Supporting Information

Perfluoroalkylphosphinic acids in northern pike (*Esox lucius*), double-crested cormorants (*Phalacrocorax auritus*), and bottlenose dolphins (*Tursiops truncatus*) in relation to other perfluoroalkyl acids

Amila O. De Silva[a*], Christine Spencer[a], Ki Chung D. Ho[a], Mohammed Al Tarhuni[a], Christopher Go[a,b], Magali Houde[c], Shane R. de Solla[a], Raphael A. Lavoie[d], Laura E. King[a], Derek C.G. Muir[a], Patricia A. Fair[e], Randall S. Wells[f, g], Gregory D. Bossart[g]

[a]Environment and Climate Change Canada, Canada Centre for Inland Waters, Burlington, ON, Canada
[b] present address: University of Toronto, Toronto, ON, Canada
[c] Environment and Climate Change Canada, Centre Saint-Laurent, Montréal, QC, Canada
[d] Biology Department, Queen's University, 116 Barrie Street, Kingston, Ontario, Canada, K7L3N6
[e] Center for Coastal Environmental Health and Biomolecular Research, NOS, NOAA, 219 Fort Johnson Road, Charleston, SC 29142
[f]Chicago Zoological Society's Sarasota Dolphin Research Program, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236 USA
[g] Georgia Aquarium, 225 Baker Street, Atlanta, GA 3031

*corresponding author at 867 Lakeshore Road, Burlington, ON, Canada, L7S 1A1. Tel.:+1 905-220-9508
Email address: amila.desilva@canada.ca

Description of Cormorant Colony Sampling Areas

Hamilton Harbour (HH) is an Area of Concern (AOC) in the eastern end of Lake Ontario. There are 3 major WWTPs. It is the heaviest used port on the Canadian side of the Great Lakes. The population of the city of Hamilton is 500,000 people. This is a heavily industrialized site with Canada's largest steel smelters.

Mohawk Island (MI) is located on the north shore of Lake Erie. The closest city is Dunnville (population 6000), located 13 km away from the colony. Mohawk Island receives inputs from the Grand River which flows through Kitchener, Cambridge and Waterloo. The Grand River receives effluent from 40 different WWTPs.

Lake Nipissing (NI) is a site with little anticipated sources of these compounds. Lake Nipissing has a surface area of 873 km^2. The town of North Bay (population 53,000) is the only major city on the lake. Industry is limited in this area and mainly focused on forestry. The lake receives WWTP effluent from North Bay.

Bergin Island (BI) is downstream of the St. Lawrence (Cornwall, population 47000) AOC. Cornwall is a small industrial town with paper mill and cotton processing (closed since 2006 and 1992 respectively). On the American side of the St. Lawrence river, there was heavy industrial activity contributing to a Superfund site.

Scotch Bonnet Island (SBI) is located in the Bay of Quinte on the north shore of Lake Ontario. The closest major city is Trenton (population 20,000) with a military base within 20 km.

Supplementary Information - Methods

Acetonitrile protein precipitation. The acetonitrile protein precipitation step is necessary for recovery of PFPAs and PFPIAs when using spike and recovery experiments in bovine plasma. When the acetonitrile was omitted, PFPA and PFPIA were not detected in the final extract. These observations are consistent with earlier methods wherein successful extraction of the same compounds from fish fillet homogenate was dependent on acetonitrile precipitation[11]. Recoveries in bovine plasma, expressed as a percentage, are in Table S1. Method blanks were clear of PFPIA, PFPAs, PFSAs, and PFCAs with the exception of PFOA. The highest method blank signal was calculated to be 0.0125 ng/ml in a 1 ml extract corresponding to 0.063 ng/g PFOA in a theoretical plasma sample. Sample concentrations were not blank-subtracted since observed levels were at least 80% higher than measured blank value.

