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Legacy and Novel Per- and Polyfluoroalkyl Substances (PFAS) in Juvenile Seabirds from the US Atlantic Coast

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Abstract

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic, globally distributed chemicals. Legacy PFAS, including perfluorooctane sulfonate (PFOS), have been regularly detected in marine fauna but little is known about their current levels or the presence of novel PFAS in seabirds. We measured 36 emerging and legacy PFAS in livers from 31 juvenile seabirds from Massachusetts Bay, Narragansett Bay, and the Cape Fear River Estuary (CFRE), USA. PFOS was the major legacy perfluoroalkyl acid present, making up 58% of concentrations observed across all habitats (range: 11 – 280 ng/g). Novel PFAS were confirmed in chicks hatched downstream of a fluoropolymer production site in the CFRE - a perfluorinated ether sulfonic acid (Nafion byproduct-2; range: 1 – 110 ng/g) and two perfluorinated ether carboxylic acids (PFO_4DA and PFO_5DoDA; PFO_5DoDA range: 5 – 30 ng/g). PFOS was inversely associated with phospholipid content in livers from CFRE and Massachusetts Bay individuals, while $\delta^{13}C$, an indicator of marine vs. terrestrial foraging, was positively correlated with some long-chain PFAS in CFRE chick livers. These results detail concentrations of legacy and novel PFAS across different marine

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Author Contributions

The manuscript was primarily drafted by A. Robuck and finalized through contributions of all authors. Sample analysis and data interpretation were carried out by A. Robuck through contributions of all authors. All authors have given approval to the final version of the manuscript.

The following files are available free of charge.

Details about sample preparation, extraction methods, instrumental analysis, quality assurance and quality control, supplementary figures (PDF)

Monitored ions, extraction performance, raw data, summary statistics, data inputs for comparison and BAF calculations (Excel workbook)

ecosystems along the US Atlantic East Coast. There is also an indication that seabird phospholipid dynamics are negatively impacted by PFAS, which should be further explored given the importance of lipids for seabirds.

Keywords

PFAS; PFEAs; PFESA; PFECA; PFOS; bioaccumulation; seabirds

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic organic contaminants used extensively in a variety of commercial, industrial, and military applications globally.[1] Continued, widespread use of PFAS with diverse formulae has resulted in the detection of multiple PFAS into the global environment[2–4]. Industrial production has shifted away from long-chain chemistries to replacements with fewer than seven fluorinated carbons, polyfluorinated structures, and/or structurally modified iterations of perfluoroalkyl acids (PFAAs) such as perfluoroalkyl ether acids (PFEAs) incorporating different numbers of ether linkages[5,6]. PFEAs include both perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs).

Both legacy PFAS and new formulations may be released to the environment via both direct or indirect discharges, or some interplay of the two[1,7–9]. The continued inputs of PFAS act in tandem with their extreme environmental persistence to sustain the proliferation of these compounds within terrestrial[10], marine[11], estuarine[12], freshwater[13], cryosphere[14], and atmospheric compartments[14,15] worldwide. PFAS compounds can permeate biota within these compartments, including marine invertebrates[16], fish[17], birds[18–21], and marine mammals[22]. This environmental and biological ubiquity presents challenges for ecological health, as substantial evidence suggests a variety of legacy and new PFAS have the potential for adverse effects across multiple taxa[23,24].

Toxicology and field studies suggest associations between PFAS concentrations and reproductive parameters, morphometric characteristics, and metabolic processes in avifauna[25–31]. Controlled studies across multiple taxa, including birds, also suggest variable associations between PFAS and lipid production, metabolism, and storage pathways[32–34]. Yet comprehensive assessments of PFAS exposure or potential toxicity in wild avifauna are stymied by a lack of foundational data detailing the environmental occurrence of diverse PFAS across multiple habitats and bird taxa. Transfer of PFAS via trophic interactions is likewise still under investigation, with habitat- and food web-specific trends apparent[35–37]. Furthermore, there is a dearth of data detailing PFAS concentrations in avifauna near industrial point sources, or potential impacts related to chronic, elevated exposure from such direct or substantial discharges[29,30,38]. This stands as a significant data gap considering that effective protection of endangered species and habitat surrounding contaminated sites relies on understanding exposure and impacts in bird species and other keystone wildlife.

The oceans are thought to be the final sink for legacy and novel PFAS compounds, receiving inputs via rivers/estuaries and atmospheric deposition[2]. Long-lived seabirds present an

opportunity to assess and compare PFAS detection and trends across a range of coastal and oceanic habitats. In their given marine habitats, seabirds act as integrative sentinels due to their generally predictable life histories and foraging strategies, long life span, top predator trophic position, sensitivity to environmental stressors on observable time scales, and physiological interconnectivity to both air and water[39,40]. They assimilate resources and related environmental conditions, and demonstrate organismal and population level responses. This responsiveness allows seabird population and individual condition to be utilized as indicators reflecting chemical contamination and/or overall ecosystem health or stress[20,41–43].

We used seabirds as sentinels to assess and contrast patterns and magnitude of PFAS exposure in three marine regions. We targeted 36 PFAS in the livers of 31 juvenile seabirds found dead in 2017 in three coastal and pelagic marine habitats with variable PFAS exposure potential. The main objectives of this study were to a) measure and compare legacy and emerging PFAS in different seabirds from marine habitats subject to variable direct or indirect PFAS discharges, and b) ascertain any association between stable isotope signatures approximating trophic habits, phospholipid levels, and PFAS concentrations.

MATERIALS AND METHODS

Chemicals and Reagents

A total of 36 PFAS were assessed in juvenile seabird livers using target and suspect screening, and 27 PFAS quantified (Table S1), including C_4–C_{14} perfluorocarboxylates (PFCAs), C_4-C_{10} perfluorosulfonates (PFSAs), three perfluoroalkyl ether carboxylic acids (PFECAs), one perfluoroalkyl ether sulfonate (PFESA), three fluorotelomer sulfonates (FTS), and three sulfonamide precursors (Tables S1,S4–S5). More details about target and suspect analytes, chemicals, and reagents can be found in the Supplementary Information (SI).

