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Chemical contaminants, health indicators, and reproductive biomarker responses in fish from rivers in the Southeastern United States

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ABSTRACT

Largemouth bass (*Micropterus salmoides*) and common carp (*Cyprinus carpio*) were collected from 13 sites located in the Mobile (MRB), Apalachicola–Flint–Chattahoochee (ARB), Savannah (SRB), and Pee Dee (PRB) River Basins to document spatial trends in accumulative chemical contaminants, health indicators, and reproductive biomarkers. Organochlorine residues, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-like activity (TCDD-EQ), and elemental contaminants were measured in composite samples of whole fish, grouped by species and gender, from each site. Mercury (Hg) and polychlorinated biphenyls (PCBs) were the primary contaminants of concern. Concentrations of Hg in bass samples from all basins exceeded toxicity thresholds for piscivorous mammals (>0.1 μg/g ww), juvenile and adult fish (>0.2 μg/g ww), and piscivorous birds (>0.3 μg/g ww). Total PCB concentrations in samples from the MRB, ARB, and PRB were >480 ng/g ww and may be a risk to piscivorous wildlife. Selenium concentrations also exceeded toxicity thresholds (>0.75 μg/g ww) in MRB and ARB fish. Concentrations of other formerly used (total chlordanes, dieldrin, endrin, aldrin, mirex, and hexachlorobenzene) and currently used (pentachlorobenzene, pentachloroanisole, dacthal, endosulfan, γ-hexachlorocyclohexane, and methoxychlor) organochlorine residues were generally low or did not exceed toxicity thresholds for fish and piscivorous wildlife. TCDD-EQs exceeded wildlife dietary guidelines (>5 pg/g ww) in MRB and PRB fish. Hepatic ethoxyresorufin *O*-deethylase (EROD) activity was generally greatest in MRB bass and carp. Altered fish health indicators and reproductive biomarker were noted in individual fish, but mean responses were similar among basins. The field necropsy and histopathological examination determined that MRB fish were generally in poorer health than those from the other basins, primarily due to parasitic infestations. Tumors were found in few fish ($n=5$; 0.01%); ovarian tumors of smooth muscle origin were found in two ARB carp from the same site. Intersex gonads were identified in 47 male bass (42%) representing 12 sites and may indicate exposure to potential endocrine disrupting compounds. Comparatively high vitellogenin concentrations (>0.35 mg/mL) in male fish from the MRB, SRB, and PRB indicate exposure to estrogenic or anti-androgenic chemicals.

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1. Introduction

The southeastern United States is known for it diverse fauna which includes many threatened and endangered aquatic species. The region also provides important habitat for endemic aquatic species, nesting and brood habitat for duck and alligator populations, and wintering areas for migratory birds and waterfowl. Species diversity in rivers and streams of the southeastern United States has declined due to dam construction, channel modifications, poor water quality, and introduction of nonindigenous species (Lydeard and Mayden, 1995). Aquatic species may also be at risk due to exposure to chemical contaminants.

The rich soils, abundant forests, and warm climate of the southeastern United States have led to the development of agriculture, forestry, mining, and manufacturing industries that are dependent on local water sources. These industries have been associated with declines in water quality in the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB). Industrial discharges from chemical manufacturing plants, military facilities, pulp and paper mills, and coal-fired power plants; urban and agricultural runoff; mine drainage; and municipal wastewater effluents have previously been associated with declines in water and habitat quality in one or more of these basins. As a result, many MRB, ARB, SRB, and PRB waters have been listed as impaired. The lower MRB has one of the largest concentrations of major industrial manufacturers along the Gulf of Mexico that have released a variety of organochlorine chemicals into the basin (USFWS, 1996), and pesticides used in agricultural and heavily-populated residential areas of MRB, ARB, SRB, and PRB enter water systems in runoff. Elevated concentrations of pesticides, polychlorinated biphenyls (PCBs), and mercury (Hg) have been reported in water, sediment, and biota in these basins (Adair et al., 2003; Atkins et al., 2004; Gilliom et al., 2006; Johnson et al., 2002; U.S. Environmental Protection Agency (USEPA), 1992; USFWS, 1996), and fish consumption advisories for Hg and PCBs have been issued for large rivers and reservoirs in the MRB, ARB, SRB, and PRB to protect human health. Livestock and poultry production is intensive in the ARB, SRB, and PRB with multiple concentrated animal feeding operations producing large amounts of animal waste and byproducts that can enter nearby streams and rivers (Burkholder et al., 2007); excess nutrients, pharmaceuticals, and synthetic and natural hormones are concerns associated with this industry. Previous contaminant studies in these basins have focused on measuring chemical contaminant concentrations in biota, but few investigations have assessed the health of aquatic biota in the MRB, ARB, SRB, and PRB.