Matrix matched vs. solvent based calibration curve. Electrospray ionization is known for matrix suppression or enhancement of analyte signals. Therefore, external calibration curves in solvent are typically not suitable for quantifying signals in sample extracts. Instead, isotopically labelled surrogates are used to correct for matrix effects or the standard addition method is employed or a matrix-matched calibration curve. Since isotopically labelled surrogates of PFPIAs are not commercially available, structurally similar isotopically labelled PFCA, PFSAs, and diPAPs were evaluated for quantifying PFPIAs using relative response. These were found to over-estimate PFPIAs. Based on instrumental response of post-extraction spikes, PFPIAs do not have an appreciable matrix effect. The C10 PFPA had slight signal suppression 10-20% matrix enhancement in the final blood plasma extract (Table S1). This is evident in PFPIA calibration curves made in solvent versus blood plasma extract (Figure S2). The curves yield equivalent response with some deviation possibly occurring above 10 ng/ml. In the current research, PFPIAs were less than 10 ng/ml in final extracts and therefore not expected to be impacted by matrix effects but as a precaution, quantification was accomplished using the matrix-matched calibration curves. No PFPIAs or PFPAs were detected in method blanks.

PFCA and PFSA Instrumental analysis. All PFCA/PFSA extracts were analyzed on the same instrument also operated in negative electrospray ionization mode. Analytes were separated on a Acquity UPLC ® BEH C18 2.1x100 mm, 1.7 um stationary phase using a water – methanol 0.1 mM ammonium acetate gradient. The initial conditions of the gradient were 75/25 water/methanol at 0.4 ml/min and held for 0.5 min before ramping to 15/85 water/methanol at 5 min. The mobile phase was further increased to 100% methanol at 5.1 min and held for 0.5 min at 0.4 ml/min before increasing speed at 7.0 min to 0.55 ml/min. At 9 min, the flow rate dropped to 0.4 ml/min and composition to 75/25 water/methanol, followed by a 3 minute equilibration period. Injection volumes were 9 µl.

PFPIA/PFPA Instrumental analysis. The mobile phase consisted of A (5% methanol in 2 mM aqueous ammonium acetate adjusted to pH 8 with ammonia) and B (100% methanol). The column was BEH C18 1.7 um, 2.1 x 50 mm. Another stationary phase (P/N 0149120181, Waters) was placed just upstream of the injector to isolate the analytical peak from background contamination. The gradient conditions began at 80% A, decreased to 5% A at 1 min, held until 1.2 min, ramped to 100% B at 1.4 min, held to 2 min, with a constant flow rate of 0.3 ml/min. From 2 min to 4 min, the mobile phase was 100% B at 0.55 ml/min, then ramped to 50% B at 4.2 min using 0.3 ml/min before returning to the initial conditions at 4.4 min with a 2.6 min equilibration time.

QA/QC. For each batch of 20 samples, the following was conducted: method blanks (n=2), spike and recovery samples (n=2), matrix spike (n=2). Method blanks consisted of extraction using the full method but with no sample. Bovine serum (Sigma Aldrich, Oakville, ON) was used for spike and recovery experiments in which 30 µl of methanolic standards (170-200 ng/ml each of 6:6, 6:8, 8:8 PFPIA and C6, C8, C10 PFPA) were added to 1 ml of bovine serum. The matrix spike entailed extracting bovine serum without addition of standards until the final step of reconstituting just prior to injection in order to ascertain matrix effects in the mass spectrometer without obscuring from extraction efficiency (Table S1). For quantitation, a 15 level calibration curve in solvent was used ranging from 0.1 ng/ml to 30 ng/ml PFCAs and PFSAs, and 0.2 ng/ml of isotopically labeled PFCAs and PFSAs in each calibration standard. Due to the absence of internal standards for PFPIAs

and PFPAs, a 15-level PFPIA and PFPA matrix-matched calibration curve was prepared using extracted bovine plasma with concentrations ranging from 0.05 ng/ml to 25 ng/ml PFPA (Figure S2). The limit of detection (LOD) was represented by the lowest calibration standard in bovine plasma extract with a signal to noise ratio (S/N) of at least 10 (Table S2). These LOD values were translated to ng PFAA per g plasma: 2-7 ng/g PFPA, 0.025 ng/g for each PFPIA, 0.07 to 0.3 ng/g PFCAs and PFSAs.

