



Contamination of Per- And Polyfluoroalkyl Substances in Freshwater Fish from Areas Adjacent to Three Former and Active Military Facilities in New York

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Abstract

This study investigates the contamination of per- and polyfluoroalkyl substances (PFAS) in freshwater fish from waterbodies near former and currently active military bases in the State of New York, USA. Three facilities with a history of long-term use and discharge of aqueous film-forming foam (AFFF) were studied. Here, we focused on previously uninvestigated areas that are outside of the base properties. Freshwater fish samples were collected from sites at different distances both downstream and upstream of the bases. Overall, 508 fish from 25 species were collected at 22 sites over 3 years, and whole-fish PFAS concentrations were quantified using LC–MS/MS. PFAS levels in fish from downstream sites have significantly higher body burden of PFAS. Perfluorooctane sulfonic acid (PFOS), the foaming agent in legacy AFFF, is the dominant PFAS component in fish from downstream sites in all waterbodies sampled and appeared to be the main driving factor differentiating fish from presumed contaminated sites and reference sites. Distance from the contaminant source, species, and hydrological conditions also significantly influenced the PFAS accumulation in fish. Temporal differences were only significant at sites where accidental discharge of AFFF occurred immediately prior to the 1st year of sampling. The current study demonstrates the extent of PFAS contamination and accumulation in biota at a distance away from the contaminant source. We also highlight the need for evaluation of potential concerns for human and ecological health in these areas as a result of historical AFFF use and release from military facilities in the US.

Aqueous film-forming foam (AFFF) has been extensively used by the United States Air Force (USAF) for decades as a fire extinguishing agent for Class B hydrocarbon fires (Center for Chemical Process Safety 2003; Geyer 1969). In order to prevent damage to aircrafts and base facilities, aircraft hangars at military bases are equipped with fire

suppression systems capable of rapidly discharging large amount of AFFF (Scheffey et al. 2000). Such systems are routinely tested, and firefighting trainings are frequently conducted at USAF bases. This practice is considered essential as valuable aircrafts and highly dangerous material may be housed in these hangars. However, it also resulted in substantial and widespread release of AFFF into the environment (Anderson & Kempisty 2018). Before January 2023, all AFFF used by USAF contained per- and polyfluoroalkyl substances (PFAS) as foaming agent because these compounds, and perfluorooctane sulfonic acid (PFOS) in particular, were arguably the only ones that satisfied the requirements defined in the Military Specification for AFFF (MIL-PRF-24385) for generating foam at specific rates (Federal Aviation Administration 2021; U. S. Dept. of Defense, 2020b).

During the past two decades, mounting evidence on the extreme persistence of PFAS in the environment as well as the health risks to both humans and wildlife from the exposure to PFAS eventually led to a series of changes globally in the management of the use and release of

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PFAS containing AFFF in the environment (Glüge et al. 2020; Wang et al. 2017). PFOS, PFOA, and PFHxS were listed in the Stockholm Convention on Persistent Organic Pollutants in 2009, 2019, and 2022, respectively. In addition, long-chain perfluorocarboxylic acids (C9–C12) are currently under consideration for listing (Stockholm Convention, n.d.). The United States Environmental Protection Agency (EPA) updated health advisory levels for PFOA and PFOS in 2016 (U. S. Environmental Protection Agency, 2016b) and then again in 2022 (U. S. Environmental Protection Agency, 2022) before proposing to designate the two chemicals as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as Superfund) (U. S. Environmental Protection Agency, 2024b). The US Department of Defense (DOD) also began monitoring PFAS in drinking water and restricting PFAS release from military installations (National Defense Authorization Act for Fiscal Year 2019; U. S. Dept. of Defense, 2020a). In 2023, the Military Specification for AFFF was updated to include fluorine-free foaming agents (U. S. Dept. of Defense, 2023). However, by then, evidence suggested that decades of PFAS containing AFFF use and unregulated discharge had already resulted in noticeable contaminated ground and surface water near former or active USAF bases (Anderson & Kempisty 2018). Consequently, there may be lasting human health and ecological risk from such contamination due to the potential toxicity and persistence of these compounds (Cousins 2015; Filipovic et al. 2015).

Chemical properties of PFAS are distinct from other common legacy organic contaminants in that their perfluorocarbon moieties are both hydrophobic and lipophobic. Consequently, the pattern of partitioning of these chemicals between different environmental media cannot be accurately predicted by their relative affinity to aqueous and organic phases (Evich et al. 2023; Glüge et al. 2020). Similarly, tendency for bioaccumulation is highly variable among different PFAS compounds and is dependent on more factors than lipid partitioning (Burkhard 2021; Munoz et al. 2022; Rayne & Forest 2009). As a result, understanding the fate of PFAS in the physical environment and biota has been particularly challenging. Nonetheless, such knowledge is essential for developing PFAS environmental quality criteria (EQC) for the protection of human and ecological health. These EQC are important for establishing cleanup goals for contaminated sites such as USAF bases and surrounding areas. While significant effort has been made to gain better understanding on the environmental fate of PFAS and their potential impact on wildlife and human populations, current available science remains inadequate for developing reliable EQC, particularly those pertaining the protection of wildlife resources (Grippo et al. 2021). Continuous effort has been made by researchers in academic and government research

institutions to expand knowledge on the behavior of PFAS in the ecosystem.

There are 12 active military installments and more than 30 formal military facilities in New York. Two of the formal Air Force Bases, the Plattsburgh Air Force Base (PAFB) in Plattsburgh, NY, and the Griffiss Air Force Base (GAFB) in Rome, NY, are federal Superfund sites due to the presence of legacy contaminants (U. S. Environmental Protection Agency, 2024a). Both of them have a known history of extensive AFFF use, and prevalent surface and ground water contamination by PFAS in the bases was confirmed by recent DOD investigations (U. S. Dept. of Air Force, 2018, 2020). In addition, the Stewart Air National Guard Base (SANGB) in Newburgh, one of the active military installments, was declared a New York State Superfund site in 2016 due to contamination of the drinking water supply for the town of Newburgh by AFFF discharge from the base (New York Air National Guard 2020; New York State Dept. of Environmental Conservation, 2016).

The areas around PAFB, GAFB, and SANGB contain a wide variety of habitats for aquatic organisms such as lakes, streams, and wetlands. These habitats are either situated in densely populated urban areas or are within State Parks and Forest with public access. Therefore, high level of human recreational activities including hunting and fishing are common in these areas. It is likely that PFAS from the bases have contaminated the areas. A healthy ecosystem provides more benefits to humans than serving as a source for food. Preserving biodiversity is culturally important and close contact with nature have been shown to correlate with improved physical and mental health in humans (Clark et al. 2014; Coutts et al. 2014; Frumkin 2001). Studying the impact of PFAS near NYS USAF sites would not only contribute significantly to the knowledge base of PFAS ecotoxicology, but also potentially provide additional benefit for humans. Based on this consideration, the Division of Fish & Wildlife (DFW) in NYSDEC analyzed fish samples from SSF and some of the areas in the vicinity of GAFB and PAFB in order to (1) determine the extent of PFAS contamination in fish near three US military facilities, and (2) identify important factors that may affect the levels of PFAS accumulation in fish (e.g., species and site conditions).

