Trophodynamics of Per- and Polyfluoroalkyl Substances in the Food Web of a Large Atlantic Slope River

Tiffany N. Penland, W. Gregory Cope,* Thomas J. Kwak, Mark J. Strynar, Casey A. Grieshaber, Ryan J. Heise, and Forrest W. Sessions

ABSTRACT: Per- and polyfluoroalkyl substances (PFASs) have attracted scientific and regulatory attention due to their persistence, bioaccumulative potential, toxicity, and global distribution. We determined the accumulation and trophic transfer of 14 PFASs (5 short-chain and 9 long-chain) within the food web of the Yadkin-Pee Dee River of North Carolina and South Carolina, US. Food web components and pathways were determined by stable isotope analyses of producers, consumers, and organic matter. Analyses of water, sediment, organic matter, and aquatic biota revealed that PFASs were prevalent in all food web compartments. Biofilm, an aggregation of bacteria, fungi, algae, and protozoans and a basal resource for the aquatic food web, showed high PFAS accumulation (in 10 of 14 compounds), particularly for perfluoroctanoic acid, with the greatest mean concentration of 463.73 ng/g. The food web compartment with the most detections and greatest concentrations of PFASs was aquatic insects; all 14 PFASs were detected in individual aquatic insect samples (range of <limit of detection [<LOD] to 1670.10 ng/g of wet weight [WW]). These findings may suggest a trophic link between biofilm PFASs and aquatic insect PFASs. Individual fish tissue samples ranged from <LOD to 797.00 ng/g of WW, where perfluorooctanesulfonate (PFOS) was the dominant PFAS among all samples (64%). The ova of an imperiled fish, the robust redhorse (Moxostoma robustum), had concentrations of 10 PFASs (range of <LOD to 482.88 ng/g of WW) and the highest PFOS concentration (482.88 ng/g of WW), indicating a likely maternal transfer. The trophic magnification factors (TMFs) calculated in this study showed that various taxa accumulated PFAS compounds differently. PFBS, a short-chain PFAS compound that would presumably exhibit lesser TMFs, had nine values among our compartments and organisms >1.0 (range of 0.57 to 2.33); it is possible that an unmeasured PFBS precursor may be accumulating in biota and metabolizing to PFBS, leading to a higher than expected TMFs for this compound. Our findings demonstrate the prevalence of PFASs in a freshwater food web with potential implications for ecological and human health.

INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a class of compounds that have attracted substantial scientific and regulatory attention as persistent contaminants in the environment. PFASs are synthetic compounds with many residential, commercial, and industrial uses that make them economically valuable, but they pose as a concern as a potential global threat to human, wildlife, and ecological health.1−4 These substances were developed to resist oil, soil, and water,5 which make for beneficial applications but also yield highly persistent chemicals when released into the environment. PFASs have been incorporated into such products as surfactants, coating additives in paints and polishes, firefighting foams, cleaning products, fabric stain repellents, and pesticides.6,7

Since the early 2000s, US and global fluorochemical manufacturers have phased out production of PFASs with longer-chain lengths and more fluorinated carbons (i.e., C ≥ 7), specifically perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), and replaced them with shorter-chain alternatives that are presumably less bioaccumulative and potentially less toxic; however, many previously produced and imported items may still contain the long-chain PFASs.5−9 The long-chain PFASs (PFOS or PFOA) are currently managed under various federal and state guidelines, advisories, and health standards.9−11 Moreover, internationally, PFOS and PFOA have been added to the list of persistent organic pollutants (POPs) of the United Nations Environment Programme.12

Received: August 19, 2019
Revised: April 8, 2020
Accepted: April 28, 2020
Published: April 28, 2020

https://dx.doi.org/10.1021/acs.est.9b05007
Environ. Sci. Technol. 2020, 54, 6800−6811
Programme Stockholm Convention, and perfluorohexanesulfonic acid (PFHxS), its salts, and PFHxS-related compounds have been recently proposed for being listed under the Convention. Despite the decrease in the production of some compounds, widespread environmental input and cycling of PFASs will continue from long-range transport, degradation of PFAS precursors, legacy products, or remobilization from other media (e.g., sediment, ice, or soil). Current major sources of PFASs include landfills, military and other locations that used aqueous film-forming foams, and industrial and municipal sewage treatment effluents. Recent ecotoxicological research has focused on PFASs as contaminants of concern, especially for aquatic biota. The concern is based on their persistence, bioaccumulative potential, toxicity, and global distribution. Carbon–fluorine bonds make these compounds chemically stable and resistant to degradation in the environment, and PFASs with longer-chain lengths and more fluorinated carbons have increased bioaccumulative potential. Among the most recognized and researched PFASs in the environment are PFOA and PFOS. Various studies have documented the presence and effects of these two compounds in aquatic environments and biota, but very few have examined their transport and dynamics in lotic ecosystems.