Analysis of Biological Parameters. A summary of the biological data for pike, cormorants, and dolphins is found in Table 1. Body length, mass, and condition were normally distributed within each site and taken together, based on Komogorov-Smirnov test where K-S distance < 1 and $p>0.05$ for pike, dolphin and cormorants. In pike, body length ($p= 0.82$), mass ($p=0.27$), body condition ($p=0.18$) and $\delta^{15}N$ ($p=0.067$) were not statistically different between the two locations. The mean ± standard deviation $\delta^{13}C$ was significantly ($p<0.05$) more negative for pike collected at the mouth of the Ottawa River (LDM; -24.1±1.12) compared to the site downstream of the St. Lawrence River (IV, -18.0±0.734). For dolphins, age ($p=0.86$) and body length ($p=0.09$) were not significantly different among the three dolphin groups (CH, SA 2004, and SA 2009), however the CH dolphins were significantly higher ($p<1.3 \times 10^{-4}$) in mass compared to both SA dolphin sampling years. CH dolphins also had a significantly greater body condition index ($p<0.0001$) than the SA dolphins. Tarsus length of the cormorants was significantly ($p<0.0001$) lower in birds from MI compared to other locations, however, body mass was not significantly different among any of the sites ($p = 0.49$). Body condition was significantly ($p<0.0079$) different between MI, which had the highest body condition index and SBI, which had the lowest body condition index. There was no significant difference in body condition index between the intermediary sites.

Stable isotopes analysis. Stable isotopes of nitrogen and carbon for all pike, Charleston dolphins, some Sarasota dolphins and some cormorants were determined using dried (45°C) and ground muscle (pike), blood (cormorants) or skin (dolphin) samples. Details regarding stable isotopes analysis of Charleston dolphins were previously published[32]. Analyses for pike muscle were completed at the University of Waterloo-Environmental

Isotope Laboratory (Waterloo, Ontario, Canada), for Charleston dolphins was by the institute of Ecology Stable Isotope Laboratory, University of Georgia, and for cormorants at the Queen's Facility for Isotope Research (Kingston, Ontario, Canada) on a Thermo Finnigan Mat Delta Plus Mass Spectrometer coupled to a Carlo Erba Elemental Analyzer (NA1500). The ratio of stable isotopes in parts per thousand (‰) are expressed as $\delta^{13}C$ or $\delta^{15}N$, where $\delta = [(R_{sample}/R_{reference})-1]\times 1000$ where $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$. Staple isotopes were not available for 2009 Sarasota dolphins nor cormorants from BI, SBI, and MI.