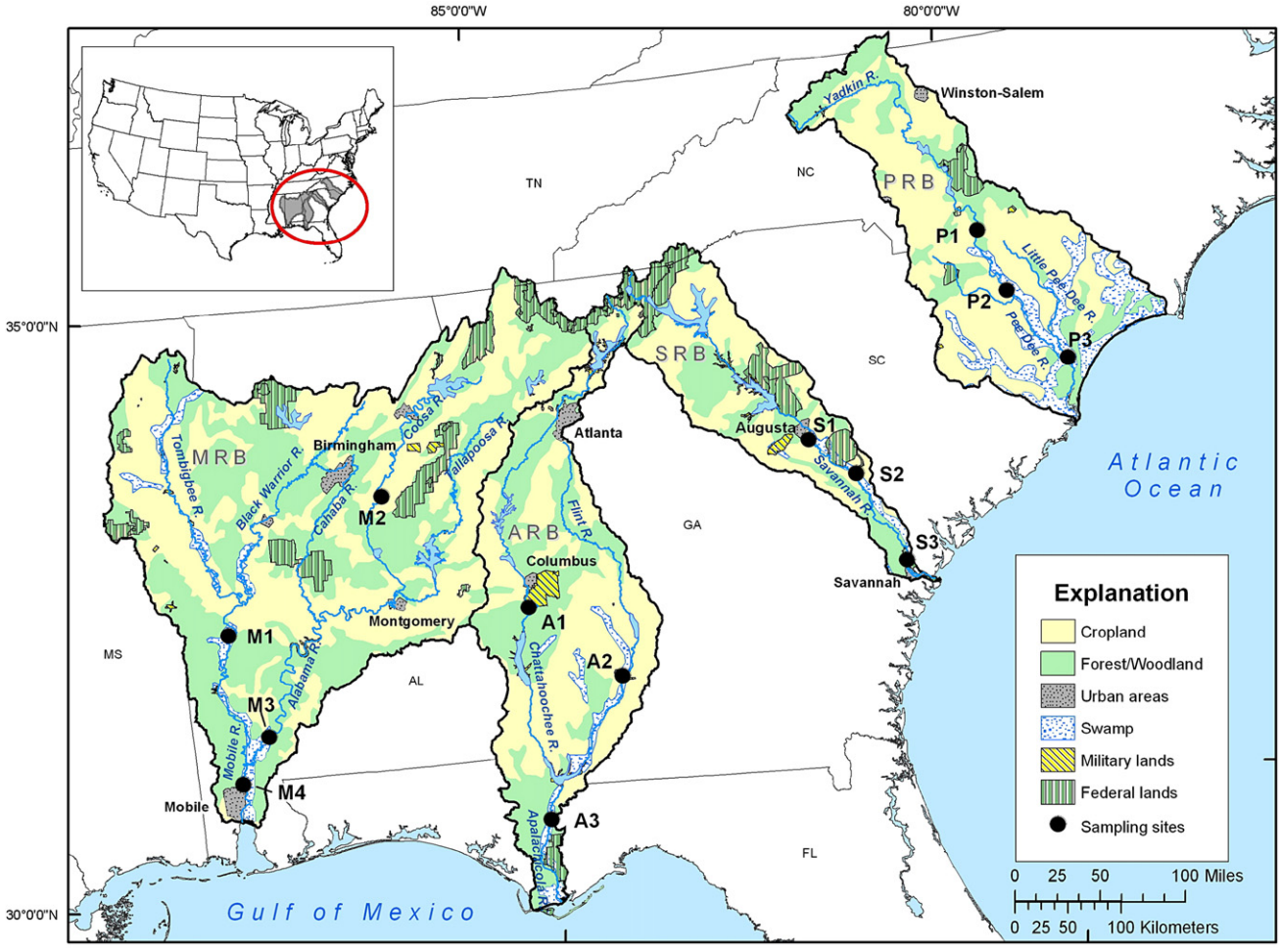

Fig. 1 – Map of the Mobile, Apalachicola–Chattahoochee–Flint, Savannah, and Pee Dee River Basins illustrating landuse, waterways, state boundaries, and locations sampled.

Previous studies in major river basins of the United States have indicated that fish populations exposed to anthropogenic stressors including chemical contaminants can be effectively evaluated using a combination of health indicators and reproductive biomarkers. Our primary objective was to document the occurrence and distribution of chemical contaminants, health indicators, and reproductive biomarkers in fish from river basins in the southeastern United States Secondary objectives were to compare results from our study to other U.S. river systems and to further refine benchmarks for quantification of long-term trends and interpretation of biomarker results. These latter objectives were achieved by building on the results of similar Large River Monitoring Network (LRMN) investigations in the Mississippi River Basin (Schmitt, 2002), Rio Grande Basin (Schmitt et al., 2005), Columbia River Basin (Hinck et al., 2006a), Colorado River Basin (Hinck et al., 2007a), and Yukon River Basin (Hinck et al., 2006b, 2007b). This paper summarizes the most pertinent findings of the study, which are reported in greater detail by Hinck et al. (2007c). Data from this and related investigations are available at <http://www.cerc.usgs.gov/data/best/search/index.htm>.

2. Materials and methods

An overview of the methods is presented here. More detail is provided by Hinck et al. (2007c).

2.1. Sampling and field procedures

Largemouth bass (*Micropterus salmoides*, henceforth bass; $n=237$) and common carp (*Cyprinus carpio*, henceforth carp; $n=209$) were collected between October and early December 2004 by electrofishing (Fig. 1; Table 1). These species were targeted because of their widespread distribution and the abundant contaminant, health indicator, and reproductive biomarker data available (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a). Fish were collected from 13 sites located in the MRB, ARB, SRB, and PRB that were chosen to represent a range of contaminant sources (e.g., chemical manufacturing, agricultural, and urban areas). Detailed site descriptions were provided by Hinck et al. (2007c). Adult fish of similar size were targeted at each site to reduce variation due to age; however, the age of fish differed (Table 2). Fish were held in aerated live-wells or in net pens until processed (usually less than 3 hours). All collection, handling, and euthanasia procedures followed animal care and use guidelines (American Fisheries Society et al., 2004).

A blood sample was obtained from the posterior caudal artery and vein using a heparinized needle and syringe and was chilled on wet ice. The fish was then weighed, measured, and killed with a blow to the head. Observations of external features were recorded, and grossly visible tissue anomalies were dissected and preserved in 10% neutral buffered formalin (NBF) for histopathological analysis. The liver (bass only; carp have a dispersed liver), spleen, and gonads were removed and weighed. The liver, gall bladder, posterior and anterior kidneys, gonads, and spleen were visually examined for abnormalities. Pieces of liver were collected and immediately flash-frozen in a dry ice-ethanol slurry for ethoxyresorufin O-deethylase (EROD) analysis. Samples (<5 g) of gill, gonad, kidney, spleen, and additional pieces of liver were collected and preserved for histopathological examination, gender confirmation (gonad), and macrophage aggregate analysis (spleen). Otoliths and scales were collected for age determination (Berg and Grimaldi, 1967; Casselman, 1990; Cowan et al., 1995). All remaining tissues (those not frozen or fixed) were wrapped in aluminum foil and frozen for analysis of organochlorine chemical residues, elemental contaminants, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-like activity (TCDD-EQ). Work surfaces and contact instruments were cleaned with ethanol and acetone (contact instruments only) between fish to prevent cross contamination. Blood samples were centrifuged, and the plasma was aspirated and frozen in a dry ice-ethanol slurry for vitellogenin (vtg) and steroid hormone analysis. Cryogenically frozen liver and plasma samples were shipped to the laboratory on dry ice and stored at −80 °C. After necropsy, whole fish were grouped by gender and site, frozen, and shipped to the analytical laboratory.

Table 1 – Location and collection dates (2004) in southeastern U.S. river basins

Site information	Collection dates	Latitude, longitude
Mobile River Basin		
M1 — Tombigbee R. at Lavaca, AL	10/12–10/13	32°15′53.60″N, 88°00′44.21″W
M2 — Coosa R. at Childersburg, AL	10/14–10/15	33°19′57.76″N, 86°21′55.87″W
M3 — Alabama R. at Eureka Landing, AL	10/6–10/7	31°23′14.06″N, 87°42′42.19″W
M4 — Mobile R. at Bucks, AL	10/8–10/9	31°03′15.85″N, 87°59′48.07″W
Apalachicola–Chattahoochee–Flint River Basin		
A1 — Chattahoochee R. at Omaha, GA	10/25–10/26	32°13′19.80″N, 84°55′35.10″W
A2 — Flint R. at Albany, GA	10/27–10/28	31°34′34.86″N, 84°08′49.80″W
A3 — Apalachicola R. at Blountstown, FL	11/2	30°25′58.20″N, 85°01′17.10″W
Savannah River Basin		
S1 — Savannah R. at Augusta, GA	11/30–12/1	33°22′00.18″N, 81°56′46.44″W
S2 — Savannah R. at Sylvania, GA	12/2–12/3	33°01′16.86″N, 81°31′04.50″W
S3 — Savannah R. at Port Wentworth, GA	12/6–12/7	32°13′26.34″N, 81°08′47.04″W
Pee Dee River Basin		
P1 — Pee Dee R. at Rockingham, NC	11/4–11/5	34°53′22.14″N, 79°51′24.89″W
P2 — Pee Dee R. at Pee Dee, SC	11/6–11/7	34°21′23.22″N, 79°41′35.19″W
P3 — Pee Dee R. at Bucksport, SC	11/8–11/9	33°42′18.09″N, 79°11′24.00″W

