Supporting Information

Characterizing the Areal Extent of PFAS Contamination in Fish Species Downgradient of AFFF Source Zones

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1. Methods & Quality Assurance/Quality Control (QA/QC)

1.1. Surface Water, Sediment, and Aquatic Biota Sampling. Surface water samples were collected in high-density polyethylene (HDPE) bottles with polypropylene (PP) caps. Bottles (1L) were pre-cleaned with liquid chromatography-mass spectrometry (LC-MS) grade methanol (MeOH) and Milli-Q (MQ) water and rinsed 3 times with sample water before filling. At least one field blank was collected at each surface water site by opening a 1L HDPE bottle filled with MQ water in the field during sample collection. All water samples were unfiltered, stored on ice in the field, then stored at -20°C until analysis. Surface water samples were taken at 12 locations across 4 surface waterbodies to assess per- and polyfluoroalkyl substances (PFAS) composition at selected riverine and lacustrine locations. Three surface waterbodies (Moody Pond, the Quashnet River, and Waquoit Bay) are located within an aqueous film-forming foam (AFFF)-contaminated watershed and one waterbody (Santuit River) is in a non-AFFF affected watershed. **Table S1** summarizes surface water sample collection and water parameters.

Sediment samples were collected in PP sediment push cores (6.3 cm diameter; 25 cm length), capped with rubber stoppers. Cores and stoppers were pre-cleaned with 1% ammonium hydroxide in MeOH, LC-MS MeOH and MQ water prior to sample collection. Sediment cores were manually pushed into the sediment deep enough that at least 10-15 cm was collected. Cores were capped on the bottom and top before removing from the ground to avoid loss of sediment and pore water. Cores were stored upright in coolers during transport and immediately frozen upright. Cores were thawed and sectioned into 5 cm depth profiles with some cores having 2 depth profiles (0-5 cm and 5-10 cm) and some having 3 depth profiles (0-5 cm, 5-10 cm, and 10-15 cm). Sediment was mixed using a pre-cleaned stainless steel spatula and some sediment was separated for fraction of organic carbon analysis using loss on ignition method, while the rest was weighed while wet, freeze-dried and then weighed again to determine % moisture content. Between 1-4 sediment cores were collected from each waterbody from the same locations as surface water collection. **Table S1** indicates location of sediment sample collection.

Aquatic biota were selected based on information about which species occupied the polluted areas, following consultation with local fishermen, Mashpee Wampanoag Tribe members, the Town of Mashpee Department of Natural Resources, and the Massachusetts (MA) Division of Fisheries and Wildlife and Division of Marine Fisheries. Samples (n=1-43 organisms per species per location) were collected in conjunction with surface water samples. Fish samples were collected using a variety of methods including electrofishing, seine fishing, eel traps, and rod and reel. Shellfish were collected using quahog rakes and crab traps. Gastropods were collected using sediment sieves. Fish were euthanized by pithing. All methods and fish handling protocols were conducted under permits by MA Department of Fish and Game. All biota samples were stored on ice in the field, and then stored at -20°C prior to dissection and analysis. **Table S2** summarizes the type and number of biotic species collected from each surface waterbody along with their habitat, fork length, weights, and tissue type measured. Whole body samples were measured individually if fish fork length was generally between 10-14 cm or composited for measurements if fish fork length was generally <10 cm. The number of samples composited for each species is indicated by the number range under the sample code names column in **Table S2**. For individual fish samples with fork lengths >14 cm (and eel > 25 cm fork length), organ tissues were dissected for additional analyses and only the muscle tissue was measured. Muscle tissue was also measured for musk turtles that were additionally collected inadvertently. For aquatic shellfish and gastropods, whole body soft tissue was measured.

Table S1. Surface water and sediment sampling locations, coordinates and date of collection, number of water and sediment samples collected, number of field blanks collected, and surface water parameters for each site location. DO is dissolved oxygen.

Sample Site	Coordinates	Date of Collection	Number of Water Samples	Number of Sediment Cores	Number of Field Blanks Collected	Sediment Organic Carbon (%)	Water Temperature (°C)	Pressure (mmHg)	DO (%)	Conductivity (µs/cm)	Salinity (psu)	pH
Moody Pond	41.6379882, -70.5130146	6/30/2021	2	1	1	0.45	26.8	761.5	87.1	66.6	0.03	6.17
		7/20/2021	2	1	1	0.80	26.9	758.8	80.3	66.2	0.03	6.33
	41.6391024, -70.5126448	6/30/2021	2	1		4.5	26.9	761.4	89.1	66.6	0.03	6.00
	41.6397937, -70.5122280	6/30/2021	2	1		4.2	26.9	761.4	90.5	66.6	0.03	5.91
Upper Quashnet River	41.6335060, -70.5049622	6/8/2021	2	1	1	27	18.6	763.5	102.2	90.5	0.05	5.86
Lower Quashnet River	41.5921514, -70.5078128	5/19/2021	2	1	1	0.96	15.4	770.5	97.3	100.0	0.06	5.30
	41.5937898, -70.5071224	5/19/2021	2	1		1.0						
Quashnet into Waquoit Bay	41.5779647, -70.5137575	6/1/2021	2	1	1	0.55	16.7	768.7	97.0	23150	17.00	7.25
	41.5729800, -70.5176159	6/1/2021	2	1		2.2						
Waquoit Bay	41.5723156, -70.5178620	6/1/2021	2	1	1	0.64	15.4	769.3	89.9	34874	28.41	7.20
	41.5791791, -70.5258868	6/23/2021	2	1								
	41.5776495, -70.5143775	8/6/2021	2	1								
Santuit River	41.6461988, -70.4535932	6/8/2021	2	1	1	0.38	14.5	762.4	107.3	160.7	0.08	6.30

Table S2. Locations of biota collection, type of species and corresponding species information, number of samples per species collected, given code names for each species, calculated lengths and weights, and the type of tissue measured for each individual sample or composite of samples. $\delta^{15}N$, $\delta^{13}C$ lipid-normalized values, and C:N ratios are provided for selected samples where measurements were conducted on dorsal muscle tissue (fishes and turtles) or total soft tissue (shellfish and gastropods). Trophic Position (TP) was calculated for species from Waquoit Bay and Quashnet into Waquoit Bay using Quahogs (TP = 2) as the baseline organism. Relative TP difference was determined for all other species using the organism with the lowest $\delta^{15}N$ as the baseline (0.00) for each site. Lengths (cm) correspond to straight fork length (fishes), carapace width (crustaceans), carapace length (turtles), shell length (gastropods) and shell length x width (bivalves). Primary diet for each species is described along with associated references.[1–42]

See attached excel file: Supplementary Information Tables: Table S2

1.2. 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), Optima™ LC-MS grade acetonitrile (Fisher Chemical, ThermoFisher Scientific, Waltham, MA), and ACS grade BDH ammonium hydroxide were purchased from VWR (Radnor, PA). Reagent grade formic acid, BioUltra ammonium acetate, ACS grade acetic acid, Supelclean ENVI-Carb (120-400 mesh, 100 m^2 g^{-1} surface area), and Sand (quartz, 50-70 mesh particle size) 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).

1.3. Sample Extraction. Surface water samples were thawed to 4˚C, warmed to room temperature, and then sonicated for 30 seconds and inverted to mix, which was repeated four times to desorb PFAS from the sample bottle walls before subsampling 300 mL of the 1L sample into precleaned 500 mL HDPE bottles. Each sample had an associated field duplicate that was also subsampled and measured, in addition to sample method duplicates. Field blanks, procedural blanks and procedural spikes consisted of Milli-Q (MQ) water. All samples were spiked with 125 µL of a 0.03 ng $µL^{-1}$ isotopically labeled extracted internal standard (EIS) mixture prior to SPE extraction. Recovery spike samples were spiked with 50 µL of a 3, 30, or 300 ng mL^{-1} native PFAS mixture prior to extraction. The pH of samples was checked prior to extraction to confirm a pH of ~7. Oasis WAX SPE cartridges were preconditioned with 5 mL of 1% ammonium hydroxide (NH_4OH) in methanol (MeOH), 5 mL of MeOH, 5 mL of MQ water, and then the 300 mL sample was added to the cartridge and placed under vacuum at a flow rate of 1 drop sec^{-1} followed by a 5 mL MQ water rinse before drying the cartridge under vacuum. The sample cartridges were eluted with 5 mL MeOH followed by 5 mL of 1% (v/v) NH_4OH in MeOH after rinsing the sample bottles first and the collected eluent was concentrated to almost dryness using ultra-high purity nitrogen gas. The extract was reconstituted in 0.75 mL MeOH, added to a 1.7 mL polypropylene (PP) microcentrifuge tube containing 25 ± 5 mg dispersive Envi-Carb (Supelclean™) and 50 µL of acetic acid, vortexed, and centrifuged at 13,000 rpm for 20 minutes. 300 µL of the extract was transferred to a 1.5 mL PP vial and stored at -20˚C for subsequent suspect screening and non-target analysis. The extract (400 µL) was transferred to a 1.7 mL PP microcentrifuge tube containing 368 µL MQ water and 32 µL of 0.0075-0.03 ng $µL^{-1}$ isotopically labeled non-extracted internal standard (NIS), vortexed, centrifuged at 13,000 rpm for 20 minutes, and transferred to a 1 mL PP vial for targeted LC-MS/MS analysis.

Freeze-dried sediment samples were removed from the freezer, homogenized by mixing with a pre-cleaned stainless-steel spatula, and weighed (1 g) into 15 mL PP tubes for extraction. Quartz sand (Sigma Aldrich) was used for procedural blanks and procedural spikes. Two procedural blanks, two procedural spikes (200 ng L^{-1} and 2,000 ng L^{-1}), three sample duplicates and three sample matrix spikes (2,000 ng L^{-1}) were included with the extraction. All samples were spiked with 125 µL of 0.03 ng $µL^{-1}$ EIS prior to extraction. Procedural and sample recovery spike samples were spiked with 100 µL of 3 ng mL^{-1} or 30 ng mL^{-1} native PFAS mixture prior to extraction. Samples were vortexed and left to equilibrate overnight prior to extraction. Samples were extracted with 5 mL of 1% NH_4OH in MeOH, vortexed, sonicated for 30 minutes, placed on a rotating mixer for 30 minutes, and then centrifuged at 4,000 rpm for 20 minutes before transferring the supernatant to a new 15 mL PP tube. The extraction was repeated 2 more times with an additional 5 mL of 1% NH_4OH in MeOH each. The final 15 mL of MeOH supernatant

was recentrifuged at 5,000 rpm for 20 minutes and decanted to new 15 mL PP tubes if significant sediment precipitate was present. Extracts were concentrated to ~0.75 mL using ultra-high purity nitrogen gas. Exact volume concentrated was measured and ranged from 0.75-0.93 mL. This exact volume was used for dilution factor determination. The ~0.75 mL MeOH extract was added to a 1.7 mL PP microcentrifuge tube containing 25 ± 5 mg dispersive Envi-Carb and 50 µL of acetic acid, vortexed, and centrifuged at 13,000 rpm for 20 minutes. The extract (300 µL) was transferred to a 1.5 mL PP vial and stored at -20˚C for subsequent suspect screening and non-target analysis. The rest of the extract (400 µL) was transferred to a 1.7 mL PP microcentrifuge tube containing 368 µL MQ water and 32 µL of 0.0075-0.03 ng μL^{-1} NIS, vortexed, centrifuged at 13,000 rpm for 20 minutes, and transferred to a 1 mL PP vial for targeted LC-MS/MS analysis.

Biota samples were kept frozen (-20˚C) prior to thawing for dissection, homogenization, and extraction. Biota biometric data regarding length, weight, and sex, if possible, were recorded prior to sample dissection. Fish samples with fork lengths >14 cm were dissected for organ tissues including liver, heart, kidney, brain, spleen, gills, stomach, intestine, and gonads that were collected in PP tubes and stored frozen for future tissue-specific analyses. Dorsal muscle tissue was subsequently collected in 15 or 50-mL PP tubes for analysis. For eel, samples with fork lengths >25 cm were dissected for muscle tissue analysis and samples <25 cm were measured as whole-body. Fish samples with fork lengths ≤ 14 cm were measured as whole-body samples and fish samples generally <10 cm with multiple individuals available per species and location, were composited together as whole-body composites. See **Table S2** for the number of samples grouped in each composite sample. Some species depending on size and number of replicates, were grouped into multiple composites for comparison measurements. Small masses of muscle tissue were taken from all fish species and locations for separate isotope analyses. Soft tissue of shellfish and gastropods were used for isotope analysis. Dissected muscle tissue samples and whole-body samples and composites were then homogenized using a pre-cleaned Black & Decker one-touch chopper and/or a hand-held OMNI International TH homogenizer.

For biotic tissue extraction, 0.5 g of wet-weight homogenized muscle or whole-body tissue was weighed into 15 mL PP tubes and spiked with 75 µL of 0.03 ng μL^{-1} EIS mixture, vortexed, and left to equilibrate for at least 1 hour prior to extraction. Procedural blanks and procedural spikes consisted of MQ water. Sample method duplicates and sample/procedural recovery spikes were included in every batch of 12 samples. Recovery spike samples were spiked with 75 µL of a 3 or 30 ng mL^{-1} native PFAS mixture prior to extraction. A surrogate reference material (SRM 1947 Lake Michigan fish tissue) was also included in every other extraction batch. Following fortification, five 4.8 mm stainless steel beads precleaned with MQ water, 0.4 M hydrochloric acid (HCl), 1% NH_4OH in MeOH, and MeOH, were added to the sample tubes, followed by 4 mL of acetonitrile. Samples were vortexed and then homogenized using a MP Biomedical FastPrep-24 Classic bead beating grinder and lysis system customized with a 12 x 15 mL tube adaptor. To thoroughly mix the tissue sample with the acetonitrile, the sample was homogenized 2x in 60 second increments at a 6.5 m/s speed. The homogenized sample was then centrifuged at 4,000 rpm for 15 minutes, the extract was transferred via Pasteur pipette to a new 15 mL PP tube and the extraction was repeated a second time with an additional 4 mL acetonitrile. Sample extracts were placed in the freezer (-20˚C) overnight to allow lipids to precipitate and were centrifuged at 4,000 rpm for 3 minutes and decanted to new 50 mL PP tubes the following day to remove the precipitate. Extracts were concentrated to ~1 mL using ultra-

high purity nitrogen gas and diluted to 50 mL with MQ water prior to SPE extraction for additional clean-up. The pH of samples was checked prior to extraction to confirm pH of ~6.5. Oasis WAX SPE cartridges were conditioned with 4 mL of 1% NH_4OH in MeOH, 4 mL of MeOH, 4 mL of MQ water, and the 50 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 rinse of 25 mM ammonium acetate buffer at pH 4 before drying the cartridge under vacuum. The sample cartridges were eluted with 4 mL MeOH and 4 mL of 1% NH_4OH in MeOH after rinsing the sample tubes and the collected eluent was concentrated to almost dryness using ultra-high purity nitrogen gas and then reconstituted in 0.75 mL MeOH. Part of the extract (400 µL) was transferred to a 1.5 mL PP vial and stored at -20˚C for subsequent suspect screening and non-target analysis. The other part of the extract (300 µL) was transferred to a 1.7 mL PP microcentrifuge tube containing 315 µL MQ water and 35 µL of 0.0075-0.03 ng μL^{-1} of NIS, vortexed, centrifuged at 13,000 rpm for 20 minutes, and transferred to a 1 mL PP vial for targeted LC-MS/MS analysis.

1.4. Targeted Analysis. For targeted analysis, sample extracts (100−300 µL) were 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 ionization 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. The LC gradient included initial conditions of 97% 2 mM ammonium acetate in water (A) and 3% 2 mM ammonium acetate in methanol (B). From 0.85 to 3.5 mins the gradient was linearly increased to 54% B. From 3.5 to 15 mins the gradient was linearly increased to 85% B and then linearly increased to 100% B at 15.5 mins and then held at 100% B until 16.5 mins. A list of targeted PFAS compounds is summarized in **Table S3**.

Instrumental blanks and the calibration curve were prepared with 55:45 MeOH:MQ water with internal standard (EIS) concentrations matching the samples. For water sample analysis, an 11-point (1-10,000 ng L^{-1}) up to 14-point calibration curve (1-25,000 ng L^{-1}) were used for quantifying PFAS concentrations. For sediment sample analysis, an 11-point calibration curve (1-10,000 ng L^{-1}) was used for quantifying PFAS. For biota sample analysis, an 11-point (1-10,000 ng L^{-1}) up to 15-point calibration curve (1-30,000 ng L^{-1}) were used for PFAS quantification. In cases where samples exceeded the highest calibration point, extracts were diluted with stock solution containing the appropriate EIS concentrations and reanalyzed. 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.

In biota tissue samples, perfluorooctane sulfonate (PFOS) was quantified using the secondary transition (498.9 → 98.9 instead of 80.0) to avoid overestimation due to cholic acid interferences in whole body samples that could not be fully removed during extraction or chromatographically. In comparing detectable PFOS concentrations in samples with minimal interference quantified using both transitions, the % difference in concentrations was <20 % (average: 7 %) indicating that using the secondary transition to quantify should not underestimate concentrations.

Table S3. Mass spectrometry acquisition parameters for targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Analyte	C#	Type	Extracted Internal Standard	Precursor Ion Mass	Product Ion Mass	Fragmentor Voltage (V)	Collision Energy (V)
Perfluoroalkyl Carboxylates							
PFBA	C3	Target	[$^{13}C_4$]PFBA	212.9792	168.9	60	2
PFPeA	C4	Target	[$^{13}C_5$]PFPeA	262.9760	218.9	60	2
PFHxA	C5	Target	[$^{13}C_5$]PFHxA	312.9728	268.9; 118.9	70	2; 14
PFHpA	C6	Target	[$^{13}C_4$]PFHpA	362.9696	318.9; 168.9; 118.9	70	2; 10; 18
PFOA	C7	Target	[$^{13}C_8$]PFOA	412.9664	368.9; 168.9	80	2; 10
PFNA	C8	Target	[$^{13}C_9$]PFNA	462.9632	418.9; 218.9; 169.0	75	2; 10; 14
PFDA	C9	Target	[$^{13}C_6$]PFDA	512.9600	468.9; 269.0; 218.9	85	6; 14; 14
PFUnDA	C10	Target	[$^{13}C_7$]PFUnDA	562.9568	518.9; 269.0; 169.0	95	6; 14; 22
PFDoDA	C11	Target	[$^{13}C_2$]PFDoDA	612.9537	569.0; 269.0; 169.0	90	6; 14; 26
PFTrDA	C12	Target	[$^{13}C_2$]PFTeDA	662.9505	618.9; 169.0	95	6; 26
PFTeDA	C13	Target	[$^{13}C_2$]PFTeDA	712.9473	669.0; 169.0	100	6; 25
Perfluoroalkyl Sulfonates							
PFBS	C4	Target	[$^{13}C_3$]PFBS	298.9430	80.0; 98.9	95	38; 30
PFPeS	C5	Target	[$^{13}C_3$]PFHxS	348.9398	80.0; 98.9	140	38; 30
PFHxS*	C6	Target	[$^{13}C_3$]PFHxS	398.9366	80.0; 98.9	135	58; 34
PFHpS	C7	Target	[$^{13}C_8$]PFOS	448.9334	80.0; 98.9	180	54; 42
PFOS*	C8	Target	[$^{13}C_8$]PFOS	498.9302	80.0; 98.9	200	60; 50
PFNS	C9	Target	[$^{13}C_8$]PFOS	548.9270	80.0; 98.9	175	60; 54
PFDS	C10	Target	[$^{13}C_8$]PFOS	598.9238	80.0; 98.9	175	60; 54
Perfluoroalkyl Sulfonamides							
FBSA	C4	Target	[$^{13}C_8$]FOSA	297.9590	78.0	140	20
FHxSA	C6	Target	[$^{13}C_8$]FOSA	397.9526	78.0	180	40
FOSA	C8	Target	[$^{13}C_8$]FOSA	497.9462	78.0	140	38
FDSA	C10	Target	[$^{13}C_8$]FOSA	597.9398	78.0	140	32
N-MeFOSA	C8	Target	d3-N-MeFOSA	511.9619	219.0; 169.0	60	26; 22
N-EtFOSA	C8	Target	d5-N-EtFOSA	525.9775	219.0; 169.0	60	26; 34
Perfluoroalkyl Sulfonamidoethanols							
N-MeFOSE	C8	Target	d7-N-MeFOSE	616.0089	59.0	55	66
N-EtFOSE	C8	Target	d9-N-EtFOSE	630.0245	59.0	55	54
Perfluoroalkyl Sulfonamidoacetic Acids							
N-MeFOSAA*	C8	Target	d3-N-MeFOSAA	569.9673	418.9; 482.9	95	14; 10
N-EtFOSAA*	C8	Target	d5-N-EtFOSAA	583.9830	418.9; 525.9	95	18; 14
FOSAA	C8	Target	[$^{13}C_8$]FOSA	555.9517	498.0; 419.0; 78.0	55	26; 26; 54
Polyfluoroalkyl Ether Carboxylates							
ADONA	C8	Target	[$^{13}C_8$]FOSA	376.9689	250.9; 85.0	80	2; 30
Fluorotelomer Sulfonates							
4:2 FTSA		Target	[$^{13}C_2$]4:2 FTSA	326.9743	307.0; 81.0	130	10; 30
6:2 FTSA		Target	[$^{13}C_2$]6:2 FTSA	426.9679	406.9; 81.0	135	18; 34
8:2 FTSA		Target	[$^{13}C_2$]8:2 FTSA	526.9615	506.9; 81.0	180	26; 42
10:2 FTSA		Target	[$^{13}C_2$]8:2 FTSA	626.9537	607.0; 81.0	180	30; 70
Fluorotelomer Carboxylates							
3:3 FTCA		Target	[$^{13}C_5$]PFPeA	241.0105	136.8; 116.8	52	10; 10
5:3 FTCA		Target	[$^{13}C_5$]PFHxA	341.0041	236.8; 216.8	72	10; 10
7:3 FTCA		Target	[$^{13}C_5$]PFHxA	440.9977	336.7; 316.7	52	10; 10
Extracted Internal Standards (EIS)							
[$^{13}C_4$]PFBA		EIS		216.9926	171.9	60	2
[$^{13}C_5$]PFPeA		EIS		267.9928	223.0	60	2
[$^{13}C_5$]PFHxA		EIS		317.9896	273.0	70	2
[$^{13}C_4$]PFHpA		EIS		366.9830	321.9	70	2

