Determination of Fish Bioaccumulation Factors (BAFs) for Selected PFAS Contaminants in Marine and Freshwater Systems

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LISTS OF ABBREVIATIONS

Site Categories; defined by NJ DEP approved QAPP

Cnt-Atl	Central Atlantic Sites
Del	Delaware River adjacent Sites
North	Northern New Jersey Sites
Upper Estuary / UE	Upper estuary sites variable flow and salinities that may range from fresh to brackish water
Open Water Bay / OWB	Lower estuary or open/bay sites with limited non-tidal flow and salinities that may range from brackish to ocean water

Site Codes; defined by the Academy of Natural Sciences

Site Code	Site Name	Latitude	Longitude
ARB	Absecon Bay	39.417604	-74.477701
BBMR	Metedeconk River	40.054048	-74.067631
BBTR	Tom's River	39.936548	-74.124311
DBCR	Cohansey River	39.344837	-75.363068
DBMR	Maurice River	39.215191	-75.028546
DROC	Oldsman's Creek	39.783882	-75.429565
DRSC	Salem Canal	39.686118	-75.509628
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SI	Shooter's Island	40.653376	-74.14809
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ASS	Assunpink Lake	40.215428	-74.517229
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LMC	Little Mantua Creek	39.824916	-75.204341
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PIL	Pine Lake	40.009228	-74.25019
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SAL	Salem Canal	39.669628	-75.466175
STL	Stewart Lake	39.841931	-75.142848
UL	Union Lake	39.408095	-75.067175

Per- and polyfluoroalkyl substances

Acronym	Compound Name	Alternate Name	Group Name
PFBA	Perfluorobutanoic acid	Perfluorobutanoate	*Perfluoroalkyl carboxylates*
PFPeA	Perfluoropentanoic acid	Perfluoropentanoate	*Perfluoroalkyl carboxylates*
PFHxA	Perfluorohexanoic acid	Perfluorohexanoate	*Perfluoroalkyl carboxylates*
PFHpA	Perfluoroheptanoic acid	Perfluoroheptanoate	*Perfluoroalkyl carboxylates*
PFOA	Perfluorooctanoic acid	Perfluorooctanoate	*Perfluoroalkyl carboxylates*
PFNA	Perfluorononanoic acid	Perfluorononanoate	*Perfluoroalkyl carboxylates*
PFDA	Perfluorodecanoic acid	Perfluorodecanoate	*Perfluoroalkyl carboxylates*
PFUnDA	Perfluoroundecanoic acid	Perfluoroundecanoate	*Perfluoroalkyl carboxylates*
PFDoA	Perfluorododecanoic acid	Perfluorododecanoate	*Perfluoroalkyl carboxylates*
PFTrDA	Perfluorotridecanoic acid	Perfluorotridecanoate	*Perfluoroalkyl carboxylates*
PFTeDA	Perfluorotetradecanoic acid	Perfluorotetradecanoate	*Perfluoroalkyl carboxylates*
PFBS	Perfluorobutanesulfonic acid	Perfluorobutanesulfonate	*Perfluotoalkyl sulfonates*
PFPeS	Perfluoropentanesulfonic acid	Perfluoropentanesulfonate	*Perfluotoalkyl sulfonates*
PFHxS	Perfluorohexanesulfonic acid	Perfluorohexanesulfonate	*Perfluotoalkyl sulfonates*
PFHpS	Perfluoroheptanesulfonic acid	Perfluoroheptanesulfonate	*Perfluotoalkyl sulfonates*
PFOS	Perfluorooctanesulfonic acid	Perfluorooctanesulfonate	*Perfluotoalkyl sulfonates*
PFNS	Perfluorononanesulfonic acid	Perfluorononanesulfonate	*Perfluotoalkyl sulfonates*
PFDS	Perfluorodecanesulfonic acid	Perfluorodecanesulfonate	*Perfluotoalkyl sulfonates*
PFDoS	Perfluorododecanesulfonic acid	Perfluorododecanesulfonate	*Perfluotoalkyl sulfonates*
4:2 FTS	1H, 1H, 2H, 2H-perfluorohexane sulfonic acid	1H, 1H, 2H, 2H-perfluorohexane sulfonate	*Fluorotelomer sulfonates*
6:2 FTS	1H, 1H, 2H, 2H-perfluorooctane sulfonic acid	1H, 1H, 2H, 2H-perfluotooctane sulfonate	*Fluorotelomer sulfonates*
8:2 FTS	1H, 1H, 2H, 2H-perfluorodecane sulfornic acid	1H, 1H, 2H, 2H-perfluorodecane sulfonate	*Fluorotelomer sulfonates*
3:3 FTCA	2H, 2H, 3H, 3H-perfluorohexanoic acid	2H, 2H, 3H, 3H-perfluorohexanoate	*Fluorotelomer carboxylates*
5:3 FTCA	2H, 2H, 3H, 3h-perfluorooctanoic acid	2H, 2H, 3H, 3H-perfluorooctanoate	*Fluorotelomer carboxylates*
7:3 FTCA	2H, 2H, 3H, 3H-perfluorodecanoic acid	2H, 2H, 3H, 3H-perfluorodecanoate	*Fluorotelomer carboxylates*
PFOSA	Perfluorooctanesulfonamide		*Perfluorooctane sulfonamides*
N-MeFOSA	N-Methylperfluorooctanesulfonamide		*Perfluorooctane sulfonamides*
N-EtFOSA	N-Ethylperfluorooctanesulfonamide		*Perfluorooctane sulfonamides*

Acronym	Compound Name	Alternate Name	Group Name
N-MeFOSAA	N-Methylperfluoro-1-octanesulfonamidoacetic acid	N-Methylperfluoro-1-octanesulfonamidoacetate	*Perfluorooctane sulfonamidoacetic acids*
N-EtFOSAA	N-Ethylperfluoro-1-octanesulfonamidoacetic acid	N-Ethylperfluoro-1-octanesulfonamidoacetate	*Perfluorooctane sulfonamidoacetic acids*
N-MeFOSE	N-Methylperfluoro-1-octanesulfonamidoethanol		*Perfluorooctane sulfonamidoethanols*
N-EtFOSE	N-Ethylperfluoro-1-octanesulfonamidoethanol		*Perfluorooctane sulfonamidoethanols*
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionic acid	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionoate	*Ether carboxylates*
ADONA DONA	Decafluoro-3H-4,8-dioxanonoate	Decafluoro-3H-4,8-dioxanonoic acid	*Ether carboxylates*
NFDHA	Perfluoro-3,6-dioxaheptanoate	Perfluoro-3,6-dioxaheptanoic acid	*Ether carboxylates*
PFMPA	Perfluoro-3-methoxypropanoate	Perfluoro-3-methoxypropanoic acid	*Ether carboxylates*
PFMBA	Perfluoro-4-methoxybutanoate	Perfluoro-4-nethoxybutanoic acid	*Ether carboxylates*
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	*Ether sulfonates*
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	*Ether sulfonates*
PFEESA	Perfluoro(2-ethoxygethane)sulfonic acid	Perfluoro(2-ethoxyethane)sulfonate	*Ether sulfonates*

EXECUTIVE SUMMARY

This technical report investigates the bioaccumulation of per- and polyfluoroalkyl substances (PFAS) in New Jersey's aquatic ecosystems, providing critical data to aid the New Jersey Department of Environmental Protection (NJDEP) in developing surface water quality standards (SWQS). PFAS, a group of over 9,000 synthetic chemicals, have been widely used since the 1950s. They are persistent in the environment, bioaccumulate in organisms, and are linked to significant human health risks, including immune suppression and cancer. Understanding PFAS behavior, particularly in fish species across various water bodies, is essential for informed regulatory decisions.

Study Objectives and Methods

The goal of this study was to calculate bioaccumulation factors (BAFs) for 40 PFAS compounds across a range of water bodies and fish taxa in New Jersey. Sampling occurred over two years, covering 16 marine and 18 freshwater sites. Fish, water, and sediment samples were analyzed for PFAS concentrations, with particular focus on species that represent different dietary and habitat exposure pathways. Both frequentist "EPA Methods" and Bayesian statistical methods were employed to calculate BAFs, with the Bayesian approach providing better handling of data uncertainties and concentrations below detection limits, but the EPA Methods providing comparable results in previous studies. Estimates via both methods are provided in full within this report.

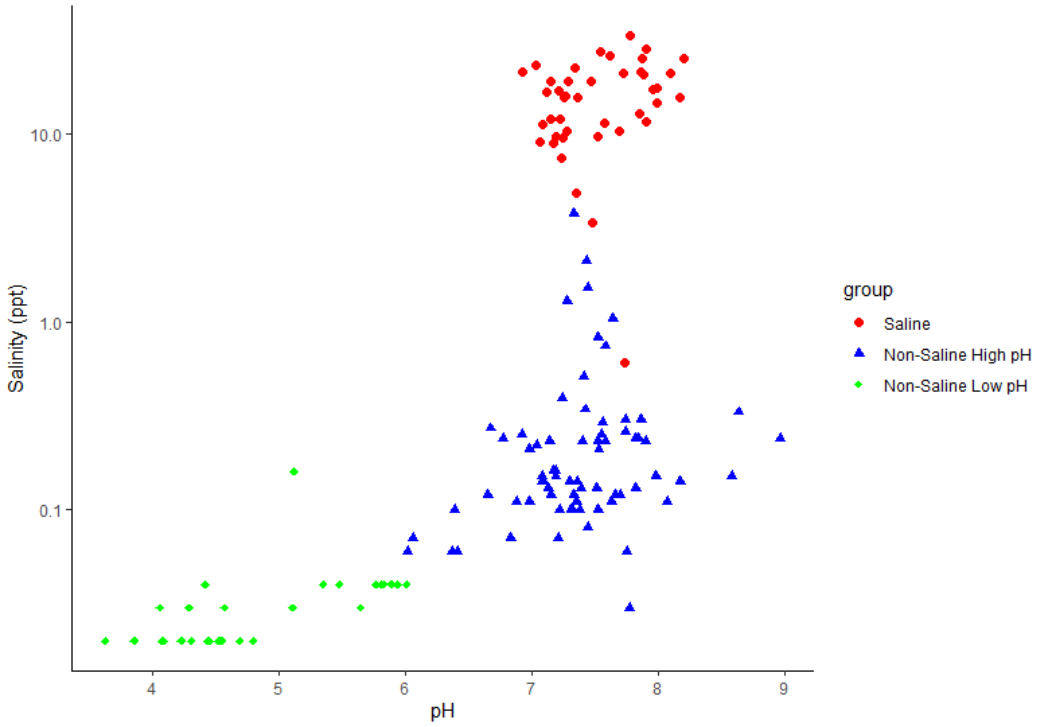
Key Findings

- EPA Method Statewide PFAS BAF Estimates: Statewide BAF estimates were calculated for all fish species across salinity and pH groups using EPA methodology for selected compounds (PFOA, PFOS, PFNA). PFOS BAFs were significantly higher in non-saline waters (1970) compared to saline waters (495), indicating a greater potential for bioaccumulation in non-saline environments. Alternatively, PFNA BAFs were lower in non-saline waters (81.4) compared to saline waters (216). For PFOA, the statewide (combined saline and non-saline sites) BAF was 18.9, reflecting more consistent bioaccumulation across environments. These differences were statistically significant for both PFNA and PFOS.

EPA methodologies were used for selected compounds (PFOA, PFOS, and PFNA) to determine aqueous BAFs for all fish species within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups. "n" represents the number of total number of unique fish species-compound-site combinations used to generate the estimates.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	34.1 (158)a	495 (681)b	216 (949)a	4	7	5
Non-Saline High pH	9.84 (112)a	1940 (2670)b	79.7 (293)b	6	11	11
Non-Saline Low pH	36.2 (455)a	3370 (5730)a	164 (1480)ab	4	5	2
Non-Saline	12.6 (109)	1970 (2770)	81.4 (295)	6	11	11
Statewide	18.9 (91.4)	1170 (1740)	106 (409)	8	15	13

- Quantitative Comparison of Site Characteristics: Among several potential grouping methods—based on hydrology, region, site type, cations, isotopes, and water quality (pH/salinity)—the water quality method, specifically using pH and salinity, proved to be the most logical, and statistically sound. Grouping by pH and salinity not only provided the clearest distinctions in PFAS bioaccumulation but also offers a straightforward approach that can be universally applied to future regulatory investigations.



Salinity and pH were used as variables for an iterative k-means grouping analysis to separate sites into logical clusters. A analysis of the total within cluster sum of squares indicated 3 groups best fit the data. The y-axis Salinity is presented on a log scale so that the non-saline groups are distinguishable by eye.

- Species-Specific Sensitivity: Certain species, such as American Eel and White Perch, exhibited higher PFAS accumulation rates.

- Sediment BAFs: Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences compared across any grouping methodology.

Methodological Innovations

The Bayesian methodologies used in this study provided several advantages over traditional methods, especially for handling observations below detection limits and ensuring more accurate BAF estimates. Bayesian models allowed for the integration of data uncertainty into the BAF calculations, improving reliability and robustness.

Implications for Surface Water Quality Standards

This study highlights several key insights for the development of SWQS in New Jersey:

- Compound-Specific BAFS: PFAS compounds, particularly PFOS, exhibited significant bioaccumulation differences and in some cases vary based on waterbody characteristics such as salinity and pH.

- Species-Specific Sensitivity: Some fish species, like American Eel and Common Carp, showed higher PFAS accumulation rates.

Conclusion

This study's findings highlight the importance of considering water quality factors, such as salinity and pH, when determining bioaccumulation factors that may be regionally specific. Of the various methods tested for analyzing and grouping data, water quality (via pH and salinity) emerged as the most robust and practical approach for regulatory research. By providing comprehensive data on PFAS bioaccumulation across New Jersey's aquatic systems, this study informs the development of more effective water quality regulations. The use of advanced Bayesian methods offers a more robust approach to handling data uncertainties and better reflects PFAS exposure risks in both saline and non-saline environments.

1. INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a chemical group that consists of over 9,000 man-made compounds (Buck et al., 2011). Since the 1950s, these compounds have been used for a wide range of industrial and commercial applications such as water/oil resistant surfaces and aqueous firefighting foams (Banks et al., 2013; Karsa, 1999; Kissa, 2001, 1994; Sunderland et al., 2019). As a result of their widespread application, PFAS have become prevalent in the environment, wildlife, and humans (Buck et al., 2011).

PFAS are bioaccumulative, with some being found in the blood, brain, kidneys, liver, lungs, and even breast milk of humans (Buck et al., 2011; Pérez et al., 2013; Sundström et al., 2011). Greater amounts of bioaccumulation occur for compounds with longer alkylchains (Martin et al., 2003a, 2003b). After intake, studies have found that PFAS tend to concentrate in the blood/plasma and highly perfused tissues (i.e., liver and heart) of organisms (Buck et al., 2011; Burkhard, 2021; Consoer et al., 2014; Martin et al., 2003a; Pérez et al., 2013; Shi et al., 2018, 2015; Wang et al., 2016).

There are multiple concerns surrounding the health impact on humans. Concentrations of PFAS in humans has been linked to immune suppression, metabolic disruption, ulcerative colitis, thyroid diseases, testicular cancer, kidney cancer, and preeclampsia (Frisbee et al., 2009; Stahl et al., 2014; Steenland et al., 2010; Sunderland et al., 2019; Watkins et al., 2013). People can be exposed to PFAS in a variety of ways: however, consumption is considered the primary mechanism (Zhang et al., 2019). Exposure pathways include food consumption, inhalation (dust/ambient air/aerosols/etc.), hand to mouth transfer following contact with PFAS, and migration of paper/cardboard into food (Trudel et al., 2008). Fish consumption is a main source of PFAS exposure in adults (Stahl et al., 2014; Zhang et al., 2019). PFAS in fish tend to accumulate in blood and liver and have lower accumulation in muscle and fillet (Lewis et al., 2022; Valsecchi et al., 2021). In one study, PFOS contributed to over 90% of PFAS found in muscle (Pan et al., 2014). Additionally, PFOS may also be a good indicator compound as there is a high correlation between it and other PFAS compounds (Pan et al., 2014).

Bioaccumulation Factors (BAFs) are one way to better understand the behavior of PFAS in the environment and to understand specific bioaccumulation and biomagnification behavior. BAFs are the ratio of the concentration of a single PFAS compound measured in fish tissue to its concentration in water. Unlike organic contaminants, PFAS do not have an affinity for lipids, thus lipid normalization is not necessary when calculating PFAS BAFs. BAF calculations can be made on a wide range of data groupings specific to the researcher's questions (e.g., individual specimen BAF, species BAF, statewide species BAF, etc.).

There are many biotic factors that influence PFAS concentrations and BAFs. Some have found sex differences among PFAS compounds (Pan et al., 2014) and significant difference in BAFs among species (Lee et al., 2020). Herbivorous and omnivorous fishes have had higher concentrations of short chain PFAS compared to piscivorous fishes who have had higher concentrations of long-chain PFAS (Ahrens et al., 2016). Also, higher trophic level aquatic organisms such as fish may have higher concentrations of PFAS due to biomagnification, a phenomenon in which a contaminant concentration increases as one moves up the food-chain.

In addition to biotic factors, there are many abiotic factors and interactions between biotic and abiotic factors that may influence PFAS concentrations and resulting BAFs. Burkhard (2021)

suggested that some PFAS may have a higher BAF for freshwater compared to marine species. Major differences between saline and non-saline waters are pH and salinity concentration. Although some researchers have observed patterns among PFAS and salinity, little is known about the influence of salinity on PFAS behavior.

Within the freshwaters of New Jersey, water chemistry differs between Pineland and non-Pineland regions. The Pineland systems have a unique pH and carbon chemistry that may result in unique PFAS partitioning and bioavailability relative to non-Pineland systems. Moreso, pH and organic carbon concentration are both known to affect PFAS sorption, bioavailability and uptake. For this reason, PFAS BAFs in the Pinelands (low pH, high DOC waters) may be distinctly different from those in the rest of the state.

Another factor that may affect PFAS bioavailability is lake depth. For lakes, shallow and deeper lakes may differ due to degrees of water-sediment interaction, groundwater interactions, and evaporation. There may be differences in bioavailability between shallow and deeper lakes due to differing degrees of water-sediment interaction as quantified by the lake volume:surface area ratio. In addition to lake depth, lake turnover time may influence environmental PFAS concentrations. Short turnover time may lead to dilution of groundwater sourced PFAS and/or more time-variable PFAS concentrations. Conversely, in shallow lakes with long turnover times evaporation is likely to be large relative to outflow, potentially concentrating PFAS in the lake waters.

Problem Statement and Objectives

A range of waterbody types and taxa exist in New Jersey. Differences in chemistry, habitat and taxa are thought to influence PFAS behavior. Therefore, our goal was to develop New Jersey-specific regional BAFs for 40 PFAS that would be used to aid NJDEP in development of surface water quality standards (SWQS). To account for the potential variability in PFAS behavior and environmental factors mentioned above, we sought to assess the influence of waterbody, habitat, and trophic groups.

Our primary objective was to calculate BAFs for PFAS that could be used across the range of New Jersey waterbodies and fish taxa. Initial discussions with NJDEP dictated site categorization (saline and non-saline; streams and lakes; Pinelands and non-Pinelands) and fish stratification. An emphasis was placed on collecting multiple water and individual fish samples to provide information on sources of variability in concentrations, and there was a recognized need for an efficient sampling design, balancing the number of sites against the breadth of data collected at each site.

2. METHODS

Sampling was conducted over two years with all 16 marine sites sampled in 2022 and all 18 freshwater sites sampled in 2023 (Figure 1). Saline sites were described by the NJ DEP approved QAPP (Appendix V) as: 1) upper estuary sites (UE) with variable flow and salinities that ranged from tidal fresh to brackish water; and 2) lower estuary or open/bay sites (OWB) with limited non-tidal flow and salinities that ranged from brackish to ocean water (Table 1). Non-saline sites included: 1) lakes (with deep and shallow hydrology) and streams, in 2) Pineland and non-Pineland areas (Table 1). Sampling was conducted based on an NJDEP approved QAPP (Appendix B).

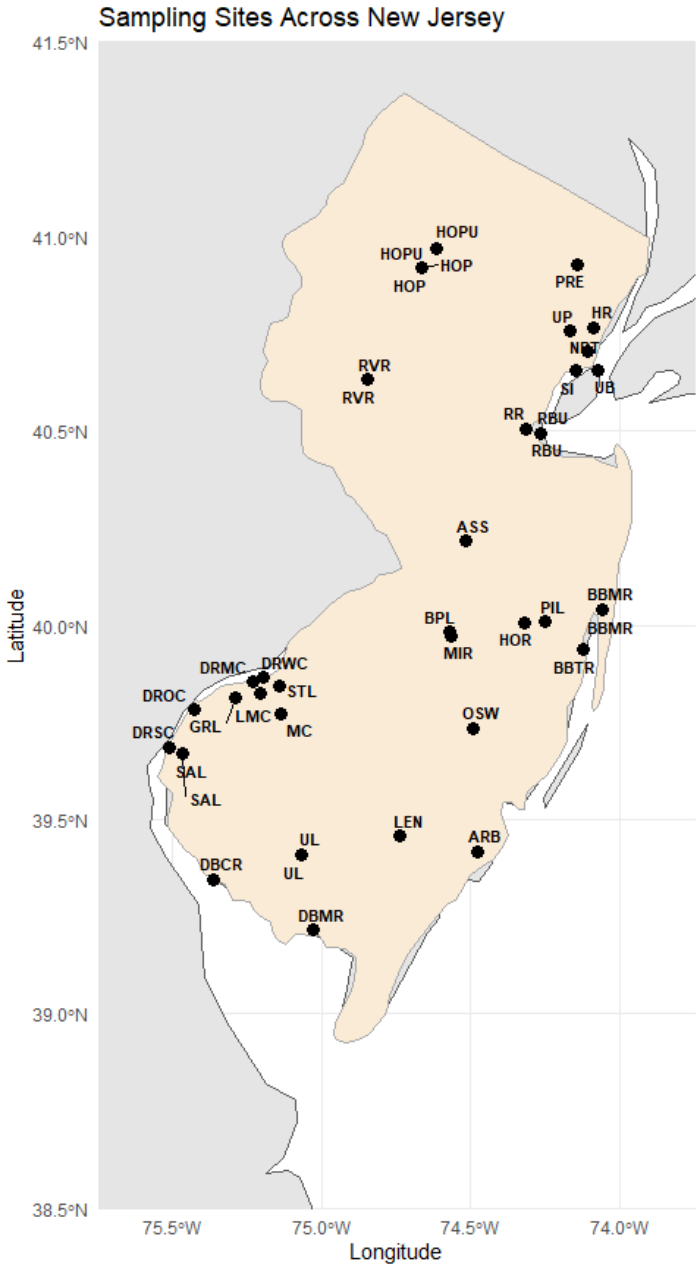


Figure 1: Site map of all sampling locations. Site codes can be found in Table 1.

Table 1: Marine and Freshwater Stations were divided by hydrology (Marine, Non-Pinelands Lake, Non-Pinelands Stream, Pinelands Lake, and Deep Lake), Region (central-Atlantic Coast [Cnt-Atl], Delaware River [Del], and Northern New Jersey [North]), site type (Upper Estuary, Open Water Bay, Lake, and Stream). These classifications were defined by the NJ DEP approved QAPP (Appendix B). Site conditions did not consistently align with these definitions and thus a series of grouping analyses were used to test for broad differences across groups of sites (Section 3; Methods; Grouping Analysis).

Region	Hydrology	Site Type	Station	Station Name	Latitude	Longitude
Cnt-Atl	Marine	Upper Estuary	ARB	Absecon Bay	39.417604	-74.477701
Cnt-Atl	Marine	Upper Estuary	BBMR	Metedeconk River	40.054048	-74.067631
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Non-Pinelands Lake		Lake	STL	Stewart Lake	39.841931	-75.142848
Deep Lake		Lake	UL	Union Lake	39.408095	-75.067175

Media collected and analyzed

Sites were pre-sampled to verify and assess the temporal variability of aqueous PFAS concentrations. Sites with no detectable PFAS were not selected for additional study. At all sites selected, we aimed to collect 3 fish species and 3 individuals of each species, representing key dietary (forager vs. predator) and habitat (pelagic vs. benthic) PFAS exposure pathways. All water, sediment, and fish filet samples were analyzed for PFAS concentrations. Additionally, fish samples were analyzed for stable isotope compositions to verify trophic level.

Sample collection in the field

Water and sediment

Temporal variability in PFAS concentrations in flowing systems (streams and upper estuary) was thought to complicate precise estimates of BAFs in streams. PFAS uptake into fish tissues has been shown to be reasonably rapid, in the order of days to weeks. Thus, accurately quantifying PFAS concentrations at analogous time scales was necessary for accurate estimation of BAFs in these systems. To address the issue of variable water concentrations, we collected stream and estuary water samples weekly in the month prior to fish sampling to characterize concentration variability on the timescale of uptake. At the estuary sites, this sampling was conducted at the same point in the tidal cycle, except for 1–2 samples collected to assess tidal variability. Water samples were kept cold for transport to the lab and then frozen until analysis. Sediment was collected on the day fish were collected by using a hand trowel or by ponar if water depths were too deep to permit hand collection. The sediment sample was homogenized, kept cold for transport to the lab and then frozen until analysis.

Fish

Fishes at freshwater sites were opportunistically sampled using boat electrofishing. Volts ranged from 50-500 using 120 DC and an average of 8.0 amps. Habitat was continually electrofished until all target species were collected. Fish at marine sites were opportunistically sampled with gill nets, angling, and eel pots. Researchers set the equipment in habitats they identified as likely to contain the target species. In estuaries, gill nets were placed parallel or perpendicular to banks along the main channel and stretched across small tributaries and side channels where possible. In open bays gill nets were placed parallel or perpendicular to banks and stretched across inlets. Angling primarily consisted of drifting across a variety of depths while using fluke rigs with mummichog as bait. Additional angling was performed to supplement unsuccessful gill netting using spot rigs with blood worms or nightcrawlers while anchoring near structure. Eel pots were baited with bunker and placed along bulk heading, rip rap, grass beds, and other structure when available. Three sites (DRMC, DROC, and DRWC) had low enough salinity that they were able to be sampled using boat electrofishing methods. Captured fish were handled with powder free nitrile gloves and directly placed into clean metal containers and put on ice. Specimens were transported to the Academy of Natural Sciences of Drexel University where they were weighed and measured, wrapped in aluminum foil with an aluminum identification tag, placed in a Ziploc bag or trash bag depending on size, and frozen for future analysis.

Collection

Sample collection occurred at a total of 16 saline and 18 non-saline sites (Table 1). The saline sampling locations were a mixture of upper estuary (n=12) and lower estuary/ open water bay sites (n=4). Locations of the sites ranged from regions in northern New Jersey, the central Atlantic, and the Delaware River. The non-saline locations were a mixture of pineland lakes (n=6), non-pineland lakes (n=5), deep lakes (n=4), and non-pineland streams (n=3). 120 water presamples were taken between all proposed saline and non-saline sites to confirm measurable PFAS concentrations in the water. 61 presamples (including field duplicates (n=3) and field blanks (n=3)) were taken during low tide, low tide was used for consistency across the study. As a part of quality control procedures, field duplicates (n=1) and field blanks (n=3) were taken in addition during the course of sampling. 22 total samples were collected within the non-saline sites, as Union Lake, Hopatcong Lake, Upper Hopatcong Lake, and Round Valley Reservoir included bottom water sample collection in addition to the surface samples. As a part of quality control procedures, field duplicates (n=1) and field blanks (n=1) were taken in addition during sampling. Deep lakes (HOPU, HOP, RVR, UL) were sampled at the surface and a depth of 13.1, 6.7, 5.1, and 10 meters, respectively.

Sediment samples were collected at each site to be analyzed for total carbon, total nitrogen and PFAS. One homogenized sample per saline site was collected, again with the exception of Raritan Bay which was sampled on two separate dates, for a total of 17 samples. In accordance with quality control procedures, equipment rinsate blanks were collected from sediment sampling equipment (steel scoops, trowels, ponars, etc.) and water sampling equipment (Van Dorn water sampler used at Passaic River) at each site for a total of 18 rinsate blank samples. Eighteen sediment samples were collected at the non-saline sites, as well as 19 equipment rinsate blank samples.

150 fish from 16 saline sites were filleted and homogenized for PFAS and stable isotope values of δ13C and δ15N. For all statistics using isotope data an $\alpha < 0.1$ was used to denote statistical significance. Fish collected by site varied by species and total number of fish collected for analysis. The saline fish species collected included American Eel (n=21), Black Sea Bass (n=6), Channel Catfish (n=6), Common Carp (n=6), Largemouth Bass (n=5), Northern Puffer (n=5), Oyster Toadfish (n=11), Pumpkinseed Sunfish (n=6), Spot (n=26), Summer Flounder (n=9), White Perch (n=46), and Yellow Perch (n=3). As a part of quality control procedures, rinsate blanks from glass cutting boards, steel blender cups, and foil used to wrap fish samples were collected at the end of each fish fileting/homogenizing day (n=30). 162 fish from 18 non-saline sites were collected for stable isotope and PFAS analysis. Non-saline fish species included American Eel (n=39), Bluegill (n=37), Chain Pickerel (n=18), Common Carp (n=6), Largemouth Bass (n=38), Pumpkinseed Sunfish (n=6), Smallmouth Bass (n=6), and White Perch (n=12). Equipment rinsate blanks were also collected at the end of each fish fileting/homogenizing event for all non-saline fish (n=24).

To model the trophic positions of fish species, we used stable isotope data for nitrogen (δ15N). The mean δ15N values for particulate matter at each site were estimated to serve as baselines, against which fish isotope values were compared. Trophic positions were estimated for each fish sample by calculating the trophic enrichment relative to the δ15N baseline, assuming a trophic enrichment factor of 3.4‰. This calculation was implemented via Equation 1.

$$Trophic\ Position = 1 + \frac{\delta^{15}N_{sample} - \delta^{15}N_{baseline}}{3.4} \qquad \textit{Equation 1}$$

A Bayesian hierarchical model was constructed using the brms package. The model included random intercepts for both Site and species to account for site-specific and species-specific variation in trophic position. The model was specified with uninformative priors: a normal prior for the intercept (mean = 0, standard deviation = 5) and a Cauchy prior for the random effect standard deviations (scale = 2). The model was run with four Markov Chain Monte Carlo chains, each with 2000 iterations. A model of trophic position will be run independently in the best preforming grouping variable as species may have a different position under differing environmental conditions.

Sample processing in laboratory

Fish were thawed out with times varying depending on size for filleting. Fish were filleted in accordance with EPA's "Guidance for Assessing Chemical Contaminant data for Use in Fish Advisories" (U.S. EPA, 2000). Fish species with scales were descaled prior to filleting and filleted with the bell flap and skin on. Fish species without scales were filleted so that only the meat was left (i.e., skin removed). Generally, the left fillet of the fish was taken, but both fillets were taken if the left fillet was less than 5 grams. After filleting, the fillet was cut up into smaller pieces and homogenized in a blender.

Frozen water, sediment, and fish tissue were sent to SGS AXYS Analytical in Sidney, British Columbia, Canada where they were processed for concentrations of 40 PFAS compounds according to SGS AXYS Method MLA-110 Rev 02 Ver 12: Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products, Blood, Serum and Solvent Extracts with LC-MS/MS by EPA Method 1633

All fish samples and one suspended particulate matter sample per site were analyzed for stable isotope values of $\delta^{13}C$ and $\delta^{15}N$, as well as total C and total N. Sample preparation and analysis was conducted at the Academy of Natural Sciences of Drexel University according to ANSDU Standard Operating Procedure No. P-16-205 "The Use of the Elementar Pyrocube Elemental Analyzer and Isoprime1000 Mass Spectrometer for the Analysis of Concentration and Stable Isotopes of Carbon, Nitrogen, and Sulfur in Tissues, Plants, Sediments and Filtered Particulate Matter." All water samples were analyzed for organic carbon concentrations at ANSDU according to the ANSDU SOP Procedure No. P-16-99r3 "Dissolved Organic Carbon and Total Organic Carbon" and polyvalent cation concentrations (Al, Ba, Ca, Fe, K, Mg, Mn, Na, Sr) at Drexel University following EPA Method 200.8 "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma—Mass Spectrometry" using an Agilent 8880 QQQ-ICP-MS. All sediment samples were analyzed for total C, and total N at the Academy of Natural Sciences of Drexel University according to ANSDU Standard Operating Procedure No. P-16-54 "The Use of Carlo Erba Model 1112 Flash Elemental Analyzer for the Analysis of Total Carbon and Nitrogen in Sediments and Filtered Particulate Matter." Additional details concerning the methodology used for this project can be found in Appendix B: "Quality Assurance and Quality Control Plan: Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems."

Data Analysis

EPA Methodology

All frequentist BAFs are reported as their geometric mean (95% upper confidence level). Due to the heteroskedastic nature of PFAS concentrations in natural waters, the diversity of individual fish concentrations of PFAS, and the limited temporal time of this field effort there are multiple instances of below detection limit samples cooccurring with high concentrations which may appear as outliers. Here, we do not eliminate any observations from our BAF estimates, instead we use a sequence of methods that can handle such observations within a frequentist method. While the Bayesian methods described in this report are more apt to handle data with these challenges, we present the US EPA methodology for discussion to ensure our data analysis is compatible with our reported BAF values.

EPA methodology reported here as:

- Defined BAFs for every available PFAS in each species
- BAFs are reported for each fish species using data from across the entire state
- BAFs are tested via ANOVA for each fish species using data from across groups of sites defined by salinity and pH (saline, non-saline high pH, non-saline low pH)
- Defined BAFs for every available PFAS considering all fish species together
- BAFs are reported for all fish species together using data from across the entire state
- BAFs are tested via ANOVA for all fish species together using data from across groups of sites defined by salinity and pH (saline, non-saline high pH, non-saline low pH)

Bayesian Methodology

All Bayesian BAF estimates are reported as the median estimate with the interquartile range (i.e., 1st to 3rd quartiles). The challenges of heteroskedastic observation, below detection limit results, and the fact that concentrations are left censored at zero (there cannot exist a PFAS negative concentration in surface waters) are addressed by Bayesian model fitting. Bayesian models are also adept at adding additional hierarchical structure to posterior distributions, however these methods are not immediately relatable to those published as EPA Methodologies and are therefore restricted to our results section.

Bayesian methodologies reported here as:

- Defined BAFs for every available PFAS in each species
- BAFs are reported for each fish species using data from across the entire state

- BAFs are tested via ANOVA within each fish species using data from groups of sites defined by salinity and pH (saline, non-saline high pH, non-saline low pH) as well as the hydrology, site type, and regions as defined in NJ DEP approved QAPP (Table 1).

EPA Methodology

PFAS Concentrations

The U.S. EPA methodology for determining BAFs is to use site specific geometric mean and geometric standard deviation to define BAFs. The EnvStats package (Millard, 2013) in program R (version 4.3.0) was developed by U.S. EPA and contains the functions for determining these parameters. Sparse data and below detection limit data require a decision tree to determine how estimates can be generated. Concentrations were estimated for water and fish at each site.

If a site had one observation and that observation was above the detection limit, its value was used as the geometric mean of that site with a standard deviation of zero. If the site had no observations greater than the detection limit that media-compound-site was omitted. If the site had less than two detected observations and any number of below detection observations the BDLs were replaced with ½ the detection limit and the geometric mean and standard deviation was estimated through EnvStats. Detection limits varied by each laboratory batch, the appropriate values per batch were used, the complete set of laboratory QA/QC is provided to NJ DEP in a separate file. If the media-

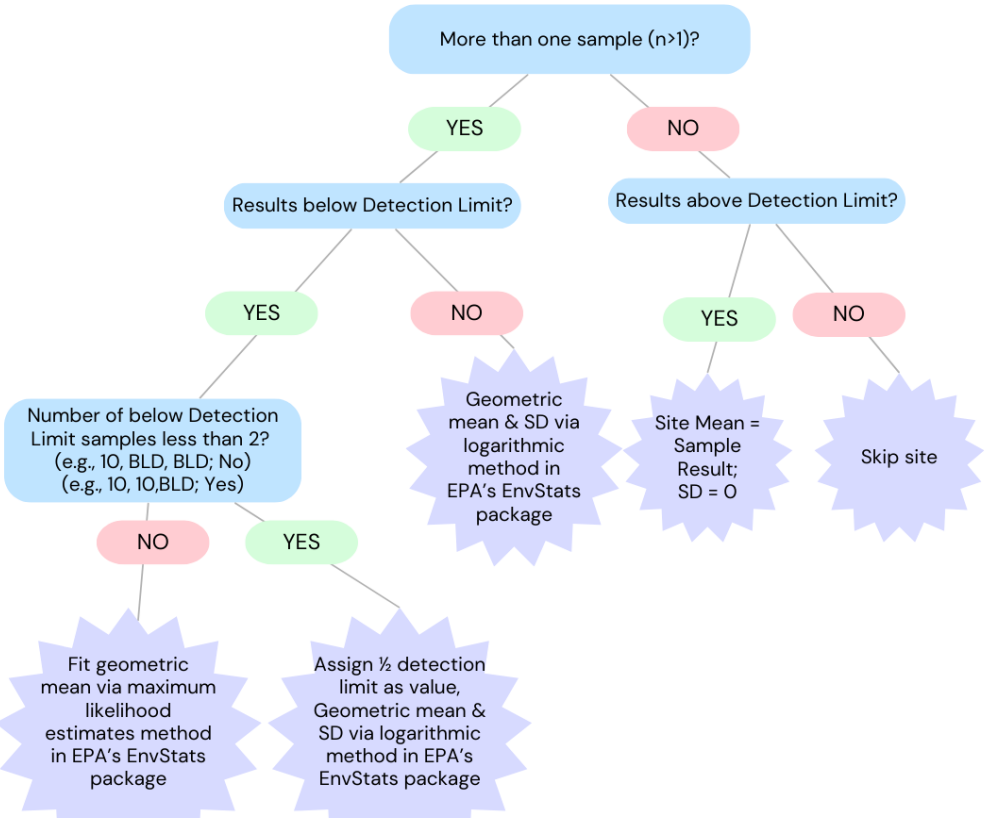

Figure 2: Decision tree used to determine which EPA methodology to use for calculating BAFs when confronted with below detection limit samples.

compound-site had two or more observations and some number of observations below detection, a maximum likelihood estimation method was used to fit geometric mean and standard deviation. If there were two or more observations and no below detection level estimates, then the geometric mean and standard deviation were estimated from observed values (Figure 2).

EPA BAFs for PFOS, PFOA, PFNA

EPA methods for PFOS, PFOA, and PFNA by site, were presented in the summary tables of the Results Section. All other PFAS compounds are presented in Appendix A. The methodology described here was the same for the full suite (Appendix A).

Concentrations of water and fish for PFOS, PFOA, and PFNA by site, were used to estimate site specific BAFs via Equation 2. Uncertainty from both sources of concentration estimates were propagated to the BAFs via Equation 3.

$$BAF = \left(\frac{Fish\ Geometric\ Mean}{Water\ Geometric\ Mean}\right) \quad \textit{Equation 2}$$

$$BAF\ SD = BAF \times \sqrt{\left(\frac{Fish\ SD}{Fish\ Mean}\right)^2 + \left(\frac{Water\ SD}{Water\ Mean}\right)^2} \quad \textit{Equation 3}$$

The BAF values and associated uncertainties (95%, UCLs) for each PFAS compound-species combination within the saline, non-saline, non-saline high pH, non-saline low pH, and state-wide were used to generate an all species BAF for each compound. For each PFAS compound, the number of sites in each group was counted to ensure sufficient data representation. Saline and non-saline groups were included in the analysis only if they contained at least two members. ANOVAs were used to test differences across groups via the same methodology for the individual fish species. The all fish combined ANOVAs used inverse variance weighting, where the weight for each species BAF was the inverse of the squared standard deviation (Equation 3) to account for uncertainty. If the ANOVA indicated significant differences between groups, a Tukey HSD (Honestly Significant Difference) post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups. For each saline and non-saline group, the geometric mean BAF was calculated using geometric mean of the species BAFs. The standard deviation of all fish BAF was computed by propagating errors, and the 95% UCL was calculated as the mean BAF plus 1.96 times the geometric standard deviation.

Table 2:The sequence of events that resulted in the estimation of the all fish species and fish species specific BAFs for each compound divided between all state wide data, saline sites, non-saline, non-saline high pH, and non-saline low pH.

Step	Nomenclature	Sample composition	Calculation
Step 1	Site Specific BAF	BAF derived from field data for each species at each site	Equation 2 & 3
Step 2	Species BAF	Site Specific BAFs combined for each species (from all sites within area of interest, i.e., non-saline or saline waters)	Geometric means of Site Specific BAFs
Step 3	Final BAF	All Species BAFs combined from all sites within area of interest, i.e., non-saline or saline waters)	Geometric mean of Species BAFs (95% UCL)

Tables summarizing BAF values (with UCLs) and highlighting statistically significant differences between groups for each PFAS compound (Appendix A) were created. A shorter table of PFOS, PFOA, and PFNA is included in the Results Section. This table provides a comprehensive view of bioaccumulation trends across different environmental conditions and facilitates the interpretation of PFAS bioaccumulation risk.

Bayesian Methodology

All Bayesian BAF estimates are reported as the median estimate with the interquartile range (i.e., 1st to 3rd quartiles).

PFAS Concentrations

Concentrations of water, sediment, and tissue PFAS compounds per site were estimated as posterior log-normal distributions using the brms R package (Bürkner, 2017). Log-normal distributions were used to estimate the posteriors because concentrations can never be negative. Each sample of water, sediment, or tissue was treated as a random draw of an underlying site distribution, allowing sample results below detection limit to be incorporated into the posterior estimates as left-right censored draws at ~0 and the PFAS compound specific detection limit, respectively. The Bayesian estimates of concentrations per site per media were fit using uninformative normal priors with a mean of 0 and variance of 1, three Markov chains were used each with a burn-in of 5,000 iterations and run for 15,000 iterations. Chain convergence was monitored via a Gelman-Rubin statistic approaching 1.

BAFs and Sediment BAFs

Bioaccumulation factors were estimated as the ratio of the per site concentration in the tissue samples to the concentrations in the water samples per compound; these ratios were converted to Kg/L. Sediment bioaccumulation factors (SBAFs) were estimated as the ratio of per site concentration in tissue to the concentration in sediments per compound; these ratios are unitless and required no conversion. Because the concentrations for all media were estimated using random posterior draws, the BAFs were calculated by dividing the respective posterior estimates (the tissue concentration distribution with the water concentration distribution). The resulting quotient represents the mean BAF and includes uncertainty. This uncertainty is additive, since it is a combination of both the tissue and media concentration estimates

Grouping Analysis

Salinity and pH

Temperature, specific conductance, dissolved oxygen, pH, and salinity screening data were collected at each site during each site visit. We used pH and salinity as conservative indicators of site conditions which would be the most insensitive to time of day the screening data were collected. Site mean pH and site mean log salinity were iteratively divided into a maximum of 10 potential groups. The sum of squared deviations from each observation and the cluster(s) centroid was tracked for each potential grouping, and a scree plot generated from these summed squares was used to determine the approximate number of water quality site clusters. This method removes the need for the investigators to establish arbitrary salinity or pH values to denote Pineland or saline, the data will group themselves into the most optimal pattern.

Cations

Cation concentrations were determined for sodium, magnesium, aluminum, potassium, calcium, manganese, strontium, and barium at each site. Cations with values below the instrument detection limit had to be imputed to complete a grouping analysis. We used the clusterMI R package to impute the missing values via a joint modelling method Dirichlet Process. We then used a cluster analysis function and pooling sum of squares after multiple imputations to choose the appropriate number of clusters according to the partition instability.

Data Models

To comprehensively assess the differences in BAFs for PFAS compounds across various environmental and biological groupings, we employed both frequentist and Bayesian approaches. A frequentist ANOVA was used to test for significant differences in BAFs by salinity and pH groups, incorporating EPA-derived estimates for PFOS, PFOA, and PFNA (the result of the PFAS are presented in Appendix A) and weighting the models by the inverse of the BAF standard deviation squared to account for error. This analysis was complemented by Bayesian methods to explore differences in BAFs based on geographical regions, salinity/pH groups, cation concentrations, and isotopic composition. The Bayesian analyses allowed for a more nuanced understanding of the uncertainty and variability within each group by modeling coefficients as posterior distributions and using credible intervals to determine statistical significance. However, the frequentist approach is reported in the Results Section tables as they more closely align with published values and EPA methodology. Collectively, these approaches provided a robust framework for examining the impact of various environmental and biological factors on PFAS bioaccumulation across New Jersey's aquatic ecosystems.

Frequentist; BAFS ~ salinity/pH groups

A frequentist analysis of variance (ANOVA) was used to test if species-compound combination BAFs by groupings assigned by salinity and pH were significantly different. EPA methodology PFOS, PFOA, and PFNA estimates were used with these frequentist ANOVAs. To incorporate the error around each site specific BAF, the models were weighted by the inverse of the BAF standard deviation squared. Groups were then subject to a Tukey HSD test to estimate which of the groups were significantly different from each other using an α-value of 0.05.

Bayesian; BAFs ~ Geographical Region

To determine if by species their PFAS compound BAFs were statistically different based on the NJDEP predefined geographical region we used a Bayesian variant of an ANOVA. Sites were categorized as Hydrology, Region, and Site Type. Hydrology was broadly defined as marine, Pineland, non-Pineland, and deep. While these designations were nominally defined by their location and depth, some sites are misclassified. Sites in the Delaware River for example are defined as marine while salinity at these locations is less than 1 part per thousand. Regions were defined as central New Jersey & Atlantic City, the Delaware River, or Northern New Jersey. As with the hydrogeography labels, these designations were arbitrary. Site types were defined as being upper estuary, open water bays, lakes, streams, or upper estuary bottom and lake bottom.

Bayesian; BAFs ~ salinity/pH groups

To determine if by species their PFAS compound BAFs were statistically different based on the water quality screening we used a Bayesian variant of an ANOVA. Sites were categorized via the above grouping analysis, and coefficients fit as posterior distributions for each group. If the 95% credible interval of difference between each coefficient did not contain zero, we estimated that there was a statistically significant difference between water quality derived groups. The Bayesian analysis was completed with the brms R package; three Markov chains were run for each model with a burn-in of 5,000 and 10,000 subsequent iterations.

Bayesian; BAFs ~ cations groups

To determine if by species their PFAS compound BAFs were statistically different based on the cation concentrations we used a Bayesian variant of an ANOVA. Sites were categorized via the above-described grouping analysis, and coefficients fit as posterior distributions for each group. If the 95% credible interval of difference between each coefficient did not contain zero, we estimated that there was a statistically significant difference between cation concentration derived groups. The Bayesian analysis was completed with the brms R package; three Markov chains were run for each model with a burn-in of 5,000 and 10,000 subsequent iterations.

Bayesian; Linear model; BAFs ~ isotopes

To determine if by species their PFAS compound BAFs were statistically different based on the isotopic composition of the fish tissues we used a Bayesian multivariant linear model. Species were categorized via N15 and C13 values, and coefficients fit as posterior distributions for each isotope. If the 95% credible interval of either coefficient did not contain zero, we estimated that there was a statistically significant difference based on either isotopic composition. The Bayesian analysis was completed with the brms R package; three Markov chains were run for each model with a burn-in of 5,000 and 10,000 subsequent iterations.

Coefficients fit as posterior distributions for each group. If the 95% credible interval of difference between each coefficient did not contain zero, we estimated that there was a statistically significant difference between cation concentration derived groups. The Bayesian analysis was completed with the brms R package; three Markov chains were run for each model with a burn-in of 5,000 and 10,000 subsequent iterations. Sediment BAF differences by group were limited to species identified as benthic piscivores or benthic foragers (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish); however, sediment BAFs statewide were determined for all species irrespective of habitat type.