2.2. Laboratory analyses

Individual fish were partly thawed, cut into pieces, and ground to a fine texture. Fifteen percent of the total body weight was sub-sampled (18–1186 g) to maintain the proportional size representation of each fish in a composite sample. The ground

Table 2 – Mean (±standard error) total length, weight, condition factor (CF), and age of bass and carp from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Species, basin	Female					Male				
	n	Length (mm)	Weight (g)	CF	Age (years)	*n*	Length (mm)	Weight (g)	CF	Age (years)
Bass										
MRB	43 [a]	421±14	1040±128	1.30±0.05	4.7±0.2	36	389±11	801±78	1.27±0.05	4.9±0.5
ARB	30	398±5	968±66	1.34±0.03	3.6±0.3	30	348±13	571±55	1.30±0.04	2.8±0.2
SRB	18	351±18	668±136	1.29±0.03	2.7±0.7	21	301±13	359±62	1.26±0.08	1.7±0.3
PRB	34	327±46	624±293	1.34±0.07	2.6±0.6	25	303±32	443±194	1.32±0.08	2.1±0.6
Carp										
MRB	37 [b]	496±53	1841±631	1.30±0.01	12±5	41 [c]	515±42	1894±415	1.26±0.01	15±8
ARB	29 [d]	586±59	3691±1081	1.57 ±0.06	28±10	24 [e]	538±48	2553±814	1.48±0.06	30±11
SRB	27 [f]	448±10	1390±160	1.41±0.02	10±2	29 [g]	460±5	1516±88	1.42±0.02	9±2
PRB	10 [h]	653±43	4352±801 [i]	1.39±0.16	21±18	12 [h]	482±65	1498±195 [i]	1.34±0.33	8±1

[a] *n*=42 for age.
[b] *n*=32 for age.
[c] *n*=39 for age.
[d] *n*=25 for age.
[e] *n*=18 for age.
[f] *n*=24 for age.
[g] *n*=21 for age.
[h] *n*=8 for age.
[i] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

sub-samples were then grouped to create a single homogenous composite sample for each site, species, and gender combination. The composite sample was then sub-sampled (200 g) and re-frozen (−20 °C). All equipment was disassembled and chemically cleaned between composite samples to prevent cross contamination. All fish collected were included in one of the 51 composite samples, which had from 1 to 13 fish in each sample. Male carp were not collected from Site P1.

Dichloromethane extracts (0.1 g) of a 10-g sub-sample were analyzed gravimetrically for lipid content. Samples were analyzed by high-resolution capillary gas chromatography with electron capture detection (GC-ECD) for 29 organochlorine pesticide residues and total PCBs after size exclusion and adsorption column cleanup procedures (Hinck et al., 2006b, 2007a). Total PCBs were reported as the sum of 139 congeners. Toxaphene residues were quantified on the basis of 20 component peaks of a technical toxaphene standard. Quality assurance (QA) measures for the organochlorine pesticide and PCB analyses included the analysis of blanks, triplicate analyses, and matrix spikes. In addition, recovery standards were added to each sample to correct for analytical losses. Pesticides were identified by dual-column GC-ECD. Recoveries (mean±SD) were 98±22% to 101±16% for organochlorine pesticides and 89±13% to 104±13% for PCBs. The limit-of-detection (LOD) for each compound was calculated by adding the average procedural blank concentration to three times the procedural blank standard deviation (Keith, 1991). The nominal LODs were ≤2.4 ng/g wet-weight (ww) for individual compounds, 61 ng/g ww for total PCBs, and 10 ng/g ww for toxaphene.

Sub-samples for elemental analyses (100 g) were freeze-dried. Percent moisture was determined as weight lost during lyophilization. One portion of the dried material was digested in nitric acid and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) for cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), and zinc (Zn). A second portion was dry-ashed (magnesium nitrate–nitric acid–HCl) and analyzed by hydride generation atomic absorption spectroscopy for arsenic (As) and selenium (Se). A third portion was analyzed directly for total mercury (Hg) using thermal combustion, amalgamation, and atomic absorption spectroscopy. QA measures for elemental determinations included the analysis of reagent blanks, replicate samples, certified reference materials, and fortified samples. Nominal LODs were 0.03 μg/g dw for As; 0.05 μg/g dw for Hg; 0.06 μg/g dw for Se; and 0.4 μg/g dw for Cd, Cu, Cr, Ni, Pb, and Zn. Elemental concentrations (including LODs) were converted from dry-weight (dw) to ww for statistical analysis and reported using the moisture content of each sample.

A third sub-sample (10 g) was solvent-extracted and subjected to reactive cleanup for use in the H4IIE bioassay (Tillitt et al., 1991; Whyte et al., 2004). Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent doses (TCDD-EQ; pg/g ww) were determined by slope ratio assay as modified by Ankley et al. (1991). QA measures for the H4IIE bioassay included analysis of duplicate samples and reference materials. Limits-of-quantification (LOQs; 1.0–2.5 pg/g) and LODs (0.3–1.2 pg/g) were computed separately for each set of samples.

Hepatic EROD activity was determined on microsomal fractions, and protein content was quantified using the fluorescamine protein assay (Hinck et al., 2007c). EROD activity was reported as the mean of triplicate determinations. The LOD was calculated by adding the average basal EROD rate to three times the standard deviation of that rate for each set of samples analyzed (*n*=14). QA measures included LODs (0.2–1.9 pmol/min/mg), LOQ (0.6–1.9 pmol/min/mg), and the analysis of reference materials and duplicate samples. Hepatic EROD activity was not determined for one male bass from P3 and was <LOQ in 66 of 445 samples.

Body and organ weights were used to compute condition factor (CF) and organosomatic indices according to the following formulae: CF = body weight in g/(length in cm)^3; hepatosomatic index (HSI) = liver weight/(total body weight − gonad weight) × 100; splenosomatic index (SSI) = spleen weight/(total body weight − gonad weight) × 100; gonadosomatic index (GSI) = gonad weight/total body weight × 100. The weight of the gonads was subtracted from the body weight to minimize the effect of the reproductive cycle on these indices.

The occurrence of gross external and internal pathological disorders was determined during field processing. To maintain consistency with previous studies (e.g., Fournie et al., 2001; Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a), only grossly visible disorders of the eye, opercles, body surface, fins, and skeleton were included. A necropsy-based health assessment index (HAI) score was calculated for each fish by assigning numerical values to gross lesions (Adams et al., 1993; Schmitt, 2002), then summing the values for all organs observed. An HAI score, which can range from 0 to 220, was computed for a fish only if observations were made for all components. In general, fish with high HAI scores were considered to be in poorer health than those with low HAI scores.