[$^{13}C_8$]PFOA		EIS		420.9933	376.0	75	2
[$^{13}C_9$]PFNA		EIS		471.9934	427.0	85	2
[$^{13}C_6$]PFDA		EIS		518.9802	474.0	90	2
[$^{13}C_7$]PFUnDA		EIS		569.9803	525.0	85	6
[$^{13}C_2$]PFDoDA		EIS		614.9604	569.9	95	6
[$^{13}C_2$]PFTeDA		EIS		714.9540	670.0	95	6
[$^{13}C_3$]PFBS		EIS		301.9531	79.9; 99.0	95	26; 26
[$^{13}C_3$]PFHxS		EIS		401.9467	79.9; 98.9	180	38; 38
[$^{13}C_8$]PFOS		EIS		506.9571	79.9; 99.0	180	50; 50
[$^{13}C_8$]FOSA		EIS		505.9730	78.0	95	38
d3-N-MeFOSA		EIS		514.9807	169.0	60	22
d5-N-EtFOSA		EIS		531.0089	169.0	55	30
d7-N-MeFOSE		EIS		623.0530	59.0	55	66
d9-N-EtFOSE		EIS		639.0811	59.0	55	30
d3-N-MeFOSAA		EIS		572.9862	418.9	100	14
d5-N-EtFOSAA		EIS		589.0144	418.9	95	14
[$^{13}C_2$]4:2 FTSA		EIS		328.9810	81.0	95	38
[$^{13}C_2$]6:2 FTSA		EIS		428.9746	81.0	95	46
[$^{13}C_2$]8:2 FTSA		EIS		528.9682	81.0	180	46
Non-Extracted Internal Standards (NIS)							
[$^{13}C_3$]PFBA		NIS		215.9893	172.0	68	2
[$^{13}C_2$]PFHxA		NIS		314.9795	270.0	60	6
[$^{13}C_4$]PFOA		NIS		416.9798	372.0	72	2
[$^{13}C_5$]PFNA		NIS		467.9800	423.0	85	2
[$^{13}C_2$]PFDA		NIS		514.9667	470.1	90	2
[$^{18}O_2$]PFHxS		NIS		402.9451	83.9	56	58
[$^{13}C_4$]PFOS		NIS		502.9436	79.9	180	50

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

1.5. Isomer Quantification. Linear and branched isomers for perfluorohexane sulfonate (PFHxS), PFOS, and N-methyl and N-ethyl sulfonamidoacetic acids (N-MeFOSAA and N-EtFOSAA) were quantified separately using available isomeric standards and individual native isomer calibration curves. Branched isomers for additional PFAS analytes were detected in many of the samples but could not be quantified separately due to unavailable branched isomer standards. Instead, to avoid underestimating PFAS concentrations, both the linear and branched isomers for those analytes were integrated together to give a total concentration. PFAS with detectable linear and branched isomers in water samples that were integrated as the total included perfluoropentane sulfonate (PFPeS), perfluoroheptane sulfonate (PFHpS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA) (**Figure S1**). PFAS with detectable linear and branched isomers in sediment samples that were integrated as the total included FHxSA and FOSA. PFAS with detectable isomers in biota tissue samples that were integrated as the total included PFHpS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), FHxSA, and FOSA (**Figure S2**).

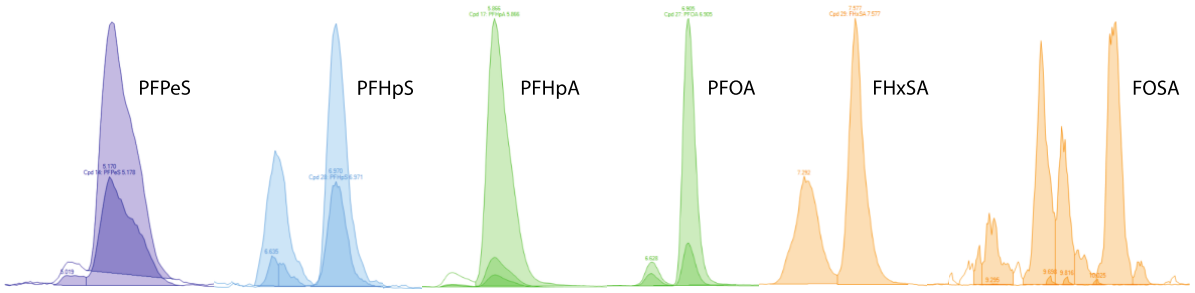

Figure S1. Chromatograms of perfluoropentane sulfonate (PFPeS), perfluoroheptane sulfonate (PFHpS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA) in a surface water sample showing presence of linear and branched isomers.

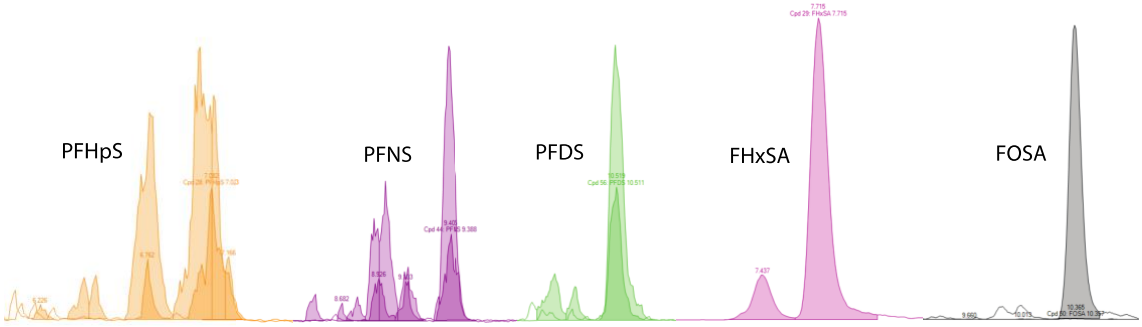

Figure S2. Chromatograms of perfluoroheptane sulfonate (PFHpS), perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA) in a fish muscle tissue sample showing presence of linear and branched isomers.

To assess the accuracy in quantifying the total by integrating linear (L-) and branched (Br-) isomers together in the absence of individual isomeric standards, we compared differences in concentration determined for total PFHxS and PFOS where L- and Br-isomers were separately quantified using standards and then added together to determine the sum versus total PFHxS and PFOS determined by integrating L- and Br-isomers together and quantifying based on the L-isomer calibration curve to get the total. In surface water samples, the percent difference between total PFHxS (isomers quantified separately) and total PFHxS (isomers integrated/quantified together) ranged between 0.3-21% (average: 3.4%; median: 2.3%) and the percent difference for total PFOS measurements ranged between 8.1-28% (average: 14%; median: 13%). In biota tissue samples, the percent difference for total PFHxS ranged between 0.0-34% (average: 2.2%; median: 0.6%) and the percent difference for total PFOS ranged between 0.0-25% (average: 4.6%; median: 3.6%). Based on this, minimal difference was observed between quantifying the sum of isomers using the linear calibration curve versus quantifying them using separate L- and Br-isomer standards and adding together. We chose to report the total PFAS quantified by integrating the L- and Br-isomers together using the L-isomer calibration curve.

Based on minimal differences in quantification of total PFAS with or without separate isomeric standards and since there were abundant Br-isomers for FHxSA and FOSA in all samples, branched, linear, and total concentrations of FHxSA and FOSA were determined. Total FHxSA and FOSA were determined by integrating L- and Br-isomers together and quantifying based on the linear calibration. L-FHxSA and L-FOSA were determined by integrating the L-isomer only and quantifying based on the linear calibration curve. To determine Br-FHxSA and Br-FOSA, the linear value was subtracted from the total to estimate the branched concentration.

1.6. Blanks, Duplicates, and Spike Recoveries. For QA/QC from targeted analysis, 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. All instrumental blanks were below the limit of detection (LOD). Procedural blanks consisting of MQ water and EIS spikes were included in every batch of 12 samples extracted for both surface water (n=4) and biota tissue samples (n=12). Procedural blanks consisting of sand and EIS spikes were included in every batch of 24 sediment samples (n=2). If PFAS concentrations were above the method detection limit (>MDL) in any blanks within each batch, then the subsequent samples within that batch were blank corrected for those analytes with detections. Field blanks (n=7) consisting of MQ water and IS spikes that were collected/stored with the surface water samples were analyzed and if PFAS concentrations were >MDL in any field blanks post procedural blank correction, then their detectable levels were additionally subtracted from the surface water samples associated with those field blanks based on site and date of collection. Procedural and field blanks were analyzed on the Orbitrap when quantifying FPeSA to assess presence of blank contamination. There were very few instances where blank detections were >MDL and required correction. See **Table S4** for average procedural and field blank detections.

Differences between duplicate measurements were assessed using the relative percent difference (RPD) statistic that determines the mean normalized difference between two replicate samples. Fourteen field duplicate and two procedural duplicate water samples were measured with RPDs ranging from 0.03 % - 95.9% (average: 11.6%, median: 5.7%) across all PFAS analyzed with detectable levels >MDL in both replicates. Three procedural duplicate sediment samples were measured with RPDs ranging from 1% - 125% (average: 20%, median: 17%) across all PFAS analyzed with detectable levels >MDL in both replicates. Twelve procedural duplicate biota tissue samples were measured with RPDs ranging from 0.07 % - 82.8% (average: 13.3%, median: 7.0%) across all PFAS analyzed with detectable levels >MDL in both replicates.

Table S4. Average concentrations of procedural blank and field blank detections (ng L^{-1} for water and ng g^{-1} for sediment and biota tissue) and detection frequencies (DF). MDL is the method detection limit. Parameter descriptions are provided in Table S3.

	Water Extractions				Sediment Extractions		Biota Extractions	
Procedure	**Procedural Blanks (n=4)**		**Field Blanks (n=7)**		**Procedural Blanks (n=2)**		**Procedural Blanks (n=12)**	
Parameters	**Mean ± SD**[1]	**DF**[2]	**Mean ± SD**	**DF**	**Mean ± SD**	**DF**	**Mean ± SD**	**DF**
PFBA	<MDL	0/4	<MDL	0/7	0.33 ± 0.001	2/2	<MDL	1/12
PFPeA	<MDL	0/4	<MDL	0/7	0.10 ± 0.003	2/2	<MDL	1/12
PFHxA	<MDL	1/4	<MDL	0/7	0.05 ± 0.007	2/2	<MDL	1/12
PFHpA	0.94 ± 1.39	4/4	0.33 ± 0.55	4/7	0.10 ± 0.007	2/2	<MDL	1/12
PFOA	0.57 ± 0.073	4/4	<MDL	2/7	0.21 ± 0.017	2/2	<MDL	0/12
PFNA	0.15 ± 0.045	2/4	<MDL	1/7	<MDL	0/2	<MDL	0/12
PFDA	<MDL	1/4	<MDL	2/7	<MDL	0/2	<MDL	0/12
PFUnDA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFDoDA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFTrDA	<MDL	0/4	<MDL	1/7	<MDL	0/2	<MDL	0/12
PFTeDA	<MDL	0/4	<MDL	1/7	<MDL	0/2	<MDL	0/12
PFBS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFPeS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
L-PFHxS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
Br-PFHxS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFHpS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
L-PFOS	0.14 ± 0.025	4/4	0.10 ± 0.072	4/7	<MDL	0/2	<MDL	1/12
Br-PFOS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFNS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
PFDS	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
FBSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
FPeSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
FHxSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
FOSA	0.10 ± 0.026	4/4	<MDL	3/7	<MDL	0/2	<MDL	0/12
FDSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
N-MeFOSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
N-EtFOSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
L-N-MeFOSAA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
Br-N-MeFOSAA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
L-N-EtFOSAA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
Br-N-EtFOSAA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
FOSAA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
N-MeFOSE	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
N-EtFOSE	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
ADONA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
4:2 FTSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
6:2 FTSA	NR[3]		NR		NR		<MDL	1/12
8:2 FTSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
10:2 FTSA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
3:3 FTCA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
5:3 FTCA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12
7:3 FTCA	<MDL	0/4	<MDL	0/7	<MDL	0/2	<MDL	0/12

[1]Mean and standard deviation (SD) – only determined if >50% of blanks have a detect, [2]DF = detection frequency, [3]NR = not reported due to variable contamination

For water samples, procedural spike recoveries (n=4) using MQ water as the matrix spiked with 0.15 ng (n=2), 1.5 ng (n=1), or 15 ng (n=1) native PFAS mixture yielded an average recovery of 98 ± 25 % for all targeted PFAS measured. Recoveries were between 39% (3:3 FTCA) to 185% (FOSAA). Matrix spikes (n=2) in surface water samples spiked with a 1.5 ng PFAS mixture yielded an average recovery of 98 ± 32 % with a range of 6% (5:3 FTCA) to 254% (FOSAA). For only PFAS detected in samples, recoveries were generally within 70-130%.

For sediment samples, procedural spike recoveries (n=2) using sand as the matrix spiked with 0.3 ng (n=1) and 3 ng (n=1) native PFAS mixture yielded an average recovery of 104±23% for all targeted PFAS. Recoveries were between 44% (10:2 FTSA) to 164% (PFTrDA). Matrix spikes (n=3) in sediment samples spiked with 3 ng PFAS mixture yielded an average recovery of 104 ± 24 % with a range of 40% (FOSAA) to 242% (PFTrDA), but again were generally within 70-130% for only PFAS that were detected in the samples.

For biota tissue samples, procedural spike recoveries (n=6) using MQ water as the matrix and 0.225 ng native PFAS mixture spike yielded an average recovery of 113 ± 32 % for all targeted PFAS. Recoveries were between 54% (4:2 FTSA) to 272% (ADONA). Matrix spikes (n=5) in biota muscle/whole body tissues spiked with 2.25 ng PFAS mixture yielded an average recovery of 106 ± 46 % with a range of 32% (5:3 FTCA) to 424% (FOSAA). For only PFAS detected in samples, recoveries were within 70-130%. Matrix spikes were conducted on 5 biotic species covering all sample locations and tissue types (Brown Bullhead whole body from Santuit River, Striped Bass muscle from Waquoit Bay, Silverside composite whole body from Waquoit Bay, Quahog whole body from Quashnet River, and Bluegill Sunfish muscle from Moody Pond).

Additional spike recovery experiments were conducted to assess procedural and matrix recovery of FPeSA following purchase of the additional standard. Analyses were conducted on the ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) Orbitrap. For surface water sample extraction, procedural spike recoveries (n=2) using MQ water as the matrix spiked with 0.11 ng (n=1) and 1.1 ng (n=1) FPeSA yielded an average recovery of 103 ± 6%. Matrix spikes (n=2) in surface water samples spiked with 0.11 ng (n=1) and 1.1 ng (n=1) FPeSA yielded an average recovery of 78 ± 4%. For fish tissue sample extraction, procedural spike recoveries (n=2) using MQ water as the matrix spiked with 0.11 ng (n=1) and 1.1 ng (n=1) FPeSA yielded an average recovery of 98 ± 13%. Matrix spikes (n=5) in fish muscle/whole body tissues spiked with 0.11 ng (n=3) and 1.1 ng (n=2) FPeSA yielded an average recovery of 75 ± 16%. Matrix spikes were conducted on 5 biotic species covering all locations and tissues (Striped Bass muscle and Silverside whole body composite from Waquoit Bay, Quahog whole body from Quashnet into Waquoit, American Eel muscle from Lower Quashnet River, and Redear Sunfish whole body from Moody Pond). Spike recovery of FPeSA for sediment extractions was not assessed since FPeSA was not detected in sediment samples. **Table S5** lists average PFAS recoveries in procedural/matrix spikes for all sample extractions.

Table S5. Average percent recovery (%) and standard deviation (SD) of per- and polyfluoroalkyl substances (PFAS) mixture spikes in procedural and matrix samples analyzed for surface water, sediment, and biota tissue extractions. MQ refers to Milli-Q water. Parameter descriptions are provided in Table S3.

	Water Extractions		Sediment Extractions		Biota Extractions	
Procedure	**Procedural Spikes (n=4)**	**Matrix Spikes (n=2)**	**Procedural Spikes (n=2)**	**Matrix Spikes (n=3)**	**Procedural Spikes (n=6)**	**Matrix Spikes (n=5)**
Matrix	**MQ Water**	**Surface Water**	**Sand**	**Sediment**	**MQ Water**	**Biota Muscle/ Whole Body**
Parameters (%)	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
PFBA	99[1]	128 ± 0	122 ± 13	115 ± 2	ND	102 ± 10
PFPeA	114 ± 20	95 ± 14	105 ± 3	103 ± 2	113 ± 5	103 ± 3
PFHxA	95 ± 26	102 ± 9	108 ± 6	107 ± 2	109 ± 7	97 ± 2
PFHpA	103 ± 15	99 ± 3	99 ± 11	103 ± 2	107 ± 4	98 ± 3
PFOA	75 ± 25	99 ± 1	95 ± 16	105 ± 4	111 ± 9	98 ± 6
PFNA	93 ± 21	98 ± 5	115 ± 8	109 ± 2	111 ± 11	97 ± 5
PFDA	91 ± 11	100 ± 6	108 ± 4	107 ± 5	109 ± 13	97 ± 7
PFUnDA	92 ± 8	96 ± 3	101 ± 7	100 ± 3	102 ± 8	101 ± 4
PFDoDA	90 ± 6	101 ± 5	101 ± 13	104 ± 0	110 ± 10	99 ± 4
PFTrDA	119 ± 113	116 ± 1	160 ± 6	208 ± 52	126 ± 12	132 ± 52
PFTeDA	93 ± 4	102 ± 11	105 ± 1	102 ± 2	106 ± 7	98 ± 4
PFBS	115 ± 12	119 ± 2	111 ± 3	1005± 3	102 ± 11	93 ± 5
PFPeS	95 ± 5	103 ± 9	97 ± 8	109 ± 4	96 ± 21	74 ± 10
L-PFHxS	121 ± 13	116 ± 3	142 ± 19	124 ± 4	121 ± 14	78 ± 12
Br-PFHxS	87 ± 10	88 ± 1	118 ± 18	103 ± 5	ND	66 ± 14
PFHpS	95 ± 8	108 ± 3	101 ± 4	107 ± 4	100 ± 13	85 ± 18
L-PFOS	124 ± 16	104 ± 15	137 ± 22	121 ± 2	122 ± 29	68 ± 5
Br-PFOS	133 ± 42	93 ± 7	137 ± 27	118 ± 4	ND	81 ± 25
PFNS	100 ± 10	104 ± 8	91 ± 6	94 ± 6	106 ± 23	105 ± 19
PFDS	89 ± 11	84 ± 5	68 ± 6	82 ± 15	94 ± 24	108 ± 8
FBSA	73 ± 3	55 ± 0	84 ± 7	78 ± 8	142 ± 33	162 ± 21
FPeSA[3]	103 ± 6	78 ± 4	NR[4]	NR	98 ± 13	75 ± 16
FHxSA	91 ± 5	67 ± 17	61 ± 1	59 ± 7	106 ± 20	123 ± 24
FOSA	91 ± 14	91 ± 4	97 ± 4	93 ± 4	113 ± 14	127 ± 18
FDSA	108 ± 10	108 ± 7	91 ± 9	92 ± 11	126 ± 35	92 ± 60
N-MeFOSA	108 ± 2	100 ± 9	110 ± 0	104 ± 8	ND	82 ± 16
N-EtFOSA	95 ± 4	117 ± 13	91 ± 6	93 ± 12	ND	117 ± 18
L-N-MeFOSAA	104 ± 9	103 ± 7	100 ± 15	113 ± 6	117 ± 11	109 ± 5
Br-N-MeFOSAA	77 ± 16	60 ± 2	121	108 ± 19	ND	103 ± 14
L-N-EtFOSAA	82 ± 10	90 ± 3	109 ± 2	102 ± 5	94 ± 14	147 ± 12
Br-N-EtFOSAA	100 ± 6	116 ± 13	129	123 ± 13	ND	111 ± 18
FOSAA	168 ± 14	254	137 ± 3	92 ± 46	202 ± 41	295 ± 94
N-MeFOSE	75 ± 12	77 ± 3	95 ± 6	103 ± 4	ND	80 ± 11
N-EtFOSE	89 ± 13	109 ± 11	101 ± 16	108 ± 8	119 ± 11	101 ± 41
ADONA	137 ± 14	137 ± 19	117 ± 13	109 ± 9	196 ± 56	195 ± 67
4:2 FTSA	96 ± 22	151	101 ± 4	104 ± 5	101 ± 29	121 ± 17
6:2 FTSA	NR	NR	NR	NR	108 ± 10	103 ± 12
8:2 FTSA	111 ± 10	112 ± 4	124 ± 13	109 ± 14	105 ± 13	86 ± 6
10:2 FTSA	82 ± 20	71 ± 6	54 ± 14	80 ± 22	88 ± 19	115 ± 15
3:3 FTCA	61 ± 30	ND	81 ± 14	91 ± 6	ND	64 ± 17
5:3 FTCA	50 ± 7	ND	94 ± 5	91 ± 7	84 ± 10	49 ± 13
7:3 FTCA	90 ± 29	36 ± 1	66 ± 10	85 ± 1	76 ± 18	75 ± 19

[1]For analytes where no SD is reported, only one spiked sample had a reportable recovery, [2]ND = spikes not detected, [3]FPeSA spike recovery was assessed separately through additional spike recovery experiments measured on the UHPLC-HRMS (n=2 procedural blanks were measured per extraction type), [4]NR = spikes not reported

Internal standard recoveries were assessed using Metric 1 to calculate % recovery for all PFAS with an available extracted internal standards (EIS) used for quantification. This was based on the average EIS area in all instrument blanks and calibration standards. Metric 2 was used to calculate % recovery for EIS of PFAS that had a corresponding NIS available. This was based on the response factor between the EIS and the non-extracted internal standard (NIS) and their associated spiked masses in the calibration standards and samples as detailed in USEPA Method 1633.[43] Biota samples were not analyzed using a matrix-matched calibration curve due to varying tissues and species measured within one analysis. To assess matrix effects, matrix spike recovery experiments were conducted, and % matrix effects were calculated based on the EIS area in the samples compared to the average EIS area in the instrumental blanks and calibration standards. **Table S6** lists average recoveries and percent matrix effects for all sample extractions.

