ABSTRACT

PENLAND, TIFFANY NICOLE. Food Web Contaminant Dynamics of a Large Atlantic Regulated River: Implications for Common and Imperiled Species. (Under the direction of Dr. Thomas J. Kwak and Dr. W. Gregory Cope).

Organic and inorganic contaminants pose threats to aquatic life in the form of over 100,000 chemicals that are released into the environment from point and non-point sources, transported through aquatic systems, and transformed by chemical and biological reactions. Aquatic organisms have the potential to accumulate contaminants from their environment to levels that exceed adverse effect thresholds. Contaminants are known to cause alterations in organism behavior and development, disruption of biological processes, reproductive abnormalities, and mortality. Water pollution also threatens the ecological integrity of aquatic ecosystems by direct toxicity to organisms and disruption of ecosystem processes.

The objectives of this research were to determine the aquatic food web structure and trophic transfer and accumulation of contaminants within a riverine food web, as well as identifying potential stressors to the health of an imperiled fish, the Robust Redhorse (*Moxostoma robustum*) and other species of conservation concern. We conducted intensive sampling of basal resources and consumers at five riverine sites along the Yadkin-Pee Dee River of North Carolina and South Carolina. Major food web components were determined by stable isotope analyses of representative producers, consumers, and organic matter. Contaminant analyses were performed on water, sediment, organic matter, and aquatic biota to assess the prevalence and accumulation potential of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), current use pesticides (CUPs), polycyclic aromatic hydrocarbons (PAHs), metals, and perfluoroalkyl acids (PFAAs).

We discovered the persistence of numerous legacy organic contaminants in the environment and food web compartments of the Yadkin-Pee Dee River. These contaminants, although relatively low in most concentrations, may pose a concern for ecological health in mixtures. PCBs were detected in 32% of biotic samples (mean 0.24 µg/g dry weight [DW], range 0.01 – 3.33 µg/g DW) and DDTs (legacy OCPs and metabolites) were detected in 90% (mean 0.014 µg/g DW, range 0.0004 – 0.29 µg/g DW). Robust Redhorse ova contaminant concentrations generally reflected mean concentrations in fish tissue; PCBs and OCPs were detected in the maternal gametes from this imperiled fish. Bioaccumulation factors (BAFs; range, 3,870 – 64,519) and trophic magnification factors (TMFs; range 0.44 – 3.75) for fish indicated that contaminant accumulation occurred from both water and dietary sources.

Analyses of essential and nonessential metals in biotic and abiotic samples revealed that metals were present in sediment and all food web compartments at various concentrations. Manganese and cadmium exceeded published threshold effect concentrations in sediment (460 and 0.99 µg/g DW, respectively). Mercury was detected in all food web samples analyzed (mean 0.13 µg/g wet weight [WW], range 0.001 – 0.6 µg/g WW). Concentrations exceeded the 0.2 µg/g WW aquatic life criteria for mercury in 38% of fish samples.

PFAAs were prevalent in all food web component samples and had the most detections and greatest concentrations in aquatic insects. All 14 PFAAs were detected in aquatic insect samples (range, below detection limit [BDL] – 1,670.1 ng/g WW) and fish tissues (range, BDL – 798 ng/g WW). Perfluorooctane sulfonate (PFOS) was the dominant PFAA among all samples (67%). Robust Redhorse ova had concentrations above detection limits for 10 PFAAs (range, BDL – 482.88 ng/g WW). Generally, the ova sample showed higher concentrations compared to fish muscle tissue. PFOS concentration in the ova sample was

exceptionally high (482.88 ng/g WW) and indicated that maternal transfer of PFAAs is likely occurring.

The important finding that both diet and water are important routes of exposure for aquatic organisms is illustrated by the contamination in all compartments of the food web in this riverine ecosystem. Our results show which basal resources support consumers and how contaminants accumulate and transfer through the riverine food web, potentially threatening the health of rare or intolerant species and ecosystem processes.

Food Web Contaminant Dynamics of a Large Atlantic Regulated River: Implications for Common and Imperiled Species

by
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DEDICATION

For my family and the individuals that encouraged me to set my goals high and face my fears.

BIOGRAPHY

I was born and raised in Georgia. I grew up with a love for the outdoors, where I spent the majority of my time running around the woods, climbing trees, playing with pets, and looking under rocks in creeks. I knew from a young age that I would pursue life sciences in school. I later attended Kennesaw State University in Kennesaw, Georgia, for my undergraduate degree in biology. This is where I met Dr. Bill Ensign. He expected more than just the average effort and taught the only aquatic courses. Dr. Ensign inspired me with his love and dedication to the field of fisheries and encouraged me to realize my potential and overcome my anxieties. This led me to North Carolina, where I landed a job with the North Carolina Wildlife Resources Commission as a fisheries technician with a great group of people. I lived in the middle of nowhere by myself and loved the adventure and independence. My time as a technician is an experience I will never forget.

After my stint as a technician, I was lucky enough to stumble into a Master's position at North Carolina State University. I was fortunate to have a great group of people in my lab and two wonderful advisors. There were definitely stressful moments (and still more to come), but I wouldn't change my time at NCSU for anything.

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CHAPTER 1

Organic Contaminant Trophodynamics of a Large Regulated River Food Web: Implications for Common and Imperiled Species

Abstract

Persistent and bioaccumulative contaminants often reach concentrations that adversely impact aquatic life and consumers. The objectives of this research were to determine the aquatic food web structure and trophic transfer and accumulation of contaminants within a riverine food web, as well as identifying potential stressors to the health of the Robust Redhorse (*Moxostoma robustum*). The Robust Redhorse is a large catostomid in critical need of conservation and recovery efforts. The current estimated spawning population is fewer than 100 individuals in the Yadkin-Pee Dee River. We conducted intensive sampling at five lotic sites along the Yadkin-Pee Dee River of North Carolina and South Carolina. Sampling sites spanned a range of diverse physical characteristics, land uses, and influx of point- and nonpoint-source pollution that facilitated longitudinal examination. Major food web components were determined by stable isotope analyses of representative producers, consumers, and organic matter. Contaminant analyses performed on biotic and abiotic samples revealed that organic contaminants were prevalent, including several of ecological and human health concern. Polychlorinated biphenyls (PCBs) were detected in 32% of biotic samples (mean 0.24 µg/g dry weight [DW], range 0.01 – 3.33 µg/g DW), and DDTs (legacy organochlorine pesticides and metabolites) were detected in 90% (mean 0.014 µg/g DW, range 0.0004 – 0.29 µg/g DW). Robust Redhorse ova

contaminant concentrations reflected the mean concentrations in fish tissue; PCBs and 15 of the 20 organochlorine pesticides were detected in the maternal gametes from this imperiled fish. Bioaccumulation factors (BAFs; range, 3,870 – 64,519) and trophic magnification factors (TMFs; range, 0.44 – 3.75) for fish samples indicated that contaminant accumulation occurred from both water and dietary sources. Our results identified basal resources that support consumers and contaminant pathways and accumulation through the riverine food web, potentially threatening the health of fish and other biota.

Introduction

A holistic understanding of ecological processes in lotic systems is critical for conservation and recovery efforts of imperiled aquatic species. Research on the effects of water quality on aquatic biota and producer and consumer relationships in aquatic systems is increasing, but remains limited (Walters et al. 2008). Because most aquatic species are restricted to conditions in their in-water environments, there is growing concern regarding the vulnerability of freshwater organisms to detrimental effects from the exposure and accumulation of persistent organic chemicals in freshwater systems (Daughton and Ternes 1999; Nilsen et al. 2014). Toxic chemicals and excess nutrients emanate from many point and nonpoint sources including agricultural field crops and animal production runoff, wastewater treatment plant effluents, industrial discharge, and storm water runoff. Widespread agricultural use of pesticides and fertilizers can result in chemical contamination of water systems due to compounds leaching from the soil or from runoff caused by precipitation (Cope 2010; Nikinmaa 2014). A nation-wide study that assessed 139 stream sites downstream of highly urbanized areas and locations of confined animal feeding operations reported that 80% of streams contained up to 38 organic wastewater contaminants within a given water sample (Kolpin et al. 2002). Previous studies have also documented detrimental effects on aquatic fauna from toxicants in freshwater ecosystems, such as increased susceptibility to disease and parasites and altered biological responses (Bringolf et al. 2007; Hinck et al. 2009; Nilsen et al. 2014). It is important to know the persistence of chemicals in freshwater systems. Some are broken down in the environment or metabolized by aquatic organisms (Nilsen et al. 2014), whereas other chemicals have been shown to

persist in water, sediment, and fish for years after being banned for use (NCDWQ 2012). Some persistent contaminants accumulate and sequester in lipid rich tissues resulting in high concentrations until those lipid stores are needed for biological processes (e.g., reproduction) causing delayed toxicity (LeBlanc and Buchwalter 2010). For example, legacy organochlorine pesticides (OCPs) such as polychlorinated biphenyls (PCBs) are some of the most hazardous organic compounds, because they are chemically stable and withstand degradation and biotransformation (Cope 2010). This study was conducted on the Yadkin-Pee Dee River in North Carolina and South Carolina, because it is a river system with notable biodiversity, but of management concern due to known water quality issues (Hinck et al. 2009; NCDWQ 2012; Fisk et al. 2014).

The Yadkin-Pee Dee River basin is a freshwater resource that provides habitat for over 50 priority aquatic species that are currently in need of conservation and management action (NCWRC 2005; SCDNR 2005). The headwaters rise in Caldwell County, North Carolina, as the Yadkin River and transitions to the Pee Dee River after the confluence of the Uwharrie and Yadkin Rivers. The river basin covers 18,702 km^2 in North Carolina making it the second largest basin in the state (NCDWQ 2009). The Pee Dee River continues through South Carolina draining one-quarter of that state before flowing into the Atlantic Ocean. The land use within the basin consists of about 32% forested upland, 23% agricultural land, and 10% developed area (MRLC 2014). River basins with a high percentage of agricultural land cover and municipal and industrial areas have greater inputs of contaminants into surface waters (Schwarzbauer 2006). There are numerous factors altering community dynamics, key species population densities, and habitat availability in the Yadkin-Pee Dee River.

Documented issues in the basin include the establishment of invasive species, habitat fragmentation and alteration due to dams, impoundments, and other anthropogenic activities such as permitted industrial discharges, and excessive nutrient, sediment, and chemical input (NCWRC 2005; Fisk et al. 2014). The Yadkin-Pee Dee River receives inputs from 1,468 permitted discharges and 776 confined animal feeding operation sites (SCDHEC 2001a, 2001b; NCDWQ 2009). The basin contains over 6,100 river kilometers that are listed as impaired, based on standards for biological integrity, turbidity, low dissolved oxygen, and fecal coliform bacteria; however, there are over 50 streams within the basin that are classified as Outstanding Resource Waters (NCDWQ 2009; SCDHEC 2015). A North Carolina statewide assessment of contaminant concentrations in fish tissue revealed that fishes sampled in the Yadkin-Pee Dee River contained concentrations of pesticides and PCBs that exceeded USEPA screening values (NCDWQ 2012). Improvements in water quality may be important to improve the conservation of fishes, mussels, and crayfishes that are currently designated as species of concern and to prevent other aquatic species from becoming imperiled. One the 53 priority species that inhabit the Yadkin-Pee Dee River, the Robust Redhorse (*Moxostoma robustum*) is a fish in critical need of conservation efforts. This species is ranked as significantly rare in North Carolina and is listed as highest priority for conservation in North Carolina and South Carolina (NCWRC 2005; SCDNR 2005).

The Robust Redhorse is the largest member of the redhorse genus *Moxostoma* and was first described from the Yadkin-Pee Dee River in 1869 by Edward Cope (1870). This large catostomid went unobserved or misidentified for over a century after its original discovery. It was not until the early 1990s, when a population was rediscovered in Georgia,

that the Robust Redhorse became a focus of the scientific community because of the species' limited distribution range and declining populations (Bryant et al. 1996; Fisk et al. 2014). Adult Robust Redhorse occupy the main stem river and migrate upstream to spawn on shallow shoals and riffles with gravel substrate (Fisk et al. 2014). They use molar-like pharyngeal teeth to crush mollusks and other macroinvertebrates as part of their diet. It is anticipated that the results of this study will reveal how their presumed food sources and any associated chemical compounds persist and interact among organisms of different trophic levels in the river. These results would indicate if diet composition is a potential limiting factor for the health and distribution of the Robust Redhorse and other aquatic consumers in the ecosystem.

Understanding community structure and dynamics within an ecosystem requires insight into food web ecology. Components of the food web provide valuable information about energy flow within a community, predator-prey interactions, and interspecific and intraspecific competition (Winemiller and Polis 1996; Morin 2011). An issue that threatens ecological health is the biomagnification of persistent contaminants within the food web, because diet is a major exposure route for many contaminants (Jardine et al. 2006). Biomagnification of contaminants increases as the food web becomes more complex (Cabana and Rasmussen 1994; Jardine et al. 2006; Walters et al. 2008). The combined use of stable isotope analysis (SIA) and environmental chemistry and toxicology analysis elucidates the transport and fate of contaminants through trophic pathways.

Stable isotope ratios are useful in ecology and environmental science for assessing diet and trophic condition and pathways. Carbon, nitrogen, and sulfur isotopes are all

involved in the cycling of organic matter and often used for food web studies (Fry 2006). The application of stable isotope analysis in food web studies is useful because different food sources have unique isotopic signatures, and the isotopic composition of consumers is proportional to the sources in their diet (Jardine et al. 2006). Carbon ($\delta^{13}C$) values are used to reveal food sources, nitrogen ($\delta^{15}N$) values are used to estimate trophic position of consumers and dietary exposure to contaminants, and sulfur ($\delta^{34}S$) values are used to determine large-scale movement patterns by differentiating between marine inputs and freshwater sources (Fry 1991). Stable isotopes also detect food web responses to environmental and anthropogenic influences (Michener and Lajtha 2008). An advantage of SIA is that it represents a time-integrated sample of diet rather than a static measure of recently ingested food (Jardine et al. 2006; Layman et al. 2012; Nithirojpakdee et al. 2014). Although there is seasonal variation within a consumer's diet, the isotopic composition of consumers exhibits low levels of inter-annual variability (Delong et al. 2001; Herwig et al. 2007; Hladyz et al. 2012). Because different tissues of vertebrates have various turnover rates, SIA can minimize seasonal variations of diet with the selection of an appropriate tissue (Perga and Gerdeaux, 2005; Layman et al. 2012). This means that tissues with slower turnover rates capture a wider range of diet over time. The white muscle tissue of fish, for example, is most widely used in food web studies, owing to its low lipid content, intermediate turnover rates, ease of homogenization, and obtainability (Jardine et al. 2005; Herwig et al. 2006; Layman et al. 2012). This study utilized the white muscle tissue of fishes for all analyses.

This study was conducted because of the documented impairment of the Yadkin-Pee Dee River and the need for conservation efforts for the Robust Redhorse population. The objectives of this research were to (1) utilize stable isotope ratios to determine the food web structure and linkages by sampling a wide variety of organic matter and aquatic biota, (2) analyze water, sediment, organic matter, and biota for organic contaminants, (3) determine contamination trends among sites along a longitudinal gradient, and (4) assess the susceptibility of consumers to bioaccumulation and biomagnification by examining the chemical concentrations within each trophic level of the food web. The overall goal of this research was to understand trophic pathways of contaminants within the riverine food web and determine potential stressors to imperiled species and overall ecological health of the river ecosystem.

Methods

Study sites

Five sites with varying watershed landscapes and anthropogenic influences were selected along the Yadkin-Pee Dee River of North Carolina and South Carolina (Figure 1, Table SI 1). The study sites span a range of physical characteristics, land uses, and influx of point and nonpoint source pollution that facilitated longitudinal examination of trophic and contaminant dynamics. Examples of non-point sources are the developed (10%) and agricultural (23%) land use coverage within the basin (Figure 2a). Point sources within the basin include over 1,400 permitted discharge facilities under the National Pollutant Discharge Elimination System (NPDES), 776 concentrated animal feeding operations, and effluent from industrial facilities (Figure 2b). The sites were also chosen to explore possible

negative effects of contaminants on the Robust Redhorse population of the river by comparing their food sources and availability. To aid in these biotic comparisons, sites included a location near where the Robust Redhorse was first described but no longer exists (site 801), where the Robust Redhorse population currently exists (Digg's Tract, Society Hill, and Pee Dee), spawns (Digg's Tract), and a potential population restoration site to reintroduce hatchery-reared Robust Redhorse (Red Hill).

Sample collection and preparation

Water and sediment samples were collected during summer 2014, and aquatic biota and organic matter were collected during spring and summer 2015. When feasible, taxa collected for trophic analyses were the same among sites. Water, sediment, organic matter, and aquatic biota samples were analyzed for selected traditional and emerging organic contaminants at each site. Biota samples consisted of fishes (10 species), mollusks (4 families), crayfishes (2 species), aquatic insects (12 families), macrophytes (5 species), and detritus from each site and analyzed for stable isotopes to determine components of the food web, trophic pathways, and bioaccumulation of contaminants. Collection and processing methods were similar to those of Hoeinghaus et al. (2007) and Pingram et al. (2014).

Water: Water quality characteristics were measured during each sampling event. Measurements included temperature (°C), conductivity (µS/cm), dissolved oxygen (mg/L), salinity (ppt), and pH, and were made by a calibrated Yellow Springs Instruments (YSI, Yellow Springs, Ohio) 556 multi-probe meter.

Passive sampling devices (PSDs) were deployed in the river at each site for 28 days to assess time-integrated water quality. PSDs accumulate the freely dissolved concentrations

of a broad range of priority pollutant organic compounds by passive drift (Vrana et al. 2005). Passively sampling the water column over time mimics the bioconcentration of hydrophobic organic contaminants by aquatic organisms from the aqueous phase contingent on the concentrations available (Heltsley et al. 2005). The PSDs used in this study consisted of a polyethylene strip (Hofelt 1998) that absorbs non-polar compounds (e.g., polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides) and three porous cartridges (Hirons 2009) that sample both polar and non-polar compounds, including current use pesticides (CUPs) which are pesticides currently registered for production and use (e.g., atrazine).

Actions were taken to avoid adventitious contamination of assembled PSDs before and during deployment and retrieval. Gloves were worn to deploy devices in water depths that allowed them to be continuously submerged in the water without resting on the river bottom by attaching a float and weight. PSDs were placed away from known sources of pollution input such as boat ramps and drainage pipes to avoid artificial increases in contaminant concentrations. After 28 days, PSDs were retrieved using gloves to remove and place cartridges and the polyethylene strip into baked aluminum foil. The foil and PSD components were placed into food grade sealable, plastic bags and held on ice until they were transferred to a freezer (-20° C) for storage.

Sediment and organic matter: Composite sediment samples from each site were collected from depositional areas and analyzed for organic contaminants. Sediment and organic matter samples consisted of multiple grabs that were collected from the top 3-5 cm of the substrate surface layer by a stainless steel scoop. Samples were typically 225-250 g and

any visible biota or debris were removed. Sediment samples were placed into labeled, sterile amber glass jars and held on ice until transported to a -20° C freezer to await processing and analysis. Detritus samples included leaf packs and suspended particulate organic matter. Leaf packs were collected by hand or with dip nets, placed into sealable plastic bags, and held on ice after visible debris and invertebrates were removed. Suspended particulate organic matter (i.e., drift) samples were collected with 500-µm mesh drift nets. Drift samples were rinsed to remove invertebrates, placed into sealable plastic bags or amber glass jars, and held on ice. Aquatic macrophytes were collected by hand, thoroughly rinsed to remove organic matter and invertebrates, and placed into plastic bags and held on ice.

Aquatic biota: Aquatic insects and crustaceans were collected with 500-µm D-frame nets, by flipping rocks, or by hand from leaf packs and woody debris. Specimens were stored in containers with filtered site water and chilled for at least 8 h to enable depuration of gut contents. Aquatic insects were sorted and classified into functional feeding guilds: collector-filterer, shredder, scraper, or predator. Insects were identified to a minimum of family taxonomic level. Mollusks were collected by hand and included native freshwater mussels (family Unionide), snails (families Pleuroceridae and Viviparidae), and clams (*Corbicula fluminea*). Snails were held in a container of filtered site water for at least 8 h to enable depuration of gut contents. All mollusks were identified to species.

Fishes were collected by boat-mounted, pulsed-DC electrofishing with efforts to retain similar size classes of the same species among sites. Fishes were euthanized by immediate placement into an ice-water slurry to induce temperature shock according to North Carolina State University approved protocols (IACUC 15-042-O). Species, total length

(mm), and wet weight (g) were recorded for all fishes. 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 bubalus*), and Whitefin Shiner (*Cyprinella nivea*) were collected to represent the variety of trophic guilds found in the Yadkin-Pee Dee River. All samples were stored frozen at -20° C until further processing.

Stable isotope analysis

All sample processing methods were performed with sterile, stainless steel utensils, and all surfaces were cleaned with lab detergent, a distilled water rinse, an acetone rinse, and another distilled water rinse between samples to avoid contamination. All samples were dried at 60° C to a constant weight in a drying oven, ground to a fine powder with a mortar and pestle, and then placed into 7-mL glass vials for storage. Processed samples were transported by overnight courier to the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Flagstaff, for analysis of carbon, nitrogen, and sulfur isotope ratios. Samples were weighed, encapsulated in tin, and then analyzed with a gas isotope-ratio mass spectrometer using their approved standard methods.

Stable isotope ratio results were expressed as delta (δ) notation in parts per thousand (‰) relative to standards according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1{,}000, \qquad (1)$$

where X is ^{13}C, ^{15}N, or ^{34}S, and R is the corresponding heavy isotope to light isotope ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$). The standard materials are Vienna Pee Dee Belemnite

limestone for carbon, atmospheric nitrogen for nitrogen, and Canyon Diablo Troilite for sulfur. Samples were not lipid normalized due to the low lipid content of animals and %C content of plants (Skinner et al. 2016). Most food sources have distinct $\delta^{13}C$ signatures that are conserved within 1‰ in consumer tissues, which makes it possible to discern sources that contribute to the consumer's diet (Finlay et al. 2002; Hoeinghaus et al. 2007).

Data were normalized using International Atomic Energy Agency (IAEA) isotope calibration standards (IAEA CH6, CH7, N1, and N2). The difference between the expected and observed isotope ratio for these standards was 0.08‰ and below for $\delta^{13}C$ and 0.11‰ and below for $\delta^{15}N$. National Institute of Standards and Technology (NIST) approved standards were used to assess drift and linearity. The precision of these standards was ±0.06‰ for $\delta^{13}C$ and $\delta^{15}N$.

Contaminant analysis

Water: PSD components were processed at the North Carolina State University, Department of Environmental and Molecular Toxicology, Chemical Exposure Assessment Laboratory. Any biofouling was removed from the polyethylene strip by gently wiping with gloved hands. Target analyte extracts (PAHs, PCBs, and OCPs) were isolated from samplers with dichloromethane (DCM), filtered, concentrated, and then analyzed using a gas chromatograph coupled with a mass selective detector. Detailed description of PSD extraction and analysis methods are described by O'Neal (2014). See Table 1 for the complete list of target analytes.

Sediment: Sediment was analyzed for PAHs, PCBs, and pesticides at the North Carolina State University, Department of Environmental and Molecular Toxicology,

Chemical Exposure Assessment Laboratory. After samples were thawed, excess water was decanted and sediment was stirred until homogenized. About 30 g of sediment was added to a glass fiber thimble with a drying agent (anhydrous Na_2SO_4), spiked with appropriate surrogate internal standards, transferred to a Soxhlet apparatus, and then extracted with acetone and DCM. The extract was concentrated by using a gentle stream of nitrogen. Clean-up methods followed EPA Method 3610B (EPA 1996). The extract was spiked with recovery internal standards and analyzed with gas chromatography with electron capture detector (GC/ECD) and gas chromatography-mass spectrometry (GC/MS). The following quality control measures were used: Matrix spikes, matrix spike duplicates, and blank spikes.

Biota and organic matter: A diverse subset of samples from all sites were selected for organic contaminant analyses. Two random, replicate samples for each representative taxon were analyzed from each site. Larger individuals were analyzed individually, whereas smaller individuals were combined to form composite samples. If composite samples were necessary, two or more individuals were combined from a particular site and consisted of the same species with similar sizes to minimize differences between age classes or life stage.

All sample processing methods were performed with sterile, stainless steel utensils, and all surfaces were cleaned between samples to avoid contamination. Contaminant analysis was conducted on white muscle tissue of fishes. Tissue samples were free of scales, skin, and bone. Whitefin Shiner samples were the only fish species to be analyzed whole. Standard fish processing protocols were used when excising muscle tissue (USEPA 2000). Crayfish exoskeletons and mollusk shells were removed. All other samples were processed whole. Samples were homogenized using a Grindomix© (Glen Mills Inc., GM200, Clifton, New

Jersey) which was triple rinsed with acetone, hexane, and DCM between each sample to avoid contamination. Wet weight of tissue was recorded (minimum of 40-50 g) then placed in sterile, amber jars and stored frozen at -80° C until ready for transport to an offsite laboratory for analysis. Samples for organic contaminants were analyzed by CompuChem Laboratories in Cary, North Carolina, and by Shealy Environmental Services, Inc. in Columbia, South Carolina, with Environmental Protection Agency (EPA) approved standard methods. All samples were prepared by Soxhlet extraction (EPA Method 3540C) and clean-up steps (EPA Methods 3660B/3665A), if necessary (USEPA 2000). PAHs were analyzed using EPA Method 8270D, a technique using GS/MS (USEPA 2000). PCBs were analyzed using EPA Method 8082A, and organochlorine pesticides were analyzed by EPA Method 8081B; both methods use a gas chromatograph equipped with an electron capture detector or electrolytic conductivity detector (USEPA 2000). Contaminant concentration (µg/kg dry weight [DW]), % lipids, and % moisture were reported for each sample. Wet weight (WW) of contaminant concentrations was calculated for comparison to published thresholds using the following equation:

$$WW = DW\left[1 - \left(\frac{\%\, moisture}{100}\right)\right]. \qquad (2)$$

Contaminant concentrations were compared to aquatic life criteria and thresholds to determine exceedances and assess the potential threat to the biota of the Yadkin-Pee Dee River ecosystem.

Quality control and assurance: A rigorous quality assurance protocol was followed with each batch of samples analyzed and consisted of procedural blanks to detect contamination, sample duplicates to assess precision, spiked samples, laboratory control

samples (LCS), and surrogate samples to measure percent recovery, and instrument calibration to ensure consistency. Background contamination was not observed in procedural blanks. Percent recoveries for all surrogate, LCS, and sample spikes fell within certified recovery limits. Relative percent difference of duplicate samples for all analyses fell below the accepted 40%.

Data analysis

Mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopic composition of taxa was compared by analysis of variance (ANOVA) to quantify differences among trophic levels and sites (Kwak and Zedler 1997; Hoeinghaus et al. 2007). $\delta^{15}N$ values were used to determine the trophic position of consumers within the food web. Asian Clams (*Corbicula fluminea*) were selected as the food web base, because they were primary consumers and abundant at every site. Because there was a significant difference in the average $\delta^{15}N$ of Asian Clams among sites, trophic position of consumers was based on the site-specific baseline. Using a trophic fractionation factor of 3.4‰, equation 3 was used to calculate trophic position (Anderson and Cabana 2007):

$$Trophic\ Position_{consumer} = \left(\frac{\delta^{15}\mathrm{N}_{consumer} - \delta^{15}\mathrm{N}_{baseline}}{3.4}\right) + 2. \qquad (3)$$

Contaminant concentrations were $\log_{10}$ transformed to normalize the data. ANOVA was performed on contaminant concentrations to determine if there were statistically significant differences within and among sites, compartments, and taxa. Tukey's HSD post-hoc test was performed after ANOVA indicated a significant difference ($p < 0.05$) to determine where differences occurred. Correlations were performed on $\log_{10}$ transformed contaminant concentrations and independent variables (e.g., lipid content, $\delta^{15}N$, and land use) to examine inherent relationships.

A bioaccumulation factor (BAF) was calculated for fishes as

$$\mathrm{BAF} = \frac{C_B}{C_W}, \qquad (4)$$

where C_B is the concentration of the contaminant in an organism, and C_W is the concentration in the water. If a contaminant did not show a site effect, the BAF was based on overall mean concentrations. A trophic magnification factor (TMF) was calculated for fishes as

$$Log[chemical_{LW}] = a + b(TP), \qquad (5)$$

$$\mathrm{TMF} = 10^b. \qquad (6)$$

The slope (b) from the $\log_{10}$-linear regression of the lipid-corrected chemical concentration ($chemical_{LW}$) versus trophic position (TP) was used to calculate the TMF. A TMF > 1 indicates that contaminants are biomagnified.

Results

Contaminant concentrations in water and sediment

Contaminant analysis of PSDs revealed that PAHs were present in water at all five sites, and 14 of the 20 PAHs analyzed were detected (Table 2). Total PAH concentrations were calculated for each site and ranged from 31.5 to 73.0 ng/L. OCPs were present at all sites and 5 of the 20 analytes were detected. Individual OCP concentrations ranged from 0.15 to 4.04 ng/L. Atrazine was the only CUP detected in water (range, 25.42 – 126.74 ng/L) and was present at all sites. PCBs were detected in water at all sites, and total PCB concentrations ranged from 0.35 to 7.31 ng/L. All sites were below the 14 ng/L chronic exposure threshold for freshwater aquatic life for total PCBs (USEPA 2016).

PAHs were present in sediment at all sites, and all 20 PAHs analyzed were detected (Table 2). Total PAH concentrations for sites ranged from 68.7 to 2,090.9 ng/g DW. Site 801

exceeded the threshold effect concentration of 1,610 ng/g DW (MacDonald 2000). Cis-chlordane, gamma-BHC, and 4,4'-DDE were the only OCPs detected in sediment. 4,4'-DDE was present at all sites with concentrations ranging from 0.49 to 2.81 ng/g DW but did not exceed the threshold effect concentration of 3.16 ng/g DW (MacDonald 2000). The only CUP detected was atrazine (3.8 ng/g DW) at the Red Hill site. Total organic carbon (TOC) was analyzed in sediment samples at each site. The TOC ranged from 0.66% (Pee Dee site) to 3.71% (Digg's Tract site).

Contaminant concentrations in biota

A total of 93 fish samples, consisting of 11 species, were analyzed for organic contaminants, with variable % moisture, % lipid, and contaminant concentrations among sites and species (Table 3). Lipid content for fishes ranged from below detection limit (BDL) to 11% for American Eel, Whitefin Shiner, and Smallmouth Buffalo, each possessing the greatest lipid content. OCPs were present at all sites, and all 20 analytes were detected. Only 9% of fish samples did not have OCP concentrations above detection limits. The three most prevalent analytes detected in fish samples were 4,4'-DDE (84%; range, BDL – 0.22 µg/g DW), trans-chlordane (75%; range, BDL – 0.05 µg/g DW), and dieldrin (69%; range, BDL – 0.05 µg/g DW. The detections of contaminants in fish samples ranged from 0% to 100% (Figure 3). The most contaminated fish species were Smallmouth Buffalo, Common Carp, and Whitefin Shiner, in which 100%, 95%, and 90% of the 20 OCPs analyzed were detected, respectively. Notchlip samples also showed 90% detections for OCPs, but contaminant concentrations were generally low and almost exclusively occurred at the Digg's Tract site. The species with the least amount of chlorinated pesticide detections were Channel Catfish

(25%), Largemouth Bass (60%), and Bluegill (60%). PCBs were detected in 38% of all fish samples analyzed. Concentrations ranged from BDL to 0.79 µg/g DW (BDL – 0.2 µg/g WW). Analyses revealed that PCBs were present in 100% of Smallmouth Buffalo and Whitefin Shiner samples. Six fish samples exceeded the published wildlife guideline concentration of 0.1 µg/g WW (NPS 2007) at the lower three sites (Digg's Tract, Society Hill, and Pee Dee). A sample of Robust Redhorse eggs was analyzed for all organic contaminants, and revealed detections for PCBs and 15 of the 20 OCPs (Table 3).

Organic analyses were performed on 17 mollusk samples comprised of 8 unionid mussel, 4 aquatic snail, and 5 clam composite samples. Lipid content ranged from 0.12 – 5.2%. OCPs were detected at all sites and 20 of the 20 analytes were detected. Fourteen of the 20 OCPs analyzed were detected in over 75% of all mollusk samples. The three most predominant analytes detected in mollusk samples were dieldrin (100%; range, 0.00032 – 0.044 µg/g DW), 4,4'-DDT (94%; range, BDL – 0.037 µg/g DW), and cis-chlordane (88%; range, BDL – 0.023 µg/g DW), but no published thresholds were exceeded (Table 4). PCBs were detected in 14 of the 17 samples with total PCB concentrations ranging from BDL – 1.4 µg/g DW. The greatest concentrations of PCBs occurred at the three most downstream sites. The only PAH detected was pyrene in one snail sample from the Digg's Tract site (0.21 µg/g DW).

