samples were warmed to room temperature before analysis, sonicated for 30 seconds, inverted to mix, and then repeated five times to desorb PFAS from the sample bottle walls before subsampling 500 mL of the 1L sample. Samples were spiked with 20 µL of a 0.1 ng μL^{-1} internal standard before SPE extraction following established methods with slight modifications.[2] Oasis WAX SPE cartridges were preconditioned with 4 mL of 0.1% ammonium hydroxide in methanol (MeOH), 4 mL of MeOH, 4 mL of Milli-Q (MQ) water, and then the 500 mL sample was added to the cartridge and placed under vacuum at a flow rate of 1 drop sec^{-1} followed by a 4 mL MQ water rinse before drying the cartridge under vacuum. The samples were eluted with 6 mL 0.1% ammonium hydroxide in MeOH and the collected eluent was concentrated to 0.5 mL using an ultra-high purity nitrogen gas stream. The 0.5 mL extract was mixed with 0.5 mL MQ water, centrifuged at 13,000 rpm for 20 minutes, and transferred to a polypropylene autosampler vial for analysis.

Fish samples were kept frozen (-20˚C) prior to analysis and fish biometric data regarding length and weight was recorded prior to sample storage. Fish extraction followed previously established methods.[3] For fish extraction, one fillet was removed completely and separated from the skin for extraction while the second fillet remained with the carcass. Fish muscle tissues were initially extracted and analyzed in 2017 as part of batch 1 for targeted PFAS (25 compounds). Additional fish muscle tissues were re-extracted for a larger suite of targeted PFAS (37 compounds) in 2021 as part of batch 2 to compare to TOP assay results. Both fish extractions used an ion-pairing extraction method but with slight differences detailed below.

For fish samples extracted in batch 1, skinless muscle tissue was weighed (3 g) and mixed with MQ water (6 g) and homogenized using an OMNI International TH homogenizer. A subsample of 1 mL homogenate (0.5 g wet-weight equivalent homogenized tissue) was fortified with 20 µL of a 0.1 ng μL^{-1} isotopically labeled internal standard. Fish samples were extracted following the ion-paring extraction method by mixing the homogenized tissue with 0.5 M tetra-butyl ammonia solution (TBAS), 0.25 M sodium carbonate-bicarbonate buffer and 4 mL of methyl-tert butyl ether (MTBE). The sample was then vortexed, mixed on a rotator and centrifuged before subsampling the MTBE layer and repeating a second time. The final 8 mL of MTBE supernatant was concentrated to dryness using ultra-high purity nitrogen and reconstituted in 0.5 mL MeOH. The extract was cleaned by filtering through a 0.45 µm polypropylene (PP) filter before mixing with 0.5 mL MQ water in a PP autosampler vial for analysis.

For fish samples extracted in batch 2, skinless muscle tissue was homogenized, and 0.5 g wet-weight was weighed into a 15 mL PP tube. The tissue was fortified with 50 µL of a 0.03 ng μL^{-1} isotopically labeled internal standard and mixed with 0.5 M TBAS, 0.25 M buffer and MTBE (4 mL). The sample was vortexed, sonicated for 30 minutes and mixed on a rotator for 20 minutes. The sample was centrifuged, the supernatant transferred to a new 15 mL PP tube and the extraction was repeated with an additional 4 mL of MTBE. The 8 mL of MTBE supernatant was kept frozen overnight in a -20˚C freezer to allow lipids to precipitate. The sample was then centrifuged for 2 minutes and decanted to remove precipitate. The decanted sample was evaporated to dryness using ultra-high purity nitrogen and reconstituted in 0.5 mL of MeOH. The extract was further cleaned by mixing with 25 mg of dispersive ENVI-Carb + 0.05 mL acetic acid and centrifuging the extract. The supernatant (0.375 mL) was mixed with 0.375 mL MQ water and centrifuged prior to transferring to a PP autosampler vial for analysis.

1.5. Targeted Analysis. LC-MS/MS analysis was conducted as described previously[2], with minor differences outlined here. Instrumental blanks and the calibration curve were prepared with 50:50 MeOH:MQ water with internal standard concentrations matching the samples. For water sample analyses, a 7-point calibration curve (2-10,000 ng L^{-1}) and 8-point calibration curve (2-60,000 ng L^{-1}) were used for quantifying PFAS concentrations. For tissue sample analyses, a 7-point calibration curve (2-

48,000 ng L^{-1}) was used for quantification for batch 1 and an 11-point calibration curve (1-10,000 ng L^{-1}) for quantification for batch 2. For all analyses conducted, all analyte calibration curves had $R^2 > 0.99$ and all calibration quality controls analyzed every 12 samples were within ± 30 % of the expected calibration concentration value. Branched and linear PFHxS and PFOS were quantified with individual native isomer calibration curves in all analyses. Branched and linear N-MeFOSAA and N-EtFOSAA were quantified separately in the batch 2 analyses with the addition of isomer standards. The gradient was modified to encompass additional compounds. Initial conditions were 97% 2 mM ammonium acetate in water (A) and 3% 2 mM ammonium acetate in methanol (B). From 0.85 to 3.5 minutes the gradient was linearly increased to 54% B. From 3.5 to 16 minutes the gradient was linearly increased to 85% B and then linearly increased to 100% B until the end of the run (16.5 minutes). This part of the gradient was later modified for the 2021 analyses to linearly increase from 3.5 to 15 minutes to 85% B and then linearly increase to 100% B at 15.5 minutes and then held at 100% B until 16.5 minutes.

Table S3. Mass spectrometry acquisition parameters for targeted LC-MS/MS analysis

Analyte	Type	Internal Standard	Precursor Ion Mass	Product Ion Mass	Fragmentor Voltage (V)	Collision Energy (V)
Perfluoroalkyl Carboxylates						
PFBA	Target	$[^{13}C_4]$PFBA	213.0	168.9	60	2
PFPeA	Target	$[^{13}C_5]$PFPeA	262.9	218.9	60	2
PFHxA	Target	$[^{13}C_5]$PFHxA	312.9	268.9; 118.9	70	2; 14
PFHpA	Target	$[^{13}C_4]$PFHpA	362.9	318.9; 168.9; 118.9	70	2; 10; 18
PFOA	Target	$[^{13}C_8]$PFOA	412.9	368.9; 168.9	80	2; 10
PFNA	Target	$[^{13}C_9]$PFNA	462.9	418.9; 218.9; 169.0	75	2; 10; 14
PFDA	Target	$[^{13}C_6]$PFDA	512.9	468.9; 269.0; 218.9	85	6; 14; 14
PFUnDA	Target	$[^{13}C_7]$PFUnDA	562.9	518.9; 269.0; 169.0	95	6; 14; 22
PFDoDA	Target	$[^{13}C_2]$PFDoDA	612.9	569.0; 269.0; 169.0	90	6; 14; 26
PFTrDA	Target	$[^{13}C_2]$PFTeDA	662.8	618.9; 169.0	95	6; 26
PFTeDA	Target	$[^{13}C_2]$PFTeDA	712.9	669.0; 169.0	100	6; 25
Perfluoroalkyl Sulfonates						
PFBS	Target	$[^{13}C_3]$PFBS	298.9	80.0; 98.9	95	38; 30
PFPeS	Target	$[^{13}C_3]$PFHxS	348.9	80.0; 98.9	140	38; 30
PFHxS	Target	$[^{13}C_3]$PFHxS	398.9	80.0; 98.9	135	58; 34
PFHpS	Target	$[^{13}C_8]$PFOS	448.9	80.0; 98.9	180	54; 42
PFOS	Target	$[^{13}C_8]$PFOS	498.9	80.0; 98.9	200	60; 50
PFNS	Target	$[^{13}C_8]$PFOS	548.9	80.0; 98.9	175	60; 54
PFDS	Target	$[^{13}C_8]$PFOS	598.9	80.0; 98.9	175	60; 54
Fluorotelomer Sulfonates						
4:2 FTSA	Target	$[^{13}C_2]$4:2 FTSA	326.9	307.0; 81.0	130	10; 30
6:2 FTSA	Target	$[^{13}C_2]$6:2 FTSA	426.9	406.9; 81.0	135	18; 34
8:2 FTSA	Target	$[^{13}C_2]$8:2 FTSA	526.9	506.9; 81.0	180	26; 42
10:2 FTSA	Target	$[^{13}C_2]$8:2 FTSA	627.0	607.0; 81.0	180	30; 70
Perfluoroalkyl Sulfonamides						
FBSA	Target	$[^{13}C_8]$FOSA	298.0	78.0	140	20
FHxSA	Target	$[^{13}C_8]$FOSA	398.0	78.0	180	40
FOSA	Target	$[^{13}C_8]$FOSA	497.9	78.0	140	38
FDSA	Target	$[^{13}C_8]$FOSA	598.0	78.0	140	32
N-MeFOSA	Target	d3-N-MeFOSA	512.0	219.0; 169.0	60	26; 22
N-EtFOSA	Target	d5-N-EtFOSA	526.0	219.0; 169.0	60	26; 34
Perfluoroalkyl Sulfonamidoethanols						
N-MeFOSE	Target	d7-N-MeFOSE	616.0	59.0	55	66
N-EtFOSE	Target	d9-N-EtFOSE	630.0	59.0	55	54

Perfluoroalkyl Sulfonamidoacetic acids						
N-MeFOSAA	Target	d3-N-MeFOSAA	569.9	418.9; 482.9	95	14; 10
N-EtFOSAA	Target	d5-N-EtFOSAA	583.9	418.9; 525.9	95	18; 14
FOSAA	Target	$[^{13}C_8]$FOSA	556.0	498.0; 419.0; 78.0	55	26; 26; 54
Polyfluoroalkyl ether carboxylates						
ADONA	Target	$[^{13}C_8]$PFOA	377.0	250.9; 85.0	80	2; 30
Fluorotelomer Carboxylates						
3:3 FTCA	Target	$[^{13}C_8]$PFOA	240.8	136.8; 116.8	52	10; 10
5:3 FTCA	Target	$[^{13}C_8]$PFOA	340.8	236.8; 216.8	72	10; 10
7:3 FTCA	Target	$[^{13}C_8]$PFOA	440.8	336.7; 316.7	52	10; 10
Internal Standards						
$[^{13}C_4]$PFBA	ISTD		216.9	171.9	60	2
$[^{13}C_5]$PFPeA	ISTD		267.9	223.0	60	2
$[^{13}C_5]$PFHxA	ISTD		317.8	273.0	70	2
$[^{13}C_4]$PFHpA	ISTD		366.8	321.9	70	2
$[^{13}C_8]$PFOA	ISTD		420.9	376.0	75	2
$[^{13}C_9]$PFNA	ISTD		472.0	427.0	85	2
$[^{13}C_6]$PFDA	ISTD		518.9	474.0	90	2
$[^{13}C_7]$PFUnDA	ISTD		569.9	525.0	85	6
$[^{13}C_2]$PFDoDA	ISTD		614.9	569.9	95	6
$[^{13}C_2]$PFTeDA	ISTD		714.8	670.0	95	6
$[^{13}C_3]$PFBS	ISTD		301.9	99.0	95	26
$[^{13}C_3]$PFHxS	ISTD		401.9	98.9	180	38
$[^{13}C_8]$PFOS	ISTD		506.9	99.0	180	50
$[^{13}C_2]$4:2 FtS	ISTD		328.9	81.0	95	38
$[^{13}C_2]$6:2 FtS	ISTD		428.9	81.0	95	46
$[^{13}C_2]$8:2 FtS	ISTD		528.9	81.0	180	46
$[^{13}C_8]$FOSA	ISTD		505.9	78.0	95	38
d3-N-MeFOSAA	ISTD		572.9	418.9	100	14
d5-N-EtFOSAA	ISTD		588.9	418.9	95	14
d3-N-MeFOSA	ISTD		515.0	169.0	60	22
d5-N-EtFOSA	ISTD		531.0	169.0	55	30
d7-N-MeFOSE	ISTD		623.0	59.0	55	66
d9-N-EtFOSE	ISTD		639.0	59.0	55	30

1.6. Blanks. For QA/QC, instrumental blanks were included in the run after every six samples, and to avoid cross-contamination and carry-over, MeOH washes were injected after high concentration sample/standard injections. Majority of the instrumental blanks were below the limit of detection (LOD), but in cases where some compounds had detectable levels, sample concentrations were corrected for this. Procedural blanks were introduced in each sample series extracted and included MQ water samples spiked with internal standards that went through the extraction procedures. Three procedural blanks were extracted with the water samples, and two procedural blanks for each batch analysis of the fish samples, in addition to two procedural blanks that went through the TOP assay extraction. Samples with concentrations at or below the average concentrations detected in the procedural blanks were reported as <MDL (method detection limit) for batch 1 analysis. Average procedural blank concentrations in batch 2 analysis were blank subtracted from the sample concentrations for calculating the TOP assay results. Average procedural blank detections are summarized in Table S4.

Table S4. Average concentrations of procedural blank detections (ng L^{-1} for water and ng g^{-1} for fish tissue) and detection frequencies (DF)

	Batch 1 Procedural Blanks				Batch 2 Procedural Blanks			
Procedure	**Water Extraction (n=3)**		**Fish Extraction (n=2)**		**Fish Extraction (n=2)**		**Fish TOP Extraction (n=2)**	
Parameters	**Mean ± SD[1]**	**DF[2]**	**Mean ± SD**	**DF**	**Mean ± SD**	**DF**	**Mean ± SD**	**DF**
PFBA	ND[3]	0/3	ND	0/2	ND	0/2	ND	0/2
PFPeA	ND	0/3	0.045 ± 0.064	1/2	ND	0/2	0.43 ± 0.34	2/2
PFHxA	ND	0/3	ND	0/2	ND	0/2	ND	0/2
PFHpA	ND	0/3	ND	0/2	0.18 ± 0.07	2/2	0.29 ± 0.24	2/2
PFOA	ND	0/3	0.20 ± 0.02	2/2	0.099 ± 0.14	1/2	0.13 ± 0.06	2/2
PFNA	ND	0/3	ND	0/2	ND	0/2	0.22 ± 0.07	2/2
PFDA	0.016 ± 0.028	1/3	ND	0/2	ND	0/2	ND	0/2
PFUnDA	0.031 ± 0.037	2/3	ND	0/2	ND	0/2	ND	0/2
PFDoDA	0.049 ± 0.043	2/3	ND	0/2	ND	0/2	ND	0/2
PFTrDA	0.32 ± 0.45	3/3	ND	0/2	ND	0/2	ND	0/2
PFTeDA	0.27 ± 0.37	3/3	0.004 ± 0.005	1/2	ND	0/2	ND	0/2
PFBS	ND	0/3	ND	0/2	ND	0/2	ND	0/2
PFPeS	ND	0/3	ND	0/2	ND	0/2	ND	0/2
L-PFHxS[5]	ND	0/3	ND	0/2	ND	0/2	0.042 ± 0.059	1/2
Br-PFHxS[6]	ND	0/3	ND	0/2	ND	0/2	ND	0/2
PFHpS	ND	0/3	ND	0/2	ND	0/2	ND	0/2
L-PFOS	ND	0/3	ND	0/2	ND	0/2	0.77 ± 0.50	2/2
Br-PFOS	ND	0/3	ND	0/2	ND	0/2	0.13 ± 0.18	1/2
PFNS	ND	0/3	ND	0/2	ND	0/2	ND	0/2
PFDS	ND	0/3	ND	0/2	ND	0/2	ND	0/2
4:2 FTSA	ND	0/3	ND	0/2	ND	0/2	ND	0/2
6:2 FTSA	0.023 ± 0.003	3/3	0.057 ± 0.081	1/2	ND	0/2	ND	0/2
8:2 FTSA	0.004 ± 0.007	1/3	ND	0/2	ND	0/2	ND	0/2
10:2 FTSA	NA[4]	NA	NA	NA	ND	0/2	ND	0/2
FBSA	NA	NA	NA	NA	ND	0/2	ND	0/2
FHxSA	NA	NA	NA	NA	ND	0/2	ND	0/2
FOSA	0.006 ± 0.011	1/3	ND	0/2	ND	0/2	ND	0/2
FDSA	NA	NA	NA	NA	0.039 ± 0.055	1/2	ND	0/2
N-MeFOSA	NA	NA	NA	NA	ND	0/2	ND	0/2
N-EtFOSA	NA	NA	NA	NA	ND	0/2	ND	0/2
L-N-MeFOSAA	0.032 ± 0.035	2/3	ND	0/2	ND	0/2	ND	0/2
Br-N-MeFOSAA	NA	NA	NA	NA	ND	0/2	ND	0/2
L-N-EtFOSAA	0.035 ± 0.035	2/3	ND	0/2	ND	0/2	ND	0/2
Br-N-EtFOSAA	NA	NA	NA	NA	ND	0/2	ND	0/2
FOSAA	NA	NA	NA	NA	ND	0/2	ND	0/2
N-MeFOSE	NA	NA	NA	NA	ND	0/2	ND	0/2
N-EtFOSE	NA	NA	NA	NA	ND	0/2	ND	0/2
ADONA	ND	0/3	ND	0/2	ND	0/2	ND	0/2
3:3 FTCA	NA	NA	NA	NA	ND	0/2	ND	0/2
5:3 FTCA	NA	NA	NA	NA	ND	0/2	ND	0/2
7:3 FTCA	NA	NA	NA	NA	ND	0/2	0.005 ± 0.007	1/2

[1]SD = standard deviation, [2]DF = detection frequency (i.e. 1/3 means 1 in 3 samples had detectable levels >MDL), [3]ND = not detected, [4]NA = not analyzed, [5]L = linear isomer, [6]Br = branched isomer

1.7. Recoveries & **Duplicates.** Differences between duplicate measurements were assessed using the relative percent difference (RPD) statistic that determines the mean normalized difference between two replicate samples. Five duplicate water samples and six duplicate fish samples were analyzed in batch 1 with relative percent differences ranging from 0% - 40% (average: 12%, median: 10%) for five water duplicates and ranging from 0% - 81% (average: 19%, median: 12%) for six fish duplicates. Recovery spikes (2 ng native PFAS spike) for surface water extractions using water as the matrix had recoveries ranging from 52% (PFPeS) to 184% (6:2 FtS) but were within 70% to 130% for all other compounds (average: 105%, standard deviation (SD): 23%). Recovery spikes (2 ng native PFAS spike) for fish tissue extractions in batch 1 using water as the matrix ranged from 31% (PFPeS) to 282% (PFHpS) but were within 70% to 130% for all compounds that were detected in the fish samples (average: 88%, SD: 10%).

Four duplicate fish samples were analyzed in batch 2 (two with the targeted analysis and two with the TOP assay analysis) with relative percent differences ranging from 0% - 178% (average: 30%, median: 15%). Recovery spikes (2 ng native PFAS spike) for fish tissue extractions analyzed in batch 2 using water as the matrix ranged from 12% (3:3 FTCA) to 148% (PFTrDA) (average: 104%, median: 107%). Recovery spikes (2 ng native PFAS spike) for fish tissue extractions analyzed in batch 2 using fish muscle as the matrix ranged from 30% (FHxSA) to 223% (L-PFOS) (average: 115%, median: 113%).

Because the recovery spikes for the two spiked fish tissue samples yielded high recoveries for L-PFOS and low recoveries for FHxSA, additional spike recovery tests were conducted separately using the same fish tissue to compare. These additional spike tests yielded L-PFOS recoveries of 82% and 96% in replicate fish spike samples and recoveries of 150% and 153% for FHxSA in replicate samples. Based on these additional spike recovery tests, we chose to use the original data generated. Average spike recoveries for individual compounds from analysis included in this study are summarized in Table S5.