Table S6. Average percent recovery (%) and standard deviation (SD) of extracted and non-extracted internal standards (EIS/NIS) in surface water, sediment, and biota tissue samples using two calculation metrics. Metric 1 is based on average area and Metric 2 is based on EIS to NIS response factors. Percent matrix effects are shown for biota tissue samples. Internal standard descriptions are provided in Table S3.

Internal Standard Analytes	**Surface Water Extractions**		**Sediment Extractions**		**Biota Tissue Extractions**		
EIS	**Metric 1**	**Metric 2**	**Metric 1**	**Metric 2**	**Metric 1**	**Metric 2**	**% Matrix Effects**
[$^{13}C_4$]PFBA	18 ± 11	99 ± 13	26 ± 6	56 ± 4	45 ± 36	119 ± 26	59 ± 33
[$^{13}C_5$]PFPeA	42 ± 23		71 ± 12		61 ± 20		39 ± 20
[$^{13}C_5$]PFHxA	73 ± 17	105 ± 6	76 ± 9	56 ± 4	72 ± 22	126 ± 12	36 ± 19
[$^{13}C_4$]PFHpA	81 ± 12		81 ± 9		64 ± 20		36 ± 20
[$^{13}C_8$]PFOA	92 ± 8	105 ± 7	81 ± 8	57 ± 4	67 ± 29	124 ± 11	40 ± 26
[$^{13}C_9$]PFNA	96 ± 10	109 ± 7	83 ± 7	56 ± 5	79 ± 24	122 ± 12	26 ± 22
[$^{13}C_6$]PFDA	102 ± 11	105 ± 9	77 ± 10	54 ± 5	107 ± 13	124 ± 13	-1 ± 12
[$^{13}C_7$]PFUnDA	109 ± 18		73 ± 14		100 ± 19		0 ± 19
[$^{13}C_2$]PFDoDA	107 ± 21		62 ± 19		110 ± 21		-10 ± 21
[$^{13}C_2$]PFTeDA	96 ± 23		31 ± 20		95 ± 34		5 ± 34
[$^{13}C_3$]PFBS	61 ± 13		75 ± 7		74 ± 48		26 ± 48
[$^{13}C_3$]PFHxS	87 ± 13	109 ± 10	70 ± 6	51 ± 6	116 ± 21	168 ± 38	-6 ± 19
[$^{13}C_8$]PFOS	98 ± 14	123 ± 14	70 ± 9	53 ± 7	129 ± 24	129 ± 45	-24 ± 23
[$^{13}C_8$]FOSA	55 ± 16		75 ± 12		50 ± 18		50 ± 18
d3-N-MeFOSA	10 ± 4		60 ± 10		28 ± 11		72 ± 11
d5-N-EtFOSA	11 ± 5		57 ± 13		14 ± 7		86 ± 7
d7-N-MeFOSE	27 ± 9		60 ± 9		34 ± 15		66 ± 15
d9-N-EtFOSE	32 ± 9		57 ± 10		20 ± 14		80 ± 14
d3-N-MeFOSAA	129 ± 31		94 ± 25		101 ± 30		-1 ± 30
d5-N-EtFOSAA	132 ± 36		93 ± 31		83 ± 31		17 ± 31
[$^{13}C_2$]4:2 FTSA	187 ± 28		200 ± 39		125 ± 37		-25 ± 37
[$^{13}C_2$]6:2 FTSA	175 ± 50		133 ± 41		180 ± 176		-80 ± 176
[$^{13}C_2$]8:2 FTSA	175 ± 58		107 ± 56		246 ± 57		-146 ± 57
NIS	**Metric 1**		**Metric 1**		**Metric 1**		
[$^{13}C_3$]PFBA	25 ± 15		37 ± 9		53 ± 40		
[$^{13}C_2$]PFHxA	98 ± 23		108 ± 13		81 ± 26		
[$^{13}C_4$]PFOA	124 ± 10		114 ± 11		76 ± 34		
[$^{13}C_5$]PFNA	124 ± 11		116 ± 11		91 ± 29		
[$^{13}C_2$]PFDA	138 ± 16		115 ± 14		121 ± 19		
[$^{18}O_2$]PFHxS	111 ± 17		107 ± 9		98 ± 21		
[$^{13}C_4$]PFOS	113 ± 11		102 ± 7		143 ± 31		

1.7. Detection Limits. For targeted PFAS analysis by LC-MS/MS, the instrument limit of detection (LOD) was calculated based on the average concentration at which the sample signal-to-noise ratio (S/N) was 3. The limit of quantification was calculated for a sample signal-to-noise ratio of 10. The method detection limit (MDL) and method quantification limit (MQL) were determined based on sample dilution volumes/weights (**Table S7**). Values >MDL are reported. The MDL for FPeSA was determined separately by targeted UHPLC-HRMS analysis. This detection limit was determined using EPA's MDL determination procedure, in which the MDL was computed as the Student's t-value for a single-tailed 99th percentile t-statistic multiplied by the sample standard deviation of the replicate spiked samples (n=7) at the lowest detectable concentration.[44] The MDL of the instrumental analysis was determined as 2.67 ng L^{-1} and was multiplied by the sample dilution factor to determine individual sample MDLs for water and biota samples, calculated as 0.017 ng L^{-1} for water samples and 0.006 ng g^{-1} for biota samples.

Table S7. Method detection limits (MDL) and method quantification limits (MQL) for surface water (ng/L), sediment (ng/g dry weight) and biota tissue (ng/g wet weight) sample extractions. (NR = not reported, NM=not measured or calculated, PFAS = per- and polyfluoroalkyl substances)

PFAS Analyte	Surface Water Extractions		Sediment Extractions		Biota Tissue Extractions	
	MDL (ng/L)	MQL (ng/L)	MDL (ng/g)	MQL (ng/g)	MDL (ng/g)	MQL (ng/g)
PFBA	5.63	18.77	0.33	1.09	2.43	8.10
PFPeA	0.61	2.02	0.025	0.084	0.18	0.60
PFHxA	0.28	0.94	0.012	0.041	0.12	0.40
PFHpA	0.16	0.54	0.012	0.039	0.061	0.20
PFOA	0.086	0.29	0.011	0.038	0.084	0.28
PFNA	0.051	0.17	0.016	0.054	0.073	0.24
PFDA	0.029	0.096	0.013	0.043	0.044	0.15
PFUnDA	0.026	0.087	0.017	0.055	0.043	0.14
PFDoDA	0.033	0.11	0.014	0.047	0.042	0.14
PFTrDA	0.036	0.12	0.034	0.11	0.045	0.15
PFTeDA	0.031	0.10	0.035	0.12	0.028	0.093
PFBS	0.34	1.14	0.016	0.18	0.082	0.27
PFPeS	0.25	0.84	0.016	0.054	0.21	0.69
L-PFHxS	0.31	1.03	0.051	0.17	0.14	0.47
Br-PFHxS	0.32	1.08	0.044	0.15	0.14	0.48
PFHpS	0.14	0.46	0.034	0.11	0.29	0.96
L-PFOS	0.10	0.35	0.043	0.14	0.15	0.51
Br-PFOS	0.14	0.46	0.056	0.19	0.36	1.21
PFNS	0.10	0.33	0.044	0.15	0.27	0.90
PFDS	0.10	0.33	0.060	0.20	0.19	0.64
FBSA	0.12	0.39	0.017	0.056	0.19	0.64
FPeSA*	0.02	NM	NM	NM	0.01	NM
FHxSA	0.16	0.54	0.035	0.12	0.36	1.19
FOSA	0.060	0.20	0.020	0.066	0.20	0.67
FDSA	0.064	0.21	0.027	0.091	0.17	0.56
N-MeFOSA	0.86	2.85	0.056	0.19	1.17	3.89
N-EtFOSA	0.35	1.18	0.039	0.13	1.05	3.51
L-N-MeFOSAA	0.15	0.48	0.022	0.072	0.086	0.29
Br-N-MeFOSAA	2.41	8.04	0.53	1.77	1.32	4.39
L-N-EtFOSAA	0.045	0.15	0.043	0.14	0.074	0.25
Br-N-EtFOSAA	1.28	4.28	0.80	2.67	1.06	3.53
FOSAA	0.19	0.63	0.062	0.21	0.29	0.97
N-MeFOSE	0.11	0.36	0.052	0.17	0.61	2.04
N-EtFOSE	0.078	0.26	0.038	0.13	0.48	1.62
ADONA	0.041	0.14	0.009	0.029	0.095	0.32

4:2 FTSA	0.13	0.44	0.027	0.092	0.19	0.64
6:2 FTSA	NR	NR	NR	NR	0.088	0.29
8:2 FTSA	0.096	0.32	0.071	0.24	0.043	0.14
10:2 FTSA	0.097	0.32	0.099	0.33	0.13	0.42
3:3 FTCA	2.74	9.14	0.23	0.77	1.18	3.94
5:3 FTCA	0.10	0.35	0.027	0.092	0.27	0.90
7:3 FTCA	0.11	0.36	0.041	0.14	0.14	0.48

*FPeSA detection limits were determined differently compared to other PFAS due to analysis on a different instrument.

1.8. SRM. Method trueness was assessed through the analysis of the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1947 reference samples (Lake Michigan Fish Tissue). Replicate extractions of SRM 1947 (n=9) were included in every other batch of twelve tissue samples. **Table S8** compares the average concentration results from these extractions with the NIST SRM 1947 reference concentrations and concentrations measured in other similar studies.[45,46]

Table S8. National Institute of Standards and Technology Standard Reference Material (NIST SRM) 1947 reference material per- and polyfluoroalkyl substances (PFAS) concentrations (ng g^{-1}). NA = not available.

	This Study	**SRM Ref**[1]	**Simonnet-Laprade et al., 2019**	**Munoz et al., 2022**
Replicates	n = 9	NA	n = 4	n = 5
PFBA	<2.43		<0.06	0.11 ± 0.04
PFPeA	<0.18		<0.10	0.07 ± 0.01
PFHxA	<0.12		0.17 ± 0.07	0.07 ± 0.01
PFHpA	0.07 ± 0.01		0.07 ± 0.01	0.07 ± 0.01
PFOA	0.12 ± 0.03		0.11 ± 0.01	0.10 ± 0.01
PFNA	0.26 ± 0.09	0.20	0.26 ± 0.06	0.25 ± 0.01
PFDA	0.27 ± 0.11	0.26	0.28 ± 0.02	0.24 ± 0.01
PFUnDA	0.31 ± 0.11	0.28	0.27 + 0.01	0.37 ± 0.03
PFDoDA	0.14 ± 0.04		0.27 ± 0.02	0.16 ± 0.01
PFTrDA	0.28 ± 0.11	0.20	0.19 ± 0.02	0.26 ± 0.03
PFTeDA	0.13 ± 0.02		0.11 ± 0.02	0.15 ± 0.02
PFBS	<0.08		<0.02	
PFPeS	<0.21			
L-PFHxS	<0.14		0.06 ± 0.01	0.03 ± 0.01
Br-PFHxS	<0.14			
PFHpS	<0.29		0.03 ± 0.01	0.03 ± 0.01
L-PFOS	4.35 ± 1.55	5.90	7.19 ± 0.56	6.01 ± 0.11
Br-PFOS	0.57 ± 0.22			
PFNS	<0.27			
PFDS	<0.19		0.20 ± 0.01	0.13 ± 0.02
FBSA	0.37 ± 0.09			0.15 ± 0.01
FHxSA	<0.36			
FOSA	0.37 ± 0.14		0.12 ± 0.01	0.27 ± 0.02
FDSA	<0.17			
N-MeFOSA	<1.17		<0.01	
N-EtFOSA	<1.05		<0.01	
L-N-MeFOSAA	<0.09		0.02 ± 0.01	0.11 ± 0.01
Br-N-MeFOSAA	<1.32			
L-N-EtFOSAA	0.10 ± 0.01		0.06 ± 0.01	0.17 ± 0.02
Br-N-EtFOSAA	<1.06			
FOSAA	<0.29		<0.01	

N-MeFOSE	<0.61			
N-EtFOSE	<0.48			
ADONA	<0.09		<0.21	
4:2 FTSA	<0.19		<0.01	
6:2 FTSA	<0.09		<0.05	
8:2 FTSA	<0.04		<0.03	
10:2 FTSA	<0.13		<0.01	
3:3 FTCA	<1.18			
5:3 FTCA	<0.27			
7:3 FTCA	<0.14			

[1]SRM Ref is the NIST SRM 1947 reference concentrations provided

1.9. Suspect Screening and Nontargeted Analysis. Surface water and all biota samples were analyzed using an ultrahigh-performance liquid chromatograph (Vanquish Flex UHPLC, ThermoFisher, U.S.) coupled with quadrupole orbitrap mass spectrometry (Orbitrap Exploris 120, ThermoFisher, U.S.) (UHPLC-HRMS) in ESI- mode. The LC system was retrofitted with a strong solvent loop to minimize solvent effects in the sample injection with needle rising pre- and post-injection to avoid contamination. Original solvent lines and mobile phase filters were replaced by ones made of PolyEtherEtherKetone (PEEK). Details on the instrumental method are provided in **Table S9**. Suspect screening was conducted using Compound Discoverer 3.3. Suspect workflow for detecting compounds included: mass range of 200-800 Da, mass tolerance of 5 ppm, minimum peak intensity of 10,000, chromatographic peak detection of 1.5, retention time tolerance of 0.2 min, peak rating threshold of 5, S/N threshold of 1.5, and background correction. Mass list included: Chemical List PFASSTRUCT-2022-04-20 (10737), PFAS_NEG (92), PFAS_NIST (4951), Fluorinated_Agrochemicals (200), and Fluorinated_Pharmaceuticals_ Metabolites (461). Additional compound filtering included a Δmass ppm range of -2 to 2, mass defect between -0.116 to 0.268 and peak rating > 3. Since FPeSA was widely detected in all samples measured and a reference standard became available, the native standard was purchased, and FPeSA was quantified (m/z 347.9558 → 78.0) in these samples on the UHPLC-HRMS using a 12-point calibration curve ranging from 2.36-18,000 ng/L using the same method detailed in **Table S9**. 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.

Sediment and some initial surface water extracts were analyzed separately at 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 MQ water. 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. An additional Phenomenex Gemini C18 column (5 μm, 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 in the SCIEX OS software based on precursor mass, isotope pattern, retention time, exact mass

accuracy (< 5 ppm), and MS/MS fragmentation matching. 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.

Surface water results on the QTOF-MS/MS were only used for comparison to UHPLC-HRMS results during method development. Suspect screening of sediment on the QTOF-MS/MS only identified PFPrS (C3 PFSA) in samples and were therefore excluded from further interpretation in this study.

Table S9. Details on the ultrahigh-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) instrumental method for suspect per- and polyfluoroalkyl substances (PFAS) analysis.

Separation Column	Thermo Acclaim RSLC 120 C18 column (2.2 µm, 2.1 × 50 mm)			
Delay Column	Thermo Hypersil Gold column (1.9 µm, 4.6 × 50 mm)			
Autosampler Temp	20°C			
Column Oven Temp	40°C			
UHPLC Mobile Phases	A: 98:2 (Milli-Q Water:Acetonitrile) with 2 mM ammonium acetate and 0.1 % acetic acid B: 98:2 (Acetonitrile:Milli-Q Water) with 2 mM ammonium acetate and 0.1 % acetic acid			
Chromatographic Gradient	Time (min)	% A	% B	Flow Rate (mL/min)
	0.0	90	10	0.4
	1.0	70	30	0.4
	5.0	54	46	0.4
	10.0	24	76	0.4
	10.5	14	86	0.4
	10.9	14	86	0.4
	11.0	90	10	0.4
	15.0	90	10	0.4
Injection Volume	5 µL			
Ion Source	Ion source type: H-ESI (electrospray ionization) Spray voltage: Static Sheath gas flow rate: 50 Arb Aux gas flow rate: 12 Arb Sweep gas flow rate: 0.5 Arb Positive spray voltage: 3500 V Negative spray voltage: 1000 V Ion transfer tube temperature: 225 °C Vaporizer temperature: 300 °C			
MS Global Settings	*Full Scan mode* Polarity: Negative Resolution: 60,000 Scan range: 200-800 m/z AGC target: Standard Maximum injection time mode: Auto Microscans: 1 Data Type: Centroid Intensity Threshold: 2.0E4 Mass Tolerance: 5 ppm RF Lens: 50%		*Data Dependent ddMS^2 mode* Resolution: 30,000 Scan range: Auto-extended AGC target: Standard Maximum injection time mode: Auto Microscans: 1 Data Type: Centroid Isolation winder: 2 m/z Collision energy type: Normalized HCD collision energies: 10, 30, 50 %	

1.10. Extractable Organofluorine Analysis. Majority of surface water, sediment, and biota tissue samples were analyzed by combustion ion chromatography (CIC) for extractable organofluorine (EOF) to assess the fraction of EOF not accounted for by targeted PFAS.

For EOF analysis of surface water samples (400 mL), samples were extracted by SPE using Oasis WAX cartridges, similar to the extraction procedure used for targeted PFAS analysis. Following sample extraction, cartridges were rinsed with 20 mL of 0.01% NH_4OH in MQ Water to remove inorganic fluorine. Cartridges were eluted with 6 mL MeOH and 6 mL 1% NH_4OH in MeOH, concentrated to almost dryness under N_2 gas, reconstituted in 0.5 mL MeOH and microcentrifuged for 20 mins at 4,000 rpm. The extract (450 µL) was transferred to a 1 mL glass vial for EOF analysis on the CIC while 50 µL of extract was combined with 450 µL MQ water, 450 µL MeOH, and 50 µL EIS for analysis of targeted PFAS on the LC-MS/MS.

For EOF analysis of sediment samples, the extraction procedure was slightly altered compared to the method used for targeted PFAS analysis. Freeze-dried sediment (5 g) was combined with 10 mL 1% NH_4OH in MeOH, vortexed, sonicated, rotated, centrifuged, decanted, and repeated 2x with a final volume of 30 mL extract that was concentrated to 2 mL under N_2 gas and then diluted to 50 mL with MQ water for SPE extraction. Oasis WAX cartridges were preconditioned, the 50 mL diluted extract was added, and the cartridges were rinsed with 20 mL of 0.01% NH_4OH in MQ Water to remove inorganic fluorine. Cartridges were eluted with 6 mL MeOH followed by 6 mL 1% NH_4OH in MeOH, concentrated to almost dryness under N_2 gas, reconstituted in 0.5 mL MeOH and microcentrifuged for 20 mins at 4,000 rpm. The extract (450 µL) was put through a 0.2 µM mini-Uniprep filter and transferred to a 1 mL glass vial for EOF analysis on the CIC while 50 µL of the extract was combined with 450 µL MQ water, 450 µL MeOH, and 50 µL EIS for analysis of targeted PFAS on the LC-MS/MS.

For EOF analysis of biota samples, the extraction procedure was the same procedure used for targeted PFAS analysis. Tissue samples (1.5 g) were mixed with 7 mL acetonitrile, 5x4.8 mm stainless steel beads, homogenized using the FastPrep-24 homogenizer and centrifuged. The extraction was repeated, and the 14 mL acetonitrile extracts were frozen overnight, concentrated to 2 mL under N_2 gas and then diluted to 50 mL with MQ water for SPE extraction. Oasis WAX cartridges were preconditioned, the 50 mL diluted extract was added, and the cartridges were rinsed with 20 mL of 0.01% NH_4OH in MQ Water to remove inorganic fluorine. Cartridges were eluted with 6 mL MeOH followed by 6 mL 1% NH_4OH in MeOH, concentrated to almost dryness under N_2 gas, reconstituted in 0.5 mL MeOH and microcentrifuged for 20 mins at 4,000 rpm. The extract (450 µL) was transferred to a 1 mL glass vial for EOF analysis on the CIC while 50 µL of the extract was combined with 450 µL MQ water, 450 µL MeOH, and 50 µL EIS for analysis of targeted PFAS on the LC-MS/MS.

EOF extracts were analyzed on a Metrohm CIC with combustion unit from Analytik Jena, 920 Absorber Module, and 930 Compact IC Flex ion chromatograph from Metrohm. Sample extracts (100 µL) were injected into the combustion unit at 1050 °C, and the anions were separated with an ion exchange column (Metrosep A Supp 5-150/4) operated at 30 °C, with sodium carbonate-bicarbonate buffer as eluent and isocratic elution. The fluorine concentration was measured via ion conductivity. For EOF analysis, a ceramic boat without sample (boat blank) was analyzed twice between each set of duplicate sample injections to determine background fluorine levels between sample injections. Samples were blank corrected using the peak areas of the boat blanks run before and after each set of injections. Methanol blanks were run during calibration and throughout analysis to account for any source of contamination from

the solvents used in the analysis. Extraction blanks from each sample extraction were used to blank correct sample concentrations and were used to determine the LOD, which was calculated as the average plus three times the standard deviation of duplicate injections of extraction blanks. Sample MDLs were calculated based on the extraction LOD multiplied by the sample dilution factor based on the final extract volume and the sample mass/volume extracted. Surface water MDLs (n=2) ranged between 0.057-0.36 ng F/mL. Sediment MDLs (n=2) ranged between 3.11-6.52 ng F/g. Biota MDLs (n=8) ranged between 7.94-317 ng F/g and the average MDL was determined as 21.5 ng F/g. Sample MDLs for surface water and sediment samples were applied based on each batch of samples and sample MDLs for biota were applied based on the average MDL across batches. All extraction blanks except for one blank from the biota analysis were <MDL. Surface water field blanks (n=2) were all <MDL. Sample concentrations were determined from the average peak areas of duplicate injections using a 12-point calibration curve (R^2>0.998) of PFOA as F^- equivalents in MeOH from 50.4 to 1,000.76 μg F/L. Quality control points (n=12) were included every 12 samples and had a variance of <19% (average of 5 ± 4 %).

