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Per- and polyfluoroalkyl substances and organofluorine in lakes and waterways of the northwestern Great Basin and Sierra Nevada

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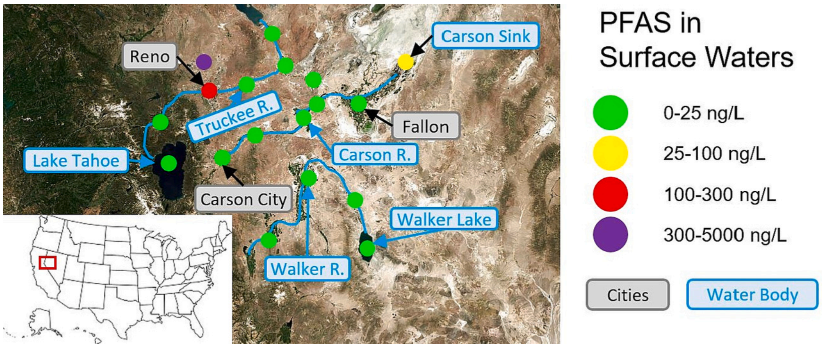
HIGHLIGHTS

- PFAS were present in 85 % of surface waters sampled.
- PFAS were present at low levels in alpine lake systems (median < 1 ng/L).
- 6:2 FTS was indicative of AFFF impacts in surface waters.
- Limited evidence indicated that PFAS may accumulate in terminal lakes.
- F⁻ breakthrough during SPE may interfere with measurements of EOF.

GRAPHICAL ABSTRACT



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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic chemicals that occur ubiquitously in the environment and have been linked to numerous adverse health effects in humans and aquatic organisms. Although numerous environmental monitoring studies have been conducted, only one has evaluated PFAS in surface waters of the northwestern Great Basin, which features unique topography that results in dozens of endorheic

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Abbreviations: PFAS, Per- and polyfluoroalkyl substances; AFFF, Aqueous film forming foam; LC-MS/MS, Liquid chromatography tandem mass spectroscopy; EOF, Extractable organofluorine; FTS, Fluorotelomer sulfonate; WWTP, Waste water treatment plant; WAX, Weak anion exchange; SPE, Solid phase extraction; OF, Organofluorine; FTA, Fire training area; RNO, Reno-Tahoe International Airport; TL1, Terminal Lake 1; TL2, Terminal Lake 2; PFBA, Perfluorobutanoic acid; PFPeA, Perfluoropentanoic acid; PFHxA, Perfluorohexanoic acid; PFHpA, Perfluoroheptanoic acid; PFOA, Perfluorooctanoic acid; PFNA, Perfluorononanoic acid; PFDA, Perfluorodecanoic acid; 6:2 FTUCA, 6:2 Fluorotelomer unsaturated carboxylic acid; 8:2 FTUCA, 8:2 Fluorotelomer unsaturated carboxylic acid; Gen-X or HFPO-DA, 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propanoic acid; PFBS, Perfluorobutane sulfonate; PFHxS, Perfluorohexane sulfonate; PFOS, Perfluorooctane sulfonate; 4:2 FTS, 4:2 Fluorotelomer sulfonate; 6:2 FTS, 6:2 Fluorotelomer sulfonate; 8:2 FTS, 8:2 Fluorotelomer sulfonate; PFOSA, Perfluorooctane sulfonamide; N-EtFOSAA, N-Ethyl perfluorooctane sulfonamidoacetic acid; N-MeFOSAA, *N*-methyl perfluorooctane sulfonamidoacetic acid; CIC, Combustion ion chromatogram; PFCA, Perfluorocarboxylic acid; PFSA, Perfluorosulfonic acid; FTOH, Fluorotelomer alcohol.

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Extractable organofluorine
Aqueous film-forming foam
Terminal lake
Endorheic

basins and terminal lakes with no natural outlet, where PFAS may accumulate. To close this knowledge gap, we evaluated the occurrence of PFAS in grab samples from 15 lakes (headwater and terminal lakes) and 10 rivers in the Great Basin located in Nevada and California of the United States. PFAS and organofluorine were quantified by liquid chromatography tandem mass spectroscopy (LC-MS/MS) and combustion ion chromatography, respectively. The highest concentrations of PFAS occurred in samples taken near sites with known or suspected prior aqueous film forming foam (AFFF) application (~20 to 4754 ng/L). Samples near wastewater treatment plants and in urban areas also tended to have PFAS concentrations greater than those measured in remote, less anthropogenically influenced areas (~2 to 15 ng/L, <3 ng/L respectively). In limited snapshot sampling events PFAS appeared to accumulate in terminal lakes to some extent; in-lake concentrations were two to five times greater than those of their inflows. Fluorotelomer sulfonates were present downstream of a known AFFF application area likely to have had fluorotelomer-based foams applied to it, and the concentrations decayed in a predictable manner, suggesting they may be used as an indicator of PFAS transport away from an AFFF source. In all but two samples, organofluorine concentrations were greater than the sum of targeted PFAS (on a F basis) (median of 0.6 % of organofluorine identified via LC-MS/MS), although there was considerable variability in organofluorine measured in replicate samples.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have been in production and use since the 1950s (ITRC, 2020). They are used in a wide range of industries, such as hydrophobic and oleophobic treatments for textiles, surfactants, non-stick coatings, and temperature resistant materials (Glüge et al., 2020). The strong carbon-fluorine bonds cause PFAS to have long environmental half-lives, earning them the moniker "forever chemicals" (Hamid and Li, 2016; Ahrens and Bundschuh, 2014). Their long environmental half-lives result in their accumulation in most environmental compartments, including biota, surface waters, groundwater, soils, and the atmosphere (ITRC, 2020; Ahrens and Bundschuh, 2014; Tokranov et al., 2021; Petre et al., 2021). Reviews of bioaccumulation literature indicate detrimental effects of PFAS on humans and wildlife, including but not limited to cancer, autoimmune disease and high cholesterol (Ahrens and Bundschuh, 2014; Evich et al., 2022). Notable environmental sources, in order of significance (i.e., environmental PFAS loading), include aqueous film forming foam (AFFF) fire training areas and firefighting events for liquid-fuel based fires, wastewater treatment plant effluent, urban runoff, and atmospheric deposition (Ahrens and Bundschuh, 2014; Evich et al., 2022; Prevedouros et al., 2006). PFAS have been detected in remote regions including snow and surface waters north of the Arctic Circle (ΣPFAS = ~300 to 2000+ pg/L), high in the Himalayas (ΣPFAS = 1 to 30 ng/L), and the deep ocean (ΣPFAS = 0 to ~100 pg/L) (Miner et al., 2020; Kwok et al., 2013; Miranda et al., 2021; Yamashita et al., 2008).

