**Supporting Information**

**Bioaccumulation and trophic magnification of emerging and legacy per- and polyfluoroalkyl substances (PFAS) in a St. Lawrence River food web**

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**Figure S2.** Full Scan UHPLC-HRMS chromatograms **(1)** and high-resolution MS/MS spectra (normalized collision energy of 35%, shown at 6.54-6.58 min) **(2)** of *m/z* 460.93447, in surface water from the St. Lawrence River and a PFECHS reference standard (Wellington Labs, Guelph, ON, Canada). Peaks at RT = 6.27 min and 6.38 min are possible PFECHS isomers (see also Figure S3) among those identified previously in literature (Stefanac et al. 2018; Wang et al. 2016).

**Figure S3.** High-resolution MS/MS spectra of *m/z* 460.93447 in a St. Lawrence River water sample at retention times 6.28, 6.37, 6.45 and 6.54 min. Peaks (1) and (2) are presumably different PFECHS isomers (Stefanac et al. 2018; Wang et al. 2016) while peak (3) is artefactual (present in the MS/MS chromatogram but not in Full Scan MS, see also Figure S2).

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**Table S13.** Biomagnification factors (BMF; average value) of PFAS for predator/prey couples of this freshwater food web. Data in yellow highlight are those above 1 (biomagnified).

**Table S14.** Trophic magnification factors (TMF) based on protein-normalized PFAS data, using a generalized linear mixed-effect model (GLMM) also accounting for censored data (R-package LMEC). TMFs not significantly different from 1 (TMF~1) are in grey font. Associated TMF confidence intervals (95% CI) and Akaike information criterion (AIC) are also provided.

**References**

**Text S1.** Chemicals and materials.

The list of 60 target PFAS with certified standards and their supplier company is provided in Table S1. Surrogate and performance internal standards are listed in Table S2. HPLC water, HPLC water containing 0.1% formic acid, methanol, and acetonitrile of HPLC grade or Optima HPLC grade were purchased from Fisher Scientific (Whitby, ON, Canada). Ammonium acetate (purity ≥ 98%), ammonium hydroxide (28–30%, NH3 basis), and formic acid (reagent grade, purity ≥ 95%) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Nitrogen (N2) (purity 99.998%) was from MEGS Inc. (St-Laurent, QC, Canada). Supelclean ENVI-Carb cartridges (250 mg/6 mL) were purchased from Supelco (Bellefonte, PA, USA) and Strata X-AW cartridges (200 mg/6 mL) from Phenomenex (Torrance, CA, USA). The high-speed LP Vortex Mixer was from Fisher Scientific (Whitby, ON, Canada).

**Text S2.** Sampling protocols.

For river water, 500 mL high-density polyethylene bottles were rinsed 3 times with the site water before collection at the subsurface (~1m depth). Sediment samples were collected using a sediment grab sampler and an aliquot was transferred to amber glass jars (100 mL at each site). A D-net was used to collect invertebrates, and the samples were stored in 50-mL polypropylene tubes. Fish were collected using a beach seine or a gillnet. Apart from yellow perch for which juveniles and adults were both sampled for analysis, only adult fish were selected for other species; juvenile bycatch were rapidly released. Fishes were euthanized in 400 mg/L clove oil, weighed, measured, and stored in polyethylene bags or polypropylene tubes. Additional information on fish size can be found in Table S3. Sampling protocols for fish were approved by Environment Climate Change Canada’s Animal Care Committee, working under the Canadian Council on Animal Care. Water, sediment, and biota samples were placed on ice until arrival at the laboratory and stored frozen (–20 °C) until further processing.

**Text S3.** Quality assurance/quality control (QA/QC).

Nonextracted solvent-based calibration curves (iCAL) and extracted matrix-matched calibration curves (mCAL) were run along each LC-MS sequence. A linear fit with inverse weighting was applied to eight- to ten-point based calibration curves.

iCAL were produced with suitable linearity, with R2 in the overall range of 0.9932-0.9999 (Table S5). After submitting the iCAL, continued calibration verification (CCV) standards (solvent-based) were run after every 10 injections to monitor the instrument state (absolute areas, retention times, and accuracy% of target PFAS). Accuracies of CCV standards were determined using iCAL. Compound-specific mean accuracy% of CCV standards (overall n = 27) ranged between 80.2-130.1%.

mCAL were constructed in matrix samples with low ambient PFAS, including river water from the St. Maurice River (R2: 0.9915-0.9997), sediment from the St. Lawrence River collected near Saint-Romuald, Lévis (R2: 0.9918-0.9998), and Pangasius (*Pangasinodon hypothalamus*) freshwater fish (R2: 0.9925-0.9999). The matrix-based calibration curves were used to determine PFAS concentrations in the actual field samples. In addition, since several types of biotic samples were included in our study, a subset of the samples (3 aquatic plant species, 1 invertebrate species, and 3 fish species) were subjected to standard additions to verify whole-method recoveries.

Procedural blanks for the water procedure (mineral bottled water, n = 6) showed detectable levels of PFAAs (generally <0.05 ng/L) and 6:2 fluorotelomer sulfonate (6:2 FTSA, ~0.01 ng/L). Procedural blanks for solids (Ottawa sand), including sediment (n = 3) and biotic (n = 7) procedures, had perfluorobutanoic acid (PFBA, ~0.05 ng and ~0.10 ng in extracts, respectively), perfluorohexadecanoic acid (PFHxDA, ~0.07 ng and ~0.20 ng), and 6:2 FTSA (~0.05 ng and ~0.03 ng) as mainly detected analytes.

Method limits of detection (LODs) were derived as three times the standard deviation of procedural blanks or from the signal intensity of low-level matrix spikes, whichever was greater. Method LODs were in the range of 0.003-0.34 ng/L for surface water, 0.0006-0.15 ng/g d.w. for sediment, and 0.004-0.59 ng/g w.w. for biota (Table S6).

Matrix spike accuracies are compiled in Table S7. PFAS accuracy% in triplicate river water samples spiked at 1 ng/L ranged between 77.7% and 121.3%, with an associated precision (RSD%) of 0.5-24.2%. PFAS accuracy% in triplicate sediment samples spiked at 1 ng/g ranged between 73.6% and 116.7%, with an RSD range of 1.0-27.0%. In Pangasius fish matrix fortified with PFAS at 15 ng/g, matrix spike accuracy ranged between 86.8% and 111.4% (RSD: 0.1-10.6%).

Method trueness was evaluated upon replicate sample preparations (n = 5) of a NIST standard reference material (SRM 1947 Lake Michigan fish tissue) (Table S8). The measured concentration for PFOS of 6.01 ± 0.11 ng/g w.w. compared well with the NIST certified value of 5.90 ± 0.39 ng/g w.w. (accuracy of 102%, Z-score of +0.28).

**Text S4.** Protein quantification.

Proteins were determined on a subsample of whole-body homogenate (i.e., subsampling of a whole-body homogenate (excluding skull for larger fish and removing viscera for all fishes) that was kept at -20°C until analysis). Further disruption/homogenization of biotic tissues was performed using a TissueLyser (Qiagen) microball mill. The samples were homogenized in 0.15M sodium chloride. Note that wet tissues were used for total protein, with the weight of the tissue used for homogenization around 30-35 mg for 500 µL of homogenization buffer. Protein quantification was performed by spectrophotometry (Bradford colorimetric test), following the standard procedure from the manufacturer, and using bovine serum albumin (BSA) to construct the calibration curve.

**Text S5.** Calculation of trophic levels and bioaccumulation and biomagnification factors.

***Trophic levels***

Trophic levels were obtained using the following equation (Fisk et al. 2001):

where a is the known trophic level of the baseline organism (set to aquatic plants in the current case, with a = 1 for heterotrophic organisms).

***Bioaccumulation and biomagnification factors***

Bioaccumulation factors (BAF, L/kg) were calculated as follows (Xu et al. 2014):

where Cbiota is the concentration of PFAS in biota (in ng/kg wet weight whole-body) and Cwater that in the river water (in ng/L) from the corresponding site.

Biota–sediment accumulation factors (BSAF, L/kg) were calculated as follows:

where Cbiota is the concentration of PFAS in benthic biota (in ng/g wet weight whole-body) and Csediment that in the sediment (in ng/g dry weight) from the corresponding site.

Biomagnification factors (BMF) were calculated by comparing the contamination level in predator ([x]) to that of its prey ([y]) (Conder et al. 2012):

Where x is the predator (or herbivore in the case of plant grazers) and y is the prey item.

***Trophic magnification factors***

Trophic magnification factors (TMF) were calculated as per the following equations (Kidd et al. 2019):

Where log(contaminant) is the logarithm of the PFAS concentration (ng/g whole-body basis) and TL is the trophic level.

Different regression approaches were tested, representing models previously used in TMF literature: parametric linear regression on all datapoints or on geomeans per taxa (balanced method) (Li et al. 2021; Madgett et al. 2021), nonparametric linear regression and nonparametric linear regression accounting for <LOD data (Munoz et al. 2017; Simonnet-Laprade et al. 2019), and generalized linear mixed-effect model also accounting for <LOD data (Munoz et al. 2017; Simonnet-Laprade et al. 2019). R scripts are summarized below, with PFOS as an example.

> Dataframe\_TMF <- read.delim("D:/Project-TMF/Dataframe\_TMF\_notepad.txt")

> install.packages(‘mblm’)

> install.packages(‘NADA’)

> install.packages(‘lmec’)

* **Method 1.**

> model <- lm(PFDoDA ~ TL, data = Dataframe\_TMF)

> summary(model)

(The TMF is then derived from the regression slope, if significant (p<0.05)).

* **Method 2.** Same script as method 1, but inputting the dataframe of geomean PFAS concentrations and geomean trophic positions per species, rather than individual data points.
* **Method 3.**

> library(mblm)

> model = mblm(PFOS~TL,data=Dataframe\_TMF)

> summary(model)

(The TMF is then derived from the regression slope, if significant (p<0.05)).

* **Method 4.**

> library(NADA)

> regression = with (Dataframe\_TMF, cenken(PFOS, PFOS\_Cen, TL, TL\_Cen))

> regression

(The TMF is then derived from the regression slope, if significant (p<0.05)).

* **Method 5.** (Munoz et al. 2017).

> library(NADA)

> library(lmec)

> cens = Dataframe\_TMF$PFOS\_Cen

> yL = Dataframe\_TMF$PFOS

> X = cbind(rep(1, length(yL)), Dataframe\_TMF$TL)

> cluster = as.numeric(Dataframe\_TMF$Taxa)

> Z = matrix(rep(1,length(yL)),ncol=1)

> fit <- lmec(yL, cens, X, Z, cluster, method = "ML", maxstep = 40)

> res <- cbind.data.frame(lowerbound = fit$beta - 1.96\*diag(fit$varFix), estimate=fit$beta, upperbound=fit$beta+1.96\*diag(fit$varFix))