For each sample extraction, MQ water samples, blank samples, and matrix samples were spiked with sodium fluoride as inorganic fluorine (IF) to assess removal efficiency or spiked with a mixture of native PFAS as organic fluorine (OF) to assess organofluorine recovery based on the extraction procedures used. The efficacy of inorganic fluoride (IF) removal for surface water sample extraction was assessed using 1,000 ng F/mL sodium fluoride (500 ng) spiked into MQ water samples (n=2) and surface water matrix samples (n=2). IF removal for sediment sample extraction was assessed using 2,000 ng F/mL sodium fluoride (1,000 ng) spiked into extraction blank samples (n=1) and sediment matrix samples (n=2). IF removal for biota sample extraction was assessed using 1,000 ng F/mL sodium fluoride (500 ng) spiked into MQ water samples (n=2) and biota matrix samples (n=6). See **Table S10** for IF percent removal results.

Table S10. Results of percent inorganic fluorine (IF) removal based on measured spike concentration of sodium fluoride in extraction spikes using Milli-Q (MQ) water and sample matrix spikes.

Sample	Matrix	Measured Spike Concentration [ppb]	% IF Removal
Surface Water Extraction			
Extraction IF Spike 1	MQ Water	936	100 %
Extraction IF Spike 2	MQ Water	936	82 %
Sample Matrix IF Spike 1	Lower Quashnet River Water	936	100 %
Sample Matrix IF Spike 2	Moody Pond Water	936	100 %
Sediment Extraction			
Extraction IF Spike 1	Blank	1748	99 %
Sample Matrix IF Spike 1	Waquoit Bay Sediment	1748	100 %
Sample Matrix IF Spike 2	Moody Pond Sediment	1748	85 %
Biota Extraction			
Extraction IF Spike 1	MQ Water	965	99 %
Extraction IF Spike 2	MQ Water	965	97 %
Sample Matrix IF Spike 1	Eel – Santuit River	965	100 %
Sample Matrix IF Spike 2	Quahog – Waquoit into Quashnet	965	63 %
Sample Matrix IF Spike 3	Mummichog – Waquoit Bay	965	97 %
Sample Matrix IF Spike 4	Silverside – Waquoit Bay	965	100 %
Sample Matrix IF Spike 5	Bluegill Sunfish – Moody Pond	965	100 %
Sample Matrix IF Spike 6	Redear Sunfish – Moody Pond	965	97 %

Table S11 details the mixture of PFAS used for the OF spike and the stock concentration in F^- equivalents used for spiking. The spiking standards were measured on the CIC to determine the spiking concentration compared to the nominal concentration. Measured concentrations were within <13 % of the nominal spike concentrations (six of seven spikes were within <6 %). Measured concentrations were used for assessing removal and recovery.

Table S11. Details of per- and polyfluoroalkyl substances (PFAS) spiking mixture (PFAC-24PAR) and spiking concentration in fluorine equivalents used for organofluorine recovery assessment.

PFAS Compounds	PFAC-24PAR Conc [ng/mL]	Molecular weight	Number of fluorines	Concentration [ng F/mL]	~3x Dilution [ng F/mL]
PFBA	2000	214	7	1242.99	410.19
PFPeA	2000	264	9	1295.45	427.50
PFHxA	2000	314	11	1331.21	439.30
PFHpA	2000	364	13	1357.14	447.86
PFOA	2000	414	15	1376.81	454.35
PFNA	2000	464	17	1392.24	459.44
PFDA	2000	514	19	1404.67	463.54
PFUnDA	2000	564	21	1414.89	466.91
PFDoDA	2000	614	23	1423.45	469.74
PFTrDA	2000	664	25	1430.72	472.14
PFTeDA	2000	714	27	1436.97	474.20
FOSA	2000	499	17	1294.59	427.21
N-MeFOSAA	2000	571	17	1131.35	373.35
N-EtFOSAA	2000	585	17	1104.27	364.41
L-PFBS	1770	300	9	1008.90	332.94
L-PFPeS	1880	350	11	1122.63	370.47
PFHxS	1824	400	13	1126.32	371.69
L-PFHpS	1900	450	15	1203.33	397.10
PFOS	1851	500	17	1195.75	394.60
L-PFNS	1920	550	19	1260.22	415.87
L-PFDS	1930	600	21	1283.45	423.54
4:2 FTS	1870	328	9	974.91	321.72
6:2 FTS	1900	428	13	1096.50	361.84
8:2 FTS	1920	528	17	1174.55	387.60
Fluorine		19	1		
SUM				30083.32	9927.50

The efficacy of organofluorine (OF) recovery for surface water sample extraction was assessed using 1985 ng F/mL PFAS (993 ng) spiked into MQ water samples (n=2) and surface water matrix samples (n=2). OF recovery for sediment sample extraction was assessed using 496 ng F/mL PFAS (248 ng) spiked into extraction blank samples (n=1) and sediment matrix samples (n=2). OF recovery for biota sample extraction was assessed using a low and high spike of 496 ng F/mL PFAS (248 ng) and 4,964 ng F/mL PFAS (2,482 ng) spiked into MQ Water samples (n=2) and biota matrix samples (n=6). See **Table S12** for OF percent recovery results.

Table S12. Results of percent organofluorine (OF) recovery based on measured spike concentration of the per- and polyfluoroalkyl substances (PFAS) mixture in extraction spikes using Milli-Q (MQ) water and sample matrix spikes.

Sample	Matrix	Measured Spike Concentration [ppb]	% OF Recovery
Surface Water Extraction			
Extraction OF Spike 1	MQ Water	1965	99 %
Extraction OF Spike 2	MQ Water	1965	100 %
Sample Matrix OF Spike 1	Lower Quashnet River Water	1965	94 %
Sample Matrix OF Spike 2	Waquoit Bay Water	1965	84 %
Sediment Extraction			
Extraction OF Spike 1	Blank	468	80 %
Sample Matrix OF Spike 1	Waquoit Bay Sediment	468	94 %
Sample Matrix OF Spike 2	Moody Pond Sediment	468	86 %
Biota Extraction			
Extraction OF Spike 1	MQ Water	483	40 %
Extraction OF Spike 2	MQ Water	4896	100 %
Sample Matrix OF Spike 1	Eel – Santuit River	483	72 %
Sample Matrix OF Spike 2	Quahog – Waquoit into Quashnet	483	79 %
Sample Matrix OF Spike 3	Mummichog – Waquoit Bay	483	48 %
Sample Matrix OF Spike 4	Silverside – Waquoit Bay	4896	80 %
Sample Matrix OF Spike 5	Bluegill Sunfish – Moody Pond	4896	90 %
Sample Matrix OF Spike 6	Redear Sunfish – Moody Pond	4896	100 %

The relative percent difference (RPD) between sample extraction and sample field duplicates was assessed for each sample type. The RPD for surface water samples (n=2) was 6% for both replicate samples measured. The RPD for sediment samples (n=2) was 5% and 18% for both replicate samples measured. The RPD for biota samples (n=6) ranged between 2% and 57% (median of 9%; average of 17 ± 22%).

Sample extract splits were also analyzed by LC-MS/MS for targeted PFAS to directly compare to the EOF extract results. Isotopically labeled internal standards were added to the LC-MS/MS fraction after extraction to avoid recovery correction since the EOF extracts are not recovery-corrected. These EOF extracts were analyzed on the LC-MS/MS in the same manner as targeted PFAS as described in **Section 1.4**. The RPD for ∑PFAS analyzed for targeted analysis (with IS added before offline extraction) and EOF (IS added after offline extraction) ranged between 4-46 % (average of 26 ± 12 %) for surface water samples, between 7-81 % (average of 49 ± 24 %) for sediment samples, and between 1-66 % (average of 24 ± 14 %) for biota samples based on the sum of all PFAS together (∑PFAS).

1.11. Stable Isotope Analysis. Most biotic samples (n=78) were prepared for stable isotope analysis (SIA) using dorsal muscle tissue for fish, eel, and turtle species and soft tissue for shellfish and gastropod species. Tissue samples were pre-homogenized using a handheld Omni homogenizer, subsamples of the homogenates were added to pre-weighed microcentrifuge tubes, the mass of the tube plus the sample were determined and then samples were dried in an oven at 60 °C for 48 hours. The mass of the dried samples was determined and % moisture was calculated (69-90%). Samples were ground into a fine homogenous powder using a stainless-steel spatula. Samples (520 ± 120 μg) were weighed in tin capsules and stable isotope ($\delta^{13}C$ and

$\delta^{15}N$) and elemental composition (%C and %N) analyses were completed using an isotope ratio mass spectrometer (Thermo Flash 2000) coupled to an elemental analyzer (EA-irMS).

Method duplicates (n=8) were assessed, and relative percent differences ranged between 0.3-1.0% for $\delta^{13}C$ values and between 0.2-6.8% for $\delta^{15}N$ values. Trueness was assessed through replicate analyses (every 4 samples) of an L-glutamic acid standard (L-glu JK) ($\delta^{15}N$ = -5.9 ‰ and $\delta^{13}C$ = -13.9 ‰) reference material and averaged -7.1 ± 0.2 ‰ (n=36) and -14.5 ± -0.2 ‰ (n=22) for $\delta^{15}N$ and $\delta^{13}C$ respectively. The scale factor was calculated from the authentic glutamic acid standards U.S. Geological Survey (USGS) 40 ($\delta^{15}N$ = -4.5 ‰ and $\delta^{13}C$ = -26.4 ‰) and USGS 41a ($\delta^{15}N$ = 47.6 ‰ and $\delta^{13}C$ = 36.6 ‰), and a tyrosine standard ($\delta^{15}N$ = 4.7 ‰ and $\delta^{13}C$ = -24.9 ‰). Size effects were calculated from a size series of L-glu JK run every 3 days of analysis.

$\delta^{13}C$ values were normalized for lipid content using the following equation:

$$\delta^{13}C_{Normalized} = \delta^{13}C + \frac{a*C:N+b}{C:N+c} \qquad \textbf{Equation S1}$$

Equation S1 is a generalized model developed by Logan et al., 2008,[47] which is based on the model from McConnaughey & McRoy, 1979 shown in **Equation S2**.[48] This model maintains the nonlinear relationship of the difference in $\delta^{13}C$ between bulk tissue and lipid extracted tissue with the assumed values aggregated into three parameters. The *y*-asymptote, or *D* in **Equation S2**, corresponds to *a* in **Equation S1**. The model estimate $C:N_{lipid\text{-}free}$ is represented by *-b/a* (*x*-intercept), whereas *b/c* (*y*-intercept) is the $\delta^{13}C$ differences corresponding to a C:N value of zero. Sample C:N is used as a proxy for lipid content as C:N values are positively correlated with lipid content in aquatic fauna.[49]

$$\delta^{13}C_{Normalized} = \delta^{13}C + D\left(\theta + \frac{3.90}{1+287/L}\right) \qquad \textbf{Equation S2}$$

$$where\ L = \frac{93}{1 + (0.246 * C:N - 0.775)^{-1}}$$

Parameter estimates were chosen based on Appendix 1 in Logan et al., 2008[47] that best represented the samples in this study. $\delta^{13}C$ values were normalized based on specific parameter values provided including values for American Eel muscle tissue, values for general muscle tissue for all other fish species, and values for general whole-body tissue for invertebrate species.

Results of $\delta^{13}C$ values normalized using **Equation S1** were compared to results using other common lipid normalization equations to assess differences. Results from **Equation S1** were compared to results for other equations provided in Logan et al., 2008,[47] including **Equation S2**, as well as the Post et al., 2007[49] equation that is commonly applied but is a more simplified version. Comparisons of results across equations yielded similar results with <6 % relative percent difference (<1 % difference in most cases) across any individual sample. Therefore, we chose to use **Equation S1** for $\delta^{13}C$ normalization since it is based on the commonly used McConnaughey & McRoy, 1979 model and tissue- and species-specific parameter values were provided. Results for $\delta^{15}N$ values and lipid-normalized $\delta^{13}C$ values are provided in **Table S2**.

Trophic positions (TPs) were calculated for fauna from Waquoit Bay and Quashnet River into Waquoit Bay using quahogs as the baseline organism with a TP of 2.0 (λ) based on filter feeding.[50] TPs were estimated by converting consumer $\delta^{15}N$ values using **Equation S3**.[51] Secondary consumer represents a higher trophic level species, base represents the quahog as the baseline organism, and Δn is the diet-tissue discrimination factor for $\delta^{15}N$ (3.2 ‰ for fishes, crabs, turtles, and 3.6 ‰ for snails).[52,53]

$$TP = \lambda + \frac{\delta^{15}N_{Secondary\ Consumer} - \delta^{15}N_{Base}}{\Delta n} \qquad \textbf{Equation S3}$$

Relative TP (ΔTP) was estimated for species from all other sites as a suitable baseline organism was not available for measurement. The organism with the lowest $\delta^{15}N$ ($\delta^{15}N_{min}$) from each site (Santuit River, Quashnet River, Moody Pond) was used as the baseline reference and the relative difference in TP from this reference sample was determined by subtracting the $\delta^{15}N$ of the secondary consumer from the $\delta^{15}N$ for the reference sample and dividing by the diet-tissue discrimination factor (3.2 ‰) (**Equation S4**). Results for TP and ΔTP are provided in **Table S2**.

$$\Delta TP = \frac{\delta^{15}N_{Secondary\ Consumer} - \delta^{15}N_{min}}{\Delta n} \qquad \textbf{Equation S4}$$

1.12. Statistical Analyses & Bioaccumulation Determination. Statistical analyses were assessed for targeted PFAS with ≥ 70 % (67 %) detection frequency. Samples were grouped by site for water samples and by site, species, and tissue type for biota samples for assessing detection frequency for statistical summaries. Samples within these groupings that had between 70-100% detection frequency were imputed with MDL/sqrt(2) for values that were <MDL (peaks were present but were below the determined detection limit) in order to calculate statistical summaries on data with 100% detection frequency. Only 2% of surface water samples (11 of 587 values), <3% of sediment samples (5 of 188 values), and <2% of biota tissue samples (26 of 1638 values) across analytes were imputed.

Statistical significance was set at $p < 0.05$. If all *p*-value results in a group were more significant, $p < 0.01$ is shown in the tables instead. Normality of data was assessed using the Shapiro-Wilk test and histograms and QQ-plot charts for visualization for water, sediment, and biota tissue concentrations grouped by sample site.

Water concentrations were normally distributed for majority (89%) of PFAS compounds across sample sites therefore parametric statistical tests including one-way analysis of variance (ANOVA) were used to determine statistical differences in PFAS concentrations across sites for water samples. Tukey's honestly significant difference (HSD) test was used to determine which groups of water sample sites were significantly different (**Table S13A**). Sediment concentrations were normally distributed for majority (80%) of PFAS compounds across sample sites therefore parametric statistical tests including one-way ANOVA were used to determine statistical differences in PFAS concentrations across sites for sediment samples. Tukey HSD results could not be determined for sediment samples due to limited sample size by site grouping.

Table S13A. Statistical results for water and sediment concentrations for individual per- and polyfluoroalkyl substances (PFAS) compounds that had >75% detections for calculation. Parametric analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) comparisons were assessed by site grouping. For paired comparisons sites are indicated as MP: Moody Pond, UQR: Upper Quashnet River, LQR: Lower Quashnet River, QWB: Quashnet into Waquoit Bay, WB: Waquoit Bay, SR: Santuit River. NA = could not be assessed.

		Water	Sediment
PFAS	**ANOVA**	**Tukey HSD**	**ANOVA**
ΣPFAS	$p < 0.01$		$p < 0.05$
PFPeA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR	NA
PFHxA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p = 0.27$
PFHpA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p = 0.26$
PFOA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB	$p = 0.48$
PFNA	$p < 0.01$	MP:UQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR	$p < 0.01$
PFDA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p < 0.05$
PFUnDA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB	$p = 0.56$
PFDoDA	NA		$p < 0.01$
PFTriDA	$p = 0.16$		$p = 0.76$
PFBS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR, WB:SR	NA
PFPeS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, UQR:LQR, UQR:QWB, UQR:WB, LQR:QWB, LQR:WB	NA
Br-PFHxS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, UQR:LQR, UQR:QWB, UQR:WB, LQR:QWB, LQR:WB	$p = 0.15$
L-PFHxS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p = 0.28$
PFHxS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p = 0.31$
PFHpS	$p < 0.01$	MP:LQR, MP:QWB, MP:WB, UQR:LQR, UQR:QWB, UQR:WB, LQR:QWB, LQR:WB, QWB:WB	NA
Br-PFOS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR	$p = 0.11$
L-PFOS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR	$p < 0.05$
PFOS	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR, UQR:LQR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR, QWB:WB, QWB:SR	$p < 0.05$
FBSA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, MP:SR	NA
FPeSA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB	NA
FHxSA	$p < 0.01$	MP:UQR, MP:LQR, MP:QWB, MP:WB, UQR:LQR, UQR:QWB, UQR:WB, LQR:QWB, LQR:WB	$p = 0.49$
FOSA	$p < 0.01$	MP:LQR, MP:QWB, MP:WB, UQR:LQR, UQR:QWB, UQR:WB	$p = 0.08$
8:2 FTSA	$p < 0.01$	MP:UQR, MP:LQR, UQR:LQR, UQR:QWB, UQR:WB, LQR:QWB, LQR:WB	$p = 0.69$
5:3 FTCA	NA		$p = 0.27$
7:3 FTCA	NA		$p = 0.25$

Biota tissue concentrations were not normally distributed for majority (60 %) of PFAS compounds therefore nonparametric statistical tests including Kruskal-Wallis were used to determine statistical differences in PFAS concentrations and compositions across sites, species, and tissue types. The Dunn-Bonferroni post hoc test was used to determine which groups of biota sample sites or sample species were significantly different for all tissues together and for each tissue type (**Tables S13B-C**).

Table S13B. Statistical results for biota concentrations for individual per- and polyfluoroalkyl substances (PFAS) compounds with at least 2 values for comparison. Non-parametric Kruskal-Wallis and post hoc Dunn test were used to assess samples grouped by site across all tissue types and grouped by site and tissue type. For paired comparisons sites are indicated as MP: Moody Pond, UQR: Upper Quashnet River, LQR: Lower Quashnet River, QWB: Quashnet into Waquoit Bay, WB: Waquoit Bay, SR: Santuit River. NA = could not be assessed.

	All Tissue Samples		Whole Body		Muscle	
PFAS	**Kruskal-Wallis**	**Post hoc Dunn test**	**Kruskal-Wallis**	**Post hoc Dunn test**	**Kruskal-Wallis**	**Post hoc Dunn test**
ΣPFAS	$p < 0.05$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p < 0.01$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB	$p < 0.01$	MP:WB, MP:SR
PFPeA	$p = 0.87$		$p = 0.87$		NA	
PFHxA	$p < 0.05$		NA		$p = 0.08$	
PFHpA	$p = 0.37$		$p = 0.40$		$p = 0.12$	
PFOA	$p = 0.38$		$p = 0.32$		$p = 0.14$	
PFNA	$p < 0.05$	MP:UQR, UQR:WB, UQR:SR, LQR:WB	$p < 0.05$	UQR:WB, UQR:SR, LQR:WB	$p < 0.05$	
PFDA	$p < 0.01$	MP:QWB, MP:WB, MP:SR, UQR:SR	$p < 0.01$	MP:QWB, MP:WB, MP:SR	$p < 0.01$	MP:WB, MP:SR
PFUnDA	$p < 0.01$	MP:WB, MP:SR	$p < 0.01$	MP:WB	$p < 0.01$	MP:WB
PFDoDA	$p < 0.01$	MP:WB, UQR:WB	$p < 0.05$	MP:QWB, MP:WB; UQR:WB	$p < 0.01$	MP:WB
PFTriDA	$p < 0.05$	MP:WB, UQR:WB, LQR:WB	$p < 0.05$	MP:QWB, MP:WB	$p < 0.05$	
PFTeDA	$p < 0.05$	UQR:WB, LQR:WB	$p < 0.05$	MP:WB	$p < 0.05$	
Br-PFHxS	$p = 0.39$		$p = 0.29$		$p = 0.96$	
L-PFHxS	$p < 0.01$	MP:WB, UQR:WB	$p < 0.05$	MP:QWB, MP:WB; UQR:WB	$p = 0.55$	
PFHxS	$p < 0.01$	MP:WB, UQR:WB	$p < 0.05$	MP:WB; UQR:WB	$p = 0.64$	
PFHpS	$p = 0.17$		$p < 0.05$		NA	
Br-PFOS	$p < 0.01$	MP:QWB, MP:WB, UQR:WB	$p < 0.05$	MP:WB	$p < 0.05$	
L-PFOS	$p < 0.05$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p < 0.01$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR	$p < 0.01$	MP:WB, MP:SR
PFOS	$p < 0.05$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR, LQR:QWB, LQR:WB, LQR:SR	$p < 0.01$	MP:QWB, MP:WB, MP:SR, UQR:QWB, UQR:WB, UQR:SR	$p < 0.05$	MP:WB, MP:SR, UQR:SR, LQR:SR
PFNS	$p = 0.77$		$p < 0.05$	MP:LQR	NA	
PFDS	$p < 0.05$	MP:LQR	$p < 0.01$	MP:LQR	$p < 0.05$	
FBSA	$p < 0.01$	MP:QWB, MP:WB	$p < 0.01$	MP:QWB, MP:WB	$p < 0.01$	MP:WB
FPeSA	$p < 0.05$	MP:LQR, MP:QWB, MP:WB	$p < 0.01$	MP:QWB, MP:WB	$p < 0.05$	MP:LQR, MP:WB
FHxSA	$p < 0.05$	MP:QWB, MP:WB, UQR:QWB, LQR:QWB	$p < 0.01$	MP:QWB, MP:WB, UQR:QWB	$p < 0.05$	MP:WB
FOSA	$p < 0.05$	MP:QWB, MP:WB, UQR:QWB, UQR:WB, LQR:QWB	$p < 0.01$	MP:QWB, UQR:QWB	$p < 0.05$	MP:WB
6:2 FTSA	$p = 0.43$		$p = 1.0$		NA	
8:2 FTSA	$p < 0.01$	MP:UQR	$p < 0.01$	MP:UQR	$p < 0.05$	MP:UQR
5:3 FTCA	$p = 0.82$		$p = 0.15$		$p = 0.44$	
7:3 FTCA	$p < 0.05$	UQR:WB	$p < 0.05$	UQR:QWB	$p < 0.05$	

Table S13C. Statistical results for biota concentrations for individual per- and polyfluoroalkyl substances (PFAS) compounds with at least 2 values for comparison. Non-parametric Kruskal-Wallis and post hoc Dunn test were used to assess samples grouped by species across all sites for whole-body and muscle tissue samples. Significant paired species comparisons are provided. NA = could not be assessed.