3. RESULTS

This study explored the spatial and temporal variability of PFAS concentrations and their influence on BAFs in aquatic ecosystems across multiple sampling sites. By integrating pre-sample data with fish collection samples, the research reveals critical insights into PFAS distribution and bioaccumulation dynamics that would otherwise be missed in a single sampling event. Key analyses include K-means clustering of water quality parameters (pH and salinity), which identifies distinct environmental groups—saline, non-saline high pH, and non-saline low pH—that influence BAF patterns. Additionally, cation concentrations were analyzed using advanced imputation methods to understand their distribution across sites. Stable isotope analysis further differentiates marine and freshwater fish by $\delta^{15}N$ and $\delta^{13}C$ values. The study's findings highlight the complexity of PFAS bioaccumulation and the role of environmental factors in shaping isotopic and chemical signatures in aquatic ecosystems.

Differences in Temporal PFAS Concentrations

At 46 of the sampling sites, water samples were collected before the fish collection date and analyzed for PFAS concentrations. Forty-eight percent of these sites had detectable concentrations of PFAS compounds in the "pre-samples" that were not detected in the samples collected concurrently with fish collection. The pre-samples were vital in determining the BAFs within sites, because they provided data which could be used to fit distributions on compounds which otherwise would have been missed. The number of PFAS compounds missed without pre-samples ranged from 1 to 9. In addition to demonstrating the temporal variability of PFAS concentrations, pre-sample observations were used in both the EPA methodology and the Bayesian methodology to define PFAS concentrations in each sites water.

Grouping Analysis

Water Quality Grouping

K-means grouping of pH and log salinity were used to group sites into similar clusters and test the hypothesis that BAFs and Sediment BAFs within fish species were different based on water quality (Figure 3. There are distinct groups identified as saline, non-saline high pH, and non-saline low pH. K-means clustering is used to categorize sites based on similar pH and salinity levels, which may help to identify patterns in water quality that might influence bioaccumulation (Figure 4).

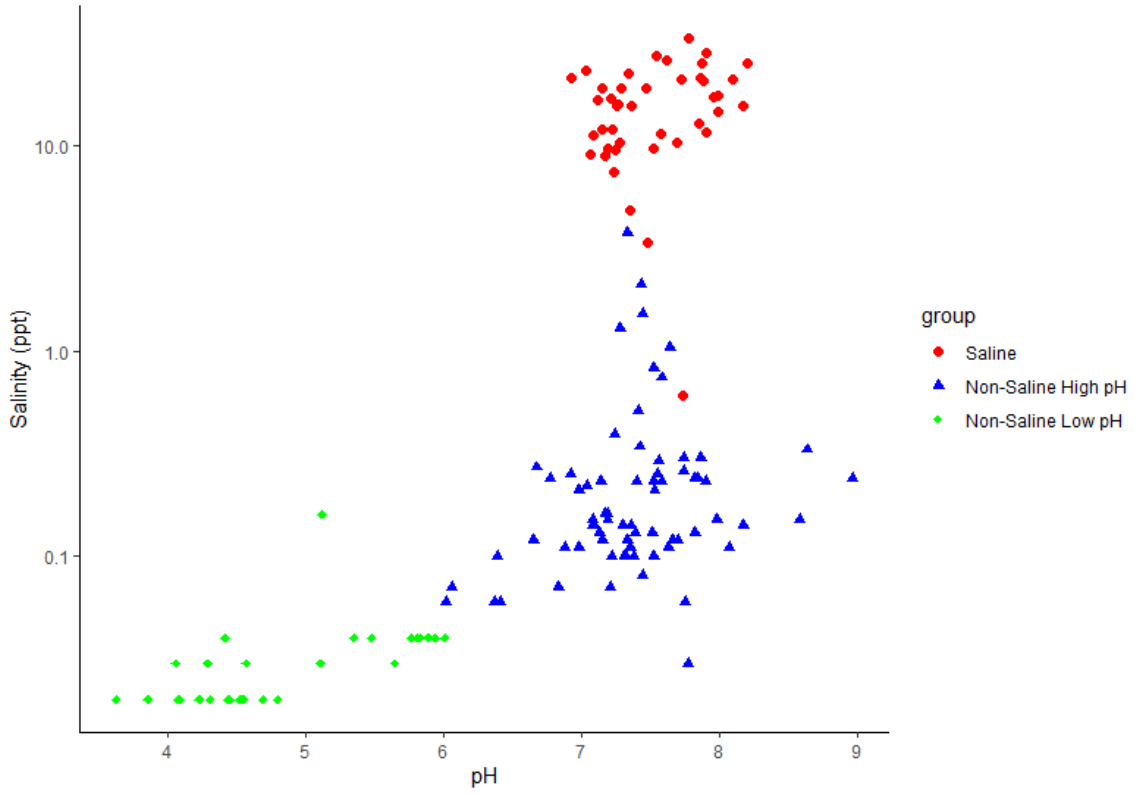

Figure 3: Salinity and pH were used as variables for an iterative k-means grouping analysis to separate sites into logical clusters. An analysis of the total within cluster sum of squares indicated 3 groups best fit the data.

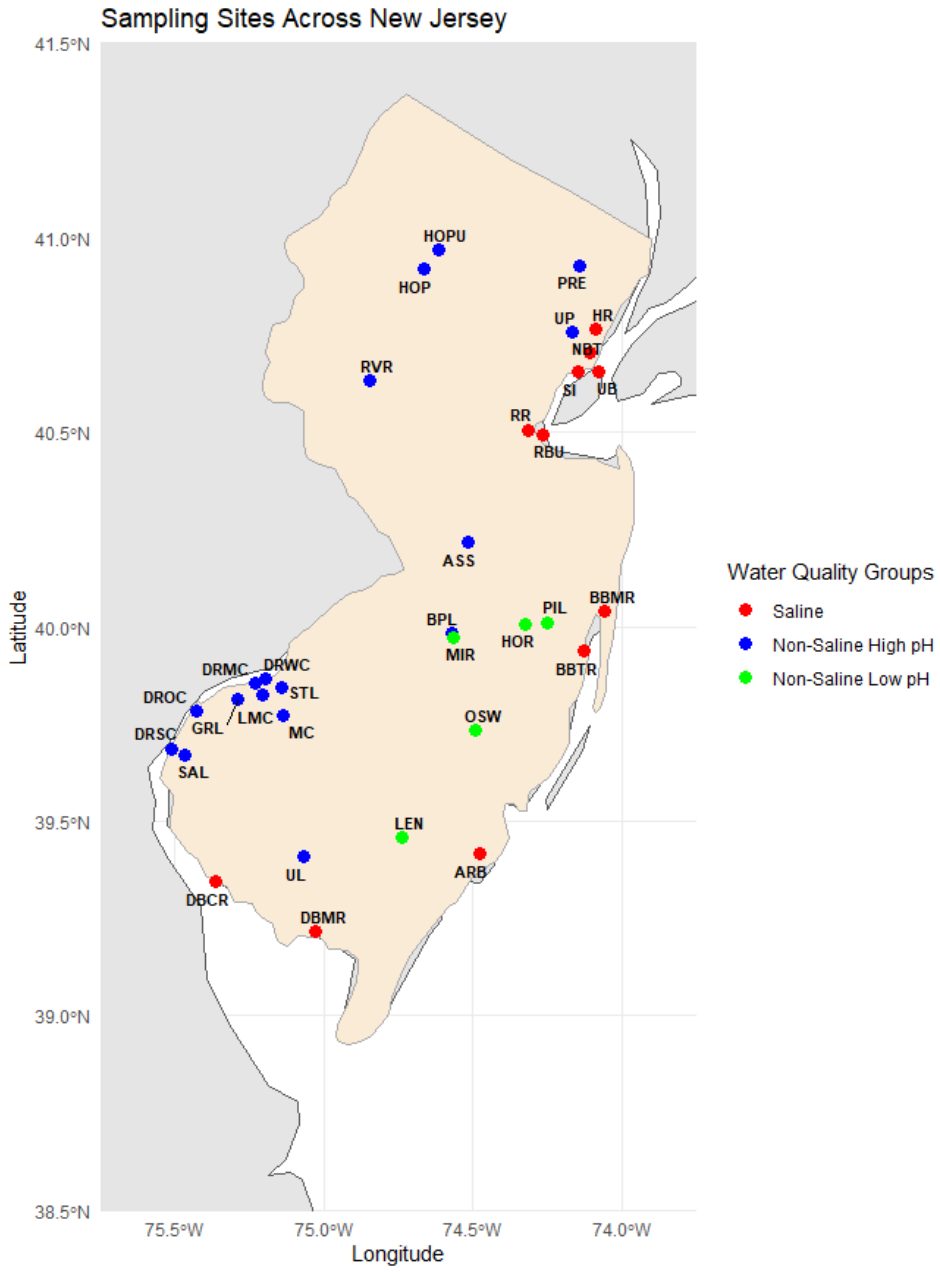

Figure 4: All sampling sites across the state were statistically grouped via K-means of salinity and pH observations. The sites grouped themselves into three, broadly defined as; saline, non-saline high pH, and non-saline low pH.

Cation Grouping

Cation concentrations were determined at sites throughout the study (Table 3). Cation concentrations were estimated by site to use as a means of comparing within and across groups of sites. Cations were grouped via a method which imputed the below detection limit values via a joint modelling method Dirichlet Process. The Dirichlet Process is a flexible statistical approach used to estimate missing values by modeling the relationships between different cations. After multiple imputations it was determined that the sites fall into three distinct groups (Figure 5).

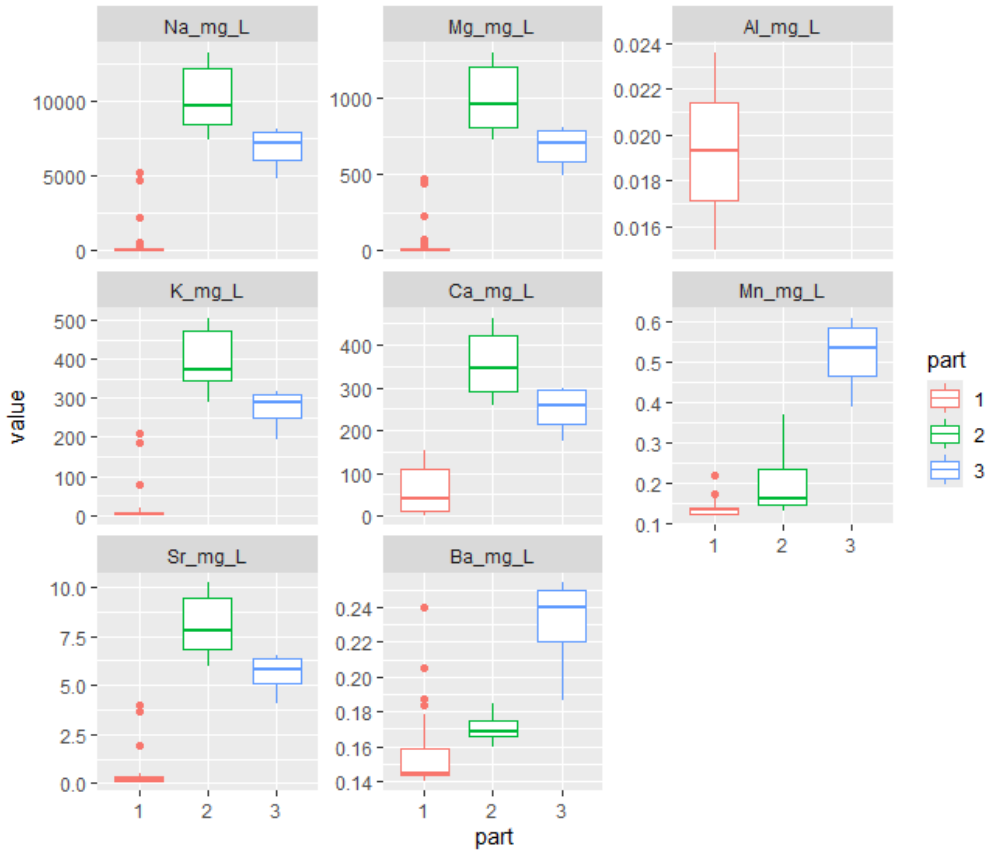

Figure 5: The grouping algorithm determined that the cation data represented three distinct groups. Median values of each analyte by group support the effectiveness of the grouping algorithm. The plots display the minimum, lower quartile (25th percentile), median (50th percentile), upper quartile (75th percentile), and maximum of a dataset, with "whiskers" extending to the smallest and largest values within 1.5 times the interquartile range from the quartiles.

Table 3: The mean cation concentration (mg/L) for each site was determined. Below detection limit (BDL) results did not exceed 0.01 mg/L. Site abbreviations are defined in Table 1.

Site	Na	Mg	Al	K	Ca	Mn	Sr	Ba
ARB	3.80	2.41	BDL	1.73	BDL	0.14	0.13	0.14
ASS	16.08	5.14	BDL	3.51	BDL	0.14	0.17	0.17
BBMR	2808.76	254.36	BDL	111.35	BDL	0.13	2.18	0.15
BBTR	4659.96	443.56	BDL	185.30	143.42	0.13	3.68	0.16
BPL	72.00	70.65	BDL	0.38	62.75	0.13	0.14	0.14
DBCR	532.23	45.24	BDL	.38	BDL	0.15	0.50	0.15
DBMR	25.66	4.14	BDL	BDL	BDL	0.12	0.15	0.15
DRCSB	43.70	11.62	BDL	5.50	10.14	0.16	0.31	0.19
DRMC	3.36	2.37	BDL	BDL	BDL	0.12	0.13	0.14
DROC	3.89	2.41	BDL	BDL	BDL	0.12	0.13	0.14
DRSC	4.89	2.58	BDL	BDL	BDL	0.12	0.13	0.14
DRWC	4.45	2.46	BDL	BDL	BDL	0.12	0.13	0.14
GRL	7388.04	733.25	BDL	288.51	258.51	0.13	5.95	0.17
HOP	13060.01	1281.92	BDL	497.17	453.75	0.27	10.05	0.17
HOPU	25.11	4.40	BDL	5.04	BDL	0.13	0.16	0.19
HOR	12081.32	1195.58	BDL	467.00	414.32	0.15	9.37	0.16
HR	6487.78	622.46	BDL	267.98	228.33	0.49	5.39	0.23
LEN	7908.39	785.13	BDL	308.38	291.66	0.58	6.30	0.25
LMC	8105.04	802.60	BDL	315.92	290.29	0.15	6.53	0.18
MC	207.38	25.92	BDL	11.33	16.08	0.17	0.43	0.18
MIR	4843.49	488.58	BDL	194.03	174.31	0.39	4.10	0.19
NBT	4.32	2.43	BDL	2.77	BDL	0.14	0.14	0.14
OSW	10197.67	1010.59	BDL	388.84	362.12	0.15	8.19	0.17
PIL	8122.26	812.75	BDL	318.15	298.57	0.61	6.53	0.25
PRE	42.01	11.20	0.01	5.02	8.71	0.16	0.30	0.18
RBU	73.16	7.63	BDL	1.81	BDL	0.12	0.18	0.14
RR	35.05	4.91	BDL	0.20	BDL	0.12	0.15	0.15
RVR	3.79	2.39	BDL	1.61	BDL	0.14	0.13	0.14
SAL	3.59	2.39	BDL	1.36	BDL	0.14	0.13	0.14
SI	8644.89	819.38	BDL	354.97	287.99	0.23	6.95	0.17
STL	31.66	7.53	BDL	5.20	0.89	0.13	0.28	0.18
UB	86.48	8.27	BDL	2.15	BDL	0.12	0.18	0.14
UL	5725.93	570.91	BDL	218.62	211.13	0.24	4.65	0.19
UP	3.98	2.41	BDL	2.48	BDL	0.14	0.13	0.14

Isotopes

Stable isotope values of $\delta^{15}N$ and $\delta^{13}C$ ranged from 5.1 to 21.7 and -43.6 to -14.7, respectively. Marine fish were generally more enriched in ^{15}N and ^{13}C than freshwater fish (Table 4; Figure 6). Marine regions were statistically distinct for both $\delta^{15}N$ and $\delta^{13}C$ regimes ($\alpha < 0.1$), except for Northern New Jersey and Central-Atlantic ($p = 0.10$). Freshwater regions were similarly distinct ($\alpha < 0.1$), except Non-Pinelands and deep samples, which did not isotopically differ (Table 4; Figure 7).

The trophic classifications of piscivore and forager do not differ in either regime.

Total N and total C were measured in fish, sediments and particulate material, which were used to calculate C:N. For aquatic animals, a C:N ratio of about 3.5 indicates that lipids, which can bias values of $\delta^{13}C$ towards depletion, are not substantial enough to warrant extraction (Post et al. 2007). Our average C:N is 3.9, which may slightly bias our $\delta^{13}C$ values, but it does not impact data interpretation.

Table 4: The mean (± standard error) stable isotope (δ15N and δ13C) and C:N (weight %) value for each site was determined.

Hydrology	Regional Description	Samples	Average $\delta^{15}N$	Average $\delta^{13}C$	Average C:N
Marine	Central-Atlantic	35	13.1 ± 1.8	-19.7 ± 1.8	3.7 ± 1.4
	Delaware River	85	16.9 ± 2.5	-22.8 ± 3.5	3.8 ± 1.2
	Northern New Jersey	48	14.2 ± 2.4	-21.4 ± 2.9	4.2 ± 1.3
Freshwater	Deepwater Hydrology	42	13.0 ± 3.4	-28.4 ± 4.3	3.9 ± 1.4
	Non-Pinelands	75	12.1 ± 3.4	-28.4 ± 3.3	3.9 ± 1.2
	Pinelands	56	10.4 ± 2.5	-34.4 ± 4.1	3.1 ± 2.1

Figure 6: Isotope values for fish species collected throughout the study differentiate between freshwater and marine environments.

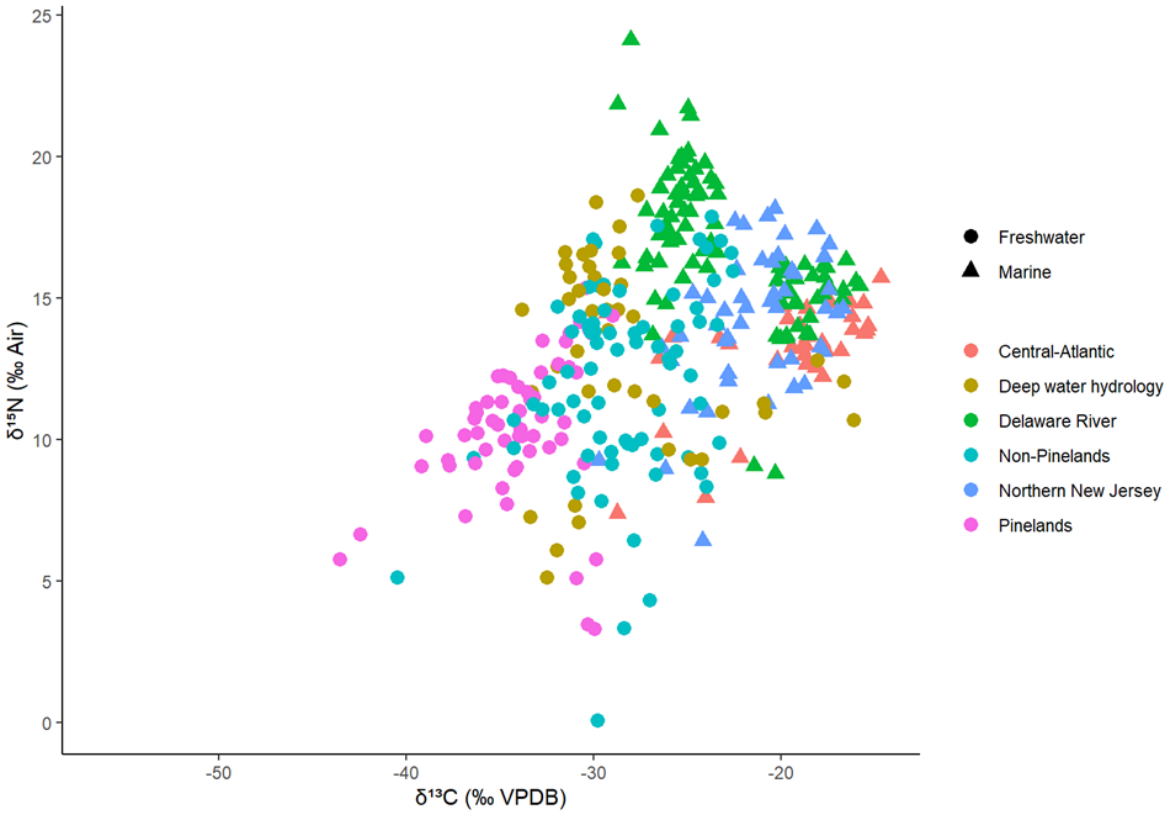

Figure 7. Isotope values for fish species collected throughout the study differentiate between freshwater and marine environments and their associated regions.

Trophic Positions

The trophic position results reveal distinct variations in trophic positions among fish species across different water quality groups (Figure 8). In the Saline group, species like the Oyster Toadfish and Summer Flounder exhibited relatively high trophic positions, with values of 2.45 and 2.42, respectively, while species such as Northern Puffer and Spot had lower trophic positions; 2.19 and 2.24, respectively. In the Non-Saline high pH group, Largemouth Bass and Smallmouth Bass showed the highest trophic positions at 2.67 and 2.65, whereas Bluegill and Spot were among the lowest, with values of 1.83 and 1.98. The Non-Saline low pH group displayed even higher trophic positions, with Largemouth Bass and Chain Pickerel reaching 3.45 and 3.36, respectively, indicating a significant shift in trophic dynamics under varying water quality conditions. These results highlight the influence of environmental factors on the feeding ecology and trophic status of different fish species.

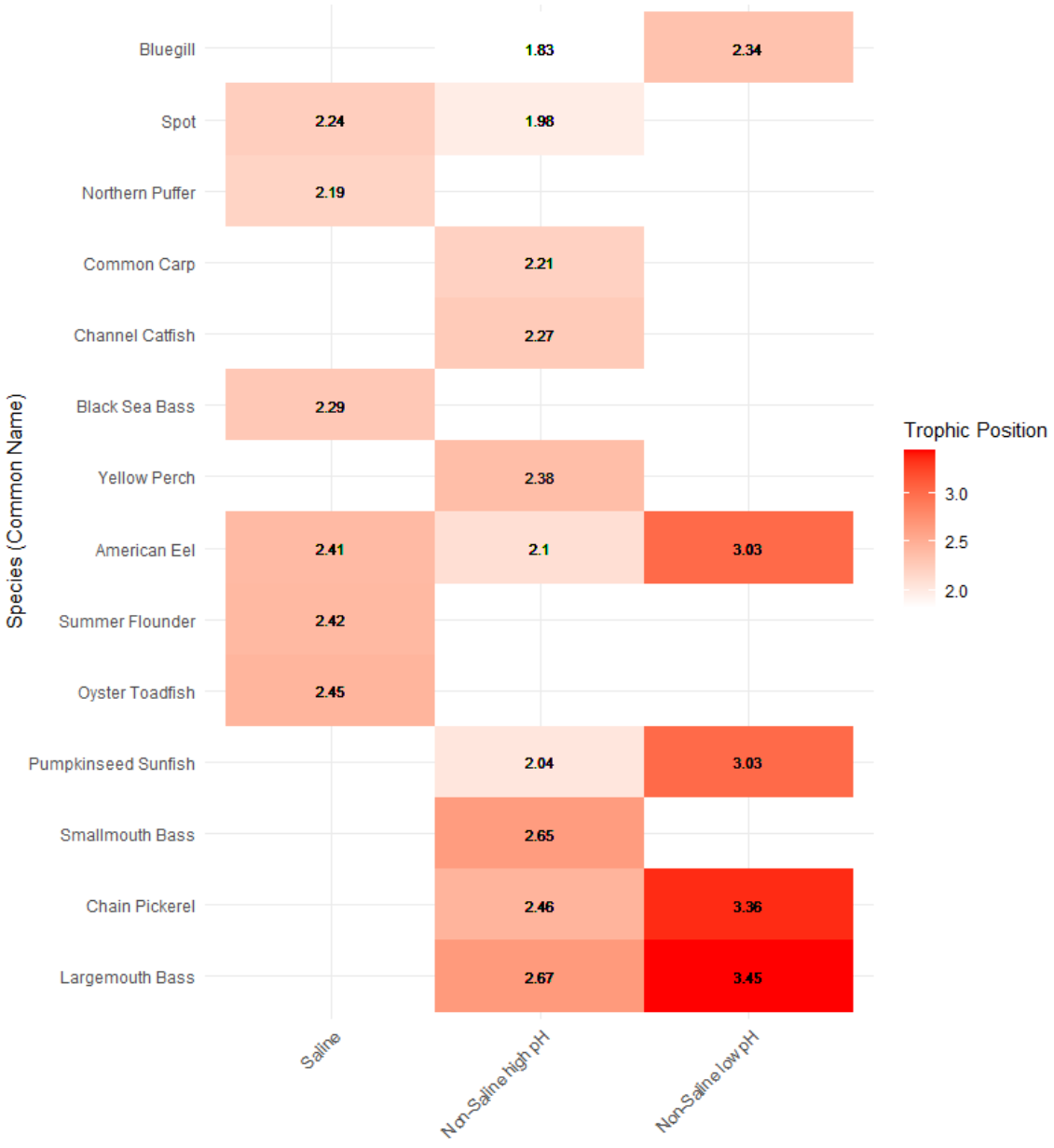

Figure 8: Trophic position was estimated with the stable isotope data for nitrogen ($\delta 15N$). The trophic position for each species was modeled independently within the three water quality groups (Saline, Non-Saline High pH, and Non-Saline Low pH).

Species-specific Bioaccumulation Factors

Species specific BAFs for state-wide, saline, non-saline, non-saline high pH, and non-saline low pH are presented for each species. For each species table, the "n" reported is the number of site specific BAFs that were available within each group. The exception is the all fish species combined table, where the "n" represents the number of total number of unique fish species-compound-group combinations used to generate the estimates.

While sediment BAFs were calculated for each species-compound combination, the discussion focuses on BAFs calculated from direct measurements of the contaminant in water and fish tissue. These are preferred because they represent and incorporate all exposure routes to an organism in the natural environment.

Frequentist ANOVA Results

For each species and for all species combined statewide BAFs, saline, non-saline, non-saline low pH, and non-saline high pH were estimated. For each species PFOA, PFOS, and PFNA are highlighted in these sections while the entire list of detected PFAS BAF is included as Appendix A. For each highlighted PFOA, PFOS, and PFNA BAF the results of the frequentist ANVOA are represented by letters which indicate grouping statistical differences at an alpha of 0.05. These ANOVAs were only generated when cooccurring species and water PFAS concentrations were observed at least 2 sites within each saline, non-saline high pH, or non-saline low pH groups. For instance, American Eel fish observations of PFOA cooccurred with water observations at 3 saline, 13, non-saline high pH, and 2 non-saline low pH sites, thus an ANOVA was used to test for there differences between groups. While Spot fish observations of PFOA cooccurred with water observations at 6 saline and 1 non-saline high pH thus no testing for differences was used.

American Eel

American Eel were collected at sites: ARB, BBTR, DBCR, DBMR, DRMC, DROC, DRWC, NBT, RR, BPL, GRL, LEN, LMC, MC, MIR, OSW, PRE, PIL, DRCSB, SAL, STL, and UL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 5: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for American Eel within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	39.5 (290)a	628 (923)b	183 (841)a	3	6	4
Non-Saline High pH	13.5 (100)b	2190 (4500)b	188 (580)a	11	12	12
Non-Saline Low pH	30.9 (857)a	3780 (12800)a	201 (1880)a	2	4	2
Non-Saline	15.3 (162)	2510 (5350)	190 (603)	13	16	14

Statewide	18.3 (146)	1720 (3790)	188 (541)	16	22	18

Table 6: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for American Eel [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DROC, DRWC, NBT	0.34 (0.25, 0.46)	0.2 (0.14, 0.34)	613.06 (359.5, 1119.46)
PFBS	STL	22.42 (19.31, 26.15)	0.21 (0.15, 0.36)	9.94 (6.72, 18.22)
PFDA	BBTR, DBCR, DBMR, DRCSB, DRMC, DROC, DRWC, GRL, LEN, LMC, MC, NBT, RR, SAL, STL	0.52 (0.43, 0.66)	0.96 (0.55, 1.51)	1751.39 (1156.67, 2843.02)
hPFHpA	GRL, MIR, STL, UL	3.04 (1.43, 4.16)	0.24 (0.15, 0.42)	97.97 (51.2, 225.96)
PFHpS	BPL, MC	0.63 (0.43, 1.09)	0.27 (0.14, 0.43)	352.77 (243.69, 561.67)
PFHxA	DRMC, DRWC, PIL, SAL	4.92 (3.03, 9.36)	0.12 (0.1, 0.16)	24.62 (11.32, 37.57)
PFHxS	ARB, BBTR, BPL, DRCSB, DRWC, GRL, LMC, MC, MIR, NBT, PIL, PRE, SAL	3.33 (1.91, 6.02)	0.31 (0.16, 0.59)	92.58 (44.73, 188.71)
PFNA	ARB, BBTR, BPL, DBCR, DBMR, DRCSB, DRMC, DROC, DRWC, GRL, LEN, LMC, MC, PIL, PRE, SAL, STL, UL	2.45 (1.39, 4.54)	0.54 (0.34, 1.01)	236.94 (135.96, 349.97)
PFOA	BBTR, BPL, DBCR, DBMR, DRCSB, DROC, DRWC, GRL, LMC, MC, MIR, PIL, PRE, SAL, STL, UL	8.02 (5.41, 12.3)	0.18 (0.14, 0.24)	22.9 (14.82, 34.32)
PFOS	ARB, BBTR, BPL, DBCR, DBMR, DRCSB, DRMC, DROC, DRWC, GRL, LEN, LMC, MC, MIR, NBT, OSW, PIL, PRE, RR, SAL, STL, UL	5.86 (3.63, 10)	9.86 (4.59, 19.34)	1665.94 (879.43, 3466.57)
PFPeS	MC, PIL	1.07 (1.07, 1.7)	0.11 (0.09, 0.13)	85.79 (56.7, 113.94)
PFUnDA	DBCR, DRMC, DROC, DRWC, GRL, LMC, MC, SAL, STL	0.55 (0.42, 0.89)	10.1 (6.02, 15.26)	16241.02 (10735.75, 24398.55)
x6_2_FTS	DRCSB, SAL	2.19 (1.58, 3.14)	0.45 (0.34, 0.56)	201.21 (134.24, 291.19)

Table 7: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for American Eel [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DROC, DRWC, NBT	0.17 (0.05, 0.5)	0.21 (0.14, 0.34)	1.32 (0.53, 3.72)
PFDA	BBTR, BPL, DBCR, DBMR, DRCSB, DROC, PRE, RR, SAL, STL	0.12 (0.07, 0.47)	0.91 (0.55, 1.36)	9.84 (3.71, 14.31)
PFDS	DRCSB, NBT, PRE, RR	0.08 (0.07, 0.15)	0.35 (0.19, 0.46)	4.68 (0.69, 6.85)
PFDoA	ARB, BPL, DBCR, DRCSB, DRMC, DROC, DRWC, GRL, MC, NBT, PIL, PRE, RR, SAL, STL	0.11 (0.05, 0.16)	1.75 (1.14, 2.51)	17.89 (8.84, 33.69)
PFHpA	STL	0.05 (0.05, 0.05)	0.16 (0.11, 0.36)	3.42 (2.32, 6.97)
PFHxA	DRMC, SAL	0.15 (0.06, 0.24)	0.16 (0.11, 0.28)	1.72 (0.6, 3.39)
PFHxS	ARB, BPL	0.7 (0.14, 1.27)	0.27 (0.16, 1.39)	1.14 (1.09, 1.14)
PFNA	ARB, BBTR, BPL, DBCR, DBMR, DRMC, DROC, DRWC, GRL, LMC, PIL, SAL, STL	0.11 (0.07, 0.29)	0.62 (0.33, 1.6)	5.58 (2.79, 11.03)
PFOA	BPL, DBCR, DBMR, DRCSB, DROC, LMC, PIL, PRE, SAL, STL	0.08 (0.06, 0.19)	0.19 (0.14, 0.29)	1.85 (1.22, 3.04)
PFOS	ARB, BBTR, BPL, DBCR, DBMR, DRCSB, DRMC, DROC, DRWC, GRL, MC, MIR, NBT, PIL, PRE, RR, SAL, STL, UL	0.32 (0.14, 0.78)	9.35 (4.14, 21.38)	33.26 (9.75, 86.99)
PFOSA	PIL	0.05 (0.05, 0.05)	0.18 (0.16, 0.23)	3.6 (3.23, 4.4)
PFTeDA	BPL, DBCR, DRCSB, DROC, DRWC, MC, NBT, PRE, RR, SAL	0.11 (0.06, 0.17)	2 (1.21, 2.82)	17.35 (11.27, 40.93)
PFTrDA	ARB, BPL, DBCR, DRCSB, DRMC, DROC, DRWC, GRL, LMC, MC, NBT, PRE, RR, SAL, STL	0.13 (0.11, 0.26)	3.35 (1.67, 5.93)	21.69 (10.84, 58)
PFUnDA	ARB, BBTR, BPL, DBCR, DBMR, DRCSB, DRMC, DROC, DRWC, GRL, LMC, MC, NBT, PRE, RR, SAL, STL, UL	0.23 (0.11, 0.52)	3.37 (1.27, 9.12)	14.25 (7.4, 32.48)

Oyster Toadfish

Oyster Toadfish were collected at sites: ARB, DBCR, DBMR, NBT, SI, and UB. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 8: EPA methodologies were used for selected compounds (PFOA, PFOS, and PFNA) to determine aqueous BAFs for Oyster Toadfish within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	46.4 (394)	716 (1180)	315 (1060)	4	6	5
Non-Saline High pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	- (-)	- (-)	0	0	0
Statewide	46.4 (394)	716 (1180)	315 (1060)	4	6	5

Table 9: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Oyster Toadfish [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	NBT	0.46 (0.35, 0.65)	0.41 (0.39, 0.47)	944.47 (648.29, 1343.52)
PFDA	DBCR, DBMR, NBT, SI	0.55 (0.47, 0.65)	0.75 (0.37, 1.5)	1477.07 (885.93, 2116.79)
PFHpS	ARB	0.56 (0.49, 0.64)	0.15 (0.1, 0.32)	274.09 (175.21, 556.13)
PFNA	ARB, DBCR, DBMR, NBT, SI	1.79 (1.11, 2.28)	0.69 (0.36, 1.36)	414.61 (274.18, 636.78)
PFOA	ARB, DBCR, NBT, SI	5.84 (4.94, 6.89)	0.46 (0.18, 0.92)	69.28 (36.7, 170.67)
PFOS	ARB, DBCR, DBMR, NBT, SI, UB	3.91 (2.82, 6.64)	3.33 (2.01, 4.1)	603.89 (379.06, 1099.91)
PFOSA	DBCR	0.4 (0.37, 0.43)	0.47 (0.47, 0.47)	1173.4 (1082.39, 1294.42)
PFUnDA	DBCR	0.44 (0.38, 0.51)	2.53 (2.53, 2.53)	5715.8 (4964.73, 6594.22)

Table 10: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Oyster Toadfish [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	NBT	0.17 (0.17, 0.17)	0.41 (0.39, 0.47)	2.47 (2.36, 2.9)
MeFOSAA	NBT	0.05 (0.05, 0.05)	0.34 (0.25, 0.59)	6.76 (4.97, 12.25)
PFDA	DBCR, DBMR	0.18 (0.15, 0.21)	1.5 (0.87, 1.5)	7.25 (5.85, 7.25)
PFDS	NBT	0.08 (0.08, 0.08)	0.48 (0.38, 0.77)	6.31 (4.96, 9.44)
PFDoA	ARB, DBCR, NBT	0.11 (0.06, 0.16)	1.81 (0.36, 1.81)	11.17 (5.26, 13.61)
PFNA	ARB, DBCR, DBMR	0.21 (0.09, 0.39)	1.31 (0.79, 1.73)	4.58 (4.47, 8)
PFOA	ARB, DBCR, NBT	0.15 (0.05, 0.17)	0.92 (0.4, 0.92)	5.99 (4.01, 8.25)
PFOS	ARB, DBCR, DBMR, NBT, SI, UB	0.25 (0.1, 0.37)	3.36 (2.01, 4.13)	15.09 (8.56, 19.14)
PFOSA	DBCR, NBT, SI	0.06 (0.06, 0.11)	1.15 (0.47, 2.05)	19.49 (4.11, 34.22)
PFTeDA	DBCR, NBT	0.11 (0.06, 0.15)	1.32 (1.32, 1.83)	22 (12.13, 22)
PFTrDA	ARB, DBCR, NBT	0.12 (0.07, 0.12)	2 (0.45, 2.43)	17.17 (5.83, 20.59)
PFUnDA	ARB, DBCR, DBMR, NBT, SI	0.1 (0.1, 0.12)	1.08 (0.52, 1.83)	9.9 (4.82, 12.02)

Summer Flounder

Summer Flounder were collected at sites: ARB, RR, UB, and RBU. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 11: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Summer Flounder within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	353 (939)	136 (3270)	0	4	2
Non-Saline High pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	- (-)	- (-)	0	0	0
Statewide	- (-)	353 (939)	136 (3270)	0	4	2

Table 12: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Summer Flounder [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	RR	0.47 (0.43, 0.52)	0.32 (0.32, 0.32)	685.62 (622.49, 752.68)
PFHxA	RBU	3.39 (2.96, 3.86)	0.26 (0.14, 0.61)	75.77 (43.22, 189.55)
PFNA	ARB, RBU	1.24 (0.6, 2.12)	0.22 (0.17, 0.28)	234.13 (91.88, 404.25)
PFOS	ARB, RBU, RR, UB	4.94 (3.09, 8.58)	1.92 (1.14, 2.08)	357.44 (199.88, 562.08)
PFOSA	RR	0.4 (0.37, 0.43)	0.81 (0.81, 0.81)	2020.69 (1864.01, 2209.12)

Table 13: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Summer Flounder [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	RBU, RR	0.12 (0.06, 0.12)	0.27 (0.12, 0.27)	2.18 (2.11, 2.18)
PFDA	RR	0.64 (0.64, 0.64)	0.32 (0.32, 0.32)	0.5 (0.5, 0.5)
PFDS	RR	0.36 (0.36, 0.36)	0.17 (0.17, 0.17)	0.47 (0.47, 0.47)
PFDoA	ARB, RBU, RR	0.12 (0.06, 0.39)	0.42 (0.24, 0.43)	2.23 (1.07, 3.84)
PFNA	ARB	0.09 (0.09, 0.09)	0.17 (0.14, 0.3)	1.96 (1.59, 3.63)
PFOS	ARB, RBU, RR, UB	2.21 (0.05, 3.78)	1.92 (1.14, 2.08)	1.18 (0.55, 20.8)
PFOSA	RR	0.09 (0.09, 0.09)	0.81 (0.81, 0.81)	9.38 (9.38, 9.38)
PFTeDA	RBU, RR	0.17 (0.07, 0.17)	0.35 (0.35, 0.53)	2.09 (2.09, 8.17)
PFTrDA	ARB, RBU, RR	0.13 (0.07, 0.27)	0.41 (0.37, 0.49)	3.25 (1.52, 4.9)
PFUnDA	ARB, RBU, RR	0.14 (0.1, 0.91)	0.32 (0.32, 0.49)	2.54 (0.35, 3.76)

White Perch

White Perch were collected at sites: ARB, RR, UB, and RBU. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 14: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for White Perch within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	19.1 (69.7)	911 (1410)a	202 (975)a	5	6	6
Non-Saline High pH	- (-)	2040 (3500)a	43.8 (746)a	0	9	7
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	2040 (3500)	43.8 (746)	0	9	7
Statewide	19.1 (69.7)	1480 (2380)	88.7 (608)	5	15	13

Table 15: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for White Perch [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DROC, DRSC, DRWC, HR, NBT, UP	0.38 (0.27, 0.6)	0.35 (0.21, 0.51)	764.93 (413.33, 1270.98)
MeFOSAA	UP	0.35 (0.26, 0.54)	0.28 (0.2, 0.51)	857.13 (478.72, 1771.83)
PFDA	DBCR, DBMR, DRMC, DROC, DRSC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	0.66 (0.49, 0.86)	0.95 (0.65, 1.61)	1469.04 (971.74, 2174.61)
PFDoA	UP	0.34 (0.23, 0.55)	1.42 (1.1, 2.07)	4350.06 (2469.11, 7509.78)
PFHpA	DBMR	1.97 (1.8, 2.14)	0.09 (0.07, 0.12)	46.14 (34.75, 62.69)
PFHpS	ARB	0.56 (0.49, 0.64)	0.18 (0.15, 0.23)	320.72 (253.3, 432.31)
PFHxA	ASS, STL	3.71 (2.01, 6.67)	0.1 (0.09, 0.11)	26 (15.44, 45.39)
PFHxS	ARB, DBCR, DBMR, HR, NBT, RR, STL	1.81 (1.06, 3.02)	0.1 (0.08, 0.14)	56.79 (34.33, 93.73)
PFNA	ARB, DBCR, DBMR, DROC, DRSC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	2.37 (1.58, 3.86)	0.23 (0.17, 0.38)	102.65 (51.31, 286.61)
PFOA	ARB, DBCR, DBMR, HR, NBT	5.98 (4.7, 8.31)	0.18 (0.12, 0.25)	30.58 (15.24, 48.49)
PFOS	ARB, ASS, DBCR, DBMR, DRMC, DROC, DRSC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	5.42 (3.51, 7.95)	7.21 (5.11, 10.26)	1309.37 (810.84, 2084.76)
PFOSA	DBCR, DRMC, DROC, DRSC, DRWC, HR, RR, STL, UP	0.41 (0.38, 0.45)	0.83 (0.68, 1.04)	1998.77 (1596.98, 2526.49)
PFPeS	ARB	1.31 (1.19, 1.43)	0.09 (0.07, 0.12)	69.92 (51.48, 97.92)
PFUnDA	DBCR, DRMC, DROC, DRSC, DRWC, HR, SAL, STL, UP	0.5 (0.41, 0.62)	2.97 (1.96, 4.26)	6053.26 (3853.94, 8647.31)
x6_2_FTS	DBMR, SAL	2.21 (1.66, 3.15)	0.43 (0.33, 0.62)	197.54 (124.2, 312.82)

Table 16: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for White Perch [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DBCR, DROC, DRWC, HR, NBT, RR, UP	0.17 (0.12, 0.5)	0.29 (0.13, 0.48)	1.2 (0.59, 3.48)
MeFOSAA	HR, NBT, UP	0.07 (0.05, 0.09)	0.18 (0.11, 0.24)	2.87 (1.28, 4.2)
PFDA	DBCR, DBMR, DROC, DRSC, HOPU, HR, RR, SAL, STL, UP	0.13 (0.09, 0.4)	1.24 (0.66, 1.87)	6.92 (4.78, 12.95)
PFDS	HR, NBT, RR, UP	0.13 (0.11, 0.19)	0.38 (0.32, 0.44)	2.98 (1.74, 4.51)
PFDoA	ARB, DBCR, DRMC, DROC, DRSC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	0.12 (0.06, 0.26)	1.59 (1.12, 2.69)	8.61 (4.24, 27.71)
PFHxS	ARB	0.14 (0.14, 0.14)	0.34 (0.28, 0.44)	2.49 (2.04, 3.27)
PFNA	ARB, DBCR, DBMR, DROC, DRWC, HR, RR, SAL, STL, UP	0.16 (0.07, 0.29)	0.25 (0.18, 0.51)	2.42 (1.12, 3.93)
PFOA	ARB, DBCR, DBMR, HR, NBT	0.08 (0.08, 0.15)	0.18 (0.12, 0.25)	1.55 (1.11, 2.91)
PFOS	ARB, DBCR, DBMR, DRMC, DROC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	0.37 (0.18, 0.78)	7.02 (4.94, 9.72)	14.07 (9.04, 37.16)
PFOSA	DBCR, DROC, HR, NBT, RR, UP	0.1 (0.09, 0.12)	0.87 (0.65, 1.24)	7.83 (5.55, 14.51)
PFTeDA	DBCR, DROC, DRWC, HOPU, HR, NBT, RR, SAL, UP	0.17 (0.06, 0.24)	1.31 (1.05, 1.65)	8.6 (5.35, 16.85)
PFTrDA	ARB, DBCR, DRMC, DROC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	0.22 (0.12, 0.28)	1.91 (1.31, 2.88)	9.21 (4.61, 18)
PFUnDA	ARB, DBCR, DBMR, DRMC, DROC, DRSC, DRWC, HOPU, HR, NBT, RR, SAL, STL, UP	0.27 (0.12, 0.59)	2.27 (0.96, 3.36)	7.44 (4.4, 12.44)
x6_2_FTS	ASS	10.5 (10.5, 10.5)	0.69 (0.67, 0.72)	0.07 (0.06, 0.07)

Black Sea Bass

Black Sea Bass were collected at sites: BBMR and SI. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 17: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Black Sea Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	138 (596)	- (-)	0	2	0
Non-Saline High pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	- (-)	- (-)	0	0	0
Statewide	- (-)	138 (596)	- (-)	0	2	0

Table 18: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Black Sea Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFOS	BBMR, SI	4.72 (3.91, 5.8)	0.6 (0.52, 0.69)	129.56 (97.75, 168)

Table 19: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Black Sea Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFOS	SI	0.1 (0.1, 0.1)	0.62 (0.5, 0.86)	5.76 (4.68, 7.84)
PFOSA	SI	0.06 (0.06, 0.06)	0.63 (0.58, 0.71)	10.72 (9.85, 12.05)

Northern Puffer

Northern Puffer were collected at sites: BBMR and BBTR. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 20: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Northern Puffer within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	301 (642)	- (-)	0	2	0
Non-Saline High pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	- (-)	- (-)	0	0	0
Statewide	- (-)	301 (642)	- (-)	0	2	0

Table 21: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Northern Puffer [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	BBMR, BBTR	0.35 (0.31, 0.39)	0.15 (0.14, 0.16)	449.2 (387.14, 521.38)
PFOS	BBMR, BBTR	7.49 (4.44, 12.18)	2.04 (1.4, 2.79)	270.99 (215.76, 338.54)
PFOSA	BBTR	0.41 (0.37, 0.44)	0.16 (0.13, 0.25)	405.63 (328.16, 647.8)

Table 22: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Northern Puffer [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFDA	BBTR	0.04 (0.04, 0.04)	0.18 (0.15, 0.31)	4.52 (3.67, 7.25)
PFOS	BBTR	0.46 (0.46, 0.46)	2.81 (2.75, 2.91)	6.1 (5.98, 6.33)
PFUnDA	BBTR	0.07 (0.07, 0.07)	0.42 (0.38, 0.56)	6.02 (5.39, 7.78)

Spot

Spot were collected at sites: BBMR, DBCR, DBMR, DRSC, RR, UB, and RBU. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 23: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Spot within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	38.7 (283)	1220 (1900)	294 (1720)	5	6	5
Non-Saline High pH	7.96 (406)	980 (1690)	107 (1360)	1	1	1
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	7.96 (406)	980 (1690)	107 (1360)	1	1	1
Statewide	29.7 (244)	1180 (1780)	248 (1460)	6	7	6

Table 24: Bayesian tatewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Spot [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DRSC	0.31 (0.24, 0.39)	0.26 (0.21, 0.4)	933.68 (645.52, 1529.5)
PFDA	BBMR, DBCR, DBMR, DRSC, RR	0.52 (0.42, 0.74)	1.01 (0.66, 1.35)	1716.47 (1204.51, 2343.85)
PFHpA	DBCR	2.88 (2.62, 3.14)	0.11 (0.08, 0.15)	37.81 (28.1, 55.11)
PFHxS	DBCR, DBMR, RR	1.01 (0.81, 1.62)	0.14 (0.11, 0.15)	128.67 (78.22, 173.29)
PFNA	BBMR, DBCR, DBMR, DRSC, RBU, RR	1.47 (0.97, 2.58)	0.3 (0.17, 1.17)	209.39 (142.12, 593.83)
PFOA	BBMR, DBCR, DBMR, DRSC, RR, UB	5.46 (4.46, 8.17)	0.18 (0.1, 0.52)	24.55 (15.83, 101.83)
PFOS	BBMR, DBCR, DBMR, DRSC, RBU, RR, UB	3.8 (2.86, 5.12)	4.39 (1.86, 6.16)	1078.71 (641.35, 1520.71)
PFOSA	DBCR, DRSC, RR	0.4 (0.37, 0.44)	0.91 (0.75, 1.03)	2238.66 (1826.16, 2655.36)
PFUnDA	DBCR, DRSC	0.52 (0.43, 0.61)	2.21 (2.11, 2.32)	4303.96 (3579.37, 5256.72)