Methods

Study Sites

Three general locations were targeted in the current study: Steward State Forest (SSF) near the SANGB, Lake Champlain near the PAFB, and streams and rivers surrounding the GAFB in Rome, NY. Multiple sites within each location were sampled (Fig. 1 & Table 1). All sites positioned

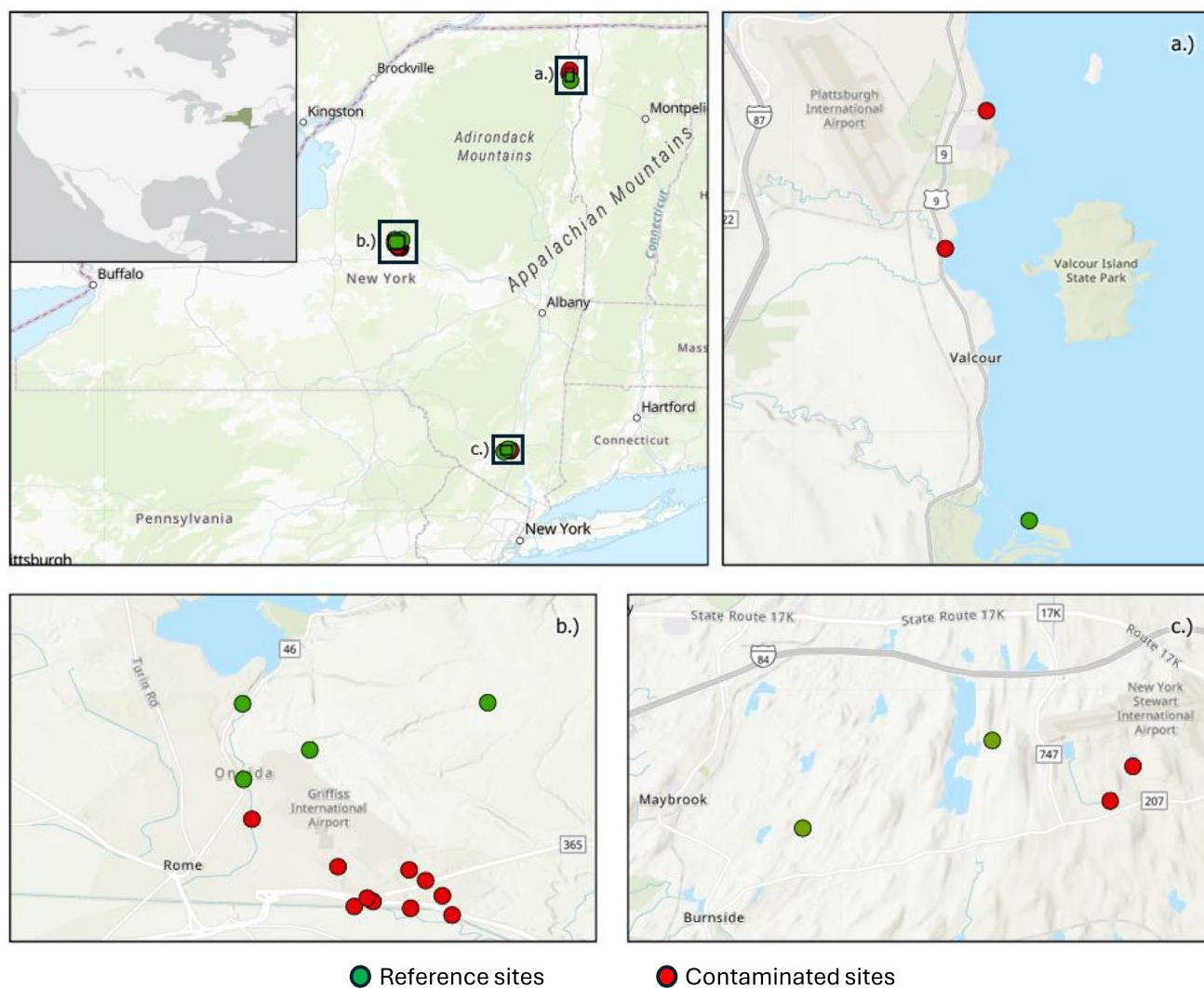


Fig. 1 General location of the study sites in New York State, US. **a** Lake Champlain at Plattsburgh, NY, **b** Griffiss International Airport (formerly Griffiss Air Force Base) at Rome, NY, and **c** Stewart State Forest in Orange County, NY

downstream to known points of PFAS discharge into the waterbody were tentatively classified as contaminated sites, and those positioned upstream to PFAS discharge points were tentatively classified as reference sites.

SSF

Immediately adjacent to SANGB is SSF. With a total area of 27 km², SSF contains diverse habitats including forest, wetlands, and meadows. A variety of aquatic and terrestrial wildlife species are found in these habitats. Many of them could have been affected by PFAS contamination from SANGB. The primary area of interest is at the eastern end of SSF between Drury Ln and Jackson Ave. This area has relatively low human activity compared to the rest of the SSF due to poor trail conditions. Several small streams originating from the main runway area of

the Stewart International Airport run through this area. Two sites were selected here: One is located at Clark Street which includes a shallow pond fed by streams originating from the airport (Figure S1 (a)). The second site contains a large wetland and a stream near a segment of the Armstrong Lane Trail off Little Britain Road (Figure S1 (b)).

Two reference sites to the west of Drury Lane also were selected for sampling: the Whalenburgh Pond off Maple Avenue (Figure S1 (c)) and the Beaver Pond near the west end of the SSF (Figure S1 (d)). These sites habitats are similar to the primary sites but experience more human activities (hiking, mountain biking, etc.) than the Clark Street and Armstrong Lane Trail sites. The paths of streams in the area indicate that direct deposition of PFAS from the airport into surface water at these sites is unlikely (Table 2).

Table 1 Lists of study sites and the species of freshwater fish collected at each site

Location	Site	Site designation	Species and number of samples collected																							
			Blacknose Dace	Common Shiner	Cut-lips Minnow	Eastern Mudminnow	Fathead Minnow	Golden Shiner	Longnose Dace	Banded Killifish	Bluegill	Pumpkinseed	Rock bass	Brown Trout	Brook Trout	Creek Chub	White Sucker	Chain Pickerel	Large-mouth Bass	Redfin Pickerel	Smallmouth Bass	Walleye	Fantail Darter	Tessellated Darter	Yellow perch	Logperch
Stewart State Forest	Beaver Pond	Reference					3																			
	Whalenburgh Pond	Reference				2			14	1									10	1						
	Armstrong Ln	Contaminated		5		3			9	13									1	1						
	Clark St. Pond	Contaminated							2	10									10	6						
	Clark St. Stream	Contaminated		6		2					13					5			4	6						6
Lake Champlain	Ausable Point	Reference				1			1	4																5
	Salmon River	Contaminated	6			6			4	2	6													6		10
	Southern Stream	Contaminated							5		2													4		1
Sixmile Creek	Camroden Rd	Reference	5												4											
	Gulf Road	Reference	3										3	3	3											
	Larry Farm	Contaminated										10	2	5	4	9									3	
Threemile Creek	Rickmeyer Rd	Contaminated	8										12	3	12	14										
	Rt 365	Contaminated	3										5	9	15	13										
	Sculpture Garden	Contaminated	2			7									10	5										
	River Rd	Contaminated													4	1										
	Pond @ HWY49	Contaminated													3	6										

Table 1 (continued)

Location	Site	Site designation	Species and number of samples collected																							
			Blacknose Dace	Common Shiner	Cutlips Minnow	Eastern Mudminnow	Fathead Minnow	Golden Shiner	Longnose Dace	Banded Killifish	Bluegill	Pumpkinseed	Rock bass	Brown Trout	Brook Trout	Creek Chub	White Sucker	Chain Pickerel	Large-mouth Bass	Redfin Pickerel	Smallmouth Bass	Wall-eye	Fantail Darter	Tessellated Darter	Yellow perch	Logperch
Mohawk River	Outfall Hatchery	Reference	1																							
	Settlement Bridge	Reference	4	6				3								3							1			
	Chestnut St. Bridge	Contaminated	1	7				1							2	1							1			
	Erie Canal	Contaminated									2	4				5				1						9
Erie Canal	Between	Contaminated																								
	Erie Canal D/S	Contaminated									2					10	1		2		9					11
	Erie Canal U/S	Contaminated								4	5	3				11	1				6					6

Lake Champlain

PAFB is sandwiched between the Saranac River to the north and the Salmon River to the south. Both rivers drain to Lake Champlain. The runway at the base is about 1 kilometer from the shore of Lake Champlain, and there are several small, unnamed streams running from the base to the lake. Lake Champlain is a large natural freshwater lake with an area of approximately 1269 km². It lies primarily between the states of New York and Vermont, but also extends north into the Canadian province of Quebec. The lake supports important cold water and warmwater fisheries.

Three sites were selected for sampling at Lake Champlain: the Salmon River inlet into the lake, a cove where an unnamed stream originating from PAFB enters the lake, and the beach area near Ausable Point Campground (Figure S2). Both Salmon River and the Unnamed Stream receive wastewater discharge and stormwater runoff from the base. Therefore, both are likely contaminated with AFFF. The Ausable Point site is about 4 km to the south of the base and is part of the Ausable River delta area. There is no known source of substantial AFFF discharge into the Ausable River that may contaminate the sampling site; therefore, this site served as a reference site for this location.