	Whole Body		Muscle	
PFAS	**Kruskal-Wallis**	**Post hoc Dunn test**	**Kruskal-Wallis**	**Post hoc Dunn test**
PFPeA	p = 0.84		NA	
PFHpA	p < 0.05		NA	
PFOA	p < 0.05	Blue Crab:Quahog	p < 0.05	American Eel:Oyster Toadfish
PFNA	p < 0.05		p < 0.05	American Eel:Redear Sunfish
PFDA	p < 0.05		p < 0.05	Yellow Perch:Oyster Toad Fish; Yellow Perch:American Eel
PFUnDA	p < 0.05	Redear Sunfish:Atlantic Silverside	p < 0.05	Bluefish:Bluegill Sunfish; Bluefish:Yellow Perch
PFDoDA	p < 0.05		p < 0.05	Yellow Perch:Oyster Toadfish; Bluefish:Yellow Perch
PFTriDA	p < 0.05	American Eel:Quahog; Redear Sunfish:Quahog	p < 0.05	Bluefish:American Eel
PFTeDA	p < 0.05		p < 0.05	American Eel:Oyster Toadfish
Br-PFHxS	p < 0.05		p < 0.05	Bluegill Sunfish:Common Musk Turtle
L-PFHxS	p < 0.05		p < 0.05	Bluegill Sunfish:Common Musk Turtle; Redear Sunfish:Common Musk Turtle
PFHxS	p < 0.05		p < 0.05	Bluegill Sunfish:Common Musk Turtle; Redear Sunfish:Common Musk Turtle
PFHpS	p = 0.35		p = 0.29	
Br-PFOS	p < 0.05		p = 0.21	
L-PFOS	p < 0.05		p < 0.05	Striped Bass:Yellow Perch; Bluefish:Yellow Perch
PFOS	p < 0.05		p < 0.05	Bluefish:Yellow Perch
PFNS	p = 0.21		p = 0.14	
PFDS	p = 0.11		p = 0.15	
FBSA	p < 0.05	Redear Sunfish:Blue Crab	p < 0.05	Striped Bass:Yellow Perch
FPeSA	p < 0.05		p < 0.05	American Eel:Yellow Perch
FHxSA	p < 0.05	Redear Sunfish:Quahog	p < 0.05	American Eel:Yellow Perch; Oyster Toadfish:Yellow Perch
FOSA	p < 0.05	White Sucker:Blue Crab	p < 0.05	Bluefish:Yellow Perch
6:2 FTSA	p = 0.09		NA	
8:2 FTSA	p = 0.12		p < 0.05	
5:3 FTCA	p = 0.45		p = 0.29	
7:3 FTCA	p = 0.08		p < 0.05	

Field-measured bioaccumulation factors were calculated as the concentration (μg kg^{-1}) in wet-weight biota tissue divided by the average surface water concentration (μg L^{-1}) for each site grouping only if 100% of samples in that grouping had a detectable value. Average surface water concentrations for each site were used to best represent the overall surface water concentration across time and space associated with the biota collection. There was also a minimal difference in PFAS concentrations (average relative percent difference < 12 %) across surface water field replicates from each site.

$$BAF = \frac{Conc_{Tissue}}{Conc_{Water}} \qquad \textbf{Equation S5}$$

Field-measured biota–sediment accumulation factors (BSAF) were calculated as the concentration (μg kg^{-1}) in wet-weight benthic biota tissue divided by the average concentration (μg kg^{-1}) in dry-weight surface (0-5 cm depth) sediment normalized to the fraction of organic carbon (f_{oc}) by dividing the sediment concentration by the f_{oc} determined by the loss on ignition method. BSAF are expressed on an organic carbon basis to account for the high organic carbon content in the Upper Quashnet River sediment sample compared to other sediment samples (**Table S1**).

$$BSAF = \frac{Conc_{Tissue}}{Conc_{Sediment}/f_{OC}} \qquad \textbf{Equation S6}$$

Principal component analysis (PCA) was used to group PFAS profiles in clusters by site and species to reduce the dimensionality of observations. Hierarchical clustering was conducted as a check on the major groupings of PFAS identified using PCA. PFAS with <70% detection frequency across all surface water samples and <60% detection frequency across all biota samples were dropped from the PCA. Compounds above this detection frequency were replaced with MDL/sqrt(2) if needed. Other replacement methods including using 0.001 times the lowest reported molar composition and zero were assessed and yielded similar results (see **Figure S13**). PFAS concentrations were converted to molarity and the molar fractional composition for each PFAS was determined. Molar fractions were transformed using the centered log ratio and data was scaled using the StandardScaler function to standardize the data to have a mean of 0 and variance of 1 to fit the PCA.

2. Results

2.1. Detection, Concentration, and Composition of PFAS

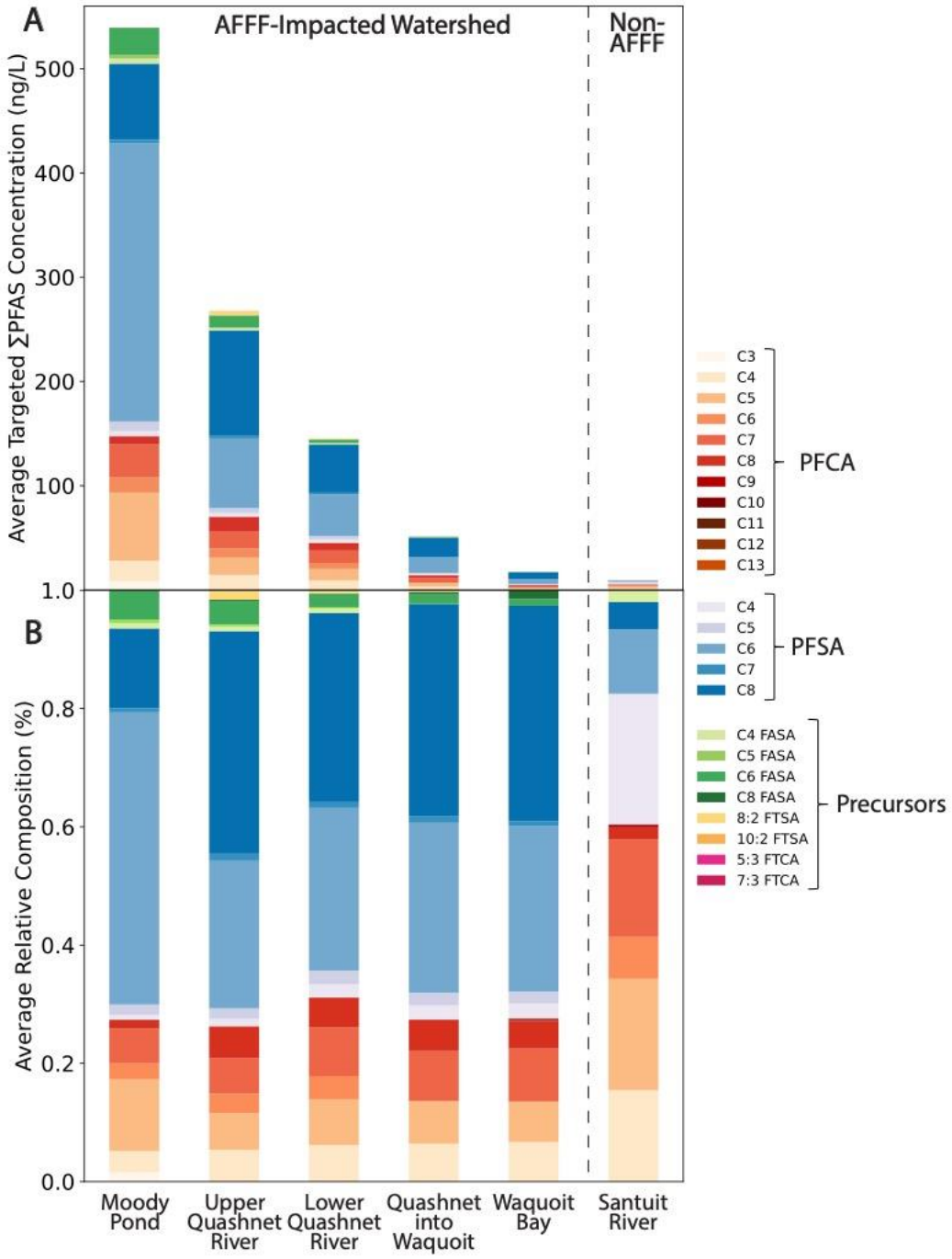

Figure S3. (A) Average concentrations (ng L^{-1}) for the sum of targeted per- and polyfluoroalkyl substances (∑PFAS) in surface water samples, grouped by location (n=2-8 samples), expressed as absolute PFAS composition. (B) Average composition of targeted PFAS in water samples, grouped by location. PFAS composition profiles include the perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursor compounds, defined by perfluorinated carbon chain length, detected in at least 70% of samples grouped by site, going from upstream closest to the aqueous film-forming foam (AFFF) source to downstream, including the background watershed site.

Table S14. Range in concentrations for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursors in surface water (ng/L), sediment (ng/g), and biological samples (ng/g) from Moody Pond, Upper and Lower Quashnet River, Quashnet into Waquoit Bay, Waquoit Bay, and Santuit River. Sediment sample ranges are based on all depth profiles (0-5 cm, 5-10 cm, and 10-15 cm). Species ranges are based on either muscle tissue, whole-body, whole-body composites, or a combination of both tissue types. Compounds with no detections in any samples are not included in the table. Some PFCA, PFSA, and perfluoroalkyl sulfonamides (FASA) were integrated as the sum of isomers if both branched and linear isomers were present. Concentrations for linear and branched isomers were calculated individually and as the sum for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA). Perfluoropentane sulfonamide (FPeSA) (sum of isomer) concentrations are based on separate measurements from the ultrahigh-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) analysis. FPeSA was measured in surface water and biota samples but not in sediment samples. NR = not reported and NM = not measured.

See attached excel file: Supplementary Information Tables: Table S14

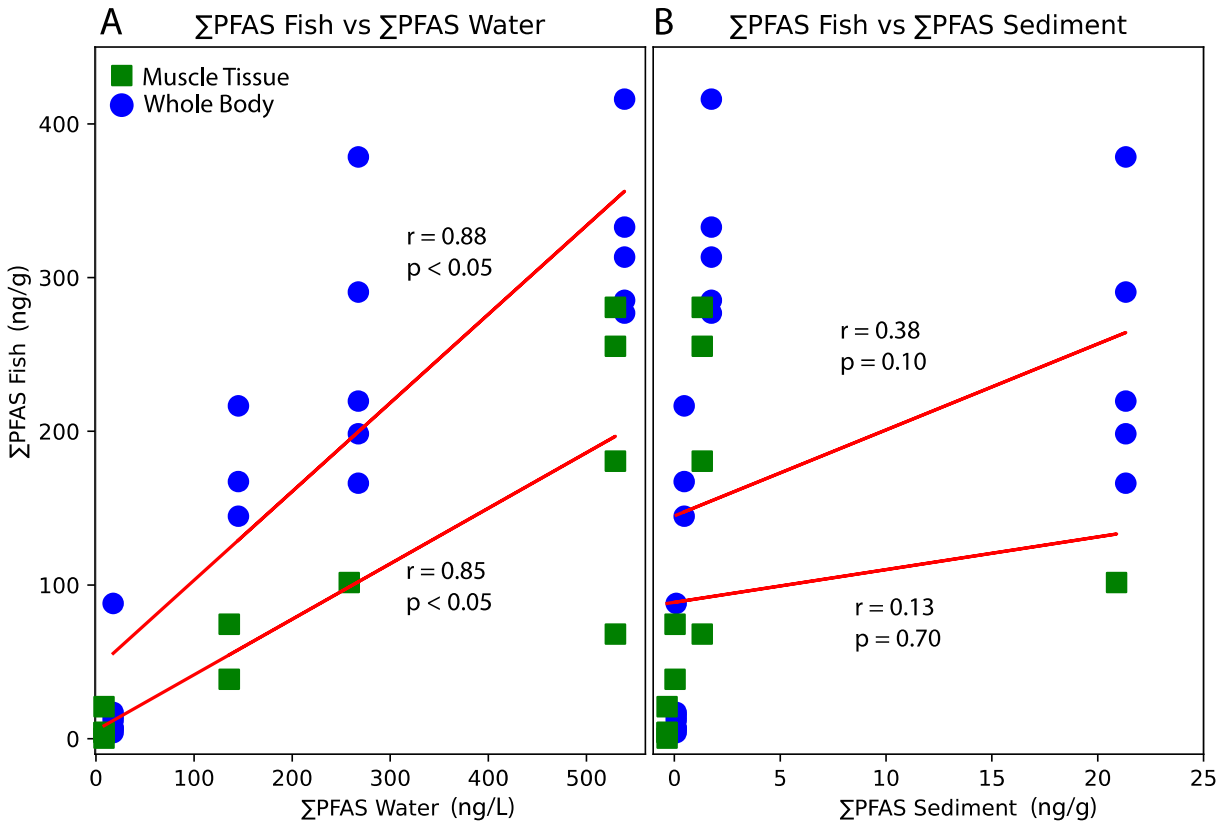

Figure S4. Pearson correlations between water (ng/L) and sediment (ng/g) sum of per- and polyfluoroalkyl substances (∑PFAS) concentrations with fish whole body and muscle tissue concentrations (ng/g). (A) Correlation between fish tissues and water sample concentrations. (B) Correlation between fish tissues and sediment sample concentrations.

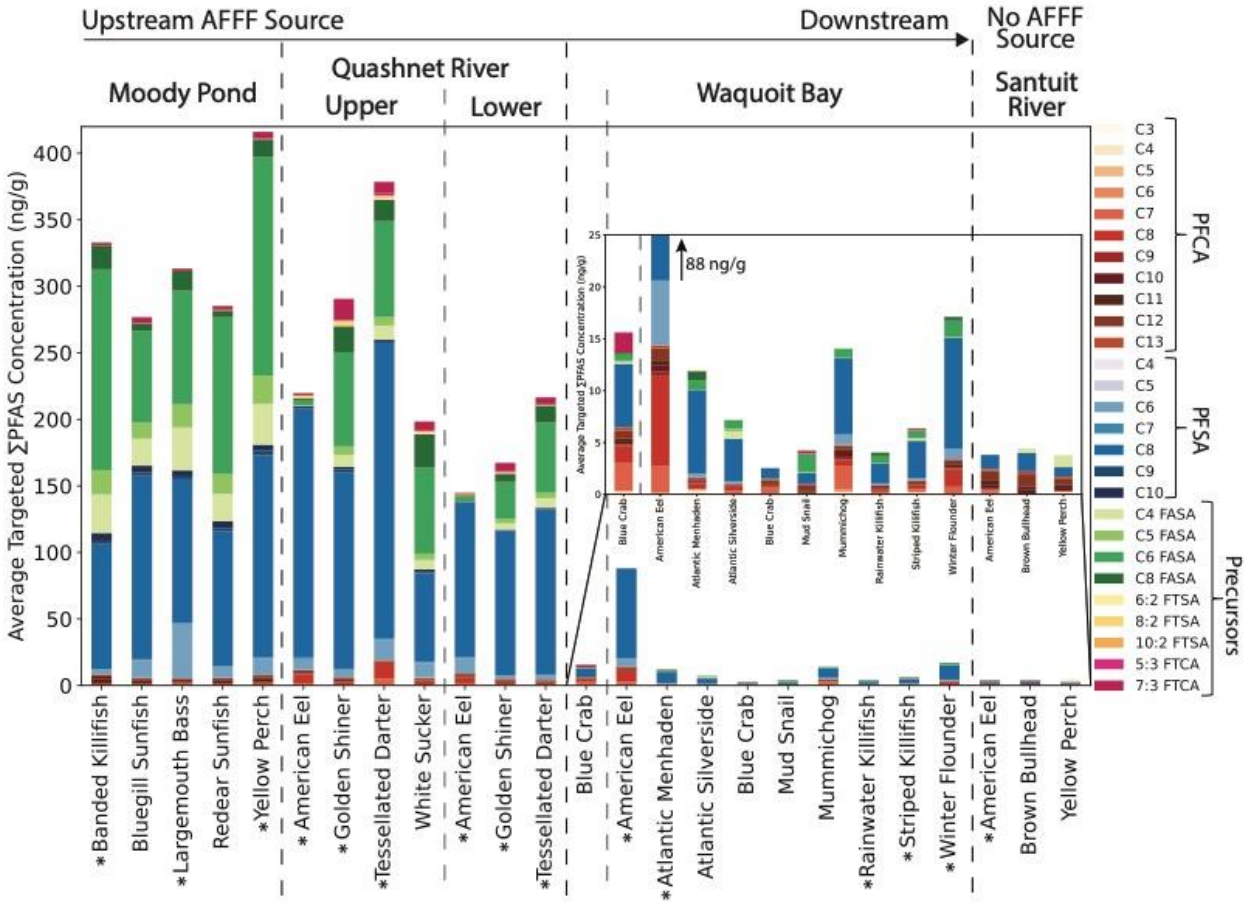

Figure S5. Average concentrations (ng g^{-1} wet weight) for the sum of targeted per- and polyfluoroalkyl substances (∑PFAS) in whole-body fish, eel, and invertebrate samples, grouped by species and location (n=1-5 samples), expressed as absolute PFAS composition. Whole body samples are either measurements of a single organism or composites of multiple organisms. Species with an asterisk are based on n=1 single or composite sample measurements. PFAS composition profiles include the perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursor compounds, defined by perfluorinated carbon chain length, detected in at least 70% of samples grouped by species and site, going from upstream closest to the aqueous film-forming foam (AFFF) source to downstream. The inset plot shows the samples from Waquoit Bay and Santuit River that have lower concentrations for visualization. The grey dashed lined indicates that Blue Crab were collected from the Quashnet River inflow into Waquoit Bay.

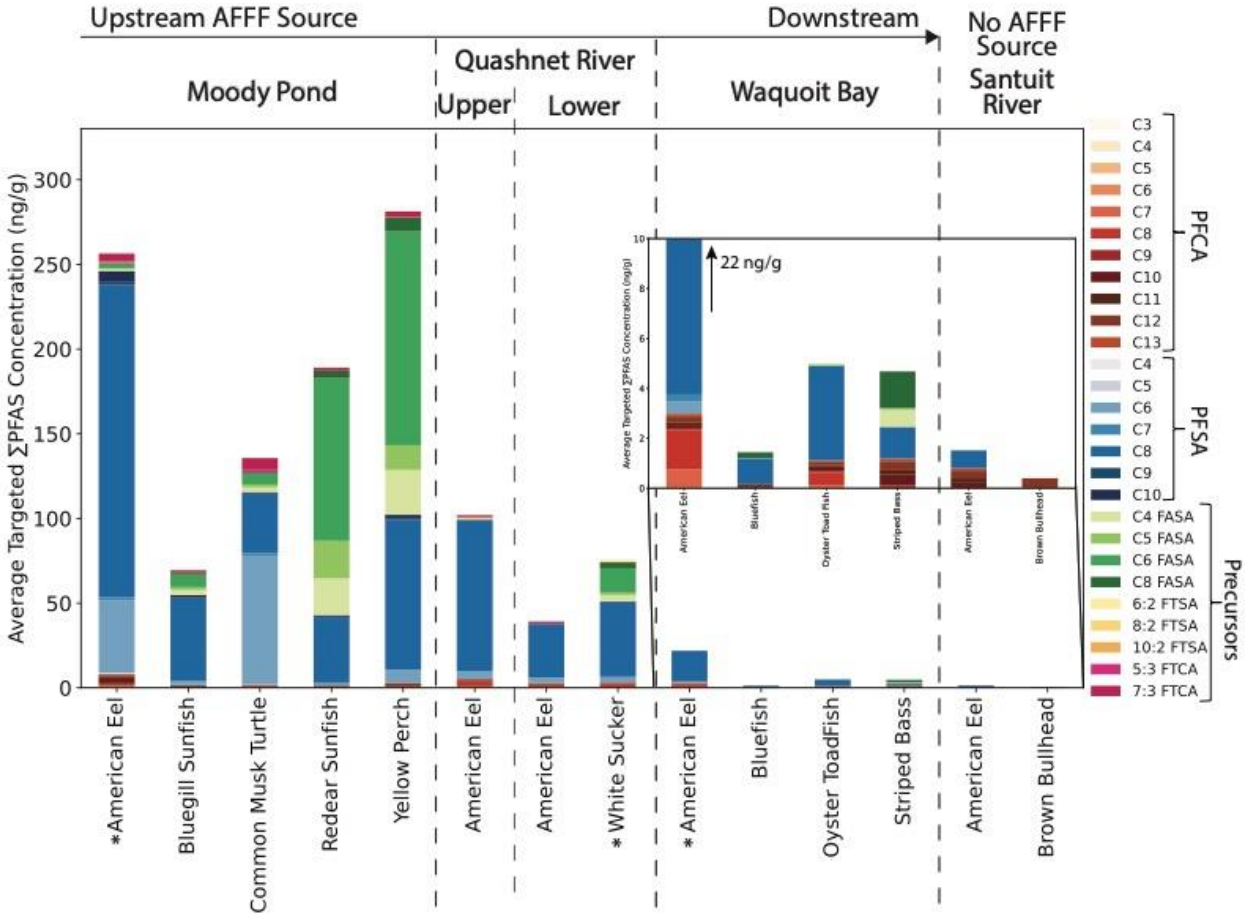

Figure S6. Average concentrations (ng g^{-1} wet weight) for the sum of targeted per- and polyfluoroalkyl substances (∑PFAS) in muscle tissue of fish, eel, and turtle samples, grouped by species and location (n=1-5 samples), expressed as absolute PFAS composition. Species with an asterisk are based on n=1 sample measurements. PFAS composition profiles include the perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursor compounds, defined by perfluorinated carbon chain length, detected in at least 70% of samples grouped by species and site, going from upstream closest to the aqueous film-forming foam (AFFF) source to downstream. The inset plot shows the samples from Waquoit Bay and Santuit River that have lower concentrations for visualization.