Table 25: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Spot [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DBCR, RBU, RR	0.12 (0.12, 0.12)	0.19 (0.16, 0.26)	1.88 (1.45, 2.68)
PFDA	DBCR, DBMR, DRSC, RR	0.18 (0.13, 0.32)	1.01 (1, 1.69)	6.82 (4.97, 12.68)
PFDS	RR	0.36 (0.36, 0.36)	0.47 (0.44, 0.5)	1.3 (1.24, 1.41)
PFDoA	DBCR, DRSC, RBU, RR	0.16 (0.06, 0.39)	1.02 (0.74, 1.76)	5.53 (3.09, 26.36)
PFNA	DBCR, DBMR, RR	0.37 (0.21, 0.39)	1.17 (0.28, 1.89)	5.27 (0.77, 5.68)
PFOA	DBCR, DBMR, RR, UB	0.12 (0.08, 0.23)	0.39 (0.15, 0.55)	2.79 (0.87, 6.6)
PFOS	DBCR, DBMR, RBU, RR, UB	0.32 (0.12, 0.59)	3.79 (1.6, 5.96)	11.73 (2.76, 18.29)
PFOSA	DBCR, RR	0.1 (0.09, 0.11)	0.82 (0.67, 0.92)	7.96 (7.37, 8.57)
PFTeDA	DBCR, RBU, RR	0.07 (0.06, 0.17)	0.61 (0.49, 1)	7.81 (6.32, 9.47)
PFTrDA	DBCR, RBU, RR	0.13 (0.12, 0.27)	0.84 (0.58, 0.97)	4.7 (3.65, 7.14)
PFUnDA	DBCR, DBMR, DRSC, RBU, RR	0.14 (0.11, 0.52)	1.83 (0.77, 2.14)	4.48 (1.35, 15.78)

Common Carp

Common Carp were collected at sites: DRMC, DRWC, HOP, and SAL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 26: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Common Carp within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	8.33 (83.3)	1670 (2160)	154 (1190)	1	4	4
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	8.33 (83.3)	1670 (2160)	154 (1190)	1	4	4
Statewide	8.33 (83.3)	1670 (2160)	154 (1190)	1	4	4

Table 27: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Common Carp [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DRWC	0.29 (0.22, 0.38)	0.18 (0.15, 0.25)	679.47 (489.22, 1017.44)
PFDA	DRMC, DRWC, HOP, SAL	0.54 (0.46, 0.7)	1.17 (0.69, 4.27)	2261.59 (1343.83, 5764.25)
PFHxS	DRMC, DRWC, HOP, SAL	1.9 (1.55, 2.55)	0.14 (0.11, 0.2)	73.74 (46.01, 113.96)
PFNA	DRMC, DRWC, HOP, SAL	3.5 (2.05, 4.26)	0.44 (0.33, 0.74)	178.18 (104.42, 317.32)
PFOA	SAL	26.22 (22.16, 31.15)	0.27 (0.25, 0.32)	11.04 (8.96, 14.21)
PFOS	DRMC, DRWC, HOP, SAL	5.62 (4.65, 6.85)	8.63 (7.07, 11.07)	1553.34 (1172.93, 2058.91)
PFOSA	DRMC, DRWC	0.41 (0.38, 0.45)	0.24 (0.21, 0.3)	587.58 (483.23, 738.44)
PFUnDA	DRMC, DRWC, SAL	0.49 (0.41, 0.59)	6.86 (4.96, 12.38)	14629.36 (10287.49, 24156.56)

Table 28: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Common Carp [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DRWC	0.05 (0.05, 0.05)	0.18 (0.15, 0.24)	3.41 (2.9, 4.65)
PFDA	HOP, SAL	0.33 (0.04, 0.62)	4.82 (1.57, 8.82)	24.55 (14.24, 36.52)
PFDoA	DRMC, DRWC, HOP, SAL	0.05 (0.04, 0.25)	1.86 (1.3, 4.96)	34.06 (20.78, 45.7)
PFNA	DRMC, DRWC, SAL	0.07 (0.04, 0.29)	0.54 (0.36, 0.99)	6.13 (4.15, 9.63)
PFOA	SAL	0.56 (0.56, 0.56)	0.27 (0.25, 0.32)	0.49 (0.44, 0.57)
PFOS	DRMC, DRWC, HOP, SAL	0.13 (0.11, 0.3)	8.62 (7.06, 10.99)	58.75 (31.48, 75.32)
PFTeDA	DRWC, SAL	0.19 (0.05, 0.34)	2.75 (1.7, 3.83)	20.86 (11.26, 32.56)
PFTrDA	DRMC, DRWC, SAL	0.21 (0.06, 0.43)	5.93 (4.75, 7.64)	33.64 (17.03, 64.47)
PFUnDA	DRMC, DRWC, HOP, SAL	0.21 (0.13, 0.42)	4.98 (3.14, 9.64)	23.29 (18.54, 29.44)

Largemouth Bass

Largemouth Bass were collected at sites: DRMC, DROC, DRWC, ASS, BPL, GRL, HOP, HOPU, LEN, MC, MIR, RVR, SAL, STL, and UL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 29: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Largemouth Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	11.2 (13.7)	4020 (6650)a	31 (357)	1	13	7
Non-Saline Low pH	49.7 (128)	5260 (10500)a	- (-)	1	2	0
Non-Saline	23.7 (62.8)	4160 (6550)	31 (357)	2	15	7
Statewide	23.7 (62.8)	4160 (6550)	31 (357)	2	15	7

Table 30: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs for Largemouth Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DRWC	0.29 (0.22, 0.38)	0.29 (0.21, 0.55)	1159.2 (715.74, 2260.64)
PFDA	DRMC, DROC, DRWC, GRL, HOP, HOPU, LEN, MC, SAL, STL	0.53 (0.41, 0.71)	2.27 (1.83, 3.52)	4791.67 (2313.3, 6612.89)
PFHpS	BPL	1.08 (0.86, 1.33)	0.14 (0.14, 0.14)	128.9 (105.25, 163.13)
PFHxS	BPL, MC, MIR	7.33 (2.5, 34.02)	0.22 (0.14, 0.4)	17.62 (11.35, 84.29)
PFNA	DROC, DRWC, GRL, MC, RVR, SAL, STL	4.52 (3.64, 9.13)	0.19 (0.14, 0.28)	36.5 (23.17, 65.53)
PFOA	GRL, MIR	5.11 (2.39, 11.71)	0.13 (0.11, 0.16)	30.02 (11.49, 57.23)
PFOS	ASS, BPL, DRMC, DROC, DRWC, GRL, HOP, HOPU, LEN, MC, MIR, RVR, SAL, STL, UL	4.94 (2.98, 7.04)	18.03 (13.87, 27.2)	3596.34 (2210.85, 6173.58)
PFOSA	DRMC, DROC, DRWC, MC, STL	0.41 (0.38, 0.45)	0.24 (0.16, 0.3)	559.44 (422.69, 734.98)
PFUnDA	DRMC, DROC, DRWC, GRL, MC, SAL, STL	0.53 (0.42, 0.79)	8.97 (5.88, 17.17)	16179.85 (11204.4, 26423.5)
x6_2_FTS	MC, SAL	2.28 (1.69, 3.24)	0.38 (0.3, 0.52)	167.48 (105.58, 263.6)

Table 31: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Largemouth Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DRWC	0.05 (0.05, 0.05)	0.29 (0.21, 0.52)	5.6 (3.92, 10.45)
PFDA	BPL, DROC, HOP, HOPU, SAL, STL	0.24 (0.07, 0.47)	2.47 (1.9, 3.65)	13.95 (6.76, 28.05)
PFDoA	BPL, DRMC, DROC, DRWC, GRL, HOP, HOPU, MC, SAL, STL	0.07 (0.04, 0.56)	2.93 (1.87, 3.84)	32.68 (7.48, 56.55)
PFHxS	BPL	1.27 (1.27, 1.27)	0.44 (0.42, 0.46)	0.34 (0.33, 0.36)
PFNA	DROC, DRWC, GRL, SAL, STL	0.11 (0.08, 0.23)	0.19 (0.13, 0.29)	1.34 (0.73, 2.87)
PFOS	BPL, DRMC, DROC, DRWC, GRL, HOP, HOPU, MC, MIR, SAL, STL, UL	0.16 (0.11, 0.79)	17.73 (13.72, 28.77)	95.24 (29.67, 210.49)
PFOSA	DROC	0.12 (0.12, 0.12)	0.32 (0.24, 0.53)	2.7 (1.99, 4.68)
PFTeDA	BPL, DROC, DRWC, HOPU, MC, SAL	0.15 (0.05, 0.31)	1.53 (1, 2.2)	13.46 (4.34, 34.86)
PFTrDA	BPL, DRMC, DROC, DRWC, GRL, HOPU, MC, SAL, STL	0.21 (0.13, 0.43)	3.58 (2.38, 5.36)	22.8 (6.82, 37.83)
PFUnDA	BPL, DRMC, DROC, DRWC, GRL, HOP, HOPU, MC, RVR, SAL, STL, UL	0.29 (0.14, 0.62)	6.6 (3.88, 12.5)	26.99 (13.02, 79.93)
x6_2_FTS	ASS	10.5 (10.5, 10.5)	0.56 (0.37, 1.02)	0.05 (0.03, 0.1)

Pumpkinseed

Pumpkinseed were collected at sites: DRMC, DRWC, PRE, and PIL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 32: EPA methodologies were used for selected compounds (PFOA, PFOS, and PFNA) to determine aqueous BAFs for Pumpkinseed within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	7.66 (368)	1810 (2490)	52.8 (826)	1	3	3
Non-Saline Low pH	14.4 (42.5)	3770 (3880)	134 (2150)	1	1	1
Non-Saline	10.5 (191)	2180 (2680)	66.7 (836)	2	4	4
Statewide	10.5 (191)	2180 (2680)	66.7 (836)	2	4	4

Table 33: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Pumpkinseed [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
EtFOSAA	DRWC	0.29 (0.22, 0.38)	0.29 (0.2, 0.54)	1062.27 (639.12, 2247.85)
PFDA	DRMC, DRWC	0.52 (0.46, 0.58)	1.15 (0.88, 1.58)	2281.91 (1673.75, 3237.72)
PFHxS	PIL	6.02 (6.02, 6.02)	1.1 (1.02, 1.21)	181.58 (168.16, 200.4)
PFNA	DRMC, DRWC, PIL, PRE	2.03 (1.29, 3.69)	0.19 (0.16, 0.23)	103.49 (48.88, 168.68)
PFOA	PIL, PRE	6.93 (6.93, 8.88)	0.14 (0.1, 0.21)	18.94 (12.29, 28.61)
PFOS	DRMC, DRWC, PIL, PRE	7.2 (5.49, 19.94)	15.37 (10.73, 36.1)	2350 (1595.63, 3453.62)
PFOSA	DRMC, DRWC	0.41 (0.38, 0.45)	0.32 (0.29, 0.36)	786.72 (687.71, 915.36)
PFUnDA	DRMC, DRWC	0.47 (0.39, 0.56)	8 (5.78, 11.57)	17187.05 (11561.87, 26615.61)

Table 34: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Pumpkinseed [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
EtFOSAA	DRWC	0.05 (0.05, 0.05)	0.29 (0.2, 0.55)	5.45 (3.69, 10.45)
PFDA	PRE	0.07 (0.07, 0.07)	0.94 (0.84, 1.09)	14.04 (12.58, 16.45)
PFDS	PRE	0.08 (0.08, 0.08)	0.6 (0.49, 0.81)	7.82 (6.49, 10.43)
PFDoA	DRMC, DRWC, PIL, PRE	0.04 (0.03, 0.08)	1.85 (1.16, 2.45)	26.36 (16.02, 56.42)
PFNA	DRMC, DRWC, PIL	0.05 (0.04, 0.07)	0.21 (0.16, 0.28)	3.99 (2.92, 5.6)
PFOA	PIL, PRE	0.05 (0.04, 0.06)	0.14 (0.1, 0.21)	3.27 (1.87, 4.91)
PFOS	DRMC, DRWC, PIL, PRE	0.27 (0.13, 0.42)	15.42 (10.71, 36.35)	100.37 (47.37, 166.88)
PFOSA	PIL	0.05 (0.05, 0.05)	3.67 (3.46, 3.94)	72.34 (68.33, 77.69)
PFTeDA	DRWC, PRE	0.1 (0.05, 0.14)	2.02 (1.59, 2.57)	23.61 (17.13, 32.57)
PFTrDA	DRMC, DRWC, PRE	0.17 (0.06, 0.21)	7.09 (5.02, 10.05)	49.47 (30.49, 94.33)
PFUnDA	DRMC, DRWC, PRE	0.15 (0.13, 0.27)	5.61 (3.9, 7.94)	31.31 (21.64, 45.42)

Yellow Perch

Yellow Perch were collected at site: DRMC. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 35: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Yellow Perch within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	- (-)	2150 (3290)	172 (1350)	0	1	1
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	2150 (3290)	172 (1350)	0	1	1
Statewide	- (-)	2150 (3290)	172 (1350)	0	1	1

Table 36: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Yellow Perch [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	DRMC	0.47 (0.42, 0.51)	1.37 (1.17, 1.7)	2960.7 (2433.65, 3827.28)
PFNA	DRMC	3.31 (2.96, 3.68)	0.77 (0.59, 1.16)	237.44 (175.77, 358.71)
PFOS	DRMC	5.56 (4.76, 6.53)	10.77 (9.12, 13.16)	1945.38 (1510.2, 2586.73)
PFOSA	DRMC	0.41 (0.37, 0.44)	0.92 (0.72, 1.27)	2330.19 (1785.7, 3403.06)
PFUnDA	DRMC	0.4 (0.35, 0.47)	6.58 (5.63, 8.07)	16793.39 (13225.74, 21369.85)

Table 37: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Yellow Perch [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFDoA	DRMC	0.03 (0.03, 0.03)	2.95 (2.69, 3.33)	105.92 (96.16, 118.18)
PFNA	DRMC	0.04 (0.04, 0.04)	0.8 (0.59, 1.2)	21.25 (15.84, 31.69)
PFOS	DRMC	0.1 (0.1, 0.1)	10.77 (9.19, 13.15)	102.37 (85.81, 125.58)
PFTrDA	DRMC	0.06 (0.06, 0.06)	6.12 (5.76, 6.55)	94.36 (88.45, 100.81)
PFUnDA	DRMC	0.15 (0.15, 0.15)	6.68 (5.73, 8.11)	43.69 (36.82, 52.35)

Channel Catfish

Channel Catfish were collected at sites: DROC and DRSC. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 38: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Channel Catfish within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	- (-)	306 (694)	42.9 (85.2)	0	2	2
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	306 (694)	42.9 (85.2)	0	2	2
Statewide	- (-)	306 (694)	42.9 (85.2)	0	2	2

Table 39: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Channel Catfish [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	DROC, DRSC	1.08 (0.82, 1.42)	0.95 (0.78, 1.13)	869.43 (717.53, 1071.57)
PFNA	DROC, DRSC	3.79 (3.28, 4.34)	0.25 (0.18, 0.4)	68.97 (47.53, 107.68)
PFOS	DROC, DRSC	5.37 (4.56, 6.33)	1.48 (1.22, 1.75)	271.85 (213.14, 347.31)
PFOSA	DROC, DRSC	0.42 (0.39, 0.46)	0.3 (0.24, 0.36)	701.59 (559.74, 868.68)
PFUnDA	DROC, DRSC	0.53 (0.45, 0.62)	1.97 (1.84, 2.11)	3690.73 (3109.96, 4442.46)

Table 40: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Channel Catfish [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFDA	DROC, DRSC	0.07 (0.06, 0.07)	0.94 (0.78, 1.15)	14.58 (10.64, 18.58)
PFDoA	DROC, DRSC	0.09 (0.06, 0.12)	2.75 (2.47, 3.13)	35.23 (22.55, 50.39)
PFNA	DROC	0.11 (0.11, 0.11)	0.24 (0.16, 0.45)	2.25 (1.5, 4.13)
PFOS	DROC	0.18 (0.18, 0.18)	1.18 (1.09, 1.33)	6.63 (6.14, 7.41)
PFOSA	DROC	0.12 (0.12, 0.12)	0.24 (0.22, 0.28)	2.03 (1.88, 2.32)
PFTeDA	DROC	0.06 (0.06, 0.06)	2.86 (2.19, 4)	46.76 (36.23, 68.67)
PFTrDA	DROC	0.23 (0.23, 0.23)	2.71 (2.46, 3.08)	11.7 (10.52, 13.21)
PFUnDA	DROC, DRSC	0.21 (0.11, 0.3)	1.96 (1.83, 2.12)	11.61 (6.56, 17.65)

Bluegill

Bluegill were collected at sites: ASS, BPL, GRL, HOR, HOP, HOPU, LEN, MC, MIR, RVR, SAL, STL, and UL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 41: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Bluegill within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	11.8 (289)	3010 (4520)a	105 (351)	5	10	8
Non-Saline Low pH	77.7 (1530)	2840 (4450)a	- (-)	1	3	0
Non-Saline	16.1 (351)	2970 (4190)	105 (351)	6	13	8
Statewide	16.1 (351)	2970 (4190)	105 (351)	6	13	8

Table 42: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Bluegill [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFBA	HOPU	1.76 (1.76, 1.76)	0.56 (0.39, 0.94)	322.94 (228.7, 531.65)
PFBS	HOPU	1.51 (1.51, 1.51)	0.14 (0.1, 0.25)	89.78 (64.88, 161.87)
PFDA	GRL, HOP, HOPU, LEN, MC, SAL, STL	0.49 (0.4, 0.69)	1.72 (1.06, 2.33)	3270.93 (2069, 4398.53)
PFHpA	ASS, HOPU	1.06 (1.06, 1.35)	0.38 (0.21, 0.89)	330.65 (178.11, 767.75)
PFHpS	BPL, MC	0.63 (0.43, 1.09)	0.12 (0.12, 0.16)	222.6 (146.1, 286.86)
PFHxA	HOPU	1.91 (1.91, 1.91)	0.28 (0.17, 0.65)	150.15 (87.32, 353.15)
PFHxS	BPL, HOPU, HOR, MC, MIR	3.54 (1.61, 8.43)	0.28 (0.19, 0.35)	64.19 (39.98, 120.66)
PFNA	GRL, HOP, HOPU, MC, RVR, SAL, STL, UL	2.7 (1.15, 6.88)	0.53 (0.33, 0.7)	162.91 (94.97, 381.44)
PFOA	GRL, HOP, HOPU, MIR, RVR, SAL	6.46 (3.3, 11.87)	0.15 (0.13, 0.18)	22.81 (11.58, 50.96)
PFOS	ASS, BPL, GRL, HOP, HOPU, HOR, LEN, MC, MIR, RVR, SAL, STL, UL	4.7 (2.66, 7.05)	12.29 (9.15, 16.38)	2667.91 (1875.79, 3772.65)
PFOSA	MC, STL	0.41 (0.37, 0.44)	0.18 (0.17, 0.18)	444.65 (399.33, 500.4)
PFUnDA	GRL, MC, SAL, STL	0.69 (0.45, 1.05)	10.24 (8.63, 13.39)	16094.11 (11804.16, 21282.5)

Table 43: Bayesian statewide estimates of sediment and fish concentrations of co-occurring PFAS compounds with accompanying SBAFs for Bluegill [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFDA	BPL, HOP, HOPU, SAL, STL	0.4 (0.09, 0.47)	1.81 (1.09, 2.66)	6.14 (3.94, 15.72)
PFDoA	BPL, GRL, HOP, HOPU, MC, SAL, STL	0.08 (0.04, 0.78)	2.02 (1.37, 2.39)	25.26 (4.76, 36.56)
PFHxS	BPL	1.27 (1.27, 1.27)	0.42 (0.37, 0.51)	0.32 (0.28, 0.4)
PFNA	GRL, SAL, STL	0.23 (0.08, 0.29)	0.52 (0.3, 1.84)	2.2 (1.14, 21.21)
PFOA	HOPU, SAL	0.37 (0.17, 0.56)	0.22 (0.13, 0.36)	0.96 (0.24, 1.83)
PFOS	BPL, GRL, HOP, HOPU, HOR, MC, MIR, SAL, STL, UL	0.19 (0.07, 0.83)	13.9 (11.5, 20.09)	74.26 (15.79, 180.83)
PFTeDA	BPL, HOPU, MC, SAL	0.28 (0.19, 0.32)	1.51 (0.7, 1.64)	5.16 (2.67, 35.73)
PFTrDA	BPL, GRL, HOPU, MC, SAL, STL	0.28 (0.13, 0.51)	2.98 (1.41, 4.23)	13.06 (2.67, 35.11)
PFUnDA	BPL, GRL, HOP, HOPU, MC, RVR, SAL, STL, UL	0.31 (0.09, 0.71)	4.69 (3.11, 8.71)	32.92 (8.42, 47.16)
x6_2_FTS	ASS	10.5 (10.5, 10.5)	0.76 (0.73, 0.8)	0.07 (0.07, 0.08)

Chain Pickerel

Chain Pickerel were collected at sites: BPL, HOR, LEN, OSW, PIL, and UL. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 44: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Chain Pickerel within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	- (-)	2520 (3590)a	63.9 (153)	0	2	1
Non-Saline Low pH	- (-)	2050 (7370)a	- (-)	0	4	0
Non-Saline	- (-)	2200 (5760)	63.9 (153)	0	6	1
Statewide	- (-)	2200 (5760)	63.9 (153)	0	6	1

Table 45: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Chain Pickerel [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	LEN	0.44 (0.38, 0.51)	0.35 (0.3, 0.47)	860.55 (659.05, 1215.97)
PFHpS	BPL	1.08 (0.86, 1.33)	0.34 (0.27, 0.48)	322.51 (232.99, 485.6)
PFHxS	BPL, PIL	8.34 (6.02, 42.99)	1.24 (1.01, 1.53)	112.19 (25.83, 227.49)
PFNA	BPL	1.8 (1.53, 2.12)	0.12 (0.1, 0.18)	70.21 (53.17, 104.01)
PFOS	BPL, HOR, LEN, OSW, PIL, UL	4.7 (2.74, 20.9)	12.88 (3.13, 75.11)	2532.44 (1255.12, 3571.93)
PFOSA	LEN	0.41 (0.38, 0.45)	0.15 (0.1, 0.3)	353.75 (236.75, 762.02)

Table 46: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Chain Pickerel [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
PFDA	BPL	0.47 (0.47, 0.47)	0.6 (0.55, 0.72)	1.28 (1.16, 1.52)
PFDoA	BPL, PIL	0.41 (0.03, 0.78)	0.67 (0.45, 0.87)	5.32 (1.1, 13.64)
PFHxS	BPL	1.27 (1.27, 1.27)	0.97 (0.83, 1.22)	0.77 (0.66, 0.96)
PFNA	BPL	1.42 (1.42, 1.42)	0.12 (0.1, 0.17)	0.08 (0.07, 0.12)
PFOS	BPL, HOR, PIL, UL	0.24 (0.07, 5.42)	40.22 (13.56, 93.8)	184.24 (36.6, 236.64)
PFOSA	PIL	0.05 (0.05, 0.05)	11.42 (10.48, 12.56)	223.07 (205.72, 244.85)
PFTeDA	BPL	0.31 (0.31, 0.31)	0.36 (0.3, 0.5)	1.14 (0.94, 1.62)
PFTrDA	BPL	0.81 (0.81, 0.81)	0.71 (0.6, 0.91)	0.89 (0.75, 1.11)
PFUnDA	BPL, UL	0.86 (0.05, 1.66)	1.56 (1.29, 1.9)	8.17 (1.04, 26.09)

Smallmouth Bass

Smallmouth Bass were collected at sites: PRE, DRCSB, and RVR. The unique combinations of observed PFAS compounds co-occurring in the fish tissue and the water were used to generate site-specific BAFs. Additionally, PFAS compounds co-occurring in fish tissue and sediment were used to generate site-specific Sediment BAFs. For each species table the "n" reported is the number of site specific BAFs that were available within each group.

Table 47: EPA methodologies were used for selected compounds (PFNA, PFOS, and PFNA) to determine aqueous BAFs for Smallmouth Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	- (-)	- (-)	- (-)	0	0	0
Non-Saline High pH	- (-)	5430 (12100)	74.5 (259)	0	3	1
Non-Saline Low pH	- (-)	- (-)	- (-)	0	0	0
Non-Saline	- (-)	5430 (12100)	74.5 (259)	0	3	1
Statewide	- (-)	5430 (12100)	74.5 (259)	0	3	1

Table 48: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Smallmouth Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Water Concentration (ng/L)	Fish Concentration (ng/g)	BAF (L/kg)
PFDA	DRCSB	0.49 (0.45, 0.54)	3.62 (3.24, 4.18)	7330.25 (6255.52, 8711.97)
PFNA	RVR	1.49 (1.28, 1.8)	0.21 (0.15, 0.37)	149.08 (101.04, 262.48)
PFOS	DRCSB, PRE, RVR	7.46 (2.55, 9.51)	26.67 (17.97, 32.69)	4191.75 (3067.76, 6500.99)
PFOSA	RVR	0.41 (0.38, 0.45)	0.17 (0.15, 0.2)	409.61 (347.8, 521.89)
x6_2_FTS	DRCSB	1.87 (1.35, 2.58)	0.41 (0.34, 0.55)	234.98 (160, 372.3)

Table 49: Bayesian statewide estimates of water and fish concentrations of co-occurring PFAS compounds with accompanying BAFs Smallmouth Bass [Median (IQR Q1, Q3)].

PFAS Compound	Sites	Sediment Concentration (ng/g)	Fish Concentration (ng/g)	SBAF
MeFOSAA	DRCSB	0.04 (0.04, 0.04)	0.11 (0.08, 0.2)	2.63 (1.98, 4.54)
PFDA	DRCSB, PRE	0.06 (0.05, 0.07)	2.8 (2.1, 3.53)	51.89 (31.67, 75.2)
PFDS	DRCSB, PRE	0.07 (0.06, 0.08)	1.19 (1, 1.49)	18.07 (14.04, 23.11)
PFDoA	DRCSB, PRE	0.13 (0.11, 0.16)	4.04 (3.26, 5.11)	31.49 (23.1, 41.82)
PFOS	DRCSB, PRE	0.41 (0.32, 0.5)	31.34 (27.18, 36.34)	78.32 (63.84, 96.59)
PFTeDA	DRCSB, PRE	0.11 (0.08, 0.14)	2.64 (2.09, 3.47)	25 (18.22, 34.32)
PFTrDA	DRCSB, PRE	0.14 (0.11, 0.17)	3.55 (2.85, 4.58)	26.56 (18.87, 36.28)

PFUnDA	DRCSB, PRE, RVR	0.11 (0.09, 0.13)	5.87 (4.52, 7.85)	53.36 (37.72, 80.06)

All Fish Combined

All fish species were combined to generate state wide estimates of BAFs by salinity/pH groupings for the EPA methodology selected PFOA, PFOS, and PFNA compounds. Differences in BAFs for PFNA and PFOS across saline and non-saline groups were statistically significant. "n" represents the number of total number of unique fish species-compound-group combinations used to generate the estimates.

Table 50: EPA methodologies were used for selected compounds (PFOA, PFOS, and PFNA) to determine aqueous BAFs for all fish species within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. If the ANOVA by group indicated significant differences between groups, a Tukey HSD post-hoc test was conducted to identify which groups differed significantly. The results of this test were used to generate superscript letters (e.g., "a," "b") indicating significant differences between groups.

Salinity pH Group	PFOA	PFOS	PFNA	PFOA n	PFOS n	PFNA n
Saline	34.1 (158)a	495 (681)b	216 (949)a	4	7	5
Non-Saline High pH	9.84 (112)a	1940 (2670)b	79.7 (293)b	6	11	11
Non-Saline Low pH	36.2 (455)a	3370 (5730)a	164 (1480)ab	4	5	2
Non-Saline	12.6 (109)	1970 (2770)	81.4 (295)	6	11	11
Statewide	18.9 (91.4)	1170 (1740)	106 (409)	8	15	13

Bayesian Differences in PFAS BAFs Among Site and Regional Strata

Frequentist differences via water quality grouping are represented in the previous sections, here the Bayesian differences via grouping variables are reported.

PFAS BAFs among Hydrology

American Eel PFOS BAFs were statistically different comparing sites in marine waters to sites in non-Pinelands (NPL) waters and marine waters to Pinelands (PL) waters. The American Eel BAFs of PFOS were 6851 (95% CI; 810-21052), 947 (95% CI; 578-1413), 2284 (95% CI; 1247-3674), and 3606 (95% CI; 1808-6045) within Deep, Marine, NPL, and PL sites, respectively. American Eel PFDA BAFs were statistically different comparing Marine to NPL. The American Eel BAFs of PFDA were 1261 (95% CI; 857-1699), 3409 (95% CI; 2163, 4824), and 2639 (95% CI; 696-5919), within Marine, NPL, and PL sites, respectively.

Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences comparing between Marine, NPL, and PL sites.

PFAS BAFs among Region

Taken across the entire dataset, American Eel PFOS BAFs were statistically different comparing Central-Atlantic to Delaware River. The American Eel BAFs of PFOS were 515 (95% CI; 117-1073), 1491 (95% CI; 720-2477), and 594 (95% CI; 172-1305), within Central-Atlantic, Delaware River, North Regions, respectively.

Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences comparing between Central-Atlantic, Delaware River, or North Regions.

PFAS BAFs among Site Type

No statistically different surface to deep comparisons existed in our dataset. American Eel PFOS BAFs were statistically different comparing lake to upper estuary and lake bottom to upper estuary. The American Eel BAFs of PFOS were 3512 (95% CI; 2075-5274), 3772 (95% CI; 2218-5684), 840 (95% CI; 90-2599), 1987 (95% CI; 784-3641), and 965 (95% CI; 557-1505), within lake, lake bottom, open water bay, stream, and upper estuary, respectively.

White Perch PFOS BAFs were statistically different comparing lake and lake bottom to upper estuary and upper estuary bottom. The White Perch BAFs of PFOS were 3245 (95% CI; 1630-5524), 3290 (95% CI; 1647-5616), 1271 (95% CI; 219-3460), 1053 (95% CI; 694-1472), and 1110 (95% CI; 748-1586), within lake, lake bottom, open water bay, upper estuary and upper estuary bottom, respectively.

White Perch PFDA BAFs were statistically different comparing lake and lake bottom to upper estuary and upper estuary bottom. The White Perch BAFs of PFDA were 3415 (95% CI; 1536-5963), 3352 (95% CI; 1463-5835), 1262 (95% CI; 217-3224), 1279 (95% CI; 826-1763), and 1331 (95% CI; 878-1858), within lake, lake bottom, open water bay, upper estuary and upper estuary bottom, respectively.

Spot PFOS BAFs were statistically different comparing open water bay to upper estuary. The Spot BAFs of PFOS were 514 (95% CI; 250-858) and 1344 (95% CI; 883-1866), within open water bay and upper estuary, respectively.

Largemouth Bass PFDA BAFs were statistically different comparing lake and lake bottom to upper estuary. The Largemouth Bass BAFs of PFDA were 5691(95% CI; 3557-8326), 5913 (95% CI; 3718-8649), 5490 (95% CI; 1223-12350), and 1610 (95% CI; 832-2741), within lake, lake bottom, stream, and upper estuary, respectively.

Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences comparing between lake, lake bottom, open water bay, upper estuary and upper estuary bottom site types.

PFAS BAFs among Water Quality Groups

Based on both the EPA methodology and the Bayesian methods, American Eel PFOS BAFs were statistically different. EPA methods comparing high salinity-high pH (Saline) to low salinity-low pH, showed statistical significance. Bayesian methods showed a difference comparing saline to both non-saline high pH and non-saline low pH. The American Eel Bayesian BAFs of PFOS were 672 (95% CI; 373-1050), 2543 (95% CI; 1725-3507), and 3508 (95% CI; 1670-6055), within saline, non-saline high pH, and non-saline low pH, respectively.

Based on the EPA methodology there was a statistical difference in PFOS BAF across all fish species combined that saline, non-saline high pH, and non-saline low pH had BAFs of 808 (UCL 997), 3766 (UCL 4583), and 3488 (UCL 6561), respectively.

Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences comparing within high salinity-high pH, low salinity-high pH, and low salinity-low pH groups.

PFAS BAFs among Cation Groups

Based on only the Bayesian methods, American Eel PFOS BAFs were statistically different comparing saline to non-saline low pH and non-saline high pH to non-saline low pH. The American Eel BAFs of PFOS were 1606 (95% CI; 1045-2288), 1627 (95% CI; 1021-2289), and 4838 (95% CI; 1170-10917), within saline, non-saline high pH, and non-saline low pH, respectively.

Sediment BAFs for the benthic species in our study (American Eel, Oyster Toadfish, Summer Flounder, Common Carp, and Channel Catfish) did not show statistical differences comparing within cation saline, non-saline high pH, and non-saline low pH.

PFAS BAFs among Isotopes

Within unique fish species PFAS compound combinations there were no linear relationships between nitrogen or carbon isotopes to BAFs nor did benthic species have a linear relationship to Sediment BAFs. The lack of any statistical relationship between the isotope observations in the fish and their respective BAFs indicates that other factors might be impacting BAF beyond feeding type or trophic position.

4. DISCUSSION

The Department engaged the Academy of Natural Sciences to evaluate the differences in chemistry, habitat, and taxa influence on PFAS bioaccumulation. We developed statewide BAFs for 17 PFAS that would be used to aid NJDEP in development of surface water quality standards (SWQS). To account for the potential variability in PFAS behavior and environmental factors mentioned above, we sought to assess the influence of waterbody, habitat, and trophic groups. We were able to calculate BAFs for PFAS that could be used across the range of New Jersey waterbodies and fish taxa.

Our analysis examined various grouping approaches, including cation concentrations, stable isotope values, and administratively defined divisions (such as hydrogeological regions, site types, and regional classifications outlined in the QAPP, Appendix B). However, these methods were ultimately not used in the broader discussion or in calculating BAFs because water quality parameters (pH and salinity) consistently provided a more robust and reliable means of comparing bioaccumulation across sites. While Bayesian methodologies were advantageous and are reported in the results for their ability to handle data below detection limits and account for uncertainty, our discussion primarily focuses on the EPA methods. This focus was chosen to align our findings with established practices in PFAS research and to facilitate comparability with other studies in the literature.

We used Bayesian methodologies to fit each site's water, fish, and sediment concentrations as a posterior using a log-normal distribution; this had several advantages including handling observations below detection limits, ensuring strictly positive estimates, and ensuring uncertainty in later BAFs explicitly account for the variation in both concentrations (Figure 9). Subsequent use of Bayesian versions of ANOVA to test if BAFs differed by grouping variables also incorporated heteroskedastic uncertainty within groups based on the inherited uncertainty of the concentration observations and allowed us to extract estimates of groups which were also strictly positive.

While the Bayesian methodologies have multiple advantages, the EPA methodology results are discussed below to align with the state of practice in PFAS research. At least one PFAS was detected in the surface water samples at all 33 sites. Mean PFOS concentrations, a particularly prevalent and bioaccumulative PFAS, ranged from 1.71 to 34.84 ng/L. The highest mean PFOS concentrations were found at Big Pine Lake, Mantua Creek, and Pine Lake with 34.8, 21.9, and 20.9 ng/L, respectively. Twenty-six sites had mean PFOS concentrations below 10 ng/L.

Administratively derived boundaries were defined generally as hydrogeological (as defined by the approved QAPP; Marine, Pineland, Non-Pineland, and Deep/Shallow), regional (Central-Atlantic, Delaware River, and North) and by site type (Upper Estuary [surface/bottom], Open Water Bay, Lake [surface/bottom], and Stream). While some BAFs for species did exhibit statistically significant separation based on these divisions, caution should be used in their interpretation. There is overlap and ambiguity in which sites are defined by the categorical divisions. For example, sites in the Delaware River are categorized as Marine even as their salinity is less than 1 ppt.

Water quality parameters pH and salinity give a much better, evidentiary categorization of sites. While dissolved oxygen, temperature, and conductivity were also collected during site visits, these parameters either vary too widely with time of day or are correlated strongly with

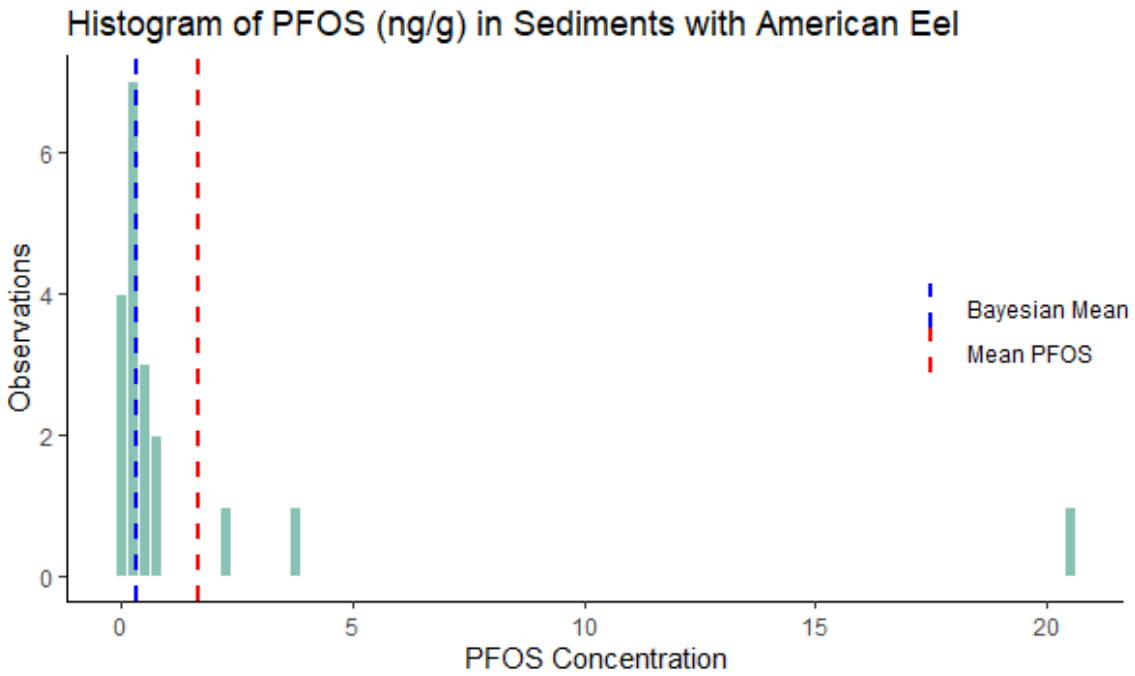

Figure 9: Nineteen sites had cooccurring concentrations of PFOS in sediments and in American Eel. Concentrations are naturally left censored at zero. Using our Bayesian methods of fitting data with a log normal distribution we better capture the mean value (blue) of the data than a simple arithmetic mean (red).

salinity. Three distinct site classes were developed by our grouping analysis, broadly defined as saline, non-salinity-high pH, and non-salinity low pH. These classes were statistically significantly different from each other based on water quality parameters, and differences existed across American Eel PFOS BAFs. American Eel PFOS saline Bayesian BAF was lower 675 (95% CI; 367-1058) than non-saline high pH Bayesian BAF 2527 (95% CI; 718-3519) and non-saline low pH Bayesian BAF 3566 (95% CI; 1683-6225). Khairy (2019) found a BAF of 9000 in the Passaic River for a single specimen of American Eel. Our American Eel Bayesian BAF for PFOS at the Passaic River was 1150 (95% CI; 512-2621), significantly lower than the Khairy (2019) result. Our BAF is derived from 5 water samples and 3 individual fish, therefore the difference between our BAF and Khairy (2019) may be a result of their analysis being limited to a single water-fish sample combination.

Cation concentrations required a more nuanced multivariate grouping analysis, but also fell into three distinct statistically different classes. Although due to the complexity of having 8 parameters, it prevents a simplistic nomenclature as in water quality classes. We performed multiple imputation for the missing cation data using the JM-DP (Joint Modeling with Dirichlet Process) method, as implemented in the NbClust package in R. The imputation was iteratively conducted with multiple cluster sizes and 20 imputed datasets. After imputation, we applied k-means clustering to the imputed datasets. The optimal number of clusters was determined using silhouette width and within-cluster sum of squares, which confirmed that a 3-cluster solution was appropriate. The cation groupings-based ANOVA estimated that American Eel PFOS was the only fish species-compound combination that resulted in statistically different BAFs groups. However, these results are less interpretable than the three-grouping structure derived by water quality.

PFOS – EPA Methods

PFOS was the only compound were BAFs available for every species. The PFOS concentration in water across the state varied from 1.67 to 35.3 ng/L (Figure 10). The highest concentrations were observed at BPL, MC, and PIL with concentrations of 35.3, 21.8, and 20.9 ng/L, respectively. Combining the observations of all fish species, the BAF within saline sites was 495 (95% UCL, 681), while non-saline sites were 1970 (95% UCL, 2770) (Table 50). The PFOS concentration in sediment across the state varied from 0.046 to 20.5 ng/g. The highest concentrations were observed at BPL, RR, and ARB with concentrations of 20.5, 3.78, and 2.21 ng/g, respectively.

Our estimates of species BAF for PFOS were less than previous estimates of BAF for four species. Khairy (2019) reported PFOS BAF for American Eel BAF of 9000; our estimates within the saline sites were 628 (95% UCL 923). Non-saline sites had a BAF of 2510 (95% UCL 5350) (Table 5). Channel catfish were reported to have a PFOS BAF of 3162 (Bhavsar et al., 2016); our estimate of PFOS BAF statewide for channel catfish was 306 (95% UCL 694) (Table 38). Previous estimates of PFOS BAF in common carp were 3600 (95% UCL 10800) (Bhavsar et al., 2016; Lee et al., 2020; Murakami et al., 2011; Pan et al., 2017; Wang et al., 2013; Zhou et al., 2012); our estimated BAF for PFOS was 1670 (95% UCL 2160). White perch PFOS BAF was previously reported at 5750 (95% UCL, 7220) (Khairy, 2019); we estimated a statewide PFOS BAF of 1480 (95% UCL, 2380) (Table 14).

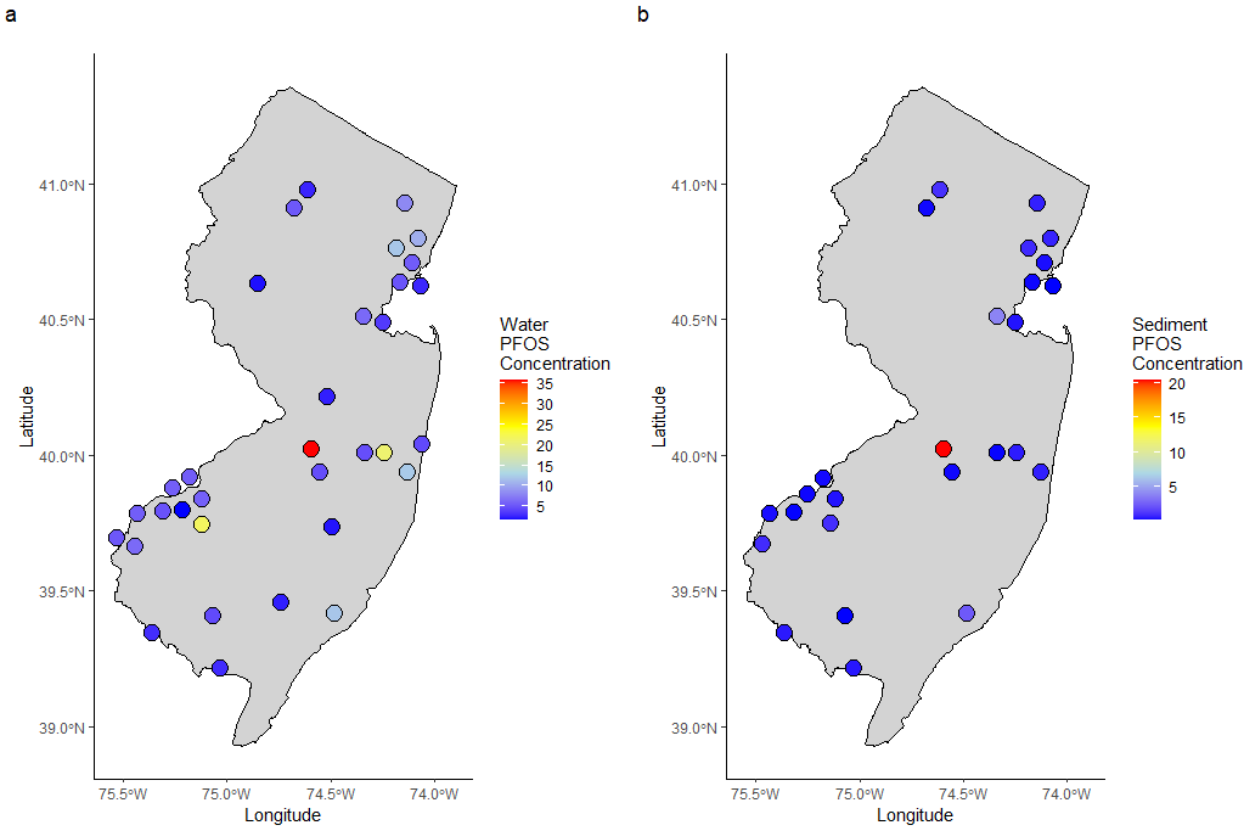

Figure 10. PFOS concentrations in water(ng/l) and sediment (ng/g) varied spatially across the State of New Jersey.

Our estimates of two species PFOS BAF were larger than existing estimates. Pumpkinseed PFOS BAF was previously reported at 631 (Bhavsar et al., 2016); our statewide estimate was 2180 (95% UCL 2680) (Table 32). Yellow perch PFOS BAF was previously reported at 794 (Bhavsar et al., 2016); our statewide estimate was 2150 (95% UCL 3290) (Table 35).

Two species estimates of PFOS BAF were similar to reported values for other species. Bhavsar et al. (2016) reports a PFOS BAF for largemouth bass of 5011, compared to our 4160 (95% UCL 6550) (Table 29). Smallmouth bass PFOS BAF was reported at 6309 (Bhavsar et al., 2016), our estimated value was 5430 (95% UCL 12100) (Table 47).

PFNA – EPA Methods

The PFNA concentration in water across the state varied from 0.512 to 28.4 ng/L (Figure 11). The highest concentrations were observed at LMC, GRL, and STL with concentrations of 28.4, 15.1, and 11.0 ng/L, respectively. Combining the observations of all fish species, the BAF within saline sites was 216 (95% UCL, 949), while non-saline sites were 81.4 (95% UCL, 295) (Table 50). The PFNA concentration in sediment across the state varied from 0.035 to 1.42 ng/g. The highest concentrations were observed at BPL, LMC, and DBCR with concentrations of 1.42, 0.91, and 0.387 ng/g, respectively.