GAFB

GAFB, located near downtown Rome in Central New York, is surrounded by mostly urban areas to the west and a mix of farmland and natural areas to the east. Several rivers and streams run through the area and are habitat for a variety of fish species including naturally reproducing brown and brook trout (*Salmo trutta* and *Salvelinus fontinalis*), creek chubs (*Semolilus atromaculatus*), and white suckers (*Catostomus commersonii*). Four streams/rivers in Rome NY were sampled because they have segments on or near the property of GAFB: Six Mile Creek, Three Mile Creek, Mohawk River, and Erie Canal (Fig. 1b).

Six Mile Creek is a small tributary which flows approximately 14 km to a confluence with the Erie Canal/Mohawk River just east of Rome, NY. A segment (~2.5 km) of the stream at approximately halfway along its course lies within the base. Most of this segment runs in an underground tunnel along the direction of the runway. The stream exits GAFB near the southeast corner of the property and continues in a southeast direction another 2.5 km to the confluence with the Erie Canal and Mohawk River system as a third order stream. Two upstream reference sites (Figure S3) and three downstream contaminated sites (Figure S4) at increasing distance away from GAFB were selected for fish sample collection.

Table 2 Average concentration of whole-fish PFAS

Location	Site	Site designation	Mean whole-fish concentration (ng/g) ¹				
			PFHxA	PFHpA	PFOA	PFNA	PFDA
Stewart State Forest	Beaver Pond	Reference	0.12 ± 0.08	0.1 ± 0	0.1 ± 0	0.27 ± 0.06	0.8 ± 0.1
	Whalenburgh Pond	Reference	0.41 ± 0.3	0.35 ± 0.28	0.45 ± 0.33	0.4 ± 0.28	0.52 ± 0.29
	Armstrong Ln	Contaminated	0.46 ± 0.33	0.3 ± 0.25	0.43 ± 0.31	0.51 ± 0.38	2.29 ± 2.09
	Clark St	Contaminated	0.47 ± 0.26	0.44 ± 0.3	0.56 ± 0.4	0.78 ± 0.57	5.96 ± 2.28
Lake Champlain	Ausable Point	Reference	0.58 ± 0.28	0.56 ± 0.36	0.46 ± 0.33	1.24 ± 0.34	1.39 ± 0.62
	Salmon River	Contaminated	0.49 ± 0.29	0.52 ± 0.3	0.63 ± 0.44	1.18 ± 0.68	1.47 ± 0.74
	Unnamed Stream	Contaminated	0.53 ± 0.31	0.69 ± 0.18	0.54 ± 0.38	1.06 ± 0.83	2.81 ± 1.42
Six Mile Creek	Camroden Rd	Reference	0.44 ± 0.21	0.44 ± 0.31	0.52 ± 0.26	0.73 ± 0.52	2.35 ± 1.43
	Gulf Road	Reference	0.47 ± 0.25	0.47 ± 0.29	0.53 ± 0.27	0.52 ± 0.36	0.46 ± 0.29
	Rickmeyer Rd	Contaminated	0.54 ± 0.31	0.66 ± 0.36	0.64 ± 0.66	1.55 ± 1.07	2.54 ± 1.76
	Rt 365	Contaminated	0.5 ± 0.36	0.67 ± 0.65	0.73 ± 0.48	1.25 ± 0.73	2.02 ± 1.24
	Larry Farm	Contaminated	0.5 ± 0.37	0.73 ± 0.63	1.3 ± 1.4	1.34 ± 1.2	1.5 ± 0.94
Three Mile Creek	Pond @ HWY49	Contaminated	0.62 ± 0.35	0.6 ± 0.35	0.83 ± 0.37	2.48 ± 1.31	3.27 ± 1.95
	River Rd	Contaminated	0.65 ± 0.37	0.27 ± 0.25	0.9 ± 0.38	2.9 ± 1.16	4.42 ± 1.45
Mohawk River	Sculpture Garden	Contaminated	0.5 ± 0.31	0.51 ± 0.31	0.96 ± 0.89	0.76 ± 0.53	2.5 ± 1.21
	Outfall @ Hatchery	Reference	0.54 ± 0.52	0.41 ± 0.01	0.7 ± 0.1	1.2 ± 0.14	1.45 ± 0.07
	Wright Settlement Rd bridge	Reference	0.56 ± 0.25	0.47 ± 0.22	0.42 ± 0.33	0.65 ± 0.39	1.32 ± 0.59
	Chestnut St bridge	Contaminated	0.5 ± 0.25	0.71 ± 0.5	0.44 ± 0.31	0.64 ± 0.42	0.94 ± 0.36
Erie Canal	Erie Canal U/S	Contaminated	0.56 ± 0.25	0.49 ± 0.29	0.53 ± 0.26	0.58 ± 0.26	0.61 ± 0.32
	Erie Canal Btw	Contaminated	0.57 ± 0.25	0.54 ± 0.26	0.54 ± 0.29	0.55 ± 0.27	0.73 ± 0.46
	Erie Canal D/S	Contaminated	0.58 ± 0.27	0.49 ± 0.28	0.36 ± 0.23	0.58 ± 0.27	0.71 ± 0.38

Location	Site	Site designation	Mean whole-fish concentration (ng/g) ^a					
			PFDoA	PFBS	PFHxS	PFOS	PFOSA	tPFAS ^b
Stewart State Forest	Beaver Pond	Reference	0.63 ± 0.06	0.1 ± 0	0.1 ± 0	2.27 ± 0.32	0.1 ± 0	6.05 ± 0.33
	Whalenburgh Pond	Reference	0.54 ± 0.37	0.68 ± 0.59	0.81 ± 0.68	2.03 ± 1.27	0.79 ± 0.69	7.83 ± 2.6
	Armstrong Ln	Contaminated	1.89 ± 1.53	0.82 ± 0.66	0.78 ± 0.63	125.02 ± 130	1.12 ± 0.96	136.55 ± 135.15
	Clark St	Contaminated	3.87 ± 1.91	1.02 ± 0.63	4.75 ± 7.17	315.7 ± 146.36	2.79 ± 1.96	339.89 ± 151.24
Lake Champlain	Ausable Point	Reference	0.68 ± 0.42	0.5 ± 0	1.03 ± 0.64	13.21 ± 5.53	1.39 ± 0.79	22.51 ± 6.35
	Salmon River	Contaminated	1.11 ± 0.65	0.5 ± 0	1.52 ± 1.17	71.35 ± 41.33	1.74 ± 1.87	81.72 ± 44.03
	Unnamed Stream	Contaminated	2.44 ± 2.59	0.5 ± 0	5.06 ± 9.14	263.8 ± 274.36	9.94 ± 7.76	289.77 ± 295.22
Six Mile Creek	Camroden Rd	Reference	1.57 ± 0.99	1 ± 0.64	1.04 ± 0.63	25.87 ± 13.35	1.11 ± 0.59	38.74 ± 16.82
	Gulf Road	Reference	0.65 ± 0.31	1.1 ± 0.63	0.81 ± 0.62	3.73 ± 1.5	1.63 ± 1.36	10.98 ± 2.5
	Rickmeyer Rd	Contaminated	2.15 ± 1.42	0.96 ± 0.54	18.2 ± 17.33	964.02 ± 1296.46	7.52 ± 3.25	1000.75 ± 1303.54
	Rt 365	Contaminated	2.44 ± 1.76	0.96 ± 0.64	21.35 ± 34.39	792.92 ± 877.64	5.81 ± 2.38	830.53 ± 878.91
	Larry Farm	Contaminated	1.75 ± 1.23	1.07 ± 0.53	15.71 ± 24.37	489.99 ± 504.43	4.21 ± 2.82	519.75 ± 512.53
Three Mile Creek	Pond @ HWY49	Contaminated	2.8 ± 1.69	1.12 ± 0.61	14.48 ± 9.35	558.24 ± 389.36	8.22 ± 4.22	594.25 ± 399.44
	River Rd	Contaminated	3.22 ± 0.96	0.76 ± 0.63	17.02 ± 7.92	754.6 ± 158.7	3.82 ± 2.47	790.93 ± 162.93
	Sculpture Garden	Contaminated	3.37 ± 1.67	1.14 ± 0.62	30.7 ± 27.16	758 ± 309.9	18.08 ± 6.94	817.94 ± 326.85
Mohawk River	Outfall @ Hatchery	Reference	0.22 ± 0.27	1.18 ± 0.69	1.14 ± 0.68	4.8 ± 1.7	1.07 ± 0.9	14.71 ± 2.77
	Wright Settlement Rd bridge	Reference	0.54 ± 0.35	1.21 ± 0.51	1.18 ± 0.58	6.85 ± 3.49	0.96 ± 0.65	16.44 ± 4.32
	Chestnut St bridge	Contaminated	0.7 ± 0.43	0.87 ± 0.68	1.18 ± 0.5	27.43 ± 46.02	1.26 ± 0.67	36.66 ± 46.39