Internal standard recoveries for the fish muscle tissue samples analyzed in batch 2 for targeted PFAS and following TOP assay, were determined based on the internal standard peak area in the sample divided by the average internal standard area in the calibration standards. Internal standard recoveries in these samples ranged from 0% (d3-N-MeFOSA and d5-N-EtFOSA) to 246% (M2-8:2 FtS). Recoveries on the very low and very high end were due to samples that were analyzed after being oxidized by the TOP assay. Average internal standard recoveries ranged from 20% (d3-N-MeFOSA) to 125% (M2-4:2 FtS). Average internal standard recoveries across samples analyzed in batch 2 are summarized in Table S6.

Since fish tissue samples were not analyzed using a matrix-matched calibration curve, tissue sample spike and recovery experiments were additionally conducted on three fish tissue samples. Analyte extraction recoveries and percent matrix effects varied quite a bit for the range of all compounds analyzed, however were within acceptable ranges for the compounds that were detected in the fish samples at concentrations above the detection limit. Analyte recovery for those compounds previously detected in the fish ranged from 50% to 89%, with median of 62%, while % matrix effects for those same compounds ranged from 6% to 328%, with median of 39%. Since most of the compounds had recoveries between 70% to 130% using a standard calibration curve approach, no further correction calculations were applied to the samples.

Table S5. Average percent recovery (%) of native PFAS spikes in water and fish tissue extractions

	Recovery Spikes (Batch 1)		Recovery Spikes (Batch 2)	
Procedure	**Water Extraction (n=3)**	**Fish Extraction (n=2)**	**Fish Extraction (n=2)**	**Fish Extraction (n=2)**
Matrix	**Water**	**Water**	**Water**	**Fish Muscle**
Parameters	**Mean ± SD[1]**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
PFBA	110 ± 5	ND[2]	113 ± 3	116 ± 3
PFPeA	105 ± 4	96 ± 5	119 ± 6	116 ± 0
PFHxA	109 ± 4	93 ± 2	110 ± 8	112 ± 1
PFHpA	108 ± 6	90 ± 4	107 ± 3	104 ± 4
PFOA	107 ± 5	94 ± 5	103 ± 3	106 ± 3
PFNA	108 ± 4	86 ± 7	113 ± 1	110 ± 17
PFDA	104 ± 8	82 ± 9	114 ± 2	113 ± 5
PFUnDA	100 ± 14	82 ± 13	111 ± 1	107 ± 18
PFDoDA	96 ± 22	80 ± 18	109 ± 0	111 ± 16
PFTrDA	109 ± 43	66 ± 11	130 ± 25	122 ± 53
PFTeDA	107 ± 3	83 ± 18	111 ± 2	113 ± 8
PFBS	112 ± 6	84 ± 2	95 ± 10	98 ± 10
PFPeS	52 ± 3	31 ± 7	108 ± 2	116 ± 62
L-PFHxS	92 ± 1	99 ± 0	104 ± 2	129 ± 25
Br-PFHxS	64 ± 3	36 ± 9	99 ± 7	143 ± 50
PFHpS	70 ± 4	282 ± 102	94 ± 23	123 ± 54
L-PFOS	109 ± 8	87 ± 9	102 ± 0	223[4] (89 ± 10)[5]
Br-PFOS	103 ± 10	78 ± 2	94 ± 20	125 ± 38
PFNS	91 ± 23	93 ± 16	102 ± 4	138 ± 17
PFDS	93 ± 31	102 ± 21	81 ± 19	130 ± 5
4:2 FTSA	124 ± 6	109 ± 8	105 ± 4	111 ± 7
6:2 FTSA	184 ± 10	89 ± 8	97 ± 4	153 ± 83
8:2 FTSA	118 ± 5	86 ± 11	127 ± 21	127 ± 6
10:2 FTSA	NA[3]	NA	89 ± 31	101 ± 33
FBSA	NA	NA	79 ± 0	82 ± 18
FHxSA	NA	NA	34 ± 6	33 ± 4 (152 ± 2)[5]
FOSA	132 ± 11	83 ± 10	99 ± 2	94 ± 3
FDSA	NA	NA	79[4]	162 ± 24
N-MeFOSA	NA	NA	116 ± 1	124 ± 28
N-EtFOSA	NA	NA	98 ± 5	129 ± 26
L-N-MeFOSAA	113 ± 11	93 ± 17	111 ± 1	120 ± 10
Br-N-MeFOSAA	NA	NA	89 ± 8	79 ± 8
L-N-EtFOSAA	118 ± 11	97 ± 19	105 ± 1	112 ± 10
Br-N-EtFOSAA	NA	NA	88 ± 32	134 ± 21
FOSAA	NA	NA	141 ± 2	128 ± 20
N-MeFOSE	NA	NA	105 ± 6	101 ± 16
N-EtFOSE	NA	NA	119 ± 0	138 ± 11
ADONA	106 ± 6	162 ± 15	99 ± 2	93 ± 3
3:3 FTCA	NA	NA	48 ± 51	31[4]
5:3 FTCA	NA	NA	123 ± 2	112 ± 30
7:3 FTCA	NA	NA	114 ± 0	119 ± 42

[1]SD = standard deviation, [2]ND = not detected, [3]NA = not analyzed, [4]For analytes where no SD is reported, only one spiked sample had a reportable recovery, [5]Recoveries in brackets were additional recovery tests conducted for PFOS and FHxSA due to high and low recoveries initially reported.

Table S6. Average percent recovery (%) of internal standards in batch 2 fish tissue extractions (includes internal standard recoveries for samples extracted by IPE for targeted PFAS and samples oxidized by TOP and analyzed for targeted PFAS increase from oxidized precursors)

Internal Standard Analytes	Mean ± SD Recoveries (%)
[$^{13}C_4$]PFBA	33 ± 11
[$^{13}C_5$]PFPeA	37 ± 15
[$^{13}C_5$]PFHxA	51 ± 12
[$^{13}C_4$]PFHpA	59 ± 11
[$^{13}C_8$]PFOA	58 ± 11
[$^{13}C_9$]PFNA	64 ± 15
[$^{13}C_6$]PFDA	62 ± 23
[$^{13}C_7$]PFUnDA	76 ± 35
[$^{13}C_2$]PFDoDA	74 ± 39
[$^{13}C_2$]PFTeDA	65 ± 37
[$^{13}C_3$]PFBS	62 ± 36
[$^{13}C_3$]PFHxS	65 ± 37
[$^{13}C_8$]PFOS	62 ± 39
[$^{13}C_2$]4:2 FtS	125 ± 43
[$^{13}C_2$]6:2 FtS	119 ± 56
[$^{13}C_2$]8:2 FtS	96 ± 50
[$^{13}C_8$]FOSA	44 ± 10
d3-N-MeFOSAA	105 ± 36
d5-N-EtFOSAA	118 ± 43
d3-N-MeFOSA	20 ± 20
d5-N-EtFOSA	18 ± 18
d7-N-MeFOSE	31 ± 11
d9-N-EtFOSE	33 ± 13

1.8. Detection Limits. The instrument limit of detection (LOD) is calculated based on the average concentration at which the sample signal-to-noise ratio (S/N) is 3. The limit of quantification is calculated for a sample signal-to-noise ratio of 10. The method detection limit (MDL) and method quantification limit (MQL) are determined based on sample dilution volumes/weights (Table S7). Values >MDL are reported in this paper. Batch 1 analysis were conducted in 2017 and Batch 2 analysis were conducted in 2021 with a larger suite of PFAS analytes.

Table S7. Method detection limits for water and fish samples analyzed in Batch 1 and Batch 2

Compound	Water Samples (Batch 1)	Fish Samples (Batch 1)	Fish Samples (Batch 2)
	MDL (ng/L)	MDL (ng/g)	MDL (ng/g)
PFBA	6.63	0.38	1.27
PFPeA	18.11	0.035	0.24
PFHxA	1.42	0.038	0.18
PFHpA	0.58	0.018	0.049
PFOA	0.074	0.017	0.061
PFNA	0.033	0.011	0.054
PFDA	0.028	0.0078	0.058
PFUnDA	0.014	0.0093	0.051
PFDoDA	0.0087	0.0041	0.064
PFTrDA	0.019	0.0011	0.074
PFTeDA	0.021	0.0014	0.13
PFBS	3.80	0.071	0.083
PFPeS	0.14	0.044	0.070
L-PFHxS	0.28	0.029	0.079
Br-PFHxS	0.18	0.014	0.10
PFHpS	0.030	0.072	0.088
L-PFOS	0.038	0.066	0.13
Br-PFOS	0.050	0.40	0.19
PFNS	0.025	0.029	0.11
PFDS	0.025	0.0098	0.13
4:2 FTSA	0.045	0.0059	0.099
6:2 FTSA	0.010	0.0013	0.048
8:2 FTSA	0.0038	0.0025	0.059
10:2 FTSA	NA*	NA	0.067
FBSA	NA	NA	0.029
FHxSA	NA	NA	0.060
FOSA	0.006	0.0033	0.019
FDSA	NA	NA	0.054
N-MeFOSA	NA	NA	0.19
N-EtFOSA	NA	NA	0.070
L-N-MeFOSAA	0.0043	0.0011	0.017
Br-N-MeFOSAA	NA	NA	0.42
L-N-EtFOSAA	0.0057	0.0034	0.017
Br-N-EtFOSAA	NA	NA	0.70
FOSAA	NA	NA	0.12
N-MeFOSE	NA	NA	0.11
N-EtFOSE	NA	NA	0.10
ADONA	0.0089	0.0005	0.016
3:3 FTCA	NA	NA	0.38
5:3 FTCA	NA	NA	0.24
7:3 FTCA	NA	NA	0.065

*NA means these compounds were not analyzed and therefore don't have an MDL associated with them.

1.9. SRM. Method trueness was assessed through the analysis of NIST SRM 1947 reference samples (Lake Michigan Fish Tissue). Duplicate extractions of SRM 1947 were included in batches 1 and 2. Table S8 compares these two extractions with the NIST SRM 1947 reference concentrations. Concentrations from the extraction conducted in batch 2 compared to the reference concentrations have a relative percent difference (RPD) within ± 30% of each other for the detectable analytes. SRM 1947 concentrations from this study were also compared to SRM 1947 samples analyzed in Simonnet-Laprade et al., 2019 that also did the TOP assay on biota, as well as a comparison to the IPE extraction for the SRM 1947 samples from University of Toronto in Reiner et al., 2012.[4,5]

Table S8. NIST SRM 1947 reference material PFAS concentrations (ng g^{-1})

	SRM Ref[1]	**This Study (Batch 1)**	**This Study (Batch 2)**	**Simonnet-Laprade et al., 2019**[2]	**Reiner et al., 2012**[3]	**RPD (%) This Study & SRM Ref**[4]
Replicates	NA	n= 2	n=2	n = 4	n = 3	
PFBA		2.54 ± 0.06	<1.27	<0.06		
PFPeA		<0.39	<0.24	<0.10		
PFHxA		0.31 ± 0.04	0.20	0.17 ± 0.07		
PFHpA		<0.14	0.21	0.07 ± 0.01	0.16 + 0.03	
PFOA		0.34 ± 0.10	0.39 ± 0.15	0.11 ± 0.01		
PFNA	0.20	<0.90	0.27 ± 0.05	0.26 ± 0.06		30%
PFDA	0.26	0.34	0.30 ± 0.01	0.28 ± 0.02	0.30 ± 0.03	14%
PFUA	0.28	0.28	0.27 ± 0.04	0.27 + 0.01	0.27 ± 0.01	4%
PFDoA		<0.19	0.14 ± 0.01	0.27 ± 0.02	0.23 ± 0.11	
PFTriA	0.20	<0.29	0.16 ± 0.01	0.19 ± 0.02		22%
PFTA		<0.46	0.16	0.11 ± 0.02		
PFBS		<0.27	<0.08	<0.02		
PFPeS		<0.36	<0.07			
L-PFHxS		<0.19	<0.08	0.06 ± 0.01	0.14 ± 0.15	
Br-PFHxS		<0.23	<0.10			
PFHpS		<0.19	<0.09	0.03 ± 0.01		
L-PFOS	5.90	8.16 ± 0.39	5.24 ± 0.09	7.19 ± 0.56	5.97 ± 0.62	12%
Br-PFOS		0.50 ± 0.03	0.58 ± 0.08			
PFNS		<0.13	<0.11			
PFDS		<0.09	<0.13	0.20 ± 0.01		
4:2 FTSA		<0.22	<0.10	<0.005		
6:2 FTSA		<0.16	<0.05	<0.05		
8:2 FTSA		<0.06	<0.06	<0.03		
10:2 FTSA		<0.09	<0.07	<0.01		
FBSA		0.25 ± 0.08	0.18 ± 0.01			
FHxSA		<0.08	<0.06			
FOSA		0.23 ± 0.02	0.28 ± 0.02	0.12 ± 0.01		
FDSA		<0.05	<0.05			
N-MeFOSA		<0.18	<0.19	<0.006		
N-EtFOSA		<0.58	<0.07	<0.009		
FOSAA		<0.09	<0.12	<0.01		
L-N-MeFOSAA		<0.07	0.08	0.02 ± 0.01		
Br-N-MeFOSAA		<0.82	<0.42			
L-N-EtFOSAA		0.09 ± 0.05	<0.02	0.06 ± 0.01		
Br-N-EtFOSAA		<0.26	<0.70			
ADONA		<0.04	<0.01	<0.21		

[1]SRM Ref is the NIST SRM 1947 reference concentrations, [2]SRM 1947 concentrations from Simonnet-Laprade et al., 2019, [3]SRM 1947 concentrations from the U of T IPE extraction in Reiner et al, 2012, [4]Relative percent difference between the SRM 1947 concentrations from batch 2 extraction in this study and the reference concentrations.

1.10. TOP Assay Extraction & QA/QC. For the TOP assay extraction, muscle tissue samples were subjected to the same ion-pairing extraction (IPE) as described above for samples extracted in batch 2, however, internal standards were not added prior to IPE extraction. Following IPE extraction with ENVI-carb clean-up, muscle tissue extracts (0.375 mL) were transferred to 50 mL PP tubes and evaporated to dryness. Then, 20 mL of MQ water and 20 mL of 0.12 M potassium persulfate and 0.25 M sodium hydroxide solution were added to the tube and vigorously shaken. Samples were heated in an 85˚C water bath overnight, for at least 12 hours and neutralized to a pH of 7 the following morning with hydrochloric acid if needed. Following oxidation, samples were then spiked with 50 µL of a 0.03 ng μL^{-1} isotopically labeled internal standard. Oasis WAX SPE cartridges were preconditioned as described above for water samples and the 40 mL TOP sample was added to the cartridge, followed by a 4 mL MQ water rinse before drying the cartridge under vacuum. The samples were eluted with 4 mL MeOH and 4 mL 0.1% ammonium hydroxide in MeOH and the collected eluent was evaporated to dryness and reconstituted in 0.375 mL MeOH. The 0.375 mL extract was mixed with 0.375 mL MQ water, centrifuged at 13,000 rpm for 20 minutes, and transferred to a PP autosampler vial for analysis. Concentrations of C3-C8 PFCA measured in the fish muscle tissue samples pre-oxidation and post-oxidation are provided in Table S9. PFCA with chain lengths < C3 were not included in the method.

Table S9. Pre-oxidation (Pre) and post-oxidation (Post) PFCA (C3-C8) concentrations (nmol L^{-1}) in fish muscle tissue samples subjected to the TOP assay procedure. Blank cells indicate that the concentration was either <MDL or <0 after blank subtraction.

Samples	**C3 (PFBA)**		**C4 (PFPeA)**		**C5 (PFHxA)**		**C6 (PFHpA)**		**C7 (PFOA)**		**C8 (PFNA)**	
Oxidation	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
LOC1BGcomp									0.0489		0.199	
LOC2BGcomp										0.0271	0.0724	
LOC3BGcomp				0.462		0.797			0.0322	0.156	0.215	0.116
LOC3PS2		3.29		3.06		2.41	0.0266		0.0559	0.399	0.173	
LOC3YP1				2.49		2.67			0.0908	0.438	0.228	0.0629
LOC4BGcomp				2.58		2.31	0.0778	0.990	1.28	4.51	0.153	0.560
LOC5BGcomp		4.24		0.0751					0.00975		0.0850	
LOC5LM1		5.18				0.683	0.0785					
LOC5SM2									0.0303	0.166	0.0784	
LOC6BGcomp									0.0663	0.270	0.0822	
LOC6DA1									0.00419	0.113		
LOC6PS1									0.0777	0.0861	0.0941	
LOC7BGcomp									0.0218	0.0356	0.110	
LOC7LM2									0.00209			
LOC7YP1						0.700	0.0541		0.0230	0.0991	0.269	
LOC8BGcomp							0.148	0.0850	0.0944	0.0294	0.378	0.0382
LOC9BGcomp									0.0731	0.0699	0.0794	
LOC9LM3				0.591			0.166		0.00233	0.130		0.520
LOC9SM1				3.56				2.69	0.0754		0.401	
LOC9YP1				3.58		0.854		0.165	0.316	0.386	0.724	0.272

Molar conversion yields of seven precursors from four PFAS classes to the corresponding PFCA, oxidized in the presence of biological tissue, were assessed, and compared to molar yields of precursors oxidized in the presence of other matrices from the literature. Oxidation of select precursors in the presence of fish muscle tissue showed similar oxidation patterns and molar yields to those reported for other matrices in previous reports.[4,6–11] See Table S10 for a summary of available molar yield information by perfluorinated carbon number from this study and other publications for all the precursor analytes included in this study. Percent molar conversion yields were determined by taking the PFCA oxidation yields of individual precursors, converting to molarity, blank subtracting any detectable levels from the fish muscle prior to spiking with precursors and dividing each PFCA molarity by the precursor spike concentration determined after extraction to account for precursor loss during IPE extraction. The percent yield of each PFCA in molarity was then divided by the sum of PFCA molarity to determine the final percent conversion yield of each precursor to the corresponding PFCA based on spike recoveries.