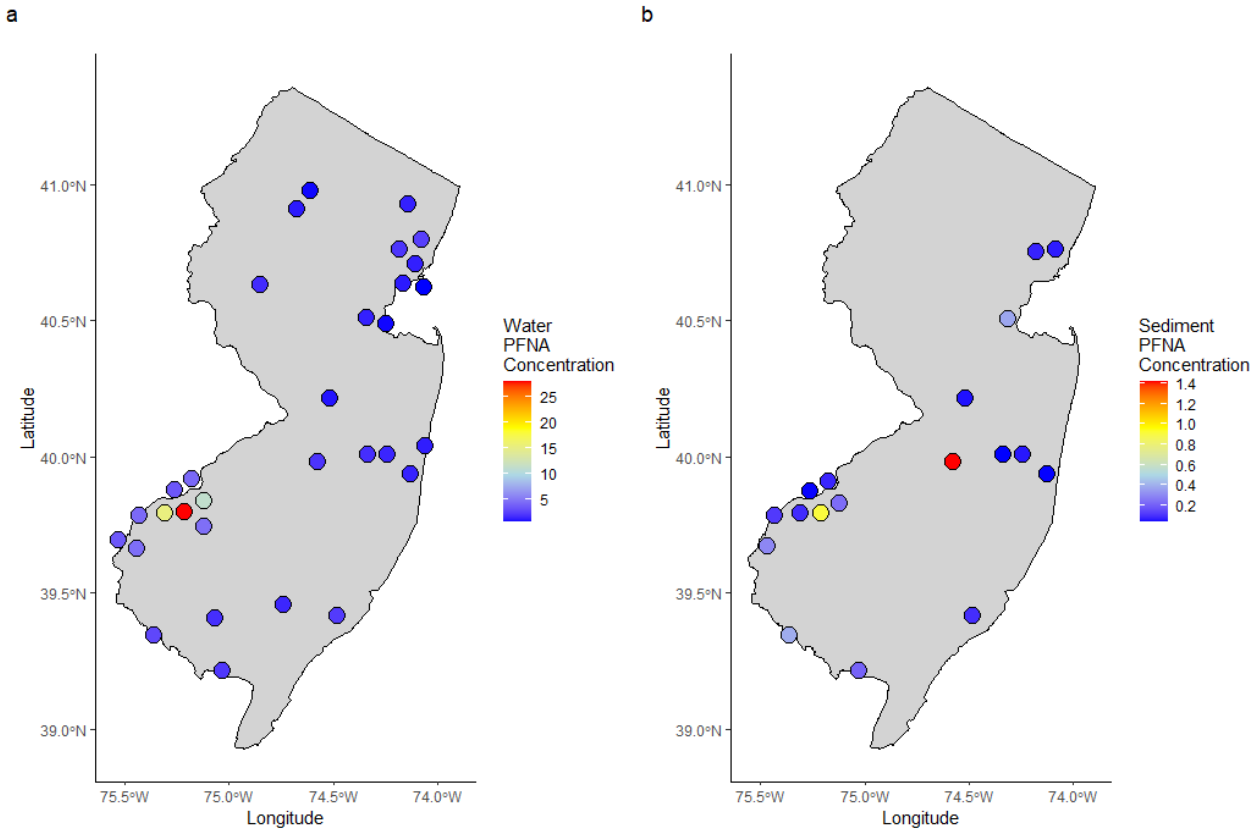

Figure 11. PFNA concentrations in water and sediment varied spatially across the State of New Jersey.

PFNA BAFs in three species were similar to the BAFs previously reported. Khairy (2019) reported PFNA BAF for American Eel of 525; our statewide estimate was 188 (95% UCL 541) (Table 5). Previous reported BAFS of PFNA in common carp were 152 (95% UCL 2104) (Meng et al., 2019; Murakami et al., 2011; Pan et al., 2017; Wang et al., 2013; Zhou et al., 2012); our estimate for statewide common carp was 154 (95% UCL 1190) (Table 26). White perch PFNA BAF was previously reported at 407 (95% UCL 530) (Khairy, 2019); our saline estimated PFNA BAF was 202 (95% UCL 975) (Table 14).

PFOA – EPA Methods

The PFOA concentration in water across the state varied from 1.45 to 25.7 ng/L (Figure 12). The highest concentrations were observed at SAL, LMC, and UP with concentrations of 25.7, 22.2, and 15.4 ng/L, respectively. Combining the observations of all fish species, the BAF within saline sites and non-saline sites were not significantly different, and estimated at 34.1 (95% UCL, 158) and 12.6 (95% UCL, 109), respectively (Table 50). The PFOA concentration in sediment across the state varied from 0.038 to 2.59 ng/g. The highest concentrations were observed at BPL, SAL, and RR with concentrations of 2.59, 0.559, and 0.47 ng/g, respectively.

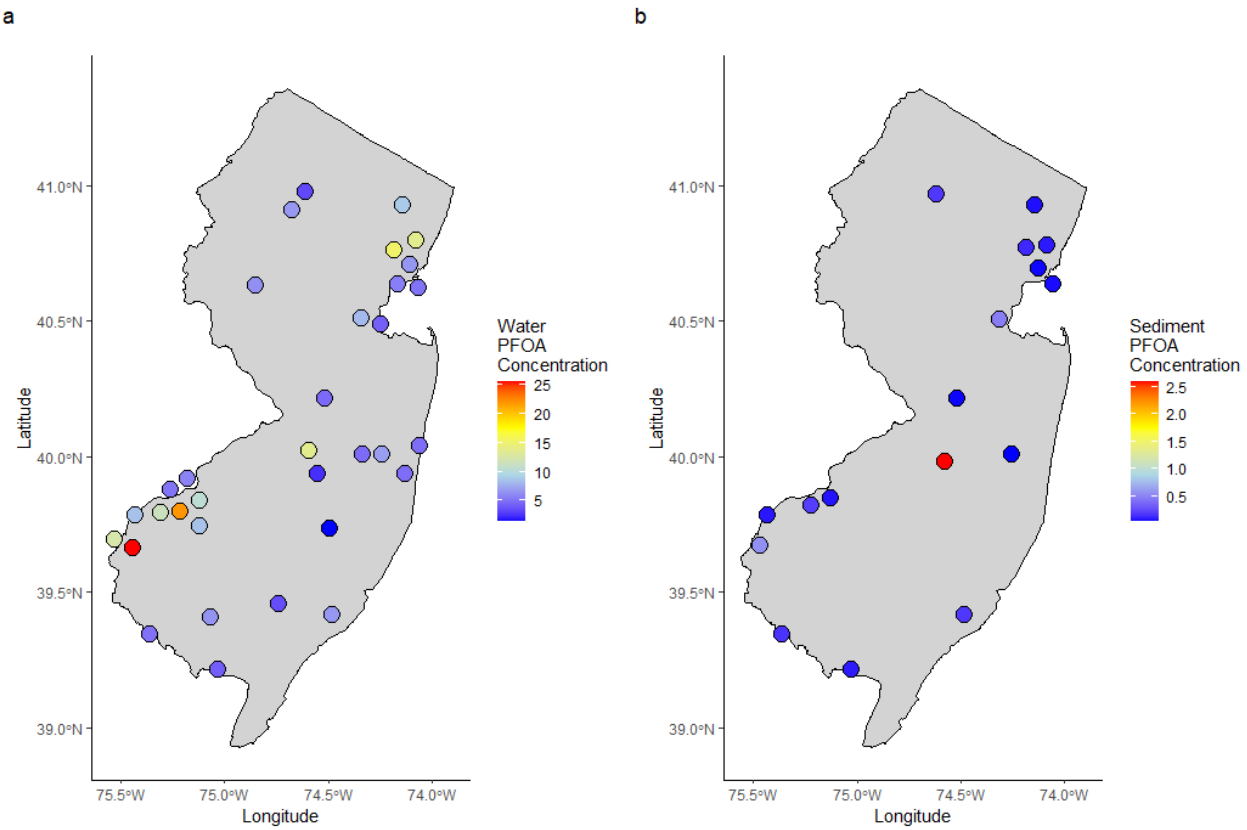

Figure 12. PFOA concentrations in water and sediment varied spatially across the State of New Jersey.

For two species of fish our estimates of PFOA BAF were less than those reported in the literature. PFOA BAFs were previously reported in American Eel at 1125 (Khairy et al., 2019); our statewide estimate was 18.3 (95% UCL 146) (Table 5). PFOA BAFs were previously reported in white perch at 544 (95% UCL 691) (Khairy et al., 2019); our statewide estimate was 19.1 (95% UCL 69.7) (Table 14).

Three species PFOA BAFs in the literature were approximate to our estimates. PFOA BAFs were previously reported in common carp at 25 (96% UCL 144) (Bhavsar et al., 2016; Meng et al., 2019; Murakami et al., 2011; Pan et al., 2017; Wang et al., 2013; Zhou et al., 2012); our statewide estimate was 8.33 (95% UCL, 83.3) (Table 26). PFOA BAFs were previously reported in largemouth bass at 8 (Bhavsar et al., 2016); our statewide estimate was 23.7 (95% UCL 62.8) (Table 29). PFOA BAFs were previously reported in pumpkinseed at 10 (Bhavsar et al., 2016); our estimate was 10.5 (95% UCL 191) (Table 32).

Sediment BAFs – Bayesian Methods

Sediment PFAS BAFs were calculated for all species irrespective of their habitat. Benthic piscivores and foragers were estimates of BAF for all compounds tested against the hypothesis that sediment BAFs would differ by water quality groups and no statistically significant differences were observed. There are values in the literature which we can use to compare our estimates. Estimates in literature were generated through frequentist techniques; however, our Bayesian methods give comparable results. Sediment BAFs are reported here as a unitless mass-mass ratio, all values reported from the literature are in the same unitless mass-mass.

Khairy et al. (2019) observed sediment BAFs for White Perch-PFNA to be 13.6 (95% UCL; 18.3); our estimate was lower, at 2.42 (IQR; 1.12, 3.93).

Common carp sediment BAFs for PFOA were previously reported at 0.73 (95% UCL; 1.36) (Meng et al 2019, Bhavsar et al 2016); our estimate was approximate at 0.49 (IQR; 0.44, 0.57). Pumpkin seed sediment BAF for PFOA in the literature is reported at 0.91, marginally lower than our estimate of 3.27 (IQR; 1.87, 4.91). White perch PFOA sediment BAF was previously estimated at 23.25 (95% UCL; 41.05) (Khairy et al., 2019); our estimated sediment BAF for PFOA in white perch was lower at 1.55 (IQR: 1.11, 2.91).

PFOS sediment BAFs are the most common in the literature and were also the most common compound-species combination in our data. Goodrow et al. (2020) reported sediment BAF for American eel-PFOS at 10.97; our estimate was approximate to this value at 33.26 (IQR; 9.75, 86.99). Bluegill sediment BAF for PFOS was reported at 20 (95% UCL; 97) (Goodrow et al., 2020; Lee et al., 2020); our estimate was approximate to the literature values at 74.26 (IQR; 15.79, 180.83). Goodrow et al. (2020) reported sediment BAF for chain pickerel-PFOS at 4.68; our estimate was larger than to this value at 184.24 (IQR; 36.6, 236.64). Channel catfish PFOS sediment BAF was previously estimated at 17.5 (95% UCL; 74.6) (Goodrow et al., 2020; Bhavsar et al., 2016); our estimate was approximate to this at 6.63 (IQR; 6.14, 7.41). Common carp PFOS sediment BAF in the literature are reported as 26 (95% UCL; 128) (Goodrow et al., 2020; Lee et al., 2020; Bhavsar et al., 2016); our estimate is approximate to this at 58.75 (IQR; 31.48, 75.32). Sediment BAF for largemouth bass has been reported at 23 (95% UCL; 90) (Goodrow et al., 2020; Bhavsar et al., 2016); our estimate of 95.24 (IQR; 29.67, 210.49) is approximate to the literature value. Pumpkinseed PFOS sediment BAF was

reported previously at 8 (95% UCL; 36) (Goodrow et al., 2020; Bhavsar et al., 2016); our estimate was larger at 100.37 (IQR; 47.37, 166.88). Bhavsar et al. (2016) estimated smallmouth bass to have a PFOS sediment BAF at 125.89; our estimate was approximate to this value at 78.32 (IQR; 63.84, 96.59). White perch PFOS sediment BAF is reported across the literature at 20.8 (95% UCL; 49.5) (Khairy et al., 2019; Goodrow et al., 2020); our estimate was approximate to this value at 14.07 (IQR; 9.04, 37.16). Lastly, yellow perch sediment BAF for PFOS in the literature is 8.3 (95% UCL; 19.6) (Goodrow et al., 2020; Bhavsar et al., 2016); our estimate was larger to this value at 102.37 (85.81, 125.58).

In the aggregate, our estimates were approximate to those values of sediment BAF reported in the literature. Differences are likely attributable to the number of individual fish used in previous studies compared to our collections. This site-by-site sample number is difficult to accommodate for in estimates and is a major advantage of our Bayesian methodologies. While we report sediment BAFs for all species collected where data allows, the only benthic species in our study were American eel, oyster toadfish, summer flounder, common carp, and channel catfish. Of these benthic species our estimates were well within what would be expected based on the literature values.

Temporal Variability

Temporal variability of water PFAS concentrations at our sites was a major factor in our study. The pre-samples were vital in determining the BAFs within sites, because they provided data which could be used to fit distributions on compounds which otherwise would have been missed. Approximately half our sites presented detectable concentrations of PFOS compounds in the pre-sample which were not present in the water collected during the fish collection two months later. Future studies must take this temporal heterogeneity into account for their sample designs. The fish species collected at a site are subject to a range of concentrations and PFAS compound mixtures, and their interaction could be a driving factor in BAF estimates. While our data do not support that kind of in-depth analysis of co-variability, it is a topic worthy of additional attention.

Implications for Water Quality Standards

This study provides key insights into the bioaccumulation of PFAS across various waterbodies and fish taxa within New Jersey, offering critical information that can inform the development of surface water quality standards (SWQS). The calculated BAFs for all fish combined across 17 PFAS compounds serves as a foundation for setting more accurate and scientifically grounded regulatory thresholds. Below are the key implications of our findings:

A key finding of this study is the significant variation in BAFs for PFNA and PFOS across salinity and pH groupings. Statewide estimates were calculated by combining data from all fish species, revealing that PFOS BAFs were significantly higher in non-saline low pH waters (3370) compared to saline waters (495) (Table 50). PFNA showed a similar pattern, with BAFs of 81.4 in non-saline environments compared to 216 in saline waters (Table 50). In contrast, PFOA demonstrated relatively consistent bioaccumulation, with a statewide BAF of 18.9 across all environments (Table 50). These findings indicate that bioaccumulation of PFAS is strongly influenced by water chemistry, particularly salinity and pH.

The variation in BAFs among different PFAS compounds complicates the estimation of uniform surface water quality standards. PFOS, for example, exhibited significantly higher bioaccumulation

in non-saline environments compared to saline settings, suggesting that there is an environmental driver for saline to non-saline differences. Similarly, the distinct bioaccumulation patterns between saline and non-saline sites of PFNA reinforce the need for compound-specific research. In contrast, PFOA BAFs showed smaller variations between 34.1 (95% UCL 158) in saline waters and 12.6 (95% UCL 109) in non-saline conditions—indicating that environmental conditions may not be as important for PFOA bioaccumulation. Further research may determine if disproportionate PFAS accumulation in sensitive water bodies provides a more nuanced approach to understanding bioaccumulation patterns.

Our findings indicate that certain species, such as American Eel and Common Carp, exhibit higher rates of PFAS bioaccumulation compared to others, underscoring the importance of understanding species-specific bioaccumulation patterns. These differences highlight how the interaction between species and environmental conditions, such as salinity, can influence BAF values. For instance, the calculated PFOS BAF for American Eel in non-saline waters is 2510 (95% UCL: 5350) (Table 5), illustrating that bioaccumulation can be significantly more pronounced in certain habitats. Moreover, the state-wide BAF for PFOS in American Eel was markedly higher than the average BAF observed across other species, emphasizing the variability in accumulation potential. Recognizing these species- and habitat-specific bioaccumulation trends provides a more nuanced understanding of how PFAS compounds interact with aquatic ecosystems and can inform strategies aimed at minimizing human exposure through fish consumption.

Our study revealed notable variations in PFAS bioaccumulation across different hydrogeological regions, such as Marine, Pineland, and Non-Pineland areas, however, the most applicable means of dividing sampling sites was water quality (Figure 3). How factors like pH and salinity can influence bioaccumulation patterns, suggesting that water quality characteristics may play a more critical role than traditional regional or regulatory boundaries. For example, elevated BAFs observed in non-saline low pH areas underscore the impact of specific environmental conditions, which may similarly affect bioaccumulation in other surface waters with comparable pH and salinity profiles. Understanding these patterns provides valuable insight into how water quality parameters can drive bioaccumulation, offering a framework for assessing PFAS impacts across diverse aquatic environments.

The use of Bayesian methods to handle observations below detection limits allowed for more accurate estimation of BAFs, particularly in cases where traditional methods might underestimate bioaccumulation potential. Additionally, the Bayesian methods provide a more accurate accounting of uncertainty and data structure (e.g., concentrations and BAFs are left censored at zero). Bayesian results also reduce the extreme values of the 95% UCLs observed in EPA method estimates for species where only a small number of individuals or few sites were observed to co-occur. We recommend that NJDEP consider reassessing methods for handling below detection limits data for PFAS compounds which could pose ecological risks. This would ensure that monitoring programs are sensitive enough to capture potential exceedances and provide a more accurate basis for compliance assessments.

The temporal variability observed in PFAS concentrations underscores the need for standards and field sampling plans that account for fluctuations over time. Seasonal changes were missed in our variations between pre-sampling and fish collection periods because of the specific sampling requirements based on fish species occurrence, however seasonality could highlight the importance

of defining temporal benchmarks for compliance. Incorporating time-based metrics into further studies, such as seasonal average concentrations or peak allowances, could improve the precision of subsequent refinements to our reported PFAS BAFs.

The findings of this study provide a robust scientific foundation for refining New Jersey's surface water quality standards for PFAS. Here we have given special attention to PFOS, PFOA, and PFNA in our discussion, however the data and estimates provided across all 43 PFAS compounds are equally valuable (Appendix A). By accounting for compound-specific, species-specific, and regionally adjusted behaviors, along with improved handling of detection limits and seasonal temporal variation, NJDEP can develop a regulatory framework that effectively addresses the complexities of PFAS bioaccumulation. This approach will help safeguard ecological health, water quality, and human health via fish consumption across New Jersey's diverse surface water environments.

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APPENDIX A:

Complete Tables of “EPA Methods” for BAFs

Table A- 1: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for American Eel within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Group 2 & 3	Statewide
PFHxA	- (-)	8.56 (437)	28.5 (70.7)	11.6 (333)	11.6 (333)
PFNA	183 (841)a	188 (580)a	201 (1880)a	190 (603)	188 (541)
PFDA	1000 (6370)a	2650 (12600)a	2980 (36900)	2680 (12200)	1930 (8550)
PFUNDA	8420 (22500)	18500 (62000)	- (-)	18500 (62000)	17000 (55600)
PFHxS	39.8 (532)a	51.7 (4700)a	227 (1250)a	69.5 (3790)	61.1 (2930)
PFOS	628 (923)b	2190 (4500)b	3780 (12800)a	2510 (5350)	1720 (3790)
PFOA	39.5 (290)a	13.5 (100)b	30.9 (857)a	15.3 (162)	18.3 (146)
EtFOSAA	233 (11000)	397 (9080)	- (-)	397 (9080)	333 (7150)
PFPeS	- (-)	38.4 (1940)	107 (119)	64 (1010)	64 (1010)
x6_2_FTS	- (-)	185 (2020)	- (-)	185 (2020)	185 (2020)
PFHpA	- (-)	30.4 (968)	112 (5700)	42.1 (1610)	42.1 (1610)
PFBS	- (-)	3.66 (11.3)	- (-)	3.66 (11.3)	3.66 (11.3)
PFHpS	- (-)	344 (11900)	- (-)	344 (11900)	344 (11900)
PFOSA	- (-)	- (-)	- (-)	- (-)	- (-)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	3	1	4	4
PFNA_n	4	12	2	14	18
PFDA_n	5	9	1	10	15
PFUNDA_n	1	8	0	8	9
PFHxS_n	3	8	2	10	13
PFOS_n	6	12	4	16	22
PFOA_n	3	11	2	13	16
EtFOSAA_n	1	2	0	2	3
PFPeS_n	0	1	1	2	2
x6_2_FTS_n	0	2	0	2	2
PFHpA_n	0	3	1	4	4
PFBS_n	0	1	0	1	1
PFHpS_n	0	2	0	2	2
PFOSA_n	0	0	0	0	0
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 2: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Common Carp within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Group 2 & 3	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	154 (1190)	- (-)	154 (1190)	154 (1190)
PFDA	- (-)	2920 (17600)	- (-)	2920 (17600)	2920 (17600)
PFUNDA	- (-)	23800 (89800)	- (-)	23800 (89800)	23800 (89800)
PFHxS	- (-)	44.8 (873)	- (-)	44.8 (873)	44.8 (873)
PFOS	- (-)	1670 (2160)	- (-)	1670 (2160)	1670 (2160)
PFOA	- (-)	8.33 (83.3)	- (-)	8.33 (83.3)	8.33 (83.3)
EtFOSAA	- (-)	667 (14500)	- (-)	667 (14500)	667 (14500)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	521 (9080)	- (-)	521 (9080)	521 (9080)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	4	0	4	4
PFDA_n	0	4	0	4	4
PFUNDA_n	0	3	0	3	3
PFHxS_n	0	4	0	4	4
PFOS_n	0	4	0	4	4
PFOA_n	0	1	0	1	1
EtFOSAA_n	0	1	0	1	1
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	2	0	2	2
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 3: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Pumpkinseed within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Group 2 & 3	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	52.8 (826)	134 (2150)	66.7 (836)	66.7 (836)
PFDA	- (-)	1860 (8300)	- (-)	1860 (8300)	1860 (8300)
PFUNDA	- (-)	25300 (82800)	- (-)	25300 (82800)	25300 (82800)
PFHxS	- (-)	- (-)	176 (547)	176 (547)	176 (547)
PFOS	- (-)	1810 (2490)	3770 (3880)	2180 (2680)	2180 (2680)
PFOA	- (-)	7.66 (368)	14.4 (42.5)	10.5 (191)	10.5 (191)
EtFOSAA	- (-)	496 (6560)	- (-)	496 (6560)	496 (6560)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	796 (11200)	- (-)	796 (11200)	796 (11200)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	3	1	4	4
PFDA_n	0	2	0	2	2
PFUNDA_n	0	2	0	2	2
PFHxS_n	0	0	1	1	1
PFOS_n	0	3	1	4	4
PFOA_n	0	1	1	2	2
EtFOSAA_n	0	1	0	1	1
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	2	0	2	2
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 4: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Yellow Perch within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Group 2 & 3	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	172 (1350)	- (-)	172 (1350)	172 (1350)
PFDA	- (-)	2720 (8730)	- (-)	2720 (8730)	2720 (8730)
PFUNDA	- (-)	37300 (132000)	- (-)	37300 (132000)	37300 (132000)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	- (-)	2150 (3290)	- (-)	2150 (3290)	2150 (3290)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	2710 (44000)	- (-)	2710 (44000)	2710 (44000)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	1	0	1	1
PFDA_n	0	1	0	1	1
PFUNDA_n	0	1	0	1	1
PFHxS_n	0	0	0	0	0
PFOS_n	0	1	0	1	1
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	1	0	1	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 5: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for White Perch within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	23.4 (859)	- (-)	23.4 (859)	23.4 (859)
PFNA	202 (975)a	43.8 (746)a	- (-)	43.8 (746)	88.7 (608)
PFDA	991 (4110)a	1720 (3830)a	- (-)	1720 (3830)	1390 (3160)
PFUNDA	5980 (17700)a	7740 (21500)a	- (-)	7740 (21500)	7310 (18400)
PFHxS	37.3 (769)	44.4 (2290)	- (-)	44.4 (2290)	38.2 (742)
PFOS	911 (1410)a	2040 (3500)a	- (-)	2040 (3500)	1480 (2380)
PFOA	19.1 (69.7)	- (-)	- (-)	- (-)	19.1 (69.7)
EtFOSAA	707 (14800)a	743 (11200)a	- (-)	743 (11200)	731 (9140)
PFPeS	40 (1960)	- (-)	- (-)	- (-)	40 (1960)
x6_2_FTS	219 (3330)	121 (978)	- (-)	121 (978)	163 (1780)
PFHpA	34 (1580)	- (-)	- (-)	- (-)	34 (1580)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	240 (812)	- (-)	- (-)	- (-)	240 (812)
PFOSA	1550 (15500)a	2430 (11500)a	- (-)	2430 (11500)	2090 (9720)
PFDoA	- (-)	5670 (108000)	- (-)	5670 (108000)	5670 (108000)
MeFOSAA	- (-)	774 (16900)	- (-)	774 (16900)	774 (16900)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	2	0	2	2
PFNA_n	6	7	0	7	13
PFDA_n	5	8	0	8	13
PFUNDA_n	2	7	0	7	9
PFHxS_n	6	1	0	1	7
PFOS_n	6	9	0	9	15
PFOA_n	5	0	0	0	5
EtFOSAA_n	2	4	0	4	6
PFPeS_n	1	0	0	0	1
x6_2_FTS_n	1	1	0	1	2
PFHpA_n	1	0	0	0	1
PFBS_n	0	0	0	0	0
PFHpS_n	1	0	0	0	1
PFOSA_n	3	6	0	6	9
PFDoA_n	0	1	0	1	1
MeFOSAA_n	0	1	0	1	1
PFBA_n	0	0	0	0	0

Table A- 6: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Largemouth Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	31 (357)	- (-)	31 (357)	31 (357)
PFDA	- (-)	4080 (23600)	6950 (83800)	4300 (23500)	4300 (23500)
PFUNDA	- (-)	22800 (133000)	- (-)	22800 (133000)	22800 (133000)
PFHxS	- (-)	9.1 (275)	109 (1560)	20.8 (537)	20.8 (537)
PFOS	- (-)	4020 (6650)a	5260 (10500)a	4160 (6550)	4160 (6550)
PFOA	- (-)	11.2 (13.7)	49.7 (128)	23.7 (62.8)	23.7 (62.8)
EtFOSAA	- (-)	851 (17300)	- (-)	851 (17300)	851 (17300)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	171 (3010)	- (-)	171 (3010)	171 (3010)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	120 (1860)	- (-)	120 (1860)	120 (1860)
PFOSA	- (-)	539 (6720)	- (-)	539 (6720)	539 (6720)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	7	0	7	7
PFDA_n	0	9	1	10	10
PFUNDA_n	0	7	0	7	7
PFHxS_n	0	2	1	3	3
PFOS_n	0	13	2	15	15
PFOA_n	0	1	1	2	2
EtFOSAA_n	0	1	0	1	1
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	2	0	2	2
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	1	0	1	1
PFOSA_n	0	5	0	5	5
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 7: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Chain Pickerel within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	63.9 (153)	- (-)	63.9 (153)	63.9 (153)
PFDA	- (-)	- (-)	955 (14800)	955 (14800)	955 (14800)
PFUNDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxS	- (-)	14.6 (60.1)	219 (776)	56.6 (336)	56.6 (336)
PFOS	- (-)	2520 (3590)a	2050 (7370)a	2200 (5760)	2200 (5760)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	227 (2760)	- (-)	227 (2760)	227 (2760)
PFOSA	- (-)	- (-)	257 (12900)	257 (12900)	257 (12900)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	1	0	1	1
PFDA_n	0	0	1	1	1
PFUNDA_n	0	0	0	0	0
PFHxS_n	0	1	1	2	2
PFOS_n	0	2	4	6	6
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	1	0	1	1
PFOSA_n	0	0	1	1	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 8: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Bluegill within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	50.1 (2730)	- (-)	50.1 (2730)	50.1 (2730)
PFNA	- (-)	105 (351)	- (-)	105 (351)	105 (351)
PFDA	- (-)	3700 (20700)	1730 (22400)	3320 (18200)	3320 (18200)
PFUNDA	- (-)	15900 (133000)	- (-)	15900 (133000)	15900 (133000)
PFHxS	- (-)	29.9 (1260)a	88.1 (487)a	46.1 (799)	46.1 (799)
PFOS	- (-)	3010 (4520)a	2840 (4450)a	2970 (4190)	2970 (4190)
PFOA	- (-)	11.8 (289)	77.7 (1530)	16.1 (351)	16.1 (351)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	91.5 (4170)	- (-)	91.5 (4170)	91.5 (4170)
PFBS	- (-)	49.6 (2270)	- (-)	49.6 (2270)	49.6 (2270)
PFHpS	- (-)	299 (1720)	- (-)	299 (1720)	299 (1720)
PFOSA	- (-)	619 (7390)	- (-)	619 (7390)	619 (7390)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	186 (2390)	- (-)	186 (2390)	186 (2390)
PFHxA_n	0	1	0	1	1
PFNA_n	0	8	0	8	8
PFDA_n	0	6	1	7	7
PFUNDA_n	0	4	0	4	4
PFHxS_n	0	3	2	5	5
PFOS_n	0	10	3	13	13
PFOA_n	0	5	1	6	6
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	2	0	2	2
PFBS_n	0	1	0	1	1
PFHpS_n	0	2	0	2	2
PFOSA_n	0	2	0	2	2
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	1	0	1	1

Table A- 9: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Northern Puffer within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	- (-)	- (-)	- (-)	- (-)
PFDA	646 (9430)	- (-)	- (-)	- (-)	646 (9430)
PFUNDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	301 (642)	- (-)	- (-)	- (-)	301 (642)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	290 (5170)	- (-)	- (-)	- (-)	290 (5170)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	0	0	0	0
PFDA_n	2	0	0	0	2
PFUNDA_n	0	0	0	0	0
PFHxS_n	0	0	0	0	0
PFOS_n	2	0	0	0	2
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	1	0	0	0	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 10: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Spot within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	294 (1720)	107 (1360)	- (-)	107 (1360)	248 (1460)
PFDA	2050 (9650)	752 (2740)	- (-)	752 (2740)	1670 (7770)
PFUNDA	7850 (21700)	4110 (24100)	- (-)	4110 (24100)	5680 (17800)
PFHxS	93.6 (885)	- (-)	- (-)	- (-)	93.6 (885)
PFOS	1220 (1900)	980 (1690)	- (-)	980 (1690)	1180 (1780)
PFOA	38.7 (283)	7.96 (406)	- (-)	7.96 (406)	29.7 (244)
EtFOSAA	- (-)	915 (16000)	- (-)	915 (16000)	915 (16000)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	4.89 (60)	- (-)	- (-)	- (-)	4.89 (60)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	3040 (33600)	1920 (5540)	- (-)	1920 (5540)	2600 (23000)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	5	1	0	1	6
PFDA_n	4	1	0	1	5
PFUNDA_n	1	1	0	1	2
PFHxS_n	3	0	0	0	3
PFOS_n	6	1	0	1	7
PFOA_n	5	1	0	1	6
EtFOSAA_n	0	1	0	1	1
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	1	0	0	0	1
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	2	1	0	1	3
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 11: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Oyster Toadfish within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	315 (1060)	- (-)	- (-)	- (-)	315 (1060)
PFDA	1370 (6250)	- (-)	- (-)	- (-)	1370 (6250)
PFUNDA	9070 (21900)	- (-)	- (-)	- (-)	9070 (21900)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	716 (1180)	- (-)	- (-)	- (-)	716 (1180)
PFOA	46.4 (394)	- (-)	- (-)	- (-)	46.4 (394)
EtFOSAA	1190 (19000)	- (-)	- (-)	- (-)	1190 (19000)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	167 (8840)	- (-)	- (-)	- (-)	167 (8840)
PFOSA	1910 (27600)	- (-)	- (-)	- (-)	1910 (27600)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	5	0	0	0	5
PFDA_n	4	0	0	0	4
PFUNDA_n	1	0	0	0	1
PFHxS_n	0	0	0	0	0
PFOS_n	6	0	0	0	6
PFOA_n	4	0	0	0	4
EtFOSAA_n	1	0	0	0	1
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	1	0	0	0	1
PFOSA_n	1	0	0	0	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 12: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Channel Catfish within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	42.9 (85.2)	- (-)	42.9 (85.2)	42.9 (85.2)
PFDA	- (-)	773 (3010)	- (-)	773 (3010)	773 (3010)
PFUNDA	- (-)	4950 (16300)	- (-)	4950 (16300)	4950 (16300)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	- (-)	306 (694)	- (-)	306 (694)	306 (694)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	578 (3930)	- (-)	578 (3930)	578 (3930)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	2	0	2	2
PFDA_n	0	2	0	2	2
PFUNDA_n	0	2	0	2	2
PFHxS_n	0	0	0	0	0
PFOS_n	0	2	0	2	2
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	2	0	2	2
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 13: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Smallmouth Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	74.5 (259)	- (-)	74.5 (259)	74.5 (259)
PFDA	- (-)	7630 (45100)	- (-)	7630 (45100)	7630 (45100)
PFUNDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	- (-)	5430 (12100)	- (-)	5430 (12100)	5430 (12100)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	296 (1430)	- (-)	296 (1430)	296 (1430)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	479 (10100)	- (-)	479 (10100)	479 (10100)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	1	0	1	1
PFDA_n	0	1	0	1	1
PFUNDA_n	0	0	0	0	0
PFHxS_n	0	0	0	0	0
PFOS_n	0	3	0	3	3
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	1	0	1	1
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	1	0	1	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 14: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Summer Flounder within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	28.2 (1600)	- (-)	- (-)	- (-)	28.2 (1600)
PFNA	136 (3270)	- (-)	- (-)	- (-)	136 (3270)
PFDA	640 (783)	- (-)	- (-)	- (-)	640 (783)
PFUNDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	353 (939)	- (-)	- (-)	- (-)	353 (939)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	3280 (46100)	- (-)	- (-)	- (-)	3280 (46100)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	1	0	0	0	1
PFNA_n	2	0	0	0	2
PFDA_n	1	0	0	0	1
PFUNDA_n	0	0	0	0	0
PFHxS_n	0	0	0	0	0
PFOS_n	4	0	0	0	4
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	1	0	0	0	1
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 15: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for Black Sea Bass within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound. Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	- (-)	- (-)	- (-)	- (-)	- (-)
PFNA	- (-)	- (-)	- (-)	- (-)	- (-)
PFDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFUNDA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOS	138 (596)	- (-)	- (-)	- (-)	138 (596)
PFOA	- (-)	- (-)	- (-)	- (-)	- (-)
EtFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFPeS	- (-)	- (-)	- (-)	- (-)	- (-)
x6_2_FTS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBS	- (-)	- (-)	- (-)	- (-)	- (-)
PFHpS	- (-)	- (-)	- (-)	- (-)	- (-)
PFOSA	- (-)	- (-)	- (-)	- (-)	- (-)
PFDoA	- (-)	- (-)	- (-)	- (-)	- (-)
MeFOSAA	- (-)	- (-)	- (-)	- (-)	- (-)
PFBA	- (-)	- (-)	- (-)	- (-)	- (-)
PFHxA_n	0	0	0	0	0
PFNA_n	0	0	0	0	0
PFDA_n	0	0	0	0	0
PFUNDA_n	0	0	0	0	0
PFHxS_n	0	0	0	0	0
PFOS_n	2	0	0	0	2
PFOA_n	0	0	0	0	0
EtFOSAA_n	0	0	0	0	0
PFPeS_n	0	0	0	0	0
x6_2_FTS_n	0	0	0	0	0
PFHpA_n	0	0	0	0	0
PFBS_n	0	0	0	0	0
PFHpS_n	0	0	0	0	0
PFOSA_n	0	0	0	0	0
PFDoA_n	0	0	0	0	0
MeFOSAA_n	0	0	0	0	0
PFBA_n	0	0	0	0	0

Table A- 16: EPA methodologies were used for all compounds to determine aqueous BAFs (L/kg) for all fish species combined within and across the groupings defined by salinity/pH grouping analysis [Geometric Mean (95% Upper Confidence Interval)]. Letter (i.e., a,b,c) indicate statistical differences based on salinity/pH groups. Rows with "_n" indicate the number of site level estimates of BAF which were available per group for each compound Entries recorded as "- (-)" represent where no unique combination of PFAS were detected in both fish and water throughout the study.

Compounds	Saline	Non-Saline High pH	Non-Saline Low pH	Non-Saline	Statewide
PFHxA	28.2 (1590)	21.6 (970)	28.5 (70.6)	23.9 (967)	24.9 (834)
PFNA	216 (949)a	79.7 (293)b	164 (1480)ab	81.4 (295)	106 (409)
PFDA	1020 (3360)a	2320 (7300)a	2420 (24300)a	2130 (6750)	1770 (5460)
PFUNDA	7740 (14300)a	14200 (37800)a	- (-)	14200 (37800)	13800 (35000)
PFHxS	51.8 (447)b	27.2 (922)b	153 (542)a	53.9 (702)	55.8 (471)
PFOS	495 (681)b	1940 (2670)b	3370 (5730)a	1970 (2770)	1170 (1740)
PFOA	34.1 (158)a	9.84 (112)a	36.2 (455)a	12.6 (109)	18.9 (91.4)
EtFOSAA	581 (8970)a	651 (5680)a	- (-)	651 (5680)	690 (5560)
PFPeS	40 (1960)	38.4 (1940)	107 (119)	64 (1010)	50.6 (1120)
x6_2_FTS	219 (3340)	183 (1100)	- (-)	183 (1100)	198 (1180)
PFHpA	12.9 (786)a	52.7 (2140)a	112 (5700)a	62.1 (2240)	28.3 (1190)
PFBS	- (-)	13.5 (1120)	- (-)	13.5 (1120)	13.5 (1120)
PFHpS	200 (4550)a	230 (3240)a	- (-)	230 (3240)	220 (2700)
PFOSA	1540 (13600)a	923 (6090)a	257 (12900)a	812 (5630)	902 (6460)
PFDoA	- (-)	5670 (108000)	- (-)	5670 (108000)	5670 (108000)
MeFOSAA	- (-)	774 (16900)	- (-)	774 (16900)	774 (16900)
PFBA	- (-)	186 (2380)	- (-)	186 (2380)	186 (2380)
PFHxA_n	1	3	1	3	4
PFNA_n	5	11	2	11	13
PFDA_n	6	10	4	11	14
PFUNDA_n	4	9	0	9	10
PFHxS_n	3	6	5	7	8
PFOS_n	7	11	5	11	15
PFOA_n	4	6	4	6	8
EtFOSAA_n	3	6	0	6	7
PFPeS_n	1	1	1	1	2
x6_2_FTS_n	1	4	0	4	4
PFHpA_n	2	2	1	2	4
PFBS_n	0	2	0	2	2
PFHpS_n	2	4	0	4	6
PFOSA_n	5	9	1	10	13
PFDoA_n	0	1	0	1	1
MeFOSAA_n	0	1	0	1	1
PFBA_n	0	1	0	1	1

APPENDIX B:

Quality Assurance and Quality Control Plan: Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems

Quality Assurance and Quality Control Plan:
Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems

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Note: all signatories will be notified of any major changes to the QAPP prior to implementation of such changes.

Project Information Page:

1. Project Name

Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems

2. Date of Project Initiation

2/15/2022 (effective date of contract between NJDEP and NJ Sea Grant).

3. ANS Co-Project Managers

David Keller, Ph.D., Fisheries Section Leader, Patrick Center for Environmental Research, The Academy of Natural Sciences of Drexel University

Marie J. Kurz, Ph.D., Research Associate, Patrick Center for Environmental Research, The Academy of Natural Sciences of Drexel University

4. Quality Assurance Officer

Joseph Heiczinger will serve as the QA officer for this project.

5. Project Description

Objective and Scope Statement

Funding for this work is provided by NJDEP and establishing SWQS for PFOA, PFNA and PFOS was the primary driving force for this project. We are planning a study based on the following: a) the primary objective of calculating water to fish bioaccumulation factors (BAFs) for per- and polyfluoroalkyl substances (PFAS) that can be used across the range of New Jersey waterbodies; b) our initial discussions with NJDEP of site categorization (marine and freshwater; streams and lakes; Pinelands and non-Pinelands) and fish stratification to better understand habitat and trophic related factors (e.g., benthic v. pelagic or forager v. piscivore); c) emphasis on multiple water and individual fish samples to provide information on sources of variability in concentrations; and d) recognition of the need for an efficient sampling design balancing the number sites against the breadth of data collected at each site. For the purposes of this proposal “marine” refers to anything in the gradient of tidal freshwater to ocean salinity.

In summary, we will sample at 16 marine sites in Year 1, these include: 1) upper estuary sites (UE) with variable flow and salinities that may range from fresh to brackish water; and 2) lower estuary or open/bay sites (OWB) with limited non-tidal flow and salinities that may range from brackish to ocean water (Table 1 and 2). In Year 2 we will sample at 18 freshwater sites including: 1) lakes (with deep and shallow hydrology) and streams, in 2) systems in Pineland and non-Pineland areas (Table 1). Select presampling will occur both years to verify and assess the temporal variability of aqueous PFAS concentrations. Candidate and final site selection will be decided in consultation with NJDEP staff. At all

sites we aim to collect 3 fish species and 3 individuals of each species, representing key dietary (forager vs. predator) and habitat (pelagic vs. benthic) PFAS exposure pathways. All environmental and fish filet samples will be analyzed for PFAS concentrations. Fish samples will be further analyzed for stable isotope compositions to verify trophic level. Water and sediment samples will be further analyzed for chemical parameters demonstrated to substantially affect the solid-water distribution and bioavailability of PFAS. The following sections outline the details and justification of this study design and the key considerations that should inform site selection.

6. Data Usage

These data will be used to develop a statewide BAF for each species selected for analysis. Depending on the data acquired, these data may be used to develop BAFs by region, site, type of site, trophic level, habitat, etc. These data may also be used to describe relationships among PFAS and environmental gradients or factors such as salinity. **The analytical data collected during this project was not measured in a NJ certified environmental laboratory. Therefore, results cannot be used for regulatory/compliance purposes.**

7. Data Quality Objectives

The data acquired under this project will be used to produce BAFs and will be used to make decisions concerning fish consumption advisories for human health. Per our conversation with the NJDEP Co-managers, a minimum of 6 individuals across a minimum of 2 sites will be used to calculate a species-specific BAF.

8. Monitoring Network Design and Rationale

Site selection will rely heavily on the criteria and specific recommendations of NJDEP staff. The following criteria will be considered when selecting sites:

- Site categorization: Sites will span a range of different aquatic environments found in the state, including marine and freshwater; streams and lakes; Pinelands and non-Pinelands. For lakes, depth and turnover time will be taken into consideration as there may be differences in PFAS bioavailability between shallow and deeper lakes due to differing degrees of water-sediment interaction, groundwater interactions, and evaporation. The Pineland systems will equally be considered separately because their unique pH and carbon chemistry may also result in unique PFAS partitioning and bioavailability relative to non-Pineland systems. Moreso, pH and organic carbon concentration are both known to effect PFAS sorption, bioavailabilty and uptake. For this reason, we predict that PFAS BAFs in the Pinelands (low pH, high DOC waters) may be distinctly different from those in the rest of the state. Also, initial conversations with NJDEP staff emphasized a Pinelands vs. non-Pinelands focus.
- Environmental PFAS concentrations: This study will focus on sites with higher environmental concentrations that are most likely to produce detectable water and fish concentrations for a number of PFAS compounds. Discharge permits and known contaminated sites will help inform where concentrations are likely high. Existing environmental PFAS data plus preliminary project sampling will be used to verify likely environmental PFAS conditions at the time of fish collection.
- Diversity of PFAS sources: The source(s) of PFAS to the environment will affect the absolute and relative concentrations of the various PFAS compounds. The selected sites should be

representative of a range of prospective PFAS sources, including sites with historic use of aqueous film forming foams on military and airport installations (esp. Joint Base McGuire-DixLakehurst), manufacturing locations (esp. Solvay Specialty Polymers USA LLC, West Deptford, NJ), and major wastewater treatment effluent inputs.

- Fish species consistency: Where possible, sites should be located to maximize the probability of sampling the same fish species across multiple sites.
- Proximity to underrepresented communities: Where possible, sites should be located in/adjacent to communities that may be disproportionately influenced by environmental contamination and/or have higher risk of PFAS exposure through environmental or dietary pathways.

9. Sampling Procedures

All samples will be collected, held and processed in such a way as to minimize PFAS contamination, as outlined in the subsections below, regardless of whether they are intended for PFAS analysis or not. Field sampling personnel for the project will be trained in the operation and use of all sampling equipment including the proper safety and handling procedures for electroshocking equipment. The sampling and processing of the samples will be carried out by trained personnel aware of the risk of sample contamination posed by incorrect handling.

9.1 Sampling materials and supplies

Materials screening will be performed on all materials that will come into direct or indirect contact with samples to ensure that all components are PFAS-free. This process will include reviews of the most current Safety Data Sheets (SDS), as the product composition may have changed over time without changing the item. PFAS-containing sampling items include the lining of hoses, tubing, and pipes, wiring and wire insulation, valves, seals, gaskets, gears, coatings on aluminum, aluminized or galvanized steel, plastic bags and bottles, and some labware. If PFAS was used as a component in the manufacturing process of a material (ex. in a mold coating or mist suppressant), it may result in PFAS contamination of the manufactured equipment. No sampling equipment containing known fluoropolymers will be used.

A tiered approach will be used for restrictions on materials used in various stages of the sampling process. First tier items will include sampling materials that come into direct contact with sample media, while second tier items will restrict materials allowed within a staging area or on sampling personnel. Prohibited sampling materials known or suspected to contain PFAS include:

- Polytetrafluoroethylene (PTFE) including Teflon® and Hostaflon®
- Polyvinylidene fluoride (PVDF) including Kynar®
- Polychlorotrifluoroethylene (PCTFE) including Neoflon®
- Ethylene-tetrafluoroethylene (ETFE) including Tefzel®
- Fluorinated ethylene propylene (FEP) including Teflon® FEP and Hostaflon® FEP, and sometimes Neoflon
- Low-density polyethylene (LDPE) (if coming into direct contact with sample media). Note, although LDPE does not contain PFAS in raw material, the manufacturing process may result in PFAS cross-contamination.

- Waterproof paper or adhesive paper including Post-it notes
- Wide-point Sharpie pens

Recommended sampling materials that should be PFAS-free include:

- High-density polyethylene (HDPE), silicone, or acetate bottles and caps (unlined)
- Powderless nitrile gloves
- LDPE bags (ex. Ziploc®) that do NOT come into direct contact with sample media
- Aluminum foil provided the shiny side is placed away from the sample or foil is tested to be PFAS-free (See section 9.7 Fish Collection)
- Fine or ultra-fine point Sharpies, ballpoint pens, pencils, and pre-printed labels, although best to avoid direct contact with sample media. Fine and Ultra-Fine point Sharpie (R) markers have been determined to be acceptable for labeling empty sample bottles, in staging area, while lid is in place and gloves are donned, and changed following sample bottle labeling. This practice is accepted protocol in the State of California (https://www.waterboards.ca.gov/pfas/docs/sept_2020_pfas_sampling_guidelines.pdf) and the State of Michigan (https://www.michigan.gov/pfasresponse/-/media/Project/Websites/PFASResponse/Sampling-Guidance/General.pdf). Sandra Goodrow and Lee Lippincott of the Division of Science and Research have confirmed that this practice is acceptable for the State of NJ.

Protective coatings on various types of clothing, shoes and leather are known to contain PFAS, due to its ability to repel water and oil. Unfortunately, clothing and PPE used for field sampling tend to be chosen as a result of these properties. When sampling for PFAS, it is important to pay particular attention to avoid wearing clothing that is waterproof, water/stain repellant or resistant, as it is likely to have used PFAS during the manufacturing process. Clothing that has been chemically treated for UV or insect resistance, or clothing that has been washed with fabric softener will also be avoided. Allowable clothing materials include polyvinyl chloride (PVC) or wax-coated fabrics, Neoprene, and synthetic or natural fibers if well laundered without using fabric softener.

Personnel should be cognizant of cross-contamination potential with PCPs and related products. Field personnel will be instructed to minimize use of topical personal care products (PCPs) like cosmetics, body lotion, hand sanitizer, bug spray, etc. on sampling days due to the well-documented presence of PFAS in these products. Mechanical protection against insects and sun exposure will be preferentially utilized to reduce the risk of PFAS contamination from chemical protection. Several insect repellants and sunscreens have been analyzed for PFAS presence and found to be PFAS-free. However, product formulations may change over time, so there is no guarantee that allowable products will remain PFASfree. Sunscreens and insect repellants will be evaluated on a case-to-case basis. The following protocols will be followed to minimize samples or sampling equipment coming in contact with PCPs.