Table 2 (continued)

Location	Site	Site designation	Mean whole-fish concentration (ng/g) ^a					
			PFD _o A	PFBS	PFHxS	PFOS	PFOSA	∑PFAS ^b
Erie Canal	Erie Canal U/S	Contaminated	0.67 ± 0.39	1.02 ± 0.57	0.96 ± 0.5	43.18 ± 23.64	0.83 ± 0.62	50.3 ± 23.87
	Erie Canal Btw	Contaminated	0.67 ± 0.45	1.18 ± 0.66	1.13 ± 0.69	72.63 ± 47.97	1.12 ± 0.68	80.61 ± 48.6
	Erie Canal D/S	Contaminated	0.57 ± 0.44	1.05 ± 0.64	1.31 ± 0.79	70.9 ± 42.94	1.36 ± 0.9	78.73 ± 44

^aConcentration of PFAS is presented as Mean ± standard deviation^bTotal PFAS concentrations, calculated as sum of all individual analytes

Three Mile Creek, located in a partially forested area in the southern part of the GAFB, is a small second-order stream (approximately 2.8 km long with a mean width of 1.5 m and a mean depth of 0.4 m) that originates in the base. It is bordered by an electrical power substation on the northwest side, landfills on the northeast side, and a former airbase housing development on the southwest side. The stream receives both surface water runoff and groundwater from the surrounding watershed and flows in a southeasterly direction into the NYS Erie Canal (about 1 km south of the former base). Consequently, the entire stream is considered contaminated. Three sites along the creek, including a small pond (100 m long and 25 m wide with a maximum depth of 2 m) about 0.5 km downstream of the base, were selected for sampling (Figure S5).

Mohawk River is a major river that runs through the city of Rome and flows past the western end of GAFB. An outfall (Outfall 003) located between the bridges at Wright Settlement Road and Chestnut Street discharges stormwater from the base into the river. Two sites upstream of the outfall at the NYSDEC Rome Fish Hatchery and Wright Settlement Road Bridge as well as one site downstream of the outfall at the Chestnut Street Bridge were selected for sampling (Figure S5).

The above three streams/rivers enter the Erie Canal, which is a large artificial waterway located to the south of the city of Rome. Three sites were selected for sampling at the Erie Canal. One is located upstream of the entry point of Three Mile Creek, one in between the entry points of Three Mile Creek and Six Mile Creek, and the third one downstream of the entry point of Six Mile Creek (Figure S6).

Sample Collection

Sampling was conducted between June and September in 2018, 2020, and 2021. Fish were caught at selected sites by electrofishing, angling, seining, or gillnetting depending on the characteristic of the sites at the time of the sampling.

The overall composition of the fish community in most sites was not known but was predicted based on the habitat characteristics. Therefore, no species were specifically targeted, and all available species were collected. Due to the high prevalence of PFAS in common products, special precautions were taken to minimize contamination during handling, transportation, and storage of all samples (e.g., PFAS free clothing for collectors and PFAS free bags and containers for sample packaging and storage). All sampled fish were measured and weighed in the field. Trout, suckers, and larger creek chubs were stored individually as separate samples. Small fish (primarily minnows) were speciated and combined into species-specific composite samples of similar size individuals to ensure sufficient mass was contained in each sample for chemical analysis. All fish samples were placed on ice immediately after collection and transferred to the laboratory by the end of the day, where they were stored frozen at -20 °C until further analysis. Collection record forms and chain-of-custody forms were completed in the field and remained with the samples until analyzed by the laboratory.

The sites within the Stewart State Forest and those from Six Mile Creek at Rome were first sampled in 2018 and then again in 2020. The Three Mile Creek sites at Rome were sampled in 2020 and 2021. The sites on the Mohawk River and Erie Canal at Rome were only sampled in 2020 while the sites on Lake Champlain were only sampled in 2021.

Chemical Analyses

Whole-fish samples were homogenized using a bench-top laboratory blender. Homogenates and Quality Control (QC) samples were fortified with labeled PFAS and extracted with basic methanol via sonication and shaking. Sample cleanup was achieved via ENVI-Carb™ followed by SPE with Waters Oasis® WAX cartridges; PFAS were eluted in two fractions: 1) neutral methanol (containing PFOSA) and 2) basic methanol (containing

all other PFAS). The basic methanol fraction was evaporated to near dryness using nitrogen, then reconstituted with the respective neutral methanol fraction and spiked with a recovery standard. Samples were then filtered and analyzed via LC/MS/MS. A gradient chromatographic method was used with 5 mM ammonium acetate (containing 0.1% acetic acid) and methanol on a C18 column, and the MS/MS was operated in negative mode. Two mass transitions were used to positively identify each native compound. PFAS concentrations were determined by an isotope dilution method (U. S. Environmental Protection Agency, 2019a, 2019b). Method detection limits (MDL) were determined following methods described in U. S. Environmental Protection Agency (2016a) and are listed in Table S4.

All samples were analyzed quantitatively for 11 PFAS (seven carboxylic acids: perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), and PFOA; three sulfonic acids: perfluorobutanesulfonic acid (PFBS), perfluorohexanesulphonic acid (PFHxS), and PFOS; and one sulfonamide: perfluorooctanesulfonamide (PFOSA)). Details of the analytical procedure are outlined in the Standard Operating Protocol developed by the Analytical Service Unit at NYSDEC (New York State Dept. of Environmental Conservation, 2021).

Statistical Analysis

All statistical procedures were performed with R version 4.3.2 (<https://www.r-project.org/>). First, a Monte Carlo simulation using the fitted probability distribution function of the dataset was performed with the threshold set at the reported detection limit for each individual PFAS. This allows substitution of non-detects with random values without affecting the overall distribution of the dataset. Values that lacked replication were removed before subsequent analyses. All data were natural log transformed after normality and equal variance tests indicated violation of parametric assumptions. Data from different years of sampling at the same sites were pooled for subsequent analysis if preliminary analysis detected no significant difference between the years. General linearized models (GLM) estimated how species, site, and location drove the differences in PFAS concentrations (ng/g) in fish. Follow-up post hoc comparisons (R package ‘emmeans’) were performed to look at pairwise comparisons across sites and species. To determine compositional differences between sites, compositional matrices were created using the Bray–Curtis dissimilarity metric. The compositional differences were plotted, and post hoc statistical analyses were done with

‘betadisper’ and ANOVA. The level of significance was set at $p < 0.05$.

Results

Fish Sample Collection

A total of 508 fish from 25 different species were caught at the selected sampling sites. The diversity of species collected at individual sites varies greatly due to the differences in the type of habitat among sites (Table 1).

Samples from small wadable creeks such as Three Mile Creek and Six Mile Creek were dominated by creek chubs, blacknose dace, and common white suckers. Healthy populations of wild brook trout and brown trout were also found in Six Mile Creek. The Mohawk River is a substantially larger stream, and fish were mostly collected from the wadable portion of the river. The dominant species in the collection were creek chubs, blacknose dace, and cutlip minnows. A few additional species were found in low numbers in these particular sections of the stream, including smaller white suckers. The Erie Canal is essentially a long narrow pool, with very limited littoral areas and steep banks. The depth of water drops to 3–4 m over a very short distance from the bank. Samples from these areas were dominated by species more typical of lentic habitats, including bluegills, pumpkinseed sunfish, larger white suckers, yellow perch, as well as walleye and bass representing the top predators in the system. Similar make up of species was also found in samples from SSF, where most study sites were big ponds within wetland areas. The samples from Lake Champlain were collected from the near-shore, wadable, littoral parts of the lake. The Unnamed Stream site was mostly unvegetated, containing a large number of killifish, and a few rock bass, yellow perch, and darters. The other two sites were more heavily vegetated and had a higher diversity of lentic fish species including bluegills, shiners, rock bass, yellow perch, and killifish. Overall, the collection included species with a wide range of morphological, physiological, and ecological characteristics.