Preserved gill, liver, gonad, spleen, anterior kidney, and posterior kidney tissues were prepared for histopathological analysis (Schmitt, 2002). Tissue samples were dehydrated, embedded in paraffin, sectioned at 6-μm, and stained with hematoxylin and eosin (H&E) for microscopic examination. In general, two to five sections of each tissue sample were examined for abnormalities in each fish. Transverse ovary sections were assigned to developmental stages 0 (immature), 1 (pre-vitellogenic), 2 (early vitellogenic), 3 (mid-vitellogenic), 4 (late vitellogenic), and 5 (spent) based on the predominant size and appearance of oocytes, and transverse testes sections were similarly classified into developmental stages 0 (immature), 1 (early spermatogenic), 2 (mid-spermatogenic), 3 (late spermatogenic), and 4 (spent; Blazer, 2002). Gonad tissue was also examined microscopically for abnormalities such as intersex and oocyte atresia (reabsorbed or degenerating eggs). Atresia was quantified by counting one hundred oocytes in each sample and reported as a percent. Fish were identified as intersex (i.e., when an ovotestis condition was detected) when individual or small foci of undeveloped oocytes were observed within testicular tissue or when spermatocytes were observed within ovarian tissue. Macrophage aggregates (MA) in spleen sections were stained using Perl's method (Luna, 1992) and quantified using computer-based image analysis. MA parameters included the number of aggregates in 2 mm^2 of tissue (MA-#) and the mean size (area) of aggregates within those 2 mm^2 (MA-A). The percentage of tissue occupied by aggregates (MA-%) was computed from these measurements.

Concentrations of vtg in bass and carp were determined by direct enzyme-linked immunosorbent assay and were reported as the mean of triplicate measurements (Denslow et al., 1999). Vitellogenin concentrations were not measured from 12 fish (1 carp; 11 bass) because the plasma sample had coagulated. QA measurements included the LOD (0.0005 mg/mL for carp; 0.001 mg/mL for bass), coefficient of variation (<10% for all samples), and inter-assay variability (<10%). Vitellogenin concentrations were <LOD in 135 of 434 (31%) plasma samples, of which 9 85(63%) were from male fish. Concentrations of 17β-estradiol (E2) and 11-ketotestosterone (KT) in plasma samples were measured by radioimmunoassay (Hinck et al., 2007c). Steroid concentrations were not obtained from one carp and 19 bass because the plasma sample had coagulated and analysis could not be performed. QA measurements included the LOD (12.7 pg/mL for E2; 15.7 pg/mL for KT), coefficient of variation (<10%), and inter-assay variability (<10%). E2 concentrations <LOD in two male bass were not included in the data analysis. Cross-reactivities of the E2 antiserum with estrone (1.32%), estriol (2.46%), 17α-estradiol (1.32%), and other steroids (<0.2%) and the KT antiserum with testosterone (9.65%), dihydrotestosterone (3.7%), androstenedione (<1.0%), and other steroids (<0.1%) were low.

2.3. *Data set composition and statistical analyses*

All results for analytes in whole-body composite samples were converted to, reported as, and analyzed statistically as ww concentrations. Arithmetic basin means and standard errors for contaminant concentrations and biomarker results were computed using a nested analysis-of-variance (ANOVA) model, with site as the class variable. Biomarker data were tested separately for male and female fish to minimize the effect of gender. A value of one-half the LOD (LOQ for EROD) was substituted for censored values in all statistical analyses and graphs. Spatial differences in contaminant concentrations and biomarker results were tested with a nested ANOVA using Fisher's restricted least significant difference (LSD; Saville, 1990). All concentration and biomarker data were log-transformed for statistical analysis. Histological descriptions of tissues were qualitative and not included in the statistical analyses. All computations and statistical analyses were performed with Version 9.1 of the Statistical Analysis System (SAS Institute, Cary, NC).

3. Results

3.1. *Lipid and moisture content (data not shown)*

Lipid content of whole-body composite samples was 2–8% for bass and 3–11% for carp. Percent moisture of whole-body composite samples was 67–75% for bass and carp.

3.2. *Exposure indicators*

3.2.1. *Elemental contaminants*

Arsenic concentrations were >LOD (0.01 μg/g) in 48 of 51 samples (94%) representing all sites and were similar in bass (<0.01–0.28 μg/g) and carp (0.03–0.18 μg/g). Cadmium concentrations were >LOD (0.01 μg/g) in 19 samples (37%) from 11 sites but were considered to be low in bass (≤0.01 μg/g) and carp (<0.01–0.19 μg/g). Chromium was detected in all samples, and concentrations were generally greater in bass (0.26–2.34 μg/g) than in carp (0.05–1.19 μg/g). Copper was detected in all samples, and concentrations were generally greater in carp (0.82–2.09 μg/g) than in bass (0.25–1.19 μg/g). Nickel concentrations were >LOD (0.11 μg/g) in 49 of 51 samples (96%) representing all sites, and concentrations were similar in bass (<0.11–1.64 μg/g) and carp (<0.11–1.60 μg/g). Lead concentrations were >LOD (0.01 μg/g) in 42 samples (82%) representing all sites and greater in carp (<0.01–0.58 μg/g) than in

Table 3 – Mean (±standard error) elemental contaminant (μg/g ww) concentrations [a] in fish from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Contaminant	Bass				$F_{3,13}$	Carp				$F_{3,12}$
	MRB	ARB	SRB	PRB		MRB	ARB	SRB	PRB	
Arsenic	0.09±0.03 a	0.08±0.03 a	0.10±0.08 a	0.06±0.03 a	0.35	0.10±0.02 a	0.13±0.02 a	0.06±0.02 a	0.10±0.03 a	2.00
Cadmium	0.01±0.00 a	0.01±0.00 a	0.01±0.00 a	0.01±0.00 a	2.89	0.01±0.01 a	0.10±0.05 a	0.02±0.00 a	0.05±0.04 a	1.80
Chromium	0.57±0.08 a	0.82±0.32 a	0.62±0.19 a	0.61±0.08 a	0.15	0.54±0.12 a	0.55±0.17 a	0.30±0.05 a	0.31±0.12 a	1.51
Copper	0.27±0.00 a	0.34±0.05 a	0.49±0.16 a	0.58±0.16 a	2.18	0.96±0.12 a	0.96±0.08 a	0.16±0.01 a	1.28±0.14 a	0.68
Mercury	0.53±0.08 a	0.37±0.11 a	0.46±0.10 a	0.46±0.14 a	0.52	0.10±0.02 a	0.12±0.03 a	0.34±0.03 a	0.19±0.03 a	1.69
Nickel	0.39±0.14 a	0.23±0.01 a	0.21±0.04 a	0.54±0.21 a	1.77	0.27±0.03 a	0.24±0.02 a	0.39±0.26 a	0.35±0.03 a	0.23
Lead	0.03±0.01 a	0.03±0.01 a	0.01±0.00 a	0.03±0.02 a	0.87	0.06±0.03 a	0.18±0.13 a	0.04±0.01 a	0.11±0.01 a	1.12
Selenium	0.46±0.01 b	0.45±0.03 b	0.48±0.01 b	0.34±0.03 a	10.0*	0.73±0.11 a	0.62±0.08 a	0.57±0.02 a	0.58±0.09 a	0.68
Zinc	11.7±0.2 a	11.5±0.2 a	11.6±0.9 a	12.4±0.3 a	0.92	54.3±1.7 a	70.8±6.7 b	54.0±0.7 a	60.2±2.4 ab	5.64*

[a]Samples sizes for contaminants in bass and carp were $n=8$ for the MRB, $n=6$ for the ARB, $n=6$ for the SRB, $n=6$ for the PRB. Also shown are results of a nested analysis-of-variance (ANOVA) as F-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P<0.05$). Censored values were replaced by one-half the LOD for the computations of basin means. Data were log-transformed for ANOVA.

bass (<0.01–0.08 μg/g). Zinc was detected in all samples, and concentrations were generally greater in carp (49.8–89.4 μg/g) than in bass (8.1–15.8 μg/g). Mean As, Cd, Cr, Cu, Ni, and Pb concentrations did not differ significantly among basins in bass or carp (Table 3). Mean Zn concentrations in carp were greater in the ARB than those in the MRB and SRB (Table 3). Overall, concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn were considered to be low and did not exceed literature-based toxicity thresholds to protect fish and piscivorous wildlife.