- Do not apply or handle PCPs in the sampling area
- Do not apply or handle PCPs while wearing PPE that will be used during sampling
- If application of PCPs is necessary, move to staging area and remove PPE
- Thoroughly wash hands with PFAS-free soap and water after handling or applying PCPs and put on new, powderless nitrile gloves when finished

9.2 Sample site activities

Within a site, efforts will be made to minimize disturbance of the physical environment and biological communities. Only essential personnel will visit the site. The water or banks will not be traversed except when necessary to collect a sample; if the water must be entered or crossed for access only, specific access areas will be marked off to limit disturbance of the broader system and samples will not be collected in disturbed areas. Samples will always be collected working from downstream to upstream. Sample collection will be conducted in the following order: surface water, surface sediment, fish.

9.3 Sample Labeling and Field Documentation

Special considerations should be taken when notetaking and labelling samples in order to prevent sample contamination. Regular or thick sized markers such as Sharpie will not be used. However, fine and ultra-fine Sharpie markers may be acceptable to label empty sample bottles within staging area (lid must remain on and gloves must be changed prior to labelling). Ballpoint pens or pencils may also be used for labelling sample containers. Pre-printed labels from the laboratory may be used, but require screening to determine if materials used are PFAS-free. Sticky notes (Post-it), waterproof paper (Rite in the Rain) and notebooks, and plastic clipboards may not be used in the sampling area. Non-waterproof, non-recycled loose paper and aluminum, polypropylene, or Masonite clipboards may be utilized instead.

9.4 Cleaning and Decontamination

Regardless of collection method, measures will be taken to minimize sampling equipment (tubing, trowels, nets, electrofishing equipment, etc.) coming into direct contact with specimens. It is imperative that proper PPE be worn at all times during sampling and all materials used have been prescreened for the presence of PFAS, pre-cleaned, and decontaminated after/between uses.

Non-disposable field sampling equipment used at multiple sampling locations or sites will be cleaned with a multi-stage decontamination process. After use, equipment shall be cleaned of any debris or solids, rinsed with deionized water, then scrubbed with phosphate free soap (Liquinox or Alconox, diluted in deionized water). The equipment should then be triple rinsed with deionized water. The final step involves triple rinsing with methanol, collecting the methanol rinsate for appropriate disposal. Equipment should then be air dried then covered or wrapped in HDPE plastic sheeting for storage. Limited reuse of essential, specialized fish sampling equipment that cannot be decontaminated may be unavoidable, including gill nets, traps, pot gear, and electrofishing equipment. These components will only come in direct contact with specimens for minimal time and will be rinsed with site water when deploying.

Hands will be well-washed with PFAS-free soap and water prior to collection of any samples. Clean, powderless nitrile gloves must be worn both before and during all sample collection, handling sampling equipment and sample containers. Gloves will be changed any time there is an opportunity for crosscontamination of the sampling including, but not limited to between sample collection and labeling, and any time after handling prohibited materials. Sample containers must remain sealed until sample collection occurs. Lids or caps will not be placed on the ground or any surface that may not be

PFASfree. Cap may be held in a clean, gloved hand or placed on clean cotton sheet or other known PFAS-free material.

9.5 Water collection

Surface water samples will be collected at each site on the same day the fish are collected and during pre-sampling. During pre-sampling, water will be collected within 2.5 hrs of low tide. During fish sampling, water will be collected without regard for tidal stage. In the absence of conditions detailed in the dynamic sampling scheme below, this project will use the explicit assumption that surface samples (~30 cm) from well mixed waters are representative of conditions experienced by the target fish species and appropriate for estimating BAFs. This assumption is based on the observed tendency for PFAS to accumulate at the air-surface boundary (Casas et al., 2020; Costanza et al., 2019; Ju et al., 2008), and previous BAFs developed in New Jersey which also used surface (~20 cm) samples in the Passaic River (Khairy et al., 2019).

Water collection during the combined fish and water collection dates will use a dynamic sampling scheme triggered by sample site depth and salinity profiles. Sample sites with greater than 3.5 m will have water samples collected at the surface and bottom. Additionally, at each sample site a profile of salinity will be measured at each 1 m increment in order to detect potential salt wedge conditions. If a salt wedge is detected, surface and bottom samples will be collected. If neither of the dynamic sampling triggers are met, a single surface sample will be collected. Note that passive collecting gear may be used to collect fish over time scales that are greater than a single tidal cycle and that we are targeting resident fish that have been at the site for multiple weeks if not longer. These fish are experiencing approximately two highs and two lows a day for multiple weeks, months or longer depending on their life history. Additionally, we are expecting most sampling will take place in areas and habitats that are relatively shallow and that support our target species', such as shoreline areas and not in the main channels of our sites. Also, we may be upstream, downstream, or in littoral areas adjacent to the salt wedge where waters may be well-mixed with regards to salinity.

Bottom water samples taken in addition to surface water samples will require additional funds or reductions in samples originally planned for freshwater sites in year 2. The chemical structure of PFAS (hydrophobic C-F tail oriented towards the air and hydrophilic head dissolved in water) contribute to their tendency to accumulate at air-water interfaces. As a result, special consideration must be taken during sampling to reduce bias of collecting PFAS from the microlayer accumulating on the water surface.

For surface water collection, the capped sample bottle will be immersed in the middle of the water column and inverted, upstream of collector in flowing systems. The cap of the bottle may then be removed to collect the sample from 1 ft below the water surface or the middle of the water column, whichever is shallower. Once a sufficient volume of water has been collected, the cap may be placed back on the sample container (while remaining submerged). If possible, the sample container will be used to collect the sample, to avoid introduction of additional sampling equipment. The sampling container will be rinsed three times with site water, following the above steps, before sample collection. Enough head space will be left to allow for expansion during freezing. The PFAS water samples will be collected in 2x 500ml methanol-rinsed, new HDPE wide-mouth bottles with unlined caps, supplied from VWR. All containers will remain sealed in LDPE resealable bags until needed for sample collection. An

additional water sample will be collected in 1x 4L new, HDPE container for non-PFAS water chemistry analyses) (see Section 12).

After collection, water samples will remain chilled (under 10°C) during transport to the laboratory. Field sample bottles will be bagged in LDPE resealable bags before placing in a dedicated cooler for PFAS aqueous samples on wet ice that has been bagged in LDPE resealable bags. Ice packs, or blue ice may only be used if it is known to be PFAS-free. Once in the laboratory, PFAS samples will be frozen and stored at ≤-20°C in the dark until preparation and analysis or analysis. PFAS water samples will be shipped to SGS AXYS within 60 days of collection (hold time before extraction is 90 days). The non-PFAS sample containers will be subsampled into separate containers for polyvalent cation and organic carbon analysis and preserved according to their respective analytical SOPs within 12 hr of collection; remaining water may be retained for additional chemical analyses.

9.6 Sediment collection

Sediment samples will be collected at each site contemporaneously with fish if water depths allow. Sediment will be collected from shore or by boat, depending on site depth and geometry, at 3 – 5 locations across the fish sampling area. Equal volume from each collection location will be combined in a stainless-steel homogenization container and mixed thoroughly to produce a single, composited sediment sample per site. Most grab sampling devices are made of stainless steel, but special precautions will be taken to ensure that sampling materials do not contain any coatings or fittings that are waterproof or contain PFAS. Pre-cleaned, PFAS-free, stainless steel ponar, trowels, shovels, spoons, and containers may be used to remove sediment from the study site and used to transfer samples to a container triple rinsed with site water. Excess water will be drained out and enough head space left to allow for expansion during freezing. The sediment/soil samples will be collected in methanol-rinsed, new 50ml HDPE centrifuge tubes with unlined caps, supplied by VWR, collected in duplicate where sufficient material exists. All containers will remain sealed in LDPE resealable bags until needed for sample collection

After collection, sediment samples will be treated similarly to water samples. Samples will remain chilled (under 10°C) during transport to the laboratory. Field sample bottles will be bagged in LDPE resealable bags before placing in a dedicated cooler for PFAS abiotic samples on wet ice or ice packs that have been bagged in LDPE resealable bags. Once in the laboratory, excess water will be decanted from sediment samples and samples homogenized. Two 5 - 10g wet weight portions of each homogenized sample will be separated for PFAS analysis. The remaining sample will be split between containers for total C and N, and polyvalent cation analysis and preserved according to their respective analytical SOPs. All sediment will be frozen and stored at -20°C in the dark. PFAS sediment samples will be shipped to SGS AXYS within 60 days of collection (hold time before extraction is 90 days). Due to water depth, sediment sampling may not be possible at all sites.

9.7 Fish collection

At all sites we aim to collect at least 3 specimens of 3 different species typically consumed by humans, representing, ideally, a benthic forager, pelagic forager and pelagic piscivore. If caught, further samples of species specifically associated with the air-water interface and deepwater lakes will be included. Resident species or migratory species believed to have been at the site for many weeks to months will be targeted. The 3 specimens will be used to provide 3 individual filet samples. Individual

samples were selected (as opposed to composited samples of many individuals) because individual variation in PFAS concentrations is of particular interest. Note, this design does not allow calculation of a whole-body BAF. If sufficient numbers of target species are not caught with reasonable collecting effort, a second species will be chosen, or additional specimens will be allocated to other sites. A single surface water sample and sediment sample will be collected at each site contemporaneously with fish.

At marine sites we will prioritize for collection: White perch, Summer flounder, and American Eel. If these species are unavailable on the day of sampling, other species may be collected. These include Black Sea Bass, Tautog, Scup, White Catfish, Atlantic Menhaden, Northern Puffer, Winter Flounder, and Weakfish. At freshwater sites we will prioritize for collection: Largemouth Bass or Chain Pickerel, Bluegill, and American Eel. If these species are unavailable other species may be collected. These include White Perch, Channel Catfish, White Catfish, Yellow Bullhead, Brown Bullhead, and Common Carp. If none of the above species are available on the day of sampling, the leader of the field crew on that day will select a suitable replacement species. Regardless of prioritization, any species selected for analysis will include a minimum of at least 6 individuals across a minimum of two sites (Table 2). If collecting the minimum number of individuals from the minimum number of sites, we will avoid to the greatest extent possible using sites of different categorization as described by our study design.

Fish specimens will be collected using standard fisheries sampling equipment and techniques. These methods include boat electrofishing when in freshwater systems and freshwater tidal portions of estuaries, and gill netting, trapping, angling, or other methods when primarily in higher salinity waters (Murphy and Willis 1996). The types of habitats where individual species will be collected such as midchannel, off-shore, or back bays are difficult to pre-determine. Therefore, field staff will collect prioritized species from habitats that those species occupy at the time of sampling. Field staff are certified in Adult First Aid/CPR/AED, trained in Electrofishing safety, and carry boat operators' licenses. Field crew members will wear Coast Guard approved personal floatation devices (PFD's) while occupying sampling vessels.

The field staff have been issued NJ Freshwater and Marine collection permits and will follow the guidelines and regulations presented in these permits. An aluminum notebook will be used with regular paper field sheets to record field notes at the time of collection. A YSI Pro-Plus Multi meter will be used by field staff to record environmental parameters 1 ft below the surface, at each collection, at a minimum. These environmental parameters will include dissolved oxygen, temperature, pH, conductivity, specific conductance, and salinity. The meter will be calibrated according to the manufacturer's guidelines specified in the owner's manual. Note that ANSDU is not a NJ certified lab for this testing. Sampling conditions, including tidal cycle, weather conditions and location (handheld GPS) of each sample will be recorded.

Captured fish will be held briefly in ambient water in pre-cleaned tubs/buckets of appropriate sizes for each targeted fish species. Fish will be handled with powderless nitrile gloves. Specimens collected for analysis will be identified to species level and measured for total length to the nearest millimeter on a pre-cleaned HDPE fish measuring board and wet weight to the nearest 100 grams, taken with either a Pesola hook scale or electronic balance covered with aluminum foil (note that balances are calibrated once per year). All specimens collected for analysis will be given a unique identifier/tag. The three targeted fish specimens/species will be, as best possible, of similar sizes and of sizes typically consumed by the public. Only intact fish specimens will be utilized for samples. Fish that have skin lacerations,

open sores, fin deterioration, or found floating dead around the sample site will not be used. If available, some additional fish specimens may be collected and archived for possible additional sampling outside this scope of work.

Collected fish will be rinsed with local site water to remove debris and vegetation, and temporarily held in HDPE containers or metal pans while at the field site. Fish will then be wrapped in aluminum foil (dull side toward the sample) and placed into appropriately sized Ziploc type bags after wrapping. Fish specimens will be wrapped individually and labelled. The field labels will be written with pencil on plain paper. Uniquely numbered aluminum tags may also be used to identify each specimen pack. The wrapped/bagged samples will be placed on wet or dry ice immediately in the field and upon arrival at the laboratory, frozen and stored at -20˚°C in the dark. If using wet ice in the field, the wrapped/bagged samples will be placed on top of a barrier (e.g., metal, or plastic tray) to keep the sample from sliding into the wet ice during transport. A field chain of custody will be prepared for samples from each sampling event (see Sample Custody Procedures section).

9.7.1 Fish tissue sample preparation

After field collection, and prior to laboratory tissue preparation, fish will be thawed overnight in a lab refrigerator in their original collection containers or bags. Typically, a single filet will be taken from a fish sample. Fish filets will be taken in accordance with EPA's "Guidance for Assessing Chemical Contaminant data for Use in Fish Advisories" (EPA 2000). For example, scaled finfish (e.g., Largemouth Bass) will be fileted with skin-on and include the belly flap, while scaleless fishes (e.g., eels and catfishes) will be fileted with skin-off. The carcass will be retained for at least one year. Separate filet and carcass weights (g) will be recorded from an electronic balance. Filet samples will be homogenized with a tissue grinder or stainless-steel blender, making sure to "pulse" samples to reduce the amount of heat generated from the spinning blades. Once a uniform consistency is achieved, a portion of the samples will be weighed and reserved for stable isotope analysis. The material for PFAS analysis will be transferred to methanol-rinsed, new 50ml HDPE centrifuge tubes with unlined caps (supplied by VWR), refrozen, and stored at -20°C in the dark. PFAS tissue samples (< 2g each) will be shipped to SGS AXYS within 60 days of collection (hold time before extraction is 90 days). Material not used for either analysis will be refrozen and held for at least one year. This material may be used for future individual sample or carcass analyses, should sample results indicate the need for such data.

10. Sample Custody Procedures

All sample containers will be enclosed in LDPE resealable bags labeled with their site, collection date, and contents. Upon return to the lab, each sample will be given a unique identifier for tracking its origination, handling, use and final disposition, placed on ice immediately following collection in the field, frozen upon arrival at the laboratory and stored at -20°C in the dark. Samples will be defrosted only as needed for preparation. Sample hold time for all PFAS matrixes is 90 days according to SGS AXYS. Sample custody and shipping procedures will follow SGS AXYS conditions and recommendations (see Appendix H for sample chain-of-custody form). PFAS samples will be packed in a cooler with sealed ice/icepacks and shipped overnight to SGS AXYS by commercial carrier (FedEx, UPS, etc.). Sample condition upon receipt should be 0-6 C and in the dark. Duplicate material will be retained at the Academy of Natural Sciences unless needed by SGS AXYS.

Sample collection and container information, and other relevant field condition information, will be recorded in field notebooks while in the field and transferred to electronic upon arrival at the laboratory. Electronic and paper chain of custody forms, supplied by SGS AXYS, will be transmitted to SGS AXYS with the shipped samples. All field, lab, and chain-of-custodies will be scanned and archived digitally. All electronic records and digital scans will be stored on the Academy of Natural Sciences' servers and/or cloud storage.

11. Monitoring Sites and Frequency of Sample Collection

11.1 Year 1 Collection

An estimated 16 marine sites will be sampled in Year 1, including: 1) sites within upper parts of estuaries of the state with variable flow and salinities that may range from fresh to brackish water; and 2) open/bay sites with limited non-tidal flow and salinities that may range from brackish to ocean water (Table 1; Table 2; Figure 1). Limited pre-sampling of water PFAS concentrations will be conducted in advance of the main fish sampling event: if no recent data is available, one sample per site will be collected ~2 months prior to the main event to verify water PFAS concentrations are above detection; up to 5 additional samples will be collected from estuarine sites in the weeks prior to the main event to assess the temporal variability in PFAS concentrations resulting from variability in inflows and tidal mixing. A single, main sampling event at each site will be conducted to collect water (single), sediment (single site composite; if depths allow), and fish samples for analysis of PFAS, isotopic and environmental chemistry necessary to calculate and interpret fish BAFs. Note that some fish collection methods may require setting collecting gear and returning the next day.

11.2 Year 2 Collection

An estimated 18 freshwater sites will be sampled in Year 2, including: 1) lakes (with deep and shallow hydrology) and streams, in 2) systems in Pineland and non-Pineland areas (Table 1). Freshwater sites will be selected before the end of year 1 and after discussion with the NJDEP Co-Project Managers. Limited pre-sampling of water PFAS concentrations will be conducted in advance of the main fish sampling event: if no recent data is available, one sample per site will be collected ~2 months prior to the main even to verify water PFAS concentrations are above detection; up to 4 additional samples will be collected weekly around the time of the main event to assess the temporal variability of aqueous PFAS concentrations at stream sites. A single, main sampling event at each site will be conducted to collect water (single), sediment (site composite), and fish samples for analysis of PFAS, isotopic and environmental chemistry necessary to calculate and interpret fish BAFs. Note that some fish collection methods may require setting collecting gear and returning the next day.

Table 1. Sampling plan summarizing the number of field visits and samples collected for water, fish and sediment. Pre= number of samples per site taken for confirming detectable levels of PFAS (i.e., screening) and for assessing temporal variability in PFAS concentrations. Main = number of samples per site taken on the day fish are collected for calculation of BAF.

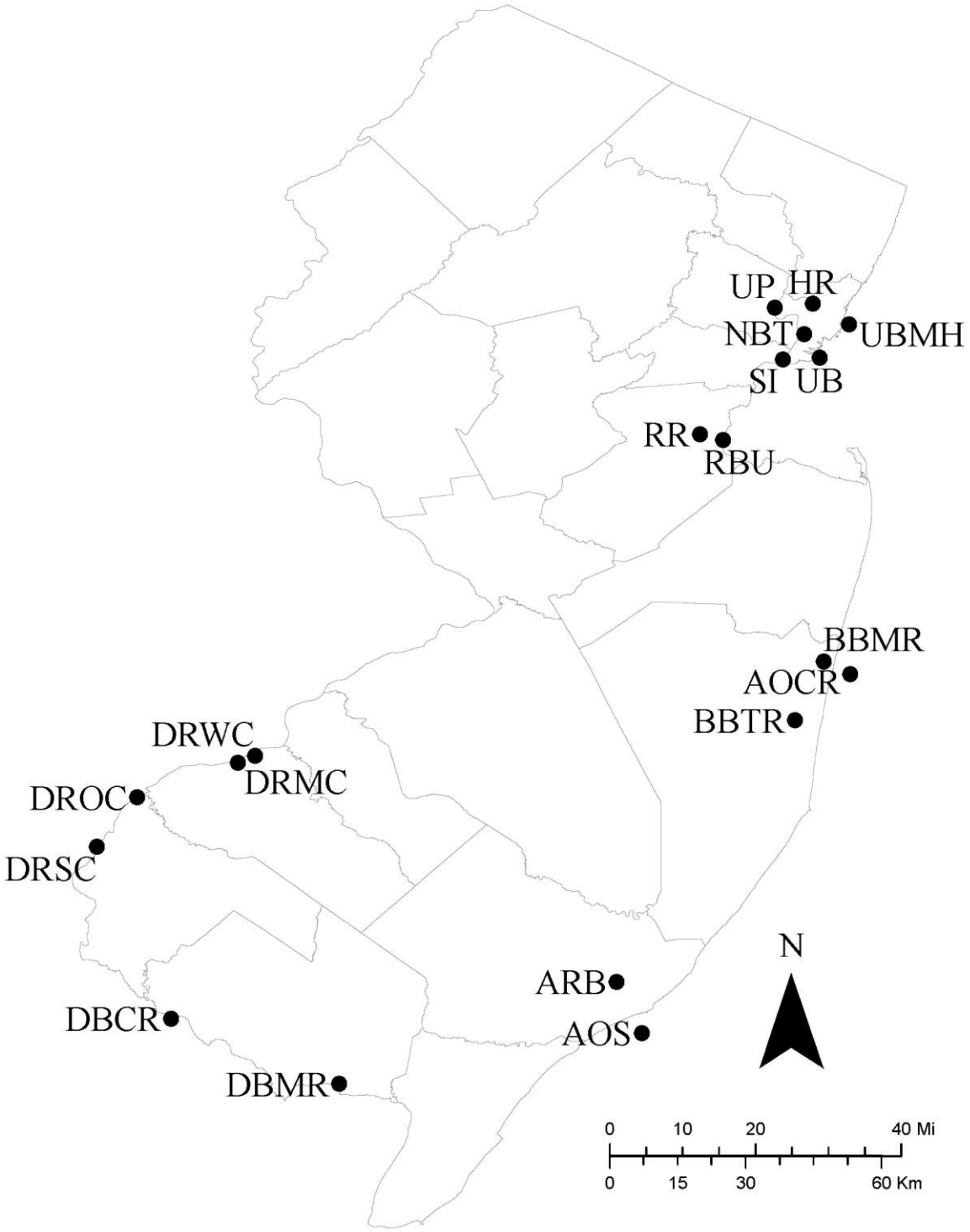
Sample Summary			Water		Sediment	Fish		
						Piscivore	Forager	
Site Classification	# Sites		Pre	Main		Pelagic	Pelagic	Benthic
Marine Sites								
Upper estuary	9	x	6	1	1	3	3	3
Open water/Bay	7	x	1	1	0.5	3	3	3
	16		61	16		48	48	48
	Total Marine Samples:		**77**		12.5		**144**	
Freshwater Sites								
Streams								
Pinelands	4	x	5	1	1	3	3	3
Non-Pinelands	4	x	5	1	1	3	3	3
Lakes								
Shallow, Pinelands	4	x	1	1	1	3	3	3
Shallow, Non-Pinelands	3	x	1	1	1	3	3	3
Deep	3	x	1	1	1	3	3	3
	18		50	18		54	54	54
	Total Freshwater Samples:		**68**		**18**		**162**	

Table 2. Marine sites selected for water, sediment, and fish sampling for analysis of PFAS, isotopic and environmental chemistry necessary to calculate and interpret fish BAFs. Del = Delaware River Drainage, Cnt-Atl = Central NJ-Atlantic Ocean, North = Hudson-Raritan Bay vicinity, UE = upper estuary, OWB = open water/bay, * denotes additional sites to be included in preliminary screening and prioritized for full analysis.

Region	Site Type	Waterbody	Lat	Long	Landmark or Surface water Discharge Point	Site Code
Del	UE	Delaware River	39.866118	-75.196415	Mouth of Woodbury Creek	DRWC
Del	UE	Delaware River	39.853051	-75.230072	Mouth of Mantua Creek	DRMC
Del	UE	Delaware River	39.783882	-75.429565	Mouth of Oldmans Creek	DROC
Del	UE	Delaware River	39.686118	-75.509628	Mouth of Salem Canal	DRSC
Del	UE	Delaware Bay/Maurice River	39.215191	-75.028546	Mouth of the Maurice River	DBMR
Cnt-Atl	OWB	Atlantic Ocean	39.315930	-74.427800	Atlantic City Sewer Line/Prime Fishing Grounds	AOS
Cnt-Atl	UE	Absecon River/Bay	39.417604	-74.477701	Absecon Bay/Mouth of the Absecon River	ARB
Cnt-Atl	UE	Toms River/Barnegat Bay	39.936548	-74.124311	Mouth of Toms River	BBTR
Cnt-Atl	OWB	Atlantic Ocean	40.029239	-74.014323	Axel Carlson Reef/NWPCF Effluent	AOCR

Cnt-Atl	UE	Barnegat Bay	40.054048 -74.067631	Mouth of Metedeconk	BBMR
North	UE	Lower Raritan River	40.504830 -74.312871	Middlesex County UA	RR
North	OWB	Raritan Bay	40.493379 -74.266932	Middlesex County UA	RBU
North	OWB	Upper Bay	40.656426 -74.075174	PVSC Discharge point	UB
North	OWB	Newark Bay (south)	40.653376 -74.148090	Shooters Island	SI
North	OWB	Newark Bay (north)	40.703825 -74.106436	Passaic/Hackensack Confluence	NBT
North	OWB	Upper Bay	40.722608 -74.016171	Mouth of Hudson River	UBMH
North	UE	Passaic River *	40.756241 -74.164585	Kearny	UP
North	UE	Hackensack River*	40.763808 -74.088631	Secaucus	HR
Del	UE	Cohansey River*	39.344837 -75.363068	Mouth of Cohansey	DBCR

Figure 1. Map of marine sites in NJ selected for water, sediment, and fish sampling for analysis of PFAS, isotopic and environmental chemistry necessary to calculate and interpret fish BAFs. Acronyms describing sites are listed in Table 2.



12. Analytical Procedures

12.1 PFAS samples

All water, sediment and fish samples will be analyzed for concentrations of 40 PFAS compounds: 11 perfluoroalkyl carboxylates, 8 perfluoroalkyl sulfonates, 3 fluorotelomer sulfonates, 3 fluorotelomer carboxylates, 3 perfluorooctane sulfonamides, 2 perfluorooctane sulfonamidoacetic acids, 2 perfluorooctane sulfonamidoethanols, 5 ether carboxylates, and 3 ether sulfonates (Appendix A). Samples will be extracted, analyzed and reported by SGS AXYS Analytical (laboratory ID CANA005) following their standard method MSU-110B (EPA Method 1633) according to their "Summary of SGS AXYS Method MLA-110 Rev 02 Ver 12: Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products, Blood, Serum and Solvent Extracts with LC-MS/MS by EPA Method 1633" (Appendix A). Note that SGS AXYS is not a NJ certified lab for this testing, however after extensive communication with NJ DEP it was determined that SGS Axys is the best analytical laboratory for performing the specialized analysis for PFAS in fish tissue as described in this project. The SGS Axys laboratory performs this analysis is in Canada and is the only lab we are aware of that does this analysis in this media. They are also the only laboratory that does this analysis for all other states that put out fish consumption advisories for PFAS in fish tissue and they are the laboratory which developed the newest Draft EPA Method 1633 for PFAS analysis. In brief, after spiking with isotopically labeled surrogate standards samples are extracted and cleaned up by Solid Phase Extraction (SPE). The extracts are then analyzed by liquid chromatography/mass spectrometry (LCMS/MS). Final sample concentrations are determined by isotope dilution/internal standard quantification as the total of linear and branched isomers. All sample condition, container, hold time, and shipping procedures will be adhered to.

NJDEP only recently began offering the one draft EPA analytical method, EPA Method 1633, that can quantify PFAS in fish tissue. Currently, there are no labs certified to analyze fish tissue in the State of NJ. The Division of Science and Research has recommended, and supports, the sole use of SGS Axys for the analysis of all environmental samples collected and analyzed for this study. SGS Axys is the one lab that has worked with US EPA to create the 1633 analytical method, and it is the only lab to date that has validated its use. SGS Axys is also the one lab that has provided many states around the country with analysis on fish tissue and other environmental samples, that states have used for a range of regulatory and guidance policies. The NJDEP Division of Science and Research has used only SGS Axys for all PFAS research up to this point and has high confidence in the quality of the data obtained from this lab. The Level IV data package provided by SGS Axys will be available for OQA review.

Shipping address of SGS AXYS Analytical:
2045 Mills Road West
Sidney, British Columbia, Canada V8L 5X2
TEL: (250) 655-5800

12.2 Isotope samples

All fish samples and one suspended particulate matter sample per site will be analyzed for stable isotopes $\delta^{13}C$ and $\delta^{15}N$. Sample preparation and analysis will be conducted at the Academy of Natural Sciences of Drexel University according to ANSDU Standard Operating Procedure No. P-16-205 "The Use of the Elementar Pyrocube Elemental Analyzer and Isoprime1000 Mass Spectrometer for the Analysis of Concentration and Stable Isotopes of Carbon, Nitrogen, and Sulfur in Tissues, Plants, Sediments and Filtered Particulate Matter" (Appendix B). Note that ANSDU is not a NJ certified lab for this testing.

12.3 Environmental chemistry samples

Field measurements of water pH, temperature, dissolved oxygen concentrations, salinity, and specific conductance will be collected in the field using a calibrated field probe, such as a YSI EXO multiparameter sonde. The meter will be calibrated according to the manufacturer's guidelines specified in the owner's manual and according to ANSDU SOP No. P-16-91r2 (Appendix G). Note that ANSDU is not a NJ certified lab for this testing. All water samples will be analyzed for organic carbon concentrations and may be analyzed for polyvalent cation concentrations. All sediment samples will be analyzed for total C, total N, and potentially for polyvalent cation concentrations. Aqueous dissolved organic carbon and sediment total C/total N analyses will be conducted at the Academy of Natural Sciences of Drexel University according to ANSDU Standard Operating Procedures No. P-16-99r2 "Dissolved Organic Carbon and Total Organic Carbon" (Appendix C), No. P-16-54 r2 "The Use of Carol Erba Model 1112 Flash Elemental Analyzer for the Analysis of Total Carbon and Nitrogen in Sediments and Filtered Particulate Matter" (Appendix D). ANSDU will partner with Temple University College of Engineering (TUCE) to analyze cation samples, note that TUCE is not a NJ certified lab for this testing. Cation analysis will be completed as per EPA Method 200.8 "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma - Mass Spectrometry", EPA Method 6020B "Inductively Coupled Plasma—Mass Spectrometry", and the internal TUCE SOP (Appendix E). If polyvalent cation concentrations in sediments or water are analyzed, samples will not be digested during metals determination and therefore results may be biased low. All polyvalent cation concentrations for sediments and water results will be reported with qualifiers stating that they may be biased low due to not having undergone digestion.

13. Data Quality Requirements

Analytical samples will be evaluated by the methods specified in this QAPP and for which the laboratory has provided method summaries and acceptable method reporting and detection limits. Quantification procedures and quality control procedures (including required calibrations, equipment cleaning, and other quality control procedures will be performed by SGS AXYS according to their specifications defined in "Summary of SGS AXYS Method MLA-110 Rev 02 Ver 12" (Appendix A).

14. Data Quality Assessments

14.1. Data Representativeness

The degree to which data from the project accurately and precisely represent characteristics of sediment, water, and fish in NJ will be determined by the data acquired, and the variability of those data for the factors included in our study design. The sites selected for analysis are spread across three large

regions of the state (see Table 2) and should provide data for calculations of statewide BAFs. Depending on the data acquired, our study design may allow us to account for regional, site-type, site-, and speciesspecific variability (Table 2).

14.2. Data Comparability

PFAS and non-PFAS chemistry samples for this project are being collected and analyzed in a manner consistent with best-available procedures and methods to ensure all data expected from this project is comparable with equivalent data from the literature. Analysis of PFAS in fish in this study is limited to filet tissue and will, therefore, have only limited comparability to published whole body or other organ tissue data. Recommended supporting data is also being collected to assist with data interpretation, site characterization and overall comparison. This includes fish weight and lengths, stable isotopic ratios to assess trophic placement, and characterization of water and sediment chemistry known to affect PFAS partitioning and bioavailability. To date, few studies have assessed PFAS bioaccumulation in marine or saline environments. Describing the relationship of PFAS bioaccumulation with salinity or testing for differences among freshwater, brackish, and saline treatments should be of great value for assessing the influence of salinity on PFAS bioaccumulation.

15. Calibration Procedures and Preventive Maintenance

Field water quality probes will be calibrated by ANSDU staff every three days and checked every field day. The meter will be (re)calibrated according to the manufacturer's guidelines specified in the owner's manual. Note that ANSDU is not a NJ certified lab for this testing. All laboratory equipment will be properly calibrated as per each method completed.

16. Quality Control Checks

Field equipment rinse blanks will be collected by rinsing field equipment that comes in contact with the sample (ex. ponar, nets, aluminum foil or other wrapping material) with lab verified water. Separate field equipment rinse blanks from water, sediment, and fish tissue sampling equipment will be collected at the end of every field day. Field blanks will be prepared in the laboratory by filling a water sampling container with lab verified water and then treating it as a sample in all respects. Field blanks will be collected every five sampling events. Preparation lab equipment blanks will be collected by rinsing laboratory equipment that comes in contact with the sample (ex. cutting boards, tissue grinder or blender) with lab verified water. Preparation lab equipment blanks will be collected at the end of every preparation day. All blanks will be collected in the same 500ml, methanol-rinsed HDEP containers as field water samples. Field duplicate samples for water, sediment and fish will be collected every 10^{th} sample. Duplicate precision will be evaluated in accordance with the applicable method SOP (See Attachments A-G for additional information).

Analytical blanks, spikes and other measures of analytical QC will be performed in accordance with the applicable method SOP (See Attachments A-G for additional information).

17. Documentation, Data Reduction, and Reporting

17.1. Documentation

All data and information obtained during this project will be kept by the laboratory in either computerized or handwritten form (i.e., notebooks and field sheets). Sample information will be recorded on field sheets while in the field and transferred to electronic form at the laboratory/office (i.e., entered into MS Excel spreadsheets or MS Access databases). Electronic and paper chain of custody forms (Appendix H) will be transmitted to SGS AXYS with the shipped samples. All datasheets, and field and lab chain-of-custodies will be scanned and archived digitally. All electronic records and digital scans will be stored on the Academy of Natural Sciences' servers and/or cloud storage and will be available for inspection on request.

17.2. Data Reduction and Reporting

Summaries of the data will be made using MS Excel spreadsheets and MS Access databases. We will provide tables of the PFAS, metals (if polyvalent cation concentrations are run for sediments or waters, all metals results will be reported with qualifiers stating that they may be biased low due to not having undergone digestion), TOC concentrations and isotopes values in water, sediment, and fish for each sample analyzed. PFAS data will be used to calculate BAFs. The data may be summarized for spatial variation or to assess the factors in our study design (See above and Appendix F). Study design factors (including results of PFAS, metals, TOC concentrations and isotopes values) may be assessed using univariate and multivariate statistics as appropriate. PFAS concentrations and associated data will be provided to NJDEP project managers (Dan Millemann, will serve as the NJDEP Data Manager for this project) in MS Excel or MS Access files for future uses yet to be fully determined but expected to include additional BAF analyses beyond the scope of this study. Files will be transferred via e-mail, MS OneDrive or similar. The data will be made available to the WQX for upload but will only be available for public viewing after completion of this research project, as is typical of other NJDEP research projects.

BAFs are the ratio of the concentration of a single PFAS compound measured in fish tissue to its concentration in contemporary water. Fish BAF calculation will follow the procedures for inorganic compounds. Unlike organic contaminants, PFAS do not have an affinity for lipids, thus lipid normalization is not necessary when calculating PFAS BAFs. BAF calculations can be made on a wide range of data groupings including per individual, per species, per site, per habitat, etc. At a minimum, we will report calculations of PFAS BAFs for each individual and each species on both a per site and global basis. Individual BAFs will be calculated as the geometric mean concentration in the same-day water samples (if multiple) divide by the concentration in a single filet. Site-specific species BAFs will be calculated as the geometric mean concentration in same-day water samples (if multiple) divided by the geometric mean concentration in the three fillets from each species. Global species BAFs will be calculated as the geometric mean of the species BAFs from each site. Depending on the samples collected and initial BAF calculations, additional BAFs may be calculated by site (using all species collected at a site), type of system (open water vs. Estuarine), geography (Delaware vs. Atlantic coast vs. Northern sites), salinity, using the geometric mean of same day and preliminary water samples, etc. Likewise, these individual, species or other BAFs can be evaluated related to trophic level, trophic placement, salinity, habitat, PFAS concentration, etc.

Reporting due dates are as follows:

1) QAPP due by 4/15/22
2) Draft Marine Report due by 12/15/22
3) Final Marine Report (or preliminary final report) due by 2/15/23
4) Draft Freshwater Report due by 12/15/23
5) Final Freshwater Report (and Final Marine) due by 2/15/24

18. Data Validation

A portion of the data transcribed from field and lab sheets will be checked against the values entered into MS Excel spreadsheets and MS Access databases to verify transcription accuracy. A subset of calculations used in the report will be reviewed to confirm the data's authenticity (i.e., we will confirm the values used are those derived from the raw data). All data will be inspected for reasonableness by experienced ANSDU scientists. As a preliminary data analysis step, we may assess the data graphically to identify outliers or data points outside of the typical pattern expected. First, we will determine if the outlier is a transcription error or a handling error by inspecting our data sheets and chain-of-custodies. If this does not resolve the issue, we may, conduct analyses with and without the outlier, use non-parametric statistics to analyze the data, or apply a criterion to the entire dataset to trim suspected outliers (e.g., remove values >3 standard deviations from the mean). In general, we will attempt to retain as much of the data as possible for analyses. Any criteria used to remove or address outliers will be detailed in our report.

19. Performance and Systems Audits

Drs. Keller and Kurz will be responsible for the overall performance of the project, field collection of sediment, water, and fish, processing and shipping of samples to the laboratory, data review and transferring data to NJ. Joseph Heiczinger will be responsible for conducting spot checks and assuring that the QAPP is being followed. Mr. Heiczinger will participate in field collection functions, assess, and make corrections when necessary to maintain the data accuracy as defined in this plan. If any major changes or significant modifications are made to this plan regarding data collection, laboratory analysis or data quality assurance as it relates to the objectives(s) and data accuracy required in this project, all original signees of the QAPP will be notified by Drs. Keller and Kurz. The NJDEP OQA will be notified of the sampling schedule in the event that the OQA has the opportunity to perform an audit to ensure compliance with the QAPP.

20. Protocol Modifications:

Minor modifications of protocol may be necessary as new requirements in the laboratory and field are identified. A memorandum (note in Progress Report) regarding any changes in procedures would be issued only after prior discussion and agreement between NJDEP and ANSDU project officers and the ANSDU QA officer.

21. References

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EPA 2000. Guidance for Assessing Chemical Contaminant data for Use in Fish Advisories. Vol 1 Fish Sampling and Analysis. Third Edition. Office of Science and Technology Office of Water U.S. Environmental Protection Agency Washington, DC.

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Khairy, M. A., Noonan, G. O., & Lohmann, R. (2019). Uptake of hydrophobic organic compounds, including organochlorine pesticides, polybrominated diphenyl ethers, and perfluoroalkyl acids in fish and blue crabs of the lower Passaic River, New Jersey, USA. *Environmental Toxicology and Chemistry*, *38*(4), 872–882.

Murphy, B.R., and D.W. Willis, editors. 1996. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

22. Project Operations and Responsibility

David Keller: Co-Principal investigator, Project design, Site selection, Oversight of sampling operations and QC, Sampling QC, Data processing QC, Data quality review, Performance evaluation/auditing, Overall QA, Data analysis, and Reporting of all aspects but especially fishes.

Marie Kurz: Co-Principal investigator, Project design, Site selection, Oversight of sampling operations and QC, Data processing QC, Data quality review, Overall QA, Data analysis, and Reporting of all aspects but especially PFAS and environmental chemistry.

Timothy Maguire: Laboratory QC, Data processing activities, Data processing QC, Data quality review, Performance evaluation/auditing, Overall QA, and Reporting of all aspects but especially PFAS and environmental chemistry.

Daniel Morrill: Sampling operations, Laboratory analysis, Data processing activities, and Data processing QC pertaining to fishes.

Paul Overbeck: Assisting with the following activities: sampling operations, Laboratory analysis, Data processing activities, pertaining to fishes.

Tracey Curran: Sampling operations, Laboratory analysis, Laboratory QC, and Data processing activities pertaining to PFAS and environmental chemistry.

Joseph Heiczinger: Field, laboratory, and report QA/QC

David Velinsky: Laboratory QC, Data processing activities, Data processing QC, Data quality review, Overall QA, and Reporting of environmental chemistry and stable isotopes.

Michelle Gannon: Laboratory analysis, Laboratory QC, Data processing activities, Data processing QC, and Data quality review pertaining to stable isotopes.

23. Appendices

Appendix A. Summary of SGS AXYS Method MLA-110 Rev 02 Ver 12, "Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products, Blood, Serum and Solvent Extracts with LC-MS/MS by EPA Method 1633"

Appendix B. ANSDU SOP No. P-16-205 "The use of the Elementar Pyrocube Elemental Analyzer and Isoprime1000 Mass Spectrometer for the Analysis of Concentration and Stabel Isotopes of Carbon, Nitrogen, and Sulfur in Tissues, Plants, Sediments and Filtered Particulate Matter"

Appendix C. ANSDU SOP No. P-16-99r2 "Dissolved Organic Carbon and Total Organic Carbon"

Appendix D. ANSDU SOP No. P-16-54 r2 "The Use of Carol Erba Model 1112 Flash Elemental Analyzer for the Analysis of Total Carbon and Nitrogen in Sediments and Filtered Particulate Matter"

Appendix E. EPA Method 200.8 "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma - Mass Spectrometry" & EPA Method 6020B "Inductively Coupled Plasma—Mass Spectrometry", and the internal Temple University College of Engineering SOP.

Appendix F. Kurz, M. and D. Keller. 2021. Proposal for Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems. Submitted to New Jersey Dept. of Environmental Protection.

Appendix G. SOP for ANSDU SOP No. P-16-91r2 "YSI Calibration and Calibration Checks"

Appendix H. Chain-of-custody form

"QQFOEJY"4VNNBSZPG4(4"9:4.FUIPE.-"3FW7FS i"OBMZUJDBM
1SPDFEVSFGPSUIF"OBMZTJTPG1FSBOE1PMZGMVPSPBMLZM4VCTUBODFT 1'"4
JO"RVFPVT
4BNQMFT 4PMJET 5JTTVFT "''1SPEVDUT #MPPE 4FSVNBOE4PMWFOU&YUSBDUTXJUI-$.4
.4CZ&1".FUIPEw

SGS AXYS Analytical Services Ltd, 2045 Mills Road W., Sidney, BC, Canada, V8L 5X2. Tel. (250) 655-5800, fax (250) 655-5811.

SGS AXYS Analytical Services Ltd.

SUMMARY OF SGS AXYS METHOD MLA-110 REV. 02 VER. 12

Analytical Procedure for the Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Samples, Solids, Tissues, AFFF Products, Blood, Serum and Solvent Extracts with LC-MS/MS by EPA Method 1633

This method describes the analysis of per- and polyfluoroalkyl substances (PFAS) in aqueous, solid, biosolid, tissue, blood/serum and AFFF product samples and solvent extracts, determined as the total of linear and branched isomers. After spiking with isotopically labeled surrogate standards samples are extracted and cleaned up by Solid Phase Extraction (SPE). The extracts are then analyzed by liquid chromatography/mass spectrometry (LC-MS/MS). Final sample concentrations are determined by isotope dilution/internal standard quantification.

The linear and branched isomers (as a total or as isomer groups) of PFOA, PFNA, PFHxS, PFOS, PFOSA, N-MeFOSA, N-MeFOSE, N-MeFOSAA, N-EtFOSA, N-EtFOSE and N-EtFOSAA can be reported separately upon request.

Typical reporting limits, based on the A CAL, are shown below, for the method default sample sizes:

Analyte groups	Aqueous sample	Leachate samples	Extract	Solid	Biosolid	Tissue	AFFF Products	Blood, Serum
Typical sample size	0.5 L	0.125 L	0.75 mL	5 g[3]	0.5 g[3]	2.0 g[3]	0.02 g	2 mL
Units	ng/L	ng/L	ng/mL	ng/g	ng/g	ng/g	ng/g	ng/g[1]
Perfluoroalkyl carboxylates								
PFBA	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
PFPeA	0.8	3.2	0.53	0.08	0.8	0.2	20	0.2
PFHxA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFHpA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFOA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFNA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFDA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFUnA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFDoA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1

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PFTrDA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFTeDA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1

Analyte groups	Aqueous sample	Leachate samples	Extract	Solid	Biosolid	Tissue	AFFF Products	Blood, Serum
Typical sample size	0.5 L	0.125 L	0.75 mL	5 g[3]	0.5 g[3]	2.0 g[3]	0.02 g	2 mL
Units	ng/L	ng/L	ng/mL	ng/g	ng/g	ng/g	ng/g	ng/g[1]
Perfluoroalkyl sulfonates								
PFBS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFPeS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFHxS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFHpS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFOS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFNS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFDS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
PFDoS	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
Fluorotelomer sulfonates								
4:2 FTS	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
6:2 FTS	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
8:2 FTS	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
Fluorotelomer carboxylates								
3:3 FTCA	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
5:3 FTCA	10	40	6.7	1	10	2.5	250	2.5
7:3 FTCA	10	40	6.7	1	10	2.5	250	2.5
Perfluorooctane sulfonamides								
PFOSA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
N-MeFOSA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1

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N-EtFOSA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
Perfluorooctane sulfonamidoacetic acids								
N-MeFOSAA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
N-EtFOSAA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1
Perfluorooctane sulfonamide ethanols								
Analyte groups	Aqueous sample	Leachate samples	Extract	Solid	Biosolid	Tissue	AFFF Products	Blood, Serum
Typical sample size	0.5 L	0.125 L	0.75 mL	5 g[3]	0.5 g[3]	2.0 g[3]	0.02 g	2 mL
Units	ng/L	ng/L	ng/mL	ng/g	ng/g	ng/g	ng/g	ng/g[1]
N-MeFOSE	4	16	2.7	0.4	4	1[2]	100	1
N-EtFOSE	4	16	2.7	0.4	4	1[2]	100	1
Per- and polyfluoroether carboxylates								
HFPO-DA	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
ADONA	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
NFDHA	0.8	3.2	0.53	0.08	0.8	0.2	20	0.2
PFMBA	0.8	3.2	0.27	0.04	0.8	0.1	10	0.1
PFMPA	1.6	6.4	0.53	0.08	1.6	0.2	20	0.2
Ether sulfonates								
9Cl-PF3ONS	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
11Cl-PF3OUdS	1.6	6.4	1.1	0.16	1.6	0.4	40	0.4
PFEESA	0.4	1.6	0.27	0.04	0.4	0.1	10	0.1

[1] Serum reporting unit default is ng/g, but units may be converted to ng/mL upon client request by using serum density.

[2] Low surrogate recoveries in tissues of D_7-N-MeFOSE D_9-N-EtFOSE typically increase the reporting limits for N-MeFOSE and NEtFOSE by a factor of 5.

[3] Upper weight limit for wet samples

Refer to Appendix B for sample storage conditions.