PFAS Concentrations in Fish

Composition of PFAS in Fish

Average total concentration of the 11 PFAS quantified (“tPFAS”) in fish ranged between 6 and 39 ng/g for reference sites, and 37–1001 ng/g for contaminated sites (Table S2). Compositionally, the contaminated sites were distinguished from and more widespread than the reference sites. Additionally, most sites from larger bodies of

water (i.e., Lake Champlain and Erie Canal) were more similar to reference sites than other contaminated streams (Fig. 2).

PFOS accounted for more than 25% of average tPFAS concentrations in fish for all sites regardless of their relative position to the source of contamination (Fig. 3). For all contaminated sites, PFOS accounted for more than 80%, and up to 95.4% of the average tPFAS concentrations except for Chestnut Street Bridge, where 55.9% of the average tPFAS concentration was PFOS. At reference sites, average tPFAS attributed to PFOS concentrations exceeded 50% only at Camroden Road on Six Mile Creek (64.6%) and Ausable Point at Lake Champlain (56.4%). For the rest of the reference sites, PFOS accounted for 25.4–39.3% of the average tPFAS concentrations. The fractions of all other PFAS in the average tPFAS concentrations were below 15% for all sites except for PFUnA in the fish from Beaver Pond at the SSF, which accounted for 24.3% of the whole-fish PFAS concentration.

PFAS concentration in Fish Relative to Locations and Sites

GLM analyses on tPFAS and PFOS yielded consistent results on the temporal and spatial differences in their

concentrations in fish, indicating PFOS is the driving factor for statistical differences of tPFAS concentrations among study sites (Table S2, Figure S7). Subsequent pairwise comparisons among sites were focused primarily on PFOS.

Of three locations investigated, fish collected from sites around GAFB had significantly higher PFOS load compared to those from SSF and Lake Champlain (p -values < 0.01). There was no significant difference in average PFOS concentrations between SSF and Lake Champlain (p -value = 0.978). Average PFOS concentrations were significantly higher at presumed contaminated sites in all three locations compared to the reference sites (p -values < 0.0001) (Fig. 4). Significant temporal differences in PFOS concentrations were only observed in fish from downstream contaminated sites on Six Mile Creek (p -values < 0.01), where average PFOS concentrations in fish were three- to five-fold higher in 2018 than 2020.

In SSF, average PFOS concentration in fish was the highest at Clark Street, followed by Armstrong Lane (p -value < 0.0001). Fish from the two reference sites had the lowest average PFOS concentrations, which were also significantly lower than the average PFOS concentrations from the contaminated sites (p -value < 0.0001). There was no significant difference between the two reference sites (p -value = 0.96).

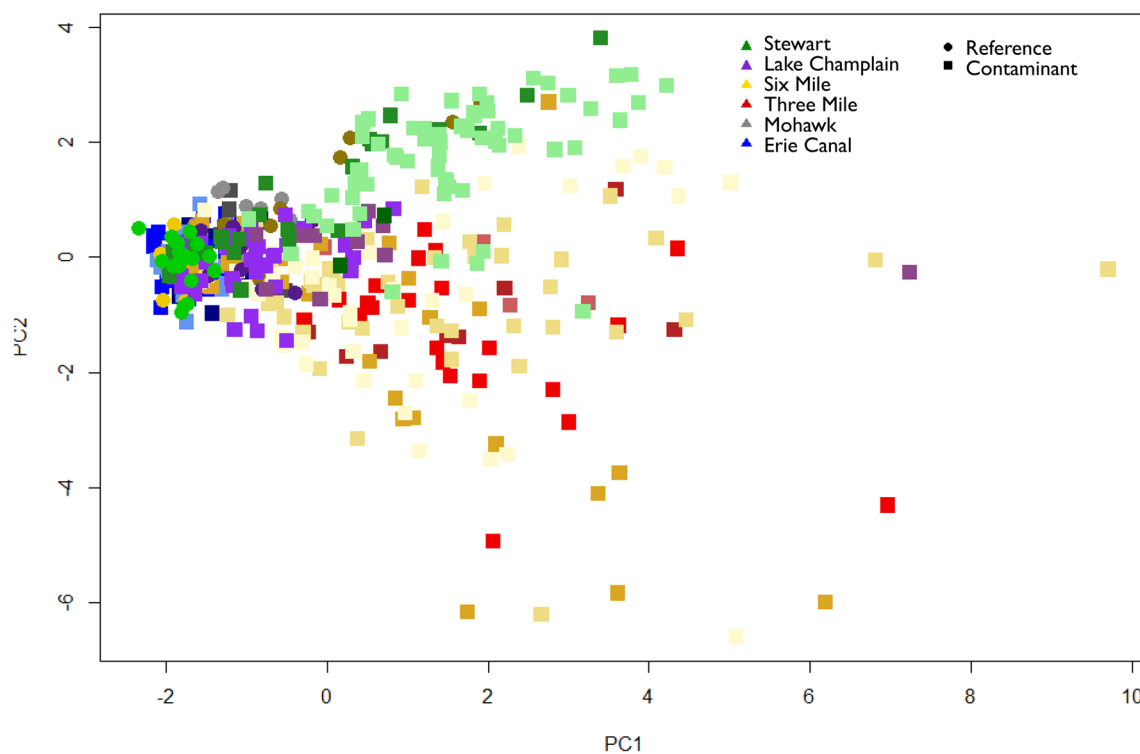


Fig. 2 Principal component analysis (Bray–Curtis distance) describing the compositional differences in PFAS concentrations between locations. The shape of each point represents contaminant (diamond)

or reference (circle) sites. The color and shade represent the location and/or site. For example, at the Six Mile Creek location, all of the points were yellow with each shade representing one of the five sites

In fish collected from Lake Champlain, PFOS concentrations were consistently higher in samples from the contaminated sites (Salmon River and Unnamed Stream) than the reference site (Ausable Point, p -value < 0.0001). Between the two contaminated sites, average whole-fish PFOS concentration is significantly higher at the Unnamed Stream site than the Salmon River site (p -value < 0.0001).

Among the rivers and streams near GAFB, downstream, contaminated sites on Six Mile Creek and Three Mile Creek had fish with the highest PFOS concentrations. Average fish PFOS concentrations from contaminated sites along the Six Mile Creek collected in 2018, and all sites along the Three Mile Creek collected in both 2020 and 2021 were significantly higher than those from any other site during any other years (p -values < 0.001). In contrast, fish collected from the Mohawk River had the lowest average PFOS concentrations among all sites sampled near GAFB. However, significant difference in the average PFOS concentrations were still observed between the presumed contaminated site

at Chestnut Street Bridge and the reference site at the Wright Settlement bridge (p -value = 0.01).

All three sites on Erie Canal were designated as contaminated because of their downstream positioning to the Mohawk River, Three Mile Creek, and Six Mile Creek. Average PFOS concentrations in fish from these three sites were significantly higher than those from the contaminated sites at the Mohawk River (p -values = 0.048) but significantly lower than the contaminated sites on Six Mile Creek and Three Mile Creek (p -values < 0.0001).

Fish PFAS concentrations in relation to size, weight, and species.