Total Hg was detected in all samples, and concentrations were greater in bass (0.22–0.78 μg/g) than in carp (0.05–0.31 μg/g; Fig. 2). Although mean Hg concentrations did not differ significantly among basins in bass or carp (Table 3), concentrations in individual samples exceeded literature-based toxicity thresholds for fish and piscivorous wildlife. Mercury concentrations in fish from all basins exceeded thresholds that pose a risk to juvenile and adult fish, piscivorous mammals, and piscivorous birds (Fig. 2).

Selenium was detected in all samples, and concentrations were greater in carp (0.43–1.29 μg/g) than in bass (0.28–0.52 μg/g; Fig. 2). Mean Se concentrations did not differ significantly among basins in carp, but concentrations in PRB bass were significantly lower than those from the other basins (Table 3). Selenium concentrations in at least one carp sample from the MRB and ARB exceeded protective thresholds for fish or piscivorous wildlife (Fig. 2).

3.2.2. *Organochlorine pesticides*

Mean concentrations of the seven chlordane-related compounds (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor, and heptachlor epoxide) were ≤14.1 ng/g and generally greatest in ARB fish (Table 4). Heptachlor was not detected (<0.1 ng/g) in any sample. Concentrations of total chlordanes (sum of seven compounds) ranged from 2 to 75 ng/g in individual samples, and *trans*-nonachlor, *trans*-chlordane, and *cis*-chlordane were the primary constituents. Mean total chlordane concentrations did not differ significantly among basins in bass or carp due to the variation among site concentrations in the ARB (Table 4), and concentrations did not exceed concentrations (300 ng/g) that may pose a threat to predatory fish and fish-eating birds (Eisler, 1990).

Concentrations of *p,p'*-DDT exceeded the LOD (>0.47 ng/g) in 37 of 51 samples (73%) from ten sites but were <12 ng/g. *p,p'*-DDD was detected in all samples, and concentrations were similar in

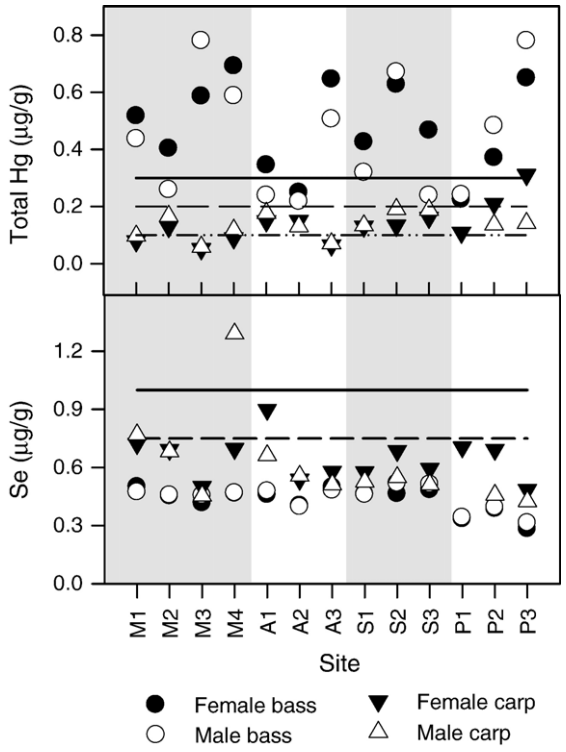

Fig. 2 – Concentrations of total mercury and selenium (all μg/g wet-weight) in whole-body composite samples of fish from the Mobile River Basin (Sites M1–M4), Apalachicola–Chattahoochee–Flint River Basin (Sites A1–A3), Savannah River Basin (Sites S1–S3), and Pee Dee River Basin (Sites P1–P3). Reference lines on mercury graph include protective thresholds for piscivorous mammals (0.1 μg/g ww; Yeardley et al., 1998), juvenile and adult fish (0.2 μg/g ww; Beckvar et al., 2005), and piscivorous birds (0.3 μg/g ww; Barr, 1986). Reference lines on the selenium graph include protective thresholds for piscivorous wildlife (0.75 μg/g ww) and larval fish (1.0 μg/g ww; Lemly, 1996, 2002).