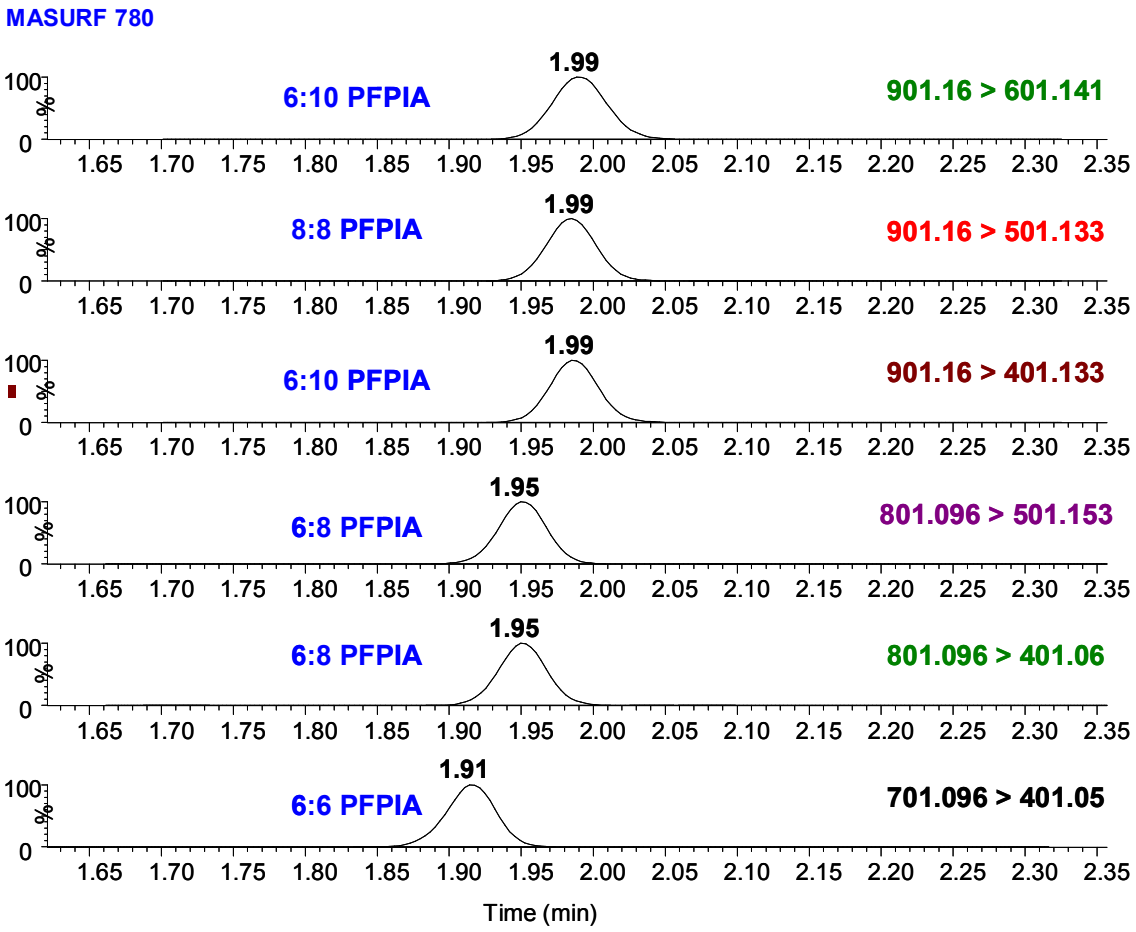

Figure S1. Separation of PFPIA congeners in commercial fluorosurfactant mixture (Masurf 780) by UHPLC-MS/MS. The numbers above the peak represent the retention time in minutes and the MRM transitions are shown on the right of the chromatograms.