A total of five plant samples, consisting of submergent macrophytes, were analyzed for organic contaminants. Sites 801 and Pee Dee lacked representative macrophyte samples. OCPs were present in all samples with 18 of the 20 analytes detected. The most prevalent OCPs detected in plant samples were beta-BHC (100%; range, 0.012 – 0.049 µg/g DW) and

heptachlor epoxide (80%; range, 0.0026 – 0.01 µg/g DW). PCBs and PAHs were not detected in plant samples. Mean OCP concentrations ranged from BDL to 0.032 µg/g DW (Table 4).

Composite leaf pack samples, representing the compartment for detritus, were analyzed for each site. OCPs were present in all samples with 16 of the 20 analytes detected. Dieldrin, endosulfan II, endosulfan sulfate, and endrin ketone were detected in 5 of the 5 detritus samples. PCBs were detected in all samples with total PCB concentrations ranging from 0.037 to 0.31 µg/g DW with the greatest concentration at Digg's Tract. PAHs, fluoranthene (0.54 µg/g DW) and pyrene (0.44 µg/g DW), were detected in a single sample at the Pee Dee site. Sites 801 and Pee Dee seem to have the most contaminated detritus samples. Mean OCP concentrations ranged from BDL to 0.18 µg/g DW (Table 4).

Spatial contaminant trends (biota, water, and sediment)

A visual general hazard assessment tool was developed to show relative longitudinal exposure and contamination by highlighting the two highest mean concentrations for each contaminant and environmental compartment sampled among sites (Figure 4). Sites 801, Digg's Tract, and Society Hill showed evidence of pervasive contamination compared to the other two sites. Mean OCP concentrations among sites ranged from 0.098 to 0.17 µg/g DW, mean PAH concentrations ranged from BDL to 0.98 µg/g DW, and mean PCB concentrations ranged from 0.099 to 0.28 µg/g DW (Table 5). Organic contaminant concentrations in water did not exhibit a distinct longitudinal trend for total PAHs, total OCPs, or atrazine. Total PCBs showed an increasing trend downstream (Figure 5). Sediment concentrations of PAHs and OCPs did not show any consistent longitudinal trends and did

not correlate with concentrations in water at the same site. ANOVA results revealed no statistically significant differences among sites for the grouped compounds except for total PCBs ($p < 0.0001$). Total PCB concentrations for fishes occurred at sites in the following ranked order: Digg's Tract > Society Hill > Pee Dee > Red Hill > 801 (Figure 5).

Stable isotopes

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ were evaluated on a total of 359 samples, and $\delta^{34}S$ was performed on a total of 224 samples (a subset of the 359 samples; Table 6, Figure 6). $\delta^{15}N$ values were significantly different among sites when comparing compartments. The Asian Clam was used as the food web base when calculating trophic position, because of their prevalence at every site and primary consumer position. Because there was a significant difference ($p < 0.0001$) in the baseline mean of $\delta^{15}N$ values among sites (801: 8.8‰, Red Hill: 15.3‰, Digg's Tract: 12.3‰, Society Hill: 8.7‰, Pee Dee: 11.2‰), individual food webs and trophic positions were constructed from the baseline of their respective site. Trophic position values for consumers ranged from 0.4‰ to 4.3‰ among sites (Table SI 2). $\delta^{13}C$ values ranged from -34.9 to -20.6‰ for all samples and -31.6 to -21.2‰ for fishes with much overlap among species. $\delta^{34}S$ values were evaluated for the three most downstream sites and exhibited a range from 1.69 to 8.71‰. $\delta^{34}S$ values were compared within taxa among sites with ANOVA, which showed that there was no statistically significant difference among sites ($p > 0.05$).

Food web contaminants

Concentrations of parent organic compounds and their metabolites or isomers were grouped and summed (Table 1). An ANOVA was performed to compare the $\log_{10}$

concentration of organic contaminants with biotic and organic matter compartments (fishes, mollusks, plants, and detritus) as model effects among all sites. Results indicated a significant difference within all contaminant groups ($p < 0.05$) among sample types (Figure 7). Fish species were also significantly different among sites for contaminant groups ($p < 0.05$) where Smallmouth Buffalo and Whitefin Shiner samples generally exhibited the greatest concentrations (Figure 8).

BAFs for total DDTs for all fishes (16,779) and each fish species (BAF range, 3,870 – 64,519) generally exceeded the established threshold value of 5,000, which indicates the contaminant is likely to bioaccumulate (USEPA 1998; Table 7). Largemouth Bass was the only species that was less than the 5,000 threshold for DDTs. Fish lipid content within species was not significantly different among sites. This allowed the contaminant concentrations to be lipid corrected and species grouped among all sites for further analysis. TMFs were calculated for total DDTs in fish due to their consistent prevalence in samples, general hazard as a persistent organic pollutant, and the positive relationship between trophic position and $\log_{10}$-transformed, lipid corrected concentrations. TMFs exceeded the established threshold value of 1.0, which indicates biomagnification, for total DDTs in 5 of 10 fish species (range, 0.21 – 3.75; Table 7).

Discussion

Contamination and bioaccumulation

We discovered that mixtures of organic chemicals were prevalent in all environmental compartments that we sampled in the Yadkin-Pee Dee River system. Chemical mixtures in the environment confound the investigation of effects from individual

contaminants on aquatic organisms (Kolpin et al. 2002; Barber et al. 2011). Contaminants, species, isotopic composition, food web compartments, trophic positions, and sites were systematically evaluated for the presence of trends or relationships. Our results indicated that organic pollution was widespread throughout the river. Dieldrin, trans-chlordane, and 4,4'-DDE were the most frequently detected individual analytes in all samples. Endosulfan I, gamma-BHC, and endosulfan sulfate were detected most infrequently. The most upstream site (site 801) was the least contaminated based upon having the least overall contaminant detections, and Digg's Tract, a site with an extant Robust Redhorse population, was the most contaminated (e.g., greatest percentage of contaminant detections and generally higher measured concentrations). Smallmouth Buffalo and Common Carp were the most contaminated fish species among all sites due to their having the highest percentage of detections and higher contaminant concentrations. Channel Catfish and Largemouth Bass were the least contaminated species in our study, a finding contrary to other similar studies of fish in the system (Hinck et al. 2008; NCDHHS 2009). Fish tissue samples did not exceed any established aquatic life criteria or benchmarks. All surface water samples exceeded the freshwater aquatic life criteria for total PCBs (NCDPH 2007). Sediment from site 801 exceeded the threshold effect concentration for aquatic life for total PAHs (MacDonald 2000). Most samples were below documented thresholds, but low concentrations are known to cause adverse effects in aquatic life (Beyer et al. 1996; Relyea 2008; Vandenberg et al. 2012; Sobolewski et al. 2014). The lack of aquatic life criteria for all analytes and the physiological differences between species makes it difficult to quantify the overall risk of exposure for many of the chemicals analyzed. Elevated concentrations of PCBs at the Digg's

Tract site and downstream are likely due to an aluminum plant upstream and other unknown sources. Other differences between sites are presumably attributable to the proximity of point and non-point sources of pollutants and their influx or cycling within the system. Current use pesticides were not significantly different among sites, but the three most downstream sites generally had greater concentrations of these compounds. This could be a result of proximity of the sites to active row crop agriculture and the absence of any major reservoirs downstream of Digg's Tract to act as a chemical sink for runoff. The majority of freshwater food web toxicology studies have focused on lentic systems (Kidd 1998; Borga et al. 2004; Law et al. 2006; Houde et al. 2008) that are subject to different biological and environmental processes. Streams are generally more heterotrophic than lakes and ponds, which are autotroph dominated (Allan 1995), providing different exposure and cycling routes for organic contaminants that result in non-uniform fate and transport (Morrissey et al. 2005). Studies that have been conducted in lotic systems are difficult to compare to our results in this regulated river system for these reasons.

BAFs and TMFs provide insight into the transport and fate of contaminants for different organisms in an aquatic ecosystem. Our regression analysis of total DDT concentrations and trophic position suggests that Largemouth Bass and Blue Catfish accumulate the majority of their DDTs through dietary exposure, as compared to other fishes that we sampled in the Yadkin-Pee Dee River. BAFs and TMFs calculated in this study indicate that contaminants are accumulated through both water and diet. Our results demonstrate that bioaccumulation and biomagnification rates vary among organisms and contaminants. Differences among species are likely due to their diversity in lipid content,

feeding strategy, exposure as a result of habitat affinity (e.g., benthic versus pelagic), size, and rates of assimilation, elimination, and biotransformation (Arnot and Gobas 2006; Hu et al. 2010).

Food web pathways

Results of this study reveal that the $\delta^{13}C$ and $\delta^{15}N$ composition of food web compartments and species are significantly different among sites. Isotopic variability is commonly found in research on lotic systems (France 1995; Finlay 2001; Woodward and Hildrew 2002). Mean $\delta^{15}N$ isotope values of common taxa in the Yadkin-Pee Dee River showed that site 801 was the most $\delta^{15}N$-depleted site, indicating among-site variation in nutrient inputs. There was an increase in $\delta^{15}N$ at the Red Hill site making it the most enriched, after which $\delta^{15}N$ decreased longitudinally downstream. The $\delta^{15}N$ enrichment of primary consumers at this site suggests anthropogenic influences from urbanization or agriculture, which have been shown to alter nitrogen input and food web structure (Fogg et al. 1998; Cabana and Rasmussen 1996; DeBruyn and Rasmussen 2002; Finlay and Kendall 2007; Atkinson et al. 2014). $\delta^{15}N$ enrichment at the Red Hill site can likely be explained by the input from the Rocky River, a tributary that drains a large metropolitan center (Charlotte-Mecklenburg County area) and the occurrence of agricultural land within the watershed. Aquatic plants were highly enriched in $\delta^{15}N$ among all sites. The enrichment of $\delta^{15}N$ in plants is not unusual, however, and could be a result of volatilization, nitrification, denitrification, or other biogeochemical processes that control the variability in dissolved inorganic nitrogen forms (Finlay and Kendall 2007). Our results did not demonstrate the expected relationship between $\delta^{15}N$ and contaminant concentrations, but there was a

moderate association with lipid content and some hydrophobic chemicals (DDTs, PCBs, and chlordanes) and agricultural land cover and $\delta^{15}N$ enrichment (Figure SI 1). Dietary sources of organic contaminants were not conclusively determined by $\delta^{13}C$, likely due to the temporal and spatial variation associated with hydrology, fish species migration, and un-sampled basal sources. $\delta^{34}S$ showed similar values among taxa and did not distinguish between marine and freshwater sources.

The overall findings from the food web components of this study provide evidence that supports the presence of disparity along a highly regulated river and the general variability in dynamic lotic systems (Finlay and Kendal 2007). The variation that we observed was likely induced by dams and resulting impoundments (Ward and Stanford 1983; Growns et al. 2014), interacting with watershed land use, various point and non-point sources including industrial effluent, inputs from tributaries, and physical and chemical properties (Poff et al. 2006; Jardine et al. 2012). Due to the variability in chemical and physical characteristics of the system, as well as variation in contaminant fate and transport, lotic ecotoxicological studies like this one should be considered as unique ecosystems and compared broadly to other non-lotic systems with caution.

Robust Redhorse implications

The Robust Redhorse population in the Yadkin-Pee Dee River is extremely low, with current estimates of breeding adults being fewer than 50 individuals (RRCC 2009). Therefore, sampling and analyzing muscle tissue from this species was not feasible. However, to make inferences about this critically imperiled species, the Notchlip Redhorse was designated as a potential surrogate based on its related taxonomy and food habits

(Jenkins and Burkhead 1994; Freeman et al. 2002). In a Georgia population, Freeman et al. (2002) found that Robust Redhorse typically fed upon mollusks (primarily Asian Clams) and other invertebrates. We were able to obtain a sample of Robust Redhorse eggs during this study from an adult female and analyzed them for organic contaminants. By examining the occurrence and concentrations of contaminants in Robust Redhorse eggs and in the Notchlip Redhorse surrogate species muscle tissue, known diet sources, and water, we were able to infer Robust Redhorse exposure. Notchlip Redhorse samples showed a substantial increase in organic contaminant detections and concentrations at the Digg's Tract site where the Robust Redhorse currently resides and spawns. Notchlip Redhorse were unfortunately not collected at the two most downstream sites. Fewer contaminant detections, but overall higher concentrations were found in Asian Clams, a food source for Notchlip Redhorse and Robust Redhorse, among all sites. The Robust Redhorse egg samples contained PCBs and 15 of the 20 OCP analytes, with a very high total DDT concentration (Figures 6 and 7). These findings provide strong evidence that contaminants are present in Robust Redhorse tissues and organs, as indicated by the results from the egg sample. This also indicates that maternal transfer of these legacy organic contaminants is likely occurring, but the potential risk to fry development and recruitment of juvenile Robust Redhorse remains unknown, but has been observed in other fish species (Hogan and Brauhn 1975; Russell et al. 1999; Metcalfe et al. 2000). Our results warrant additional study into the potential influence of organic contaminants on Robust Redhorse reproduction and development; controlled laboratory experiments with Robust Redhorse eggs and PCBs and OCPs seem logical next steps.

Conclusions

We discovered the persistence of many legacy organic contaminants in the environment and food web compartments of the Yadkin-Pee Dee River. These contaminants, although relatively low in most concentrations, may pose a serious concern for ecological health. The effects of chemical mixtures, even at low concentrations, may exert chronic adverse effects in aquatic organisms (Beyer et al. 1996; Relyea 2008; Sobolewski et al. 2014). Chemicals that were banned from use decades ago remain prevalent in water, sediment, and biota, and elicit concern for water quality and organismal health. The significance of understanding that both diet and water are important routes of exposure for aquatic organisms is illustrated by the contamination in all compartments of the food web and ecosystem. Our results also confirm the importance of understanding the differences in fish species exposure and accumulation to organic compounds, with clear implications for their potential susceptibility and toxicity. Through this study, we have provided essential information that was previously lacking for the Yadkin-Pee Dee River ecosystem, most importantly for the Robust Redhorse and other imperiled taxa. We suggest that this new knowledge will enable resource managers to develop strategies and management practices for the protection and conservation of this river system and its biota.

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Tables

Table 1. List of organic contaminants and their metabolites or isomers analyzed in samples of water, sediment, detritus, and food web biota from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina.

Polycyclic aromatic hydrocarbons (PAHs)	Current Use Pesticides (CUPs)	Organochlorine Pesticides (OCPs)	Polychlorinated biphenyls (PCBs)
1,1'-Biphenyl	Phorate	Aldrin	Aroclor-1016
1-Methylnaphthalene	Dimethoate	Dieldrin	Aroclor-1221
2-Methylnaphthalene	Pronamide	Hexachlorobenzene	Aroclor-1232
Acenaphthene	Disulfoton	Methoxychlor	Aroclor-1242
Acenaphthylene	Methyl Parathion	Benzene Hexachloride (BHC)	Aroclor-1248
Anthracene	Atrazine	alpha-BHC	Aroclor-1254
Benzo (a) anthracene		beta-BHC	Aroclor-1260
Benzo (a) pyrene		delta-BHC	Aroclor-1262
Benzo (b) fluoranthene		gamma-BHC (Lindane)	Aroclor-1268
Benzo (g,h,i) perylene		Chlordane compounds	
Benzo(k)fluoranthene		alpha-Chlordane (cis)	
Chrysene		gamma-Chlordane (trans)	
Dibenzo(a,h)anthracene		DDT metabolites	
Dibenzofuran		4,4'-DDD	
Fluoranthene		4,4'-DDE	
Fluorene		4,4'-DDT	
Indeno(1,2,3-cd)pyrene		Endosulfan compounds	
Naphthalene		Endoslfan I	
Phenanthrene		Endoslfan II	
Pyrene		Endosulfan sulfate	
		Endrin compounds	
		Endrin	
		Endrin aldehyde	
		Endrin ketone	
		Heptachlor compounds	
		Heptachlor	
		Heptachlor epoxide	

Table 2. Contaminant concentrations in water and sediment samples from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. BDL: below detection limit. Superscript letter indicates corresponding threshold was exceeded.

	Water (ng/L)								Sediment (ng/g dw)					
Site	Total PAHs	Atrazine (CUP)	Total PCBs	Total OCPs	Heptachlor epoxide	Chlordane	4, 4' DDD	4, 4' DDE	Total PAHs	Atrazine (CUP)	Total PCBs	Total OCPs	Chlordane	4, 4' DDE
801	73.03	25.42	0.35	4.06	0.37	2.05	0.43	1.21	2090.86[a]	BDL	BDL	1.94	0.81	1.13
Red Hill	35.19	94.81	1.36	4.01	0.30	1.65	BDL	2.06	318.19	3.78	BDL	1.06	BDL	1.06
Digg's Tract	31.49	50.82	4.92	2.72	0.22	1.55	BDL	0.95	667.96	BDL	BDL	2.37	0.89	1.47
Society Hill	52.94	140.39	6.67	4.25	0.23	1.70	BDL	2.32	105.01	BDL	BDL	0.74	BDL	0.74
Pee Dee	52.33	126.74	7.31	4.02	0.14	1.11	BDL	2.55	68.74	BDL	BDL	0.49	BDL	0.49

a: TEC (threshold effect concentration), sediment concentrations below which harmful effects are unlikely to be observed (MacDonald 2000).

Table 3. Mean, standard deviation (±), sample size (n), and range for contaminant concentrations, percent moisture, and percent lipids in fish muscle tissue and fish ova from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. Contaminant concentrations are reported in µg/g DW.

	n	Mean % moisture	Mean % lipids	PCBs (total)*	4,4'-DDTs (total)	Aldrin	BHCs (total)	Chlordanes (total)
All fishes	87	77.93 69.3 - 81.1	1.68 0.01 - 11	0.218 (± 0.187) BDL - 0.79	0.037 (± 0.066) BDL - 0.421	0.003 (± 0.002) BDL - 0.013	0.009 (± 0.008) BDL - 0.048	0.01 (± 0.013) BDL - 0.079
American Eel (*Anguilla rostrata*)	8	73.7 69.3 - 77.4	5.5 0.22 - 10	0.188 (± 0.051) BDL - 0.24	0.039 (± 0.023) 0.016 - 0.083	0.0015 (± 0.0005) BDL - 0.002	0.006 (± 0.006) 0.003 - 0.011	0.008 (± 0.004) BDL - 0.012
Blue Catfish (*Ictalurus furcatus*)	10	79.7 78.4 - 81	0.43 0.13 - 0.89	0.14 (± 0.021) BDL - 0.17	0.012 (± 0.008) 0.003 - 0.027	0.003 (± 0.001) BDL - 0.004	0.002 (± 0.0004) BDL - 0.002	0.005 (± 0.003) 0.002 - 0.012
Bluegill (*Lepomis macrochirus*)	10	79 77.7 - 80.4	0.66 0.12 - 2.4	BDL –	0.011 (± 0.003) 0.003 - 0.014	BDL –	0.008 (± 0.002) BDL - 0.011	0.002 (± 0.001) BDL - 0.004
Channel Catfish (*Ictalurus punctatus*)	10	79 78.2 - 80.8	0.71 0.32 - 1.44	0.061 (± 0.01) BDL - 0.076	0.007 (± 0.002) BDL - 0.008	BDL –	0.026 (± 0.031) BDL - 0.048	0.003 (± 0.0) BDL - 0.003
Common Carp (*Cyprinus carpio*)	10	77 72.5 - 79.5	2.3 0.6 - 7.1	0.283 (± 0.214) BDL - 0.53	0.031 (± 0.032) 0.005 - 0.095	0.004 (± 0.004) BDL - 0.013	0.013 (± 0.008) 0.006 - 0.035	0.017 (± 0.019) 0.001 - 0.056
Largemouth Bass (*Micropterus salmoides*)	10	78.8 77.5 - 79.9	0.23 0.01 - 0.48	BDL –	0.006 (± 0.003) 0.002 - 0.01	0.001 –	0.003 (± 0.003) BDL - 0.007	0.002 (± 0.0007) BDL - 0.003
Notchlip Redhorse (*Moxostoma collapsum*)	6	79.9 79.2 - 80.4	0.38 0.26 - 0.54	0.047 (± 0.001) BDL - 0.047	0.008 (± 0.003) BDL - 0.006	0.001 –	0.009 (± 0.004) BDL - 0.014	0.003 (± 0.001) BDL - 0.004
Shorthead Redhorse (*Moxostoma macrolepidotum*)	10	78.6 75.4 - 80.3	0.59 0.18 - 1.1	0.244 (± 0.113) BDL - 0.42	0.026 (± 0.018) 0.004 - 0.056	0.003 (± 0.001) BDL - 0.004	0.01 (± 0.006) 0.0007 - 0.022	0.011 (± 0.005) 0.002 - 0.016
Smallmouth Buffalo (*Ictiobus bubalus*)	8	78.7 74.4 - 81.1	2.82 0.79 - 10.8	0.332 (± 0.301) 0.032 - 0.79)	0.143 (± 0.157) 0.032 - 0.421	0.002 (± 0.002) BDL - 0.005	0.004 (± 0.004) BDL - 0.012	0.021 (± 0.026) 0.001 - 0.079
Whitefin Shiner** (*Cyprinella nivea*)	5	72.1 71 - 73.3	5.43 3.12 - 7.17	0.25 (± 0.107) 0.15 - 0.41	0.097 (± 0.018) 0.073 - 0.123	0.003 (± 0.0008) BDL - 0.004	0.018 (± 0.005) 0.013 - 0.025	0.017 (± 0.002) 0.015 - 0.02
Robust Redhorse ova (*Moxostoma robustum*)	1	65.2	–	0.17	0.065	0.0007	0.009	0.006

*PCB concentrations were signicantly different among sites

**Whitefin Shiners are reported as whole-body concentrations

Table 3-continued

	n	Mean % moisture	Mean % lipids	Dieldrin	Endosulfans (total)	Endrins (total)	Heptachlors (total)	Methoxychlor
All fishes	87	77.93 69.3 - 81.1	1.68 0.01 - 11	0.007 (± 0.009) BDL - 0.052	0.005 (± 0.004) BDL - 0.015	0.006 (± 0.007) BDL - 0.043	0.005 (± 0.003) BDL - 0.015	0.007 (± 0.003) BDL - 0.014
American Eel (*Anguilla rostrata*)	8	73.7 69.3 - 77.4	5.5 0.22 - 10	0.006 (± 0.003) 0.003 - 0.01	0.004 (± 0.004) BDL - 0.01	0.007 (± 0.006) 0.002 - 0.016	0.008 (± 0.003) 0.004 - 0.012	BDL –
Blue Catfish (*Ictalurus furcatus*)	10	79.7 78.4 - 81	0.43 0.13 - 0.89	0.002 (± 0.001) BDL - 0.004	0.002 (± 0.0005) BDL - 0.003	0.004 (± 0.003) BDL - 0.01	0.003 (± 0.001) 0.001 - 0.004	0.005 (± 0.0004) BDL - 0.005
Bluegill (*Lepomis macrochirus*)	10	79 77.7 - 80.4	0.66 0.12 - 2.4	0.001 (± 0.0001) BDL - 0.001	BDL –	0.003 (± 0.001) BDL - 0.005	0.004 (± 0.002) 0.001 - 0.007	BDL –
Channel Catfish (*Ictalurus punctatus*)	10	79 78.2 - 80.8	0.71 0.32 - 1.44	0.005 (± 0.0008) BDL - 0.006	BDL –	BDL –	BDL –	BDL –
Common Carp (*Cyprinus carpio*)	10	77 72.5 - 79.5	2.3 0.6 - 7.1	0.005 (± 0.004) 0.002 - 0.016	0.005 (± 0.004) 0.001 - 0.015	0.005 (± 0.003) BDL - 0.009	0.006 (± 0.005) 0.001 - 0.015	0.009 (± 0.003) BDL - 0.014
Largemouth Bass (*Micropterus salmoides*)	10	78.8 77.5 - 79.9	0.23 0.01 - 0.48	0.002 (± 0.0007) BDL - 0.003	BDL –	0.002 –	BDL –	0.007 (± 0.001) 0.005 - 0.009
Notchlip Redhorse (*Moxostoma collapsum*)	6	79.9 79.2 - 80.4	0.38 0.26 - 0.54	0.0009 (± 0.0001) BDL - 0.001	0.002 –	0.002 (± 0.002) BDL - 0.003	0.001 (± 0.0003) BDL - 0.0003	0.001 –
Shorthead Redhorse (*Moxostoma macrolepidotum*)	10	78.6 75.4 - 80.3	0.59 0.18 - 1.1	0.004 (± 0.001) BDL - 0.005	0.005 –	0.008 (± 0.007) BDL - 0.021	0.004 (± 0.002) BDL - 0.007	BDL –
Smallmouth Buffalo (*Ictiobus bubalus*)	8	78.7 74.4 - 81.1	2.82 0.79 - 10.8	0.017 (± 0.017) 0.002 - 0.052	0.004 (± 0.004) 0.001 - 0.012	0.011 (± 0.015) 0.001 - 0.043	0.005 (± 0.003) BDL - 0.012	0.003 (± 0.0008) BDL - 0.003
Whitefin Shiner** (*Cyprinella nivea*)	5	72.1 71 - 73.3	5.43 3.12 - 7.17	0.016 (± 0.0008) 0.015 - 0.017	0.006 (± 0.002) BDL - 0.009	0.008 (± 0.005) BDL - 0.015	0.004 (± 0.002) 0.002 - 0.008	BDL –
Robust Redhorse ova (*Moxostoma robustum*)	1	65.2	–	0.006	0.001	0.004	0.002	BDL

*PCB concentrations were signicantly different among sites

**Whitefin Shiners are reported as whole-body concentrations

Table 4. Mean, standard deviation (±), sample size (n), and range for contaminant concentrations, percent moisture, and percent lipids in organic matter, plant, and mollusk samples from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. Organic matter is represented by conditioned leaf packs. Plants are represented by submergent macrophytes. Concentrations are reported in µg/g DW. BDL: below detection limit.

	n	Mean % moisture	Mean % lipids	PCBs (total)*	4,4'-DDTs (total)	Aldrin	BHCs (total)	Chlordanes (total)	Dieldrin	Endosulfans (total)	Endrins (total)	Heptachlors (total)	Methoxychlor
Organic matter	5	83	–	0.183 (± 0.11)	0.013 (± 0.006)	BDL	0.008 (± 0.004)	0.005 (± 0.015)	0.007 (± 0.001)	0.02 (± 0.01)	0.012 (± 0.003)	0.004 (± 0.001)	0.01
		79.9 - 85	–	0.037 - 0.31	0.007 - 0.018	–	BDL - 0.013	BDL - 0.006	0.006 - 0.008	0.011 - 0.035	0.008 - 0.016	BDL - 0.005	–
Plants	5	90.4	–	BDL	0.019 (± 0.006)	0.005 (± 0.003)	0.032 (± 0.028)	0.017 (± 0.009)	0.011 (± 0.006)	0.056 (± 0.048)	0.016 (± 0.008)	0.006 (± 0.003)	0.014 (± 0.005)
		85.7 - 96.4	–	–	BDL - 0.024	BDL - 0.007	0.012 - 0.08	BDL- 0.027	BDL - 0.015	BDL - 0.11	BDL - 0.02	BDL - 0.01	BDL - 0.019
Mollusks	16	83.4	0.98	0.248 (± 0.367)	0.017 (± 0.028)	0.004 (± 0.005)	0.013 (± 0.006)	0.008 (± 0.008)	0.007 (± 0.01)	0.007 (± 0.007)	0.006 (± 0.006)	0.006 (± 0.005)	0.008 (± 0.009)
		76.2 - 87.5	0.12 - 5.19	BDL - 1.4	0.001 - 0.121	BDL - 0.019	0.004 - 0.021	0.0005 - 0.023	0.0003 - 0.044	BDL - 0.017	BDL - 0.021	BDL - 0.016	BDL - 0.014
Asian Clam	5	82.7	1.32	0.277 (± 0.188)	0.016 (± 0.006)	0.01 (± 0.007)	0.012 (± 0.006)	0.017 (± 0.006)	0.011 (± 0.003)	0.012 (± 0.005)	0.009 (± 0.004)	0.009 (± 0.004)	BDL
		79.5 - 85.2	1.13 - 1.56	0.063 - 0.54	0.009 - 0.022	BDL - 0.019	0.006 - 0.021	0.011 - 0.023	0.006 - 0.015	BDL - 0.018	0.005 - 0.016	BDL - 0.014	–
Snails	3	79.2	2.1	0.775 (± 0.884)	0.051 (± 0.061)	0.002	0.014 (± 0.008)	0.009 (± 0.01)	0.018 (± 0.022)	0.015 (± 0.001)	0.016 (± 0.007)	0.016	0.014
		76.2 - 82.9	0.12 - 5.19	BDL - 1.4	0.01 - 0.121	–	0.005 - 0.021	0.003 - 0.021	0.004 - 0.044	BDL - 0.016	BDL - 0.021	–	–
Unionid mussels	8	85.3	0.35	0.084 (± 0.116)	0.007 (± 0.005)	0.001 (± 0.001)	0.013 (± 0.005)	0.002 (± 0.002)	0.002 (± 0.001)	0.001 (± 0.001)	0.002 (± 0.001)	0.003 (± 0.001)	0.002
		81.2 - 87.5	0.17 - 1.1	BDL - 0.32	0.001 - 0.017	BDL - 0.002	0.006 - 0.019	0.0005 - 0.007	0.0003 - 0.004	BDL - 0.004	BDL - 0.004	0.002 - 0.005	–

*PCB concentrations were signicantly different among sites

Table 5. Mean and standard deviation of target analytes in organic matter and biota samples from five sites in the Yadkin-Pee Dee River of North Carolina and South Carolina. Concentrations reported in µg/g DW. BDL: below detection limit.