Sample Collection

Liver tissue was obtained from six species of deceased juvenile seabirds. Juvenile Great Shearwaters (*Ardenna gravis*) originated from Massachusetts Bay (n = 10) and Herring Gull chicks (*Larus argentatus smithsonianus*) from Narragansett Bay (n = 10). Royal Tern (*Thalasseus maximus*), Sandwich Tern (*Thalasseus sandvicensis*), Laughing Gull (*Leucophaeus atricilla*), and Brown Pelican (*Pelecanus occidentalis*) chicks originated from the Cape Fear River Estuary (n = 11) (CFRE) (Fig. S1). These birds, while unique species with nuanced life histories and food web roles, reflect broadly similar foraging preferences and strategies in coastal and pelagic habitats (Table S2). Literature also implies similar PFAS bioaccumulative capacity between different bird species, enabling a comparison of similar avifauna[44–46].

The individuals analyzed here span several months in age, ranging from 2–4 week old chicks to ~6 month old juveniles. Based on the long tissue half-life of PFAS, these young birds predominantly reflect PFAS derived from maternal offloading, and thus the highest internal

concentrations experienced by each individual over their lifetime[46–48]. More details supporting our use of a multi-species, variable age sample set can be found in the SI.

The selected habitats represent a continuum of potential PFAS exposure, with the highest likelihood of exposure in the CFRE downstream from a fluoropolymer production facility. Narragansett Bay represents an intermediate potential for PFAS exposure; it is a well-mixed coastal embayment adjacent to a large urban center, but lacks major PFAS production facilities. The lowest likelihood of exposure, based on increased distance from direct human sources of PFAS, is reflected by seabird juveniles collected from Massachusetts Bay. Massachusetts Bay is a productive offshore marine habitat encompassing Stellwagen Bank National Marine Sanctuary, and representative of the offshore pelagic environment of the North Atlantic (Fig. S1).

All individuals were necropsied in a standardized manner[49]. Liver tissue was used for PFAS analysis and stable isotope analysis, while muscle was only used for stable isotope analysis. Additional details about sample condition, sample procurement, and individual sample details can be found in the SI.

Analysis of PFAS

Complete extraction methods are provided in the SI. Briefly, liver samples were lyophilized and solvent-extracted in methanol using sonication, centrifugation, and freezing, paired with graphitized non-porous carbon solid phase extraction. Measurement and quantification of 25 PFAS was achieved using liquid chromatography tandem-mass spectrometry (UPLC-MS/MS) experiments in negative electrospray ionization mode. Further details about quantification and instrumental parameters are provided in the SI. Estimates of PFAS concentrations were obtained by quantification using isotope dilution. Method recovery ranged from 14 – 112% with a mean recovery of 62% across all compounds, similar to recoveries reported in other work using avian liver[47,50,51] (Table S6).

Liver tissue was also assessed via suspect screening using high resolution mass spectrometry (HRMS). Fresh tissue aliquots were extracted for PFEAs of interest by means of protein precipitation and dilution[19,52]. Sample extracts were measured using a ThermoFisher Orbitrap Fusion (Thermo Fisher Scientific, Waltham, MA, USA) operated in heated electrospray ionization in negative mode as previously described[19,52].

Concentrations of PFEAs were derived from these HRMS experiments. Nafion BP2 and PFO_5DoDA were assessed using native standards and reported here quantitatively while all other PFEAs were assessed based on previously determined accurate mass and spectral information using a semi-quantitative approach (Tables S1, S5)[8,53]. PFO_4DA was the only PFEA detected in samples that lacked a native standard; PFO_4DA was reported as raw abundance and excluded from concentration calculations due to the lack of an authentic native standard. Mass-labelled surrogates of similar molecular weight and retention time were used in the absence of matched surrogates for quantitation of Nafion BP2 and PFO_5DoDA (Table S5).

For those compounds analyzed via both UPLC-MS/MS and HRMS, UPLC-MS/MS concentrations are reported here due to more rigorous quality assurance and increased sensitivity (Table S1). Further details about sample preparation, analysis, and quality assurance for both UPLC-MS/MS and HRMS are provided in the SI.

Stable Isotope and Phospholipid Analysis

Stable isotope analysis was used to evaluate trophic transfer of PFAS, as well as to ensure trophic comparability of the sample set. $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ were measured in muscle; $\delta^{15}N$ and $\delta^{13}C$ were also measured in liver to facilitate comparison to a tissue with a faster isotopic turnover rate[42], given the unknown rate of PFAS turnover in avian tissues.

Total lipid was extracted from liver tissue aliquots using a modified Folch method, and phospholipid content of liver tissue assessed colorimetrically using an EnzyChrom Phospholipid Assay Kit (BioAssay Systems, Hayward, CA)[54]. More details about stable isotope and phospholipid analysis are available in the SI.

Statistical Analysis

All data manipulation and statistical analyses were performed in R version 3.6.1 (R Core Team, 2020)[55]. Concentrations were converted to a wet weight basis for comparability to other literature values. Responses not detected or below the linear dynamic range of the curve were labeled as “nd” and assigned a value of zero. Observations below method reporting limits with a detection frequency higher than 50% were replaced with the half the method reporting limit for statistical analyses, and included as such in calculation of summed concentrations ($\sum_{19}$PFAS) and statistical analysis[56]. Data were checked for normality and homoscedasticity using the Shapiro-Wilk test and Levene’s test. Concentration data were non-normal despite log transformation and therefore treated non-parametrically for statistical analyses; habitat groups displayed no significant differences in variance. Differences between habitats were assessed using Kruskal-Wallis tests with post hoc application of Dunn’s test with Bonferroni correction for multiple testing, or with the Wilcoxon rank sum test. Relationships between concentrations were assessed using Spearman rank correlation coefficients (R_s^2). R_s^2 presents the proportion of the rank variance explained by the correlation between variables with test assumptions more suitable for this dataset, providing insight about the relationship similar to the Pearson R^2. Liver-water bioaccumulation factors (BAFs) were calculated by dividing geometric mean liver PFAS levels by measured or estimated surface water concentrations adjacent to nesting or collection locations, followed by log transformation; more details about BAF calculations can be found in the SI.

RESULTS AND DISCUSSION

Observed detection frequencies and patterns by habitat

Samples were screened for 36 analytes using target and suspect screening; 27 analytes were quantified using native standards. Only one semi-quantitative compound was detected via suspect screening, PFO_4DA. 19 of the 27 quantifiable analytes were measured above

detection limits in at least one sample, and detection frequencies varied by habitat. PFOS, PFNA, PFDA, and PFUnDA were present in at least 97% of individuals (Table 1).