Terminal lakes, also called endorheic or sink lakes, represent a unique ecosystem where runoff from within a closed (endorheic) basin runs off into a lake with no evident outlet. The unique geologic setting makes the Great Basin prone to the development of terminal basins and lakes that have no hydrologic outflows, aside from evaporation and infiltration, allowing them to accumulate salts and potentially to accumulate recalcitrant organic compounds, a phenomenon that would be exacerbated by climate change and additional water diversions (Wurtsbaugh et al., 2017; Sharma and Hanigan, 2021). We are not aware of any peer-reviewed publications assessing PFAS occurrence in the terminal lakes of the Great Basin nor the adjacent Sierra Nevada Mountain range, a region that is home to over five million people, and multiple endemic and endangered wildlife species (Torregrosa and Devoe, 2008; Nevada Division of Natural Heritage, 2021; Chambers et al., 2008). Despite numerous studies documenting the widespread occurrence of PFAS around the world, we are aware of only one publication studying surface water occurrence in the northwestern Great Basin (Bai and Son, 2021). The study included 17 targeted PFAS species (ΣPFAS = ~15 to ~200 ng/L), but did not include broader organofluorine measurements which include non-targeted species (i.e., extractable organofluorine [EOF]), and only sampled two waterways in Nevada, one of which was the Truckee River in the Northwestern Great Basin.

Our goal was to understand the occurrence of PFAS and organofluorine in surface waters of the Northwestern Great Basin, Central Sierra Nevada, and Upper Sacramento River watershed where there is currently little to no occurrence data. To do so, we measured 19 PFAS via liquid chromatography tandem mass spectrometry (LC-MS/MS) and extractable organofluorine (EOF) in 10 waterways and 15 lakes including Great Basin terminal and non-terminal lakes, rivers, and urban streams, and alpine lakes. Collectively, these water ways represent major sources of water to human urban and rural populations in California and the northwestern Great Basin, in addition to providing habitat for numerous endemic and threatened wildlife species (Nevada Division of Natural Heritage, 2021). Given the limited resources and the lack of information on PFAS occurrence in the region, we chose to sample as many sites as possible, understanding that there is a tradeoff between breadth and depth. For example, an in-depth evaluation of groundwater flows was not conducted for any individual lake because our focus was breadth, and because it is not likely to be important for the terminal lakes, which, as a requirement for formation, have limited groundwater outflows.

2. Materials and methods

2.1. Study area

Sampled sites and potential contaminant sources are described here, and in Table S1 (sampling site metadata), Table S2 (source locations), and Table S3 (watershed landcover). Four sites were sampled along the Carson River (August 2022), two in Lahontan Reservoir (January 2022) and five in the Carson Sink (January 2022) at the terminus of the Carson River. Wastewater treatment plants (WWTPs) in the Carson River watershed include the Carson City Water Resource Recovery Facility (Carson City, NV), the Rolling-A Wastewater Treatment Plant (Dayton, NV), and the Fallon Wastewater Treatment Plant. A known AFFF fire training area (FTA) exists at the Fallon Naval Air Station (Fallon, NV), with a documented PFAS groundwater plume (EWG, 2022). Additionally, the Carson River watershed receives flows from the Truckee River watershed via the Truckee Canal.

In the Truckee River watershed, five sites were sampled offshore near the southern and eastern banks of Lake Tahoe and one in the Tahoe Keys Marina (September 2021). 14 sites were sampled across 2 sampling events (October 2021, June 2022) in the Truckee River using Lagrangian sampling, with the Lockwood site being sampled in both events. Travel times between sampling sites were calculated using the Truckee River flow rates and tracer studies conducted by others (Bohman, 2000). Sampling times are given in Table S1. Other rivers were not sampled in a Lagrangian manner as there are no existing tracer studies. The Truckee Meadows Water Reclamation Facility effluent was sampled once (October 2021), and 2 samples were taken from the Truckee Canal (June 2021). The Tahoe-Truckee Sanitation Agency discharges wastewater effluent to the groundwater in Truckee, CA.

In Reno, NV, five additional sites were sampled, targeting surface water near the Reno-Tahoe International Airport (RNO) (June 2021). The Truckee Meadows Water Reclamation Facility discharges to Steamboat Creek ~210 m upstream from the confluence with the Truckee River (EPA, 2012). RNO has an AFFF FTA with a documented PFAS groundwater plume (EWG, 2022). Swan Lake was also sampled in Reno at the surface on the eastern shore and from the engineered inflow channel (canal with urban drainage and effluent from the Reno Stead Water Reclamation Facility) (August 2021). Swan Lake also likely receives flow from the upgradient Dodd/Beal Fire Training Academy, which closed in the mid-1990's and may have employed PFAS-containing AFFF (although it was not possible to confirm this). This site was also remediated for soil and groundwater hydrocarbon contamination (McGinley and Associates, Inc., 2002; Army National Guard Bureau, 2020). Additionally, Swan Lake may receive flow from the Reno Stead Airport (RSA), which is known to have some PFAS contamination (Army National Guard Bureau, 2020).

The Walker River was sampled at three points (June 2022). Walker Lake was sampled at 3 m depth at three locations and from the surface at one location (November 2021). We are unaware of any wastewater discharges or AFFF FTAs in this watershed.

Mono Lake was sampled at the surface at two points and one sample was collected from an inflow, Lee Vining Creek, below the town of Lee Vining, CA (January 2021). We did not identify any wastewater discharges or AFFF FTAs which may directly influence the sampling locations.

Two terminal lakes have been anonymized at the landowners' request. Terminal Lake 1 (TL1) was sampled at four locations on the lake, and from the primary inflow (March 2022). The lake is influenced by upstream urban development, including wastewater discharge(s), and a known subsurface PFAS plume resulting from AFFF use. Terminal Lake 2 (TL2) was sampled at two points on the lake in addition to one of its source creeks (October 2021). TL2 is in a remote region, and we are unaware of any wastewater discharges or AFFF FTAs in the watershed.

Serene Lake, in the Upper American River watershed, was sampled at two sites (October 2021). Two lakes were also sampled in the Upper Sacramento watershed; Cliff Lake and Castle Lake, each sampled at two points from the surface (September 2021). We are unaware of any wastewater discharges or AFFF FTAs in these watersheds.

2.2. Sampling

Samples from each site were collected in triplicate for conductivity and fluoride analysis, EOF, and LC-MS/MS. Surface water grab samples were collected at the surface of the water. Samples taken at depth were collected using a van-dorn sampler that was rinsed with sample water on-site prior to sample collection. Sampling depths are described in Table S1; initially samples were collected at multiple depths to assess the effects of depth on PFAS concentration, later in the study samples were taken at the surface to ease sampling logistics and create a more consistent sampling approach. All sample containers were borosilicate glass baked at 500 °C for a minimum of 3 h or high-density polyethylene triple rinsed with methanol. Containers for samples used for conductivity and fluoride analysis and EOF measurements were triple rinsed with sample water prior to filling. Sample containers for LC-MS/MS were not rinsed with sample as they contained ascorbic acid to quench any residual oxidant (most samples were not expected to contain residual oxidant but sampling and analytical conditions were kept constant for all samples, further discussion in Addition Notes section of the Supplemental Information). All field samples were stored on ice after collection and transferred to a refrigerator at 5 °C upon returning to the University of Nevada, Reno.