Analyte / SiteA2:K23	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
PAHs					
1,1'-Biphenyl	BDL	BDL	BDL	BDL	BDL
1-Methylnaphthalene	BDL	BDL	BDL	BDL	BDL
2-Methylnaphthalene	BDL	BDL	BDL	BDL	BDL
Acenaphthene	BDL	BDL	BDL	BDL	BDL
Acenaphthylene	BDL	BDL	BDL	BDL	BDL
Anthracene	BDL	BDL	BDL	BDL	BDL
Benzo (a) anthracene	BDL	BDL	BDL	BDL	BDL
Benzo (a) pyrene	BDL	BDL	BDL	BDL	BDL
Benzo (b) fluoranthene	BDL	BDL	BDL	BDL	BDL
Benzo (g,h,i) perylene	BDL	BDL	BDL	BDL	BDL
Benzo(k)fluoranthene	BDL	BDL	BDL	BDL	BDL
Chrysene	BDL	BDL	BDL	BDL	BDL
Dibenzo(a,h)anthracene	BDL	BDL	BDL	BDL	BDL
Dibenzofuran	BDL	BDL	BDL	BDL	BDL
Fluoranthene	BDL	BDL	BDL	BDL	0.54
Fluorene	BDL	BDL	BDL	BDL	BDL
Indeno(1,2,3-cd)pyrene	BDL	BDL	BDL	BDL	BDL
Naphthalene	BDL	BDL	BDL	BDL	BDL
Phenanthrene	BDL	BDL	0.29	0.52	BDL
Pyrene	BDL	BDL	0.21	BDL	0.44
Total PAHs	BDL	BDL	0.5	0.52	0.98
OCPs					
Aldrin	0.0038 ± 0.0013	0.0019 ± 0.0014	0.0025 ± 0.0026	0.0046 ± 0.0056	0.0027 ± 0.0016
Dieldrin	0.0063 ± 0.0059	0.0046 ± 0.0039	0.0095 ± 0.0129	0.0069 ± 0.0068	0.0057 ± 0.0072
Hexachlorobenzene	BDL	BDL	BDL	BDL	BDL
Methoxychlor	0.0094 ± 0.0028	0.0087 ± 0.0036	0.0062 ± 0.0056	0.0098 ± 0.003	0.0061 ± 0.0026
alpha-BHC	0.0045 ± 0.0037	0.002 ± 0.0015	0.0031 ± 0.0036	0.0027 ± 0.0014	0.0025 ± 0.0018
beta-BHC	0.0084 ± 0.0018	0.0077 ± 0.0062	0.0097 ± 0.0117	0.0116 ± 0.012	0.0068 ± 0.0048
delta-BHC	0.006 ± 0.0045	0.003 ± 0.0019	0.0036 ± 0.0025	0.0048 ± 0.0031	0.0051 ± 0.0032
gamma-BHC (Lindane)	0.0036 ± 0.0031	0.0013 ± 0.0008	0.0038 ± 0.0049	0.0014 ± 0.0009	0.0017 ± 0.0014
alpha-Chlordane (cis)	0.0092 ± 0.0081	0.0056 ± 0.0066	0.0065 ± 0.0073	0.0046 ± 0.0028	0.0033 ± 0.0016
gamma-Chlordane (trans)	0.008 ± 0.0087	0.0027 ± 0.0022	0.0072 ± 0.0099	0.0068 ± 0.0093	0.0057 ± 0.0054

Table 5-continued

Analyte / Site	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
Heptachlor	0.0033 ± 0.0018	0.0047 ± 0.0034	0.0029 ± 0.0027	0.0032 ± 0.0021	0.0032 ± 0.0016
Heptachlor epoxide	0.0042 ± 0.0026	0.0023 ± 0.0016	0.004 ± 0.0027	0.0046 ± 0.003	0.0027 ± 0.0016
4,4'-DDD	0.0079 ± 0.0048	0.004 ± 0.0037	0.0043 ± 0.0042	0.0057 ± 0.0037	0.0036 ± 0.0038
4,4'-DDE	0.0246 ± 0.0307	0.0142 ± 0.0164	0.0316 ± 0.0487	0.0247 ± 0.0393	0.0178 ± 0.0224
4,4'-DDT	0.0117 ± 0.0083	0.011 ± 0.0083	0.0212 ± 0.04	0.0231 ± 0.0458	0.014 ± 0.0202
Endoslfan I	0.0109 ± 0.0055	0.0042 ± 0.003	0.0055 ± 0.0048	0.0321 ± 0.0485	0.0072 ± 0.0093
Endoslfan II	0.003 ± 0.0026	0.0039 ± 0.0033	0.0045 ± 0.0035	0.0039 ± 0.0039	0.0025 ± 0.002
Endosulfan sulfate	0.0053	0.0042 ± 0.0044	0.0066 ± 0.0055	0.0056 ± 0.0041	0.0041 ± 0.004
Endrin	0.0024 ± 0.001	0.0035 ± 0.0031	0.0027 ± 0.0019	0.0033 ± 0.0028	0.0025 ± 0.0015
Endrin aldehyde	0.0063 ± 0.0057	0.0043 ± 0.0022	0.0056 ± 0.0021	0.0053 ± 0.0031	0.0052 ± 0.004
Endrin ketone	0.0042 ± 0.0035	0.0038 ± 0.0036	0.0047 ± 0.0043	0.007 ± 0.0081	0.0031 ± 0.003
Total OCPs	0.143	0.0976	0.1457	0.1717	0.1055
CUPs					
Phorate	BDL	BDL	BDL	BDL	BDL
Dimethoate	BDL	BDL	BDL	BDL	BDL
Pronamide	BDL	BDL	BDL	BDL	BDL
Disulfoton	BDL	BDL	BDL	BDL	BDL
Methyl Parathion	BDL	BDL	BDL	BDL	BDL
Atrazine	BDL	BDL	BDL	BDL	BDL
Total CUPs	BDL	BDL	BDL	BDL	BDL
Total PCBs	0.165 ± 0.0071	0.0985 ± 0.0659	0.1957 ± 0.1863	0.2794 ± 0.2316	0.2838 ± 0.1946

Table 6. Stable isotope ratio means and standard deviations of aquatic food web biota and organic matter from five sites of the Yadkin-Pee Dee River of North Carolina and South Carolina.

	801		Red Hill		Digg's Tract		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.62 ± 1.05	2.83 ± 0.21	-30.0 ± 0.64	3.83 ± 1.03	-30.26 ± 0.76	5.75 ± 0.17	3.69 ± 0.3
Plants	-27.86 ± 0.99	12.52 ± 2.52	-31.42	13.7	-26.38 ± 1.24	12.08 ± 1.99	5.17 ± 0.96
Mollusks	-24.72 ± 2.36	10.07 ± 1.43	-28.58 ± 1.97	15.55 ± 0.75	-27.05 ± 3.15	12.41 ± 1.6	4.49 ± 1.39
Asian Clam	-26.76 ± 0.34	8.84 ± 0.25	-28.68 ± 0.04	15.33 ± 0.27	-28.66 ± 0.03	12.34 ± 0.06	5.02 ± 1.52
Snails	-22.69 ± 0.06	11.3 ± 0.11	-25.56 ± 0.27	14.68 ± 0.01	-24.34 ± 1.87	12.28 ± 2.35	3.49 ± 1.01
Unionid mussels	–	–	-30.04 ± 0.36	16.1 ± 0.57	-30.30 ± 0.36	12.65 ± 0.32	5.73 ± 0.56
Insects	-27.49 ± 1.31	9.97 ± 1.18	-27.46 ± 1.52	14.82 ± 2.22	-27.59 ± 1.5	12.68 ± 2.2	4.2 ± 0.81
Collector	-29.41 ± 2.59	9.53 ± 0.72	-29.34 ± 0.71	11.98 ± 0.85	-29.13 ± 0.47	10.46 ± 0.06	2.55 ± 1.05
Filterer	-27.4 ± 0.26	9.65 ± 0.57	–	–	-31.12	14.02	4.65
Predator	-27.02 ± 0.76	10.08 ± 1.47	-26.99 ± 1.35	14.59 ± 0.67	-27.12 ± 1.29	13.05 ± 1.37	4.36 ± 0.57
Predator/Parasite	–	–	-25.89 ± 0.04	18.26 ± 3.34	-26.06 ± 0.29	15.85 ± 2.36	4.44 ± 0.74
Scraper	-27.62 ± 0.67	10.63 ± 0.15	-28.57 ± 0.4	14.9 ± 0.21	-27.65 ± 0.43	11.15 ± 2.35	4.48 ± 0.3
Crayfishes	-24.55 ± 0.6	10.40 ± 0.01	-24.9 ± 0.87	13.45 ± 0.69	-24.64 ± 0.57	12.89 ± 1.53	5.21 ± 0.19
Fishes	-25.95 ± 0.87	13.07 ± 1.54	-25.32 ± 1.38	16.15 ± 2.63	-27.13 ± 1.46	15.88 ± 1.47	5.84 ± 0.73
American Eel	–	–	-27.09 ± 0.72	17.87 ± 2.3	-30.15 ± 1.28	16.6 ± 0.54	6.22 ± 0.29
Blue Catfish	-26.69 ± 0.71	14.89 ± 0.81	-26.05 ± 0.71	18.06 ± 1.03	-27.16 ± 1.26	17.06 ± 1.17	5.61 ± 0.36
Bluegill	-25.42 ± 0.7	12.18 ± 0.32	-25.03 ± 0.64	14.75 ± 1.1	-26.12 ± 0.31	15.0 ± 0.34	5.57 ± 0.14
Channel Catfish	-26.01 ± 0.54	12.23 ± 1.1	-25.82 ± 0.82	14.82 ± 2.69	-28.69 ± 0.4	16.1 ± 0.11	5.89 ± 0.32
Common Carp	-26.19 ± 0.98	12.84 ± 1.86	-26.27 ± 0.73	11.83 ± 1.42	-26.92 ± 0.52	14.39 ± 0.87	5.43 ± 1.38
Largemouth Bass	-26.13 ± 1.28	14.64 ± 1.33	-25.93 ± 0.99	17.33 ± 4.01	-26.0 ± 0.45	17.47 ± 1.18	6.44 ± 0.63
Notchlip Redhorse	-27.32 ± 1.32	11.6 ± 0.19	-24.66 ± 0.17	16.5 ± 0.62	-26.99 ± 0.73	14.5 ± 2.42	5.08 ± 1.19
Shorthead Redhorse	-25.46 ± 0.42	13.34 ± 0.17	-22.81 ± 1.18	18.13 ± 0.18	-27.69 ± 0.99	16.9 ± 0.41	6.29 ± 0.15
Smallmouth Buffalo	–	–	-25.05 ± 1.07	15.05 ± 2.05	-28.09 ± 1.3	15.87 ± 0.95	5.91 ± 0.48
Whitefin Shiner*	-25.32 ± 0.09	11.45 ± 0.97	-24.36 ± 0.83	17.21 ± 1.15	-25.0 ± 0.34	15.28 ± 0.57	6.14 ± 0.47

*Whitefin Shiners are reported as whole-body concentrations

Table 6-continued

	Society Hill			Pee Dee		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.9 ± 0.52	5.48 ± 0.06	4.07 ± 1.4	-30.04 ± 0.46	5.54 ± 0.66	3.57 ± 1.42
Plants	-26.36 ± 0.91	14.97 ± 0.6	3.83 ± 1.24	–	–	–
Mollusks	-28.07 ± 1.78	9.41 ± 0.55	4.5 ± 0.8	-32.0 ± 0.41	11.35 ± 0.4	4.95 ± 0.36
Asian Clam	-28.92 ± 0.32	8.68 ± 0.45	3.87 ± 0.57	-32.10 ± 0.02	11.18 ± 0.59	4.72 ± 0.27
Snails	-25.28 ± 0.01	9.4 ± 0.17	4.98 ± 0.11	–	–	–
Unionid mussels	-29.04 ± 0.65	9.78 ± 0.32	4.59 ± 0.98	-31.96 ± 0.52	11.43 ± 0.35	5.06 ± 0.37
Insects	-28.36 ± 2.11	10.13 ± 2.13	4.27 ± 1.01	-30.72 ± 2.54	10.90 ± 1.41	3.96 ± 0.9
Collector	-29.95 ± 2.08	9.1 ± 0.74	3.53 ± 1.41	-31.94 ± 3.19	10.83 ± 0.48	3.64 ± 1.16
Filterer	–	–	–	-34.03 ± 0.06	11.69 ± 0.25	4.4 ± 0.76
Predator	-28.75 ± 0.86	10.02 ± 0.92	4.55 ± 0.75	-29.91 ± 1.38	10.55 ± 1.76	4.0 ± 0.86
Predator/Parasite	-24.58 ± 0.36	15.18 ± 0.22	4.73 ± 0.19	-25.99	13.04	3.51
Scraper	-26.61 ± 0.06	8.11 ± 0.42	4.61 ± 0.52	-30.79	10.94	4.77
Crayfishes	-25.16 ± 0.42	10.98 ± 1.22	4.86 ± 0.56	-28.09 ± 0.84	13.13 ± 0.07	5.97 ± 0.11
Fishes	-26.16 ± 1.5	13.14 ± 1.39	5.13 ± 1.33	-27.09 ± 1.37	12.87 ± 0.95	5.02 ± 1.29
American Eel	-25.62 ± 0.61	13.19 ± 0.56	4.58 ± 0.77	-27.15 ± 0.42	12.59 ± 0.77	4.69 ± 0.75
Blue Catfish	-27.04 ± 0.75	13.59 ± 0.95	4.8 ± 1.26	-26.56 ± 0.63	13.26 ± 0.43	4.59 ± 0.45
Bluegill	-27.25 ± 3.14	12.54 ± 0.5	5.21 ± 0.77	-26.71 ± 1.77	12.79 ± 0.79	3.84 ± 0.92
Channel Catfish	-25.78 ± 0.55	12.4 ± 0.89	4.7 ± 0.61	-26.34 ± 0.6	12.85 ± 0.74	5.19 ± 0.6
Common Carp	-26.02 ± 0.6	12.58 ± 0.66	4.59 ± 0.74	-27.73 ± 0.8	11.87 ± 1.42	4.54 ± 0.74
Largemouth Bass	-25.53 ± 0.39	15.75 ± 0.79	5.93 ± 0.8	-27.04 ± 0.62	14.17 ± 0.92	5.63 ± 0.52
Notchlip Redhorse	–	–	–	–	–	–
Shorthead Redhorse	-27.12 ± 2.48	13.14 ± 1.56	4.72 ± 0.97	-28.21 ± 0.81	13.4 ± 0.36	5.48 ± 0.18
Smallmouth Buffalo	-26.43 ± 0.42	11.86 ± 1.6	3.83 ± 0.8	-29.29 ± 0.73	12.4 ± 0.13	3.72 ± 0.73
Whitefin Shiner*	-24.7 ± 0.67	13.22 ± 1.01	7.84 ± 0.59	-25.08 ± 0.6	12.64 ± 0.87	7.63 ± 0.94

*Whitefin Shiners are reported as whole-body concentrations

Table 7. Calculated trophic position (TP), bioaccumulation factors (BAFs), and trophic magnification factors (TMFs) for total DDTs. Total DDT concentrations are reported in µg/g LW (lipid corrected weight).

Species	n	BAF	TMF	Mean (TP)	Mean (DDTs LW)
American Eel	8	18,798	0.44	2.89	0.039
Blue Catfish	10	6,010	3.75	3.20	0.013
Bluegill	10	5,577	0.47	2.64	0.012
Channel Catfish	10	5,529	0.96	2.71	0.012
Common Carp	10	15,288	1.04	2.42	0.032
Largemouth Bass	10	3,870	2.22	3.35	0.008
Notchlip Redhorse	6	5,000	1.64	2.58	0.010
Shorthead Redhorse	10	14,327	1.89	3.11	0.028
Smallmouth Buffalo	8	64,519	0.96	2.56	0.143
Whitefin Shiner	5	46,971	0.65	2.79	0.098

Figures

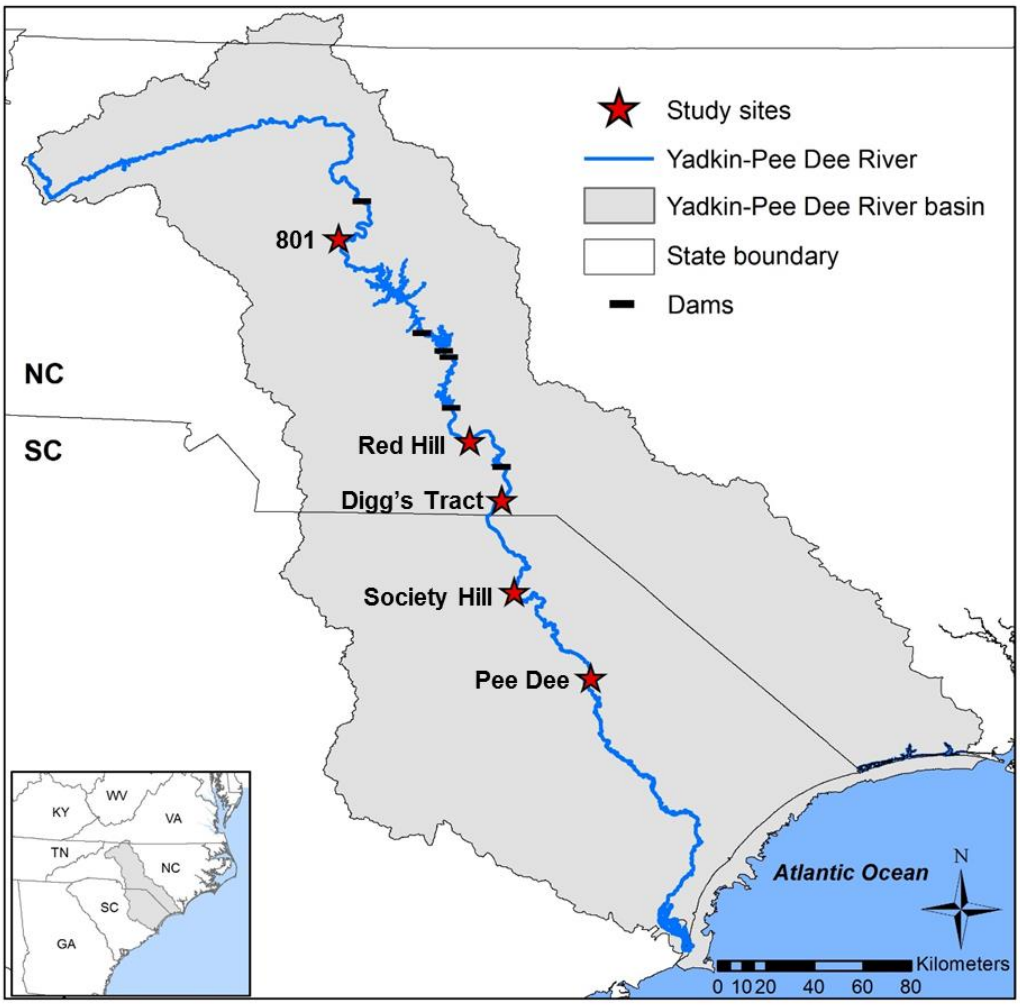

Figure 1. Study sites and major dams along the Yadkin-Pee Dee River of North Carolina and South Carolina.

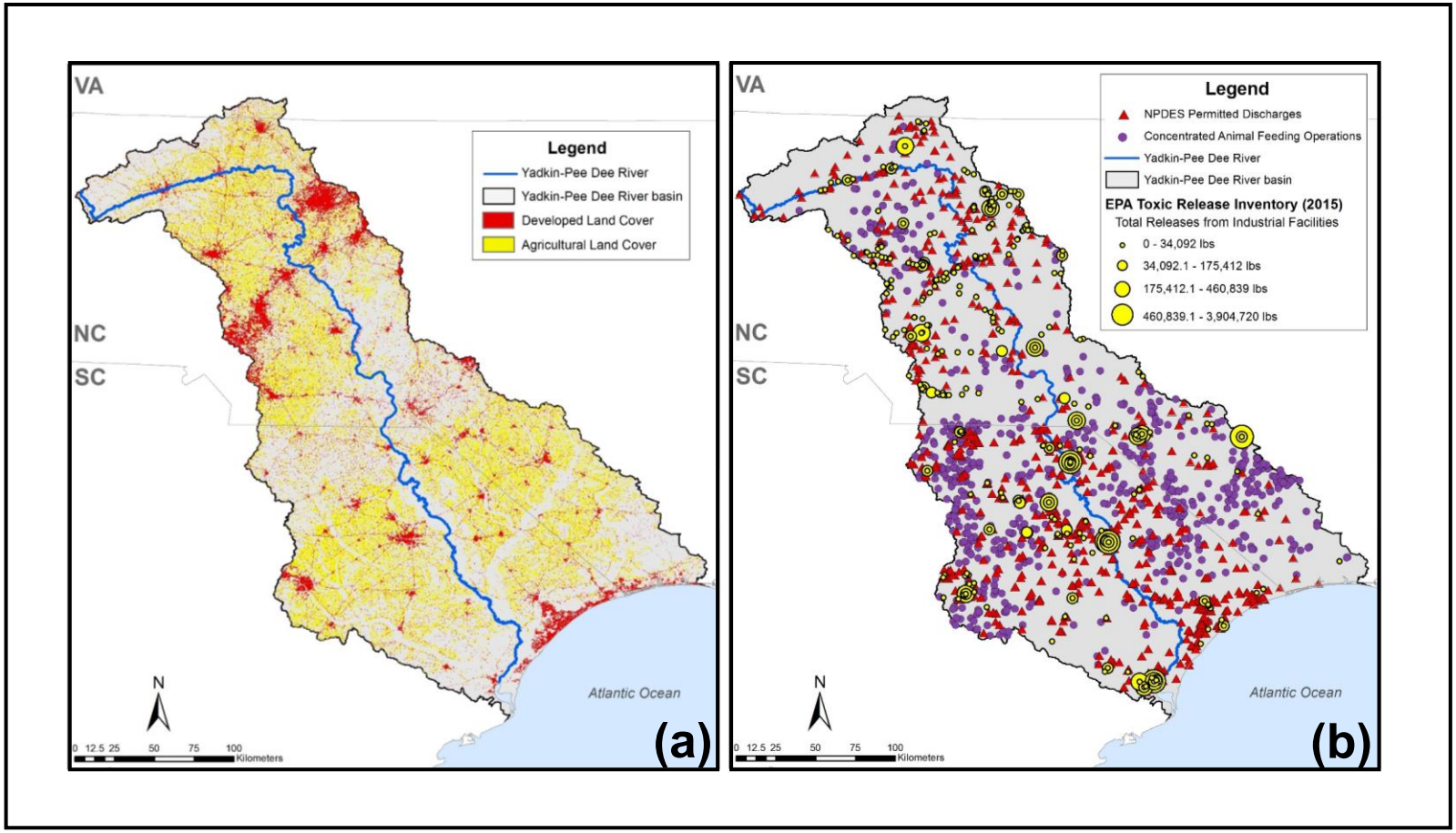

Figure 2. Non-point (a) and point (b) sources of organic contaminants in the Yadkin-Pee Dee River basin of North Carolina and South Carolina.

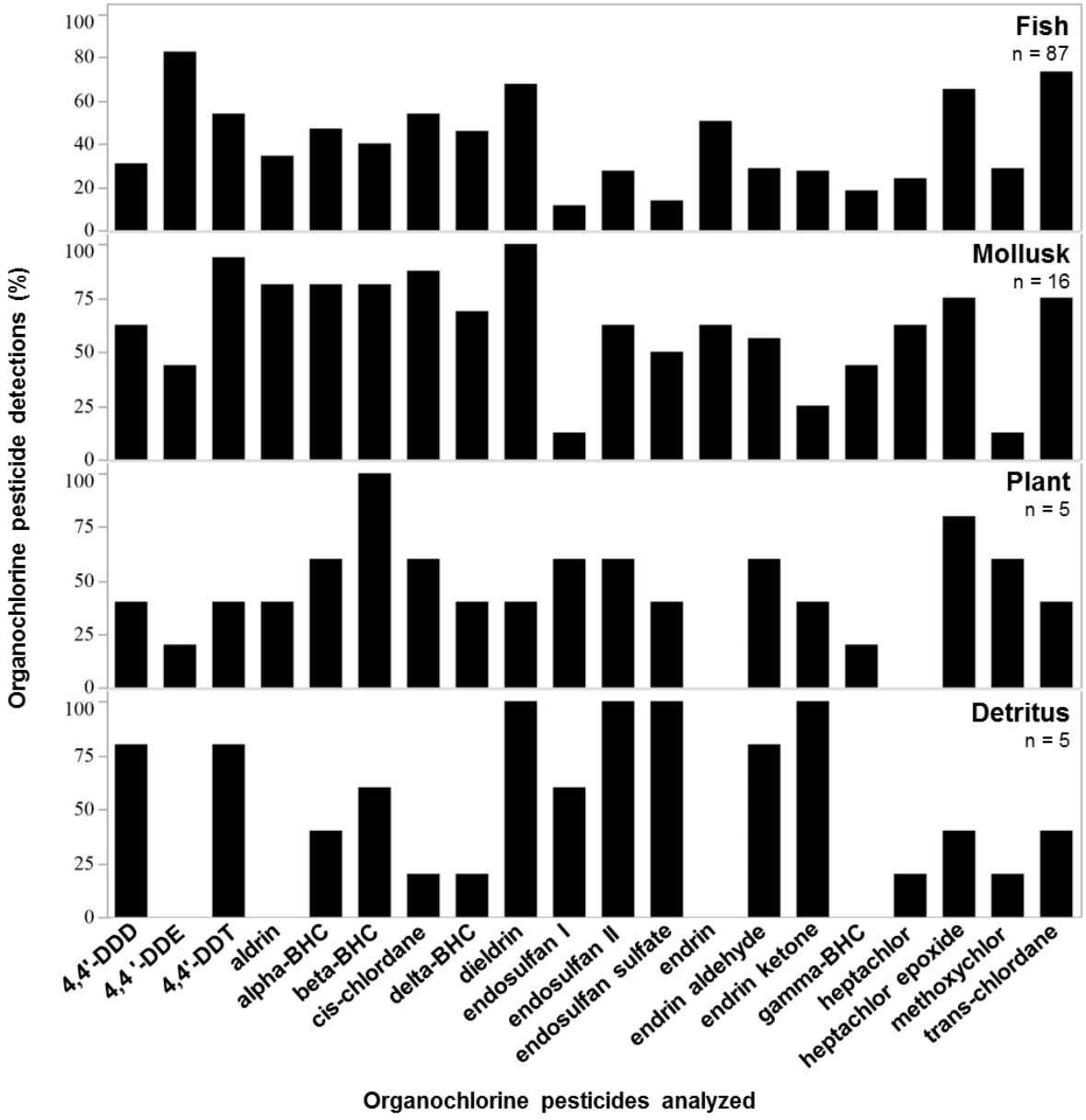

Figure 3. Detections (%) of organochlorine pesticides in samples of fishes, mollusks, plants, and detritus collected from five sites on the Yadkin-Pee Dee River in North Carolina and South Carolina, n = number of samples analyzed.

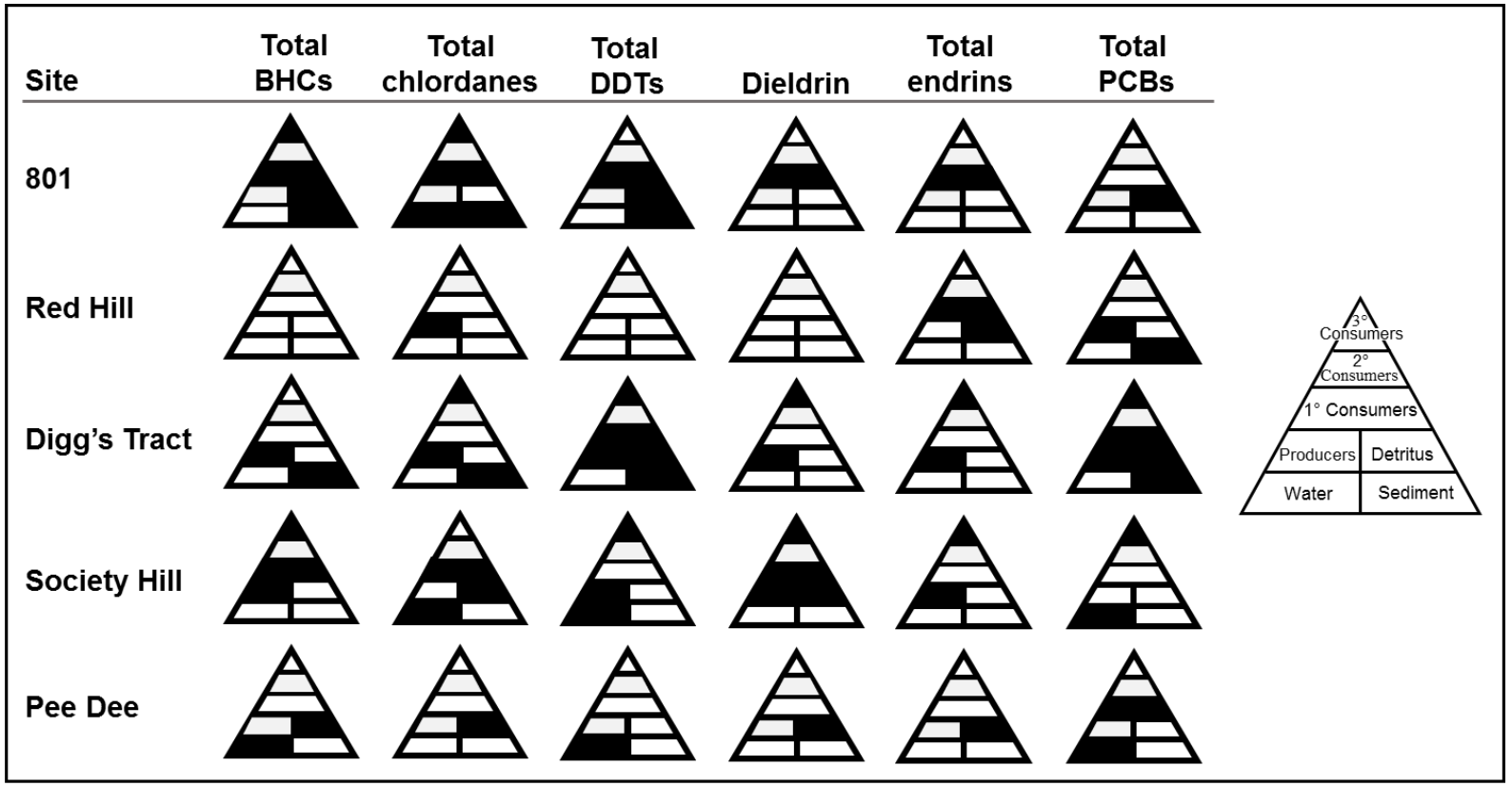

Figure 4. Summary of selected organic compounds among sites in the Yadkin-Pee Dee River of North Carolina and South Carolina. For each triangle, a solid black section represents the greatest measured mean concentration of an organic contaminant for the corresponding food web compartment among sites. A gray filled section indicates that there were no samples in that compartment that were analyzed.

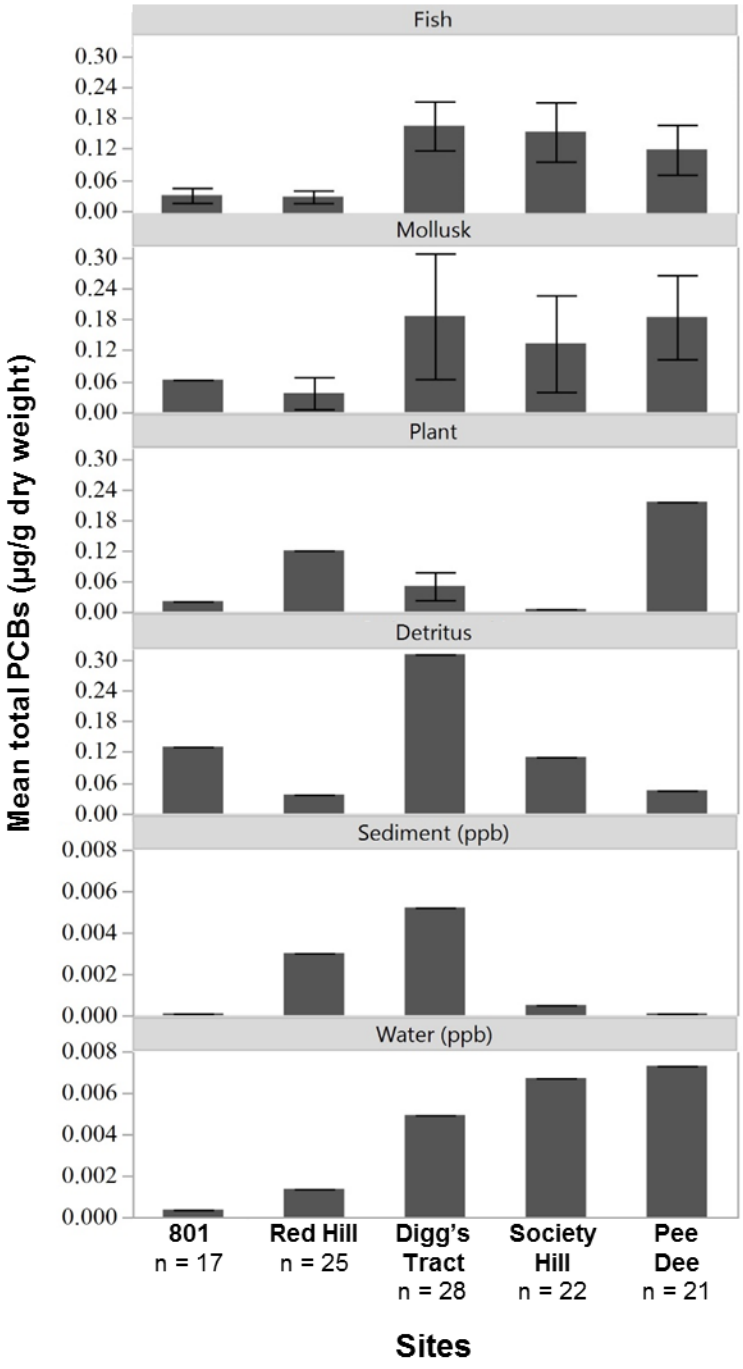

Figure 5. Mean total PCB concentrations (µg/g dry weight) and standard error for fish, mollusk, plant, detritus, sediment, and water at five sites (listed from upstream to downstream) on the Yadkin-Pee Dee River of North Carolina and South Carolina, n = number of samples analyzed.