PFASs have been detected in wildlife worldwide. PFOS and PFOA are typical dominant compounds found in surface waters, sediment, aquatic organisms, and their consumers. Published toxicological effects are limited, but experimental research has indicated moderate acute and chronic effects in a variety of aquatic organisms. Empirical evidence has demonstrated that PFOS and related substances bioaccumulate in fish tissue to concerning levels that pose a threat to the health of fishes, piscivorous wildlife, and humans. Other research has indicated that maternal transfer of PFASs can lead to high concentrations in fish eggs and embryos, causing potential adverse effects.

While many freshwater lacustrine, estuarine, and marine food web studies of PFAS have been performed, few have been undertaken in freshwater lotic ecosystems. The Yadkin-Pee Dee River of North Carolina and South Carolina, US, is subject to numerous anthropogenic contaminant inputs (e.g., municipal, industrial, and agricultural sources) but has no identifiable industrial production or military-related point discharge of PFASs, and thus, it is a model ecosystem to study associated distribution and toxicological effects. Over 6115 river kilometers are classified as impaired waters within the basin due to high levels of sediment and contaminants in surface water and fish tissue. The Yadkin-Pee Dee River basin is an important aquatic ecosystem that provides habitat for over 50 imperiled aquatic species. An imperiled fish species in critical need of conservation and recovery in the Yadkin-Pee Dee River is the robust redhorse (Moxostoma robustum). The current estimated spawning population for the Yadkin-Pee Dee River is 62 reproducing adults (95% CI 48–77). Currently, sufficient information on the occurrence, abundance, and dynamics of PFASs is lacking to guide management strategies for robust redhorse population recovery in this ecosystem. To our knowledge, PFASs have not been analyzed and assessed in this river’s food web prior to our study.

Stable isotope techniques can assess the trophic transfer of PFASs and other contaminants and the extent of exposure through dietary routes. Stable isotopes provide dietary information from assimilated food sources integrated over...
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*Detected were reported when concentrations were equal to or greater than the limit of quantitation (LOQ) of 5 ng/g of WW; n = number of samples analyzed; <LOQ = less than limit of quantitation. Values <LOQ were not included in calculation of means; values with no SD had only 1 sample ≤LOQ; na = not analyzed. *Whole body concentrations.
time and detect changes in trophic linkages associated with environmental and anthropogenic influences.\textsuperscript{36−38} Combining stable isotope analyses and chemical analyses of biota can reveal biomagnification or biodilution of contaminants within the food web.

Due to the bioaccumulative characteristics and widespread occurrence of PFASs, this study aimed to analyze a wide range of biotic and abiotic components of the Yadkin-Pee Dee River aquatic food web. Samples spanned all major food web components, some of which are known to be linked through ecological and trophic pathways, and included water, sediment, detritus, algae, biofilm, macrophytes, aquatic insects, crayfishes, mollusks, fishes, and fish ova. The specific objectives of this research were to (1) utilize stable isotope ratios to determine the food web structure by sampling a variety of organic matter and aquatic biota, (2) analyze water, sediment, organic matter, and biota for PFAS presence and concentration, (3) determine contamination trends among the sites along a longitudinal gradient, and (4) assess bioaccumulation and biomagnification of PFASs by examining chemical concentrations among trophic compartments of the food web. The overall goal of this research was to understand PFAS dynamics within a riverine food web and reveal potential stressors to common and imperiled species in the Yadkin-Pee Dee River.

\section*{METHODS}

\textbf{Study Sites.} Five riverine sites with variable topography and anthropogenic influences were selected along the Yadkin-Pee Dee River of North Carolina and South Carolina (Figure 1, Table S1). Physical characteristics, land use, hydrology, and influx of point and nonpoint source pollution differed among the sites, which facilitated longitudinal examination of trophic and PFAS dynamics. Site selection was also based on associations with the Yadkin-Pee Dee River robust redhorse population so that potential environmental stressors could be identified. To aid in the investigation of food sources and availability for robust redhorse, the sites included the location near where the robust redhorse was first described in 1870 but no longer exists (site 801),\textsuperscript{39} where the robust redhorse population is extant (Digg’s Tract, Society Hill, and Pee Dee), and a proposed reintroduction site to stock hatchery-propagated robust redhorse (Red Hill).