The highest $\sum_{19}$PFAS measured was 390 ng/g w.w. liver comprised of 14 quantifiable analytes, found in a CFRE Royal Tern chick. The lowest $\sum_{19}$PFAS concentration was observed in a juvenile Great Shearwater from Massachusetts Bay containing $\sum_{19}$PFAS of 26 ng/g, comprised of 5 analytes above detection limits (Fig. 1). Chicks from the CFRE system contained significantly greater concentrations and number of PFAS than juveniles from Massachusetts Bay or Narragansett Bay (Dunn's test; $p < 0.001$) (Figs. 1a, 3a). There was no significant difference between mean $\sum_{19}$PFAS levels observed in individuals from Narragansett Bay and Massachusetts Bay (Fig. 3a), though Great Shearwater individuals were older than Narragansett Bay chicks, and may underestimate levels found in chicks of this species. Within each habitat, concentrations were not significantly different between male and female chicks, though the sample size of sexed individuals was small (Table S17).

Multiple PFEAs were not detected, including novel PFECAs PMPA, PEPA, PFO_2HxA, and PFO_3OA, and novel PFESAs NVHOS, Nafion byproduct 4 and 1. No fluorotelomer sulfonates were detected above reporting limits, nor N-EtFOSAA or N-MeFOSAA. The lack of bioaccumulation of shorter-chain PFEAs may be analogous to the reduced bioaccumulation potential of short-chain PFCAs in upper trophic level homeotherms[57]. The lack of detection of these compounds may also denote *in vivo* biotransformation. Research in other biota has shown fluorotelomer sulfonates are precursors to some PFCAs[58] while N-EtFOSAA and N-MeFOSAA are PFOS precursors[7]. Further work is required to deduce if the non-detects observed in this study are indicative of reduced bioavailability, rapid biotransformation, or the true absence of a compound in these environments and food webs. FOSA, a perfluoroalkane sulfonamide, was only detectable in three birds from the CFRE. These detections may be the result of continuous, high levels of FOSA or FOSA-precursors related to production activities or legacy PFAS sources in the region[53]. Such continuous inputs could exceed metabolic capacity and cause tissue residues of FOSA.

Continued dominance of PFOS in juvenile seabirds

PFOS was the most abundant compound in all sampled livers, making up 58% of total liver concentrations across the sample set. The proportion of PFOS measured in each individual varied by habitat, with the CFRE and Narragansett Bay individuals containing the highest geometric mean of 61% and 67% PFOS, respectively (Fig. 2).

These data align well with previous literature that determined a high proportion of PFOS in seabird livers, eggs, and serum in species from multiple ecological provinces[20,21,59–63]. The highest PFOS concentration measured in this study was 280 ng/g w.w. liver in a Sandwich Tern chick from the CFRE, similar or higher than levels measured within the past ten years in other long-lived temperate seabirds[20,21,60,61]. Average avian toxicity reference values (TRVs) for PFOS reported by Newsted et al. of 600 ng PFOS/g liver were approximately 2–30 times higher than PFOS levels reported in these young birds[64]. Seven of eleven CFRE chicks exceeded the female-specific liver TRV of 140 ng PFOS/g liver; these birds were female or not yet able to be sexed visually[64]. One female from Mass. Bay and one unsexed bird from Narra. Bay also exceeded the female-specific TRV.

Our data indicate the continued occurrence of PFOS in juvenile avifauna at levels of toxicological concern despite a production phase-out of perfluorooctanesulfonyl fluoride (POSF), PFOS, and PFOS precursors in the US in the early 2000s[1]. The phase-out in 2000–2002 resulted in decreased PFOS and/or precursor concentrations in select environmental matrices[22,65]. Modeling and empirical results suggest decreased availability of volatile precursor compounds like FOSA, whose environmental occurrence responded quickly to the phase-out, likely caused any decreasing trends of PFOS in wildlife observed after 2002[22]. Yet biotic trends in PFOS vary based on spatial habits, proximity to local sources, and with trophic strategy, and there is no consistent global pattern of continually decreasing PFOS across multiple avian matrices[18,60,63,66]. Current PFOS concentrations in wildlife reflect exposure to extant precursor compounds that may transform *in situ* or *in vivo* to PFOS, *in vivo* depuration of PFOS, and sustained transfer of PFOS itself via environmental or trophic interactions[65,67,68]. Our results highlight the continued biological occurrence of PFOS as a function of these exposures.

Variable contribution of PFCAs by Habitat

Concentrations of PFCAs including PFDA, PFNA, and PFUnDA reported in this study were similar to or elevated compared to concentrations previously reported in temperate species, as well as in birds from Great Lakes and Arctic environments[4,60–62,66,69]. In CFRE, mean concentrations of PFCAs increased up to PFDA, where PFOA < PFNA < PFDA > PFUnDA, whereas in Massachusetts Bay PFNA < PFDA < PFUnDA and in Narragansett Bay PFNA < PFDA ≈ PFUnDA.

The proportion of ∑PFCAs to ∑PFSAs varied between habitats, with individuals from CFRE and Narragansett Bay dominated by PFSAs while individuals from Massachusetts Bay contained a significantly higher proportion of PFCAs (Dunn's test; $p < 0.001$) (Fig. 3b). The dominance of PFCAs in offshore Massachusetts Bay individuals was driven partly by significantly higher concentrations of the C_{11} PFCA, PFUnDA, in offshore individuals (Dunn's test; $p < 0.05$) (Fig. S2). Mean concentrations of other long-chain (C_nF_{2n-1} COOH, $n \geq 7$) PFCAs found in both Narragansett Bay and Massachusetts Bay were not significantly different.

The preferential dominance of PFUnDA seen here in seabirds from offshore Massachusetts Bay habitat has been observed in Arctic marine mammals as well as Arctic and temperate seabirds[4,20,21,70]. These studies exemplify a broader pattern in which PFCAs with odd chain lengths are more abundant in biota than PFCAs with even chain lengths. This pattern is a result of preferential bioaccumulation of longer-chain homologues, coupled to substantial atmospheric and water-borne transport of PFCAs and PFCA precursors of variable chain length[15,23,70–72]. Notably, our data suggests this pattern only applies to biota exposed to diffuse PFAS sources, as CFRE birds subject to localized point sources of PFAS actually contained greater concentrations of PFDA (C_{10}) compared to PFUnDA (C_{11}).