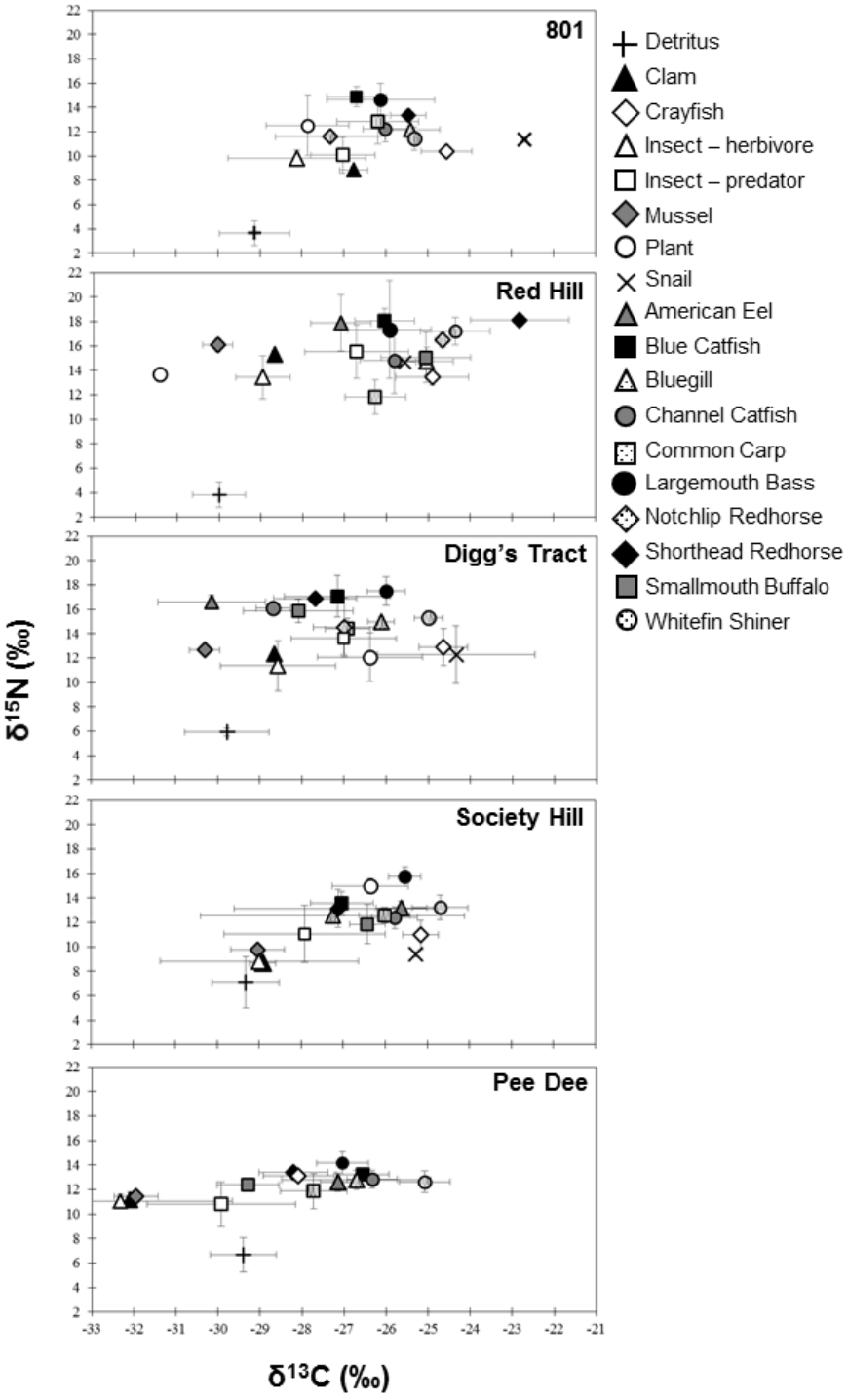

Figure 6. $\delta^{13}C$ and $\delta^{15}N$ biplots and standard errors of consumers and food sources from five sites (listed from upstream to downstream) on the Yadkin-Pee Dee River of North Carolina and South Carolina.

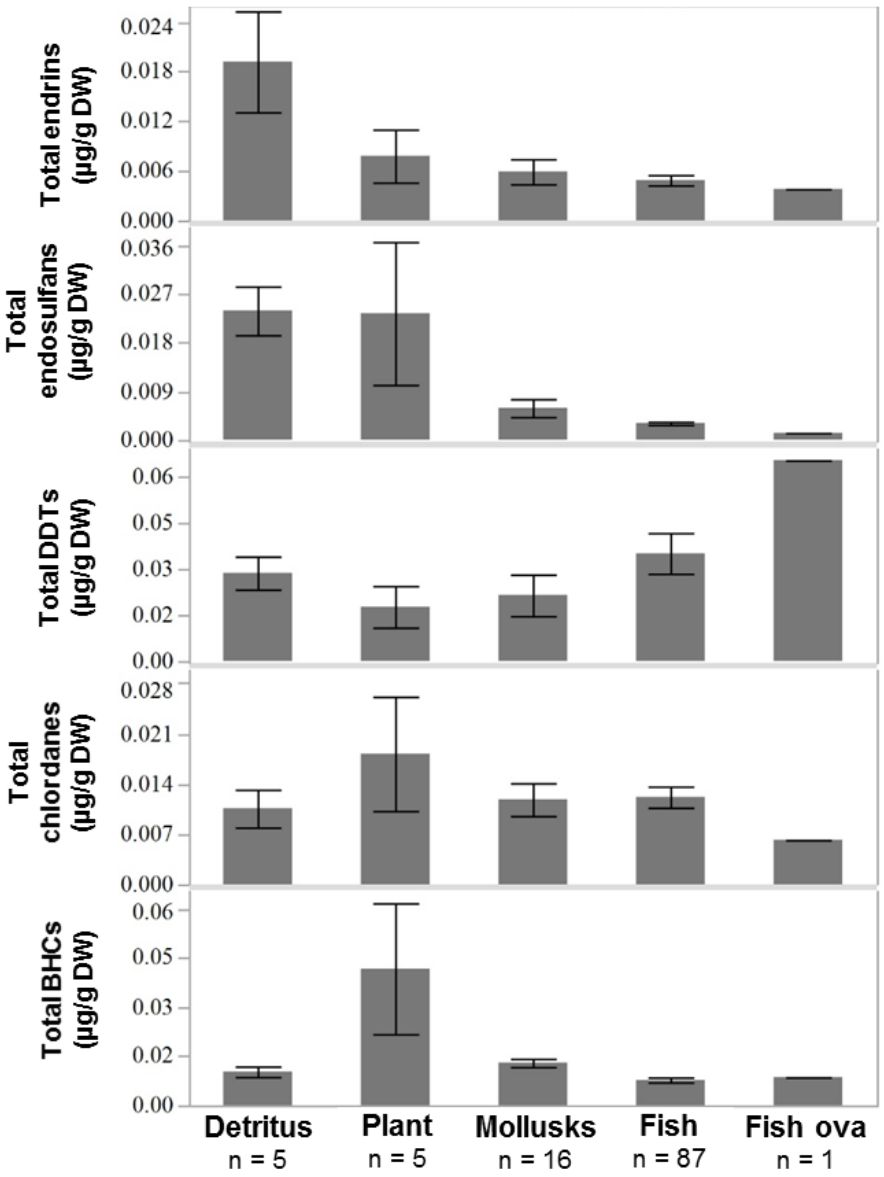

Figure 7. Mean contaminant concentrations and standard errors for select organochlorine pesticides by taxa from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. Fish ova are from a Robust Redhorse (*Moxostoma robustum*) mortality, n = number of samples analyzed.

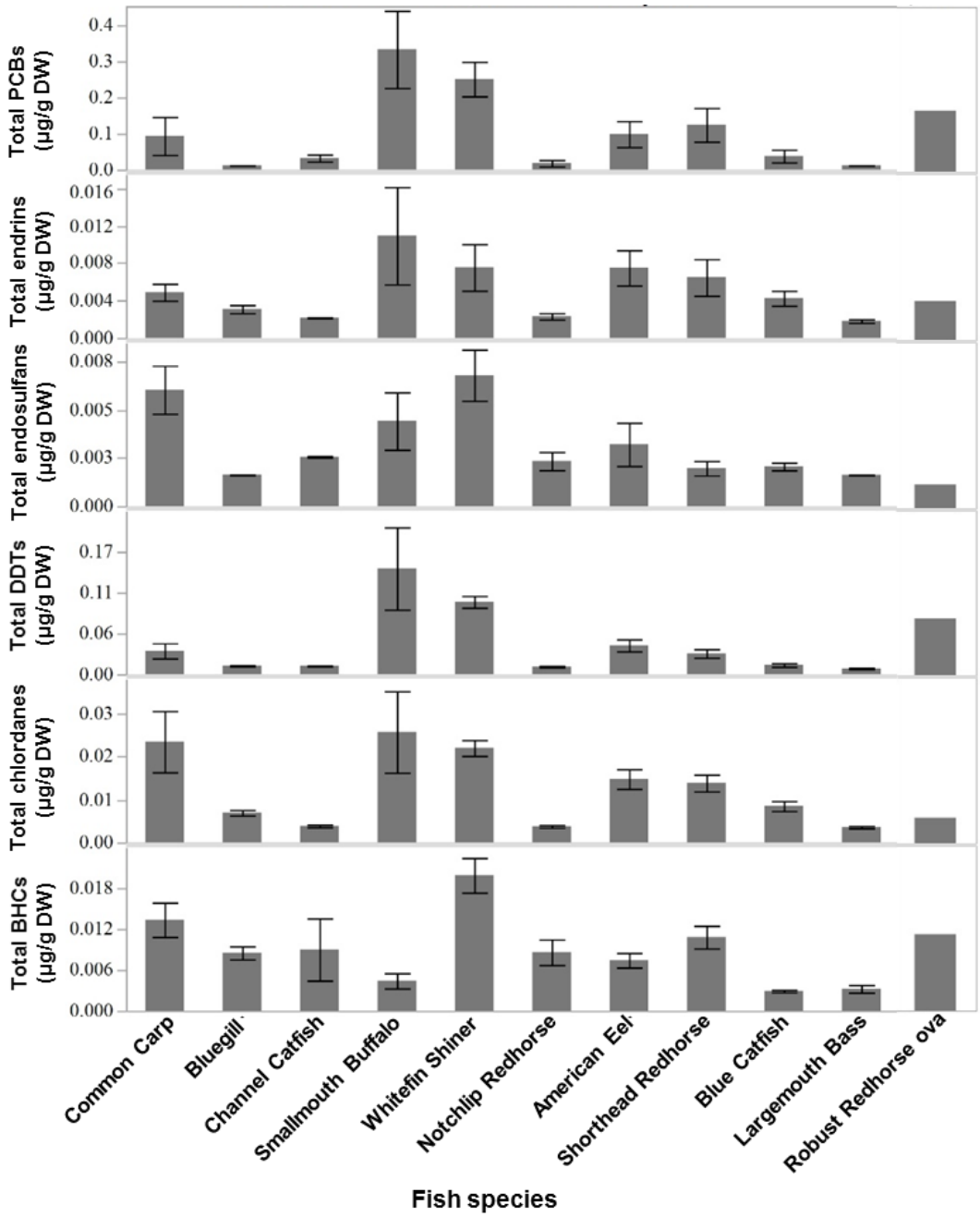

Figure 8. Mean contaminant concentrations and standard errors of select organochlorine pesticides for fish species and Robust Redhorse (*Moxostoma robustum*) ova from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. Species are ordered by ascending $\delta^{15}N$ enrichment.

CHAPTER 2

Trophodynamics of Metals in a Large River Food Web

Abstract

Widespread impairment of freshwater ecosystems, caused by increasing landscape development is of utmost concern. Metals are known toxicants that have the capability to cause adverse effects in aquatic life when concentrations exceed critical thresholds. Metals enter the environment through natural and anthropogenic processes and accumulate in aquatic organisms through water and dietary sources. The objectives of this research were to determine the aquatic food web structure and trophic transfer and accumulation of metals within the food web of an imperiled sucker species, the Robust Redhorse (*Moxostoma robustum*), inhabiting a southeastern U.S. river. We conducted intensive sampling at five riverine sites along the Yadkin-Pee Dee River of North Carolina and South Carolina. The sampling sites spanned a range with diverse physical characteristics, land uses, and influx of point and nonpoint source pollution that facilitated longitudinal examination. Major food web components and pathways were determined by stable isotope analyses of representative producers, consumers, and organic matter. Contaminant analyses performed on biotic and abiotic samples revealed that metals were present in sediment and all food web components at various concentrations. Manganese and cadmium exceeded published threshold effect concentrations in sediment (460 and 0.99 µg/g dry weight [DW], respectively). Mercury was detected in all food web samples analyzed (mean 0.13 µg/g wet weight [WW], range 0.001 – 0.6 µg/g WW). Concentrations of mercury in fish samples exceeded the 0.2 µg/g WW

aquatic life criterion. Our results show that metals accumulate and transfer through the riverine food web, potentially threatening the health of rare or intolerant species.

Introduction

Understanding the transport and fate of contaminants in freshwater ecosystems and their potential effects on aquatic organisms are critical for assessing hazards posed, especially in areas where agriculture or urbanization are dominant. Urban and industrial areas cause ecosystem disturbances that can influence contaminant trophic dynamics (Hershey et al. 2007). Anthropogenic activities are a major contributing source for chemical contaminants and excess nutrients into surface waters (Nriagu 1990). When contaminant and nutrient concentrations reach high levels, they can cause adverse effects in aquatic organisms and become a threat to ecological health. Chemicals that tend to bioaccumulate, persist in the environment, and exhibit elevated toxicity pose major threats to ecological health in freshwater ecosystems (Rainbow 1996; LeBlanc and Buchwalter 2010). Metals are an example of persistent pollutants in aquatic environments due to their tendency to accumulate in biota and their inability to biodegrade (LeBlanc and Buchwalter 2010). Some metals are considered essential for life (e.g., chromium, iron, nickel, and zinc) whereas others are designated nonessential (e.g., cadmium, mercury, and lead). Essential metals are required for the proper function of physiological processes, such as enzyme catalysts or cofactors, but both essential and nonessential metals have the capability to cause toxicity when concentrations exceed optimum thresholds (Heath 1995).

Metals enter the environment through natural and anthropogenic processes. Natural sources include the weathering of rocks and soils, volcanic emissions, sea spray, forest fires, and atmospheric deposition (Nriagu 1990; Cope and Hodgson 2010). Major anthropogenic sources include mining and smelting operations, municipal and industrial effluents, and

agricultural runoff (Nriagu 1990; Nikinmaa 2014). Aquatic organisms are exposed to metals through multiple routes including direct uptake from water, sediment, and through their diet (LeBlanc and Buchwalter 2010). Diet is a well-documented route of exposure for many metals (Suedel et al. 1994; Croteau 2005; LeBlanc and Buchwalter 2010). The other form of uptake is through water by transport across membranes by ion channels, carrier proteins, or amino acids (LeBlanc and Buchwalter 2010; Nikinmaa 2014). The bioavailability, uptake, and toxicity of metals in aquatic systems are affected by biological and physiochemical mechanisms (Luoma 1983; Wang 1987; Deforest et al. 2007). Metals tend to sequester in sediment, reducing bioavailability in the water column, and become a major source of exposure for benthic organisms and bottom feeding consumers (Luoma 1983). Previous research suggests that aquatic invertebrates tend to have higher concentrations of some metals compared to fish (Ikemoto et al. 2008; LeBlanc and Buchwalter 2010).

Excess amounts of metals can lead to detrimental effects in aquatic organisms when the rate of uptake is greater than the rate of excretion or detoxification. Toxicity of metals is dependent on uptake mode, species, and biochemical properties of the water (Wang 1987). Sublethal effects from metals can include enzyme inhibition or activation, nephrotoxicity, neurotoxicity, hepatotoxicity, immunotoxicity, osmoregulation interference, reproductive effects, and developmental effects (Heath 1995; Eisler 2000; Cope and Hodgson 2010; Authman et al. 2015).

Stable isotope ratios are a useful technique to assess the transfer of metals by trophic pathways and the extent of exposure through dietary routes. Stable isotopes provide integrated dietary information from assimilated food sources over time. Carbon ($\delta^{13}C$)

isotopic composition is used to reveal changes in food sources, nitrogen ($\delta^{15}N$) values are used to estimate trophic position of consumers and dietary exposure to contaminants, and sulfur ($\delta^{34}S$) values are used to determine large-scale movement patterns by differentiating between marine and freshwater sources (Fry 1991). Stable isotopes also detect food web responses to environmental and anthropogenic influences (Michener and Lajtha 2008). Combining stable isotope analysis (SIA) and chemical analyses of biota can reveal biomagnification or biodilution of metals within the food web.

The Yadkin-Pee Dee River of North Carolina and South Carolina is subject to a multitude of anthropogenic contaminant inputs and a model ecosystem for associated effects. The Yadkin-Pee Dee River basin provides habitat for species of imperiled fishes (31), mussels (21), and crayfish (1) that are in need of protection and conservation (NCWRC 2005; SCDNR 2005). This is why it was crucial to examine the entire food web to better understand contaminant dynamics in an ecosystem. Included in the 53 priority species that inhabit the Yadkin-Pee Dee River, the Robust Redhorse (*Moxostoma robustum*), a large riverine fish, is in critical need of conservation and recovery efforts. This large catostomid is classified as endangered in North Carolina and listed as a highest priority for conservation in North Carolina and South Carolina (NCWRC 2005; SCDNR 2005). The current estimated spawning population is fewer than 50 individuals in the Yadkin-Pee Dee River (RRCC 2009). Risks from pollutant exposure are significant for aquatic life and their consumers, and large portions of the river are classified as impaired due to high levels of metals in sediment and fish tissue (SCDNR 2009; NCDEQ 2012). Excessive levels of metals in the riverine

environment pose a likely ongoing threat to fish, wildlife, and ultimately to humans, who rely on the river for drinking water, food, and recreation.

The documented impairment of the Yadkin-Pee Dee River and the need for conservation efforts for the Robust Redhorse and other priority species were the motivation and study system for this research. The objectives of this research were to (1) utilize stable isotope ratios to determine the food web structure and linkages by sampling a variety of organic matter and aquatic biota, (2) analyze water, sediment, organic matter, and biota for trace metals, (3) determine contamination trends among sites along a longitudinal gradient, and (4) assess the susceptibility of consumers to bioaccumulation and biomagnification by examining the chemical concentrations within each trophic level of the food web. The overall aim was to understand trophic pathways of metals within the riverine food web and determine likely stressors to imperiled species and overall ecological health of the Yadkin-Pee Dee River ecosystem.

Methods

Study sites

Five riverine sites with a wide spatial range and variable topography and anthropogenic influences were selected along the Yadkin-Pee Dee River in North Carolina and South Carolina (Figure 1, Table SI 1). The study sites spanned a range of physical characteristics, land uses, and influx of point and nonpoint source pollution that facilitated longitudinal examination of trophic and contaminant 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, sites included the location near where the Robust Redhorse was first described but no longer occurs (site 801), where populations are currently extant (Digg's Tract, Society Hill, and Pee Dee) and a proposed reintroduction site to stock future hatchery-reared Robust Redhorse (Red Hill).

Sample collection and preparation

Intensive sample collection for sediment, organic matter, and aquatic biota was conducted at all five sites during spring and summer. When feasible, taxa collected for trophic and contaminant analyses were the same among sites. Sediment, organic matter, and aquatic biota from each site were analyzed for selected trace metals [aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn)]. Fishes (10 species), mollusks (4 families), crayfishes (2 species), aquatic insects (12 families), macrophytes, and detritus were collected from each site for SIA to determine the major components of the food web, trophic pathways, and bioaccumulation of contaminants. Collection and processing methods were similar to those of Hoeinghaus et al. (2007) and Pingram et al. (2014).

Water: Water quality characteristics were measured using a Yellow Springs Instrument (YSI, Yellow Springs, Ohio) 556 multi-purpose meter during each sampling event. Measurements included: temperature (°C), conductivity (µS/cm), dissolved oxygen (mg/L), salinity (ppt), and pH.

Sediment and organic matter: Composite sediment samples from each site were collected from depositional areas and analyzed for inorganic contaminants. Sediment and organic matter samples consisted of multiple grabs that were collected from the top 3-5 cm of the substrate surface layer by a stainless steel scoop. Samples were typically 225-250 g, and any visible biota or debris were removed. Sediment samples were placed into labeled, sterile amber glass jars and held on ice until transported to a -20° C freezer to await processing and analysis. Detritus samples were represented by leaf packs and suspended particulate organic matter. Leaf packs were collected by hand or with dip nets, placed into sealable plastic bags, and held on ice after visible debris and invertebrates were removed. Suspended particulate organic matter (i.e., drift) samples were collected with 500-µm mesh drift nets. Drift samples were rinsed to remove invertebrates, placed into sealable plastic bags or amber glass jars, and held on ice. Macrophytes were collected by hand, thoroughly rinsed to remove organic matter and invertebrates, and placed into sealable plastic bags and held on ice. The sites at Red Hill and Pee Dee lacked representative macrophyte samples.

Aquatic consumers: Aquatic insects and crustaceans were collected with 500-µm D-frame nets, by flipping rocks, or by hand from leaf packs and woody debris. Specimens were stored in containers with filtered site water and chilled for at least 8 h to enable depuration of gut contents. Aquatic insects were sorted and classified into functional feeding groups: collector-filterer, shredder, scraper, or predator. When feasible, insects were identified to a minimum of family taxonomic level (Brachycentridae, Corydalidae, Elmidae, Gerridae, Glossiphoniidae, Gyrinidae, Heptageniidae, Hydropsychidae, Limnephilidae, and Perlidae). Odonates were grouped by suborder (Anisoptera and Zygoptera). Mollusks were collected by

hand and represented native freshwater mussels (family Unionidae), snails (families Pleuroceridae and Viviparidae), and the non-native Asian Clam (*Corbicula fluminea*). Snails were held in a container of filtered site water for at least 8 h to enable depuration of gut contents.

Fishes were collected by pulsed-DC boat-mounted electrofishing with efforts to retain comparable size classes of the same species among sites. Fishes were euthanized by immediate placement into an ice-water slurry to induce temperature shock according to North Carolina State University approved protocols (IACUC 15-042-O). Species, total length (mm), and weight (g) were measured for all fishes. 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 bubalus*), and Whitefin Shiner (*Cyprinella nivea*) were collected to represent the variety of trophic guilds found in the Yadkin-Pee Dee River. All samples were stored in a -20° C freezer in the laboratory until further processing.

Stable isotope analysis

All sample processing methods were performed with sterile, stainless steel utensils and all surfaces were cleaned with lab detergent, a distilled water rinse, an acetone rinse, and another distilled water rinse between samples to avoid contamination. All samples were dried at 60° C to a constant weight in a drying oven, ground to a fine powder with a mortar and pestle, and then placed into 7-mL scintillation glass vials for storage. Processed samples were transported by overnight carrier to the Colorado Plateau Stable Isotope Laboratory, Northern

Arizona University, Flagstaff, for analysis of carbon, nitrogen, and sulfur isotope ratios. Samples were weighed, encapsulated in tin, and then analyzed with a gas isotope-ratio mass spectrometer using approved standard methods.

Stable isotope ratio results were expressed as delta (δ) notation in parts per thousand (‰) comparative to standards according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1000, \qquad (1)$$

where *X* is ^{13}C, ^{15}N, or ^{34}S, and *R* is the corresponding heavy isotope to light isotope ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$). The standard materials are Vienna Pee Dee Belemnite limestone for carbon, atmospheric nitrogen for nitrogen, and Canyon Diablo Troilite for sulfur. Samples were not lipid normalized due to the low lipid content of animals and %C content of plants (Skinner et al. 2016). Most food sources have distinct $\delta^{13}C$ signatures that are conserved within 1‰ in consumer tissues, which makes it possible to discern the sources that contribute to the consumer's diet (Finlay et al. 2002; Hoeinghaus et al. 2007).

Data were normalized using International Atomic Energy Agency (IAEA) isotope calibration standards (IAEA CH6, CH7, N1, and N2). The difference between the expected and observed isotope ratio for these standards was 0.08‰ and below for $\delta^{13}C$ and 0.11‰ and below for $\delta^{15}N$. National Institute of Standards and Technology (NIST) approved standards were used to assess drift and linearity. The precision of these standards was ± 0.06‰ for $\delta^{13}C$ and $\delta^{15}N$.

Contaminant analysis

Sediment: Samples were transported frozen to the RTI International laboratory (Durham, North Carolina) for analysis of 23 metals. Mercury (Hg) concentration in sediment

samples was determined using a Milestone DMA-80 direct mercury analyzer. Other metals were measured with a modified version of Method 3050B (USEPA 1996) and a Thermo X-Series II ICP-MS or a Thermo iCAP6500 ICP-OES depending on the concentration of the analyte present in the sample according to their standard protocols.

Biota and organic matter: A subset of samples was analyzed for 23 trace metals at RTI International (Durham, North Carolina). Larger individuals were analyzed separately, whereas smaller individuals were combined to form composite samples. If composite samples were necessary, two or more individuals were combined from a particular site and consisted of the same species with similar sizes to minimize differences between age classes or life stage.

All sample processing methods were performed with sterile, stainless steel utensils, and all surfaces were cleaned between samples to avoid contamination. Metal analysis was conducted on white muscle tissue of fish samples. Tissue samples were free of scales, skin, and bone. Whitefin Shiner was the only fish species to be analyzed whole. Standard fish processing protocols were used when excising muscle tissue (USEPA 2000). Crayfish exoskeletons and mollusk shells were removed. All other samples were processed whole. Samples were homogenized using a Grindomix© (Glen Mills Inc., GM200, Clifton, New Jersey) which was triple-rinsed with acetone, hexane, and DCM between every sample to avoid contamination. Wet weight of tissue was recorded (minimum of 10 g) then placed into sterile, amber jars, and stored frozen in a -80° C freezer until analysis.

All samples were lyophilized and manually homogenized prior to the analysis of metals. Samples analyzed for mercury were weighed into nickel boats and placed into a

Milestone DMA-80 direct mercury analyzer autosampler for analysis. A 1-g aliquot from each sample was processed for the other 22 metals with a modified version of Method 3050B (USEPA 1996). Samples were analyzed with a Thermo X-Series II ICP-MS or a Thermo iCAP6500 ICP-OES. Samples with insufficient material were not analyzed for all 23 metals.

Metal concentrations (µg/g dry weight (DW) and % moisture were measured for every sample. Wet weight (WW) of metal concentration was calculated for comparison to published thresholds using the following equation:

$$WW = DW\left[1 - \left(\frac{\%\, moisture}{100}\right)\right]. \qquad (2)$$

Metal concentrations were compared to aquatic life criteria to determine exceedances and assess the potential threat to the biota of the Yadkin-Pee Dee River ecosystem.

Quality control and assurance: A rigorous quality control protocol was followed with each batch of samples analyzed and consisted of procedural blanks to detect contamination, sample duplicates to assess precision, spiked samples, and standard reference materials to measure percent recovery, and instrument and standard calibration to ensure consistency. Percent recoveries of reference standards, calibration checks, and sample spikes for Hg averaged 98% (range, 89–114%). The relative percent difference of duplicate samples for Hg averaged 3% (range, 0.7–7.7%). Percent recoveries of spiked samples for all other metals averaged 98% (range, 70-120%). Relative percent difference (RPD) of duplicate samples for all other metals averaged 11% (range, 0–60%). Certified reference materials (CRMs) were used throughout analyses to assess potential matrix effect and as calibration check standards. Lobster hepatopancreas tissue, dogfish muscle tissue, and dogfish liver tissue were analyzed to assess method accuracy. All CRM analyses yielded values within the certified range. For

three metals (Al, Cu, and Sb) and one batch of samples, spiked samples had recoveries (135%, 520% and 159%, respectively) greater than the acceptable range (80-120%) and a duplicate sample had an RPD of 109%; the measured values for this metal were censored from statistical analysis.

Data analysis

Mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopic composition of taxa was compared by analysis of variance (ANOVA) to quantify differences among trophic levels and sites (Kwak and Zedler 1997; Hoeinghaus et al. 2007). $\delta^{15}N$ values were used to determine the trophic position of consumers within the food web. Asian Clams (*Corbicula fluminea*) were selected as the food web baseline because it was a primary consumer and abundant at every site. Because there was a significant difference ($p < 0.05$) in the average $\delta^{15}N$ of Asian Clams among sites, trophic position of consumers was based on the respective site baseline. Using a fractionation factor of 3.4‰, Equation 3 was used to calculate trophic position (Anderson and Cabana 2007):

$$Trophic\ Postion_{consumer} = \left(\frac{\delta^{15}\mathrm{N}_{consumer} - \delta^{15}\mathrm{N}_{\ baseline}}{3.4}\right) + 2. \qquad (3)$$

Metal concentrations were $\log_{10}$-transformed to normalize the data. ANOVA was performed on contaminant concentrations to detect statistically significant differences within and among sites, compartments, and species. Tukey's HSD post hoc test was performed if an ANOVA indicated a significant difference ($p < 0.05$) to determine where the differences occurred. Simple linear regression was performed on log-transformed metal concentrations and calculated trophic position to examine relationships. Trophic magnification factors (TMFs) were calculated for fishes with the following equations:

$$Log[chemical_{LW}] = a + b(TP), \qquad (4)$$

$$TMF = 10^{b}. \qquad (5)$$

The slope (b) from the log-linear regression of the wet weight chemical concentration versus trophic position (TP) was used to calculate the TMF. TMFs > 1 indicate that contaminants are biomagnified.

Results

Metal concentrations in sediment

Of the 23 metals analyzed in sediment, 19 were detected. Mn exceeded the lowest effect concentration of 460 µg/g DW at 4 of the 5 sites and exceeded the severe effect concentration of 1,100 µg/g DW at 1 site, Digg's Tract (Persaud and Jaagumagi 1993; Table 1). Cd exceeded the threshold effect concentration (TEC) of 0.99 µg/g DW at all sites, but did not exceed the predicted effect concentration (PEC) of 4.98 µg/g DW at any site (MacDonald et al. 2000). No other metal concentration in sediment exceeded published aquatic life benchmarks.

Metal concentrations in biota

A total of 87 fish samples consisting of 10 different species were analyzed for metals. Of the 23 metals, 19 were detected in fish samples. Be, Cd, Co, and Ni were the only metals not detected in fishes (Table 2). Hg was detected in 100% of samples and was the only metal detected in fish tissue that exceeded published aquatic life thresholds (0.2 µg/g WW, Fuchsman et al. 2016). Hg concentrations for all fish samples ranged from 0.024 to 0.597 µg/g WW. Hg exceeded the 0.2 µg/g WW criteria in 38% of fish samples. Common Carp, Largemouth Bass, and Blue Catfish held the greatest average Hg concentrations (0.29, 0.26,

and 0.24 µg/g WW, respectively). Like Hg, As and Pb are nonessential, bioaccumulative metals considered environmentally hazardous at elevated concentrations (Authman et al. 2015). Arsenic was detected in only 8% of fish samples including Notchlip Redhorse (100%; range, 0.51 – 0.71 µg/g WW) and Whitefin Shiner (20%; range, below detection limit (BDL) – 0.74 µg/g WW) samples. Pb was detected in 48% of fish samples, including all species except Notchlip Redhorse, with a range from BDL to 0.18 µg/g WW.

Metal analyses were performed on 18 mollusk samples comprised of 8 unionid mussel, 5 aquatic snail, and 5 clam composite samples. Metals were present in all samples with 22 of the 23 metals detected (Table 2). Sb was the only metal not detected in mollusks. Hg was detected in all mollusk samples with a range from 0.003 to 0.17 µg/g WW. Pb was detected in 94% of mollusk samples (range, BDL – 1.12 µg/g WW). As was detected in 89% of mollusk samples (range, BDL – 5.56 µg/g WW) with Asian Clams possessing relatively greater concentrations (range, 1.49 – 5.31 µg/g WW). Concentrations varied for each metal and did not exceed published thresholds.

A total of 31 composite invertebrate samples, aquatic insects and crayfishes, were analyzed for metals. Only a subset of 10 samples were analyzed for Hg due to a lack of sufficient tissue mass. All metals excluding Se were detected in invertebrates. Hg was detected in all 10 samples analyzed (range, 0.018 – 0.029 µg/g WW; Table 2). Pb was detected in 74% of invertebrate samples (range, BDL – 1.51 µg/g WW), whereas As was detected in only 10% of samples (range, BDL – 0.62 µg/g WW).

A total of six plant samples, consisting of submergent macrophytes, were analyzed for all 23 metals with 21 detected. Sb and Se were the only metals not detected in plant

samples. Hg in plants ranged from 0.001 to 0.003 µg/g WW, Pb ranged from 0.51 to 1.89 µg/g WW, and As ranged from 0.66 to 2.47 µg/g WW (Table 2). Five conditioned leaf pack samples, representing the compartment for detritus, were analyzed for all metals with all but Sb and Se detected. Hg concentrations in detritus ranged from 0.001 to 0.007 µg/g WW, Pb ranged from 0.77 to 12.63 µg/g WW, and As ranged from 0.31 to 3.28 µg/g WW.

Spatial trends of metals (biota, water, and sediment)

A visual general hazard assessment tool was developed to show relative longitudinal exposure and contamination by highlighting the greatest mean concentration for each selected metal and trophic compartment sampled among sites (Figure 2). The Red Hill and Pee Dee sites held the majority of the greatest compartment concentrations compared to the other three sites. Site differences in $\log_{10}$-transformed mean metal concentrations were evaluated by ANOVA which detected no significant difference among sites when examining all samples ($p > 0.05$). Hg and Pb exhibited a general increase in concentrations from upstream to downstream.