\textbf{Sample Collection and Preparation.} The collection of samples for organic matter and aquatic biota was conducted at all five sites during spring and summer 2015. When feasible, taxa collected for food web and PFAS analyses were kept constant among the sites. Fishes (10 species), mollusks (4 families), crayfishes (2 species), aquatic insects (12 families), macrophytes, and detritus (each described in detail below) were collected from each site for stable isotope analysis (SIA) to determine the major components of the food web, trophic pathways, and bioaccumulation of PFASs. Collection and processing methods were similar to those of Hoeinghaus et al. and Pingram et al.\textsuperscript{30,41}

\textbf{Watter.} Grab water samples were collected from all five sites on two different occasions in 2016 (February 25 and March 10; offset in time from organic matter and biota collection due to scheduling logistics). Surface water was collected midstream in precleaned 1 L polypropylene bottles and placed into a cooler for transport to the laboratory for processing, extraction, and analysis according to the methods of Nakayama et al. and Strynar et al.\textsuperscript{32,43}

\textbf{Sediment.} Composite samples of the sediment were collected from all five sites in February 2016. Each sample was collected with a precleaned stainless steel scoop from the top 3−5 cm of the sediment surface layer from several locations within a 1 m\(^2\) area to form a single composite sample per site. Sediment samples were placed into labeled sterile amber glass jars and held in a cooler for transportation to the laboratory, where they were stored in a −80 °C freezer until processing and analysis.

\textbf{Biota and Organic Matter.} Detritus was sampled from conditioned leaf packs. Leaf packs were collected by hand or with dip nets, placed into sealable plastic bags, and then placed on ice after visible debris and invertebrates were removed. Biofilm was collected by brushing the surface of rocks with a firm bristled brush and rinsing into a container and held on ice. Samples were vacuum filtered through glass fiber filters in the laboratory and stored frozen at −20 °C. Aquatic macrophytes and algae were collected by hand, thoroughly rinsed to remove organic matter and invertebrates, and placed into sealable plastic bags and held on ice. The Pee Dee site lacked sufficient macrophytes to obtain a sample.

Conventional techniques were used to collect aquatic macroinvertebrates and included using 500 μm mesh D-frame nets, flipping rocks, or hand separating from leaf packs and woody debris. Insect and crayfish specimens were held chilled for at least 8 h in filtered river water to enable depuration of gut contents. Aquatic insects were classified into functional feeding guilds and separated taxonomically. Feeding guilds included collector−filterer, shredder, scraper, or predator, and taxa included the families Brachycentridae, Corydalidae, Elmidae, Gerridae, Glossiphoniidae, Gyrinidae, Heptageniidae, Hydropsychidae, Limnephilidae, and Perlidae as well as Odonata suborders Anisoptera and Zygoptera. Mollusks were collected by hand and identified into species. Native freshwater mussels (family Unionidae), snails (Pleuroceridae and Viviparidae), and non-native Asian clams (Corbicula fluminea) were included. Snails were held in filtered river water for at least 8 h to enable depuration of gut contents.

Standard boat-mounted, pulsed-DC electrofishing was employed to collect fish. The fish collection goal was to maintain consistency in numbers, size classes, and species among the sites. Following North Carolina State University approved protocols (IACUC 15-042-O), fish were immersed into an ice−water slurry to induce temperature shock and euthanasia. Fish were identified into species, and total length (mm) and wet weight (WW) (g) were measured for each individual. The collected fish species represented the major trophic guilds and families found in the Yadkin-Pee Dee River. These included American eel (Anguilla rostrata), blue catfish (Ictalurus furcatus), bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), common carp (Cyprinus carpio), largemouth bass (Micropterus salmoides), notchlip redhorse (Moxostoma collapsum), shorthead redhorse (Moxostoma macrolepidotum), smallmouth buffalo (Ictiobus bubalis), and whitefin shiner (Cyprinella nivea) (Table 1). In addition, one sample of ova from the imperiled robust redhorse was serendipitously collected during sampling associated with related research (lethal sampling of the fish was prohibited) but was relevant to our PFAS objectives and was analyzed to provide insight related to accumulation in this species and the other two closely related redhorse species sampled. All fish samples were stored frozen at −20 °C until further processing.
Stable Isotope Analysis. Standard laboratory practices were followed to avoid cross-contamination of organic matter samples. Sterile, stainless steel utensils were used for handling material, and all surfaces were cleaned with laboratory detergent, a distilled water rinse, an acetone rinse, and another distilled water rinse between samples. Samples of aquatic food web biota and organic matter were processed for stable isotope analysis following the same methods described below for PFAS analysis. Organic matter samples were individually dried at 60 °C to a constant weight and were then ground to a fine powder by mortar and pestle. Processed samples were inserted into 7 mL glass scintillation vials for storage and shipping to the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Flagstaff, Arizona, US, where they were analyzed for carbon, nitrogen, and sulfur isotope ratios with a gas and a vacuum manifold for solid-phase extraction. Extracts of sample was spiked with 30 ng of each isotopically labeled standard (PFHxA, PFHxS, PFOA, PFNA, PFDA, and PFOS) overnight. Samples were lyophilized then homogenized by sedimentation samples, and then, the sediment was frozen. Dilution analysis (See Supporting Information (SI) for details).