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6. PFAS TARGET ANALYTES

Perfluoroalkyl carboxylates
Perfluorobutanoic acid (PFBA, Perfluorobutanoate)
Perfluoropentanoic acid (PFPeA, Perfluoropentanoate)
Perfluorohexanoic acid (PFHxA, Perfluorohexanoate)
Perfluoroheptanoic acid (PFHpA, Perfluoroheptanoate)
Perfluorooctanoic acid (PFOA, Perfluorooctanoate)
Perfluorononanoic acid (PFNA, Perfluorononanoate)
Perfluorodecanoic acid (PFDA, Perfluorodecanoate)
Perfluoroundecanoic acid (PFUnA, Perfluoroundecanoate)
Perfluorododecanoic acid (PFDoA, Perfluorododecanoate)

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Perfluorotridecanoic acid (PFTrDA, Perfluorotridecanoate)

Perfluorotetradecanoic acid (PFTeDA, Perfluorotetradecanoate)

Perfluoroalkyl sulfonates

Perfluorobutanesulfonic acid (PFBS, Perfluorobutanesulfonate)

Perfluoropentanesulfonic acid (PFPeS, Perfluoropentanesulfonate)

Perfluorohexanesulfonic acid (PFHxS, Perfluorohexanesulfonate)

Perfluoroheptanesulfonic acid (PFHpS, Perfluoroheptanesulfonate)

Perfluorooctanesulfonic acid (PFOS, Perfluorooctanesulfonate)

Perfluorononanesulfonic acid (PFNS, Perfluorononanesulfonate)

Perfluorodecanesulfonic acid (PFDS, Perfluorodecanesulfonate)

Perfluorododecanesulfonic acid (PFDoS, Perfluorododecanesulfonate)

Fluorotelomer sulfonates

1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS, 1H, 1H, 2H, 2H-perfluorohexane sulfonate)

1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS, 1H, 1H, 2H, 2H-perfluorooctane sulfonate)

1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS, 1H, 1H, 2H, 2H-perfluorodecane sulfonate)

Fluorotelomer carboxylates

2H, 2H, 3H, 3H-perfluorohexanoic acid (3:3 FTCA, 2H, 2H, 3H, 3H-perfluorohexanoate)

2H, 2H, 3H, 3H-perfluorooctanoic acid (5:3 FTCA, 2H, 2H, 3H, 3H-perfluorooctanoate)

2H, 2H, 3H, 3H-perfluorodecanoic acid (7:3 FTCA, 2H, 2H, 3H, 3H-perfluorodecanoate)

Perfluorooctane sulfonamides

Perfluorooctanesulfonamide (PFOSA) [1]

N-Methylperfluorooctanesulfonamide (N-MeFOSA)

N-Ethylperfluorooctanesulfonamide (N-EtFOSA)

Perfluorooctane sulfonamidoacetic acids

N-Methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA, N-Methylperfluoro-1octanesulfonamidoacetate)

N-Ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA, N-Ethylperfluoro-1octanesulfonamidoacetate)

Perfluorooctane sulfonamidoethanols

N-Methylperfluoro-1-octanesulfonamidoethanol (N-MeFOSE)

N-Ethylperfluoro-1-octanesulfonamidoethanol (N-EtFOSE)

Ether carboxylates

2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionic acid (HFPO-DA, 2,3,3,3-Tetrafluoro2-(1,1,2,2,3,3,3-heptafluoropropoxy)propionoate)

Decafluoro-3H-4,8-dioxanonoate (ADONA, DONA, Decafluoro-3H-4,8-dioxanonoic acid)

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Perfluoro-3,6-dioxaheptanoate (NFDHA, Perfluoro-3,6-dioxaheptanoic acid)

Perfluoro-3-methoxypropanoate (PFMPA, Perfluoro-3-methoxypropanoic acid)
Perfluoro-4-methoxybutanoate (PFMBA, Perfluoro-4-methoxybutanoic acid)
Ether sulfonates
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS, 9-chlorohexadecafluoro-3oxanonane-1-sulfonate)
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS, 11-chloroeicosafluoro-3oxaundecane-1-sulfonate)
Perfluoro(2-ethoxyethane)sulfonic acid (PFEESA, Perfluoro(2-ethoxyethane)sulfonate

[1] PFOSA also called FOSA

The carboxylic and sulfonic acid analyte concentrations can be reported as either the acid or the anion forms. The anion and corresponding acid forms and their CAS Registry Numbers are shown in Appendix A of this summary.

Each compound is determined as the total of linear and branched isomers where branched standards are available to confirm their retention time.

7. 1.0 EXTRACTION AND CLEANUP PROCEDURES

Aqueous samples size may be up to 1000 mL for aqueous samples analyzed by this method, and up to 0.75 mL for extracts/solvents. Leachate samples are typically 125 mL. The OPR and BLK (reagent water) must be of the same volume as a typical sample in the batch. Samples are stored in HDPE (high density polyethylene) containers. All samples are spiked with surrogate standards. Aqueous samples are extracted by solid phase extraction (SPE) using weak anion exchange cartridges; wash and elution procedures are chosen to meet various analysis requirements. Sample extracts are then treated with carbon powder, spiked with recovery standards and analyzed by LC-MS/MS. For aqueous samples that clog the SPE cartridge during loading, the project manager may approve matters related to change in sample size and the spiking of MS/MSD samples. A second, non-standard procedure may be used to filter samples using the entire sample bottle. This option measures dissolved PFAS. It does not include PFAS bound to particulate. It does not give equivalent results to the standard procedure above.

Extract/solvent samples don't undergo solid phase extraction. The samples are spiked with surrogate and recovery standards, and analyzed by LC-MS/MS.

The sample sizes may be up to 5 g dry weight or up to 10 g wet weight for solid samples and up to 0.5 g dry weight or up to 5 g wet weight for biosolid samples. After addition of isotopically labelled surrogate standards the sample is extracted by shaking three times with methanolic ammonium hydroxide solution, each time collecting the supernatants. The supernatants are combined, treated with ultra pure carbon powder and evaporated to remove methanol. The resulting solution is diluted with water and cleaned up by solid phase extraction (SPE) using disposable cartridges containing a weak anion exchange sorbent. For a batch, either

PromoChrom automated SPE or vacuum manifold SPE equipment will be used. The eluate is spiked with recovery standards and analyzed by LC-MS/MS.
Tissue sample size may be up to 2 g wet weight. After addition of isotopically labelled surrogate standards the sample is extracted with methanolic potassium hydroxide solution, with acetonitrile, and finally with methanolic potassium hydroxide solution, each time collecting the supernatants. The supernatants are combined, treated with ultra pure carbon powder and evaporated to remove methanol. The resulting solution is diluted with water and cleaned up by solid phase extraction (SPE) on a weak anion exchange sorbent. The eluate is spiked with recovery standards and analyzed by LC-MS/MS.

All AFFF samples are pre-screened before analysis to determine the appropriate amount of sample to analyze. A suitable subsample is dissolved in water, spiked with surrogate standards and extracted by solid phase extraction (SPE). The extracts are treated with carbon powder, spiked with recovery standards and analyzed by LC-MS/MS.

Blood/Serum sample size may be up to 2 mL. After the addition of isotopically labelled surrogate standards the sample is extracted with 50% formic acid. The resulting solution is cleaned up by solid phase extraction (SPE) on a weak anion exchange sorbent. The eluent tubes are spiked with recovery standards and then eluent collected and analyzed by LC-MS/MS.

8. 2.0 INSTRUMENTATION

Analysis of the sample extract is performed on a UPLC-MS/MS (ultrahigh performance liquid chromatography) reversed phase C18 column using a solvent gradient. The column is coupled to a triple quadrupole mass spectrometer run at unit mass resolution in the Multiple Reaction Monitoring (MRM) mode, using negative electrospray ionization.

9. 3.0 CALIBRATION

Initial calibration of the UPLC-MS/MS instrument is performed by the analysis of five or more calibration solutions. A mid-level calibration standard is analyzed to verify the initial calibration and injected after: x at least every 12 hours.

x For DoD accredited work after every 10 client samples or every 12 hours, whichever occurs first, and at the end of the instrumental run sequence.

10. LIST OF SURROGATE AND RECOVERY STANDARDS

Surrogate Standards	Recovery Standards
$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA

$^{13}C_5$-PFHxA	$_{13}C_4$-PFOA
$^{13}C_4$-PFHpA	$_{13}C_5$-PFNA
$^{13}C_8$-PFOA	$_{13}C_2$-PFDA
$^{13}C_9$-PFNA	$_{18}O_2$-PFHxS
$^{13}C_6$-PFDA	$_{13}C_4$-PFOS
$^{13}C_7$-PFUnA	$^{13}C_2$-D_4-6:2-FTS
$^{13}C_2$-PFDoA	
$^{13}C_2$-PFTeDA	
$^{13}C_3$-PFBS	
$^{13}C_3$-PFHxS	
$^{13}C_8$-PFOS	
$^{13}C_2$-4:2 FTS	
$^{13}C_2$-6:2 FTS	
$^{13}C_2$-8:2 FTS	
$_{13}C_8$-PFOSA	
D_3-N-MeFOSA	
D_5-N-EtFOSA	
D_3-N-MeFOSAA	
D_5-N-EtFOSAA	
D_7-N-MeFOSE	
D_9-N-EtFOSE	
$^{13}C_3$-HFPO-DA	

4.0 QUANTIFICATION PROCEDURES

11. 4.1 CALCULATIONS

Target compounds are quantified using the isotope dilution/internal standard method, comparing the area of the primary (quantifying) transition product ion of the target compound to that of the ^{13}C-labelled or deuterium labeled standard and correcting for response factors. Mean relative response factors (RRF), determined from the multi-level initial calibration series are used to convert raw peak areas in sample chromatograms to final concentrations as follows:

§area of Target· §weight of Qt Std· § 1 ·

Concentration of Target $= \frac{\text{area of Qt Std}}{\text{RRF} \times \text{weight of sample}}$

where $\text{RRF} = \frac{\text{area of Target}}{\text{area of Qt Std}} \times \frac{\text{weight of Qt Std}}{\text{weight of Target}}$

and the Qt Std is either the surrogate or the internal standard

The isotopically labeled analog of an analyte (surrogate) is used for quantitation (Isotope Dilution Quantitation). If a labeled analog is not commercially available, a surrogate with chemical similarity and close retention time is used for quantitation (internal standard quantitation). Final target concentrations are recovery corrected by this method of quantification.

12. 4.2 REPORTING LIMITS

Sample Specific Detection Limits (SDL) are determined by converting the area equivalent to 3.0 times the estimated chromatographic noise height to a concentration in the same manner that target peak responses are converted to final concentrations. The SDL accounts for any effect of matrix on the detection system and for recovery achieved through the analytical work-up.

Results are reported to the greater of the SDL or the concentration equivalent to CAL A. The lowest method calibration limit (LMCL) is CAL B. Results below the LOQ are J flagged.

The EPA defines ML (minimum level of detection) as the lowest concentration at which an analyte can be measured with a known level of confidence. EPA considers the terms, “reporting limit”, “quantitation limit”, “limit of quantitation” and “minimum level” to be synonymous. For SGS AXYS purposes, the ML is established by multiplying the MDL by 3.18 and rounding the result to the nearest integer of 1, 2 or 5 X 10^n, where n is an integer.

A client may choose to report field samples to the ML. If this is chosen, the blank should be reported to the MDL.

13. ANALYTES AND QUANTIFICATION REFERENCES

Target analytes	Quantified using
PFBA	$^{13}C_4$-PFBA
PFPeA	$^{13}C_5$-PFPeA
PFHxA	$^{13}C_5$-PFHxA
PFHpA	$^{13}C_4$-PFHpA

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PFOA	$^{13}C_8$-PFOA
PFNA	$^{13}C_9$-PFNA
PFDA	$^{13}C_6$-PFDA
PFUnA	$^{13}C_7$-PFUnA
PFDoA	$^{13}C_2$-PFDoA
PFTrDA	avg. $^{13}C_2$-PFTeA and $^{13}C_2$-PFDoA
PFTeDA	$^{13}C_2$-PFTeDA
PFBS	$^{13}C_3$-PFBS [5]
PFPeS	$^{13}C_3$-PFHxS
PFHxS	$^{13}C_3$-PFHxS
PFHpS	$^{13}C_8$-PFOS
PFOS	$^{13}C_8$-PFOS
PFNS	$^{13}C_8$-PFOS
PFDS	$^{13}C_8$-PFOS
PFDoS	$^{13}C_8$-PFOS
4:2 FTS	$^{13}C_2$-4:2 FTS
6:2 FTS	$^{13}C_2$-6:2 FTS
8:2 FTS	$^{13}C_2$-8:2 FTS
PFOSA	$^{13}C_8$-PFOSA
N-MeFOSA	D_3-N-MeFOSA
N-EtFOSA	D_5-N-EtFOSA
N-MeFOSAA	D_3-N-MeFOSAA
N-EtFOSAA	D_5-N-EtFOSAA
N-MeFOSE	D_7-N-MeFOSE
N-EtFOSE	D_9-N-EtFOSE
HFPO-DA	$^{13}C_3$-HFPO-DA
ADONA	$^{13}C_3$-HFPO-DA
9Cl-PF3ONS	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	$^{13}C_3$-HFPO-DA
3:3 FTCA	$^{13}C_5$-PFPeA
5:3 FTCA	$^{13}C_5$-PFHxA

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Target analytes	Quantified using
7:3 FTCA	$^{13}C_5$-PFHxA
PFMPA	$^{13}C_5$-PFPeA
PFMBA	$^{13}C_5$-PFPeA
NFDHA	$^{13}C_5$-PFHxA
PFEESA	$^{13}C_5$-PFHxA

[1] If the quantifying standard $^{13}C_3$-PFBS is unavailable PFBS may optionally be quantified against $^{13}C_3$-PFHxS.

Surrogate standards	Quantified using
$^{13}C_4$-PFBA	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	$^{13}C_4$-PFOA
$^{13}C_8$-PFOA	$^{13}C_4$-PFOA
$^{13}C_9$-PFNA	$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	$^{13}C_2$-PFDA
$_{13}C_3$-PFBS	$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	$^{13}C_4$-PFOS
$^{13}C_2$-4:2 FTS	$^{13}C_2$-D_4-6:2 FTS
$^{13}C_2$-6:2 FTS	$^{13}C_2$-D_4-6:2 FTS
$^{13}C_2$-8:2 FTS	$^{13}C_2$-D_4-6:2 FTS
$^{13}C_8$-PFOSA	$^{13}C_4$-PFOS
D_3-N-MeFOSA	$^{13}C_4$-PFOS
D_5-N-EtFOSA	$^{13}C_4$-PFOS

D_3-N-MeFOSAA	$^{13}C_2$-D_4-6:2 FTS
D_5-N-EtFOSAA	$^{13}C_2$-D_4-6:2 FTS
D_7-N-MeFOSE	$^{13}C_4$-PFOS
D_9-N-EtFOSE	$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	$_{13}C_2$-PFHxA
Recovery standards	**Quantified using**
$^{13}C_3$-PFBA	External
$_{13}C_2$-PFHxA	External
$_{13}C_4$-PFOA	External
$^{13}C_5$-PFNA	External
$_{13}C_2$-PFDA	External
$_{18}O_2$-PFHxS	External
$_{13}C_4$-PFOS	External
$^{13}C_2$-D_4-6:2 FTS	External

14. 5.0 QUALITY ACCEPTANCE CRITERIA

Samples are analyzed in batches consisting of a maximum of twenty samples, one procedural blank and one spiked matrix (OPR) sample. A duplicate is analyzed, provided there is sufficient sample, with batches containing 7-20 samples. Matrix spike/matrix spike duplicate (MS/MSD) pairs may be analyzed on an individual contract basis. The batch is carried through the complete analytical process as a unit. For sample data to be reportable, the batch QC data must meet the established acceptance criteria presented on the analysis reports.

15. QC SPECIFICATION TABLE FOR AQUEOUS, SOLID, AFFF AND TISSUE SAMPLES: PROCEDURAL BLANK LEVELS AND OPR RECOVERY RANGES, INCLUDES DOD QSM RECOVERY RANGES

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Compound	Procedural Blank Level (ng/sample)	OPR Recovery Range for Aqueous and AFFF Samples (%)	OPR Recovery Range for Solid Samples (%)	OPR Recovery Range for Tissue Samples (%)	OPR Recovery Range for Blood/ Serum Samples (%)
PFBA	≤ 1.6	70-130	70-130	70-130	70-130
PFPeA	≤ 0.8	70-130	70-130	70-130	70-130
PFHxA	≤ 0.4	70-130	70-130	70-140	70-130
PFHpA	≤ 0.4	70-130	70-130	70-133	70-130
PFOA	≤ 0.4	70-130	70-130	70-130	70-130
PFNA	≤ 0.4	70-130	70-130	54-159	70-130
PFDA	≤ 0.4	70-130	70-130	70-130	70-150
PFUnA	≤ 0.4	70-130	70-130	70-130	70-140
PFDoA	≤ 0.4	70-130	70-130	70-130	70-140
PFTrDA	≤ 0.4	70-130	70-130	60-140	70-130
PFTeDA	≤ 0.4	70-130	70-130	70-130	70-130
PFBS	≤ 0.4	70-131	70-130	70-133	70-130
PFPeS	≤ 0.4	70-130	70-130	60-130	70-130
PFHxS	≤ 0.4	70-130	70-130	70-130	70-130
PFHpS	≤ 0.4	70-130	70-130	60-130	70-130
PFOS	≤ 0.4	70-130	70-130	70-130	70-130
PFNS	≤ 0.4	70-130	70-130	60-130	40-140
PFDS	≤ 0.4	70-130	70-130	60-130	70-130
PFDoS	≤ 0.4	70-130	70-130	42-130	70-130
4:2 FTS	≤ 1.6	70-130	70-130	70-130	70-130
6:2 FTS	≤ 5	70-130	70-130	70-130	70-130
8:2 FTS	≤ 1.6	70-130	70-130	70-130	70-130
PFOSA	≤ 0.4	70-130	70-130	70-130	70-130
N-MeFOSA	≤ 0.4	70-130	70-130	70-150	70-130
N-EtFOSA	≤ 0.4	70-130	70-130	70-130	70-130
N-MeFOSAA	≤ 0.4	70-130	70-130	70-130	70-150
N-EtFOSAA	≤ 0.4	70-130	70-130	70-130	60-130

N-MeFOSE	≤ 4	70-130	70-130	n.a.[1]	70-130
N-EtFOSE	≤ 4	70-130	70-130	62-157	70-160
HFPO-DA	≤ 1.6	49-150	70-130	62-149	70-130
ADONA	≤ 1.6	70-132	70-132	70-219	70-130
9Cl-PF3ONS	≤1.6	70-131	70-130	70-197	60-130
11Cl-PF3OUdS	≤ 1.6	68-130	59-130	68-188	70-130
3:3 FTCA	≤ 1.6	70-130	68-130	47-130	n.a.[2]
5:3 FTCA	≤ 10	70-130	69-130	70-198	60-140
7:3 FTCA	≤ 10	70-130	59-130	70-164	60-140
PFMPA	≤ 0.8	70-130	70-130	67-140	60-140
PFMBA	≤ 0.4	70-130	70-130	70-130	60-140
NFDHA	≤ 0.8	45-130	39-130	n.a[1]	n.a.[2]
PFEESA	≤ 0.4	70-130	70-130	53-140	60-140

[1] N-MeFOSE and NFDHA in tissues are for information only [2]

3:3 FTCA and NFDHA in serums are for information only.

Marginal exceedance allowance – results for one compound may fall outside of these limits by a maximum of 10% of the value. Note that for AFFF products, these are interim specifications and data outside the specifications may be acceptable based on application and professional judgment. DoD specification for procedural blanks: No analytes detected >½ LOQ or > 1/10th the amount measured in any sample or 1/10th the regulatory limit, whichever is greater.

16. QC SPECIFICATIONS FOR AQUEOUS, SOLID, AFFF, TISSUE AND BLOOD/SERUM SAMPLES: SURROGATE STANDARD RECOVERIES, OPR AND SAMPLES

Surrogate Standard	**OPR and Sample Recovery Range [1] for Aqueous and AFFF Samples (%)**	**OPR and Sample Recovery Range [1] for Solid Samples (%)**	**OPR and Sample Recovery Range [1] for Tissue Samples (%)**	**OPR and Sample Recovery Range [1] for Blood /Serum Samples (%)**
$^{13}C_4$-PFBA	50-150	50-150	50-150	50-150
$^{13}C_5$-PFPeA	50-150	50-150	50-150	50-150

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$^{13}C_5$-PFHxA	50-150	50-150	50-150	50-150
$^{13}C_4$-PFHpA	50-150	50-150	50-150	50-150
$^{13}C_8$-PFOA	50-150	50-150	50-150	50-150
$^{13}C_9$-PFNA	50-150	50-150	50-150	50-150
$^{13}C_6$-PFDA	50-150	50-150	50-150	50-150
$^{13}C_7$-PFUnA	50-150	50-150	50-150	50-160
$^{13}C_2$-PFDoA	50-150	50-150	50-150	50-150
$^{13}C_2$-PFTeDA	50-150	50-150	34-150	40-150
$^{13}C_3$-PFBS	50-150	50-150	50-150	50-150
$^{13}C_3$-PFHxS	50-150	50-150	50-150	50-150
$^{13}C_8$-PFOS	50-150	50-150	50-150	50-150
$^{13}C_2$-4:2 FTS	50-150	47-186	50-157	50-150
$^{13}C_2$-6:2 FTS	50-150	50-154	50-150	30-180
$^{13}C_2$-8:2 FTS	50-150	50-150	50-156	30-180
$^{13}C_8$-PFOSA	50-150	50-150	50-155	50-150
D_3-N-MeFOSA	43-150	25-150	15-150[2]	50-150
D_5-N-EtFOSA	41-150	24-150	15-150[2]	50-150
D_3-N-MeFOSAA	49-150	47-200	50-150[2]	40-200
D_5-N-EtFOSAA	50-150	50-200	50-150[2]	40-200
D_7-N-MeFOSE	50-150	34-150	n.a	50-150
D_9-N-EtFOSE	50-150	30-150	9-150[2]	30-150
$^{13}C_3$-HFPO-DA	50-150	50-150	43-150	50-150

[1] Lower surrogate recoveries may be accepted based on application and professional judgment. Note that for AFFF products, these are interim specifications and data outside the specifications may be acceptable based on application and professional judgment.

[2] These surrogates used only to quantify the analogous native compounds. Surrogate recoveries that fall within method specifications are considered to be reliable. Surrogate recoveries that fall outside of method specifications may result in the qualification of analyte data, if surrogate recoveries are below 10% or in analyte data being non-quantifiable if below 1%. Professional judgement applies in all cases. Surrogate recoveries of D_5-N-EtFOSAA in tissue samples may be high, with increased uncertainty in the analyte concentration when the surrogate recovery is above 200%. Under these conditions, N-EtFOSAA is for information only. Likewise,

surrogate recoveries of D_7-N-MeFOSE and D_9-N-EtFOSE in tissue samples may be low, with increased uncertainty in the analyte concentration when the surrogate recovery is below 8%. Under these conditions, N-Et-FOSE and N-Me-FOSE are for information only.

17. QC SPECIFICATION TABLE: OTHER PARAMETERS

QC Parameter	Specification[1]
MS Acquisition Rate	Minimum acquisition rate for every native analyte and labeled compound peak: At least 10 data points per peak.
Mass Calibration	Instrument must have a valid mass calibration following the manufacturer specified procedure prior to any sample analysis. The mass calibration is updated on an as-needed basis (e.g. QC failures, ion masses fall outside of the ±0.5 amu of the true value, major instrument maintenance, or if the instrument is moved.) Refer to SIN-033. The entire range (bracketing all the masses of the target analytes) must be mass calibrated. The maximal allowed residual error is ≤ 0.1 Da for each mode with no more than two calibration points missed.
Mass Calibration Verification	Mass calibration must be verified after each mass calibration, prior to any sample analysis. Mass calibration must be performed per the instrument manufacturer's instructions. A mass calibration verification must be performed using standards whose mass range brackets the masses of interest (quantitative and qualitative ions).
Initial Calibration (I-CAL)	Run initially, and as required to maintain compliance with calibration verification and instrument sensitivity. The isotopically labeled analog of an analyte (surrogate standard) must be used for quantitation if commercially available (Isotope Dilution Quantitation). If a labeled analog is not commercially available, the surrogate standard with the closest retention time or chemical similarity to the native standard must be used for quantitation. Quantification is achieved by the constant RRF method. The I-CAL specifications (CAL B to CAL J) for the RRF are <20% RSD of mean RRFs and 70-130% recovery of analytes and surrogates at each concentration level from Cal C and above. For concentrations at or above the method LOQ, the total (branched and linear isomer) quantification ion response to the total (branched and linear isomer) confirmation ion response ratio must fall within ±50% (50-150%) of the ratio observed in the I-CAL, CAL E. CAL B to CAL J must meet a 3:1 S/N specification in

QC Parameter	Specification[1]
	the quantification ion and a 3:1 S/N in the confirmation ion. The A CAL (sensitivity CAL) must achieve 3:1 S/N for the quantification ion. Peak Asymmetry, SGS AXYS guidance: 0.8-1.5 for PFBA and PFPeA measured in CAL E (mid cal point) at 10% of the peak height. If this is not achieved, perform instrument maintenance and re-run I-CAL.
Initial Calibration Verification (ICV):	After each Initial Calibration (I-CAL) and prior to sample analysis; analyze a second source standard (similar concentration to the CAL E); quantify against I-Cal, results must meet Cal/Ver accuracy specifications of 70% to 130%. Ion ratios must be within 50-150% of the ratios determined from ICAL CAL E.
Retention Time (RT) window	Relative retention times (RRT) for linear and branched isomers vs, the surrogates are determined from the qualitative standard run every 12 hours. Maximum RRTs windows are 0.1 minutes.
Surrogate Standards	Must be added to every field sample, standard, blank, and QC sample. Recoveries of the surrogate standard analytes (EPA term for Surrogate Standard is Extracted Internal Standard-EIS; EPA term for Recovery Standard is Injection Internal Standard-IIS) are calculated by internal standard quantification against the IIS using an average RRF. Recovery criteria for surrogate standard analytes in instrument blanks and standards is 70% to 130%. Recovery criteria for surrogate standard in field samples and preparatory QC samples are listed in the table above.
Recovery Standards	Must be added to every prepared field sample, standard, blank, and QC sample prior to instrumental analysis. Recovery standard analyte recovery is calculated by external standards by evaluation of the mean RF from the I-CAL; specification is 50% to 200%. Professional judgement applies.
Calibration Verification (Cal/Ver or CCV)	CAL E. Prior to sample analysis and at the end of the analytical sequence, or every 12 hours. Quantify against I-CAL. Native standard analyte and surrogate standard concentrations must be within ±30% of their true value. Recovery standard analyte concentrations must be within 50-200% of their true value. Ion ratios must be within 50150% of the ratios determined from I-CAL CAL E. If the CCV criterion are not met, an instrument re-calibration is performed. *Additional requirement for DoD: Run Cal/Ver every 10 client samples or every 12 hours, whichever occurs first, and at the end of the analytical sequence; quantify against I-CAL. If the CCV criterion are not met, immediately analyze 2 additional CCVs. If both CCVs meet the criteria, samples may be reported without re-analysis. If either CCV exceed the criteria, or if two successive CCVs cannot be analyzed immediately following the failing CCV, corrective action must be taken. Once*

QC Parameter	Specification[1]
	correction has been made and a CCV has been analyzed and met the criteria, all samples bracketed by the failing CCV must be re-analyzed. For internal purposes monitor Peak Asymmetry for every Cal/Ver
Instrument Sensitivity Check (ISC)	Run every 12 hours. CAL A – S:N ≥ 3:1 for quantification ion. CAL C – S:N ≥ 3:1 for quantification and confirmation ion. Native compound recoveries 70-130%. Ion ratios must be within 50-150% of the ratios determined from I-CAL CAL E.
Instrument Background	For DoD work an instrument blank containing surrogates is run immediately after every Initial Calibration (highest standard) and Calibration Verification and daily thereafter. The concentration of each analyte in the instrument blank must be ≤ ½ of the LOQ (C CAL; B CAL for DoD AFFF PFOA and PFOS only). In this case (assuming Cal J would fail this test) the instrument blank can be run after CAL I and before the CAL J. Cal J is not included in the ICAL for DoD samples but can be included for other work. If any sample concentrations exceed the highest allowed standard and the sample(s) following exceed this acceptance criteria (>½ LOQ), they must be reanalyzed. The percent recoveries of both the surrogate and recovery standards in the instrument blank must be 50-200% of their true value.
Instrument Carryover	The specification is d 0.3 % carryover from the Cal Ver standard into following instrument blank or from any sample into the following injection.
Duplicate Samples	If conc. ≥ 5 times R.L., RPD ≤ 40% If conc. < 5 times R.L., guideline RPD ≤ 100% *Requirement for DoD AFFF PFOS and PFOA only: for concentrations that are ≥ the LOQ (CAL B), RPD* d *30%.*
Ongoing Precision and Recovery (OPR or LCS)	Ongoing Precision and Recovery (OPR) or Laboratory Control Sample (LCS) is spiked at the same level as CAL E. Refer to the tables above for native analytes for OPRs and for OPR surrogate acceptance limits.
Low-Level Ongoing Precision and Recovery (LLOPR)	Spiked at 2X the LOQ (CAL-C) and serves to verify the LOQ and must be analyzed with a minimum frequency of 5% of client samples (i.e., each batch of up to 20 client samples contains an LLOPR), prior to sample injection in the same manner as the OPR.

Method Blank (MB)	One per preparatory batch. No analyte can be detected > ½ LOQ or >1/10th the amount measured in field samples in the batch, whichever is greater. If any PFAS is detected, greater than these levels, sample analysis must be halted, and the problem corrected. If PFAS contribution to the blank is traced to the analysis batch, samples affected by the blank must be re-extracted and analyzed, provided there is enough sample
QC Parameter	**Specification**[1]
	available and is still within holding times. If continued testing results in continued blank detects, the blank results are flagged (unless not required by the client).
Bile Salts Interference check standard (TDCA)	Run after every I-CAL, and also prior to the analysis of tissue samples, on that day, to check for interferences caused by bile salts
MS/MSD (required for DOD, optional for others)	Native standard concentration must be spiked at concentrations ≥ LOQ and ≤ the mid-level calibration concentration. If an MS/MSD cannot be used, an OPR duplicate must be run instead. This is optional for non-DoD work. *Additional requirement for DoD: For all matrices analyzed by SPE one Matrix Spike and one Matrix Spike Duplicate shall be included with every analysis batch. MS/MSD recoveries are evaluated against project limits if prescribed by the client, otherwise MS/MSD recoveries are evaluated against the DOD specific acceptance ranges for OPRs listed in the table above, or against the MLA-110 OPR method recovery limits for analytes not listed . RPDs are evaluated against project limits if prescribed by the client, otherwise RPDs are evaluated against the DoD specific limit of ≤30%.*

18. APPENDIX A: NAMING CONVENTION AND CAS NUMBERS

PFAS: Naming Convention and CAS Numbers		
Abbreviation	***Name - Anion Form***	***CAS#***
PFBA	Perfluorobutanoate	45048-62-2
PFPeA	Perfluoropentanoate	45167-47-3

[1] Corrective actions, stated in the current version of DoD QSM, Appendix B, Table B-15, Table B-24 and EPA 1633, must be utilized when QC parameter fails to meet the specification. Any requirement to meet ion ratios only applies to Authentics in samples, as per DoD requirement. This requirement does not apply to authentics in calibration standards but does apply to OPRs, blanks and MS/MSDs and does not apply to any surrogates.

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PFHxA	Perfluorohexanoate	92612-52-7
PFHpA	Perfluoroheptanoate	120885-29-2
PFOA	Perfluorooctanoate	45285-51-6
PFNA	Perfluorononanoate	72007-68-2
PFDA	Perfluorodecanoate	73829-36-4
PFUnA	Perfluoroundecanoate	196859-54-8
PFDoA	Perfluorododecanoate	171978-95-3
PFTrDA	Perfluorotridecanoate	862374-87-6
PFTeDA	Perfluorotetradecanoate	365971-87-5
PFBS	Perfluorobutanesulfonate	45187-15-3
PFPeS	Perfluoropentanesulfonate	175905-36-9
PFHxS	Perfluorohexanesulfonate	108427-53-8
PFHpS	Perfluoroheptanesulfonate	146689-46-5
PFOS	Perfluorooctanesulfonate	45298-90-6
PFNS	Perfluorononanesulfonate	474511-07-4
PFDS	Perfluorodecanesulfonate	126105-34-8
PFDoS	Perfluorododecanesulfonate	343629-43-6
4:2 FTS	4:2 fluorotelomersulfonate	414911-30-1
6:2 FTS	6:2 fluorotelomersulfonate	425670-75-3
8:2 FTS	8:2 fluorotelomersulfonate	481071-78-7
3:3 FTCA	3:3 perfluorohexanoate	1169706-83-5
5:3 FTCA	5:3 perfluoroctanoate	1799325-94-2
7:3 FTCA	7:3 perfluorodecanoate	1799325-95-3
N-MeFOSAA	N-Methylperfluorooctanesulfonamidoacetate	n.a.
N-EtFOSAA	N-Ethylperfluorooctanesulfonamidoacetate	n.a.
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3heptafluoropropoxy)propanoate	122499-17-6
ADONA	Dodecafluoro-3H-4,8-dioxanonanoate	2127366-90-7

PFAS: Naming Convention and CAS Numbers

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9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	1621485-21-9
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1sulfonate	2196242-82-5
NFDHA	Perfluoro-3,6-dioxaheptanoate	39187-41-2
PFMPA	Perfluoro-3-methoxypropanoate	n.a.
PFMBA	Perfluoro-4-methoxybutanoate	1432017-36-1
PFEESA	Perfluoro(2-ethoxyethane)sulfonate	220689-13-4
Abbreviation	***Name - Acid Form***	***CAS#***
PFBA	Perfluorobutyric acid	375-22-4
PFPeA	Perfluoropentanoic acid	2706-90-3
PFHxA	Perfluorohexanoic acid	307-24-4
PFHpA	Perfluoroheptanoic acid	375-85-9
PFOA	Perfluorooctanoic acid	335-67-1
PFNA	Perfluorononanoic acid	375-95-1
PFDA	Perfluorodecanoic acid	335-76-2
PFUnA	Perfluoroundecanoic acid	2058-94-8
PFDoA	Perfluorododecanoic acid	307-55-1
PFTrDA	Perfluorotridecanoic acid	72629-94-8
PFTeDA	Perfluorotetradecanoic acid	376-06-7
PFBS	Perfluorobutanesulfonic acid	375-73-5
PFPeS	Perfluoropentanesulfonic acid	2706-91-4
PFHxS	Perfluorohexanesulfonic acid	355-46-4
PFHpS	Perfluoroheptanesulfonic acid	375-92-8
PFOS	Perfluorooctanesulfonic acid	1763-23-1
PFNS	Perfluorononanesulfonic acid	68259-12-1
PFDS	Perfluorodecanesulfonic acid	335-77-3
PFDoS	Perfluorododecanesulfonic acid	79780-39-5
4:2 FTS	4:2 fluorotelomersulfonic acid	757124-72-4
6:2 FTS	6:2 fluorotelomersulfonic acid	27619-97-2
8:2 FTS	8:2 fluorotelomersulfonic acid	39108-34-4
3:3 FTCA	3:3 perfluorohexanoic acid	356-02-5

5:3 FTCA	5:3 perfluorooctanoic acid	914637-49-3
PFAS: Naming Convention and CAS Numbers		
7:3 FTCA	7:3 perfluorodecanoic acid	812-70-4
N-MeFOSAA	N-Methylperfluorooctanesulfonamidoacetic acid	2355-31-9
N-EtFOSAA	N-Ethylperfluorooctanesulfonamidoacetic acid	2991-50-6
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3heptafluoropropoxy)propanoic acid	13252-13-6
ADONA	Dodecafluoro-3H-4,8-dioxanonanoic acid	919005-14-4
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	756426-58-1
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9
NFDHA	Perfluoro-3,6-dioxaheptanoic acid	151772-58-6
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7
Abbreviation	***Name - Sulfonamide***	***CAS#***
PFOSA	Perfluorooctanesulfonamide	754-91-6
N-MeFOSA	N-Methylperfluorooctanesulfonamide	31506-32-8
N-EtFOSA	N-Ethylperfluorooctanesulfonamide	4151-50-2
Abbreviation	***Name - Sulfonamidoethanol***	***CAS#***
N-MeFOSE	N-Methylperfluorooctanesulfonamidoethanol	24448-09-7
N-EtFOSE	N-Ethylperfluorooctanesulfonamidoethanol	1691-99-2

APPENDIX B: SAMPLE STORAGE CONDITIONS

19. SAMPLE STORAGE REQUIREMENTS

Matrix	Sample Size (per analysis)	Sample Container [1]	Sample Condition Upon Receipt	Storage Condition [2]	Sample Hold Time [3]	Extract Hold Time [4]	Preservation
Aqueous	Up to 1000 mL, but typically 500 mL or less (max. 50 mg solids). Leachate samples are up to 125mL	High density polyethylene (HDPE)	0-6 °C, dark	≤ -20 °C, dark	90 days	30 days	None Required
Solvent extracts[7]	Typically 0.75 mL	High density polyethylene (HDPE)	0-6 °C, dark	0-4 °C, dark	60 days	30 days	None Required
Solid[8]	Up to 5 g dry but not more than 10 g wet.	High density polyethylene (HDPE)	0-6 °C, dark	≤ -20 °C, dark	90 days[11]	30 days	None required
Biosolid[9]	Up to 0.5 g dry but not more than 5 g wet.	High density polyethylene (HDPE)	0-6 °C, dark	≤ -20 °C, dark	90 days[12]	30 days	None required
Tissue[10]	Up to 2 g (wet)	High density polyethylene (HDPE) or amber glass jar	0-6 °C, dark	≤ -20 °C, dark	90 days[11]	30 days	None Required
AFFF	Up to 0.02 g	High density polyethylene (HDPE)	Room temperature.	0-4 °C, dark	90 days	30 days	None Required
Whole Blood [5] or Serum[6]	Up to 2 mL	HDPE or Glass, not polypropylene.	0-6 °C, dark	≤ -20 °C, dark	1 Year	30 days	None Required

[2] Storage temperatures quoted are nominal temperatures.

[3] Hold times are from time of sampling. Client negotiated requests for specific holding times or other method-specific holding times are adhered to. This 90-day holding time on freezing of aqueous samples is based on SGS AXYS storage stability studies.

[4] Hold times for sample extracts are from time of extraction with storage at 4°C. This 30-day holding time is a guideline, longer hold times may be accepted based on professional judgement.

[5] Whole blood must be treated with anticoagulant such as heparin or sodium citrate at time of collection.

[6] Glass vacutainers should be used for blood or serum collection. Plastic vacutainers (such as PET plastic) are not suitable as long chain PFAS compounds may be adsorbed onto the vacutainer surface.

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[1] HDPE containers are preferred; amber glass containers are also acceptable. All containers should be organically clean; i.e. solventrinsed or purchased 'certified' clean. All containers should be tightly sealed with screw cap lids.

[7] Detection limits for extract samples depend on the samples size processed. The reporting limits provided here are based on the typical extract sample size listed above.

[8] A maximum of 10 g wet, or 5 g dry, solid may be analyzed.

[9] A maximum of 5 g wet, or 0.5 g dry, biosolid may be analyzed.

[10] A maximum of 2 g of tissue may be analyzed.

[11] Samples may need to be extracted as soon as possible if NFDHA is required by the client.

[12] Samples may need to be frozen if storage is required for more than a few days.

20. STABILITY OF PFAS IN AQUEOUS SAMPLES

SGS AXYS has completed an extended-time storage study for the 29 PFAS listed in the table below. The study was conducted in reagent water, surface water and two wastewater treatment plant (WWTP) effluents. PFAS concentrations were measured at 0, 7, 14, 30, 90, and 180-day timepoints with data analysis of a 180-day timepoint in progress. Data analysis up to the 90-day timepoint shows that precursors present in samples containing matrix and biological activity can transform under room temperature and cold storage conditions within 7 days. At this time, only freezing of aqueous samples was demonstrated to stabilize the analytes over a period of up to 90 days. We are recommending freezing non-potable aqueous samples as soon as practicable if not analyzed within 3-4 days.

21. SUMMARY OF ANALYTE STABILITY IN AQUEOUS SAMPLES BY STORAGE CONDITION

	Stability (days)		**Remarks**
Analyte	**4°C**	**-20°C**	
C_4-C_{14} Perfluorinated carboxylates including PFOA	90	90	
C_4-C_{10} perfluorinated sulfonates including PFOS	90	90	
PFDoS	90	90	
4:2 FTS	90	90	
6:2 FTS	90	90	
8:2 FTS	14	90	Decreasing trend seen from day 7 at 4°C
PFOSA	14	90	Increasing trend seen day 14 onwards at 4°C
N-MeFOSA	7	90	Decreasing trend seen at first stability point 4°C
N-EtFOSA	7	90	Decreasing trend seen at first stability point 4°C
N-MeFOSAA	<7	90	Increase from transformation of MeFOSE
N-EtFOSAA	7	90	Increase from transformation of EtFOSE
N-MeFOSE	<7	90	Loss seen at first stability point 4°C
N-EtFOSE	<7	90	Loss seen at first stability point 4°C

"QQFOEJY#"/4%6401/P1i5IFVTFPGUIF&MFNFOUBS1ZSPDVCF&MFNFOUBM
"OBMZ[FSBOE*TPQSJNF.BTT4QFDUSPNFUFSGPSUIF"OBMZTJTPG$PDFOUSBUJPOBOE4UB
CFM *TPUPQFTPG$BSCPO /JUSPHFO BOE4VMGVSJO5JTTVFT 1MBOUT
4FEJNFOUTBOE'JMUFSFE 1BSUJDVMBUF.BUUFSw

ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-205
Rev. 1

THE USE OF THE ELEMENTAR PYROCUBE ELEMENTAL ANALYZER AND ISOPRIME100 MASS SPECTROMETER FOR THE ANALYSIS OF CONCENTRATION AND STABLE ISOTOPES OF CARBON, NITROGEN, AND SULFUR IN TISSUES, PLANTS, SEDIMENTS AND FILTERED PARTICULATE MATTER

Prepared By: Paula Zelanko
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Approved By: _____ Date:_____

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THE USE OF THE ELEMENTAR PYROCUBE ELEMENTAL ANALYZER AND ISOPRIME100 MASS SPECTROMETER FOR THE ANALYSIS OF CONCENTRATION AND STABLE ISOTOPES OF CARBON, NITROGEN, AND SULFUR IN TISSUES, PLANTS, SEDIMENTS AND FILTERED PARTICULATE MATTER

1. METHOD

1.1. This is a method for analyzing stable isotopes of total (and organic) carbon and/or nitrogen and/or sulfur content in tissues, plants, sediment, or filtered particulate matter using an Elementar Pyrocube and Isoprime100 Isotope Ratio Mass Spectrometer.

2. SUMMARY

2.1. The homogenized sample is carefully placed into a tin capsule based on weight (mg) or by filtered volume (ml). The capsule is closed and following the manufacturers guidelines, the sample is combusted at 1115°C. The resultant evolved CO_2, N_2, and SO_2 gases are separated via gas chromatograph and elemental concentration is determined with a thermal conductivity detector. Further, gases are ionized and separated with an electro-magnet and the mass:charge is measured. Ratios of the heavy to light isotope are calculated within IonVantage software and reported in terms of $\delta^{X}Y$ ‰, where ^{X} is the mass number of the rare isotope and Y is the associated element.

3. APPARATUS

3.1. Elementar Pyrocube Elemental Analyzer

3.1.1. Combustion Tube

3.1.2. Reduction Tube

3.1.3. Drying Tubes (3x)

3.1.4. Ash Finger (2x)

3.1.5. Helium (Airgas: CY-HE ISP300 or equivalent)

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3.1.6. Oxygen (Airgas: CY-OX UHP200 or equivalent)

3.2. Isoprime100 Isotope Ratio Mass Spectrometer

3.2.1. Carbon Dioxide (Airgas: CY-CD R200 or equivalent)

3.2.2. Nitrogen Gas (Airgas: CY-NI HP300 or equivalent)

3.2.3. Sulfur Dioxide (Airgas: CY-SD AH35 or equivalent)

3.2.4. Two Reference Gas Control Boxes

3.2.5. Diluter box

3.2.6. Air compressor

3.3. IonVantage and varioPYROcube Software installed on a compatible computer, connected with fiber optic cables

3.4. Freeze Dryer

3.5. Ball Mill

3.6. Constant temperature drying oven, capable of maintaining 60 ± 5°C

3.7. Water filtering apparatus

3.8. Glass Fiber Filters (25 mm) Whatman or equivalent

3.9. Micro-balance accurate to at least 3 decimal places (e.g. 0.000 mg)

3.10. Drying desiccator (glass or plastic)

3.11. Acid-washed glass or plastic petri dishes (preferably glass)

3.12. Tin capsules (3.5 x 5 mm)

3.13. Acetone cleaned pelletizer die and assorted micro-forceps

3.14. Muffled aluminum foil

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4. REAGENTS

4.1. Hydrochloric Acid (concentrated)

4.2. Acetone (reagent grade)

4.3. Aluminum Oxide Wool

4.4. Granulated Tungsten (VI) Oxide

4.5. Aluminum Oxide Spheres

4.6. Quartz Wool

4.7. Copper Turnings

4.8. Silver Wool

4.8. Sicapent

4.9. Cotton Wool

5. STANDARDS

5.1. IAEA NO-3 (Potassium Nitrate)

5.2. IAEA N-1 (Ammonium Sulfate)

5.3. USGS26 (Ammonium Sulfate)

5.4. NBS18 (Calcite)

5.5. NBS19 (TS-Limestone)

5.6. IAEA-CH-6 (Sucrose)

5.7. IAEA-S-1 (Silver Sulfide)

5.8. IAEA-S-2 (Silver Sulfide)

5.9. IAEA-SO-6 (Barium Sulfate)

5.10. Calibrated in-house standards

6. PROCEDURE

6.1. Sample Preparation

6.1.1. Filtration and collection of samples should follow SOPs for water filtration of organic particles or sediment collection. Filters must be pre-combusted, and all material should be cleaned prior to and during filtration. Filters should be dried in a 60 ± 5°C oven until maintain consistent weight.

6.1.2. Sediment samples should be collected with cleaned sediment sampler. Sediment and plant material should be dried in a 60 ± 5°C oven until maintain consistent weight.

6.1.3. Tissues should be freeze-dried until all moisture is removed. Duration depends on volume of material and will take between 24 and 72 hours.

6.2. Homogenization: Samples are homogenized in a ball mill.

6.3. Unless $\delta^{13}C$ values of total (organic and inorganic) carbon are desired, inorganic carbon, is removed by acid fumigation.

6.3.1. Homogenized samples are placed in acid-washed glass petri dishes, which are then placed in a large glass desiccator (without desiccant).

6.3.2. Fill a glass petri dish halfway with concentrated hydrochloric acid, carefully place dish in the center of the desiccator and quickly replace lid.

6.3.3. Samples are left overnight in the fuming HCl, in order to remove the inorganic C from the sample. The next day the filters and sediments are placed in a 60 5°C drying oven overnight.

6.4. Tin boat/instrument cleaning.

6.4.1. Place an appropriate number of tin boats in a clean 100-ml beaker, add enough acetone to completely cover the boats, swirl, decant, repeat two more times, and place the beaker in the drying oven for a minimum of three hours. Remove the beaker, and cover with muffled aluminum foil.

forceps, shake off excess, place on muffled foil and dry in the drying over for a minimum of three hours. Upon removal from the oven, wrap in muffled foil.

6.5. Capsule Preparation

6.5.1. Remove a glass petri dish containing sample from the desiccator. Every other sample regardless of type will be a duplicate. Place a cleaned tin boat in the pelletizer die.

6.5.1.1. If analyzing particulate filters, using the pelletizer die as a work surface, the filter must be folded twice to fit the filter into the boat. Using forceps place the folded filter into the boat and pack it down. Be careful not to lose any material from filter.

6.5.1.2. If analyzing sediments, plants, or tissue, tare the tin boat on the microbalance, place in die, using scoop tool, place a small amount of sediment in boat (low mg quantities), weigh, and record weight.

6.5.2. Remove boat from die, z-fold the top, and fold over the top and pack the capsule until it is a three-dimensional cube or sphere.

6.5.3. Place boat in labeled tray or autosampler and record sample location. Dip all forceps used into a 100-ml acetone filled beaker, to clean them. Using a Kimwipe, dipped in acetone, clean the pelletizer die. Repeat procedure for next sample. Be sure all acetone has evaporated away.

6.6. Blank and Standard Preparation

6.6.1. Two types of blanks; gas, and filter (when applicable), will be used during the analysis.

6.6.1.1. Gas blanks will consist of nothing more than an empty chamber on the autosampler rack. This will represent the baseline.

6.5.1.2. Filter blanks, during particulate analysis represent the lab/field blank (DI filtered through), sample preparation is the same as all of the other samples. This value will be subtracted from the samples as the blank.

6.5.2. Using a tared capsule, weigh out a representative amount of the calibrated inhouse standard. Prepare blanks and standards at intervals as outlined in the project protocol, usually every 10 samples.

6.7. Sample Analysis

6.7.1. Sample runs should consist of the following:

6.7.1.1. A standard curve with a Bypass, Blank, and five standards

6.7.1.2. Unknown samples run in duplicate

6.7.1.3. Run standard unknowns for all the samples (~ every 10 samples)

6.7.2. Follow manufacturers guidelines for the operation and set up of instrument.

7. References

7.1. See “Isoprime user’s guide v1.02” and “Isoprime100 Elemental Analyser User Guide” for Pyrocube and Isotope Ratio Mass Spectrometer set up and operation.