Stable isotopes

$\delta^{13}C$ and $\delta^{15}N$ were measured in a total of 359 samples. $\delta^{34}S$ isotope analysis was performed on a total of 224 samples (a subset of the 359 samples). $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopic composition means were variable among food web compartments, species, and sites (Table 3). $\delta^{15}N$ values were significantly different among sites when comparing compartments ($p < 0.05$). Asian Clams were used as the baseline when calculating trophic position, and because there was a significant difference ($p < 0.0001$) with baseline mean $\delta^{15}N$ values among sites (801: 8.8‰, Red Hill: 15.3‰, Digg's Tract: 12.3‰, Society Hill: 8.7‰,

Pee Dee: 11.2‰), individual food webs and trophic positions were constructed from the baseline at their respective site. Trophic position values for consumers ranged from 0.4‰ to 4.3‰ among sites (Table SI 2).

Food web and metals

An ANOVA followed by Tukey's HSD post hoc test was performed to assess differences in means of $\log_{10}$-transformed metal concentrations among the different compartment types (fishes, mollusks, aquatic insects, crayfishes, plants, and detritus) as model effects. There were statistically significant differences for all metals among compartments ($p < 0.0001$). Variation in mean metal concentrations occurred among food web compartments (Figure 3). The same method was used to compare mean metal concentrations among fish species, and all metals except Pb and Sb demonstrated a significant difference ($p < 0.05$). The greatest Hg concentrations occurred in top predators and large, long-lived fish species (Figure 4). Overall, metal concentrations were generally low.

Simple linear regression was used to examine the slopes between $\log_{10}$-transformed As, Cr, Hg, and Pb concentrations and trophic position. Hg showed a positive relationship with trophic position, whereas As, Cr, and Pb held the inverse relationship with trophic position (Figure 5). TMFs were calculated for As, Cr, Hg, and Pb using Equations 4 and 5. Cr, Hg, and Pb TMFs for fishes showed a value greater than 1.0 for 5, 2, and, 4 species, respectively, indicating diet as a major route of exposure (Table 4). Mollusk was the only other food web compartment that exhibited TMF values greater than 1 (As: 2.37, Pb: 10.94).

Discussion

Contamination and bioaccumulation

We examined the accumulation of metals in aquatic biota, organic matter, and sediment of the Yadkin-Pee Dee River. Our analysis of metal concentrations, isotopic composition, food web compartments, species, and sites revealed that metals were pervasive in the river. Both diet and the surrounding environment (water and sediment) influenced bioaccumulation. The Digg's Tract and Pee Dee sites possessed generally greater metal concentrations than other sites, but analyses detected no significant differences among sites. Hg concentrations showed a longitudinal increase downstream, likely induced by a change in biogeochemical properties. Many factors influence the bioavailability and accumulation of mercury and other metals in aquatic environments; such influences may include water temperature, pH, salinity, oxygenation, alkalinity, dissolved organic carbon, hydrologic alterations, and interactions with other metals (Snodgrass et al. 2000; Chen et al. 2005; Kidd et al. 2012; Nikinmaa 2014).

Hg, more specifically methylmercury (CH_3Hg^+), is known to cause toxicity in wildlife (Weiner and Spry 1996; Wolfe et al. 1998; Boening 2000; Hammerschmidt et al. 2002; Scheuhammer 2007). Findings for this study showed that Hg was detected in every sample, and various fish samples exceeded aquatic life thresholds, indicating a potential risk for fish and piscivorous consumers. The mean Hg concentration in fish in this study (0.19 µg/g WW) is similar to the national mean concentration in river systems (0.21 µg/g WW, Hinck et al. 2009). All other metals were present in samples but at generally low

concentrations; however, Cd and Mn concentrations in sediment exceeded aquatic life thresholds. Cd is a nonessential metal that tends to bioaccumulate in food webs and has exhibited adverse effects in aquatic life even at low concentrations (Cearley and Coleman 1974; Woodworth and Pascoe 1982; Jarvinen and Ankley 1999). There is a lack of information on the toxicity of Mn in aquatic organisms in the current literature.

Food web pathways

Our results revealed that metal accumulation was variable among food web compartments and fish species. Factors that may influence metal concentrations in fishes includes body size, trophic position, age, growth rate, habitat affinity, and diet (Weech et al. 2004; Jezierska and Witeska 2006; Kidd et al. 2012). Most of our results showed weak to no correlations between metal concentrations and trophic position, but the majority of those that did show an association exhibited an inverse relationship (Figure 5). The inverse relationships suggest that the majority of the metals measured do not biomagnify up the food web in this river system. Hg results positively correlated with trophic position and showed a TMF greater than 1 for consumers, supporting previous findings that Hg tends to biomagnify in aquatic food webs (Kidd et al. 2012; Lavoie et al. 2013). Largemouth Bass is a top predator in the Yadkin-Pee Dee River and exhibited a TMF of 0.76 for Hg (< 1.0), contrary to expected results. The difference in observed and expected TMF is likely due to the different contributions from a variety of food sources (Dallinger et al. 1987; Cabana and Rasmussen 1994).

Robust Redhorse implications

Notchlip Redhorse, the closest surrogate species for Robust Redhorse (based on habitat, diet, and taxonomy), showed generally low metal concentrations; however, As and Se, known hazardous metals, were detected, and at notably high concentrations (Figure 4). Asian Clams, a food source for Notchlip Redhorse and Robust Redhorse, exhibited high concentrations of As, Cr, and Se. The detected concentrations in Notchlip Redhorse and food sources suggested that some metals (As, Cr, and Se) are a potential threat to Robust Redhorse health in the Yadkin-Pee Dee River.

Conclusions

Our findings showed that essential and nonessential metals are present in the Yadkin-Pee Dee River ecosystem. Metal concentrations varied among food web compartments and species. Hg notably exhibited biomagnification potential within the food web, but other metals did not. Hg is a major cause of impairment in freshwater systems and will continue to be of concern (USEPA 2011). The significance of understanding that both diet and water are important routes of exposure for aquatic organisms is illustrated by the contamination in all compartments of the food web in this riverine ecosystem. Our results also confirm the importance of understanding the differences in fish species exposure and accumulation of metals, with direct implications for their potential susceptibility and toxicity. These findings highlight the dynamics and importance of monitoring metals classified as nonessential for aquatic life, and that all metals can pose a threat to ecological health.

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Tables

Table 1. Sediment metal concentrations (µg/g DW) at each site in the Yadkin-Pee Dee River of North Carolina and South Carolina. Lightly shaded values indicate a TEC/LEL threshold has been exceed. Darkly shaded values indicate a PEC/SEL threshold has been exceeded. DL: detection limit (µg/g DW). TEC: threshold effect concentration (µg/g DW; MacDonald et al. 2000). LEL: lowest effect level (µg/g DW Persaud and Jaagumagi 1993). PEC: predicted effect concentration (µg/g DW; MacDonald et al. 2000). SEL: severe effect level (µg/g DW Persaud and Jaagumagi 1993). BDL: below detection limit.

				Site				
Metals	DL	TEC/ LEL*	PEC/ SEL*	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
Aluminum (Al)	0.5	NA	NA	1,506.4	1,192.8	1,619.8	859.4	837.0
Barium (Ba)	0.25	NA	NA	113.8	85.9	177.2	95.7	73.6
Beryllium (Be)	0.25	NA	NA	0.7	0.5	0.9	0.5	0.5
Cadmium (Cd)	0.25	0.99	4.98	2.7	2.9	3.6	1.9	1.8
Cobalt (Co)	0.25	NA	NA	10.3	8.5	15.9	8.4	7.4
Chromium (Cr)	0.25	43.4	111	24.5	19.5	26.1	15.5	15.6
Copper (Cu)	0.25	31.6	149	17.8	21.1	25.8	12.5	10.1
Iron (Fe)	0.5	20,000*	NA	2,447.8	2,617.8	3,154.0	1,693.8	1,598.3
Mercury (Hg)	0.001	0.18	1.06	0.024	0.053	0.062	0.029	0.014
Potassium (K)	0.5	NA	NA	2,184.4	554.1	906.2	747.5	890.6
Magnesium (Mg)	0.5	NA	NA	3,265.7	1,908.7	2,414.9	1,550.0	1,797.9
Manganese (Mn)	0.25	460*	1,100*	540.5	724.3	2,593.9	920.8	458.4
Nickel (Ni)	0.25	22.7	48.6	5.0	7.9	11.8	5.9	4.7
Lead (Pb)	0.25	35.8	128	13.3	9.8	15.2	8.1	8.7
Antimony (Sb)	0.25	NA	NA	<0.25	0.4	0.6	<0.25	<0.25
Silicon (Si)	0.5	NA	NA	1,315.4	544.1	1,262.8	1,117.1	916.5
Strontium (Sr)	0.25	NA	NA	16.3	12.2	21.1	10.1	8.1
Vanadium (V)	0.25	NA	NA	38.4	37.8	46.8	26.7	24.8
Zinc (Zn)	0.25	NA	NA	65.2	28.3	71.3	38.0	35.6

* indicates a LEL or SEL threshold.

Table 2. Mean metal concentration (µg/g DW) and standard deviation (±) of analyzed samples of food web biota and organic matter from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. BDL: below detection limit. n = number of samples analyzed.

Samples	n	% moisture	Al	As	Ba	Be	Cd	Co	Cr	Cu
Detritus	5	84.5	3,234 (± 1,156)	13.7 (± 6.2)	188 (± 23)	1.5 (± 0.6)	2.3 (± 0.5)	31.1 (± 1.2)	37.9 (± 13.1)	15.0 (± 1.2)
Plants	6	86.6	1,852 (± 1,269)	12.4 (± 11.5)	289 (± 74.2)	0.4 (± 0.2)	1.5 (± 0.3)	34.0 (± 11.7)	18.0 (± 11.0)	16.3 (± 4.5)
Mollusks	18	82.4	239 (± 210)	11.4 (± 9.7)	427 (± 642)	0.2 (± 0.04)	0.8 (± 0.5)	3.5 (± 4.2)	4.6 (± 3.3)	61.2 (± 79.8)
Asian Clam	5	82.2	368 (± 348)	18.4 (± 6.1)	18.7 (± 6.5)	0.15 (± 0.04)	1.4 (± 0.5)	4.8 (± 1.4)	8.5 (± 2.5)	50.1 (± 9.9)
Snails	5	78.6	173 (± 163)	16.1 (± 9.3)	14.8 (± 10.0)	BDL	0.8 (± 0.4)	7.0 (± 6.2)	2.0 (± 1.0)	159 (± 95.5)
Unionid mussels	8	85	199 (± 77)	1.8 (± 0.9)	939 (± 678)	BDL	0.5 (± 0.3)	0.6 (± 0.2)	3.8 (± 2.4)	7.3 (± 1.7)
Aquatic insects	27	79.5	925 (± 947)	1.3 (± 1.0)	54.6 (± 114)	0.1 (± 0.05)	0.6 (± 1.0)	3.4 (± 3.5)	1.9 (± 1.6)	25.9 (± 10.6)
Crayfishes	4	74.5	133 (± 41)	BDL	15.9 (± 3.9)	BDL	0.3 (± 0.1)	0.7 (± 0.7)	0.4 (± 0.1)	130 (± 14)
Fishes	87	77.7	4.4 (± 16)	3.2 (± 0.5)	1.5 (± 4.4)	BDL	BDL	BDL	0.3 (± 0.1)	0.9 (± 0.6)
American Eel	8	74.9	1.4 (± 0.6)	BDL	0.4 (± 0.2)	BDL	BDL	BDL	0.2 (± 0.06)	0.7 (± 0.1)
Blue Catfish	10	73.6	1.6 (± 0.9)	BDL	0.2 (± 0.04)	BDL	BDL	BDL	0.2 (± 0.05)	0.6 (± 0.1)
Bluegill	10	80.1	1.0 (± 0.5)	BDL	0.2 (± 0.1)	BDL	BDL	BDL	0.2 (± 0.06)	0.7 (± 0.1)
Channel Catfish	10	81	1.1 (± 0.6)	BDL	0.1 (± 0.04)	BDL	BDL	BDL	0.4 (± 0.2)	0.7 (± 0.1)
Common Carp	10	74	2.2 (± 2.6)	BDL	0.3 (± 0.2)	BDL	BDL	BDL	0.2 (± 0.05)	1.5 (± 0.7)
Largemouth Bass	10	78.5	1.1 (± 0.5)	BDL	0.2 (± 0.1)	BDL	BDL	BDL	0.2 (± 0.07)	0.8 (± 0.2)
Notchlip Redhorse	6	82.1	1.4 (± 0.8)	3.3 (± 0.4)	0.4 (± 0.1)	BDL	BDL	BDL	0.6 (± 0.08)	0.9 (± 0.2)
Shorthead Redhorse	10	78.9	1.6 (± 0.9)	BDL	0.2 (± 0.1)	BDL	BDL	BDL	0.2 (± 0.05)	0.8 (± 0.2)
Smallmouth Buffalo	8	80.9	2.3 (± 1.5)	BDL	0.5 (± 0.4)	BDL	BDL	BDL	0.3 (± 0.09)	0.6 (± 0.1)
Whitefin Shiner *	5	71.9	46.1 (± 51.9)	2.5	15.9 (± 5.7)	BDL	BDL	BDL	0.5 (± 0.04)	3.0 (± 0.3)

Table 2-continued.

Samples	n	% moisture	Fe	Hg	K	Mg	Mn	Mo	Na	Ni
Detritus	5	84.5	20,215 (± 5,607)	0.03 (± 0.01)	855 (± 431)	1,742 (± 211)	2,402 (± 740)	3.0 (± 1.4)	225 (± 52)	22 (± 4.3)
Plants	6	86.6	5,568 (± 4,644)	0.01 (± 0.01)	21,589 (± 10,000)	3,001 (± 615)	7,734 (± 3,036)	5.8 (± 4.0)	2,257 (± 632)	13.3 (± 4.9)
Mollusks	18	82.4	2,306 (± 3,484)	0.2 (± 0.1)	2,324 (± 1,900)	2,370 (± 2,339)	2,895 (± 4,019)	1.4 (± 1.1)	2,025 (± 586)	2.5 (± 2.2)
Asian Clam	5	82.2	659 (± 463)	0.1 (± 0.04)	1,416 (± 96)	809 (± 107)	63 (± 27)	2.1 (± 0.6)	2,052 (± 407)	2.1 (± 0.9)
Snails	5	78.6	400 (± 231)	0.2 (± 0.3)	4,735 (± 2,284)	5,606 (± 2,118)	228 (± 225)	2.2 (± 0.9)	2,267 (± 768)	4.6 (± 3.6)
Unionid mussels	8	85	4,528 (± 4,377)	0.2 (± 0.04)	1,385 (± 188)	1,322 (± 496)	6,332 (± 3,859)	0.3 (± 0.1)	1,856 (± 572)	1.6 (± 0.5)
Aquatic insects	27	79.5	1,643 (± 1,219)	0.07 (± 0.02)	6,388 (± 1,983)	1,008 (± 294)	955 (± 945)	0.9 (± 0.6)	4,213 (± 1,987)	1.5 (± 0.7)
Crayfishes	4	74.5	381 (± 73)	0.09 (± 0.01)	8,399 (± 1,139)	1,168 (± 167)	159 (± 31)	0.2	6,607 (± 649)	1.1 (± 0.4)
Fishes	87	77.7	16 (± 25)	0.9 (± 0.6)	17,639 (± 3,419)	1,326 (± 236)	3.1 (± 7.3)	0.09 (± 0.02)	1,882 (± 547)	BDL
American Eel	8	74.9	7.1 (± 2.6)	0.5 (± 0.2)	12,334 (± 1,802)	865 (± 138)	3.1 (± 1.9)	0.08 (± 0.02)	2,241 (± 611)	BDL
Blue Catfish	10	73.6	7.8 (± 2.6)	0.9 (± 0.6)	18,722 (± 2,649)	1,226 (± 217)	1.1 (± 0.3)	0.1 (± 0.01)	1,743 (± 441)	BDL
Bluegill	10	80.1	8.2 (± 2.9)	0.7 (± 0.6)	17,939 (± 1,130)	1,448 (± 44.5)	1.1 (± 0.5)	0.1 (± 0.02)	2,520 (± 364)	BDL
Channel Catfish	10	81	7.2 (± 1.7)	0.6 (± 0.2)	20,992 (± 1,037)	1,382 (± 48.0)	0.9 (± 0.3)	0.1 (± 0.01)	1,902 (± 242)	BDL
Common Carp	10	74	31.2 (± 16.1)	1.2 (± 0.6)	17,598 (± 2,064)	1,226.5 (± 124)	0.9 (± 0.3)	0.1 (± 0.02)	1,233 (± 252)	BDL
Largemouth Bass	10	78.5	6.3 (± 1.3)	1.2 (± 0.7)	19,003 (± 2,190)	1,395 (± 158)	0.3 (± 0.1)	0.1 (± 0.02)	1,781 (± 423)	BDL
Notchlip Redhorse	6	82.1	11.4 (± 2.3)	0.7 (± 0.4)	18,284 (± 1,247)	1,715 (± 55.2)	1.1 (± 0.4)	BDL	2,184 (± 274)	BDL
Shorthead Redhorse	10	78.9	9.6 (± 3.5)	1.0 (± 0.5)	19,626 (± 3,126)	1,374 (± 177)	1.3 (± 0.7)	0.1 (± 0.01)	1,733 (± 396)	BDL
Smallmouth Buffalo	8	80.9	11.3 (± 5.2)	1.3 (± 0.7)	16,819 (± 2,719)	1,335 (± 229)	3.1 (± 2.6)	0.1 (± 0.01)	1,369 (± 278)	BDL
Whitefin Shiner *	5	71.9	93.0 (± 59.3)	0.2 (± 0.1)	10,578 (± 968)	1,389 (± 130)	31.6 (± 5.0)	BDL	2,524 (± 379)	BDL

*Whole body concentrations

Table 2-continued.

Samples	n	% moisture	Pb	Sb	Se	Si	Sr	V	Zn
Detritus	5	84.5	27 (± 26)	BDL	BDL	384 (± 169)	105 (± 31)	71 (± 22)	62 (± 29)
Plants	6	86.6	9.1 (± 3.6)	BDL	BDL	336 (± 211)	109 (± 20.5)	24.2 (± 10.2)	92.9 (± 50.3)
Mollusks	18	82.4	1.9 (± 1.9)	BDL	5.9 (± 2.6)	88.0 (± 38.5)	161 (± 177)	2.6 (± 2.1)	198 (± 138)
Asian Clam	5	82.2	1.4 (± 1.4)	BDL	7.7 (± 1.6)	82 (± 50)	16 (± 3.8)	3.3 (± 2.7)	173 (± 47)
Snails	5	78.6	1.4 (± 0.8)	BDL	3.6 (± 1.6)	57.2 (± 26.6)	68.2 (± 45.9)	1.8 (± 1.2)	237 (± 192)
Unionid mussels	8	85	2.5 (± 2.6)	BDL	BDL	107 (± 26)	309 (± 169)	BDL	189 (± 148)
Aquatic insects	27	79.5	2.1 (± 1.9)	0.4 (± 0.2)	BDL	71 (± 56)	6.8 (± 4.1)	3.0 (± 2.1)	161 (± 94)
Crayfishes	4	74.5	BDL	BDL	BDL	29 (± 4)	48 (± 14)	0.4 (± 0.1)	135 (± 10)
Fishes	87	77.7	0.1 (± 0.1)	0.1	1.8 (± 2.2)	8.4 (± 8.4)	7.9 (± 28)	0.2 (± 0.2)	30.1 (± 43)
American Eel	8	74.9	0.1 (± 0.06)	BDL	0.2	3.8 (± 0.6)	2.3 (± 1.7)	0.1 (± 0.01)	52.8 (± 9.9)
Blue Catfish	10	73.6	5.5 (± 0.02)	BDL	BDL	5.5 (± 2.1)	0.8 (± 0.2)	BDL	15.1 (± 2.7)
Bluegill	10	80.1	0.1 (± 0.04)	BDL	0.7 (± 0.6)	8.2 (± 2.5)	1.4 (± 1.3)	BDL	24.6 (± 3.8)
Channel Catfish	10	81	0.1 (± 0.03)	BDL	BDL	5.6 (± 1.5)	0.9 (± 0.2)	BDL	18.4 (± 0.8)
Common Carp	10	74	0.1 (± 0.01)	BDL	0.5 (± 0.3)	6.7 (± 1.7)	1.4 (± 1.0)	BDL	15.9 (± 2.5)
Largemouth Bass	10	78.5	0.1 (± 0.04)	BDL	0.6	7.6 (± 2.1)	0.7 (± 0.9)	BDL	15.0 (± 3.1)
Notchlip Redhorse	6	82.1	0.1	BDL	4.7 (± 2.6)	BDL	1.0 (± 0.5)	BDL	17.8 (± 1.4)
Shorthead Redhorse	10	78.9	0.1 (± 0.03)	0.1	0.2 (± 0.02)	7.7 (± 3.0)	1.3 (± 0.8)	BDL	16.2 (± 3.2)
Smallmouth Buffalo	8	80.9	0.1 (± 0.02)	BDL	1.2 (± 0.6)	9.9 (± 3.2)	2.0 (± 2.1)	BDL	13.3 (± 2.2)
Whitefin Shiner *	5	71.9	0.6	BDL	2.7	37.9 (± 21.8)	116 (± 32.9)	0.2 (± 0.2)	193 (± 40.1)

*Whole body concentrations

Table 3. Stable isotope ratio means and standard deviations of aquatic food web biota and organic matter from five sites of the Yadkin-Pee Dee River of North Carolina and South Carolina.

	801		Red Hill		Digg's Tract		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.62 ± 1.05	2.83 ± 0.21	-30.0 ± 0.64	3.83 ± 1.03	-30.26 ± 0.76	5.75 ± 0.17	3.69 ± 0.3
Plants	-27.86 ± 0.99	12.52 ± 2.52	-31.42	13.7	-26.38 ± 1.24	12.08 ± 1.99	5.17 ± 0.96
Mollusks	-24.72 ± 2.36	10.07 ± 1.43	-28.58 ± 1.97	15.55 ± 0.75	-27.05 ± 3.15	12.41 ± 1.6	4.49 ± 1.39
Asian Clam	-26.76 ± 0.34	8.84 ± 0.25	-28.68 ± 0.04	15.33 ± 0.27	-28.66 ± 0.03	12.34 ± 0.06	5.02 ± 1.52
Snails	-22.69 ± 0.06	11.3 ± 0.11	-25.56 ± 0.27	14.68 ± 0.01	-24.34 ± 1.87	12.28 ± 2.35	3.49 ± 1.01
Unionid mussels	–	–	-30.04 ± 0.36	16.1 ± 0.57	-30.30 ± 0.36	12.65 ± 0.32	5.73 ± 0.56
Insects	-27.49 ± 1.31	9.97 ± 1.18	-27.46 ± 1.52	14.82 ± 2.22	-27.59 ± 1.5	12.68 ± 2.2	4.2 ± 0.81
Collector	-29.41 ± 2.59	9.53 ± 0.72	-29.34 ± 0.71	11.98 ± 0.85	-29.13 ± 0.47	10.46 ± 0.06	2.55 ± 1.05
Filterer	-27.4 ± 0.26	9.65 ± 0.57	–	–	-31.12	14.02	4.65
Predator	-27.02 ± 0.76	10.08 ± 1.47	-26.99 ± 1.35	14.59 ± 0.67	-27.12 ± 1.29	13.05 ± 1.37	4.36 ± 0.57
Predator/Parasite	–	–	-25.89 ± 0.04	18.26 ± 3.34	-26.06 ± 0.29	15.85 ± 2.36	4.44 ± 0.74
Scraper	-27.62 ± 0.67	10.63 ± 0.15	-28.57 ± 0.4	14.9 ± 0.21	-27.65 ± 0.43	11.15 ± 2.35	4.48 ± 0.3
Crayfishes	-24.55 ± 0.6	10.40 ± 0.01	-24.9 ± 0.87	13.45 ± 0.69	-24.64 ± 0.57	12.89 ± 1.53	5.21 ± 0.19
Fishes	-25.95 ± 0.87	13.07 ± 1.54	-25.32 ± 1.38	16.15 ± 2.63	-27.13 ± 1.46	15.88 ± 1.47	5.84 ± 0.73
American Eel	–	–	-27.09 ± 0.72	17.87 ± 2.3	-30.15 ± 1.28	16.6 ± 0.54	6.22 ± 0.29
Blue Catfish	-26.69 ± 0.71	14.89 ± 0.81	-26.05 ± 0.71	18.06 ± 1.03	-27.16 ± 1.26	17.06 ± 1.17	5.61 ± 0.36
Bluegill	-25.42 ± 0.7	12.18 ± 0.32	-25.03 ± 0.64	14.75 ± 1.1	-26.12 ± 0.31	15.0 ± 0.34	5.57 ± 0.14
Channel Catfish	-26.01 ± 0.54	12.23 ± 1.1	-25.82 ± 0.82	14.82 ± 2.69	-28.69 ± 0.4	16.1 ± 0.11	5.89 ± 0.32
Common Carp	-26.19 ± 0.98	12.84 ± 1.86	-26.27 ± 0.73	11.83 ± 1.42	-26.92 ± 0.52	14.39 ± 0.87	5.43 ± 1.38
Largemouth Bass	-26.13 ± 1.28	14.64 ± 1.33	-25.93 ± 0.99	17.33 ± 4.01	-26.0 ± 0.45	17.47 ± 1.18	6.44 ± 0.63
Notchlip Redhorse	-27.32 ± 1.32	11.6 ± 0.19	-24.66 ± 0.17	16.5 ± 0.62	-26.99 ± 0.73	14.5 ± 2.42	5.08 ± 1.19
Shorthead Redhorse	-25.46 ± 0.42	13.34 ± 0.17	-22.81 ± 1.18	18.13 ± 0.18	-27.69 ± 0.99	16.9 ± 0.41	6.29 ± 0.15
Smallmouth Buffalo	–	–	-25.05 ± 1.07	15.05 ± 2.05	-28.09 ± 1.3	15.87 ± 0.95	5.91 ± 0.48
Whitefin Shiner*	-25.32 ± 0.09	11.45 ± 0.97	-24.36 ± 0.83	17.21 ± 1.15	-25.0 ± 0.34	15.28 ± 0.57	6.14 ± 0.47

*Whitefin Shiners are reported as whole-body concentrations

Table 3-continued.

	Society Hill			Pee Dee		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.9 ± 0.52	5.48 ± 0.06	4.07 ± 1.4	-30.04 ± 0.46	5.54 ± 0.66	3.57 ± 1.42
Plants	-26.36 ± 0.91	14.97 ± 0.6	3.83 ± 1.24	–	–	–
Mollusks	-28.07 ± 1.78	9.41 ± 0.55	4.5 ± 0.8	-32.0 ± 0.41	11.35 ± 0.4	4.95 ± 0.36
Asian Clam	-28.92 ± 0.32	8.68 ± 0.45	3.87 ± 0.57	-32.10 ± 0.02	11.18 ± 0.59	4.72 ± 0.27
Snails	-25.28 ± 0.01	9.4 ± 0.17	4.98 ± 0.11	–	–	–
Unionid mussels	-29.04 ± 0.65	9.78 ± 0.32	4.59 ± 0.98	-31.96 ± 0.52	11.43 ± 0.35	5.06 ± 0.37
Insects	-28.36 ± 2.11	10.13 ± 2.13	4.27 ± 1.01	-30.72 ± 2.54	10.90 ± 1.41	3.96 ± 0.9
Collector	-29.95 ± 2.08	9.1 ± 0.74	3.53 ± 1.41	-31.94 ± 3.19	10.83 ± 0.48	3.64 ± 1.16
Filterer	–	–	–	-34.03 ± 0.06	11.69 ± 0.25	4.4 ± 0.76
Predator	-28.75 ± 0.86	10.02 ± 0.92	4.55 ± 0.75	-29.91 ± 1.38	10.55 ± 1.76	4.0 ± 0.86
Predator/Parasite	-24.58 ± 0.36	15.18 ± 0.22	4.73 ± 0.19	-25.99	13.04	3.51
Scraper	-26.61 ± 0.06	8.11 ± 0.42	4.61 ± 0.52	-30.79	10.94	4.77
Crayfishes	-25.16 ± 0.42	10.98 ± 1.22	4.86 ± 0.56	-28.09 ± 0.84	13.13 ± 0.07	5.97 ± 0.11
Fishes	-26.16 ± 1.5	13.14 ± 1.39	5.13 ± 1.33	-27.09 ± 1.37	12.87 ± 0.95	5.02 ± 1.29
American Eel	-25.62 ± 0.61	13.19 ± 0.56	4.58 ± 0.77	-27.15 ± 0.42	12.59 ± 0.77	4.69 ± 0.75
Blue Catfish	-27.04 ± 0.75	13.59 ± 0.95	4.8 ± 1.26	-26.56 ± 0.63	13.26 ± 0.43	4.59 ± 0.45
Bluegill	-27.25 ± 3.14	12.54 ± 0.5	5.21 ± 0.77	-26.71 ± 1.77	12.79 ± 0.79	3.84 ± 0.92
Channel Catfish	-25.78 ± 0.55	12.4 ± 0.89	4.7 ± 0.61	-26.34 ± 0.6	12.85 ± 0.74	5.19 ± 0.6
Common Carp	-26.02 ± 0.6	12.58 ± 0.66	4.59 ± 0.74	-27.73 ± 0.8	11.87 ± 1.42	4.54 ± 0.74
Largemouth Bass	-25.53 ± 0.39	15.75 ± 0.79	5.93 ± 0.8	-27.04 ± 0.62	14.17 ± 0.92	5.63 ± 0.52
Notchlip Redhorse	–	–	–	–	–	–
Shorthead Redhorse	-27.12 ± 2.48	13.14 ± 1.56	4.72 ± 0.97	-28.21 ± 0.81	13.4 ± 0.36	5.48 ± 0.18
Smallmouth Buffalo	-26.43 ± 0.42	11.86 ± 1.6	3.83 ± 0.8	-29.29 ± 0.73	12.4 ± 0.13	3.72 ± 0.73
Whitefin Shiner*	-24.7 ± 0.67	13.22 ± 1.01	7.84 ± 0.59	-25.08 ± 0.6	12.64 ± 0.87	7.63 ± 0.94

*Whitefin Shiners are reported as whole-body concentrations

Table 4. Arsenic (As), chromium (Cr), mercury (Hg), and lead (Pb) trophic magnification factors (TMFs) for consumers in the Yadkin-Pee Dee River of North Carolina and South Carolina, n = number of samples analyzed.

Species	n	As TMF	Cr TMF	Hg TMF	Pb TMF
All consumers	136	0.60	0.60	1.62	0.54
Mollusks	18	2.37	0.11	0.76	10.94
Aquatic insects	27	0.25	0.39	0.49	0.21
Crayfishes	4	–	–	0.98	0.77
Fishes	87	1.00	0.98	0.90	1.04
American Eel	8	–	1.27	0.50	1.75
Blue Catfish	10	–	1.01	1.19	0.90
Bluegill	10	–	1.05	1.19	0.78
Channel Catfish	10	–	1.14	0.84	1.19
Common Carp	10	–	0.77	0.73	0.92
Largemouth Bass	10	–	1.18	0.76	0.72
Notchlip Redhorse	6	0.91	0.82	0.72	–
Shorthead Redhorse	10	–	0.92	0.95	1.06
Smallmouth Buffalo	8	–	0.88	0.78	1.09
Whitefin Shiner*	5	–	0.98	0.92	–

* Based on whole-body concentrations

Figures

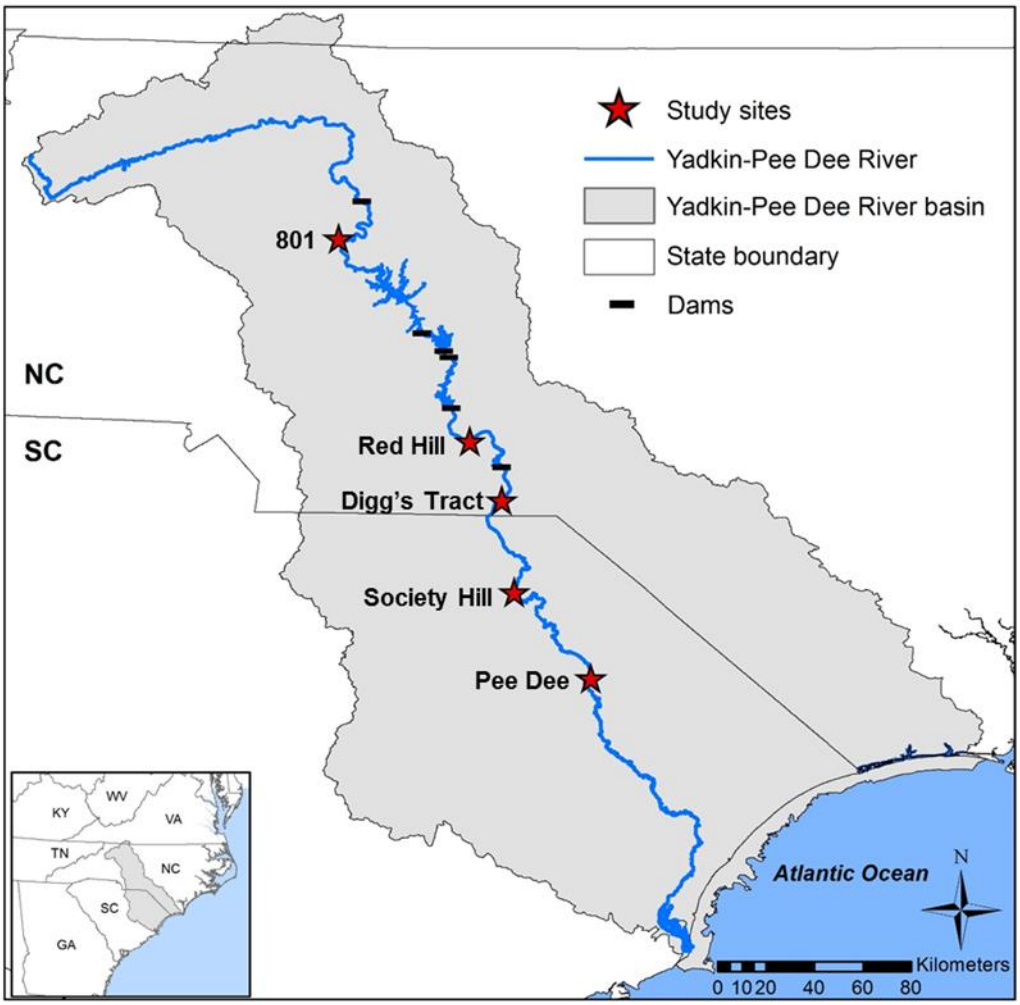

Figure 1. Study sites and major dams along the Yadkin-Pee Dee River of North Carolina and South Carolina.