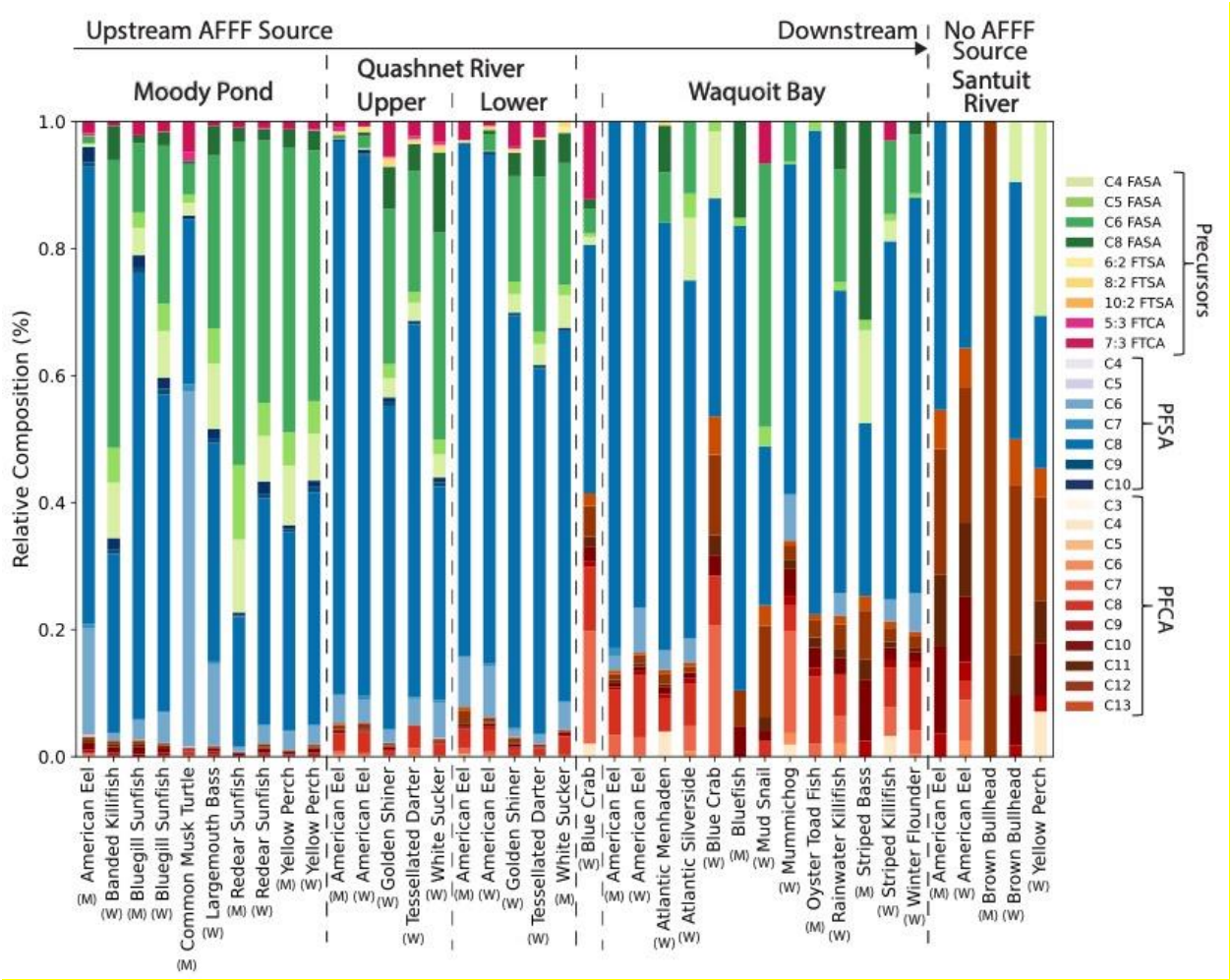

Figure S7. Average relative composition (%) of targeted per- and polyfluoroalkyl substances (PFAS) with ≥70 % detection frequency in individual aquatic biota grouped by site and tissue type going downstream of aqueous film-forming foam (AFFF) source zones and background site. Tissue sample types are indicated with a letter below the species name with both muscle (M) and whole-body (W) samples shown for certain fish species, eel, and shellfish. Perfluorooctane sulfonate (PFOS; C8 PFSA) is shown as the blue bars that account for the greatest fraction of the PFAS composition across samples.

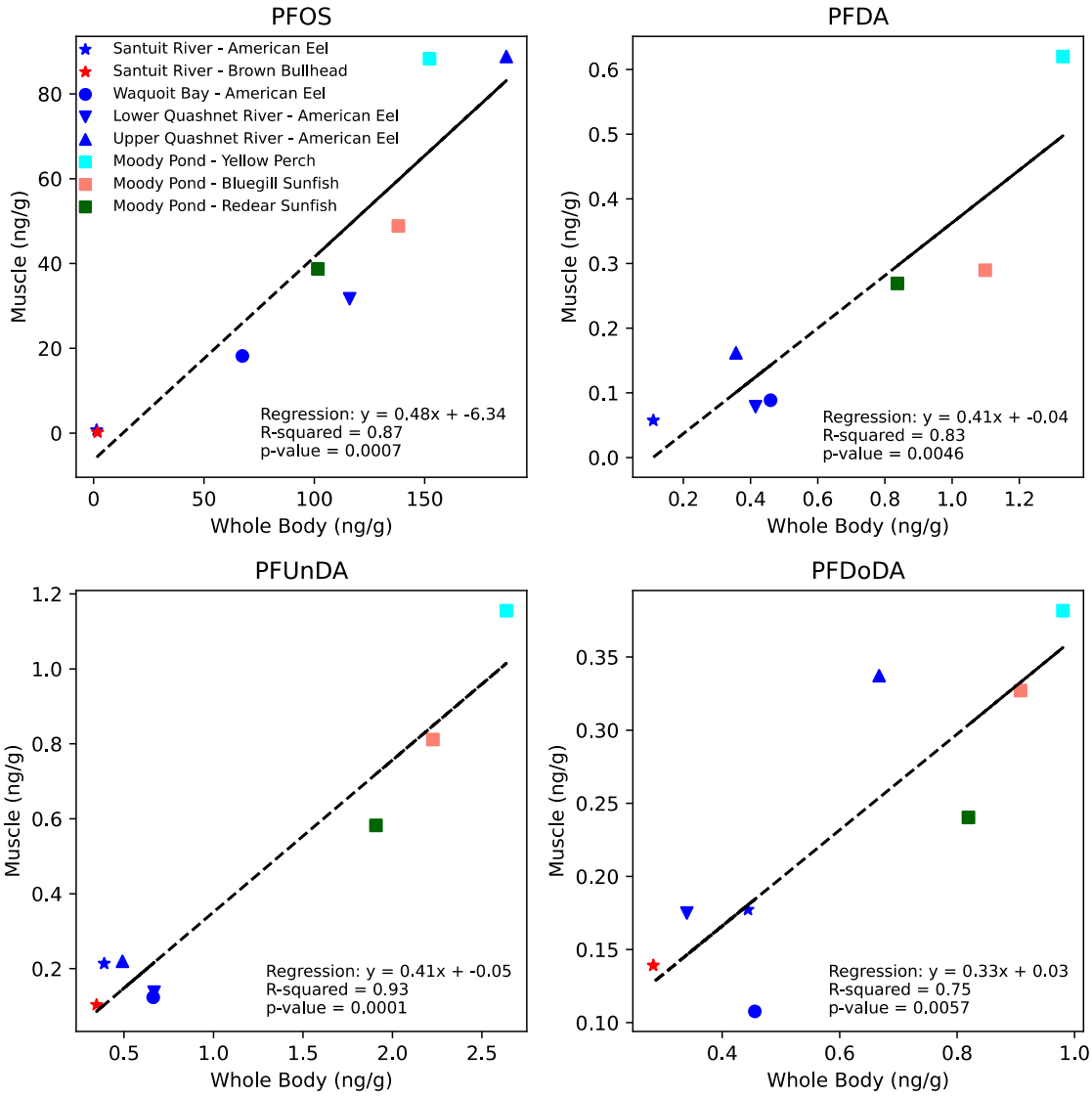

Figure S8. Whole-body to muscle fillet correlation for fish species and eel samples from sites with measurements for both muscle tissue and whole-body for perfluorooctane sulfonate (PFOS) and C9-C11 perfluorocarboxylates (PFCA) (perfluorodecanoate: PFDA; perfluoroundecanoate: PFUnDA; and perfluorododecanoate: PFDoDA). Markers represent average concentrations per species per site for each tissue correlation.

Table S15. Detection frequencies of targeted per- and polyfluoroalkyl substances (PFAS) > method detection limit (MDL) measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in surface water, sediment and biological samples from Moody Pond, Upper and Lower Quashnet River, Quashnet into Waquoit Bay, Waquoit Bay, and Santuit River. Species detection frequencies are based on either muscle tissue, whole body, or a combination. Sediment detection frequencies are based on the surface layer (0-5 cm) only. Sample number indicates the number of samples measured with an asterisk (*) indicating composites of multiple organisms. Some perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and perfluoroalkyl sulfonamides (FASA) were integrated as the sum of isomers if both branched and linear isomers were present. Detection frequencies for perfluoropentane sulfonamide (FPeSA) are based on separate measurements on the ultrahigh-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) instrument. Detection frequencies for linear and branched isomers were calculated individually and as the sum for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA). NR = not reported for 6:2 fluorotelomer sulfonate (FTSA) due to blank contamination.

See attached excel file: Supplementary Information Tables: Table S15

Table S16. Average (± standard deviation) concentrations for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursors in surface water (ng/L), sediment (ng/g), and biological samples (ng/g) from Moody Pond, Upper and Lower Quashnet River, Quashnet into Waquoit Bay, Waquoit Bay, and Santuit River. Averages are based on samples with 100 % detection frequency and if no standard deviation is provided that means only one sample was measured. Biological samples are separated into tissue specific (muscle vs whole body) measurements and averages were only determined for the surface layer (0-5 cm) sediment samples. Some PFCA, PFSA, and perfluoroalkyl sulfonamides (FASA) were integrated as the sum of isomers if both branched and linear isomers were present. Concentrations for linear and branched isomers were calculated individually and as the sum for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonamide (FHxSA), and perfluorooctane sulfonamide (FOSA). Perfluoropentane sulfonamide (FPeSA) (sum of isomer) concentrations are based on separate measurements from the ultrahigh-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) instrument. FPeSA was measured in surface water and biota samples but not in sediment samples. NR = not reported.

See attached excel file: Supplementary Information Tables: Table S16

2.2. Extractable Organofluorine & Suspect PFAS

Table S17. Average extractable organofluorine (EOF) concentrations measured in surface water (ng F/L), sediment (ng F/g), and biological (ng F/g) samples across all sites. Relative error in EOF concentrations are based on replicate injections for each sample. Uncorrected sum of targeted per- and polyfluoroalkyl substances (∑PFAS) concentrations quantified by targeted analysis from the EOF extract split is shown for comparison to EOF. Average ± standard deviation ∑PFAS concentrations given if n > 1 measurements per sample. Range in percent EOF explained by targeted ∑PFAS are shown for samples with detectable EOF and PFAS. MDL = method detection limit.

See attached excel file: Supplementary Information Tables: Table S17

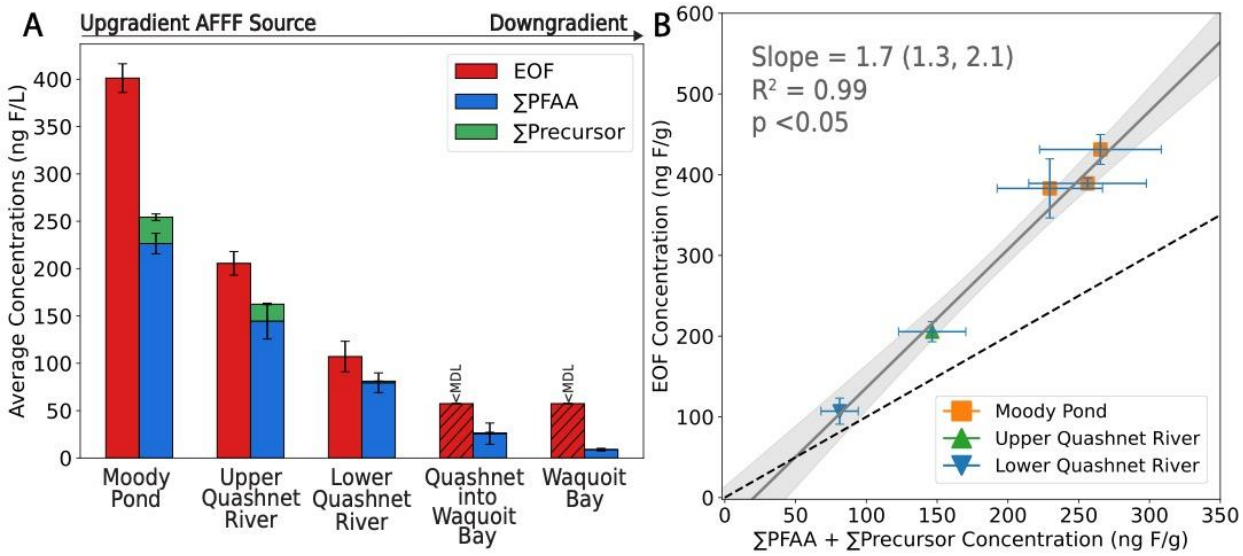

Figure S9. Organofluorine mass budget in surface water samples. Panel (A) compares average extractable organofluorine concentrations (EOF, ng F L^{-1}) in surface water samples grouped by site compared to summed concentrations (ng F L^{-1}) of targeted per- and polyfluoroalkyl substances (PFAS) grouped by perfluoroalkyl acids (∑PFAA) and precursors (∑Precursor). Hatched bars indicate >70% of samples were below the method detection limit (<MDL). Error bars represent the standard deviation across samples grouped by site. Panel (B) compares ∑PFAA + ∑Precursor to EOF concentrations in water samples. Each marker represents an individual sample by location. EOF error bars represent the standard deviation of replicate measurements, and targeted PFAS error bars represent the average weighted error based on relative percent difference between sample method replicates. Weighted least squares linear regression (gray solid line) with 95% confidence intervals (shaded gray area) for water samples are compared to the 1:1 line (black dash).

Table S18. Suspect per- and polyfluoroalkyl substances (PFAS) identified in surface water, sediment, and biological tissue (muscle and whole body) samples. Detection frequencies are indicated based on the number of samples analyzed (n=13 surface water samples, n=9 surface sediment samples, n=83 biota samples) with a peak area >10,000 after extraction blank correction. If two retention times and mass errors are given, the first value represents results for water analysis and the second value represents results for biotic tissue analysis or sediment analysis for perfluoropropance sulfonate (PFPrS) only. KMD is the Kendrick Mass Defect and tandem mass spectrometry (MS/MS; MS2) fragments are provided if detected. Confidence levels (CL) are based on the Schymanski and Charbonnet scales.[54,55]

See attached excel file: Supplementary Information Tables: Table S18

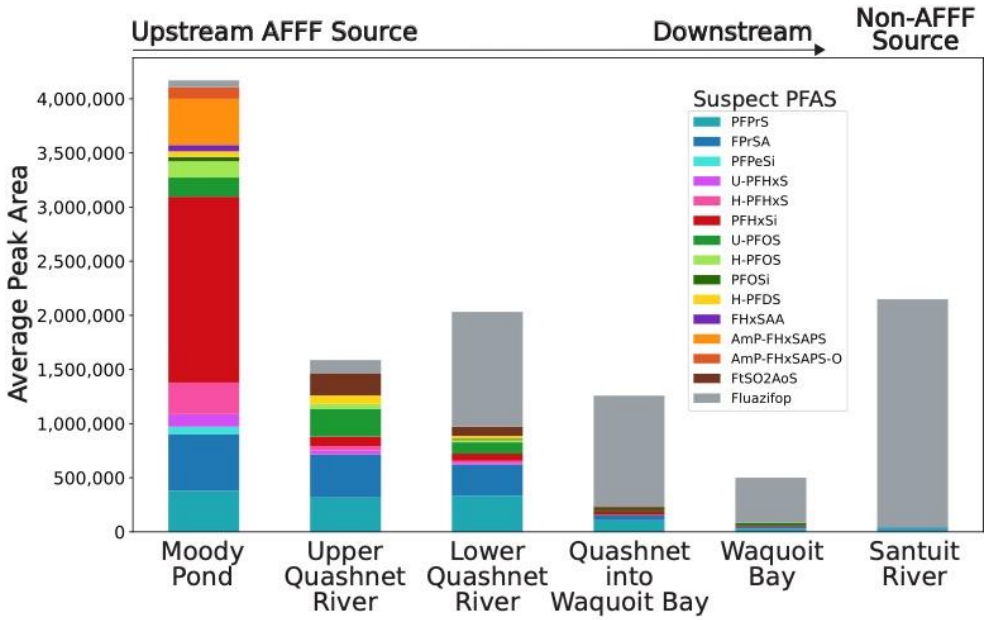

Figure S10. Stacked bar chart of average peak area [counts] of suspect per- and polyfluoroalkyl substances (PFAS) identified by suspect screening analysis in surface water samples grouped by site. See Table S18 for suspect PFAS names, chemical formulas, and structures.

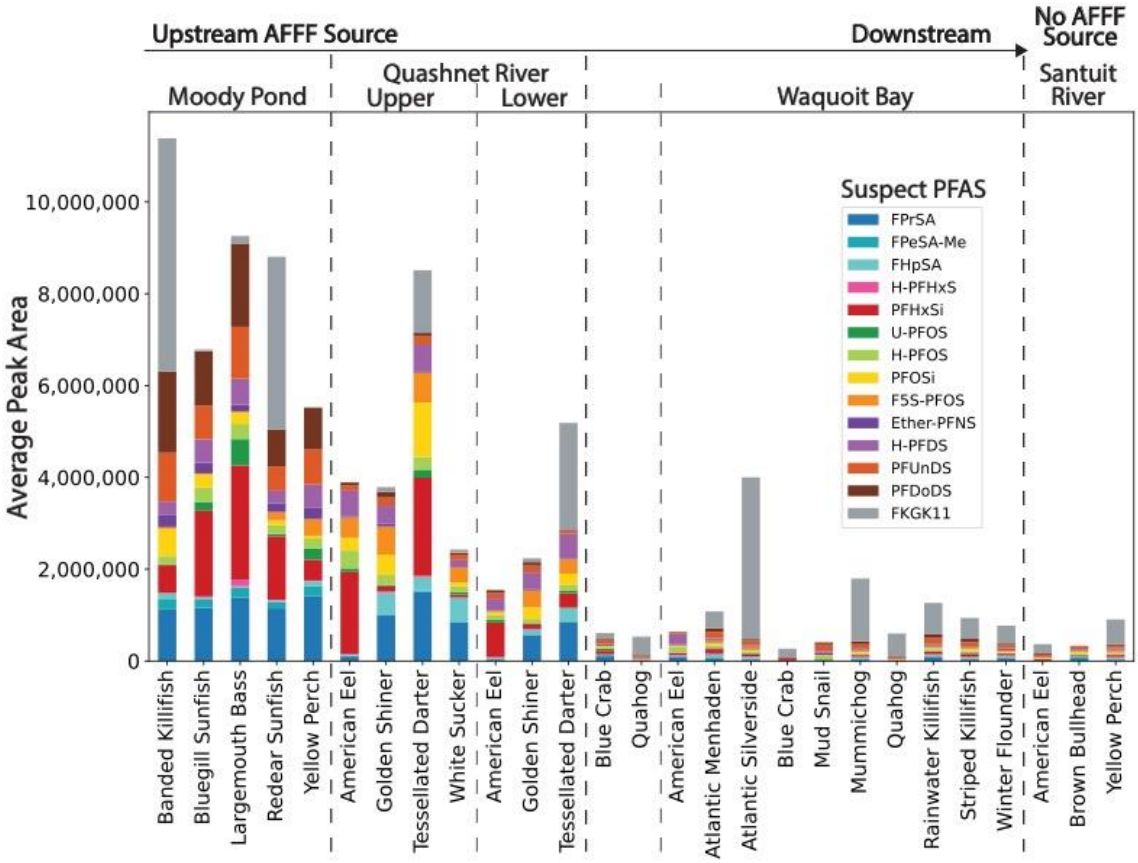

Figure S11. Stacked bar chart of average peak area [counts] of suspect per- and polyfluoroalkyl substances (PFAS) identified by suspect screening analysis in whole-body fish and invertebrate samples grouped by site. See Table S18 for suspect PFAS names, chemical formulas, and structures.