Fish PFAS concentrations were not significantly affected by length and weight of the fish analyzed (p -values > 0.05). Comparison of PFAS accumulation among different fish species was only done with data from the contaminated sites due to the low concentrations of PFAS in fish from the reference sites. Average whole-fish concentrations were found to be significantly different among different species

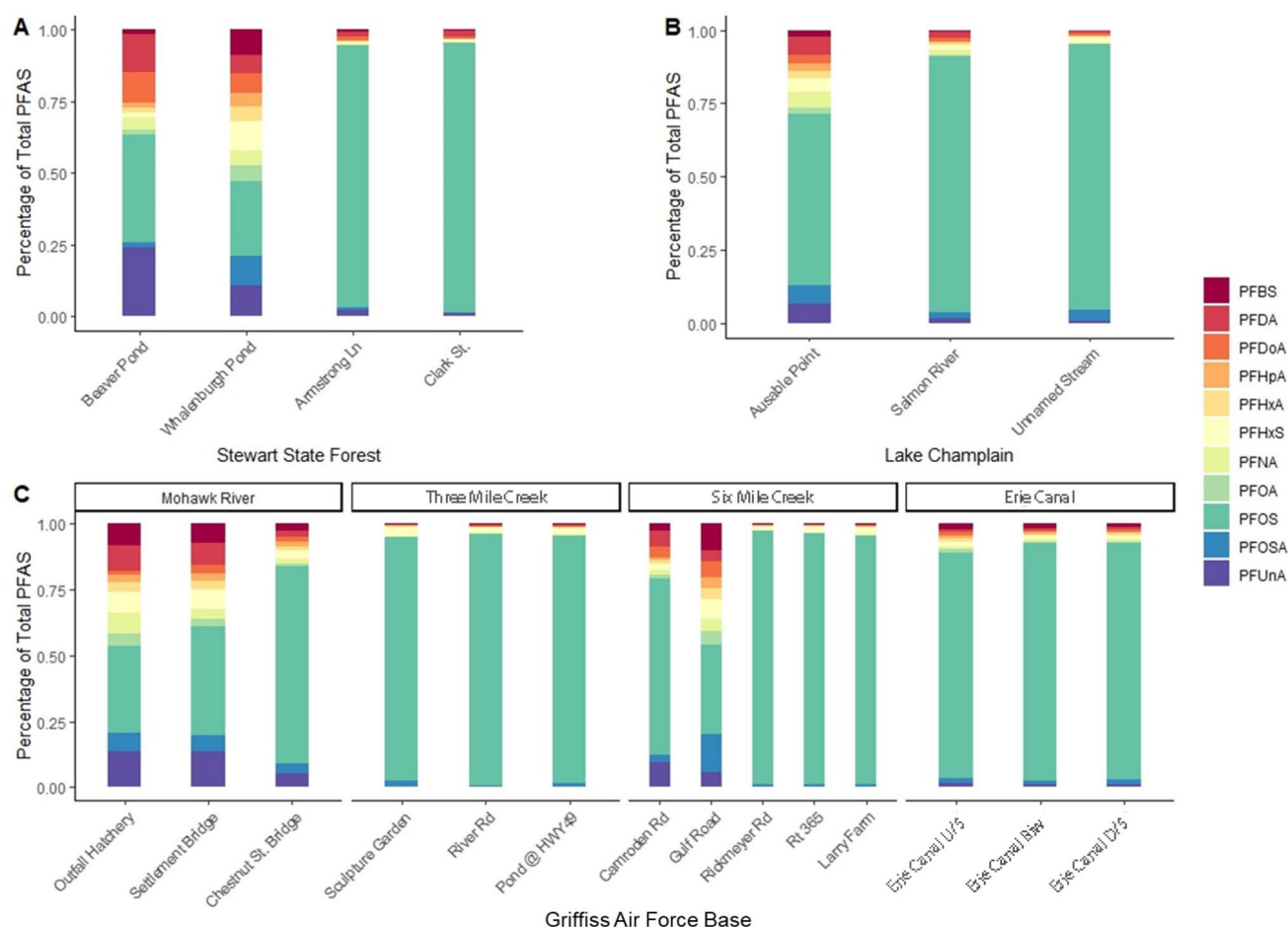


Fig. 3 Proportion of PFAS components in total PFAS in freshwater fish from all study sites. **A** Sites at the Stewart State Forest, **B** Sites at Lake Champlain, and **C** Sites at Rome, NY. The X-axis displays the

names of the study sites at each location, and the Y-axis shows the percentage of each PFAS component in the total average concentration of PFAS found in the fish samples from each individual site

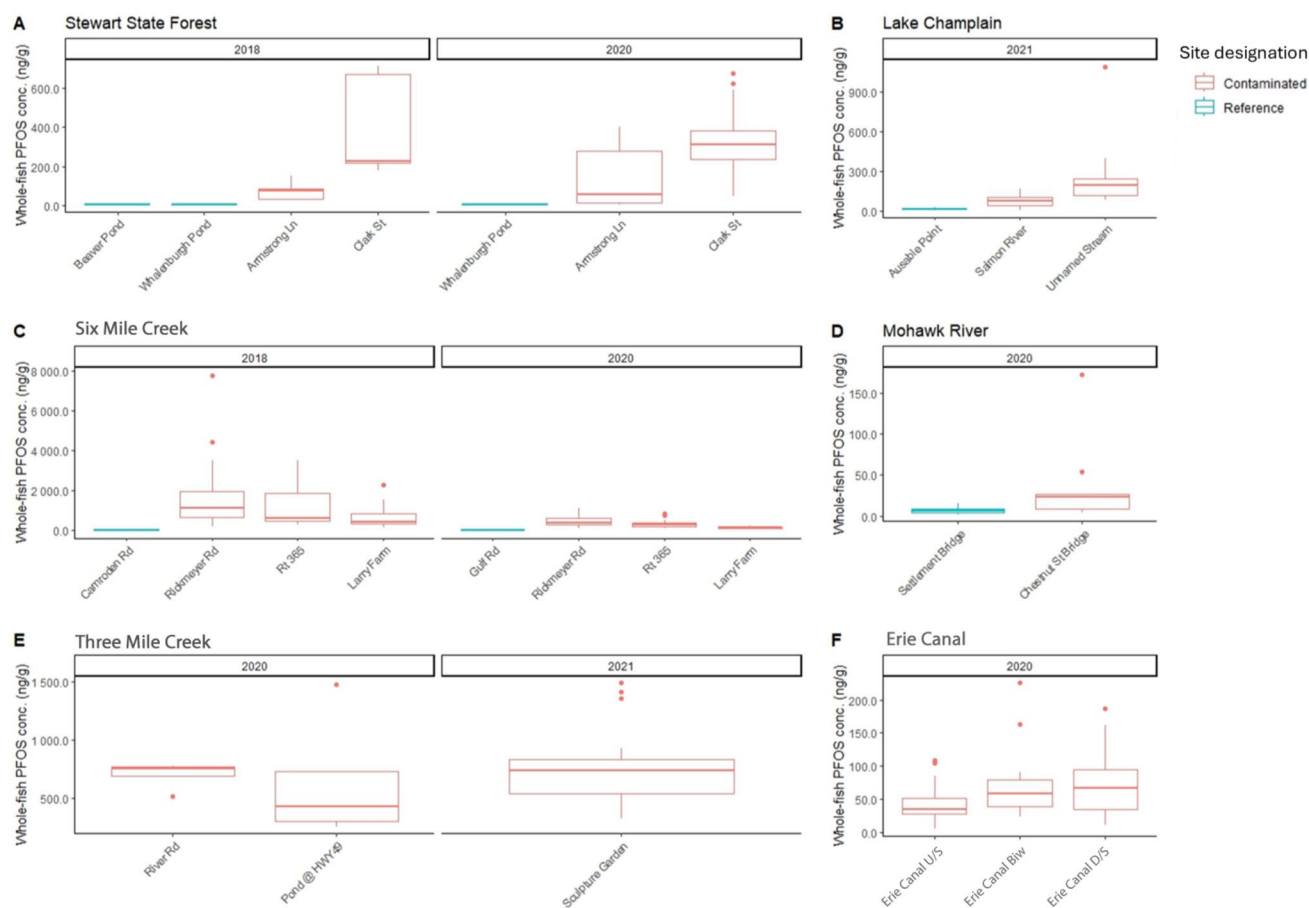


Fig. 4 Boxplot of whole-fish PFOS concentration from different study sites. **A** Stewart State Forest, **B** Lake Champlain, **C** Six Mile Creek, **D** Mohawk River, **E** Three Mile Creek, and **F** Erie Canal. The

X-axis displays the names of the study sites, and the Y-axis shows the whole-fish PFOS concentration (ng/g). Within each location, data from different years of sampling are presented in separated panels

for all PFAS analyzed (p -values < 0.05) except for PFHxA and PFHpA (p -values > 0.05). However, excluding PFOS, PFHxS, and PFOSA, the highest concentration of any other PFAS in any species tested was 10.5 ng/g (Fig. 5).

The highest concentrations of PFHxS were found in brown trout and brook trout, averaged at 42.4 ng/g and 51.7 ng/g, respectively. Only five out of the 24 species had average PFHxS concentrations higher than 10 ng/g while half of the species had average PFHxS concentrations lower than 2 ng/g. Similarly, the highest average PFOSA concentration was 21.6 ng/g found in fathead minnow while 22 out of the 24 species had average PFOSA concentrations below 10 ng/g. In comparison, average PFOS concentration was lower than 10 ng/g in only one species but higher than 100 ng/g in 15 out of the 24 species analyzed. The highest average PFOS concentration was 1491.5 ng/g found in blacknose dace.