7.2. US. Environmental Protection Agency. 1992. Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis. Method 440.0 *In:* Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples. EPA/600/R-92/121 ORD, Washington DC.

7.3. http://isotopes.usgs.gov/lab/methods/RSIL_SOP_1832.pdf

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ACADEMY OF NATURAL SCIENCES OF DREXEL UNIVERSITY
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-99r3
Rev. 3 (05/22)

DISSOLVED ORGANIC CARBON AND TOTAL ORGANIC CARBON

Prepared By: Jennifer DeAlteris/Karen Bushaw-Newton

Approved By: _____ Date: _____
Ms. Robin Davis, QA Officer

DISSOLVED ORGANIC CARBON AND TOTAL ORGANIC CARBON

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to surface water, groundwater, drinking water, wastewater, and seawater with organic carbon in the range of 0.001 to 100.0 mg/L. The range of analysis can be extended by sample dilution, volume injected or calibration at higher concentrations.

2.0 SUMMARY OF METHOD

2.1 A specified sample volume is transferred to a reaction chamber with a pre-programmed volume of phosphoric acid (H_3PO_4.) The acidified sample is sparged with a stream of inert gas (Helium or ultra-pure air) at a flow rate of 100mL/min to allow bicarbonates in the sample to dissociate to CO2. The resulting gas flow is vented for a pre-programmed sparge time as purgeable organic carbon (POC) in the sample is removed.

Total organic carbon (TOC) is determined by measuring the CO_2 released by chemical oxidation of the organic carbon in the sample. After the removal of total inorganic carbon (TIC), a preprogrammed volume of sodium persulfate ($Na_2S_2O_8$) is added to the sample as an oxidizer. $Na_2S_2O_8$ reacts with organic carbon in the sample at a temperature of 100 ^oC to form CO_2. When the oxidation reaction is complete, the CO_2 is purged from solution and routed to the nondispersive infrared (NDIR) detector that is sensitive to the specific absorption for the wavelength of CO_2. The NDIR outputs a detection signal which generates a peak whose area is calculated by the instrument's software. When the TOC in the sample has been calculated, results are reported as ppm or ppb TOC.

3.0 INTERFERENCES

3.1 This method is for analysis of NPOC (non-purgeable organic carbon) which refers to organic carbon present in a non-volatile form. In most literature for water analysis, the terms are used interchangeably because the amount of purgeable organic substances in natural waters is small. Because purgeable organic substances may be lost during sparging, true TOC may also be determined by calculating the difference between TC and TIC.

3.2 Any contact with organic material may contaminate a sample. Care must be taken in sample handling and storage to minimize exposure.

3.3 Inorganic carbon is considered an interference in the analysis and must be removed or accounted for in the final calculation.

3.4 Removal of inorganic carbon by acidification and sparging may result in the loss of volatile organic substances.

4.0 SAMPLE PREPARATION

4.1 Samples are stored in pre-cleaned (HCl and DI rinsed and muffled at 450ºC for 4 hours) glass vials. The whole water sample is used for TOC analysis and an aliquot filtered through a clean, muffled (450ºC for 4 hours) 0.45 Pm glass fiber filter is used for DOC analysis. The samples should be frozen (-18 ºC) after collection and filtering and taken out to thaw completely before analysis can occur.

5.0 **APPARATUS**

5.1 OI Analytical Aurora 1030w Wet Oxidation TOC analyzer and 1088 Autosampler

5.2 High purity compressed air (ultra zero grade)

5.3 Muffled 40mL borosilicate glass vials

5.4 Pre-cleaned lids to 40mL glass vials

5.5 High-purity deionized water

5.6 10% $Na_2S_2O_8$ reagent

5.7 5% H_3PO_4 reagent

5.8 Muffle furnace

5.9 Balance

5.10 Volumetric flasks and pipettes

6.0 **STANDARDS**

6.1 Stock standard, Certipur TOC standard solution 1000 mg/L (1000 ppm):

Standards are prepared by dilution of a single element standard purchased from a vendor that provides traceability to NIST standards. Working standards for calibration are prepared at concentrations which range from 0.5 mg/L to 20mg/L depending on sample type.

6.2 Antipyrene Reference Sample:

Prepare 1.0g antipyrene to 200mL with deionized water in a volumetric flask, this makes approximately a 700mg/L C solution. This solution is then further diluted by 1mL in a 100mL volumetric flask with deionized water to make approximately a 7mg/L solution which is the reference sample.

7.0 **QUALITY CONTROL**

7.1 Preparation of stock standards is recorded on worksheets and documented by volume of stock standard added to a given flask before dilution to volume with DI water. All records of certification are kept on file.

7.2 Blank: DI water samples run at the beginning and end of the sequence, after check standards, and every 10 samples.

7.3 Antipyrene check standard: Run at the beginning and end of the sequence and every ten samples to monitor drift and recovery.

7.4 Lab fortified blank/Spike: 0.1mL 1000ppm KHP in 20mL DI run every 10 samples to monitor drift and recovery.

7.5 Analytical Duplicate: Separate analysis from the same sample aliquot. One per ten samples.

7.6 Standard recoveries are tracked over time to monitor overall performance.

7.7 Samples with concentration greater than the highest calibration standard are diluted and reanalyzed.

8.0 **CALIBRATION AND STANDARDIZATION**

8.1 Balances: calibrated yearly by external vendor.

8.2 Pipette delivery checked by weight to within 1% of theoretical weight of aliquot volume.

8.3 The R^2 value of each calibration curve should be greater than 0.999.

8.4 Check standard recovery must be within 10% of theoretical value.

9.0 **PROCEDURE**

9.1 Turn on the power switch on the autosampler and Aurora and open the 1030w control software on the PC (log in on instrument interface screen and PC).

9.2 Allow Start-Up mode to bring heaters to temperature and initiate operating gas flow rate.

9.3 Check levels of reagents, DI reservoir, needle rinse DI, and gas.

9.4 From the Monitor menu, navigate to the Sequence tab and Load Active Sequence. Select and run Clean-Up sequence, which will flush the instrument with DI and measure TIC, TOC and establish a baseline.

9.5 Create a Sequence (see pages 150-156 of the Aurora 1030w TOC Analyzer's Operator's Manual).

9.5.1 To create a calibration curve, from the Editor menu, navigate to the Method tab and select New to create a new method. Input desired mode, sparge time, reagent volumes, and number of rinses. In the Calibration tab, determine the number of samples, reps and concentrations. Follow instructions as listed on pages 137-150 of the Aurora 1030w TOC Analyzer's Operator's Manual. Samples are run based on old curves, so it is important that quality calibration methods are maintained.

9.5.2 To create a new sequence of samples (see pages 150-156 of the Aurora 1030w TOC Analyzer's Operator's Manual), navigate to the Sequence tab in the Editor menu to view the Sequence Editor screen. Use the Add/Insert function to automatically fill a number of rows in the sample table using the same method for all samples.

9.6 Prepare standards or samples in the muffled glass vials and place in the autosampler. When the tray is filled, replace the carousel and the cover of the autosampler. A Clean-Up sequence must be run before running any samples or standards to make sure the baseline is stabilized and the machine is running well. A pre-determined Clean-Up sequence can be set up and used before every run.

9.7 From the Monitor menu, choose Start (green arrow). To watch the injections, navigate to the real-time peak button at the bottom of the menu. Peak results will also be saved in the Reporter file for the sequence.

9.8 When the instrument is finished running, the user can navigate to the Reporter application found in the TOC 1030w Launchpad on the PC. There, the user can find area, mass, concentration, standard deviation, and %RSD information. All raw data is copied into a template for sample concentration and standard recovery determination.

10.0 **REFERENCES**

OI Analytical. (2012). Aurora 1030 Wet Oxidation TOC Analyzer Operator's Manual Rev. 2.1. College Station, Texas.

ASTM. American Society for Testing and Materials. Standard Specifications for Reagent Water. D1193-77 (Reapproved 1983). Annual Book of ASTM Standards, Vol. 11.01. ASTM: Philadelphia, PA, 1991.

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ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-54 r2

Rev. 2 (5/2004)

THE USE OF CARLO ERBA MODEL 1112 FLASH ELEMENTAL ANALYZER FOR THE ANALYSIS OF TOTAL CARBON AND NITROGEN IN SEDIMENTS AND FILTERED PARTICULATE MATTER

Prepared By: Nathan Saxe

Approved By: _____ Date:_____

Robin S. Davis
Quality Assurance Unit

The Use of Carlo Erba Model 1112 Flash Elemental Analyzer for the Analysis of Total Carbon and Nitrogen in Sediments and Filtered Particulate Matter

1. METHOD.

1.1. This is a method for analyzing total organic carbon and/or nitrogen content in sediment/algae or filtered particulate matter using a Carlo Erba Model 1112 Flash Elemental Analyzer or equivalent.

2. SUMMARY.

2.1. The sample is carefully placed into a tin boat either by weight or by filtered volume. The boat is closed and, following the manufacturers guidelines, the sample is combusted at 1000(C. The resultant CO_2 and N_2 gas evolved is separated via GC and the mass is determined with a thermal conductivity detector. If the sample contains inorganic carbon, the inorganic carbon is removed by fuming HCl overnight, dried, then the sample is placed into a cleaned tin boat for combustion and analysis in the CHN.

3. APPARATUS.

3.1. Carlo Erba Model 1112 Flash Elemental Analyzer or equivalent, autosampler rack and cover, combustion and reduction columns, and gases (UHP Helium and Oxygen). The instrument is set up following manufacturers guidelines.

3.2. Eager 300 Software installed on a compatible computer.

3.3. A constant temperature drying oven, capable of maintaining 60 5°C.

3.4. Micro-balance accurate to at least 3 decimal places (e.g. 0.000 mg).

3.5. Hydrochloric Acid (concentrated).

3.6. Drying desiccator (glass or plastic).

3.7. Acid-washed glass or plastic petri dishes (preferably glass).

3.8. Acetone (reagent grade) and squirt bottle.

3.9. Acetone cleaned tin boats (3.5 x 5 mm).

3.10. Acetone cleaned pelletizer die and assorted micro-forceps.

3.11. Muffled aluminum foil.

3.12. Aspartic Acid, crystal form (dried in a low temperature oven and in desiccator).

3.13. SRM-CE Soil or Spinach (1570a).

4. PROCEDURE.

4.1. Filtration and collection of samples should follow SOPs for water filtration of organic particles or sediment collection. Filters need to be pre-combusted glass fiber filters (e.g., Whatman GFF) and all material should be cleaned prior to and during filtration.

Sediment samples should be collected with cleaned sediment sampler.

4.2. Removal of inorganic carbon.

4.2.1. Filters or sediments are placed in acid-washed glass petri dishes, which are then placed in a large glass desiccator (without desiccant).

4.2.2. Fill a glass petri dish halfway with concentrated hydrochloric acid, carefully place dish in the center of the desiccator and quickly replace lid.

4.2.3. Samples are left overnight in the fuming HCl, in order to remove the inorganic C from the sample. The next day the filters and sediments are placed in a 60 5°C drying oven overnight.

4.3. Tin boat/instrument cleaning.

4.3.1. Place an appropriate number of tin boats in a clean 100-ml beaker, add enough acetone to completely cover the boats, swirl, decant, repeat two more times

and place the beaker in the drying oven for a minimum of three hours. Remove the beaker, and cover with muffled aluminum foil.

4.3.2. Using acetone filled squirt bottle, liberally squirt pelletizer die and each micro-forceps, shake off excess, place on muffled foil and dry in the drying over for a minimum of three hours. Upon removal from the oven, wrap in muffled foil.

4.4. Sample/boat Preparation.

4.4.1. Remove a glass petri dish containing sample from the desiccator. Every other sample regardless of type will be a duplicate. Place a cleaned tin boat in the pelletizer die.

4.4.1.1. If analyzing particulate filters, using the pelletizer die as a work surface, the filter must be folded twice in order to fit the filter into the boat. Using forceps place the folded filter into the boat and pack it down. Be careful not to lose any material from filter.

4.4.1.2. If analyzing sediments, tare the tin boat on the microbalance, place in die, using scoop tool, place a small amount of sediment in boat (low mg quantities), weigh, and record weight.

4.4.2. Remove boat from die, z-fold the top, and fold over the top and pack the boat until it is a three-dimensional cube or sphere.

4.4.3. Place boat in labeled tray or autosampler and record sample location. Dip all forceps used into a 100-ml acetone filled beaker, to clean them. Using a Kimwipe, dipped in acetone, clean the pelletizer die. Repeat procedure for next sample. Be sure all acetone has evaporated away.

4.5. Blank and Standard Preparation.

4.5.1. Three types of blanks, gas, boat and filter, will be used during the analysis.

4.5.1.1. Gas blanks will consist of nothing more than an empty chamber on the autosampler rack. This will represent baseline C and N.

4.5.1.2. Boat blanks will consist of empty folded boats placed in the autosampler.

4.5.1.3. Filter blanks, during particulate analysis represent the lab/field blank (DI filtered through), sample preparation is the same as all of the other samples. This value will be subtracted from the samples as the blank.

4.5.2. Using a tared boat, weigh out a representative amount of the Aspartic Acid standard (~1mg)and record the weight. Also using a tared boat, weigh out a representative amount (~25 mg) of the SRM to be used and record the weight. Run blanks, SRMs and standards at intervals as outlined in the project protocol, usually every 10 samples.

4.5.3. Standard amounts for the curve and the standard unknowns should be calculated from the C and N expected in the samples. Here are typical weights used for calibration:

mg of Aspartic Acid	N (g)	C (g)
0.50	53	180
1.00	105	361
1.50	158	541
2.00	210	722
2.50	263	902

4.6. Sample Analysis.

4.6.1. Sample runs should consist of the following:

- A standard curve with a Bypass, Blank, and five standards
- An Aspartic, SRM and Boat blank as standard unknowns
- Unknown samples run in duplicate
- Run standard unknowns for all the samples (~ every 10 samples)

4.6.2. Follow manufacturers guidelines for the operation and set up of instrument.

5. REFERENCES.

5.1. Carlo Erba Manuals.

5.2. US. Environmental Protection Agency. 1992. Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis. Method 440.0 *In:* Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples. EPA/600/R-92/121 ORD, Washington DC.

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METHOD 6020B

INDUCTIVELY COUPLED PLASMA—MASS SPECTROMETRY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests (Refs. 1 and 2). When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required. The analyst should insure that a sample digestion method is chosen that is appropriate for each analyte and the intended use of the data. Refer to Chapter Three for the appropriate digestion procedures.

1.2 ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which the acceptability of Method 6020 has been demonstrated through multi-laboratory testing on solid and aqueous wastes are listed below.

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Aluminum	Al	7429-90-5	Magnesium	Mg	7439-95-4
Antimony	Sb	7440-36-0	Manganese	Mn	7439-96-5
Arsenic	As	7440-38-2	Mercury	Hg	7439-97-6
Barium	Ba	7440-39-3	Nickel	Ni	7440-02-0

Beryllium	Be	7440-41-7	Potassium	K	7440-09-7

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Cadmium	Cd	7440-43-9	Selenium	Se	7782-49-2
Calcium	Ca	7440-70-2	Silver	Ag	7440-22-4
Chromium	Cr	7440-47-3	Sodium	Na	7440-23-5
Cobalt	Co	7440-48-4	Thallium	Tl	7440-28-0
Copper	Cu	7440-50-8	Vanadium	V	7440-62-2
Iron	Fe	7439-89-6	Zinc	Zn	7440-66-6
Lead	Pb	7439-92-1			

[a]Chemical Abstract Service Registry Number

The performance acceptability of ICP-MS for the determination of the listed elements was based upon comparison of the multi-laboratory testing results with those obtained from either furnace atomic absorption spectrophotometry or inductively coupled plasma—optical emission spectrometry. It should be noted that one multi-laboratory study was conducted in 1988. As advances in ICP-MS instrumentation and software have been made since that time, other elements have been added through validation and with additional improvements in performance of the method. Performance, in general, presently exceeds the original multi-laboratory performance data for the listed elements (and others) that are provided in Sec. 13.0. Instrument detection limits (IDLs), lower limits of quantitation (LLOQs) and linear ranges will vary with the matrices, instrumentation, and operating conditions. In relatively simple matrices, IDLs will generally be < 0.1 µg/L. For less sensitive elements (e.g., Se and As) and desensitized major elements, IDLs may be ≥ 1.0 µg/L.

1.3 If Method 6020 is used to determine any analyte not listed in Sec. 1.2, it is the responsibility of the analyst to demonstrate the precision and bias of the method for the waste to be analyzed. The analyst must always monitor potential sources of interferences and take appropriate action to ensure data of known quality (see Sec. 9.0). Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in

the matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.4 Use of this method should be restricted to spectroscopists who are knowledgeable in the recognition and correction of spectral, chemical, and physical interferences in ICP-MS analysis.

1.5 An appropriate internal standard is necessary for each analyte determined by ICP-MS. Recommended internal standards are ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{165}Ho, and ^{209}Bi. The lithium internal standard should have an enriched abundance of ^{6}Li, so that

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interference from lithium native to the sample is minimized. Other elements may need to be used as internal standards when samples contain significant native amounts of the recommended internal standards as indicated by high bias of internal standard recoveries.

Note: Other potential causes of a high bias should also be considered before a final decision is made that the internal standard high bias is caused by an excessive concentration of the internal standard isotope in the sample.

1.6 Prior to employing this method, analysts are advised to consult the preparatory method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.7 This method is restricted to use by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, aqueous and solid samples are solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary, if the samples

are filtered and acid-preserved prior to analysis (e.g., Methods 3005, 3010, 3015, 3031, 3050, 3051 and 3052). For oils, greases, or waxes, use the solvent dissolution procedure in method 3040 to prepare the samples.

2.2 This method describes multi-element determinations using ICP-MS in environmental samples. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species in liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge (*m/z*) ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 DEFINITIONS

Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal *m/z* ratio. A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal.

4.2 Isobaric molecular and doubly charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature (Refs. 3 and 4). Examples include $^{75}ArCl^{+}$ ion on the ^{75}As signal and MoO^{+} ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature (Ref. 5), the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals *observed* for a standard solution of the interfering element at a concentration which produces sufficient interference at the isotopes of interest that a reliable measurement can be made. Because the ^{35}Cl natural abundance of 75.77% is 3.13 times the ^{37}Cl abundance of 24.23%, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^{+}$ contribution at *m/z* 75 is a negligible 0.06% of the $^{40}Ar^{35}Cl^{+}$ signal):

Corrected arsenic signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 75 signal) - (3.13) [(*m/z* 77 signal) - (0.87) (*m/z* 82 signal)]

where, the final term adjusts for any selenium contribution at 77 *m/z*,

NOTE: Arsenic values can be biased high by this type of equation when the net signal at *m/z* 82 is caused by ions other than $^{82}Se^{+}$, (e.g., $^{81}BrH^{+}$ from bromine wastes [Ref. 6]).

NOTE: The coefficients should be verified experimentally using the procedures or coefficients provided by the instrument manufacturer.

Similarly,

Corrected cadmium signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 114 signal) - (0.027)(*m/z* 118 signal) - (1.63)(*m/z* 108 signal)

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where, the last 2 terms adjust for any $^{114}Sn^{+}$ or $^{114}MoO^{+}$ contributions at *m/z* 114.

NOTE: Cadmium values will be biased low by this type of equation when $^{92}ZrO^{+}$ ions contribute at *m/z* 108, but use of *m/z* 111 for Cd is even subject to direct ($^{94}ZrOH^{+}$) and indirect ($^{90}ZrO^{+}$) additive interferences when Zr is present.

NOTE: With respect to the arsenic equation above, the coefficients could be improved. For example, the coefficient to modify “3.13” (in the equation above) for a particular instrument can be determined from the observed ratio of the m/z 75 to the m/z 77 net isotope signals for a solution of hydrochloric acid. The concentration of HCl used should provide enough signal at the measured isotopes to ensure that a reliable measurement can be made, while not exceeding the linear range of the detector.

The accuracy of these types of equations is based upon the constancy of the *observed* isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found (Ref. 7) to be reliable, e.g., oxide levels can vary with operating conditions. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. For example, this type of correction has been reported (Ref. 7) for oxide-ion corrections using ThO^{+}/Th^{+} for the determination of rare earth elements. The use of aerosol desolvation and/or mixed gas plasmas have been shown to greatly reduce molecular interferences (Ref. 8). These techniques can be used, provided that IDL, bias, and precision specifications for analysis of the samples can be met.

4.3 As technology continues to develop, modifications to existing ICP-MS instrumentation can reduce or completely remove common interferences thus eliminating the need for reliance on correction equations. Instruments must be able to demonstrate successful freedom from interferences. Examples of such modifications are discussed in more detail below:

4.3.1 Recent ICP-MS instruments may include collision or reaction cells for removal of molecular isobaric interferences. This type of interference removal is effective, and highly recommended for complex and/or varying matrices. The systems work either by collision of molecular species with an inert gas (usually helium) or by reaction of molecular species or the target analyte with reactive gases (e.g., ammonia or methane). Manufacturer recommendations should be followed for the configuration of the collision/reaction cell. This technique may eliminate the need for most correction equations, but freedom from interference still needs to be demonstrated using the spectral interference check (SIC) solutions described in sections 7.23 and 9.9.

4.3.2 High resolution ICP-MS instruments are available based on several mass analyzer designs with much higher mass resolution within the mass range of traditional ICP-MS instruments. These mass analyzers are not based on quadrupole mass analyzers and have orders of magnitude resolution above quadrupoles, which helps reduce or eliminate interference from polyatomic ions with the same nominal mass. These mass analyzers reduce or eliminate the need for most correction equations, but the instrument needs to be operated at sufficient resolution to remove the expected

interference. For example, resolving ^{52}Cr from $^{40}Ar^{12}C$ requires a resolution of around 4000, while resolving ^{75}As from $^{40}Ar^{35}Cl$ requires a resolution of around 8000. Freedom from interferences needs to be demonstrated for the particular higher resolution mass analyzers ICP-MS.

4.4 Additionally, solid-phase chelation may be used to eliminate isobaric interferences from both element and molecular sources. An on-line method has been demonstrated for environmental waters such as sea water, drinking water and acid decomposed samples. Acid decomposed samples refer to samples decomposed by methods similar to methods 3052, 3051, 3050 or 3015. Samples with% levels of iron and aluminum should be avoided. The method also provides a method for preconcentration to enhance detection limits simultaneously with elimination of isobaric interferences. The method relies on chelating resins such as imminodiacetate or other appropriate resins and selectively concentrates the elements of interest while eliminating interfering elements from the sample matrix. By eliminating the elements that are direct isobaric interferences or those that form isobaric interfering molecular masses, the mass region is simplified and these interferences cannot occur. The method has been proven effective for the certification of reference materials and validated using reference materials (Refs. 13-15). The method has the potential to be used on-line or off-line as an effective sample preparation method specifically designed to address interference problems.

4.5 Since commercial quadrupole ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could need resolution improvement, matrix separation, or analysis using another verified and documented isotope, or otherwise the use of another method.

4.6 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement (Ref. 9). Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Dissolved solid levels below 0.2% (2,000 mg/L) have been currently recommended (Ref. 10) to minimize solid deposition, although currently-available ICP-MS systems may be able to tolerate much higher levels. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes (Ref. 11). When intolerable physical interferences are present in a sample, a significant suppression of the internal standard signals (to less than 30% of the signals in the calibrations standard) will be observed. Dilution of the sample five-fold (i.e., dilute one part sample with four parts diluent [1:5 = 1+4]) will usually eliminate the problem.

4.7 Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the

extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

4.8 Reagents and sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents may be necessary. Refer to each method to be used for specific guidance on QC procedures.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

5.3 **Hydrofluoric acid is a very toxic acid and penetrates the skin and tissues deeply if not treated immediately.** Injury occurs in two stages: firstly, by hydration that induces tissue necrosis; and secondly, by penetration of fluoride ions deep into the tissue and thereby reacting with calcium. Boric acid and/or other complexing reagents and appropriate treatment agents should be administered immediately.

WARNING: Consult appropriate safety literature for determining the proper protective eyewear, clothing and gloves to use when handling hydrofluoric acid. **Always have appropriate treatment materials readily available prior to working with this acid.** See Method 3052 for additional recommendations for handling hydrofluoric acid from a safety and an instrument standpoint.

5.4 Many metal salts, are extremely toxic if inhaled or swallowed.

WARNING: Exercise extreme care to ensure that samples and standards are handled safely and properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma-mass spectrometer:

6.1.1 The system must be capable of providing resolution, better than or equal to 1.0 u (unified atomic mass unit) at 10% peak height. The system must have a mass range from at least 6 to 240 u and a data system that allows corrections for isobaric interferences and the application of the internal standard technique. Use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended.

6.1.2 Argon gas, high-purity grade (99.99%).

6.2 Volumetric flasks of suitable material composition, precision and accuracy

6.3 Volumetric pipets of suitable material composition, precision and accuracy

This section does not list all common laboratory ware (e.g., beakers) that might be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade, and whenever necessary, ultra-high purity-grade chemicals, must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water - Reagent water must be interference free. All references to water in this method refer to reagent water unless otherwise specified.

7.3 Ultra high-purity or equivalent acids must be used in the preparation of standards and for sample processing. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid at less than 2% (v/v) is necessary for ICP-MS to minimize damage to the interface and to minimize isobaric molecular-ion interferences with the analytes. Many more molecular-ion interferences are observed when hydrochloric and sulfuric acids are used (Refs. 3 and 4). The use of 1% (v/v) HCl is necessary for the stability of antimony and silver concentrations in the range of 50 - 500 µg/L. For concentrations greater than 500 µg/L silver, additional HCl will be needed. As a consequence, the accuracy of analytes that need significant chloride molecular-ion corrections (e.g., As and V) will degrade.

7.3.1 Nitric acid (concentrated), HNO_3

7.3.2 Nitric acid (50% [v/v]), HNO_3 - Prepare by adding 500 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.3 Nitric acid (1% [v/v]), HNO_3 - Prepare by adding 10 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.4 Hydrochloric acid (concentrated), HCl

7.3.5 Hydrochloric acid (37%), HCl - Prepare by adding 370 mL concentrated HCl to 400 mL water and diluting to 1L.

7.3.6 Hydrofluoric acid (concentrated), HF

7.3.7 Phosphoric acid (concentrated), H_3PO_4

7.3.8 Phosphoric acid (85% [v/v]), H_3PO_4 - Prepare by adding 850 mL concentrated H_3PO_4 to 100 mL water and diluting to 1 L.

7.3.9 Sulfuric acid (concentrated), H_2SO_4

7.3.10 Sulfuric acid (96% [v/v]) H_2SO_4, - Prepare by adding 40 mL water to a 2 L glass beaker. While gently stirring, carefully add 960 mL concentrated H_2SO_4 to the beaker. Mix until combined. Allow to cool. Carefully, quantitatively transfer solution to a 1-L volumetric flask. Bring to volume with additional water if necessary. Mix thoroughly through inversion to combine.

WARNING: Considerable heat is generated upon combining sulfuric acid and water. The use of appropriate personal protection (e.g. proper gloves, safety glasses and protective clothing) is necessary to avoid personal injury such as thermal burns or acid burns due to solution splatter. Also, always add acid to water (rather than water to acid) to reduce splatter.

7.3.11 Citric acid, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$

7.4 Bismuth(III) oxide, Bi_2O_3

7.5 Holmium(III) carbonate pentahydrate, $Ho_2(CO_3)_3 \cdot 5H_2O$

7.6 Indium (powder), In

7.7 Lithium[^{6}Li] carbonate (95 atom % ^{6}Li), $^6Li_2CO_3$

7.8 Ammonium hexachlororhodate(III), $(NH_4)_3RhCl_6$

7.9 Scandium(III) oxide, Sc_2O_3

7.10 Terbium(III) carbonate pentahydrate, $Tb_2(CO_3)_3 \cdot 5H_2O$

7.11 Yttrium(III) carbonate, $Y_2(CO_3)_3 \cdot 3H_2O$

7.12 Ammonium hexafluorotitanate(IV), $(NH_4)_2TiF_6$

7.13 Ammonium molybdate(VI) $(NH_4)_2MoO_4$

7.14 Aluminum(III) nitrate nonahydrate, $Al(NO_3)_3 \cdot 9H_2O$

7.15 Calcium carbonate, $CaCO_3$

7.16 Iron powder, Fe

7.17 Magnesium oxide, MgO

7.18 Sodium carbonate, Na_2CO_3

7.19 Potassium carbonate, K_2CO_3

7.20 Standard stock solutions - Purchase standard stock solutions from an appropriate commercial source. Otherwise, prepare them manually in the laboratory using only ultra, highpurity grade chemicals or metals (≥ 99.99% purity). See Method 6010 for instructions on preparing standard solutions from solids. Replace stock standards when succeeding dilutions for the preparation of calibration standards cannot be verified.

7.20.1 Bismuth internal standard stock solution (100 µg/mL Bi) - Dissolve 0.1115 g Bi_2O_3 in a minimum amount of dilute HNO_3. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.2 Holmium internal standard stock solution (100 µg/mL Ho) - Dissolve 0.1757 g $Ho_2(CO_3)_3 \cdot 5H_2O$ in 10 mL reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.3 Indium internal standard stock solution (100 µg/mL In) - Dissolve 0.1000 g indium in 10 mL concentrated HNO_3. Dilute to 1 L with reagent water.

7.20.4 Lithium internal standard stock solution (100 µg/mL ^6Li) - Dissolve 0.6312 g $^6Li_2CO_3$ (95% atomic abundance) in 10 mL of reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.5 Rhodium internal standard stock solution (100 µg/mL Rh) - Dissolve 0.3593 g $(NH_4)_3RhCl_6$ in 10 mL reagent water. Add 100 mL concentrated HCl and dilute to 1 L with reagent water.

7.20.6 Scandium internal standard stock solution (100 µg/mL Sc) - Dissolve 0.15343 g Sc_2O_3 in 10 mL 50% hot HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.7 Terbium internal standard stock solution (100 µg/mL Tb) - Dissolve 0.1828 g $Tb_2(CO_3)_3 \cdot 5H_2O$ in 10 mL 50% HNO_3. After dissolution is complete, warm the solution to degas. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.8 Yttrium internal standard stock solution (100 µg/mL Y) - Dissolve 0.2316 g $Y_2(CO_3)_3 \cdot 3H_2O$ in 10 mL 50% HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.9 Titanium interference stock solution (100 µg/mL Ti) - Dissolve 0.4133 g $(NH_4)_2TiF_6$ in reagent water. Add 2 drops concentrated HF and dilute to 1 L with reagent water.

7.20.10 Molybdenum interference stock solution (100 µg/mL Mo) - Dissolve 0.2043 g $(NH_4)_2MoO_4$ in reagent water. Dilute to 1 L with reagent water.

7.20.11 Gold preservative stock solution for mercury (100 µg/mL Au) - Purchase as a commercially prepared, high-purity solution of $AuCl_3$ in dilute HCl matrix.

7.21 Mixed-calibration standard solutions - Prepare by diluting stock standard solutions to levels in the linear range for the instrument, using the same combination and concentrations of acids used in the preparation of the sample digestates (approximately 1% HNO_3). The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. Internal standards may be added on-line at the time of analysis using a second channel of the peristaltic pump and an appropriate mixing manifold. Generally, an internal standard should be no more than 50 u removed from the analyte. Recommended internal standards include ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{169}Ho, and ^{209}Bi. Prior to preparing the mixed standards, each stock standard solution must be analyzed separately to determine possible spectral interferences or the presence of impurities.

NOTE: Care should be taken when preparing the calibration standards to ensure that the elements are compatible and stable when mixed together. Standards which interfere with another analyte, or which are contaminated with another analyte, may not be included in the same calibration standard as that analyte.

Transfer the mixed-standard solutions to an appropriate container for storage. Freshly mixed standards must be prepared as needed with the realization that concentrations can change upon aging. Calibration standards must be initially verified using a QC standard (see Sec. 7.24).

7.22 Blanks - Three types of blanks are necessary for analysis: (1) the calibration blank, which is used in establishing the calibration curve; (2) the method blank, which is used to monitor for possible contamination resulting from the sample preparation procedure; and (3) the rinse blank, which is used to flush the system between all samples and standards.

7.22.1 Calibration blank - Prepare by acidifying reagent water using the same combination and concentrations of acids used in the preparation of the matrix-matched calibration standards (Sec. 7.21) along with the selected concentrations of internal standards, such that there is an appropriate internal standard element for each of the target analytes. The use of HCl for antimony

and silver is discussed in Sec. 7.3. The calibration blank will also be used for all initial calibration blank (ICB) and continuing calibration blank (CCB) determinations.

7.22.2 Method blank — Prepare by a processing either a volume of reagent water equal to that used for actual aqueous samples, or, otherwise, a clean, empty container, equivalent to that used for actual solid samples through all of the preparatory and instrument determination steps used for making ICP-MS determinations in samples. These steps may include, but are not limited to, pre-filtering, digestion, dilution, filtering, and analysis (refer to Sec. 9.5).

7.22.3 Rinse blank - Prepare as a 1 - 2% HNO_3 solution. Prepare a sufficient quantity such that it may be used to flush the system in between standards and samples.

If mercury is to be analyzed, the rinse blank should also contain 2 µg/mL $AuCl_3$.

7.23 Spectral interference check (SIC) solutions - Prepare so as to contain known concentrations of interfering elements that will demonstrate the appropriate magnitude of interferences and provide an adequate test of any corrections. Chloride in the SIC solution provides a means to evaluate software corrections for chloride-related interferences such as $^{35}Cl^{16}O^{+}$ on $^{51}V^{+}$ and $^{40}Ar^{35}Cl^{+}$ on $^{75}As^{+}$. Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese. Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various molecular-ion isobaric interferences. The SIC is used to verify that the interference levels are corrected by the data system within appropriate QC limits.

NOTE: The final SIC solution concentrations in Table 1 are intended to evaluate corrections for known interferences on only the analytes identified in Sec. 1.0. If the test method is to be used to determine other element(s), it is the responsibility of the analyst to modify the SIC solution accordingly, or prepare an alternative SIC solution, so as to allow adequate verification of interference corrections on the additional element(s) (see Sec. 9.9).

7.23.1 Mixed stock SIC solutions - Prepare the SIC stock solutions using only ultra-pure reagents. They can be obtained commercially or prepared using the following procedures:

7.23.1.1 Mixed SIC stock solution I - Prepare by adding 13.903 g $Al(NO_3)_3 \cdot 9H_2O$, 2.498 g $CaCO_3$ (previously dried at 180 EC for 1 hr), 1.000 g Fe, 1.658 g MgO, 2.305 g Na_2CO_3 and 1.767 g K_2CO_3 to 25 mL of reagent water. Slowly add 40 mL of (50%) HNO_3. After dissolution is complete, warm the solution to degas. Cool and dilute to 1 L with reagent water.

7.23.1.2 Mixed SIC stock solution II - Prepare by slowly adding 7.444 g 85% H_3PO_4, 6.373 g 96% H_2SO_4, 40.024 g 37% HCl, and 10.664 g citric acid ($C_6O_7H_8$) to 100 mL of reagent water. Dilute to 1 L with reagent water.

7.23.2 Mixed working SIC solution - Prepare by combining 10.0 mL of SIC stock solution I, 2.0 mL each of 100-µg/mL titanium stock solution and 100-µg/mL molybdenum stock solution, and 5.0 mL of SIC stock solution II. Dilute to 100 mL with reagent water. Prepare fresh weekly.

7.24 Initial calibration verification (ICV) standard - Prepare by combining compatible metals from standard stock solution sources that differ from those used for the preparation of the calibration standards. The ICV should be prepared so as to contain metal concentrations that are near, but not equal to, the midpoint concentration level of the calibration curve.

7.25 Continuing calibration verification (CCV) standard - Prepare using the same acid matrix and stock standards employed when preparing the calibration standards. The CCV should be prepared so as to contain metal concentrations equal or nearly equivalent to the midpoint concentration of the calibration curve.

7.26 Mass spectrometer tuning solution - Prepare so as to contain elements that represent all of the mass regions of interest (i.e., 10 µg/L Li, Co, In, and Tl) in order to verify that the resolution and mass calibration of the instrument are within the designated specifications (see Sec. 10.1).

7.27 If the determination of one or more metals using a non-aqueous solvent is required, then all standards and quality control samples must be prepared on a weight/weight basis in the non-aqueous solvent since the density of non-aqueous solvents is not uniform. Standards and quality control materials containing organometallic materials that are soluble in non-aqueous solvents are available from a variety of vendors.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

See Chapter Three, Inorganic Analytes, for sample collection and preservation instructions.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over those criteria given in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800 for QC procedures to ensure the proper operation of the various sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800.

9.3 Instrument Detection Limits

Instrument detection limits (IDLs) are useful means to evaluate the instrument noise level and response changes over time for each analyte from a series of reagent blank analyses to obtain a calculated concentration. They are not to be confused with the lower limit of quantitation, nor should they be used in establishing this limit. It may be helpful to compare the calculated IDLs to the established lower limit of quantitation, however, it should be understood that the lower limit of quantitation needs to be verified according to the guidance in Sec. 9.8. IDLs in µg/L can be estimated as the mean of the blank result plus three times the standard deviation of 10 replicate analyses of the reagent blank solution. (Use zero for the mean if the mean is negative). Each measurement should be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs should be determined at least once using new equipment, after major instrument maintenance such as changing the detector, and/or at a frequency designated by the project. An instrument log book should be kept with the dates and information pertaining to each IDL performed.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination by generating data of acceptable precision and bias for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. It is recommended that the laboratory should repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment that come into direct contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are digested and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If an interference is observed that would prevent the determination of the target analyte, determine the source and eliminate it, if possible, before processing the samples. The method blank should be carried through all stages of sample preparation and instrument determination procedures. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Linear range

The linear range establishes the highest concentration that may be reported without diluting the sample. Following calibration, the laboratory may choose to analyze a standard at a higher concentration than the high standard in the calibration. The standard must recover within 10% of the true value, and if successful, establishes the linear range. The linear range standards must be analyzed in the same instrument run as the calibration they are associated with (i.e., on a daily basis) but may be analyzed anywhere within that run. If a linear range standard is not analyzed for any specific element, the highest standard in the calibration becomes the linear range.

9.7 Sample QC for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, and sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike (MS), a laboratory control sample (LCS), and a duplicate sample in each analytical batch. Any method blanks, LCS, MS samples, and duplicate samples should be subjected to the same preparatory and instrument determination procedures as those used on actual samples (see Sec. 11.0).

9.7.1 For each batch of samples analyzed, at least one method blank must be carried throughout the entire sample preparation and instrument determination process, as described in Chapter One. The importance of the method blank is to aid in identifying when and/or if sample contamination is occurring. The method blank is considered to be acceptable if it does not contain the target analytes at concentration levels that exceed the acceptance limits defined in Chapter One or in the project-specific DQOs. The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is not reliable because it is based on a single method blank value rather than a statistically determined blank concentration.

Blanks are generally considered to be acceptable if target analyte concentrations are less than ½ the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e. targets are not present in samples or sample concentrations **are ≥10X** the blank). Other criteria may be used depending on the needs of the project.

If the method blank fails to meet the necessary acceptance criteria, it should be reanalyzed once. If still unacceptable, then all samples associated with the method blank must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples. If the method blank results do not meet the acceptance criteria and reanalysis is not practical, then the laboratory should report the sample results along with the method blank results, and provide a discussion of the potential impact of the contamination on the sample results. However, if an analyte of interest is found in a sample in the batch near its concentration confirmed in the blank, the presence and/or concentration of that analyte should be considered suspect and may require qualification. Refer to Chapter One for additional guidance regarding the proper protocol when analyzing method blanks.

9.7.2 Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair for each batch of samples processed, at a minimum frequency of one per every 20 samples, as described in Chapter One. An MS/MSD pair is used to document the bias and precision of a method in a given sample matrix. The decision on whether to prepare and analyze duplicate samples or an MS/MSD pair must be based on knowledge of the samples in the analysis batch. If samples are expected to contain target analytes above the LLOQ, laboratories may choose to use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes above the LLOQ, the laboratories should use an MS/MSD pair.

MS/MSD samples should be spiked with each target element at the project-specific action levels, or, when lacking project-specific action levels, between the low- and midlevel standards, as appropriate. Acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses, for each matrix type being analyzed. However, historically derived acceptance limits must not exceed ± 25%

recovery of the target element spike values for bias, and ≤ 20 relative percent difference (RPD) for precision. In the absence of historical data, MS/MSD acceptance limits should be set at ± 25% recovery and ≤ 20 RPD. Refer to Sec. 4.0 of Chapter One for further guidance. If the bias and precision indicators in an analytical batch fail to meet the acceptance criteria, then the interference test discussed in Sec. 9.10 should be performed. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable. To assess data precision with duplicate analyses, it is preferable to use a high concentration field sample to prepare unspiked laboratory duplicates for metals analyses.

Calculate the RPD between duplicate or MS determinations as follows:

$$RPD = \frac{\left| D_1 - D_2 \right|}{\left(\frac{D_1 + D_2}{2} \right)} \times 100$$

where:

RPD = relative percent difference
D_1 = MS or first sample analysis value
D_2 = MSD or duplicate sample analysis value

9.7.3 At least one LCS should be prepared and analyzed with each batch of analytical samples processed, at a minimum frequency of one LCS per every 20 samples, as described in Chapter One. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS should be spiked at the same levels and using the same spiking materials as the corresponding MS/MSD (see above Sec. 9.7.2). When the results of the MS analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can acceptably perform the analysis in a clean matrix.

LCS acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses. However, historically derived acceptance limits must not exceed ± 20% of the target element spike values. In the absence of historical data, LCS acceptance limits should be set at ± 20%. If the result of an LCS does not meet the established acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the LCS must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples.

9.7.4 Reference materials containing known amounts of target elements are recommended when an appropriately similar medium of interest are available as one type of QC after appropriate sample preparation. The reference material may be used as the LCS. For soil reference materials, the manufacturers' established acceptance criterion should be used. For solid reference materials, ± 20% (see Sec. 9.7.3) recovery of the reported manufacturers' target element values may not be achievable. Refer to Chapters One and Three for additional information.

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9.8 Lower Limit of Quantitation (LLOQ) check standard

9.8.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve. The LLOQ is initially verified by the analysis of at least 7 replicate samples, spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases the mean recovery should be +/- 35% of the true value and RSD should be $\leq$ 20%. In-house limits may be calculated when sufficient data points exist. Monitoring recovery of LLOQ over time is useful for assessing precision and bias. Refer to a scientifically valid and published method such as Chapter 9 of Quality Assurance of Chemical Measurements (Taylor 1987) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (http://water.epa.gov/scitech/methods/cwa/det/index.cfm) for calculating precision and bias for LLOQ.

9.8.2 Ongoing LLOQ verification, at a minimum, is on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix (free of target compounds). Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated project-specific requirements.

9.9 Verify the magnitude of elemental and molecular-ion isobaric interferences and the adequacy of any corrections at the beginning of an analytical run or once every 12 hours of continuing sample analysis, whichever is more frequent. Do this by analyzing the SIC solution. Results for the unspiked elements in the SIC solution should be less than 2 times the LLOQ. Note that it may not be possible to obtain SIC spiking solutions that are completely free of the unspiked elements. If the presence and concentration of an unspiked element can be confirmed via vendor documentation and/or determination of multiple isotopes of the element in the correct ratios, the concentration actually present may be subtracted from the determined value prior to comparing to the LLOQ limits. Refer to Sec. 4.0 for a discussion on interferences and potential solutions to those interferences if additional guidance is needed.

9.10 The intensities of each internal standard must be monitored for every analysis to ensure that it does not decrease below 30%, with respect to its intensity during the initial

calibration. If this occurs, a significant matrix effect must be suspected. Under these conditions, the IDL has degraded, and therefore the correction capability of the internalstandardization technique must then be questioned. If this happens, perform the following procedure:

9.10.1 Make sure the instrument has not drifted by observing the internal standard intensities in the nearest clean matrix, i.e., the calibration blank. If the low internal standard intensities are also observed in the nearby calibration blank, terminate the analysis, correct the problem, recalibrate the instrument, verify the new calibration, and reanalyze the affected samples.

9.10.2 If drift has not been demonstrated to occur as outlined in Sec. 9.10.1, matrix effects need to be removed by diluting the affected sample. Dilute the sample fivefold (1:5), taking into consideration the need to add the appropriate amounts of internal standards, and reanalyze. If the first dilution does not eliminate the problem, repeat the dilution procedure in an iterative fashion, using ever-increasing dilutions, until the internalstandard intensities exceed the 30% acceptance limit. Correct the reported results using the appropriate dilution factors.

9.11 To obtain analyte data of known quality, it is necessary to measure more than the analytes of interest in order to apply corrections or to determine whether interference corrections are necessary. For example, tungsten oxide molecular-ion species can be very difficult to distinguish from mercury isotopes. If the concentrations of interference sources (such as C, Cl, Mo, Zr, W) are such that, at the correction factor, the analyte is less than the LLOQ and the concentration of interferents are insignificant, then the data may go uncorrected.

NOTE: Monitoring the interference sources does not inevitably necessitate monitoring of the interferant itself, but that a molecular species may be monitored to indicate the presence of the interferent.

When correction equations are used, all QC criteria must also be met. Extensive QC for interference corrections is needed at all times. The monitored masses must include those elements whose hydrogen, oxygen, hydroxyl, chlorine, nitrogen, carbon and sulfur molecular ions could impact the analytes of interest. Unsuspected interferences may be detected by adding pure major matrix components to a sample to observe any impact on the analyte signals. When an interference source is present, the sample elements impacted must be flagged to indicate (a) the percentage interference correction applied to the data; or (b) an uncorrected interference, by virtue of the elemental equation used for quantitation. The isotope proportions for an element or molecular-ion cluster provide information useful for QA.

NOTE: Only isobaric elemental, molecular, and doubly charged interference corrections, which employ the observed isotopic-response ratios or parent-to-oxide ratios (provided an oxide internal standard is used as described in Sec. 4.2) for each instrument system, are acceptable corrections for use in this method.

9.12 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the

laboratory and the nature of the samples. Whenever possible, the laboratory should analyze reference materials and participate in relevant performance evaluation (PE) studies.

9.13 If less than acceptable bias and precision data are generated for the matrix spike(s), the additional QC protocols in Sections 9.13.1 and/or 9.13.2 should be performed prior to reporting concentration data for the elements in this method. At a minimum these tests should be performed with each batch of samples prepared/analyzed with corresponding unacceptable data quality results. If matrix interference effects are confirmed, then an alternative test method should be considered or the current test method modified, so that the analysis is not affected by the same interference. The use of a standard-addition analysis procedure may also be used to compensate for this effect (refer to Method 7000).

9.13.1 Dilution test

If the analyte concentration is within the linear range of the instrument and sufficiently high (minimally, a factor of 25 times greater than the LLOQ), an analysis of a 1:5 dilution should agree to within ± 20% of the original determination. If not, then a chemical or physical interference effect must be suspected. The matrix spike is often a good choice of sample for the dilution test, since reasonable concentrations of most analytes are present. Elements that fail the dilution test are reported as estimated values.

9.13.2 Post-digestion MS

If a high concentration sample is not available for performing the dilution test, then a post-digestion MS should be performed. The test only needs to be performed for the specific elements that failed original matrix spike limits, and only if the spike concentration added was greater than the concentration determined in the unspiked sample. Following preparation, which may include, but is not limited to, pre-filtration, digestion, dilution and filtration, an aliquot, or dilution thereof, should be obtained from the final aqueous, unspiked-analytical sample, and spiked with a known quantity of target elements. The spike addition should be based on the indigenous concentration of each element of interest in the sample. The recovery of the post-digestion MS should fall within a ± 25 % acceptance range, relative to the known true value, or otherwise within the laboratoryderived acceptance limits. If the post-digestion MS recovery fails to meet the acceptance criteria, the sample results must be reported as estimated values.