Limited data suggest environmentally relevant exposures of long-chain PFCAs are associated with changes in metabolic rate, oxidative stress, and reproductive behaviors in Arctic black-legged kittiwakes[25,73]. We highlight the need for further research about the formation, long-range transport, occurrence, and effects of PFUnDA and other long-chain

PFCAs in marine systems supporting seabirds. Such a comprehensive understanding is necessary given the sustained or increasing occurrence of long-chain PFCAs in remote wildlife[21,63], the substantial suite of stressors currently impacting marine species globally, and the continued importance of marine food resources to human communities[74].

Potentially Confounding Factors

The opportunistic sample set of dead chicks and juveniles enabled us to measure PFAS in liver tissue, allowed the acquisition of unique samples from the CFRE before the cessation of certain industrial PFAS discharges, and allowed assessment of PFAS during a critical development window in immature individuals from data-deficient habitats. Yet this opportunistic sampling also introduced potentially confounding factors related to the variable species and ages of collected individuals.

The older Great Shearwater juveniles in this study were self-feeding for approximately two months, while the chicks from other habitats were still being provisioned by their parents. Great Shearwater PFAS profiles may therefore reflect increased input from dietary sources. Immature Shearwaters are thought to eat similar items as adult Great Shearwaters[75]; hence – the PFAS profile should be conserved, allowing their comparison between habitats. More likely, the older juveniles reflect growth dilution, and may underestimate PFAS concentrations present in chicks of this species.

Literature to date has yet to identify significant differences between uptake, metabolism, or elimination pathways and rates between similar bird species[20,44–46]; bioaccumulation in birds appears driven by habitat and trophic exposures. However, species-specific toxicokinetics have been identified between multiple mammal species[76]. We also have not investigated the possibility of developmental changes in molecular receptors of PFAS in birds or other wildlife, although PFAS have been found at higher levels in juveniles than adults across multiple taxa[48,77,78]. We suggest additional research on the toxicokinetic behavior of PFAS in different bird species and across developmental stages, given their utility and importance as sentinel predators.

Association with phospholipid content

Lipid moieties play key roles in organismal metabolism, reproduction, migration, and other basic functions key to wildlife health and fitness. Phospholipid levels were significantly ($p<0.05$) associated with PFOS in Massachusetts Bay and CFRE individuals ($R_s^2 = 0.52$ and 0.45, respectively) (Fig. 4a). $\sum_{19}$PFAS was more weakly associated with measured phospholipids ($R_s^2 = 0.14$ and 0.45 for Massachusetts Bay and CFRE individuals, respectively and $R_s^2 = 0.51$ when assessed as a total sample set, n = 31). There was also a statistically significant correlation between PFNA and phospholipid in CFRE chicks ($R_s^2 = 0.53$, $p < 0.05$).

The importance of phospholipids in the accumulation of long-chain PFAAs has been supported by both empirical observations from marine mammals as well as modeling results[54,79–81]. Conversely, controlled studies in chickens suggest PFOS may impact lipid metabolism and production via gene suppression, suggesting PFOS may instead indirectly

mediate lipid levels[32,33,68,82]. Currently, our understanding of phospholipid-PFAS associations and relationships lacks substantial field-derived data beyond marine mammals.

These data, though derived from a small sample set, suggest an association between environmentally relevant concentrations of PFOS (and possibly PFNA) and decreased liver phospholipid content in wild seabirds, a previously unreported phenomenon (Fig. 4). Lipid levels in seabird livers may be influenced by a variety of physiological and nutritional constraints not measured within the scope of this study; we note the lack of comparative baseline data describing liver phospholipid levels in the species examined within this work as a possible limiting factor. However, lines of evidence from multiple disciplines suggest a high conservation of non-diet lipid composition in a given tissue between similar avian species[83–86]. Controlled animal studies also point to the same relationship between PFOS exposure and changes in phospholipid content, lipid profiles, and lipid metabolism alongside altered expression of genes related to lipid dynamics in the livers of domestic chickens[32,33,68,82]. Most relevant to the results seen here, subcutaneous delivery of 0.02 mg/ml and 0.1mg/ml PFOS resulted in decreased phospholipid content in domestic chicken plasma after 28 days of exposure and 28 days of depuration[68].

Laboratory-based studies using model phospholipid bilayers, liposomes, and bacterial membranes exhibit an inverse relationship between PFAS levels and phospholipids, manifested via increased incorporation of PFAS into bilayers and subsequently decreased lipid content. These studies also found increased bilayer disruption by PFAS based on chain-length and functional group[87,88]. The results from these controlled membrane studies are not easily translated to realistic biological conditions, yet in combination with other evidence (our data, previous work in pilot whales[54], and controlled animal studies referenced above) we highlight a potential and currently undefined relationship between PFOS and phospholipid responses in wild organismal systems at environmentally relevant exposure levels. Further research is warranted to better describe relationships between PFAS-driven lipid responses in combination with the role of lipids in PFAS partitioning.

Relationship to stable isotope data

$\delta^{15}N$ is frequently used as a proxy for trophic level across terrestrial and aquatic systems; while system and prey-base specific, enrichment of $\delta^{15}N$ typically indicates foraging at a higher trophic level[42]. Legacy persistent organic pollutants (POPs) like polychlorinated biphenyls or DDT frequently increase in concentration with trophic level as approximated by $\delta^{15}N$ values, due to bioaccumulation of hydrophobic POPs in lipid-rich consumer matrices[89]. Here, we found $\delta^{15}N$ values and calculated trophic level were not significantly associated with concentrations of the majority of individual PFAS in each habitat (Fig. S6, Table S23). PFOS and $\sum_{19}$PFAS were positively associated with $\delta^{15}N$ only in Massachusetts Bay individuals (Table S23).

$\delta^{13}C$ values reflect basal sources of primary production supporting trophic networks[42] and exhibit significantly less step-wise change with prey-consumer interactions. This allows bulk differentiation between inshore and offshore food chains based on characteristic enrichment or depletion of $\delta^{13}C$ associated with terrestrial vs marine primary production (Fig. S8)[42]. $\delta^{13}C$ ranged from −19.8 to −17.0 in CFRE individuals, and from −22.5 to −19.0 in

Narragansett and Massachusetts Bay individuals. In contrast to previous studies, concentrations of C_9, C_{10}, C_{11}, and C_{13} PFCAs, PFOS, and $\sum_{19}$PFAS in CFRE birds were associated with enriched $\delta^{13}C$ values in seabird muscle and liver, likely reflecting the unique geomorphology of the CFRE system and energy-saving coastal foraging habits of CFRE seabird species (Fig. S7). PFTrDA and PFDA were negatively associated with $\delta^{13}C$ values in Mass. Bay individuals, which may reflect increased exposure to long-chain PFCAs in offshore environments via long-range transport and transformation of PFCA precursors. More details about relationships between PFAS and stable isotopes are available in the SI.