2.3. Sample preparation

Samples for conductivity, fluoride, and EOF measurements were filtered as soon as possible after collection, typically within seven days (Table S1 contains dates of collection and filtration). Logistical reasons inhibited a shorter delay between collection and filtration. No comparison or pre-testing was done to verify that the delay would not interfere with detection as the recalcitrance of most PFAS was believed to make degradation negligible. Samples for measurement of conductivity and fluoride, and EOF were filtered separately using stacked 0.5 μm (Advantec GC-50) and 2.7 μm (Whatman GF/D) glass fiber filters that had been baked at 500 °C for a minimum of 3 h. For EOF sample filtration, the hopper, filter and flask of the vacuum filtration apparatus were rinsed with ~10 mL of MeOH that was then recombined with the filtered sample, to capture PFAS that might have sorbed to the walls of the apparatus. LC-MS/MS samples were shipped, unprocessed, to the Southern Nevada Water Authority (SNWA) for filtration, extraction, and analysis, within one month of collection. Upon receipt, the samples were filtered using prewashed glass fiber filters by SNWA staff, as described in Gonzalez et al. (Gonzalez et al., 2021).

2.4. PFAS extraction

Samples for LC-MS/MS were extracted using methods described elsewhere (Gonzalez et al., 2021). Briefly, samples were adjusted to pH <2 and spiked with isotopically-labeled standards. Automated SPE was carried out using pre-packed 200 mg, 6 mL hydrophilic-lipophilic balance (HLB) cartridges and a Dionex Autotrace 280. Cartridges were preconditioned with MTBE, methanol, and reagent water and samples were loaded at 5 mL/min. Cartridges were dried for 30 min with N_2 gas, PFAS eluted with 15 mL methanol, and concentrated to 500 μL using N_2 gas.

EOF was extracted similar to Miyake, et al., modified for use with a Dionex Autotrace 280 solid-phase extraction instrument according to Thermo-Scientific (Zhang et al., 2020; Miyake et al., 2007). Briefly, 250 mL of sample was adjusted to pH 4 ± 0.2 using glacial acetic acid. 200 mg, 6 mL Oasis weak anion exchange (WAX) cartridges were conditioned with 5 mL 0.1 % (*v*/*v*) NH_4OH/MeOH, followed by 5 mL milli-Q water, prior to sample loading. Samples were loaded at 5 mL/min. Cartridges were then sequentially rinsed with 20 mL of 0.01 % (*v*/*v*) NH_4OH in water, 10 mL of milli-Q water, 10 mL of 25 mM ammonium acetate buffer (pH 4) and 10 mL of 20 % (*v*/*v*) MeOH in water. The cartridges were then dried with N_2 gas for 20 min, PFAS were eluted with 10 mL of 0.1 % (v/v) NH_4OH/MeOH and concentrated to 1 mL under N_2 gas.

HLB was selected as the solid phase for LC-MS/MS extractions because dissolved salts present in the sample do not interfere with the sorption of PFAS, and terminal lakes have relatively high salinity (compared to EPA Method 537, specific to drinking water, which uses anion exchange). Further, it is consistent with the demonstrated method for the analytical lab. However, no demonstrated method exists for HLB EOF, leading us to use WAX for our EOF analysis to allow for comparability with other studies.

2.5. Analytical

Target analytes were separated using an Agilent 1260 Infinity quaternary LC pump and HTC-PAL auto sampler (CTC Analytics) with a 50 × 4.6 mm Kinetex C18 column (Phenomenex, Torrance, CA) of 2.6 μm pore size. Potential PFAS leached by the LC system were chromatographically separated from analyte peaks by installing a 50 × 4.6 mm, 2.6 μm pore-size Kinetex C18 column in-line upstream from the injector valve. A gradient of 5.0 mM ammonium acetate in water (A) and 100 % methanol (B) at a flow rate of 0.5 mL/min was used with a 2 μL injection volume. Tandem mass spectrometry was performed using a Sciex API 4000 triple-quadrupole mass spectrometer in ESI-negative mode. Instrumental response for target analytes and added isotopes in samples were matched against the relative response ratio from calibrators and the concentration of the unlabeled analyte determined. Minimum reporting limits were 0.25 to 5 ng/L for each PFAS, a list of detection limits was reported elsewhere, quality assurance data is included in Table S4 (Gonzalez et al., 2021) in addition to a description of quality assurance measures taken by SNWA. 19 PFAS were quantified: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), 8:2 fluorotelomer unsaturated carboxylic acid (8:2 FTUCA), 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (Gen-X or HFPO-DA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), 4:2 fluorotelomer sulfonate (4:2 FTS), 6:2 fluorotelomer sulfonate (6:2 FTS), 8:2 fluorotelomer sulfonate (8:2 FTS), perfluorooctane sulfonamide (PFOSA), N-ethyl-N-[(heptadecafluorooctyl)sulphonyl]glycine (N-EtFOSAA), *N*-methyl perfluorooctane sulfonamidoacetic acid (N-MeFOSAA).

Total fluorine analysis of EOF concentrates was conducted with a combustion ion chromatogram (CIC) (Metrohm) operated at 1050 °C. Samples were combusted under 300 mL/min O_2 to react organofluorine compounds to HF in the presence of excess water vapor. HF was carried to a deionized water absorber by 100 mL/min argon. The combustion duration was 5 min. HF, as F^-, was then analyzed by a Metrohm 930 Compact IC Flex equipped with a 1 mL injection loop and a 4 mm × 250 mm A Supp5 analytical column heated at 55 °C. Description of quality assurance measures for EOF analysis are included in the supplementing information.

Because the published and adopted EOF method employs anion exchange to concentrate OF, and because anion exchange is salt sensitive (competition for ion exchange sites) and multiple samples were from sources with high salinity (e.g., 30,000 to 65,533 uS/cm), we report EOF as a likely conservative measure of OF present. Experiments conducted by Miyake et al. demonstrated that 99.995 % of F^- present in a seawater sample was removed and thus we initially believed that salts did not substantially interfere with OF analysis (Miyake et al., 2007). However, to further understand the potential for F^- to interfere with OF analysis our samples, we spiked NaF and PFOA to milli-Q water at varying concentrations, intended to mimic F^- and OF present in these samples. Samples were extracted and analyzed for EOF in the same manner as field samples. NaF was spiked to make solutions with 0, 0.05×10^6, 0.5×10^6, and 1×10^6 ngF/L. PFOA was spiked such that the solutions had 0, 10, 100, 1000 ngF/L. Each solution was made in bulk and separated into triplicates, which were extracted and analyzed separately.

Sample conductivity was analyzed using a TitraLab AT-1000 series workstation after filtration. Fluoride was quantified using a Dionex ICS-6000 ion chromatogram in samples diluted between 1 and 1000×, to reduce chloride interference.

3. Results and discussion

3.1. Occurrence of targeted species

PFAS were ubiquitous in samples that were near known or suspected AFFF application sites and/or urban areas (46 of 65 sites fit this definition and PFAS was detected at all 46 sites, median sum of species =~14 ng/L), and were also detectable in more remote regions, although less frequently and at lower concentrations (19 of 65 sites with PFAS detected at 12 of the 19 sites, median sum of species ≤1 ng/L) (Figs. 1 and 2, Table S1). For example, near AFFF FTAs, sums of the 19 targeted species ranged from ~20 to 4754 ng/L. Lower concentrations, from ~2 to 15 ng/L, were observed at sites near urban areas and wastewater treatment plants, and the lowest concentrations detected were in areas with minimal or no anthropogenic activity (<MDL to 3 ng/L).