Table S10. Average molar conversion yields (%) ± standard error for PFAS precursors by perfluorinated carbon number (n)

				Mean Perfluorinated Carbon Number (n) Percent Molar Yield (%)							
Precursor	**Reference**	**Matrix**	**# C-F**	**n**	**n-1**	**n-2**	**n-3**	**n-4**	**n-5**	**n-6**	**n-7**
Fluorotelomer-Based Precursors (FT)											
4:2 FTSA	Martin et al., 2019	water	4	3 ± 1	24 ± 2						
5:3 FTCA	Martin et al., 2019	water	5	4 ± 1	12 ± 1	42 ± 1					
6:2 FTUCA	Martin et al., 2019	water	5	31 ± 1	17 ± 1	21 ± 1					
6:2 FTSA	Houtz & Sedlak, 2012	water	6	2 ± 1	22 ± 2	27 ± 2	22 ± 5				
6:2 FTSA	Martin et al., 2019	water	6	2 ± 0.1	17 ± 1	24 ± 1	21 ± 1				
6:2 FTAB	Martin et al., 2019	water	6	1.4 ± 0.1	8 ± 1	33 ± 2	21 ± 1				
6:2 diPAP	Simonnet-Laprade et al., 2019	water	6	21 ± 4	51 ± 7	61 ± 7	24 ± 6				
6:2 diPAP	Gockener et al., 2020	egg yolk	6	12.4 ± 1.7	27.7 ± 3.8	23.9 ± 4.3	16.5 ± 0.8				
6:2 diPAP	Houtz & Sedlak, 2012	water	6	15 ± 3	33 ± 2	47 ± 3	27 ± 3				
8:2 diPAP	Gockener et al., 2020	egg yolk	8	12.1 ± 0.7	21.6 ± 0.7	23.4 ± 0.3	15.1 ± 0.4	9.1 ± 0.1	11.1 ± 1		
8:2 diPAP	Houtz & Sedlak, 2012	water	8	13 ± 1	38 ± 2	43 ± 2	24 ± 1	17 ± 1	10 ± 2		
7:3 FTCA	Martin et al., 2019	water	7	3 ± 1	8 ± 1	36 ± 3	18 ± 2	15 ± 1			
8:2 FTUCA	Martin et al., 2019	water	7	26 ± 2	14 ± 1	16 ± 1	19 ± 1	14 ± 1			
8:2 FTSA	Houtz & Sedlak, 2012	water	8	3 ± 0.1	21 ± 2	27 ± 3	19 ± 3	12 ± 4	11 ± 4		
8:2 FTSA	Simonnet-Laprade et al., 2019	water	8	3 ± 0.5	33 ± 6	32 ± 8	22 ± 4	13 ± 3	9 ± 4		
8:2 FTSA	Martin et al., 2019	water	8	2 ± 1	20 ± 4	25 ± 4	19 ± 5	16 ± 2	9 ± 1		
8:2 FTSA	Wang et al., 2021	water	8	2.2 ± 0.2	25 ± 2.7	34 ± 2	15 ± 0.6	11 ± 1	12 ± 1.4		
8:2 FTSA	This Study	fish muscle	8	0.6 ± 1.1	12.8 ± 1.8	40 ± 1	29.9 ± 1.9	16.7 ± 0.5	0		
8:2 FTSA	This Study	fish muscle	8	1.3 ± 2.2	9.3 ± 0.2	41.1 ± 1.9	30.4 ± 4	16.2 ± 3.3	0		
10:2 FTUCA	Martin et al., 2019	water	9	29 ± 6	18 ± 4	16 ± 4	17 ± 3	13 ± 1	9 ± 1	6 ± 1	
10:2 FTSA	Martin et al., 2019	water	10	3 ± 2	28 ± 8	29 ± 6	16 ± 1	14 ± 3	6 ± 2	3 ± 1	2 ± 1
10:2 FTSA	This Study	fish muscle	10	4	23	36	16	7	13	0	0

Electrochemical Fluorination Based Precursors (ECF)											
FBSA	Janda et al., 2019	soil	4	0	65 ± 7						
FHxSA	Janda et al., 2019	soil	6	0	84 ± 6	0	0				
FHxSA	Martin et al., 2019	water	6	0	96 ± 3	1.3 ± 0.1	0				
PFHxSAm	Ruyle et al., 2021	water	6	0	88 ± 3	0	0				
PFHxSAmS	Ruyle et al., 2021	water	6	0	87 ± 5	0	0				
FOSA	Simonnet-Laprade et al., 2019	water	8	0	128 ± 16	1.1 ± 0.4	0.4 ± 0.5	0	0		
FOSA	Janda et al., 2019	soil	8	0	103 ± 16	0	0	0	0		
FOSA	Houtz & Sedlak, 2012	water	8	0	97 ± 3	0	0	0	0		
FOSA	Martin et al., 2019	water	8	0	88 ± 1	1.5 ± 0.1	0	0	0		
FOSA	Wang et al., 2021	water	8	0	98 ± 4	0	0	0	0		
FOSA	This Study	fish muscle	8	0	97.4 ± 1.4	2.6 ± 1.4	0	0	0		
FOSA	This Study	fish muscle	8	1.8 ± 1.6	92.6 ± 2.8	3 ± 0.6	0	0	0		
FOSA	This Study	fish muscle	8	0.5 ± 0.5	90.2 ± 2.9	5.3 ± 1.5	3.3 ± 2.8	0	0		
FOSA	This Study	fish muscle	8	1	84	6	7	0	0		
MeFOSA	Martin et al., 2019	water	8	0	84 ± 2	0.7 ± 0.4	0	0	0		
EtFOSA	Martin et al., 2019	water	8	0	76 ± 2	0.7 ± 0.1	0	0	0		
FOSAA	Martin et al., 2019	water	8	0	88 ± 14	2 ± 0.5	0	0	0		
FOSAA	This Study	fish muscle	8	0	70	0	28	0	0		
N-MeFOSAA	Simonnet-Laprade et al., 2019	water	8	0	121 ± 24	4.1 ± 1	1.8 ± 0.2	0.6 ± 0.6	0		
N-MeFOSAA	Houtz & Sedlak, 2012	water	8	0	110 ± 8	0	0	0	0		
N-MeFOSAA	Martin et al., 2019	water	8	0	94 ± 10	1.7 ± 0.2	0	0	0		
N-MeFOSAA	This Study	fish muscle	8	1.3 ± 2.3	77.3 ± 6.9	11.5 ± 2.2	9.5 ± 8.3	0.4 ± 0.7	0		
N-MeFOSAA	This Study	fish muscle	8	2 ± 1.8	49.3 ± 0.6	15.1 ± 1.3	22.4 ± 2.6	11.1 ± 2.5	0		
N-MeFOSAA	This Study	fish muscle	8	6.9 ± 0.8	77.1 ± 12.9	4.6 ± 0.2	10.1 ± 14.3	0	0		
N-EtFOSAA	Houtz & Sedlak, 2012	water	8	0	92 ± 4	0	0	0	0		
N-EtFOSAA	Gockener et al., 2020	egg yolk	8	0	62.8 ± 1.8	7.1 ± 0.1	3.4 ± 0.7	5.5 ± 1.5	3.1 ± 3.1		
N-EtFOSAA	Martin et al., 2019	water	8	0	95 ± 6	1.2 ± 0.1	0	0	0		
PFOAB	Martin et al., 2019	water	8	0	71 ± 1	2.3 ± 0.1	0	0	0		
PFOSB	Martin et al., 2019	water	8	0	73 ± 5	2 ± 0.7	0	0	0		
PFOANO	Martin et al., 2019	water	8	0	79 ± 4	2 ± 0.4	0	0	0		
PFOSNO	Martin et al., 2019	water	8	0	73 ± 9	2 ± 0.2	0	0	0		
PFOSAmS	Martin et al., 2019	water	8	0	68 ± 4	1.3 ± 0.2	0	0	0		
PFOSAm	Martin et al., 2019	water	8	0	89 ± 3	2.5 ± 0.3	0	0	0		
FDSA	This Study	fish muscle	10	1	83	5	0	0	0	9	0

The effectiveness of the TOP assay on fish muscle tissue was assessed in this study. Prior work in our lab has assessed the effectiveness of the TOP assay on aqueous samples.[2,9] The efficacy of the TOP assay to oxidize precursors in the presence of biological fish tissue was evaluated with each sample batch in duplicate. Both individual precursor standards and a mixture of the nineteen precursor standards included in this study (see Table S11) were assessed for their ability to fully oxidize with this method. Complete oxidation of precursors (both individual and as a mixture) were observed with every batch of samples as indicated by precursor concentrations <MDL after oxidation.

The same extraction and oxidation procedure was carried out on fish muscle spiked with the full suite of analytes prior to extraction and oxidation, with IS added after oxidation. In this scenario, the oxidation solution was not added to the blown down extract, but rather the extract was reconstituted in only MQ-water, heated overnight and SPE extracted as if being oxidized, to assess the recovery of all analytes (both PFAA and precursors) after both the extraction and oxidation procedures (see Table S11 for fish extraction recoveries from this spike test). Recoveries of precursors that went through the full procedure minus the oxidant solution showed generally low recoveries compared to the assessment of recoveries after only the IPE extraction procedure, suggesting loss of precursors during the nitrogen blow down step and/or the heating process and subsequent SPE extraction. Individual precursors were also assessed in this manner with each sample batch to determine potential variability in precursor spike recoveries due to the lower recoveries (Table S11).

Since IS are added after oxidation to the samples, additional spike recovery checks were tested on fish muscle tissue with only PFAA native standards and PFAA IS (these IS are the MPFAC-MXA and PFCA-MXB Wellington standards) to determine recoveries when IS are added at the start, before extraction and oxidation, versus when IS are added at the end, after extraction and oxidation but prior to SPE extraction. The recoveries for these two methods shown in Table S11 indicate that loss of PFAS analytes occurs between the IPE extraction and the SPE extraction during either nitrogen blow down and/or the oxidation heating process. Since we did not have access to the full suite of PFAA internal standards that were not mixed with precursor internal standards to be able to add all the IS prior to oxidation, we chose to use the common method of adding IS after oxidation to the samples. But because there are additional recovery issues in the presence of these tissue matrices, we additionally chose to recovery-correct the PFCA concentrations after making sure that the spike recoveries for IS addition after extraction were repeatable. We used the PFCA spike recovery values from 03-03-2021 that were analyzed with all the samples, to correct for the original concentrations to account for the losses that occur in between the two extraction procedures and IS addition.

Table S11. TOP assay spike recoveries (%) for PFAS in fish muscle tissue

Procedure	All PFAS Spike[1] (n=1)	Individual Precursor Spikes[2] (n=1)		TOP IS After[3] (n=4)	TOP IS Before[4] (n=4)
Analysis Date	**03-03-2021**	**03-03-2021**	**02-22-2021**	**03-23-2021**	**03-23-2021**
PFBA	129			121 ± 79	114 ± 43
PFPeA	73			53 ± 6	100 ± 33
PFHxA	62			63 ± 1	116 ± 5
PFHpA	65			60 ± 1	118 ± 22
PFOA	61			54 ± 2	108 ± 9
PFNA	58			53 ± 3	112 ± 5
PFDA	42			44 ± 7	109 ± 4
PFUnDA	28			37 ± 13	120 ± 7
PFDoDA	20			29 ± 14	114 ± 4
PFTrDA	15			17 ± 9	76 ± 10
PFTeDA	10			8 ± 4	45 ± 5
PFBS	53			17 ± 10	35 ± 17
PFPeS	47				
L-PFHxS	36			25 ± 2	46 ± 7
Br-PFHxS	38				
PFHpS	47				
L-PFOS	31			49 ± 6	166 ± 17
Br-PFOS	54				
PFNS	35				
PFDS	26			33 ± 5	76 ± 3
4:2 FTSA	66				
6:2 FTSA	94				
8:2 FTSA	53		61		
10:2 FTSA	28	38			
FBSA	43				
FHxSA	29				
FOSA	9	12	9		
FDSA	2	5			
N-MeFOSA	0				
N-EtFOSA	0				
L-N-MeFOSAA	5		12		
Br-N-MeFOSAA	0				
L-N-EtFOSAA	3				
Br-N-EtFOSAA	0				
FOSAA	15	19			
N-MeFOSE	0				
N-EtFOSE	0				
ADONA	55				
3:3 FTCA	36				
5:3 FTCA	0				
7:3 FTCA	0				

[1]Percent recovery of fish muscle spiked with native standard that went through the TOP extraction without the addition of oxidant solution and IS spiked after oxidation, [2]Individual precursor spike recoveries that went through the TOP extraction without the addition of oxidant solution and IS spiked after oxidation, [3]Average recovery of fish muscle spiked with PFAA standards and IS spiked after oxidation with TOP extraction including oxidant (n=2) and no oxidant (n=2), [4]Average recovery of fish muscle spiked with PFAA standards and IS spiked before extraction and oxidation with TOP extraction including oxidant (n=2) and no oxidant (n=2).

1.11. Bayesian Inference Method. A full description of the Bayesian inference is in Ruyle et al. 2021 a,b.[9,12] Source code available at: https://github.com/SunderlandLab/oxidizable-pfas-precursor-inference.

For all fish, a measurement error ranging between 5-45% in pre- and post-TOP assay PFCA (C3-C8) concentrations was included based on relative percent differences between sample duplicates. To perform the inference, we used a non-informative Jeffrey's prior[13] with variance equal to 3, upper bound equal to 1000 nM, and lower bound equal to the sum of targeted precursors of that class when available. For example, C4 ECF precursors were bounded by measured concentrations of FBSA. C5 ECF precursors had no lower bound since commercially available analytical standards don't include any C5 ECF precursors. We sampled from the posterior distribution using Markov chain Monte Carlo (MCMC) implemented in *emcee* 3.0.2 in Python 3.7.4.[14] Sequential steps in the Markov chain were determined using a differential evolution algorithm[12] with mean equal to 0.595 (2.38/SQRT[2*ndim]) and standard deviation equal to 1.01, following recommendation of the software.[13] The MCMC was run until the Monte Carlo standard error was 1/SQRT(5000) of the standard deviation of the posterior distribution.

1.12. Suspect Screening & Non-Targeted Analysis. For suspect screening and non-targeted analysis, the concentrated tissue extracts in methanol were reconstituted with 10mM ammonium acetate in Milli-Q water to reach a final ratio of 40/60 (methanol/water). The sample was loaded onto a Phenomenex Gemini C18 analytical column (3 µm, 110 Å, 50 × 2 mm) using 10 mM ammonium acetate in Milli-Q water (A) and 10 mM ammonium acetate in methanol (B) and eluted with a solvent gradient. Initial conditions were 60 % A and 40 % B which gradually increased to 80 % B from 1 to 5.5 minutes and to 100 % B from 5.5 to 7 minutes. The gradient was held for 1 minute, dropped to 40 % B from 8 to 8.5 minutes and held constant for 6.5 minutes for a total run time of 15 minutes.

Table S12. Mass spectrometry acquisition parameters for the targeted QTOF-HRMS/MS analysis

Analyte	Internal Standard	Precursor Mass (Da)	Product Mass (Da)	Declustering Potential (V)	Collision Energy (V)
Perfluoroalkyl Carboxylates					
PFEA (TFA)	[$^{13}C_4$]PFBA	112.99	68.9954	-60	-35
PFPrA	[$^{13}C_4$]PFBA	163.08	119.0865	-60	-35
PFBA	[$^{13}C_4$]PFBA	212.98	168.9890	-25	-12
PFPeA	[$^{13}C_5$]PFPeA	262.98	218.9858	-20	-12
PFHxA	[$^{13}C_5$]PFHxA	312.97	268.9824	-25	-12
PFHpA	[$^{13}C_4$]PFHpA	362.97	318.9791	-25	-12
PFOA	[$^{13}C_8$]PFOA	412.97	368.9761	-25	-14
PFNA	[$^{13}C_9$]PFNA	462.96	418.9732	-25	-14
PFDA	[$^{13}C_6$]PFDA	512.96	468.9700	-25	-16
PFUnDA	[$^{13}C_7$]PFUnDA	562.96	518.9673	-25	-18
PFDoDA	[$^{13}C_2$]PFDoDA	612.95	568.9634	-25	-18
PFTrDA	[$^{13}C_2$]PFDoDA	662.95	618.9617	-30	-19
PFTeDA	[$^{13}C_2$]PFTeDA	712.95	668.9579	-30	-22
Perfluoroalkyl Sulfonates					
PFBS	[$^{13}C_3$]PFBS	298.94	79.9572	-55	-58
PFPeS	[$^{13}C_3$]PFBS	348.94	79.9572	-60	-66
PFHxS	[$^{13}C_3$]PFHxS	398.94	79.9572	-60	-74
PFHpS	[$^{13}C_3$]PFHxS	448.93	79.9572	-65	-88
PFOS	[$^{13}C_8$]PFOS	498.93	79.9573	-65	-108
PFNS	[$^{13}C_8$]PFOS	548.93	79.9571	-50	-120
PFDS	[$^{13}C_8$]PFOS	598.92	79.9569	-45	-120

Fluorotelomer Sulfonates					
4:2 FTSA	$[^{13}C_2]$4:2 FTSA	326.97	306.9676	-50	-28
6:2 FTSA	$[^{13}C_2]$6:2 FTSA	426.97	406.9615	-50	-32
8:2 FTSA	$[^{13}C_2]$8:2 FTSA	526.96	506.9557	-50	-40
10:2 FTSA	$[^{13}C_2]$8:2 FTSA	626.95	606.9479	-50	-40
Perfluoroalkyl Sulfonamides					
FPrSA	$[^{13}C_3]$PFBS	247.96	77.9653	-60	-35
FBSA	$[^{13}C_3]$PFBS	297.96	77.9653	-40	-50
FPeSA	$[^{13}C_3]$PFHxS	347.95	77.9653	-60	-35
FHxSA	$[^{13}C_3]$PFHxS	397.95	77.9654	-10	-70
FOSA	$[^{13}C_8]$FOSA	497.94	77.9654	-60	-85
MeFBSA	$[^{13}C_3]$PFBS	311.97	77.9654	-40	-25
N-MeFOSA	d3-N-MeFOSA	511.96	168.9889	-95	-36
N-EtFOSA	d5-N-EtFOSA	525.98	168.9890	-90	-36
Perfluoroalkyl Sulfonamidoacetic acids					
N-MeFOSAA	d3-N-MeFOSAA	569.97	418.9734	-75	-28
N-EtFOSAA	d5-N-EtFOSAA	583.98	418.9731	-50	-36
Polyfluoroalkyl ether carboxylates					
ADONA	$[^{13}C_4]$PFHpA	376.97	250.9754	-55	-16
HFPO-DA	$[^{13}C_3]$HFPO-DA	329.05	284.9784	-48	-6
9ClPF3ONS	$[^{13}C_8]$PFOS	530.89	350.9450	-120	-30
11ClPF3OUdS	$[^{13}C_8]$PFOS	630.89	450.9383	-160	-40
PFECHS	$[^{13}C_3]$PFHxS	460.93	98.9557	-10	-70
Fluorotelomer Carboxylates					
5:3 FTCA	$[^{13}C_9]$PFNA	341.00	286.9924	-70	-14
7:3 FTCA	$[^{13}C_4]$PFHpA	441.00	336.9892	-70	-14
9:3 FTCA	$[^{13}C_7]$PFUnDA	540.99	416.9766	-70	-14
Internal Standards					
$[^{13}C_4]$PFBA	$[^{13}C_3]$PFBA	215.99 / 216.99	171.9994 / 171.9989	-25	-12
$[^{13}C_5]$PFPeA		267.99	222.9995	-20	-12
$[^{13}C_5]$PFHxA		317.99	272.9961	-25	-12
$[^{13}C_4]$PFHpA		366.98	321.9900	-25	-12
$[^{13}C_8]$PFOA	$[^{13}C_2]$PFOA	420.99 / 414.97	376.0003 / 369.9799	-25	-14
$[^{13}C_9]$PFNA		471.99	427.0009	-25	-14
$[^{13}C_6]$PFDA		518.98	473.9867	-25	-16
$[^{13}C_7]$PFUnDA		569.98	524.9864	-25	-18
$[^{13}C_2]$PFDoDA		614.96	569.9673	-25	-18
$[^{13}C_2]$PFTeDA		714.96	669.9630	-55	-25
$[^{13}C_3]$PFBS		301.95	79.9574	-55	-58
$[^{13}C_3]$PFHxS		401.95	79.9572	-60	-50
$[^{13}C_8]$PFOS	$[^{13}C_4]$PFOS	506.96 / 502.94	79.9572 / 79.9574	-65	-108
$[^{13}C_2]$4:2 FtS		328.98	80.9649	-50	-40
$[^{13}C_2]$6:2 FtS		428.97	80.9651	-55	-60
$[^{13}C_2]$8:2 FtS		528.97	80.9648	-55	-50
$[^{13}C_8]$FOSA		505.97	77.9654	-60	-85
d3-N-MeFOSAA		572.98	418.9744	-75	-28
d5-N-EtFOSAA		589.01	418.9727	-75	-37
d3-N-MeFOSA		514.98	168.9896	-90	-36
d5-N-EtFOSA		531.01	168.9882	-90	-34
$[^{13}C_3]$HFPO-DA		331.98	286.9843	-40	-10

Two novel PFAS, FPrSA and FPeSA, the C3 and C5 perfluoroalkyl sulfonamides were tentatively identified using the suspect screening approach. The raw data were screened using the SCIEX Fluorochemical HRMS/MS Spectral Library 2.0 in the SCIEX OS software based on precursor mass, isotope pattern, retention time, exact mass accuracy (< 5 ppm), and MS/MS fragmentation matching (Table S13). Following the conventions from Charbonnet et al.,[15] both compounds were assigned the confidence level of identification 2a (MS/MS spectral library match). Concentrations of the tentatively identified compounds were estimated semi-quantitatively by applying a relative response factor of a closely related native standard (PFBS used for FPrSA and PFHxS used for FPeSA) and its mass labelled internal standard. The response factor was assigned based primarily on the functional group, retention time and chain length. Consecutively, using the non-targeted approach, the data was searched for additional compounds not included in the targeted list or suspects library by examining compounds with the negative CF_2-normalized Kendrick mass defect and peak intensity greater than 1000 counts.