9.14 Ultra-trace analysis necessitates the use of clean chemistry practices. Several suggestions for the reduction of contaminants in the analytical blank are provided in Chapter Three, Inorganic Analytes.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Conduct mass calibration and resolution verification checks in the mass regions of interest using the mass spectrometer tuning solution (Sec. 7.26). The mass calibration and resolution verification acceptance criteria must be met prior to the analysis of samples. If the mass calibration differs by more than 0.1 u from the true value, then the mass calibration must

be adjusted to the correct value. The resolution must also be verified to be less than 0.9 u full width at 10% peak height.

10.2 At a minimum, the elements required for the project plus any required for interference correction must be calibrated. Recommended isotopes for the analytes in Sec. 1.2 are provided in Table 2. Flush the system in between each standard and sample using the rinse blank (Sec. 7.22.3). The rinse time needs to be sufficient to ensure that analytes present in the linear range are effectively cleaned out prior to analysis of the subsequent sample. Use the average of at least three readings (of a single injection) for both calibration standard and sample analyses.

10.3 Calibration standards should be prepared on an as-needed basis unless stability warrants preparing fresh daily, (or each time a batch of samples is analyzed). If the ICV standard is prepared daily and the results of the ICV analyses meet the acceptance criteria, then the calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV fails to meet the acceptance criteria, trouble shoot the situation, and then prepare a new set of calibration standards if needed and recalibrate the instrument

10.4 A calibration curve must be analyzed daily. The instrument may be calibrated using a single point standard and a calibration blank (ICB) or a multipoint calibration curve. If a multipoint curve is used a minimum of three standards are required and the correlation coefficient (r) should be $\geq$ 0.995 or the coefficient of determination (r^2) should be $\geq$ 0.990. Relative Standard Error may be used as an alternative to r or r^2, and should be $\leq$ 20%. If a multipoint calibration is used the low standard must be at or below the LLOQ.

NOTE: Inversely weighted linear regressions or other methods may be used in order to minimize curve fitting errors at the low end of the calibration curve.

10.5 After the initial calibration is completed it is verified using several checks.

10.5.1 Initial Calibration Verification (ICV) - The ICV is a standard prepared from a different source than the initial calibration standards. It is analyzed at approximately the mid-level of the calibration and serves as a check that the initial calibration standards are at the correct concentrations. The acceptance range is 90-110% of the true value.

10.5.2 Low-level readback or verification - For a multi-point calibration, the low level standard should quantitate to within 80-120% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at or below the LLOQ is analyzed and should recover within 80-120% of the true value.

10.5.3 Mid-level readback or verification - For a multi-point calibration, the mid-level standard should quantitate to within 90-110% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at the midpoint of the linear range is analyzed and should recover within 90-110% of the true value.

10.5.4 Initial Calibration blank (ICB) - If a multi-level calibration is used, an ICB is analyzed immediately after the calibration (or after the ICV) and must not contain target analytes above half the LLOQ. If a single point calibration is used, the calibration is forced through the ICB, but a second ICB is analyzed as a check and must not contain target analytes above half the LLOQ. If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.

NOTE: After cleaning the sampler and skimmer cones, improved performance in calibration stability has been observed by method users if the instrument is exposed to the SIC solution. Improved performance has also been observed if the instrument is allowed to rinse for 5 - 10 minutes before starting the calibration process.

10.5.5 Verify the ongoing validity of the calibration curve after every 10 samples, and at the end of each analysis batch run, through the analysis of a CCV standard (Sec. 7.25) and a CCB (Sec. 7.22.1). For the curve to be considered valid the analysis result of the CCV standard must be within ± 10% of its true value and the CCB must not contain target analytes above the LLOQ. If the calibration cannot be verified, sample analysis must be discontinued, the cause of the problem determined and the instrument recalibrated. All samples following the last acceptable CCV standard must be reanalyzed Flow-injection systems may be used as long as they can meet the performance criteria of the method.

11.0 PROCEDURE

11.1 Preliminary treatment of most samples is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix-matched with the standards (i.e., acid concentrations should match). Solubilization and digestion procedures are presented in Chapter Three, Inorganic Analytes.

NOTE: If mercury is to be analyzed, the digestion procedure must use mixed nitric and hydrochloric acids through all steps of the digestion. Mercury will be lost if the sample is digested when hydrochloric acid is not present. If it has not already been added to the sample as a preservative, Au should be added to give a final concentration of 2 mg/L (use 2.0 mL of gold preservative stock (Sec. 7.20.11) per 100 mL of sample) to preserve the mercury and to prevent it from plating out in the sample introduction system.

11.2 Initiate an appropriate operating configuration of the instrument computer according to the instrument manufacturer's instructions.

11.3 Set up the instrument with the proper operating parameters according to the instrument manufacturer's instructions.

11.4 Operating conditions

Tune the instrument by following the instructions provided by the instrument manufacturer. Allow at least 30 minutes for the instrument to equilibrate before analyzing samples.

NOTE: The instrument should have features that protect it from high ion currents. If not, precautions must be taken to protect the detector. A channel electron multiplier or active film multiplier will suffer from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this time period, response factors are constantly changing, which invalidates the calibration curve, causes instability, and invalidates sample analyses.

11.5 Calibrate the instrument following the procedure outlined in Sec. 10.0.

11.6 Flush the system with the rinse blank solution (Sec. 7.22.3) until the signal levels return to the data quality objectives or method LLOQs (usually about 30 seconds) before the analysis of each sample. Nebulize each sample until a steady-state signal is achieved (usually about 30 seconds) prior to collecting data.

11.7 Dilute and reanalyze samples that exceed the linear range for an analyte (or species needed for a correction) or measure an alternate, but less-abundant, isotope. The linearity at the alternate mass must be confirmed by appropriate calibration (see Sec. 10.4). Alternatively apply solid-phase chelation chromatography to eliminate the matrix as described in Sec. 4.3.

11.8 Determination of percent dry weight

When sample results are to be calculated on a dry-weight basis, a separate portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

CAUTION: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

11.8.1 Immediately after weighing the sample aliquot to be digested, weigh an additional 5- to 10-g aliquot of the sample to the nearest 0.01g into a tared crucible. Dry this aliquot overnight at 105 EC. Allow the sample to cool in a desiccator before weighing.

11.8.2 Calculate the % dry weight as follows:

$$\% \text{ dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

This oven-dried aliquot is not used for the extraction and should be appropriately disposed of once the dry weight is determined.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 If dilutions were performed, apply the appropriate corrections to the sample values.

12.2 If appropriate, or required by the project or regulation for data reporting, calculate results for solids on a dry-weight basis as follows:

$$Concentration_{DW} = \frac{VC \times}{SW \times}$$

where:

Concentration$_{DW}$ = Concentration on a dry weight basis (mg/kg)
C = Digest concentration (mg/L)
V = Final volume after sample preparation (L)
W =Wet sample mass (kg)
S = % Solids/100 = % dry weight/100

Calculations must include appropriate interference corrections (see Sec. 4.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 *m/z* for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

13.0 METHOD PERFORMANCE

Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

Table 3 summarizes the method performance data for aqueous and sea water samples with interfering elements removed and samples preconcentrated prior to analysis. Table 4 summarizes the performance data for a simulated drinking water standard. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The pages to follow contain the tables, and figures referenced by this method.

TABLE 1

RECOMMENDED SPECTRAL INTERFERENCE CHECK (SIC) SOLUTION COMPONENTS AND CONCENTRATIONS

Solution Component		SIC Concentration (mg/L)
Al		100.0
Ca	300.0 Fe	250.0
Mg		100.0
Na	250.0 P	100.0
K		100.0
S		100.0
C		200.0
Cl		2000.0
Mo		2.0
Ti		2.0

TABLE 2

RECOMMENDED ELEMENTAL ISOTOPES FOR SELECTED ELEMENTS

Element of Interest	Mass of Isotope
Aluminum	**27**
Antimony	121, **123**
Arsenic	**75**
Barium	138, 137, 136, **135**, 134
Beryllium	**9**
Bismuth (IS)	209
Cadmium	**114**, 112, **111**, 110, 113, 116, 106
Calcium (I)	42, 43, **44**, 46, 48
Chlorine (I)	35, 37, (77, 82)[a]
Chromium	**52**, **53**, **50**, 54
Cobalt	**59**
Copper	**63**, **65**
Holmium (IS)	165
Indium (IS)	**115**, 113
Iron (I)	**56**, **54**, **57**, 58
Lanthanum (I)	139
Lead	**208**, **207**, **206**, 204
Lithium (IS)	6[b], 7
Magnesium (I)	24, **25**, **26**
Manganese	**55**
Mercury	202, **200**, 199, 201
Molybdenum (I)	98, 95, 96, 92, **97**, 94, (108)[a]
Nickel	58, **60**, 62, **61**, 64
Potassium (I)	**39**
Rhodium (IS)	103
Scandium (IS)	45
Selenium	80, **78**, **82**, **76**, **77**, 74
Silver	**107** **23** , **109**
Sodium (I)	
Terbium (IS)	159
Thallium	**205**, 203
Vanadium	**51**, **50**
Tin (I)	120, **118**
Yttrium (IS)	89
Zinc	64, **66**, **68**, **67**, 70

NOTE: Method 6020 is recommended for only those analytes listed in Sec.1.2. Other elements are included in this table because they are potential interferents (labeled I) in the determination of recommended analytes, or because they are commonly used internal standards (labeled IS). Isotopes are listed in descending order of natural abundance. The most generally useful isotopes are underlined and in boldface, although certain matrices may necessitate the use of alternative isotopes.

[a] These masses are also useful for interference correction (Sec. 4.2).

[b] ^{6}Li Internal standard must be enriched in the Li isotope. This minimizes interference from indigenous lithium.

TABLE 3

METHOD PERFORMANCE DATA FOR AQUEOUS AND SEA WATER SAMPLES [a]
WITH INTERFERING ELEMENTS REMOVED AND SAMPLES PRECONCENTRATED PRIOR TO ANALYSIS

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]		
		9.0 mL	27.0 mL	CERTIFIED
Manganese	55	1.8±0.05	1.9±0.2	1.99±0.15
Nickel	58	0.32±0.018	0.32±0.04	0.30±0.04
Cobalt	59	0.033±0.002	0.028±0.003	0.025±0.006
Copper	63	0.68±0.03	0.63±0.03	0.68±0.04
Zinc	64	1.6±0.05	1.8±0.15	1.97±0.12
Copper	65	0.67±0.03	0.6±0.05	0.68±0.04
Zinc	66	1.6±0.06	1.8±0.2	1.97±0.12
Cadmium	112	0.020±0.0015	0.019±0.0018	0.019±0.004
Cadmium	114	0.020±0.0009	0.019±0.002	0.019±0.004
Lead	206	0.013±0.0009	0.019±0.0011	0.019±0.006
Lead	207	0.014±0.0005	0.019±0.004	0.019±0.006
Lead	208	0.014±0.0006	0.019±0.002	0.019±0.006

NOTE: Data obtained from Ref. 12.

[a] The dilution of the sea-water during the adjustment of pH produced 10 mL samples containing 9 mL of sea-water and 30 mL samples containing 27 mL of sea-water. Samples containing 9.0 mL of CASS-2, n=5; samples containing 27.0 mL of CASS-2, n=3.

[b] 95% confidence limits

TABLE 4

ANALYSIS OF NIST SRM 1643b - TRACE METALS IN WATER [a]

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]	
		DETERMINED	CERTFIED
Manganese	55	30±1.3	28±2
Nickel	58	50±2	49±3
Cobalt	59	27±1.3	26±1
Nickel	60	51±2	49±3
Copper	63	23±1.0	21.9±0.4
Zinc	64	67±1.4	66±2
Copper	65	22±0.9	21.9±0.4
Zinc	66	67±1.8	66±2
Cadmium	111	20±0.5	20±1
Cadmium	112	19.9±0.3	20±1
Cadmium	114	19.8±0.4	20±1
Lead	206	23±0.5	23.7±0.7
Lead	207	23.9±0.4	23.7±0.7
Lead	208	24.2±0.4	23.7±0.7

NOTE: Data obtained from Ref. 12.

[a] 5.0 mL samples, n=5

[b] 95% confidence limits

TABLE 5

COMPARISON OF TOTAL MERCURY RESULTS IN HEAVILY CONTAMINATED SOILS

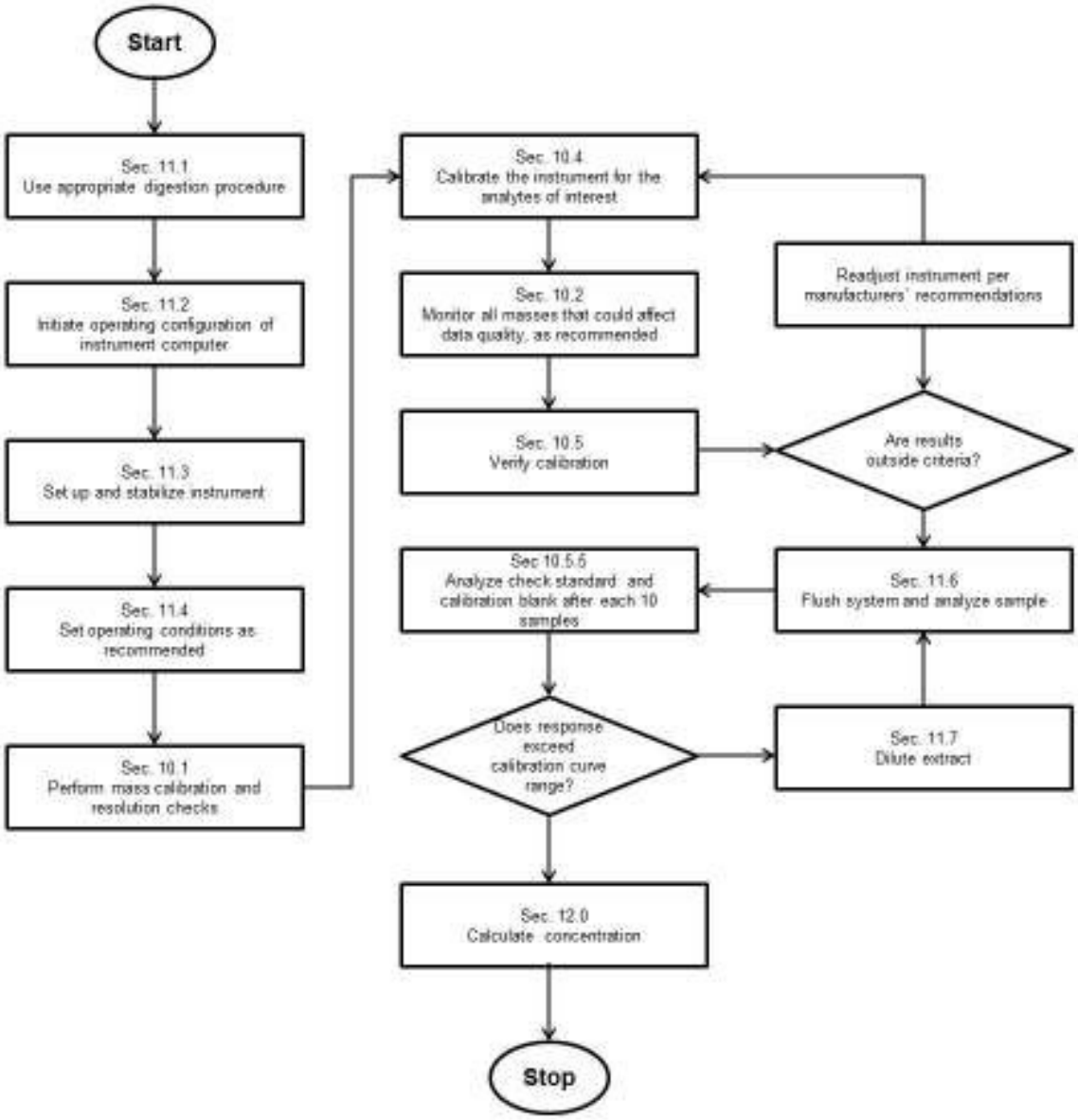
Soil Sample	Mercury in µg/g	
	ICP-MS	CVAA
1	27.8	29.2
2	442	376
3	64.7	58.2
4	339	589
5	281	454
6	23.8	21.4
7	217	183
8	157	129
9	1670	1360
10	73.5	64.8
11	2090	1830
12	96.4	85.8
13	1080	1190
14	294	258
15	3300	2850

16	301	281
17	2130	2020
18	247	226
19	2630	2080

NOTE: Data obtained from Ref. 16.

METHOD 6020A

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY



Appendix A

Summary of Revisions to Method 6020 (From Revision 1, February 2007):

1. Improved overall method formatting for consistency with new SW-846 methods style guidance.
2. Section 1.2 – Changed “Inductively coupled plasma—atomic emission spectrometry” to “Inductively coupled plasma—optical emission spectrometry”.

3. Section 1.6 - inserted references to additional 3000 series preparatory methods to ICP analysis. Also added method 6800 to sections 1.6 and 9.2 as a preparatory method.
4. Inserted additional safety guidance regarding the use of HF.
5. Inserted new section (7.27) regarding analysis of non-aqueous solvents.
6. Reformatted certain paragraphs with the heading "NOTE" or "WARNING" to better denote the importance of the recommendations provided therein.
7. Extensively reformatted “REAGENTS AND STANDARDS” section and to meet current SW-846 method guidelines.
8. Significantly updated and expanded “QUALITY CONTROL” section for better adherence to current SW-846 method guidelines and for improved alignment with current universal practices for published analytical methods.
9. Inserted new sections (Sections 7.23 and 9.9) to describe the preparation and use of the spectral interference check (SIC) solution; also added instructions to match the matrix of this solution to that of the calibration standards.
10. Renamed "QC standard" as "ICV standard" in Sec. 7.24.
11. Added new Sec. 7.25 describing the preparation of a "CCV" standard, consistent with the equivalent section in 6010.
12. Replaced the term “unity” with “uniform” in Section 7.27.
13. Removed all references to method 7000 except for guidance regarding the method of standard addition.
14. The term “accuracy” was replaced by “bias” where appropriate.
15. In Section 9.4, the requirement to repeat the demonstration of proficiency for new staff and instrumentation changes was changed to a recommendation.
16. Section 9.7.2 – Added a note regarding MS/MSD spike concentrations and unspiked laboratory duplicates.
17. The section regarding analysis of reference materials (Sec. 9.7.4) was revised for clarity and the term “Standard Reference Material” was replaced with “reference material” throughout the method.
18. Inserted new section (Sec. 9.8) describing the preparation and use of an LLOQ standard. This section includes two new references for guidance on assessing precision and bias.
19. The section describing matrix interference check samples (Sec. 9.13) has been revised for clarity. The post-digestion MS is only recommended if a high concentration sample is not available for performing the dilution test.
20. Substituted certain terms with new terms (i.e. “must” in place of “shall”) to conform with the Performance-based Methods Approach goal of flexibility.
21. Removed reference to “linear dynamic range” as noted by the Inorganic Methods Work Group. Section 9.6 regarding the linear range was added.
22. Mid-level read back or verification standard added to Section 10.5.3.
23. Moved the sentence “If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.” From Section 10.5.5 to Section 10.5.4.
24. Added 95 as mass of isotope for molybdenum.

25. Tables 3 and 4 from 6020A presenting example precision and accuracy data for aqueous and solid matrices were removed.
26. Language was updated in Section 9.7.1 regarding method blanks.

McKenzie Environmental Engineering Research Group -- Agilent 7900 SOP

For ANS samples

1. Instrument startup – startup tune is done <u>every time the instrument is turned on (i.e., daily)</u>
    a. Turn on argon, chiller, and ignite plasma
    b. Allow instrument to go through programed startup routine including (warmup, no gas initial tune)
        i. A 1 ug/L tuning solution is used for the no-gas start up tune – Li (7), Y (89), and Tl (205) ii. The startup tune requires ~10 minutes for the instrument to make parameter adjustments and recorded for an extended time (0.1 s integration time).
    c. Compare the initial tune to manufacturer expectation and historical start-up tune performance
        i. Criteria – considers Li (7), Y (89), and Tl (205)
            1. Background < 25 cps
            2. Axis resolution ± 0.05 of nominal mass
            3. Signal relative standard deviation (RSD%) < 5%
            4. Sensitivity (counts per second) consistent with previous batches and exceeds manufacturer's specifications
        ii. If manufacturer standards are not met or performance is notably different than historical startup tun performance -> initiate troubleshooting
2. Batch –
    a. ANS sample considerations
        i. Vials: plastic centrifuge tubes; acid washing centrifuge tubes can be implements if background contamination is an issue.
        ii. Surface water samples – generally low to moderate salinity
        iii. Elements of interest:
            1. Aluminum (Al, 27)
            2. Barium (Ba, 137)
            3. Calcium (Ca, 44)
            4. Iron (Fe, 56)
            5. Potassium (K, 39)
            6. Magnesium (Mg, 24)
            7. Manganese (Mn, 55)
            8. Sodium (Na, 23)
            9. Strontium (Sr, 88) iv. Acid matrix: 2% HNO_3 and 0.5% HCl (v/v); trace metal or sub-boiled acids are used to minimize contamination potential
            1. Acid matrix should be same in all samples and QA/QC samples
            2. Direct analysis of acid preserved samples (i.e., 2% HNO_3 and 0.5% HCl (v/v)) is acceptable for low NTU samples (i.e., <1 NTU) (EPA 200.8)

3. Samples are centrifuges at 1000 g for 15 minutes to remove turbidity

b. Calibration curve and QA/QC samples

i. Standards are made within 7 days of analysis and stored at 4°C in the dark up till use

ii. Calibration curve is analyzed with every batch

iii. Stock standards are purchased from Inorganic Ventures and typically include:

1. Trace elements (71A) – 43 elements including the listed interests:

a. Al, Ba, Ca, Fe, K, Mg, Mn, Na, Sr

2. Major elements (IV-Stock-2): Ca, Mg, K, and Na

3. Individual elements standards as needed

4. Internal standard (6020 stock): Bi, Ho, In, ^{6}Li, Rh, Sc, Tb, Y iv. A calibration blank is used to identify and account for background concentrations

v. standards are made using the same acid concentration as used to preserve samples (2% HNO_3 and 0.5% HCl (v/v)). Typical calibration curve values are shown below (adjusted based on project needs):

1. Al: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 ug/L

2. Ba: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 ug/L

3. Ca: 0.1, 0.3, 1, 3, 10, 30, 100 mg/L

4. Fe: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000, 10000 ug/L

5. K: 0.1, 0.3, 1, 3, 10, 30, 100 mg/L

6. Mg: 0.1, 0.3, 1, 3, 10, 30, 100 mg/L

7. Mn: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 ug/L

8. Na: 0.1, 0.3, 1, 3, 10, 30, 100 mg/L

9. Sr: 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 ug/L vi. Determination of detection limit – 7 diluent samples (2% HNO_3 and 0.5% HCl)

vii. NIST standard reference material (SRM; currently 1643f)

1. Assessment of accuracy and continuous calibration verification (CCV)

2. Analyzed at least once every 20 samples, with a minimum of three analyses per batch

viii. Spectral interference check (SIC)

1. Inorganic Ventures 6020 ICP interference check A – contains Al, Ca, C, Cl, Fe, Mg, Mo, P, K, Na, S, Ti

2. Analyzed at least once every 20 samples ix. Lab fortified sample matrix (LFM-D1) and Lab fortified sample matrix duplicate (LFM-D2)

1. Included as the greater of: one LFM-D1 and LFM-D2 set per batch; one LFM(D1) and LFM(D2) set per 20 samples

2. Spike concentration will be quantified through inclusion of a lab fortified blank (LFB) at the same concentration

3. These will be compared to the unspiked sample (S)
4. Spike recovery will be calculated as shown below and will be considered quantitative if it is between 75 – 125%
    a. $Recovery\ (\%) = \frac{(LMF(D1) - S)}{LFB} * 100\%$
5. Precision will be calculated based on the relative percent difference (RDP) of duplicates (LFM(D1) and LFM(D2)), and will be considered acceptable if it is < 20%
    a. $RPD\ (\%) = \frac{|LMF(D1) - LMF(D2)|}{\{\{LMF(D1) + LMF(D2)\}/2\}} * 100\%$

x. Lab reagent blank (LRB) – diluent blanks (2% HNO_3 and 0.5% HCl (v/v))
    1. Included as the greater of: one LRB per batch; one (2% HNO_3 and 0.5% HCl (v/v)) per 20 samples

c. Method and batch tune - done <u>prior to each batch</u>
    i. High Matrix Introduction (HMI) selected based on sample water quality, notably salinity; for freshwater surface samples, HMI 25 is often used; HMI 50 or 100 is used for more saline samples
    ii. Tune mode reflect potential inclusion of He or H2 in the collision reaction cell
    iii. ANS samples are analyzed in He and H2 tune modes to limit polyatomic interferences; He is used as the primary analytical approach, and H2 as a backup option
    iv. Run auto-tune for selected introduction and source configuration
    v. Criteria – generally considers Y (89) and Tl (205)
        1. Background < 25 cps
        2. Axis resolution ± 0.05 of nominal mass
        3. Signal relative standard deviation (RSD%) < 5%
        4. Oxide ratio (156/140) < 2% but lower is better
        5. Doubly charged (70/140) between 1.5 – 3.5%
        6. Sensitivity (counts per second) consistent with previous batches and exceeds manufacturer's specifications
        7. If manufacturer standards are not met or performance is notably different than historical startup tun performance -> initiate troubleshooting
    vi. Target elements are paired with internal standards elements based on similar ionization energies
    vii. Analysis via autosampler; “vial” is used as the generic term to indicate sample, standard, qa/qc etc.
        1. Three rinses are included between each vial
        2. Stabilization period included to limit artifact from transitions (15 s)

3. Three analyses are collected per element per tune mode viii. Internal standards are included as an online addition via a t- connection prior to the nebulizer

d. Typical batch order
    i. 4 blanks
    ii. 2 calibration blanks
    iii. Calibration curve (number of vials varies, but is often ~12) iv. 7 diluent blanks
    v. SRM/CCV
    vi. 1 blank vii. 1 LRB viii. Repeat the following block to complete all samples
        1. 10 samples
        2. 2 blanks
        3. 10 samples
        4. 1 blank
        5. SRM/CCV
        6. 1 blank
        7. 1 LRB ix. 1 blank
    x. SRM/CCV
    xi. 3 blank

3. Data analysis
    a. Agilent MassHunter employed all data considering the response = analyte cps/internal standard cps
    b. QA/QC criteria are shown in the table below and discussed below
    c. Detection limit is assessed two ways and the greater value is reported
        i. MassHunter estimate based on the calibration blank
        ii. Evaluated from 7 blanks as the 99% upper confidence value (mean plus three standard deviations)
    d. Linear calibration curve fit – only retain points that meet the following
        i. At least 2x the calibration blank (cps)
        ii. RSD% < 30%
        iii. Assessed concentration is within 30% of the true concentration
        iv. The lowest retained calibration curve point is the limit of quantification (LOQ)
        v. Valid calibration curve must contain at least 5 points and have an $r > 0.995$
    e. LRB values will be considered valid if they are below the lowest retained point on the calibration curve.
    f. SRM recovery is considered accurate if it is within 10% of known value
    g. All vials (blanks, calibration standards, SRM/CCV, samples) – separate consideration for each vial, each element, and each tune mode: <u>a value is only considered quantitative if all of the below are met</u>

i. Target element concentration is within the linear range of the calibration curve (> LOQ and below highest retained point) and stable (i.e., 30% for within sample measurements)
ii. Internal standard recovery within 70 - 125% of initial value and stable (i.e., 5% for within sample measurements)
iii. SRM recovery is considered accurate (i.e., within 10% of known value)

h. Values that do not meet all of the above criteria are reported with a notification that they are estimates, rather than accurate quantification

i. Elemental correction equation (from 200.8)

1. Aluminum (Al, 27): no correction (from 200.8)
2. Barium (Ba, 137): no correction (from 200.8)
3. Calcium (Ca, 44): no correction
4. Iron (Fe, 56): no correction
5. Potassium (K, 39): no correction
6. Magnesium (Mg, 24): no correction
7. Manganese (Mn, 55): no correction (from 200.8)
8. Sodium (Na, 23): no correction
9. Strontium (Sr, 88): no correction ii.

Parameter	Explanation	Determination
Batch stability	Determination of batch-wide matrix has affected ICP-MS performance	Internal standard recovery: 100 ± 30%
Analysis stability	Determination of instrument is stable (consistent) for a sample	Internal standard RSD < 5%
Sample stability	Determination of analysis is stable (consistent) for a sample	< 30%
Detection limit (DL)	Quantification of the lowest value that can be differentiated from 0	MassHunter© DL determination based on replicate analysis of calibration blank
		Analysis of seven blank vials: mean + 3*stdev
Limit of quantification (LOQ)	Determination of the lowest concentration that can be quantified (30% precision)	Estimated concentration is ± 30% of known standard concentration
Accuracy	Accurate analysis of a known sample	Measured value ± 10% of known standard reference material concentrations*
Max concentration	Upper limit of quantification based on calibration curve	Maximum value of standard that is retained in the calibration curve
*NIST SRM 1640a was used for verification of aqueous sample accuracy; only applies to relevant elements		

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Proposal for Estimation of Fish BAF for Selected PFAS Contaminants in Marine and Freshwater Systems

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Prepared for:
New Jersey Dept. of Environmental Protection

Revised June 1, 2021

We are proposing a study based on the following: a) the primary objective of calculating water to fish bioaccumulation factors (BAFs) for p (PFAS) that can be used across the range of New Jersey waterbodies; b) our initial discussions with NJDEP of site categorization (marine and freshwater; -Pinelands) and fish stratification; c) emphasis on multiple water and individual fish samples to provide information on sources of variability in concentrations; and d) recognition of the need for an efficient sampling design balancing the number sites against the breadth of data collected at each site. For the purposes of this proposal "marine" refers to anything in the gradient of tidal freshwater to ocean salinity.

In summary, we propose sampling at 16 marine sites in Year 1, these include: 1) upper estuary sites with variable flow water; and 2) lower estuary or open/bay sites with limited non-tidal flow and salinities that may range from to ocean water (Table 1). In Year 2 we propose sampling at 18 freshwater sites including: 1) treams, in 2) systems in Pineland and non-Pineland areas (Table 1). Select pre-sampling will occur both years to verify and assess the temporal variability of aqueous PFAS concentrations. Candidate and final site selection will be decided in consultation with NJDEP staff. At all sites we aim to collect 3 species and 3 individuals of each species,
vs. benthic) PFAS exposure pathways. All environmental and fish filet samples will be analyzed for PFAS concentrations. Fish samples will be further analyzed for stable isotope compositions to verify trophic level. Water and sediment samples will be further analyzed for chemical parameters demonstrated to substantially affect the solid-water distribution and bioavailability of PFAS. The following sections outline the details and justification of this study design and the considerations that should inform site selection.

Experimental Approach and Considerations

Factors influencing BAF determination - The following results from NJDEP's SR15-010 Report are pertinent to estimation of BAFs and influenced our proposed study design: 1) Short chain PFAS were frequently detected in water and rarely detected in fish; 2) PFOS was frequently

detected in fish, and also detected in water; 3) PFOA, PFHxS, and PFNA were frequently detected in fish, but only detected in water from a few sites; 4) other longer-chain PFAS were frequently detected in fish, but not in water; Thus, BAFs could only be estimated for PFOS at several sites and PFOA and PFNA at a few sites. These are the only compounds with consumption criteria, so these are the most important for development of surface water criteria. In addition, where the same compound was detected in both fish and water, the estimated BAFs were highly variable, ranging over an order of magnitude for some sites and species. The variability of BAFs may reflect variation in concentrations in water, concentrations in fish (since PFAS have a relatively short residence time compared to fish longevity), and/or variations in processes determining BAF including exposure pathways, metabolic differences between species, and concentration- . Therefore, multiple sampling of both water and fish will be important in estimating BAFs. Notably, BAF data are limited for marine species and additional data are needed to determine if marine and freshwater systems warrant different BAFs due to apparent salinity effects on PFAS bioavailability and species metabolism. That said, given the many variables potentially influencing fish BAFs it is important to be selective of which variables to target or control for relative to the number of sampling sites.

Proximity to known or suspected sources - Given the results cited above, a geographically extensive survey of different waterbodies may be inefficient, as it may produce data useful for BAF estimation at relatively few sites. Thus, we recommend this study focus on sites with higher ly to produce detectable water and fish concentrations for a number of PFAS compounds, especially PFNA. To ensure this, we will propose candidate sites from where existing environmental PFAS data are available, including those previously sampled by NJDEP for PFAS in water and/or fish. Regionally, PFAS concentrations are generally declining. Thus, unless PFAS data for water is available within the past year, we propose conducting pre-sampling at candidate sites to ensure sufficient detection of PFAS in water, especially for longer- in fish. Within this template and our study design, we recommend site selection consider the following: proximity to PFAS sources; diversity of PFAS sources including historic use of aqueous film forming foams on military and airport installations (esp. Joint Base McGuire-Dix-), manufacturing locations (esp. Solvay Specialty Polymers USA LLC, West Deptford, NJ), and major waste water treatment effluent inputs; and proximity to underrepresented communities that may be disproportionately influenced by environmental contamination. Candidate and final site selection will be decided in consultation with NJDEP staff.

Lake depth - We recommend a categorization depth because there may be due to differing degrees of water- In addition to

environmental PFAS concentrations. Short turnover time may lead to dilution of ground-water sourced PFAS and/or more time-variable PFAS concentrations. Conversely, in with long turnover time potentially concentrating PFAS in water.

g turnover-

We that m would fit into this group. these conditions may be present in impoundments of small streams (e.g. Atlantic City Reservoir), formed from gravel pits or dredging

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Variability in PFAS concentrations - Temporal variability in PFAS concentrations in flowing systems (streams and upper estuary) precise estimates of BAFs in streams. PFAS into fish tissues has been shown to be reasonably rapid, on the order of days to PFAS concentrations at analogous time scales would be necessary for accurate estimation of BAFs in these systems. To address the issue of variable water concentrations, we will collect stream and estuary water samples ~ prior to fish sampling in order to characterize concentration variability on the timescale of . At the estuary sites, this sampling will be conducted at the same point in the tidal cycle, except for 1 – 2 samples collected to assess tidal variability. Quarterly sampling could, similarly, help to characterize long-term variability in environmental PFAS concentrations. However, in the current design we judged an increased site number to be more relevant to the study objectives than extensive, long-term temporal sampling other than the initial pre-sample.

Methodology

Sample Collection - We propose sampling 3 species of fish at all sites representing, ideally, a benthic forager, pelagic forager and pelagic piscivore. If caught, further samples of species specifically associated with the air-water interface and will be included. This selection represents the transfer pathways of PFAS to fish, trophic transfer and environmental exposure through water and sediment. All species would be susceptible to direct bioconcentration from water contact and some biomagnification from food. Piscivores would have greater potential biomagnification. Benthic fish may have greater sediment contact and more benthic food sources, signific -chain PFAS in aquatic ecosystems.

We will collect at least three specimens of each species, each of which would be used to provide 3 individual filet samples. We propose analyzing individual rather than composite specimen samples in order to target the highest, not an average, BAF for each species and site. Note, this design does not allow calculation of a whole-body BAF. If sufficient numbers of target species are not caught with reasonable collecting effort, a second species will be chosen, or additional specimens will be allocated to other sites. Selection will target species and specimen sizes most

Fish specimens will be collected with appropriate equipment, primarily by boat-electrofishing in freshwater systems and freshwater tidal portions of estuaries, and gill netting, angling, or other methods in higher salinity waters. Specimens will be held on ice in the field and frozen on return to the laboratory. If available, some additional specimens may be collected and archived for measured and weighed. Separate filet and carcass weights will be recorded. Material not used for analyses will be refrozen and held for at least one year. This material may be used for future individual sample or carcass analyses, should sample results indicate the need for such data.

Surface water and sediment samples will be collected at each site contemporaneously. PFAS bioavailability has been shown to be substantially affected by solid-water distribution coefficients, which in turn vary with different solid phase characteristics and solution chemistry, including temperature, carbon concentrations, and polyvalent cation concentrations. Thus, data on sediment PFAS concentrations may be useful if benthic fish are found to have higher concentrations of PFAS. We have included a minimal amount of sediment sampling in the present scope. An alternative may be to collect and archive sediment samples for future analysis, depending on the results of the water and fish analyses.

Sample Analysis - PFAS extraction and analysis will be sub-contracted to SGS AXYS. AXYS has been performing PFAS analyses since 2002 on a variety of matrices including water, soil/sediment and biota/tissues and has analyzed prior NJDEP PFAS samples. Analysis of water and sediment samples will be done using AXYS' PFAS method MLA-110, which covers list of 29 PFAS compounds (11 perfluorinated carboxylates, 8 perfluorinated sulfonates, 3 fluorotelomer sulfonates, 3 perfluorooctane sulfonamides, 2 perfluorooctane sulfonamide ethanols, and 2 perfluococtane sulfonamideacetic acids) and has generally improved reporting limits. In addition to analyzing for PFAS concentrations, we propose analyzing all fish samples and one suspended particulate matter sample per site ^{13} ^{15}N in order to verify trophic level and assess the potential for confounding effect of trophic PFAS pathways on BAF calculations. All water samples will be analyzed for field parameters (pH, temperature, dissolved oxygen concentrations, and specific conductivity), polyvalent cation concentrations and organic carbon concentrations, and all sediment samples will be analyzed for total C and N, and polyvalent cation concentrations (all demonstrated to substantially affect the solid-water partitioning of PFAS). All non-PFAS analysis will be conducted in-house at the Academy.

Timeline, Deliverables and Budget

Sample and project timing will depend on the timing of project approval. If approved by June 2021, marine site sampling would be conducted in the fall of 2021 and freshwater site sampling in 2022. Decisions on primary and alternate sampling sites will be made in concert with NJDEP staff.

Data analysis will include estimation of BAFs, analyses of differences in PFAS concentrations among fish species, among water sampling periods, and among sampling strata. We will submit a draft report of the 2021 marine results by late spring 2022, and a draft report of the 2022 freshwater results by late spring 2023. We will plan a meeting or conference call following the

submission of each draft report to discuss the results and requested edits by NJDEP before revising and submitting the final reports.

In total we request $500 , $247,742 for the marine component in Year 1 and $252,259 for the freshwater component in Year 2 (Table 2). Of the total request, $200,860 (40%) will support the sub-contract to SGS AXYS and $5,000 will cover the fee for New Jersey Sea Grant Consortium. Indirect is charged on the first $25,000 of the SGS AXYS sub-contract.

Table 1. Sampling plan summarizing the number of field visits and samples collected for water, fish and sediment.

Sample Summary		**Water**			**Sediment**	**Fish**		
						Piscivore	**Forager**	
Site Classification		**Pre**	**Main**			**Pelagic**		**Benthic**
Marine Sites	**# Sites**	6 1	1 1		1 0.5	3 3	3 3	3 3
Upper estuary	8							
Open water/Bay	8							
		16		56		48	48	
16								48
		Total Samples:		**72**	**12**			**144**
Freshwater Sites								
Streams								
Pinelands	4			5	1	1	3	3	3
Non-Pinelands	4			5	1	1	3	3	3
Lakes								
Shallow, Pinelands	4			1	1	1	3	3	3
Shallow, Non-Pinelands	3			1	1	1	3	3	3
Deep	3			1	1	1	3	3	3
		18		50	18		54	54	54
		Total Samples:			**68**	**18**			**162**

Table 2. Budget summary, including total costs based on an indirect rate of 50%, as requested by

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Budget Summary	**Total Cost (incl. labor, fringe & supplies)**		
	Year 1	**Year 2**	**Total**

Field Work						
- Fish	$ 18,815	$ 23,096	$ 41,910	- Chemistry	$ 26,694	$ 28,721
$ 55,415						
ANSDU Laboratory						
- Fish	$ 8,243	$ 9,422	$ 17,665			
- Chemistry	$ 14,874	$ 16,068	$ 30,942			
Management, data analysis and reporting			$ 20,603	$ 21,225	$ 41,828	
ANSDU Indirect			$ 57,114	$ 49,266	$ 106,380	
		ANSUD subtotal	$ 146,342	$ 147,799	$ 294,140	SGS
Axys Laboratory*	$ 96,400	$ 104,460	$ 200,860			
21.1 New Jersey Sea Grant Consortium*			$ 5,000		$ 5,000	
		Total	**$ 247,742**	**$ 252,259**	**$ 500,000**	

*Indirect charged on the first $25,000 of sub-contracts but not on NJSGC contracting fee

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ACADEMY OF NATURAL SCIENCES
PATRICK CENTER FOR ENVIRONMENTAL RESEARCH

Procedure No. P-16-91
Rev. 2 (03/2012)

YSI 6920V2, Professional Plus, and 556 MPS Calibration and Calibration Check

Prepared By: Paul Kiry and David Velinsky

Approved By: _____ Date:_____
Robin S. Davis
Quality Assurance Unit

22. YSI 6920V2, PROFESSIONAL PLUS, AND 556 MPS CALIBRATION AND CALIBRATION CHECK

1. EQUIPMENT.

1.1. This SOP applies to any of the units above. For more detailed information refer to each unit manual. Be sure that the units have fresh batteries and are at least at 25% capacity. New batteries should be taken as a backup. Calibration standards include: pH 4.0, 7.0 and 10.0 buffers, de-ionized water (DIW), conductivity solution (1.412 mS/cm or higher for marine and brackish waters) either bought or made with a salt standard (e.g., KCl). All standards can be obtained commercially and certified.

2. CALIBRATIONS.

2.1.All calibrations are done using the calibration menu (D.O., pH, conductivity-salinity, etc.). Press Power to turn on the unit—it will be in the Run mode when it comes on or you may have to select Run mode. Check battery condition (dark bar at bottom of screen). Pressing ESC will take you to the main menu. The arrow keys are used to scroll and to select calibration from the main menu. The enter key is used to enter the selections. (Refer to operation manual for more information.)

2.2. D.O. Calibration.

2.2. 1.Rinse probe and cup with DI water. Shake excess water from the probe and place the protective cup over the probe (s). Do this in the evening or 30 min before checking calibration. This is needed so that the atmosphere in the cup has time to equilibrate with the oxygen and water vapor. Only one or two threads of the cup should be engaged. Be sure no droplets of water are on the probe and it should not be immersed in water. Turn on the unit at least 20 minutes before calibration, and wait until temperature and DO are stabilized. Go to calibration screen by pressing Esc to get to the main menu- use arrows to move to calibration-use arrows to select DO %. Enter the barometric pressure reading (this is automatic) wait for a steady DO reading then press enter. The DO value should be accepted and wait for the Calibration Successful message. If no more calibrations are required, return to the Run mode by moving back through the menu using the Esc key to the main menu and select Run mode. If you need to continue calibrating use the arrows to scroll up and down the calibration list.

2.3. pH Calibration.

2.3. 1.Remove any probe guard. Rinse the probe with DIW; shake off excess, blot dry with a kimwipe**. Be careful not to scratch the tip of the probe**. Go to pH calibration and select 2 or 3 point calibration. Rinse the probes with buffer. Using a two point calibration place the pH probe and conductivity / temperature probe into the first pH buffer (4.0), enter the value and wait for the "successful" message. Rinse the probes with DIW and blot dry. Rinse with the second buffer place the probes into the second pH buffer (10.0), enter the value and wait for "successful" message. Rinse the probe with DI and return to Run mode or continue with a third buffer as above if required.

2.4. Conductivity.

2.4.1.Remove the probe guard. Rinse the probe with DIW; shake off excess, blot dry with a kimwipe. Rinse probe with a little standard. Rinse the probe with some standard. Go to Sp Cond. (mS/cm) and place the probe in the container containing the standard of choice and enter its value. Wait for the "successful" message. If calibrations are complete, return to the run mode.

3. CALIBRATION CHECK.

3.1. The calibration checks are done in the Run mode and should be completed after calibration and after using the unit in the field. All data should be recorded and any variances should be noted.

3.2. pH.

3.2. 1.Remove the probe guard. Rinse the probe with DIW; shake off excess, blot dry with a kimwipe. **Be careful not to scratch the tip of the pH probe**. Place the probe in the pH buffer (7.0) and record the value. Rinse the probe and replace the probe guard if calibration checks are completed. NOTE: If calibration check is substantially off (outside a 6.9 to 7.1 range), the meter must be re-calibrated. This is the maximum range, if more accuracy is needed for the specific project; recalibrate the meter.

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3.3. Conductivity (Salinity).

3.3.1. Remove the probe guard. Rinse the probe with DIW, shake off excess water and blot dry with a kimwipe. Place the probe in the bottle containing the conductivity standard or a suitable salinity standard and record the value. Rinse the probe and replace the probe guard if calibration checks are completed. NOTE: If calibration check is substantially off (1% standard), the meter must be re-calibrated.

3.4. Dissolved Oxygen.

3.4.1 This check can be done in the laboratory before and after field use. Place the probe in a beaker of well aerated DIW. Measure the temperature and determine the DO saturation at that temperature (use tables posted in the lab) or by the Winkler titration. Compare the measured DO value with the theoretical value. They should agree within 0.3 mg/L. If they do not, membrane replacement may be required. (Consult with the unit manual for the procedure). Then, recalibrate and check again. If a problem persists a new probe or further maintenance may be needed.

4. Technical Support Numbers.

800-765-4974 ext. 358; David Kasenheiser

513-767-7241 Option #3 YSIE-mail: support@ysi.com

800-363-3269 ext. 247; Mike Lazotte

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Patrick Center Biogeochemistry Section Chain of Custody

Academy of Natural Sciences
1900 Benjamin Franklin Parkway
Philadelphia, PA 19103-1195
ph: 215-299-1076

Project: NJ TOXICS

Date Collected	Time Collected	Collected By	Sample ID	Type of Sample (water/sed)	Container (4L/whirlpak)	Preserved (chilled/frozen)	Notes	For Chem Only
4/27/22	0530	TC/CR	SALEM CANAL (DDCA)	H_2O	250mL (2x)	CHILLED		2527
↓	0610	↓	OLDMAN'S CREEK (DUCA)	↓	↓	↓		2528
	0655		MANTUA CREEK (DDSA)				* LITTLE TINICUM ISLAND	2529
↓	0725	↓	WOODBURY CREEK (DUSA)	↓	↓	↓		2530

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4/27/22	1130	JMH/DPM	ABSECON RIVER (ABSEA)	H_2O	250mL (2x)	CHILLED		2531
↓	1253	↓	MAURICE RIVER (MAURA)	↓	↓	↓		2532
↓	1411	↓	COHANSEY (COHA)	↓	↓	↓		2533
↓	1615	↓	TOMS RIVER (TRBB)	↓	↓	↓		2534
↓	1640	↓	METEDECONK (BBA)	↓	↓	↓		2535

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4/28/22	1218	JMH/DPM	UPPER BAY (UPBAYB)	H_2O	250mL (2x)	CHILLED		2536
↓	1305	↓	HACKENSACK CONFLUENCE	↓	↓	↓		2537
↓	1335	↓	HUDSON RIVER	↓	↓	↓		2538
↓	1423	↓	HACKENSACK UPSTREAM	↓	↓	↓		2539
↓	1500	↓	PASSAIC (PASK)	↓	↓	↓		2540

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4/28/22	1215	TC/CR	RARITAN BAY (RBA)	H_2O	250mL (2x)	CHILLED		2541
↓	1245	↓	RARITAN RIVER/ LOWER RARITAN (RARR)	↓	↓	↓		2542
↓	1505	↓	NEWARK BAY SOUTH (NWBAYL)	↓	↓	↓	* USED BACK-UP COORDINATES	2543

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5/3/22	0830	TC	AC SEWER LINE / (OOAC) PRIME FISHING GROUNDS	H_2O	250mL (2x)	CHILLED		2544

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Date Collected	Time Collected	Collected By	Sample ID	Type of Sample (water/sed)	Container (4L/whirlpak)	Preserved (chilled/frozen)	Notes	For Chem Only
5/3/22	0849	JMH	AXEL CARLESON REEF/ UWPCF EFFLUENT (000cc)	H_2O	250mL(2x)	CHILLED		2545

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