ABSTRACT

GRIESHABER, CASEY ALYSON. Relation of Fish Intersex and Survival to Contaminants in a Riverine System. (Under the direction of Dr. Thomas J. Kwak and Dr. W. Gregory Cope)

Contaminants from human activities have been recognized for decades as harmful to natural ecosystems, and especially to aquatic habitats and organisms. These contaminants, including endocrine active compounds, are known to affect the survival and health of fishes in impacted aquatic ecosystems. The objectives of this research were to determine if and how aquatic contaminant stressors in a river ecosystem affect the survival and health of fishes by sampling adult, wild fish and by conducting 28-day *in situ* bioassays with juvenile hatchery-propagated fish. Organic and inorganic environmental contaminants were measured in the sediment, water, and fish. The presence of intersex, a condition where female germ cells are detected in a predominantly male gonad, and a known biomarker of endocrine disruption, was assessed in both wild, adult and bioassay fishes.

Occurrence and severity of the intersex condition in wild, adult black bass (*Micropterus*), sunfish (*Lepomis*), and catfish (Ictaluridae) species at 11 sites located on the Yadkin-Pee Dee River, North Carolina and South Carolina, were evaluated. Relationships among health parameters, intersex condition, and measured contaminants were evaluated using principal component analysis (PCA) and all subsets regression modeling. Fish intersex condition was most frequently observed in black basses (mean 40%, range 7–100%) and was less frequently detected in sunfishes (mean 7%, range 0–20%) and catfishes (mean 1%, range 0–7%). Among intersex fish, the among-site mean severity index was 2.7 for black bass, 1.8 for sunfish, and 1.0 for catfish, with the detected range spanning the entire index range of 1–4. The occurrence of the intersex condition in fish did not show any longitudinal trend in the

river. PCA identified waterborne polycyclic aromatic hydrocarbons (PAHs) as the most correlated environmental variable for intersex occurrence and severity in black bass and sunfish. All subsets regression modeling and model selection procedures revealed that black bass intersex was associated with waterborne organochlorine pesticides and sediment-associated mercury. Sunfish intersex was related to waterborne PAHs in regression models.

Survival, intersex condition, and contaminant accumulation of three species of fish, Largemouth Bass *Micropterus salmoides* (LMB), Fathead Minnow *Pimephales promelas* (FHM), and Robust Redhorse *Moxostoma robustum* (RRH) was assessed with *in situ* bioassays. Juvenile hatchery-propagated fish were placed into cages located along the length of the Yadkin-Pee Dee River for a 28-day period, and contaminants were measured in the sediment and surface water near the cages. No apparent longitudinal trends in fish survival were detected, and contaminant concentrations varied among sites. Juvenile LMB and RRH did not survive past 13 and 23 days with corresponding Kaplan-Meier (K-M) median survival estimates of 9.7 d and 12.1 d, respectively. In contrast, survival of LMB and RRH in reference cages deployed at fish hatcheries were much greater with 70% of LMB and 67% of RRH surviving, validating the bioassay procedure. Survival of adult FHM deployed in cages alongside the juvenile LMB or RRH averaged 43% survival at the end of the 28-d exposure with a 22-d K-M median survival estimate. Intersex was not observed in any bioassay fish individuals. Contaminant accumulation in bioassay FHM tissue was apparent for almost all contaminants measured, with highest accumulated concentrations in polychlorinated biphenyls, organochlorine pesticides, and mercury.

Water quality stressors and contaminants in this river system appear to adversely impact juvenile and adult fish. The occurrence of intersex, contaminant accumulation within

organisms, and low survival of juvenile fish all have presumed assemblage- and community-level effects, which may impact fish sustainability. These findings enhance the understanding of the relationship between contaminants and fish health and provide information that can guide ecologically comprehensive conservation and management decisions.

Relation of Fish Intersex and Survival to Contaminants in a Riverine System

by
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DEDICATION

For my parents and family. Thank you for all your support, encouragement, and love.

BIOGRAPHY

Casey Alyson Grieshaber was born on August 29, 1991, in Livermore, California. In 1999, she moved across the country to Mooresville, North Carolina. She attended Lake Norman High School where she exceled in science classes and as a member of the soccer team. Through scuba certifications, trips to the beach, and summer camps, she found a love of the ocean and being in the water. In 2009, she began her college career at University of North Carolina Wilmington (UNCW). She quickly declared her major as Marine Biology and took many classes involving everything from organic chemistry to ichthyology. During her sophomore year Ecology class, Casey learned of the problems facing the world's fisheries and their importance for people around the globe. This sparked her interest in the fisheries field. Soon after, she completed independent research in a clownfish aquaculture lab and then found a position in Dr. Fred Scharf's lab. In the Scharf lab, she completed research investigating Southern Flounder *Paralichthys lethostigma* reproductive physiology. During the summer of 2012, she was selected as a National Science Foundation Research Experience for Undergraduates participant and traveled to Port Aransas, Texas, to work with Dr. Benjamin Walther. She spent the summer examining the ability to mark fish otoliths through a barium-enriched diet to help with aquaculture practices. The following year, Casey completed her undergraduate research at UNCW and was awarded honors in Marine Biology and was selected as a distinguished undergraduate research scholar. After graduation, Casey moved to Puerto Rico to work for North Carolina State University (NCSU) as a fisheries technician. For five months, she assisted in field collections of amphidromous fishes. Following her time in Puerto Rico, Casey moved back to North Carolina and continued to

work for NCSU. Within a few months, a graduate position became available that matched

Casey's research interests and would allow her to further her education. While at NCSU,

Casey served as the Co-President for the Student Fisheries Society, presented research

findings at national, regional, and state professional conferences, and was awarded travel

grants by both the Student Fisheries Society and the NCSU Graduate School. The research

Casey completed at NCSU has allowed her to become a more educated and well-rounded

scientist and has cultivated her interests in the fields of toxicology and fisheries. In the

future, Casey hopes to continue conducting sound, applicable science that helps to ensure

healthy fish populations and natural ecosystems.

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TABLE OF CONTENTS

LIST OF TABLES

APPENDIX B

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

APPENDIX A

LIST OF ABBREVIATIONS

Fish Species
Largemouth Bass…………………………………………………………………………... LMB
Robust Redhorse………………………………………………………………………… RRH
Fathead Minnow………………………………………………………………………… FHM

Contaminants
Endocrine active compounds……………………………………………………..….EACs
Polycyclic aromatic hydrocarbons………………………………………………………PAHs
Polychlorinated biphenyls……………………………………………………….….…PCBs
Organochlorine pesticides……………………………………………………...…OCPs
Current use pesticides………………………………………………………….....CUPs
Bisphenol-A………………………………………………….…………....BPA
17α-Ethinyl estradiol……………………………………………………….EE2
17β- estradiol equivalent……………………………………………….E2β-Eq
Cadmium……………………………………………………………………....Cd
Mercury…………………………………………………………………..………Hg
Manganese……………………………………………………………………….Mn

Health Parameters
Relative weight………………………………………………………...….…W_r
Gonadosomatic index………………………………………………………………GSI
Hepatosomatic index…………………………………………………….……HSI

Other
Passive sampling devices……………………………………………………………….PSDs
Dry weight…………………………………………………………………….…DW
Wet weight…………………………………………………………………...WW
Detection limit…………………………………………………………...….DL
Below detection limit……………………………………………………...…BDL
Predicted effect concentration……………………………………………….…PEC
Threshold effect concentration…………………………………………….…TEC
Predicted no effect concentration…………………………………………...PNEC
Equilibrium partitioning sediment benchmark toxic unit……………………….…ESBTU
Principal component analysis……………………………………………………....PCA

Relation of Fish Intersex to Contaminants in Riverine Sport Fishes

Abstract

Endocrine active compounds (EACs) are a group of pollutants that have been recognized as an emerging and widespread threat to aquatic ecosystems globally. Intersex, the presence of female germ cells within a predominantly male gonad, is considered a biomarker of endocrine disruption caused by EACs. I measured a suite of inorganic and organic EACs and assessed their associated impacts on fish intersex occurrence and severity in a river system in North Carolina and South Carolina. The specific objective was to assess the relation of contaminants in water, sediment, and fish tissue to the occurrence and severity of the intersex condition in wild, adult black bass (*Micropterus*), sunfish (*Lepomis*), and catfish (Ictaluridae) species at 11 sites located on the Yadkin-Pee Dee River. Polycyclic aromatic hydrocarbons (PAHs), ethinylestradiol (EE2), and heavy metals were the most prevalent contaminants that exceeded various thresholds or effect levels for the protection of aquatic organisms. Fish intersex condition was most frequently observed in black basses (mean 40%, range 6.7–100%) and was less frequently detected in sunfishes (mean 7%, range 0–20%) and catfishes (mean 1%, range 0–7%). Among intersex fish, the among site mean severity index was 2.7 for black bass, 1.8 for sunfish, and 1.0 for catfish, with the detected range spanning the entire range of the index (1–4). The occurrence of the intersex condition in fish did not show any

longitudinal trend in the river. There were no significant differences in condition or health parameters (gonadosomatic index, relative weight, etc.) between intersex and non-intersex black bass. Mean black bass and catfish tissue contaminant concentrations were higher than that of sunfish, likely because of their higher trophic position in the food web. Principal component analysis determined that waterborne PAHs was the most correlated environmental contaminant for intersex occurrence and severity in black bass and sunfish. All subsets regression modeling revealed that black bass intersex was associated with waterborne organochlorine pesticides and sediment-associated mercury. Sunfish intersex was related to waterborne PAHs. EACs are having an adverse effect on the health of wild fish species, as indicated by intersex condition, with variable effects throughout the river due to fluctuations in EAC inputs and the dynamic nature of the riverine system. These findings enhance the understanding of the relationship between contaminants and fish health and provide information that can guide ecologically comprehensive conservation and management decisions.

1. Introduction

Aquatic ecosystems are under threat because of their susceptibility to act as a "sink" by accumulating numerous chemical contaminants that are being released from industrial, agricultural, and municipal sources (Scholz and Mayer, 2008). Anthropogenic impacts may become increasingly problematic for aquatic species within ecosystems due to continued human population growth and pollution. One class of contaminants that has been identified as problematic is endocrine active contaminants (EACs). The endocrine system is important to maintain health and reproductive success of organisms, and alterations to this system can be detrimental (Solomon, 2015). EACs have the ability to interfere with the endocrine system by disrupting normal synthesis, storage, release, metabolism, transport, binding action, and elimination of endogenous hormones (Kavlock et al., 1996). With exposures at sufficient levels, or during critical times of development, EACs also have the potential to cause toxicity to an organism (Barton and Andersen, 1998).

EACs are often introduced into aquatic systems through agriculture, industrial, and municipal effluents and include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, bisphenol A (BPA), and heavy metals (Hinck et al., 2009; Muthumbi et al., 2003). Originating from numerous routes of entry and anthropogenic influences, EACs have been detected in aquatic environments throughout the world and are likely to remain ever-present (Abdel-moneim et al., 2015; Kolpin et al., 2002; Ternes et al., 1999).

The estrogenic potency of EACs has been cause for concern because of the potential for negative effects on the reproduction and sustainability of wildlife populations. EACs

have been linked to skewed sex ratios, reduced fecundity, production of vitellogenin (an egg-yolk precursor) in male fish, intersex condition, and population collapse in fish (Bhandari et al., 2015; Brian et al., 2007; Jobling et al., 1998; Kidd et al., 2007; Puy-Azurmendi et al., 2013; Williams et al., 2009; Woodling et al., 2006). Intersex, the presence of female germ cells within a predominantly male gonad (Nolan et al., 2001), has been suggested as a biomarker of endocrine disruption from environmental, and especially estrogenic, EACs (Bahamonde et al., 2013; Barnhoorn et al., 2004; Leino et al., 2005). To assess the impacts of EACs on wildlife, intersex has been evaluated in various wild fish populations worldwide (Adeogun et al., 2016; Allen et al., 1999; Bizarro et al., 2014; Blazer et al., 2007; Tetreault et al., 2011). By examining intersex and EACs researchers are able to better understand the dynamics and health of an ecosystem. Fish are particularly applicable for this area of study because they are susceptible to EACs through the aquatic environment, can act as sentinel species, and are indicators of ecosystem health.

In the southeastern United States, intersex has been documented in several studies (Hinck et al., 2009; Kellock et al., 2014; Lee Pow et al., 2016). This region is especially relevant for the study of EAC effects on fish because of previous research and the impacts caused by dense human populations, agriculture, industry, wastewater treatment, and concentrated animal feeding operations, which have all been described as significant sources of EACs (Kolpin et al., 2002; Mills and Chichester, 2005; Vajda et al., 2008). Recently, a nation-wide study of intersex was completed by Hinck et al. (2009) in which researchers assessed the intersex condition in black basses at 111 sites in nine river basins. This study found that the highest incidence of intersex, within the United States, occurred in the Yadkin-

Pee Dee River basin. Another study, by Sackett et al. (2015), detected high levels of EACs at the same sites where Hinck found high occurrence of intersex. These studies prompted research to understand what was causing high incidences of intersex in the Yadkin-Pee Dee River and associated EAC relationships. To better understand the association between intersex and EACs within a southeastern U.S. river system, I investigated intersex occurrence and severity associated with EAC concentrations. The primary objectives of my research were to (1) determine intersex occurrence and severity in common sport fishes in a southeastern U.S. river, (2) measure and evaluate contaminants in surface water and sediments of the same river, (3) investigate relationships between intersex and EACs, and (4) determine trends in EAC concentrations, intersex occurrence, and intersex severity, longitudinally within the Yadkin-Pee Dee river system. The ultimate goal of this research is to enhance the understanding of the relationship between contaminants and fish health to facilitate ecologically comprehensive conservation and management decisions.

2. Methods

2.1. Study System

The Yadkin-Pee Dee River of North Carolina and South Carolina was chosen as my study system. A total of 11 sites were selected longitudinally along the river in North Carolina (8 sites) and South Carolina (3 sites; Figure 1). Coordinates of site locations and site descriptions are included in Table SI 1. Sites exhibited varying levels of anthropogenic influence, land use, and habitat types and were also selected for ease of boat access and sampling logistics. Eight sites were located in riverine habitats and three were located in

reservoirs impounded by high dams. Three of the sites we studied were previously evaluated

by Hinck et al. (2009; Hinck et al. sites PRB 336, PRB 337, PRB 338; this study sites 74

Bridge, Pee Dee, Bucksport). Their research and finding of relatively high occurrence of

intersex in black bass among sites sampled nationwide also motivated the selection of this

southeastern U.S., impacted river for my study.

2.2. Fish Collection and Histopathology

   In April and May of 2014, boat-mounted electrofishing was conducted to catch wild, adult

fish at all sites (within 2 km upstream or downstream of a river site or within the sampled

reservoir). Additional sampling was conducted in June 2015 at the Bucksport, SC, site

because of the high incidence of intersex observed by Hinck et al. (2009) at this site.

Collections from 2014 and 2015 catches from the Bucksport site were combined for analyses.

Up to 10 male black bass (*Micropterus* spp.), 10 sunfish (*Lepomis* spp.), and 10 catfish

(Ictaluridae) were collected at each site whenever possible. These taxa represent fish with

varying life history strategies and are recreationally important sport fish within the river.

Black bass have special relevance, because of previous related research conducted on both

Largemouth Bass (*Micropterus salmoides*) and Smallmouth Bass (*Micropterus dolomieu*)

examining intersex occurrence (Blazer et al., 2014; Hinck et al., 2009; Yonkos et al., 2014).

Fish, at sizes that were indicative of sexual maturity, were collected, and any obvious

females (eggs apparent when pressure was applied to the abdomen) were released back into

the river. Euthanasia of male fish was completed following IACUC guidelines, with a lethal

overdose of pH-buffered tricaine methanosulfate (MS-222, Sigma Aldrich). Length (TL,

mm) and weight (g) of each individual was measured, as well as observations of any external

lesions and abnormalities. Relative weight (W_r), an index of individual fish condition, was

determined for each individual according to the formula (Neumann et al., 2012)

$$W_r = \left(\frac{body\ weight}{10^a \times length\ (mm)^b} \right) \times 100.$$

Intercept (a) and slope (b) parameters for each species are in Table SI 2. Following

measurements, sex was verified macroscopically and male internal organs were removed.

Testes were weighed and gonadosomatic index (GSI; Strange, 1996) was calculated as

$$GSI = \left(\frac{gonad\ weight}{body\ weight} \right) \times 100.$$

 Liver was removed, weighed, and hepatosomatic index (HSI; Tveranger, 1985) was

calculated as

$$HSI = \left(\frac{liver\ weight}{body\ weight} \right) \times 100.$$

A gill segment and the spleen were excised and organs (including liver and testes) were

preserved in modified Davidson's fixative (35.15% distilled water, 31.35% ethanol, 22%

formalin [37-40%], and 11.5% glacial acidic acid). Macroscopic internal parasites and

abnormalities were observed and noted. Fish carcasses were placed in food-grade plastic

bags, labeled, and stored on ice until frozen at -20°C for later muscle tissue collections.

Tissues were left in fixative for 24 h and then were transferred to 70% ethanol until

histological processing occurred. Sections of the spleen, liver, and gill were cut and placed

into a suitable labeled cassette (perforated basket). Testis tissue was either embedded whole

or cross sections were cut with a microtome (depending on testis size) creating a

representative tissue sample. Preserved tissues were embedded in paraffin, sectioned at 5

μm, and stained using hematoxylin and eosin by the North Carolina State University (NCSU)

College of Veterinary Medicine Histopathology Laboratory (Raleigh, NC). Following

histological preparation, a certified fish pathologist examined testis tissue slides using light

microscopy for the occurrence and severity of intersex, which for this study, was defined as

the presence of oocytes within the testis tissue. Intersex occurrence was evaluated and

severity was determined using the method developed by Blazer et al. (2007), where severity

is ranked 1 to 4, with 4 being the most severe ranking. Spleen, gill, and liver tissue slides

were examined for abnormalities using light microscopy.

2.3. Water Estrogenic Activity Evaluation

Water samples were collected using solvent rinsed, and baked 2-L amber glass

bottles. At each site, a subsurface sample was collected and acidified to a pH of 2. Samples

were placed on ice and transported to NCSU where they were stored at 4°C for a maximum

of 72 h before processing. Water samples were then filtered and solid phase extraction was

completed. Extracts were analyzed for total estrogenic activity by a T47D-Kbluc bioassay,

which uses human breast adenocarcinoma cells and an E2β standard to determine E2β

equivalent concentrations (E2β Eq–ng/L). The bioassay and extraction process were detailed

by Yost (2014) and Lee Pow (2015).

2.4. Contaminant Analyses

We sampled organic contaminants in water using passive sampling devices (PSDs; O'Neal, 2014) and organic and inorganic contaminants in sediments with grab samples. In addition to measuring contaminant loading in the abiotic environment, we analyzed fish white muscle tissue for PCBs, OCPs, and metals to determine whether contaminant concentrations exceeded published thresholds for the protection of aquatic wildlife. We also examined the relationship between fish muscle contaminants and environmental contaminants from the water and sediment compartments. Specific contaminants analyzed can be found in Table SI 3.

2.5. Muscle Tissue Metal Analysis and Organic Contaminant Extraction

A subsample of the fish, representing all of the sites and major genera, were selected for muscle tissue contaminant analyses. Composite samples of individuals of the same species and site location were made when adequate tissue from an individual was not available (this was only necessary for sunfish). Using standard fish processing protocols, muscle tissue samples were dissected and homogenized (US EPA, 2000). Following homogenization, samples were frozen at -80°C until further processing was conducted. In preparation for contaminant analyses, muscle tissue samples were lyophilized and manually homogenized. RTI International (Durham, NC) analyzed the fish tissue samples for 22 metals (Table SI 3). Mercury (Hg) concentration in tissue samples was determined using a Milestone DMA-80 direct mercury analyzer. Other metals were analyzed with a modified version of Method 3050B (US EPA, 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. The Analytical Toxicology Laboratory at NCSU (Raleigh, NC) analyzed the fish samples for PCBs and OCPs (Table SI 3) and determined percentage of lipids in each muscle tissue sample. For organics, lyophilized muscle tissue was extracted with DCM by means of pressurized solvent extraction using a Buchi Speed Extractor E-916. Extracts were then cleaned using gel permeation chromatography (GPC) and were processed through Florisil solid phase extraction cartridges.

2.6. Sediment Metal Analysis and Contaminant Extraction

Sediment samples were collected at each site during May and June of 2014. Samples were taken by wading from the shoreline, in an upstream direction from the boat access. Two composite samples, each consisting of 3-5 grabs of surficial (top 5 cm) sediment were collected using a stainless steel scoop and tray and were placed into 250 mL amber glass jars. Sediment was transported on ice and stored at -20°C until extraction. RTI International (Durham, NC) analyzed samples for 22 metals. Mercury (Hg) concentration in sediment samples was determined using a Milestone DMA-80 direct mercury analyzer. Other metals were evaluated with a modified version of Method 3050B (US EPA, 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. NCSU determined the concentrations of current use pesticides (CUPs), OCPs, PAHs, and PCBs (Table SI 3). Sediment samples were extracted with dichloromethane (DCM) by means of pressurized fluid extraction using a Buchi Speed

extractor E-916. Extracts were cleaned using GPC. Before chemical analysis extracts were concentrated to 0.5 mL under a gentle stream of nitrogen.

2.7. Water Contaminant Extraction

PSDs were deployed at each site for approximately 28 days to determine time-weighted estimated concentrations of waterborne contaminants. Two types of PSDs were deployed. Low-density polyethylene strips (PEPSD) were utilized to measure OCPs, PCBs, and PAHs. A sorbent containing cartridge, universal passive sampling device (UPSD), was used to assess CUPs, hormones, and industrial EACs (Table SI 3). A weighted cage containing both types of PSDs was connected to the riverbank upstream and on the opposite bank of the boat access (to reduce impacts directly from boaters and deter vandalism of cages) or at least 100 m away from the boat access in reservoirs. Each cage was suspended in the water column by a buoy and kept from moving downstream by attaching a brick. After approximately 28 days, PSDs were retrieved from each site, removed from their cages, wrapped in baked aluminum foil, placed on ice, transported to NCSU, and stored at -20°C until extraction. PEPSDs were serially extracted three times over a 24-h period with a total of 150 mL of DCM. UPSDs analyzed for CUPs were placed in 20-mL vials and serially extracted two times over a 4-h period with a total of 40 mL DCM. Extracts were concentrated, filtered, and stored at -20°C until analysis. Extracts were further concentrated under a gentle stream of nitrogen to approximately 0.5 mL just prior to analysis. UPSDs analyzed for hormones and industrial EACs were placed in 20-mL vials and extracted with 10 mL of ethyl acetate by shaking for 1-h at 150 rpm. Nitrogen evaporation at 35°C under

34.5 kPa was then conducted to reduce extracts to 0.5 mL. Extracts for hormone and

industrial EAC analysis were additionally filtered through a 0.45-μm polytetrafluoroethylene

filter into a 1.5-mL microvial, evaporated with a nitrogen stream at ambient temperature until

dry, and cap sealed with argon gas. Complete description of PSD protocols, extraction

procedures, and analytical instrumentation are detailed by O'Neal (2014) and Lee Pow

(2015).

2.8. Analysis of Sediment, PSD, and Muscle Extracts

Extracts from PSDs and sediment samples were analyzed for 42 PAHs, 28 OCPs, 21

PCBs, and 47 CUPs. PSD extracts were also analyzed for concentrations of 7 estrogen-

related hormones and 2 industrial EACs. Muscle tissue extracts were analyzed for 21 PCBs

and 28 OCPs (Table SI 3). CUPs, OCPs, PAHs, and PCBs were measured using an Agilent

6890 gas chromatograph (GC) connected to an Agilent 5973 mass selective detector (MSD)

operated in Select Ion Monitoring (SIM) mode. Analytes were separated on a Restek Rtx-

5MS column with a 5 m integrated guard column. Hormones and industrial EACs were

analyzed on an Agilent 7890 GC connected to an Agilent 7000 MSD operated in SIM mode,

with back flushing following a blank injection of pyridine to condition the column. All

analyses adhered to rigorous quality assurance protocol and included procedural blanks,

replicate samples, spiked samples, and data correction using surrogate recoveries, if

necessary. Water contaminant results are presented in ng/L and sediment and muscle tissue

contaminant results are presented in ng/g dry weight (DW). Detection limits (DL) for

waterborne contaminants were 0.2 ng/L for PAHs, PCBs, and OCPs, 0.5 ng/L for CUPs, and

0.1 ng/L for hormones, nonylphenol, and BPA (based on an equivalent 30-day PSD deployment). DLs for sediment and muscle tissue contaminants were 0.1 ng/g for OCPs and PCBs, 1.0 ng/g for PAHs, and 2.0 ng/g for CUPs (all DW). Sediment DLs, for metals that were detected, are listed in Table 3. DLs for metals in muscle tissue are identical to the DLs for sediment. Muscle tissue results, when necessary for comparisons with thresholds, are presented in wet weight by conversions using percent moisture in each sample.

Chemicals were evaluated both as individual analytes and as total concentrations of chemical classes, when appropriate. For PAHs, individual analytes were examined and a total sum of analytes was determined to compare to predicted effect concentrations and threshold effect concentrations (PECs; TECs; MacDonald et al., 2000). PAHs in water and sediment samples were also analyzed using the equilibrium partitioning sediment benchmark toxicity units (ESBTU; US EPA, 2012) method, which incorporates the additive nature of PAH toxicity and the bioavailability of PAHs due to organic carbon in the sediment. Of the 42 PAHs analyzed, 34 have PAH potency divisors published by the US EPA (2012). Using the potency divisor, the determined concentration and, for the sediment sample, the carbon content of the sediment sample, a toxic unit (TU) was determined for each PAH (acute and chronic values). Then, all 34 PAH toxic units were summed to determine an overall PAH toxic unit value, with a value less than 1.0 indicating that it is unlikely for the PAHs to cause adverse effects to aquatic life and a value greater than 1.0 indicating that aquatic life is possibly being negatively affected.

2.9. Statistical Analysis

All statistical analyses were completed using JMP Pro 12 (SAS, Cary, NC). The Shapiro-Wilk normality test was conducted on all continuous variables to assess normality. The majority (>50%) of variables did not conform to a normal distribution and the condition was not remedied by transformation in most variables, thus, non-parametric statistical procedures were applied. Intersex occurrence is presented as a percentage of fish sampled at each site with the intersex condition, and intersex severity is presented as the average severity index (1-4) among intersex individuals at each site. A Kruskal-Wallis test was applied to assess relationships between species and fish health parameters, intersex severity ranking/occurrence and genera, intersex condition and fish health parameters, and fish families and muscle tissue contaminants. Linear regression was utilized to determine relationships between environmental concentrations of contaminants and fish muscle tissue contaminant concentrations. Logistic regression was performed to explain intersex condition by fish muscle tissue contaminant concentrations. Principal component analysis (PCA) was used to assess the trends among environmental contaminants, intersex occurrence, and intersex severity within the river. Analyses were weighted by the number of individuals collected at each site. All significance levels were set to $\alpha = 0.05$.

We adopted an information theoretic approach to model contaminant-intersex relationships. All subsets regression was performed to identify plausible relationships between environmental contaminants and intersex occurrence and severity. The candidate model included waterborne and sediment contaminant concentrations. The sum of chemical contaminants within a class, or a representative contaminant from each class, was selected as

14

independent variables for model development. Atrazine (representative CUP, water, ng/L), industrial EACs (sum of BPA and nonylphenol, water, ng/L), ethinylestradiol (EE2, water, ng/L), PCBs (sum of all congeners, water, ng/L), OCPs (sum of all OCPs, water, ng/L), PAHs (sum of all PAHs, water, ng/L), PAHs (sum of all PAHs, sediment, ug/g DW), OCPs (sum of all OCPs, sediment, ug/g DW), and Hg (representative metal, sediment, ug/g DW) were the predictors selected based on their occurrence in the river, co-linearity, environmental relevance, and background knowledge of EACs and their influence on intersex in fish. If a contaminant concentration was not detected the value used for statistical procedures was expressed as 50% of the DL. Akaike's information criteria corrected for small-sample bias (AICc) was determined for each candidate model (Akaike, 1973; Burnham and Anderson, 2002). Candidate models were arranged from lowest to highest AICc and a ΔAICc was calculated for each model. Akaike weight (w_i) was calculated for each model to determine the relative likelihood of each model, with the highest w_i indicating the most plausible model (Burnham and Anderson, 2002). Then, a set of confidence models was identified by selecting models with w_i within 10% of the best-model w_i following Ruiz and Peterson (2007), Thompson and Lee (2000), and similar to Royall (1997), to evaluate model strength. To determine relative variable importance, variables were ranked by summing all w_i values of models that included a specific variable. Total w_i for variables were compared and ranked, and the variable with the highest w_i was deemed the greatest importance. The all subsets regression modeling process was completed for black bass intersex occurrence, black bass intersex average severity, sunfish intersex occurrence, and sunfish intersex average severity.

3. Results

3.1. Fish Health Parameters

In 2014 and 2015, I collected a total of 268 male black bass, sunfish, and catfish (n=80, n=112, n=76; respectively, 12 species total). Species and the number of individuals collected at each site varied (Table 1). Individuals from each genus and family were captured at each site except for the Route 801 site, where only Ictaluridae were collected. Largemouth Bass, Bluegill *Lepomis macrochirus*, and Blue Catfish *Ictalurus furcatus*, were collected most frequently in the black bass, sunfish, and catfish groups, respectively. Seventy-eight individual fish were collected from reservoirs and 190 from riverine sites. Of all individuals from all sites, there was no significant difference ($p > 0.05$) in the length or weight among the different black bass species (Figure SI 1A, B). There were significant differences among black bass species in GSI, HSI, and W_r values ($p<0.05$; Figure SI 1C, D, E). Length, weight, GSI, and W_r were significantly different among sunfish species ($p<0.05$; Figure SI 1A, B, C, E). No significant difference in HSI was detected among sunfish species (Figure SI 1D). Length, weight, HSI, and W_r differed significantly among catfish species, but GSI was not significantly different among catfish species (Figure SI 1A, B, C, D, E). Species within each group were combined for further statistical analysis.