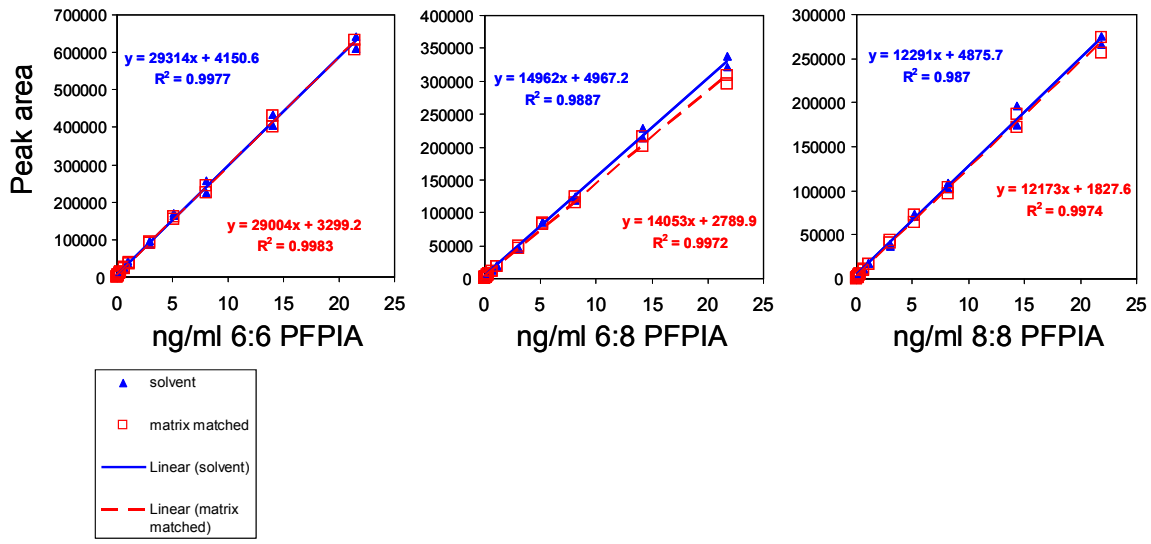

Figure S2. Calibration curves for 6:6 PFPIA, 6:8 PFPIA, and 8:8 PFPIA made in solvent (filled triangle, solid line, equation in upper left) and bovine plasma extract (open square, dashed line, equation in bottom right).

Table S1. Mean (standard deviation) extraction recovery and matrix recovery for PFPA, PFPIA, PFCAs, and PFSAs in bovine plasma.

Analyte	pre-extraction spike recovery (%)	post-extraction spike recovery (%)
6:6 PFPIA	93 (6)	104 (5)
6:8 PFPIA	95 (7)	108 (5)
8:8 PFPIA	96 (5)	107 (5)
C6 PFPA	61 (7.4)	86 (5.4)
C8 PFPA	62 (1.3)	69 (11)
C10 PFPA	83 (13)	78 (5.2)
PFBA	106 (13)	111 (12)
PFPeA	129 (8.8)	130 (9.1)
PFHxA	120 (6.6)	125 (5.8)
PFHpA	120 (5.0)	114 (1.5)
PFOA	143 (7.3)	140 (6.1)
PFNA	81 (12)	85 (13)
PFDA	114 (5.1)	112 (5.1)
PFUnA	118 (6.9)	123 (5.7)
PFDoA	121 (6.9)	111 (2.3)
PFTrA	96 (1.3)	96 (4.0)
PFTeA	107 (7.5)	106 (6.2)
PFBS	95 (4.0)	92 (6.3)
PFHxS	109 (4.0)	111 (4.7)
PFOS	107 (8.7)	111 (7.3)
PFDS	102 (2.2)	97 (7.9)
PFECHS	97 (2.5)	97 (2.8)

[a] Recovery = peak area$_{sample}$ ÷ peak area$_{standard}$ x 100

Table S2. Limit of Detection of PFPA, PFPIA, PFCA, and PFSA based on signal to noise of standard spiked into bovine plasma extract.

Analyte	LOD (ng/g)	S/N
C6 PFPA	3.3	14
C8 PFPA	2.3	10
C10 PFPA	6.7	42
6:6 PFPIA	0.025	14
6:8 PFPIA	0.025	44
8:8 PFPIA	0.025	26
PFBA	0.26	10
PFPeA	0.18	10
PFHxA	0.22	13
PFHpA	0.20	10
PFOA	0.067	10
PFNA	0.093	10
PFDA	0.18	11
PFUnA	0.21	11
PFDoA	0.093	10
PFTrA	0.34	10
PFTeA	0.18	10
PFOS	0.10	30
PFDS	0.17	10
PFECHS	0.17	22
PFHxS	0.16	10
PFBS	0.20	10