The most frequently detected species were perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs). Other species were detected somewhat less frequently (Table 1). This may be attributable to degradation of precursors and unknown legacy PFAS to terminal degradation products (Ahrens and Bundschuh, 2014; Lenka et al., 2021). The most frequently detected PFCA was PFOA and the most frequently detected PFSA was PFHxS. Outside of PFSAs and PFCAs, 6:2 FTS was most frequently detected and at the highest concentrations of the compounds measured. This is likely due to the degradation of fluorotelomer chemistries used in modern AFFF following the phase out of PFOS-based foams in the early 2000's, to 6:2 FTS (Ruyle et al., 2021; Place and Field, 2012). PFCAs present at the highest concentrations were PFPeA and PFHxA, which are known to be primary transformation products of 6:2 FTS (Zhang et al., 2016). N-MeFOSAA, N-EtFOSAA and PFOSA were detected less frequently than 6:2 FTS and have been shown to undergo long range transport and degradation in the atmosphere, indicating that they may originate in diffuse PFAS contamination from atmospheric deposition (Stock et al., 2007).

3.2. Alpine and terminal lakes

Alpine lakes ($n = 4$) had concentrations at or near the detection limit (<1 ng/L for most species), demonstrating that headwaters with no known direct PFAS sources have low concentrations of PFAS. A notable exception to this was Lake Tahoe, in which the Tahoe Keys Marina had the highest concentration of PFAS of all the alpine lake sites sampled. The Tahoe Keys Marina has substantially more anthropogenic influence than others sampled, receiving runoff from the City of South Lake Tahoe. Further, mixing at the sampling location, inside the marina, is limited. The second highest concentration in the samples from alpine lakes were also taken in Lake Tahoe, offshore of the inflow of the Upper Truckee River. Again, the elevated concentration is likely attributable to PFAS transported into the lake from the City of South Lake Tahoe by the river that has not yet been diluted into the lake. The other two adjacent sites near South Lake Tahoe were below detection limits.

Great Basin lakes ($n = 11$) had concentrations from ~2 to 4754 ng/L. Among them, the lowest concentrations were observed at sites that did not have known nearby AFFF use sites, wastewater treatment plants, or substantial urban development (e.g., Mono Lake, TL2, Walker Lake). Great Basin lakes with the highest concentrations were associated with known or likely AFFF application areas (e.g., Swan Lake and Carson Sink sites: Harmon Reservoir, Stillwater Point Reservoir, Foxtail Reservoir, East Alkali Lake No. 1). Concentrations in terminal lakes directly impacted by PFAS sources were somewhat lower than expected given the long residence time in the lakes and known recalcitrance of PFAS. It is possible that salinity led to “salting out” resulting in higher PFAS sediment concentrations. Sampling of sediments was beyond the scope of this study but would be valuable in future efforts.

For four of the five terminal lakes sampled, concentrations in the body of the lake were two to five times greater than their respective primary inflow (Fig. 2, Mono Lake, Walker Lake, TL2, and Swan Lake), indicating some accumulation of PFAS may occur in the water column. In one terminal lake, TL1, the concentrations in the body of the lake were ~ 10× less than in the primary inflow (~15 ng/L vs ~2 ng/L). PFAS in the inflow likely originated in the sources described in the methods (e.g., wastewater discharge, AFFF use) but it is not clear why they do not appear to accumulate in this lake.

Further demonstration that PFAS are likely to accumulate to some extent in terminal lakes is provided by samples taken from the Carson Sink. Lower Foxtail Reservoir and East Alkali Lake No. 1 receive water from Stillwater Point Reservoir and are managed as intermittent wetlands, with naturally intermittent outflows which are further limited by human controls (C. Lunderstadt, personal communications, October 25, 2022). Thus, their flow characteristics are similar to terminal lakes. Further evidence of their similarity to terminal lakes is demonstrated by increasing conductivity from the Carson River (source water) through Stillwater Point Reservoir to East Alkali Lake No. 1 and Lower Foxtail