3.2. Fish Intersex Occurrence and Severity

Intersex was observed in 40% of black bass (32 of 80 collected), 7% of sunfish (8 of 112 collected), and 1% of catfish (1 of 76 collected). Specific intersex occurrence for each species at each site is found in Table 1. Intersex occurrence in black bass ranged 6.3–100.0%

16

among the 10 sites where they were collected (Figure 2A). Average severity of black bass

intersex fish was 2.7. Sunfish intersex occurrence ranged 0.0–16.7% among the 10 sites

from which they were collected (Figure 2B). Severity of intersex sunfish averaged 1.8

(Table 1). Intersex in catfish was only observed at the most downstream site (Bucksport,

SC), with only one individual displaying the condition (7.7% occurrence, severity 1; Figure

2C; Table 1). Due to the low occurrence of catfish with the intersex condition they were

excluded from further statistical analyses. Intersex occurrence and fish health parameters

were only compared for black bass because they were the only fish group with sufficient

sample sizes of both intersex and non-intersex individuals. When comparing black bass

length (mm), weight (g), W_r, GSI, and HSI between intersex and non-intersex individuals

there were no significant differences (p>0.05). There were also no differences in fish health

parameters among the severity ranking groups (p>0.05).

3.3. Muscle Tissue Analyses

A total of 30 black bass, 28 sunfish (14 composite samples), and 29 catfish muscle

tissue samples were analyzed for organic and inorganic contaminants, and results for each

species at each site can be found in Table SI 4. Black bass samples had a mean percent

moisture of 79.8%, catfish had a mean 78.6%, and sunfish had a mean 80.0% moisture.

OCPs were present in the muscle tissue of all three taxa, with 6 of the 28 OCPs detected.

OCPs detected in black bass tissue were hexachlorobenzene, trans-chlordane, cis-chlordane,

trans-nonachlor, 4,4'-DDE, and 4,4'-DDD (21% of the 28 OCPs analyzed).

Hexachlorobenzene and 4,4'-DDD were present in only 1 and 2 samples, respectively. Total

OCP concentration (sum of all OCPs detected) in black bass tissue samples ranged 2.2–88.0 ng/g dry weight (DW) (0.6–18.4 ng/g wet weight (WW)). OCPs detected in sunfish were heptachlor epoxide, trans-chlordane, cis-chlordane, trans-nonachlor, and 4,4'-DDE (18% of the 28 OCPs analyzed). Heptachlor epoxide was detected in only one sample. OCP concentrations in sunfish ranged from below detection limits (BDL) to 27.1 ng/g DW (BDL–5.2 ng/g WW). Trans-chlordane, cis-chlordane, trans-nonachlor, 4,4'-DDE, and 4,4'-DDD were OCPs detected in catfish muscle tissue. 4,4'-DDD was detected in only four samples. Concentrations of OCPs in catfish ranged BDL–127.6 ng/g DW (BDL–39.1 ng/g WW). OCP concentrations among the black bass, sunfish, and catfish were significantly different ($p<0.0002$) with catfish and black bass having higher concentrations of OCPs than sunfish (Figure 3A). There were no significant differences detected in OCP concentrations between species of black bass, species of sunfish, or species of catfish when individuals from all sites were pooled ($p>0.05$). OCP concentrations in fish tissue varied among sites, with highest OCP tissue concentrations occurring at the 74 Bridge, Digg's Tract, Badin Lake, and Route 801 sites (Figure 4A). When examining differences among sites (with all species pooled) there was a significant difference between sites ($p=0.0007$) with the Route 801 site having significantly higher concentrations of OCPs than the rest of the sites (Figure 4A). This significant difference is most likely influenced by low sample size ($n=2$) and that only catfish were collected at this site.

Fish muscle tissue samples were analyzed for 21 PCB congeners, and 14 (67%) of those congeners were detected (Table SI 3). Concentrations of individual PCB congeners were summed as a total PCB concentration for each muscle tissue sample. Total PCB

concentration in black bass ranged 1.37–202.40 ng/g DW (0.31–42.5 ng/g WW). In sunfish

samples, it ranged BDL–42.3 ng/g DW (BDL–12.8 ng/g WW), and in catfish it ranged 4.6–

215.2 ng/g DW (0.89–65.9 ng/g WW). PCB concentrations were significantly different

among black bass, sunfish, and catfish (p<0.0001; Figure 3B) with lower PCB concentrations

in sunfish. Significant differences in PCB concentrations were detected among black bass

species (p=0.029) and catfish species (p=0.045); however, some sample sizes were small

(n≤5). No significant differences were detected in sunfish concentrations of PCBs. Among

sites, average PCB concentrations (DW of black bass, sunfish, and catfish) varied and were

highest at the 74 Bridge, Digg's Tract, and Society Hill sites (Figure 4B). There was no

significant difference detected in PCBs, with all species pooled among sites (p>0.05).

Among all fish tissue sampled, the NC Department of Public Health total PCB action level

for human consumption of freshwater fish of 50 ng/g WW was exceeded in only one catfish,

at the 74 Bridge site (NC DPH, 2007).

Metal analysis revealed that 14 of the 22 metals were predominant (above DL in at

least 20% of samples) in black bass, sunfish, and catfish tissue (Table SI 3). These metals

included aluminum (Al), barium (Ba), copper (Cu), iron (Fe), mercury (Hg), potassium (K),

magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), silicon (Si),

strontium (Sr), and zinc (Zn). The only metal that was detected in fish tissue that was of

concern (greater than the human health consumption advisory and/or aquatic wildlife

thresholds, when available) was Hg (Figure 4C; NC human health consumption threshold=

400 ng/g, muscle tissue WW). Concentrations of Hg were highest (exceeded 400 ng/g WW)

in black bass and catfish at the two most downstream sites, Pee Dee and Bucksport. Hg

muscle tissue concentration was significantly lower in sunfish when compared to both black bass and catfish.

3.4. Water E2β Equivalence and Contaminants

E2β equivalence was determined for each of the 11 sites and ranged from 0.10 to 1.26 ng/L (Table 2). No sample exceeded the 2.0 ng/L predicted-no-effect concentration for E2 (Caldwell et al., 2012); however, if estrogenicity was caused by other, more potent estrogens not measured by the assay, the levels could be of concern. E2β equivalence concentration varied among sites and was highest at the Route 801 and 74 Bridge sites (Figure 5A).

PAHs were present in the water at 100% of the sites, and 33 of the 42 PAHs tested were detected. PAHs were summed and a total PAH value was determined (ng/L) for each site. Total PAH concentrations ranged from 72.6 to 238.2 ng/L (Table 2). Concentrations fluctuated among sites with the highest levels occurring at the Route 801 and Society Hill sites. Downstream of these sites, concentrations seemed to attenuate (Figure 5B). ESBTU values ranged 0.009–0.035 TU for acute toxicity, all below 1. For chronic toxicity, TUs ranged 0.017–0.071 TU, all below 1, indicating a low likelihood for negative effects of waterborne PAHs on aquatic life (Table SI 5).

OCPs were present in water at 100% of the sites and 9 of the 28 OCPs tested were detected. OCPs were summed to get a total OCP concentration (ng/L) for each site. OCP concentrations ranged 0.26–12.29 ng/L (Table 2). No individual OCP concentration exceeded published aquatic life protection thresholds. No combined OCP concentration

threshold or aquatic life thresholds exist. OCPs were notably elevated at the Red Hill, Route 801, and Society Hill sites (Figure 5C).

PCB congeners were present in water above detection limits at 8 of the 11 sites and of the 21 PCB congeners evaluated, 10 were present in the river. All PCB congeners were summed and a total PCB concentration for each site was calculated (ng/L). Concentrations ranged BDL–7.31 ng/L (Table 2). Total PCB concentrations did not exceed the 14.0 ng/L chronic exposure threshold for freshwater aquatic life at any of the sites (US EPA, 2016). Concentrations were low (<1.5 ng/L) at all sites upstream of Digg's Tract and the most downstream site, Bucksport, but they were elevated (4.5–7.5 ng/L) at the Digg's Tract, Society Hill, and Pee Dee sites (Figure 5D).

CUPs were present in water at 10 of the 11 sites, with no CUPs detected at Kerr Scott Reservoir, the most upstream site. Out of the 47 CUPs evaluated, 8 were present in the river. CUPs were summed to get a total CUP concentration (ng/L) for each site (Table 2). The CUPs ranged from BDL to 315.6 ng/L. CUP concentrations were low at the uppermost sites, variable at intermediate sites, and decreased at the most downstream site, Bucksport (Figure 5E).

From the PSDs, two industrial EACs and 7 hormones were evaluated; both industrial EACs (nonylphenol and BPA) were detected. Nonlyphenol was detected at all 11 sites and ranged 0.40–4.60 ng/L (Table 2). Concentrations of nonylphenol were low (<1 ng/L) at upstream sites and higher (>1 ng/L) at all sites downstream of Badin Lake (Figure 5F). None of the measured concentrations exceeded the 6,600 ng/L chronic exposure threshold determined by the US EPA (2005). BPA was present at 9 of the 11 sites, with no detections

at the two most upstream sites. Concentrations of BPA ranged BDL–2.27 ng/L (Table 2).

Concentrations of BPA were similar to nonylphenol, with concentrations low upstream (<1

ng/L) and higher (>1 ng/L) at downstream sites below Blewett Lake (Figure 5G). BPA

concentrations did not exceed the 1,500 ng/L predicted-no-effect-concentration (PNEC), set

by the European Union (Aschberger et al., 2010) for the protection of freshwater aquatic

species, at any site. EE2 was the only hormone detected and was above detection limits at 7

of 11 sites. Concentrations of EE2 ranged BDL–1.97 ng/L (Table 2) and at all sites where

EE2 was detected, the concentrations exceeded the 0.1 ng/L PNEC for aquatic organisms

(Caldwell et al., 2012). The DL for EE2 was 0.1 ng/L, so any sites without detections should

not be considered absent of EE2, similar to all other BDL instances, and was supplemented

with half of the DL for modeling analysis. EE2 was detected at the 7 most downstream sites

(Figure 5H).

3.5. Sediment Contaminants

Of the 28 OCPs evaluated, 5 were detected in sediment samples. OCPs were summed

to get a total OCP concentration (ng/g DW) at each site. OCP concentrations ranged from

BDL–101.49 ng/g DW with concentrations highest at the Badin Lake site (36 times higher

than any other site, Table 2, Figure 6A). This high level of OCPs at the Badin Lake site was

due to the detection of hexachlorobenzene, which was not detected at any other site and

accounted for 100% of the OCP concentration. Hexachlorobenzene does not have any

known thresholds for human or aquatic wildlife health, and in a study by Fuchsman et al.

(1998), limited toxicity to benthic invertebrates was observed. 4'4-DDE was the most

frequently detected OCP with detections at 6 of 11 sites. 4'4-DDE levels did not exceed the threshold of 3.16 ng/g DW for freshwater ecosystems at any site (MacDonald et al., 2000). Chlordane was also detected at two sites (Route 801 and Digg's Tract) but was below the 4.5 ng/g DW interim sediment quality guideline that corresponds to threshold level effects, below which adverse biological effects are not expected (Environment Canada, 1999).

PCBs were detected in sediment at only 5 of the sites and a maximum of 4 of 21 congeners were present at any site (Table 2; Table SI 3). Overall, concentrations were low (<5 ng/g DW Total PCB concentration) and did not exceed the 59.8 ng/g DW threshold (MacDonald et al., 2000). Sites with PCB detections were Badin Lake and four more downstream sites (Figure 6B). Atrazine was the only CUP detected in the sediment (Table 2). It was present at only 4 of the sites and never exceeded the 6.62 ng/g DW screening benchmark developed by the US EPA (US EPA, 2006; Figure 6C).

Thirty-nine of the 42 PAHs evaluated were detected in sediment (Table SI 3). PAHs were summed to get a total PAH concentration in ng/g DW at each site. Total PAH concentrations ranged 1.66–11,068.05 ng/g DW (Table 2). PAH concentrations were similar at all sites except for Route 801, which was slightly elevated, and Badin Lake where PAH concentrations were eight times higher than average (Figure 6D). Total PAH levels exceeded the 1,610 ng/g DW threshold effect concentration (TEC) for freshwater ecosystems (MacDonald et al., 2000) at two sites, Route 801 and Badin Lake. PAH concentration did not exceed the PEC of 22,800 ng/g DW (MacDonald et al., 2000) at any site. ESBTU values ranged 0.0002–0.069 for acute toxicity and 0.001–0.281 for chronic toxicity (Table SI 6).

No sediment TUs exceeded 1, indicating little likelihood of sediment PAHs negatively affecting aquatic life.

Of the 22 metals analyzed in sediment, 18 were detected. Concentrations at each site and thresholds for each metal (when available) are listed in Table 3. Mn exceeded the lowest effect level of 460 ug/g DW at 5 of the 11 sites and exceeded the severe effect level of 1,100 ug/g DW at 1 site, Digg's Tract (Persaud and Jaagumagi, 1993; Figure 7A). Cd exceeded the TEC of 0.99 ug/g DW at all sites (Figure 7B), but did not exceed the PEC of 4.98 ug/g DW at any site (MacDonald et al., 2000). No other metal concentrations exceeded known benchmarks.

3.6. Environmental and Fish Muscle Tissue Contaminant Relationships

PCB and OCP concentrations in the water were not significantly correlated to the muscle tissue concentrations of PCBs and OCPs found in black bass ($\rho < 0.6$). Sediment PCB and OCP concentrations were also unrelated to black bass PCB and OCP muscle tissue concentrations. There was no significant relationship between Hg in the sediment and black bass tissue. Sunfish muscle tissue contaminant concentrations were not significantly correlated to OCPs or PCBs in water, or OCPs or PCBs in sediment, or sediment Hg. Catfish muscle tissue contaminant concentration was not significantly related to sediment Hg, PCBs, or OCPs. There was also no significant relationship between catfish muscle tissue PCBs or OCPs and PCBs or OCPs in water.

3.7. Intersex and Contaminants

Significant relationships between environmental contaminants and intersex occurrence and severity were evaluated for the black bass and sunfish taxa (catfish did not have enough intersex individuals for statistical analysis, n=1). Black bass intersex occurrence (% of fish at each site with the intersex condition) was significantly correlated with Cu (sediment, µg/g DW, Spearman's correlation coefficient, ρ=0.72), Hg (sediment, µg/g DW, ρ=0.67), Pb (sediment, µg/g DW, ρ=0.64), Mn (sediment, µg/g DW, ρ=0.78), Sr (sediment, µg/g DW, ρ=0.66), PAHs (sediment, µg/g DW, ρ=0.64), PCBs (sediment, µg/g DW, ρ=0.88) and OCPs (sediment, µg/g DW, ρ=0.89). Black bass intersex severity was not correlated to any environmental variable. Sunfish intersex occurrence was significantly correlated with Ba (sediment, µg/g DW, ρ=0.65) and Si (sediment, µg/g DW, ρ=0.70). Sunfish severity was correlated with Ba (sediment, µg/g DW, ρ=0.78), Cu (sediment, µg/g DW, ρ=0.64), Hg (sediment, ng/g DW, ρ=0.69), Mn (sediment, µg/g DW, ρ=0.67), Si (sediment, µg/g DW, ρ=0.83), and Sr (sediment, µg/g DW, ρ=0.64).

Adequate sample size of intersex (n=32) and non-intersex (n=48) black bass individuals allowed analysis of relationships between intersex condition and environmental contaminant concentrations. PAHs, OCPs, CUPs, and PCBs (water, ng/L) were significantly higher in relation to the intersex black bass, relative to those without the condition. PAHs, OCPs, and PCBs were significantly higher in the sediment (µg/g DW) at sites with intersex black bass individuals. Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si, Sr, and V concentrations (sediment, ng/g DW) were also significantly higher at sites with intersex individuals. K and Mg (sediment, µg/g DW) were significantly lower at sites with intersex individuals.

3.8. Principal Components Analysis

Principal component analysis was applied to assess the relationship of environmental contaminants on the intersex occurrence and severity of black bass and sunfish. Two principal components accounted for 68.9% of data variability for black bass intersex occurrence and severity (Figure 8A, Table SI 7). Sites with high intersex occurrence and severity clustered most closely to PAHs in the water. Occurrence and severity were similarly related to the other waterborne and sediment contaminants. Sites with lower intersex occurrence (<20%) were clustered and less correlated with any EACs. Two principal components accounted for 63.2% of data variability in sunfish intersex occurrence and severity (Figure 9B, Table SI 7). Occurrence closely clustered with PAHs in the water. Severity grouped evenly between Hg in the sediment and PAHs in the water. Most lower (<8%) intersex occurrence sites clustered together, away from EAC variables. Intersex occurrence and severity in sunfish and black bass both grouped between waterborne and sediment sequestered EACs, with PAHs in the water and Hg in the sediment being the most correlated for black bass and sunfish, respectively.

3.9. Intersex Models

A total of 10 confidence models were developed for bass intersex occurrence using the AIC_c analysis for model selection. The most plausible candidate model weight (w_i) was 0.244. All candidate models with w_i greater than 0.0244 (10% of the best-fitting model) were selected as confidence models (Table 4). Top models included OCPs (water) and Hg (sediment) as explanatory variables. Variable ranking also identified OCPs (water) and Hg

(sediment) in the top two rankings for the bass intersex occurrence model (Table 5). Bass

intersex severity modeling yielded three confidence models. The most plausible candidate

model weight was 0.4317, and two more models were selected within the 10% w_i range

(Table 6). Only two variables were included within these three models, atrazine (water) and

industrial EACs (water). These variables were also highly ranked (Table 7).

A total of five confidence models were developed for sunfish intersex occurrence.

The most plausible model w_i was 0.3452. Additional confidence models were selected with

$w_i \geq 0.03452$ (Table 4). All confidence models were comprised of one variable, with PAHs

(water) and PCBs (water) being most plausible. PAHs (water) and Hg (sediment) were the

highest-ranking model variables (Table 5). Sunfish intersex severity modeling developed 10

confidence models. The most plausible model included PAHs (water, ng/L, w_i=0.2645).

Nine additional confidence models were selected with $w_i \geq 0.02645$ (Table 6). PAHs (water)

and Hg (sediment) were the top ranking variables for this model set (Table 7).

4. Discussion

4.1. Intersex Occurrence and Severity

My results indicate that the intersex condition occurs throughout the Yadkin-Pee Dee

River at varying frequencies, is dependent on species, and is related to EACs. Black bass

displayed the intersex condition most frequently, which is similar to other studies that

examined intersex in riverine fishes (Lee Pow et al., 2016; Hinck et al., 2006; Hinck et al.,

2009). The percentage of black bass with intersex (40%) lies within ranges of intersex

occurrence in previous studies (Blazer et al., 2007; Blazer et al., 2014; Kellock et al., 2014;

Hinck et al., 2009). Intersex was also found in sunfish, but to a lesser extent (7% compared to 40% in black bass), which is similar to findings of Lee Pow et al. (2016). Intersex was almost non-existent in catfish (1% of individuals at 9% of sites), which differs from findings by Barnhoorn et al. (2004), who found 20% intersex occurrence in Sharptooth Catfish (*Clarias gariepinus*) and Hinck et al. (2009), who found 7% intersex in Channel Catfish (*Ictalurus punctatus*) at 50% of sites. This variation in catfish intersex may be due to species differences or varying EAC exposures. Another study of Sharptooth Catfish (Brink et al., 2012) found subtle changes in EAC biomarkers, but no intersex condition, indicating limited catfish susceptibility to EACs.

Intersex severity also varied among sites and species. Black bass severity was, on average, higher than both sunfish and catfish. For example, 60% of black bass were identified as severity 3 or 4 and the majority (75%) of intersex sunfish were classified as either severity 1 or 2. One catfish (Channel Catfish) displayed the intersex condition and was identified as a severity "2". Most related studies do not include a severity index, and there is little known regarding severity among different species; however, black bass may be more susceptible to intersex based on frequent higher severity rankings.

Differences in fish health parameters between intersex and non-intersex black bass were not detected. There were also no significant distinctions in health parameters between the severities. This lack of variation in fish health parameters and intersex condition is consistent with other studies that found little to no significant relations (Hinck et al., 2009; Kellock et al., 2014). Hinck et al. (2009), however, found significant differences in age and reproductive stage of fish that displayed the intersex condition in the Yadkin-Pee Dee River.

I did not determine age or reproductive stage and thus could not assess these influences in my study. Overall, the intersex condition was not related to general fish health parameters such as length, weight, or GSI of black bass.

4.2. EACs

EACs were prevalent at all 11 sites examined on the Yadkin-Pee Dee River. In total, 169 contaminants were analyzed and many (58%, n=98) were detected in fish muscle tissue, sediment, or surface water (Table SI 3). Contaminant detection and concentration in muscle tissue of fish were generally lowest in sunfish. This may be due to the sunfishes' lower trophic level in the food web and consumption of primary consumers, compared to black bass, which are piscivorous secondary consumers and may, therefore, be exposed to more EACs through their diet (Keast, 1978; Walter III and Austin, 2003). This lower level of dietary exposure in sunfish may help to explain the observed differences in intersex occurrence between sunfish and black bass. As opportunistic omnivores, it is difficult to infer a relationship between catfish diet and the intersex condition (Graham, 1999).

PAHs were detected at every site in water and sediment. Sediment PAHs were the only EAC that exceeded published thresholds for aquatic life in sediment. Although levels did not exceed an ESBTU value of 1, these levels only refer to acute and chronic toxicity and may not account for reproductive impairment or reduction in overall health. PAHs are ubiquitous in the environment and reach higher levels through anthropogenic sources such as coal- and gas-fired power plants, and many industrial processes (NRC, 1983). When PAHs enter aquatic environments, they adsorb onto organic particulate matter with most PAHs

adsorbing rapidly onto the sediment particles (Tuvikene, 1995). In our study, intersex black bass were associated with higher PAH concentrations in sediment (p=0.003) and water (p=0.0002). PAHs adversely impact organisms through suppression of the immune system, acting as anti-estrogens, and altering reproduction in fish (Tuvikene, 1995). Water PAHs were identified as the top explanatory variable in the sunfish intersex model and were associated with occurrence and severity in the PCA model. Sediment PAHs were also important in explaining the variation in black bass intersex. Negative effects of PAHs on fish endocrine and immune systems helps explain the intersex condition that I detected in the Yadkin-Pee Dee River and suggest that PAH exposure and effects be investigated further.

OCPs were detected at every site in the water and sediment compartments of the nine most downstream sites. However, none of the OCPs exceeded aquatic life thresholds, when they were available. Chlordanes and 4'4-DDE were the most prevalent OCPs in water and sediment. Hexachlorobenzene was also detected in water and in the sediment at one site (Badin Lake). OCPs were used extensively in agriculture and mosquito control from the 1940s to the 1960s and are, for the most part, now banned from use in the U.S. OCPs and their metabolites are extremely persistent in the environment and can bioaccumulate in organisms and biomagnify through the food webs (Skaar et al., 1981). The OCPs that I detected have been shown to bind and compete for androgen and estrogen receptors, inhibit androgen production, and block estradiol binding, thus possibly interrupting normal endocrine function (Mnif et al., 2011). Associated concentrations of OCPs in water and sediment were significantly higher in intersex black bass. Water concentration of OCPs were the highest ranking variables in the black bass intersex occurrence model and this may be due

to bioaccumulation and the black bass' position as an apex predator. Sediment OCPs were also important for black bass intersex occurrence, but were less important than waterborne OCPs. Water and sediment OCPs were not very relevant in sunfish intersex modeling and PCA, possibly due to the fishes' lower trophic position.

PCBs were detected in water at downstream sites and in the sediment of five sites. All PCB levels were low and did not exceed published aquatic life thresholds. PCBs are known persistent EACs and cause endocrine disruption and alter reproductive capabilities in fish (Baldigo et al., 2006; Hillis et al., 2015; Simmons et al., 2014). Elevated concentrations of PCBs in sediment and water were associated with black bass intersex. Waterborne PCBs were equally important in both the black bass and sunfish intersex occurrence models, and this is due to their endocrine disruption capabilities, persistence, and ability to bioaccumulate into all trophic levels of a food web.

CUPs were detected at 10 of the 11 sites in water and at 4 of the sites in sediment. Atrazine, simazine, and metolachlor were frequently detected in the water and atrazine was the only CUP detected in sediment. CUPs are used on agricultural lands and in the Yadkin-Pee Dee River basin, agriculture accounts for 24% of land use (Homer et al., 2015). CUPs are capable of endocrine disruption by acting as anti-androgens, strong estrogens, and receptor activators (Mnif et al., 2011). With existing pesticide use, and probable future use in NC and SC, CUPs will most likely remain in the aquatic environment for the foreseeable future. The toxicity of many CUPs has not been fully investigated for aquatic organisms and even less is known about the effects of pesticide mixtures or interactions with other EACs. In the PCA, CUPs tracked closely with industrial EACs, but were not explicitly related to

intersex occurrence or severity in black bass or sunfish. Atrazine (the representative CUP for modeling) was not identified as particularly important for explaining intersex occurrence in black bass or sunfish and was not significantly different between intersex and non-intersex black bass. This may be because of the low concentrations and overall detections of atrazine or CUPs in general. However, an important limitation was that water (PSD) sampling occurred for a one-month duration and CUP application times may vary throughout the year. A more comprehensive CUP field assessment coupled with laboratory testing during critical windows of fish development may bring to light CUP effects on fish health in natural environments. Atrazine was a predominant variable in black bass intersex severity, and this may indicate that CUPs have a more influential role on EAC effects in areas where initial disruptions are occurring (areas of intersex occurrence).

Industrial EACs and estrogens have been increasingly investigated because of their potential to cause endocrine disruption at low concentrations (ng/g, ng/L; Mills and Chichester, 2005). In my study system, industrial EACs (BPA and nonylphenol), E2β-Eq, and EE2 were detected at most sites and were often found in higher concentrations downstream of, or in association with, urbanized areas. Six other estrogens were not detected in the river, but this may be due to instrument and method detection limits. In the future, improved methods for detecting estrogens and other EACs in environmental samples will be critical because EACs are thought to be capable of disruption at very low concentrations or within mixtures (Brian et al., 2007; Kolpin et al., 2002). EE2 is a synthetic estrogen and is >10 fold more potent than estradiol for several estrogen receptors and has a low threshold (0.1 ng/L), making its presence in the river of great concern (Caldwell et al.,

2012; Thorpe et al., 2003). Industrial EACs and estrogens were not important explanatory variables in PCA or modeling of black bass or sunfish intersex occurrence. Similar to CUPs, these compounds may fluctuate in the environment due to flow and input.

Within fish muscle tissue and sediment samples, up to 18 different metals were detected at almost all sites. Most metal concentrations did not exceed thresholds; however, Mn and Cd exceeded thresholds for aquatic life in sediment, and Hg in fish exceeded human health consumption thresholds in several specimens. Even at low concentrations, Cd enters the food web and bioaccumulates. It can interfere with steroid hormones of both male and female reproductive organs by disrupting biosynthesis of hormones and by binding to both androgen and estrogen receptors (Georgescu et al., 2011). Mn is a naturally occurring metal but can be found at higher levels in the environment because of anthropogenic influences such as industrial waste discharges, combustion of fossil fuels, mining, and leaching from landfills (ATSDR, 2012). Mn has been studied very little in fish; however, a study on rats found that Mn altered the production and secretion of reproductive hormones (Pine et al., 2005). Hg, and associated methylmercury, can cause adverse reproductive effects through suppressed and altered hormone levels (Tan et al., 2009). The presence of these heavy metals in both sediment and fish tissue is of concern because of the direct impacts they may exert on the endocrine system. Sediment Hg (the representative metal chosen for PCA and modeling) tracked with intersex severity in sunfish and, to a lesser extent, intersex occurrence in black bass when examining PCA results. Modeling identified Hg as high importance in black bass and sunfish intersex occurrence and sunfish intersex severity.

4.3. Riverine Contaminants and Intersex Trends

No distinct longitudinal trend in the fish intersex condition was observed within the Yadkin-Pee Dee River, which may be due to variable inputs of EACs, fluctuations in habitat characteristics and fish assemblages, and the dynamic nature of the river system. Some waterborne EACs (PCBs, nonylphenol, EE2, BPA) had elevated concentrations at downstream sites, and these may be due to influx from the confluence of smaller rivers, that may be influenced by wastewater effluent from several major cities. Sediment EACs did not exhibit any longitudinal trend and this may be due to sediment accumulation occurring locally from pollution sources and the possible lack of EAC degradation in sediment. Fish tissue concentrations also did not exhibit longitudinal trends, again presumably because of varying species and EAC inputs along the river. Hg concentrations in muscle tissue were elevated at the most downstream sites and this is likely due to the stimulation of methylmercury production in the sediments of low pH areas (i.e., swamps) that drain into the lowland rivers (Jernelov, 1972).

By examining EACs and the fishes in freshwater ecosystems, these organisms may function as sentinels for human populations and to investigate human health. EACs, and particularly estrogenic EACs, have been found in sediment, surface water, ground water, wastewater, aquatic life, and even the atmosphere (Campbell et al., 2006). To protect human health and ecosystems, it is imperative that contaminant influx be reduced and best practices for doing so are determined. Improved wastewater technologies, reduced outputs from industrial and power production facilities, low impact agriculture practices, and public awareness of EAC effects on the environment may be beneficial first steps.

4.4. Future Implications

Long-term exposure to EACs, even at low concentrations, has raised concern for population-level effects of fishes (Nash et al., 2004). Although population level threats were not examined in this study, EACs have been shown to cause population collapse of Fathead Minnow (*Pimephales promelas*) in a lake environment (Kidd et al., 2007). Monitoring of intersex condition, overall fish health, and EAC concentrations in the Yadkin-Pee Dee River will be required to detect reproductive failure or other population-level impacts. With probable increased anthropogenic impacts and new chemical contaminants being produced and released into the environment, it will be vital to investigate EACs in the environment and their subsequent influences on fish health and populations. In addition to contaminant monitoring, it may be necessary to improve wastewater treatment, reduce runoff, and mitigate overall contaminant pollution to reduce inputs of EACs into the aquatic environment.