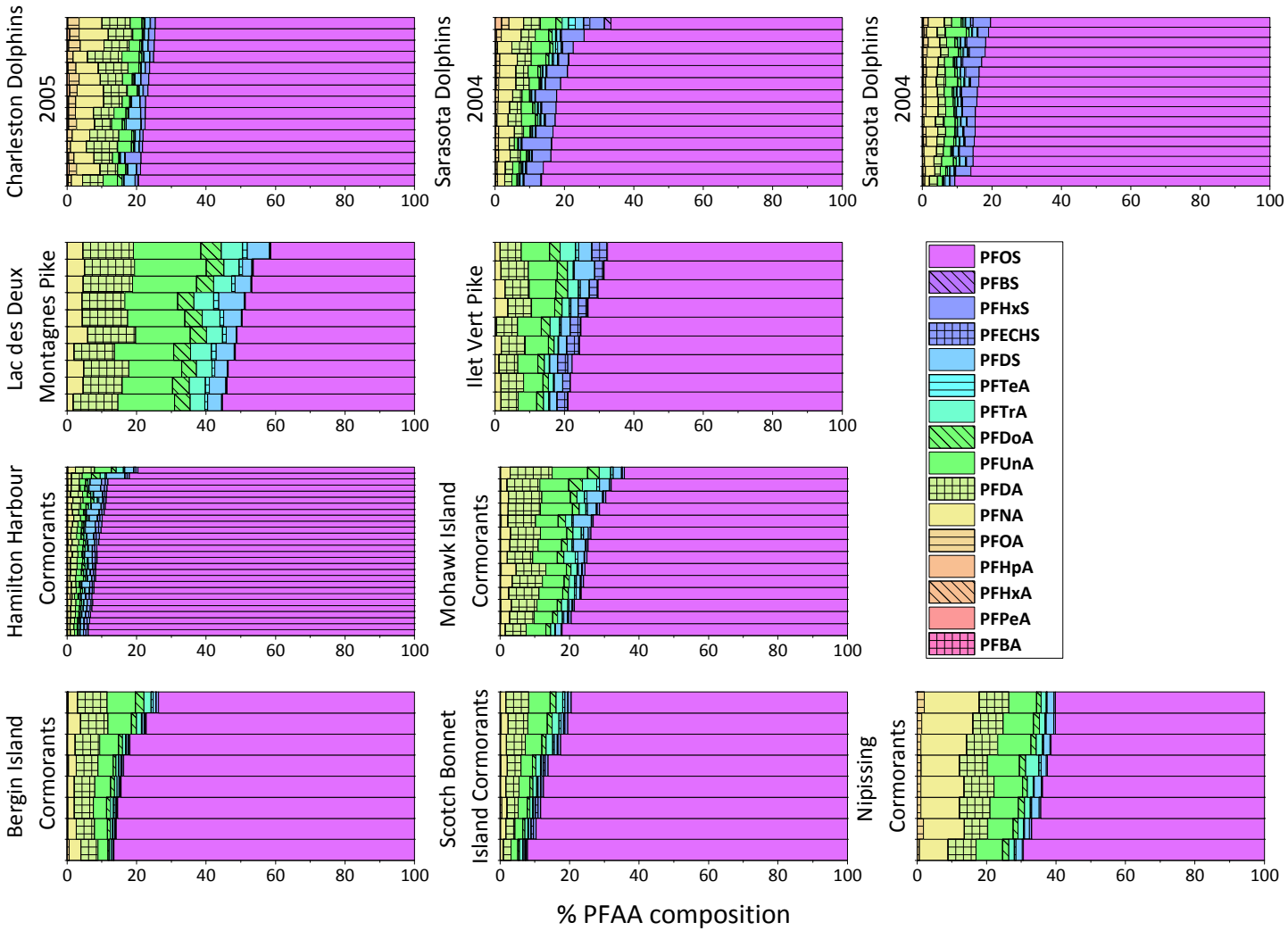

Figure S3. Distribution of PFCAs and PFSAs in dolphins (row 1), pike (row 2) and cormorants (rows 3 to 4).

Table S3. Statistical parameters assessing correlation ($p<0.05$) for log normalized ΣPFCAs and ΣPFSAs with age, length, and body mass in dolphins. R^2 and Pearson Correlation Coefficient (R_P) shown for significant correlations ($p<0.05$).