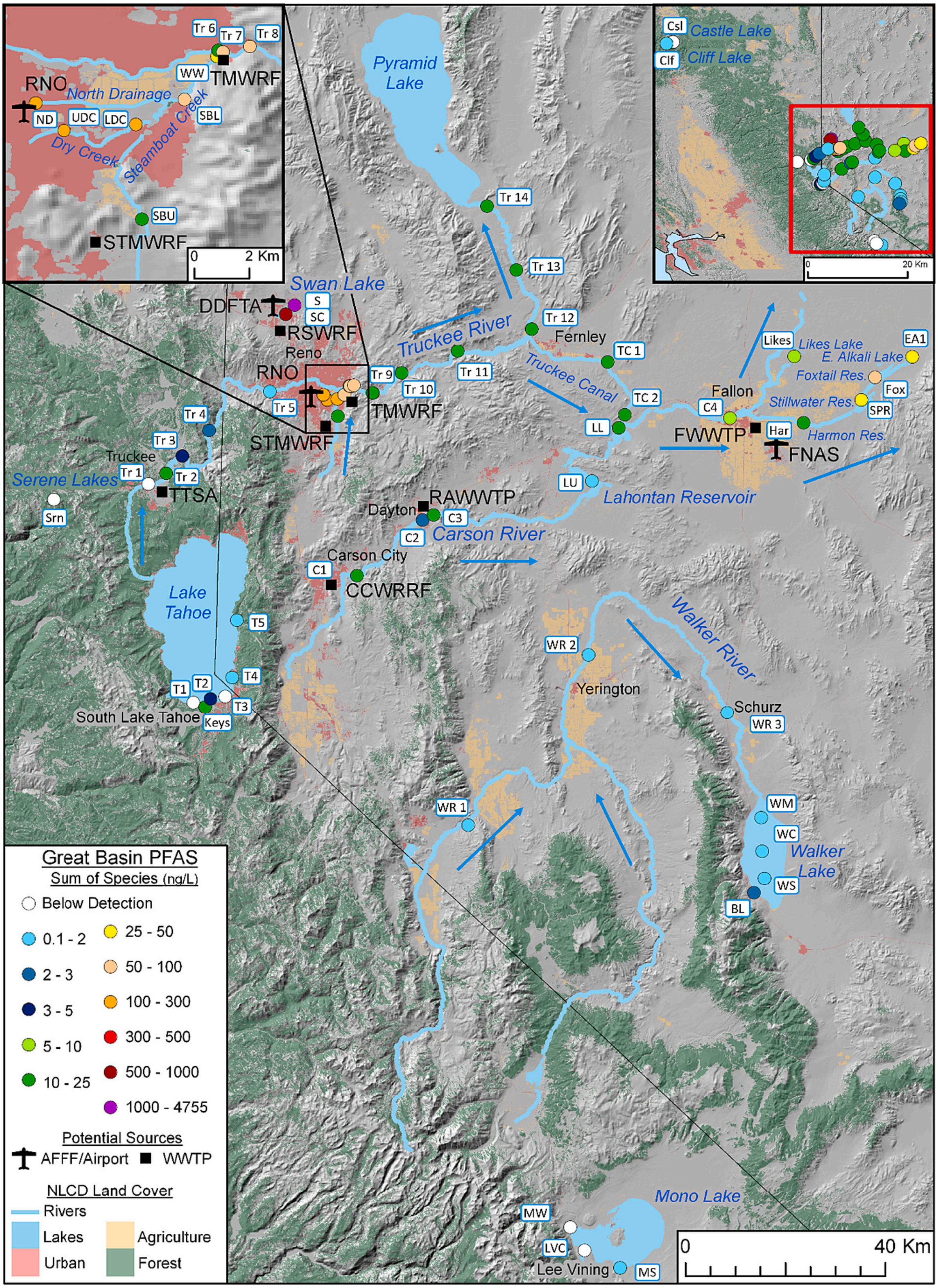

Fig. 1. Concentrations of PFAS at sampled sites, displayed as the sum of 19 targeted species (average of triplicate samples). Acronyms: DDFTA (Dodd/Beal Fire Academy), RSA (Reno-Stead Airport), (RNO) Reno-Tahoe International Airport, FNAS (Fallon Naval Air Station), RSWRF (Reno Stead Water Reclamation Facility), TTSA (Tahoe-Truckee Sanitation Agency), FWWTP (Fallon Wastewater Treatment Plant), TMWRF (Truckee Meadows Water Reclamation Facility), STMWRF (South Truckee Meadows Water Reclamation Facility), CCWRRF (Carson City Water Resource Recovery Facility), RAWWTP (Rolling-A Wastewater Treatment Plant). Blue arrows indicate direction of flow, site IDs in blue boxes correspond to labels in Fig. 2.

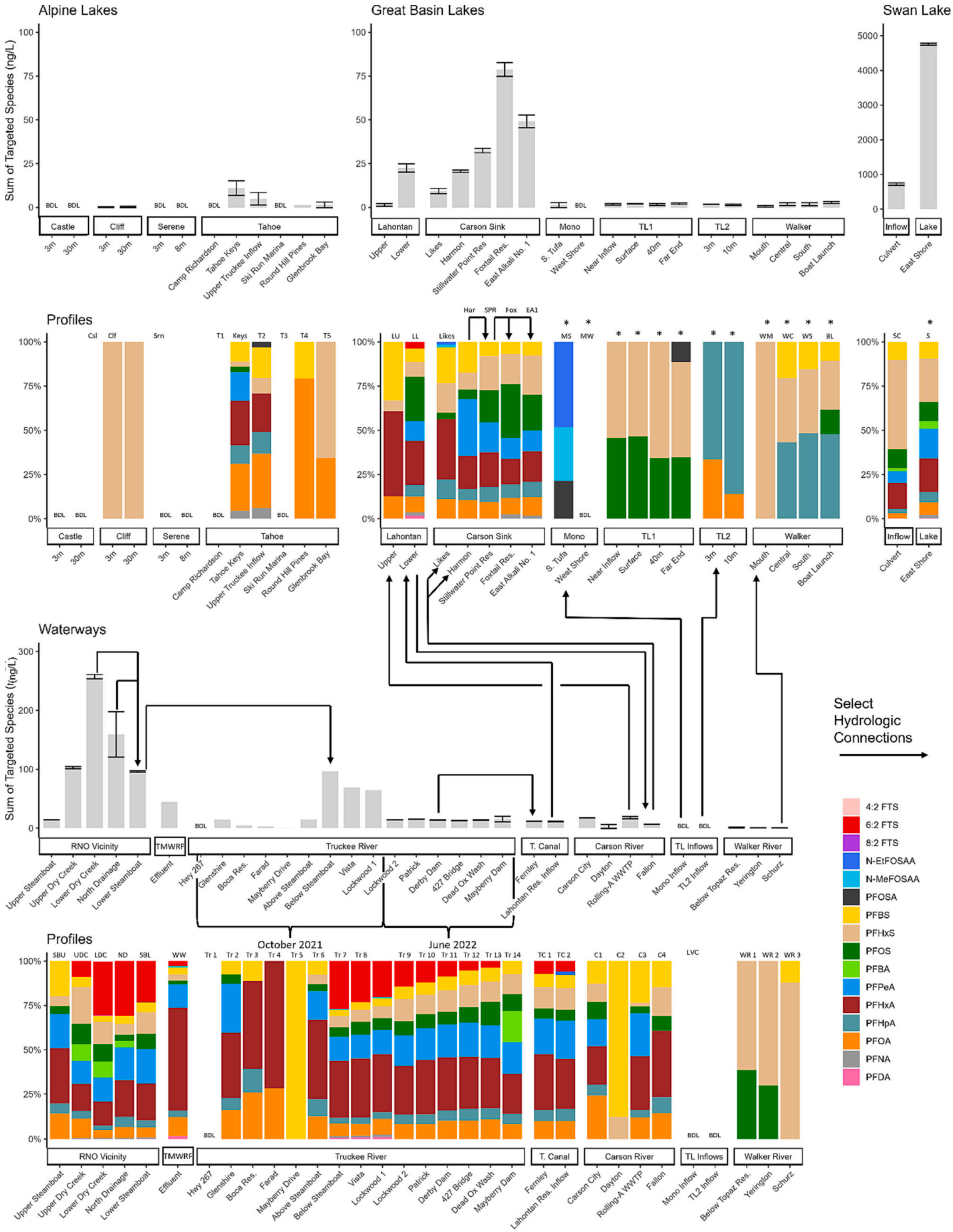

Fig. 2. LC-MS/MS results for the 19 targeted species presented as the sum of the averages of field triplicate samples (only the 16 detected species are included in the legend). Tabular concentrations of all species are included in Table S5. Error bars represent one standard deviation of the average of the triplicates, including propagated error from each species. Relative abundance profiles were produced using the average of individual species' concentrations. Sites within the same water body are presented left to right from upstream to downstream. Labels above profile bars correspond to site IDs in Fig. 1. Asterisks above profile bars indicate terminal lakes. Acronyms: (RNO) Reno-Tahoe International Airport.

Table 1
LC-MS/MS targeted species detection frequency and ranges. Each PFAS was below the detection limit in all replicates in at least one set of triplicates (i.e., min is less than MDL for all PFAS).

	Species	Detection Frequency	Max (ng/L)	Average (ng/L)	Median (ng/L)
Perfluorocarboxylic acids	PFOA	60 %	390	15	2
	PFHpA	59 %	310	12	1
	PFHxA	54 %	990	44	5
	PFPeA	45 %	930	42	4
	PFNA	22 %	90	9	1
	PFDA	12 %	19	4	1
	PFBA	10 %	230	53	13
Perfluorosulfonic acids	PFHxS	72 %	1400	42	2
	PFBS	63 %	500	18	2
	PFOS	59 %	570	22	1
Other	6:2 FTS	26 %	80	15	2
	N-MeFOSAA	9 %	1	1	1
	N-EtFOSAA	8 %	3	1	1
	PFOSA	6 %	1	1	1
	4:2 FTS	2 %	1	1	1
	8:2 FTS	2 %	1	1	1
	6:2 FTUCA	0 %	NA	NA	NA
	8:2 FTUCA	0 %	NA	NA	NA
	Gen X	0 %	NA	NA	NA

Reservoir (Table S1). Notably, summed PFAS concentrations also increased in the same order. PFAS species profiles observed within Stillwater Point Reservoir, East Alkali Lake No. 1, and Lower Foxtail Reservoir were similar, indicating that new sources of PFAS are not likely to be the cause of the increase in concentration (Fig. 2). Together, this evidence suggests that PFAS in all three lakes are likely to originate in the same source and increase in concentration as the lake water evaporates.