In conclusion, the intersex condition appears to be correlated with both sediment and waterborne EACs, which are taxon-specific. There is a possibility that mixtures of EACs, working in an additive or synergistic manner, may increase their toxicity to organisms (Rajapakse et al., 2002). This mixture of EACs was also evident in the Lee Pow et al. (2016) study where it appeared that different EACs influenced the intersex condition and its severity. Further, the absence of intersex does not necessarily indicate that a fish is reproductively viable and thus, monitoring other reproductive biomarkers and reproductive success should be considered when evaluating the health of fish populations. Using modeling and PCA, we identified which variables are most plausible for explaining intersex

occurrence in black bass and sunfish. Although modeling was limited by sample sizes, this

method is a valuable initial exploratory exercise for identifying relationships between EACs

and the intersex condition. Our findings document a strong correlation between EACs and

intersex and provide the basis for future research to examine molecular mechanisms involved

with the development of the intersex condition. Such mechanistic research, along with

additional field research, may further elucidate individual- and population-level effects of

contaminants in aquatic systems toward sustainability of wild fish populations and help

determine best management practices.

REFERENCES

Abdel-moneim, A., Coulter, D.P., Mahapatra, C.T., Sepúlveda, M.S., 2015. Intersex in fishes and amphibians: population implications, prevalence, mechanisms and molecular biomarkers. J. Appl. Toxicol. 35, 1228–1240. doi:10.1002/jat.3204

Adeogun, A.O., Ibor, O.R., Adeduntan, S.D., Arukwe, A., 2016. Intersex and alterations in reproductive development of a cichlid, *Tilapia guineensis*, from a municipal domestic water supply lake (Eleyele) in Southwestern Nigeria. Sci. Total Environ. 541, 372–382. doi:10.1016/j.scitotenv.2015.09.061

Agency for Toxic Substances and Disease Registry (ATSDR), 2012. Toxicological profile for manganese. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. 2nd Int. Symp. Inf. Theory 267–281. doi:10.1016/j.econlet.2011.12.027

Allen, Y., Matthiessen, P., Scott, A.P., Haworth, S., Feist, S., Thain, J.E., 1999. The extent of oestrogenic contamination in the UK estuarine and marine environments - Further surveys of flounder. Sci. Total Environ. 233, 5–20. doi:10.1016/S0048-9697(99)00175-8

Aschberger, K., Munn, S., Olsson, H., Pakalin, S., Pellegrini, G., Vegro, S., Paya Perez, A.B., 2010. European Union Risk Assessment Report - BPA (4,4'– Isopropylidenediphenol (Bisphenol – A)), Assessment. doi:10.2788/40195

Bahamonde, P.A., Munkittrick, K.R., Martyniuk, C.J., 2013. Intersex in teleost fish: Are we distinguishing endocrine disruption from natural phenomena? Gen. Comp. Endocrinol. 192, 25–35. doi:10.1016/j.ygcen.2013.04.005

Baldigo, B.P., Sloan, R.J., Smith, S.B., Denslow, N.D., Blazer, V.S., Gross, T.S., 2006. Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. Aquat. Sci. 68, 206–228. doi:10.1007/s00027-006-0831-8

Barnhoorn, I.E.J., Bornman, M.S., Pieterse, G.M., Van Vuren, J.H.J., 2004. Histological evidence of intersex in feral Sharptooth Catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. Environ. Toxicol. 19, 603–608. doi:10.1002/tox.20068

Barton, H.A., Andersen, M.E., 1998. Endocrine active compounds: from biology to dose response assessment. Crit. Rev. Toxicol. 28, 363–423. doi:10.1080/10408449891344227

Bhandari, R.K., Deem, S.L., Holliday, D.K., Jandegian, C.M., Kassotis, C.D., Nagel, S.C.,

    Tillitt, D.E., vom Saal, F.S., Rosenfeld, C.S., 2015. Effects of the environmental

    estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development

    and adult behaviors in aquatic wildlife species. Gen. Comp. Endocrinol. 214, 195–219.

    doi:10.1016/j.ygcen.2014.09.014

Bizarro, C., Ros, O., Vallejo, A., Prieto, A., Etxebarria, N., Cajaraville, M.P., Ortiz-

    Zarragoitia, M., 2014. Intersex condition and molecular markers of endocrine disruption

    in relation with burdens of emerging pollutants in Thicklip Grey Mullets (*Chelon*

    labrosus) from Basque estuaries (South-East Bay of Biscay). Mar. Environ. Res. 96,

    19–28. doi:10.1016/j.marenvres.2013.10.009

Blazer, V.S., Iwanowicz, L.R., Iwanowicz, D.D., Smith, D.R., Young, J. A., Hedrick, J.D.,

    Foster, S.W., Reeser, S.J., 2007. Intersex (testicular oocytes) in Smallmouth Bass from

    the Potomac River and selected nearby drainages. J. Aquat. Anim. Health 19, 242–253.

    doi:10:1577/H07-031.1

Blazer, V.S., Iwanowicz, D.D., Walsh, H.L., Sperry, A.J., Iwanowicz, L.R., Alvarez, D.A.,

    Brightbill, R.A., Smith, G., Foreman, W.T., Manning, R., 2014. Reproductive health

    indicators of fishes from Pennsylvania watersheds: association with chemicals of

    emerging concern. Environ. Monit. Assess. 6471–6491. doi:10.1007/s10661-014-38685

Brian, J.V., Harris, C.A., Scholze, M., Kortenkamp, A., Booy, P., Lamoree, M., Pojana, G., Jonkers, N., Marcomini, A., Sumpter, J.P., 2007. Evidence of estrogenic mixture effects on the reproductive performance of fish. Environ. Sci. Technol. 41, 337–344. doi:10.1021/es0617439

Brink, K., Jansen van Vuren, J., Bornman, R., 2012. The lack of endocrine disrupting effects in Catfish (*Clarias gariepinus*) from a DDT sprayed area. Ecotoxicol. Environ. Safety. 79, 256–263. doi:10.1016/j.ecoenv.2012.01.006

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference, second ed. Springer-Verlag, New York.

Caldwell, D.J., Mastrocco, F., Anderson, P.D., Länge, R., Sumpter, J.P., 2012. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. Environ. Toxicol. Chem. 31, 1396–1406. doi:10.1002/etc.1825

Campbell, C.G., Borglin, S.E., Green, F.B., Grayson, A., Wozei, E., Stringfellow, W.T., 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. Chemosphere 65, 1265–1280. doi:10.1016/j.chemosphere.2006.08.003

Environment Canada, 1999. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life; Chlordane. Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment, Winnipeg, MB.

Fuchsman, P.C., Barber, T.R., Sheehan, P.J., 1998. Sediment toxicity evaluation for
hexachlorobenzene: Spiked sediment tests with *Leptocheirus plumulosus*, *Hyalella
azteca*, and *Chironomus tentans*. Arch. Environ. Contam. Toxicol. 35, 573–579.
doi:10.1007/s002449900418

Georgescu, B., Georgescu, C., Dărăban, S., Bouaru, A., Paşcalău, S., 2011. Heavy metals
acting as endocrine disrupters. Anim. Sci. Biotechnol. 44, 89–93.

Graham, K., 1999. A review of the biology and management of Blue Catfish. Amer. Fish.
Soc. Sympos. 24, 37–49.

Hillis, J.J., Garvey, J.E., Lydy, M.J., 2015. Contaminants reduce male contribution to
reproduction at the population scale. Ecosphere 6 (4), 66. doi:10.1890/ES14-00391.1

Hinck, J.E., Schmitt, C.J., Blazer, V.S., Denslow, N.D., Bartish, T.M., Anderson, P.J., Coyle,
J.J., Dethloff, G.M., Tillitt, D.E., 2006. Environmental contaminants and biomarker
responses in fish from the Columbia River and its tributaries: Spatial and temporal
trends. Sci. Total Environ. 366, 549–578. doi:10.1016/j.scitotenv.2005.11.008

Hinck, J.E., Blazer, V.S., Schmitt, C.J., Papoulias, D.M., Tillitt, D.E., 2009. Widespread
occurrence of intersex in black basses (*Micropterus* spp.) from U.S. rivers, 1995-2004.
Aquat. Toxicol. 95, 60–70. doi:10.1016/j.aquatox.2009.08.001

Homer, C.G., Dewitz, J.A., Yang, L., Jin, S., Danielson, P., Xian, G., Coulston, J., Herold, N.D., Wickham, J.D., Megown, K., 2015. Completion of the 2011 national land cover database for the conterminous United States- Representing a decade of land cover change information. Photogram. Engineer. Rem. Sens. 81, 345–354.

Jernelov, A. 1972. Factors in the transformation of mercury to methylmercury; in: Bertram, D.D., Hartung, R. (Eds.), Environmental Mercury Contamination. Ann Arbor Science Publishers, Inc. Ann Arbor, Michigan. p. 167–172.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498–2506. doi:10.1021/es9710870

Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D.M., Sinks, T., Tilson, H.A., 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ. Health Perspect. 104 (Suppl 4), 715–40. doi:10.1289/ehp.96104s4715

Keast, A., 1978. Feeding interrelations between age-groups of Pumpkinseed (*Lepomis gibbosus*) and comparisons with Bluegill (*L. macrochirus*). J. Fish. Res. Board Can. 35, 12–27. doi:10.1139/f78-003

Kellock, K.A., Trushel, B.E., Ely, P.C., Jennings, C.A., Bringolf, R.B., 2014. Survey of

    intersex Largemouth Bass from impoundments in Georgia USA. Trans. Am. Fish. Soc.

    143, 565–572. doi:10.1080/00028487.2013.824922

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick,

    R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc.

    Natl. Acad. Sci. U. S. A. 104, 8897–8901. doi:10.1073/pnas.0609568104

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., 2002.

    Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams,

    1999–2000: A national reconnaissance. Environ. Sci. Technol. 36, 1202–1211.

    doi:10.1021/es011055j

Lee Pow, C.S.D., 2015. Biological effects of complex estrogenic and other endocrine active

    mixtures in aquatic systems. North Carolina State University Dissertation. Raleigh,

    North Carolina.

Lee Pow, C.S.D., Law, J.M., Kwak, T.J., Cope, W.G., Rice, J.A., Kullman, S.W., Aday,

    D.D., 2016. Endocrine active contaminants in aquatic systems and intersex in common

    sport fishes. Environ. Toxicol. Chem. Accepted Article. doi:10.1002/etc.3607

Leino, R.L., Jensen, K.M., Ankley, G.T., 2005. Gonadal histology and characteristic

    histopathology associated with endocrine disruption in the adult Fathead Minnow

    (*Pimephales promelas*). Environ. Toxicol. Pharmacol. 19, 85–98.

    doi:10.1016/j.etap.2004.05.010

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of

    consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ.

    Contam. Toxicol. 39, 20–31. doi:10.1007/s002440010075

Mills, L.J., Chichester, C., 2005. Review of evidence: Are endocrine-disrupting chemicals in

    the aquatic environment impacting fish populations? Sci. Total Environ. 343, 1–34.

    doi:10.1016/j.scitotenv.2004.12.070

Mnif, W., Hassine, A.I.H., Bouaziz, A., Bartegi, A., Thomas, O., Roig, B., 2011. Effect of

    endocrine disruptor pesticides: A review. Int. J. Environ. Res. Public Health 8, 2265–

    2303. doi:10.3390/ijerph8062265

Muthumbi, W., De Boever, P., Pieters, J.G., Siciliano, S., D'Hooge, W., Verstraete, W.,

    2003. Polycyclic aromatic hydrocarbons (PAHs) and estrogenic compounds in

    experimental flue gas streams. J. Environ. Qual. 32, 417–422.

Nash, J.P., Kime, D.E., Van der Ven, L.T.M., Wester, P.W., Brion, F., Maack, G., Stahlschmidt-Allner, P., Tyler, C.R., 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ. Health Perspect. 112, 1725–1733. doi:10.1289/ehp.7209

National Resource Council (NRC), 1983. Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects. National Academy Press, Washington D.C.

North Carolina Department of Public Health (NC DPH), 2007. North Carolina Risk-Based Approach for PCB Advisories. Raleigh, NC

Neumann, R.M., Guy, C.S., Willis, D.W., 2012. Length, Weight, and Associated Indices, in: Zale, A.V., Parrish, D.L., Sutton, T.M. (Eds.), Fisheries Techniques 3rd ed. American Fisheries Society, Bethesda, Maryland; p. 637–676.

Nolan, M., Jobling, S., Brighty, G., Sumpter, J.P., Tyler, C.R., 2001. A histological description of intersexuality in the Roach. J. Fish Biol. 58, 160–176. doi:10.1006/jfbi.2000.1431

O'Neal, K.L., 2014. Use of passive sampling to measure the bioavailability of chemical contaminants in surface waters and soil. North Carolina State University Dissertation, Raleigh, NC.

Persaud, D., Jaagumagi, R., Hayton, A., 1993. Guidelines for the protection and management of aquatic sediment quality in Ontario. Minist. Environ. Energy. Ontario. doi:10.1080/00431672.1993.9930274

Pine, M., Lee, B., Dearth, R., Hiney, J.K., Les Dees, W., 2005. Manganese acts centrally to stimulate luteinizing hormone secretion: A potential influence on female pubertal development. Toxicol. Sci. 85, 880–885. doi:10.1093/toxsci/kfi134

Puy-Azurmendi, E., Ortiz-Zarragoitia, M., Villagrasa, M., Kuster, M., Aragón, P., Atienza, J., Puchades, R., Maquieira, A., Domínguez, C., López de Alda, M., Fernandes, D., Porte, C., Bayona, J.M., Barceló, D., Cajaraville, M.P., 2013. Endocrine disruption in Thicklip Grey Mullet (*Chelon labrosus*) from the Urdaibai Biosphere Reserve (Bay of Biscay, Southwestern Europe). Sci. Total Environ. 443, 233–244. doi:10.1016/j.scitotenv.2012.10.078

Rajapakse, N., Silva, E., Kortenkamp, A., 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health Perspect. 110, 917–921.

Royall, R.M., 1997. Statistical Evidence: A Likelihood Paradigm. Chapman and Hall, New York.

Ruiz, J.C., Peterson, J.T., 2007. An evaluation of the relative influence of spatial, statistical, and biological factors on the accuracy of stream fish species presence models. Trans. Am. Fish. Soc. 136, 1640–1653. doi:10.1577/T06-086.1

Sackett, D.K., Lee Pow, C., Rubino, M.J., Aday, D.D., Cope, W.G., Kullman, S., Rice, J.A., Kwak, T.J., Law, M., 2015. Sources of endocrine-disrupting compounds in North Carolina waterways: A geographic information systems approach. Environ. Toxicol. Chem. 34, 437–445. doi:10.1002/etc.2797

Scholz, S., Mayer, I., 2008. Molecular biomarkers of endocrine disruption in small model fish. Mol. Cell. Endocrinol. 293, 57–70. doi:10.1016/j.mce.2008.06.008

Simmons, D.B.D., McMaster, M.E., Reiner, E.J., Hewitt, L.M., Parrott, J.L., Park, B.J., Brown, S.B., Sherry, J.P., 2014. Wild fish from the Bay of Quinte Area of Concern contain elevated tissue concentrations of PCBs and exhibit evidence of endocrine-related health effects. Environ. Int. 66, 124–137. doi:10.1016/j.envint.2014.01.009

Solomon, E.P., 2015. Endocrine Regulation, in: Introduction to Human Anatomy and Physiology. Saunders, Maryland Heights, Missouri, p. 149–166.

Strange, R.J., 1996. Field examination of fishes, in: Murphy, B.R., Willis, D.W. (Eds.), Fisheries Techniques 2nd ed. American Fisheries Society, Bethesda, Maryland; p. 433–446.

Skaar, D.R., Johnson, B.T., Jones, J.R., Huckins, J.N., 1981. Fate of kepone and mirex in a model aquatic environment: sediment, fish, and diet. Can. J. Fish. Aquat. Sci. 38, 931–938. doi:10.1139/f81-126

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39, 228–269. doi:10.1080/10408440802233259

Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., Servos, M., 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. Sci. Total Environ. 225, 81–90. doi:10.1016/S0048-9697(98)00334-9

Tetreault, G.R., Bennett, C.J., Shires, K., Knight, B., Servos, M.R., McMaster, M.E., 2011. Intersex and reproductive impairment of wild fish exposed to multiple municipal wastewater discharges. Aquat. Toxicol. 104, 278–290. doi:10.1016/j.aquatox.2011.05.008

Thompson, W.L., Lee, D.C., 2000. Modeling relationships between landscape-level attributes and snorkel-counts of Chinook Salmon and Steelhead parr in Idaho. Can. J. Fish. Aquat. Sci. 57, 1834–1842.

Thorpe, K.L., Cummings, R.I., Hutchinson, T.H., Scholze, M., Brighty, G., Sumpter, J.P., Tyler, C.R., 2003. Relative potencies and combination effects of steroidal estrogens in fish. Environ. Sci. Technol. 37, 1142–1149. doi:10.1021/es0201348

Tuvikene, A., 1995. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Annu. Zool. Fenn. 32, 295–309. doi:10.2307/23735700

Tveranger, B., 1985. Variation in growth rate, liver weight and body composition at first sexual maturity in Rainbow Trout. Aquaculture. 49, 89–99.

US Environmental Protection Agency (EPA), 1996. Method 3050B - Acid digestion of sediments, sludges, and soils. 1996 12. doi:10.1117/12.528651

US EPA, 2000. Guidance for assessing chemical contaminant data for use in fish advisories. EPA-823-B-00-007.

US EPA, 2005. EPA-822-R-05-005: Aquatic life ambient water quality criteria nonylphenol.

US EPA, 2006. Freshwater Sediment Screening Benchmarks. Region III BTAG. 1–6.

US EPA, 2012. Equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: procedures for the determination of the freely dissolved interstitial water concentrations of nonionic organics. EPA-600-R-02-012 65.

US EPA, 2016. National Recommended Water Quality Criteria- Aquatic Life Criteria Table. hhtp://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table#table (accessed 06.06.16)

Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E.M., Woodling, J.D., Norris, D.O., 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ. Sci. Technol. 42, 3407–3414. doi:10.1021/es0720661

Walter III, J.F., Austin, H.M., 2003. Diet composition of large Striped Bass (*Morone saxatilis*) in Chesapeake Bay. Fish Bull. 101, 414–423.

Williams, R.J., Keller, V.D.J., Johnson, A.C., Young, A.R., Holmes, M.G.R., Wells, C., Gross-Sorokin, M., Benstead, R., 2009. A national risk assessment for intersex in fish arising from steroid estrogens. Environ. Toxicol. Chem. 28, 220–230. doi:10.1897/08-047.1

Woodling, J.D., Lopez, E.M., Maldonado, T.A., Norris, D.O., Vajda, A.M., 2006. Intersex and other reproductive disruption of fish in wastewater effluent dominated Colorado streams. Comp. Biochem. Physiol. Part C. Toxicol. Pharmacol. 144, 10–15. doi:10.1016/j.cbpc.2006.04.019

Yonkos, L.T., Friedel, E. A., Fisher, D.J., 2014. Intersex (testicular oocytes) in Largemouth Bass (*Micropterus salmoides*) on the Delmarva Peninsula, USA. Environ. Toxicol. Chem. 33, 1163–9. doi:10.1002/etc.2544

Yost, E.E., 2014. Fate and biological activity of endocrine disrupting compounds in the waste of a commercial swine operation. North Carolina State University Dissertation. Raleigh, North Carolina.

Tables

Table 1. Fish collected at each site by species and group. Numbers within parentheses are the number of individuals with intersex and the mean intersex severity. LMB: Largemouth Bass. SMB: Smallmouth Bass. SPB: Spotted Bass. RES: Redear Sunfish. WAR: Warmouth. BLG: Bluegill. RBS: Redbreast Sunfish. PKS: Pumpkinseed. CHC: Channel Catfish. WHC: White Catfish. BLC: Blue Catfish. FHC: Flathead Catfish.

Site	LMB	SMB	SPB	RES	WAR	BLG	RBS	PKS	CHC	WHC	BLC	FHC
Kerr Scott R.	3		7 (1,4)	1	2	10 (1,1)	1		1	6		
Ronda		2	5 (1,2)			1	4		1			
Route 801									1		2	1
Badin Lake	10 (8, 2.6)			3 (1,2)		9	1			2		
Red Hill	2 (2,3)			2		8			2		4	1
Blewett Lake	10 (2,2)					10			2			
74 Bridge	3 (1,4)			2			8 (1,1)		5		5	
Digg's Tract	5 (4,2.3)			4		4	4 (2,2)		6		4	
Society Hill	8 (6,3.7)			1 (1,4)		9 (1,1)			2		6	2
Pee Dee	10 (6,2.3)			2		8			1		5	3
Bucksport	15 (1,1)			9 (1,1)	1	6	1	1			11 (1,2)	3
Total Collected Per Species	66	2	12	24	3	65	19	1	21	8	37	10
Total Collected	Black Bass 80			Sunfish 112					Catfish 76			
Intersex (%)	40 (n=32)			7.14 (n=8)					1 (n=1)			
Mean Severity	2.72			1.75					2			

Table 2. Water (ng/L) and sediment (ug/g DW) contaminant concentrations at each site. Values in bold exceed published thresholds for aquatic life (Caldwell et al., 2012; US EPA, 2005; Aschberger et al., 2010; MacDonald et al., 2000; US EPA, 2006). NA: no known thresholds exist. BDL: below detection limits.

Threshold and Site	Water (ng/L)								Sediment (ug/g DW)			
	PAHs	OCPs	PCBs	CUPs	EE2	Nonyl-phenol	BPA	E2β-Eq.	PAHs	OCPs	PCBs	Atrazine
Threshold	NA	NA	14	NA	0.1	6600	1500	2.0	1610	NA	59.8	6.62
Kerr Scott R.	128.60	0.26	0.03	BDL	BDL	0.85	BDL	0.269	1.66	BDL	BDL	BDL
Ronda	105.22	1.64	0.04	3.16	BDL	0.56	BDL	0.507	349.73	BDL	BDL	BDL
Route 801	219.00	5.01	0.35	39.36	BDL	0.7	0.62	0.895	**2960.20**	2.28	BDL	BDL
Badin Lake	152.47	1.67	0.04	24.04	BDL	0.4	0.4	0.212	**11068.05**	101.49	1.03	BDL
Red Hill	118.86	12.29	1.36	153.99	**0.62**	0.21	0.89	0.103	595.33	1.06	2.97	3.8
Blewett Lake	72.60	2.83	0.63	20.18	**1.35**	0.97	0.65	0.282	7.34	BDL	BDL	0.7
74 Bridge	95.23	3.05	0.92	315.55	**1.97**	3.1	1.4	1.259	383.48	0.99	0.15	0.85
Digg's Tract	134.48	3.46	4.92	74.07	**0.84**	2.4	1.9	0.118	1343.62	2.81	5.18	BDL
Society Hill	238.25	5.29	6.67	205.05	**0.33**	4.6	2.51	0.351	237.26	0.74	0.48	BDL
Pee Dee	159.44	4.53	7.31	190.66	**1.62**	3.5	1.05	0.533	210.94	0.49	BDL	BDL
Bucksport	113.70	1.85	1.47	29.38	**0.27**	1.4	2.27	0.32	336.66	BDL	BDL	3.4

Table 3. Sediment metal concentrations (ug/g DW) at each site. Lightly shaded values indicate a TEC/LEL threshold has been exceeded. Darkly shaded values indicate a PEC/SEL threshold has been exceeded. DL: detection limit (ug/g DW). TEC: threshold effect concentration (ug/g DW; MacDonald et al., 2000). LEL: lowest effect level (ug/g DW; Persaud and Jaagumagi, 1993). PEC: predicted effect concentration (ug/g DW; MacDonald et al., 2000). SEL: severe effect level (ug/g DW; Persaud and Jaagumagi, 1993). * indicates a LEL or SEL threshold. BDL: below detection limit.

				Site										
Metal	DL	TEC/LEL*	PEC/SEL*	Kerr Scott R.	Ronda	Route 801	Badin Lake	Red Hill	Blewett Lake	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Buck-sport
Aluminum (Al)	0.5	NA	NA	1656.0	659.0	1505.8	1011.6	1192.8	393.7	687.3	1620.8	859.1	836.8	1086.3
Barium (Ba)	0.25	NA	NA	98.3	49.4	113.8	108.7	85.9	34.2	64.1	177.3	95.7	73.6	90.2
Berylium (Be)	0.25	NA	NA	0.4	BDL	0.7	BDL	0.5	BDL	BDL	0.9	0.5	0.5	0.6
Cadmium (Cd)	0.25	0.99	4.98	2.3	1.1	2.7	4.2	2.9	1.2	1.7	3.6	1.9	1.8	2.0
Cobalt (Co)	0.25	NA	NA	8.7	4.8	10.3	15.7	8.5	4.2	6.2	15.9	8.4	7.4	6.9
Chromium (Cr)	0.25	43.4	111	6.8	10.6	24.5	14.4	19.5	8.2	12.3	26.1	15.5	15.6	19.1
Copper (Cu)	0.25	31.6	149	8.0	6.4	17.8	41.5	21.1	2.7	10.3	25.8	12.5	10.1	12.3
Iron (Fe)	0.5	20,000*	NA	2226.6	1032.9	2446.8	3591.5	2617.8	1109.4	1435.6	3155.8	1693.3	1597.8	1797.3
Mercury (Hg)	0.001	0.18	1.06	0.016	0.006	0.024	0.076	0.053	0.003	0.022	0.062	0.029	0.014	0.028
Potassium (K)	0.5	NA	NA	4424.6	1475.6	2183.5	274.3	554.0	1879.9	711.4	906.7	747.2	890.3	812.9
Magnesium (Mg)	0.5	NA	NA	3500.5	1829.9	3264.2	945.9	1908.6	1457.1	1218.1	2416.2	1549.5	1797.3	1929.6
Manganese (Mn)	0.25	460*	1100*	210.0	107.4	540.3	759.1	724.3	100.0	424.3	2595.3	920.5	458.2	226.1
Nickel (Ni)	0.25	22.7	48.6	BDL	BDL	5.0	4.8	7.9	BDL	3.3	11.8	5.9	4.7	6.4
Lead (Pb)	0.25	35.8	128	6.4	4.4	13.3	13.2	9.8	3.7	6.9	15.2	8.1	8.7	9.0
Silicon (Si)	0.5	NA	NA	1041.5	638.2	1314.8	1573.2	544.0	478.7	809.1	1263.5	1116.7	916.2	858.8
Strontium (Sr)	0.25	NA	NA	9.9	4.0	16.3	40.3	12.2	2.3	7.9	21.1	10.1	8.1	8.5
Vanadium (V)	0.25	NA	NA	25.6	14.6	38.4	83.0	37.8	17.6	22.4	46.8	26.7	24.8	28.4
Zinc (Zn)	0.25	121	459	44.2	30.8	65.2	31.0	28.3	16.2	28.9	71.3	38.0	35.6	42.7

Table 4. Intersex occurrence (%) confidence models for black bass and sunfish. Confidence models are models with AICc weights 10% or more of the top model. (+) indicates a positive relationship with severity. (–) indicates a negative relationship with severity.

Taxon	Model	r^2	AICc	ΔAICc	AICc Weight
Black Bass	OCPs (Water, +); Hg (Sediment, +)	0.82	97.44	0.00	0.244
	PCBs (Water, +); OCPs (Water, +); Hg (Sediment, +)	0.92	98.22	0.78	0.165
	PCBs (Water, +); OCPs (Water, +); PAHs (Sediment, +)	0.91	98.79	1.35	0.124
	OCPs (Water, +); PAHs (Sediment, +)	0.76	100.15	2.71	0.063
	Hg (Sediment, +)	0.55	100.25	2.80	0.060
	PCBs (Water, +); Hg (Sediment, +)	0.74	100.90	3.45	0.043
	OCPs (Water, +); OCPs (Sediment, +)	0.73	101.11	3.67	0.039
	PCBs (Water, +); OCPs (Water, +); OCPs (Sediment, +)	0.89	101.13	3.69	0.039
	OCPs (Water, +); PAHs (Water, +); Hg (Sediment, +)	0.89	101.68	4.23	0.029
	OCPs (Water, +)	0.48	101.74	4.30	0.028
Sunfish	PAHs (Water, +)	0.38	72.04	0.00	0.3452
	PCBs (Water, +)	0.16	75.15	3.11	0.0729
	Mercury (Sediment, +)	0.14	75.33	3.29	0.0667
	Industrial EACs (Water, +)	0.09	75.98	3.93	0.0483
	Atrazine (Water, +)	0.08	76.01	3.97	0.0474

Table 5. Intersex occurrence confidence model variable rankings for black bass and sunfish.

Taxon	Variable	Rank	Sum AICc Weight
Black Bass	OCPs-Water	1	0.7965
	Hg-Sediment	2	0.6436
	PCBs-Water	3	0.3896
	PAHs-Sediment	4	0.2193
	OCPs-Sediment	5	0.1027
	PAHs-Water	6	0.0699
	Atrazine-Water	7	0.0366
	EE2-Water	8	0.0190
	Industrial EACs-Water	9	0.0004
Sunfish	PAHs-Water	1	0.5167
	Hg-Sediment	2	0.1364
	PCBs-Water	3	0.1304
	Industrial EACs-Water	4	0.1017
	Atrazine-Water	5	0.0996
	OCPs-Water	6	0.0894
	EE2-Water	7	0.0839
	PAHs-Sediment	8	0.0738
	OCPs-Sediment	9	0.0727

Table 6. Intersex severity (average per site) confidence models for black bass and sunfish. Confidence models are models with AICc weights 10% or more of the top model. (+) indicates a positive relationship with severity. (–) indicates a negative relationship with severity.