Table 4 – Means (±standard error) organochlorine residues (ng/g ww) concentrations [a] in fish from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Contaminant	Bass				$F_{3,13}$	Carp				$F_{3,12}$
	MRB	ARB	SRB	PRB		MRB	ARB	SRB	PRB	
Dieldrin	1.46±0.19 a	9.13±3.15 b	2.81±0.43 a	1.76±0.54 a	7.90*	1.94±0.90 a	6.67±1.99 a	3.65±1.61 a	1.70±0.03 a [b]	1.89
HCB	0.66±0.30 a	0.81±0.40 a	1.03±0.62 a	0.37±0.10 a	0.42	2.48±1.55 a	2.38±1.56 a	2.73±2.06 a	0.43±0.04 a [b]	0.64
PCA	0.38±0.11 b	0.27±0.10 b	0.06±0.01 a	0.28±0.11 b	8.04*	1.99±0.52 b	1.69±0.54 ab	0.72±0.14 a	3.18±0.39 b	4.72*
cis-chlordane	2.70±1.56 a	7.48±3.32 a	1.01±0.19 a	3.42±1.68 a	1.25	3.79±3.10 a	11.1±4.19 a	1.92±0.39 a	3.24±0.78 a	2.34
trans-chlordane	0.66±0.19 a	2.03±0.79 a	0.54±0.14 a	0.74±0.41 a	1.58	2.19±1.39 a	5.62±2.39 a	1.42±0.39 a	1.12±0.26 a	1.20
cis-nonachlor	2.79±0.87 a	8.20±4.01 a	1.24±0.37 a	2.47±1.39 a	2.96	1.62±1.03 a	6.22±2.63 a	1.27±0.23 a	1.34±0.41 a	2.66
trans-nonachlor	4.98±1.21 a	14.1±5.29 a	2.68±0.84 a	4.13±2.42 a	3.05	4.04±2.99 a	9.97±4.28 a	2.67±0.66 a	2.22±0.63 a	1.17
Heptachlor epoxide	0.58±0.15 a	2.23±0.88 b	0.26±0.06 a	0.59±0.22 ab	4.44*	1.02±0.73 a	2.51±0.96 a	0.36±0.07 a	0.63±0.10 a	2.65
Oxychlordane	0.77±0.24 a	3.83±1.40 b	0.80±0.16 ab	0.93±0.49 a	4.17*	0.56±0.43 a	1.53±0.66 a	0.41±0.18 a	0.36±0.05 a	1.12
ΣChlor	12.6±4.0 a	38.1±15.2 a	6.6±1.6 a	12.3±6.6 a	2.65	13.3±9.7 a	36.8±14.8 a	8.07±1.83 a	8.94±2.19 a	1.67
o,p'-DDE	3.60±2.03 b	0.84±0.23 ab	0.41±0.00 a	0.59±0.10 a	3.98*	5.06±2.83 a	1.22±0.49 a	0.95±0.54 a	0.63±0.13 a	2.58
o,p'-DDD	0.67±0.40 a	0.85±0.13 a	0.66±0.22 a	0.36±0.08 a	0.85	1.16±0.57 a	1.37±0.46 a	0.98±0.43 a	0.41±0.12 a	0.72
o,p'-DDT	0.05±0.00 a	0.27±0.22 a	0.50±0.27 a	0.05±0.00 a	3.03	0.05±0.00 a	0.05±0.00 a	0.78±0.47 a	0.19±0.11 a	3.34
p,p'-DDE	46.8±14.2 a	73.0±46.5 a	15.4±6.1 a	34.3±22.6 a	1.23	35.1±14.1 a	79.7±57.7 a	12.4±1.7 a	17.4±6.9 a	0.79
p,p'-DDD	5.01±1.25 ab	8.88±2.67 b	2.74±0.71 a	2.87±0.54 a	4.29*	5.71±2.06 a	9.97±3.34 a	3.31±1.26 a	2.78±0.18 a	1.41
p,p'-DDT	2.03±0.31 bc	5.57±2.53 c	0.84±0.19 ab	0.67±0.21 a	6.53*	0.77±0.09 a	0.97±0.40 a	0.71±0.31 a	0.24±0.00 a	1.74
ΣDDT	53.7±15.4 a	87.4±51.1 a	19.0±6.9 a	37.7±23.4 a	1.66	41.6±15.8 a	90.4±60.2 a	16.5±2.9 a	20.5±7.1 a	0.93
Mirex	5.64±1.67 ab	10.7±3.21 b	6.59±5.22 b	0.37±0.01 a	5.23*	3.07±1.97 ab	12.3±5.49 b	5.58±2.71 b	0.32±0.10 a	4.54*
PCB	683±473 a	448±262 a	125±37 a	311±78 a	1.08	481±366 a	533±160 a	106±15 a	196±53 a	1.36
Toxaphene	21.9±3.7 a	45.0±10.0 a	15.0±5.0 a	29.2±0.8 a	2.88	23.1±4.7 ab	51.7±19.2 b	12.5±4.3 a	36.0±6.8 b	4.14*

Note. HCB, hexachlorobenzene; PCA, pentachloroanisole; ΣChlor, sum of *cis*- and *trans*-chlordanes and nonachlors; oxychlordane; heptachlor; and heptachlor epoxide; ΣDDT, sum of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE.

[a] Samples sizes for contaminants in bass and carp were $n=8$ for the MRB, $n=6$ for the ARB, $n=6$ for the SRB, $n=6$ for the PRB; the sample size for TCDD-EQ in carp was $n=5$ for the PRB. Also shown are results of a nested analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P<0.05$). Censored values were replaced by one-half the LOD for the computations of basin means. Data were log-transformed for ANOVA.

[b] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

bass (1–16 ng/g) and carp (1–19 ng/g). Mean *p,p'*-DDT and *p,p'*-DDD concentrations were greatest in ARB bass (Table 4). *p,p'*-DDE, the most persistent metabolite of *p,p'*-DDT, was detected in all samples, and concentrations were similar in bass (7–180 ng/g) and carp (5–310 ng/g; Fig. 3). Mean *p,p'*-DDE concentrations did not differ significantly among basins in bass or carp (Table 4), but concentrations in Site A1 samples exceeded protective thresholds for sensitive avian wildlife (Fig. 3). Concentrations of *o,p'*-DDE were generally low with the exception of samples from Site M4 (Table 4; Fig. 3).

Toxaphene was detected in 43 of 51 samples (84%) representing all sites, and concentrations were similar in bass (10–70 ng/g) and carp (10–100 ng/g; Fig. 3). Mean toxaphene concentrations did not differ significantly among basins in bass, but concentrations were significantly greater in ARB and PRB carp than SRB carp (Table 4). Mean toxaphene concentrations did not exceed toxicity thresholds for piscivorous wildlife, but concentrations in Site A2 samples were relatively high and may affect fish reproduction (Fig. 3).

Concentrations of other organochlorine residues and their metabolites including aldrin (<0.2 ng/g), dieldrin (<19 ng/g), endrin (<1.7 ng/g), mirex (<22 ng/g), hexachlorobenzene (<13 ng/g), pentachlorobenzene (<1.2 ng/g), pentachloroanisole (PCA;<4 ng/g), hexachlorocyclohexane isomers (α-, β-, γ-, δ-HCH;<0.54 ng/g), dacthal (<2.8 ng/g), endosulfans (endosulfan I, endosulfan II, and endosulfan sulfate;<1.2 ng/g), and methoxychlor (<LOD; 0.10 ng/g) were low in individual samples. Spatial trends in mean concentrations were not examined in many of these residues because of the high number of censored (<LOD) values (Table 4). Mean concentrations of these contaminants did not exceed toxicity thresholds for fish or piscivorous wildlife or protective thresholds were not available.

3.2.3. *Total PCBs and TCDD-EQ*

Total PCBs were detected in all samples, and concentrations were similar in bass (64–2700 ng/g) and carp (66–2200 ng/g; Fig. 4). Mean PCB concentrations did not differ significantly among basins in bass or carp (Table 4), but concentrations in individual samples exceeded thresholds that may pose a risk to piscivorous mammals (Fig. 4). Specifically, PCB concentrations in Site M2 samples were high (>950 ng/g).

TCDD-EQs were >LOD (1.0–2.5 pg/g) in 11 of 50 samples (22%) representing six sites (Fig. 4); a TCDD-EQ could not be calculated for Site P2 male carp. Spatial differences were not statistically tested because of the low detection frequency of TCDD-EQ concentrations in the composite samples. Concentrations ranged from <1.0 to 33.6 pg/g in individual samples, and concentrations in several MRB samples exceeded protective thresholds for fish and piscivorous wildlife (Fig. 4).