There appeared to be little consistency across species in terms of which PFAS was preferentially accumulated. However, the five species with the highest average PFOS

concentrations, blacknose dace, creek chub, fathead minnow, brown trout, and brook trout, also tended to have high average concentration of PFHxS and PFOSA. No clear pattern between PFAS accumulation and diet preference could be observed. When grouped by general knowledge of their dietary selection, it was found that piscivorous species had significantly higher whole-body PFOS concentrations than insectivorous species (p -value < 0.05), while both piscivorous and insectivorous species had significantly higher whole-body PFOS concentrations than omnivorous species (p -values < 0.0001).

Discussion

Widespread PFAS contamination of surface and ground water near active and former USAF bases as a result of long-term, frequent use of AFFF has been well documented by the DOD (New York Air National Guard 2020; U. S. Dept. of Air Force, 2018, 2020). In New York State, information

on the extent and degree of contamination is mostly limited to areas within the property line of the bases. Our results demonstrated high probability of widespread surface water PFAS contamination, demonstrated by high concentrations of the chemicals in fish tissues, in areas near and adjacent to two former USAF bases and one currently active Air National Guard base. Moreover, the results indicate that the extent of PFAS accumulation in the aquatic ecosystems is significantly affected not only by their relative distance to the source of contamination but also by the type of the habitat present at the contaminated sites.

The spatial patterns observed in fish PFAS concentrations from all three locations are very similar: All sites located downstream from the bases consistently had fish with higher levels of PFAS contamination than those located either upstream from or in distant positions without direct runoff from the bases. This agrees with our speculation that the USAF bases are a source of PFAS contamination. PFOS is the most prevalent and most abundant PFAS in fish tissues

at both contaminated and reference sites. The detection of PFOS in the reference sites is not surprising since it has been known to be ubiquitous in the environment (Cousins et al. 2022; Wee & Aris 2023). However, the noticeably high concentrations of PFOS in fish from presumed contaminated sites compared to the rest of the PFAS concentrations (i.e., PFHxA, PFOSA, etc.) strongly implies that it is the main driver for the difference in concentrations of total PFAS between fish from contaminated sites and reference sites. Moreover, PFHxS is also significantly higher in contaminated sites than in reference sites. PFOS and PFHxS are the most frequently found PFAS in known AFFF contaminated water (East et al. 2021); therefore, the results from our study strongly suggest that AFFF use at the bases is a major contributor to the PFAS accumulation in the fish from the contaminated sites.

Many studies have found that PFOS and PFOA are the most common PFAS in the environment (Wee & Aris 2023). The fish PFOA concentrations in our study were very low

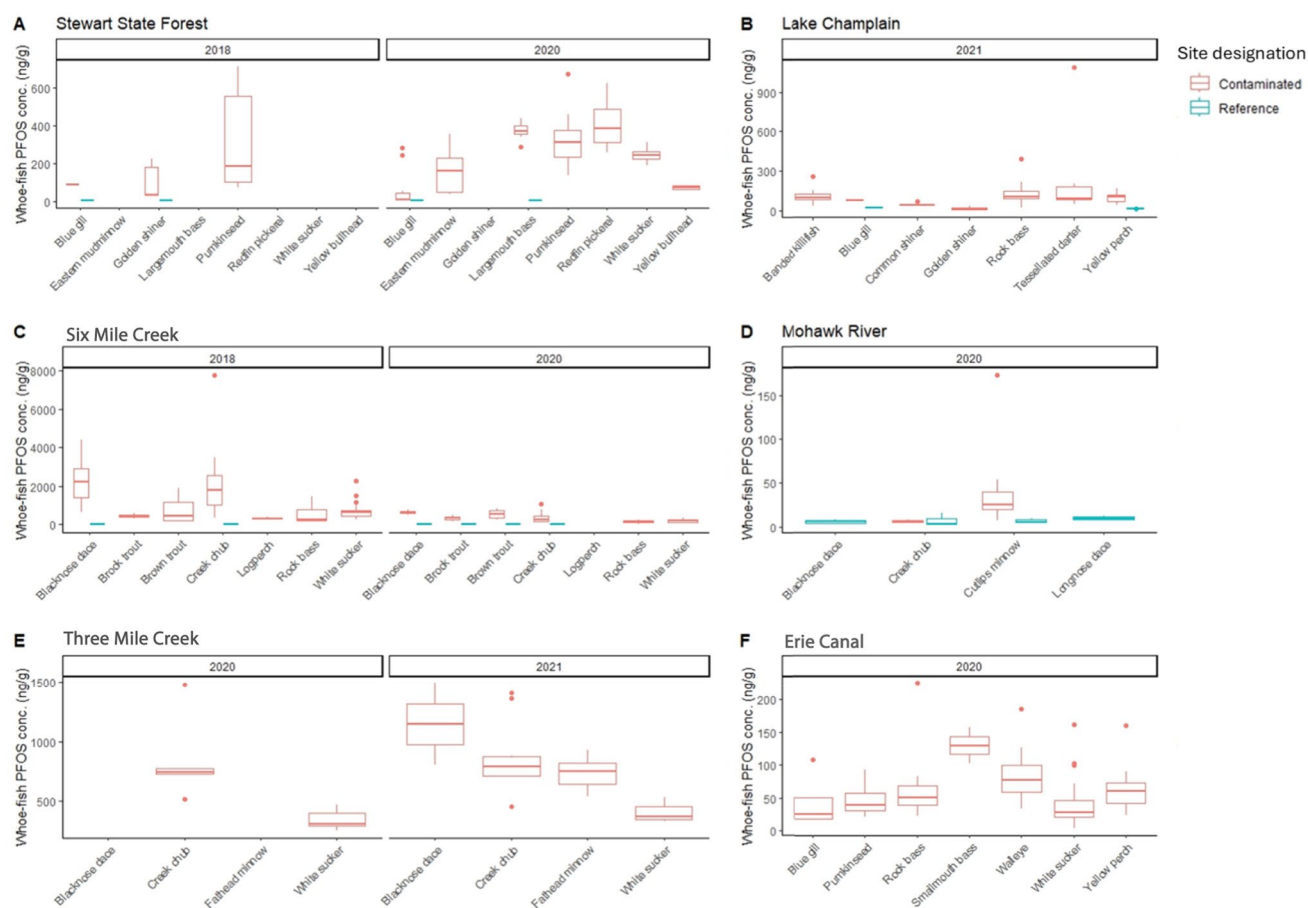


Fig. 5 Boxplot of whole-fish PFOS concentration in different species of freshwater fish. **A** Stewart State Forest, **B** Lake Champlain, **C** Six Mile Creek, **D** Mohawk River, **E** Three Mile Creek, and **F** Erie Canal. The X-axis displays the common names of the species of fish

collected at each site, and the Y-axis shows the whole-fish PFOS concentration (ng/g). Within each location, data from different years of sampling are presented in separated panels

and do not appear to differ significantly among species (Figure S8). It is well demonstrated in the literature that PFOA does not bioaccumulate as effectively as PFOS (Burkhard 2021). Consequently, the profile of PFAS in fish tissue may not accurately represent the profile of PFAS contamination in the environmental media at the sites investigated.

