

1 **Supporting Information**

2 **Isolating the AFFF Signature in Coastal Watersheds using Oxidizable PFAS Precursors**  
3 **and Unexplained Organofluorine**

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## 60 **Supplemental Methods.**

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

## 63 **Supplemental site information.**

64 The former fire-training area is in the western part of the *Childs* and *Quashnet R.*  
65 watersheds on Joint Base Cape Cod (JBCC). AFFF containing PFAS were used at the fire-  
66 training area between 1970 and 1985 and again for one fire emergency in 1997.<sup>1,2</sup> Additional  
67 locations of historical AFFF releases on JBCC have recently been identified in the eastern part of  
68 the *Quashnet R.* watershed<sup>3</sup> that discharges directly to the river<sup>4</sup> and to *Moody P.* (Fig. 1), a  
69 small groundwater-fed pond downgradient from the JBCC and about 0.4 km upgradient from the  
70 river. For more information about the Installation Restoration Program at JBCC, see  
71 <https://www.massnationalguard.org/JBCC/afcec.html>. The *Mill Creek* watershed contains a fire-  
72 training area at the Barnstable County Fire and Rescue Training Academy in the Town of  
73 Barnstable, MA, U.S.A that reportedly was active until approximately 2009.

## 74 **Sampling Protocol.**

75 All surface-water samples were collected at about half the water depth at the sampling  
76 sites (0.2 to 0.5 m below the water surface at most sites) in 1 L HDPE bottles. Prior to sampling,  
77 each bottle was rinsed twice with Milli-Q (Thermo Scientific™ Barnstead™, Lake Balboa, CA)  
78 water, ACS grade methanol (Macron Fine Chemicals, Radnor, PA), LC-MS grade methanol (J.T.  
79 Baker, Center Valley, PA), Milli-Q water, and air-dried in a clean laboratory. In the field, sample  
80 bottles were rinsed three times with surface water before sampling. We collected 15 field  
81 duplicates and 12 field blanks. Each field blank contained Milli-Q water exposed to the ambient

82 environment during sample collection. All samples were stored on ice in the field and at 4°C in  
83 the laboratory before extraction for PFAS analysis.

#### 84 **PFAS Extraction and Analysis.**

85 Samples were removed from the refrigerator and equilibrated to room temperature before  
86 sonicating for 20 seconds and vigorous shaking three times. Subsamples of surface water (200  
87 mL) were taken gravimetrically by pouring into pre-cleaned 500 mL HDPE bottles. Samples  
88 were spiked with 40 µL of 0.03 ng µL<sup>-1</sup> mass-labeled PFAS internal standard mixture  
89 (Wellington, Guelph, Canada; Table S2) and equilibrated for twelve hours. Samples were  
90 extracted in batches of twelve (nine samples, one sample duplicate, one extraction blank, and  
91 either a sample spike or blank spike) using weak anion exchange cartridges (Waters Oasis®  
92 WAX, 6 mL, 150 mg sorbent) conditioned by sequential application of 4 mL 0.1% NH<sub>4</sub>OH  
93 (28.0-30.0%, ACS grade, BDH® VWR International, Radnor, PA) in LC-MS grade methanol, 4  
94 mL of LC-MS grade methanol, and 4 mL of Milli-Q water. Samples were shaken vigorously and  
95 loaded onto the SPE cartridges with a flow rate of 1 drop per second. After sample loading, SPE  
96 cartridges were rinsed with 4 mL of Milli-Q water. PFAS were eluted into 15 mL polypropylene  
97 centrifuge tubes (Corning, Corning, New York) with 4 mL of LC-MS grade methanol used to  
98 rinse the sample bottles followed by 4 mL 0.1% NH<sub>4</sub>OH in LC-MS grade methanol. The extracts  
99 were blown to dryness using a N-EVAP (Organomation, Berlin, MA) nitrogen evaporator,  
100 reconstituted in 750 µL LC-MS grade methanol, vortexed, and heated at 40°C for 30 minutes.  
101 Samples were vortexed again and transferred to 1.5 mL microcentrifuge tubes filled with 25 mg  
102 Supelclean™ ENVI-Carb™ (Supelco, Bellefonte, PA) for clean-up, vortexed, and centrifuged  
103 for 20 minutes at 13,000 RPM. 500 µL subsamples was transferred to another 1.5 mL  
104 microcentrifuge tube and mixed with 500 µL Milli-Q water before instrumental analysis.

105 The total oxidizable precursor (TOP) assay was performed on surface-water (200 mL)  
 106 samples by combining equal parts by volume of 120 mM potassium persulfate (ACS-grade  
 107 K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, Honeywell, Charlotte, NC) and 250 mM sodium hydroxide (ACS-grade NaOH, Macron  
 108 Fine Chemicals, Radnor, PA) and heated for twelve hours at 85°C in a water bath. Samples were  
 109 cooled, neutralized with hydrochloric acid (ACS-Plus grade 37% HCl, Fisher Scientific,  
 110 Hampton, NH), and spiked with 40 µL of 0.03 ng µL<sup>-1</sup> mass-labeled PFAS internal standard  
 111 mixture. Samples were extracted in a similar manner as targeted analysis extraction. The 4 mL  
 112 Milli-Q water rinse after sample loading was added to the sample bottle before the SPE cartridge  
 113 to prevent precipitation of residual potassium persulfate during the methanol rinse.

114 The efficacy of precursor oxidation was evaluated once per batch of samples analyzed by  
 115 spiking 3 ng of FOSA, 6:2 FTSA, 8:2 FTSA, N-MeFOSAA, and N-EtFOSAA in Milli-Q water  
 116 before performing the TOP assay (Supp Methods Table 1). Concentrations of precursors were  
 117 below method detection limit (MDL) in all of these samples, indicating complete oxidation.

118 **Supplementary Methods Table 1. Average Recovery (%) and Relative Standard Deviation**  
 119 **(RSD %) of TOP Assay Analysis.<sup>1</sup>**

PFAS	1000 ng L <sup>-1</sup> sample spike (n = 4)		100 ng L <sup>-1</sup> DI spike (n = 2)		1000 ng L <sup>-1</sup> DI Spike (n = 2)		10000 ng L <sup>-1</sup> DI spike (n = 1)
	Avg (%)	RSD (%)	Avg (%)	RSD (%)	Avg (%)	RSD (%)	Avg (%)
	PFBA	104	9	88	14	115	0
PFPeA	116	15	95	5	104	3	118
PFHxA	94	13	99	2	108	4	109
PFHpA	96	13	95	6	105	7	118
PFOA	96	18	108	7	104	2	100

120 <sup>1</sup>Spiking mixture was added after samples were removed from water bath and neutralized at the  
 121 same time as the addition of the internal standard  
 122

123 LC-MS/MS analysis was conducted with minor modifications outlined in prior work  
 124 from our lab.<sup>1</sup> LC-MS/MS blanks and the calibration curve were prepared with 50:50  
 125 methanol:DI water and internal standard concentrations matching the samples. The 13-point

126 calibration curve ranged from 1 to 40,000 ng L<sup>-1</sup>. Calibration quality controls ranged were  
127 included throughout the sample run and ranged between 70 and 130% for all analytes. Branched  
128 and linear PFOS and PFHxS were quantified with individual native isomer calibration curves.  
129 Initial conditions were 97% 2 mM ammonium acetate in DI water (A) and 3% mM ammonium  
130 acetate in methanol (B). From 0.85 to 3.5 min the gradient was linearly increased to 54% B.  
131 From 3.5 to 16 min the gradient was linearly increased to 85% B and then from 16 to 16.5 min  
132 the gradient was linearly increased to 100% B and maintained until the end of the run (17.5 min).  
133 The column temperature was 50 °C. Throughout each run, mass spectrometry parameters are  
134 detailed in Table S2.