PFAS Analysis. University personnel analyzed the samples at the U.S. Environmental Protection Agency’s (EPA) Office of Research and Development Laboratory (Durham, North Carolina, US). All supplies used in processing and analysis were previously verified to be free of PFASs. Water samples were processed within 24 h after collection. Biota, organic matter, and sediment samples were stored at −80 °C until further processing. Samples were analyzed for perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorobutanesulfonate (PFBS), PFOS, perfluorohexanesulfonate (PFHxS), perfluorotridecanoic acid (PFTrA), and perfluorotetradecanoic acid (PFTeA). Only organic matter, plants, aquatic insects, and a subset of fish were analyzed for PFUnA, PFDoA, PFTrA, and PFTeA compounds because they are generally not observed in water samples. The long-chain PFASs are generally classified as perfluorinated sulfonic acids containing >6 fluorinated carbons and perfluorinated carboxylic acids containing >7 fluorinated carbons. Thus, in this study, we investigated five short-chain PFASs (PFBA, PFPeA, PFBS, PFHxA, and PFHxS) and nine long-chain PFASs (PFNA, PFDA, PFUnA, PFDoA, PFOS, PFOA, PFHxS, PFTrA, and PFTeA).

Water. Analytical methods followed the procedures of Nakayama et al. and Strynar et al. In brief, samples were extracted for PFAS using weak anion exchange (WAX) solid-phase extraction followed by an UPLC MS/MS isotope dilution analysis (See Supporting Information (SI) for additional details).

Sediment. After collection, excess water was decanted from sediment samples, and then, the sediment was frozen overnight. Samples were lyophilized then homogenized by grinding to a fine powder with a mortar and pestle. A 1 g aliquot of sample was spiked with 30 ng of each isotopically labeled standard (PFHxA, PFHxS, PFOA, PFNA, PFDA, and PFOS) in 7 mL of MeOH. Samples were vortexed, sonicated in a water bath for 30 min, and centrifuged at 10000 rpm for 5 min. The supernatant was transferred into Supelco Supelclean ENVI-Carb cartridges (Supelco, Bellefonte, Pennsylvania, US) and a vacuum manifold for solid-phase extraction. Extracts were concentrated under nitrogen to approximately 1 mL. Then 100 μL of the sample was mixed with 300 μL of an ammonium formate buffer in liquid chromatography (LC) vials to be analyzed by UPLC-MS/MS according to Strynar et al.

Biota and Organic Matter. Algae, macrophyte, and organic matter samples were processed after excess water was removed. Biofilm samples were scraped from filters and placed into 15 mL tubes. Samples (~100 mg) were digested and extracted with 5 mL of MeOH containing the internal standards (PFOS, PFNA, PFDA, and PFBS), vortexed, sonicated for 30 min, and centrifuged at 10000 rpm for 3.5 min. The solid-phase extraction was performed on the supernatant using Supelco Supelclean ENVI-Carb cartridges and a Waters vacuum manifold. Extracts were concentrated under nitrogen, and aliquots were added to polypropylene LC autosampler vials with 2-mM ammonium acetate buffer and analyzed by UPLC-MS/MS.

Aquatic insect samples with a tissue mass ≥1.0 g of WW were lyophilized and manually homogenized as composite samples and were grouped by order: Coleoptera, Ephemeroptera, Megaloptera, Odonata (Anisoptera and Zygoptera), and Trichoptera. Samples were placed in 50 mL Falcon tubes, and a 3:1 ratio of deionized water to tissue was homogenized. A 2 mL aliquot was removed from the homogenized sample, placed into a 15 mL tube, weighed, and then placed into a −80 °C freezer until further processing. The homogenate was digested and extracted with 28% ammonium hydroxide (NH₄OH) in a MeOH solution containing internal standards (PFOS, PFNA, PFDA, and PFBS). Samples were treated similarly to the above biota samples (see SI for additional details).

For fish samples, white muscle tissue was excised and freed of scales, skin, and bone and was analyzed. Individual fish were analyzed when there was sufficient tissue mass (≥1.0 g WW) to meet the minimum required for analysis; otherwise, composite samples of a species from a given site were used when tissue was lacking. Whitefin shiners were the only fish that were processed whole due to their relatively small size. Crayfish exoskeletons and mollusk shells were removed and excluded from analyses. Aquatic insects, plants, and organic matter were processed whole. Samples were homogenized with 3 mL of deionized (DI) water for every gram of tissue using a Polytron PT 10/35 homogenizer (Brinkmann Instruments, Westbury, New York, US).

