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TEMPORAL MONITORING OF PERFLUOROOCTANE SULFONATE ACCUMULATION IN AQUATIC BIOTA DOWNSTREAM OF HISTORICAL AQUEOUS FILM FORMING FOAM USE AREAS

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Abstract: Perfluoroalkyl substances (PFAS) have recently received increased research attention, particularly concerning aquatic organisms and in regions of exposure to aqueous film forming foams (AFFFs). Air Force bases historically applied AFFFs in the interest of fire training exercises and have since expressed concern for PFAS contamination in biota from water bodies surrounding former fire training areas. Six PFAS were monitored, including perfluorooctane sulfonate (PFOS), in aquatic species from 8 bayou locations at Barksdale Air Force Base in Bossier City, Louisiana (USA) over the course of 1 yr. The focus was to evaluate temporal and spatial variability in PFAS concentrations from historic use of AFFF. The PFOS concentrations in fish peaked in early summer, and also increased significantly downstream of former fire training areas. Benthic organisms had lower PFOS concentrations than pelagic species, contrary to previous literature observations. Bioconcentration factors varied with time but were reduced compared with previously reported literature values. The highest concentration of PFOS in whole fish was 9349 ng/g dry weight, with 15% of samples exceeding what is believed to be the maximum whole fish concentration reported to date of 1500 ng/g wet weight. Further studies are ongoing, to measure PFAS in larger fish and tissue-specific partitioning data to compare with the current whole fish values. The high concentrations presently observed could have effects on higher trophic level organisms in this system or pose a potential risk to humans consuming contaminated fish. *Environ Toxicol Chem* 2017;36:2022–2029. © 2016 SETAC

Keywords: Perfluorooctane sulfonate Bioconcentration Aqueous film forming foam

INTRODUCTION

Perfluoroalkyl substances (PFAS) have received considerable research attention in the last decade because of classification as emerging chemicals of concern by the US Environmental Protection Agency [1], with the most studied PFAS to date, perfluorooctane sulfonate (PFOS), listed as a persistent organic pollutant (POP) by the Stockholm Convention in 2009 [2–4]. Perfluoroalkyl substances are long-chained carbon structures containing either a carboxylate or sulfonate hydrophilic head group. The carbon backbone, with the number of carbons varying from 4 to up to 15 [5], is completely fluorinated, resulting in high thermal stability and extreme resistance to degradation in the environment and via metabolism [2]. Because of the hydrophobic, fluorine-saturated backbone and hydrophilic head group, the dual nature of PFAS has allowed for their widespread usage in consumer products including grease- and stain-resistant coatings, surfactants, and lubricants, and as components in aqueous film forming foams (AFFFs) [2,4,6].

Aqueous film forming foams were developed in the 1960s [7] and used extensively on military bases and at airports for training exercises and in emergencies. After a voluntary phase-out of PFOS by the leading manufacturer (3M) in 2002, PFOS-laden AFFFs were stockpiled, with an estimated 2.84 million gallons held by the US military (1.33 million of that by the US Air Force alone) in 2004 [8]. Regulations are in place in Canada and the European Union on the import of PFOS-containing AFFFs and the usage of stockpiled foams; however, no such restrictions are currently in place in the United States, Australia, or Japan [9].

Theoretically, PFOS input through fire-fighting activities ceased after the phase-out of PFOS-containing foams; however, it is possible that stockpiled foams are still in use, or at least were used since 2002. Moody and Field detected PFAS at Air Force bases in Nevada and Florida (USA), despite the non-use of AFFFs at these locations for over 7 yr [10]. This emphasizes the persistence of these compounds and may have implications for continued detections in habitats in close proximity to former fire training areas. Unfortunately, because of the restrictiveness of AFFF usage information, particularly at individual bases, PFOS inputs cannot be determined quantitatively.

Perfluoalkyl substance accumulation has been studied globally, including areas of historic AFFF use [7,11–13] and arctic environments [5,14–16]. Because of their unique chemical properties, a primary route of exposure of PFAS (as opposed to other POPs) is through contaminated drinking water [17–19] in addition to biomagnification through diet [20,21]. Fish PFAS burdens, however, are considered to be dominated by bioconcentration directly from water, with limited incorporation via biomagnification [22]. Furthermore, PFAS differ from other legacy POPs in that they tend to partition to regions of high protein density like liver and serum instead of lipid-rich regions.

Regarding PFAS concentrations in wildlife, peak literature concentrations include snapping turtle plasma from Ontario, Canada, with a maximum value of 5392 ng/g wet weight [13], polar bear livers averaging 3377 ng/g wet weight from Greenland [14], and mink livers from Michigan (USA)

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averaging 18 000 ng/g wet weight, with a maximum observed concentration of nearly 60 000 ng/g wet weight [23]. To our knowledge, maximum observed PFOS concentrations in fish were determined by Moody et al. [6] to be 72.9 μg/g in common shiner livers sampled 21 d after an accidental spill of AFFF in Etobicoke Creek in Ontario, Canada [6]. Oliaei et al. observed PFOS concentrations of 6350 ng/g in smallmouth bass livers and just under 30 000 ng/mL in white bass blood from Minnesota [3] sampled in 2004 and 2005, 2 yr to 3 yr after the expected cessation of input from 3M manufacturing processes.