Environmental PFAS data are not available for most of the sites at the time of sampling. Exceptions are the Sculpture Garden site at Three Mile Creek, the two downstream sites on the Mohawk River, and the Clark Street site at SSF, for which the water and sediment PFAS concentrations were reported by DOD and NYSDEC (U. S. Dept. of Air Force, 2018) (Table S4). For the rest of the study sites, status of PFAS contamination can only be speculated using environmental PFAS data from areas upstream from the sampling location and within the property lines of the bases. The DOD results showed that surface water PFOS and PFHxS concentrations are consistently higher than PFOA concentrations in areas contaminated with AFFF (e.g., firefighting training facilities, aircraft hangers, and wastewater outfalls), with ratios of PFOS vs. PFOA concentrations ranging between 2 and 40. However, environmental PFOA concentrations in these areas are still considerably high compared to the non-contaminated areas (e.g., concentrations in the on-base segment of the Unnamed Stream at PAFB reached more than 0.08 µg/g). On the other hand, the average ratio of PFOS vs. PFOA concentrations in fish from our study is 39 for reference sites and 4021 for contaminated sites. The DOD and NYSDEC Site Investigations on the three bases were conducted a few years earlier than our study, and the available results only allowed a rough estimate of bioaccumulation factors (BAFs) for a few of our study sites. Using the site-specific average whole-fish PFAS concentrations, we estimated that the whole-fish logBAFs are between 0.778 and 1.56 for PFOA and 2.39 and 3.53 for PFOS for the contaminated sites in our study. These values, although should be updated with new measurements of water and biota concentrations from samples collected concurrently, are in agreement with those reported in Burkhard (2021), which calculated the median whole-fish logBAFs as 2.16 ± 0.85 and 3.55 ± 0.83 for PFOA and PFOS, respectively. Consequently, the lack of significant difference in fish PFOA concentrations between contaminated and reference sites in our study may be mainly because PFOS and PFHxS are the dominant component in AFFF but could also be the result of a low tendency of PFOA to bioaccumulate.

The pattern of whole-fish PFOS concentrations across contaminated sites strongly indicates that hydrological conditions of the waterbodies play important roles in the extent of PFOS accumulation in the aquatic food web. Conditions of our study sites are quite diverse but can be roughly divided into the following common categories: (a) small, slow flowing streams and creeks (e.g., Six Mile Creek and

Three Mile Creek at GAFB); (b) shallow ponds and wetland areas (e.g., Clark Street and Armstrong Lane sites in SSF), (c) larger rivers with high water volume and flowrate (e.g., Mohawk River and Erie Canal at GAFB), and (d) delta areas of large lake inlets (all sites at Lake Champlain). Fish from sites in categories (a) and (b) appear to have the highest PFOS body load, and fish from contaminated category (d) sites also have significantly elevated tissue PFOS accumulation. PFOS in fish from category (c) sites, however, are only marginally higher compared to the reference sites.

Data from DOD showed that PFOS concentration in Six Mile Creek was 0.289 µg/L near the end of the section within GAFB and between 0.214 and 0.289 µg/L in the stormwater at GFAB near the Mohawk River. However, at the outfall where stormwater is released into the Mohawk River, PFOS concentration was only 0.105 µg/L. Then at the Chestnut St Bridge, one of our sampling sites and only less than 250 m downstream the outfall, PFOS concentration in water dropped to below detection limit (0.0064 µg/L) (U. S. Dept. of Air Force, 2018). It appeared that the higher water volume and flowrate in the Mohawk River resulted in almost immediate dilution and dispersion of PFOS after discharged from the outfall. PFOS concentrations at our sampling sites in Six Mile Creek are not available. However, Six Mile Creek is a small stream and with multiple beaver dams in the section between GAFB and the Erie Canal. During the years of the sampling, water flow was slow, and several flooded areas were present. Consequently, PFOS concentration may not have decreased as drastically away from the contaminant source as it did in the Mohawk River. Therefore, it is not surprising that the most contaminated fish samples were found in Six Mile Creek while the least contaminated ones came from the Mohawk River. Similarly, fish from the Erie Canal, a deep artificial canal, also have relatively low tissue PFOS concentrations (average 60.4 ng/g and maximum 225.5 ng/g) despite being downstream from two of the most contaminated streams sampled in this study (Six Mile Creek and Three Mile Creek). In contrast, Lake Champlain is the largest waterbody sampled but the sampling sites are located at stream and river inlets, where water is mostly shallow. The average PFOS concentrations in fish from the contaminated inlet areas (average 115 ng/g and maximum 1090 ng/g) are also higher than those from the Erie Canal, though not statistically significant. Overall, the results suggest that fish in deep and fast-flowing rivers tend to accumulate less PFOS in their tissues, likely due to the quick dilution and transportation of the contaminants after their release from the source.

The highest fish PFOS concentrations were observed at contaminated sites on Six Mile Creek during the 2018 sampling. PFOS concentrations were higher than 1000 ng/g in 25 out of 69 downstream fish samples from 2018 but in only one out of 60 in those from 2020. The much higher level of PFOS in fish from the 2018 collection was most

likely caused by an accidental spill of AFFF at GAFB in July 2017. A failure in the fire suppression/water-foam sprinkler system, caused by a faulty valve, resulted in the discharge of large amount of AFFF into the Six Mile Creek through a drainage system. NYSDEC staff observed foam in the creek reaching more than 3 meters above the water surface (New York State Dept. of Environmental Conservation, 2017). A number of studies in the literature have monitored the temporal pattern of environmental and biota PFAS concentrations after incidents of major AFFF release, such as accidental spills and deployment of AFFF against a large-scale fire. Sharp increases in PFAS levels in the surrounding water and sediment after such incidents were consistently observed but these levels also started to decline quickly within a few months after the discharge (Aly et al. 2020; Nolen et al. 2022). Despite such a quick decline, environmental PFAS concentrations still may remain significantly elevated after several years (Awad et al. 2011; Marchiandi et al. 2021). Similar patterns were also observed in the biota, with PFAS body burden in fish and other aquatic organisms spiking to unusually high levels after an AFFF discharge followed by a gradual decline over the subsequent months. This decline may continue for years (Awad et al. 2011; Kwadijk et al. 2014; Moody et al. 2002; Nolen et al. 2022; Oakes et al. 2010). Consequently, for Six Mile Creek, the 2018 results likely represent a transient effect from the spill at GAFB, and the 2020 results may be an indicator for long-term PFAS persistence in the creek.

Accumulation of environmental contaminants in aquatic organisms is known to be affected by many factors including habitat and diet (Baron 2002). Dietary selection and consequently trophic level position have been shown to affect PFAS bioaccumulation more than morphological characteristics such as length and weight (Babut et al. 2017; Koponen et al. 2015; Macorps et al. 2022; Pan et al. 2018). Our study included a total of 25 freshwater species with a wide variety of habitat and dietary preferences. However, compared to the distance of the sites from the source of contamination, these preferences turned out to be significant but relatively inconsistent predictors for concentrations of the most dominant PFAS (PFOS and PFHxS) in fish from contaminated sites. This agrees with the findings by Macorps et al. (2022) and Semerád et al. (2022), who demonstrated that the accumulation of PFAS in fish was more or about equally influenced by geographical location relative to a contamination source than trophic positions. Significant inter-specific differences were also detected in a few PFAS other than PFOS, but the concentrations for these contaminants were also extremely low. This suggests that the significance may be a result of very small changes in the absolute concentrations and their biological relevance needs to be further evaluated. Additionally, the current study focused on fish species

only. Whereas information on PFAS accumulation in other aquatic and terrestrial species from the same study sites and concurrent concentrations in the environmental media would provide a relatively complete picture of PFAS movement in the biota. A future study will be focused on the predator–prey relationship among the species that more accurately with methods like stable isotope analysis.

Finally, the current New York State Fish Consumption Numerical Guidelines for PFOS advises against consumption of fish with PFOS concentrations greater than 200 ng/g for the general population, or 150 ng/g for sensitive population. Consequently, the results from the current study led to new fish consumption advisory for several of the contaminated sites (New York State Dept. of Health, 2024).

Conclusions

The data we have collected in the current study provide a general picture of PFAS accumulation in freshwater fish from waterbodies near three former and active DOD bases. The results demonstrate that fish from sites that are close in proximate and downstream to the bases have the highest PFAS body burdens. Moreover, PFOS is the most predominant component of all PFAS analyzed thus is the most determining factor for the differences in total PFAS in fish among study sites. These findings implicate that the main source of PFAS contamination in these waterbodies is historical AFFF deployment on the bases. Our results also show that hydrological conditions of the waterbodies have a significant influence on the distribution of PFAS in the environmental media and the biota. Animals in small streams and flooded wetlands may accumulate more PFAS than those in rivers with a large volume and fast flow rate. Moreover, the highest concentrations of PFAS, and PFOS in particular, were observed in small forage fish from small streams. Although larger waterbodies that typically host more sport fish species are often prioritized by environmental monitoring programs, the minor streams and wetlands can be equally important due to their biodiversity and ecological benefits, and as the current study suggests, may be more at risk from high level of PFAS contamination.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00244-025-01116-x>.

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Declarations

Conflict of interest The authors declare that there is no financial or non-financial interest to disclose.

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