Fish and crayfish samples were digested and extracted with 0.01 N sodium hydroxide (NaOH) in a MeOH solution containing internal standards (PFOS, PFNA, and PFDA). Mollusk samples were digested and extracted with internal standards (PFOS, PFNA, and PFDA). Samples were analyzed following the methods of Delinsky et al. (See Supporting Information (SI) for additional details).

Quality Control. Rigorous quality control measures were followed during all analyses. Quality control and assurance consisted of solvent blanks, method blanks, and matrix blanks to detect contamination at different stages of processing; sample duplicates to assess precision; and spiked samples to measure percent recovery. Solvent-based calibration curves were used for quantitation. Seven-point calibration curves containing native and internal standards were prepared in a range from 5 to 125 ng. The accuracy fell within 90–110% for compounds that had reference internal standards, and those that did not have an internal standard fell within 80–120%. PFAS detections were reported when the concentrations were estimated to be free of PFASs. Water samples were analyzed following the methods of Nakayama et al. and Strynar et al.
equal to or greater than the limit of quantitation (LOQ). The LOQs for our analyses were compound- and media-specific and were defined as the lowest point on the calibration curve capable of back calculating the theoretical concentration within ±30% precision; the average LOQ was 5 ng/g or 5 ng/L. The limit of detection (LOD) was set at 1% of the average LOQ (0.05 ng/g or ng/L). Our LOQs and LODs were similar to those of recently published studies analyzing various environmental matrices.19

Data Analysis. The mean δ13C, δ15N, and δ34S isotopic composition of biota was compared by an analysis of variance (ANOVA) to detect the differences among trophic levels and sites.37,40 δ15N values were interpreted to estimate the trophic position of consumers within the food web. Asian clams were selected as the food web base because they were primary consumers and abundant at every site, and their use as such has been validated in other research.49−51 Because there was a significant difference (p < 0.05) in the average δ15N of Asian clams among the sites, the trophic position of consumers was based on the site-specific baseline. A trophic fractionation factor of 3.4‰ was incorporated into eq 1 to calculate the trophic position:52

\[
\text{Trophic Position}_{\text{consumer}} = \left( \frac{\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}}}{3.4} \right) + 2
\]

PFAS concentrations in water, sediment, and organic matter. Ten PFASs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, and PFOS) were analyzed in water, sediment, and organic matter samples at each site. PFAS concentrations in water from both sampling events were averaged for each site. PFOA (8.07 ng/L) and PFHpA (5.79 ng/L) were the only two compounds detected in water samples above the LOQ (Table SI2). Both of these compounds occurred at the Pee Dee site in South Carolina. All 10 PFASs were below the LOQ for sediment and organic matter samples at the five sites (Table SI2).

PFAS concentrations in biota. PFPeA, PFHxA, PFOS, and PFTeA were not detected above the LOQ in biofilm samples. Concentrations in biofilm were generally high with the greatest mean concentration of 463.73 ng/g for PFOA (Table 1). Plant samples, including algae and submergent macrophytes, had only 4 of the 14 PFAS compounds detected (PFBA, PFBS, PFHpA, and PFDoA), and the greatest mean

![Figure 2. Summary of selected per- and polyfluoroalkyl substances (PFASs) among the sites in the Yadkin-Pee Dee River of North Carolina and South Carolina, US, listed in order from upstream to downstream. For each triangle, a solid black section represents the greatest measured mean concentration of a PFAS for the corresponding food web compartment among the sites.](https://dx.doi.org/10.1021/acs.est.9b05007)
concentration occurred for PFDoA (55.10 ng/g of WW; Table 1). Only five of the 14 PFASs (PFPeA, PFBS, PFPO, PFOS, and PFDoA) were detected in mollusk samples at or above the LOQ. All other compounds were detected at low concentrations that fell below the LOQ. PFAS mean concentrations in mollusk samples ranged from <LOQ to 11.71 ng/g of WW (Table 1). All 14 PFASs were detected in individual aquatic insect samples (range of <LOD to 1,670.10 ng/g of WW). PFOSA, PFDA, PFU, and PFDoA were detected in all of the individual samples. Two compounds with notably high mean concentrations (Table 1) were PFOS (132.82 ng/g of WW) and PFDoA (174.81 ng/g of WW). Consistently high concentrations occurred for PFOS in insect samples, in which 65% of detections were over 100 ng/g of WW. Individual crayfish samples exhibited detections from 6 of the 10 PFASs analyzed and had means ranging from <LOQ to 23.99 ng/g of WW (Table 1). PFHpA was the only compound detected in all crayfish samples (range of 9.85 to 51.80 ng/g of WW). All 14 PFASs were detected in fish tissues, in which mean concentrations ranged from 11.50 to 242.14 ng/g of WW (Table 1). PFOS was detected in 92% of individual aquatic insect samples (range of <LOD to 23.99 ng/g of WW), PFDA was detected in 67% (range of <LOD to 59.00 ng/g of WW), Bluegill samples showed the most PFAS detections (56%) but contained generally low concentrations among fish species. However, bluegill muscle and whitefin shiner whole body samples showed the greatest mean concentrations in PFOS, 20.42 and 37.36 ng/g of WW, respectively. The robust redhorse ova sample was analyzed for 14 PFASs, and 10 were detected (range of <LOQ to 482.88 ng/g of WW; Table 1), with the PFOS concentration being the greatest.