135         The recovery of internal standards was monitored throughout each run and ranged  
136 between 70-130% of the average peak area of the calibration standards consistent with EPA  
137 Method 533.<sup>5</sup>

138         Consistent with previous work from our lab,<sup>1,6</sup> limits of detection (LOD) were calculated  
139 as the average concentration at which the sample signal-to-noise ratio was three. Samples  
140 included in calculating the LOD included all environmental samples with qualified ratios  
141 between 70-130% of the 10,000 ng L<sup>-1</sup> calibration standard, spikes, and extraction blanks.  
142 Concentrations of all PFAS in the LC-MS/MS blanks were below LOD. The MDL was 0.0075-  
143 times the LOD because 200 mL subsamples were extracted into 750 µL methanol (Table S4).  
144 Concentration of PFAS were below MDL in most extraction blanks except for infrequent  
145 contamination by PFBA, N-MeFOSAA, and N-EtFOSAA. In instances where concentrations of  
146 PFAS in the extraction blank exceeded MDL, the blank value was subtracted from the  
147 concentrations of the other samples in the extraction batch. All concentrations above the MDL  
148 after blank subtraction are reported in this study, except when the qualifier ratio was less than

149 70% or more than 130% of the 10,000 ng L<sup>-1</sup> calibration standard. In those instances, the  
 150 concentration was assumed to be <MDL.

151 **Extractable Organofluorine (EOF) Analysis.**

152 Extractable organofluorine (EOF) analysis was performed at Harvard University  
 153 following five steps. First, samples were extracted via offline SPE using the same method as  
 154 targeted analysis. Next, inorganic fluoride was removed by rinsing cartridges with 10 mL of  
 155 0.01% (v/v) NH<sub>4</sub>OH in Milli-Q water. The efficacy of fluoride removal was tested by spiking 20  
 156 mL of Milli-Q water with sodium fluoride (NaF, 99+%, for analysis, ACROS Organics) at  
 157 26,300 nM F in triplicate and performing the extraction. We observed complete removal of the  
 158 26,300 nM F spike. The extract EOF concentrations in this work were <23,000 nM F (Supp  
 159 Methods Table 2), therefore we conclude the unexplained fraction of EOF in this work is not due  
 160 to incomplete removal of inorganic fluoride.

161 **Supplementary Methods Table 2. EOF Extract Concentrations in Surface Water Samples**  
 162 **from the Quashnet R. Watershed, Cape Cod, MA, U.S.A. [nM]**

Site Name	MPSC	QR01	QR03	QR09	QR09	QR09	QR09
Date	7/11/18	7/10/18	7/10/18	7/11/18	10/20/18	4/9/19	7/1/19
Description	Moody Pond	Quashnet River					
Extract EOF <sup>1</sup>	22,700	17,100	20,900	<LOD <sup>2</sup>	16,300	17,200	<LOD <sup>2</sup>
Sample EOF	29.0	14.8	24.2	<LOD	14.7	26.3	<LOD

163 <sup>1</sup>Concentration of EOF in extracted sample

164 <sup>2</sup>Sample was below LOD (10,580 nM F) after blank subtraction

165  
 166 All samples for CIC analysis were eluted into centrifuge tubes using methanol and 0.1%  
 167 NH<sub>4</sub>OH. They were blown to dryness using an N-EVAP nitrogen evaporator and reconstituted in  
 168 1 mL of LC-MS grade methanol. Reconstituted samples were split for analysis by combustion



169 ion chromatography (CIC) and LC-MS/MS. An isotopically labeled internal standard (Table S2)  
170 was added to the LC-MS/MS fraction after the extract was split between the two fractions.  
171 Samples were analyzed on the LC-MS/MS in the same manner as targeted PFAS. The relative  
172 percent difference of PFAS analyzed for targeted analysis (with internal standard added before  
173 offline extraction) and EOF (internal standard added after offline extraction) ranged between 2%  
174 and 12%, except for PFBA which was 22%.

175 All samples for EOF determination were combusted at 1050°C with a combustion unit  
176 from Analytik Jena (Jena, Germany). Adsorption and detection were performed using a 920  
177 Absorber Module and 930 Compact IC Flex ion chromatograph from Metrohm (Herisau,  
178 Switzerland). Two boat blanks were run between each set of triplicate injections of 100 µL  
179 methanol extracts and samples were blank corrected using the peak areas of the boat blanks run  
180 before and after each set of injections. Two methanol blanks were run before and after the  
181 calibration and after every six samples to account for any source of contamination from the  
182 solvents used in the analysis. Concentrations were determined from the average peak areas of  
183 triplicate injections using an eight-point calibration curve ( $R^2 = 0.999$ ) from 2,630 to 526,300 nM  
184 F.

185 The limit of detection (LOD) was 10,580 nM F and was calculated as the average plus  
186 three times the standard deviation of triplicate injections of the extraction blank. Concentrations  
187 above the LOD were adjusted for the dilution factor (3,200-5,400x) and corrected by subtracting  
188 the field blank. The concentrations of EOF in the samples were 11-21 times larger than in the  
189 field blank. The concentration of EOF in the extraction blank was below LOD. The MDL ranged  
190 from 1.95 to 3.26 nM F and was calculated as three times the standard deviation of the extraction  
191 blanks adjusted for the dilution factor. The relative percent difference of field duplicates was 4%

192 and extraction duplicates was 2%, although they were below LOD after blank subtraction. The  
193 percent recovery (128%;  $n = 1$ ) was calculated from the concentration of organofluorine  
194 measured by the CIC divided by the concentration of organofluorine measured by the LC-  
195 MS/MS using a 66 nM F as PFOA (95% purity, Sigma-Aldrich, St. Louis, MO) spike in Milli-Q  
196 water. Recovery of PFOA in this study was similar to that for cosmetics spiked with PFOS  
197 ( $69 \pm 14\%$ ) by Schultes et al.<sup>7</sup>

### 198 **Bayesian Inference Method.**

199 Bayesian inference is a widely used statistical procedure that estimates the conditional  
200 probability of an unknown variable given all available observations and their uncertainties. For  
201 this study, Bayesian inference was used to estimate PFAS precursor concentrations prior to  
202 oxidation by the TOP assay given measured changes in concentrations of the terminal PFCA  
203 following the TOP assay and published laboratory data on oxidative yields, and their respective  
204 uncertainties.<sup>8-10</sup>

205 We implement the Bayesian inference method to estimate precursors from the TOP assay  
206 described in Ruyle et al.,<sup>11</sup> with slight modifications to the assumptions of the prior distribution  
207 for environmental samples. The model (Eq. 1) predicts the original concentration of unknown  
208 PFAS grouped by perfluorinated chain length and manufacturing source ( $\theta$ ) given measured  
209 concentrations of oxidation products ( $x$ ) in the TOP assay:

$$210 \quad \pi(\theta|x) \propto \pi(\theta)p(x|\theta) \quad (\text{Eq. 1})$$

211 where:

212  $\pi(\theta|x)$  is the posterior, the log<sub>10</sub>-normal distribution of unknown PFAS concentrations.