Given the widespread use and distribution of PFAS-containing AFFFs and the apparent exposure of wildlife species and fish near former AFFF use areas, there is ongoing concern regarding the potential exposure and effects of PFAS to wildlife and fish. The objective of the present study was to measure and characterize PFAS exposure to freshwater fish and invertebrates inhabiting a bayou in close proximity to former fire training areas at Barksdale Air Force Base in Louisiana (USA). We focused on measuring 6 PFAS (perfluorobutane sulfonate [PFBS], perfluorohexane sulfonate [PFHxS], PFOS, perfluoroheptanoic acid [PFHpA], perfluorooctanoic acid [PFOA], and perfluorononanoic acid [PFNA]) in aquatic organisms collected from several bayous adjacent to former AFFF use areas as well as 2 nearby reference locations. By sampling over 1 yr at multiple locations, we obtained insights into the temporal and spatial variability of PFAS concentrations in biota. To our knowledge, the present study contains the largest data set on PFAS concentrations in fish that also includes spatially and temporally co-occurring water samples and tissue-specific analyses on individual organisms (H. Lanza, unpublished data). Simultaneous monitoring of environmental and tissue concentrations is important for estimating and characterizing risk at this location, with potential ecological effects via biomagnification of PFOS up through the food web, as well as potential exposures to humans via fish consumption [21]. We discuss our results in light of ongoing concerns regarding risks of PFAS to wildlife and fish.

MATERIALS AND METHODS

Site characteristics

Barksdale Air Force Base, established in the 1930s in Bossier City, Louisiana, is just 1 example of a base in which fire-fighting training with AFFFs occurred. Located in northwest Louisiana, Barksdale Air Force Base has 2 fire training areas along the east edge of the runways that are adjacent to natural Coopers Bayou (Figure 1). Surface water flows southward along Flat River and Coopers Bayou, and east along Macks Bayou. Previously conducted sampling demonstrated a north–northwestern groundwater flow from the northern fire training area toward Coopers Bayou, and a southeastern flow from the southern fire training area toward the intersection of Coopers Bayou and Macks Bayou. Samples were collected from the confluence of these 2 bayous, and slightly upstream along each of the converging bayous. Two reference locations were identified east of Coopers Bayou: Flat River, which had a comparable physical habitat to Coopers Bayou, and Flag Lake. Two additional sites were sampled along Coopers Bayou between the northern and southern fire training areas for a total of 6 sample locations of interest. Sample location IDs from upstream to downstream along Coopers Bayou are as follows: Upstream, Weapons Bridge, FTA-1 (close to the fire training area), South Coopers, and Confluence, with the Macks Bayou sample location being just west of the Confluence location along Macks Bayou.

Reference sites are important to determine baseline levels of PFAS at Barksdale Air Force Base, without direct influence from the historic fire training areas. Because PFAS, particularly PFOS, are present in many consumer products, Coopers Bayou and Flat River likely receive input of PFAS from the surrounding community upstream of Barksdale Air Force Base. Therefore, monitoring concentrations at reference locations enabled us to account for loadings to this system from other, non-AFFF–related inputs. We expected the relatively high water solubility of PFAS to result in an extended residence time in the slow-moving water associated with the bayou environment [24], and thus a long exposure window to aquatic organisms.

Sample collection

The majority of specimens collected in the present study were fish from the Centrarchidae family (bass and sunfishes), accounting for 77% of whole fish samples. Other species collected in the present study included representatives from Poeciliidae (gambusia and mollies, 8%), Cambaridae (crayfish, 5%), Cyprinidae (carps and minnows, 5%), and Ictaluridae (catfish, 5%). Opportunistic biota sampling via electrofishing co-occurred with water collection at 5 time points between August 2013 and September 2014. Aquatic biota were collected from up to 6 locations at each sampling event, for a total of 831 individuals (74 of which were set aside for tissue specific partitioning) captured over the course of 13 mo: August ($n = 235$ individuals) and November of 2013 ($n = 147$ individuals), and March ($n = 226$ individuals), June ($n = 104$ individuals), and September ($n = 15$ individuals) of 2014. The global positioning coordinates and water quality measurements were recorded at all sampled sites at all time periods. At least 1 reference location was represented during each sampling event (sites R1 and/or R2) with the exception of September because of poor fishing conditions. Organisms were euthanized using tricaine methanesulfonate buffered with sodium bicarbonate and were stored on ice. After return to Texas Tech University they were stored at –10 °C until further processing. All methods followed Texas Tech University Institutional Animal Care and Use Committee (IACUC) protocol 13047-05.

Water samples were collected more frequently than biota, with grab sample collections occurring in August and November of 2013, and in March, early May (coded as April), late May, June, July, and September of 2014. Water was collected (1 grab sample/location) from up to 8 locations during each of the 8 sampling events. Details of water sampling activities and results are reported elsewhere (R.S. Cochran, 2015, Master's thesis, Texas Tech University, Lubbock, TX, USA).

Chemicals and standards

All PFAS analyte standards (L-PFBS, L-PFHxS, L-PFOS, PFHpA, PFOA, PFNA), surrogate standards (Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid [$^{13}C_2$-PFHxA], Perfluoro-n-[1,2-$^{13}C_2$] decanoic acid [$^{13}C_2$-PFDA], N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid [d_5-N-EtFOSAA]), and internal standards (Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate [$^{13}C_4$-PFOS], Perfluoro-n-[1,2-$^{13}C_2$]octanoic acid [$^{13}C_2$-PFOA], N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid [d_3-N-MeFOSAA]) were purchased from Wellington Laboratories. All solvents used were high-performance liquid chromatrography (HPLC) grade and were obtained from Fisher Scientific. Ammonium acetate used in the mobile phase had a purity of >99%.