Taxon	Model	r^2	AICc	ΔAICc	AICc Weight
Black Bass	Atrazine (Water, +); Industrial EACs (Water, –)	0.69	31.12	0.00	0.4317
	Atrazine (Water, +)	0.25	34.01	2.89	0.1018
	Industrial EACs (Water, +)	0.23	34.38	3.26	0.0845
Sunfish	PAHs (Water, +)	0.42	30.95	0.00	0.2645
	Hg (Sediment, +)	0.33	32.46	1.51	0.1243
	PAHs (Water, +); Hg (Sediment, +)	0.59	33.59	2.64	0.0707
	PAHs (Sediment, +)	0.18	34.47	3.51	0.0456
	OCPs (Sediment, +)	0.16	34.71	3.75	0.0405
	OCPs (Water, –); PAHs (Water, +)	0.53	34.87	3.91	0.0374
	PAHs (Water, +); PAHs (Sediment, +)	0.52	34.99	4.03	0.0352
	PAHs (Water, +); OCPs (Sediment, +)	0.51	35.32	4.37	0.0298
	EE2 (Water, –)	0.10	35.40	4.45	0.0286
	PCBs (Water, –); PAHs (Water, +)	0.50	35.54	4.59	0.0267

Table 7. Intersex severity confidence model variable rankings for black bass and sunfish.

| Taxon	Variable	Rank	Sum AICc Weight
Black Bass	Atrazine- Water	1	0.6549
	Industrial EACs- Water	2	0.6278
	PAHs- Water	3	0.1035
	EE2- Water	4	0.0634
	PCBs- Water	5	0.0548
	Hg- Sediment	6	0.0507
	OCPs- Sediment	7	0.0505
	OCPs- Water	8	0.0504
	PAHs- Sediment	9	0.0496
Sunfish	PAHs- Water	1	0.5403
	Hg- Sediment	2	0.2924
	OCPs- Water	3	0.1211
	PAHs- Sediment	4	0.1144
	OCPs- Sediment	5	0.1012
	PCBs- Water	6	0.0904
	EE2- Water	7	0.0767
	Industrial EACs- Water	8	0.0589
	Atrazine- Water	9	0.0579

Figures
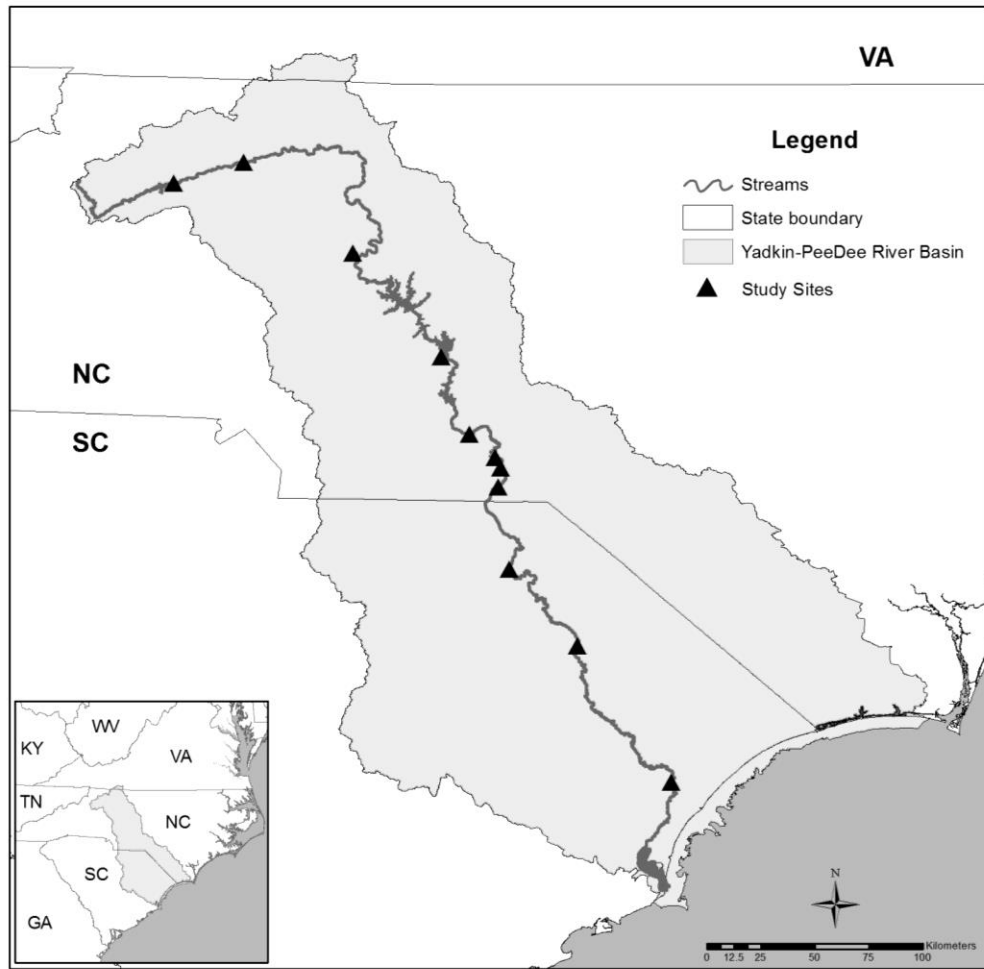
Figure 1. Map of the Yadkin-Pee Dee River basin in North Carolina and South Carolina and the 11 study sites.
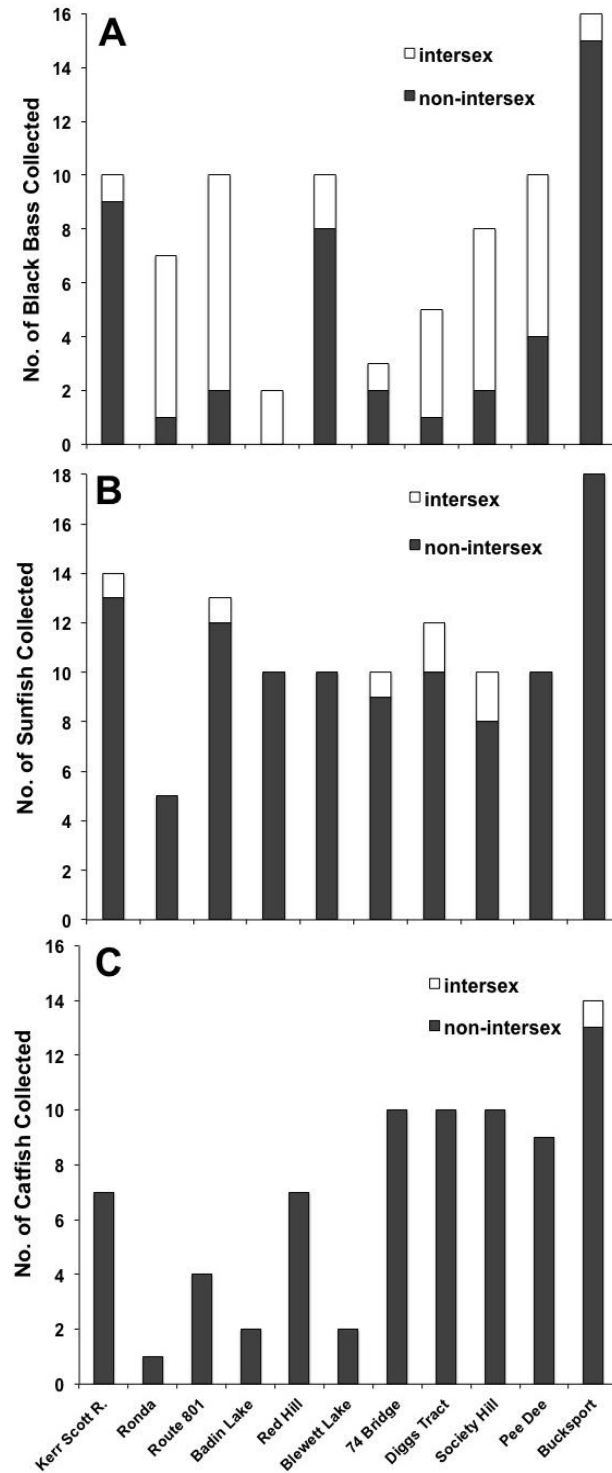
Figure 2. Total number of black bass (A), sunfish (B), and catfish (C) analyzed for intersex at each site. Sites are arranged (left to right) from upstream to downstream.
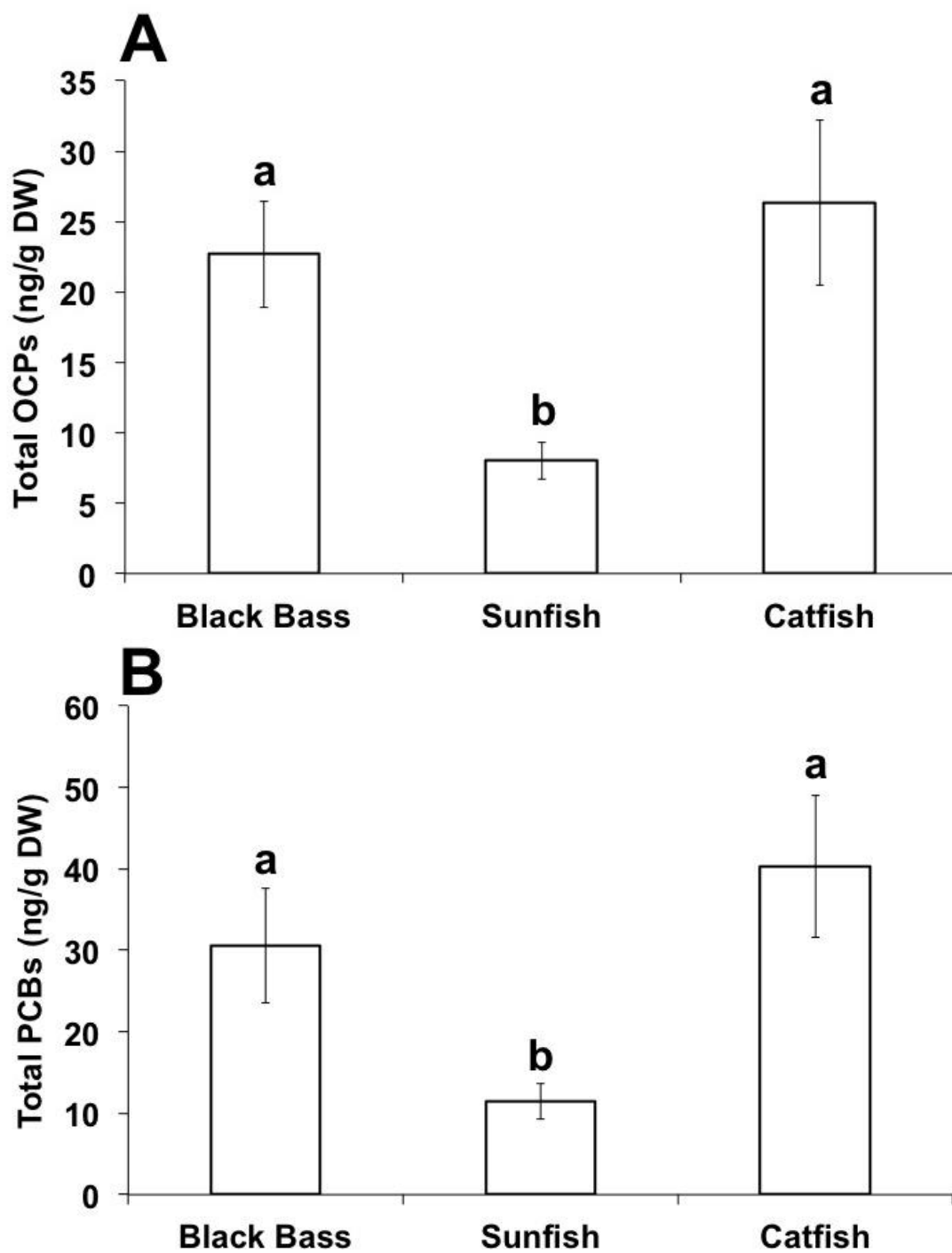
Figure 3. Total OCP (A) and total PCB (B) concentration in black bass, sunfish, and catfish muscle tissue (mean ± standard error). Sites having the same letter indicate no significant difference ($p > 0.05$).

Figure 4. Total OCP (A), total PCB (B), and Hg (C) muscle tissue concentrations at each site (mean ± standard deviation).
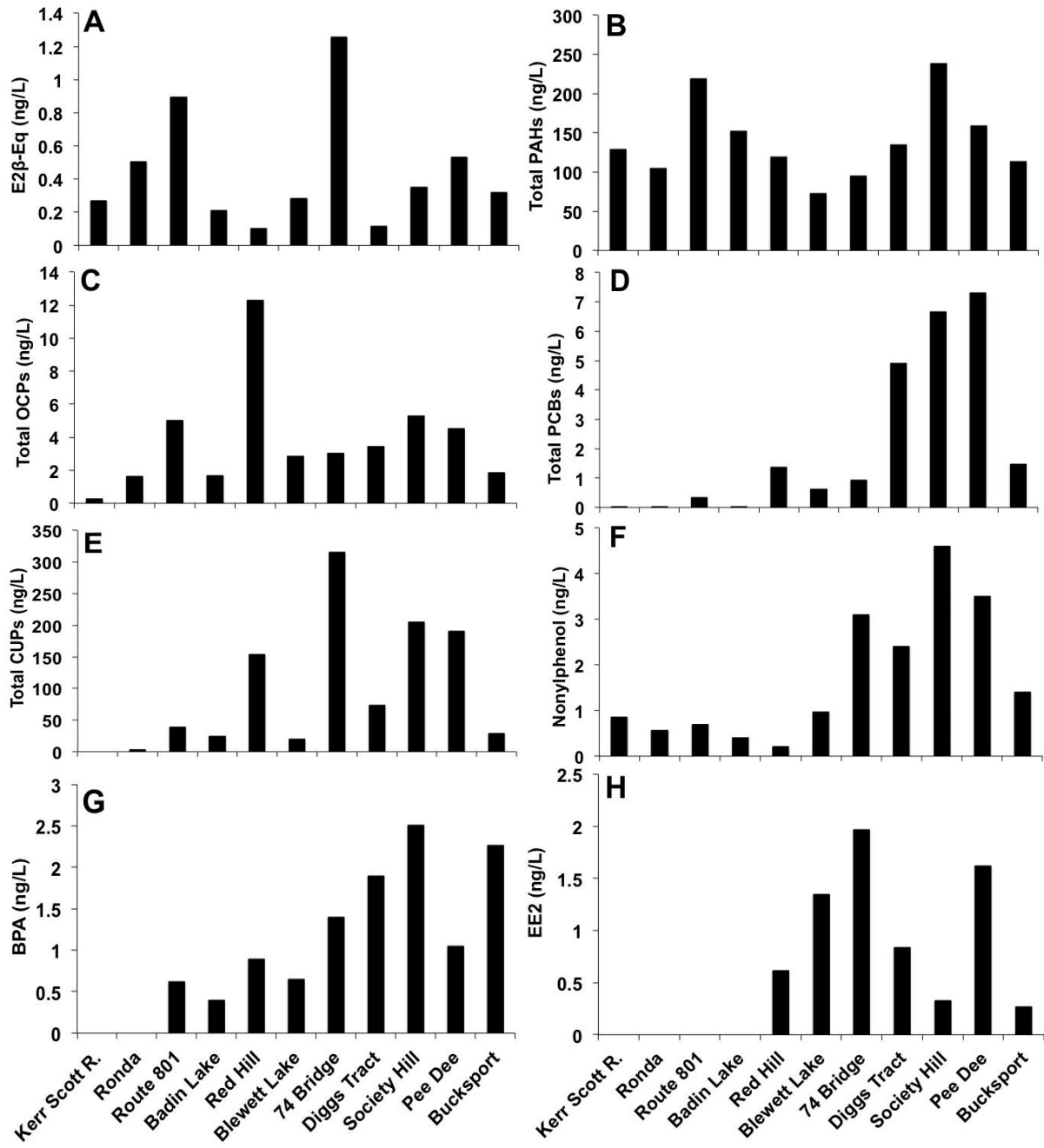
Figure 5. Concentrations of E2β-Eq. (A), PAHs (B), OCPs (C), PCBs (D), CUPs (E), nonylphenol (F), BPA (G), and EE2 (H) in ng/L at each site.

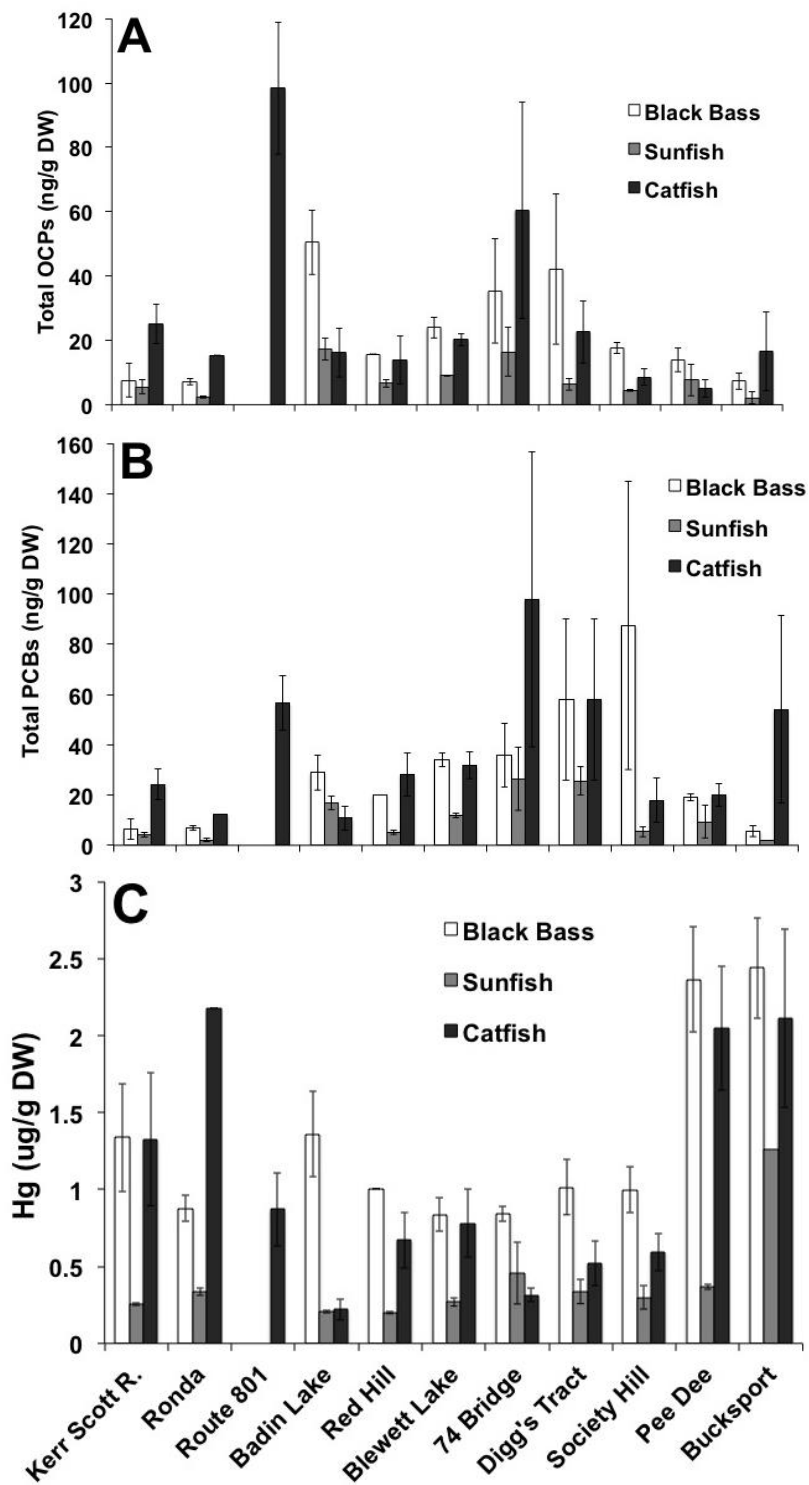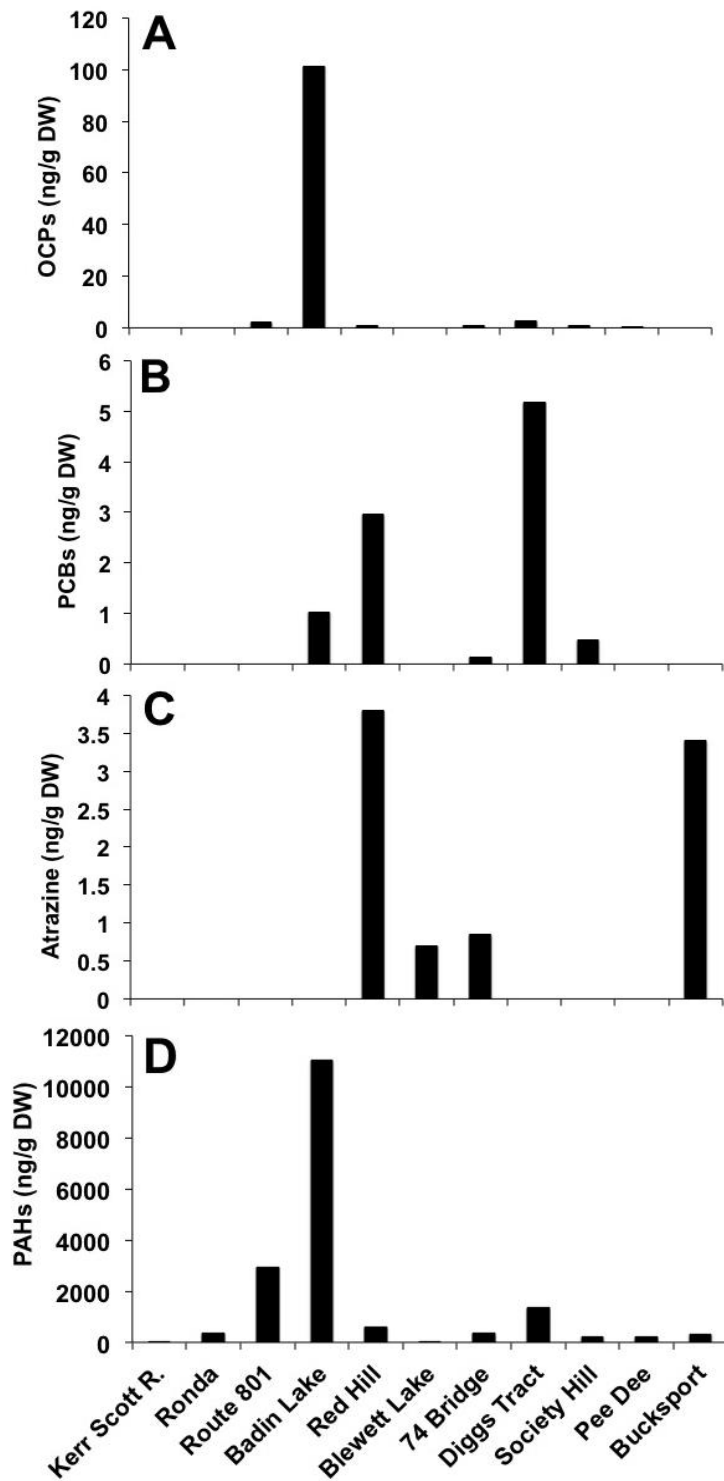Figure 6. Sediment concentrations of OCPs (A), PCBs (B), atrazine (C), the only CUP detected), and PAHs (D) in ng/g DW at each site.
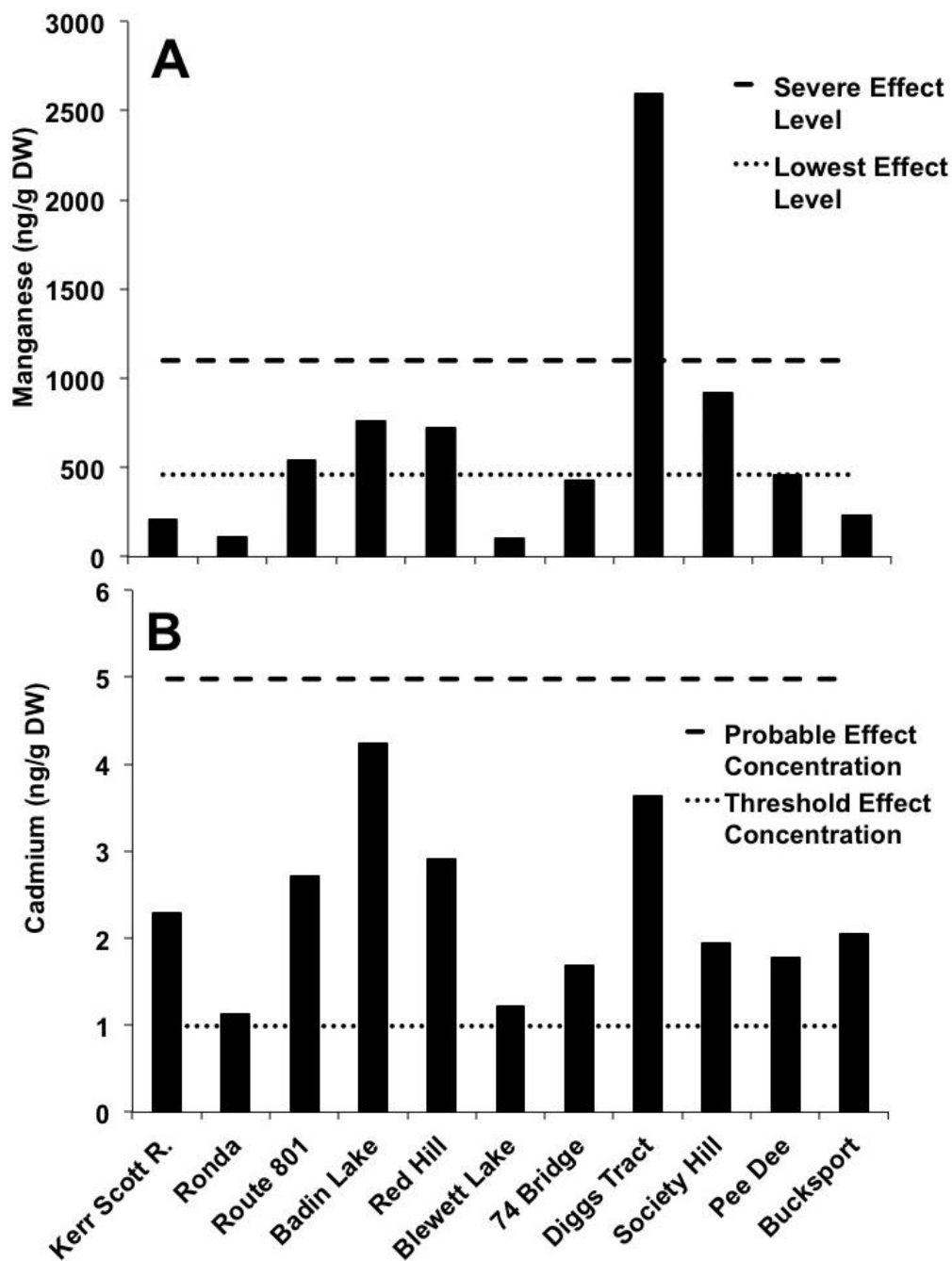
Figure 7. Manganese (A) and cadmium (B) sediment concentrations at each site (ng/g DW), with severe effect and lowest effect manganese threshold level concentrations (Persaud and Jaagumagi, 1993) and probable effect (i.e., above which harmful effects are likely to be observed) and threshold effect (i.e., below which harmful effects are unlikely to be observed) cadmium concentrations (MacDonald et al., 2000).
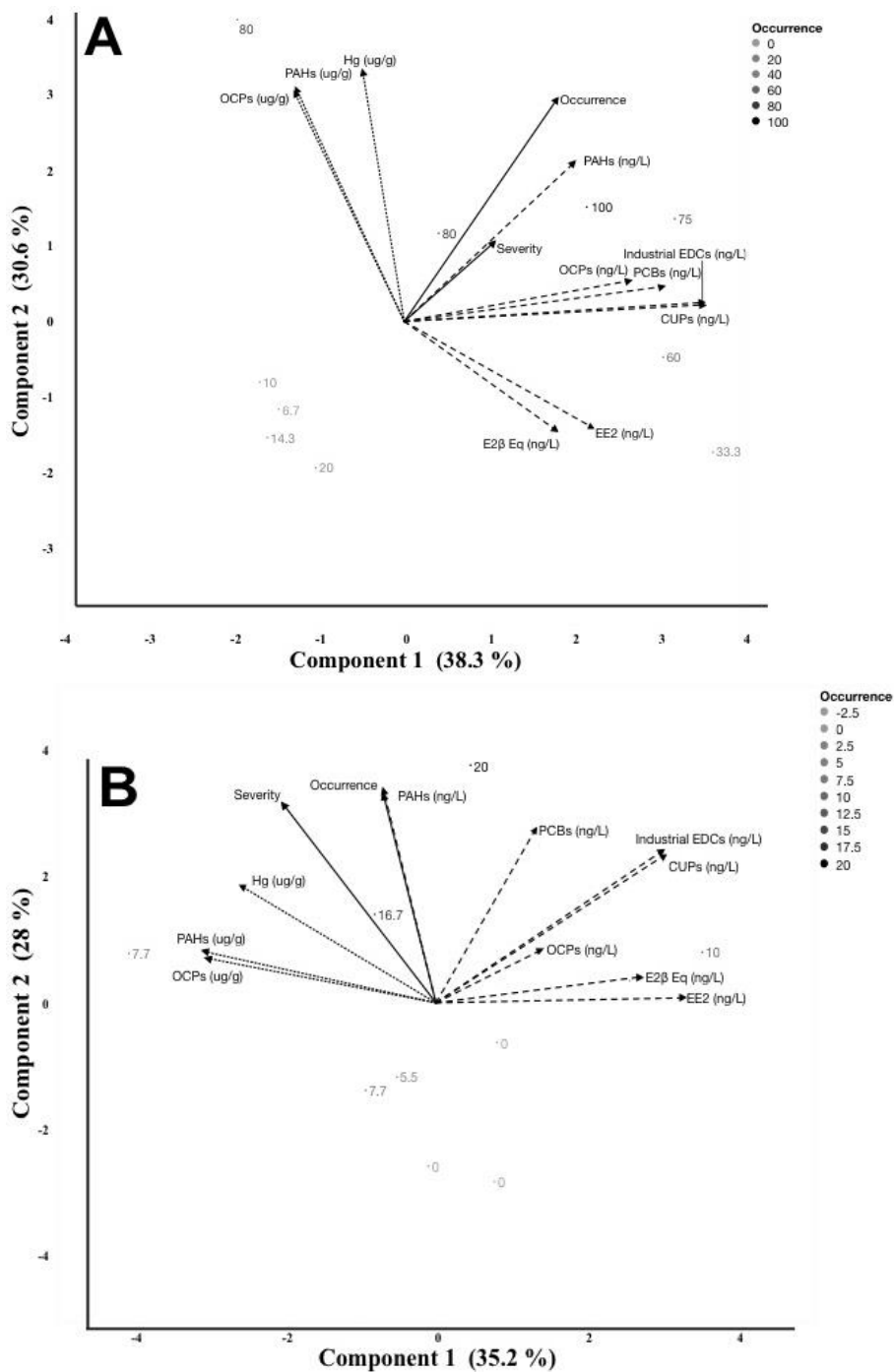
Figure 8. Principal components analysis for black bass (A) and sunfish (B) intersex and severity (solid lines) with sediment PAHs, OCPs, and Hg (ug/g DW, dotted lines) and water CUPs, industrial EACs, PCBs, OCPs, EE2, PAHs, and E2β-Eq (ng/L, dashed lines). Labels are percent intersex occurrence per site (see legend).