213  $\pi(\theta)$ , is the prior, the log<sub>10</sub> uncertainty in concentrations of unknown PFAS based on known

214 information regarding the concentrations of these compounds. For samples in watersheds with an

215 AFFF source, we use a prior for ECF precursors based on their expected range of concentrations  
216 in AFFF based on concentrations of PFOS (Table S8) similar to that used in Ruyle et al.<sup>11</sup> To  
217 account for potential modifications of these ratios due to precursor transformations in the  
218 environment, we use relax this assumption by expanding the confidence interval to the second  
219 standard deviation. For samples from watersheds without an AFFF source, no prior information  
220 for ECF precursors was available. No prior information for fluorotelomer (FT) PFAS was  
221 available for any sample.  $p(x|\theta)$  is the likelihood, the log10 sum of least squares estimator:

$$222 \quad p(x|\theta) = \sum_i [(\mu_{A,i}\theta_i - x) / \varepsilon_i]^2 \quad (\text{Eq. 2})$$

223 where:

224  $\mu_{A,i}$  represent the average molar oxidation yields of unknown PFAS  $i$  into perfluoroalkyl  
225 carboxylates reported in the literature (see Table S5 in Ruyle et al.).<sup>8-11</sup>  $\varepsilon_i$  is the total error of the  
226 comparison for PFAS  $i$ :

$$227 \quad \varepsilon_i = [(\sigma_{A,i} / \mu_{A,i})^2 + \Delta_{x,i}^2]^{0.5} \quad (\text{Eq. 3})$$

228 Where,  $\sigma_{A,i}$  is the standard deviation of the average molar oxidation yields of unknown PFAS  $i$   
229 into perfluoroalkyl carboxylates reported in the literature and  $\Delta_{x,i}$  the relative error in the  
230 measurement.

231 The posterior distribution was sampled by Markov chain Monte Carlo (MCMC) analysis  
232 using 32 ensemble samplers. Sequential steps in the Markov chain were determined using the  
233 differential evolution algorithm<sup>12</sup> with the mean equal to 0.595 (2.38/SQRT[2\*ndim]) and  
234 standard deviation equal to 1.01, following the recommendation of the software.<sup>13</sup> The MCMC  
235 was run until the Monte Carlo standard error was 1/SQRT(2,500) of the standard deviation of the  
236 posterior distribution.

237 Measured changes of C3-C7 PFCA were used in the inference because prior work from  
238 our lab showed no generation of >C7 PFCA in legacy ECF and contemporary FT AFFF. Houtz  
239 et al.<sup>14</sup> similarly found no generation of PFCA with greater than C7 in ECF AFFF from 1988-  
240 2001, but measured very small amounts of C8 PFCA (<4% of all PFCA generated by TOP  
241 assay) after the TOP assay in fluorotelomer AFFF from 1986-2010. While non-targeted HRMS  
242 has identified longer chained precursors in AFFF,<sup>15-17</sup> data from the TOP assay suggest their  
243 concentrations are negligible.

244 Grouping of precursors by manufacturing origin (ECF vs. FT) is performed solely on the  
245 unique yields of ECF and FT precursors.<sup>11</sup> Prior work has hypothesized that ECF and FT  
246 precursors could also be distinguished based on the linear and branched isomer ratios of PFCA  
247 produced in the TOP assay.<sup>14</sup> However, linear and branched isomer ratios are have chain length  
248 and functional group dependencies, and there are no currently available data on these ratios for  
249 the precursors found in AFFF.<sup>18</sup>

## 250 **Supplementary Statistical Methods.**

251 We tested two imputation methods for concentrations below the MDL (regression on  
252 order statistics (ROS) and replacement with MDL/SQRT(2)). ROS is a semi-parametric method  
253 that assumes only the censored data below detection are normal or lognormal and is a commonly  
254 used imputation method for PFAS.<sup>19-21</sup> All targeted PFAS concentrations with > 70% detection  
255 and above the MDL were used directly in statistical analyses without any modification.

256 Since the concentrations and composition of PFAS was neither normally nor log-  
257 normally distributed, non-parametric statistics were used in this work. The Mann-Whitney *U* test  
258 was selected to compare the concentrations and composition of PFAS in watersheds with and  
259 without an AFFF source zone because we compare one dependent continuous variable (PFAS

260 concentration) from two independent groups (watersheds with and without AFFF source zones)  
261 using independent observations within each watershed. The p-value was corrected for using false  
262 discovery rate (FDR) correction to account for multiple comparisons.

263 Significant temporal variability at the mouth of the *Quashnet R.* over the study period  
264 from August 2017 to July 2019 was tested using Kruskal-Wallis one-way analysis of variance  
265 (ANOVA) (Table S12). The Kruskal-Wallis test is the extension of the Mann-Whitney *U* test for  
266 comparing more than two independent groups. Samples, extraction duplicates, and field  
267 duplicates were grouped by sampling date to determine whether the observed variability between  
268 dates was greater than error introduced by the sampling and extraction procedure.

269 PCA is a data analytical technique used to reduce the dimensionality of observations by  
270 grouping interrelated variables into combinations of linearly independent basis vectors.<sup>21</sup> In this  
271 case, we use PCA to group PFAS profiles in clusters with common sources. While PCA only  
272 uses a subset of the data to identify dominant components, hierarchical clustering using the  
273 UPGMA algorithm (which is ideal for generating close knit collectives of data)<sup>22</sup> uses 100% of  
274 the observations and does not require any a priori assumptions regarding the number of clusters.  
275 It therefore provides a check on the major groupings of PFAS identified using PCA. Measured  
276 chemical composition profiles in combination with multivariate statistical analysis has been used  
277 to fingerprint contamination sources for many organic pollutants.<sup>23</sup> PCA assumes linearity by  
278 using a linear mapping of high dimensional data into a lower dimensional space. Inspection of  
279 relationships between transformed PFAS concentrations and compositions in two dimensions did  
280 not yield clear non monotonic relationships between variables and conclude that our data is  
281 suitable for PCA. Neither PCA nor hierarchical clustering requires data to be univariate or  
282 multivariate normal distributed.<sup>24</sup>