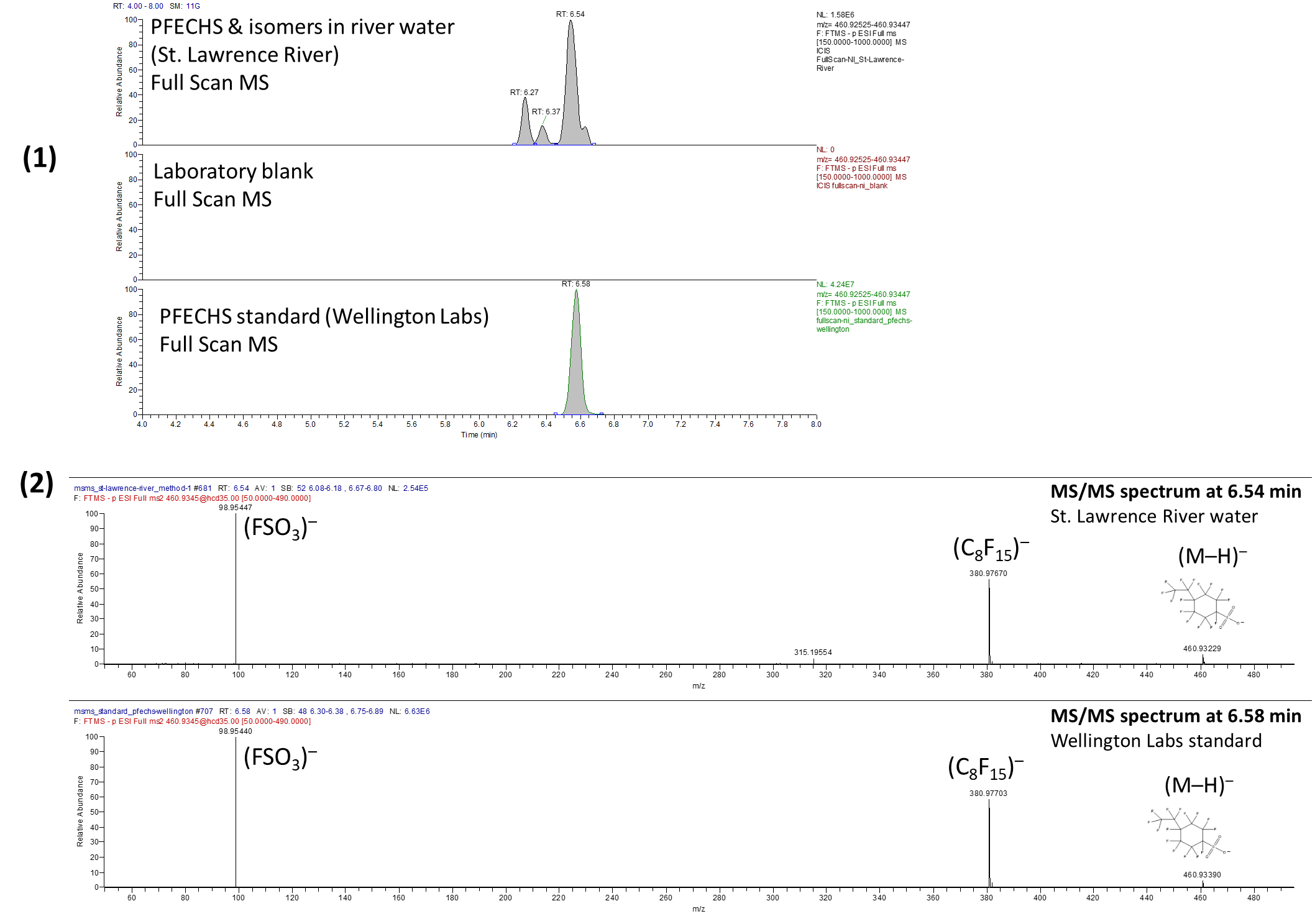
> TMF = 10^res[2,]

> TMF

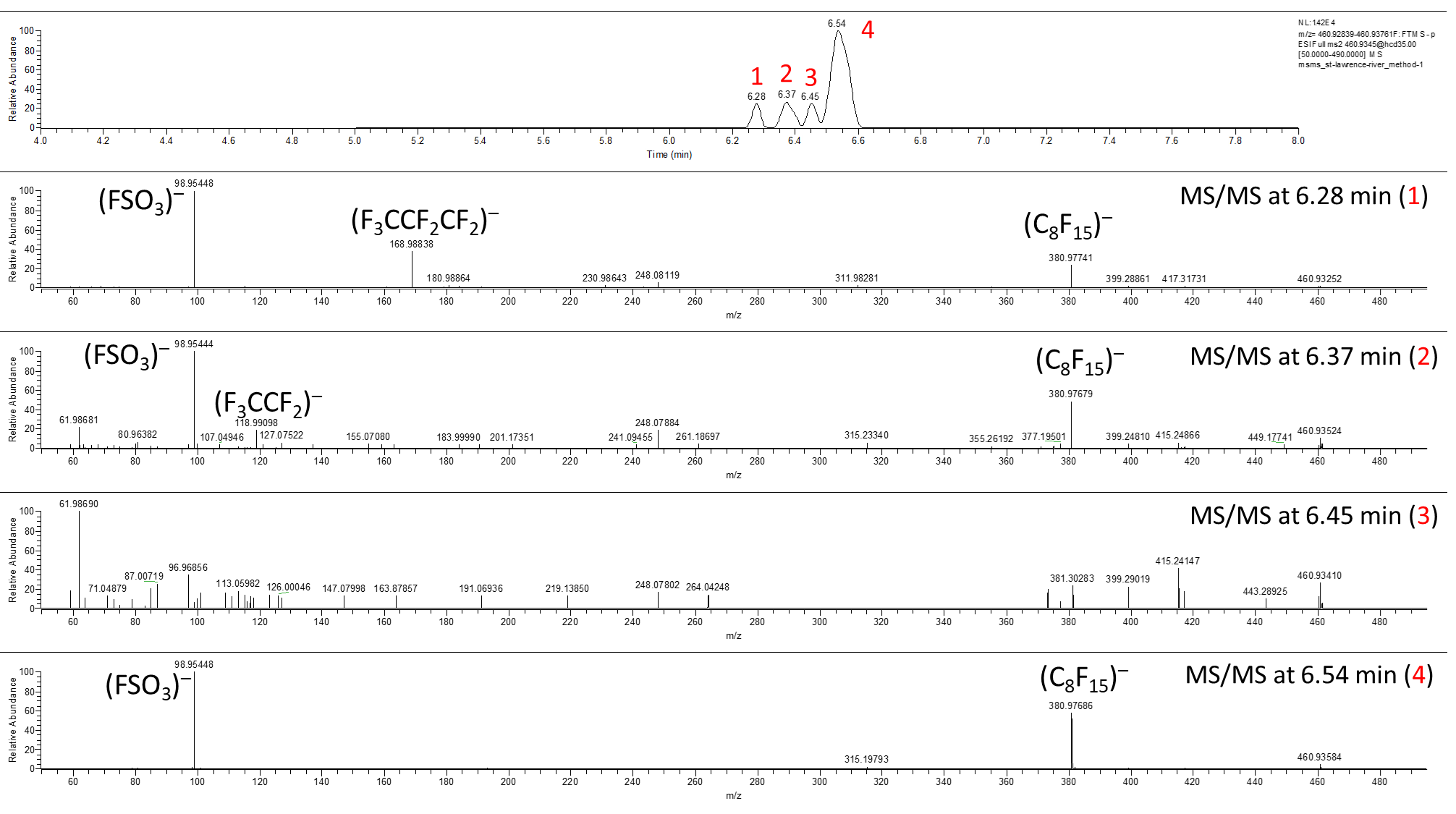
Une image contenant carte

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**Figure S1.** Map of the study area and geographical location of sampling sites (square symbols) in the St. Lawrence River near the island of Montreal, QC, Canada (1: Ile à Pinard, Iles de Boucherville; 2: Ile à la Truie, Ile aux Cerfeuils, Ilet Vert; 3: Ile Robinet).



**Figure S2.** Full Scan UHPLC-HRMS chromatograms **(1)** and high-resolution MS/MS spectra (normalized collision energy of 35%, shown at 6.54-6.58 min) **(2)** of *m/z* 460.93447, in surface water from the St. Lawrence River and a PFECHS reference standard (Wellington Labs, Guelph, ON, Canada). Peaks at RT = 6.27 min and 6.38 min are possible PFECHS isomers (see also Figure S3) among those identified previously in literature (Stefanac et al. 2018; Wang et al. 2016).

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**Figure S3.** High-resolution MS/MS spectra of *m/z* 460.93447 in a St. Lawrence River water sample at retention times 6.28, 6.37, 6.45 and 6.54 min. Peaks (1) and (2) are presumably different PFECHS isomers (Stefanac et al. 2018; Wang et al. 2016) while peak (3) is artefactual (present in the MS/MS chromatogram but not in Full Scan MS, see also Figure S2).

**Table S1.** Name and acronym of the 60 targeted PFAS with certified standards. Suppliers and ion mode used for analysis are also indicated.

|  |  |  |  |
| --- | --- | --- | --- |
| **Acronym** | **Name** | **Supplier** | **Ion mode** |
| PFBA | Perfluorobutanoic acid | Wellington Labs | ESI(-) |
| PFPeA | Perfluoropentanoic acid | Wellington Labs | ESI(-) |
| PFHxA | Perfluorohexanoic acid | Wellington Labs | ESI(-) |
| PFHpA | Perfluoroheptanoic acid | Wellington Labs | ESI(-) |
| PFOA | Perfluorooctanoic acid | Wellington Labs | ESI(-) |
| PFNA | Perfluorononanoic acid | Wellington Labs | ESI(-) |
| PFDA | Perfluorodecanoic acid | Wellington Labs | ESI(-) |
| PFUnA | Perfluoroundecanoic acid | Wellington Labs | ESI(-) |
| PFDoA | Perfluorododecanoic acid | Wellington Labs | ESI(-) |
| PFTrDA | Perfluorotridecanoic acid | Wellington Labs | ESI(-) |
| PFTeDA | Perfluorotetradecanoic acid | Wellington Labs | ESI(-) |
| PFHxDA | Perfluorohexadecanoic acid | Wellington Labs | ESI(-) |
| PFPrS | Perfluoropropane sulfonate | Wellington Labs | ESI(-) |
| PFBS | Perfluorobutane sulfonate | Wellington Labs | ESI(-) |
| PFPeS | Perfluorohexane sulfonate | Wellington Labs | ESI(-) |
| PFHxS | Perfluorohexane sulfonate | Wellington Labs | ESI(-) |
| PFHpS | Perfluoroheptane sulfonate | Wellington Labs | ESI(-) |
| PFOS | Perfluorooctane sulfonate | Wellington Labs | ESI(-) |
| PFNS | Perfluorononane sulfonate | Wellington Labs | ESI(-) |
| PFDS | Perfluorodecane sulfonate | Wellington Labs | ESI(-) |
| PFDoS | Perfluorododecane sulfonate | Wellington Labs | ESI(-) |
| FBSA | Perfluorobutane sulfonamide | Wellington Labs | ESI(-) |
| FHxSA | Perfluorohexane sulfonamide | Wellington Labs | ESI(-) |
| FOSA | Perfluorooctane sulfonamide | Wellington Labs | ESI(-) |
| MeFBSA | N-methyl-perfluorobutane sulfonamide | Wellington Labs | ESI(-) |
| MeFOSA | N-methyl-perfluorooctane sulfonamide | Wellington Labs | ESI(-) |
| EtFOSA | N-ethyl-perfluorooctane sulfonamide | Wellington Labs | ESI(-) |
| FOSAA | Perfluorooctane sulfonamidoacetic acid | Wellington Labs | ESI(-) |
| MeFOSAA | N-methyl-perfluorooctane sulfonamidoacetic acid | Wellington Labs | ESI(-) |
| EtFOSAA | N-ethyl-perfluorooctane sulfonamidoacetic acid | Wellington Labs | ESI(-) |
| 3:3 acid | 3:3 fluorotelomer carboxylate | Synquest | ESI(-) |
| 5:3 acid | 5:3 fluorotelomer carboxylate | DuPont USA | ESI(-) |
| 7:3 acid | 7:3 fluorotelomer carboxylate | DuPont USA | ESI(-) |
| 6:2 FTCA | 6:2 fluorotelomer carboxylate | Wellington Labs | ESI(-) |
| 8:2 FTCA | 8:2 fluorotelomer carboxylate | Wellington Labs | ESI(-) |
| 10:2 FTCA | 10:2 fluorotelomer carboxylate | Wellington Labs | ESI(-) |
| 4:2 FTSA | 4:2 fluorotelomer sulfonate | Wellington Labs | ESI(-) |
| 6:2 FTSA | 6:2 fluorotelomer sulfonate | Wellington Labs | ESI(-) |
| 8:2 FTSA | 8:2 fluorotelomer sulfonate | Wellington Labs | ESI(-) |
| 10:2 FTSA | 10:2 fluorotelomer sulfonate | Wellington Labs | ESI(-) |
| 6:2 diPAP | Bis-(1H,1H,2H,2H-perfluorooctyl)phosphate | Wellington Labs | ESI(-) |
| 6:2 Cl-PFESA | 6:2 chlorinated perfluoroalkylether sulfonate | Wellington Labs | ESI(-) |
| 8:2 Cl-PFESA | 8:2 chlorinated perfluoroalkylether sulfonate | Wellington Labs | ESI(-) |
| Gen-X (HFPO-DA) | Hexafluoropropylene oxide dimer acid | Wellington Labs | ESI(-) |
| DONA | Dodecafluoro-3H-4,8-dioxanonanoate | Wellington Labs | ESI(-) |
| Cl-PFOS | Chloroperfluorooctane sulfonate | Wellington Labs | ESI(-) |
| PFECHS | Perfluoro-4-ethylcyclohexane sulfonate | Wellington Labs | ESI(-) |
| PFHxPA | Perfluorohexylphosphonic acid | Wellington Labs | ESI(-) |
| PFOPA | Perfluorooctylphosphonic acid | Wellington Labs | ESI(-) |
| AmPr-FHxSA (PFHxSAm) | Perfluorohexane sulfonamidopropyl amine | Wellington Labs | ESI(+) |
| AmPr-FOSA (PFOSAm) | Perfluorooctane sulfonamidoalkyl amine | Fluobon | ESI(+) |
| T-AmPr-FHxSA (PFHxSAmS) | Perfluorohexane sulfonamidopropyl ammonium | Wellington Labs | ESI(+) |
| T-AmPr-FOSA (PFOSAmS) | Perfluorooctane sulfonamidopropyl ammonium | Fluobon | ESI(+) |
| CMeAmPr-FOAd (PFOAB) | Perfluorooctane amidopropyl betaine | Fluobon | ESI(+) |
| CMeAmPr-FOSA (PFOSB) | Perfluorooctane sulfonamidopropyl betaine | Fluobon | ESI(+) |
| OAmPr-FOAd (PFOANO) | Perfluorooctane amidopropyl amine oxide | Fluobon | ESI(+) |
| OAmPr-FOSA (PFOSNO) | Perfluorooctane sulfonamidopropyl amine oxide | Fluobon | ESI(+) |
| 5:3 FTB | 5:3 fluorotelomer betaine | Wellington Labs | ESI(+) |
| 5:1:2 FTB | 5:1:2 fluorotelomer betaine | Wellington Labs | ESI(+) |
| 6:2 FTSA-PrB (6:2 FTAB) | 6:2 fluorotelomer sulfonamidopropyl betaine | Wellington Labs | ESI(+) |