Table S13. Suspect screening identification of perfluoroalkyl sulfonamide precursors

Analyte Acronym	Analyte Name	Internal Standard	Precursor Ion m/z	Average Mass Error (ppm)	Detection Rate (%)	Confidence Level
FPrSA	Perfluoropropane sulfonamide	[$^{13}C_3$]PFBS	247.9622	0.33 ± 0.75	75	2a
FPeSA	Perfluoropentane sulfonamide	[$^{13}C_3$]PFHxS	347.9558	-0.16 ± 1.0	100	2a

1.13. Statistical Analyses. 7:3 FTCA was detected in >90% of fish samples measured by LC-MS/MS in unit resolution but was excluded from further interpretation due to the presence of an interference identified by high resolution analysis. In unit resolution, a nominal mass of 441 is used for 7:3 FTCA. In high resolution, 7:3 FTCA has an exact precursor mass of 440.9979. The HRMS spectra for 7:3 FTCA showed an additional mass at 441.2263 with high intensity. There were interfering fragments for the two product masses as well (336.9899 (7:3 FTCA) vs 337.2381 and 316.9842 (7:3 FTCA) vs 317.2114) indicating this interference can't be distinguished from 7:3 FTCA in unit resolution using either m/z ratio.

Due to limited sample sizes, non-parametric hierarchical clustering was conducted to identify commonalities in PFAS profiles detected among water sampling locations (Figure S2). Statistical significance analyses on fish concentrations were then tested using the water clusters.

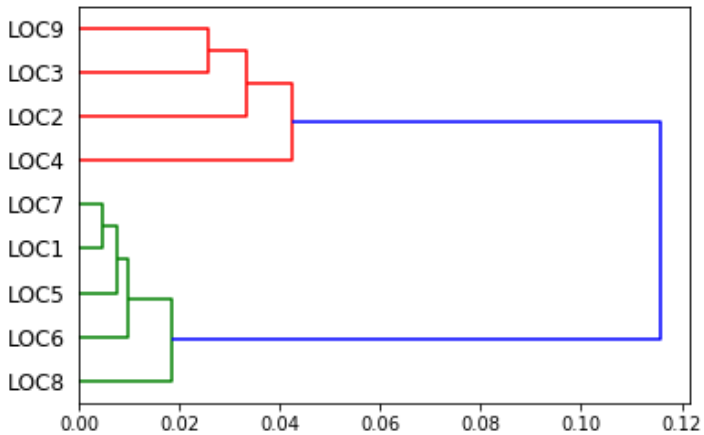


Figure S2. Hierarchical clustering of surface water locations. Two distinct clusters shown. Cluster 1 includes locations 2, 3, 4, and 9. Cluster 2 includes locations 1, 5, 6, 7, and 8.

For comparisons of more than two populations of parametric data, data were first log-transformed and the Type-II ANOVA with Tukey HSD *post hoc* test was used to identify groups of fish that were significantly different for PFAS analytes detected in ≥70% of samples (Table S14).

Table S14. Statistical analysis results for fish concentrations across water clusters and fish species[1]

Compound	Fish Concentrations	
	ANOVA (Type II)	Tukey HSD (Species pairs)
PFOA	p=0.0116*	
PFNA	p=0.0005*	YP-LB, YP-SB
PFDA	p=0.0004*	
PFUnDA	p=0.0021*	
PFDoDA	p=0.0021*	
PFTrDA	p=0.0214*	
PFTeDA	p=0.0085*	LW-SB
∑PFOS	p=0.0112*	
PFDS	p=0.1310	
FOSA	p=0.0377*	YP-LB
L-N-MeFOSAA	p=0.0880	
L-N-EtFOSAA	p=0.6414	

*Significantly different ($p<0.05$). [1]Fish species include species with more than one fish sample: yellow perch (YP), smallmouth bass (SB), largemouth bass (LB), lake whitefish (LW).

2. Results

2.1. Estimated Fish Consumption Limits. The state of New Hampshire Department of Environmental Services (NHDES) developed guidelines for fish consumption in 2019 modeled after those developed for mercury (1 meal per month for high risk populations (pregnant/nursing women and children) and 4 meals per month for low risk populations (all other adults)).[16,17] The state has been developing surface water quality standards and fish consumption advisories based on PFAS levels.

We estimated the magnitudes of fish that could be consumed before exceeding the reference dose (RfD) based on the average body weights (BW) for children and adults and average fish meal sizes for each group (fish consumption triggers). Equation S1 defines the calculation for a daily trigger concentration (ng g^{-1}):

$$\text{Consumption Trigger}_{\text{daily}} = \left(\frac{\text{RfD x BW}}{\text{Meal Size}}\right) \quad \text{Equation S1}$$

Here we use their recommended available RfD data to derive our own fish consumption triggers for four PFAS. NHDES derived RfD values in 2019 for PFOA, PFOS, PFNA and PFHxS based on other Agency's proposed values.[17] The RfD values adopted by NHDES for these four PFAS are 6.1 ng kg^{-1} d^{-1} for PFOA, 3.0 ng kg^{-1} d^{-1} for PFOS, 4.3 ng kg^{-1} d^{-1} for PFNA, and 4.0 ng kg^{-1} d^{-1} for PFHxS. For PFOA and PFNA the RfD is based on liver effects (hepatotoxicity). For PFHxS and PFOS, the RfD is based on female reproduction and immunotoxic effects, respectively.[17] We used an average body weight for an adult in the U.S. of 80 kg and average body weight for child<7 years of age of 16.9 kg.[18] Average meal sizes for adult is 227 g (8oz) and for child is 113.4 g (4oz).

For less frequent consumption triggers, the daily consumption trigger is multiplied by the appropriate timeframe (7-fold for weekly, 30.4-fold for monthly, 365-fold for yearly). Table S15 shows the consumption frequency trigger concentrations for the PFAS with RfD values derived by NHDES. PFOS has the most conservative consumption trigger due to its lower RfD compared to the other PFAS.

Comparing these consumption trigger values to the mean concentrations detected in the fish species in this study, the only targeted PFAS of concern in these fish species based on concentration is PFOS.

Table S15. Daily, weekly, monthly, and yearly fish consumption advisory triggers (ng g^{-1}) for four PFAS

	PFOA	PFNA	PFHxS	PFOS	PFOA	PFNA	PFHxS	PFOS
Low-Risk Population (Adults (8 oz meal size))					**High-Risk Population (Children (4 oz meal size))**			
Daily	2.15	1.52	1.41	1.06	0.91	0.64	0.60	0.45
Weekly	15.1	10.6	9.87	7.40	6.36	4.49	4.17	3.13
Monthly	65.4	46.1	42.9	32.1	27.6	19.5	18.1	13.6
Yearly	785	553	515	386	332	234	218	163

Daily consumption for adults of <1.06 ng g^{-1} PFOS is exceeded for all species analyzed except Brown Bullhead and Chain Pickerel which only had one sample each collected. Based on a weekly adult consumption trigger of <7.4 ng g^{-1}, smallmouth bass (n=5) is the only species above this limit. But for high-risk populations, a weekly child consumption trigger of <3.13 ng g^{-1} is additionally exceeded by bluegill (n=10), largemouth bass (n=6), pumpkinseed (n=9) and yellow perch (n=8). For PFOA, PFNA, and PFHxS, no fish samples analyzed exceed the consumption triggers for low or high-risk populations.

2.2. Concentrations and Detection. A summary of PFAS concentrations in the surface water and fish muscle tissue samples and associated summary metrics are detailed in the following tables:

Table S16. PFAS concentrations, means, medians and detection frequencies for fish tissue samples.

See attached excel file: Table S16 Fish Concentrations

Table S17. PFAS concentrations, means, medians and detection frequencies for surface water samples.

See attached excel file: Table S17 Water Concentrations

Detection frequency of PFAS in paired fish muscle tissue and surface water samples is shown in Figure S3. Additional precursors, FBSA and 7:3 FTCA, are included in this figure as they had a high detection frequency in the fish muscle but were not analyzed (NA) in the surface water samples due to sample volume/inadequate storage for further extraction and analysis of additional PFAS compounds.

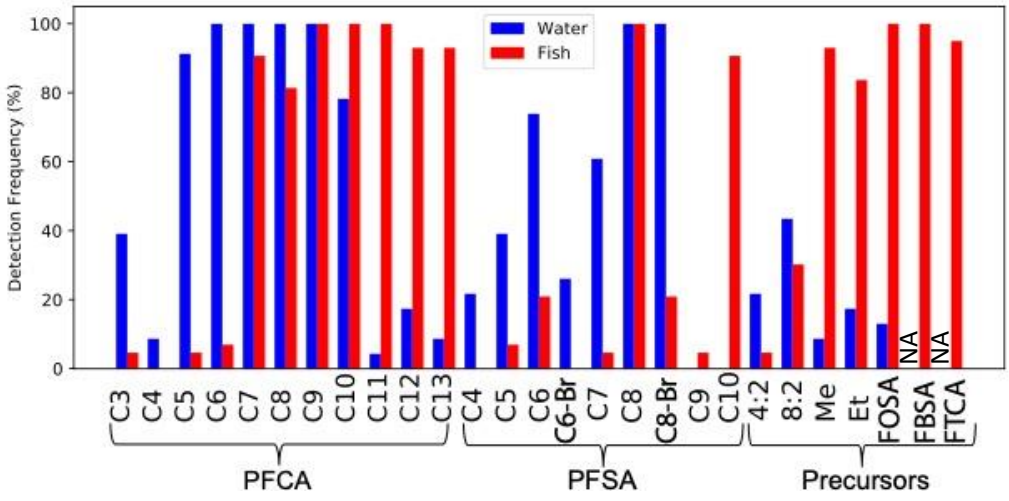


Figure S3. Detection frequency of PFAS detected in paired fish and water samples. PFAS are labeled by perfluorinated carbon chain length: PFCA range from C3-C13, PFSA range from C4-C10 with C6 and C8 separated into linear and branched (Br), and precursors include 4:2 and 8:2 FTSA, L-N-MeFOSAA (Me), L-N-EtFOSAA (Et), FOSA, FBSA, and 7:3 FTCA (FTCA).

2.3. Bioaccumulation Factors. Field-measured bioaccumulation factors (BAF) were calculated for all PFAS in all the samples from each location. Measured BAF were determined for samples with detectable concentrations in both matrices (fish muscle and surface water). Potential BAF were determined for samples when there were only detectable levels in one matrix using the MDL as the concentration for the other matrix. Minimum potential BAF were determined if the concentration in the water was <MDL and the concentration in the fish muscle was >MDL. Maximum potential BAF were determined if the concentration in the fish muscle was <MDL and the concentration in the water was >MDL. Figure S4 illustrates the range in log BAF values for individual fish species for each PFAS from all nine sampling locations. Table S18 details the average BAF ± standard deviation for each species.

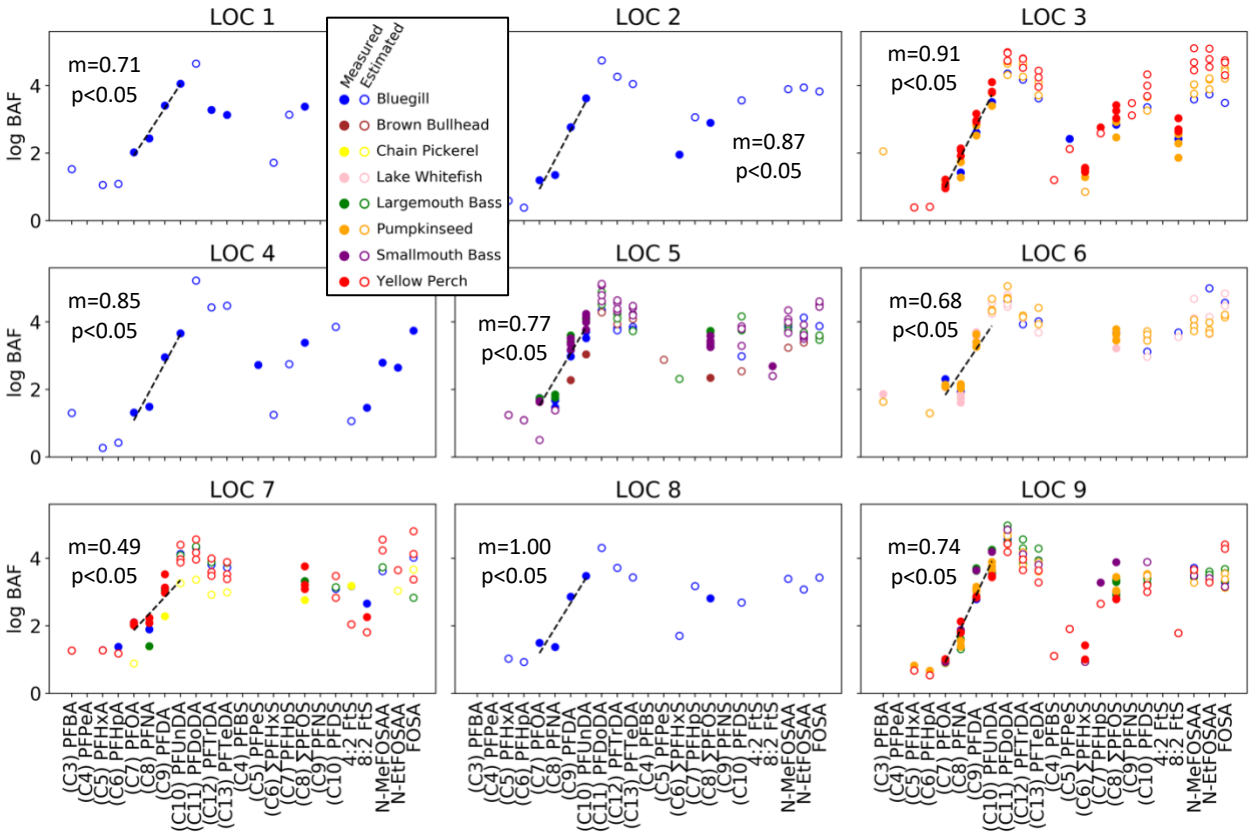


Figure S4. Range of log BAF values for individual species for each PFAS from all nine sampling locations. Colors represent individual species. Closed markers represent measured BAF values calculated when fish and water samples had concentrations >MDL in both. Open markers represent minimum/maximum potential BAF values calculated when only one phase had a detectable level, and the other phase used the MDL value for calculation. The regression line (black dashed line) is based on measured BAF data for C7-C10 PFCA and is significant for all locations ($p < 0.05$).

Table S18. Empirically derived bioaccumulation factors (BAF) (L kg^{-1} ww) (average ± standard deviation when replicates). Average BAFs only calculated when >50% of species had individual BAFs calculated. Values highlighted gray are measured BAFs calculated when both samples were >MDL. Italicized average BAF values include minimum potential values calculated when only fish muscle was >MDL.