A visual general hazard assessment tool was developed to show the relative longitudinal exposure and contamination by highlighting the greatest mean concentrations for five selected PFASs and each environmental and food web compartment among the sites (Figure 2). For these compounds, the Red Hill site had 10 of the greatest mean concentrations among all environmental and food web compartments, with producers being consistently the greatest. The Pee Dee site (farthest downstream) followed with 8 of the greatest mean concentrations measured, with the water compartment being predominant. However, an ANOVA performed on log10-transformed PFAS concentrations failed to detect a significant difference among all sites (p < 0.05). Furthermore, there were no apparent consistent increasing longitudinal trends among the sites from upstream to downstream.

Stable Isotopes. Stable isotopes of δ13C and δ15N were measured on a total of 359 samples and a δ34S isotopic analysis was performed on a total of 224 samples (a subset of the 359 samples). δ13C, δ15N, and δ34S isotopic composition means varied among the food web compartments, species, and sites (Table S1 3). δ15N values were significantly different among the sites for each respective food web compartment (p < 0.05). The Asian clam was used as the food web base when estimating the trophic position because of their prevalence at every site and their primary consumer position. Because there was a significant difference (p < 0.0001) in the baseline mean of δ15N values among the sites (801, 8.8%e; Red Hill, 15.3%e; Digg’s Tract, 12.3%; Society Hill, 8.7%e; Pee Dee, 11.2%), site-specific food webs and trophic positions were constructed from the baseline at their respective site. Trophic positions for consumers ranged from 0.4 to 4.3 among the sites (Table S1 4).

Food Web Contamination. There were significant differences in log10-transformed PFAS concentrations among food web compartments, and Tukey’s HSD post-hoc test identified those differences (p < 0.0001) among all food web compartments (fishes, mollusks, aquatic insects, crayfishes, plants, and detritus). The variation in mean PFAS concentrations occurred among food web compartments, in which aquatic insects exhibited relatively high concentrations of PFHpA, PFOS, PFDA, PFBS, and PFDoA (Figure 3). The same method was used to compare mean PFAS concentrations among fish species, in which PFPeA, PFHpA, PFOSA, PFNA, PFDA, PFBS, PFHxS, and PFOS demonstrated a significant difference (p < 0.05). Figure 4 depicts the variation of PFASs that were detected in over 50% of samples among fish species and the lack of consistent trends among compounds. Figure 5 presents the distribution percentage of the 10 PFAS sum-total concentrations measured within each tax group, showing a discernible difference in PFAS detections among all groups. TMF values that incorporated PFAS regression slopes among food web compartments demonstrated that both water and dietary sources likely contributed to the accumulation of PFASs. PFHpA and PFOA exhibited negative slopes, whereas PFBS, PFOS, and PFDA showed positive slopes. The PFBS TMF for all consumers was greater than 1.0, indicating diet as a major route of exposure and as a potential for biomagnification (1.08; Table 2). TMFs showed wide-ranging variation when calculated for consumer groups or species. PFHpA, PFOA, PFOS, and PFDA showed TMFs less than 1.0 for all consumers but exhibited a biomagnification potential for specific taxa and fish species. For example, the calculated PFOS
TMF for all consumers was less than 1.0 but revealed the potential for biomagnification for bluegill (1.12), channel catfish (1.07), common carp (1.30), shorthead redhorse (1.19), and whitefin shiner (1.67) (Table 2).

**DISCUSSION**

**Distribution and Accumulation.** Our systematic analysis of PFASs, isotopic composition, aquatic taxa, trophic position, and sites indicated widespread contamination in the Yadkin-Pee Dee River and evidence that both diet and water likely contributed to bioaccumulation. PFASs were detected in water, sediment, organic matter, and aquatic biota, and PFOS and PFDA were the most prevalent PFASs in samples among all sites. The greatest PFAS concentrations and most enriched baseline $\delta^{15}$N values occurred at the Red Hill site, which occurs 10.7 km downstream of the mouth of the Rocky River. The Rocky River drains a large metropolitan center (City of Charlotte, Mecklenburg County), and municipal and industrial inputs and the occurrence of agricultural land within the watershed\textsuperscript{28,30,32} likely explain these findings. The producer

Figure 4. Mean per- and polyfluoroalkyl substance (PFAS) concentration and standard error for fish species in the Yadkin-Pee Dee river of North Carolina and South Carolina, US. For a given PFAS, species with different letters had significantly different concentrations, as determined by Tukey’s HSD.