Survival and Contaminant Accumulation in Riverine Fish Assessed with an *In Situ* Bioassay Approach

Abstract

The aim of this research was to determine if aquatic contaminant stressors in a river ecosystem affect the survival and health of juvenile fish using an *in situ* bioassay approach. With two sequential 28-d *in situ* bioassays, we assessed the survival of three species of fish, Largemouth Bass *Micropterus salmoides* (LMB), Fathead Minnow *Pimephales promelas* (FHM), and Robust Redhorse *Moxostoma robustum* (RRH). Hatchery-propagated fish were placed into cages (20 of each species in 3 replicated cages at each site; LMB and FHM in bioassay 1 and RRH and FHM in bioassay 2) located along the length of the Yadkin-Pee Dee River in North Carolina and South Carolina, and contaminants were measured in the sediment and surface water near the cages. No apparent longitudinal trends in fish survival were detected, and contaminant concentrations varied among sites. Juvenile LMB and RRH did not survive past 13 and 23 days with corresponding Kaplan-Meier (K-M) median survival estimates of 9.7 d and 12.1 d, respectively. In contrast, survival of LMB and RRH in reference cages at their respective hatchery ponds were much greater with 70% of LMB and 67% of RRH surviving to the end of the 28-d bioassay, validating the bioassay

procedure. Survival of adult FHM deployed in cages alongside the juvenile LMB or RRH

averaged 43% survival at the end of the 28-d exposure with a 22-d K-M median survival

estimate. Intersex (a biomarker of endocrine disruption) was not observed in any adult FHM

(baseline or post-exposure). Contaminant accumulation in bioassay FHM was apparent,

when compared to baseline concentrations, for almost all contaminants measured, with

highest accumulated concentrations in polychlorinated biphenyls, organochlorine pesticides,

and mercury. Contaminants and other water quality stressors in this river system appear to

detrimentally impact juvenile fish survival and overall health with presumed effects at the

fish assemblage and community levels.

1. Introduction

With increasing human populations and associated anthropogenic influences, understanding and protecting aquatic ecosystems is critical. Aquatic ecosystems are especially vulnerable to anthropogenic impacts because they receive and sequester many contaminants (Scholz and Mayer, 2008). Contaminants are being released from industrial, agricultural, and municipal activities, and the magnitude and frequency of releases is likely to grow as human sprawl continues (Daughton and Ternes, 1999). In the southeastern United States, where this study was conducted, many diverse assemblages of animals are at risk, and threats continue to increase (Warren et al., 2000), associated with substantial human population and infrastructure growth. The many contaminants that enter aquatic ecosystems can be detrimental to the health and survival of organisms. Contaminants which are commonly introduced into aquatic systems include polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, bisphenol A (BPA), and heavy metals, among others (Hinck et al., 2009; Muthumbi et al., 2003).

Contaminants cause reproductive problems through endocrine disruption, high levels of stress and reduced ability to combat stress, altered behaviors, and ultimately death (Colman et al., 2009; Kidd et al., 2007; Tan et al., 2009). Exposure during critical periods of development can also be important in determining how an organism will respond to contaminants (Barton and Andersen, 1998). In addition to causing impaired health and mortality, contaminants, and especially contaminants with estrogenic properties, cause the occurrence of the intersex condition (Bahamonde et al., 2013). Intersex, in this study, is

defined as the presence of female germ cells within a predominantly male gonad (Nolan et al., 2001). Research on fish intersex has previously been conducted throughout the United States by Hinck et al. (2009). Their survey involved examining intersex in adult male black bass and included three sites on the Yadkin-Pee Dee River of North Carolina and South Carolina. They found that fish intersex occurrence in this river was the highest detected of any location they sampled throughout the United States. That study, along with the knowledge of impacts caused by dense human populations, industry, wastewater treatment, agriculture, and concentrated animal feeding operations in this area, motivated our research to examine contaminant impacts on the survival and health of riverine fish. Fish are regularly considered an indicator of aquatic ecosystem health, because they are continuously exposed to the contaminants in their environments (Blazer et al., 2012). By examining fish health and survival, researchers are able to assess water quality stressors and the overall condition of the river ecosystem.

The Yadkin-Pee Dee River is an important study system, because it is home to a rare fish species, the Robust Redhorse *Moxostoma robustum*. Robust Redhorse (RRH) is a long-lived sucker species that only inhabits three drainages in the southern United States, and in the Yadkin-Pee Dee River, the species is further impacted by habitat alteration (Fisk et al., 2015). Population estimates of this species in the Yadkin-Pee Dee River are very low (estimated 35-58 adult, spawning individuals, RRCC, 2014). Past research suggested that adequate physical habitat (i.e., water depth and velocity, substrate, and cover) for this species exists in the Yadkin-Pee Dee River (Fisk et al., 2015), indicating that water quality may be

more problematic than physical habitat availability. The low population numbers and limited recruitment of this imperiled species further motivated our study.

To study the effects of contaminants on fish in a field setting, our research employed the use of *in situ* bioassays, which allow for ecologically relevant toxicity testing, in a time- and cost-effective manner. Hewitt et al. (2006) and Cope et al. (2011) have previously applied this exposure method with other fish species in river ecosystems, and I employed similar procedures. These bioassay exposures allow juvenile and adult, captively-propagated fish to be exposed to the river environment in a controlled manner, while giving investigators the ability to monitor survival and determine contaminant accumulation at the end of the exposure. This allows researchers to provide a better understanding of what environmentally relevant conditions exist, their impacts on organism health and survival, and overall water and sediment quality (Goulden, 1999). Research has examined intersex and contaminant effects in adult, wild fish (black bass, sunfish, and catfish); however, less has been done to examine contaminant effects on juvenile fish or on fish through *in situ* bioassays (i.e., controlled exposures of organisms to ambient field conditions).

The objectives of this study utilized *in situ* bioassays to (1) examine survival of three fish species in the river ecosystem, (2) determine whether the intersex condition or other obvious health abnormalities develop in exposed fish, (3) assess water and sediment quality, and (4) determine whether contaminant accumulation occurs in river-exposed fish over a relatively short (28 d) duration.

2. Methods

2.1. Site Selection and Test Design

Eight riverine sites located on the Yadkin-Pee Dee River in North Carolina and South Carolina were selected for our *in situ* bioassays. Five sites were located in North Carolina and three in South Carolina (Figure 1). Sites exhibited varying levels of anthropogenic influence, land use, and habitat types (Sackett et al., 2015) and were also selected for availability of boat access and sampling logistics. Three of the sites we assessed were previously evaluated by Hinck et al. (2009) (Hinck: PRB 336, PRB 337, PRB 338; this study: 74 Bridge, Pee Dee, Bucksport). The Digg's Tract site is a known area of RRH spawning. Bioassays were conducted at these sites to assess potential associations between poor RRH recruitment and contaminants.

The experimental test included the use of three species of fish, Fathead Minnow *Pimephales promelas* (FHM), Largemouth Bass *Micropterus salmoides* (LMB), and RRH. Two separate and sequential *in situ* bioassays were each conducted for 28 days. The first bioassay utilized adult FHM and juvenile LMB, and the second included adult FHM and juvenile RRH. The bioassays were conducted at the eight selected sites located on the Yadkin-Pee Dee River. At each site, three cages per species were deployed containing 20 fish each. Survival and physicochemical properties were monitored every 3 days for the duration of the 28-d bioassay. During each bioassay, in addition to riverine bioassay cages, three cages of juvenile LMB and three cages of juvenile RRH were placed at the Watha State Fish Hatchery and the McKinney Lake State Fish Hatchery, respectively. These cages

allowed us to determine how well the fish survived in hatchery ponds compared to riverine sites.

2.2. Fish Transport and Deployment

All FHM used in the bioassays were purchased from Aquatic Biosystems Inc. (Fort Collins, CO), were approximately 8 to 10 months old, were all presumptive males (based on presence of breeding tubercles), and were housed in 'clean systems' with minimal exposure to contaminants. Using a subsample of baseline individuals, which were anesthetized, average lengths and weights were determined. FHM were 65.44 mm ± 0.77 mm and 63.60 mm ± 1.10 mm total length for the first and second bioassays, respectively (mean ± standard error). FHM were 3.16 g ± 0.14 g and 2.91 g ± 0.15 g weight for the first and second bioassays, respectively (mean ± standard error). LMB were obtained from the Watha State Fish Hatchery, North Carolina, and were approximately 30 days old at the beginning of testing. LMB were 27.9 mm ± 0.34 mm (total length, mean ± standard error) and 0.199 g ± 0.013 g (weight, mean ± standard error). RRH were acquired from the McKinney Lake State Fish Hatchery, North Carolina, and were the captively propagated offspring of adults collected from the Yadkin-Pee Dee River. RRH were approximately 45 days old at the beginning of testing. RRH were 38.60 mm ± 0.26 mm (total length, mean ± standard error) and 0.519 g ± 0.011 (weight, mean ± standard error).

At the initiation of each bioassay, fish were obtained in-person from the respective state hatchery or shipped via overnight courier from Aquatic Biosystems Inc. and placed into a fish transport box equipped with a compressed oxygen tank that provided aeration, with

adequate temperature acclimation. Water quality was routinely monitored for dissolved

oxygen (DO) and temperature (°C) in the fish transport box, and mortality was monitored

throughout transportation during deployment to bioassay cages in the river. Fish were

transported to each riverine site where 60 of each species (60 LMB and 60 FHM, for the first

bioassay and 60 RRH and 60 FHM, for the second bioassay) were removed from the fish

transport box and placed into a 5 g bucket for a brief (1 h) acclimation period. Over the 1-h

acclimation period, river water (approximately 1 L) was added to the bucket every 15

minutes. Twenty fish (of the same species) were placed into each cage with three replicate

cages per species, per site. Cages were held in buckets with river water and transported by

boat to the riverine locations and deployed. The first i*n situ* bioassay was conducted during

June 2014, and the second was conducted during July 2014.

Cages were constructed with a plexiglass tube (length, 25 cm; outer diameter, 15 cm)

with 1.6-mm holes drilled into the sides to ensure flow. Nitex screen (1-mm mesh) was

secured to each end of the cage with stainless steel circular clamps. Cages were fastened to a

concrete block using cable. Concrete blocks ensured that cages were elevated approximately

25 cm off the river bottom. Cages were secured to solid structure on the riverbank with

parachute cord leads. Each replicate cage was uniquely identified by different colors and

numbers of zip ties. We attempted to place cages nearly parallel to the riverbank to reduce

drag and allow water flow through the cages.

Additional fish, which served as the baseline subsample, were returned to North

Carolina State University (NCSU) where they were euthanized using a lethal overdose of pH

buffered tricaine methanesulfonate (MS-222). Each fish was weighed (g) and measured

(total length, mm) and for FHM, the presence or absence of nuptial tubercles was recorded.

A subsample of fish from this group was then preserved in modified Davidson's fixative

(35.15% distilled water, 31.35% ethanol, 22% formalin [37-40%], and 11.5% glacial acidic

acid) for histological evaluation. Remaining fish were randomly allocated into groups of

approximately 25 fish each and stored frozen at -20°C for contaminant analysis.

2.3. Bioassay Monitoring and Termination

Approximately every three days throughout the *in situ* bioassays, cages were

monitored for fish mortality, biofouling, and sediment accumulation. Each cage was

carefully pulled to the surface, and the cage was assessed and cleaned, as necessary. If a

large mortality event had occurred, dead fish were removed from the cage. Any vandalism

or abnormal conditions were noted. During each monitoring event, water chemistry variables

including water temperature, conductivity, salinity, DO, and pH were measured using a YSI

Model 556 multiprobe system and a Beckman Coulter Phi 400 series, model 410 pH meter.

If complete mortality in a replicate occurred before the end of the planned 28-d

bioassay, the cage was removed from the river. At the end of the 28-d duration, any

remaining cages were retrieved, the total number of live fish was recorded, and cages with

live fish were labeled and transported to NCSU using an oxygenated fish transport box. Any

additional mortality during transportation was noted. Remaining fish were euthanized with

MS-222 and subsamples of fish from each site (3-10 depending on survival) were preserved

in modified Davidson's fixative. All other fish were frozen at -20°C for further contaminant

analysis. Presence or absence of nuptial tubercles was noted on FHM.

2.4. Fish Histopathology

Preserved fish were transported to the NCSU College of Veterinary Medicine for microscopic evaluation. Whole fish (some with head or tail excluded depending on size) were embedded in paraffin wax, sectioned at 5 μm with a microtome, and stained with hematoxylin and eosin. Following histological preparation, a certified fish pathologist examined the slides with light microscopy for the occurrence and severity of intersex, as well as any other abnormalities.

2.5. Contaminant Analyses

Contaminants were examined in the water and sediment of the Yadkin-Pee Dee River at each of the eight sites. Surface water was analyzed using grab samples (2-L bottles) and passive sampling devices (PSDs). Sediment was analyzed using grab samples of bottom sediments. FHM whole body composite samples were analyzed for contaminants; both baseline and river-exposed individuals were assessed.

To determine estrogenicity in the water, grab water samples were collected using solvent rinsed and baked 2-L amber glass bottles during the fish bioassay period of 2014. At each site, a subsurface sample was collected and acidified to a pH of 2 to prevent bacterial degradation. Samples were placed on ice and transported to NCSU where they were stored at 4°C for a maximum of 72 h before processing. Water samples were then filtered and solid phase extraction was completed. Extracts were analyzed for total estrogenic activity by a T47D-Kbluc assay, which uses human breast adenocarcinoma cells and 17β-estradiol (E2β)

standard to determine E2β equivalent concentrations (E2β Eq–ng/L). Full descriptions of the assay and extraction process were detailed by Yost et al. (2014).

Passive sampling devices (PSDs; O'Neal, 2014) were deployed at each site for approximately 28 days to determine time-weighted estimated concentrations of waterborne contaminants. Two types of PSDs were deployed. Low-density polyethylene strips (PEPSD) were utilized to sample OCPs, PCBs, and PAHs. A sorbent containing cartridge, universal passive sampling device (UPSD), was used to sample CUPs, hormones, and industrial contaminants (Table SI 1). A cage containing both types of PSDs was connected to the shoreline upstream and on the opposite bank of any boat access. Each PSD cage was suspended in the water column by a buoy and kept stationary by attaching a brick. After approximately 28 days, PSDs were collected from each site, removed from their cages, wrapped in baked aluminum foil, placed on ice, transported to NCSU, and stored at -20°C until extraction. PEPSDs were serially extracted three times over a 24 h period with a total of 150 mL of dichloromethane (DCM). UPSDs analyzed for CUPs were placed in 20 mL vials and serially extracted two times over a 4 h period with a total of 40 mL DCM. Extracts were concentrated, filtered, and stored at -20°C until analysis. Extracts were further concentrated under a gentle stream of nitrogen to approximately 0.5 mL just prior to analysis. UPSDs analyzed for hormones and industrial contaminants were placed in 20 mL vials and extracted with 10 mL ethyl acetate by shaking for 1 h at 150 rpm. Nitrogen evaporation at 35°C under 34.5 kPa as then conducted to reduce extracts to 0.5 mL. Extracts for hormone and industrial contaminant analysis were additionally filtered through a 0.45 μm polytetrafluoroethylene filter into a 1.5 mL microvial, evaporated with a nitrogen stream at

ambient temperature until dry, and cap sealed with argon gas. Complete detail of PSD

protocols and extraction procedures are explained by O'Neal (2014) and Lee Pow (2015).

PSDs were deployed once during the bioassay period, from mid-June to mid-July.

Sediment samples were collected at each site within two months of the bioassays.

Samples were taken from the shoreline, upstream of boating access points. Two composite

samples (3-5 grabs each) of surficial (top 5 cm) sediment were collected using a stainless

steel scoop and tray and were stored in 250 mL amber glass jars. Sediment was transported

on ice and stored at -20°C until extraction. RTI International (Durham, NC) analyzed

samples for metals. Mercury (Hg) concentration was determined using a Milestone DMA-80

direct mercury analyzer. Other metals were evaluated with a modified version of Method

3050B (US EPA, 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. The Analytical

Toxicology Laboratory at NCSU determined the concentrations of current use pesticides

(CUPs), OCPs, PAHs, and PCBs (Table SI 1). Sediment samples were extracted with

dichloromethane (DCM) by means of pressurized fluid extraction using a Buchi Speed

Extractor E-916. Extracts were cleaned using GPC. Before chemical analysis extracts were

concentrated to 0.5 mL under a gentle stream of nitrogen.

Whole-fish samples were homogenized and stored frozen at -80°C until further

processing. One FHM sample was prepared for each site during each bioassay, as well as 3

baseline samples for each bioassay. In preparation for contaminant analyses, samples were

lyophilized and manually homogenized. RTI International (Durham, NC) analyzed fish

tissue samples for 22 metals (Table SI 1). Hg concentration in tissue samples was

determined using a Milestone DMA-80 direct mercury analyzer. Other metals were analyzed

with a modified version of Method 3050B (US EPA, 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. The Analytical Toxicology Laboratory at North Carolina State University

(NCSU, Raleigh, NC) analyzed samples for PCBs and OCPs (Table SI 1).   Lyophilized

muscle tissue was extracted with DCM by means of pressurized solvent extraction using a

Buchi Speed Extractor E-916. Extracts were then cleaned using gel permeation

chromatography (GPC) and were processed through Florisil solid phase extraction cartridges.

Extracts from PSDs and sediment samples were analyzed for 42 PAHs, 28 OCPs, 21 PCBs,

and 47 CUPs. PSDs were also used to determine concentrations of seven estrogen hormones

and two industrial contaminants. Whole-fish composite sample extracts were analyzed for

21 PCBs and 28 OCPs (Table SI 1). CUPs, OCPs, PAHs, and PCBs were measured using an

Agilent 6890 gas chromatograph (GC) connected to an Agilent 5973 mass selective detector

(MSD) operated in Select Ion Monitoring (SIM) mode. Analytes were separated on a Restek

Rtx-5MS column with a 5m integrated guard column. Hormones and industrial contaminants

were analyzed on an Agilent 7890 GC connected to an Agilent 7000 MSD operated in SIM

mode, with back flushing following a blank injection of pyridine to condition the column.

All analyses adhered to rigorous quality assurance protocols and included procedural blanks,

replicate samples, spiked samples, and data correction using surrogate recoveries, if

necessary. Water contaminant results are expressed as ng/L, and sediment and whole-fish

contaminant results are expressed as ng/g dry weight (DW) for organics and ug/g DW for

metals. Detection limits (DL) for waterborne contaminants were 0.2 ng/L for PAHs, PCBs,

and OCPs, 0.5 ng/L for CUPs, and 0.1 ng/L for hormones and contaminants (based on an equivalent 30 day PSD deployment). DLs for sediment and whole-fish contaminants were 0.1 ng/g for OCPs and PCBs, 1.0 ng/g for PAHs, and 2.0 ng/g for CUPs (all DW).

Chemicals were evaluated both as individual analytes and as total concentrations of classes of chemicals, when appropriate. For PAHs, individual analytes were examined and a total sum of analytes was calculated to compare to predicted effect concentrations (PECs). PAHs in water and sediment samples were also analyzed using the equilibrium partitioning sediment benchmark toxicity units (ESBTU) method because it incorporates the additive nature of PAH toxicity and the bioavailability of PAHs due to organic carbon in the sediment. Of the 42 PAHs analyzed, 34 have PAH potency divisors published by the US EPA (2012). Using the potency divisor, the determined concentration, and, for the sediment sample, the carbon content of the sediment sample, a toxic unit (TU) was determined for each PAH (acute and chronic values). Then, all 34 PAH toxic units were summed to an overall PAH toxic unit value; a value below 1.0 indicates that it is unlikely for the PAHs to be causing adverse effects to aquatic life, and a value above 1.0 indicates that aquatic life is possibly negatively affected.

2.6. Statistical Analysis

Statistical analyses were completed using JMP Pro 12 and SAS 9.4 (SAS, Cary, NC). All significance levels were set to $\alpha=0.05$. Fish survival was modeled using the Kaplan-Meier Proc Lifetest in SAS to estimate median survival within the 28-d bioassay (Kaplan and Meier, 1958). Differences in survival among test sites were evaluated with the log-rank test.

Significant relationships between environmental conditions, contaminants, and fish survival were assessed using a pairwise correlation matrix and correlation probabilities. To determine significant differences in length and weight of fish between baseline and test sites ANOVA and Tukey's tests were performed.

3. Results

3.1. Fish Survival and Growth

Mean survival among all river sites during the first bioassay was 58% for FHM and 0% for LMB. FHM survival remained high (>90%) for the first 20 days of the bioassay at most sites. Ronda, the most upstream site exhibited the lowest survival with only 15% of fish surviving to day 28. FHM survival remained above 50% for all other sites (Figure 2). FHM K-M survival functions were significantly different among sites (χ^2 =30.65, df=7, p< 0.0001). LMB survival was lower than FHM, and almost no fish survived after day 9 at most sites. By day 16, no surviving LMB were present and all cages were removed from the river. Median survival for LMB was 9.7 d and 24.9 d for FHM, at river sites within the 28 d test (Figure 3A). LMB survival at the hatchery site was 70%. LMB K-M survival functions were significantly different among river and hatchery sites (χ^2=148.18, df=8, p<0.0001).

Survival trends during the second bioassay were similar to those of the first. However, higher mortality at the beginning of the bioassay and overall lower survival of FHM was observed when compared to FHM survival in the first bioassay. Mean FHM survival was 28.1% and 0% for RRH among river sites. By day 12, FHM at four of the sites had experienced ≥ 50% mortality. Survival of FHM was lowest at the Ronda, 74 Bridge, and

Digg's Tract sites (Figure 2). FHM K-M survival functions were significantly different among river sites (χ^2=140.38, df=7, p<0.0001). RRH in the river did not survive to the end of the planned 28-d test. RRH survival was >60% before day 9, but following day 9, survival at most sites declined precipitously. All RRH cages were pulled from the river on or before day 27 due to complete mortality. Median survival for RRH was 12.1 d and 19.5 d for FHM, at river sites within the 28 d period (Figure 3B). Survival of RRH (28 d) at the hatchery site was 67%. RRH K-M survival functions were significantly different among river and hatchery sites (χ^2=139.86, df=8, p<0.0001).

FHM were the only species to survive to the end of the 28-d bioassay and were collected for comparison to baseline fish measurements. In the first bioassay, river-exposed fish length was significantly lower at two sites, and weight was significantly lower at all sites, relative to baseline measurements (Figure 4A). In the second bioassay, there were no significant differences in lengths of fish at river sites and baseline fish, but there were significantly differences in weights of fish at river sites compared to baseline fish, with exposure fish weighing less (Figure 4B).

3.2. Fish Intersex and Histopathology

From the first bioassay, a total of 12 baseline FHM individuals were assessed for the intersex condition. Another 76 FHM (5–15 from each river site) were assessed for intersex, post river exposure. Intersex was not detected in any FHM. Baseline LMB (n=7) and hatchery site exposed LMB (n=5) were also examined for intersex condition, and it was not found. This result was expected because these juvenile LMB were sexually immature.

From the second bioassay, a total of 11 baseline FHM were assessed for the intersex condition. Another 60 FHM, (2–16 from 7 river sites; not enough FHM survived at the Digg's Tract site to create a composite sample) were assessed for intersex, post river exposure. Intersex was not detected in any FHM. Baseline RRH (n=5) and hatchery site RRH (n=5) were also examined, and no intersex was observed. Likewise, this result was expected because these juvenile RRH were sexually immature.

Using light microscopy, fish gastrointestinal (GI) tracts were assessed for the presence of food items to determine whether starvation was a potential factor in the observed mortality. The GI tracts of FHM, from all riverine sites, and RRH and LMB taken from the hatchery sites, were filled with organic material, indicating feeding was occurring. Mineralization deposits were seen in the testes of FHM, although these deposits were not considered to represent any adverse health condition, and were present in both baseline and river-exposed fish. In general, there was no discernible evidence of confounding disease or infection in the river-exposed FHM.

The presence or absence of breeding tubercles on the male FHM snout was noted for baseline and river-exposed fish. All baseline FHM had noticeable to large tubercles, and some had enlarged dorsal epithelial fat pads (male characteristics). Upon termination of the bioassay, all river-exposed FHM had no tubercles or had very small, light tubercles and light or non-existent dorsal epithelial fat pads.

3.3. Contaminants Analysis

E2β-Eq was detected in the water at all eight sites with concentrations ranging 0.20–0.44 ng/L (Table SI 2). All concentrations detected were below the 2.0 ng/L predicted no-effect concentration (PNEC) for E2β (Caldwell et al., 2012); however, if some estrogenecity was caused by other, more potent estrogens not measured by the assay, the levels could be of concern.

Of the 7 hormones assessed with PSDs only ethinylestradiol (EE2) was detected. EE2 was detected at the 6 most downstream sites and concentrations ranged 0.27–1.62 ng/L, all above the 0.1 ng/L PNEC for aquatic organisms (Caldwell et al., 2012). Two industrial contaminants, nonylphenol and BPA, were measured. Both were detected at all sites. Nonylphenol concentrations ranged 0.2–3.5 ng/L and was higher at downstream sites (>1 ng/L downstream of 74 Bridge). None of the concentrations measured exceeded the 6,600 ng/L chronic exposure threshold determined by the US EPA (2005). BPA concentrations ranged 0.16–2.27 ng/L, and similar to nonylphenol, concentrations were higher downstream (Table SI 2). BPA concentrations did not exceed the 1,500 ng/L predicted no-effect concentration (PNEC, freshwater), set by the European Union (Aschberger et al., 2010) for the protection of aquatic species, at any site.

PAHs were present in the water at 100% of the sites, and 31 of the 42 PAHs analyzed were detected. Total PAH concentrations ranged from 85.8 to 242.36 ng/L (Table SI 2). Concentrations fluctuated between sites with the highest levels occurring at the Route 801, Pee Dee, and Society Hill sites (Table 1). ESBTU values ranged 0.0009–0.035 TU for acute

toxicity, all < 1. For chronic toxicity, TUs ranged 0.017–0.071 TU, all < 1, indicating low

likelihood for any negative effects of water PAHs on aquatic life (Table SI 3).

OCPs were present in the water at all of the sites and 9 of the 28 OCPs analyzed were

detected. Total OCP concentrations ranged 1.9–12.3 ng/L (Table SI 2). No individual OCP

concentrations exceeded published thresholds, when they existed. No combined OCP

concentration threshold or aquatic life thresholds exist. OCPs were noticeably elevated at the

Red Hill, Route 801, and Society Hill sites (Table 1).

PCBs were present in the water at all but one site (Ronda, most upstream site) and 9

of the 21 congeners analyzed were detected. Concentrations ranged BDL–7.3 ng/L and were

highest at the Pee Dee site (Table SI 2; Table 1). Total PCB concentrations did not exceed

the 14.0 ng/L chronic exposure threshold for freshwater aquatic life at any of the sites (US

EPA, 2016).

CUPs were present in the water at all of the sites and 8 of the 47 CUPs analyzed were

detected. Concentrations ranged 1.4–190.7 ng/L and were highest at the Pee Dee, Red Hill,

and Digg's Tract sites (Table SI 2; Table 1).

PAHs were present in the sediment at all sites, and 37 of the 42 PAHs analyzed were

detected. Total PAH concentrations ranged 211–2,960 ng/g DW and were highest at the

Route 801, Digg's Tract, and Red Hill sites (Table SI 2; Table 1). Total PAH levels

exceeded the 1,610 ng/g DW threshold effect concentration (TEC) for freshwater ecosystems

(MacDonald et al., 2000) at the Route 801 site. PAH levels did not exceed the PEC of

22,800 ng/g DW (MacDonald et al., 2000) at any site. ESBTU values ranged 0.004–0.069

for acute toxicity and 0.015–0.281 for chronic toxicity (Table SI 4). No sediment TUs exceeded 1, indicating little likelihood of negative sediment PAH effect on aquatic life.

OCPs were detected in the sediment at 6 of the 8 sites and were not present at Ronda, the most upstream site, and Bucksport, the most downstream site. 4 of the 28 OCPs analyzed were detected. Concentrations ranged BDL–2.8 ng/g DW and were highest at the Route 801, Digg's Tract, and Red Hill sites (Table SI 2; Table 1). 4,4'-DDE was the most frequently detected OCP with detections at 6 of the 8 sites. 4,4'-DDE levels did not exceed the threshold of 3.16 ng/g DW for freshwater ecosystems at any site (MacDonald et al., 2000). Chlordane was also found at two sites (Route 801 and Digg's Tract) but was below the 4.5 ng/g DW interim sediment quality guideline that corresponds to threshold level effects below which adverse biological effects are not expected (Environment Canada, 1999).

PCBs were detected in the sediment at 4 of the 8 sites and 6 of the 21 congeners analyzed were detected. All congeners were summed to calculate a total PCB concentration (ng/g DW), and concentrations ranged BDL–5.2 ng/g DW (Table SI 2). Levels were overall low (<6 ng/g DW total PCB concentration) and did not exceed the 59.8 ng/g DW threshold (MacDonald et al., 2000). PCB concentrations were highest at the Digg's Tract, Red Hill, and Society Hill sites (Table 1).

Atrazine was the only CUP detected in sediment and was found at 3 of the 8 sites. Concentrations were 3.8 ng/g DW at Red Hill, 3.4 ng/g DW at Bucksport, and 0.9 ng/g DW at the 74 Bridge site (Table SI 2; Table 1). Atrazine detections did not exceed the 6.62 ng/g DW screening benchmark developed by the EPA (US EPA, 2006).