Unique occurrence of emerging compounds

HRMS analysis helped us confirm the presence of (previously identified) PFEAs in chick livers for the first time: Nafion BP2 and PFO_5DoDA were found in livers of all CFRE individuals (n = 11) while PFO_4DA was found in 7 of 11 CFRE individuals (Fig. 1b, Table S16).

The maximum Nafion BP2 concentration was 110 ng/g w.w. in a Laughing Gull chick and a maximum PFO_5DoDA concentration of 30 ng/g w.w. in a Royal Tern chick (Fig. 1b). Nafion BP2 was also detected in two Great Shearwaters and one Herring Gull from outside the CFRE, or 15% of the non-CFRE sample set (Figs. 1b, S10).

The detection of these PFEAs in CFRE chicks is due to proximity to an industrial point source and the then-ongoing discharge of PFEAs to surface water. CFRE chicks were hatched in a well-mixed estuary ~145 km downstream from a fluoropolymer production facility in Fayetteville, NC. “Gen X” or HFPO-DA, along with other PFEAs were detected at high levels in Cape Fear River surface water and Wilmington, NC drinking water as a result of industrial wastewater discharges into the mainstem Cape Fear River[53,90,91]. Increased research attention following the 2017 termination of the industrial discharge has revealed the presence of multiple PFEAs in downstream environments, fish, and humans[52,92].

Notably, concentrations of Nafion BP2, PFO_5DoDA, and PFO_4DA reported here are the highest biotic measurements of these PFEAs at the greatest distance from the production plant recorded to date, similar to measured PFOS concentrations and PFOS bioaccumulation factors in chick livers (Figs. 1, S9). Average PFEA concentrations reported here from seabird chick liver tissue (ppb, ng/g) are approximately 20 times higher than PFEA concentrations found in striped bass serum (ppb, ng/mL)[52].

Observed PFEA concentrations in CFRE chicks are likely not a result of riverine foraging proximate to the plant discharge or incorporation of freshwater prey items by seabird parents. The species sampled in this study are strictly marine, and do not inhabit or use freshwater, riverine habitats based on colony observations and their foraging preferences (marine and estuarine prey including forage fish, squid, and crustaceans)[93–95]. PFEAs were previously measured in river water upstream from the breeding colony at very high concentrations while the industrial discharge was ongoing[90,96]. Their detection in seabirds here suggests these emerging PFEAs are environmentally persistent and capable of

significant downstream transport and biotic uptake via undefined water-borne, particulate, and/or atmospheric pathways.

The confirmed presence of Nafion BP2 in three individuals outside of the CFRE is the first identification of Nafion BP2 in biota outside of the CFRE region, reiterating the environmental persistence and mobility of Nafion BP2. These detections are difficult to explain because virtually no data exists describing Nafion BP2 environmental occurrence beyond the CFRE region. Nafion BP2 detections in non-CFRE chicks may be due to migratory proximity to the CFRE; we consider this unlikely due to the lack of evidence indicating use or reliance on the CFRE system by non-CFRE populations sampled in this study.

More likely, Great Shearwater juveniles and Herring Gull chicks accumulated Nafion BP2 as a result of exposure to indirect discharges contaminated with Nafion products or related degradation byproducts. Nafion is the brand name of a perfluorinated ionic polymer first discovered in the 1960s via modification of the Teflon polymer. The Fayetteville, NC production facility has produced this ionic polymer since the 1980s. Nafion byproduct-2 is a side product from the reaction of the polymer precursors.[92]. Little data exist describing how production, use, or disposal of this perfluorinated polymer may contribute to PFAS to the environment. Evidence from the CFRE suggests Nafion production waste streams may contribute substantial loads of Nafion byproducts to receiving environments[52,53,90,92]. Feng et al. (2015) investigated the thermolysis products of Nafion 117, a typical Nafion membrane and suggested high-temperature uses or disposal of Nafion via incineration may produce multiple perfluorinated structures as a result of incomplete combustion[97]; Nine groups of fluorinated analogues were identified as a result of thermal treatment of Nafion 117 membranes, including breakdown products structurally similar to Nafion BP2[97]. Additional research documents chemical and mechanical degradation pathways relevant to Nafion membrane function and efficiency[98]. The (albeit limited) detection of Nafion BP2 beyond CFRE individuals warrants additional screening of Nafion byproducts in diverse environmental matrices, investigation into the life-cycle of Nafion technologies, and potential environmental persistence of PFESAs.

Divergent sources of emerging and legacy PFAS in the CFRE system

Ratios of PFEAs to PFOS varied between individuals, with PFEA levels on the same order of magnitude as PFOS in select individuals (Fig. 1b inset). PFEA concentrations were not correlated with PFOS or long-chain PFCAs in CFRE chicks, while PFO_5DoDA displayed a weak positive correlation with PFDA (Fig. S5). This lack of correlation suggests legacy PFAAs like PFOS were contributed to the system via different pathways unassociated with the industrial facility producing PFEAs, in-line with their previous detection in surface waters from the region[53]. Prior research indicates concentrations of PFCAs and PFSAs in surface water are similar above and below the fluoropolymer facility in Fayetteville, NC while concentrations of PFEAs varied starkly upstream and downstream of the facility while active discharges from the facility were ongoing[53,90].