Targeted PFAS species in lakes sampled at multiple depths had little variance in PFAS concentration with depth, as shown in Fig. 2 and in Table S5. These lakes included Castle (3 m and 30 m ≤MDL; 9/21), Cliff (3 m = 0.17 ± 0.29 ng/L, 30 m = 0.22 ± 0.38 ng/L, 9/21), TL1 (Surface = 1.99 ± 0.11 ng/L, 40 m = 1.61 ± 0.49 ng/L, 3/22), Serene (3 m and 30 m ≤MDL, 10/21), and TL2 (3 m = 1.75 ± 0.16 ng/L, 10 m = 1.47 ± 0.36 ng/L, 10/21). Due to the seasons in which these samples were collected (early fall and early spring) it is likely that the lakes were well mixed, reducing the effect of stratification. Additionally, no clear correlation was observed between sample conductivity and PFAS concentration, likely due to the stronger influence of local sources overpowering the effects of ionic strength when comparing samples from multiple sites.

3.3. Waterways

Waterways ($n = 10$) had summed PFAS species concentrations from below detection limits to 722 ng/L (Fig. 2). The highest concentrations were observed in samples taken near sites impacted by AFFF (96 to 722 ng/L). Lower concentrations were observed in sites near urban areas and wastewater treatment plants (7 to 17 ng/L) and the lowest concentrations were observed in remote and rural areas (<3 ng/L).

Continuity of summed concentrations along the sampled reaches varied between waterways. The Truckee River was sampled using Lagrangian sampling informed by previous tracer studies; no such studies were found for the other rivers sampled (Bohman, 2000). PFAS concentrations were remarkably similar in the Walker River (coefficient of variation (CV) between summed PFAS concentrations for all Walker River samples = 21 %), Truckee Canal (CV = 2 %), and in the Truckee River reach from Lockwood 2 to Marble Bluff dam (i.e., downstream of Reno, NV, CV = 7 %). These similarities could be attributed to minimal inflows and a lack of additional PFAS sources.

At the other sites, notable variance in the concentrations were observed along the reach. For example, the Truckee, CA, to Reno, NV, reach of the Truckee River, and the sampled reach of the Carson River, had CVs of 124 % and 68 %, respectively. Concentrations both increased and decreased from site to site along each of these reaches (Fig. 2). Increases in concentrations may be reflective of the presence of additional PFAS sources, or a confluence with a waterway that had higher PFAS concentrations. This is particularly evident when accompanied by a substantial shift in species profile, such as the addition of new species or dramatic changes in the ratio of species present. An example of this can be seen at the confluence of the Truckee River and Steamboat Creek in Fig. 2 (see profile change from "Above Steamboat" to "Below Steamboat"). Steamboat Creek contains TMWRF effluent, which discharges to the creek approximately 100 m upstream from the confluence, and is also impacted by AFFF use at RNO. Wastewater and AFFF are known to contain PFAS and thus the substantial change in concentration and species profile is expected (Lenka et al., 2021). Less clear are the mechanisms leading to decreases in concentration. In montane sites such as the Truckee River from Truckee, CA, to Reno, NV, the observable decrease in concentration may be explained by dilution with less contaminated water (e.g., numerous alpine creeks and reservoirs feed into the river along this reach). However, the mechanism(s) which caused the decrease observed from Carson City to Dayton, along the Carson River, are less clear as we are unaware of any inflows from surface flow or reservoirs. However, dilution from an unidentified source, such as a groundwater discharge, is supported by the modest 10–15 % reduction in conductivity observed at the Dayton site relative to the Carson City site (715 μS/cm at Carson City to 630 μS/cm in Dayton). It is also possible that there is temporospatial variability in concentration along the Carson River, but we are unaware of any tracer study to inform Lagrangian sampling. Upper Dry Creek also had greater PFAS concentrations than Lower Dry Creek, and Upper Dry Creek is in closer proximity to RNO, an airport with known groundwater PFAS contamination in the aquifer below it (EWG, 2022). It is not clear why this occurred, though it may be because of an increasing ratio of PFAS-containing groundwater-derived flows to less impacted surface-derived flows along the reach. Further investigation is needed but is beyond the scope of this study.

The species profile at the Lockwood site on the Truckee River was very similar across two sampling events, nearly a year apart, indicating that the source(s) are unlikely to change temporally (Fig. 2). However, the concentration across the two sampling events changed by nearly fourfold (64 ng/L in Fall 2021 to 14 ng/L in Summer 2022), which appears to be caused almost entirely by dilution; river flow at the nearby Tracy, NV gaging station was 100 and 520 cfs on the sampling dates (US Geolgic Survey, n.d.). Bai and Son also measured PFAS in the Truckee River, and the highest concentrations were present in the City of Reno near the identified AFFF FTA (Bai and Son, 2021). Our measurements in samples from Lake Tahoe and the Truckee River had generally lower concentrations than those of Bai and Son, further indicating that temporal variance in PFAS concentration is likely to occur in the region.

3.4. Fluorotelomer sulfonate as an indicator of aqueous film forming foam impacts

Following the discovery of significant human health and environmental impacts of PFOS based AFFF in the early 2000's AFFF manufacturers began seeking less harmful alternatives (Sontake and Wagh, 2014). 6:2 fluorotelomers, such as 6:2 fluorotelomer sulfinyl amido sulfonic acid, are used in many of these alternative AFFF formulations and can degrade to 6:2 FTS and 6:2 FTOH in aerobic systems (Ruyle et al., 2021; Choi et al., 2022). Thus, it is no surprise that 6:2 FTS is frequently present at AFFF impacted sites, though data on its occurrence is limited as many studies do not include it in their targeted species assay

(Gonzalez et al., 2021; Karrman et al., 2011; Brusseau and Guo, 2020). 6:2 FTS has also been demonstrated to be degraded to PFPeA and PFHxA in aerobic sediments, which, over time, reduces 6:2 FTS concentrations to near or below detection limits, although these terminal products take some time to form through multiple intermediates (Zhang et al., 2016). While other sources of 6:2 FTS have been documented such as electroplating and fluoropolymer production facilities, we are unaware of the presence of such facilities within the study area (Glüge et al., 2020). For these reasons, we believed 6:2 FTS may be useful as an indicator of PFAS transport from an AFFF impacted site with recent (in the last ~20 years) training and firefighting activities.