	Fish Species BAF values							
Compound	**Pumpkinseed (n=9)**	**Bluegill (n=10)**	**Yellow Perch (n=8)**	**Brown Bullhead (n=1)**	**Smallmouth Bass (n=5)**	**Lake Whitefish (n=3)**	**Chain Pickerel (n=1)**	**Largemouth Bass (n=6)**
PFOA (C7)	55 ± 62	63 ± 64	50 ± 53	43	35 ± 18	123 ± 5		45 ± 40
PFNA (C8)	67 ± 49	69 ± 80	125 ± 38			56 ± 16		52 ± 20
PFDA (C9)	1565 ± 1224	1339 ± 1114	1290 ± 888	189	2720 ± 1109	3580 ± 1500	192	2955 ± 1762
PFUnDA (C10)	5502 ± 1822	4771 ± 2726	6288 ± 4026	1089	12561 ± 4652			12615 ± 5525
PFDoDA (C11)	*>51279 ± 26465*	*>45090 ± 44419*	*>43277 ± 34874*	*>19154*	*>85164 ± 39606*	*>42004 ± 18497*	*>2308*	*>54312 ± 29457*
PFTrDA (C12)	*>21055 ± 14935*	*>10940 ± 7672*	*>23676 ± 25983*	*>8683*	*>27893 ± 12395*		*>833*	*>16678 ± 10784*
PFTeDA (C13)	*>10044 ± 6964*	*>8713 ± 8198*	*>9247 ± 8978*	*>12133*	*>18836 ± 9254*		*>972*	*>10325 ± 6305*
∑PFHxS (C6)			27 ± 10					
∑PFOS (C8)	2247 ± 1910	1854 ± 1250	1917 ± 1678	220	3649 ± 2318	3432 ± 1923	579	2955 ± 1871
PFDS (C10)	*>3651 ± 1091*	*>2363 ± 2029*	*>6092 ± 7501*	*>348*	*>5835 ± 2667*	*>2430 ± 1351*		*>4845 ± 5021*
8:2 FTSA			552 ± 379					
L-N-MeFOSAA	*>6826 ± 3545*	*>6010 ± 3551*	*>43511 ± 43600*	*>1727*	*>10300 ± 7432*	*>22909 ± 22058*		*>5053 ± 1990*
L-N-EtFOSAA	*>7907 ± 5480*	*>16801 ± 31054*	*>45323 ± 49981*	*>2439*	*>4333 ± 2226*	*>7725 ± 5437*	*>1088*	*>3996 ± 634*
FOSA	*>14124 ± 10807*	*>8057 ± 10440*	*>31018 ± 21943*	*>3705*	*>26397 ± 14781*	*>38481 ± 27399*	*>4656*	*>3022 ± 1461*
	Fish Species Log BAF values							
PFOA (C7)	1.42 ± 0.57	1.59 ± 0.46	1.43 ± 0.53	1.63	1.46 ± 0.36	2.09 ± 0.02		1.45 + 0.51
PFNA (C8)	1.71 ± 0.33	1.67 ± 0.36	2.08 + 0.15			1.74 ± 0.12		1.69 + 0.19
PFDA (C9)	3.08 ± 0.33	3.02 + 0.30	3.05 ± 0.23	2.28	3.41 ± 0.18	3.52 + 0.20	2.28	3.38 + 0.34
PFUnDA (C10)	3.72 ± 0.17	3.64 ± 0.19	3.73 ± 0.27	3.04	4.07 ± 0.19			4.05 ± 0.26
PFDoDA (C11)	*>4.66 ± 0.21*	*>4.54 + 0.29*	*>4.50 ± 0.38*	*>4.28*	*>4.89 ± 0.21*	*>4.60 ± 0.18*	*>3.36*	*>4.67 ± 0.26*
PFTrDA (C12)	*>4.23 ± 0.29*	*>3.92 ± 0.34*	*>4.10 ± 0.54*	*>3.94*	*>4.41 ± 0.21*		*>2.92*	*>4.15 ± 0.27*
PFTeDA (C13)	*>3.93 ± 0.25*	*>3.80 ± 0.37*	*>3.80 ± 0.41*	*>4.08*	*>4.22 ± 0.26*		*>2.99*	*>3.95 ± 0.26*
∑PFHxS (C6)			1.39 ± 0.22					
∑PFOS (C8)	3.20 + 0.41	3.18 ± 0.29	3.17 ± 0.32	2.34	3.50 ± 0.24	3.49 ± 0.26	2.76	3.39 + 0.31
PFDS (C10)	*>3.54 + 0.14*	*>3.25 + 0.29*	*>3.50 ± 0.54*	*>2.54*	*>3.71 ± 0.28*	*>3.32 ± 0.31*		*>3.53 + 0.37*
8:2 FTSA			2.66 ± 0.32					
L-N-MeFOSAA	*>3.77 ± 0.26*	*>3.68 ± 0.37*	*>4.45 ± 0.48*	*>3.24*	*>3.91 ± 0.34*	*>4.23 ± 0.41*		*>3.68 ± 0.17*
L-N-EtFOSAA	*> 3.79 ± 0.35*	*>3.76 ± 0.68*	*>4.27 ± 0.77*	*>3.39*	*>3.60 ± 0.19*	*>3.82 ± 0.28*	*>3.04*	*>3.60 + 0.07*
FOSA	*>3.97 ± 0.49*	*>3.72 ± 0.38*	*>4.34 ± 0.46*	*>3.57*	*>4.24 ± 0.61*	*>4.51 ± 0.32*	*>3.67*	*>3.41 ± 0.31*

2.4. Inferred Oxidizable Precursors. Results from seven of twenty (35%) fish tissue samples oxidized using the TOP assay contained at least one C3-C8 PFCA analyte with > 1 nmol L^{-1} increase in PFCA concentration after oxidation. Unknown precursors accounted for an increase in molarity > 1 nmol L^{-1}, after subtracting measured concentrations of targeted precursors. Targeted precursors accounted for molarity increases ranging from 2.4 to 6.2 nmol L^{-1} for the sum of C3-C8 PFCA. These molarity increases determined as the estimated total amount of unknown precursors not yet accounted for, make up between 26% to 99% of the molar increase in C3-C8 PFCA concentration after oxidation in these samples. Figure S5 illustrates these increases in C3-C8 PFCA post-oxidation for the seven samples.

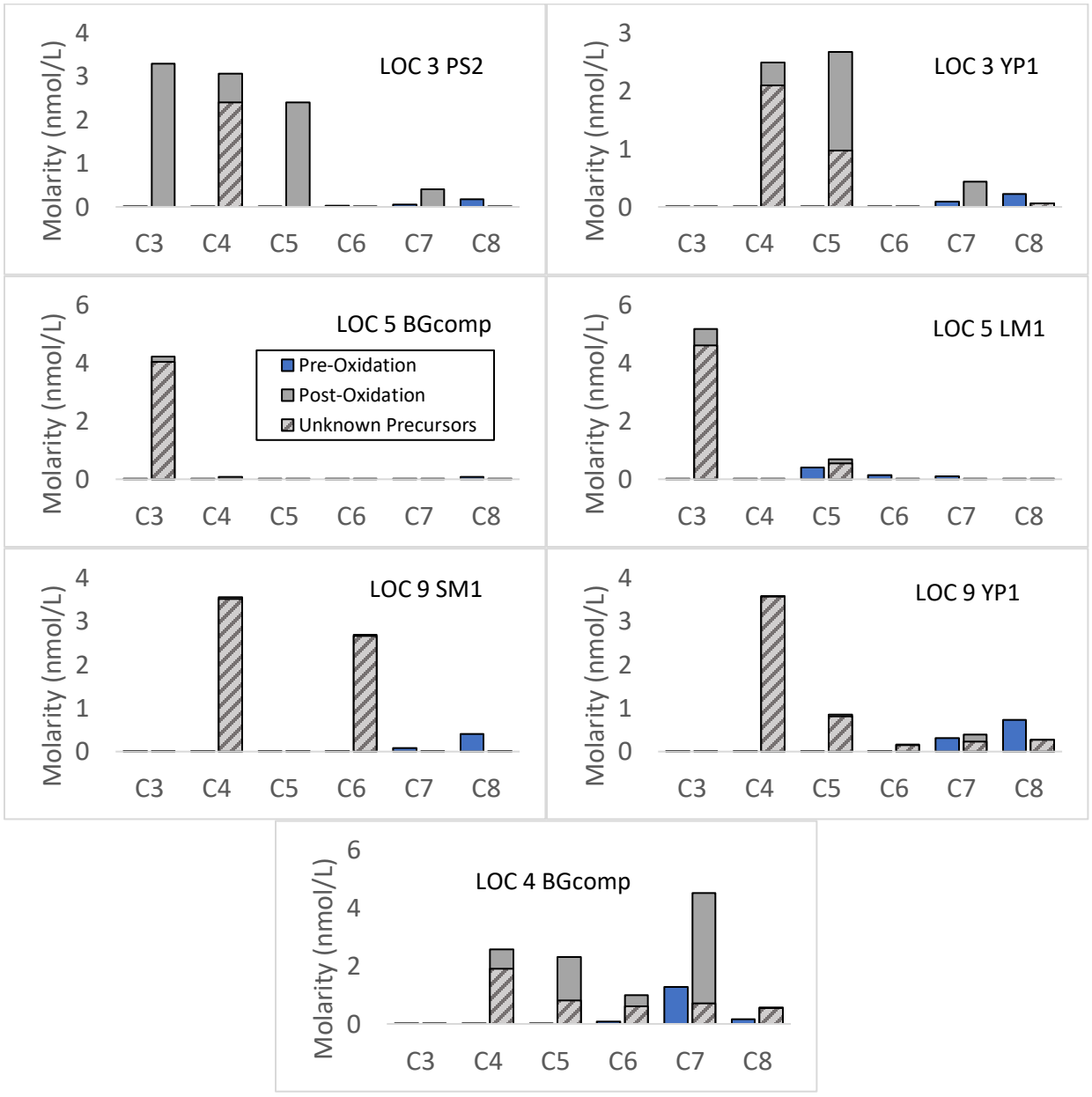


Figure S5. Samples with an increase in C3-C8 PFCA molarity > 1 nmol/L after oxidation. C3-C8 PFCA molarities pre-oxidation (blue bars), C3-C8 PFCA molarities post-oxidation (grey bars), and C3-C8 PFCA molarities post-oxidation not accounted for by targeted precursor measurements (grey dashed bars).

Precursor concentrations categorized by chain length and manufacturing origin were inferred using Bayesian inference. Figure S6 shows probability density functions of the inferred precursors for LOC 5 and LOC 9 samples. In this figure the probability density (y-axis) indicates the probability of occurrence of a given precursor group, while the x-axis shows the expected concentrations (nM) given constraints from PFCA produced in the TOP assay.

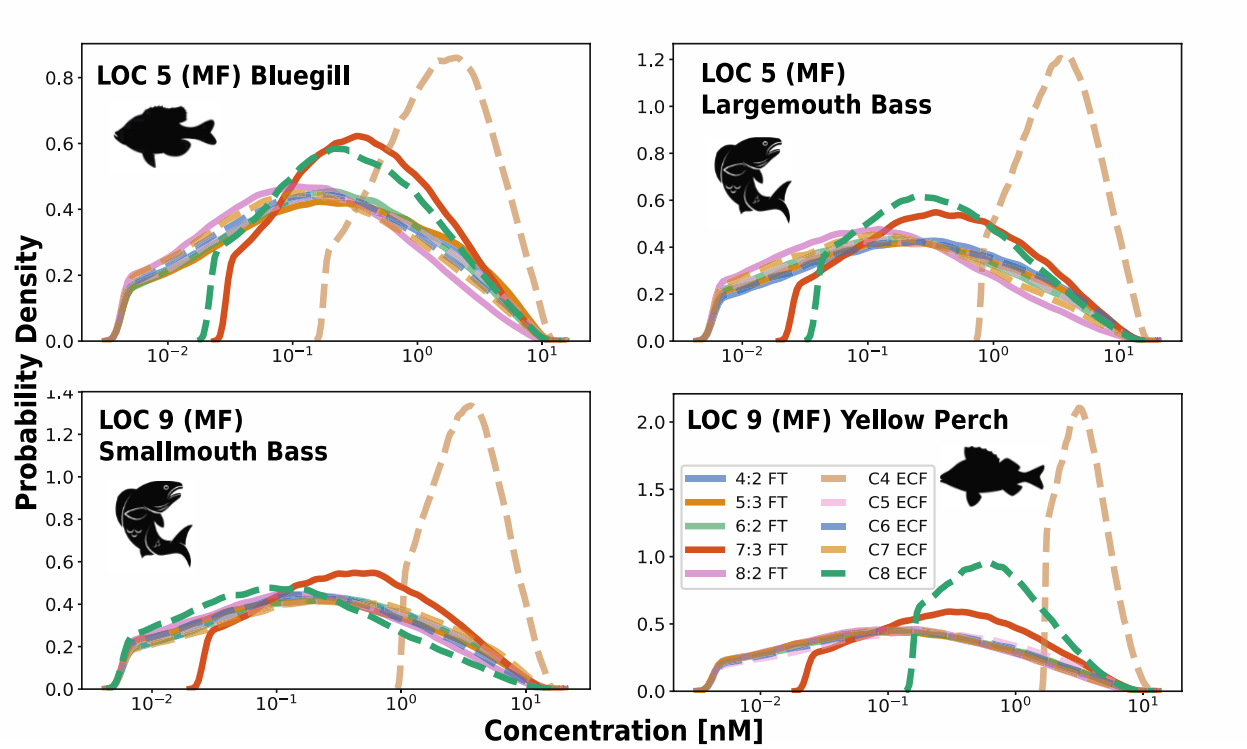


Figure S6. Inferred concentrations of oxidizable precursors and their perfluorinated carbon chain length using Bayesian inference on TOP assay results. Panels show probability density functions estimated by the nonparametric kernel density of the concentrations of oxidizable precursors for bluegill (BGcomp) and largemouth bass (LM1) from location 5 (LOC 5) where manufacturing (MF) is the potential source and for smallmouth bass (SM1) and yellow perch (YP1) from location 9 (LOC 9) where manufacturing (MF) is also a potential source. A high probability density indicates greater probability of the estimate.

Table S19. Bayesian inference average median and expected mean ± standard deviation molar concentrations (nmol L^{-1} (nM)) and 95% confidence interval (CI) results for replicate (n=4) simulations of FT and ECF precursors in seven fish tissue samples with covariance <52%.

Fish	BI Results	4:2 FT	5:3 FT	6:2 FT	7:3 FT	8:2 FT	C4 ECF	C5 ECF	C6 ECF	C7 ECF	C8 ECF	Sum
LOC3PS2	Median (nM)	0.15 ± 0.03	0.15 ± 0.01	0.75 ± 0.02	0.82 ± 0.03	0.37 ± 0.01	13 ± 0.10	0.17 ± 0.01	3.3 ± 0.02	0.11 ± 0.01	1.1 ± 0.04	20.0
	Covariance (%)	17%	7%	3%	4%	2%	1%	4%	1%	8%	4%	
	Mean (nM)	0.47 ± 0.07	0.45 ± 0.01	1.1 ± 0.05	1.2 ± 0.04	0.64 ± 0.02	13 ± 0.10	0.53 ± 0.03	3.7 ± 0.04	0.34 ± 0.02	1.4 ± 0.04	23.1
	Covariance (%)	15%	3%	4%	3%	4%	1%	5%	1%	5%	3%	
	95 % CI	0.011-3.06	0.011-2.97	0.197-4.31	0.239-4.17	0.079-2.96	10.9-17.4	0.012-3.35	1.96-7.41	0.011-2.24	0.423-4.34	13.9-52.2
LOC3YP1	Median (nM)	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.38 ± 0.002	0.42 ± 0.005	10 ± 0.07	0.08 ± 0.03	1.8 ± 0.03	0.05±0.008	0.73 ± 0.03	13.9
	Covariance (%)	15%	29%	16%	0%	1%	1%	38%	2%	15%	4%	
	Mean (nM)	0.19 ± 0.03	0.20 ± 0.07	0.17 ± 0.01	0.50 ± 0.007	0.52 ± 0.005	10 ± 0.27	0.24 ± 0.13	1.9 ± 0.02	0.14 ± 0.01	0.84 ± 0.03	14.9
	Covariance (%)	18%	36%	7%	1%	1%	3%	52%	1%	8%	4%	
	95 % CI	0.007-1.07	0.007-1.12	0.006-1.00	0.139-1.52	0.175-1.45	9.58-11.7	0.007-1.31	1.29-3.23	0.006-0.84	0.397-1.94	11.6-25.1
LOC4BGcomp	Median (nM)	0.22 ± 0.02	0.23 ± 0.02	0.22 ± 0.02	1.3 ± 0.01	0.24 ± 0.02	2.3 ± 0.04	0.23 ± 0.02	1.1 ± 0.03	0.22 ± 0.01	8.4 ± 0.10	14.5
	Covariance (%)	8%	7%	9%	1%	8%	2%	10%	3%	7%	1%	
	Mean (nM)	0.81 ± 0.05	0.85 ± 0.04	0.84 ± 0.04	2.1 ± 0.01	0.88 ± 0.04	3.0 ± 0.09	0.85 ± 0.08	1.8 ± 0.05	0.78 ± 0.03	9.1 ± 0.07	21.0
	Covariance (%)	7%	5%	4%	1%	5%	3%	9%	3%	4%	1%	
	95 % CI	0.012-5.69	0.012-5.96	0.012-5.91	0.292-8.41	0.012-5.99	0.875-9.42	0.012-5.77	0.215-7.74	0.012-5.30	5.34-16.9	6.80-77.1
LOC5BGcomp	Median (nM)	0.18 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.36 ± 0.02	0.10 ± 0.02	1.5 ± 0.14	0.16 ± 0.009	0.14 ± 0.01	0.12 ± 0.01	0.24 ± 0.04	3.16
	Covariance (%)	12%	10%	14%	4%	24%	9%	6%	9%	10%	16%	
	Mean (nM)	0.78 ± 0.07	0.77 ± 0.05	0.62 ± 0.02	0.86 ± 0.04	0.42 ± 0.09	2.2 ± 0.15	0.71 ± 0.03	0.61 ± 0.03	0.55 ± 0.04	0.69 ± 0.10	8.20
	Covariance (%)	9%	7%	4%	5%	22%	7%	4%	5%	7%	14%	
	95 % CI	0.006-5.26	0.006-5.13	0.006-4.39	0.036-4.80	0.005-3.09	0.218-7.75	0.006-4.91	0.006-4.34	0.006-4.01	0.026-4.26	0.32-47.9
LOC5LM1	Median (nM)	0.22 ± 0.01	0.21 ± 0.01	0.19 ± 0.02	0.38 ± 0.02	0.13 ± 0.007	3.2 ± 0.07	0.18 ± 0.02	0.21 ± 0.01	0.15 ± 0.02	0.35 ± 0.02	5.22
	Covariance (%)	6%	6%	9%	4%	5%	2%	9%	7%	11%	7%	
	Mean (nM)	0.91 ± 0.04	0.89 ± 0.03	0.82 ± 0.05	1.1 ± 0.03	0.54 ± 0.03	3.9 ± 0.09	0.80 ± 0.03	0.87 ± 0.02	0.67 ± 0.05	0.91 ± 0.04	11.4
	Covariance (%)	5%	3%	6%	3%	6%	2%	4%	3%	8%	4%	
	95 % CI	0.008-6.32	0.008-6.23	0.008-5.81	0.031-6.18	0.007-4.05	0.903-11.0	0.008-5.68	0.008-6.10	0.007-4.96	0.045-5.46	1.03-61.8
LOC9SM1	Median (nM)	0.22 ± 0.004	0.20 ± 0.01	0.21 ± 0.01	0.41 ± 0.02	0.15 ± 0.008	3.3 ± 0.07	0.22 ± 0.01	0.16 ± 0.01	0.24 ± 0.02	0.11±0.008	5.26
	Covariance (%)	2%	7%	7%	5%	5%	2%	6%	8%	7%	7%	
	Mean (nM)	0.91 ± 0.03	0.85 + 0.03	0.86 ± 0.04	1.1 ± 0.05	0.66 ± 0.03	4.0 ± 0.06	0.91 ± 0.03	0.71 ± 0.03	0.93 ± 0.05	0.53 ± 0.02	11.5
	Covariance (%)	3%	4%	5%	4%	5%	2%	4%	4%	5%	5%	
	95 % CI	0.008-6.21	0.008-6.03	0.008-5.93	0.032-6.25	0.007-4.80	1.12-10.8	0.008-6.21	0.007-5.17	0.008-6.27	0.007-4.09	1.21-61.8
LOC9YP1	Median (nM)	0.12 ± 0.006	0.12 ± 0.003	0.13±0.003	0.29 ± 0.01	0.11 ± 0.003	3.3 ± 0.02	0.14 ± 0.009	0.12±0.005	0.11±0.004	0.66 ± 0.02	5.11
	Covariance (%)	6%	3%	2%	5%	3%	1%	6%	4%	4%	4%	
	Mean (nM)	0.48 ± 0.01	0.50 ± 0.008	0.51 ± 0.01	0.70 ± 0.03	0.42 ± 0.007	3.7 ± 0.02	0.54 ± 0.03	0.48 ± 0.01	0.43±0.005	1.0 ± 0.04	8.81
	Covariance (%)	3%	2%	3%	5%	2%	1%	5%	3%	1%	4%	
	95 % CI	0.005-3.43	0.005-3.50	0.006-3.57	0.028-3.94	0.005-2.97	1.78-7.80	0.006-3.65	0.006-3.38	0.005-3.05	0.173-4.19	2.02-39.5

2.5. Evaluation in precursor results across analytical methods. In comparing individual and total precursor concentrations across methods, targeted analysis accounts for a large proportion of total precursor amount based on expected mean estimates for LOC 3 (75% for LOC3 PS2, 92% for LOC3 YP1) and LOC 4 (46% for LOC4 BGcomp) samples, with less accounted for in LOC 5 (8% for LOC5 BGcomp, 11% for LOC5 LM1) and LOC 9 (11% for LOC9 SM1, 22% for LOC9 YP1) samples.