PFAA	Parameter	p	R^2, R_P	Degrees of freedom
Location: CH				
ΣPFCA	Age	0.00188	0.600, -0.775	11
ΣPFSA	Age	0.00260	0.577, -0.759	11
ΣPFCA	Length	0.296		
ΣPFSA	Length	0.344		
ΣPFCA	Mass	0.0599		
ΣPFSA	Mass	0.0892		
Location and sampling year: SA 2009				
ΣPFCA	Age	1.08×10^{-4}	0.726, -0.852	12
ΣPFSA	Age	1.59×10^{-4}	0.709, -0.842	12
ΣPFCA	Length	0.0067	0.472, -0.687	12
ΣPFSA	Length	1.66×10^{-4}	0.707, -0.841	12
ΣPFCA	Mass	0.0464	0.314, -0.560	11
ΣPFSA	Mass	0.00267	0.575, -0.758	11
Location and sampling year: SA 2004				
ΣPFCA	Age	0.00160	0.548, -0.740	13
ΣPFSA	Age	0.00228	0.524, -0.724	13
ΣPFCA	Length	0.00346	0.445, -0.667	15
ΣPFSA	Length	0.00363	0.441, -0.664	15
ΣPFCA	Mass	0.00383	0.437, -0.661	15
ΣPFSA	Mass	0.00316	0.451, -0.672	15

Table S4. Mean (± standard deviation) PFPIA compositional analysis expressed as a percentage in cormorants.

Sampling area	**6:6 PFPIA**	**6:8 PFPIA**	**8:8 PFPIA**	**6:10 PFPIA**
HH	30 (5.2)	61 (3.6)	6.2 (2.1)	3.3 (1.2)
MI	35 (3.8)	54 (2.6)	7.6 (1.7)	3.9 (1.0)
NI	40 (5.9)	55 (4.1)	3.3 (1.4)	1.8 (0.86)
BI	41 (5.5)	50 (5.8)	5.6 (1.9)	3.6 (2.1)
SBI	35(2.0)	57 (1.8)	4.5 (0.35)	2.7 (0.32)
p-value	2.0×10^{-4}	8.7×10^{-10}	1.1×10^{-5}	0.0045
Ranking from Highest to Lowest Proportion*	$BI_{a\,c}$, $NI_{a\,c}$, $SBI_{a\,c}$, $MI_{a\,d}$, HH_b	HH_a, $SBI_{a\,c}$, MI_b, NI_b, $BI_{b\,d}$	MI_a, $HH_{a\,c}$, BI_b, SBI_b, $NI_{b\,d}$	MI_a, BI_a, $HH_{a\,b}$, SBI_a, $NI_{b\,c}$

* Letter subscripts indicate statistical differences. One-way ANOVA using Tukey post hoc test.