**Table S2.** Surrogate and injection internal standards used in this study, including acronyms, names, *m/z*, supplier company, and ion mode.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Use** | **Acronym** | **Name** | ***m/z*** | **Supplier** | **Ion mode** |
| Surrogate | **13C4-PFBA** | Perfluoro-n-[1,2,3,4-13C4]butanoic acid | 216.99177 | Wellington | ESI– |
| Surrogate | **13C5-PFPeA** | Perfluoro-n-[13C5]pentanoic acid | 267.99345 | Wellington | ESI– |
| Surrogate | **13C5-PFHxA** | Perfluoro-n-[1,2,3,4,6-13C5]hexanoic acid | 317.99046 | Wellington | ESI– |
| Surrogate | **13C4-PFHpA** | Perfluoro-n-[1,2,3,4-13C4]heptanoic acid | 366.98249 | Wellington | ESI– |
| Surrogate | **13C8-PFOA** | Perfluoro-n-[13C8]octanoic acid | 420.99272 | Wellington | ESI– |
| Surrogate | **13C9-PFNA** | Perfluoro-n-[13C9]nonanoic acid | 471.99288 | Wellington | ESI– |
| Surrogate | **13C6-PFDA** | Perfluoro-n-[1,2,3,4,5,6-13C6]decanoic acid | 518.97962 | Wellington | ESI– |
| Surrogate | **13C7-PFUnA** | Perfluoro-n-[1,2,3,4,5,6,7-13C7]undecanoic acid | 569.97978 | Wellington | ESI– |
| Surrogate | **13C2-PFDoA** | Perfluoro-n-[1,2-13C2]dodecanoic acid | 614.95981 | Wellington | ESI– |
| Surrogate | **13C2-PFTeDA** | Perfluoro-n-[1,2-13C2]tetradecanoic acid | 714.95342 | Wellington | ESI– |
| Surrogate | **13C3-PFBS** | Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate | 301.95251 | Wellington | ESI– |
| Surrogate | **13C3-PFHxS** | Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate | 401.94612 | Wellington | ESI– |
| Surrogate | **13C8-PFOS** | Sodium perfluoro-1-[13C8]octanesulfonate | 506.95641 | Wellington | ESI– |
| Surrogate | **13C8-FOSA** | Perfluoro-1-[13C8]octanesulfonamide | 505.97249 | Wellington | ESI– |
| Surrogate | **d3-N-MeFOSA** | N-methyl-d3-perfluoro-1-octanesulfonamide | 514.98013 | Wellington | ESI– |
| Surrogate | **d5-N-EtFOSA** | N-ethyl-d5-perfluoro-1-octanesulfonamide | 531.00830 | Wellington | ESI– |
| Surrogate | **d3-N-MeFOSAA** | N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid | 572.98561 | Wellington | ESI– |
| Surrogate | **d5-N-EtFOSAA** | N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid | 589.01382 | Wellington | ESI– |
| Surrogate | **13C2-6:2 FTSA** | 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-octane sulfonate | 428.97537 | Wellington | ESI– |
| Surrogate | **13C2-8:2 FTSA** | 1H,1H,2H,2H-perfluoro-1-[1,2-13C2]-decane sulfonate | 528.96898 | Wellington | ESI– |
| Surrogate | **13C4-6:2 diPAP** | 13C4-Bis-(1H,1H,2H,2H-perfluorooctyl)phosphate | 792.98792 | Wellington | ESI– |
| Surrogate | **13C3-HFPO-DA** | 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-13C3-propanoic acid | 286.98406 | Wellington | ESI– |
| Surrogate | **TAmPr-FOAd** | Perfluorooctane amidoalkyl ammonium | 513.10176 | Fluobon | ESI+ |
| Injection | **13C3-PFBA** | Perfluoro-n-[2,3,4-13C3]butanoic acid | 215.98871 | Wellington | ESI– |
| Injection | **13C2-PFHxA** | Perfluoro-n-[1,2-13C2]hexanoic acid | 314.98039 | Wellington | ESI– |
| Injection | **13C2-PFOA** | Perfluoro-n-[1,2-13C2]octanoic acid | 414.97258 | Wellington | ESI– |
| Injection | **13C5-PFNA** | Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid | 467.97969 | Wellington | ESI– |
| Injection | **13C2-PFDA** | Perfluoro-n-[1,2-13C2]decanoic acid | 514.96640 | Wellington | ESI– |
| Injection | **13C2-PFUnA** | Perfluoro-n-[1,2-13C2]undecanoic acid | 564.96326 | Wellington | ESI– |
| Injection | **18O2-PFHxS** | Sodium perfluoro-1-hexane[18O2]sulfonate | 402.94505 | Wellington | ESI– |
| Injection | **13C4-PFOS** | Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate | 502.94308 | Wellington | ESI– |

**Table S3.** Species andmorphometric measurements (length (cm) and weight (g)) of fish specimens sampled in the St. Lawrence River in 2019.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample code** | **Species** | **Length (cm)** | **Weight (g)** |
| AMRU-1 | *Amblolites rupestris* | 15.8 | 95.2 |
| AMRU-2 | *Amblolites rupestris* | 18 | 135 |
| AMRU-3 | *Amblolites rupestris* | 19.7 | 161 |
| AMRU-4 | *Amblolites rupestris* | 9.3 | 14 |
| AMRU-5 | *Amblolites rupestris* | 9.6 | 17.2 |
| AMRU-6 | *Amblolites rupestris* | 9.8 | 17.7 |
| CACO-1 | *Catostomus commersoni* | 44.6 | 985 |
| CACO-2 | *Catostomus commersoni* | 50.2 | 1505 |
| ESLU-2 | *Esox lucius* | 37.5 | 310 |
| ESLU-3 | *Esox lucius* | 42.6 | 445 |
| ESLU-4 | *Esox lucius* | 46.8 | 555 |
| ESLU-5 | *Esox lucius* | 55.2 | 900 |
| ESLU-6 | *Esox lucius* | 75.1 | 2345 |
| ESLU-7 | *Esox lucius* | 66 | 1000 |
| ESLU-9 | *Esox lucius* | 51.3 | 835 |
| LEGI-1 | *Lepomis gibossus* | 17.9 | 115 |
| LEGI-2 | *Lepomis gibossus* | 14.9 | 71 |
| LEGI-3 | *Lepomis gibossus* | 16.6 | 115 |
| LEGI-4 | *Lepomis gibossus* | 19.4 | 180 |
| LEGI-5 | *Lepomis gibossus* | 9.85 | 18.5 |
| MIDO-1 | *Micropterus dolomieu* | 18.9 | 101 |
| MIDO-2 | *Micropterus dolomieu* | 29.5 | 385 |
| MIDO-3 | *Micropterus dolomieu* | 19.9 | 118 |
| MIDO-4 | *Micropterus dolomieu* | 18.1 | 80 |
| MIDO-5 | *Micropterus dolomieu* | 38.2 | 685 |
| MIDO-6 | *Micropterus dolomieu* | 44 | 1105 |
| NEME-2 | *Neogobius melanostomus* | 10.6 | 15 |
| NEME-3 | *Neogobius melanostomus* | 11.3 | 22 |
| NEME-4 | *Neogobius melanostomus* | 9.9 | 15 |
| NEME-5 | *Neogobius melanostomus* | N/A | N/A |
| NEME-7 | *Neogobius melanostomus* | N/A | N/A |
| NEME-8 | *Neogobius melanostomus* | N/A | N/A |
| NEME-9 | *Neogobius melanostomus* | N/A | N/A |
| NOAT-1 | *Notropis atherinoides* | 8.9 | 4.35 |
| NOAT-2 | *Notropis atherinoides* | 9.4 | 5.25 |
| NOAT-3 | *Notropis atherinoides* | 9.45 | 6.5 |
| NOAT-4 | *Notropis atherinoides* | 9.2 | 6 |
| NOAT-5 | *Notropis atherinoides* | 9.8 | 6 |

**Table S3.** (Continued).

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample code** | **Species** | **Length (cm)** | **Weight (g)** |
| NOCR-1 | *Notemigonus crysoleucas* | 11.85 | 15.05 |
| NOCR-2 | *Notemigonus crysoleucas* | 10.75 | 12.75 |
| NOCR-3 | *Notemigonus crysoleucas* | 10.1 | 9.2 |
| NOCR-4 | *Notemigonus crysoleucas* | 10 | 8.45 |
| NOCR-5 | *Notemigonus crysoleucas* | 10.85 | 11.9 |
| NOCR-6 | *Notemigonus crysoleucas* | 9.5 | 7.45 |
| NOCR-7 | *Notemigonus crysoleucas* | 10 | 8.65 |
| NOST/VO-1 | *Notropis volucellus/straminueus* | 9.7 | 8 |
| NOST/VO-2 | *Notropis volucellus/straminueus* | 9.25 | 7 |
| NOST/VO-3 | *Notropis volucellus/straminueus* | 9.25 | 6 |
| NOST/VO-4 | *Notropis volucellus/straminueus* | 9.35 | 7 |
| NOST/VO-5 | *Notropis volucellus/straminueus* | 9 | 6 |
| NOST/VO-6 | *Notropis volucellus/straminueus* | 9.45 | 6.05 |
| NOST/VO-7 | *Notropis volucellus/straminueus* | 9.75 | 7.5 |
| NOST/VO-8 | *Notropis volucellus/straminueus* | 8.05 | 4.05 |
| PEFL-A-1 | *Perca flavescens* | 22 | 129 |
| PEFL-A-2 | *Perca flavescens* | 18.8 | 86 |
| PEFL-A-3 | *Perca flavescens* | 21.2 | 112 |
| PEFL-J-1 | *Perca flavescens* | 13.5 | 50 |
| PEFL-J-2 | *Perca flavescens* | 14 | 35 |
| PEFL-J-3 | *Perca flavescens* | 15.9 | 70 |
| PEFL-J-4 | *Perca flavescens* | 15.5 | 50 |
| PEFL-J-5 | *Perca flavescens* | 15.2 | 55 |
| PEFL-J-6 | *Perca flavescens* | 14 | 30 |
| PEFL-J-7 | *Perca flavescens* | 14.5 | 30 |
| PEFL-J-9 | *Perca flavescens* | 14.1 | 29.7 |
| PINO-1 | *Pimephales notatus* | 12.5 | 15 |
| PINO-2 | *Pimephales notatus* | 13 | 21 |
| PINO-3 | *Pimephales notatus* | 7.45 | 4.4 |
| PINO-4 | *Pimephales notatus* | 7.65 | 3.5 |
| PINO-5 | *Pimephales notatus* | 11.7 | 14 |
| Redhorse-1 | *Moxostoma spp.* | 48 | 1255 |
| Redhorse-2 | *Moxostoma spp.* | 44 | 880 |
| Redhorse-3 | *Moxostoma spp.* | 57.2 | 1900 |
| Redhorse-4 | *Moxostoma spp.* | 46.5 | 1025 |
| TITI-1 | *Tinca tinca* | 48.1 | 1325 |
| TITI-2 | *Tinca tinca* | 33.6 | 600 |