Sediment metals were detected at each site, and specific metals and concentrations varied among sites. Eighteen of the 22 metals analyzed were detected. Many metals did not exceed published thresholds or thresholds do not exist, and their concentrations can be found in Table SI 2. Selected metals known for their endocrine disruption capabilities are highlighted in Table 1. Mn exceeded the lowest effect level of 460 ug/g DW at 4 of the 8 sites and exceeded the severe effect level of 1,100 ug/g DW at 1 site, Digg's Tract (Persaud and Jaagumagi, 1993).

One composite FHM whole-body tissue sample was analyzed for contaminants from each site, for each bioassay. The baseline concentration of each contaminant was determined by averaging the 6 baseline FHM sample concentrations. Fourteen of the 21 PCB congeners analyzed were detected in the FHM tissue samples. Baseline average total PCB concentration was 29.55 ng/g DW. Total PCB concentrations in FHM surviving to the end of the first bioassay ranged 39.7–93.4 ng/g DW (Figure 5A; Table SI 2). The total PCB concentrations in FHM from the end of the second bioassay ranged 34.6–92.0 ng/g DW (Figure 5A; Table SI 2). All FHM PCB concentrations were greater in the river-exposed fish when compared to the baseline fish PCB concentrations. No PCB action levels or thresholds were exceeded in FHM tissue.

OCPs were detected in FHM tissue samples at every site. Seven of the 28 OCPs analyzed were detected and average baseline concentration was 31.64 ng/g DW. Total OCP concentrations for the first bioassay ranged 42.3–66.1 ng/g DW. Total OCP concentrations for the second bioassay ranged 19.9–64.5 ng/g DW (Table SI 2). With the exception of two

sites in the second bioassay, all riverine FHM samples had higher OCP concentrations than baseline FHM samples (Figure 5B).

Seventeen of the 22 metals analyzed were detected in fish tissue samples (Cd was detected in sediment but not in fish tissue). Concentrations of each metal varied and many did not exceed published thresholds or thresholds do not exist (Table SI 2). Hg was evaluated because of its known endocrine disrupting capabilities (Georgescu et al., 2011). FHM baseline Hg concentration averaged 0.19 μg/g DW. Hg concentrations in FHM from the first bioassay ranged 0.25–0.38 μg/g DW. Hg concentrations in FHM from the second bioassay ranged 0.17–0.63 μg/g DW (Figure 6A). With the exception of one sample, Hg concentrations in FHM increased in every river-exposed fish sample. Mn was also examined because of its high rate of accumulation in fish tissue. The FHM Mn baseline average concentration was 4.5 μg/g DW. Mn concentrations in FHM from the first bioassay ranged 8.2–61.0 μg/g DW. Mn concentrations in FHM from the second bioassay ranged 15.4–49.1 μg/g DW (Table SI 2; Figure 6B). Mn concentrations in river-exposed FHM samples exceeded concentrations in baseline samples.

3.4. Water Physicochemical Characteristics

In general for a given bioassay, water temperatures (ºC) were warmer at downstream sites, but were similar between bioassays one and two (Table 2). Conductivity (μS cm^{-1}) was also higher downstream and similar between bioassays. Salinity (ppt) remained fairly constant and was slightly higher downstream. Dissolved oxygen (DO, mg/L) was higher at upstream sites and during the first bioassay. Although over-night DO was not measured,

cages were placed in areas with water flow likely to allow for adequate oxygenation. pH was

consistent between bioassays and was similar among sites, with Bucksport, the most

downstream site, exhibiting the lowest pH. At the Watha State Fish Hatchery pond with the

LMB cages, temperatures were slightly higher than the river, conductivity was more than two

times higher than any river site, salinity was more than double that of any river site, and DO

was higher (Table 2). At the McKinney Lake State Fish Hatchery pond with the RRH cages,

temperatures were higher, conductivity was two times lower than any river sites, salinity was

low, DO was higher than any river site, and pH was also higher than any river site (Table 2).

3.5. Correlations of Survival to Contaminants and Physicochemical Characteristics

Pairwise correlations were examined between FHM survival and contaminants and

water physicochemical characteristics. In the first bioassay, survival of FHM at each site was

negatively correlated with water temperature (ºC, $p=0.0232$) and conductivity ($\mu S\ cm^{-1}$,

$p=0.0139$). In the second bioassay, survival of FHM at each site was not correlated with any

contaminant or physicochemical properties.

4. Discussion

I examined the effects of contaminants on survival and health of caged fish in two

longitudinal riverine bioassays, and results indicated that water and sediment quality may

have adversely affected the survival of RRH, LMB, and FHM. No RRH or LMB survived to

the end of the planned 28-d bioassay, and a combined average of 43% of FHM survived.

FHM survival among sites followed similar spatial patterns between bioassay one and two,

with low survival at Ronda, decreasing survival from Route 801 downstream to Digg's Tract, increasing survival at Society Hill and Pee Dee, and lower survival at Bucksport. LMB survival was slightly less than RRH and this may have been due to their smaller size or their sensitivity to riverine contaminants and water quality because they survived in cages held in the hatchery pond. FHM survival was significantly, negatively correlated with water temperature and conductivity. Fluctuations in temperature have been shown to cause mortality, reduced growth, and the development of skin ulcers in fish (Coulter et al., 2015). Although we did not monitor temperature continuously, it may be a direct or indirect contributing factor to the mortality that we observed. Water temperature is strongly tied to DO dynamics that possibly influenced the observed mortality of FHM.

Although contaminants did not significantly correlate with survival of fish, there were trends of high contaminant loading and low survival observed throughout the river. For example, the Digg's Tract site yielded low survival of all fish species in both bioassays, associated with consistently high levels of contaminants in the sediment, water, and fish tissue. Contaminants were present at every site, to varying degrees, and may have caused toxicant-induced stress to the fish. Previous studies have shown that when FHM are exposed to contaminated effluent there are impacts on the individuals swimming performance and aerobic energy metabolism (Goertzen et al., 2011). Metal contaminants in effluents have also been linked to reduced reproductive success, significant metal accumulation into fish tissues, and overall metal toxicity (Rozon-Ramilo et al., 2011). Many contaminants detected did not exceed aquatic life thresholds; however, these thresholds, and our ability to measure contaminants, did not account for the possibility of toxicity from 'pulse' (high concentrations

for short periods of time which are difficult to measure) exposures, which may be associated with greater risk than continuous exposures (Diamond et al., 2005).

Accumulation of contaminants in fish tissue over the short duration of the bioassay (<28 days) was substantial. OCP concentrations in FHM tissue increased from baseline concentrations in all but two samples. PCB concentrations increased at all sites, and although exposure with no associated significant effects on survival, growth, or reproduction has been shown in FHM (Suedel et al., 1997), mixture effects with other contaminants are possible. Mn and Hg concentrations in fish tissue increased at almost every site. Mn and Hg toxicity, as well as toxicity to the other metals detected and their ability to act in an additive response, may have caused mortality. Additional research to examine the effects of heavy metal accumulation on fish survival would be required to elucidate biotic responses.

Although evidence revealed that FHM were feeding within the cages, weights of surviving FHM were significantly reduced in bioassay individuals at the end of the exposures relative to baseline samples. In addition to water quality stress, this may have been partially due to inadequate nutrition of food resources in the cages or greater energy expenditures in flowing river water (compared to relatively static hatchery ponds), which influenced their weight loss. However, the presence of FHM feeding (material in the GI tracts) and the accumulation of biofilm within the cages suggest that starvation may not have been a primary factor in the FHM mortality. Whether this was also the case for the LMB and RRH mortality is uncertain. The higher percentages of survival of RRH and LMB in cages in hatchery ponds and positive evidence of feeding occurring while inside hatchery cages suggests that

87

they could have fed and survived similarly in the cages in the river, had contaminant and water quality conditions been suitable.

The intersex condition was not observed in any assayed FHM, RRH, or LMB, but it was not expected in the juvenile RRH or LMB because of their sexual immaturity and absence of developed gonads. Exposed adult FHM did not develop the intersex condition, which has been shown to exist in this species under ecologically relevant concentrations of estrogenic contaminants (Kidd et al., 2007). The absence of the intersex condition in FHM may have been due to low concentrations of contaminants, the short duration or developmental timing of exposure, or some other unknown factor. The near complete loss of breeding tubercles in FHM exposed to the river is a possible indicator of endocrine effects and exposure to estrogenic contaminants (Vajda et al., 2011).

Low survival rates of caged fish and the rapid accumulation of contaminants into fish tissue, as well as high sediment and water contaminant concentrations, indicate that pollutants may be exerting an adverse impact on juvenile and adult fish within the Yadkin-Pee Dee River. The complete mortality of juvenile LMB and RRH in the riverine bioassays may indicate a critical recruitment bottleneck in these (and perhaps other) species at early life stages, which may directly reflect the low population size and limited recruitment of RRH in the river. Contaminants accumulated within 28 d in FHM tissues, indicate that contaminants may pose a substantial risk to fish health in the system, especially considering that wild fish are chronically exposed to ambient river conditions during all life stages, including those where critical reproductive changes occur. In the future, effective management actions exist that could be implemented to improve water quality conditions and reduce contaminant

influx within the river system. Improved wastewater treatment technologies, reduced discharges from industrial and power production facilities, reduced agricultural and urban runoff, and public awareness of pollutant impacts on the environment may be beneficial first steps. This research provides important findings that may inform and guide those actions toward maintaining and enhancing habitat for aquatic life.

REFERENCES

Aschberger, K., Munn, S., Olsson, H., Pakalin, S., Pellegrini, G., Vegro, S., Paya Perez, A.B., 2010. European Union Risk Assessment Report - BPA (4,4' – Isopropylidenediphenol (Bisphenol – A)), Assessment. doi:10.2788/40195

Bahamonde, P.A., Munkittrick, K.R., Martyniuk, C.J., 2013. Intersex in teleost fish: Are we distinguishing endocrine disruption from natural phenomena? Gen. Comp. Endocrinol. 192, 25–35. doi:10.1016/j.ygcen.2013.04.005

Barton, H.A., Andersen, M.E., 1998. Endocrine active compounds: from biology to dose response assessment. Crit. Rev. Toxicol. 28, 363–423. doi:10.1080/10408449891344227

Blazer, V.S., Iwanowicz, L.R., Henderson, H., Mazik, P.M., Jenkins, J.A., Alvarez, D.A., Young, J.A., 2012. Reproductive endocrine disruption in Smallmouth Bass (*Micropterus dolomieu*) in the Potomac River basin: Spatial and temporal comparisons of biological effects. Environ. Monit. Assess. 184, 4309–4334. doi:10.1007/s10661-011-2266-5

Caldwell, D.J., Mastrocco, F., Anderson, P.D., Länge, R., Sumpter, J.P., 2012. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. Environ. Toxicol. Chem. 31, 1396–1406. doi:10.1002/etc.1825

Colman, J.R., Baldwin, D., Johnson, L.L., Scholz, N.L., 2009. Effects of the synthetic estrogen, 17α-ethinylestradiol, on aggression and courtship behavior in male Zebrafish (*Danio rerio*). Aquat. Toxicol. 91, 346–354. doi:10.1016/j.aquatox.2008.12.001

Cope, W.G., Holliman, F.M., Kwak, T.J., Oakley, N.C., Lazaro, P.R., Shea, D., Augspurger, T., Cope, W.G., Law, J.M., Henne, J.P., Ware, K.M., 2011. Assessing water quality suitability for Shortnose Sturgeon in the Roanoke River, North Carolina, USA with an in situ bioassay approach. J. Appl. Ichthyol. 27, 1–12. doi: 10.1111/j.1439-0426.2010.01570.x

Coulter, D.P., Sepúlveda, M.S., Troy, C.D., Höök, T.O., 2015. Species-specific effects of subdaily temperature fluctuations on consumption, growth and stress responses in two physiologically similar fish species. Ecol. Freshw. Fish. 25, 465–475. doi:10.1111/eff.12227

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environ. Health Perspect. 107 (suppl 6): 907–938. doi: 10.1021/bk-2001-0791

Diamond, J., Bowersox, M., Latimer, H., Barbour, C., Bearr, J., Butcher, J., 2005. Effects of pulsed contaminant exposures on early life stages of the Fathead Minnow. Arch. Environ. Contam. Toxicol. 49, 511–519. doi:10.1007/s00244-005-7023-8

Environment Canada, 1999. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life - Chlordane. Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment. Ontario, Canada.

Fisk, J.M., Kwak, T.J., Heise, R.J., 2015. Effects of regulated river flows on habitat suitability for the Robust Redhorse. Trans. Am. Fish. Soc. 144, 792–806. doi:10.1080/00028487.2015.1042557

Georgescu, B., Georgescu, C., Dărăban, S., Bouaru, A., Paşcalău, S., 2011. Heavy metals acting as endocrine disrupters. Sci. Pap. Anim. Sci. Biotechnol. 44, 89–93.

Goertzen, M.M., Driessnack, M.K., Janz, D.M., Weber, L.P., 2011. Swimming performance and energy homeostasis in juvenile laboratory raised Fathead Minnow (*Pimephales promelas*) exposed to uranium mill effluent. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 154, 420–426. doi:10.1016/j.cbpc.2011.07.012

Goulden, C.E., 1999. A critique of effluent bioassays. in: Measures of environmental performance and ecosystem condition. Ed: Schultze, P.E. National Academy Press, Washington, DC, pp.191–198.

Hewitt, A.H., Cope, W.G., Kwak, T.J., Augspurger, T., Lazaro, P.R., Shea, D., 2006. Influence of water quality and associated contaminants on survival and growth of the endangered Cape Fear Shiner (*Notropis mekistocholas*). Environ. Toxicol. Chem. 25, 2288–2298. doi:10.1897/05-569R.1

Hinck, J.E., Blazer, V.S., Schmitt, C.J., Papoulias, D.M., Tillitt, D.E., 2009. Widespread occurrence of intersex in black basses (*Micropterus* spp.) from U.S. rivers, 1995-2004. Aquat. Toxicol. 95, 60–70. doi:10.1016/j.aquatox.2009.08.001

Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53, 457–481. doi: 10.2307/2281868

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick, R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. U.S.A. 104, 8897–8901. doi:10.1073/pnas.0609568104

Lee Pow, C.S.D., 2015. Biological effects of complex estrogenic and other endocrine active mixtures in aquatic systems. North Carolina State University Dissertation. Raleigh, North Carolina.

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31. doi:10.1007/s002440010075

Muthumbi, W., De Boever, P., Pieters, J.G., Siciliano, S., D'Hooge, W., Verstraete, W., 2003. Polycyclic aromatic hydrocarbons (PAHs) and estrogenic compounds in experimental flue gas streams. J. Environ. Qual. 32, 417–422.

Nolan, M., Jobling, S., Brighty, G., Sumpter, J.P., Tyler, C.R., 2001. A histological
     description of intersexuality in the Roach. J. Fish Bio 58, 160–176.
     doi:10.1006/jfbi.2000.1431

O'Neal, K.L., 2014. Use of passive sampling to measure the bioavailability of chemical
     contaminants in surface waters and soil. North Carolina State University Dissertation,
     Raleigh, NC.

Persaud, D., Jaagumagi, R., 1993. Guidelines for the protection and management of aquatic
     sediment quality in Ontario. Minist. Environ. Energy.
     doi:10.1080/00431672.1993.9930274

RRCC (Robust Redhorse Conservation Committee), 2014. Report of the Robust Redhorse
     Conservation Committee annual meeting. Available: http://www.robustredhorse.com.
     (March 2015).

Rozon-Ramilo, L.D., Dubé, M.G., Squires, A.J., Niyogi, S., 2011. Examining waterborne
     and dietborne routes of exposure and their contribution to biological response patterns in
     Fathead Minnow (*Pimephales promelas*). Aquat. Toxicol. 105, 466–481.
     doi:10.1016/j.aquatox.2011.07.006

Sackett, D.K., Pow, C.L., Rubino, M.J., Aday, D.D., Cope, W.G., Kullman, S., Rice, J.A.,
Kwak, T.J., Law, M., 2015. Sources of endocrine-disrupting compounds in North
Carolina waterways: A geographic information systems approach. Environ. Toxicol.
Chem. 34, 437–445. doi:10.1002/etc.2797

Scholz, S., Mayer, I., 2008. Molecular biomarkers of endocrine disruption in small model
fish. Mol. Cell. Endocrinol. 293, 57–70. doi:10.1016/j.mce.2008.06.008

Suedel, B.C., Dillon, T.M., Benson, W.H., 1997. Subchronic effects of five di-ortho PCB
congeners on survival , growth and reproduction in the Fathead Minnow *Pimephales
Promelas*. Environ. Toxicol. 16, 1526–1532.

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans
and wildlife. Crit. Rev. Toxicol. 39, 228–269. doi:10.1080/10408440802233259

US Environmental Protection Agency (EPA), 1996. Method 3050B - Acid digestion of
sediments, sludges, and soils. Revision 2. Washington, DC.

US EPA, 2005. EPA-822-R-05-005: Aquatic life ambient water quality criteria nonylphenol.
Office of Water. Office of Science and Technology. Washington, DC.
http://www.epa.gov/waterscience/criteria/aqlife.html (accessed 06.06.16)

US EPA, 2006. Freshwater Sediment Screening Benchmarks. Region III BTAG. 1–6.
http://www.epa.gov/risk/freshwater-sediment-screening-benchmarks (accessed
06.06.16)

US EPA, 2012. Equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: procedures for the determination of the freely dissolved interstitial water concentrations of nonionic organics. EPA-600-R-02-012 65.

US EPA, 2016. National Recommended Water Quality Criteria- Aquatic Life Criteria Table. http://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table#table (accessed 06.06.16)

Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E.M., Bolden, A.M., Schoenfuss, H.L., Norris, D.O., 2011. Demasculinization of male fish by wastewater treatment plant effluent. Aquat. Toxicol. 103, 213–221. doi:10.1016/j.aquatox.2011.02.007

Warren, M.L. Jr., Burr, B.M., Walsh, S.J., Bart, H.L.J., Cashner, R.C., Etnier, D.A., Freeman, B.J., Kuhajda, B.R., Mayden, R.L., Robison, H.W., Ross, S.T., Starnes, W.C., 2000. Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries. 25:10, 7–31. doi:10.1577/1548-8446(2000)025<0007

Yost, E.E., Meyer, M.T., Dietze, J.E., Williams, C.M., Worley-Davis, L., Lee, B., Kullman, S.W., 2014. Transport of steroid hormones, phytoestrogens, and estrogenic activity across a swine lagoon/sprayfield system. Environ. Sci. Technol. 48, 11600–11609. doi:10.1021/es5025806

Tables

Table 1. Summary of selected contaminants among sites during the *in situ* bioassays. For each triangle, a filled section represents a measured contaminant concentration among the highest three for a given analyte at all sites. Top = water; middle = sediment; bottom left = FHM, first bioassay; bottom right = FHM, second bioassay. If more than three sections are filled this was because the third highest concentration was present in multiple samples.

Site	Analyte								
	Cd^a	Cr^a	Hg^a	Mn^a	Zn^a	PCBs	OCPs	$PAHs^b$	$CUPs^b$
Ronda	△	△	△	△	△	△	△	△	△
Route 801	△	▲	△	△	△	△	▲	▲	△
Red Hill	△	◮	◮	▲	△	◮	◮	△	▲
74 Bridge	△	◮	△	▲	▲	△	△	△	◮
Digg's Tract	▲	◮	◮	◮	◮	◮	◮	△	△
Society Hill	△	◮	▲	◮	△	▲	◮	△	△
Pee Dee	△	△	◮	△	◮	▲	◮	△	△
Bucksport	△	△	△	◮	◮	◮	△	△	▲

[a] Metals not measured in water.
[b] PAHs and CUPs not measured in fish tissue.

Table 2. Mean physicochemical characteristics of river water (standard error in parentheses) measured at each site during the 28-d bioassay.

Site	Bioassay 1					Bioassay 2				
	Temperature (°C)	Conductivity (µS/cm)	Salinity (ppt)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (µS/cm)	Salinity (ppm)	Dissolved Oxygen (mg/L)	pH
Ronda	22.3 (0.5)	61 (2)	0.03 (0.00)	7.6 (0.2)	7.4 (0.0)	22.1 (0.3)	66 (3)	0.03 (0.00)	6.0 (0.7)	7.1 (0.1)
Route 801	25.7 (0.6)	83 (2)	0.04 (0.00)	7.2 (0.2)	7.4 (0.0)	24.9 (0.6)	94 (6)	0.04 (0.00)	5.9 (0.6)	7.3 (0.1)
Red Hill	25.5 (0.8)	99 (3)	0.04 (0.00)	5.2 (0.2)	7.2 (0.0)	26.3 (0.7)	118 (15)	0.05 (0.01)	5.0 (0.6)	7.1 (0.1)
74 Bridge	26.7 (0.8)	97 (3)	0.04 (0.00)	5.7 (0.3)	7.2 (0.0)	27.5 (0.4)	100 (4)	0.05 (0.00)	5.1 (0.3)	7.2 (0.1)
Diggs Tract	27.6 (0.4)	96 (1)	0.04 (0.00)	5.6 (0.5)	7.3 (0.0)	27.1 (0.4)	96 (8)	0.04 (0.00)	4.7 (0.4)	7.1 (0.1)
Society Hill	27.4 (0.9)	111 (11)	0.05 (0.00)	6.5 (0.3)	6.9 (0.0)	27.5 (0.3)	113 (5)	0.05 (0.00)	6.2 (0.3)	7.2 (0.1)
Pee Dee	27.7 (0.7)	106 (4)	0.05 (0.00)	6.1 (0.2)	7.0 (0.0)	27.6 (0.4)	113 (4)	0.05 (0.00)	5.5 (0.7)	7.2 (0.1)
Bucksport	27.8 (2.5)	97 (0)	0.05 (0.01)	5.1 (0.3)	6.7 (0.0)	27.1 (0.4)	96 (3)	0.04 (0.00)	4.7 (0.4)	6.8 (0.1)
Watha State Fish Hatchery	28.2 (0.6)	278 (2)	0.13 (0.00)	8.6 (1.1)	–	–	–	–	–	–
McKinney Lake Fish Hatchery	–	–	–	–	–	28.2 (0.7)	31 (1)	0.01 (0.0)	7.4 (0.1)	8.4 (0.4)

Figures

Site	Latitude	Longitude	State
Ronda	36.21601	-80.937164	NC
Rt. 801	35.838287	-80.484752	NC
Red Hill	35.0847222	-79.9986111	NC
74 Bridge	34.9455556	-79.8691667	NC
Diggs Tract	34.8652778	-79.8791667	NC
Society Hill	34.524154	-79.832815	SC
Pee Dee	34.203845	-79.547676	SC
Bucksport	33.661734	-79.153058	SC

Figure 1. Map of study sites located on the Yadkin-Pee Dee River for the *in situ* bioassays with Fathead Minnow, Largemouth Bass, and Robust Redhorse, June-August 2014.
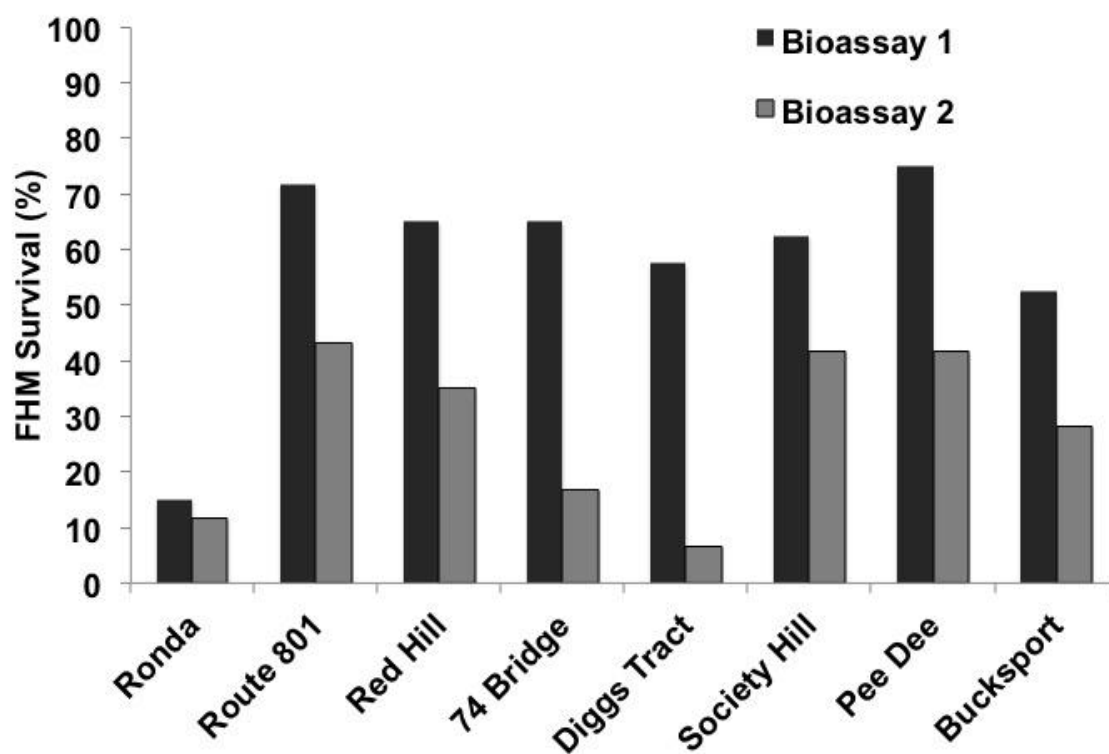
Figure 2. Fathead minnow survival at each site, at day 28 of *in situ* bioassays 1 and 2.
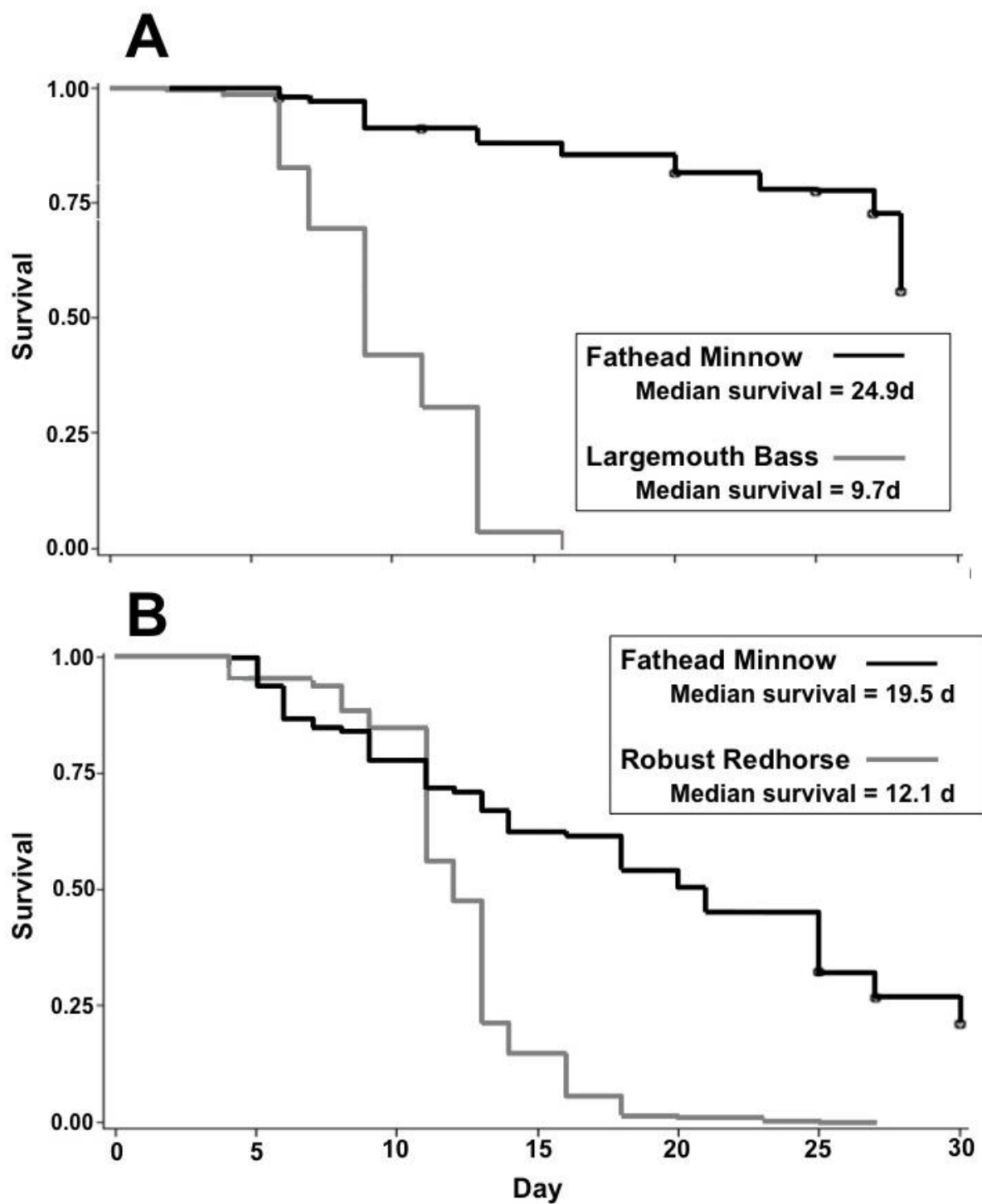
Figure 3. Kaplan-Meier survival distribution function for Fathead Minnow, Largemouth Bass, and Robust Redhorse at all river sites during *in situ* bioassays 1 (A) and 2 (B).
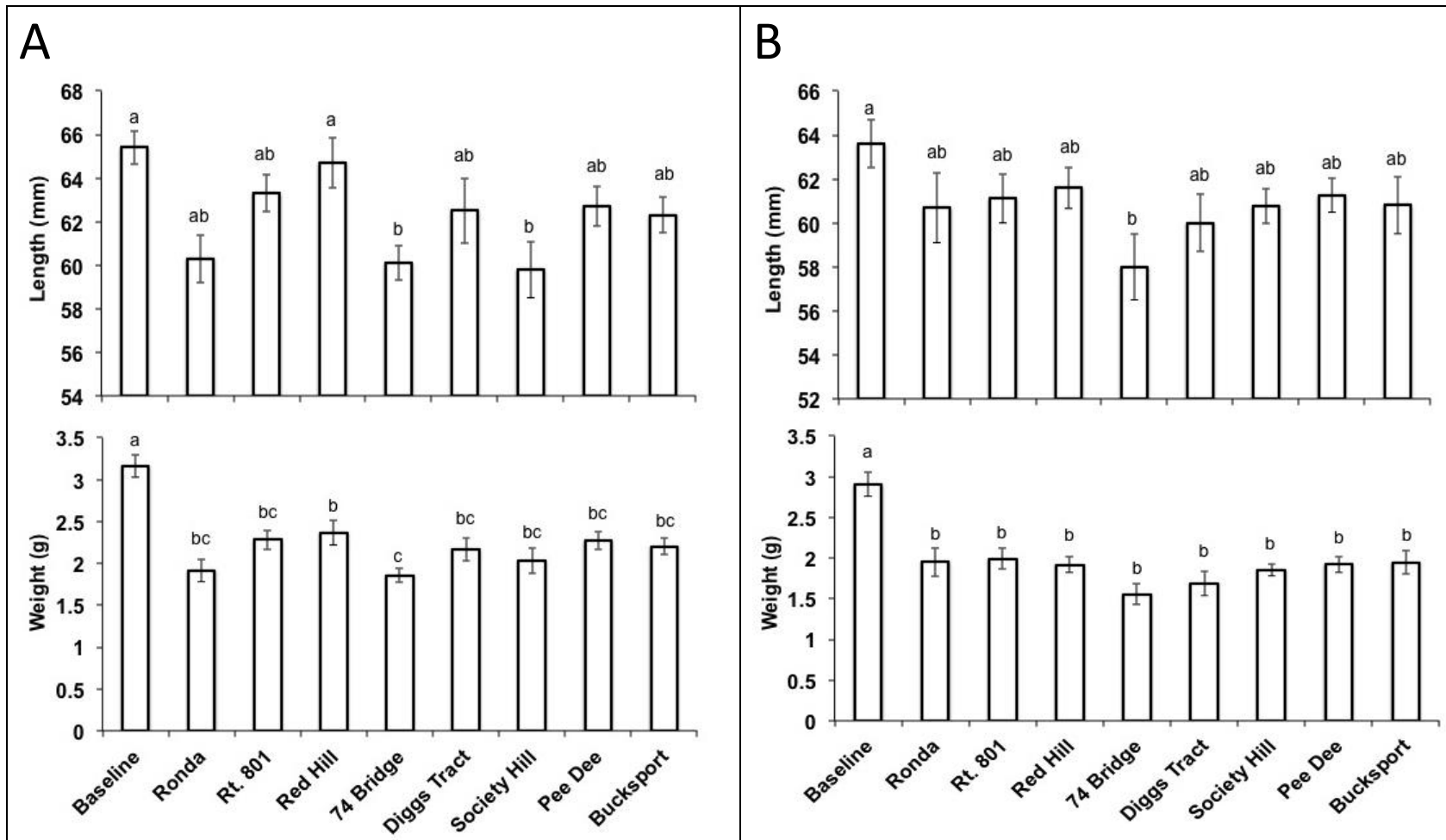
Figure 4. Mean (± SE) fish total length (mm) and weight (g) of baseline Fathead Minnow and those recovered from cages at each site for bioassay 1 (A) and bioassay 2 (B). Sites with the same letter indicate no significant difference (p>0.05).