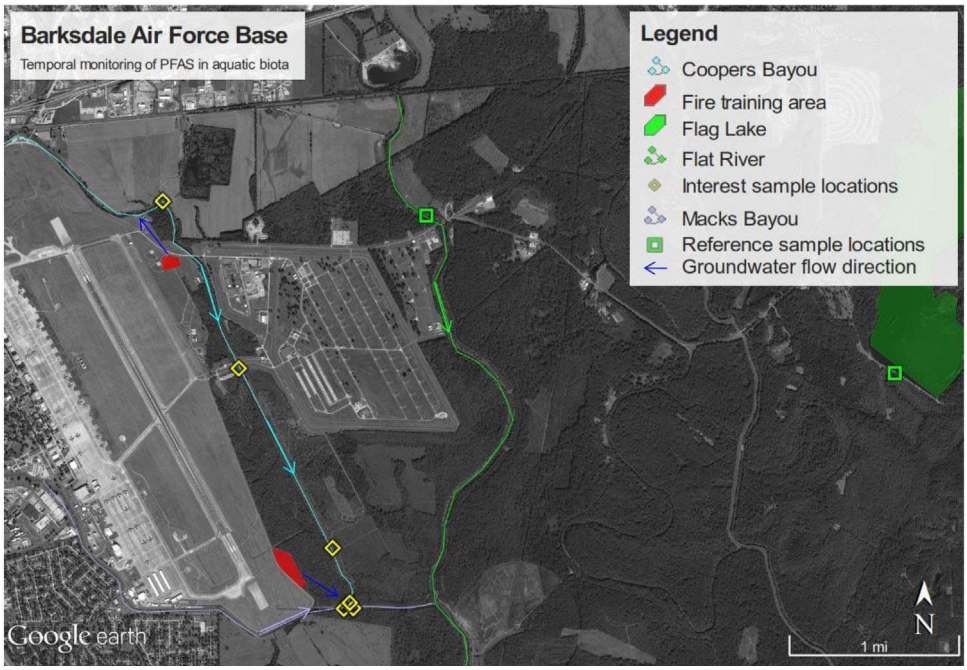

Figure 1. Site details of sampling locations at Barksdale Air Force Base. Interest locations (yellow diamonds) were collected along Coopers Bayou (light blue) and at the confluence of Coopers and Macks Bayou (purple). Reference samples were collected from Flat River and Flag Lake (green squares). Approximate groundwater flows from the historic fire training areas are indicated by blue arrows.

Whole fish processing method

After returning to the laboratory, fish were identified to species level and lengths and weights were recorded. Individuals greater than 30 g wet weight ($n = 74$) were set aside for tissue-specific partitioning analysis. The remaining smaller fish were processed individually, or grouped into composites containing multiples of the same species from the same locations at the same time points. Whole fish sample sizes were as follows: August ($n = 49$ composites) and November 2013 ($n = 37$ composites), March ($n = 109$ composites), June ($n = 53$ composites), and September 2014 ($n = 9$ composites), for a total of 257 composites.

Whole fish composites were processed via the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method similar to Haljasorg et al. [25]. Briefly, composites were desiccated under laboratory fume hoods for approximately 1 wk. The desiccated samples were then homogenized prior to being added to a polypropylene centrifuge tube containing 4:1 g $MgSO_4$:NaCl (United Chemical Technologies) and spiked with 100 ppb of a mixture of the 3 surrogate standards. Desiccation was important to remove variability in final, mass-adjusted concentrations based on varying water mass within the samples. This enabled a more accurate comparison among different species and different sized individuals. Tubes received 10 mL of acetonitrile and 2 mL of MilliQ water, were vortexed, and were left on a shaker plate overnight. The following morning, tubes were revortexed and centrifuged at 0 °C for 15 min. Supernatant was transferred to a second tube containing 900 mg anhydrous $MgSO_4$, 300 mg primary secondary amine (PSA), and 150 mg endcapped C18 (United Chemical Technologies). Tubes were vortexed and centrifuged as before. Samples were then held at –20 °C for several hours to remove any lipid interferences and were filtered cold into a 15-mL collection vial via 0.2-μm cellulose acetate syringe filters (GE Whatman); recovered volumes were recorded to determine a factor of recovery. We expected the 2 mL of MilliQ water added at the beginning of the procedure to be completely absorbed by the QuEChERS sorbent and desiccated fish tissue, meaning that only acetonitrile solvent would be recovered. Therefore, a recovery of 6 mL (of the original 10 mL added), would correspond to 60% recovery of associated PFAS. Final concentrations for each sample were adjusted according to the sample-specific factor of recovery to account for this variability in recovered solvent. Samples were evaporated to dryness and reconstituted in 0.5 mL methanol, transferred to microcentrifuge tubes with 0.2-μm cellulose acetate filters (Sigma Aldrich), filtered for 2 min at 4500 rpm, and stored at –20 °C until analysis.

Instrumental analysis and quality assurance/control

Prior to analysis, samples were transferred to a polypropylene LC vial (Thermo Scientific), and spiked with 100 ppb of a mixture of the 3 internal standards. A Thermo Fisher Scientific Triple Stage Quadrupole Quantum liquid chromatography tandem mass spectrometer was used to quantify PFAS residues. A 6-point calibration curve was acquired for all compounds of interest, with $^{13}C_2$-PFOA, $^{13}C_4$-PFOS, and d_3-NMeFOSAA as the internal standards for PFHpA/PFOA/PFNA/$^{13}C_2$-PFHxA/$^{13}C_2$-PFDA, PFBS/PFHxS/PFOS, and d_5-NEtFOSAA, respectively. Perfluorinated species were separated using a Gemini-NX C18 column (150 mm × 2.0 mm, 3 μm; Phenomenex), via gradient elution with a mobile phase consisting of methanol and 20 mM ammonium acetate in water, at a flow rate of 0.3 mL/min. Blanks and quality control samples were run after approximately every 3 biota samples to reduce instrument clogging and to check for instrumental error and matrix effects.