**Table S4.** UHPLC-HRMS method for PFAS analysis.

|  |  |
| --- | --- |
| **UHPLC-HRMS system** | Dionex Ultimate 3000 UHPLC chain  Heated electrospray ionization source  Thermo Q-Exactive Orbitrap high-resolution mass spectrometer |
| **Separation column** | Thermo Hypersil Gold C18 column (100 mm × 2.1 mm; 1.9 μm) |
| **Delay column** | Thermo Hypercarb column (20 mm × 2.1 mm; 7 μm) |
| **Column oven temperature** | 40°C |
| **UHPLC mobile phases** | A: 0.1% formic acid in HPLC-water  B: 0.1% formic acid in ACN  Mobile phase flow rate 550 μL/min |
| **Chromatographic gradient** | |  |  |  |  | | --- | --- | --- | --- | | Time (min) | % A | % B |  | | 0.0 | 90 | 10 |  | | 7.0 | 27.5 | 72.5 |  | | 8.5 | 0 | 100 |  | | 12.5 | 0 | 100 |  | | 12.6 | 90 | 10 |  | | 14.6 | 90 | 10 |  | |
| **Injection Volume** | Variable (20 μL for extracts of river water and sediments; 12 μL for extracts of biotic samples) |
| **Source** | Sheath gas flow rate 50 a.u.  Aux gas flow rate 15 a.u.  Sweep gas flow rate 0 a.u.  Positive spray voltage (|V|) 3500  Negative spray voltage (|V|) 3000  Capillary temperature (°C) 320  Vaporizer temperature (°C) 350  S-lens RF level 55 |
| **Q-Exactive Orbitrap settings** | Full Scan MS mode  Scan range (*m/z*) 150-1000  Resolution (FWHM) 70,000 at *m/z* 200  AGC target 3e6  Max. Inject Time (ms) 50 |

**Table S5.** Linearity performance (determination coefficients R2) of solvent-based (iCAL, multibatch replicates) and matrix-based (mCAL) calibration curves.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **iCAL (Solvent)** | **mCAL (River water)** | **mCAL (Sediment)** | **mCAL (Fish)** |
|  | R2 | R2 | R2 | R2 |
| PFBA | 0.9996-0.9997 | 0.9946 | 0.9968 | 0.9990 |
| PFPeA | 0.9992-0.9997 | 0.9973 | 0.9992 | 0.9996 |
| PFHxA | 0.9993-0.9999 | 0.9970 | 0.9996 | 0.9998 |
| PFHpA | 0.9984-0.9999 | 0.9972 | 0.9994 | 0.9997 |
| PFOA | 0.9993-0.9997 | 0.9994 | 0.9988 | 0.9991 |
| PFNA | 0.9990-0.9996 | 0.9991 | 0.9983 | 0.9998 |
| PFDA | 0.9987-0.9999 | 0.9996 | 0.9991 | 0.9995 |
| PFUnDA | 0.9994-0.9999 | 0.9997 | 0.9984 | 0.9992 |
| PFDoDA | 0.9983-0.9997 | 0.9997 | 0.9989 | 0.9991 |
| PFTrDA | 0.9976-0.9994 | 0.9993 | 0.9989 | 0.9972 |
| PFTeDA | 0.9979-0.9998 | 0.9995 | 0.9984 | 0.9982 |
| PFHxDA | 0.9971-0.9996 | 0.9994 | 0.9976 | 0.9933 |
| PFPrS | 0.9993-0.9997 | 0.9997 | 0.9998 | 0.9978 |
| PFBS | 0.9993-0.9999 | 0.9978 | 0.9997 | 0.9997 |
| PFPeS | 0.9977-0.9998 | 0.9989 | 0.9990 | 0.9992 |
| PFHxS | 0.9993-0.9999 | 0.9995 | 0.9997 | 0.9999 |
| PFHpS | 0.9965-0.9991 | 0.9963 | 0.9985 | 0.9987 |
| PFOS | 0.9995-0.9999 | 0.9996 | 0.9972 | 0.9996 |
| PFNS | 0.9971-0.9994 | 0.9977 | 0.9987 | 0.9967 |
| PFDS | 0.9958-0.9994 | 0.9946 | 0.9995 | 0.9991 |
| PFDoS | 0.9933-0.9992 | 0.9978 | 0.9994 | 0.9955 |
| FBSA | 0.9957-0.9989 | 0.9947 | 0.9989 | 0.9986 |
| FHxSA | 0.9986-0.9997 | 0.9948 | 0.9998 | 0.9964 |
| FOSA | 0.9996-0.9999 | 0.9997 | 0.9992 | 0.9991 |
| MeFBSA | 0.9987-0.9998 | 0.9983 | 0.9925 | 0.9993 |
| MeFOSA | 0.9959-0.9997 | 0.9978 | 0.9998 | 0.9998 |
| EtFOSA | 0.9958-0.9999 | 0.9982 | 0.9983 | 0.9995 |
| FOSAA | 0.9959-0.9978 | 0.9987 | 0.9997 | 0.9977 |
| MeFOSAA | 0.9983-0.9999 | 0.9992 | 0.9997 | 0.9993 |
| EtFOSAA | 0.9981-0.9994 | 0.9989 | 0.9994 | 0.9997 |
| 3:3 Acid | 0.9979-0.9999 | 0.9915 | 0.9989 | 0.9977 |
| 5:3 Acid | 0.9978-0.9997 | 0.9990 | 0.9976 | 0.9993 |
| 7:3 Acid | 0.9986-0.9998 | 0.9996 | 0.9993 | 0.9945 |
| 6:2 FTCA | 0.9982-0.9997 | 0.9952 | 0.9984 | 0.9985 |
| 8:2 FTCA | 0.9955-0.9998 | 0.9995 | 0.9994 | 0.9925 |
| 10:2 FTCA | 0.9963-0.9992 | 0.9997 | 0.9984 | 0.9927 |
| 4:2 FTSA | 0.9993-0.9998 | 0.9975 | 0.9997 | 0.9992 |
| 6:2 FTSA | 0.9997-0.9999 | 0.9993 | 0.9990 | 0.9996 |
| 8:2 FTSA | 0.9991-0.9999 | 0.9994 | 0.9988 | 0.9995 |
| 10:2 FTSA | 0.9942-0.9993 | 0.9940 | 0.9991 | 0.9992 |
| Gen-X | 0.9986-0.9995 | 0.9947 | 0.9993 | 0.9991 |
| ADONA | 0.9987-0.9993 | 0.9992 | 0.9962 | 0.9991 |
| 6:2 Cl-PFESA | 0.9981-0.9998 | 0.9985 | 0.9995 | 0.9976 |
| 8:2 Cl-PFESA | 0.9980-0.9996 | 0.9976 | 0.9997 | 0.9988 |
| Cl-PFOS | 0.9977-0.9997 | 0.9983 | 0.9996 | 0.9971 |
| PFECHS | 0.9953-0.9998 | 0.9987 | 0.9996 | 0.9994 |
| PFHxPA | 0.9932-0.9981 | 0.9937 | 0.9964 | 0.9958 |
| PFOPA | 0.9976-0.9998 | 0.9958 | 0.9918 | 0.9996 |
| 6:2 diPAP | 0.9988-0.9998 | 0.9985 | 0.9981 | 0.9995 |
| PFHxSAm | 0.9935-0.9997 | 0.9959 | 0.9995 | 0.9990 |
| PFOSAm | 0.9988-0.9993 | 0.9921 | 0.9978 | 0.9957 |
| PFHxSAmS | 0.9959-0.9990 | 0.9988 | 0.9985 | 0.9987 |
| PFOSAmS | 0.9986-0.9994 | 0.9978 | 0.9995 | 0.9954 |
| PFOANO | 0.9977-0.9996 | 0.9964 | 0.9964 | 0.9991 |
| PFOSNO | 0.9979-0.9997 | 0.9959 | 0.9951 | 0.9962 |
| PFOAB | 0.9962-0.9989 | 0.9960 | 0.9973 | 0.9979 |
| PFOSB | 0.9981-0.9985 | 0.9982 | 0.9981 | 0.9970 |
| 5:3 FTB | 0.9981-0.9996 | 0.9985 | 0.9990 | 0.9996 |
| 5:1:2 FTB | 0.9987-0.9996 | 0.9971 | 0.9964 | 0.9997 |
| 6:2 FTAB | 0.9986-0.9994 | 0.9946 | 0.9920 | 0.9994 |

**Table S6.** Method limits of detection (LODs) of PFAS for river water, sediment, and biota samples.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **River water** | **Sediment** | **Biota samples** |
|  | **LOD (ng/L)** | **LOD (ng/g dry weight)** | **LOD (ng/g wet weight)** |
| PFBA | 0.119 | 0.0468 | 0.126 |
| PFPeA | 0.058 | 0.0069 | 0.030 |
| PFHxA | 0.086 | 0.0050 | 0.016 |
| PFHpA | 0.017 | 0.0036 | 0.020 |
| PFOA | 0.188 | 0.0025 | 0.013 |
| PFNA | 0.006 | 0.0038 | 0.010 |
| PFDA | 0.011 | 0.0039 | 0.007 |
| PFUnDA | 0.008 | 0.0038 | 0.008 |
| PFDoDA | 0.007 | 0.0042 | 0.015 |
| PFTrDA | 0.011 | 0.0025 | 0.009 |
| PFTeDA | 0.011 | 0.0086 | 0.022 |
| PFHxDA | 0.021 | 0.0709 | 0.591 |
| PFPrS | 0.008 | 0.0015 | 0.017 |
| PFBS | 0.004 | 0.0010 | 0.005 |
| PFPeS | 0.003 | 0.0008 | 0.006 |
| PFHxS | 0.005 | 0.0012 | 0.005 |
| PFHpS | 0.003 | 0.0007 | 0.007 |
| PFOS | 0.012 | 0.0038 | 0.004 |
| PFNS | 0.005 | 0.0008 | 0.007 |
| PFDS | 0.008 | 0.0010 | 0.011 |
| PFDoS | 0.009 | 0.0006 | 0.005 |
| FBSA | 0.012 | 0.0012 | 0.006 |
| FHxSA | 0.007 | 0.0012 | 0.016 |
| FOSA | 0.007 | 0.0013 | 0.023 |
| MeFBSA | 0.022 | 0.0032 | 0.020 |
| MeFOSA | 0.028 | 0.0019 | 0.010 |
| EtFOSA | 0.019 | 0.0019 | 0.013 |
| FOSAA | 0.006 | 0.0050 | 0.161 |
| MeFOSAA | 0.029 | 0.0066 | 0.011 |
| EtFOSAA | 0.020 | 0.0061 | 0.008 |
| 3:3 Acid | 0.154 | 0.0313 | 0.163 |
| 5:3 Acid | 0.043 | 0.0059 | 0.071 |
| 7:3 Acid | 0.036 | 0.0068 | 0.032 |
| 6:2 FTCA | 0.071 | 0.0208 | 0.108 |
| 8:2 FTCA | 0.053 | 0.0236 | 0.127 |
| 10:2 FTCA | 0.091 | 0.0139 | 0.034 |
| 4:2 FTSA | 0.004 | 0.0029 | 0.014 |
| 6:2 FTSA | 0.036 | 0.0498 | 0.054 |
| 8:2 FTSA | 0.007 | 0.0018 | 0.014 |
| 10:2 FTSA | 0.014 | 0.0024 | 0.086 |
| Gen-X | 0.069 | 0.0385 | 0.414 |
| ADONA | 0.006 | 0.0021 | 0.016 |
| 6:2 Cl-PFESA | 0.006 | 0.0013 | 0.014 |
| 8:2 Cl-PFESA | 0.013 | 0.0011 | 0.011 |
| Cl-PFOS | 0.005 | 0.0014 | 0.009 |
| PFECHS | 0.003 | 0.0013 | 0.009 |
| PFHxPA | 0.013 | 0.0179 | 0.023 |
| PFOPA | 0.017 | 0.0094 | 0.016 |
| 6:2 diPAP | 0.347 | 0.0196 | 0.004 |
| PFHxSAm | 0.012 | 0.0022 | 0.035 |
| PFOSAm | 0.032 | 0.0023 | 0.036 |
| PFHxSAmS | 0.015 | 0.0012 | 0.021 |
| PFOSAmS | 0.035 | 0.0024 | 0.034 |
| PFOANO | 0.033 | 0.0033 | 0.058 |
| PFOSNO | 0.036 | 0.0036 | 0.047 |
| PFOAB | 0.030 | 0.0192 | 0.081 |
| PFOSB | 0.092 | 0.0208 | 0.161 |
| 5:3 FTB | 0.011 | 0.0381 | 0.027 |
| 5:1:2 FTB | 0.042 | 0.1559 | 0.030 |
| 6:2 FTAB | 0.022 | 0.0063 | 0.053 |