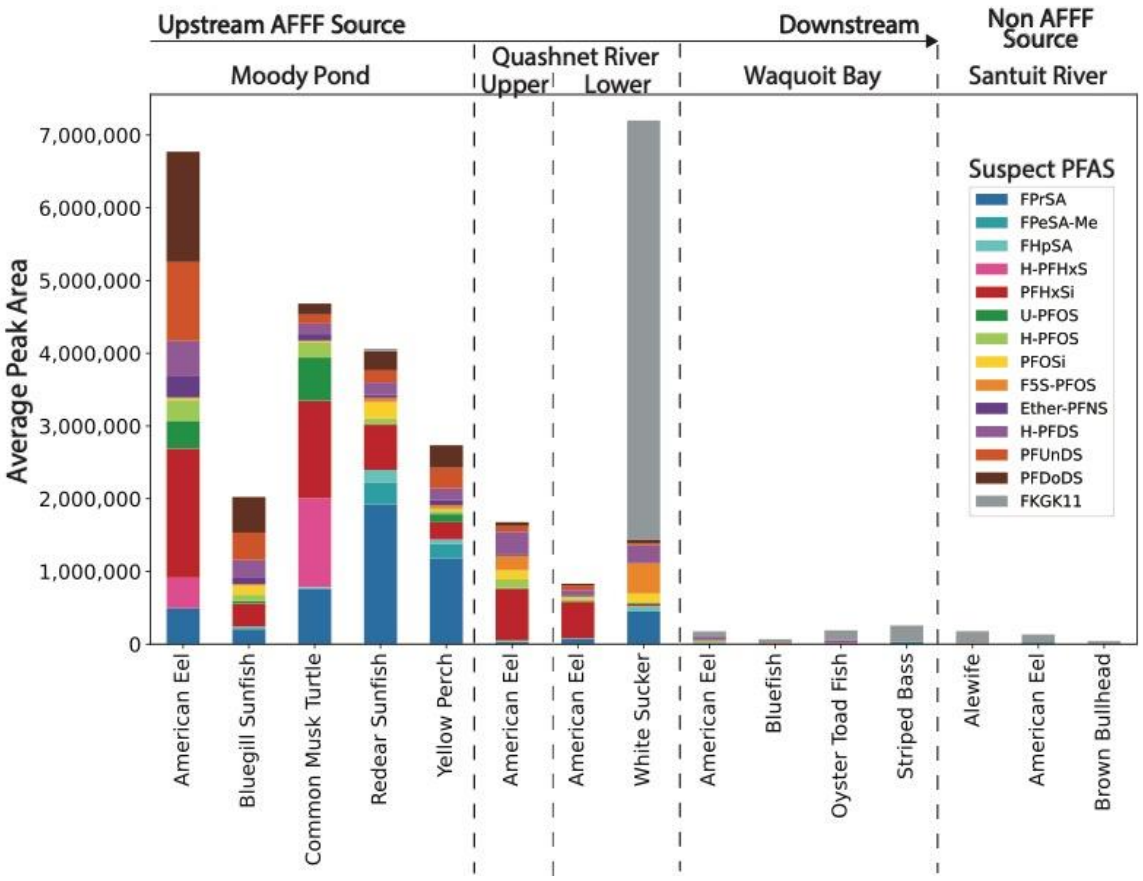

Figure S12. Stacked bar chart of average peak area [counts] of suspect per- and polyfluoroalkyl substances (PFAS) identified by suspect screening analysis in muscle tissue of fish and turtle samples grouped by sample site. See Table S18 for suspect PFAS names, chemical formulas, and structures.

2.3. Principal Component Analysis.

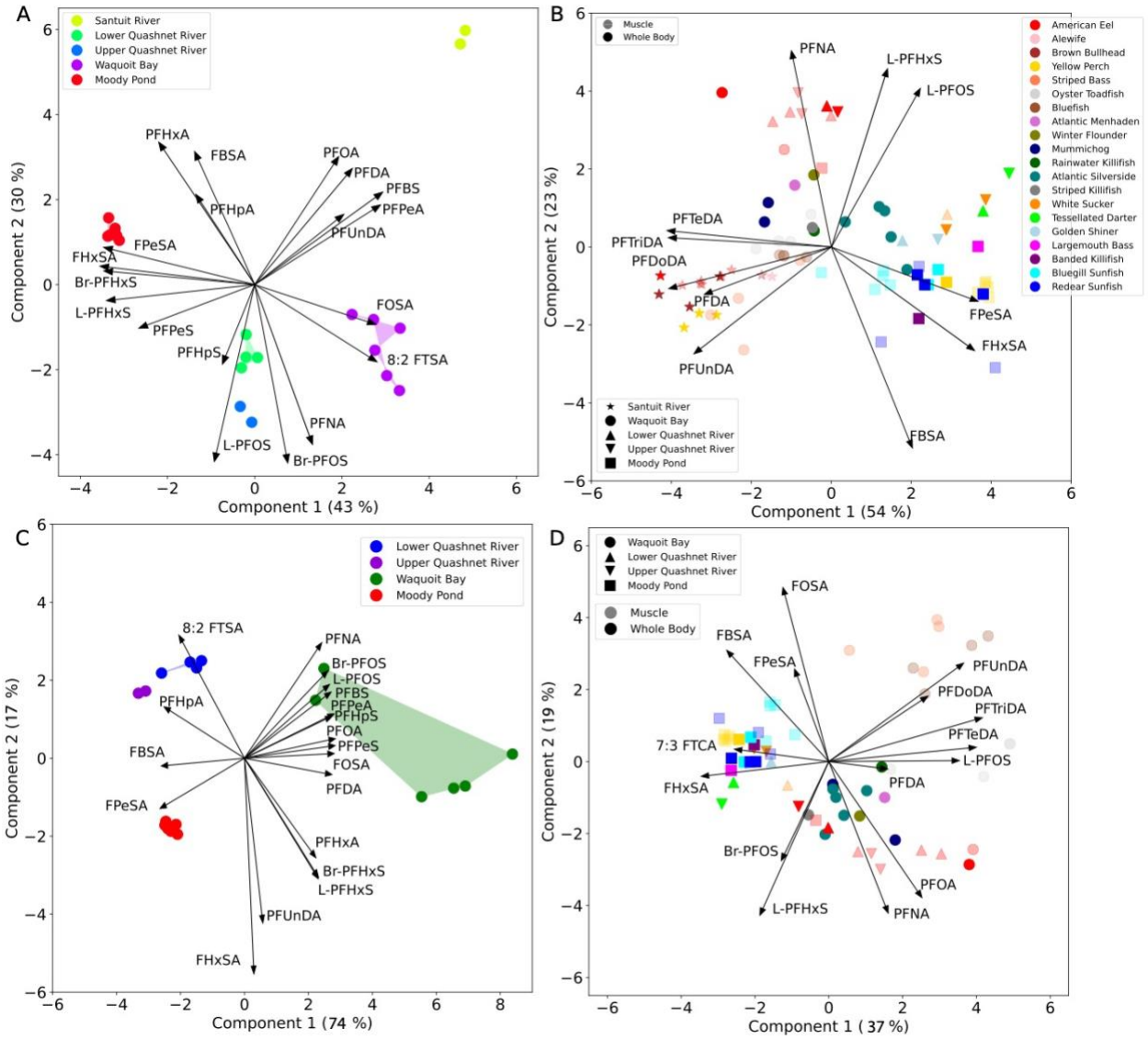

Figure S13. Results from principal component analysis (PCA) using method detection limit (MDL)/√2 imputation method for targeted per- and polyfluoroalkyl substances (PFAS) in surface waters (A) and fish species (B) from locations (Moody Pond, Upper and Lower Quashnet River, and Waquoit Bay) downgradient of AFFF-contamination source zones and from the background site (Santuit River). PFAS compounds for each component are shown as vectors with magnitudes scaled by a factor of 10. (A) First and second components of surface water data points plotted against each other and colored by site. Shaded areas represent the minimum convex hull that encircles all data within the group. Enrichment of C4 PFSA shown as perfluorobutane sulfaonte (PFBS) vector positively enriched in both components towards Santuit River. (B) First and second components of fish sample data points plotted against each other and colored by species. Marker type denotes site of species collection and lighter shaded markers refer to muscle tissue samples whereas darker shaded markers refer to whole-body samples. Results from PCA using 0.001 times the lowest reported molar composition imputation method (same results if using zero) are shown for targeted PFAS in surface waters (C) and fish species (D) from locations (Moody Pond, Upper and Lower Quashnet River, and Waquoit Bay) downgradient of aqueous film-forming foam (AFFF)-contamination source zones.

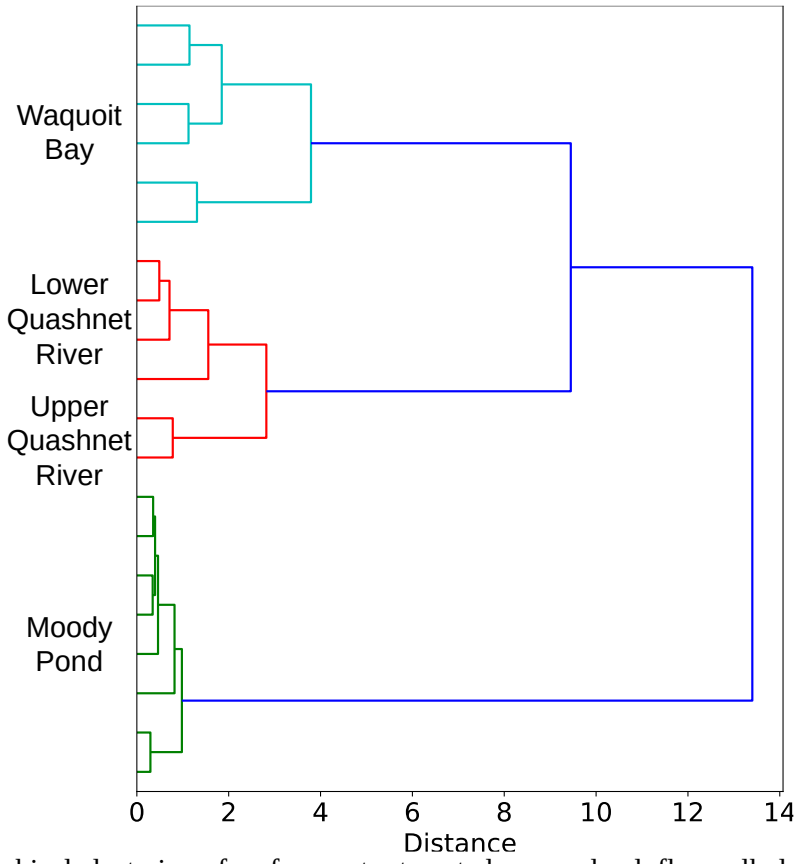

Figure S14. Hierarchical clustering of surface water targeted per- and polyfluoroalkyl substances (PFAS) data using the Euclidean distance metric and the Ward linkage method. Data are based on log transformed molarity with non-detects replaced by method detection limit (MDL)/√2 for compounds with >70-100% detection frequency.

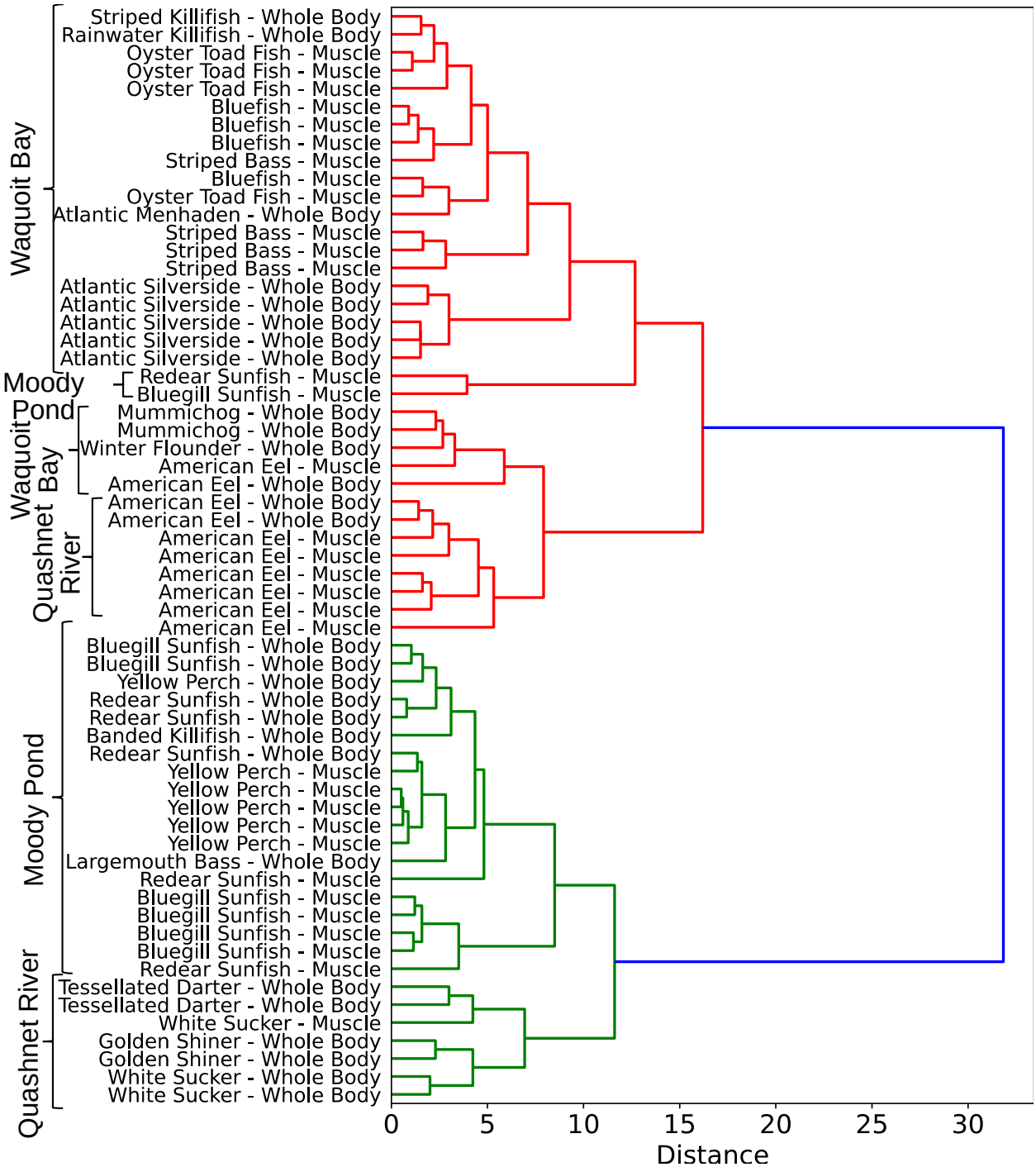

Figure S15. Hierarchical clustering of fish targeted per- and polyfluoroalkyl substances (PFAS) data using the Euclidean distance metric and the Ward linkage method. Data are based on log transformed molarity with non-detects replaced by method detection limit (MDL)/√2 for compounds with >60-100% detection frequency.

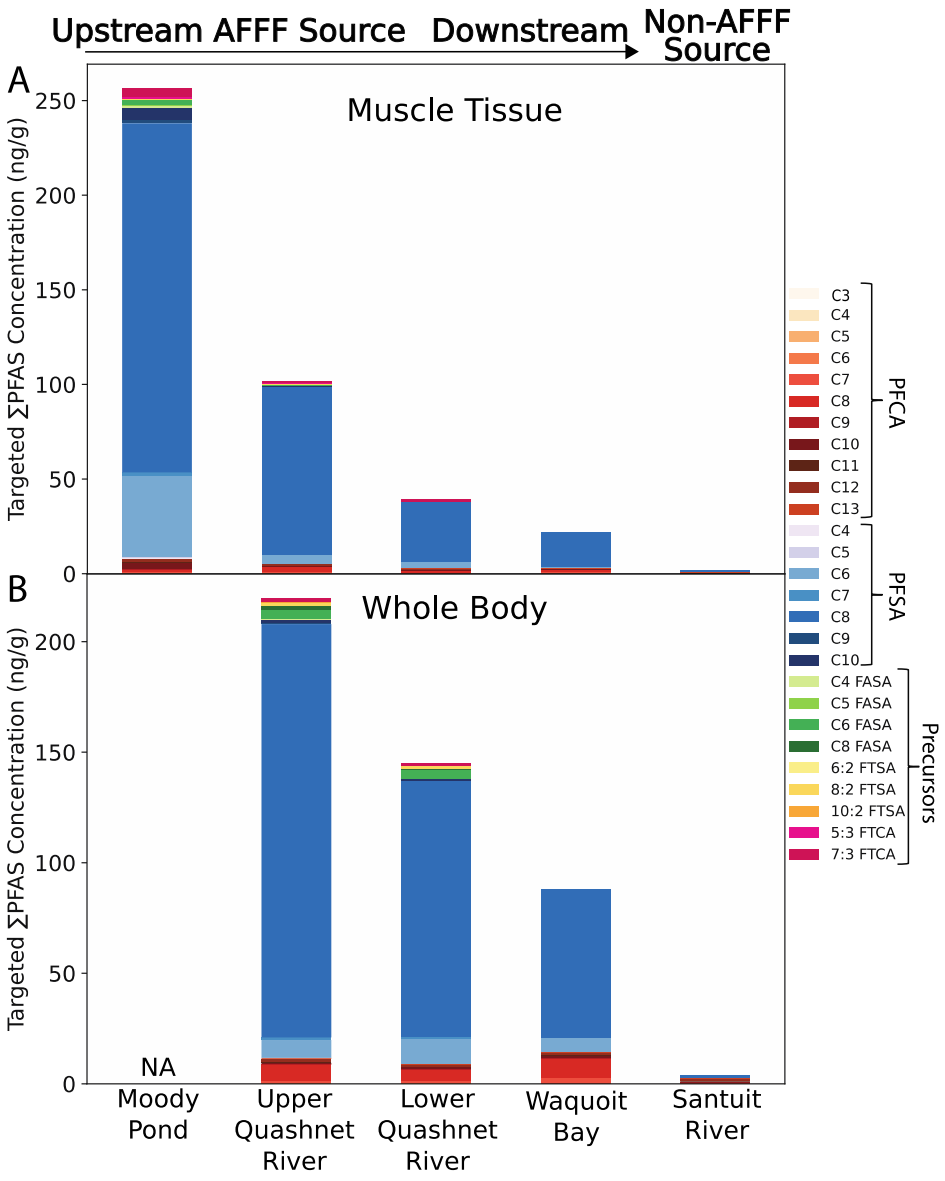

Figure S16. Average concentrations (ng g^{-1} wet weight) for the sum of targeted per- and polyfluoroalkyl substances (∑PFAS) in (A) muscle tissue and (B) whole-body samples for American Eel, grouped by location (n=1-5 samples), expressed as absolute PFAS composition. PFAS composition profiles include the perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursor compounds, defined by perfluorinated carbon chain length, detected in at least 70% of samples, going from upstream closest to the aqueous film-forming foam (AFFF) source to downstream. NA = not assessed.

2.4. Stable Isotope Values

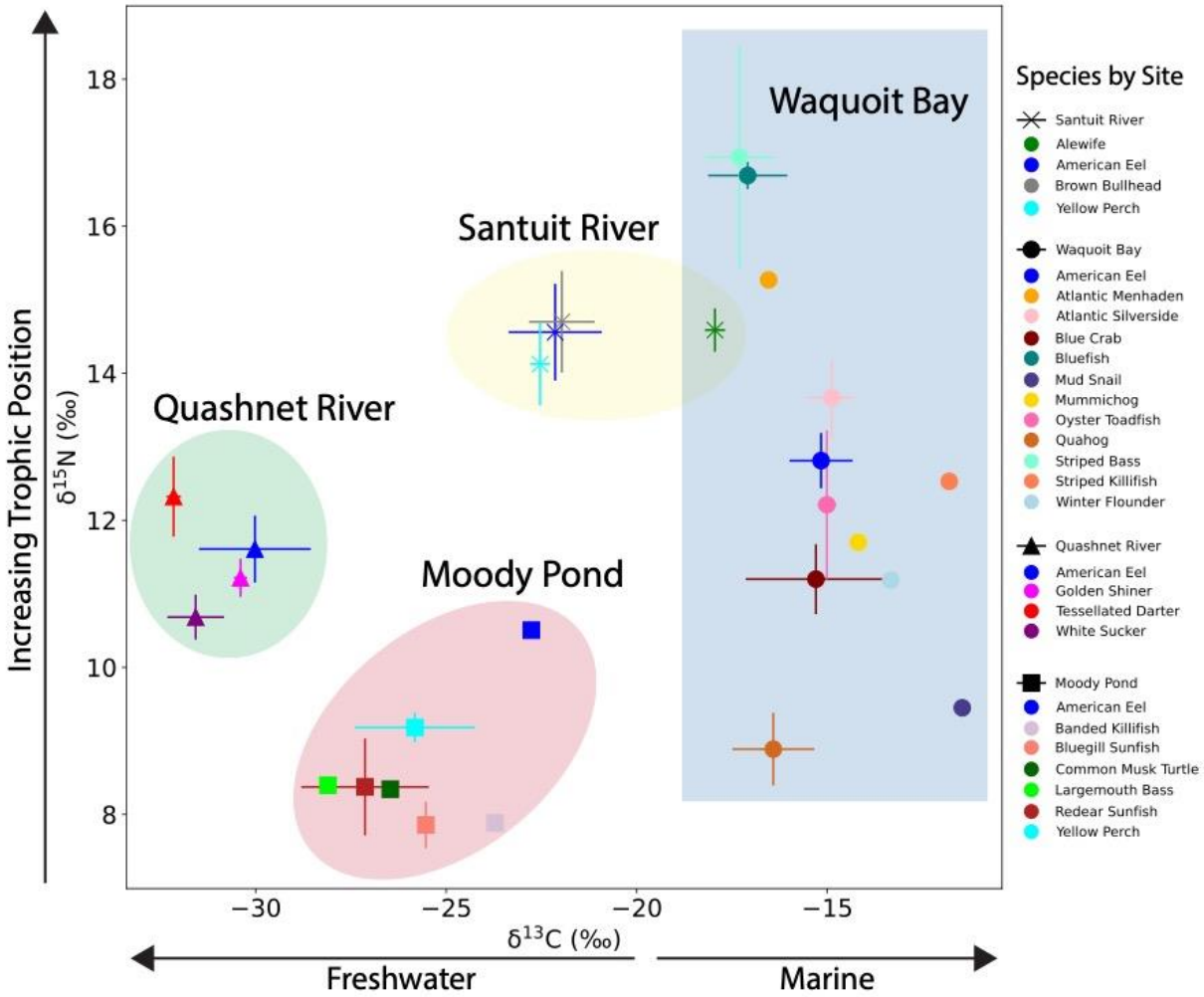

Figure S17. Mean nitrogen (N) and carbon (C) stable isotope values ($\delta^{15}N$ vs lipid-normalized $\delta^{13}C$) in fish/shellfish/gastropod/turtle species collected from the four surface water sites. Error bars represent the standard deviation. Marker type denotes site of sample collection and marker color denotes type of species. Samples of the same species collected from the Upper and Lower Quashnet River are grouped together and shellfish samples of the same species from Quashnet into Waquoit Bay and Waquoit Bay are grouped together. Shaded areas encircle samples from the same sites to represent distinct separation in isotope signatures based on location. The Alewife sample from the Santuit River falls within the Waquoit Bay stable isotope space as it was collected during the spring spawning immigration and so recently migrated from the marine environment.