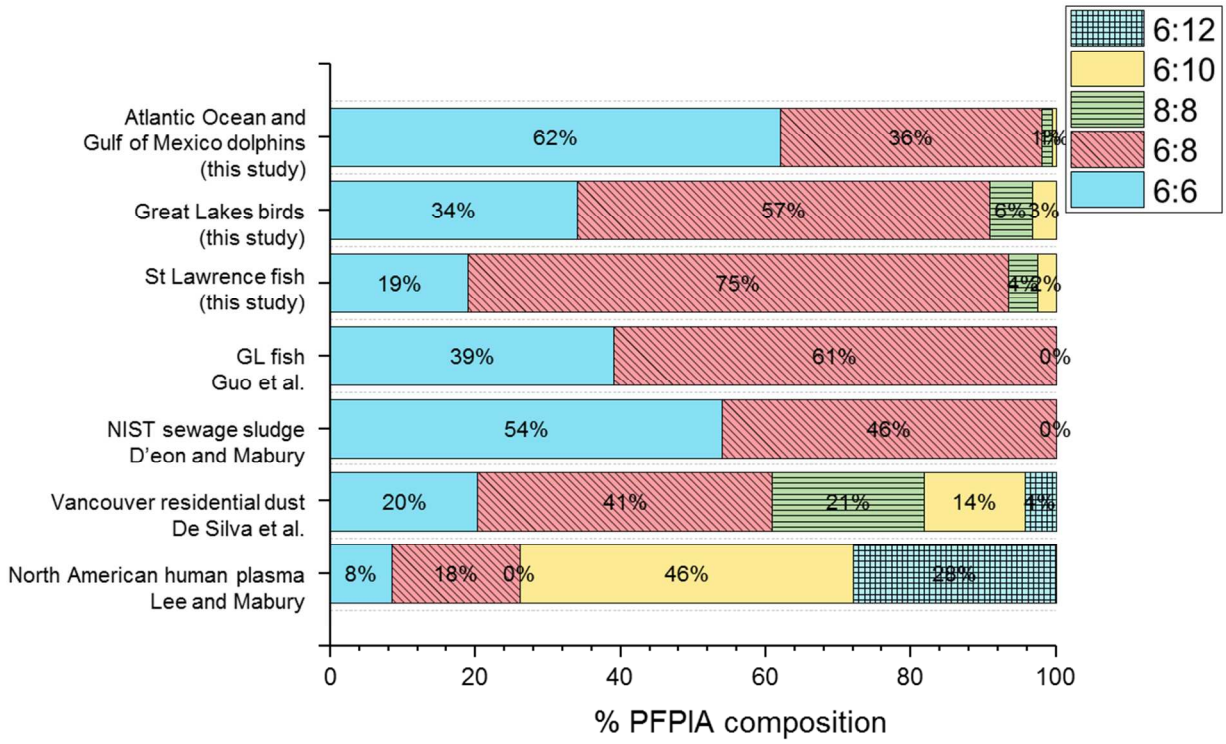

Figure S4. Comparison of PFPIA homolog patterns in biota from this study with published studies in Great Lakes (GL) trout, NIST SRM sewage sludge, residential dust from Vancouver, and human plasma. Note: Guo et al. did not monitor 6:10 or 6:12 PFPIA.

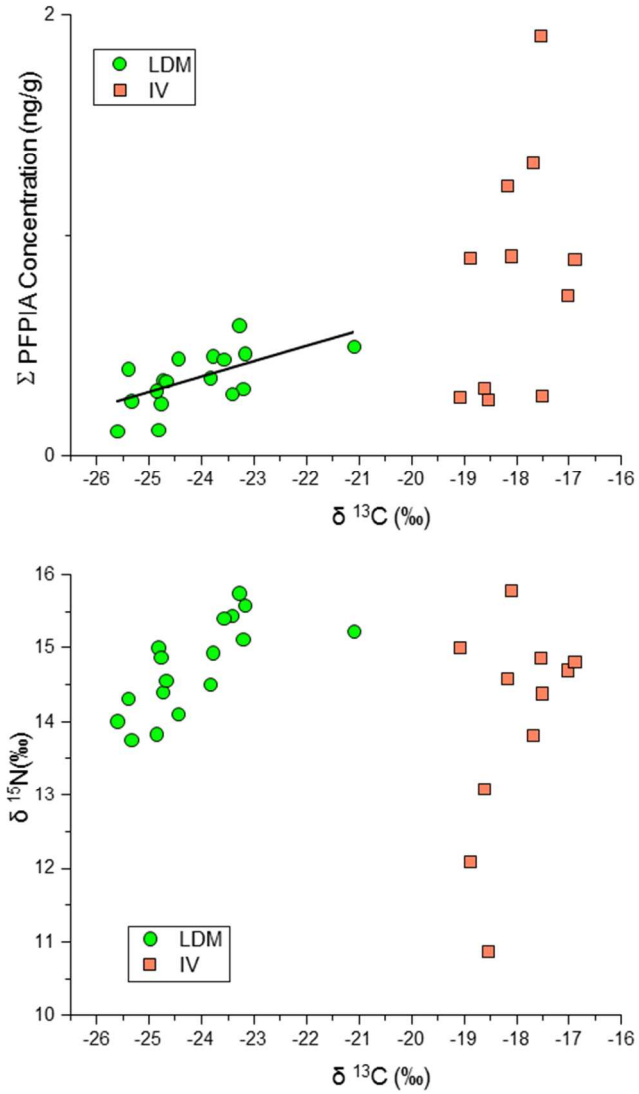

Figure S5. Sum of PFPIA concentration in pike plasma from LDM (circles) and IV (squares) with carbon isotope ratio, top, and carbon and nitrogen stable isotope plot for pike, bottom.