Implications for further research

Our results highlight the potential role of seabirds as key sentinels of marine environments, and confirm the sustained presence of legacy PFAS in marine systems along the US East Coast. We also document PFEAs in seabirds for the first time, reflecting the shifting suite of PFAS in production and in environmental matrices. As our current understanding of PFAS effects in wildlife is limited, future biomonitoring in seabirds and other wildlife should derive responses and effects related to ambient PFAS levels. Understanding PFAS levels and effects in marine food webs and biota ultimately stands to benefit public health and commerce as we continue to rely on marine food webs for economic, nutritional, and aesthetic services.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PFAS	per- and polyfluoroalkyl substances
CFRE	Cape Fear River and Estuary
PFCAs	perfluoroalkyl carboxylate(s)
PFSAs	perfluoroalkyl sulfonates(s)
PFEAs	perfluoroalkyl ether acid(s)
PFECAs	perfluoroalkyl ether carboxylate(s)
PFESAs	perfluoroalkyl ether sulfonate(s)
BCF	bioconcentration factor
EPA	US Environmental Protection Agency

See SI Table S1 for the full names of all individual PFAS discussed in the scope of this analysis

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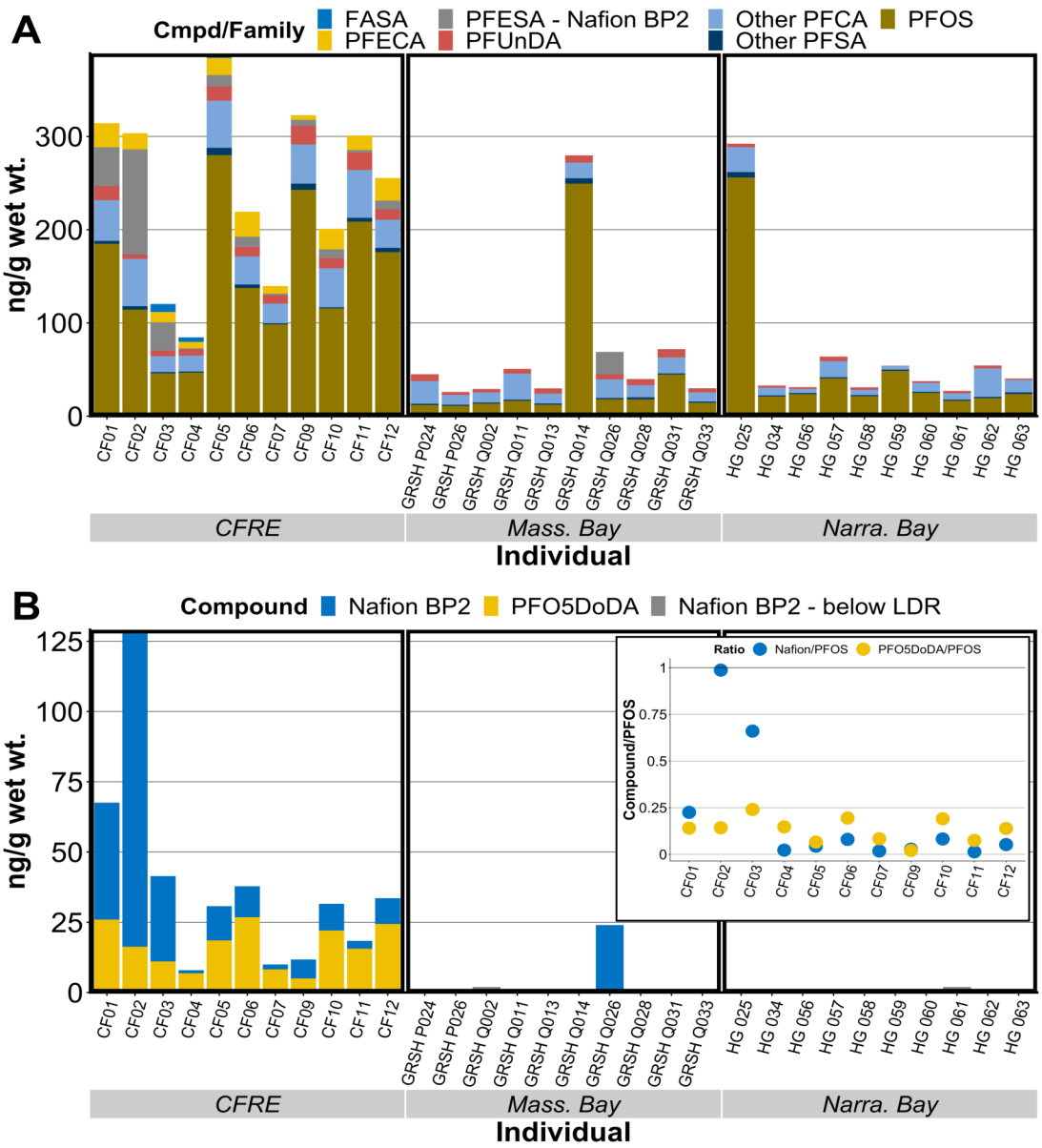

Figure 1.

Measured concentrations of A) PFAS in juvenile seabird livers measured via LC-MS/MS, B) two emerging PFAS measured via targeted HRMS, alongside B-inset) ratios of emerging PFEAs to PFOS in CFRE chicks. Nafion BP2 concentrations positively identified in non-CFRE chicks but below the linear dynamic curve range are graphed in panel B as half the reporting limit; grey arrows are used to distinguish these data points.