Surrogate recovery

Surrogate recovery varied widely. Overall, detection of surrogates was high (>90%). Quantifications of the 3 species, however, held between 11% and 36%, with recovery

averaging approximately 30% of the spiked value for the 3 surrogates. The reduced surrogate recovery for whole fish was, we believe, a result of the desiccation process, not the QuEChERS method, which showed high recovery of surrogate in both blank tests and preliminary laboratory tests. We hypothesize that the reduced surrogate recovery is an artifact of desiccating the samples, which resulted in reduced extraction solvent recovery, and lipid residues in whole fish carcasses that were removed after storing samples cold prior to filtration. Sample-specific factor of recoveries were applied to account for a loss of extraction solvent; however, this could be the dominant reason why so many whole fish samples had merely detectable and not quantifiable surrogate levels, because this determination is made prior to adjustment calculations. Regardless, we believe desiccation is a valuable step to reduce variability that would occur with varying water levels in the samples and thus providing us with more consistent, accurate, and comparable final concentrations. In addition, cold storage of the samples, although necessary to further clean samples prior to instrumental analysis, could have resulted in surrogates (and likely other analytes) remaining in the lipid precipitates, resulting in reduced surrogate recovery and a possible underestimation of overall PFAS concentrations in whole fish.

We did not adjust our final concentrations for surrogate recovery because recovery was so variable within these samples. Therefore, the concentrations reported presently are likely conservative estimates and possibly underestimate total PFAS body burdens in this system. We did not believe it was reasonable to apply a blanket adjustment factor of 3× to all whole fish samples because of the high variability.

Statistical analysis

The PFAS concentrations in whole-organism homogenate data generally remained non-normal and heteroscedastic even after transformation. Therefore, we tested for differences in concentrations in whole-body homogenates with location, time, and species as factors using the Kruskal–Wallis nonparametric test. Welch's 2-sample t tests were used for comparisons of 2 groups (e.g., PFOS in fish from interest sites vs reference sites). Welch's t tests are robust to samples with unequal variance and sample sizes and, hence, are well suited to analyzing field samples. Significance was set at $\alpha = 0.05$.

Variability

As with any environmental sampling, our results showed high variability across time, space, and species. The major explanation for this variability was likely the ability of biota to move between locations. In addition, the temporal sampling approach was another contributor to variability. For example, rainfall immediately prior to sampling or sampling after a period of drought may have affected groundwater influx and PFAS input to surface water. Finally, the variability in species captured, including benthic invertebrates in conjunction with pelagic carnivores, was likely responsible for additional variability around our sample averages. In summary, interpretation of the collected data was complicated by low analytical recovery in surrogates as well as complicating environmental factors, and thus it is difficult to draw any specific conclusions on temporal or spatial trends in the analytes. However, the data do provide some unique information on the presence of some contaminants that are very difficult to analyze.

RESULTS AND DISCUSSION

Occurrence of 6 PFAS

All 6 PFAS were detected at 3 of the 5 sampling times; PFBS was not detected in March or September, and PFOA and PFHpA were not detected in September. In all samples, PFOS was detectable, and PFHxS was detected in approximately 95% of samples. Perfluorooctane sulfonate was quantifiable in 99% (255 of 257 composites) of samples. The quantification of PFHxS across time varied, and overall, was quantifiable in approximately 38% of all samples. By comparison, carboxylic acid derivatives (PFHpA, PFOA, and PFNA) and the 4-carbon sulfonate (PFBS) were detected in less than a third of samples and were almost never quantifiable (once each for PFOA and PFNA). The following results will focus on the 2 dominant PFAS: PFOS and PFHxS.

Spatial trends of PFOS and PFHxS

Reference locations (Flat River and Flag Lake) had the lowest concentrations of analytes, as expected. Reference PFOS averages were significantly lower than interest averages (Welch's 2-sample t test: $t_{101.8} = 12.583$, $p << 0.001$). The same was true for PFHxS (Welch's 2-sample t test: $t_{17.54} = 12.993$, $p << 0.001$). Average PFOS concentrations were significantly greater than PFHxS concentrations (2-sample t test: $t_{348} = 20.255$, $p << 0.01$).

Spatial trends varied by each PFAS along Coopers Bayou. Generally speaking, both PFOS and PFHxS concentrations were highest near the intersection of Macks Bayou and Coopers Bayou (South Coopers, Macks Bayou, and Confluence). The spatial variation of PFHxS most closely followed the expected trend of PFAS partitioning to this bayou (Figure 2; bayou downstream flow ordered from left to right). Because of the groundwater flow from the northern fire training area, we expected PFAS concentrations at the Upstream location to be greater than the concentrations at Weapons Bridge and FTA-1 because of depuration and dilution with movement further downstream. We expected concentrations to peak at the area where the 2 bayous intersect (Coopers, Macks, and Confluence) because of the proximity to the southern fire training area, and

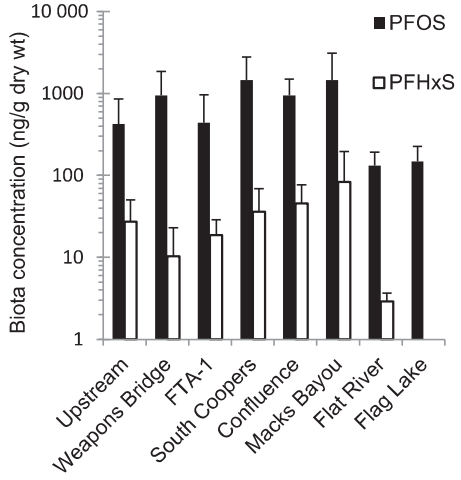

Figure 2. Spatial distribution of perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) to the system, irrespective of time. Downstream flow ordered left to right. Flat River and Flag Lake represent reference locations. Error bars represent standard error. FTA = fire training area.

direction of groundwater flow toward this location. One possible explanation for relatively high PFOS concentrations at the Weapons Bridge location could be its location under a bridge. The bayou pools in this area, providing a slow moving region where PFOS could stall, thus potentially increasing exposure time to individuals in this location.