Figure 5. Percentage of sum total concentration contribution for per- and polyfluoroalkyl substance (PFAS) compounds that were measured in biota above the limit of quantitation (LOQ) from the Yadkin-Pee Dee river of North Carolina and South Carolina, US.
Table 1). However, between insects and aquatic biota except for fresh source of PFAS. Aquatic insects exhibited relatively high concentrations, and the water compartment there was noticeably Carolina had the second greatest measured PFAS concentration at this site consistently had the greatest mean concentrations of the five predominant PFASs among the sites (Figure 2), which may indicate that the PFASs delivered by the Rocky River had already bioaccumulated into the lower trophic level of the food web. Interestingly, the Pee Dee site that is the farthest downstream in South Carolina had the second greatest measured PFAS concentrations, and the water compartment there was noticeably contaminated, indicating the possibility of a nearby upstream fresh source of PFAS. Aquatic insects exhibited relatively high mean concentrations of all 14 PFASs compared to other aquatic biota except for fishes (measured in 13 of 14 PFASs; Table 1). However, between insects and fishes, mean PFAS concentrations were greatest in insects for 5 of the compounds and greatest in fishes for 9 of the compounds. Although there are published aquatic life benchmarks for several of the PFASs, the physiological differences among taxa make it difficult to compare the overall hazard of exposure to specific biotic groups from the various compounds and exposure routes. However, this study revealed the accumulation potential within many taxa residing in a large lotic ecosystem. For comparative purposes, a directive of the European Parliament and the European Council established an environmental quality standard (EQS) for PFOS and its derivatives in surface waters (0.65 ng/L) and biota (9.1 ng/g) for the protection of aquatic life and overall ecosystem health. Considering their values in the absence of similar standards for these compounds in the United States, we found that all of our water samples exceeded (>LOD) the annual allowance EQS for PFOS and that an average of 61% of biotic samples exceeded their EQS for aquatic life. The biotic samples that exceeded the EQS in our study were for insects (76%) and fish (46%); crayfish and mollusks never exceeded the EQS. In contrast, a draft standard with total PFOS for the protection of aquatic ecosystems in Australia with 95% species protection has a freshwater toxicity guideline value of 130 ng/L (range of 0.23 ng/L for 99% species protection to 2000 ng/L for 90% species protection). The federal environmental quality guideline for PFOS in Canadian surface water is 6.8 μg/L (https://www.ec.gc.ca/ese-ees/38E6993C-76AA-4486-BAE8-D3828B430A6E/PFOS_En.pdf, accessed 03/11/2020).

### Food Web Exposure.

Our results demonstrated variable PFAS accumulations among food web compartments and fish species. Detritus was the only compartment with no PFAS detections. Plant samples exhibited the lowest frequency of PFAS detections (16%). Biofilm, an aggregation of bacteria, fungi, algae, and protozoans and a basal resource for the aquatic food web, showed high PFAS accumulation (in 10 of 14 compounds), particularly for PFOA. Aquatic insects exhibited the greatest accumulation of PFASs relative to other taxa, as in other studies. Our findings may suggest a trophic link between biofilm PFAS and aquatic insect PFAS. Moreover, as shown in MacDonald et al., certain aquatic insects like *Chironomus tentans* may be quite sensitive to some PFAS compounds, with adverse effects of PFOX upon emergence occurring at <2.3 μg/L. Benthic invertebrates are important diet sources for aquatic organisms and are likely to transfer contaminants to their consumers through trophic pathways. The TMFs calculated in this study (Table 2) showed that various taxa accumulated PFAS compounds differently. Although there can be variability in TMFs related to the characteristics of ecosystems, the biology and ecology of organisms, the experimental design and timing of sampling, and the statistical methods used, our TMFs for specific PFAS compounds, organisms, and compartments are relatively similar to those in the recently published literature. Interestingly, PFBS, which is one of the short-chained PFAS compounds that we studied and would presumably exhibit lesser TMFs, had nine values among our compartments and organisms >1.0 (range of 0.57 to 2.33; Table 2). It is possible that an unmeasured PFBS precursor is accumulating in biota and metabolizing to PFBS, leading to a higher than expected TMF. Recent studies have demonstrated the precursor FBSA in lake trout (*Salvelinus namaycush*) and other PFBS precursors in water. Fishes exhibited dissimilar detections of PFASs among species. This could be a result from differences in size, age, physiology, and feeding strategy of the species or individual. The bioaccumulation of PFASs in consumers is likely a combination of diet and water exposure.