283 **Composition of AFFFs in the Principal Component Analysis.**

284 The composition of AFFFs used as supplementary independent variables in the principal  
285 component analysis (Table S14) were from the AFFF reported in Ruyle et al.<sup>11</sup> and in Tables S5  
286 and S6 in Houtz et al.<sup>14</sup> ECF AFFFs were given a weight of 0.69 and fluorotelomer AFFFs were  
287 given a weight of 0.21 in the PCA due to the mean ECF fraction of precursors in watersheds with  
288 a fire-training area. The PFAS included in the principal component analysis (PFAS detected  $\geq$   
289 80% of surface water) composed  $96 \pm 4\%$  (min = 86%) of the PFAS reported in the AFFFs.  
290 Compounds below detection were uniformly replaced by 0.001 times the lowest reported  
291 composition in each AFFF; PFPeA and PFNA were not detected above the detection limit in any  
292 AFFF. Linear and branched isomers of PFHxS and PFOS were estimated from the total reported  
293 values based on the ratios (linear to branched: PFHxS = 0.75:0.25, PFOS = 0.74:0.26) measured  
294 in the 3M AFFF reported in Ruyle et al.<sup>11</sup> These ratios agree with the ratios of linear to branched  
295 isomers produced by electrochemical fluorination and used in 3M AFFF.<sup>25</sup>

296 **PFAS Fluxes from the *Quashnet R.* to *Waquoit Bay.***

297 Fluxes of PFAS and EOF from the *Quashnet R.* to *Waquoit Bay* were calculated as the  
298 product of the average concentration of six temporal samples taken at QR09 from August 2017  
299 to July 2019 and the total volumetric discharge of the river over the same time period.<sup>26</sup>  
300 Temporal variability of in measured concentrations for most PFAS at that site was insignificant  
301 (Table S12). The volumetric discharge was calculated from the midpoint Reimann sum of  
302 instantaneous discharge measurement taken every 15 minutes at QR09 by USGS Gage  
303 011058837 (Figure S3).

304

305 **Table S1. Auxiliary Information for Surface Water Sampling Locations on Cape Cod, MA,**  
 306 **U.S.A.**

307 See accompanying Excel Spreadsheet.

308 **Table S2. PFAS LC-MS/MS parameters.**

Analyte	Type	Internal Standard	Precursor Ion	Quantifier Ion	Quantifier Collision Energy (V)	Qualifier Ion	Qualifier Collision Energy (V)	Fragmentor Voltage (V)
PFBA	Target	[ <sup>13</sup> C <sub>4</sub> ] PFBA	213.0	168.9	2			60
PFPeA	Target	[ <sup>13</sup> C <sub>5</sub> ] PFPeA	262.9	218.9	2			60
PFHxA	Target	[ <sup>13</sup> C <sub>5</sub> ] PFHxA	312.9	268.9	2	118.9	14	70
PFHpA	Target	[ <sup>13</sup> C <sub>4</sub> ] PFHpA	362.9	318.9	2	168.9/118.9	10/18	70
PFOA	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOA	412.9	368.9	2	168.9	10	80
PFNA	Target	[ <sup>13</sup> C <sub>9</sub> ] PFNA	462.9	418.9	2	218.9/169.0	10/14	75
PFDA	Target	[ <sup>13</sup> C <sub>6</sub> ] PFDA	512.9	468.9	6	269.0/218.9	14/14	85
PFUnDA	Target	[ <sup>13</sup> C <sub>7</sub> ] PFUnDA	562.9	518.9	6	269.0/169.0	14/22	95
PFDoDA	Target	[ <sup>13</sup> C <sub>2</sub> ] PFDoDA	612.9	569.0	6	269.0/169.0	14/26	90
PFTeDA	Target	[ <sup>13</sup> C <sub>2</sub> ] PFTeDA	662.8	618.9	6	169.0	26	95
PFTeDA	Target	[ <sup>13</sup> C <sub>2</sub> ] PFTeDA	712.9	669.0	6	169.0	25	100
PFBS	Target	[ <sup>13</sup> C <sub>3</sub> ] PFBS	298.9	80.0	38	98.9	30	95
PFPeS	Target	[ <sup>13</sup> C <sub>3</sub> ] PFHxS	348.9	80.0	38	98.9	30	140
PFHxS	Target	[ <sup>13</sup> C <sub>3</sub> ] PFHxS	398.9	80.0	58	98.9	34	135
PFHpS	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOS	448.9	80.0	54	98.9	42	180
PFOS	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOS	498.9	80.0	60	98.9	50	200
PFNS	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOS	548.9	80.0	60	98.9	54	175
PFDS	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOS	598.9	80.0	60	98.9	54	175
4:2 FTSA	Target	[ <sup>13</sup> C <sub>2</sub> ] 4:2 FTSA	326.9	307.0	10	81.0	30	130
6:2 FTSA	Target	[ <sup>13</sup> C <sub>2</sub> ] 6:2 FTSA	426.9	406.9	18	81.0	34	135
8:2 FTSA	Target	[ <sup>13</sup> C <sub>2</sub> ] 8:2 FTSA	526.9	506.9	26	81.0	42	180
FBSA	Target	[ <sup>13</sup> C <sub>8</sub> ] FOSA	298.0	78.0	20			140
FHxSA	Target	[ <sup>13</sup> C <sub>8</sub> ] FOSA	398.0	78.0	40			180
FOSA	Target	[ <sup>13</sup> C <sub>8</sub> ] FOSA	497.9	78.0	38			140
N-EtFOSAA	Target	d5-N-EtFOSAA	583.9	418.9	18	525.9	14	95
N-MeFOSAA	Target	d3-N-MeFOSAA	569.9	418.9	14	482.9	10	95
DONA	Target	[ <sup>13</sup> C <sub>8</sub> ] PFOA	377.0	250.9	2	85.0	30	80
[ <sup>13</sup> C <sub>4</sub> ] PFBA	ISTD		216.9	171.9	2			60
[ <sup>13</sup> C <sub>5</sub> ] PFPeA	ISTD		267.9	223.0	2			60
[ <sup>13</sup> C <sub>5</sub> ] PFHxA	ISTD		317.8	273.0	2			70
[ <sup>13</sup> C <sub>4</sub> ] PFHpA	ISTD		366.8	321.9	2			70
[ <sup>13</sup> C <sub>8</sub> ] PFOA	ISTD		420.9	376.0	2			75

[ <sup>13</sup> C <sub>9</sub> ] PFNA	ISTD	472.0	427.0	2	85
[ <sup>13</sup> C <sub>6</sub> ] PFDA	ISTD	518.9	474.0	2	90
[ <sup>13</sup> C <sub>7</sub> ] PFUnDA	ISTD	569.9	525.0	6	85
[ <sup>13</sup> C <sub>2</sub> ] PFDoDA	ISTD	614.9	569.9	6	95
[ <sup>13</sup> C <sub>2</sub> ] PFTeDA	ISTD	714.8	670.0	6	95
[ <sup>13</sup> C <sub>3</sub> ] PFBS	ISTD	301.9	99.0	26	95
[ <sup>13</sup> C <sub>3</sub> ] PFHxS	ISTD	401.9	98.9	38	180
[ <sup>13</sup> C <sub>8</sub> ] PFOS	ISTD	506.9	99.0	50	180
[ <sup>13</sup> C <sub>2</sub> ] 4:2 FTSA	ISTD	328.9	81.0	38	95
[ <sup>13</sup> C <sub>2</sub> ] 6:2 FTSA	ISTD	428.9	81.0	46	95
[ <sup>13</sup> C <sub>2</sub> ] 8:2 FTSA	ISTD	528.9	81.0	46	180
[ <sup>13</sup> C <sub>8</sub> ] FOSA	ISTD	505.9	78.0	38	95
d5-N-EtFOSAA	ISTD	588.9	418.9	14	95
d3-N-MeFOSAA	ISTD	572.9	418.9	14	100