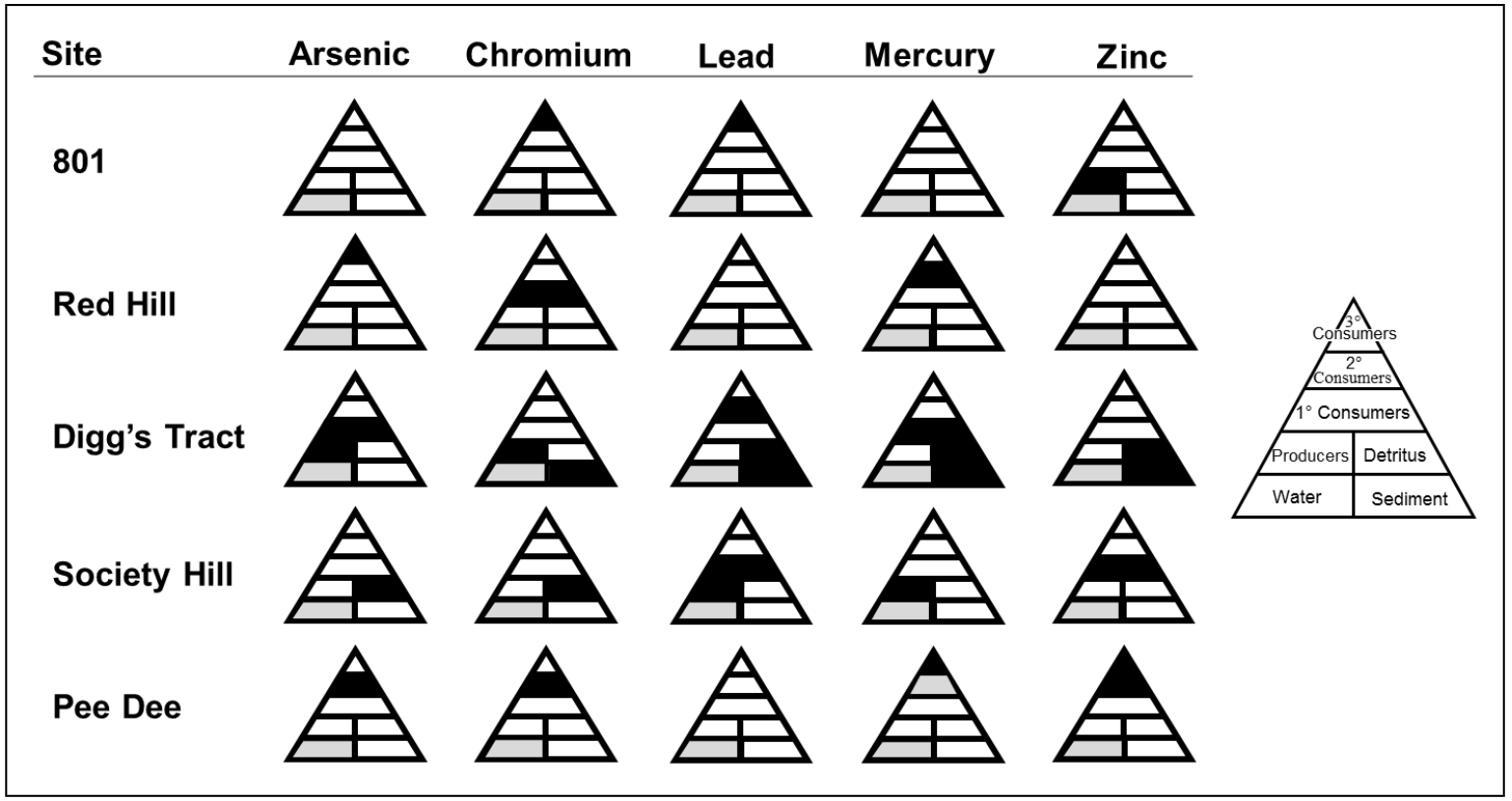

Figure 2. Summary of selected metals among sites in the Yadkin-Pee Dee River of North Carolina and South Carolina. For each triangle, a solid black section represents the greatest measured mean concentration of an organic contaminant for the corresponding food web compartment among sites. A gray filled section indicates that there were no samples in that compartment that were analyzed.

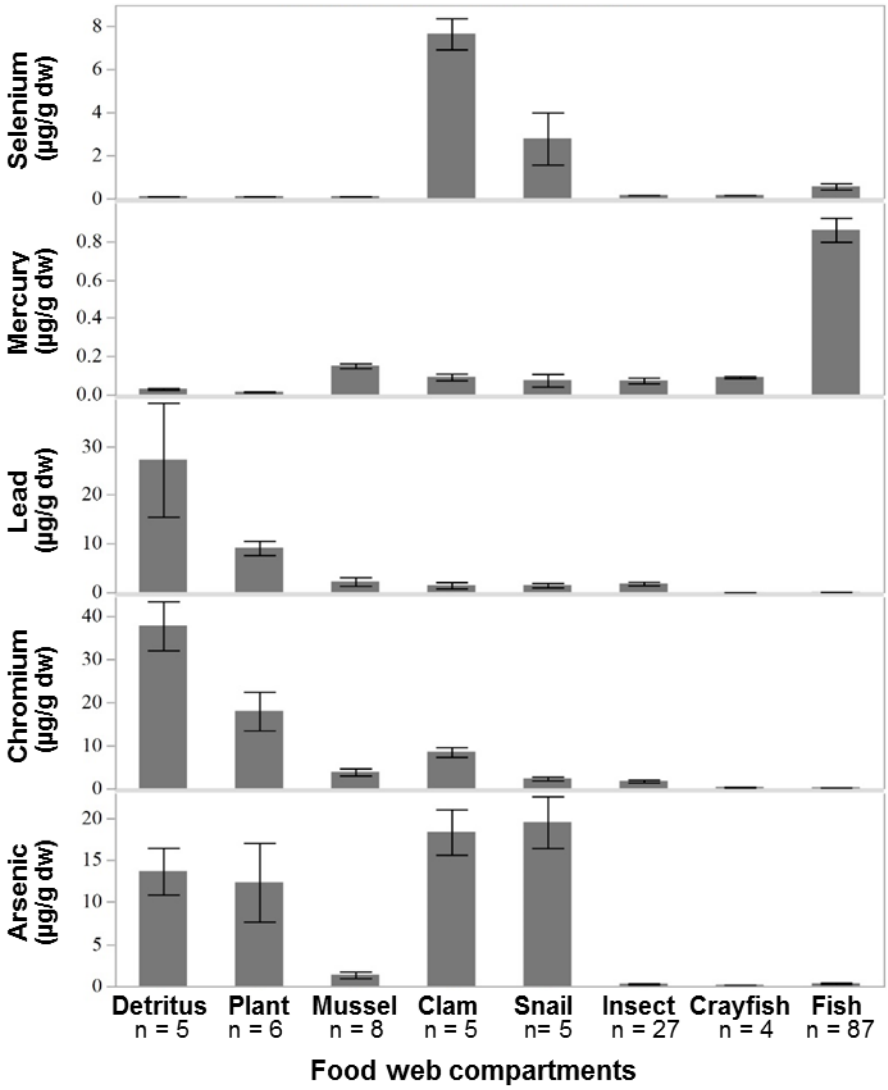

Figure 3. Mean (± SE) metal concentrations (µg/g dry weight) for detritus, plant, mussel, clam, snail, insect, crayfish, and fish samples at five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina, n = number of samples analyzed.

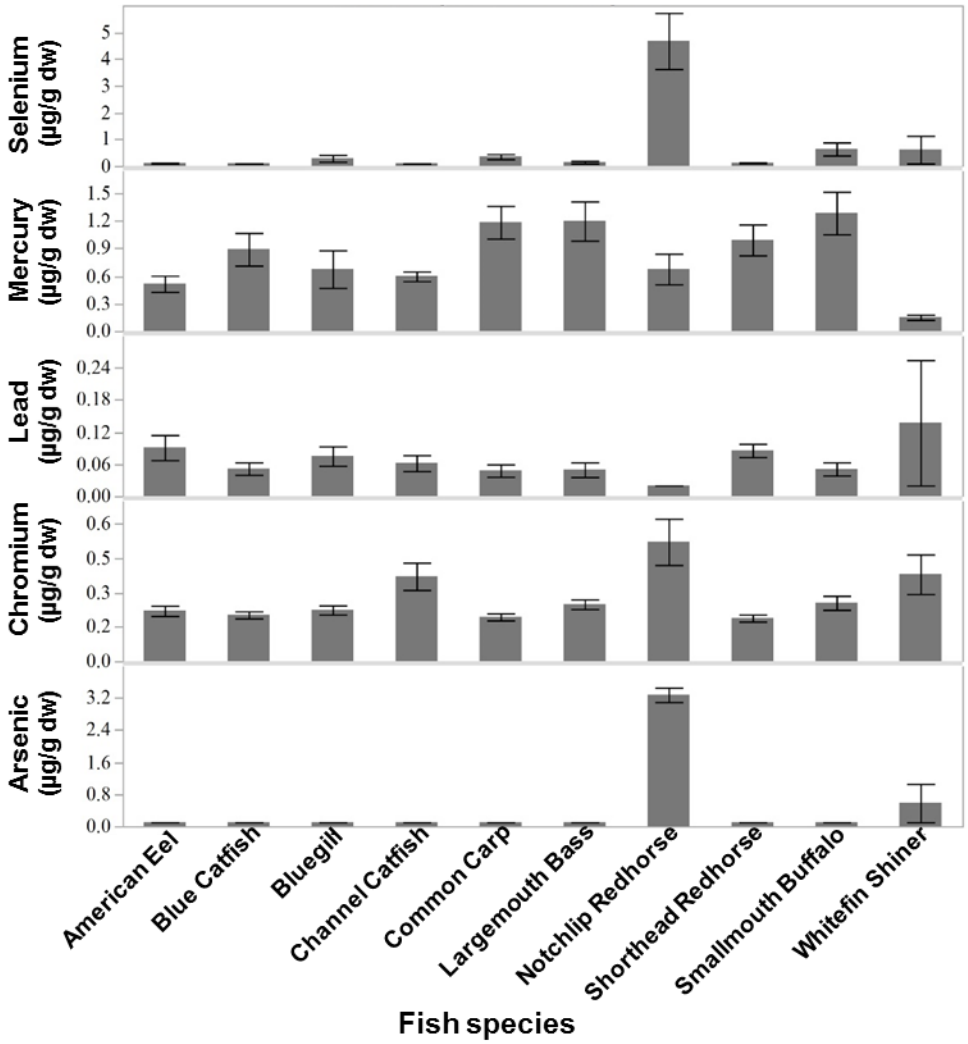

Figure 4. Mean (± SE) metal concentrations (µg/g dry weight) for fish species at five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina.

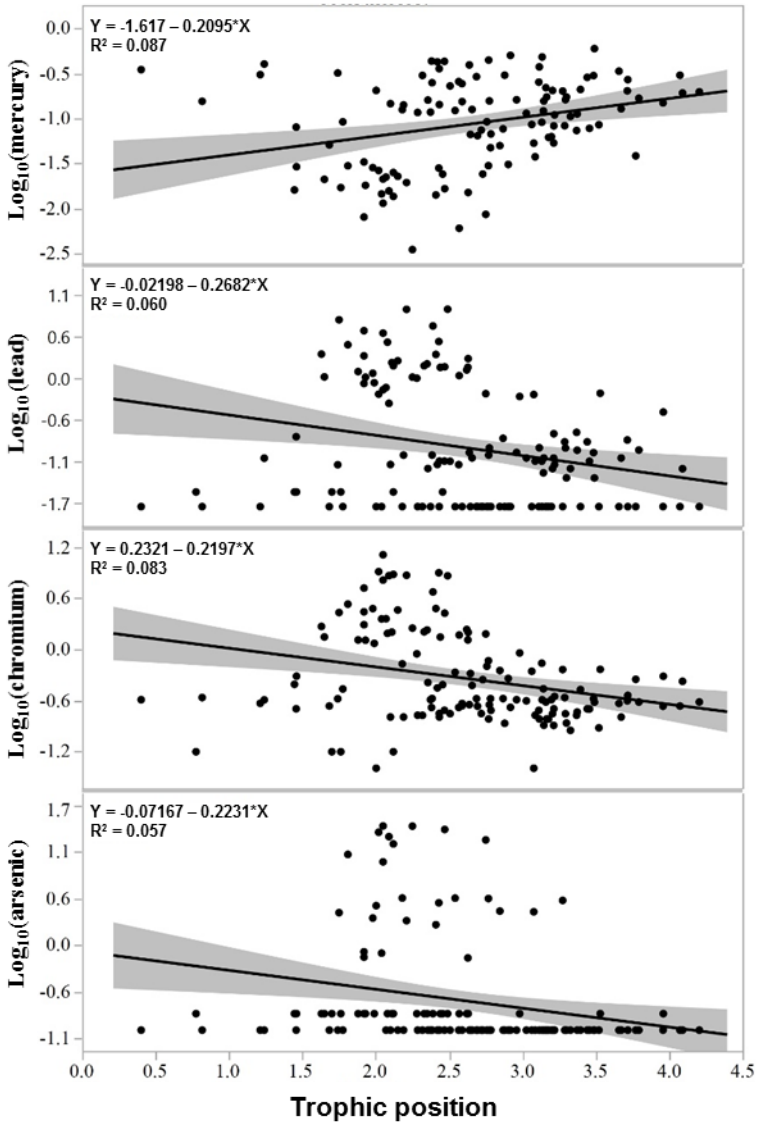

Figure 5. Simple linear regression of $\log_{10}$ transformed metal concentrations (µg/g dry weight) and trophic position of consumer samples from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. Regression equation and R^2 value given for each metal. Gray area indicates the 95% confidence interval of the fitted line.

CHAPTER 3

Trophodynamics of Perfluorinated Compounds in a Large River Food Web

Abstract

Contaminants of emerging concern are present globally in water, sediment, and aquatic biota and pose a potential threat to human, wildlife, and ecological health. Understanding the sources, fate, and transport of new classes of contaminants is essential to characterize ecological exposure and risk. Perfluoroalkyl acids (PFAAs) are one such class of compounds that have attracted substantial scientific and regulatory attention due to their persistence, bioaccumulative potential, toxicity, and global distribution. The objectives of this research were to determine the trophic transfer and accumulation of 14 PFAAs within a riverine food web. Major food web components and pathways were determined by stable isotope analyses of representative producers, consumers, and organic matter. Contaminant analyses performed on water, sediment, organic matter, and aquatic biota revealed that PFAAs were prevalent in samples of all food web components, with the most detections and greatest concentrations occurring in aquatic insects. All 14 PFAAs were detected in aquatic insect samples (range, below detection limit [BDL] – 1,670.1 ng/g wet weight [WW]) and fish tissues (range, BDL – 798 ng/g WW). Perfluorooctane sulfonate (PFOS) was the dominant PFAA among all samples (67%). Robust Redhorse (*Moxostoma robustum*) ova had concentrations above detection limits for 10 PFAAs (range, BDL – 482.88 ng/g WW). Generally, the ova sample showed higher concentrations compared to fish muscle tissue.

PFOS concentration in the ova sample was exceptionally high (482.88 ng/g WW). Ova sample results indicated that maternal transfer of PFAAs is likely occurring. Our results demonstrated the prevalence of PFAAs in a freshwater environment and aquatic biota. The observed contamination of PFAAs in all compartments of the food web confirms the importance of examining routes of exposure to better understand contaminant dynamics in freshwater lotic systems.

Introduction

New classes of contaminants are being measured in the environment and are an emerging concern as a potential global threat to human, wildlife, and ecological health. Perfluoroalkyl acids (PFAAs) are one such class of compounds that have attracted substantial scientific and regulatory attention as persistent contaminants in the environment. PFAAs are artificial compounds with many residential, commercial, industrial, and pharmaceutical uses that make these compounds economically valuable (Giesy and Kannan 2001; Houde et al. 2006; Ahrens and Bundschuh 2014). These fluorinated compounds were developed to resist oil, soil, and water (OECD 2002), which makes for beneficial applications, but also yields highly persistent chemicals in the environment. In the past, PFAAs were used in such products as surfactants, coating additives in paints and polishes, firefighting foams, cleaning products, stain repellents (e.g., Scotchgard^{TM}), and pesticides (Kissa 2001; OECD 2002).

Recent ecotoxicological research has focused on PFAAs as contaminants of concern, especially for aquatic biota (Houde et al. 2011). PFAAs are known for their persistence, bioaccumulative potential, toxicity, and global distribution (Giesy and Kannan 2002; Houde et al. 2006; Ahrens and Bundschuh 2014). Carbon-fluorine bonds make these compounds chemically stable and resistant to degradation in the environment (Kissa 2001; Giesy and Kannan 2002; Houde et al. 2011). PFAAs with longer chain lengths and more fluorinated carbons (i.e., $C \geq 7$) tend to have increased bioaccumulation potential (Martin et al. 2003; Giesy et al. 2010; Houde et al. 2011). The most recognized and researched PFAAs in the environment are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Various studies have examined the presence and effects of PFOS and PFOA in aquatic

environments and biota, but very few have examined their transport, and effects in lotic ecosystems (Ding and Peijnenburg 2013).

Beginning in 2001, companies began voluntarily phasing out the production of PFOA, PFOS, and many related compounds (OECD 2002; EPA 2009), but PFOS-related substances continue to be used for metal plating, hydraulic fluids, and in the photography industry (EPA 2009). Long chain PFAAs are under strict regulations, and the complete elimination of long chain PFAA production is in progress for major manufacturing companies (USEPA 2015). Despite the decrease in production, widespread input and cycling of PFAAs will continue from long range transport, degradation of PFAA precursors, legacy products, or remobilization from other media (e.g., sediment, ice, or soil) (Houde et al. 2001; Buck et al. 2011; Ahrens and Bundschuh 2014). Current major sources of PFAAs are landfills and industrial and municipal sewage treatment effluent (Ahrens 2011; Ahrens and Bundschuh 2014).

PFAAs have been detected in wildlife worldwide (Giesy and Kannan 2001). PFOS and PFOA were typically the dominant compounds found in surface waters, sediment, aquatic organisms, and their consumers (Giesy and Kannan 2001; Higgins et al. 2005; Prevedouros et al. 2006; EPA 2009; Houde et al. 2011). Published toxicological effects are limited, but various experimental research indicated moderate acute and chronic effects in a variety of aquatic organisms (Ding and Peijnenburg 2013). Evidence has shown 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 (Giesy et al. 2010; Houde et al. 2011; Ahrens and Bundschuh 2014). Other studies have indicated that maternal transfer of PFAAs

can lead to high concentrations in eggs and embryos causing potential adverse effects (Peng et al. 2010; Sharpe et al. 2010; Houde et al. 2011).

The Yadkin-Pee Dee River of North Carolina and South Carolina is subject to a multitude of anthropogenic contaminant inputs and a model ecosystem to study associated effects. Over 6,115 river kilometers are classified as impaired waters within this basin due to high levels of sediment and contaminants in surface water and fish tissue (NCDEQ 2012; SCDHEC 2015). The Yadkin-Pee Dee River basin is an important aquatic ecosystem that provides habitat for over 50 imperiled aquatic species in need of conservation management (NCWRC 2005; SCDNR 2005). A fish species in critical need of conservation and recovery effort in the Yadkin-Pee Dee River is the Robust Redhorse (*Moxostoma robustum*). The current estimated spawning population for the Yadkin-Pee Dee River is less than 50 individuals (RRCC 2009). Currently sufficient information for PFAAs is lacking to guide management strategies for Robust Redhorse population recovery for the Yadkin-Pee Dee River ecosystem. To our knowledge, PFAAs had not been analyzed and assessed previously to the extent required to inform decisions on river and fisheries management, ecological integrity, and public health.

Stable isotope ratios are a useful technique to assess the transfer of PFAAs by trophic pathways and the extent of exposure through dietary routes. Stable isotopes provide dietary information from assimilated food sources integrated over time. Carbon ($\delta^{13}C$) values are used to reveal food sources, nitrogen ($\delta^{15}N$) values are used to estimate trophic position of consumers and dietary exposure to contaminants, and sulfur ($\delta^{34}S$) values are used to determine large-scale movement patterns by differentiating between marine inputs and

freshwater sources (Fry 1991). Stable isotopes also detect food web responses to environmental and anthropogenic influences (Michener and Lajtha 2008). 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 PFAAs, this study aimed to analyze a wide range of biotic and abiotic components of the Yadkin-Pee Dee River aquatic food web. Samples included water, sediment, detritus, algae, biofilm, macrophytes, aquatic insects, crayfishes, mollusks, fishes, and fish ova. The 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 PFAAs, (3) determine contamination trends among sites along a longitudinal gradient, and (4) assess bioaccumulation and biomagnification of PFAAs by examining chemical concentrations within each trophic compartment of the food web. The overall aim of this research was to understand contaminant dynamics within a riverine food web and reveal potential stressors to common and imperiled species in the Yadkin-Pee Dee River.

Methods

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 SI 1). Physical characteristics, land use, hydrology, and influx of point and nonpoint source pollution differed among sites, which facilitated longitudinal examination of trophic

and contaminant dynamics. Site selections were 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, sites included the location near where the Robust Redhorse was first described but no longer exists (site 801), where the Robust Redhorse population is extant (Digg's Tract, Society Hill, and Pee Dee), and a proposed reintroduction site to stock future hatchery-propagated Robust Redhorse (Red Hill).

Sample collection and preparation

Intensive sample collection for organic matter and aquatic biota was conducted at all five sites during spring and summer 2015. When feasible, taxa collected for trophic and contaminant analyses were the same among sites. Fishes (10 species), mollusks (4 families), crayfishes (2 species), aquatic insects (12 families), macrophytes, and detritus were collected from each site for SIA to determine the major components of the food web, trophic pathways, and bioaccumulation of contaminants. Collection and processing methods were similar to those of Hoeinghaus et al. (2007) and Pingram et al. (2014).

Water: Water grab samples were collected at all five sites on the same day in February 2016. Water was collected mid-stream with pre-cleaned polypropylene bottles and placed into a cooler for transport to the laboratory for processing, extraction, and analysis.

Sediment: Composite samples of sediment were collected from all five sites in February 2016. Each sample was collected with pre-cleaned stainless steel utensils from the top 3-5 cm of the sediment surface layer from several locations within a 1 m^2 area. Sediment

samples were placed into labeled sterile amber glass jars and held in a cooler for transportation to the laboratory where samples were stored in a -80° C freezer.

Biota and organic matter: Detritus samples included leaf packs and suspended particulate organic matter. 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. Suspended particulate organic matter (i.e., drift) samples were collected with 500-µm mesh drift nets. Drift samples were rinsed to remove invertebrates, and placed into sealable plastic bags or amber glass jars and held on ice. 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 lab 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 site at Pee Dee lacked sufficient macrophytes for a sample.

Aquatic insects and crayfishes were collected with 500-µm mesh D-frame nets, by flipping rocks, or by hand from leaf packs and woody debris. Specimens were stored in containers with filtered site water and chilled for at least 8 h to enable depuration of gut contents. Aquatic insects were sorted and classified into functional feeding guilds: collector-filterer, shredder, scraper, or predator. When feasible, insects were identified to a minimum of family taxonomic level (Brachycentridae, Corydalidae, Elmidae, Gerridae, Glossiphoniidae, Gyrinidae, Heptageniidae, Hydropsychidae, Limnephilidae, and Perlidae). Odonates were grouped by suborder (Anisoptera and Zygoptera). Mollusks were collected by hand and included native freshwater mussels (family Unionidae), snails (Pleuroceridae and

Viviparidae), and clams (*Corbicula fluminea*). Snails were held in a container of filtered site water for at least 8 h to enable depuration of gut contents. Mollusks were identified to species.

Fishes were collected by boat-mounted, pulsed-DC electrofishing with efforts to retain similar size classes of the same species among sites. Fishes were euthanized by immediate placement into an ice-water slurry to induce temperature shock according to North Carolina State University approved protocols (IACUC 15-042-O). Species, total length (mm), and wet weight (g) were recorded for all fishes. Fish species, 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 bubalus*), and Whitefin Shiner (*Cyprinella nivea*), were collected to represent the variety of trophic guilds found in the Yadkin-Pee Dee River. All samples were stored frozen at -20° C until further processing.

Stable isotope analysis

All sample processing methods were performed with sterile, stainless steel utensils and all surfaces were cleaned with lab detergent, a distilled water rinse, an acetone rinse, and another distilled water rinse between samples to avoid contamination. All samples were dried at 60° C to a constant weight, ground to a fine powder with a mortar and pestle, and then placed into 7-mL scintillation glass vials for storage. Processed samples were transported by overnight carrier to the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Flagstaff, for analysis of carbon, nitrogen, and sulfur isotope ratios. Samples

were weighed, encapsulated in tin, and then analyzed with a gas isotope-ratio mass spectrometer using their approved standard methods.

Stable isotope ratio results were expressed as delta (δ) notation in parts per thousand (‰) relative to standards according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1{,}000. \qquad (1)$$

X is ^{13}C, ^{15}N, or ^{34}S, and *R* is the corresponding heavy isotope to light isotope ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$). The standard materials are Vienna Pee Dee Belemnite limestone for carbon, atmospheric nitrogen for nitrogen, and Canyon Diablo Troilite for sulfur. Samples were not lipid normalized due to the low lipid content of animals and %C content of plants (Skinner et al. 2016). Most food sources have distinct $\delta^{13}C$ signatures that are conserved within 1‰ in consumer tissues, which makes it possible to discern the sources that contribute to the consumer's diet (Finlay et al. 2002; Hoeinghaus et al. 2007).

Contaminant analysis

All samples were analyzed at the U.S. Environmental Protection Agency's (EPA) Office of Research and Development laboratory (Durham, North Carolina). All supplies used in processing and analysis were previously verified to be free of PFAAs. 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 perfluorobutric acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUA), perfluorododecanoic acid (PFDoA), perfluorobutane sulfonate (PFBS), PFOS, perfluorohexane sulfonate (PFHS),

perfluorotridecanoate acid (PFTrA), and perfluorotetradecanoic acid (PFTA). Only aquatic insects, organic matter, plants, and a subset of fish were analyzed for PFUA, PFDoA, PFTrA, and PFTA compounds.

Water: Water samples were transferred into a methanol-rinsed (MeOH) graduated cylinder to record volume. Ten mL of MeOH was added into emptied sample bottles and shaken. Samples were poured back into the bottles and mixed with the MeOH. A solution of isotopically labeled standards PFHxA, PFHxS, PFNA, PFDA, and PFOS was added to each sample and mixed well. Samples were filtered through glass fiber filters under gentle vacuum. PFAAs were extracted using a conditioned and equilibrated dual piston pump system and 3-mL Oasis WAX solid phase extraction cartridges (Waters, Milford, Massachusetts). Eluate was concentrated under nitrogen, combined with 2-mM ammonium acetate buffer in autosampler vials, vortexed, and analyzed with ultra-high performance liquid chromatography (UPLC) with tandem mass spectrometry (MS/MS).

Sediment: After field collection, excess water was decanted from sediment samples then the sediment was frozen overnight. Samples were lyophilized then homogenized by grinding to a fine power 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 10,000 rpm for 5 min. Supernatant was transferred into Supelco Supelclean ENVI-Carb cartridges (Supelco, Belefonte, Pennsylvania) and a vacuum manifold for solid phase extraction. Extracts were concentrated under nitrogen to approximately 1 mL. Then

100 µL of sample was mixed with 300 µL ammonium formate buffer in LC vials to be analyzed by UPLC-MS/MS.

Biota and organic matter: Alga, 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 were digested and extracted with MeOH containing perfluorinated internal standards (PFOS, PFNA, PFDA, and PFBS), vortexed, sonicated for 30 min, and centrifuged. Solid phase extraction was performed on the supernatant using Supelco Supelclean ENVI-Carb cartridges and a Waters vacuum manifold. Extracts were concentrated under nitrogen, aliquots were added to polypropylene LC autosampler vials with 2-mM ammonium acetate buffer, and analyzed by UPLC-MS/MS.

Aquatic insects were lyophilized and manually homogenized as composite samples grouped by Order: Coleoptera, Ephemeroptera, Megaloptera, Odonata (Anisoptera and Zygoptera), and Trichoptera. The homogenate was digested and extracted with 28% ammonium hydroxide (NH_4OH) in MeOH solution containing perfluorinated internal standards (PFOS, PFNA, PFDA, and PFBS). Samples were vortexed, sonicated for 30 min, and centrifuged at 10,000 rpm for 5 min. Supernatant was transferred to Supelco Supelclean ENVI-Carb cartridges and a vacuum manifold for solid phase extraction. Extracts were concentrated under nitrogen, added to autosampler vials with 2 mM ammonium acetate, and then analyzed by UPLC-MS/MS.

For fish samples, analysis was conducted on white muscle tissue. Tissue samples were free of scales, skin, and bone. Standard fish processing protocols were used when excising muscle tissue (USEPA 2000). Whitefin Shiners were the only fish that were

processed whole. 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).

Fish and crayfish samples were digested and extracted with 0.01 N sodium hydroxide (NaOH) in MeOH solution containing perfluorinated internal standards (PFOS, PFNA, and PFDA). Mollusk samples were digested and extracted with internal standards, PFOS, PFNA, PFDA, and PFBS. Eight milliliters of the NaOH and MeOH solution was added to 2 mL of sample homogenate in a 15-mL polypropylene Falcon tube (Becton Dickinson, Franklin Lakes, New Jersey) and sonicated for 30 min. Samples were centrifuged at 10,000 rpm for 5 min and 3 mL of supernatant was removed and diluted with DI water (1:10 sample:water). The diluted extract was subjected to solid phase extraction using 3-mL Waters Oasis WAX cartridges and a vacuum manifold. The extract was concentrated under nitrogen and then an aliquot was added to a polypropylene LC autosampler vial with 2-mM ammonium acetate buffer. Sample extracts were analyzed by HPLC-MS/MS.

Quality control: Rigorous quality control measures were followed during all analyses. Quality control and assurance consisted of double blanks, method blanks, and matrix blanks to detect contamination at different stages of processing, sample duplicates to assess precision, spiked samples to measure percent recovery, and calibration curves to ensure consistency. Internal standards were also used for quality control by correcting for minor among-sample differences.