### Robust Redhorse Implications.

The robust redhorse population in the Yadkin-Pee Dee River is extremely rare and imperiled. Consequently, direct sampling for contaminants in adults or juveniles of this species was not feasible, but one sample of robust redhorse ova that was serendipitously collected during sampling associated with related research was analyzed for the 14 PFASs and compared to the closely related notchlip redhorse, a potential surrogate species based on its similar taxonomy and food habits. This comparison facilitated inferences about robust redhorse exposure based on PFAS concentrations in water, notchlip redhorse muscle tissue, and robust redhorse ova and diet sources. All 14 PFASs except PFPeA and PFHxS were detected in notchlip redhorse tissue samples >LOQ. Asian clams and aquatic insects are known primary diet sources of robust redhorse, and aquatic insects exhibited high concentrations of PFASs whereas Asian clams showed much lower concentrations. Our findings provide the plausibility that PFASs are accumulating in robust redhorse tissues and organs (as directly indicated by the results from the single ova sample) and that their exposure likely comes from their diet of aquatic insects, which showed the greatest contamination of PFASs. The ova sample results also indicate that the maternal transfer of PFASs may occur. The maternal

<table>
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*a* number of samples analyzed. 
*On the basis of whole-body concentrations.
transfer of PFOS and potential reproductive effects in fishes have been documented in other studies.26,27,62−64 Due to the high concentrations of PFASs in robust redhorse ova, adverse effects from these compounds have the potential to affect early life stages and overall fecundity. Sharpe et al.27 estimated that 10% of the adult PFOS body burden transferred to ova and reduced fecundity in zebra fish (Danio rerio). Wang et al.63 observed impaired embryonic development and larval survival from maternally transferred PFOS. Another study showed decreased fecundity and altered sex ratios in fathead minnow (Pimephales promelas) that would alter and delay ova development. Such reproductive effects of PFASs and potentially other contaminants present in the system26,27,62 may be a concern for the already low population size and imperilment of the robust redhorse in the Yadkin-Pee Dee River. Moreover, the Red Hill site that is considered a potential reintroduction site to stock hatchery-propagated robust redhorse for species restoration had 10 of the greatest mean measured PFAS concentrations among all environmental and food web compartments in our study. Thus, additional study is warranted on this imperiled fish, its habitat, and its life history to aid in conservation and management.

No previous study has analyzed such a wide variety of food web samples for PFASs from a lotic ecosystem like the Yadkin-Pee Dee River, and very few have investigated their transfer through freshwater riverine aquatic food webs. Our results demonstrate the prevalence of PFASs in the environment and biota of the river. The observed contamination of PFASs in all compartments of the food web confirms the importance of examining routes of exposure to better understand contaminant dynamics in freshwater lotic systems. Our results also showed the potential of certain PFASs to biomagnify in the food web.

Our findings provide essential information that was previously lacking for fish and other biota to inform conservation and public health decisions and actions. The tendency of PFOS to maternally transfer to ova causes concern for the implications of reproductive health for imperiled native fish species, including the robust redhorse in this system. The presence of persistent and bioaccumulative chemical compounds can affect the success of recovery efforts for habitat and imperiled species populations. Further toxicological testing of PFASs is crucial for better understanding the risks to aquatic organisms and overall ecological health.

**ASSOCIATED CONTENT**

*Supporting Information* The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.9b05007.

Analytical methods, GPS coordinates of study sites, PFAS concentrations in water and sediment, mean stable isotope ratios in aquatic food web biota, and mean trophic position for fishes (PDF)

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This research was funded by the North Carolina Wildlife Resources Commission and South Carolina Department of Natural Resources through a competitive state wildlife grant (NC-U2-F14AP00075). We thank James Wehbie, Spencer Gardner, Bobby Cope, and Seth Newton for field and laboratory support. Bryn Tracy and Victor Holland of the North Carolina Department of Environmental Quality provided assistance with identification of specimens. David Buchwalter, Andrew Gillespie, Amanda Jarvis, James McCord, Owen McDonough, Joachim Pleil, Leanne Stahl, and three anonymous reviewers provided constructive comments on an earlier draft of the manuscript. The North Carolina Cooperative Fish and Wildlife Research Unit is jointly supported by North Carolina State University, North Carolina Wildlife Resources Commission, US Geological Survey, US Fish and Wildlife Service, and Wildlife Management Institute. The views expressed in this article are those of the author(s) and do not necessarily represent the views or policies of the US Environmental Protection Agency. Any use of trade, firm, or product names is for descriptive purposes only and does not imply an endorsement by the US Government.

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