3.2.4. *Hepatic ethoxyresorufin O-deethylase (EROD) activity*

Hepatic EROD activity was similar in female (6.3–191.3 pmol/min/mg) and male (9.6–277.4 pmol/min/mg) bass. Mean EROD

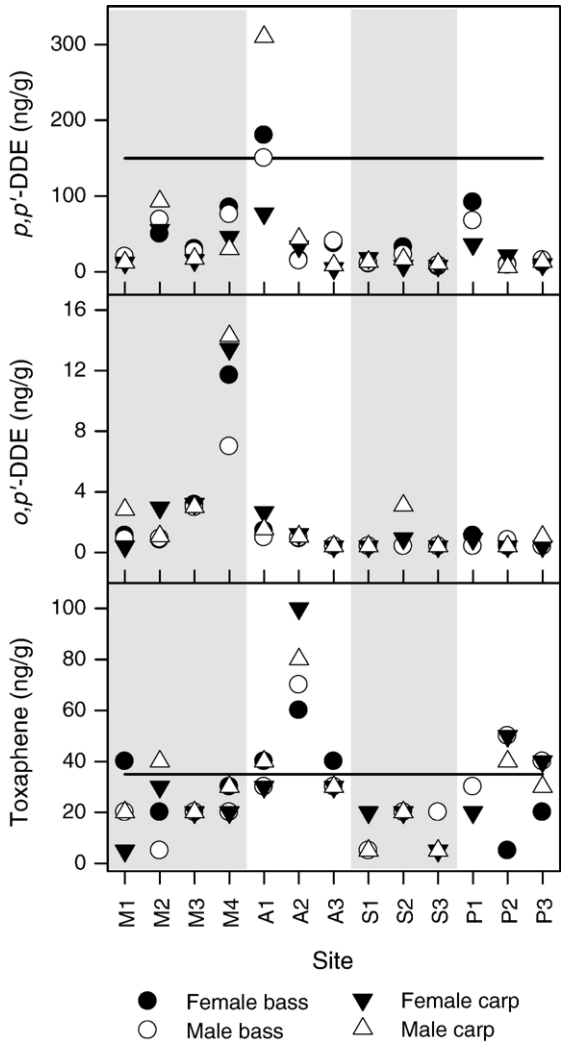

Fig. 3 – Concentrations of *p,p'*-DDE, *o,p'*-DDE, and toxaphene (all ng/g wet-weight) in whole-body composite samples of fish from the Mobile River Basin (Sites M1–M4), Apalachicola–Chattahoochee–Flint River Basin (Sites A1–A3), Savannah River Basin (Sites S1–S3), and Pee Dee River Basin (Sites P1–P3). Censored values are plotted as 50% of LOD. Literature-based toxicity thresholds were plotted if concentrations in one or more samples exceeded the threshold. Reference lines on the *p,p'*-DDE graph include toxicity thresholds for sensitive avian wildlife (150 ng/g; Anderson et al., 1975). The reference line on the toxaphene graph represents the lowest threshold where reproductive effects have been documented in fish (35 ng/g; Mayer et al., 1975).

activity did not differ significantly among basins but was generally greatest in MRB and PRB bass (Table 5). Hepatic EROD activity was lower in female (0.3–203.2 pmol/min/mg) and male (0.5–103.6 pmol/min/mg) carp when compared to female and male bass. Mean EROD activity did not differ significantly among basins in male carp, but EROD activity in female MRB carp were significantly greater than female ARB and SRB carp (Table 5).

3.3. *Fish health indicators*

3.3.1. *Health assessment index (HAI) and histology*

HAI scores differed significantly among basins for both bass and carp and were greater for bass than for carp (Table 6). Individual HAI scores ranged from 0 to 160 in female bass and 0 to 190 in male bass. Mean HAI scores were greater in MRB bass than those from the other basins, but the differences were only significant in female bass (Table 6). Liver discoloration; granular liver, kidney, and spleen; and body surface lesions were the primary contributors of the elevated HAI scores in both female and male MRB bass. Individual HAI scores ranged from 0 to 150 in female carp and 0 to 130 in male carp. Similar to bass, mean HAI scores were significantly greater in female and male MRB carp than ARB and SRB carp (Table 6). Frayed fins, gill abnormalities, and liver discoloration were the main contributors of elevated HAI scores in carp.

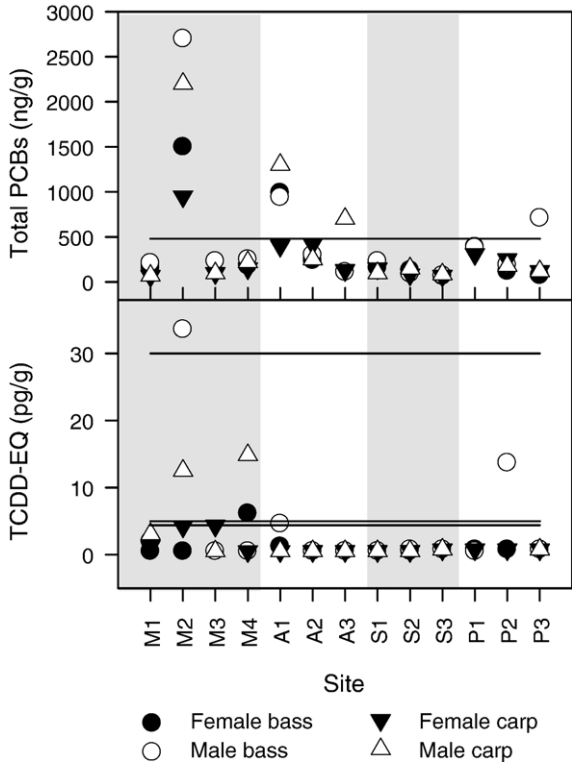

Fig. 4 – Concentrations of total PCB (ng/g wet-weight) and H4IIE bioassay-derived TCDD-EQ (pg/g wet-weight) in whole-body composite samples of fish from the Mobile River Basin (Sites M1–M4), Apalachicola–Chattahoochee–Flint River Basin (Sites A1–A3), Savannah River Basin (Sites S1–S3), and Pee Dee River Basin (Sites P1–P3). Literature-based toxicity thresholds were plotted if concentrations in one or more samples exceeded the threshold. The reference line on the PCB graph represents the protective threshold for mink (480 ng/g; Hornshaw et al., 1983). Reference lines on the TCDD-EQ graph include dietary thresholds for mammals (4.4 pg/g; Heaton et al., 1995; Tillitt et al., 1996), birds (5 pg/g; Nosek et al., 1992), and fish (30 pg/g; Walker et al., 1996; Whyte et al., 2004).

Table 5 – Mean (±standard error) hepatic ethoxyresorufin O-deethylase (EROD) activity [a] in bass and carp from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Species, basin	Female		Male	
	n	EROD (pmol/min/mg)	*n*	EROD (pmol/min/mg)
Bass				
MRB	43	52.9±7.2 a	36	62.7±11.8 a
ARB	30	36.6±9.4 a	30	39.3±9.2 a
SRB	18	46.4±3.1 a	21	61.3±14.7 a
PRB	34	63.4±16.2 a	24	76.2±23.1 a
ANOVA		$F_{3,9}=1.23$		$F_{3,9}=1.45$
Carp				
MRB	37	21.4±3.8 b	41	24.9±3.4 a
ARB	29	2.9±0.3 a	34	14.1±4.6 a
SRB	27	2.2±0.7 a	29	5.8±1.5 a
PRB	10	4.7±1.5 ab [b]	12	9.4±2.4 a [b]
ANOVA		$F_{3,9}=14.98^*$		$F_{3,8}=2.64$

[a] Also shown are results of a nested analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P<0.05$). Censored values were represented by 50% of the limit-of-quantification in all computations. Data were log-transformed for ANOVA.
[b] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