In the Truckee River watershed, the presence of 6:2 FTS was observable from the surface waters surrounding RNO, dilution in Steamboat creek, further dilution in the Truckee River, transport along the Truckee River into the Truckee Canal, and finally in the lower end of Lahontan Reservoir (where the Truckee Canal discharges to the reservoir). 6:2 FTS was not detectable above RNO in Steamboat Creek, in the Truckee River above the Steamboat Creek confluence, in the Carson River above Lahontan Reservoir, nor in the upper end of the Reservoir (furthest from the Truckee Canal discharge). The presence/absence of 6:2 FTS in these relevant tributaries and waterways alone indicates that the 6:2 FTS present in the lower end of the reservoir and in the Truckee River is derived from the Steamboat Creek flow (i.e., RNO) and demonstrates an instance in which 6:2 FTS can be used as an indicator of PFAS sources.

Concentrations and 6:2 FTS abundance normalized to summed PFAS concentrations ("relative abundance") from the confluence of the Truckee River and Steamboat Creek to Lahontan Reservoir are highlighted in Fig. 3 (also in Fig. 2 and Fig. S1). 6:2 FTS relative abundance decreased exponentially with river distance, similar to the findings of Zhang et al., which documented a logarithmic decay of 6:2 FTS in aerobic sediments (Zhang et al., 2016). While it is possible that the mass loss could be attributed to partitioning to sediments or dilution from groundwater discharge this is unlikely given the relatively strong fit to the relative abundance data (over the raw data) and the relatively unchanged concentrations of other terminal species such as PFOS and PFOA. Therefore, given that RNO has documented contamination with PFAS (although no data on 6:2 FTS was available), the observed similarity of the degradation kinetic profile to the findings of Zhang et al. (assuming river miles is approximately equivalent to time and that 6:2 FTS relative abundance across two separate sampling campaigns is similar to concentration), and the presence/absence of 6:2 FTS in the relevant tributaries discussed above, the source of 6:2 FTS in the system appears to be RNO (EWG, 2022). Finally the half-life of 6:2 FTS in aerobic sediments as demonstrated by Zhang et al. was "<5 days" (although it is somewhat unclear how this was arrived upon based on the first order fit of the same data shown in Fig. S2 with resulting $t_{1/2} = 16$d but concentration falling to ~50 % of the initial spike within 1d, and what appears to be a logarithmic fit shown in the main text of the publication without a supporting exponential fit) and this data appears to be consistent (~20 to 40 h travel time between the sites (Bohman, 2000) (Crompton and Bohman, 2000)).

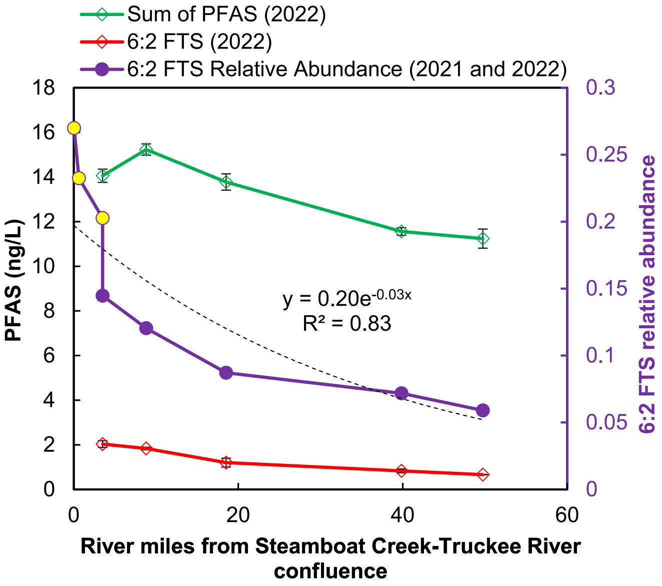

Fig. 3. Sum of concentrations of PFAS ($n = 19$), concentration of 6:2 FTS, and correlation between 6:2 FTS relative abundance (6:2 FTS / sum of PFAS concentrations, right axis) and river miles along the Truckee River from its confluence with Steamboat Creek to Lahontan Reservoir (Sites Tr 9 – Tr 14 on Figs. 1 and 2). Fall 2021 6:2 FTS relative abundance data included with yellow fill (Sites Tr 7, Tr 8, and Lockwood 2 in Figs. 1 and 2). All other data shown is from 2022. 2021 concentrations are only shown as relative abundance for clarity (scale). Non-normalized concentrations of 6:2 FTS and summed PFAS are not shown here but are present in Fig. 2. Logarithmic fit of this data and exponential fit for Zhang et al. data shown in Fig. S2.

Swan Lake and its inflow also contained a relatively small fraction of 6:2 FTS. Although total precursors accounted for <0.1 % of the PFAS mass detected, 6:2 FTS presence may indicate AFFF contributed to the total PFAS load. This is corroborated by the presence of two potential AFFF sources near the lake (described in Materials and Methods).

Samples from the Stillwater National Wildlife Refuge (Carson Sink) did not contain detectable concentrations of 6:2 FTS, despite proximity to a documented AFFF FTA at the Fallon Naval Air Station and the presence of a drainage canal leading from the Air Station to the Refuge. Further data on 6:2 FTS presence in groundwater below the Fallon Naval Air Station was not available. This may be due to degradation of 6:2 FTS to PFHxA and PFPeA, which is supported by the fact that PFPeA does not occur upstream of the Fallon Naval Air Station, but does occur downstream (See Figs. 1 and 2) (Zhang et al., 2016). Alternatively, this may also indicate that the Refuge was not receiving a significant quantity of water from the FTA, but rather another source such as reclaimed water from the WWTP in Fallon.

3.5. Extractable organofluorine

Targeted PFAS analysis methods, using LC-MS/MS, are limited by the availability of analytical standards. Non-target methods attempt to capture species outside of those amenable to mass spectrometry and we attempted to understand the total organofluorine loading by measuring EOF in 34 sampling sites covering a range of targeted species concentrations and environmental settings (Fig. 4). The sum targeted PFAS (as F equivalents) as a percentage of EOF were highly variable, ranging from ~0.1 to ~600 % with median value of only 0.6 % (standard deviation was ±0.9 %; $n = 34$). The occurrence of summed targeted PFAS being greater than the EOF indicates that EOF may not perfectly capture all PFAS or that assumptions regarding the capacity of the resin for PFAS may be inaccurate. Other research has also demonstrated that the total EOF load is much greater than what is captured by LC-MS/MS, indicating the presence of unknown organofluorine species (Miyake et al., 2007; Yeung et al., 2013; Forster et al., 2023). Sites with a higher fraction of identified EOF (i.e., LC-MS/MS targeted species) were associated with AFFF FTAs and other point sources; the percentage of EOF comprised of targeted species for different samples were Swan Lake: 27 %, Lower Dry Creek: 99.9 %, Harmon Reservoir: 74 %. Samples with the lowest percent of identified EOF were remote (e.g. Cliff Lake: 0.03 %). Other publications have also shown that the percentage of identified organofluorine is typically higher at sites with a distinct point source such as AFFF application (Miyake et al., 2007; Koch et al., 2019).