Table S20. Comparison between precursor group concentrations (nM) quantified using the three methods (<DL = below detection limit).

Sample	Method Concentration (nmol L^{-1})	C4 ECF (FBSA)	C6 ECF (FHxSA)	C8 ECF (FOSA, N-MeFOSAA, N-EtFOSAA)	∑Precursors All precursors measured
LOC3 PS2	TOP+BI: 95 % CI Range	10.9-17.4	1.96-7.41	0.42-4.34	13.9-52.2
	(Expected Mean)	(13)	(3.7)	(1.4)	(23.1)
	Targeted Analysis	10.8	1.88	0.39	17.4
	Suspect Screening Analysis	10.6	2.94	0.42	49
LOC 3 YP1	TOP+BI: 95 % CI Range	9.58-11.7	1.29-3.23	0.40-1.94	11.6-25.1
	(Expected Mean)	(10)	(1.9)	(0.84)	(14.9)
	Targeted Analysis	9.54	1.26	0.38	13.7
	Suspect Screening Analysis	8.65	2.57	0.41	35
LOC 4 BGcomp	TOP+BI: 95 % CI Range	0.88-9.42	0.22-7.74	5.34-16.9	6.80-77.1
	(Expected Mean)	(3.0)	(1.8)	(9.1)	(21.0)
	Targeted Analysis	0.81	0.19	5.17	9.76
	Suspect Screening Analysis	1.11	0.27	7.28	11
LOC 5 BGcomp	TOP+BI: 95 % CI Range	0.22-7.75	0.006-4.34	0.026-4.26	0.32-47.9
	(Expected Mean)	(2.2)	(0.61)	(0.69)	(8.20)
	Targeted Analysis	0.18	<DL	0.02	0.66
	Suspect Screening Analysis	0.18	<DL	0.03	0.24
LOC 5 LM1	TOP+BI: 95 % CI Range	0.91-11.0	0.008-6.10	0.045-5.46	1.03-61.8
	(Expected Mean)	(3.9)	(0.87)	(0.91)	(11.4)
	Targeted Analysis	0.79	<DL	0.04	1.21
	Suspect Screening Analysis	0.88	<DL	0.02	1.1
LOC 9 SM1	TOP+BI: 95 % CI Range	1.12-10.8	0.007-5.17	0.007-4.09	1.21-61.8
	(Expected Mean)	(4.0)	(0.71)	(0.53)	(11.5)
	Targeted Analysis	1.01	<DL	<DL	1.30
	Suspect Screening Analysis	1.08	<DL	0.02	1.9
LOC 9 YP1	TOP+BI: 95 % CI Range	1.78-7.80	0.006-3.38	0.17-4.19	2.02-39.5
	(Expected Mean)	(3.7)	(0.48)	(1.0)	(8.81)
	Targeted Analysis	1.70	<DL	0.16	1.97
	Suspect Screening Analysis	1.75	0.49	0.26	4.4

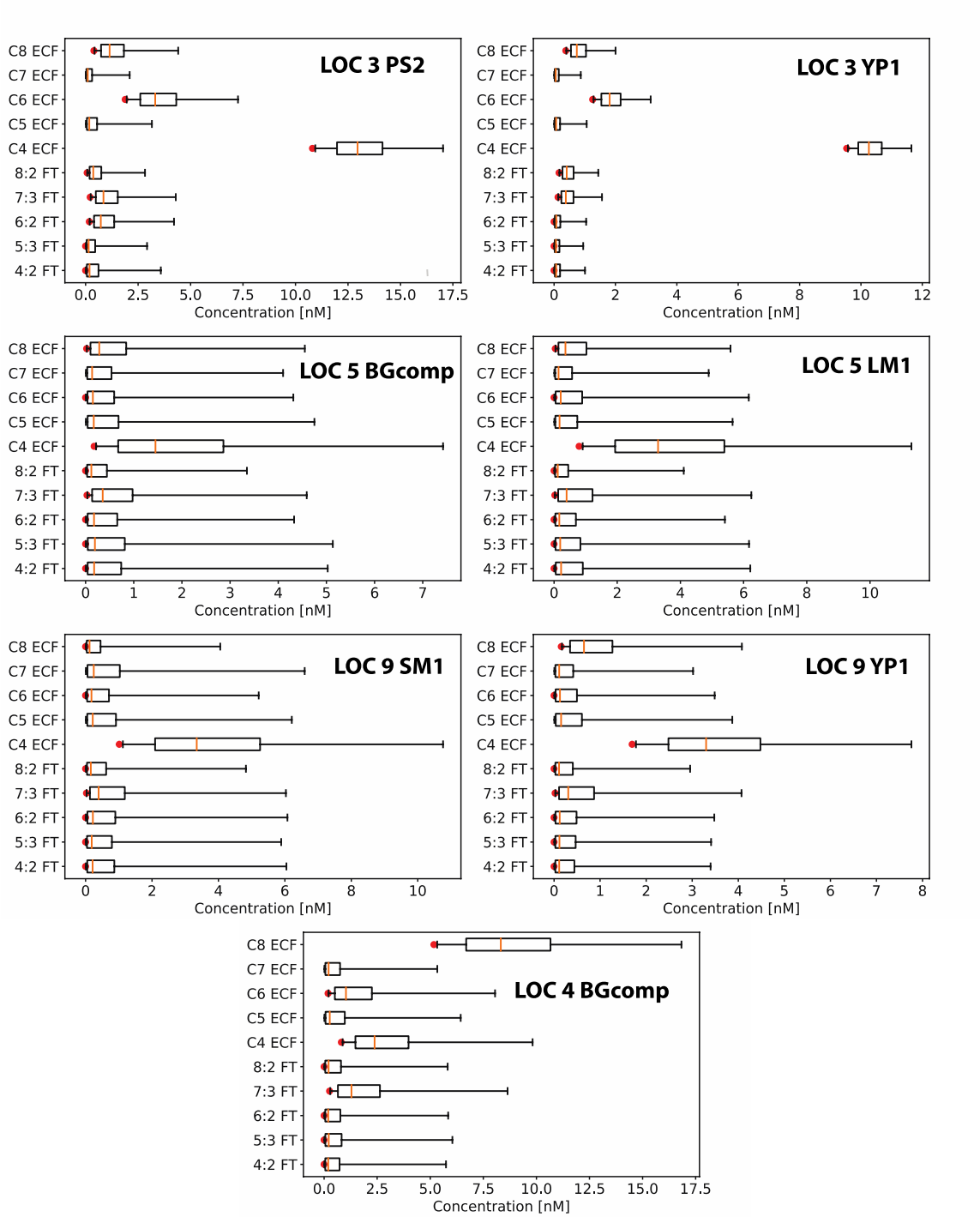


Figure S7. Comparison of inferred and measured precursor concentrations. Measured concentrations of targeted precursors of a given chain-length and manufacturing origin are shown as red circles. Box and whisker plots show the inferred precursor concentration ranges. The median is shown as the orange line. The interquartile ranges (25th and 75th percentiles) are represented by boxes and the whiskers show upper and lower 95 % confidence intervals (CI). Outliers have been omitted from the plot to aid in visualizing results.

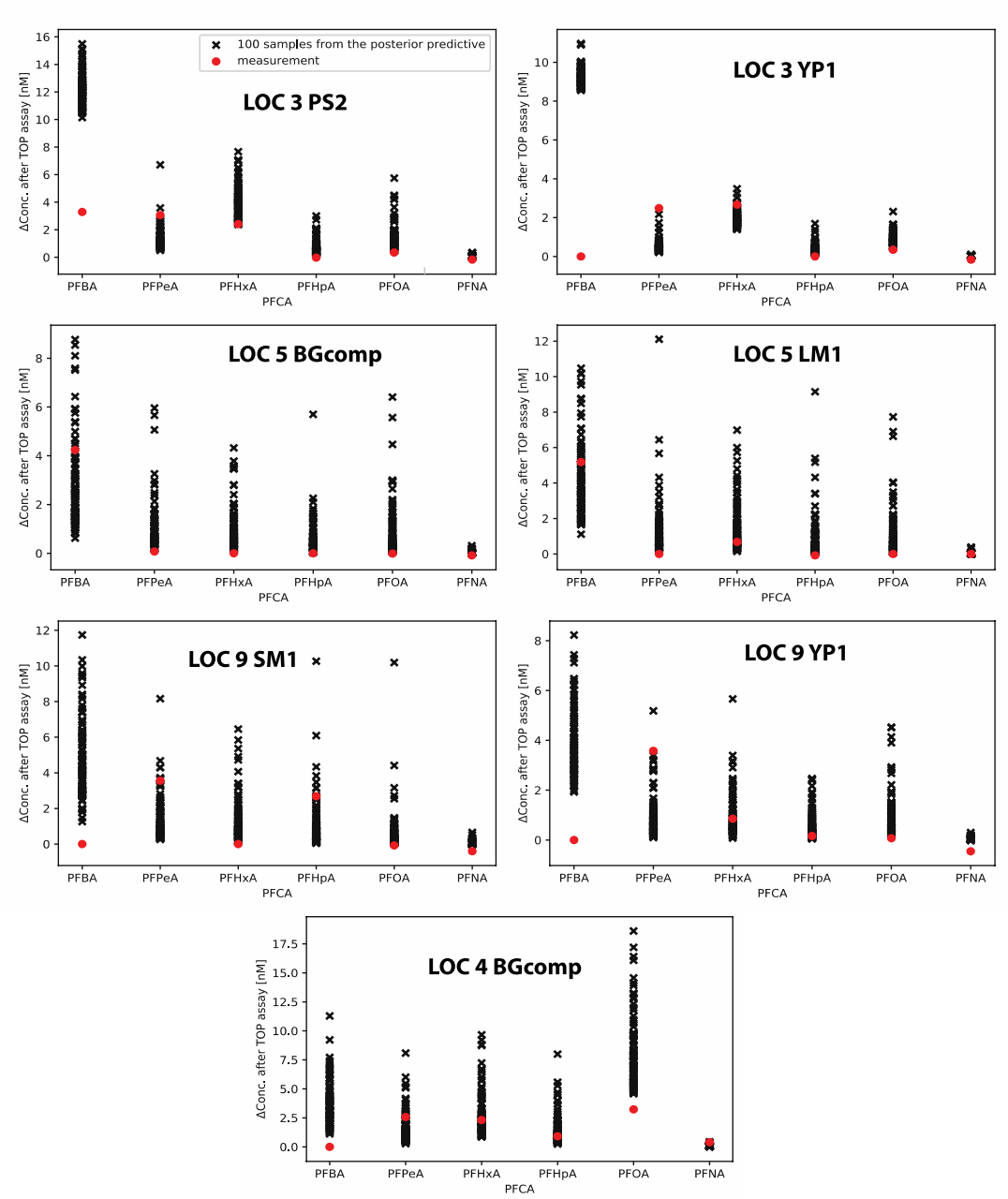


Figure S8. Posterior predictive plots for the seven fish samples showing where the measured value lies within the inferred prediction for the C3-C8 PFCA.

Table S21. Peak areas of PFAS compounds determined by HRMS analysis in fish samples

	LOC 3 PS2	LOC 3 YP1	LOC 4 BGcomp	LOC 5 BGcomp	LOC 5 LM1	LOC 9 SM1	LOC 9 YP1
FPrSA	15585	7829	880		582	3433	4071
FBSA	105356	48900	10636	1996	7761	10893	17504
FPeSA	205518	84675	9619	379	829	820	8374
FHxSA	41664	23395	4311	181			8234
FOSA	4556	3377	40758	488		282	3859
L-N-EtFOSAA			16447				
6:2 FTSA	1819	1858					215389
PFHpA (C6)	5910	2773	4471	5171	22137	13366	15545
PFOA (C7)	14643	5593	10546	8146	98168	13724	23189
PFNA (C8)	8238	8297	5031	1721	1467	6292	10931
PFDA (C9)	23575	18267	73912	10789	24356	65147	16338
PFUnDA (C10)	40211	33622	47133	11673	22808	55573	16204
PFDoDA (C11)	10101	7866	84619	5971	13698	20132	9507
PFTrDA (C12)	15111	12530	23455	4487	5475	9203	6218
L-PFHxS (C6)	2724	1885	1413			1214	3219
Br-PFHxS (C6)	224	168	236			203	125
PFHpS (C7)		645	409			1025	834
L-PFOS (C8)	77732	126617	410379	19326	53361	372630	42205
Br-PFOS (C8)	8868	12922	64786	2835	5235	24972	11812
PFDS (C10)		708	2497	276	810	1582	546

Table S22. Semi-quantified/quantified suspect screening results in molarity (nmol L^{-1}) for PFOS and PFAS precursors.

Fish	∑PFOS	6:2 FTSA	8:2 FTSA	10:2 FTSA	FPrSA	FBSA	FPeSA	FHxSA	FOSA	N-MeFOSAA	N-EtFOSAA	7:3 FTCA	9:3 FTCA	∑Precursor Target (9)	∑Precursor All (12)
LOC3PS2	14.19	0.06	0.09	0.04	2.88	10.60	31.90	2.94	0.42			0.22	0.15	14.38	49.30
LOC3YP1	33.78	0.15	0.21	0.05	2.54	8.65	20.41	2.57	0.41			0.13	0.02	12.17	35.14
LOC4BGcomp	68.21			0.20		1.11	1.32	0.27	2.89	0.23	4.16	0.26	0.11	9.11	10.53
LOC5BGcomp	3.03					0.18			0.03			0.03		0.24	0.24
LOC5BGcomp Dup	3.00					0.21			0.04			0.03		0.27	0.27
LOC5LM1	8.80					0.88	0.13		0.02			0.02		0.92	1.06
LOC9SM1	62.98				0.62	1.08	0.13		0.02			0.03		1.12	1.87
LOC9YP1	6.56	9.40			0.75	1.75	1.10	0.49	0.26			0.02	0.01	2.53*	4.39*

*Excluding 6:2 FTSA in sum precursor calculations since it may be an outlier and was not detected in the targeted analysis.

The percent difference between detectable precursor concentrations quantified in both targeted analysis and suspect screening analysis ranged from 0 – 61 % (average: 22 %, median: 23 %). This is excluding 7:3 FTCA due to an interference with 7:3 FTCA that was distinguishable using HRMS but not using LC-MS/MS unit resolution, leading to quantified concentrations an order of magnitude higher in the targeted analysis compared to the suspect screening analysis. Results for 7:3 FTCA were updated in the model to reflect the lower concentrations quantified by suspect screening as to not overestimate inferred concentrations for this precursor compound due to the interference.

References

(1) Esri. "State Map" [basemap]. Scale Not Given. "World Light Gray Base". June 30, 2021. https://services.arcgisonline.com/ArcGIS/rest/services/Canvas/World_Light_Gray_Base/MapServer. (September 13, 2022)

(2) Weber, A. K.; Barber, L. B.; LeBlanc, D. R.; Sunderland, E. M.; Vecitis, C. D. Geochemical and Hydrologic Factors Controlling Subsurface Transport of Poly- and Perfluoroalkyl Substances, Cape Cod, Massachusetts. *Environ. Sci. Technol.* **2017**, *51* (8), 4269–4279. https://doi.org/10.1021/acs.est.6b05573.

(3) Dassuncao, C.; Pickard, H.; Pfohl, M.; Tokranov, A. K.; Li, M.; Mikkelsen, B.; Slitt, A.; Sunderland, E. M. Phospholipid Levels Predict the Tissue Distribution of Poly- and Perfluoroalkyl Substances in a Marine Mammal. *Environ. Sci. Technol. Lett.* **2019**, *6* (3), 119–125. https://doi.org/doi.org/10.1021/acs.estlett.9b00031.

(4) Simonnet-Laprade, C.; Budzinski, H.; Maciejewski, K.; Le Menach, K.; Santos, R.; Alliot, F.; Goutte, A.; Labadie, P. Biomagnification of Perfluoroalkyl Acids (PFAAs) in the Food Web of an Urban River: Assessment of the Trophic Transfer of Targeted and Unknown Precursors and Implications. *Environ. Sci. Process. Impacts* **2019**, *21* (11), 1864–1874. https://doi.org/10.1039/C9EM00322C.

(5) Reiner, J. L.; O'Connell, S. G.; Butt, C. M.; Mabury, S. A.; Small, J. M.; De Silva, A. O.; Muir, D. C. G.; Delinsky, A. D.; Strynar, M. J.; Lindstrom, A. B.; Reagen, W. K.; Malinsky, M.; Schäfer, S.; Kwadijk, C. J. A. F.; Schantz, M. M.; Keller, J. M. Determination of Perfluorinated Alkyl Acid Concentrations in Biological Standard Reference Materials. *Anal. Bioanal. Chem.* **2012**, *404* (9), 2683–2692. https://doi.org/10.1007/s00216-012-5943-5.

(6) Martin, D.; Munoz, G.; Mejia-Avendaño, S.; Duy, S. V.; Yao, Y.; Volchek, K.; Brown, C. E.; Liu, J.; Sauvé, S. Zwitterionic, Cationic, and Anionic Perfluoroalkyl and Polyfluoroalkyl Substances Integrated into Total Oxidizable Precursor Assay of Contaminated Groundwater. *Talanta* **2019**, *195*, 533–542. https://doi.org/10.1016/j.talanta.2018.11.093.

(7) Houtz, E. F.; Sedlak, D. L. Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff. *Environ. Sci. Technol.* **2012**, *46* (17), 9342–9349. https://doi.org/10.1021/es302274g.

(8) Göckener, B.; Eichhorn, M.; Lämmer, R.; Kotthoff, M.; Kowalczyk, J.; Numata, J.; Schafft, H.; Lahrssen-Wiederholt, M.; Bücking, M. Transfer of Per- and Polyfluoroalkyl Substances (PFAS) from Feed into the Eggs of Laying Hens. Part 1: Analytical Results Including a Modified Total Oxidizable Precursor Assay. *J. Agric. Food Chem.* **2020**, *68* (45), 12527–12538. https://doi.org/10.1021/acs.jafc.0c04456.

(9) Ruyle, B. J.; Pickard, H. M.; LeBlanc, D. R.; Tokranov, A. K.; Thackray, C. P.; Hu, X. C.; Vecitis, C. D.; Sunderland, E. M. Isolating the AFFF Signature in Coastal Watersheds Using Oxidizable PFAS Precursors and Unexplained Organofluorine. *Environ. Sci. Technol.* **2021**, *55* (6), 3686–3695. https://doi.org/10.1021/acs.est.0c07296.

(10) Janda, J.; Nödler, K.; Scheurer, M.; Happel, O.; Nürenberg, G.; Zwiener, C.; Lange, F. T. Closing the Gap – Inclusion of Ultrashort-Chain Perfluoroalkyl Carboxylic Acids in the Total Oxidizable Precursor (TOP) Assay Protocol. *Environ. Sci. Process. Impacts* **2019**, *21* (11), 1926–1935. https://doi.org/10.1039/C9EM00169G.

(11) Wang, B.; Yao, Y.; Wang, Y.; Chen, H.; Sun, H. Per- and Polyfluoroalkyl Substances in Outdoor and Indoor Dust from Mainland China: Contributions of Unknown Precursors and Implications for Human Exposure. *Environ. Sci. Technol.* **2022**, *56* (10), 6036–6045. https://doi.org/10.1021/acs.est.0c08242.