Data analysis

Mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopic composition of biota was compared by analysis of variance (ANOVA) to detect differences among trophic levels and sites (Kwak and Zedler 1997; Hoeinghaus et al. 2007). $\delta^{15}N$ values were used to determine the trophic position of consumers within the food web. Asian Clams (*Corbicula fluminea*) were selected as the food web base because they were primary consumers and abundant at every site. Because there was a significant difference ($p < 0.05$) in the average $\delta^{15}N$ of Asian Clams among sites, trophic position of consumers was based on the site-specific baseline. Using a trophic fractionation factor of 3.4‰, Equation 3 was used to calculate trophic position (Anderson and Cabana 2007):

$$Trophic\ Position_{consumer} = \left(\frac{\delta^{15}\mathrm{N}_{consumer} - \delta^{15}\mathrm{N}_{baseline}}{3.4}\right) + 2. \qquad (3)$$

PFAA concentrations were $\log_{10}$ transformed to normalize the data. ANOVA was performed on contaminant concentrations to detect statistically significant differences within and among sites, compartments, and taxa. Tukey's HSD post hoc test was performed after ANOVA indicated a significant difference ($p < 0.05$) to determine where differences occurred. Simple linear regression was performed on log-transformed PFAA concentrations and calculated trophic position to examine relationships. Trophic magnification factors (TMFs) were calculated for consumers as

$$Log[chemical_{WW}] = a + b(TP), \qquad (4)$$

$$TMF = 10^{b}. \qquad (5)$$

The slope (*b*) from the $\log_{10}$-linear regression of the wet weight (WW) chemical concentration ($chemical_{WW}$) versus trophic position (*TP*) was used to calculate TMFs. A TMF > 1 indicates that contaminants are biomagnified.

Results

Contaminant concentrations in water, sediment, and organic matter

Ten PFAAs (PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHS, and PFOS) were analyzed in water, sediment, and organic matter samples at each site. All 10 PFAAs were detected in water samples ranging from below detection limit (BDL) to 9.1 ng/L (Table 1). Generally, greater concentrations occurred at the Pee Dee site among sites. PFOA was detected at the greatest concentration at every site (range, 4.3 – 9.1 ng/L). Six PFAAs (PFPA, PFHpA, PFHS, PFOA, PFOS, and PFDA) were detected at low levels in sediment and inconsistently detected among sites (Table 1). PFOS was the only compound detected in sediment at all sites (range, 0.04 – 0.62 ng/g DW). All 10 PFAAs were below detection limits for organic matter samples.

Contaminant concentrations in biota

Plant samples, including algae and submergent macrophytes, were analyzed for all 14 PFAAs. Only 5 of the 14 compounds were detected (PFBA, PFBS, PFNA, PFDA, and PFDoA), and the greatest mean concentration occurred for PFDoA (38.52 ng/g WW; Table 2).

Only three PFAAs (PFNA, PFTrA, and PFTA) were not detected in mollusk samples. All other compounds were detected at relatively low concentrations with means ranging from 1.40 to 8.98 ng/g WW (Table 2). PFHpA was detected in all mollusk samples (range, 0.34 –

3.24 ng/g WW) and PFOA was detected in 15 of the 19 samples (range, BDL – 7.41 ng/g WW).

All 14 PFAAs were detected in aquatic insect samples (range, BDL – 1,670.1 ng/g WW). PFOA, PFDA, PFUA, and PFDoA were detected in 100% of the samples. Two compounds with notably high mean concentrations were PFOS (125.17 ng/g WW) and PFDoA (166.65 ng/g WW). Consistently high concentrations occurred for PFOS in insect samples, in which 65% of detections were over 100 ng/g WW. Crayfish samples exhibited detections from 6 of the 10 PFAAs analyzed with means ranging from BDL to 23.99 ng/g WW (Table 2). PFHpA was the only compound detected in all crayfish samples (range, 9.85 – 51.83 ng/g WW).

All 14 PFAAs were detected in fish tissues, in which mean concentrations ranged from 1.16 to 242.14 ng/g WW (Table 2). PFOS was detected in 92% of fish samples (range, BDL – 53.81 ng/g WW), PFBS was detected in 67% (range, BDL – 16.91 ng/g WW), and PFDA was detected in 67% (range, BDL – 59.02 ng/g WW). Bluegill samples showed the most PFAAs detections (56%), but contained generally low concentrations among fish species. However, Bluegill and Whitefin Shiner samples showed the greatest mean concentrations in PFOS, 20.44 and 37.36 ng/g WW, respectively. The Robust Redhorse ova sample was analyzed for 14 PFAAs and 10 were detected (range, BDL – 482.88 ng/g WW; Table 2). Generally, the ova sample showed high concentrations compared to fish muscle tissue. PFOS concentration in the ova sample was notably high (482.88 ng/g WW).

A visual general hazard assessment tool was developed to show relative longitudinal exposure and contamination by highlighting the greatest mean concentrations for selected

PFAAs and each environmental and food web compartment among sites (Figure 2). Our results showed that the majority of the greatest concentrations occurred at the Red Hill site. An ANOVA performed on $\log_{10}$-transformed PFAA concentrations failed to detect a significant difference among sites ($p > 0.05$). Further, there were no apparent consistent longitudinal trends among sites.

Stable isotopes

Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ were measured for a total of 359 samples, and $\delta^{34}S$ isotope analysis was performed on a total of 224 samples (a subset of the 359 samples). $\delta^{15}N$ values were significantly different among sites when comparing respective compartments ($p < 0.05$). The Asian Clam was used as the food web base when calculating trophic position, because of their prevalence at every site and primary consumer position. Because there was a significant difference ($p < 0.0001$) in baseline mean of $\delta^{15}N$ values among sites (801: 8.8‰, Red Hill: 15.3‰, Digg's Tract: 12.3‰, Society Hill: 8.7‰, Pee Dee: 11.2‰), individual 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 sites (Table SI 2).

Food web contamination

An ANOVA detected significant differences in $\log_{10}$-transformed PFAA concentrations among food web compartments and Tukey's HSD post hoc test identified those differences between all food web compartments (fishes, mollusks, aquatic insects, crayfishes, plants, and detritus). There were statistically significant differences for all PFAAs among compartments ($p < 0.0001$). Variation in mean PFAA concentrations occurred among

food web compartments in which aquatic insects exhibited high contaminant levels (Figure 3). The same method was used to compare mean PFAA concentrations among fish species in which PFPA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHS, and PFOS demonstrated a significant difference ($p < 0.05$). Figure 4 demonstrates the variation of PFAAs that were detected in over 50% of samples among fish species and the lack of consistent trends among compounds.

TMF values that incorporated PFAA regression slopes among food web compartments showed that water and dietary sources contributed to the accumulation of PFAAs. PFHpA and PFOA exhibited negative slopes, whereas PFBS, PFOS, and PFDA showed positive slopes (Figure SI 1). TMFs greater than one indicate diet as a major route of exposure and the potential for biomagnification. PFBS, PFOS, and PFDA TMFs for all consumers were greater than 1.0 indicating diet as a major route of exposure and the potential for biomagnification (1.09, 1.53, and 1.16, respectively; Table 4). TMFs showed wide-ranging variation when calculated for consumer groups or species. PFHpA only exhibited a TMF greater than 1.0 for mollusk, Blue Catfish, Channel Catfish, and Whitefin Shiner samples. PFOA showed a TMF less than 1.0 for all consumers but exhibited biomagnification potential for aquatic insect (1.22), American Eel (1.15), Channel Catfish (1.11), Largemouth Bass (14.45), Smallmouth Buffalo (1.9), and Whitefin Shiner (1.93) samples.

Discussion

Contamination and accumulation

Our systematic analysis of PFAAs, isotopic composition, aquatic taxa, trophic positions, and sites indicated widespread contamination in the Yadkin-Pee Dee River and evidence that both diet and water contributed to bioaccumulation. PFAAs were detected in water, sediment, organic matter, and aquatic biota at various concentrations. PFOS and PFDA were the most prevalent PFAAs in samples among all sites. Aquatic insects exhibited high contaminant loads of PFAAs relative to all other aquatic biota. The lack of published aquatic life thresholds for PFAAs and the physiological differences among taxa make it difficult to quantify the overall risk of exposure. However, this study revealed the accumulation potential within a lotic ecosystem.

Food web exposure

Our results demonstrated variable PFAA accumulations among food web compartments and fish species. Detritus was the only compartment with no PFAA detections. Plant samples exhibited the least amount of PFAA detections (16%). Biofilm, an aggregation of bacteria, algae, and protozoans, is a basal resource for the aquatic food web. Our findings showed that PFAA accumulation occurred at high levels in biofilm samples, particularly for PFOA. Aquatic insects exhibited the greatest accumulation of PFAAs relative to other taxa, as seen in other studies (Fernández-Sanjuan et al. 2010; Lescord et al. 2015). 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 showed that various taxa accumulated PFAA compounds differently. Fishes exhibited

dissimilar detections of PFAAs among species. This could be a result from differences in size, age, physiology, and feeding strategy of the species or individual. Bioaccumulation of PFAAs 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 sparse and imperiled (RRCC 2009). Consequently, direct sampling for contaminants in adults or juveniles of this species is not feasible. For this reason, we investigated the Notchlip Redhorse as a potential surrogate species based on its related taxonomy and food habits (Freeman et al. 2002). This facilitated inferences about Robust Redhorse exposure based on contaminant concentrations in water, Notchlip Redhorse muscle tissue, and Robust Redhorse ova and diet sources. All PFAAs except PFPA were detected in Notchlip Redhorse tissue samples. Asian Clams and aquatic insects are known primary diet sources of Robust Redhorse (Freeman et al. 2002), and aquatic insects exhibited extremely high concentrations of PFAAs while clams showed much lower concentrations. Our findings provided strong cumulative evidence that PFAAs are present in Robust Redhorse tissues and organs, as directly indicated by the results from the ova sample. Redhorse PFAA exposure likely comes from their diet of aquatic insects, which showed the greatest contamination of PFAAs. Ova sample results also indicated that maternal transfer of PFAAs is likely to occur. Maternal transfer of PFOS and potential reproductive effects in fishes have been documented in other studies (Ankley et al. 2005; Ji et al. 2008; Peng et al. 2010; Sharpe et al. 2010). Due to the high concentrations of PFAAs in Robust Redhorse ova, adverse effects from these compounds have great potential to affect early life stages and overall fecundity. Ankley et al.

(2005) observed histopathological alterations in the ovaries of female Fathead Minnows (*Pimephales promelas*) that would alter and delay ova development. Sharpe et al. (2010) observed an estimated 10% of the adult PFOS body burden transferred to ova and a reduction in fecundity in Zebrafish (*Danio rerio*). Such reproductive effects of PFAAs are a likely contributing influence on the low population size and imperilment of the Robust Redhorse in the Yadkin-Pee Dee River.

Conclusions

No previous study has analyzed such a wide variety of food web samples for PFAAs from a lotic ecosystem like the Yadkin-Pee Dee River, and very few have investigated their transfer through aquatic food webs. Our results demonstrated the prevalence of PFAAs in the environment and biota of the Yadkin-Pee Dee River. The observed contamination of PFAAs 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 PFAAs to biomagnify in the food web, up to extreme degrees in an apex predator.

Our findings provided essential information that was previously lacking for fish and other biota. The tendency of PFOS to maternally transfer to ova causes concern for the implications of reproductive health for imperiled 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 PFAAs is crucial for better understanding the risks to aquatic organisms and overall ecological health.

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Tables

Table 1. Perfluoroalkyl acid (PFAA) concentrations in water and sediment samples from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. BDL: Below detection limit.

	Site				
PFAA and samples	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
Water (ng/L)					
PFBA	0.94	0.61	0.68	0.63	1.20
PFPA	BDL	BDL	0.24	0.07	0.90
PFBS	0.79	0.94	1.49	1.83	2.80
PFHxA	2.61	2.75	3.43	3.66	5.53
PFHpA	2.88	0.78	2.13	2.10	5.77
PFHS	1.93	1.63	1.64	1.77	2.29
PFOA	4.73	4.92	5.25	4.30	9.10
PFNA	1.01	0.87	1.63	0.77	1.52
PFOS	1.21	1.76	2.48	2.22	3.02
PFDA	1.86	1.71	2.56	1.51	2.22
Sediment (ng/g)					
PFBA	BDL	BDL	BDL	BDL	BDL
PFPA	0.02	BDL	BDL	BDL	BDL
PFBS	BDL	BDL	BDL	BDL	BDL
PFHxA	BDL	BDL	BDL	BDL	BDL
PFHpA	0.02	0.03	BDL	BDL	BDL
PFHS	0.02	BDL	0.03	BDL	BDL
PFOA	0.07	0.22	BDL	BDL	BDL
PFNA	BDL	BDL	BDL	BDL	BDL
PFOS	0.56	0.62	0.04	0.05	0.10
PFDA	0.07	BDL	0.04	BDL	BDL

Table 2. Mean perfluoroalkyl acid concentration (ng/g WW) and standard deviation (±) in samples of food web biota and organic matter from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina, n = number of samples analyzed. BDL: Below detection limit.

Sample	n	PFBA	PFPA	PFBS	PFHxA	PFHpA	PFHS	PFOA
Detritus	5	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Biofilm	3	108.54	BDL	194.31 (± 259.73)	BDL	112.04	19.06	463.73 (± 601.16)
Plants	11	6.46	BDL	3.71 (± 1.36)	BDL	BDL	BDL	BDL
Algae	3	BDL	BDL	5.18	BDL	BDL	BDL	BDL
Submergent macrophytes	8	6.46	BDL	2.98 (± 0.68)	BDL	BDL	BDL	BDL
Mollusks	19	3.08 (± 2.60)	1.50 (± 1.74)	2.21 (± 1.61)	1.93 (± 0.98)	1.92 (± 0.69)	2.14 (± 1.52)	1.40 (± 1.94)
Asian Clam	5	1.24	0.87 (± 0.46)	1.26 (± 0.21)	1.34 (± 0.37)	2.17 (± 0.37)	BDL	0.48 (± 0.21)
Snails	6	BDL	1.99 (± 2.49)	2.22 (± 1.23)	1.14	2.22 (± 0.52)	2.14 (± 1.52)	1.57 (± 1.64)
Unionid mussels	8	4.92	1.38	4.11 (± 3.18)	2.58 (± 1.01)	1.53 (± 0.82)	BDL	2.16 (± 2.94)
Aquatic insects	21	7.80 (± 1.31)	10.05 (± 2.80)	16.01 (± 3.64)	9.25 (± 2.32)	10.50 (± 3.02)	15.22 (± 4.16)	10.71 (± 3.27)
Crayfishes	4	23.81 (± 1.03)	17.49 (± 3.49)	1.70 (± 1.68)	5.43	23.99 (± 19.77)	BDL	BDL
Fishes	85	107.92 (± 240.06)	242.14 (± 342.36)	4.94 (± 4.09)	39.58 (± 127.19)	6.12 (± 7.97)	1.16 (± 1.00)	2.54 (± 8.06)
American Eel	8	21.14	BDL	4.68 (± 2.38)	3.10 (± 0.21)	6.61 (± 5.23)	0.1	0.69 (± 0.44)
Blue Catfish	10	BDL	BDL	7.32	BDL	BDL	BDL	BDL
Bluegill	10	6.15 (± 4.91)	BDL	3.65 (± 2.83)	2.20 (± 0.21)	2.72	BDL	0.88 (± 0.51)
Channel Catfish	10	210.85 (± 391.41)	777.44	3.97 (± 4.61)	277.51 (± 388.64)	32.84	BDL	10.61 (± 14.96)
Common Carp	10	BDL	BDL	2.44 (± 0.75)	2.35	BDL	BDL	0.25 (± 0.16)
Largemouth Bass	10	BDL	BDL	5.32 (± 3.10)	2.50 (± 0.29)	0.73	BDL	0.77 (± 1.04)
Notchlip Redhorse	6	13.04 (± 11.59)	BDL	6.45 (± 5.93)	4.07 (± 2.05)	3.93 (± 5.16)	2.1	15.50 (± 26.13)
Shorthead Redhorse	11	22.72	13.37	7.57 (± 5.71)	2.44 (± 0.01)	4.93 (± 9.09)	BDL	0.720 (± 0.003)
Smallmouth Buffalo	7	235.60 (± 304.85)	139.97 (± 222.11)	7.30 (± 6.20)	132.68 (± 183.67)	7.40 (± 2.73)	BDL	0.46
Whitefin Shiner *	3	BDL	BDL	3.55 (± 0.79)	BDL	BDL	1.27	2.32 (± 1.39)
Robust Redhorse ova	1	11.78	BDL	BDL	BDL	0.72	14.51	4.85

*Whole body concentrations

Table 2-continued.

Sample	n	PFNA	PFOS	PFDA	PFUA	PFDoA	PFTrA	PFTA
Detritus	5	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Biofilm	3	91.80 (± 115.24)	3.64	21.01 (± 26.01)	20.96 (± 24.46)	79.75 (± 58.31)	65.75	BDL
Plants	11	0.25	BDL	0.24	BDL	38.52 (± 41.78)	BDL	BDL
Algae	3	BDL	BDL	BDL	BDL	20.10	BDL	BDL
Submergent macrophytes	8	0.25	BDL	0.24	BDL	44.66 (± 48.91)	BDL	BDL
Mollusks	19	BDL	4.31 (± 3.03)	2.40 (± 0.96)	0.65	8.98 (± 6.45)	BDL	BDL
Asian Clam	5	BDL	BDL	1.85 (± 0.04)	–	–	–	–
Snails	6	BDL	4.31 (± 3.03)	2.95 (± 1.17)	–	–	–	–
Unionid mussels	8	BDL	BDL	BDL	0.65	8.98 (± 6.45)	BDL	BDL
Aquatic insects	21	8.52 (± 2.33)	125.17 (± 82.39)	18.69 (± 13.51)	5.46 (± 2.52)	166.65 (± 419.34)	5.13 (± 6.23)	16.65 (± 16.65)
Crayfishes	4	BDL	4.12 (± 0.91)	BDL	–	–	–	–
Fishes	85	3.23 (± 3.05)	12.17 (± 10.52)	3.64 (± 7.64)	8.62 (± 3.83)	13.63 (± 8.36)	21.90 (± 11.05)	15.84 (± 0.81)
American Eel	8	BDL	8.63 (± 6.88)	0.76 (± 0.27)	–	–	–	–
Blue Catfish	10	BDL	4.02 (± 5.84)	0.96 (± 0.93)	–	–	–	–
Bluegill	10	2.85 (± 0.26)	20.44 (± 10.82)	3.45 (± 0.54)	–	–	–	–
Channel Catfish	10	BDL	5.23 (± 5.01)	0.50	–	–	–	–
Common Carp	10	2.35 (± 1.32)	12.42 (± 7.36)	2.41 (± 1.32)	–	–	–	–
Largemouth Bass	10	2.83 (± 0.36)	13.34 (± 6.98)	3.55 (± 0.51)	–	–	–	–
Notchlip Redhorse	6	9.26 (± 10.27)	4.85 (± 5.72)	16.32 (± 28.52)	7.38 (± 4.84)	14.54 (± 11.67)	25.66 (± 9.91)	15.84 (± 0.80)
Shorthead Redhorse	11	1.91 (± 0.16)	11.79 (± 11.39)	2.94 (± 2.31)	10.48 (± 0.45)	12.26 (± 0.77)	10.61	BDL
Smallmouth Buffalo	7	BDL	12.40 (± 5.43)	1.57	–	–	–	–
Whitefin Shiner *	3	BDL	37.36 (± 15.75)	4.33 (± 0.16)	–	–	–	–
Robust Redhorse ova	1	6.85	482.88	34.62	52.24	29.60	19.97	BDL

*Whole body concentrations

Table 3. Stable isotope ratio means and standard deviations of aquatic food web biota and organic matter from five sites of the Yadkin-Pee Dee River of North Carolina and South Carolina.

	801		Red Hill		Digg's Tract		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.62 ± 1.05	2.83 ± 0.21	-30.0 ± 0.64	3.83 ± 1.03	-30.26 ± 0.76	5.75 ± 0.17	3.69 ± 0.3
Plants	-27.86 ± 0.99	12.52 ± 2.52	-31.42	13.7	-26.38 ± 1.24	12.08 ± 1.99	5.17 ± 0.96
Mollusks	-24.72 ± 2.36	10.07 ± 1.43	-28.58 ± 1.97	15.55 ± 0.75	-27.05 ± 3.15	12.41 ± 1.6	4.49 ± 1.39
Asian Clam	-26.76 ± 0.34	8.84 ± 0.25	-28.68 ± 0.04	15.33 ± 0.27	-28.66 ± 0.03	12.34 ± 0.06	5.02 ± 1.52
Snails	-22.69 ± 0.06	11.3 ± 0.11	-25.56 ± 0.27	14.68 ± 0.01	-24.34 ± 1.87	12.28 ± 2.35	3.49 ± 1.01
Unionid mussels	–	–	-30.04 ± 0.36	16.1 ± 0.57	-30.30 ± 0.36	12.65 ± 0.32	5.73 ± 0.56
Insects	-27.49 ± 1.31	9.97 ± 1.18	-27.46 ± 1.52	14.82 ± 2.22	-27.59 ± 1.5	12.68 ± 2.2	4.2 ± 0.81
Collector	-29.41 ± 2.59	9.53 ± 0.72	-29.34 ± 0.71	11.98 ± 0.85	-29.13 ± 0.47	10.46 ± 0.06	2.55 ± 1.05
Filterer	-27.4 ± 0.26	9.65 ± 0.57	–	–	-31.12	14.02	4.65
Predator	-27.02 ± 0.76	10.08 ± 1.47	-26.99 ± 1.35	14.59 ± 0.67	-27.12 ± 1.29	13.05 ± 1.37	4.36 ± 0.57
Predator/Parasite	–	–	-25.89 ± 0.04	18.26 ± 3.34	-26.06 ± 0.29	15.85 ± 2.36	4.44 ± 0.74
Scraper	-27.62 ± 0.67	10.63 ± 0.15	-28.57 ± 0.4	14.9 ± 0.21	-27.65 ± 0.43	11.15 ± 2.35	4.48 ± 0.3
Crayfishes	-24.55 ± 0.6	10.40 ± 0.01	-24.9 ± 0.87	13.45 ± 0.69	-24.64 ± 0.57	12.89 ± 1.53	5.21 ± 0.19
Fishes	-25.95 ± 0.87	13.07 ± 1.54	-25.32 ± 1.38	16.15 ± 2.63	-27.13 ± 1.46	15.88 ± 1.47	5.84 ± 0.73
American Eel	–	–	-27.09 ± 0.72	17.87 ± 2.3	-30.15 ± 1.28	16.6 ± 0.54	6.22 ± 0.29
Blue Catfish	-26.69 ± 0.71	14.89 ± 0.81	-26.05 ± 0.71	18.06 ± 1.03	-27.16 ± 1.26	17.06 ± 1.17	5.61 ± 0.36
Bluegill	-25.42 ± 0.7	12.18 ± 0.32	-25.03 ± 0.64	14.75 ± 1.1	-26.12 ± 0.31	15.0 ± 0.34	5.57 ± 0.14
Channel Catfish	-26.01 ± 0.54	12.23 ± 1.1	-25.82 ± 0.82	14.82 ± 2.69	-28.69 ± 0.4	16.1 ± 0.11	5.89 ± 0.32
Common Carp	-26.19 ± 0.98	12.84 ± 1.86	-26.27 ± 0.73	11.83 ± 1.42	-26.92 ± 0.52	14.39 ± 0.87	5.43 ± 1.38
Largemouth Bass	-26.13 ± 1.28	14.64 ± 1.33	-25.93 ± 0.99	17.33 ± 4.01	-26.0 ± 0.45	17.47 ± 1.18	6.44 ± 0.63
Notchlip Redhorse	-27.32 ± 1.32	11.6 ± 0.19	-24.66 ± 0.17	16.5 ± 0.62	-26.99 ± 0.73	14.5 ± 2.42	5.08 ± 1.19
Shorthead Redhorse	-25.46 ± 0.42	13.34 ± 0.17	-22.81 ± 1.18	18.13 ± 0.18	-27.69 ± 0.99	16.9 ± 0.41	6.29 ± 0.15
Smallmouth Buffalo	–	–	-25.05 ± 1.07	15.05 ± 2.05	-28.09 ± 1.3	15.87 ± 0.95	5.91 ± 0.48
Whitefin Shiner*	-25.32 ± 0.09	11.45 ± 0.97	-24.36 ± 0.83	17.21 ± 1.15	-25.0 ± 0.34	15.28 ± 0.57	6.14 ± 0.47

*Whitefin Shiners are reported as whole-body concentrations

Table 3-continued.

	Society Hill			Pee Dee		
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)
Detritus	-29.9 ± 0.52	5.48 ± 0.06	4.07 ± 1.4	-30.04 ± 0.46	5.54 ± 0.66	3.57 ± 1.42
Plants	-26.36 ± 0.91	14.97 ± 0.6	3.83 ± 1.24	–	–	–
Mollusks	-28.07 ± 1.78	9.41 ± 0.55	4.5 ± 0.8	-32.0 ± 0.41	11.35 ± 0.4	4.95 ± 0.36
Asian Clam	-28.92 ± 0.32	8.68 ± 0.45	3.87 ± 0.57	-32.10 ± 0.02	11.18 ± 0.59	4.72 ± 0.27
Snails	-25.28 ± 0.01	9.4 ± 0.17	4.98 ± 0.11	–	–	–
Unionid mussels	-29.04 ± 0.65	9.78 ± 0.32	4.59 ± 0.98	-31.96 ± 0.52	11.43 ± 0.35	5.06 ± 0.37
Insects	-28.36 ± 2.11	10.13 ± 2.13	4.27 ± 1.01	-30.72 ± 2.54	10.90 ± 1.41	3.96 ± 0.9
Collector	-29.95 ± 2.08	9.1 ± 0.74	3.53 ± 1.41	-31.94 ± 3.19	10.83 ± 0.48	3.64 ± 1.16
Filterer	–	–	–	-34.03 ± 0.06	11.69 ± 0.25	4.4 ± 0.76
Predator	-28.75 ± 0.86	10.02 ± 0.92	4.55 ± 0.75	-29.91 ± 1.38	10.55 ± 1.76	4.0 ± 0.86
Predator/Parasite	-24.58 ± 0.36	15.18 ± 0.22	4.73 ± 0.19	-25.99	13.04	3.51
Scraper	-26.61 ± 0.06	8.11 ± 0.42	4.61 ± 0.52	-30.79	10.94	4.77
Crayfishes	-25.16 ± 0.42	10.98 ± 1.22	4.86 ± 0.56	-28.09 ± 0.84	13.13 ± 0.07	5.97 ± 0.11
Fishes	-26.16 ± 1.5	13.14 ± 1.39	5.13 ± 1.33	-27.09 ± 1.37	12.87 ± 0.95	5.02 ± 1.29
American Eel	-25.62 ± 0.61	13.19 ± 0.56	4.58 ± 0.77	-27.15 ± 0.42	12.59 ± 0.77	4.69 ± 0.75
Blue Catfish	-27.04 ± 0.75	13.59 ± 0.95	4.8 ± 1.26	-26.56 ± 0.63	13.26 ± 0.43	4.59 ± 0.45
Bluegill	-27.25 ± 3.14	12.54 ± 0.5	5.21 ± 0.77	-26.71 ± 1.77	12.79 ± 0.79	3.84 ± 0.92
Channel Catfish	-25.78 ± 0.55	12.4 ± 0.89	4.7 ± 0.61	-26.34 ± 0.6	12.85 ± 0.74	5.19 ± 0.6
Common Carp	-26.02 ± 0.6	12.58 ± 0.66	4.59 ± 0.74	-27.73 ± 0.8	11.87 ± 1.42	4.54 ± 0.74
Largemouth Bass	-25.53 ± 0.39	15.75 ± 0.79	5.93 ± 0.8	-27.04 ± 0.62	14.17 ± 0.92	5.63 ± 0.52
Notchlip Redhorse	–	–	–	–	–	–
Shorthead Redhorse	-27.12 ± 2.48	13.14 ± 1.56	4.72 ± 0.97	-28.21 ± 0.81	13.4 ± 0.36	5.48 ± 0.18
Smallmouth Buffalo	-26.43 ± 0.42	11.86 ± 1.6	3.83 ± 0.8	-29.29 ± 0.73	12.4 ± 0.13	3.72 ± 0.73
Whitefin Shiner*	-24.7 ± 0.67	13.22 ± 1.01	7.84 ± 0.59	-25.08 ± 0.6	12.64 ± 0.87	7.63 ± 0.94

*Whitefin Shiners are reported as whole-body concentrations

Table 4. PFBS, PFHpA, PFOA, PFOS, and PFDA trophic magnification factor (TMF) for consumers in the Yadkin-Pee Dee River of North Carolina and South Carolina, n = number of samples analyzed.

Species	n	PFBS TMF	PFHpA TMF	PFOA TMF	PFOS TMF	PFDA TMF
All consumers	136	1.09	0.23	0.57	1.53	1.16
Mollusks	18	67.61	2.34	0.2	0.0	0.07
Aquatic insects	27	1.77	0.05	1.22	0.0	31623
Crayfishes	4	0.01	0.0	1.0	0.0	1.0
Fishes	85	1.88	0.05	0.06	2.05	0.24
American Eel	8	1.42	0.2	1.15	0.36	1.02
Blue Catfish	10	1.11	1.0	1.0	0.24	0.06
Bluegill	10	0.57	0.81	0.95	1.12	1.22
Channel Catfish	10	1.2	1.0	1.11	1.2	1.2
Common Carp	10	1.53	0.36	0.4	2.11	1.23
Largemouth Bass	10	1.29	0.32	14.45	0.6	1.18
Notchlip Redhorse	6	0.07	0.03	0.17	0.78	0.78
Shorthead Redhorse	11	0.29	0.68	0.73	1.54	0.77
Smallmouth Buffalo	7	2.82	0.47	1.9	1.44	4.79
Whitefin Shiner*	3	3.66	1.28	1.93	1.67	1.07

* Based on whole-body concentrations

Figures

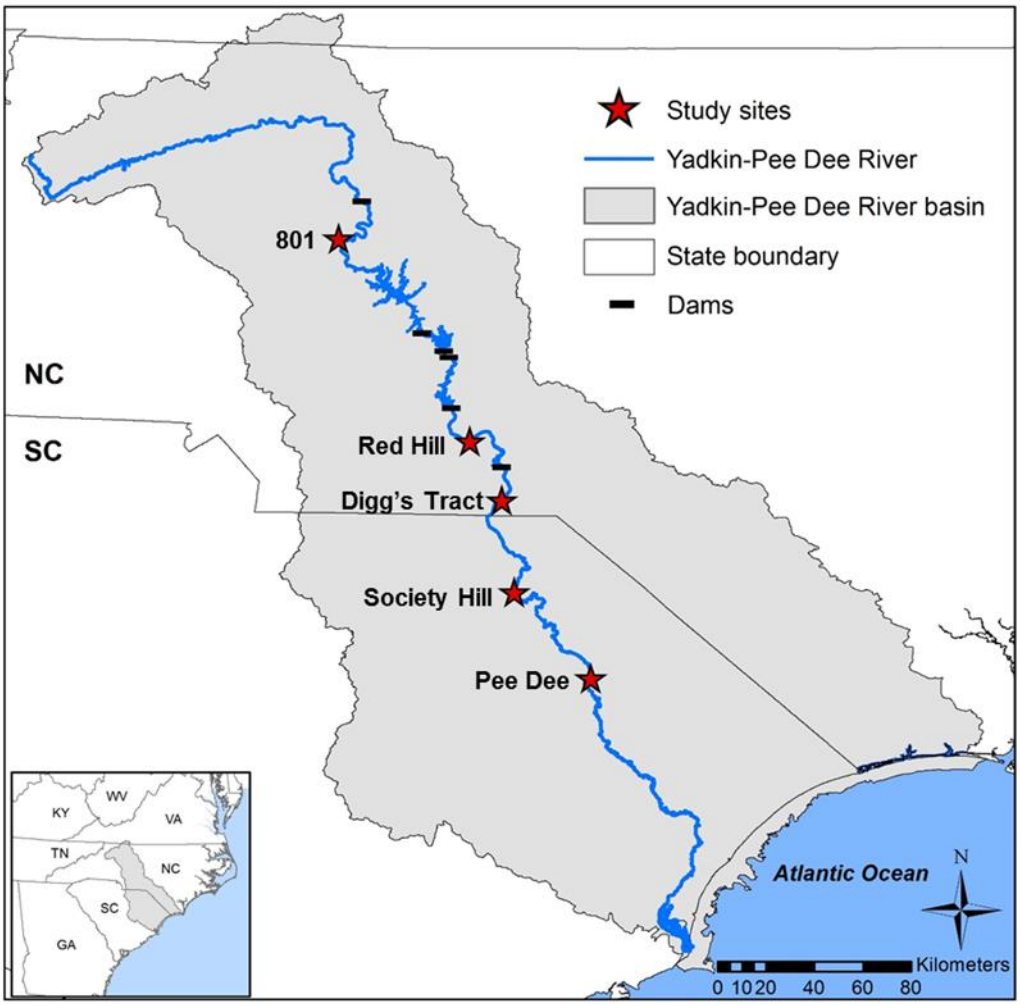

Figure 1. Study sites and major dams along the Yadkin-Pee Dee River of North Carolina and South Carolina.