There was a higher standard deviation in EOF concentrations (Fig. 4 error bars), compared to LC-MS/MS concentrations, among field triplicates. Therefore, additional experiments were conducted to understand the potential for environmental F^- to be inconsistently rinsed from the ion exchange column during the washing step, and be eluted into the

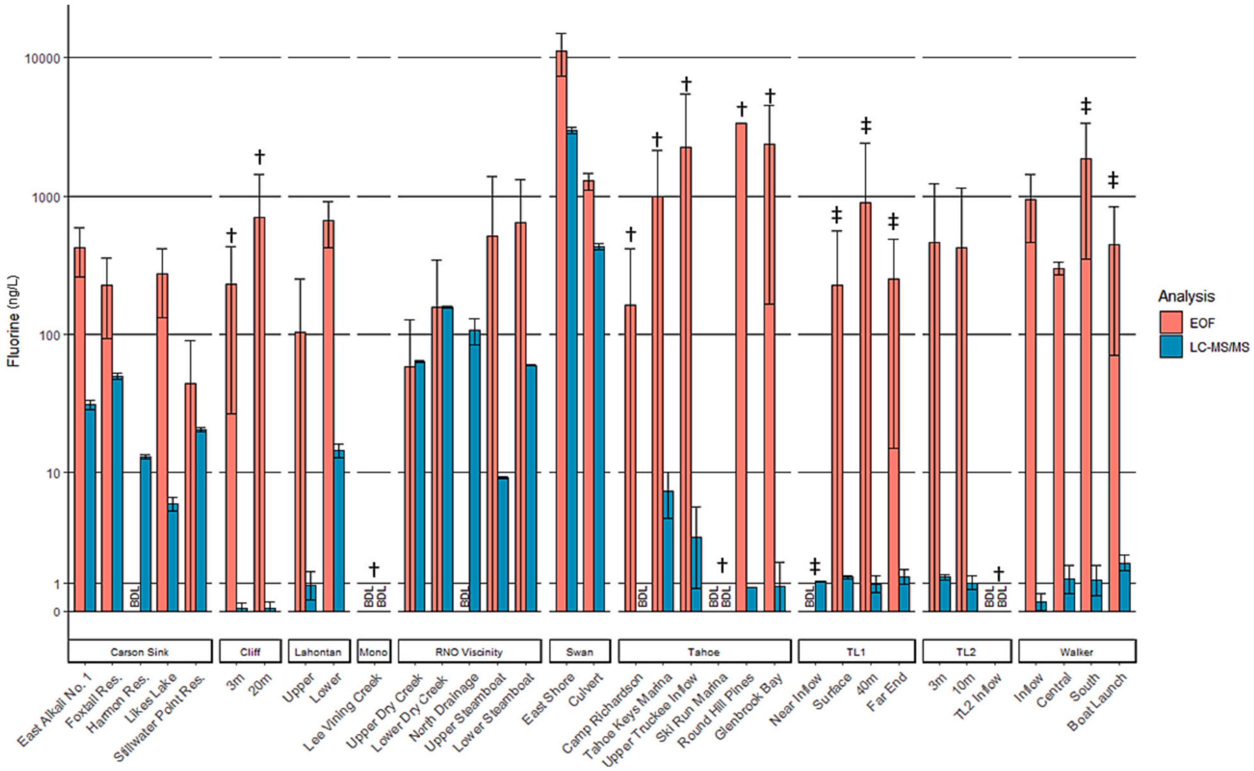

Fig. 4. Comparison of EOF and summed, targeted PFAS captured by LC-MS/MS, tabular concentrations are included in Table S5. Both are presented as the average of field triplicates with error bars representing the standard deviation. Sites with <0.05 ppm F^- (F^- was analyzed via ion chromatography) are marked with †, and those with >1 ppm F^- are marked with ‡.

EOF isolate. A series of solutions containing spiked PFOA and NaF salt were extracted and analyzed. EOF was 2 to 10× greater than spiked OF, and the interference with EOF measurement increased as spiked NaF increased (Fig. S3). This indicates the potential for F^- to be retained on the ion exchange cartridge during the rinse steps described by Miyake et al., be eluted into the isolate, and interfere with measurements of EOF, resulting in potentially greater reported EOF concentrations than are present (Miyake et al., 2007). This phenomenon would be more problematic in samples with low organofluorine concentrations, but high F^- concentrations, likely resulting in a relationship between measured EOF and F^-. However, there was no correlation ($R^2 = 0.0027$) between F^- and EOF concentrations in field samples from this research (Fig. S4), indicating that F^- breakthrough may be somehow mitigated in environmental samples. F^- was, however, positively correlated with ionic strength ($R^2 = 0.8$, Fig. S5) and high concentrations of other anions may lead to binding site competition with F^- and preferential sorption of other, non-interfering anions, potentially explaining the lack of correlation. Determining precise concentrations of ionic strength and F^- in which EOF is applicable versus subject to strong F^- interference is outside the scope of this study, but inspection of Fig. S3 results in a rough estimate of maximum F^- concentrations (maximum of $50\ \mu gF^-/L$) in which EOF is subject to reduced interference. Samples with less than this concentration of F^- have been identified (†) in Fig. 4 and all others should be interpreted with caution. This challenge is not likely to be specific to only this sample set, and we urge caution in interpretation of all environmental EOF measurements in samples where it is likely that F^- is present (e.g., groundwater).

4. Conclusions

PFAS were detected in nearly every sample collected in the study area. The highest PFAS concentrations were at sites near AFFF FTAs (~20 to 4754 ng/L), lower concentrations were observed near WWTPs and urban areas (~2 to 15 ng/L), and the lowest concentrations observed were at remote sites (3 ng/L or less). At 2 of 4 alpine lakes, all 19 measured PFAS species were below their respective detection limits. We found that PFAS may accumulate in terminal lakes, which may be exacerbated in the future as lake levels decline due to increased evaporation and reduced inflows. We showed that 6:2 FTS can be used as an indicator of PFAS transport away from AFFF-impacted sites in forensic analysis of PFAS sources and transport pathways. We also demonstrated the potential for EOF measurements to be subject to interference from environmentally occurring F^-, complicating the current understanding and interpretation of EOF measurements.

Due to limited resources, the scope of this study was intentionally restricted and thus the conclusions drawn here are similarly limited. Future studies should consider additional sampling and variables such as lake mixing, seasonal variability in both rivers and lakes, time history of source inputs, groundwater flows, precipitation effects on loading and surface water concentrations and evaporation rates among others.

The Lahontan cutthroat trout, cui-ui, and several other threatened or endangered species are endemic to terminal lakes and drainages of the Great Basin and PFAS have been shown to accumulate in fish, making these species likely to be vulnerable to bioaccumulation (Burkhard, 2021). Terminal lakes are exceptionally sensitive ecosystems due to the potential for accumulation of all inputs. Given that PFAS tended to accumulate in terminal lakes, special attention should be given to their protection.

CRediT authorship contribution statement

Michael DeNicola: Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization. **Zunhui Lin:** Investigation, Writing – review & editing. **Oscar Quiñones:** Investigation, Writing – review & editing. **Brett Vanderford:** Investigation, Data curation. **Mingrui Song:** Investigation, Writing – review & editing. **Paul Westerhoff:** Writing – review & editing, Supervision. **Eric Dickenson:** Writing – review & editing, Supervision. **David Hanigan:** Conceptualization, Writing – review & editing, Resources, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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