102
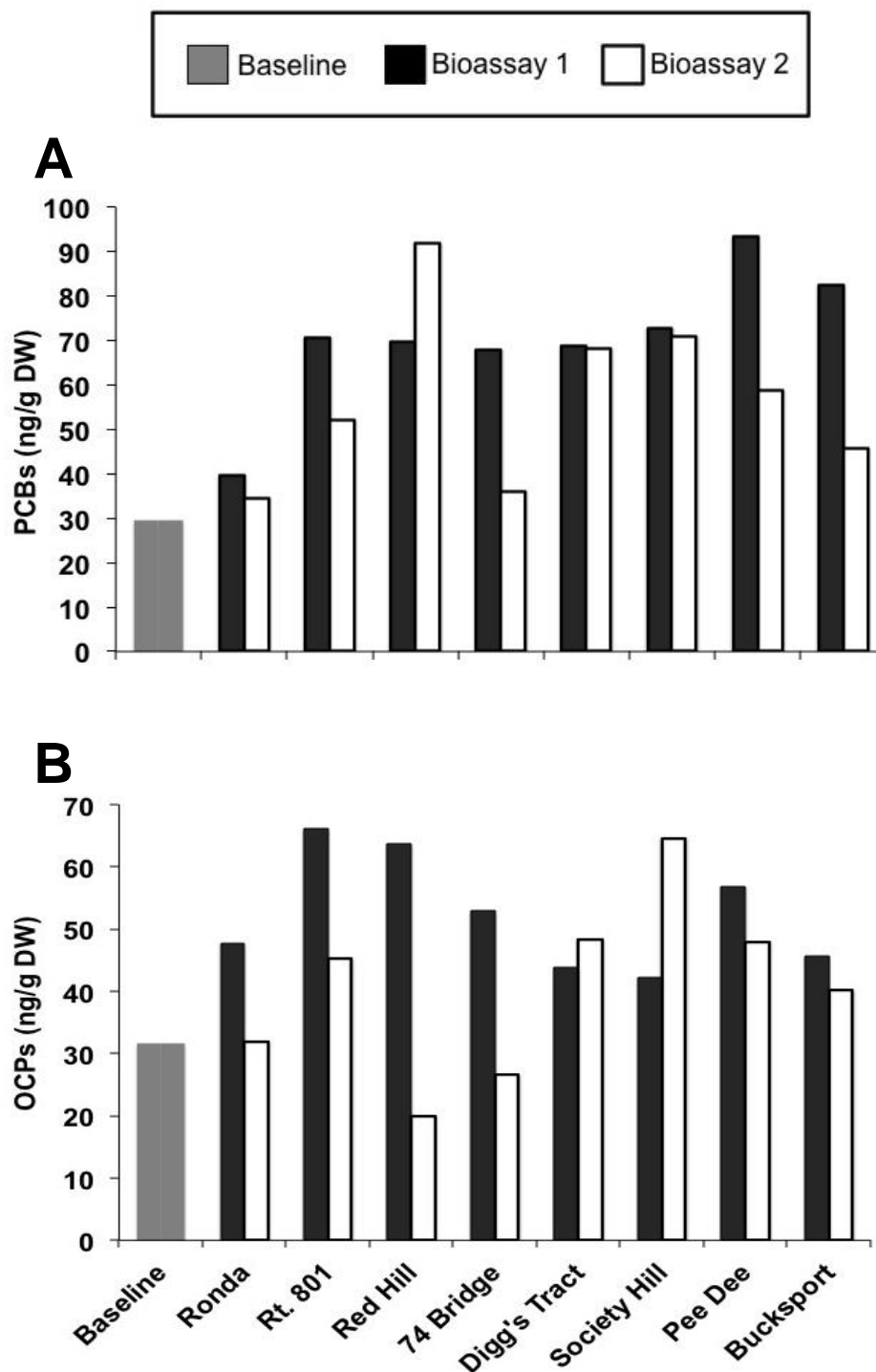
Figure 5. PCB (A; ng/g DW) and OCP (B; ng/g DW) Fathead Minnow whole-fish concentrations (composite samples) from each site following the *in situ* bioassay (1 and 2) and baseline samples.
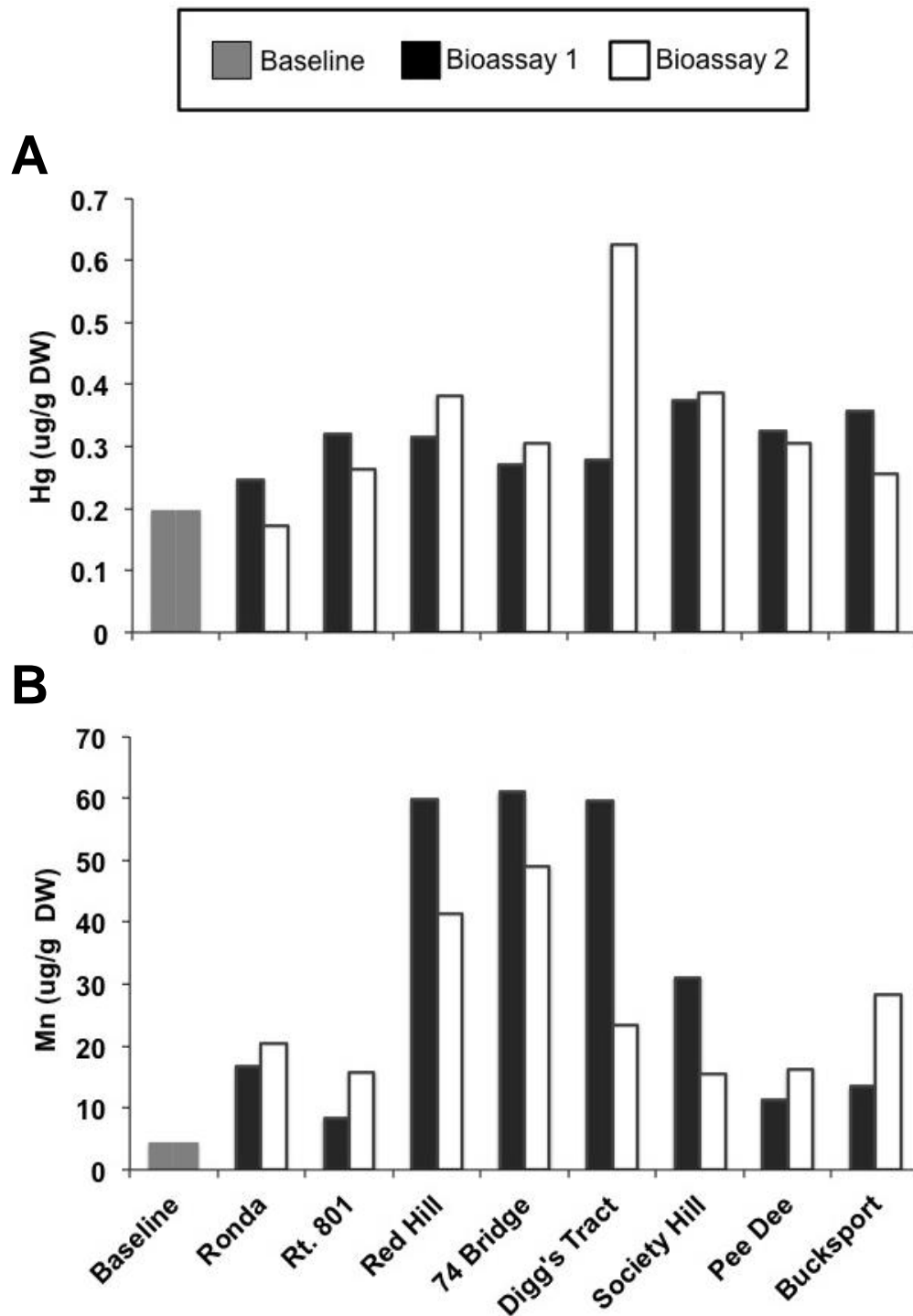
Figure 6. Hg (A; ug/g DW) and Mn (B; ug/g DW) Fathead Minnow whole-fish concentrations (composite samples) from each site following the *in situ* bioassay (1 and 2) and baseline samples.

APPENDICES

Supporting Information for Chapter 1

Table SI 1. Yadkin-Pee Dee River site descriptions. NC: North Carolina. SC: South Carolina.

Site	Latitude	Longitude	Type	State
Kerr Scott Reservoir	36.130355	-81.229061	Reservoir	NC
Ronda	36.21601	-80.937164	River	NC
Route 801	35.838287	-80.484752	River	NC
Badin Lake	35.407817	-80.114984	Reservoir	NC
Red Hill	35.0847222	-79.9986111	River	NC
Blewett Lake	34.9877778	-79.8911111	Reservoir	NC
74 Bridge	34.9455556	-79.8691667	River	NC
Diggs Tract	34.8652778	-79.8791667	River	NC
Society Hill	34.524154	-79.832815	River	SC
Pee Dee	34.203845	-79.547676	River	SC
Bucksport	33.661734	-79.153058	River	SC

Table SI 2. Fish species common name, scientific name, family, and associated abbreviation along with species-specific standard weight equation parameters for determining relative weights.

Common Name	Scientific Name	Family	Abbreviation	Intercept (a)	Slope (b)	Minimum TL (mm)
Largemouth Bass	*Micropterus salmoides*	Centrarchidae	LMB	–5.528	3.273	150
Smallmouth Bass	*Micropterus dolomieu*	Centrarchidae	SMB	–5.329	3.2	150
Spotted Bass	*Micropterus punctulatus*	Centrarchidae	SPB	–5.392	3.215	100
Redear Sunfish	*Lepomis microlophus*	Centrarchidae	RES	–4.968	3.119	70
Warmouth	*Lepomis gulosus*	Centrarchidae	WAR	–5.180	3.241	80
Bluegill Sunfish	*Lepomis macrochirus*	Centrarchidae	BLG	–5.374	3.316	80
Redbreast	*Lepomis auritus*	Centrarchidae	RBS	–4.755	2.997	NA
Pumpkinseed	*Lepomis gibbosus*	Centrarchidae	PKS	–5.179	3.237	50
Channel Catfish	*Ictalurus punctatus*	Ictaluridae	CHC	–5.800	3.294	70
White Catfish	*Ameiurus catus*	Ictaluridae	WHC	–5.851	3.395	100
Blue Catfish	*Ictalurus furcatus*	Ictaluridae	BLC	–6.067	3.4	160
Flathead Catfish	*Pylodictis olivaris*	Ictaluridae	FHC	–5.542	3.23	130

Table SI 3. Full list of all contaminants analyzed. PAHs, OCPs, PCBs, CUPs, hormones, and industrial EACs were evaluated in water. PAHs, PCBs, OCPs, CUPs, and metals were analyzed in sediment. PCBs, OCPS, and metals were evaluated in fish muscle tissue. (*) indicates water detection; (+) indicates fish muscle tissue detection; (•) indicates sediment detection.

PAHs	OCPs	PCBs	CUPs	Hormones	Industrial EACs	Metals
Naphthalene *•	2,4'-DDD	Cl2(08)	Acetochlor	17_-Estradiol (aE2)	BisphenolA *	Aluminum (Al) +•
Acenaphthylene •	2,4'-DDE	Cl3(18) *	alachlor	17_-Estradiol (bE2)	Nonylphenol *	Antimony (Sb)
Acenaphthene *•	2,4'-DDT	Cl3(28)*	Atrazine*•	17_-Testosterone (bT)		Arsenic (As)
C1 – Naphthalenes *•	4,4'-DDD *+	Cl4(52) *+	Atrazine-desethyl *	Epitestosterone (aT)		Barium (Ba) +•
C2 – Naphthalenes *•	4,4'-DDE *+•	Cl4(44) *+	Atrazine-desisopropyl *	Estriol (E3)		Berylium (Be) •
C3 – Naphthalenes *•	4,4'-DDT	Cl4(66) *+	Benfluralin	Estrone (E1)		Cadmium (Cd) •
C4 – Naphthalenes *•	Aldrin	Cl5(101) *+	Bifenthrin	Ethinylestradiol (EE2) *		Cobalt (Co) •
Fluorene *•	alpha-BHC	Cl4(77)	Butylate			Chromium (Cr) •
C1 – Fluorenes *•	betaBHC	Cl5(118) *+	Chlorothalonil			Copper (Cu) +•
C2 – Fluorenes *	Chlorothalonil	Cl6(153) *+	Chlorpyrifos			Iron (Fe) +•
C3 – Fluorenes *	Chlorpyrifos *	Cl5(105) *+	Cyfluthrin			Mercury (Hg) +•
Dibenzothiophene •	cis-Chlordane *+•	Cl6(138) *+	Cypermethrin			Potassium (K) +•
C1 – Dibenzothiophene *•	Delta-BHC	Cl5(126)	Dacthal			Magnesium (Mg) +•
C2 – Dibenzothiophene *•	Dieldrin	Cl7(187) +	Deltamethrin			Manganese (Mn) +•
C3 – Dibenzothiophene *•	Endosulfan I	Cl6(128) +	Diazinon			Molybdenum (Mo)
Phenanthrene *•	Endosulfan II	Cl8(201)	Dimethenamid *			Nickel (Ni) +•
Anthracene *•	Endosulfan Sulfate	Cl7(180) +	Dimethoate			Lead (Pb) +•
C1 - Phenanthrenes/Anthracenes *•	Endrin	Cl7(170) +	EPTC			Selenium (Se) +
C2 - Phenanthrenes/Anthracenes *•	Endrin Aldehyde	Cl8(195)	Esfenvalerate			Silicon (Si) +•
C3 - Phenanthrenes/Anthracenes *•	Endrin Ketone	Cl9(206) +	Ethalfluralin			Strontium (Sr) +•
C4 - Phenanthrenes/Anthracenes *•	gamma-BHC (Lindane)	Cl10(209) +	Ethopropyl			Vanadium (V) •

Table SI 3-continued.

PAHs	OCPs	PCBs	CUPs	Hormones	Industrial EACs	Metals
Fluoranthene *•	Heptachlor		Fenpropathrin			Zinc (Zn) +•
Pyrene *•	Heptachlor epoxide *		Flumetralin			
C1 - Fluoranthenes/Pyrene *•	Hexachlorobenzene *+•		Fonofos			
C2 - Fluoranthrene/Pyrene *•	Methoxychlor		lambda cyhalothrin			
C3 - Fluoranthrene/Pyrene •	Mirex		Malathion			
Retene *•	Trans-Chlordane *+•		Methidathion			
Benz[a]anthracene *•	Trans-Nonachlor *+•		Methyl parathion			
Chrysene *•			Metolachlor *			
C1 - Chrysenes *•			Metribuzin			
C2 - Chrysenes *•			Napropamide			
C3 – Chrysenes •			Pebulate			
C4 – Chrysenes •			Pendimethalin			
Benzo[b]fluoranthene *•			Permethrin			
Benzo[k]fluoranthene *•			Phorate			
Benzo[e]pyrene *•			Phosmet			
Benzo[a]pyrene •			Prometon *			
Perylene *•			Propachlor			
Indeno[1,2,3-cd]pyrene •			Propazine *			
Dibenz[a,h,]anthracene •			Propiconazole			
Benzo[g,h,i]perylene *•			Simazine *			
Coronene •			Tebuthiuron			
			Terbufos			
			Triallate			
			Tribufos			
			Trichlorfon			
			Trifluralin			

Table SI 4. Average contaminant concentrations and average percent moisture in fish muscle tissue for each species at each site. PCBs, 4'4-DDE, and OCPs measured in ng/g WW. Hg in ug/g WW. NA=not applicable. SD=standard deviation. n=sample size.

Site	Species	n	% Moisture Mean	% Moisture SD	PCBs Mean	PCBs SD	OCPs Mean	OCPs SD	4'4-DDE Mean	4'4-DDE SD	Hg Mean	Hg SD
Kerr Scott R.	*Micropterus salmoides*	3	75.706	6.385	1.420	1.490	1.679	1.864	0.661	0.919	0.344	0.209
	Lepomis microlophus	1	80.024	NA	1.123	NA	0.476	NA	0.285	NA	0.049	NA
	Lepomis macrochirus	2	81.515	0.530	0.621	0.249	1.308	0.613	0.78	0.452	0.048	0.004
	Ictalurus catus	3	83.212	1.047	4.018	1.643	4.159	1.749	2.125	0.783	0.218	0.111
Ronda	*Micropterus punctulatus*	3	79.931	0.483	1.407	0.310	1.441	0.366	0.944	0.275	0.176	0.028
	Lepomis auritus	2	81.709	1.603	0.368	0.203	0.443	0.124	ND	NA	0.062	0.01
	Ictalurus punctatus	1	82.272	NA	2.163	NA	2.720	NA	1.623	NA	0.386	NA
Route 801	*Ictalurus punctatus*	1	78.421	NA	14.566	NA	25.675	NA	15.542	NA	0.174	NA
	Ictalurus furcatus	1	79.715	NA	9.258	NA	15.795	NA	9.615	NA	0.21	NA
Badin Lake	*Micropterus salmoides*	3	73.942	10.652	6.688	0.856	12.035	1.606	9.241	1.067	0.326	0.041
	Lepomis macrochirus	3	81.045	0.116	3.188	0.834	3.262	1.135	2.823	1.054	0.039	0.003
	Ictalurus catus	2	85.260	4.945	1.763	1.561	2.646	2.375	1.93	1.688	0.034	0.008
Red Hill	*Micropterus salmoides*	1	79.180	NA	4.174	NA	3.265	NA	2.713	NA	0.209	NA
	Lepomis macrochirus	2	80.007	0.232	1.070	0.277	1.313	0.308	1.011	0.155	0.04	0.003
	Ictalurus furcatus	3	78.980	5.505	6.421	5.042	3.422	3.910	2.526	2.503	0.129	0.044
Blewett Lake	*Micropterus salmoides*	5	76.472	2.463	8.022	1.674	5.664	1.963	4.559	1.440	0.198	0.064
	Lepomis macrochirus	3	81.111	0.959	2.243	0.282	1.712	0.102	1.364	0.083	0.051	0.007
	Ictalurus punctatus	2	78.120	4.291	7.137	3.039	4.475	1.476	3.955	1.565	0.178	0.103

Table SI 4-continued.

Site	Species	n	% Moisture Mean	% Moisture SD	PCBs Mean	PCBs SD	OCPs Mean	OCPs SD	4'4-DDE Mean	4'4-DDE SD	Hg Mean	Hg SD
74 Bridge	*Micropterus salmoides*	3	80.238	2.017	7.25	4.988	7.262	6.416	5.67	4.501	0.167	0.03
	Lepomis auritus	3	81.034	0.0652	7.389	0.931	4.201	0.83	3.427	0.745	0.047	0.005
	Lepomis microlophus	1	81.456	NA	7.376	NA	0.351	NA	0.351	NA	0.158	NA
	Ictalurus punctatus	3	72.437	5.639	28.879	32.2	17.817	18.558	11.129	12.085	0.084	0.004
Digg's Tract	*Micropterus salmoides*	3	79.033	0.508	10.834	9.996	8.799	8.46	6.536	5.918	0.22	0.071
	Lepomis microlophus	3	75.290	8.791	6.882	5.122	1.369	0.378	0.888	0.775	0.091	0.07
	Ictalurus furcatus	3	70.641	8.403	54.452	36.258	6.896	4.711	4.547	3.179	0.171	0.083
	Ictalurus punctatus	1	71.773	NA	71.773	NA	3.825	NA	2.578	NA	0.085	NA
Society Hill	*Micropterus salmoides*	3	78.985	0.134	18.396	20.874	3.726	0.677	3.171	0.348	0.209	0.053
	Lepomis macrochirus	3	80.580	0.129	1.065	0.703	0.877	0.1	0.745	0.095	0.058	0.026
	Ictalurus furcatus	3	78.141	6.769	3.377	2.465	1.665	0.6	1.357	0.419	0.121	0.017
Pee Dee	*Micropterus salmoides*	3	77.938	1.291	4.187	0.461	3.069	1.472	2.494	1.06	0.526	0.159
	Lepomis macrochirus	3	79.882	2.204	1.898	2.333	1.525	1.781	1.022	1.209	0.074	0.009
	Ictalurus furcatus	3	79.921	0.709	4.933	0.766	0.987	0.876	0.641	1.11	0.362	0.209
Bucksport	*Micropterus salmoides*	3	72.487	4.391	1.638	1.184	2.123	1.457	1.777	1.142	0.666	0.16
	Lepomis macrochirus	3	78.665	0.422	0.376	0.458	0.446	0.666	0.348	0.602	0.27	0.092
	Ictalurus furcatus	3	80.627	1.498	10.898	13.634	3.343	4.483	2.787	3.539	0.406	0.184

Table SI 5. PAH ESBTU calculations for water at each site.

PAH	Kerr S. R.	Ronda	Rt. 801	Badin Lake	Red Hill	Blewett Lake	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Naphthalene	0.0	0.6	0.4	0.5	1.1	0.4	0.4	0.3	1.0	1.6	0.9
Acenaphthylene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acenaphthene	0.0	1.0	1.2	0.0	0.5	0.1	0.1	0.7	0.7	0.3	0.1
C1 - Naphthalenes	1.0	0.7	0.8	1.1	1.0	0.4	0.4	0.6	1.0	1.7	0.7
C2 - Naphthalenes	5.4	3.1	3.5	5.2	2.6	1.1	1.4	2.2	2.9	1.9	1.6
C3 - Naphthalenes	25.0	8.3	8.2	17.4	6.0	3.2	4.2	5.2	7.8	3.5	3.9
C4 - Naphthalenes	22.1	7.9	8.7	21.4	12.1	4.7	6.4	6.5	14.8	7.3	7.0
Fluorene	0.3	1.1	1.3	0.4	0.4	0.1	0.3	0.9	0.7	0.3	0.2
C1 - Fluorenes	7.9	2.8	3.1	5.1	3.0	1.7	1.7	2.5	3.5	1.6	1.4
C2 - Fluorenes	15.6	5.8	8.4	14.2	7.7	3.9	4.8	5.1	16.6	6.6	5.0
C3 - Fluorenes	12.0	5.2	8.3	10.1	5.4	2.8	4.1	5.4	25.8	12.4	5.3
Dibenzothiophene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C1 - Dibenzothiophene	0.4	0.8	2.8	0.0	0.9	0.4	0.6	0.9	1.4	0.0	0.0
C2 - Dibenzothiophene	0.0	1.5	2.5	0.0	2.4	1.1	1.6	2.3	7.8	2.5	1.1
C3 - Dibenzothiophene	0.0	1.7	2.5	0.0	2.0	1.3	1.4	2.1	8.7	4.2	1.7
Phenanthrene	1.0	6.8	9.9	1.0	3.8	1.0	1.3	5.8	3.8	1.3	0.7
Anthracene	0.2	0.5	0.8	0.5	0.5	0.3	0.3	0.6	0.6	0.4	0.2
C1 - P/A	8.5	5.3	15.2	7.7	6.3	2.7	3.6	7.5	8.8	4.4	3.4
C2 - P/A	11.5	6.5	14.2	12.5	6.9	3.3	5.1	7.8	18.9	11.0	5.9
C3 - P/A	5.2	0.0	11.6	7.7	6.1	3.7	5.2	5.9	18.1	12.0	7.6
C4 - P/A	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fluoranthene	5.0	15.3	30.2	9.9	16.0	8.9	10.9	21.1	20.0	13.0	6.6
Pyrene	2.7	11.6	19.8	12.3	9.7	5.5	8.0	15.2	15.2	10.7	5.7
C1 - F/P	1.9	2.8	5.9	5.2	3.8	2.4	3.0	4.9	8.8	6.4	3.3
C2 - F/P	0.0	2.0	3.3	3.5	2.5	1.6	2.2	3.1	4.4	4.1	2.4

Table SI 5-continued.

PAH	Kerr S. R.	Ronda	Route 801	Badin Lake	Red Hill	Blewett Lake	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
						Site					
C3 - F/P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Retene	1.6	7.9	41.9	1.7	8.2	4.7	6.5	8.4	23.0	26.4	28.2
Benz[a]anthracene	0.0	1.1	1.5	1.4	0.0	0.0	0.8	1.5	1.8	1.2	0.8
Chrysene	0.3	1.6	3.9	1.5	0.0	0.0	2.8	4.1	5.1	4.7	1.7
C1 - Chrysenes	0.3	0.6	1.3	1.1	0.0	0.0	0.9	1.1	1.6	1.2	0.0
C2 - Chrysenes	0.0	0.0	0.7	0.6	0.0	0.0	0.0	0.6	0.8	0.7	0.0
C3 - Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4 - Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene	0.0	0.0	2.6	2.7	1.9	1.1	1.4	1.7	2.0	2.1	0.9
Benzo[k]fluoranthene	0.0	0.0	0.6	0.6	0.3	0.2	0.3	0.4	0.4	0.3	0.1
Benzo[e]pyrene	0.3	0.8	1.6	1.9	1.2	0.0	0.0	1.1	1.5	1.9	0.0
Benzo[a]pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Perylene	0.3	1.8	2.3	1.6	6.4	15.8	15.5	8.7	10.8	13.9	17.1
Indeno[1,2,3-cd]pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dibenz[a,h,]anthracene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[g,h,i]perylene	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coronene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum of PAH 42	128.60	105.22	219	152.47	118.86	72.60	95.23	134.48	238.25	159.44	113.70
Sum of PAH 34	126.58	91.27	165.96	147.21	102.84	63.47	83.00	117.64	192.98	122.24	80.23
Sum of ESBTUs											
Acute	0.014	0.009	0.023	0.024	0.015	0.014	0.018	0.019	0.035	0.026	0.018
Chronic	0.026	0.017	0.046	0.047	0.030	0.029	0.036	0.038	0.071	0.054	0.037

Table SI 6. PAH ESBTU calculations for sediment at each site.