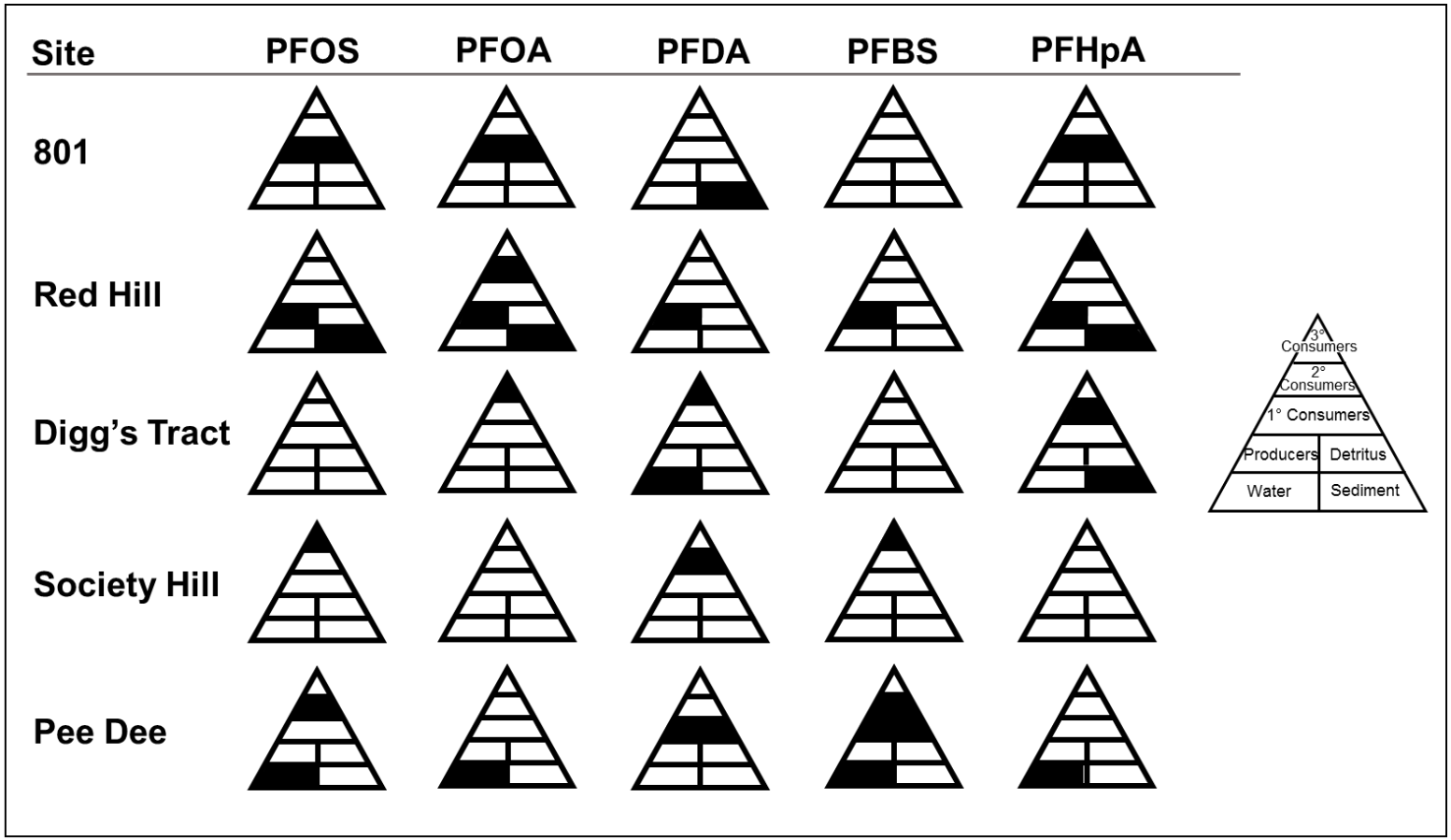

Figure 2. Summary of selected organic compounds among sites in the Yadkin-Pee Dee River of North Carolina and South Carolina. For each triangle, a solid black section represents the greatest measured mean concentration of a perfluoroalkyl acid for the corresponding food web compartment among sites.

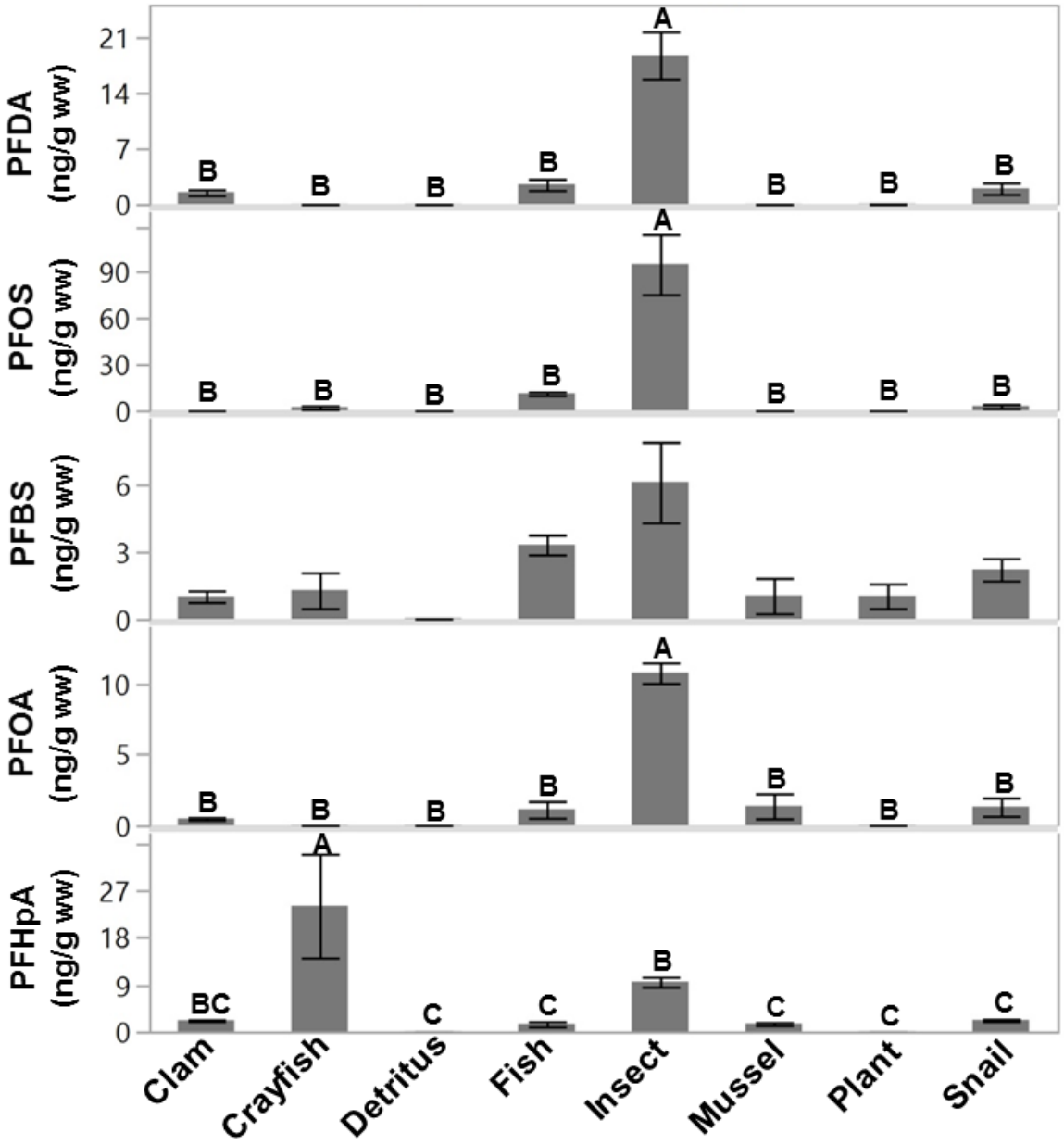

Figure 3. Mean pefluoroalkyl acid (PFAA) concentration and standard error for food web compartments in the Yadkin-Pee Dee of North Carolina and South Carolina. Tukey's HDS post-hoc test was performed for PFAAs that showed a significant ANOVA ($p < 0.05$). Species not connected by the same letter are significantly different within each PFAA.

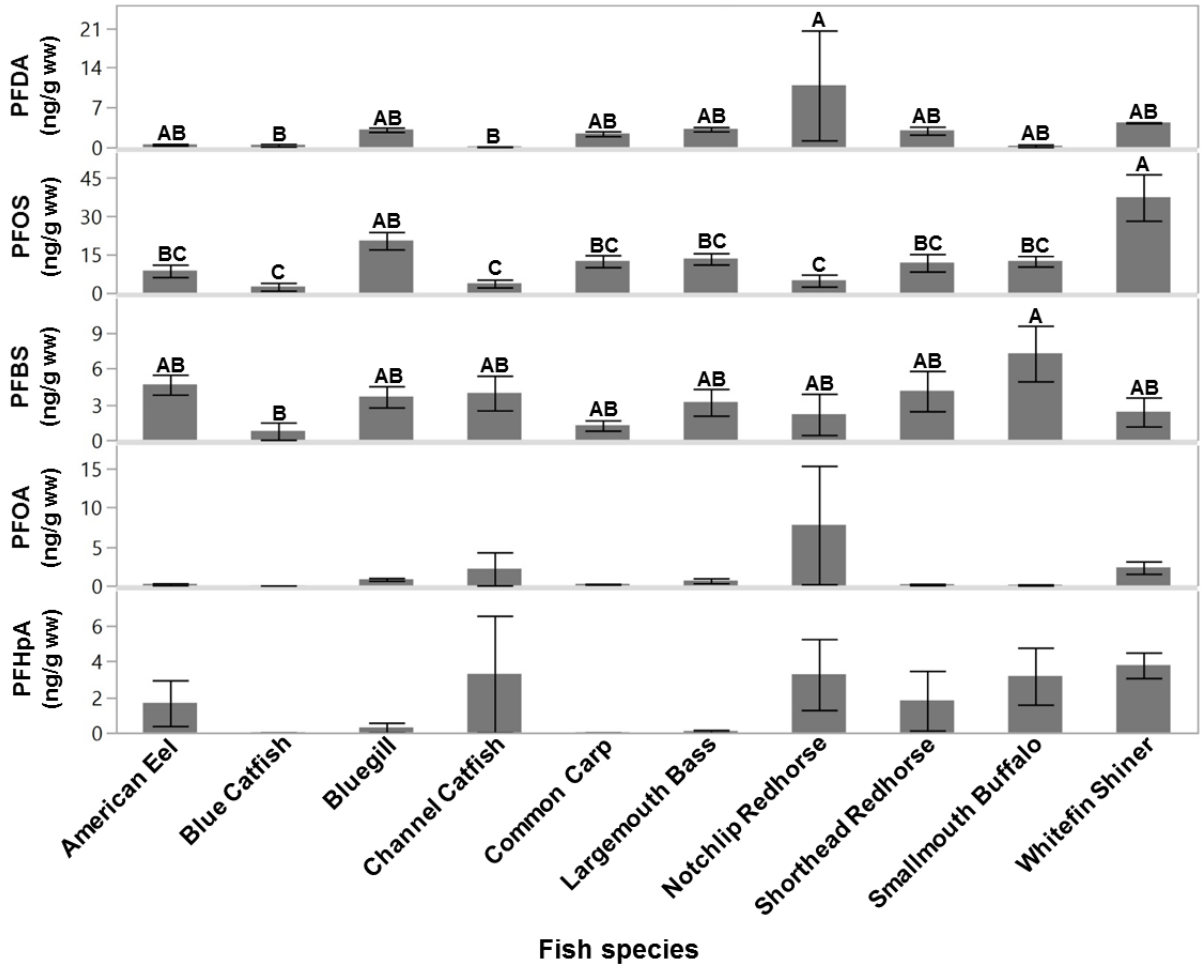

Figure 4. Mean pefluoroalkyl acid (PFAA) concentration and standard error for fish species in the Yadkin-Pee Dee of North Carolina and South Carolina. Tukey's HDS post-hoc test was performed for PFAAs that showed a significant ANOVA ($p < 0.05$). Species not connected by the same letter are significantly different within each PFAA.

APPENDICES

Appendix A

Supporting Information for Chapter 1

Table SI 1. GPS coordinates, location, and land use of study sites of the Yadkin-Pee Dee River. Sites are arranged from an upstream to downstream order. NC: North Carolina. SC: South Carolina.

				Watershed Land Use						
Site	Latitude (degrees)	Longitude (degrees)	State	Forested Upland (%)	Planted and Cultivated (%)	Developed (%)	Herbaceuous (%)	Shrub and Woodland (%)	Wetlands (%)	Barren Land (%)
801	35.83829	-80.48475	NC	57.0	22.3	13.3	3.6	2.9	0.3	0.1
Red Hill	35.08722	-79.99861	NC	51.1	25.6	13.8	4.9	2.5	0.7	0.1
Digg's Tract	34.86528	-79.87917	NC	52.2	23.7	13.1	5.4	3.0	1.1	0.2
Society Hill	34.52412	-79.83271	SC	50.8	23.0	12.5	5.9	3.5	2.9	0.2
Pee Dee	34.20385	-79.54768	SC	47.5	23.2	11.9	5.8	4.1	6.0	0.2

Table SI 2. Mean trophic position (± standard deviation) and range for fishes from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina.

	Site				
Species	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
American Eel					
Mean (± SD)	–	2.75 (± 0.68)	3.26 (± 0.16)	3.33 (± 0.17)	2.42 (± 0.23)
Range	–	2.10 - 3.60	3.14 - 3.37	3.20 - 3.56	2.09 - 2.59
Blue Catfish					
Mean (± SD)	3.78 (± 0.24)	2.80 (± 0.30)	3.39 (± 0.51)	3.45 (± 0.28)	2.61 (± 0.13)
Range	3.54 - 4.10	2.49 - 3.21	2.88 - 4.06	3.04 - 3.66	2.50 - 2.75
Bluegill					
Mean (± SD)	2.98 (± 0.09)	1.83 (± 0.32)	2.79 (± 0.10)	3.14 (± 0.15)	2.47 (± 0.23)
Range	2.87 - 3.09	1.46 - 2.19	2.71 - 2.93	2.94 - 3.27	2.29 - 2.81
Channel Catfish					
Mean (± SD)	3.00 (± 0.32)	1.85 (± 0.79)	3.11 (± 0.04)	3.10 (± 0.26)	2.49 (± 0.22)
Range	2.66 - 3.29	0.82 - 2.71	3.06 - 3.14	2.76 - 3.37	2.19 - 2.70
Common Carp					
Mean (± SD)	3.18 (± 0.55)	0.97 (± 0.42)	2.61 (± 0.26)	3.15 (± 0.19)	2.20 (± 0.42)
Range	2.38 - 3.57	0.40 - 1.34	2.38 - 2.96	2.94 - 3.41	1.58 - 2.48
Largemouth Bass					
Mean (± SD)	3.71 (± 0.39)	2.59 (± 1.18)	3.51 (± 0.35)	4.08 (± 0.23)	2.88 (± 0.27)
Range	3.24 - 4.09	0.85 - 3.46	3.05 - 3.83	3.76 - 4.29	2.50 - 3.13
Notchlip Redhorse		-			
Mean (± SD)	2.81 (± 0.06)	2.35 (± 0.18)	2.64 (± 0.71)	–	–
Range	2.77 - 2.85	2.18 - 2.54	2.00 - 3.27	–	–
Shorthead Redhorse					
Mean (± SD)	3.32 (± 0.05)	2.83 (± 0.06)	3.34 (± 0.12)	3.32 (± 0.46)	2.65 (± 0.10)
Range	3.28 - 3.39	2.77 - 2.89	3.21 - 3.48	2.68 - 3.71	2.57 - 2.77
Smallmouth Buffalo					
Mean (± SD)	–	1.92 (± 0.60)	3.04 (± 0.28)	2.94 (± 0.48)	2.36 (± 0.04)
Range	–	1.24 - 2.69	2.62 - 3.20	2.43 - 3.40	2.31 - 2.40
Whitefin Shiner					
Mean (± SD)	2.77 (± 0.28)	2.55 (± 0.34)	2.87 (± 0.17)	3.34 (± 0.3)	2.43 (± 0.26)
Range	2.57 - 3.18	2.25 - 2.90	2.68 - 3.08	3.11 - 3.77	2.09 - 2.65

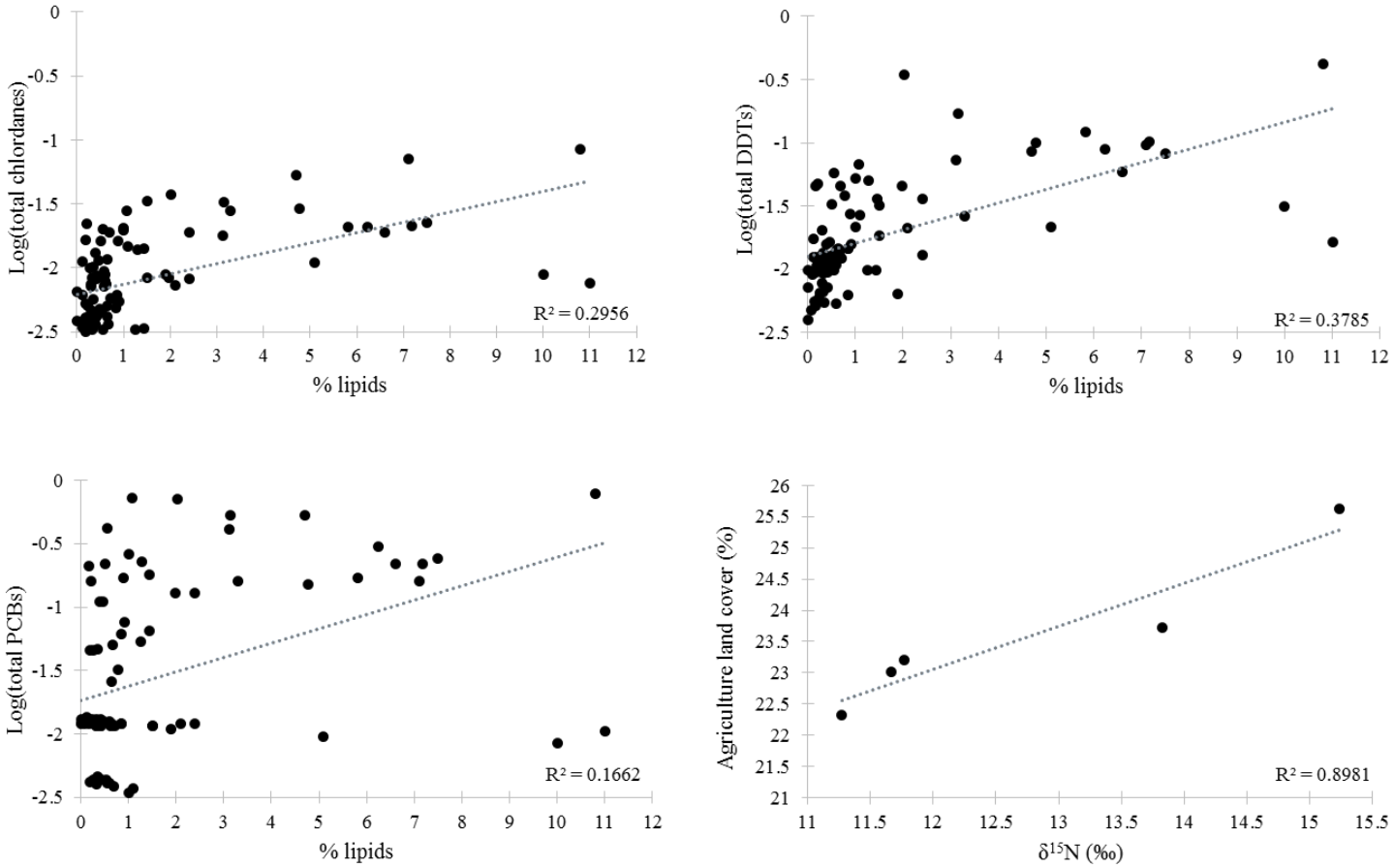

Figure SI 1. Associations between lipid content and select $\log_{10}$-transformed contaminant concentrations and $\delta^{15}N$ and agricultural land use.

Appendix B

Supporting Information for Chapter 2

Table SI 1. GPS coordinates, location, and land use of study sites of the Yadkin-Pee Dee River. Sites are arranged from an upstream to downstream order. NC: North Carolina. SC: South Carolina.

				Watershed Land Use						
Site	Latitude (degrees)	Longitude (degrees)	State	Forested Upland (%)	Planted and Cultivated (%)	Developed (%)	Herbaceuous (%)	Shrub and Woodland (%)	Wetlands (%)	Barren Land (%)
801	35.83829	-80.48475	NC	57.0	22.3	13.3	3.6	2.9	0.3	0.1
Red Hill	35.08722	-79.99861	NC	51.1	25.6	13.8	4.9	2.5	0.7	0.1
Digg's Tract	34.86528	-79.87917	NC	52.2	23.7	13.1	5.4	3.0	1.1	0.2
Society Hill	34.52412	-79.83271	SC	50.8	23.0	12.5	5.9	3.5	2.9	0.2
Pee Dee	34.20385	-79.54768	SC	47.5	23.2	11.9	5.8	4.1	6.0	0.2

Table SI 2. Mean trophic position (± standard deviation) and range for fishes from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina.

	Site				
Species	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
American Eel					
Mean (± SD)	–	2.75 (± 0.68)	3.26 (± 0.16)	3.33 (± 0.17)	2.42 (± 0.23)
Range	–	2.10 - 3.60	3.14 - 3.37	3.20 - 3.56	2.09 - 2.59
Blue Catfish					
Mean (± SD)	3.78 (± 0.24)	2.80 (± 0.30)	3.39 (± 0.51)	3.45 (± 0.28)	2.61 (± 0.13)
Range	3.54 - 4.10	2.49 - 3.21	2.88 - 4.06	3.04 - 3.66	2.50 - 2.75
Bluegill					
Mean (± SD)	2.98 (± 0.09)	1.83 (± 0.32)	2.79 (± 0.10)	3.14 (± 0.15)	2.47 (± 0.23)
Range	2.87 - 3.09	1.46 - 2.19	2.71 - 2.93	2.94 - 3.27	2.29 - 2.81
Channel Catfish					
Mean (± SD)	3.00 (± 0.32)	1.85 (± 0.79)	3.11 (± 0.04)	3.10 (± 0.26)	2.49 (± 0.22)
Range	2.66 - 3.29	0.82 - 2.71	3.06 - 3.14	2.76 - 3.37	2.19 - 2.70
Common Carp					
Mean (± SD)	3.18 (± 0.55)	0.97 (± 0.42)	2.61 (± 0.26)	3.15 (± 0.19)	2.20 (± 0.42)
Range	2.38 - 3.57	0.40 - 1.34	2.38 - 2.96	2.94 - 3.41	1.58 - 2.48
Largemouth Bass					
Mean (± SD)	3.71 (± 0.39)	2.59 (± 1.18)	3.51 (± 0.35)	4.08 (± 0.23)	2.88 (± 0.27)
Range	3.24 - 4.09	0.85 - 3.46	3.05 - 3.83	3.76 - 4.29	2.50 - 3.13
Notchlip Redhorse		-			
Mean (± SD)	2.81 (± 0.06)	2.35 (± 0.18)	2.64 (± 0.71)	–	–
Range	2.77 - 2.85	2.18 - 2.54	2.00 - 3.27	–	–
Shorthead Redhorse					
Mean (± SD)	3.32 (± 0.05)	2.83 (± 0.06)	3.34 (± 0.12)	3.32 (± 0.46)	2.65 (± 0.10)
Range	3.28 - 3.39	2.77 - 2.89	3.21 - 3.48	2.68 - 3.71	2.57 - 2.77
Smallmouth Buffalo					
Mean (± SD)	–	1.92 (± 0.60)	3.04 (± 0.28)	2.94 (± 0.48)	2.36 (± 0.04)
Range	–	1.24 - 2.69	2.62 - 3.20	2.43 - 3.40	2.31 - 2.40
Whitefin Shiner					
Mean (± SD)	2.77 (± 0.28)	2.55 (± 0.34)	2.87 (± 0.17)	3.34 (± 0.3)	2.43 (± 0.26)
Range	2.57 - 3.18	2.25 - 2.90	2.68 - 3.08	3.11 - 3.77	2.09 - 2.65

Appendix C

Supporting Information for Chapter 3

Table SI 1. GPS coordinates, location, and land use of study sites of the Yadkin-Pee Dee River. Sites are arranged from an upstream to downstream order. NC: North Carolina. SC: South Carolina.

				Watershed Land Use						
Site	Latitude (degrees)	Longitude (degrees)	State	Forested Upland (%)	Planted and Cultivated (%)	Developed (%)	Herbaceuous (%)	Shrub and Woodland (%)	Wetlands (%)	Barren Land (%)
801	35.83829	-80.48475	NC	57.0	22.3	13.3	3.6	2.9	0.3	0.1
Red Hill	35.08722	-79.99861	NC	51.1	25.6	13.8	4.9	2.5	0.7	0.1
Digg's Tract	34.86528	-79.87917	NC	52.2	23.7	13.1	5.4	3.0	1.1	0.2
Society Hill	34.52412	-79.83271	SC	50.8	23.0	12.5	5.9	3.5	2.9	0.2
Pee Dee	34.20385	-79.54768	SC	47.5	23.2	11.9	5.8	4.1	6.0	0.2

Table SI 2. Mean trophic position (± standard deviation) and range for fishes from five sites on the Yadkin-Pee Dee River of North Carolina and South Carolina.

	Site				
Species	801	Red Hill	Digg's Tract	Society Hill	Pee Dee
American Eel					
Mean (± SD)	–	2.75 (± 0.68)	3.26 (± 0.16)	3.33 (± 0.17)	2.42 (± 0.23)
Range	–	2.10 - 3.60	3.14 - 3.37	3.20 - 3.56	2.09 - 2.59
Blue Catfish					
Mean (± SD)	3.78 (± 0.24)	2.80 (± 0.30)	3.39 (± 0.51)	3.45 (± 0.28)	2.61 (± 0.13)
Range	3.54 - 4.10	2.49 - 3.21	2.88 - 4.06	3.04 - 3.66	2.50 - 2.75
Bluegill					
Mean (± SD)	2.98 (± 0.09)	1.83 (± 0.32)	2.79 (± 0.10)	3.14 (± 0.15)	2.47 (± 0.23)
Range	2.87 - 3.09	1.46 - 2.19	2.71 - 2.93	2.94 - 3.27	2.29 - 2.81
Channel Catfish					
Mean (± SD)	3.00 (± 0.32)	1.85 (± 0.79)	3.11 (± 0.04)	3.10 (± 0.26)	2.49 (± 0.22)
Range	2.66 - 3.29	0.82 - 2.71	3.06 - 3.14	2.76 - 3.37	2.19 - 2.70
Common Carp					
Mean (± SD)	3.18 (± 0.55)	0.97 (± 0.42)	2.61 (± 0.26)	3.15 (± 0.19)	2.20 (± 0.42)
Range	2.38 - 3.57	0.40 - 1.34	2.38 - 2.96	2.94 - 3.41	1.58 - 2.48
Largemouth Bass					
Mean (± SD)	3.71 (± 0.39)	2.59 (± 1.18)	3.51 (± 0.35)	4.08 (± 0.23)	2.88 (± 0.27)
Range	3.24 - 4.09	0.85 - 3.46	3.05 - 3.83	3.76 - 4.29	2.50 - 3.13
Notchlip Redhorse		-			
Mean (± SD)	2.81 (± 0.06)	2.35 (± 0.18)	2.64 (± 0.71)	–	–
Range	2.77 - 2.85	2.18 - 2.54	2.00 - 3.27	–	–
Shorthead Redhorse					
Mean (± SD)	3.32 (± 0.05)	2.83 (± 0.06)	3.34 (± 0.12)	3.32 (± 0.46)	2.65 (± 0.10)
Range	3.28 - 3.39	2.77 - 2.89	3.21 - 3.48	2.68 - 3.71	2.57 - 2.77
Smallmouth Buffalo					
Mean (± SD)	–	1.92 (± 0.60)	3.04 (± 0.28)	2.94 (± 0.48)	2.36 (± 0.04)
Range	–	1.24 - 2.69	2.62 - 3.20	2.43 - 3.40	2.31 - 2.40
Whitefin Shiner					
Mean (± SD)	2.77 (± 0.28)	2.55 (± 0.34)	2.87 (± 0.17)	3.34 (± 0.3)	2.43 (± 0.26)
Range	2.57 - 3.18	2.25 - 2.90	2.68 - 3.08	3.11 - 3.77	2.09 - 2.65

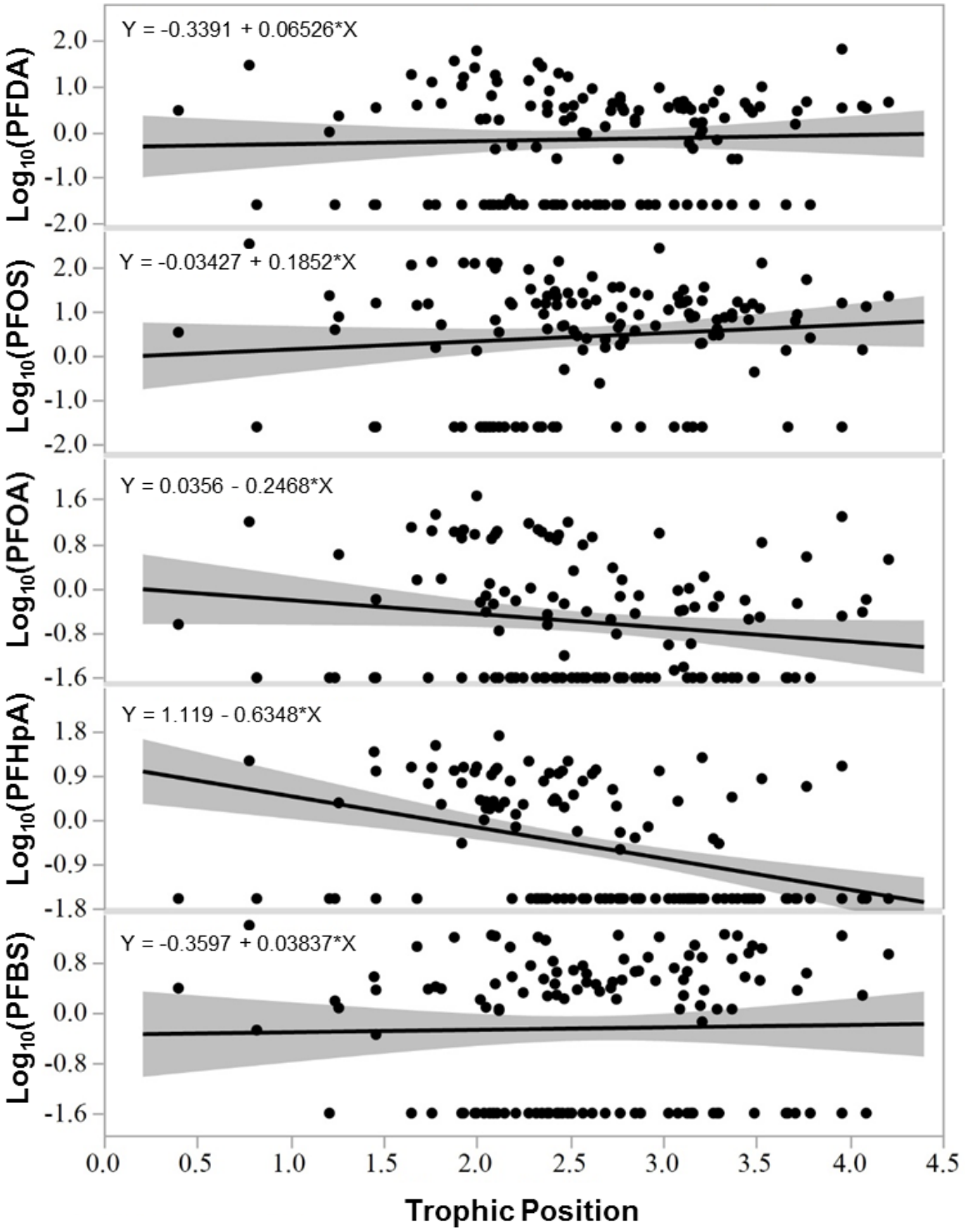

Figure SI 1. Log_{10}-transformed pefluoroalkyl acid concentrations and trophic position of consumers in the Yadkin-Pee Dee of North Carolina and South Carolina.