(12) Ruyle, B. J.; Thackray, C. P.; McCord, J. P.; Strynar, M. J.; Mauge-Lewis, K. A.; Fenton, S. E.; Sunderland, E. M. Reconstructing the Composition of Per- and Polyfluoroalkyl Substances in Contemporary Aqueous Film-Forming Foams. *Environ. Sci. Technol. Lett.* **2021**, *8* (1), 59–65. https://doi.org/10.1021/acs.estlett.0c00798.

(13) Gelman, A.; Carlin, J. B.; Stern, H. S.; Rubin, D. B. *Bayesian Data Analysis, 2nd Ed.*, 2nd Edition.; Chapman & Hall/CRC: Boca Raton, Florida, 2004.

(14) Foreman-Mackey, D.; Hogg, D. W.; Lang, D.; Goodman, J. Emcee : The MCMC Hammer. *Publ. Astron. Soc. Pacific* **2013**, *125* (925), 306–312. https://doi.org/10.1086/670067.

(15) Charbonnet, J. A.; McDonough, C. A.; Xiao, F.; Schwichtenberg, T.; Cao, D.; Kaserzon, S.; Thomas, K. V; Dewapriya, P.; Place, B. J.; Schymanski, E. L.; Field, J. A.; Helbling, D. E.; Higgins, C. P. Communicating Confidence of Per- and Polyfluoroalkyl Substance Identification via High-Resolution Mass Spectrometry. *Environ. Sci. Technol. Lett.* **2022**, *9* (6), 473–481. https://doi.org/10.1021/acs.estlett.2c00206.

(16) NHDES. *Status and Trends of Mercury in Fish Tissue in New Hampshire Waterbodies, 1992 – 2016*; 2018.

(17) New Hampshire Department of Environmental Services (NHDES). *Technical Background Report for Proposed Maximum Contaminant Levels (MCLs) and Ambient Groundwater Quality Standards (AGQSs) for Perfluorooctane Sulfonic Acid (PFOS), Perfluorooctanoic (PFOA), Perfluorononanoic Acid (PFNA), and Perfluorohexane Sulfonic Ac*; 2019.

(18) USEPA. *Exposure Factors Handbook: 2011 Edition*; 2011.

Site	Fish Species	Fish Sample ID	Analysis Batch	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFPeS	L-PFHxS	Br-PFHxS	∑PFHxS	PFHpS	L-PFOS	Br-PFOS	∑PFOS	PFNS	PFDS	4:2 FTSA	6:2 FTSA	8:2 FTSA	10:2 FTSA	FBSA	FHxSA	FOSA	FDSA	N-MeFOSA	N-EtFOSA	FOSAA	L-N-MeFOSAA	Br-N-MeFOSAA	L-N-EtFOSAA	Br-N-EtFOSAA	N-MeFOSE	N-EtFOSE	ADONA	3:3 FTCA	5:3 FTCA	7:3 FTCA	∑PFAS
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.329	0.158	0.480	0.666	0.348	0.397	0.252	<MDL	<MDL	<MDL	<MDL	<MDL	QH	3.230	<MDL	3.230	<MDL	0.052	<MDL	NI	<MDL	NA	NA	NA	0.015	NA	NA	NA	NA	0.058	NA	0.092	NA	NA	NA	<MDL	NA	NA	NA	6.077
LOC 1	Bluegill	LOC1BGcomp	Batch 2	<MDL	<MDL	<MDL	0.120	0.220	0.194	0.624	0.732	0.390	0.573	0.191	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.772	0.363	4.135	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.048	<MDL	0.023	<MDL	<MDL	<MDL	<MDL	0.017	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.145	7.412
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.231	0.034	0.363	0.567	0.433	0.329	0.234	<MDL	<MDL	0.067	<MDL	0.067	<MDL	2.125	<MDL	2.125	<MDL	0.144	<MDL	NI	<MDL	NA	NA	NA	0.041	NA	NA	NA	NA	0.035	NA	0.050	NA	NA	NA	<MDL	NA	NA	NA	4.652
LOC 2	Bluegill	LOC2BGcomp	Batch 2	<MDL	<MDL	<MDL	QL	0.154	0.066	1.106	1.568	1.100	1.188	0.587	<MDL	<MDL	0.443	<MDL	0.443	<MDL	3.501	0.412	3.913	<MDL	QL	<MDL	<MDL	<MDL	<MDL	0.681	<MDL	0.074	<MDL	<MDL	<MDL	<MDL	0.028	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.089	10.998
			Batch 1	<MDL	<MDL	QL	<MDL	0.281	0.059	0.197	0.532	0.177	0.272	0.088	<MDL	0.088	<MDL	<MDL	<MDL	QH	5.302	<MDL	5.302	<MDL	0.090	<MDL	NI	0.083	NA	NA	NA	0.019	NA	NA	NA	NA	0.017	NA	0.031	NA	NA	NA	<MDL	NA	NA	NA	7.235
LOC 3	Bluegill	LOC3BGcomp	Batch 2	<MDL	<MDL	<MDL	QL	0.225	0.199	0.785	QL	0.644	1.226	0.235	<MDL	<MDL	QL	<MDL	<MDL	<MDL	19.955	1.245	21.199	<MDL	0.169	<MDL	QL	0.441	<MDL	0.904	<MDL	0.055	<MDL	<MDL	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.751	1.816	28.648
LOC 3	Pumpkinseed	LOC3PS1	Batch 1	<MDL	<MDL	<MDL	<MDL	0.256	0.042	0.159	0.411	0.160	0.331	0.108	<MDL	<MDL	QL	<MDL	<MDL	<MDL	2.253	<MDL	2.253	<MDL	0.072	<MDL	NI	0.023	NA	NA	NA	0.098	NA	NA	NA	NA	0.047	NA	0.086	NA	NA	NA	<MDL	NA	NA	NA	4.045
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.327	0.120	0.350	1.065	0.350	0.763	0.196	<MDL	<MDL	0.079	<MDL	0.079	<MDL	7.526	0.467	7.993	<MDL	0.179	<MDL	NI	0.114	NA	NA	NA	0.199	NA	NA	NA	NA	0.048	NA	0.094	NA	NA	NA	<MDL	NA	NA	NA	11.877
LOC 3	Pumpkinseed	LOC3PS2	Batch 2	<MDL	<MDL	<MDL	0.203	0.244	0.158	0.574	1.446	0.461	1.568	0.242	<MDL	<MDL	QL	<MDL	<MDL	<MDL	7.370	0.777	8.147	<MDL	<MDL	<MDL	0.149	0.072	QL	6.379	1.485	0.370	<MDL	<MDL	<MDL	<MDL	0.022	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.569	1.562	24.651
LOC 3	Pumpkinseed	LOC3PS3	Batch 1	0.862	<MDL	QL	<MDL	0.254	0.126	0.316	0.987	0.407	0.837	0.269	<MDL	<MDL	0.127	<MDL	0.127	<MDL	6.409	<MDL	6.409	<MDL	0.186	<MDL	NI	0.061	NA	NA	NA	0.166	NA	NA	NA	NA	0.025	NA	0.044	NA	NA	NA	<MDL	NA	NA	NA	11.074
			Batch 1	<MDL	<MDL	<MDL	QL	0.411	0.184	0.442	1.078	0.756	1.144	0.582	<MDL	<MDL	0.136	<MDL	0.136	<MDL	13.005	0.821	13.826	0.080	0.845	<MDL	NI	0.159	NA	NA	NA	0.341	NA	NA	NA	NA	0.559	NA	0.704	NA	NA	NA	<MDL	NA	NA	NA	21.247
LOC 3	Yellow Perch	LOC3YP1	Batch 2	<MDL	<MDL	<MDL	0.182	0.274	0.212	0.586	QL	0.361	0.927	0.220	<MDL	<MDL	QL	<MDL	<MDL	<MDL	16.472	1.153	17.625	<MDL	0.239	<MDL	<MDL	0.174	<MDL	5.723	1.009	0.384	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.710	1.187	29.812
LOC 3	Yellow Perch	LOC3YP2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.227	0.270	0.711	2.086	0.720	1.109	0.368	<MDL	<MDL	0.149	<MDL	0.149	0.108	19.162	1.028	20.191	0.034	0.394	<MDL	NI	0.340	NA	NA	NA	0.298	NA	NA	NA	NA	0.213	NA	0.348	NA	NA	NA	<MDL	NA	NA	NA	27.566
LOC 3	Yellow Perch	LOC3YP3	Batch 1	<MDL	<MDL	<MDL	<MDL	0.280	0.304	0.406	1.036	0.429	0.610	0.194	<MDL	<MDL	0.113	<MDL	0.113	<MDL	7.777	0.432	8.209	<MDL	0.193	<MDL	NI	0.138	NA	NA	NA	0.123	NA	NA	NA	NA	0.126	NA	0.204	NA	NA	NA	<MDL	NA	NA	NA	12.364
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.307	0.052	0.677	0.626	1.299	0.485	0.638	<MDL	0.088	<MDL	<MDL	<MDL	QH	16.743	1.498	18.242	<MDL	0.278	<MDL	NI	0.010	NA	NA	NA	1.339	NA	NA	NA	NA	0.128	NA	1.787	NA	NA	NA	<MDL	NA	NA	NA	25.954
LOC 4	Bluegill	LOC4BGcomp	Batch 2	<MDL	<MDL	<MDL	0.240	1.245	0.140	1.655	1.331	2.575	1.383	1.337	<MDL	<MDL	0.107	<MDL	0.107	<MDL	27.734	3.023	30.758	<MDL	0.161	<MDL	<MDL	<MDL	QL	0.479	0.149	2.184	<MDL	<MDL	<MDL	<MDL	QH	<MDL	2.141	1.283	<MDL	<MDL	<MDL	<MDL	<MDL	3.139	50.305
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.300	0.013	0.123	0.177	0.162	0.117	0.150	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.710	<MDL	1.710	<MDL	QL	<MDL	NI	<MDL	NA	NA	NA	0.046	NA	NA	NA	NA	0.032	NA	0.077	NA	NA	NA	<MDL	NA	NA	NA	2.905
LOC 5	Bluegill	LOC5BGcomp	Batch 2	<MDL	<MDL	<MDL	0.158	0.206	0.078	0.291	0.351	0.174	0.288	0.192	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.514	<MDL	1.514	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.104	<MDL	0.021	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.404	3.782
LOC 5	Bluegill	LOC5BGcompJ	Batch 1	<MDL	<MDL	<MDL	<MDL	0.258	0.022	0.187	0.279	0.173	0.102	0.127	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.787	<MDL	1.787	<MDL	0.038	<MDL	NI	<MDL	NA	NA	NA	0.023	NA	NA	NA	NA	0.034	NA	0.025	NA	NA	NA	<MDL	NA	NA	NA	3.052
LOC 5	Brown Bullhead	LOC5CF1	Batch 1	<MDL	<MDL	<MDL	<MDL	0.228	<MDL	0.024	0.058	0.149	0.156	0.256	<MDL	0.114	<MDL	<MDL	<MDL	<MDL	0.205	<MDL	0.205	<MDL	0.014	<MDL	NI	<MDL	NA	NA	NA	0.023	NA	NA	NA	NA	0.008	NA	0.014	NA	NA	NA	<MDL	NA	NA	NA	1.249
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.252	0.032	0.513	0.667	0.594	0.311	0.354	<MDL	<MDL	0.067	<MDL	0.067	QH	5.042	<MDL	5.042	<MDL	0.224	<MDL	NI	<MDL	NA	NA	NA	0.018	NA	NA	NA	NA	0.038	NA	0.027	NA	NA	NA	<MDL	NA	NA	NA	8.139
LOC 5	Largemouth Bass	LOC5LM1	Batch 2	4.804	0.801	0.252	0.331	0.289	<MDL	0.854	0.720	0.562	0.572	0.345	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	7.306	0.608	7.914	<MDL	QL	<MDL	<MDL	<MDL	<MDL	0.354	<MDL	0.021	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	QL	17.819
LOC 5	Largemouth Bass	LOC5LM2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.296	0.026	0.338	0.634	0.261	0.228	0.113	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	4.769	<MDL	4.769	<MDL	0.576	<MDL	NI	<MDL	NA	NA	NA	0.024	NA	NA	NA	NA	0.024	NA	0.022	NA	NA	NA	<MDL	NA	NA	NA	7.310
LOC 5	Smallmouth Bass	LOC5SM1	Batch 1	<MDL	<MDL	<MDL	0.0183	0.236	<MDL	0.300	0.774	0.943	0.669	0.554	<MDL	QL	<MDL	<MDL	<MDL	QH	2.556	<MDL	2.556	<MDL	0.294	<MDL	NI	0.005	NA	NA	NA	0.204	NA	NA	NA	NA	0.053	NA	0.018	NA	NA	NA	<MDL	NA	NA	NA	6.623
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.231	<MDL	0.419	0.928	1.035	0.788	0.625	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.565	<MDL	3.565	<MDL	0.247	<MDL	NI	QH	NA	NA	NA	0.176	NA	NA	NA	NA	0.043	NA	0.021	NA	NA	NA	<MDL	NA	NA	NA	8.077
LOC 5	Smallmouth Bass	LOC5SM2	Batch 2	<MDL	<MDL	<MDL	QL	0.223	0.072	0.585	QL	1.231	0.991	0.634	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	5.056	0.337	5.393	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.472	<MDL	0.237	<MDL	<MDL	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.188	11.025
LOC 5	Smallmouth Bass	LOC5SM3	Batch 1	<MDL	<MDL	<MDL	QL	<MDL	<MDL	0.193	0.314	0.319	0.376	0.345	<MDL	QL	<MDL	<MDL	<MDL	<MDL	1.680	<MDL	1.680	<MDL	0.077	<MDL	NI	<MDL	NA	NA	NA	0.171	NA	NA	NA	NA	0.021	NA	0.023	NA	NA	NA	<MDL	NA	NA	NA	3.518
LOC 5	Smallmouth Bass	LOC5SM4	Batch 1	<MDL	<MDL	<MDL	<MDL	0.241	<MDL	0.283	0.526	0.485	0.439	0.329	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.127	<MDL	2.127	<MDL	QH	<MDL	NI	QL	NA	NA	NA	0.246	NA	NA	NA	NA	0.096	NA	0.047	NA	NA	NA	<MDL	NA	NA	NA	4.820
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.386	0.029	0.313	0.279	0.318	0.154	0.221	<MDL	QH	<MDL	QL	<MDL	QH	3.449	<MDL	3.449	<MDL	0.051	<MDL	NI	0.015	NA	NA	NA	0.224	NA	NA	NA	NA	0.034	NA	0.561	NA	NA	NA	<MDL	NA	NA	NA	6.034
LOC 6	Bluegill	LOC6BGcomp	Batch 2	<MDL	<MDL	<MDL	0.140	0.253	0.076	0.573	0.474	0.478	0.380	0.315	<MDL	<MDL	QL	<MDL	<MDL	<MDL	6.318	QH	6.318	<MDL	QL	<MDL	<MDL	<MDL	<MDL	0.223	<MDL	0.254	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.408	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.677	10.567
			Batch 1	0.638	<MDL	<MDL	<MDL	0.238	0.025	0.294	0.488	0.492	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.283	<MDL	2.283	<MDL	0.140	<MDL	NI	QH	NA	NA	NA	0.184	NA	NA	NA	NA	0.056	NA	0.025	NA	NA	NA	<MDL	NA	NA	NA	4.861
LOC 6	Lake Whitefish	LOC6DA1	Batch 2	<MDL	<MDL	<MDL	0.140	0.202	<MDL	0.622	QL	0.486	0.557	0.202	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.397	0.214	2.610	<MDL	0.128	<MDL	<MDL	<MDL	<MDL	0.490	<MDL	0.237	<MDL	<MDL	<MDL	<MDL	QL	<MDL	0.027	<MDL	<MDL	<MDL	<MDL	<MDL	0.339	1.031	7.072
LOC 6	Lake Whitefish	LOC6DA2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.221	0.014	0.158	0.229	0.220	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.184	<MDL	1.184	<MDL	0.037	<MDL	NI	<MDL	NA	NA	NA	0.099	NA	NA	NA	NA	0.035	NA	0.027	NA	NA	NA	<MDL	NA	NA	NA	2.222
LOC 6	Lake Whitefish	LOC6DA3	Batch 1	<MDL	<MDL	<MDL	<MDL	0.240	0.019	0.392	0.303	0.272	0.236	0.102	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.927	<MDL	3.927	<MDL	0.111	<MDL	NI	0.011	NA	NA	NA	0.421	NA	NA	NA	NA	0.212	NA	0.080	NA	NA	NA	<MDL	NA	NA	NA	6.325
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.227	0.046	0.338	0.633	0.886	0.278	0.549	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	4.348	<MDL	4.348	<MDL	0.207	<MDL	NI	<MDL	NA	NA	NA	0.085	NA	NA	NA	NA	0.052	NA	0.033	NA	NA	NA	<MDL	NA	NA	NA	7.680
LOC 6	Pumpkinseed	LOC6PS1	Batch 2	<MDL	<MDL	<MDL	QL	0.262	0.086	0.447	0.571	0.752	0.702	0.472	<MDL	QL	<MDL	<MDL	<MDL	<MDL	4.462	0.351	4.814	<MDL	0.169	<MDL	<MDL	<MDL	<MDL	0.220	<MDL	0.070	<MDL	<MDL	<MDL	<MDL	QL	<MDL	0.022	<MDL	<MDL	<MDL	<MDL	<MDL	0.281	1.000	9.866
LOC 6	Pumpkinseed	LOC6PS2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.253	0.050	0.199	0.301	0.405	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.055	<MDL	3.055	<MDL	0.165	<MDL	NI	QH	NA	NA	NA	0.085	NA	NA	NA	NA	0.033	NA	0.026	NA	NA	NA	<MDL	NA	NA	NA	4.571
LOC 6	Pumpkinseed	LOC6PS3	Batch 1	<MDL	<MDL	<MDL	<MDL	0.261	0.038	0.143	0.275	0.370	0.253	0.180	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.027	<MDL	2.027	<MDL	0.110	<MDL	NI	<MDL	NA	NA	NA	0.100	NA	NA	NA	NA	0.023	NA	0.055	NA	NA	NA	<MDL	NA	NA	NA	3.834
			Batch 1	<MDL	<MDL	0.038	0.028	0.283	0.035	0.160	0.176	0.163	0.115	0.112	<MDL	<MDL	QL	<MDL	<MDL	<MDL	2.622	<MDL	2.622	<MDL	0.049	0.080	NI	0.018	NA	NA	NA	0.063	NA	NA	NA	NA	0.018	NA	QL	NA	NA	NA	<MDL	NA	NA	NA	3.961
LOC 7	Bluegill	LOC7BGcomp	Batch 2	<MDL	<MDL	<MDL	0.172	0.216	0.102	0.348	0.249	0.237	0.126	0.227	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.836	0.370	4.207	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.485	<MDL	0.221	<MDL	<MDL	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	QL	7.590
LOC 7	Chain Pickerel	LOC7PICK	Batch 1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.025	0.024	0.018	0.015	0.021	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.729	<MDL	0.729	<MDL	QH	0.081	NI	QH	NA	NA	NA	0.028	NA	NA	NA	NA	<MDL	NA	0.006	NA	NA	NA	<MDL	NA	NA	NA	0.946
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.233	0.011	0.143	0.160	0.175	0.137	0.156	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	2.644	<MDL	2.644	<MDL	0.055	<MDL	NI	<MDL	NA	NA	NA	0.004	NA	NA	NA	NA	0.024	NA	QH	NA	NA	NA	<MDL	NA	NA	NA	3.741
LOC 7	Largemouth Bass	LOC7LM2	Batch 2	<MDL	<MDL	<MDL	0.171	0.200	<MDL	0.207	0.245	0.190	0.136	0.253	<MDL	<MDL	0.083	<MDL	0.083	<MDL	4.200	0.306	4.505	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.175	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.416	6.581
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.230	0.077	0.175	0.123	0.114	0.070	0.071	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.531	<MDL	1.531	<MDL	QH	<MDL	NI	<MDL	NA	NA	NA	0.082	NA	NA	NA	NA	0.075	NA	QH	NA	NA	NA	<MDL	NA	NA	NA	2.547
LOC 7	Yellow Perch	LOC7YP1	Batch 2	<MDL	<MDL	<MDL	0.223	0.217	0.248	0.590	QL	0.233	0.192	0.157	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.935	0.544	4.479	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.991	<MDL	0.226	<MDL	<MDL	<MDL	<MDL	0.082	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.190	7.827
LOC 7	Yellow Perch	LOC7YP2	Batch 1	<MDL	<MDL	<MDL	QL	0.278	0.076	0.434	0.332	0.282	0.176	0.164	<MDL	<MDL	<MDL	<MDL	<MDL	QH	6.609	0.598	7.207	<MDL	0.118	<MDL	NI	0.007	NA	NA	NA	0.384	NA	NA	NA	NA	0.156	NA	0.025	NA	NA	NA	<MDL	NA	NA	NA	9.640
LOC 7	Yellow Perch	LOC7YP3	Batch 1	<MDL	<MDL	<MDL	<MDL	0.253	0.054	0.122	0.099	0.071	0.055	0.051	<MDL	<MDL	<MDL	<MDL	<MDL	QH	2.036	<MDL	2.036	<MDL	0.027	<MDL	NI	<MDL	NA	NA	NA	0.014	NA	NA	NA	NA	QL	NA	QH	NA	NA	NA	<MDL	NA	NA	NA	2.782
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.271	0.022	0.302	0.379	0.157	0.094	0.057	<MDL	QH	<MDL	<MDL	<MDL	<MDL	1.535	<MDL	1.535	<MDL	0.019	<MDL	NI	<MDL	NA	NA	NA	0.016	NA	NA	NA	NA	0.011	NA	0.007	NA	NA	NA	<MDL	NA	NA	NA	2.870
LOC 8	Bluegill	LOC8BGcomp	Batch 2	<MDL	<MDL	<MDL	0.291	0.276	0.350	1.121	1.079	0.457	0.336	0.142	<MDL	<MDL	0.244	<MDL	0.244	<MDL	3.732	0.286	4.018	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.153	<MDL	0.034	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.185	8.687
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.240	QH	0.137	0.265	0.288	0.268	0.183	<MDL	QL	<MDL	<MDL	<MDL	<MDL	3.635	<MDL	3.635	<MDL	0.116	<MDL	NI	<MDL	NA	NA	NA	0.012	NA	NA	NA	NA	0.023	NA	0.018	NA	NA	NA	<MDL	NA	NA	NA	5.183
LOC 9	Bluegill	LOC9BGcomp	Batch 2	<MDL	<MDL	<MDL	QH	0.258	0.073	QL	0.398	0.334	0.393	0.222	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	7.121	0.558	7.679	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.152	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.215	9.723
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.271	0.072	0.163	0.238	0.209	0.166	0.091	<MDL	<MDL	0.088	<MDL	0.088	QH	2.290	<MDL	2.290	<MDL	0.063	<MDL	NI	<MDL	NA	NA	NA	0.155	NA	NA	NA	NA	0.020	NA	0.011	NA	NA	NA	<MDL	NA	NA	NA	3.837
LOC 9	Yellow Perch	LOC9YP1	Batch 2	<MDL	<MDL	<MDL	QL	0.460	0.673	0.353	0.397	0.288	0.247	<MDL	<MDL	<MDL	0.364	<MDL	0.364	<MDL	4.963	QH	4.963	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.014	<MDL	0.156	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.104	9.017
LOC 9	Yellow Perch	LOC9YP2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.267	0.034	0.149	0.214	0.119	0.080	0.041	<MDL	<MDL	0.034	<MDL	0.034	QH	2.397	<MDL	2.397	<MDL	0.040	<MDL	NI	<MDL	NA	NA	NA	0.117	NA	NA	NA	NA	QH	NA	<MDL	NA	NA	NA	<MDL	NA	NA	NA	3.490
LOC 9	Largemouth Bass	LOC9LM1	Batch 1	<MDL	<MDL	<MDL	<MDL	0.215	QL	0.165	0.310	0.231	0.123	0.093	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.036	<MDL	3.036	<MDL	0.069	<MDL	NI	<MDL	NA	NA	NA	0.013	NA	NA	NA	NA	0.012	NA	<MDL	NA	NA	NA	<MDL	NA	NA	NA	4.266
LOC 9	Largemouth Bass	LOC9LM2	Batch 1	<MDL	<MDL	<MDL	<MDL	0.207	0.037	1.121	1.330	0.728	0.650	0.412	<MDL	<MDL	<MDL	<MDL	<MDL	QH	8.052	0.467	8.519	<MDL	0.097	<MDL	NI	<MDL	NA	NA	NA	0.029	NA	NA	NA	NA	0.019	NA	0.023	NA	NA	NA	<MDL	NA	NA	NA	13.173
			Batch 1	<MDL	<MDL	<MDL	<MDL	<MDL	0.021	0.947	1.392	0.553	0.353	0.180	<MDL	<MDL	<MDL	<MDL	<MDL	QH	7.039	0.406	7.446	<MDL	0.124	<MDL	NI	<MDL	NA	NA	NA	0.022	NA	NA	NA	NA	0.016	NA	0.019	NA	NA	NA	<MDL	NA	NA	NA	11.073
LOC 9	Largemouth Bass	LOC9LM3	Batch 2	<MDL	<MDL	<MDL	0.304	0.200	QH	1.417	1.760	0.537	0.452	0.159	<MDL	QL	<MDL	<MDL	<MDL	<MDL	7.122	QH	7.122	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.182	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.472	12.604
LOC 9	Pumpkinseed	LOC9PS1	Batch 1	<MDL	<MDL	0.053	<MDL	<MDL	0.014	0.317	0.607	0.459	0.188	0.148	<MDL	<MDL	<MDL	<MDL	<MDL	QH	4.117	<MDL	4.117	<MDL	0.135	<MDL	NI	<MDL	NA	NA	NA	0.021	NA	NA	NA	NA	0.018	NA	0.011	NA	NA	NA	<MDL	NA	NA	NA	6.088
LOC 9	Pumpkinseed	LOC9PS2	Batch 1	<MDL	<MDL	<MDL	0.025	0.261	0.019	0.238	0.450	0.361	0.272	0.162	<MDL	<MDL	<MDL	<MDL	<MDL	QH	3.860	<MDL	3.860	<MDL	0.123	<MDL	NI	<MDL	NA	NA	NA	0.015	NA	NA	NA	NA	0.016	NA	0.012	NA	NA	NA	<MDL	NA	NA	NA	5.812
LOC 9	Pumpkinseed	LOC9PS3	Batch 1	<MDL	<MDL	<MDL	<MDL	0.211	0.013	0.269	0.346	0.203	0.110	0.086	<MDL	<MDL	<MDL	<MDL	<MDL	QH	10.407	<MDL	10.407	<MDL	0.120	<MDL	NI	<MDL	NA	NA	NA	0.008	NA	NA	NA	NA	0.008	NA	QH	NA	NA	NA	<MDL	NA	NA	NA	11.781
			Batch 1	<MDL	<MDL	<MDL	<MDL	0.220	0.040	0.970	1.204	0.540	0.238	0.135	<MDL	<MDL	<MDL	<MDL	<MDL	0.308	27.466	0.851	28.317	<MDL	0.302	<MDL	NI	<MDL	NA	NA	NA	0.009	NA	NA	NA	NA	0.014	NA	0.015	NA	NA	NA	<MDL	NA	NA	NA	32.310
LOC 9	Smallmouth Bass	LOC9SM1	Batch 2	<MDL	<MDL	<MDL	QL	0.260	0.371	1.536	1.528	0.709	0.383	0.159	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	52.371	1.458	53.828	<MDL	0.140	<MDL	<MDL	<MDL	<MDL	0.603	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.252	59.767
	Median (n=43) (ng/g)		Batch 1					0.253	0.037	0.294	0.411	0.319	0.260	0.172							3.230		3.230		0.118							0.082					0.033		0.027								5.812
	Median (n=20) (ng/g)		Batch 2					0.234	0.149	0.590	0.720	0.469	0.504	0.227							5.009	0.478	5.178		0.165					0.485		0.189														0.410	10.217
	Mean (n=43) (ng/g)		Batch 1					0.262	0.063	0.329	0.548	0.391	0.337	0.225							5.052		5.205		0.158							0.134					0.062		0.130								7.833
	Mean (n=20) (ng/g)		Batch 2					0.294	0.194	0.751	0.857	0.610	0.631	0.331							9.657	0.750	10.257		0.167					1.092		0.285														0.726	16.688
	DF (n=43) (%)		Batch 1	5%	0%	5%	7%	91%	81%	100%	100%	100%	93%	93%	0%	7%	21%	0%	21%	5%	100%	21%	100%	5%	91%	5%	NI	30%	NA	NA	NA	100%	NA	NA	NA	NA	93%	NA	84%	NA	NA	NA	0%	NA	NA	NA	
	DF (n=20) (%)		Batch 2	5%	5%	5%	65%	100%	80%	95%	75%	100%	100%	95%	0%	0%	25%	0%	25%	0%	100%	80%	100%	0%	30%	0%	5%	15%	0%	100%	15%	80%	0%	0%	0%	0%	20%	0%	20%	5%	0%	0%	0%	0%	25%	90%	