**Table S7.** Accuracy (%) and precision (RSD%) of matrix spikes to river water (PFAS spike level: 1 ng/L), sediment (1 ng/g), and biota samples (15 ng/g).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | River water | | Sediment | | Fish | |
|  | Accuracy% (n = 3) | RSD% (n = 3) | Accuracy% (n = 3) | RSD% (n = 3) | Accuracy% (n = 3) | RSD% (n = 3) |
| PFBA | 121.3 | 19.2 | 108.3 | 3.4 | 97.7 | 2.8 |
| PFPeA | 96.1 | 6.6 | 110.1 | 2.7 | 100.2 | 1.6 |
| PFHxA | 103.5 | 8.5 | 106.4 | 4.4 | 94.8 | 0.3 |
| PFHpA | 97.2 | 7.7 | 109.2 | 1.7 | 99.7 | 1.9 |
| PFOA | 100.2 | 3.3 | 108.7 | 2.6 | 97.4 | 2.0 |
| PFNA | 96.8 | 2.6 | 109.3 | 2.7 | 101.3 | 0.1 |
| PFDA | 97.8 | 2.0 | 109.8 | 1.0 | 99.1 | 1.8 |
| PFUnDA | 99.3 | 2.5 | 109.2 | 2.5 | 101.9 | 1.6 |
| PFDoDA | 103.7 | 1.9 | 110.5 | 2.0 | 101.8 | 2.4 |
| PFTrDA | 102.2 | 2.8 | 113.1 | 6.9 | 94.7 | 4.0 |
| PFTeDA | 99.6 | 1.3 | 112.5 | 2.2 | 109.4 | 1.1 |
| PFHxDA | 98.3 | 1.4 | 110.6 | 1.3 | 101.6 | 1.0 |
| PFPrS | 95.6 | 4.7 | 102.6 | 2.6 | 93.2 | 4.6 |
| PFBS | 91.8 | 4.5 | 109.1 | 2.7 | 96.1 | 2.8 |
| PFPeS | 98.1 | 5.5 | 104.7 | 2.0 | 94.6 | 1.6 |
| PFHxS | 98.3 | 1.7 | 109.1 | 1.0 | 98.3 | 1.4 |
| PFHpS | 96.0 | 4.8 | 113.1 | 4.6 | 97.6 | 3.2 |
| PFOS | 97.2 | 1.9 | 110.1 | 4.0 | 98.7 | 2.3 |
| PFNS | 91.3 | 1.8 | 109.2 | 2.8 | 98.0 | 5.1 |
| PFDS | 90.1 | 1.7 | 104.0 | 1.0 | 95.9 | 10.3 |
| PFDoS | 93.3 | 1.7 | 103.5 | 1.4 | 88.8 | 8.1 |
| FBSA | 106.1 | 10.9 | 106.0 | 5.5 | 95.5 | 7.7 |
| FHxSA | 111.7 | 1.7 | 106.5 | 4.2 | 98.8 | 5.7 |
| FOSA | 97.4 | 0.5 | 109.3 | 3.0 | 100.5 | 4.8 |
| MeFBSA | 93.6 | 8.1 | 103.4 | 9.0 | 105.5 | 0.9 |
| MeFOSA | 97.9 | 3.4 | 107.3 | 2.1 | 97.7 | 1.6 |
| EtFOSA | 103.3 | 5.6 | 108.4 | 1.7 | 94.3 | 4.0 |
| FOSAA | 99.6 | 6.1 | 89.0 | 27.0 | 87.3 | 2.1 |
| MeFOSAA | 95.2 | 3.5 | 111.1 | 1.1 | 95.1 | 0.9 |
| EtFOSAA | 95.9 | 2.5 | 108.3 | 4.5 | 101.2 | 1.7 |
| 3:3 Acid | 82.7 | 14.1 | 102.1 | 5.1 | 94.7 | 2.5 |
| 5:3 Acid | 97.3 | 5.9 | 110.1 | 4.6 | 103.1 | 4.1 |
| 7:3 Acid | 95.0 | 9.9 | 116.7 | 6.5 | 98.2 | 4.8 |
| 6:2 FTCA | 105.3 | 5.1 | 103.4 | 4.0 | 106.1 | 3.0 |
| 8:2 FTCA | 94.2 | 5.1 | 106.0 | 4.4 | 106.9 | 6.3 |
| 10:2 FTCA | 101.6 | 4.5 | 115.3 | 3.6 | 107.0 | 2.0 |
| 4:2 FTSA | 101.3 | 6.0 | 106.4 | 1.8 | 90.9 | 2.1 |
| 6:2 FTSA | 99.6 | 2.3 | 106.7 | 1.5 | 99.4 | 1.4 |
| 8:2 FTSA | 97.3 | 2.6 | 109.9 | 2.6 | 96.5 | 2.0 |
| 10:2 FTSA | 88.9 | 3.4 | 107.8 | 2.5 | 98.0 | 3.4 |
| Gen-X | 85.6 | 24.2 | 105.4 | 4.6 | 86.8 | 10.6 |
| ADONA | 97.1 | 13.3 | 111.9 | 6.6 | 97.6 | 3.1 |
| 6:2 Cl-PFESA | 93.3 | 2.3 | 106.6 | 1.2 | 94.3 | 2.9 |
| 8:2 Cl-PFESA | 89.3 | 4.8 | 105.0 | 2.5 | 98.4 | 4.0 |
| Cl-PFOS | 92.6 | 3.9 | 106.7 | 3.0 | 95.2 | 3.0 |
| PFECHS | 97.2 | 2.2 | 99.0 | 1.6 | 93.4 | 1.9 |
| PFHxPA | 94.9 | 3.9 | 73.6 | 9.0 | 93.0 | 2.9 |
| PFOPA | 94.5 | 5.9 | 78.1 | 6.5 | 111.4 | 3.5 |
| 6:2 diPAP | 83.5 | 8.5 | 109.6 | 4.0 | 103.9 | 0.7 |
| PFHxSAm | 99.1 | 5.2 | 96.6 | 2.5 | 88.4 | 1.7 |
| PFOSAm | 96.2 | 10.1 | 104.6 | 1.2 | 87.1 | 1.8 |
| PFHxSAmS | 99.9 | 4.2 | 94.0 | 2.2 | 100.2 | 1.9 |
| PFOSAmS | 93.6 | 1.2 | 105.6 | 1.2 | 93.9 | 1.3 |
| PFOANO | 99.1 | 6.8 | 101.9 | 4.6 | 93.4 | 3.8 |
| PFOSNO | 93.8 | 3.5 | 103.9 | 5.0 | 88.4 | 1.9 |
| PFOAB | 93.0 | 12.3 | 94.0 | 5.4 | 90.5 | 2.0 |
| PFOSB | 77.7 | 7.8 | 105.1 | 3.4 | 92.4 | 3.1 |
| 5:3 FTB | 95.1 | 4.2 | 104.0 | 5.3 | 98.5 | 2.6 |
| 5:1:2 FTB | 95.6 | 3.7 | 108.4 | 4.2 | 97.5 | 1.8 |
| 6:2 FTAB | 92.4 | 3.2 | 91.0 | 2.5 | 110.1 | 2.6 |

**Table S8.** Absolute recovery % of the biota sample preparation method, validated by standard additions to 7 species from the present survey. Data in red highlight are those outside the target performance scope of 70-130%. **(a) Aquatic vegetation.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Water sedge (CAREX) | |  | Water stargrass (HEDU) | |  | Pondweed (PORI) | |
|  | Mean | Stdev |  | Mean | Stdev |  | Mean | Stdev |
| PFBA | 87.8 | 3.6 |  | 88.6 | 3.4 |  | 88.1 | 3.9 |
| PFPeA | 90.4 | 2.1 |  | 91.6 | 2.4 |  | 89.2 | 3.1 |
| PFHxA | 93.4 | 3.7 |  | 88.0 | 1.4 |  | 90.8 | 2.1 |
| PFHpA | 90.9 | 1.0 |  | 88.9 | 2.3 |  | 88.2 | 1.8 |
| PFOA | 91.5 | 1.4 |  | 85.6 | 2.7 |  | 91.3 | 2.2 |
| PFNA | 90.2 | 2.4 |  | 88.7 | 0.6 |  | 92.6 | 2.4 |
| PFDA | 90.8 | 2.4 |  | 88.0 | 0.3 |  | 92.1 | 2.8 |
| PFUnDA | 90.6 | 1.1 |  | 90.0 | 0.8 |  | 92.1 | 1.0 |
| PFDoDA | 90.2 | 1.6 |  | 90.1 | 1.3 |  | 92.7 | 1.9 |
| PFTrDA | 90.4 | 2.0 |  | 86.8 | 2.9 |  | 93.0 | 1.7 |
| PFTeDA | 92.4 | 1.0 |  | 90.1 | 1.2 |  | 94.5 | 1.5 |
| PFHxDA | 91.9 | 1.0 |  | 90.0 | 0.7 |  | 92.9 | 1.4 |
| PFPrS | 93.1 | 1.2 |  | 93.8 | 2.9 |  | 90.6 | 2.7 |
| PFBS | 91.0 | 1.4 |  | 88.8 | 2.8 |  | 92.9 | 2.1 |
| PFPeS | 90.9 | 7.2 |  | 94.0 | 0.6 |  | 95.5 | 5.6 |
| PFHxS | 90.5 | 1.2 |  | 89.8 | 0.8 |  | 92.5 | 1.1 |
| PFHpS | 88.7 | 3.9 |  | 90.2 | 0.9 |  | 91.7 | 4.0 |
| PFOS | 92.1 | 1.6 |  | 91.3 | 2.3 |  | 94.2 | 2.5 |
| PFNS | 89.5 | 5.5 |  | 89.7 | 2.3 |  | 97.2 | 1.4 |
| PFDS | 90.9 | 5.6 |  | 92.6 | 0.3 |  | 95.5 | 2.2 |
| PFDoS | 95.7 | 3.1 |  | 83.7 | 1.6 |  | 96.9 | 2.9 |
| FBSA | 98.3 | 3.8 |  | 83.5 | 6.1 |  | 81.7 | 5.1 |
| FHxSA | 95.7 | 3.5 |  | 86.0 | 5.4 |  | 82.3 | 6.8 |
| FOSA | 87.4 | 2.7 |  | 88.2 | 2.1 |  | 86.8 | 3.4 |
| MeFBSA | 79.2 | 1.3 |  | 71.0 | 5.0 |  | 79.4 | 7.3 |
| MeFOSA | 77.8 | 3.2 |  | 74.5 | 3.7 |  | 77.8 | 2.8 |
| EtFOSA | 84.1 | 5.4 |  | 75.0 | 2.5 |  | 76.7 | 9.7 |
| FOSAA | 73.9 | 2.5 |  | 94.4 | 4.9 |  | 92.5 | 3.5 |
| MeFOSAA | 90.9 | 1.0 |  | 91.1 | 3.3 |  | 94.7 | 2.5 |
| EtFOSAA | 88.4 | 3.2 |  | 92.2 | 3.6 |  | 98.1 | 6.1 |
| 3:3 Acid | 79.2 | 1.2 |  | 80.8 | 5.1 |  | 76.9 | 9.0 |
| 5:3 Acid | 78.9 | 4.0 |  | 88.4 | 1.6 |  | 78.0 | 8.5 |
| 7:3 Acid | 78.3 | 3.6 |  | 86.9 | 0.8 |  | 76.0 | 2.0 |
| 6:2 FTCA | 84.1 | 3.7 |  | 86.6 | 3.3 |  | 79.6 | 6.1 |
| 8:2 FTCA | 83.7 | 1.8 |  | 85.9 | 1.5 |  | 80.5 | 5.8 |
| 10:2 FTCA | 96.5 | 1.1 |  | 81.6 | 3.9 |  | 84.6 | 4.9 |
| 4:2 FTSA | 95.0 | 1.9 |  | 87.8 | 0.4 |  | 91.0 | 3.2 |
| 6:2 FTSA | 93.2 | 2.1 |  | 87.1 | 1.0 |  | 89.2 | 3.0 |
| 8:2 FTSA | 92.9 | 0.9 |  | 92.7 | 1.9 |  | 93.3 | 3.3 |
| 10:2 FTSA | 106.4 | 4.9 |  | 88.4 | 2.2 |  | 84.3 | 1.6 |
| PFHxPA | 77.1 | 1.7 |  | 80.1 | 4.7 |  | 69.5 | 5.3 |
| PFOPA | 86.3 | 4.9 |  | 79.2 | 4.2 |  | 69.7 | 3.1 |
| Cl-PFOS | 90.2 | 3.3 |  | 91.4 | 1.9 |  | 95.9 | 3.8 |
| PFECHS | 90.4 | 5.9 |  | 91.2 | 3.0 |  | 91.2 | 3.9 |
| Gen-X | 82.5 | 4.2 |  | 82.3 | 9.1 |  | 85.5 | 2.6 |
| ADONA | 93.7 | 5.3 |  | 88.2 | 7.5 |  | 92.8 | 9.7 |
| 6:2 Cl-PFESA | 88.0 | 6.2 |  | 93.1 | 3.0 |  | 97.8 | 3.8 |
| 8:2 Cl-PFESA | 92.3 | 5.9 |  | 92.9 | 2.3 |  | 98.3 | 3.4 |
| PFHxSAm | 90.7 | 4.2 |  | 74.7 | 4.6 |  | 102.3 | 3.2 |
| PFOSAm | 91.7 | 8.6 |  | 77.5 | 2.9 |  | 97.6 | 3.9 |
| PFHxSAmS | 97.7 | 4.5 |  | 89.0 | 3.8 |  | 96.3 | 3.2 |
| PFOSAmS | 94.9 | 1.5 |  | 86.5 | 3.1 |  | 101.2 | 1.7 |
| PFOANO | 91.8 | 2.5 |  | 89.9 | 2.4 |  | 100.5 | 3.2 |
| PFOSNO | 88.1 | 3.2 |  | 88.4 | 0.5 |  | 102.8 | 4.3 |
| PFOAB | 89.2 | 3.9 |  | 92.5 | 3.5 |  | 100.6 | 0.6 |
| PFOSB | 91.8 | 3.1 |  | 92.2 | 1.0 |  | 103.9 | 2.5 |
| 5:3 FTB | 96.4 | 2.6 |  | 85.1 | 5.1 |  | 94.2 | 6.9 |
| 5:1:2 FTB | 93.1 | 2.0 |  | 91.0 | 6.1 |  | 95.6 | 5.6 |
| 6:2 FTAB | 96.6 | 0.5 |  | 89.4 | 7.0 |  | 96.7 | 5.0 |