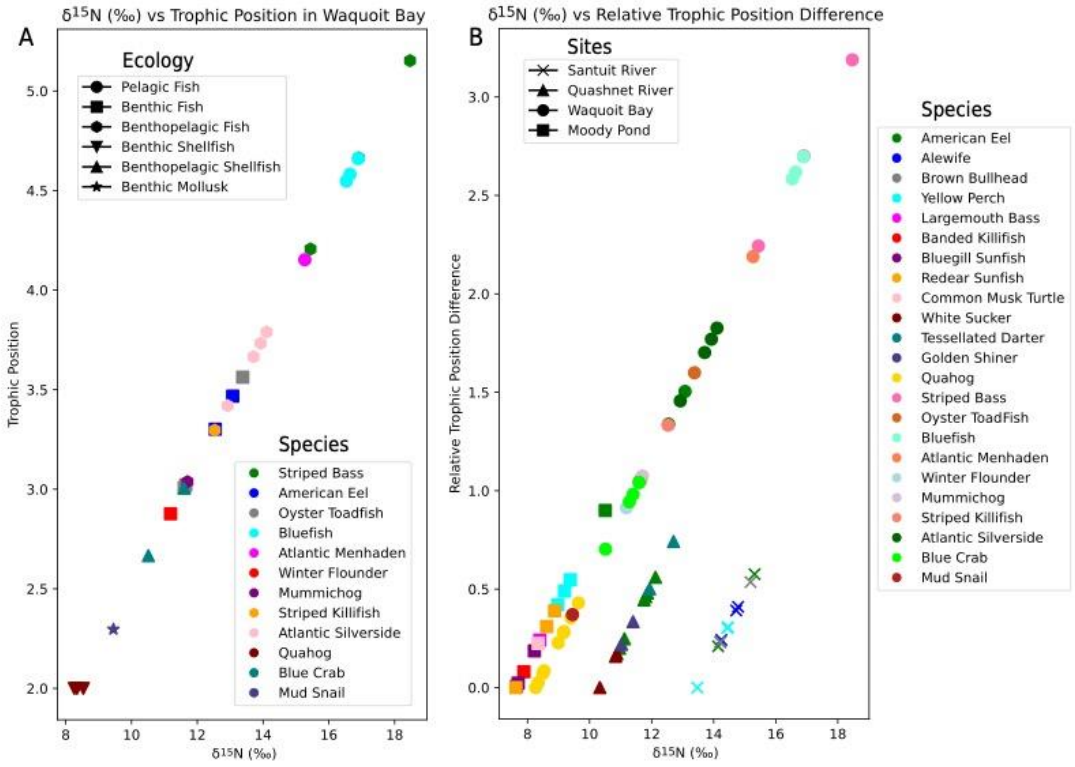

Figure S18. (A) Sample $\delta^{15}N$ (‰) versus calculated trophic position (TP) for species from Waquoit Bay using quahogs as baseline organism with a set TP=2.0. (B) Sample $\delta^{15}N$ (‰) versus relative TP difference for species across all sites. Trophic position estimates based on tissue bulk $\delta^{15}N$ values are sensitive to variable trophic enrichment among species and trophic levels as well as potential baseline differences among trophic pathways (e.g., benthic vs. pelagic).[56,57] E.g. Atlantic menhaden, an obligate filter feeder that consumes particulate organic matter and phytoplankton as juveniles,[32] had among the highest $\delta^{15}N$ values (**Table S2**) resulting in an estimated TP>4.0 similar to its predator,[24] juvenile striped bass. This discrepancy could be due to greater trophic enrichment for menhaden associated with low protein diet.[58]

Table S19. Linear regression relationship (slope, R^2, and p-value) between sum of targeted per- and polyfluoroalkyl substances (∑PFAS) concentrations (ng g^{-1}) in fish and eel whole-body and muscle tissue samples compared to size (length or weight), $\delta^{15}N$ (‰), $\delta^{13}C$ (‰), carbon-to-nitrogen (C:N) ratio, and trophic position difference (ΔTP) for fishes grouped by collection site. NA means not enough samples associated with a site to assess statistically. Italicized values have $p < 0.05$.

		Moody Pond			**Quashnet River**			**Waquoit Bay**			**Santuit River**		
Parameter	**Tissue**	**slope**	**R^2**	**p-value**	**slope**	**R^2**	**p-value**	**slope**	**R^2**	**p-value**	**slope**	**R^2**	**p-value**
Fish Length (cm)	Whole-Body	5.7	0.12	0.38	-6.6	0.29	0.14	-0.65	0.19	0.16	-0.13	0.03	0.67
	Muscle	*25*	*0.34*	*<0.05*	-15	0.23	0.29	*0.33*	*0.46*	*<0.05*	*0.04*	*0.40*	*<0.05*
Fish Weight (g)	Both	-0.04	0.0	0.79	-0.78	0.17	0.13	-0.008	0.01	0.57	-0.003	0.05	0.34
$\delta^{15}N$ (‰)	Whole-Body	NA	NA	NA	24	0.06	0.54	-1.6	0.24	0.16	-4.6	0.83	0.20
	Muscle	*90*	*0.65*	*<0.05*	0.83	0.0	0.99	-1.2	0.19	0.19	0.29	0.02	0.70
$\delta^{13}C$ (‰)	Whole-Body	NA	NA	NA	-25	0.06	0.53	-6.1	0.11	0.33	3.71	0.54	0.37
	Muscle	-0.6	0.0	0.98	-18	0.41	0.19	2.3	0.22	0.16	-0.24	0.34	0.11
C:N	Whole-Body	NA	NA	NA	-32	0.07	0.50	-150	0.18	0.19	NA	NA	NA
	Muscle	35	0.07	0.44	-2.2	0.0	0.91	6.5	0.09	0.36	*1.8*	*0.84*	*<0.05*
ΔTP difference	Whole-Body	NA	NA	NA	76	0.06	0.54	-5.1	0.24	0.16	NA	NA	NA
	Muscle	*289*	*0.65*	*<0.05*	2.6	0.0	0.99	-3.8	0.19	0.19	0.93	0.02	0.70

2.5. Bioaccumulation.

Table S20. Range in log bioaccumulation factors (BAF; L kg^{-1} wet weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursors if measured in more than one sample per species and tissue type per site. Results for linear and branched isomers separately are provided for C6 and C8 PFSA and perfluoroalkyl sulfonamides (FASA), as well as for the sum of isomers.

See attached excel file: Supplementary Information Tables: Table S20

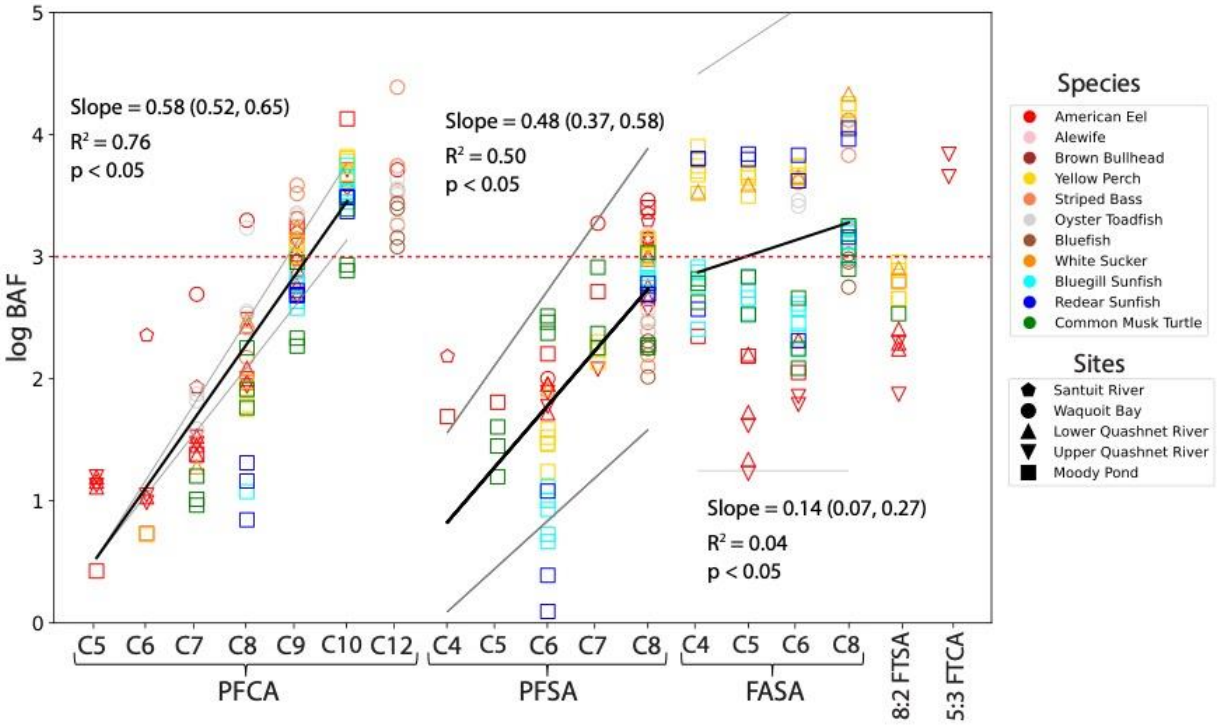

Figure S19. Field-measured bioaccumulation factors (log BAF, L kg^{-1} wet-weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), perfluoroalkyl sulfonamides (FASA), and other targeted precursors in muscle tissue samples for fish and turtle species from surface waters downgradient of aqueous film-forming foam (AFFF) source zones and from the background site. Each marker indicates an individual measurement. Marker type denotes sample site collection and marker color denotes species. R^2 values and p-values are based on linear regression (shown as solid lines with 95% confidence interval) of BAF data for C5-C10 PFCA (PFHxA-PFUnDA), C4-C8 PFSA (PFBS-PFOS), and C4-C8 FASA (FBSA-FOSA). The red dotted line indicates the threshold of log BAF ≥ 3.0 at which PFAS have a tendency to bioaccumulate.

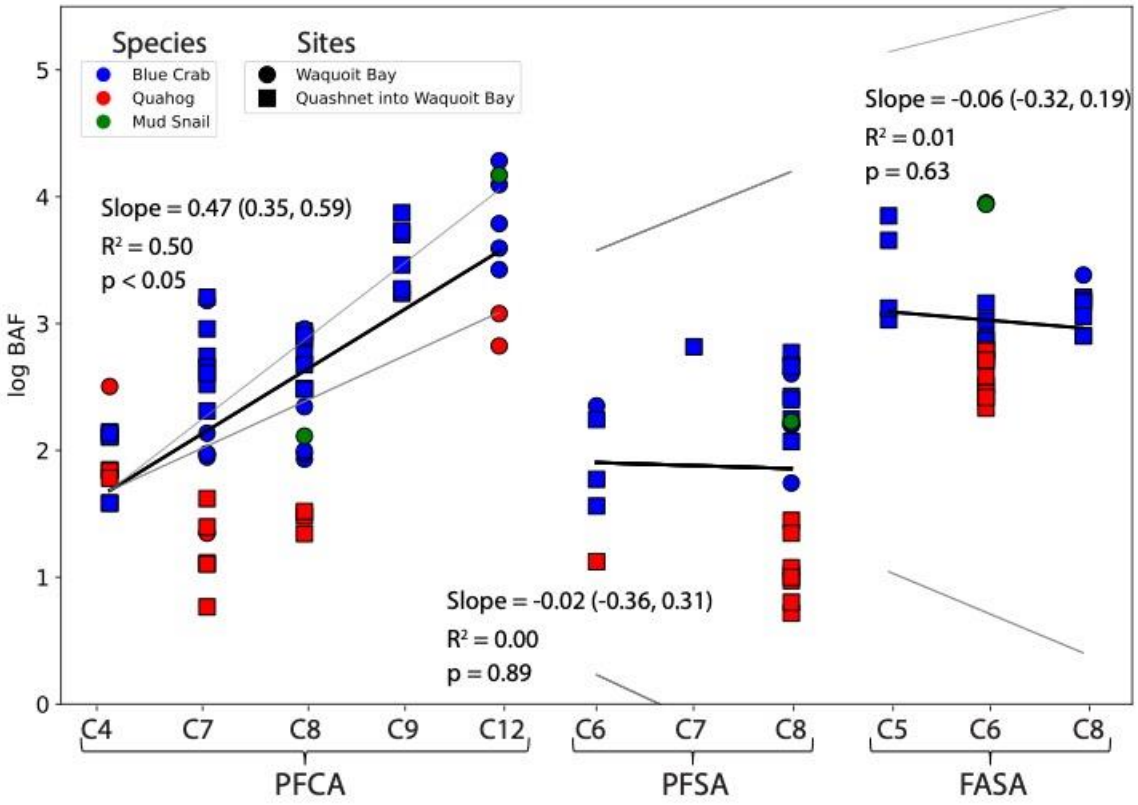

Figure S20. Field-measured bioaccumulation factors (log BAF, L kg^{-1} wet-weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and perfluoroalkyl sulfonamides (FASA) in whole-body invertebrate samples from sites downstream of aqueous film-forming foam (AFFF) source zone. Each marker indicates an individual measurement. Marker type denotes sample site collection and marker color denotes species. R^2 values and p-values are based on linear regression (shown as solid lines with 95% confidence interval) of BAF data for C4-C12 PFCA (PFPeA-PFTriDA), C6-C8 PFSA (PFHxS-PFOS), and C5-C8 FASA (FPeSA-FOSA).

Table S21. Range in log biota-sediment accumulation factors (BSAF; kg organic carbon/kg wet weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and targeted precursors if measured in more than one sample per benthic species and tissue type per site. Results for linear and branched isomers separately are provided for C6 and C8 PFSA, as well as for the sum of isomers.

See attached excel file: Supplementary Information Tables: Table S21

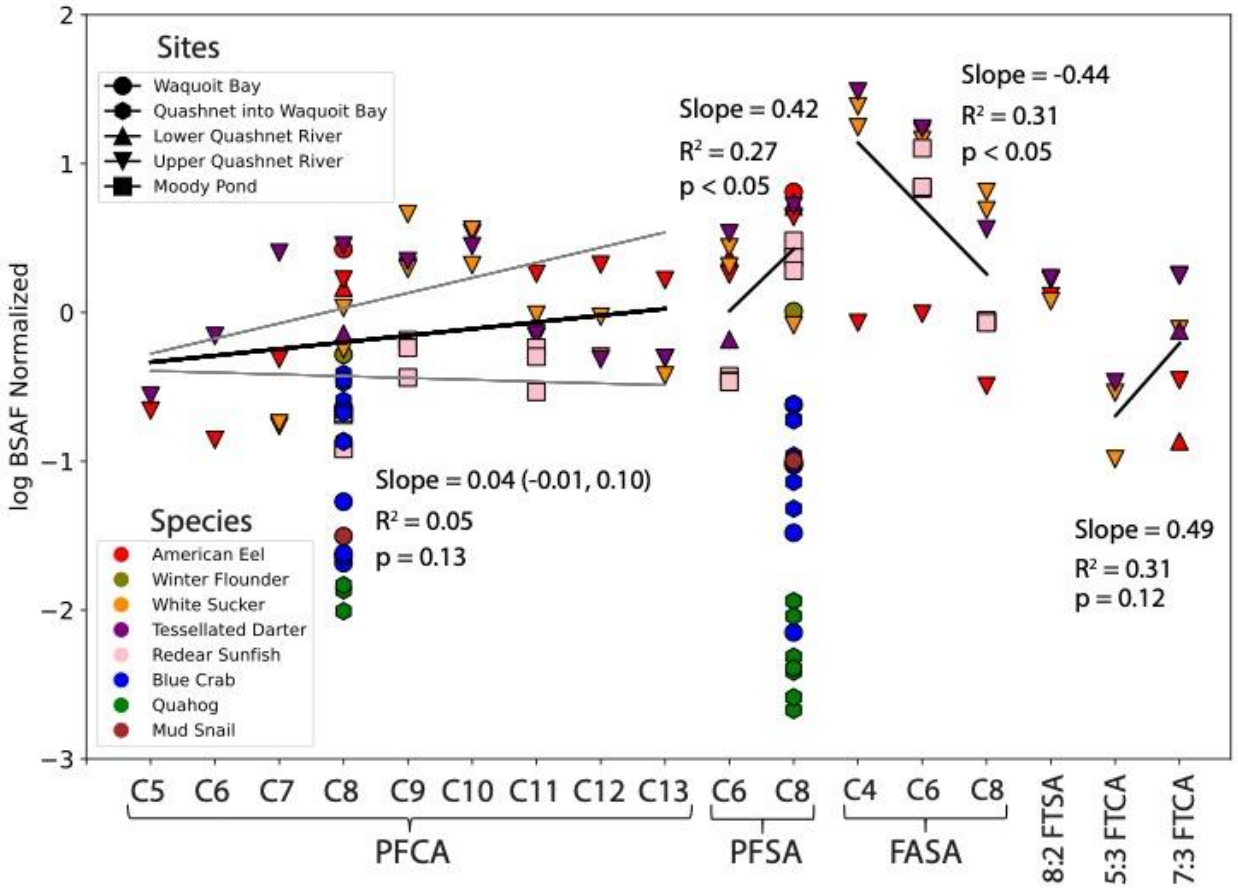

Figure S21. Field-measured organic-carbon normalized biota-sediment accumulation factors (log BSAF Normalized, kg OC kg^{-1} wet-weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and perfluoroalkyl sulfonamides (FASA) in whole-body benthic fish and invertebrate samples from sites downstream of aqueous film-forming foam (AFFF) source zone. Each marker indicates an individual measurement. Marker type denotes sample site collection and marker color denotes species. R^2 values and p-values are based on linear regression (shown as solid lines with 95% confidence interval) of BAF data for fish species only for C5-C13 PFCA (PFHxA-PFTeDA), C6-C8 PFSA (PFHxS-PFOS), and C4-C8 FASA (FBSA-FOSA).

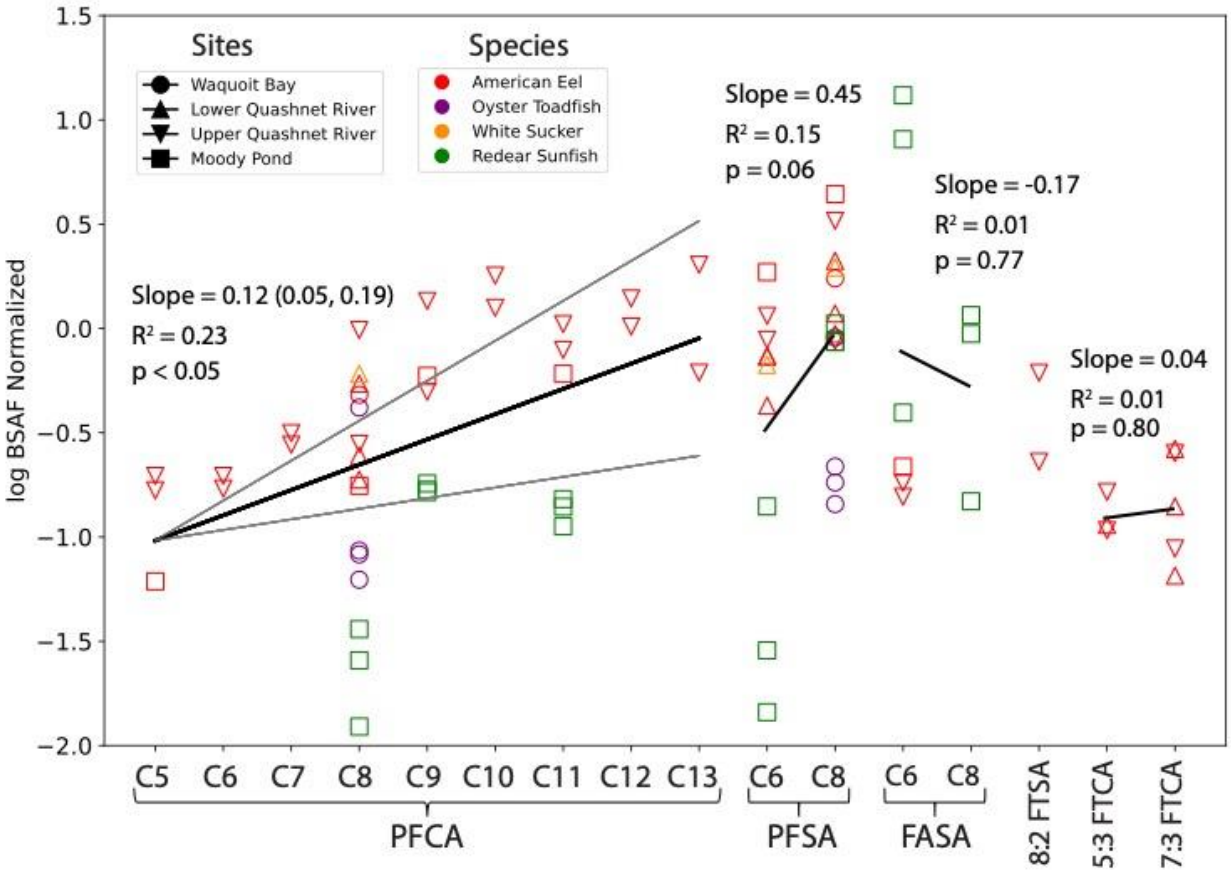

Figure S22. Field-measured organic-carbon normalized biota-sediment accumulation factors (log BSAF Normalized, kg OC kg^{-1} wet-weight) for perfluorocarboxylates (PFCA), perfluorosulfonates (PFSA), and perfluoroalkyl sulfonamides (FASA) in muscle tissue of benthic fish from sites downstream of aqueous film-forming foam (AFFF) source zone. Each marker indicates an individual measurement. Marker type denotes sample site collection and marker color denotes species. R^2 values and p-values are based on linear regression (shown as solid lines with 95% confidence interval) of BAF data for C5-C13 PFCA (PFHxA-PFTeDA), C6-C8 PFSA (PFHxS-PFOS), and C4-C8 FASA (FBSA-FOSA).

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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