PAH	Site										
	Kerr S. R.	Ronda	Route 801	Badin Lake	Red Hill	Blewett Lake	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Naphthalene	0.4	1.2	3.4	15.1	2.1	0.2	1.1	5.9	1.2	0.9	1.0
Acenaphthylene	0.0	0.0	5.4	13.7	5.7	0.0	2.6	5.7	1.1	0.0	0.8
Acenaphthene	0.0	0.0	12.3	22.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C1 - Naphthalenes	0.0	0.0	4.6	14.7	4.2	0.0	0.0	5.2	1.4	0.0	1.3
C2 - Naphthalenes	0.0	0.0	0.0	67.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Naphthalenes	0.0	0.0	0.0	43.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4 - Naphthalenes	0.0	0.0	0.0	29.3	0.0	0.0	0.0	3.7	0.0	0.0	0.0
Fluorene	0.0	0.6	17.6	29.9	1.7	0.0	1.2	3.3	0.7	0.6	0.8
C1 - Fluorenes	0.0	0.0	10.7	22.8	9.7	0.0	0.0	12.1	0.0	0.0	0.0
C2 - Fluorenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Fluorenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dibenzothiophene	0.0	0.0	11.5	17.6	1.9	0.0	0.0	2.1	0.0	0.0	0.0
C1 - Dibenzothiophene	0.0	0.0	9.0	10.2	3.2	0.0	0.0	0.0	0.0	0.0	0.0
C2 - Dibenzothiophene	0.0	0.0	10.5	15.2	4.4	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Dibenzothiophene	0.0	0.0	9.8	0.0	4.3	0.0	0.0	0.0	0.0	0.0	0.0
Phenanthrene	0.5	10.1	237.7	281.7	19.5	0.4	12.8	35.7	6.5	5.0	5.5
Anthracene	0.0	2.1	27.3	94.1	5.7	0.0	3.9	10.0	1.8	1.1	1.6
C1 - P/A	0.0	5.3	88.9	124.5	21.2	0.0	12.9	29.1	6.7	5.3	6.2
C2 - P/A	0.0	0.0	73.1	128.5	29.7	0.0	0.0	0.0	0.0	0.0	0.0
C3 - P/A	0.0	0.0	44.1	87.6	23.9	0.0	0.0	0.0	0.0	0.0	0.0
C4 - P/A	0.0	0.0	26.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fluoranthene	0.4	43.0	399.8	710.3	50.3	0.5	30.3	107.6	16.7	13.2	12.5
Pyrene	0.3	35.5	313.5	611.6	45.0	0.5	28.2	91.2	14.1	10.8	10.7
C1 - F/P	0.0	9.5	142.2	270.0	24.8	0.9	14.1	41.9	8.1	6.0	7.9
C2 - F/P	0.0	10.7	80.9	372.4	17.9	0.0	12.7	36.9	8.2	6.1	0.0
C3 - F/P	0.0	2.9	26.3	94.6	6.7	0.0	4.3	12.4	3.2	0.0	0.0

Table SI 6-continued.

PAH	Kerr S. R.	Ronda	Route 801	Badin Lake	Red Hill	Blewett Lake	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
						Site					
Retene	0.0	20.9	21.7	119.7	38.5	2.4	9.8	233.3	30.8	15.3	48.4
Benz[a]anthracene	0.0	14.1	132.7	551.8	21.9	0.0	15.2	37.1	6.2	4.2	6.5
Chrysene	0.0	25.8	204.0	956.2	32.7	0.6	18.5	67.5	10.3	7.5	9.1
C1 - Chrysenes	0.0	7.7	59.8	237.1	15.5	0.0	11.5	27.5	5.0	3.5	0.0
C2 - Chrysenes	0.0	0.0	23.7	77.2	7.0	0.0	0.0	14.9	0.0	0.0	0.0
C3 - Chrysenes	0.0	0.0	12.3	112.9	3.7	0.0	0.0	8.8	0.0	0.0	0.0
C4 - Chrysenes	0.0	0.0	0.0	56.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene	0.0	36.0	237.2	1812.1	42.8	0.8	23.5	99.0	15.3	10.7	14.5
Benzo[k]fluoranthene	0.0	13.1	80.0	472.0	14.6	0.0	8.5	32.9	5.0	3.2	4.7
Benzo[e]pyrene	0.0	20.7	127.2	1068.1	23.5	0.0	12.8	54.6	8.3	5.2	7.2
Benzo[a]pyrene	0.0	20.5	143.0	612.5	22.8	0.0	14.7	51.7	7.5	0.0	0.0
Perylene	0.0	15.2	65.4	193.9	35.7	1.1	115.7	186.6	59.2	98.9	187.0
Indeno[1,2,3-cd]pyrene	0.0	20.4	117.3	769.1	21.7	0.0	11.9	53.5	8.0	5.2	7.7
Dibenz[a,h,]anthracene	0.0	4.2	28.1	237.3	5.2	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[g,h,i]perylene	0.0	21.0	120.0	584.3	20.6	0.0	13.6	57.8	9.5	6.3	0.0
Coronene	0.0	9.2	32.2	130.3	7.3	0.0	3.6	15.6	2.4	1.9	3.3
Sum of PAH 42	1.66	349.73	2960.20	11068.05	595.33	7.34	383.48	1343.62	237.26	210.94	336.66
Sum of PAH 34	1.66	306.08	2758.21	10308.10	511.15	4.98	353.11	1043.34	192.66	187.65	285.01
Sum of ESBTUs											
Acute	0.0002	0.022	0.069	0.024	0.017	0.001	0.013	0.008	0.005	0.008	0.004
Chronic	0.001	0.088	0.281	0.095	0.068	0.003	0.052	0.032	0.020	0.032	0.015

Table SI 7. Principal component analysis loading coefficients and variance explained for black bass and sunfish.

	Loading Coefficients	Black Bass		Sunfish	
		PC1	PC2	PC1	PC2
Intersex	Occurrence (%)	0.49	0.81	-0.17	0.80
	Severity Ranking (1-4)	0.29	0.29	-0.50	0.77
Water (ng/L)	E2β-Eq.	0.49	-0.40	0.66	0.10
	EE2	0.61	-0.39	0.81	0.02
	Industrial EACs	0.97	0.07	0.74	0.59
	CUPs	0.97	0.06	0.74	0.57
	PCBs	0.84	0.13	0.32	0.67
	OCPs	0.73	0.15	0.34	0.20
	PAHs	0.55	0.58	-0.17	0.82
Sediment (ug/g DW)	Hg	-0.13	0.91	-0.63	0.45
	PAHs	-0.35	0.85	-0.75	0.20
	OCPs	-0.35	0.83	-0.74	0.17
Variance Explained		38.30	30.60	35.20	28.00
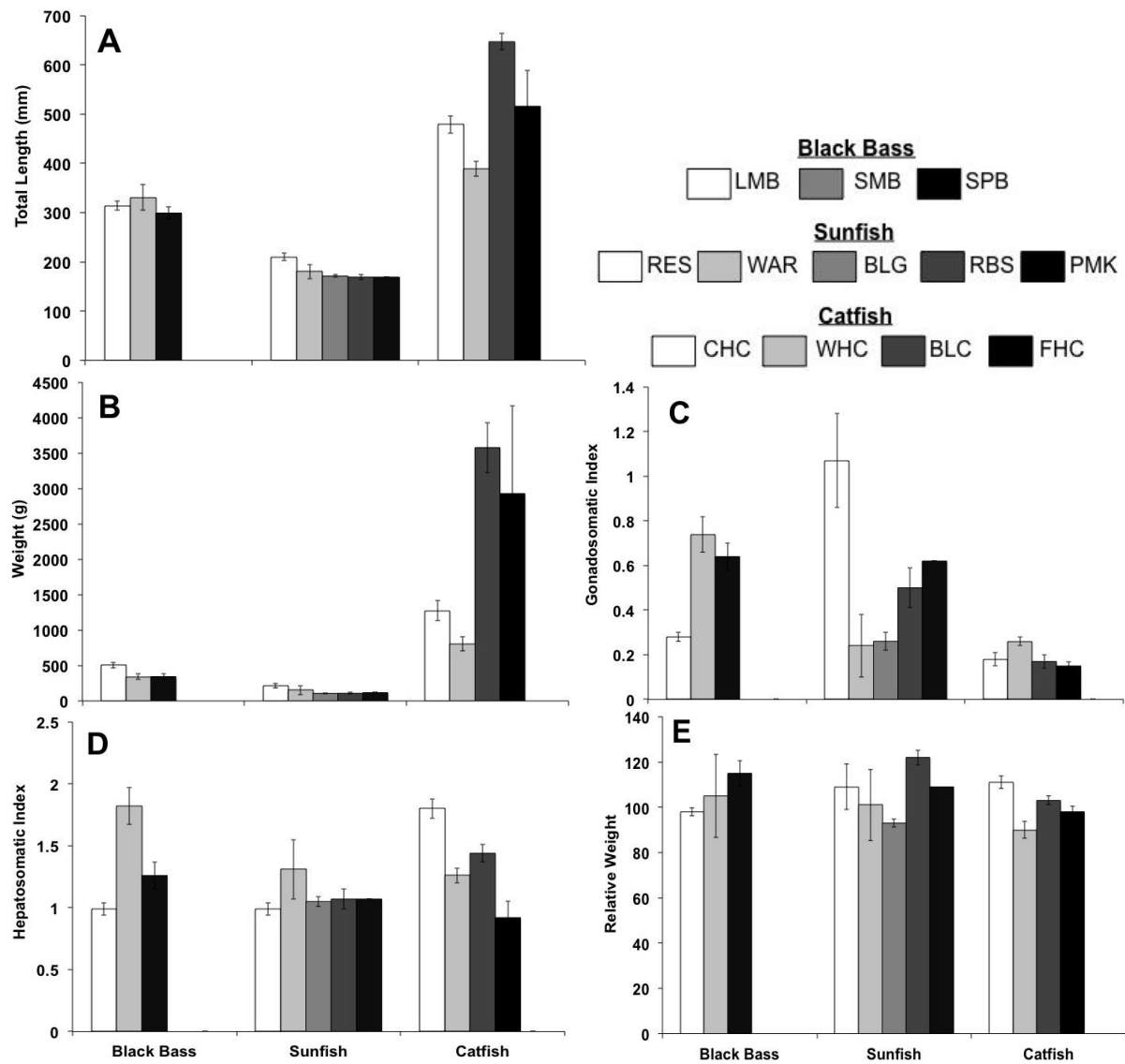
Figure SI 1. Total length (A), weight (B), gonadosomatic index (C), hepatosomatic index (D), and relative weight (E) by species (mean ± standard error). Largemouth bass (LMB, n=66), Smallmouth Bass (SMB, n=2), Spotted Bass (SPB, n=12), Redear Sunfish (RES, n=24), Warmouth (WAR, n=3), Bluegill (BLG, n=65), Redbreast (RBS, n=19), Pumpkinseed (PKS, n=1), Channel Catfish (CHC, n=21), White Catfish (WHC, n=8), Blue Catfish (BLC, n=37), and Flathead Catfish (FHC, n=10).

Supporting Information for Chapter 2

Table SI 1. Complete list of contaminants analyzed. PAHs, OCPs, PCBs, CUPs, hormones, and industrial contaminants were analyzed in water. PAHs, PCBs, OCPs, CUPs, and metals were analyzed in sediment. PCBs, OCPS, and metals were analyzed in whole-fish composite samples.   (*) indicates water detection; (+) indicates whole-fish detection; (•) indicates sediment detection.

PAHs	OCPs	PCBs	CUPs	Hormones	Industrial	Metals
Naphthalene *•	2,4'-DDD	Cl2(08)	Acetochlor	17_-Estradiol (aE2)	BisphenolA *	Aluminum (Al) +•
Acenaphthylene •	2,4'-DDE	Cl3(18) *	alachlor	17_-Estradiol (bE2)	Nonylphenol *	Antimony (Sb)
Acenaphthene *•	2,4'-DDT	Cl3(28)*•	Atrazine*•	17_-Testosterone (bT)		Arsenic (As)
C1 – Naphthalenes *•	4,4'-DDD *+	Cl4(52) *+	Atrazine-desethyl *	Epitestosterone (aT)		Barium (Ba) +•
C2 – Naphthalenes *•	4,4'-DDE *+•	Cl4(44) *+	Atrazine-desisopropyl *	Estriol (E3)		Beryllium (Be) •
C3 – Naphthalenes *•	4,4'-DDT	Cl4(66) *+•	Benfluralin	Estrone (E1)		Cadmium (Cd) •
C4 – Naphthalenes *•	Aldrin	Cl5(101) *+	Bifenthrin	Ethinylestradiol (EE2) *		Cobalt (Co) •
Fluorene *•	alpha-BHC	Cl4(77)	Butylate			Chromium (Cr) •
C1 – Fluorenes *•	betaBHC	Cl5(118) *+	Chlorothalonil			Copper (Cu) +•
C2 – Fluorenes *	Chlorothalonil	Cl6(153) *+•	Chlorpyrifos			Iron (Fe) +•
C3 – Fluorenes *	Chlorpyrifos *	Cl5(105) *+ •	Cyfluthrin			Mercury (Hg) +•
Dibenzothiophene •	cis-Chlordane *+•	Cl6(138) *+	Cypermethrin			Potassium (K) +•
C1 – Dibenzothiophene *•	Delta-BHC	Cl5(126)	Dacthal			Magnesium (Mg) +•
C2 – Dibenzothiophene *•	Dieldrin	Cl7(187) +	Deltamethrin			Manganese (Mn) +•
C3 – Dibenzothiophene *•	Endosulfan I	Cl6(128) +	Diazinon			Molybdenum (Mo)
Phenanthrene *•	Endosulfan II	Cl8(201)	Dimethenamid *			Nickel (Ni) +•
Anthracene *•	Endosulfan Sulfate	Cl7(180) +	Dimethoate			Lead (Pb) +•
C1 - Phenanthrenes/Anthracenes *•	Endrin	Cl7(170) +	EPTC			Selenium (Se) +
C2 - Phenanthrenes/Anthracenes *•	Endrin Aldehyde	Cl8(195)	Esfenvalerate			Silicon (Si) +•
C3 - Phenanthrenes/Anthracenes *•	Endrin Ketone	Cl9(206) +•	Ethalfluralin			Strontium (Sr) +•
C4 - Phenanthrenes/Anthracenes •	gamma-BHC (Lindane)	Cl10(209) +•	Ethoprop			Vanadium (V) •

Table SI 1-continued.

PAHs	OCPs	PCBs	CUPs	Hormones	Industrial	Metals
Fluoranthene *•	Heptachlor *		Fenpropathrin			Zinc (Zn) +•
Pyrene *•	Heptachlor epoxide *		Flumetralin			
C1 - Fluoranthenes/Pyrene *•	Hexachlorobenzene *+•		Fonofos			
C2 - Fluoranthrene/Pyrene *•	Methoxychlor		lambda cyhalothrin			
C3 - Fluoranthrene/Pyrene •	Mirex		Malathion			
Retene *•	Trans-Chlordane *+•		Methidathion			
Benz[a]anthracene *•	Trans-Nonachlor *+•		Methyl parathion			
Chrysene *•			Metolachlor *			
C1 - Chrysenes *•			Metribuzin			
C2 - Chrysenes *•			Napropamide			
C3 – Chrysenes •			Pebulate			
C4 – Chrysenes •			Pendimethalin			
Benzo[b]fluoranthene *•			Permethrin			
Benzo[k]fluoranthene *•			Phorate			
Benzo[e]pyrene *•			Phosmet			
Benzo[a]pyrene •			Prometon *			
Perylene *•			Propachlor			
Indeno[1,2,3-cd]pyrene •			Propazine *			
Dibenz[a,h,]anthracene •			Propiconazole			
Benzo[g,h,i]perylene *•			Simazine *			
Coronene •			Tebuthiuron			
			Terbufos			
			Triallate			
			Tribufos			
			Trichlorfon			
			Trifluralin			

Table SI 2. Contaminants detected in whole fish (DW, ug/g=metals, ng/g=organics), water (ng/L), and sediment (ug/g=metals, ng/g=organics) among sites and baseline fish.

Analyte Compartment	Baseline	Site							
		Ronda	Rt. 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Al									
FHM 1/FHM 2	45.4/45.1	147/572	124/326	149/218	220/283	204/114	142/114	121/125	124/183
Water	–	–	–	–	–	–	–	–	–
Sediment	–	659.0	1505.8	1192.8	687.4	1620.8	859.1	836.8	1086.3
Ba									
FHM 1/FHM 2	2.0/1.8	6.7/9.5	7.5/7.8	7.4/8.1	10.8/11.6	8.9/7.7	7.3/7.8	7.6/8.2	8.1/8.4
Water	–	–	–	–	–	–	–	–	–
Sediment	–	49	114	86	64	177	96	74	90
Cr									
FHM 1/FHM 2	0.02/0.1	0.3/0.4	0.37/0.74	0.46/BDL	0.3/0.49	0.91/0.1	0.15/0.66	0.31/0.36	0.37/0.15
Water	–	–	–	–	–	–	–	–	–
Sediment	–	10.6	24.5	19.5	12.3	26.1	15.5	15.6	19.1
Cu									
FHM 1/FHM 2	0.02/0.10	0.30/0.42	0.37/0.74	0.46/BDL	0.30/0.49	0.91/0.10	0.15/0.66	0.31/0.36	0.37/0.15
Water	–	–	–	–	–	–	–	–	–
Sediment	–	6.4	17.8	21.1	10.3	25.8	12.5	10.1	12.3
Fe									
FHM 1/FHM 2	133/92	197/489	187/383	211/231	244/267	274/148	211/157	163/166	159/208
Water	–	–	–	–	–	–	–	–	–
Sediment	–	1032	2447	2618	1436	3156	1693	1598	1797
Hg									
FHM 1/FHM 2	0.21/0.18	0.25/0.17	0.32/0.26	0.31/0.38	0.27/0.31	0.28/0.63	0.38/0.39	0.32/0.31	0.36/0.26
Water	–	–	–	–	–	–	–	–	–
Sediment	–	0.01	0.02	0.05	0.02	0.06	0.03	0.01	0.03
K									
FHM 1/FHM 2	12242/ 15146	12494/ 15146	14345/ 15465	13900/ 12032	10978/ 12782	13282/ 12915	13605/ 13906	13772/ 13980	13675/ 12252
Water	–	–	–	–	–	–	–	–	–
Sediment	–	1476	2183	554	711	907	747	890	813
Mg									
FHM 1/FHM 2	1204/ 1137	1418/ 1552	1618/ 1380	1463/ 1669	1860/ 1712	1668/ 1515	1598/ 1477	1497/ 1488	1570/ 1430
Water	–	–	–	–	–	–	–	–	–
Sediment	–	1830	3264	1909	1218	2416	1549	1797	1930
Mn									
FHM 1/FHM 2	3.8/5.1	16.6/20.4	8.21/15.8	59.8/41.4	61/49.1	59.6/23.4	31.1/15.4	11.2/16.3	13.4/28.2
Water	–	–	–	–	–	–	–	–	–
Sediment	–	107.4	540.3	724.3	424.3	2595.4	920.5	458.2	226.1
Ni									
FHM 1/FHM 2	BDL/ BDL	0.2/0.24	0.2/0.34	0.34/BDL	0.23/0.24	0.53/BDL	BDL/0.33	BDL/ BDL	0.27/BDL
Water	–	–	–	–	–	–	–	–	–
Sediment	–	0.1	5.0	7.9	3.3	11.8	5.9	4.7	6.4
Pb									
FHM 1/FHM 2	BDL/0.2	BDL/0.48	BDL/ BDL	0.05/0.51	0.41/0.27	0.26/0.06	0.11/BDL	BDL/0.08	BDL/0.37
Water	–	–	–	–	–	–	–	–	–
Sediment	–	4.39	13.28	9.8	6.89	15.19	8.12	8.69	8.96

[a] BDL= below detection limits. – = not applicable. FHM 1= Fathead Minnow composite, whole-body fish samples from bioassay 1. FHM 2= Fathead Minnow composite, whole-body fish samples from bioassay

Table SI 2-continued.

Analyte Compartment	Baseline	Ronda	Rt. 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Se									
FHM 1/FHM 2	1.0/BDL	0.96/BDL	BDL/1.18	0.72/BDL	BDL/BDL	BDL/BDL	BDL/BDL	BDL/1.48	BDL/BDL
Water	–	–	–	–	–	–	–	–	–
Sediment	–	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Si									
FHM 1/FHM 2	51/35	83/222	77/144	97/116	110/144	119/79	89/72	76/76	86/104
Water	–	–	–	–	–	–	–	–	–
Sediment	–	638	1315	544	809	1263	1117	916	859
Sr									
FHM 1/FHM 2	54/47	93/83	99/68	90/109	145/121	104/103	90/80	94/88	90/87
Water	–	–	–	–	–	–	–	–	–
Sediment	–	15	38	38	22	47	27	25	28
Zn									
FHM 1/FHM 2	118/131	134/184	162/177	170/191	221/229	221/201	194/262	150/224	220/200
Water	–	–	–	–	–	–	–	–	–
Sediment	–	659.0	1505.8	1192.8	687.4	1620.8	859.1	836.8	1086.3
PCBs									
FHM 1/FHM 2	30/29	39.7/34.6	70.7/52.0	69.7/92.0	68.0/36.1	68.7/68.2	72.8/70.9	93.4/58.7	82.3/45.8
Water	–	0.1	1.1	1.4	0.8	1.4	3.4	7.3	1.5
Sediment	–	BDL	BDL	3.0	0.3	5.2	0.5	BDL	BDL
OCPs									
FHM 1/FHM 2	31.2/32.0	47.7/31.8	66.1/45.3	63.6/19.9	52.9/26.5	43.9/48.3	42.3/64.5	56.9/47.9	45.7/40.2
Water	–	2.6	11.5	12.3	2.8	3.6	11.9	4.5	1.9
Sediment	–	BDL	2.8	1.1	1.0	2.8	0.7	0.5	BDL
PAHs									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	97	242	119	92	86	137	159	114
Sediment	–	350	2960	595	383	1344	237	211	337
CUPs									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	1.4	7.2	154.0	44.6	71.1	61.1	190.7	29.4
Sediment	–	BDL	BDL	3.8	0.9	BDL	BDL	BDL	3.4
Nonylphenol									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	0.4	0.3	0.2	1.6	1.2	2.3	3.5	1.4
Sediment	–	–	–	–	–	–	–	–	–
BPA									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	0.16	0.30	0.89	1.60	1.50	0.98	1.05	2.27
Sediment	–	–	–	–	–	–	–	–	–
EE2									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	BDL	BDL	0.62	0.60	0.41	0.85	1.62	0.27
Sediment	–	–	–	–	–	–	–	–	–
E2β- Eq.									
FHM 1/FHM 2	–	–	–	–	–	–	–	–	–
Water	–	0.27	0.20	0.25	0.44	0.28	0.34	0.35	0.21
Sediment	–	–	–	–	–	–	–	–	–

Table SI 3. PAH ESBTU calculations for water at each site.

PAH	Site							
	Ronda	Rt. 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Naphthalene	0.6	0.4	1.1	0.4	0.3	1.0	1.6	0.9
Acenaphthylene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acenaphthene	1.0	1.2	0.5	0.1	0.7	0.7	0.3	0.1
C1 - Naphthalenes	0.7	0.8	1.0	0.4	0.6	1.0	1.7	0.7
C2 - Naphthalenes	3.1	3.5	2.6	1.4	2.2	2.9	1.9	1.6
C3 - Naphthalenes	8.3	8.2	6.0	4.2	5.2	7.8	3.5	3.9
C4 - Naphthalenes	7.9	8.7	12.1	6.4	6.5	14.8	7.3	7.0
Fluorene	1.1	1.3	0.4	0.3	0.9	0.7	0.3	0.2
C1 - Fluorenes	2.8	3.1	3.0	1.7	2.5	3.5	1.6	1.4
C2 - Fluorenes	5.8	8.4	7.7	4.8	5.1	16.6	6.6	5.0
C3 - Fluorenes	5.2	8.3	5.4	4.1	5.4	25.8	12.4	5.3
Dibenzothiophene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C1 - Dibenzothiophene	0.8	2.8	0.9	0.6	0.9	1.4	0.0	0.0
C2 - Dibenzothiophene	1.5	2.5	2.4	1.6	2.3	7.8	2.5	1.1
C3 - Dibenzothiophene	1.7	2.5	2.0	1.4	2.1	8.7	4.2	1.7
Phenanthrene	6.8	9.9	3.8	1.3	5.8	3.8	1.3	0.7
Anthracene	0.5	0.8	0.5	0.3	0.6	0.6	0.4	0.2
C1 - P/A	5.3	15.2	6.3	3.6	7.5	8.8	4.4	3.4
C2 - P/A	6.5	14.2	6.9	5.1	7.8	18.9	11.0	5.9
C3 - P/A	0.0	11.6	6.1	5.2	5.9	18.1	12.0	7.6
C4 - P/A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fluoranthene	15.3	30.2	16.0	10.9	21.1	20.0	13.0	6.6
Pyrene	11.6	19.8	9.7	8.0	15.2	15.2	10.7	5.7
C1 - F/P	2.8	5.9	3.8	3.0	4.9	8.8	6.4	3.3
C2 - F/P	2.0	3.3	2.5	2.2	3.1	4.4	4.1	2.4
C3 - F/P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Retene	7.9	41.9	8.2	6.5	8.4	23.0	26.4	28.2
Benz[a]anthracene	1.1	1.5	0.0	0.8	1.5	1.8	1.2	0.8
Chrysene	1.6	3.9	0.0	2.8	4.1	5.1	4.7	1.7

Table SI 3-continued.

PAH	Ronda	Route 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
C1 - Chrysenes	0.6	1.3	0.0	0.9	1.1	1.6	1.2	0.0
C2 - Chrysenes	0.0	0.7	0.0	0.0	0.6	0.8	0.7	0.0
C3 - Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4 - Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene	0.0	2.6	1.9	1.4	1.7	2.0	2.1	0.9
Benzo[k]fluoranthene	0.0	0.6	0.3	0.3	0.4	0.4	0.3	0.1
Benzo[e]pyrene	0.8	1.6	1.2	0.0	1.1	1.5	1.9	0.0
Benzo[a]pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Perylene	1.8	2.3	6.4	15.5	8.7	10.8	13.9	17.1
Indeno[1,2,3-cd]pyrene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dibenz[a,h,]anthracene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[g,h,i]perylene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coronene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sum of PAH 42	105.22	219.00	118.86	95.23	134.48	238.25	159.44	113.70
Sum of PAH 34	91.27	165.96	102.84	83.00	117.64	192.98	122.24	80.23
Sum of ESBTUs								
Acute	0.001	0.023	0.015	0.018	0.019	0.035	0.026	0.018
Chronic	0.017	0.046	0.030	0.036	0.038	0.071	0.054	0.037

Table SI 4. PAH ESBTU calculations for sediment at each site.

PAH	Site							
	Rond a	Route 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
Naphthalene	1.2	3.4	2.1	1.1	5.9	1.2	0.9	1.0
Acenaphthylene	0.0	5.4	5.7	2.6	5.7	1.1	0.0	0.8
Acenaphthene	0.0	12.3	0.0	0.0	0.0	0.0	0.0	0.0
C1 - Naphthalenes	0.0	4.6	4.2	0.0	5.2	1.4	0.0	1.3
C2 - Naphthalenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Naphthalenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C4 - Naphthalenes	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0
Fluorene	0.6	17.6	1.7	1.2	3.3	0.7	0.6	0.8
C1 - Fluorenes	0.0	10.7	9.7	0.0	12.1	0.0	0.0	0.0
C2 - Fluorenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C3 - Fluorenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dibenzothiophene	0.0	11.5	1.9	0.0	2.1	0.0	0.0	0.0
C1 - Dibenzothiophene	0.0	9.0	3.2	0.0	0.0	0.0	0.0	0.0
C2 - Dibenzothiophene	0.0	10.5	4.4	0.0	0.0	0.0	0.0	0.0
C3 - Dibenzothiophene	0.0	9.8	4.3	0.0	0.0	0.0	0.0	0.0
Phenanthrene	10.1	237.7	19.5	12.8	35.7	6.5	5.0	5.5
Anthracene	2.1	27.3	5.7	3.9	10.0	1.8	1.1	1.6
C1 - P/A	5.3	88.9	21.2	12.9	29.1	6.7	5.3	6.2
C2 - P/A	0.0	73.1	29.7	0.0	0.0	0.0	0.0	0.0
C3 - P/A	0.0	44.1	23.9	0.0	0.0	0.0	0.0	0.0
C4 - P/A	0.0	26.9	0.0	0.0	0.0	0.0	0.0	0.0
Fluoranthene	43.0	399.8	50.3	30.3	107.6	16.7	13.2	12.5
Pyrene	35.5	313.5	45.0	28.2	91.2	14.1	10.8	10.7
C1 - F/P	9.5	142.2	24.8	14.1	41.9	8.1	6.0	7.9
C2 - F/P	10.7	80.9	17.9	12.7	36.9	8.2	6.1	0.0
C3 - F/P	2.9	26.3	6.7	4.3	12.4	3.2	0.0	0.0
Retene	20.9	21.7	38.5	9.8	233.3	30.8	15.3	48.4
Benz[a]anthracene	14.1	132.7	21.9	15.2	37.1	6.2	4.2	6.5
Chrysene	25.8	204.0	32.7	18.5	67.5	10.3	7.5	9.1

Table SI 4-continued.

PAH	Ronda	Route 801	Red Hill	74 Bridge	Digg's Tract	Society Hill	Pee Dee	Bucksport
C1 - Chrysenes	7.7	59.8	15.5	11.5	27.5	5.0	3.5	0.0
C2 - Chrysenes	0.0	23.7	7.0	0.0	14.9	0.0	0.0	0.0
C3 - Chrysenes	0.0	12.3	3.7	0.0	8.8	0.0	0.0	0.0
C4 - Chrysenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo[b]fluoranthene	36.0	237.2	42.8	23.5	99.0	15.3	10.7	14.5
Benzo[k]fluoranthene	13.1	80.0	14.6	8.5	32.9	5.0	3.2	4.7
Benzo[e]pyrene	20.7	127.2	23.5	12.8	54.6	8.3	5.2	7.2
Benzo[a]pyrene	20.5	143.0	22.8	14.7	51.7	7.5	0.0	0.0
Perylene	15.2	65.4	35.7	115.7	186.6	59.2	98.9	187.0
Indeno[1,2,3-cd]pyrene	20.4	117.3	21.7	11.9	53.5	8.0	5.2	7.7
Dibenz[a,h,]anthracene	4.2	28.1	5.2	0.0	0.0	0.0	0.0	0.0
Benzo[g,h,i]perylene	21.0	120.0	20.6	13.6	57.8	9.5	6.3	0.0
Coronene	9.2	32.2	7.3	3.6	15.6	2.4	1.9	3.3
Sum of PAH 42	349.73	2960.20	595.33	383.48	1343.62	237.26	210.94	336.66
Sum of PAH 34	306.08	2758.21	511.15	353.11	1043.34	192.66	187.65	285.01
Sum of ESBTUs								
Acute	0.022	0.069	0.017	0.013	0.008	0.005	0.008	0.004
Chronic	0.088	0.281	0.068	0.052	0.032	0.020	0.032	0.015