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310 **Table S3. Targeted Analytes for LC-MS/MS.**

Number of perfluorinated carbons	Name	Acronym	Molecular weight
3	Perfluorobutanoate	PFBA	213
4	Perfluoropentanoate	PFPeA	263
5	Perfluorohexanoate	PFHxA	313
6	Perfluoroheptanoate	PFHpA	363
7	Perfluorooctanoate	PFOA	413
8	Perfluorononanoate	PFNA	463
9	Perfluorodecanoate	PFDA	513
10	Perfluoroundecanoate	PFUnDA	563
11	Perfluorododecanoate	PFDoDA	613
12	Perfluorotridecanoate	PFTriDA	663
13	Perfluorotetradecanoate	PFTeDA	713
4	Perfluorobutane sulfonate	PFBS	299
5	Perfluoropentane sulfonate	PFPeS	349
6	Linear perfluorohexane sulfonate isomer	l-PFHxS	399
6	Branched perfluorohexane sulfonate isomers	br-PFHxS	399
7	Perfluoroheptane sulfonate	PFHpS	449
8	Linear perfluorooctane sulfonate isomer	l-PFOS	499
8	Branched perfluorooctane sulfonate isomers	br-PFOS	499
9	Perfluorononane sulfonate	PFNS	549
10	Perfluorodecane sulfonate	PFDS	599
4	4:2 fluorotelomer sulfonate	4:2 FTSA	327
6	6:2 fluorotelomer sulfonate	6:2 FTSA	427
8	8:2 fluorotelomer sulfonate	8:2 FTSA	527
4	Perfluorobutane sulfonamide	FBSA	299
6	Perfluorohexane sulfonamide	FHxSA	399
8	Perfluorooctane sulfonamide	FOSA	499
8	N-ethyl perfluorooctane sulfonamido acetate	N-EtFOSAA	584
8	N-methyl perfluorooctane sulfonamido acetate	N-MeFOSAA	571
5	4,8-dioxa-3H-perfluorononanoate	DONA	377

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312 **Table S4. PFAS Method Detection Limits (MDLs).**

313 See accompanying Excel Spreadsheet.

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315 **Table S5. PFAS Concentrations in Surface Water Samples from Cape Cod, MA, U.S.A.**

316 See accompanying Excel Spreadsheet.

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320 **Table S6. Average Recovery (%) and Relative Standard Deviation (RSD %) of Targeted**  
 321 **Analysis.**

PFAS	1000 ng L <sup>-1</sup> sample spike (n = 3)		100 ng L <sup>-1</sup> DI spike (n = 3)		1000 ng L <sup>-1</sup> DI Spike (n = 1)	10000 ng L <sup>-1</sup> DI spike (n = 1)
	Avg	RSD	Avg	RSD	Avg	Avg
	[%]	[%]	[%]	[%]	[%]	[%]
PFBA	112	6	112	12	94	87
PFPeA	111	6	101	2	100	96
PFHxA	90	12	110	15	100	92
PFHpA	101	5	140	59	95	91
PFOA	103	10	101	2	99	94
PFNA	104	5	101	8	97	92
PFDA	106	6	99	1	100	98
PFUnDA	103	7	105	10	106	97
PFDoDA	106	6	100	9	92	86
PFTTrDA	128	10	107	13	100	86
PFTeDA	106	7	108	17	94	88
PFBS	98	15	107	3	97	98
PFPeS	99	8	96	8	100	97
1-PFHxS	96	29	119	21	112	98
br-PFHxS	113	37	90	3	89	93
PFHpS	109	13	106	5	92	83
1-PFOS	94	28	107	7	106	90
br-PFOS	62	85	111	5	77	84
PFNS	100	13	99	3	100	75
PFDS	92	17	101	15	89	84
4:2 FTSA	105	8	105	14	90	86
6:2 FTSA	103	10	106	1	73	73
8:2 FTSA	106	1	115	10	82	89
FBSA	72	NA	113	NA	NA	NA
FHxSA	83	NA	120	NA	NA	NA
FOSA	107	6	105	4	87	91
N-MeFOSAA	100	7	137	28	96	84
N-EtFOSAA	116	15	140	21	97	84
NaDONA	85	6	92	4	92	75

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323 **Table S7. Change in PFCA Observed Following the TOP Assay in Surface Water Samples**  
 324 **from Cape Cod, MA, U.S.A.**

325 See accompanying Excel Spreadsheet.

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327 **Table S8. Molar PFAS Concentrations in Surface Water Samples from Cape Cod, MA,**  
 328 **U.S.A.**

329 See accompanying Excel Spreadsheet.

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331 **Table S9. ECF Precursor Prior in Watersheds with AFFF Source Based on Measured**  
 332 **PFOS Levels.**

Sample <sup>1</sup>	$\Delta$ C3:PFOS <sup>2</sup>	$\Delta$ C4:PFOS	$\Delta$ C5:PFOS	$\Delta$ C6:PFOS	$\Delta$ C7:PFOS
3M 1988	0.13	0.06	0.75	0.02	0.03
3M 1989	0.17	0.02	0.76	0.02	0.03
3M 1992	0.22	0.10	0.65	0.01	0.02
3M 1993	0.22	0.11	0.64	0.01	0.02
3M 1993	0.25	0.11	0.61	0.01	0.02
3M 1998	0.21	0.14	0.61	0.01	0.02
3M 1998	0.26	0.07	0.64	0.01	0.01
3M 1999	0.00	0.26	0.67	0.05	0.02
3M 2001	0.20	0.09	0.67	0.02	0.03
<b>mean<sup>3</sup></b>	0.19	0.11	0.67	0.02	0.02
<b>standard deviation<sup>3</sup></b>	0.08	0.07	0.05	0.01	0.01

333 <sup>1</sup>Sample names and data correspond to Table S5 and Table S6 in Houtz et al.<sup>14</sup>

334 <sup>2</sup> $\Delta$ Cn:PFOS = the ratio of the molar change in Cn PFCA in the TOP assay to the molar  
 335 concentration of PFOS measured using targeted LC-MS/MS

336 <sup>3</sup>Random samples from a normal distribution parametrized by mean ( $\mu$ ) and standard deviation  
 337 ( $\sigma^2$ ) are implemented in the prior in the Bayesian inference as  $\pi(\theta=N(\mu,2*\sigma^2))$  in Eq S1.