**Table S8.** Absolute recoveries of the biota sample preparation method, validated by standard additions to 7 species from the present survey. Data in red highlight are those outside the target performance scope of 70-130%. **(b) Invertebrate and fishes.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Crayfish** | |  | **Redhorse (Moxostoma)** | |  | **Northern Pike (ESLU)** | |  | **Sand/Mimic shiner (NOST/VO)** | |
|  | **Mean** | **Stdev** |  | **Mean** | **Stdev** |  | **Mean** | **Stdev** |  | **Mean** | **Stdev** |
| PFBA | 87.8 | 1.7 |  | 93.9 | 2.3 |  | 97.0 | 0.7 |  | 94.6 | 2.2 |
| PFPeA | 95.4 | 0.9 |  | 97.3 | 1.3 |  | 94.4 | 2.1 |  | 101.6 | 3.4 |
| PFHxA | 100.1 | 0.7 |  | 98.6 | 2.1 |  | 93.6 | 2.4 |  | 98.9 | 4.7 |
| PFHpA | 94.8 | 0.3 |  | 99.2 | 3.9 |  | 93.6 | 3.7 |  | 96.3 | 4.2 |
| PFOA | 94.9 | 2.1 |  | 100.4 | 3.1 |  | 95.1 | 1.2 |  | 96.4 | 0.4 |
| PFNA | 96.5 | 1.4 |  | 100.3 | 1.2 |  | 96.0 | 1.6 |  | 100.8 | 2.7 |
| PFDA | 97.1 | 0.9 |  | 100.5 | 1.5 |  | 95.9 | 0.6 |  | 99.0 | 3.3 |
| PFUnDA | 92.8 | 0.4 |  | 99.1 | 2.1 |  | 95.4 | 1.6 |  | 97.5 | 2.2 |
| PFDoDA | 96.2 | 0.4 |  | 98.0 | 1.7 |  | 96.2 | 0.9 |  | 95.6 | 2.7 |
| PFTrDA | 110.1 | 6.1 |  | 105.1 | 2.2 |  | 98.2 | 2.1 |  | 100.3 | 7.1 |
| PFTeDA | 95.9 | 0.7 |  | 99.2 | 1.4 |  | 96.3 | 1.0 |  | 98.2 | 0.9 |
| PFHxDA | 96.4 | 0.5 |  | 100.5 | 0.9 |  | 93.8 | 0.6 |  | 98.7 | 1.3 |
| PFPrS | 97.8 | 4.5 |  | 100.8 | 2.3 |  | 91.3 | 1.6 |  | 97.8 | 4.4 |
| PFBS | 97.1 | 1.6 |  | 99.4 | 2.9 |  | 96.4 | 0.6 |  | 102.2 | 1.1 |
| PFPeS | 96.7 | 3.0 |  | 96.7 | 1.7 |  | 94.3 | 4.4 |  | 97.8 | 2.6 |
| PFHxS | 97.0 | 1.3 |  | 99.5 | 0.6 |  | 94.7 | 1.2 |  | 99.6 | 0.4 |
| PFHpS | 96.3 | 1.6 |  | 95.7 | 2.1 |  | 96.9 | 1.5 |  | 102.4 | 5.1 |
| PFOS | 95.3 | 1.8 |  | 103.8 | 2.7 |  | 95.4 | 2.6 |  | 97.9 | 1.7 |
| PFNS | 97.7 | 2.0 |  | 95.2 | 2.3 |  | 94.3 | 4.1 |  | 92.0 | 3.0 |
| PFDS | 104.5 | 4.9 |  | 105.7 | 2.6 |  | 96.6 | 3.3 |  | 96.7 | 6.7 |
| PFDoS | 110.9 | 12.0 |  | 103.2 | 1.2 |  | 100.1 | 4.3 |  | 94.6 | 14.9 |
| FBSA | 91.0 | 3.2 |  | 95.2 | 8.1 |  | 86.6 | 3.4 |  | 82.9 | 2.9 |
| FHxSA | 95.0 | 2.9 |  | 99.4 | 5.9 |  | 89.8 | 1.5 |  | 88.0 | 2.7 |
| FOSA | 95.5 | 2.8 |  | 97.8 | 3.7 |  | 93.1 | 2.9 |  | 91.6 | 2.4 |
| MeFBSA | 74.3 | 2.3 |  | 74.2 | 3.8 |  | 77.3 | 4.1 |  | 73.3 | 3.0 |
| MeFOSA | 80.3 | 3.2 |  | 79.2 | 3.0 |  | 83.5 | 2.5 |  | 81.0 | 1.8 |
| EtFOSA | 78.2 | 4.6 |  | 73.5 | 3.5 |  | 93.5 | 1.6 |  | 97.5 | 3.3 |
| FOSAA | 82.6 | 2.8 |  | 92.9 | 5.8 |  | 86.3 | 2.8 |  | 91.5 | 4.4 |
| MeFOSAA | 95.8 | 0.6 |  | 97.7 | 2.1 |  | 94.6 | 2.1 |  | 98.3 | 2.8 |
| EtFOSAA | 98.2 | 3.6 |  | 107.5 | 1.2 |  | 88.9 | 2.2 |  | 95.4 | 2.4 |
| 3:3 Acid | 97.7 | 5.9 |  | 71.2 | 34.1 |  | 95.7 | 4.7 |  | 43.9 | 4.8 |
| 5:3 Acid | 95.9 | 1.9 |  | 93.8 | 2.3 |  | 97.6 | 2.9 |  | 82.0 | 6.8 |
| 7:3 Acid | 100.1 | 4.1 |  | 102.1 | 4.4 |  | 108.6 | 2.2 |  | 89.9 | 10.9 |
| 6:2 FTCA | 106.5 | 3.1 |  | 92.2 | 2.9 |  | 92.3 | 2.8 |  | 97.2 | 2.7 |
| 8:2 FTCA | 97.8 | 0.7 |  | 99.8 | 6.4 |  | 84.3 | 4.4 |  | 107.1 | 10.3 |
| 10:2 FTCA | 95.9 | 5.1 |  | 103.5 | 8.9 |  | 71.2 | 6.3 |  | 97.9 | 5.3 |
| 4:2 FTSA | 98.8 | 3.9 |  | 97.6 | 3.9 |  | 103.0 | 6.1 |  | 95.7 | 2.3 |
| 6:2 FTSA | 90.4 | 1.9 |  | 96.7 | 1.1 |  | 94.8 | 0.7 |  | 98.3 | 3.8 |
| 8:2 FTSA | 98.8 | 2.0 |  | 101.0 | 2.9 |  | 95.4 | 0.6 |  | 98.9 | 0.9 |
| 10:2 FTSA | 86.1 | 1.2 |  | 90.7 | 1.7 |  | 80.0 | 2.9 |  | 124.0 | 7.8 |
| PFHxPA | 64.7 | 4.5 |  | 88.8 | 5.5 |  | 66.9 | 3.1 |  | 88.8 | 4.7 |
| PFOPA | 68.3 | 2.4 |  | 81.0 | 0.8 |  | 78.2 | 2.2 |  | 87.0 | 3.7 |
| Cl-PFOS | 95.6 | 2.4 |  | 97.4 | 4.2 |  | 91.2 | 1.2 |  | 100.0 | 4.4 |
| PFECHS | 98.6 | 1.6 |  | 96.2 | 1.3 |  | 92.5 | 3.7 |  | 98.1 | 7.3 |
| Gen-X | 88.1 | 7.5 |  | 83.8 | 5.4 |  | 97.8 | 2.8 |  | 107.5 | 3.9 |
| ADONA | 99.8 | 3.6 |  | 101.7 | 3.0 |  | 91.8 | 3.9 |  | 83.5 | 2.7 |
| 6:2 Cl-PFESA | 99.3 | 3.1 |  | 100.4 | 3.7 |  | 94.8 | 5.3 |  | 99.8 | 4.4 |
| 8:2 Cl-PFESA | 115.0 | 7.9 |  | 101.0 | 3.7 |  | 97.5 | 3.4 |  | 97.1 | 6.7 |
| PFHxSAm | 86.4 | 3.6 |  | 99.1 | 5.2 |  | 80.7 | 5.9 |  | 91.2 | 2.0 |
| PFOSAm | 91.0 | 1.2 |  | 95.3 | 6.4 |  | 83.3 | 7.4 |  | 89.6 | 0.5 |
| PFHxSAmS | 100.9 | 3.4 |  | 101.1 | 5.3 |  | 90.9 | 8.6 |  | 96.6 | 2.9 |
| PFOSAmS | 106.7 | 3.9 |  | 102.5 | 2.3 |  | 87.9 | 5.0 |  | 104.9 | 2.3 |
| PFOANO | 100.9 | 2.3 |  | 105.7 | 1.5 |  | 87.7 | 2.8 |  | 97.1 | 3.2 |
| PFOSNO | 107.7 | 3.7 |  | 103.5 | 2.8 |  | 93.5 | 4.1 |  | 95.6 | 4.0 |
| PFOAB | 102.0 | 4.2 |  | 101.2 | 3.1 |  | 95.2 | 8.3 |  | 99.8 | 5.5 |
| PFOSB | 107.4 | 2.7 |  | 103.5 | 6.7 |  | 92.2 | 4.0 |  | 104.6 | 2.3 |
| 5:3 FTB | 99.5 | 3.8 |  | 100.4 | 1.4 |  | 95.0 | 3.8 |  | 90.2 | 1.3 |
| 5:1:2 FTB | 100.1 | 7.0 |  | 103.6 | 3.7 |  | 98.1 | 6.0 |  | 83.2 | 1.6 |
| 6:2 FTAB | 94.8 | 4.9 |  | 103.9 | 8.5 |  | 96.4 | 3.4 |  | 97.8 | 3.0 |

**Table S9.** Analysis of NIST standard reference material SRM 1947 (SRM 1947 Lake Michigan fish tissue). Bold numbers are those for which a NIST reference or information value is available.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Concentration (ng/g wet weight) in NIST SRM 1947** | | | |
|  | **This study** | | ***NIST*** | |
|  | Average (n = 5) | STDEV (n = 5) | *Reference value* | *Information value* |
| PFBA | 0.11 | 0.044 | *-* | *-* |
| PFPeA | 0.07 | 0.007 | *-* | *-* |
| PFHxA | 0.07 | 0.007 | *-* | *-* |
| PFHpA | 0.07 | 0.010 | *-* | *-* |
| PFOA | 0.10 | 0.007 | *-* | *-* |
| **PFNA** | **0.25** | **0.008** | *-* | *0.20* |
| **PFDA** | **0.24** | **0.006** | *-* | *0.26* |
| **PFUnDA** | **0.37** | **0.030** | *-* | *0.28* |
| PFDoDA | 0.16 | 0.009 | *-* | *-* |
| **PFTrDA** | **0.26** | **0.026** | *-* | *0.20* |
| PFTeDA | 0.15 | 0.015 | *-* | *-* |
| PFHxS | 0.03 | 0.004 | *-* | *-* |
| PFHpS | 0.03 | 0.004 | *-* | *-* |
| **PFOS** | **6.01** | **0.113** | *5.90 ± 0.39* | *-* |
| PFDS | 0.13 | 0.017 | *-* | *-* |
| FBSA | 0.15 | 0.013 | *-* | *-* |
| FOSA | 0.27 | 0.023 | *-* | *-* |
| MeFOSAA | 0.11 | 0.014 | *-* | *-* |
| EtFOSAA | 0.17 | 0.016 | *-* | *-* |
| PFECHS | 0.15 | 0.014 | *-* | *-* |

**Table S10.** Feeding ecology of the fish species of interest belonging to the same St. Lawrence freshwater food web (Boivin et al. 2021; Gouvernement du Québec 2007; <https://mffp.gouv.qc.ca/faune/peche/poissons/>).