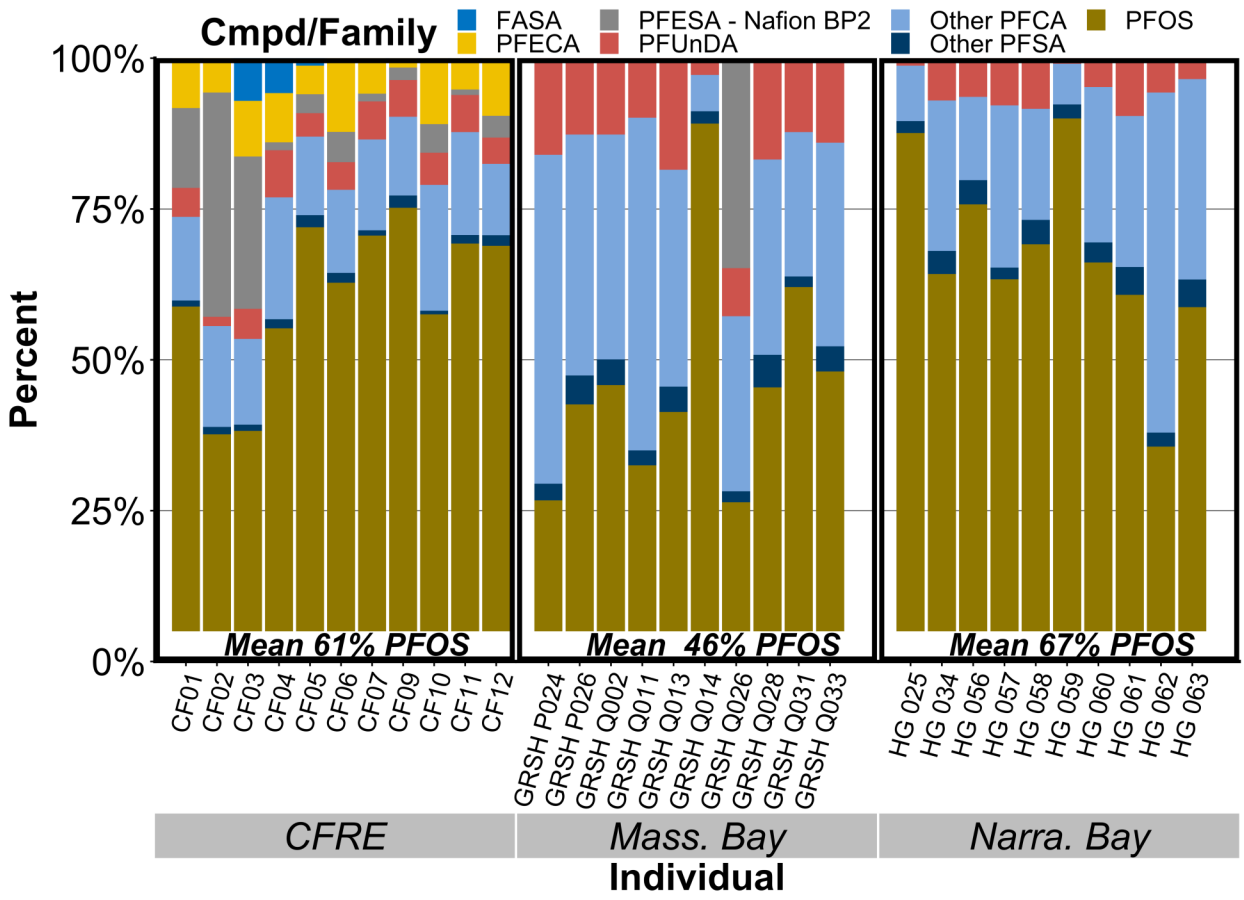

Figure 2.
The composition of PFAS in liver tissue, presented by individual and grouped by habitat.

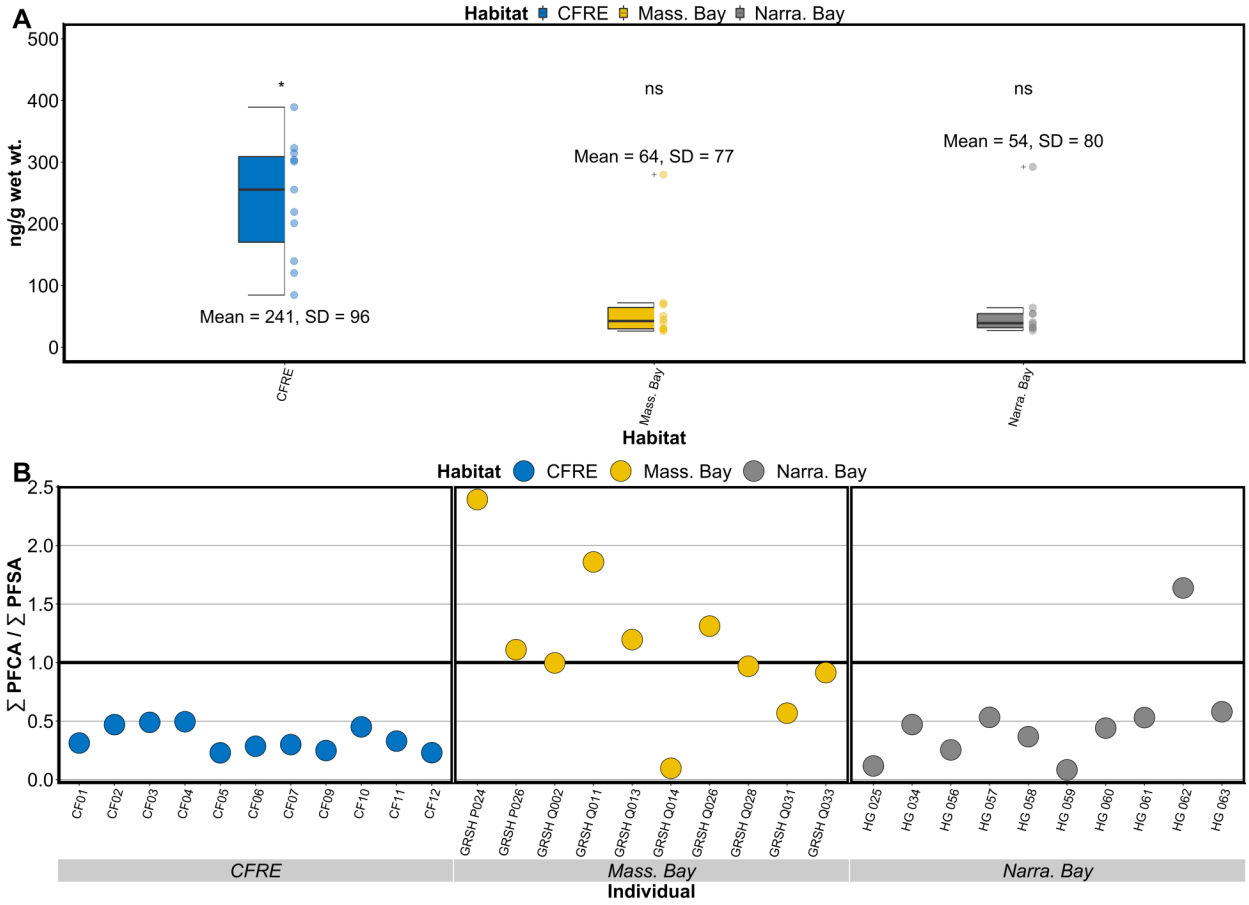

Figure 3.

$\sum_{19}$PFAS presented in A) as a boxplot, with the dark line representing the median, box limits representing the first and third quartiles, whiskers denoting 1.5 times the interquartile range, and crosses denoting outliers. The asterisk indicates a statistically significant difference between habitat mean $\sum_{19}$PFAS compared via Wilcoxon rank sum test using the CFRE as the reference group, while B) presents ratios of ∑PFCAs to ∑PFSAs in each individual. Ratios above 1 indicate PFCA dominance, while ratios below 1 indicate PFSA dominance.