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357 **Table S10. EOF in Surface Water Samples from the *Quashnet R.* Watershed, Cape Cod,**  
 358 **MA, U.S.A.**

Site Name	MPSC	QR01	QR03	QR09	QR09	QR09	QR09
Date	7/11/18	7/10/18	7/10/18	7/11/18	10/20/18	4/9/19	7/1/19
Description	Moody Pond	Quashnet River					
EOF	29.0	14.8	24.2	<LOD <sup>1</sup>	14.7	26.3	<LOD <sup>1</sup>
Quantified EOF	18.3	9.17	12.5	6.46	8.98	6.18	6.18
PFBA	0.21	0.13	0.15	0.10	0.10	0.09	0.11
PFPeA	0.73	0.38	0.61	0.35	0.31	0.26	0.35
PFHxA	1.53	0.51	0.66	0.42	0.44	0.36	0.46
PFHpA	0.59	0.29	0.47	0.27	0.34	0.28	0.22
PFOA	0.95	0.49	0.63	0.45	0.56	0.54	0.48
PFNA	0.31	0.78	0.52	0.23	0.27	0.28	0.29
PFBS	0.12	0.10	0.12	0.09	0.09	0.08	0.11
PFPeS	0.19	0.12	0.11	0.10	0.13	0.11	0.09
PFHxS	6.27	1.32	1.83	1.20	1.72	1.18	1.19
PFHpS	0.09	0.05	0.09	0.04	0.05	0.04	0.04
PFOS	2.42	2.58	3.62	1.49	1.98	1.53	1.57
PFNS	0.01	0.00	0.00	0.00	0.00	0.00	0.00
ΣPrecursors	4.86	2.42	3.69	1.72	2.99	1.43	1.27
Unexplained EOF Conc. [nM F]	10.7	5.63	11.7		5.72	20.1	
Unexplained EOF Comp. [%]	37	38	48		39	77	

<sup>1</sup>Sample was below LOD (10,580 nM F) after blank subtraction

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375 **Table S11. PFAS Detected in Less Than 70% of Samples.**

PFAS	Detection frequency <sup>1</sup> [%]	AFFF watershed (n = 41)		non-AFFF watershed (n = 13)	
		Min [pM]	Max [pM]	Min [pM]	Max [pM]
PFNS	13	0.06	0.53	ND <sup>2</sup>	ND
PFDS	7	0.19	0.49	ND	ND
PFDA	57	0.12	8.41	0.13	0.21
PFUA	31	0.05	9.48	0.07	0.22
PFDoA	9	0.06	0.17	ND	ND
PFTriA	9	0.04	0.16	ND	ND
PFTA	9	0.03	0.12	0.04	0.04
4:2 FTSA	48	0.17	2.14	ND	ND
6:2 FTSA	43	1.82	86.39	ND	ND
8:2 FTSA	41	0.11	8.32	ND	ND
FBSA	22	0.55	15.84	0.30	1.72
FHxSA	7	1.57	22.44	ND	ND
FOSA	46	0.07	7.76	ND	ND
N-MeFOSAA	2	0.11	0.11	ND	ND
N-EtFOSAA	9	0.08	0.21	0.12	0.12
NaDONA	0	ND	ND	ND	ND

376 <sup>1</sup>Across all samples in the study377 <sup>2</sup>ND = not detected

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379 **Table S12. Fluxes of PFAS from the *Quashnet R.* to *Waquoit Bay*, Cape Cod, MA, U.S.A.**

PFAS	Concentration [pM]	Flux [kmol yr <sup>-1</sup> ]	Kruskal-Wallis <i>p</i> -value <sup>1</sup>
PFBA	25.8±21.3	0.70±0.53	0.13
PFPeA	34.3±3.11	0.93±0.08	0.23
PFHxA	38.8±2.03	1.05±0.05	0.24
PFHpA	17.5±3.34	0.47±0.08	0.16
PFOA	29.6±2.40	0.80±0.06	0.16
PFNA	14.5±4.01	0.39±0.1	0.10
PFBS	11.0±1.79	0.30±0.04	0.29
PFPeS	9.08±0.87	0.25±0.02	0.46
1-PFHxS	83.5±8.29	2.25±0.20	0.42
br-PFHxS	14.7±1.66	0.40±0.04	0.51
ΣPFHxS	98.3±9.23	2.65±0.23	0.54
PFHpS	3.20±0.39	0.09±0.01	0.39
1-PFOS	75.4±25.2	2.04±0.62	0.19
br-PFOS	33.0±6.38	0.89±0.16	0.21
ΣPFOS	108±31.7	2.93±0.78	0.19
Σ <sub>13</sub> PFAAs	390±40.2	10.53±0.99	0.35
4:2 FT precursors	3.70±0.73	0.10±0.02	0.35
6:2 FT precursors	4.71±2.21	0.13±0.05	0.36
8:2 FT precursors	6.49±9.10	0.18±0.22	0.23
C4 ECF precursors	14.9±5.84	0.40±0.14	0.23
C5 ECF precursors	8.14±3.28	0.22±0.08	0.23
C6 ECF precursors	56.7±22.6	1.53±0.56	0.23
C7 ECF precursors	0.99±0.41	0.03±0.01	0.23
C8 ECF precursors	1.60±0.68	0.04±0.02	0.24
Σprecursors	140±53.7	3.77±1.32	0.23
Total PFAS	530±91.9	14.30±2.27	0.32
EOF <sup>2</sup>	20.5±8.20	9.86±3.95	NA

380 <sup>1</sup>Performed on all samples, field duplicates, and extraction duplicates grouped by sampling date  
381 to determine significant differences in concentration between sampling dates (08-2017, 07-2018,  
382 10-2018, 02-2019, 04-2019, 07-2019). P-values corrected for multiple comparison using false  
383 discovery rate (FDR) correction.

384 <sup>2</sup>Calculated from the two (of four) samples above LOD

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390 **Table S13. Results of the Principal Component Analysis on all Surface Water Samples.**

	Surface waters PC 1	AFFF and AFFF- impacted surface water PC 1	AFFF and AFFF- impacted surface water PC 2
Eigenvalue	7.13	8.22	5.56
Variance explained	34%	40%	17%
<i>loadings</i>			
PFBA	0.78	-0.75	-0.33
PFPeA	0.26	0.51	-0.20
PFHxA	0.02	-0.24	0.10
PFHpA	0.22	-0.54	-0.50
PFOA	-0.08	0.05	-0.20
PFNA	-0.48	-0.46	-0.59
PFBS	0.91	0.30	-0.68
PFPeS	0.46	-0.14	-0.86
1-PFHxS	-0.86	0.90	-0.35
br-PFHxS	0.24	0.78	-0.36
PFHpS	0.15	0.76	-0.44
1-PFOS	-0.95	0.97	-0.05
br-PFOS	-0.92	0.96	-0.19
4:2 FT	0.82	-0.77	0.53
6:2 FT	0.09	-0.78	0.57
8:2 FT	0.11	-0.54	0.35
C4 ECF	0.38	0.46	0.85
C5 ECF	-0.57	0.55	0.76
C6 ECF	-0.89	0.45	0.79
C7 ECF	0.71	0.57	0.24
C8 ECF	0.33	0.58	0.61

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392 **Table S14. Fractional AFFF Composition Used in Principal Component Analysis.**

393 See accompanying Excel Spreadsheet.

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395 **Table S15. Estimation of Unexplained PFAS Measured Using <sup>19</sup>F Nuclear Magnetic**  
 396 **Resonance (NMR) in Moody et al.<sup>1</sup>**