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Common name** | **Family** | **Environment & feeding ecology** |
| *Micropterus dolomieu* | Smallmouth bass | Centrarchidae - sunfishes | Benthopelagic invertivore/piscivore  Top predator that consumes a wide range of preys. Feeds primarily on crayfish and round goby. Other prey items include fish (yellow perch, crappies, cyprinids, young basses) and insects. Occasionally feeds on amphibians, fish eggs and aquatic vegetation. |
| *Esox lucius* | Northern pike | Esocidae - pikes | Benthopelagic piscivore/omnivore  Opportunistic top predator described as a ''carnivorous omnivore'', consuming fish, crustaceans, amphibians, small mammals, and young waterfowl. Feeds primarily on yellow perch and round goby. Other prey items include other fish (suckers, crappies, cyprinids), insects, crayfish, frogs, mice, muskrats, and ducklings. |
| *Perca flavescens* | Yellow perch | Percidae - perches and darters | Benthopelagic invertivore/piscivore  Feeds on aquatic insects, crayfish, and other invertebrates (gammarids, gasteropods), small fish (e.g., round goby), and fish eggs. |
| *Lepomis gibbosus* | Pumpkinseed | Centrarchidae - sunfishes | Benthopelagic invertivore/piscivore  Diet mainly based on aquatic insects. Also feeds on small fish, small mollusks, zooplankton, amphibians (larval stage), and polychaetes. |
| *Neogobius melanostomus* | Round goby | Gobiidae - gobies | Benthic invertivore  Diet composed of benthic organisms: crustaceans, mollusks (including zebra mussels), polychaetes, small fish, goby eggs and chironomid larvae. |

**Table S10.** (Continued).

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Common name** | **Family** | **Environment & feeding ecology** |
| *Tinca tinca* | Tench | Tincidae - tenches | Benthic invertivore/herbivore  Feeds on insect larvae, mollusks and aquatic vegetation. |
| *Ambloplites rupestris* | Rock bass | Centrarchidae - sunfishes | Benthopelagic invertivore/piscivore  Feeds on aquatic insects, crayfish and small fish (minnows, yellow perch, young sunfish). |
| *Notemigonus crysoleucas* | Golden shiner | Leuciscidae - minnows | Benthopelagic invertivore/herbivore  Feeds on plankton, insects, and mollusks. |
| *Catostomus commersonii* | White sucker | Catostomidae - suckers | Benthic invertivore/detritivore  Diet composed of worms, clams, insect larvae and occasionally fish eggs. |
| *Notropis atherinoides* | Emerald shiner | Leuciscidae - minnows | Benthopelagic planktivore  Feeds on microcrustaceans, chironomid larvae, and algae. |
| *Pimephales notatus* | Bluntnose minnow | Leuciscidae - minnows | Benthopelagic invertivore/detritivore  Feeds on aquatic insects and their larvae, small crustaceans, detritus, occasionally fish eggs, small fish, algae. |
| *Moxostoma spp.* | Redhorses | Catostomidae - suckers | Benthic invertivore  Feeds on mussels, snails, crustaceans, and immature aquatic insects. |
| *Notropis stramineus/*  *Notropis volucellus* | Sand shiner /  Mimic shiner | Leuciscidae - minnows | Benthopelagic invertivore/detritivore  Feeds on insects, macrocustaceans, detritus, and diatoms. |

**Table S11.** Bioaccumulation factors (BAF; average value) for a) PFCAs, b) PFSAs, and c) other compounds in aquatic plants, invertebrates, and fish. Data in red highlight are above the very bioaccumulative (vB) criterion (BAF > 5000).

1. Perfluoroalkyl carboxylic acids – PFCAs.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PFBA** | **PFPeA** | **PFHxA** | **PFHpA** | **PFOA** | **PFNA** | **PFDA** | **PFUnDA** | **PFTrDA** |
| ***Aquatic plants (mean)*** | *16* | - | - | *79* | *27* | *225* | *1128* | *3977* | *2457* |
| Carex sp. | - | - | - | 79 | 7.5 | 74 | 201 | 1197 | 2457 |
| H. dubia | 25 | - | - | - | 48 | 411 | 2021 | 7117 | - |
| P. richardsonii | 6.7 | - | - | - | 25 | 189 | 1163 | 3616 | - |
| ***Invertebrates (mean)*** | *135* | *31* | *515* | *132* | *521* | *3314* | *19814* | *82024* | *44948* |
| Crayfish | 70 | 25 | - | 199 | 402 | 1983 | 13572 | 83770 | 77927 |
| Gammarids | - | 37 | - | - | 444 | 3921 | 15206 | - | - |
| Insects | 199 | - | 515 | 66 | 894 | 6887 | 46309 | 141757 | 47822 |
| Molluscs (snails) | - | - | - | - | 344 | 467 | 4169 | 20544 | 9096 |
| ***Fishes (mean)*** | *13* | - | *86* | - | *60* | *2447* | *37690* | *149185* | *96791* |
| A. rupestris | - | - | - | - | 50 | 2546 | 56708 | 153987 | 112733 |
| C. commersonii | 1.6 | - | 162 | - | - | 4461 | 31283 | 122297 | 63154 |
| E. lucius | 1.9 | - | - | - | 16 | 1499 | 20540 | 72598 | 59266 |
| L. gibbosus | 2.6 | - | - | - | 44 | 2831 | 58097 | 225861 | 180905 |
| M. dolomieu | - | - | - | - | - | 1224 | 59687 | 240440 | 125406 |
| Moxostoma sp. | - | - | - | - | 241 | 6499 | 45036 | 196036 | 105500 |
| N. atherinoides | - | - | - | - | 12 | 1574 | 25989 | - | - |
| N. crysoleucas | - | - | - | - | - | 400 | 18892 | 86916 | - |
| N. melanostomus | 27 | - | - | - | - | 4666 | 69599 | - | - |
| N. volucellus | 42 | - | - | - | - | 508 | 14137 | - | - |
| P. flavescens | 12 | - | - | - | 52 | 3200 | 39829 | 147267 | 99063 |
| P. notatus | - | - | 8.6 | - | - | 361 | 28323 | 143400 | 70322 |
| T. tinca | 2.5 | - | - | - | 6.7 | 2039 | 21852 | 103043 | 54773 |

**Table S11.** Bioaccumulation factors (BAF; average value) of PFAS in aquatic plants, invertebrates, and fish. Data in red highlight are those above the very bioaccumulative (vB) criterion (BAF > 5000). (Continued).

1. Perfluoroalkane sulfonates – PFSAs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **PFBS** | **PFPeS** | **PFHxS** | **PFHpS** | **PFOS** | **PFDS** |
| ***Aquatic plants (mean)*** | - | - | *9.5* | - | *272* | - |
| Carex sp. | - | - | - | - | 62 | - |
| H. dubia | - | - | 9.5 | - | 444 | - |
| P. richardsonii | - | - | - | - | 309 | - |
| ***Invertebrates (mean)*** | *230* | *467* | *1189* | *1308* | *5439* | *6393* |
| Crayfish | - | - | 130 | - | 1607 | 1110 |
| Gammarids | - | - | 92 | 241 | 4249 | - |
| Insects | 333 | - | 269 | 1494 | 14805 | 16549 |
| Molluscs (snails) | 127 | 467 | 4263 | 2190 | 1093 | 1521 |
| ***Fishes (mean)*** | *38* | - | *44* | *851* | *14305* | *24695* |
| A. rupestris | - | - | 33 | 1420 | 23929 | 12409 |
| C. commersonii | - | - | - | 1067 | 11489 | 16845 |
| E. lucius | - | - | 40 | 629 | 10085 | 14515 |
| L. gibbosus | - | - | 33 | 792 | 24159 | 42401 |
| M. dolomieu | - | - | 33 | 698 | 29043 | 45020 |
| Moxostoma sp. | - | - | 124 | 1626 | 14758 | 25982 |
| N. atherinoides | - | - | 27 | 395 | 10714 | - |
| N. crysoleucas | - | - | 18 | - | 4237 | - |
| N. melanostomus | - | - | - | 266 | 13508 | - |
| N. volucellus | - | - | 20 | - | 6256 | - |
| P. flavescens | 38 | - | 71 | 1332 | 15932 | 16522 |
| P. notatus | - | - | - | - | 14286 | 35138 |
| T. tinca | - | - | - | 280 | 7568 | 13425 |

**Table S11.** Bioaccumulation factors (BAF; average value) in aquatic plants, invertebrates, and fish. Data in red highlight are those above the very bioaccumulative (vB) criterion (BAF > 5000). (Continued).

1. Other compounds.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **FBSA** | **FHxSA** | **FOSA** | **EtFOSAA** | **6:2 FTSA** | **8:2 FTSA** | **PFECHS** | **6:2 diPAP** |
| ***Aquatic plants (mean)*** | - | - | - | *358* | - | - | - | - |
| Carex sp. | - | - | - | - | - | - | - | - |
| H. dubia | - | - | - | 358 | - | - | - | - |
| P. richardsonii | - | - | - | - | - | - | - | - |
| ***Invertebrates (mean)*** | *168* | *2458* | *7267* | *3381* | *2796* | *2847* | *564* | - |
| Crayfish | 145 | 1710 | 3196 | - | - | 727 | 37 | - |
| Gammarids | - | - | - | 3381 | - | - | - | - |
| Insects | 192 | 3870 | 11339 | - | - | - | 216 | - |
| Molluscs (snails) | - | 1795 | - | - | 2796 | 4968 | 1438 | - |
| ***Fishes (mean)*** | *1010* | *6796* | *7866* | *4201* | *483* | - | *95* | *575* |
| A. rupestris | 521 | - | - | 4550 | - | - | 159 | 575 |
| C. commersonii | 1296 | 7030 | 4438 | - | - | - | 51 | - |
| E. lucius | 1612 | 9579 | 8529 | - | - | - | 80 | - |
| L. gibbosus | 1460 | 7359 | 9845 | - | - | - | 30 | - |
| M. dolomieu | 1245 | 11667 | 7563 | - | 345 | - | 182 | - |
| Moxostoma sp. | 1547 | 7441 | 14667 | 1139 | - | - | 124 | - |
| N. atherinoides | 435 | - | - | - | 707 | - | - | - |
| N. crysoleucas | - | - | - | - | - | - | - | - |
| N. melanostomus | 110 | - | - | - | - | - | - | - |
| N. volucellus | 696 | - | - | 10869 | - | - | 33 | - |
| P. flavescens | 751 | 3583 | - | 1818 | - | - | 160 | - |
| P. notatus | - | - | - | 2629 | 396 | - | - | - |
| T. tinca | 1443 | 913 | 2156 | - | - | - | 37 | - |