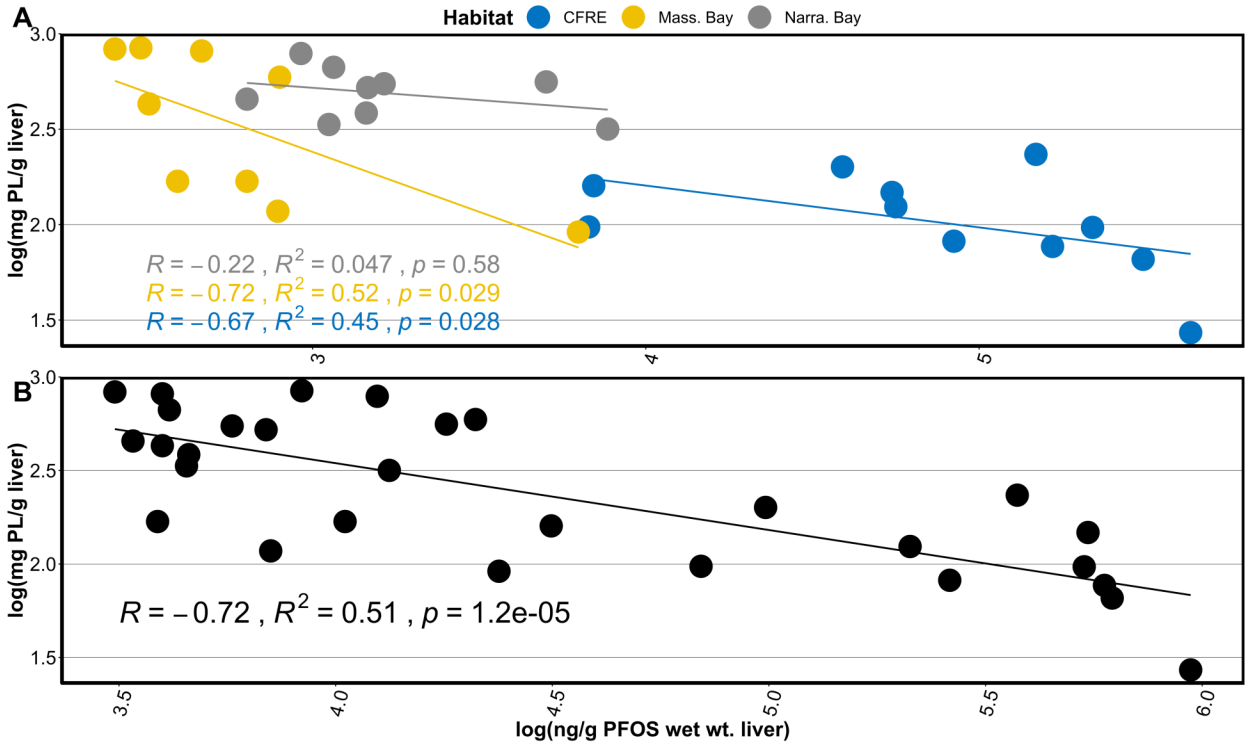

Figure 4.

A) presents log-transformed concentrations of PFOS vs phospholipid (PL) grouped by habitat, while B) displays log-transformed PFOS and phospholipid (PL), assessed as a total sample set (n = 31). Text annotation presents R, R_s^2, and p-value derived from Spearman rank correlation analysis.

Table 1.

Detection frequency of quantifiable and semi-quantitative analytes in seabird juveniles from each habitat and as a total sample set across all habitats combined. Mass. Bay = Massachusetts Bay, Narra. Bay = Narragansett Bay, and CFRE = Cape Fear River Estuary. Family names are from Buck et al. 2011. Compounds highlighted in gray are those compounds detected above reporting levels in at least 97% of individuals via LC-MS/MS.

% Detection by Ecosystem						
Compound	**Family**	**# Fluorinated Carbons**	**Mass. Bay**	**Narra. Bay**	**CFRE**	**All**
N-MeFOSAA	FASAA	8	0	0	0	0
N- EtFOSAA	FASAA	8	0	0	0	0
FOSA[a]	FASA	8	0	0	27	10
4:2 FTS	FTS	4	0	0	0	0
6:2 FTS	FTS	6	0	0	0	0
8:2 FTS	FTS	8	0	0	0	0
PFBA	PFCA	3	100	80	73	84
PFPeA	PFCA	4	0	0	0	0
PFHxA	PFCA	5	0	30	27	19
PFHpA	PFCA	6	0	0	0	0
PFOA	PFCA	7	0	0	64	23
PFNA	PFCA	8	100	100	100	100
PFDA	PFCA	9	90	100	100	97
PFUnDA	PFCA	10	100	90	100	97
PFDoA	PFCA	11	0	10	27	13
PFTrDA	PFCA	12	70	20	27	39
PFTeDA	PFCA	13	0	10	0	3
PMPA	PFECA	3	0	0	0	0
PFO_2HxA	PFECA	3	0	0	0	0
PEPA	PFECA	4	0	0	0	0
PFO_3OA	PFECA	4	0	0	0	0
HFPO-DA	PFECA	5	0	0	9	3
PFO_4DA[b]	PFECA	5	0	0	70	23
PFO5DODA	PFECA	6	0	0	100	36
Nafion BP4	PFESA	6	0	0	0	0
Nafion BP2	PFESA	7	20	10	100	45
Nafion BP1	PFESA	7	0	0	0	0
NVHOS	PFESA	4	0	0	0	0
PFBS	PFSA	4	0	10	0	3
PFPeS	PFSA	5	20	0	0	7
PFHxS	PFSA	6	10	10	55	26
PFHpS	PFSA	7	0	0	55	19

% Detection by Ecosystem						
Compound	**Family**	**# Fluorinated Carbons**	**Mass. Bay**	**Narra. Bay**	**CFRE**	**All**
PFOS	PFSA	8	100	100	100	100
PFNS	PFSA	9	0	0	0	0
PFDS	PFSA	10	0	10	9	7
PFECHS	Cyclic PFSA	8	0	0	0	0

[a]Detection based on raw abundances in comparison to blank raw abundances due to lack of authentic standards.

[b]Low recovery (14%) related to sample preparation