Sample	Reported in Moody et al.		Estimated total PFAS <sup>2</sup> [ug/L]		Estimated unexplained PFAS <sup>3</sup> [%]	
	PFOS [ug/L]	<sup>19</sup> F NMR [ug/L]	if 30% PFOS <sup>4</sup>	if 50 % PFOS <sup>5</sup>	if 30% PFOS	if 50 % PFOS
2-1	0	0	0	0	NA	NA
2-2	89.2	311	297	178	4	43
2-3	113	417	377	226	10	46
2-4	126	539	420	252	22	53
2-5	174	900	580	348	36	61
2-6	2210	17000	7370	4420	57	74
3-1	0	0	0	0	NA	NA
3-3	201	931	670	402	28	57
3-4	66.7	267	222	133	17	50
Average					25±18	56±11

397 <sup>1</sup>Data from Moody et al.<sup>27</sup>

398 <sup>2</sup>Estimated detectable PFAS = Concentration of PFOS/Composition of PFOS

399 <sup>3</sup>Estimated unexplained PFAS =100 - Estimated detectable PFAS/Concentration of PFAS  
 400 measured by <sup>19</sup>F NMR

401 <sup>4</sup>Lower bound of PFOS composition in 3M AFFF (Table S14)

402 <sup>5</sup>Upper bound of PFOS composition in 3M AFFF (Table S14)

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404 **Table S16. Fluorinated Pharmaceuticals Characterized in the Broader Cape Cod,**  
 405 **MA,U.S.A Region.**

Fluorinated pharmaceutical	MW [g mol <sup>-1</sup> ]	number of fluorines	Private wells <sup>1</sup>		Public wells <sup>2</sup>	
			MRL <sup>3</sup> [ng L <sup>-1</sup> ]	EOF <sup>4</sup> [pM F]	MRL <sup>3</sup> [ng L <sup>-1</sup> ]	EOF <sup>4</sup> [pM F]
Ciprofloxacin	331.0	1	18.0	52.6	50.0	153
Dexamethasone	392.5	1	2.0	5.26		
Enrofloxacin	359.4	1	32.0	89.5	50.0	137
Fluoxetine (Prozac)	309.3	3	0.3	5.26	1.0	10.5
Norfloxacin	319.3	1	24.0	73.7	50.0	158
<b>Sum</b>				<b>226</b>		<b>458</b>

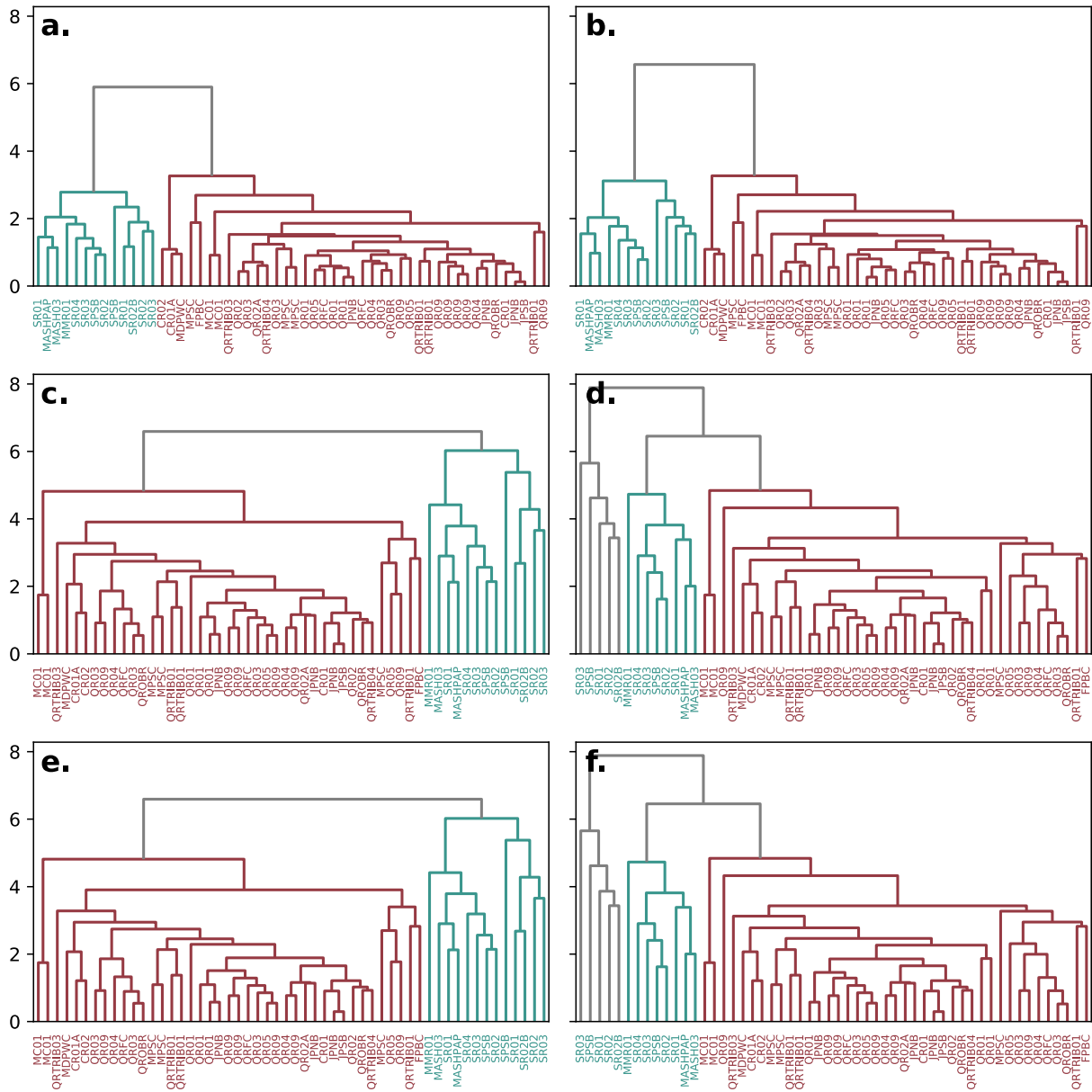
406 <sup>1</sup>Reported in Schaider et al. 2016<sup>28</sup>

407 <sup>2</sup>Reported in Schaider et al. 2014<sup>29</sup>

408 <sup>3</sup>No fluorinated pharmaceutical was detected above the method reporting limit (MRL) in either  
 409 study