Several factors could explain the expected spatial distribution for PFHxS but not for PFOS. First, PFOS is ubiquitous and more readily bioconcentrated than PFHxS in biota, but, more importantly, it is present in more consumer products than PFHxS. We believe that we are observing the expected spatial distribution of PFHxS because input is only from the historic usage of AFFFs, whereas PFOS, although likely dominated by the historic AFFF usage, likely also enters Coopers Bayou from the upstream community of Bossier City. There is even greater evidence of support for this hypothesis when we consider that there is no quantifiable PFHxS at Flag Lake, and very low concentrations at Flat River (Figure 2), our 2 reference locations. It is evident that PFHxS contamination in this system primarily exists at interest locations, further substantiating that exposure to this particular PFAS is likely originating from the historic fire training areas [9].

Temporal trends of PFOS

Concentrations of PFOS in fish were greatest in June 2014, with interest sites averaging 1929 ± 1685 ng/g dry weight. September PFOS concentrations (802 ± 366 ng/g dry wt) returned to those observed around the previous August (interest sites averaging 560 ± 711 ng/g dry wt) and November (interest sites average 734 ± 543 ng/g dry wt; Figure 3). The maximum concentration observed presently was 9349 ng/g dry weight in a longear sunfish (*Lepomis megalotis*) collected during June 2014 from our Macks Bayou location. To our knowledge, the highest PFOS concentrations observed in whole fish prior to the present study was measured in perch at 1500 μg/kg wet weight collected downstream of the Schiphol Amsterdam Airport (The Netherlands), 3 yr after an accidental spill of AFFFs [26], 1250 ng/g wet weight in a threadfin shad from the Ohio River (USA) [27], and 508 ng/g wet weight in sunfish and 351 ng/g wet weight in bullhead collected downstream of a Canadian airport with historic AFFF usage [13]. Higher concentrations have been observed in specific tissues like liver, however [3]. Note that the concentrations from other studies listed above were calculated in terms of tissue-wet weight. If we consider 1500 ng/g (wet wt or dry wt) burdens to be the maximum literature value for whole fish, in the present study we observed 39 composites, or approximately 15% of total samples and nearly 19% of composites from interest sites that exceeded this level. Furthermore, because of our reduced recovery of surrogates in whole fish, these concentrations are conservative estimates of body burdens and may be underestimating total PFOS in this system.

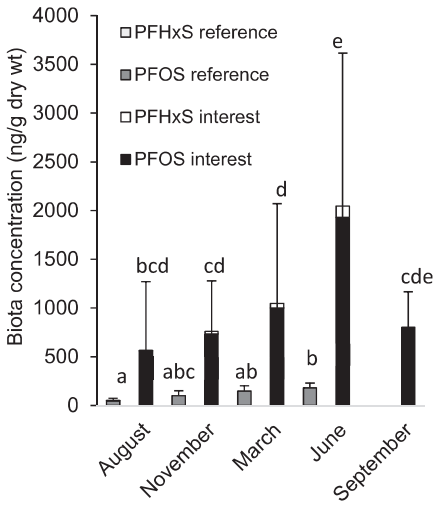

Figure 3. Temporal variation of perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS). Concentrations for both compounds increased with time through June, prior to returning to previous year levels in September. Error bars (standard error) only shown for PFOS. Perfluorohexane sulfonate was only quantifiable at reference sites in August. Significance indicated only for PFOS.

Average June interest site concentrations for fish were significantly greater than at all other time points, with the exception of September interest sites, likely because of the reduced number of biota samples collected in September. Each reference average was significantly lower than its respective interest average for each time point, with the exception of November reference versus November interest, again likely because of a smaller sampling event at this time point. Overall, we observed a distribution of PFOS concentrations that approximated a Gaussian distribution at interest locations through time. Reference values showed a significant linear increase in PFOS concentrations with time from August to June ($F_{1,2} = 202.8$, $p < 0.005$); [PFOS] $= 41.7 + 13.04\times$ month(#); $r^2 = 0.9902$), but unfortunately, because of difficulty sampling at these locations in September, we could not determine whether levels of PFOS declined at reference sites in September as they did overall (Figure 3).

Concentrations of PFHxS followed this same general trend from August to June (stacked bars, Figure 3); however, with the exception of August 2013, PFHxS was never quantifiable at reference locations. In addition, PFHxS concentrations were not quantifiable in September 2014 samples, suggesting, albeit not quantifiably, a return to low PFHxS exposure in fall.

We believe the observed increase in fish tissue concentrations through time was predominantly the result of a greater influx of groundwater (under the fire training areas) to bayou surface water. An additional hypothesis is that increased runoff from the surrounding community or through contaminated soil in the area could have increased PFAS in Coopers Bayou biota. However, because PFOS in whole fish followed a similar temporal trend as PFHxS in whole fish and we do not believe PFHxS runoff from the upstream community occurs to any great degree, an influx of PFAS-contaminated groundwater likely explains the increased concentrations in fish through time. Regardless of mechanism, the return of PFOS concentrations in fish in September 2014 to concentrations similar to those observed the previous August and November suggests that PFOS levels in fish are following a yearly, cyclical trend of exposure, perhaps as a result of seasonality, but more collections in subsequent years would be required to substantiate this hypothesis.