Concentrations in ng/g
See Table S7 in SI for MDL values for PFAS analytes
<MDL = below method detection limit
QH = qualifier peak flagged high
QL = qualifier peak flagged low
NI = not included (6:2 FTSA only) due to unreliable QA/QC
NA = not analyzed (only 25 PFAS analyzed in batch 1 vs 37 in batch 2)
Median/Mean calculated for compounds if >75% of samples have concentration >MDL

Site	Sample Location	Latitude	Longitude	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFPeS	L-PFHxS	Br-PFHxS	∑PFHxS	PFHpS	L-PFOS	Br-PFOS	∑PFOS	PFNS	PFDS	4:2 FTSA	8:2 FTSA	L-N-MeFOSAA	L-N-EtFOSAA	FOSA	ADONA	∑PFAS
LOC 1	Great Pond, Kingston, INFLOW	42.9166	-71.0814	<MDL	<MDL	2.48	1.49	3.34	0.53	0.15	0.05	<MDL	<MDL	<MDL	<MDL	<MDL	0.52	<MDL	0.52	QH	0.63	0.68	1.31	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	9.87
LOC 1	Great Pond, Kingston, CENTER	42.9185	-71.0621	11.29	<MDL	3.31	1.48	3.12	0.58	0.19	0.06	<MDL	0.21	0.19	<MDL	<MDL	0.56	<MDL	0.56	0.05	0.64	0.72	1.36	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	22.39
LOC 1	Great Pond, Kingston, OUTFLOW	42.9136	-71.0618	11.29	<MDL	2.98	1.45	3.03	0.61	0.22	0.07	<MDL	0.17	0.16	<MDL	<MDL	0.50	<MDL	0.50	QH	0.73	0.77	1.50	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	21.98
LOC 2	Hedgehog Pond, Salem	42.7623	-71.2439	<MDL	<MDL	9.75	7.46	14.68	1.48	0.62	0.13	<MDL	<MDL	<MDL	<MDL	<MDL	0.74	<MDL	0.74	0.06	1.71	1.00	2.71	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	37.63
LOC 3	Pine Island Pond, Manchester, INFLOW	42.9406	-71.4443	21.22	<MDL	12.48	6.02	19.87	1.61	0.35	0.09	<MDL	<MDL	<MDL	<MDL	0.29	2.86	0.21	3.07	0.12	3.40	2.20	5.60	<MDL	<MDL	QL	0.21	<MDL	0.06	<MDL	<MDL	71.00
LOC 3	Pine Island Pond, Manchester, CENTER	42.9342	-71.4488	<MDL	<MDL	15.37	6.95	24.90	2.21	0.48	0.16	<MDL	<MDL	<MDL	4.39	0.33	3.57	0.49	4.06	0.19	4.45	3.21	7.66	<MDL	<MDL	QL	0.31	<MDL	<MDL	<MDL	<MDL	67.02
LOC 3	Pine Island Pond, Manchester, Cohas Brook OUTFLOW	42.9316	-71.4514	<MDL	31.16	20.53	10.32	51.65	10.00	1.61	0.80	0.08	<MDL	<MDL	4.17	0.48	8.68	1.07	9.75	0.53	18.10	10.28	28.38	<MDL	<MDL	QL	1.97	<MDL	0.07	0.08	<MDL	171.58
LOC 4	Nashua River, Mine Falls Dam	42.7494	-71.5059	18.80	<MDL	20.29	6.78	14.86	1.68	0.76	0.14	<MDL	<MDL	<MDL	<MDL	0.17	1.62	<MDL	1.62	0.13	4.77	2.76	7.53	<MDL	<MDL	0.51	0.36	0.21	4.06	0.24	0.02	78.16
LOC 4	Nashua River, Mine Falls Dam BELOW	42.7510	-71.5041	10.29	<MDL	13.68	6.25	13.14	1.50	0.57	0.11	<MDL	<MDL	<MDL	<MDL	<MDL	1.03	<MDL	1.03	0.09	3.44	2.53	5.97	<MDL	<MDL	0.40	0.43	0.13	0.98	0.15	QL	54.70
LOC 5	Merrimack River, upstream St. Gobain	42.8970	-71.4588	<MDL	<MDL	<MDL	0.79	1.59	0.37	0.08	<MDL	<MDL	0.09	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.33	0.31	0.64	<MDL	<MDL	QL	QL	<MDL	<MDL	<MDL	<MDL	3.55
LOC 5	Merrimack River, downstream St. Gobain	42.8845	-71.4676	<MDL	<MDL	2.16	1.47	5.36	0.46	0.13	0.05	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.51	0.42	0.93	<MDL	<MDL	QL	0.01	<MDL	<MDL	<MDL	<MDL	10.58
LOC 5	Merrimack River, St. Gobain Brook	42.8870	-71.4659	33.84	117.57	136.93	108.42	526.78	7.55	2.56	0.16	<MDL	<MDL	<MDL	8.72	1.68	6.84	0.82	7.66	1.02	19.04	10.87	29.91	<MDL	<MDL	QL	0.03	<MDL	<MDL	<MDL	<MDL	982.83
LOC 6	Greeley Launch Merrimack River	42.7836	-71.4571	8.75	<MDL	<MDL	0.91	1.90	0.35	0.08	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.37	0.35	0.72	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	12.69
LOC 6	Penninchuck Brook @Rt3	42.7944	-71.4694	<MDL	<MDL	4.86	2.62	12.76	0.70	0.18	0.05	<MDL	<MDL	<MDL	<MDL	0.17	1.20	<MDL	1.20	0.06	1.45	1.30	2.75	<MDL	<MDL	QL	QL	<MDL	<MDL	<MDL	<MDL	25.34
LOC 6	Merrimack River @ Nanocomp	42.8064	-71.4738	<MDL	<MDL	2.24	1.44	4.00	0.50	0.14	0.05	<MDL	<MDL	<MDL	<MDL	<MDL	0.29	<MDL	0.29	<MDL	0.56	0.48	1.04	<MDL	<MDL	0.11	<MDL	<MDL	<MDL	<MDL	<MDL	9.82
LOC 7	Cocheco River, Rochester, Riverview Dr, Downstream	43.3185	-70.9911	<MDL	<MDL	1.87	0.85	1.87	0.36	0.10	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.61	0.56	1.17	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	6.22
LOC 7	Cocheco River, Rochester, Hanson Pines	43.3137	-70.9845	20.43	<MDL	2.03	1.19	2.23	0.45	0.13	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.71	0.55	1.26	<MDL	<MDL	0.05	0.04	<MDL	<MDL	<MDL	<MDL	27.81
LOC 7	Cocheco River, Rochester, Sagamore Lane, Upstream	43.3268	-70.9969	<MDL	<MDL	1.72	0.76	1.80	0.27	0.07	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.50	0.45	0.95	<MDL	<MDL	0.08	<MDL	<MDL	<MDL	<MDL	<MDL	5.65
LOC 8	Baboosic Lake, Amherst, OUTFLOW	42.8931	-71.5807	14.93	<MDL	3.71	2.09	7.72	0.85	0.43	0.13	<MDL	<MDL	<MDL	<MDL	<MDL	0.53	<MDL	0.53	0.04	1.28	0.98	2.25	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	32.68
LOC 8	Baboosic Lake, Amherst, CENTER	42.8827	-71.5770	<MDL	<MDL	3.56	2.13	8.66	0.91	0.42	0.13	<MDL	<MDL	<MDL	<MDL	<MDL	0.57	<MDL	0.57	0.05	1.19	1.18	2.37	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	18.80
LOC 9	Horseshoe Pond, Merrimack, Naticook INFLOW	42.8445	-71.4917	<MDL	<MDL	19.93	10.79	52.95	1.44	0.33	0.05	<MDL	<MDL	<MDL	8.00	0.26	2.08	0.19	2.26	0.20	3.40	2.98	6.38	<MDL	<MDL	QL	0.02	<MDL	<MDL	<MDL	<MDL	102.60
LOC 9	Horseshoe Pond, Merrimack, Naticook OUTFLOW	42.8421	-71.4873	<MDL	<MDL	4.43	3.09	14.52	0.42	0.11	0.05	<MDL	0.11	<MDL	<MDL	0.40	0.65	<MDL	0.65	0.04	0.71	0.69	1.40	<MDL	<MDL	QL	<MDL	<MDL	<MDL	<MDL	<MDL	25.22
LOC 9	Horseshoe Pond, Merrimack, CENTER	42.8496	-71.4914	<MDL	<MDL	7.97	5.26	26.12	0.54	0.22	0.08	<MDL	<MDL	<MDL	5.50	0.54	2.93	0.43	3.36	0.16	2.08	1.66	3.74	<MDL	<MDL	QL	0.04	QL	<MDL	<MDL	<MDL	53.53
	Median (n=9) (ng/L)					5.76	2.13	8.66	0.58	0.22	0.13										1.19	1.00	2.37									27.81
	Mean (n=9) (ng/L)					8.06	3.74	11.31	0.96	0.34	0.11										1.83	1.32	3.14									36.51
	DF (n=9) (%)			44%	0%	89%	100%	100%	100%	100%	78%	0%	11%	11%	22%	33%	67%	22%	67%	67%	100%	100%	100%	0%	0%	22%	56%	11%	11%	11%	11%	100%

Concentrations in ng/L

Surface water locations highlighted grey are the paired water samples where the fish were collected

Mean, median and detection frequency (DF) were determined for only the nine samples where fish were subsequently collected

Median/Mean calculated for compounds if >75% of samples have concentration >MDL

See Table S7 in SI for MDL values for PFAS analytes

<MDL = below method detection limit

QH = qualifier peak flagged high

QL = qualifier peak flagged low