**Table S12.** Sediment-biota accumulation factors (BSAF; average value) calculated for benthic species collected in the St. Lawrence River.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Biota-sediment accumulation factors (BSAFs)** | | | | | | |
|  | **Crayfish** | **Gammarids** | **Molluscs** | ***C. commersonii*** | ***Maxostoma spp.*** | ***N. melanogobius*** | ***T. tinca*** |
| PFBA | - | - | - | 1.2 | - | - | 1.8 |
| PFPeA | 0.8 | - | - | - | - | - | - |
| PFHxA | - | - | - | 38 | - | - | - |
| PFHpA | 16 | - | - | - | - | - | - |
| PFOA | 27 | 122 | 14 | - | 14 | - | 0.3 |
| PFNA | 60 | - | 9.1 | 118 | 220 | - | 57 |
| PFDA | 43 | - | 9.0 | 101 | 170 | - | 71 |
| PFUnDA | 29 | - | 5.1 | 59 | 72 | - | 48 |
| PFDoDA | 27 | - | 3.3 | 44 | 48 | - | 30 |
| PFTrDA | 37 | - | 2.4 | 73 | 53 | - | 40 |
| PFTeDA | 13 | - | 4.3 | 15 | 12 | - | 14 |
| PFBS | - | - | 13 | - | - | - | - |
| PFPeS | - | - | 40 | - | - | - | - |
| PFHxS | 9.4 | 40 | 158 | - | 25 | - | - |
| PFHpS | - | - | 64 | 36 | 53 | - | 10 |
| PFOS | 11 | 319 | 5.2 | 103 | 340 | 1015 | 64 |
| PFDS | 0.9 | 99 | 0.9 | 21 | 212 | 376 | 21 |
| FHxSA | 12 | - | 12 | 69 | 28 | - | - |
| FOSA | 4.8 | 26 | - | 20 | 50 | 63 | 5.1 |
| FOSAA | - | - | - | 18 | 101 | - | - |
| MeFOSAA | - | - | - | 7.4 | - | - | - |
| EtFOSAA | - | - | 2.0 | 3.1 | 1.4 | - | 0.4 |
| PFECHS | 3.0 | - | 53 | 3.9 | 15 | - | 1.4 |

**Table S13.** Biomagnification factors (BMF; average value) of a) PFCAs, b) PFSAs, and c) other compounds for predator/prey couples of a St. Lawrence River freshwater food web. Data in yellow highlight BMF > 1 (biomagnification observed).

1. Perfluoroalkyl carboxylic acids – PFCAs.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Predator/Prey** | **PFBA** | **PFOA** | **PFNA** | **PFDA** | **PFUnDA** | **PFDoDA** | **PFTrDA** | **PFTeDA** |
| Smallmouth bass/Crayfish | - | - | 0.6 | 4.1 | 3.6 | 2.1 | 1.5 | 1.6 |
| Smallmouth bass/Yellow perch | - | - | 0.4 | 1.5 | 1.8 | 1.3 | 1.2 | 0.9 |
| Smallmouth bass/Pumpkinseed | - | - | 0.4 | 1.0 | 1.1 | 1.0 | 0.7 | 0.6 |
| Smallmouth bass/Round goby | - | - | 0.4 | 1.1 | 1.2 | 1.1 | 1.1 | 1.0 |
| Northern pike/Yellow perch | 1.0 | 0.3 | 0.5 | 0.6 | 0.5 | 0.4 | 0.5 | 0.5 |
| Northern pike/Suckers | 1.2 | 0.07 | 0.3 | 0.5 | 0.6 | 0.5 | 0.7 | 0.6 |
| Northern pike/Pumpkinseed | 0.7 | 0.4 | 0.5 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 |
| Northern pike/Crayfish | 0.2 | 0.04 | 0.7 | 1.5 | 1.1 | 0.7 | 0.7 | 0.8 |
| Northern pike/Round Goby | 1.0 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 | 0.5 |
| Yellow perch/Insects | 0.01 | 0.06 | 0.4 | 0.8 | 1.0 | 1.5 | 2.4 | 7.8 |
| Yellow perch/Gammarids | - | 0.1 | 0.9 | 2.7 | 2.8 | 2.2 | 2.9 | 2.8 |
| Yellow perch/Molluscs | - | 0.2 | 5.5 | 8.0 | 6.5 | 7.0 | 9.6 | 5.6 |
| Yellow perch/Round Goby | 1.0 | 1.7 | 1.0 | 0.7 | 0.7 | 0.8 | 0.9 | 1.1 |
| Rock bass/Insects | - | 0.06 | 0.3 | 1.3 | 1.5 | 2.0 | 2.7 | 8.2 |
| Rock bass/Crayfish | - | 0.1 | 1.1 | 4.3 | 3.2 | 2.1 | 1.5 | 1.8 |
| Rock bass/Yellow Perch (Young) | - | 0.8 | 0.7 | 1.6 | 1.5 | 1.2 | 1.0 | 1.0 |
| Rock bass/Minnows | - | 4.0 | 3.6 | 3.1 | 2.7 | 3.2 | 3.3 | 2.8 |
| Pumpkinseed/Insects | 0.01 | 0.05 | 0.4 | 1.3 | 1.6 | 2.1 | 3.8 | 12.1 |
| Pumpkinseed/Molluscs | - | 0.2 | 5.2 | 12.7 | 10.9 | 9.6 | 14.8 | 8.7 |
| Minnows/Insects | 0.01 | 0.01 | 0.09 | 0.4 | 0.6 | 0.6 | 0.8 | 3.0 |
| Minnows/Gammarids | - | 0.03 | 0.2 | 1.4 | 1.6 | 0.9 | 1.0 | 1.1 |
| Suckers/Insects | 0.01 | 0.3 | 0.9 | 0.9 | 0.9 | 1.2 | 1.8 | 5.8 |
| Suckers/Molluscs | - | 0.8 | 11.0 | 8.9 | 6.0 | 5.6 | 7.0 | 4.1 |

**Table S13.** Biomagnification factors (BMF; average value) of PFAS for predator/prey couples of this freshwater food web. Data in yellow highlight are those above 1 (biomagnified). (Continued).

(b) Perfluoroalkane sulfonates – PFSAs.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Predator/Prey** | **PFHxS** | **PFHpS** | **PFOS** | **PFDS** |
| Smallmouth bass/Crayfish | 0.2 | - | 16.2 | 32.5 |
| Smallmouth bass/Yellow perch | 0.4 | 0.6 | 1.6 | 1.5 |
| Smallmouth bass/Pumpkinseed | 1.0 | 0.9 | 1.1 | 1.1 |
| Smallmouth bass/Round goby | - | 3.0 | 2.3 | 1.7 |
| Northern pike/Yellow perch | 0.5 | 0.5 | 0.6 | 0.5 |
| Northern pike/Suckers | 0.3 | 0.4 | 0.7 | 0.4 |
| Northern pike/Pumpkinseed | 1.2 | 0.8 | 0.4 | 0.3 |
| Northern pike/Crayfish | 0.3 | - | 6.1 | 10.1 |
| Northern pike/Round Goby | - | 2.7 | 0.9 | 0.5 |
| Yellow perch/Insects | 0.3 | 0.8 | 1.1 | 1.9 |
| Yellow perch/Gammarids | 0.7 | 5.9 | 4.0 | 3.8 |
| Yellow perch/Molluscs | 0.02 | 0.6 | 11.2 | 15.9 |
| Yellow perch/Round Goby | - | 5.4 | 1.4 | 1.1 |
| Rock bass/Insects | 0.1 | 1.1 | 1.6 | 2.8 |
| Rock bass/Crayfish | 0.3 | - | 14.6 | 31.8 |
| Rock bass/Yellow Perch (Young) | 0.5 | 1.4 | 1.5 | 1.3 |
| Rock bass/Minnows | 1.5 | 4.6 | 2.8 | 2.2 |
| Pumpkinseed/Insects | 0.1 | 0.5 | 1.6 | 2.6 |
| Pumpkinseed/Molluscs | 0.01 | 0.4 | 16.5 | 21.9 |
| Minnows/Insects | 0.09 | 0.2 | 0.6 | 1.3 |
| Minnows/Gammarids | 0.2 | 1.6 | 2.1 | 2.6 |
| Suckers/Insects | 0.5 | 0.9 | 1.0 | 2.1 |
| Suckers/Molluscs | 0.03 | 0.6 | 10.2 | 17.8 |

**Table S13.** Biomagnification factors (BMF; average value) of PFAS for predator/prey couples of this freshwater food web. Data in yellow highlight are those above 1 (biomagnified). (Continued).

(c) Other compounds.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Predator/Prey** | **FBSA** | **FHxSA** | **FOSA** | **MeFOSAA** | **EtFOSAA** | **PFECHS** |
| Smallmouth bass/Crayfish | 8.2 | 6.0 | 2.3 | - | - | 4.2 |
| Smallmouth bass/Yellow perch | 1.4 | 2.3 | 2.2 | - | 1.1 | 1.0 |
| Smallmouth bass/Pumpkinseed | 0.8 | 1.4 | 0.8 | - | - | 6.0 |
| Smallmouth bass/Round goby | 4.6 | 1.6 | 1.4 | 1.1 | 1.3 | - |
| Northern pike/Yellow perch | 2.0 | 2.2 | 2.5 | - | 0.7 | 0.5 |
| Northern pike/Suckers | 1.0 | 0.8 | 0.7 | 0.2 | 0.4 | 0.7 |
| Northern pike/Pumpkinseed | 1.1 | 1.3 | 0.9 | - | - | 2.6 |
| Northern pike/Crayfish | 11.2 | 5.6 | 2.6 | - | - | 1.9 |
| Northern pike/Round Goby | 6.3 | 1.5 | 1.6 | 0.4 | 0.8 | - |
| Yellow perch/Insects | 4.3 | 1.1 | 0.3 | - | 0.2 | 0.8 |
| Yellow perch/Gammarids | - | - | 2.2 | - | 0.5 | - |
| Yellow perch/Molluscs | - | 2.4 | 3.8 | - | 0.3 | 0.1 |
| Yellow perch/Round Goby | 3.2 | 0.7 | 0.7 | - | 1.2 | - |
| Rock bass/Insects | 3.0 | - | - | 0.6 | 0.5 | 0.8 |
| Rock bass/Crayfish | 4.0 | - | - | - | - | 4.2 |
| Rock bass/Yellow Perch (Young) | 0.8 | - | - | - | 2.7 | 1.0 |
| Rock bass/Minnows | 1.0 | - | - | - | 1.0 | 4.9 |
| Pumpkinseed/Insects | 7.6 | 1.9 | 0.9 | - | - | 0.1 |
| Pumpkinseed/Molluscs | - | 4.1 | 11.0 | - | - | 0.02 |
| Minnows/Insects | 3.2 | - | - | - | 0.5 | 0.2 |
| Minnows/Gammarids | - | - | - | - | 1.5 | - |
| Suckers/Insects | 8.0 | 3.0 | 1.0 | 0.4 | 0.3 | 0.6 |
| Suckers/Molluscs | - | 6.5 | 13.0 | - | 0.6 | 0.08 |

**Table S14.** Trophic magnification factors (TMF) based on protein-normalized PFAS data, using a generalized linear mixed-effect model (GLMM) also accounting for censored data (R-package LMEC). Having low protein content, aquatic plants were not included in the regressions. TMFs not significantly different from 1 (TMF~1) are in grey font. Associated TMF confidence intervals (95% CI) and Akaike information criterion (AIC) are also provided.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **TMF** | **95% CI** | **AIC** |
| ΣPFAS | 1.71 | [1.68; 1.75] | 89.5 |
| PFOA | 0.40 | [0.25; 0.63] | 357.6 |
| PFNA | 1.09 | [1.00; 1.18] | 231.5 |
| PFDA | 1.78 | [1.74; 1.81] | 96.0 |
| PFUnDA | 1.59 | [1.56; 1.62] | 94.6 |
| PFDoDA | 1.25 | [1.22; 1.28] | 116.9 |
| PFTrDA | 1.24 | [1.19; 1.28] | 148.2 |
| PFTeDA | 1.20 | [1.14; 1.26] | 188.3 |
| PFHxS | 0.60 | [0.41; 0.86] | 354.2 |
| PFHpS | 2.72 | [2.09; 3.54] | 312.8 |
| PFOS | 1.86 | [1.82; 1.89] | 96.3 |
| PFDS | 1.28 | [1.24; 1.32] | 137.9 |
| FBSA | 1.14 | [0.85; 1.52] | 323.9 |
| FOSA | 3.02 | [1.97; 4.63] | 328.4 |
| EtFOSAA | 0.27 | [0.18; 0.41] | 350.8 |
| PFECHS | 1.40 | [1.04; 1.88] | 321.3 |
| 6:2 diPAP | 0.05 | [0.02; 0.10] | 409.1 |

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