Family-level variation in PFOS in fish

Unfortunately, variability among teleost families remains relatively inconclusive because most of the samples are representative of the centrachid family (199 of 257 composites). Furthermore, the majority of samples were collected from interest locations compared with reference locations (209 of 257 composites). However, we observed a significant difference in PFOS concentrations in reference ($n = 46$) versus interest ($n = 153$) centrachids (Welch's 2-sample t test: $t_{80.2} = 15.051$,

$p << 0.001$), and this relationship is illustrated by the cumulative distribution function in Figure 4. In addition, a Kruskal–Wallis test revealed a significant difference between Cambaridae averages against Centrachidae, Cyprinidae, and Poeciliidae family averages (Figure 5).

Important to note is that although average PFOS body burdens in Ictaluridae were not significantly different from the pelagic fish families (Centrarchidae, Cyprinidae, and Poeciliidae), concentrations in ictalurids were lower than in these other families, more closely matching concentrations observed in crayfish (Cambaridae). Hence, there appears to have been less PFAS accumulation in catfish and benthic invertebrates compared with colocated pelagic species. This could be because of differences in metabolism or prey items, or perhaps the ability of catfish to tolerate pollution [28] has resulted in reduced incorporation or increased excretion of these compounds. Regardless of mechanisms, the observation of reduced contamination in benthic individuals is similar to results reported by de Solla et al. [13], but the opposite of observations by Gewurtz et al. [7].

Relationship to observed water concentrations

Because of the opportunistic nature of biota sampling, some sampling events were conducted only for water (and sediment; R.S. Cochran, 2015, Master's thesis, Texas Tech University, Lubbock, TX, USA). Thus the present study reports temporal concentrations of water not co-occurring with biota collection. In comparing water concentrations of PFOS and PFHxS with those found in biota, it should be noted that water and biota averages for both compounds only included interest location samples to reduce variability. Water PFOS concentrations peaked in late May (Figure 6), with subsequent fish sample PFOS concentrations peaking during the June sampling event. Because of the proteophilic nature of PFOS, this observed lag period likely reflects the time required between when exposure occurs and whole-body PFAS distribution and accumulation, as well as potential seasonal movement of fish.

In comparison with PFOS, the trends in water concentrations varied slightly for PFHxS, for which maximum water concentrations occurred in July (only marginally higher than March concentrations) as opposed to May, although

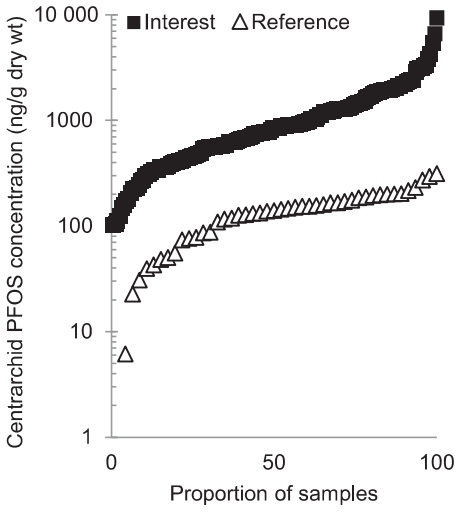

Figure 4. Cumulative distribution function of centrarchid perfluorooctane sulfonate (PFOS) concentrations at reference versus interest locations, irrespective of time.

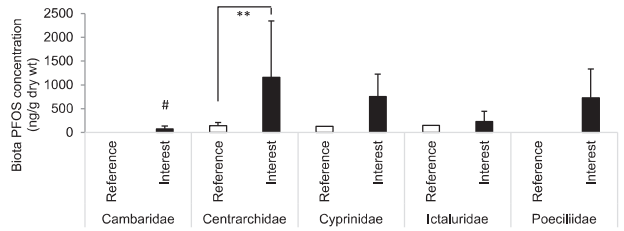

Figure 5. Family perfluorooctane sulfonate (PFOS) concentrations, irrespective of time. Cambaridae averages were significantly lower (#) than those for all other families except Ictaluridae, and there was a significant difference between reference and interest centrarchids (**).

concentrations were similar at all 3 time points (Figure 6). Biota concentrations still peaked during June, although it is possible that if there had been additional biota sampling in April and May, we might have observed maximum fish PFHxS concentrations at 1 of these time periods, closer to the higher water concentrations in March.

Generally, water concentrations of PFHxS and PFOS were comparable throughout the duration of the present study. August and November 2013 and March 2014 PFHxS water concentrations were marginally greater than PFOS concentrations at these same time points, but PFOS became the dominant PFAS in water in May and July 2014. Despite similar water concentrations for both PFAS, PFOS remained the dominant PFAS in biota. This was likely because of the relative lipophilicity of these 2 compounds, with the longer carbon backbone of PFOS making it slightly more hydrophobic than PFHxS. Interestingly, the octanol/water coefficient (K_{OW}) values for these compounds are not considered to be measurable, because of their ability to "form multiple layers in an octanol–water mixture" [29]. This characteristic further complicates the determination of fate and accumulation patterns in the environment; however, previous data suggest that bioconcentration factors (BCFs) increase approximately 100-fold with each carbon (and its accompanying 2 fluorines) added to the carbon backbone [30].