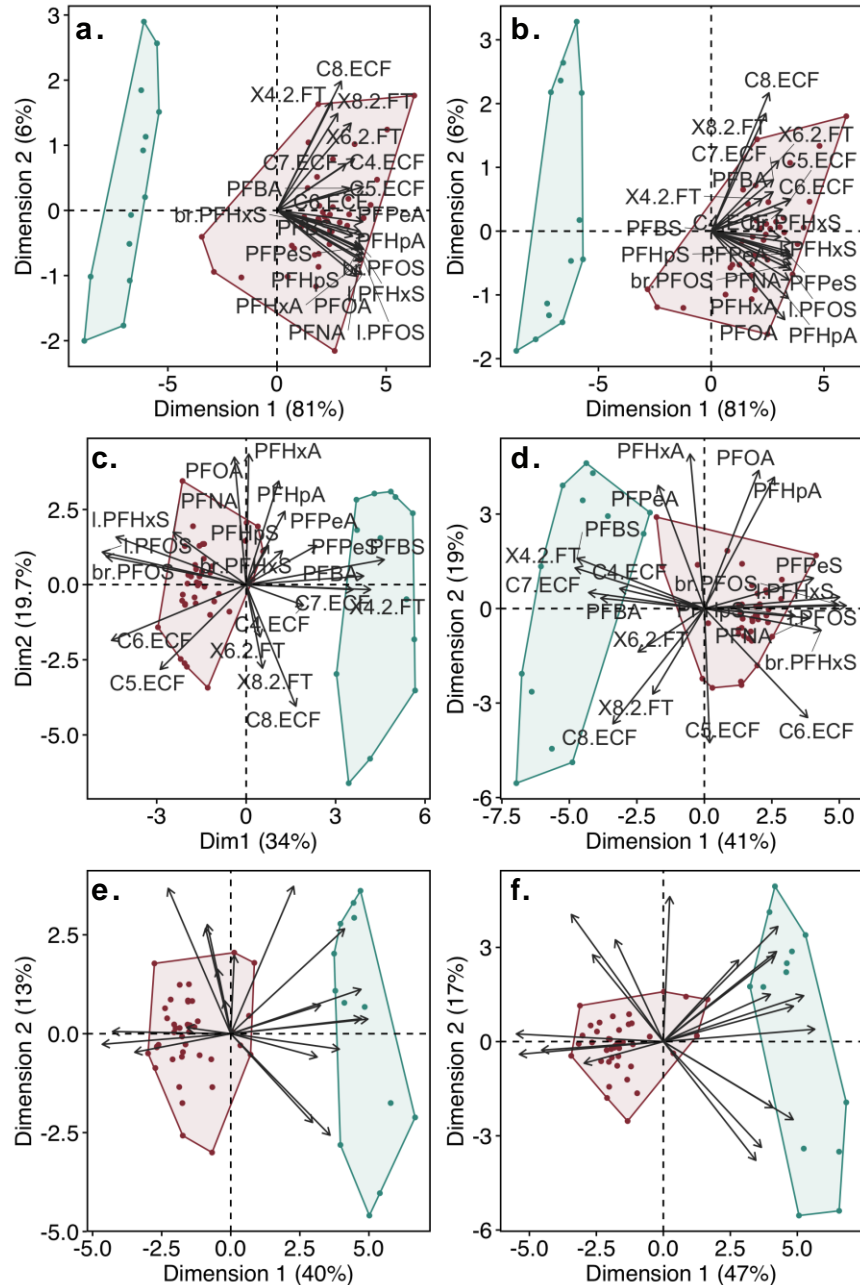
410 <sup>4</sup>EOF<sub>i</sub> = MRL<sub>i</sub>/MW<sub>i</sub>\*n<sub>fluorines,i</sub>

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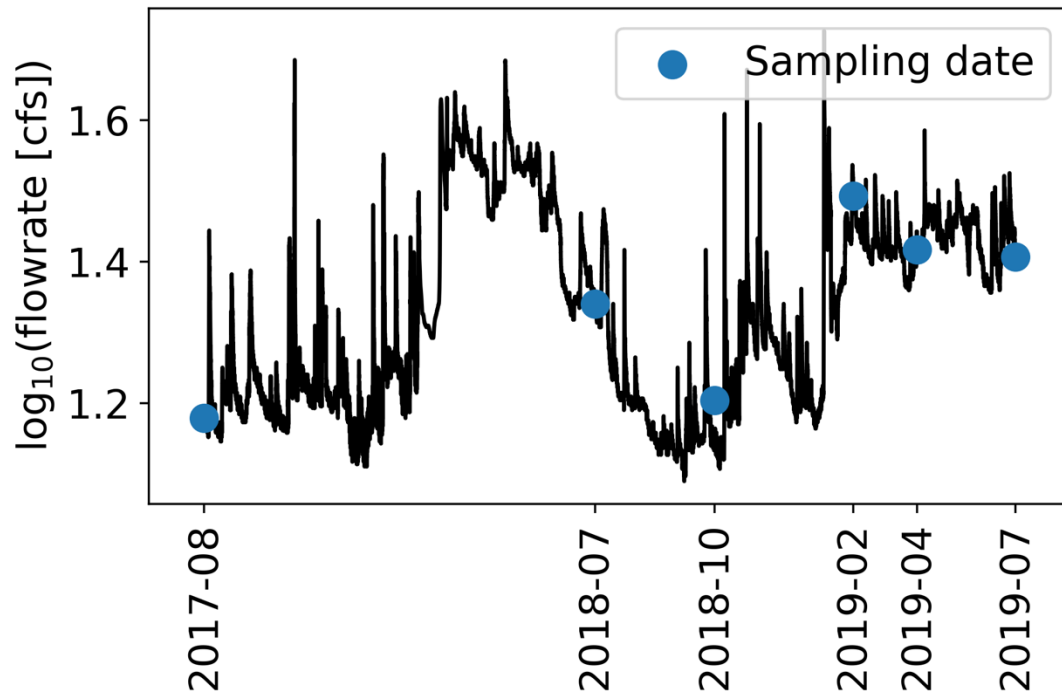


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 413 **Figure S1. Hierarchical Clustering of Surface Water Data Using the UPGMA Algorithm.**  
 414 Red indicates samples from FTA watersheds and blue indicates samples from non-FTA  
 415 watersheds. Panel (a.): log transformed molarity, non-detects imputed using regression on order  
 416 statistics (ROS). Panel (b.): log transformed molarity, non-detects replaced by  $MDL/\sqrt{2}$ . Panel  
 417 (c.): molar composition transformed using the centered log-ratio method, non-detects imputed  
 418 using ROS. Panel (d.): molar composition transformed using the centered log-ratio method, non-  
 419 detects imputed with  $MDL/\sqrt{2}$ . Panel (e.): molar composition transformed using the isometric  
 420 log-ratio method, non-detects imputed using ROS. Panel (f.): molar composition transformed  
 421 using the isometric log-ratio method, non-detects imputed with  $MDL/\sqrt{2}$ .

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 426 **Figure S2. Loading Vectors and Scores for the First Two Principal Component Axes of**  
 427 **Surface Water Samples.** Samples from watersheds with (red) and without (blue) an AFFF  
 428 source zone are distinguished by the minimum convex hull encircling all data within the group.  
 429 (a) Log transformed molarity and loading vectors where non-detects imputed using ROS. (b) Log  
 430 transformed molarity and loading vectors where non-detects imputed with MDL/ $\sqrt{2}$ . (c)  
 431 Compositional data transformed using the centered log-ratio method and loading vectors where  
 432 non-detects imputed using ROS. (d) Compositional data transformed using the centered log-ratio  
 433 method and loading vectors where non-detects imputed with MDL/ $\sqrt{2}$ . (e) Compositional data  
 434 transformed using the isometric log-ratio method and loading vectors where non-detects imputed  
 435 using ROS. (f) Compositional data transformed using the isometric log-ratio method and loading  
 436 vectors where non-detects imputed with MDL/ $\sqrt{2}$ .



437  
 438 **Figure S3. Flowrate at QR09 (USGS Gage 011058837) in the *Quashnet R.*** Flowrate at the  
 439 site during the duration of the study ranged from 12.3 to 53.2 cubic feet per second (cfs) with  
 440 mean 22.4 cfs.  
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