Because of the apparent lag period in incorporation of PFOS into whole carcasses after high exposure events, our BCFs were highly variable by month; November and June BCFs were 10 805 and 10 310 respectively, whereas August and March BCFs were much lower, 3965 and 2172, respectively (September BCF was 8902). Despite this variability, this range of BCFs fell

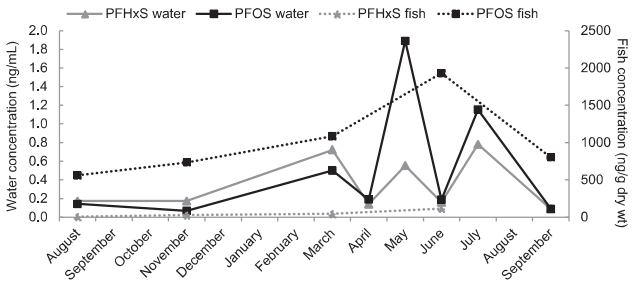

Figure 6. Average water concentrations of perfluorooctane sulfonate (PFOS; black) and perfluorohexane sulfonate (PFHxS; gray; primary *y*-axis, solid) from interest locations versus whole fish concentrations (secondary *y*-axis, dotted) from interest locations. Maximum fish PFOS concentrations (June) occur after maximum water PFOS concentrations are reported (May), despite water concentrations in June declining to levels observed at other time points. This lag period suggests a delay in whole-body PFOS distribution and accumulation.

within the range determined for whole fish from Korea by Naile et al. (despite their much lower PFOS body burdens) [31]. Similar to previous studies, August and November PFOS BCFs were nearly 100-fold larger than PFHxS (110-fold and 70-fold for August and November, respectively). However, March and June PFOS BCFs were less than 40-fold greater than PFHxS BCFs: 37.5-fold and 14-fold, respectively. Because water grab samples are snapshots of immediate contamination in the system and are not representative of temporal exposure to organisms, in a subsequent study we considered water concentrations against concentrations in gill and liver, both of which would show more immediate effects of exposure (H. Lanza, unpublished data).

CONCLUSIONS

Our data show PFAS contamination at Barksdale Air Force Base likely resulting from the use of AFFF in former fire training exercises. Our monitoring efforts encompassed a complete year of data, allowing us to consider impacts of environmental and temporal variability. Whole fish concentrations provided valuable insights into temporal, spatial, and species-specific variation in PFAS whole-body burdens; we observed increasing concentrations of PFOS from upstream to downstream, a possible cyclical annual partitioning pattern of PFOS, peaking in late spring/early summer, and benthic invertebrates having significantly lower concentrations of PFOS than pelagic fish families. We also observed a lag period between whole fish concentrations of PFOS in relation to water concentrations from the same locations, likely as a result of the time required for PFOS to incorporate into whole carcasses.

Temporal PFOS concentrations approximated a Gaussian distribution, with concentrations peaking during June and remaining low in the autumns of 2013 and 2014, suggesting a potential yearly cyclical exposure trend. Unfortunately, because of the limited samples size in September 2014, we cannot conclusively say this trend was the case for all locations or for the system as a whole, and additional sampling would be needed to further elucidate temporal trends.

Because of the non-uniform distribution of PFAS in this system (a uniform distribution could suggest PFAS contamination is a result of runoff from the surrounding upstream community from other consumer products), and the cessation of fire training activities with AFFF containing PFAS of interest at this location, we believe that there is varying retention of PFAS in groundwater. The temporal variation observed in the present study suggests that groundwater is reaching the surface in a heterogeneous manner, likely as a result of differences in groundwater sediment attenuation [12], water level variation, and gaining stream differences [32]. We observed the expected PFAS distribution in this system for PFHxS but, interestingly, not for PFOS.

A probable contributor to the variability of PFAS concentrations in our system is likely the fact that fish are not sessile. They are able to move freely throughout the bayou and therefore may move from areas of high PFAS contamination to areas of low contamination and vice versa. Hence, the location of capture is not necessarily representative of where they were located in the time leading up to their capture.

To our knowledge, the present study reports the highest concentrations of PFOS in whole fish to date, and supports the surrounding literature in that PFOS concentrations may pose a potential exposure risk to fishermen [21]. However, because of the difficulty accessing our sampling areas, human exposure to highly contaminated fish at Barksdale Air Force Base is unlikely, although, because fish are mobile species, some individuals are likely to move off-site where human exposure is more likely. There is also concern for other ecological receptors, specifically larger predators in this ecosystem like alligators and bird species, and also mammals like deer, which may be drinking contaminated water and then traveling to a more accessible hunting area.

In conclusion, the present study highlights the importance of monitoring for PFAS in environmental samples, and the magnitude of samples analyzed supports a robust risk assessment and site characterization effort at Barksdale Air Force Base. The present study's results may be used for extrapolation of risk to other locations of known historic use of AFFFs, and may spur additional studies to consider risk to the surrounding communities via transport of these PFAS off-site.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (heather.a.lanza@gmail.com).

REFERENCES

1. US Environmental Protection Agency. 2012. Emerging contaminants—Perfluorooctane sulfonate (PFOS) and perflu-orooctanoic acid (PFOA). EPA 505-F-14-001. Washington, DC.
2. Paul AG, Jones KC, Sweetman AJ. 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ Sci Technol* 43:386–392.
3. Oliaei F, Kriens D, Weber R, Watson A. 2013. PFOS and PFC releases and associated pollution from a PFC production plant in Minnesota (USA). *Environ Sci Pollut Res* 20:1977–1992.
4. Lindstrom AB, Strynar MJ, Libelo EL. 2011. Polyfluorinated compounds: Past, present, and future. *Environ Sci Technol* 45:7954–7961.
5. Houde M, De Silva AO, Muir DCG, Letcher RJ. 2011. Monitoring of perfluorinated compounds in aquatic biota: An updated review of PFCs in aquatic biota. *Environ Sci Technol* 45:7962–7973.
6. Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SC. 2002. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environ Sci Technol* 36:545–551.
7. Gewurtz SB, Bhavsar SP, Petro S, Mahon CG, Zhao X, Morse D, Reiner EJ, Tittlemier SA, Braekevelt E, Drouillard K. 2014. High levels of perfluoroalkyl acids in sport fish species downstream of a firefighting training facility at Hamilton International Airport, Ontario, Canada. *Environ Int* 67:1–11.
8. Darwin RL. 2004. Estimated quantities of aqueous film forming foam (AFFF) in the United States. The Fire Fighting Foam Coalition, Arlington, VA, USA.
9. Place BJ, Field JA. 2012. Identification of novel fluorochemicals in aqueous film-forming foams used by the US military. *Environ Sci Technol* 46:7120–7127.
10. Moody CA, Field JA. 2000. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ Sci Technol* 34:3864–3870.
11. Moody CA, Hebert GN, Strauss SH, Field JA. 2003. Occurrence and persistence of perfluorooctanesulfonate and other perfluorinated surfactants in groundwater at a fire-training area at Wurtsmith Air Force Base, Michigan, USA. *J Environ Monit* 5:341–345.
12. Guelfo JL, Higgins CP. 2013. Subsurface transport potential of perfluoroalkyl acids at aqueous film-forming foam (AFFF)-impacted sites. *Environ Sci Technol* 47:4164–4171.
13. de Solla SR, De Silva AO, Letcher RJ. 2012. Highly elevated levels of perfluorooctane sulfonate and other perfluorinated acids found in biota and surface water downstream of an international airport, Hamilton, Ontario, Canada. *Environ Int* 39:19–26.
14. Greaves AK, Letcher RJ. 2013. Linear and branched perfluorooctane sulfonate (PFOS) isomer patterns differ among several tissues and blood of polar bears. *Chemosphere* 93:574–580.

15. Riget F, Bossi R, Sonne C, Vorkamp K, Dietz R. 2013. Trends of perfluorochemicals in Greenland ringed seals and polar bears: Indications of shifts to decreasing trends. *Chemosphere* 93:1607–1614.
16. Butt CM, Berger U, Bossi R, Tomy GT. 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci Total Environ* 408:2936–2965.
17. Perez F, Nadal M, Navarro-Ortega A, Fabrega F, Domingo JL, Barcelo D, Farre M. 2013. Accumulation of perfluoroalkyl substances in human tissues. *Environ Int* 59:354–362.
18. Trudel D, Horowitz L, Wormuth M, Scheringer M, Cousins IT, Hungerbuhler K. 2008. Estimating consumer exposure to PFOS and PFOA. *Risk Anal* 28:251–269.
19. Domingo JL, Ericson-Jogsten I, Perello G, Nadal M, Van Bavel B, Karrman A. 2012. Human exposure to perfluorinated compounds in Catalonia, Spain: Contribution of drinking water and fish and shellfish. *J Agric Food Chem* 60:4408–4415.
20. Holzer J, Goen T, Just P, Reupert R, Rauchfuss K, Kraft M, Muller J, Wilhelm M. 2011. Perfluorinated compounds in fish and blood of anglers at Lake Mohne, Sauerland Area, Germany. *Environ Sci Technol* 45:8046–8052.
21. Berger U, Glynn A, Holmstrom KE, Berglund M, Ankarberg EH, Tornkvist A. 2009. Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden—Analysis of edible fish from Lake Vattern and the Baltic Sea. *Chemosphere* 76:799–804.
22. Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 22:196–204.
23. Kannan K, Tao L, Sinclair E, Pastva SD, Jude DJ, Giesy JP. 2005. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. *Arch Environ Contam Toxicol* 48:559–566.
24. Nyman JA, Baltz DM, Kaller MD, Leberg PL, Richards CP, Romaire RP, Soniat TM. 2013. Likely changes in habitat quality for fish and wildlife in coastal Louisiana during the next fifty years. *J Coastal Res* 67:60–74.
25. Haljasorg T, Saame J, Kipper K, Teearu A, Herodes K, Reinik M, Leito I. 2014. Alternative eluent composition for LC-MS analysis of perfluoroalkyl acids in raw fish samples. *J Agric Food Chem* 62:5259–5268.
26. Kwadijk CJAF, Kotterman M, Koelmans AA. 2014. Partitioning of perfluorooctanesulfonate and perfluorohexanesulfonate in the aquatic environment after an accidental release of aqueous film forming foam at Schiphol Amsterdam Airport. *Environ Toxicol Chem* 33:1761–1765.
27. Ye X, Strynar MJ, Nakayama SF, Varns J, Helfant L, Lazorchak J, Lindstrom AB. 2008. Perfluorinated compounds in whole fish homogenates from the Ohio, Missouri, and Upper Mississippi Rivers, USA. *Environ Pollut* 156:1227–1232.
28. Walden HT. 1964. *Familiar Freshwater Fishes of America*. Harper & Row, New York, NY, USA.
29. US Agency for Toxic Substances and Disease Registry. 2009. Chemical and physical information—Draft toxicological profile for perfluoroalkyls. Atlanta, GA, USA.
30. Giesy JP, Naile JE, Khim JS, Jones PD, Newsted JL. 2010. Aquatic toxicology of perfluorinated chemicals. *Rev Environ Contam Toxicol* 202:1–52.
31. Naile JE, Khim JS, Hong S, Park J, Kwon B-O, Ryu JS, Hwang JH, Jones PD, Giesy JP. 2013. Distributions and bioconcentration characteristics of perfluorinated compounds in environmental samples collected from the west coast of Korea. *Chemosphere* 90:387–394.
32. Alley WM, Franke OL, Reilly TE. 1999. Sustainability of ground-water resources. US Department of the Interior, US Geological Survey, Denver, CO, USA.