Histopathological examinations identified anomalies in gill, liver, gonad, kidney, and spleen tissues of individual fish. In bass, liver discoloration was due to differential storage of lipid/glycogen within hepatocytes and also many large MAs. Granular or nodular livers and granular kidney and spleens were due to a variety of parasites. Helminth parasites were present in all MRB bass tissues examined that were identified as granular or nodular during the field necropsy; severe infestations were found in some fish (Fig. 5A). Cysts containing myxozoan parasites were also occasionally present within the kidney (Fig. 5B). Small, focal granulomas were also present in liver, kidney, and spleen tissues but were more extensive in Site M1 bass. In one Site M1 bass, the granulomas were large and replaced much of the normal tissue (Fig. 5C). Cysts of a microsporidia were observed in head kidney tissue of Sites M1 and M3 fish and were most common and severe in Site M3 bass (Fig. 5D). Frayed gills and parasites including large metacercarial stages of digenetic trematodes, monogenetic trematodes, and myxozoan cysts were the most common gill lesions, and most of these were observed on MRB and PRB bass. Frayed fins and parasites were also common fin lesions in bass, and black spots from the accumulation of melanin around the metacercariae of digenetic trematodes were identified on fins of MRB and PRB bass. In carp, gill lesions including abnormal cartilage; proliferation of lamellar epithelial cells, leading to fusion of lamellae; congestion or telangiectasis; and myxozoan parasites were most common in MRB and PRB fish. Abnormalities specific to Site M2 carp included fluid-filled abdominal cavities, calcified follicles or fibrosis of the gonads, and thick asterisci otoliths. Granular spleen and kidney tissue in MRB carp were from small granulomas whose etiology was congestion, fibrosis, or unidentified.

Histopathological examination of external lesions revealed that most nodules on the body surface and fins were areas of thickened epidermis or dermal inflammation, which often included congestion or hemorrhage, consistent with secondary bacterial infections or trematode metacercariae. Several

Table 6 – Mean (±standard error) health assessment index (HAI) score, splenosomatic index (SSI), and hepatosomatic index (HSI) [a] in bass and carp from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Species, basin	Female				Male			
	n	HAI	HSI (%) [b]	SSI (%)	*n*	HAI	HSI (%)	SSI (%)
Bass								
MRB	43	110.6±1.8 b	0.67±0.04 a	0.11±0.01 a	36	99.3±12.4 a	0.70±0.07 a	0.09±0.01 a
ARB	30	44.8±1.8 a	0.60±0.03 a	0.10±0.00 a	30	41.1±4.2 a	0.56±0.02 a	0.09±0.00 a
SRB	18	50.7±8.7 a	0.70±0.08 a	0.12±0.07 a	21	38.2±8.5 a	0.69±0.06 a	0.08±0.02 a
PRB	34	59.2±11.2 a	0.68±0.01 a	0.14±0.01 a	25	50.5±5.6 a [d]	0.79±0.10 a	0.11±0.01 a [d]
ANOVA		$F_{3,9}=10.63^*$	$F_{3,9}=1.15$	$F_{3,9}=1.74$		$F_{3,9}=1.62$	$F_{3,9}=1.70$	$F_{3,9}=0.67$
Carp								
MRB	37	39.8±3.8 b	NA	0.32±0.09 a	41	45.2±6.2 c	NA	0.37±0.04 a
ARB	29 [c]	4.6±3.0 a	NA	0.25±0.02 a	24	5.9±5.9 a	NA	0.30±0.04 a
SRB	27	11.2±3.9 a	NA	0.24±0.03 a	29	15.3±2.9 ab	NA	0.28±0.01 a
PRB	10	34.1±18.8 ab	NA	0.36±0.03 a [d]	12	48.4±18.9 bc	NA	0.45±0.21 a
ANOVA		$F_{3,9}=9.84^*$	NA	$F_{3,9}=1.25$		$F_{3,8}=12.60^*$	NA	$F_{3,8}=2.07$

NA, not applicable.
[a] Also shown are results of a nested analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P<0.05$). Data were log-transformed for ANOVA.
[b] Not measured for carp hepatopancreas.
[c] $n=28$ for SSI.
[d] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

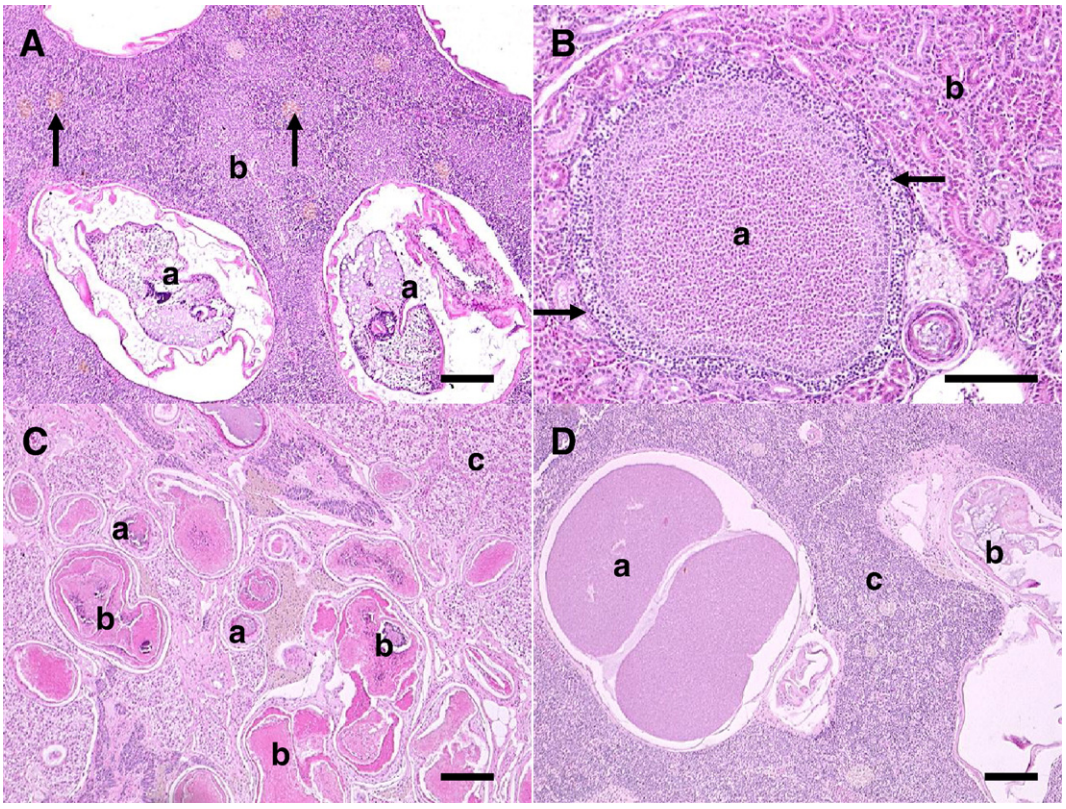

Fig. 5 – A. Helminth parasites (a) encysted in the anterior kidney (b) of a bass. Arrows illustrate macrophage aggregates. Scale bar=150 μm. B. A cyst (a) containing spores of a myxozoan parasite within the posterior kidney (b) of a bass. The encysted parasites are surrounded by a cuff of inflammatory cells (arrows). Scale bar=100 μm. C. Granulomas (a and b), a chronic inflammatory reaction, replacing much of the normal kidney (c) in a Site M1 bass. Small, focal granulomas (a) were most common; however, larger, coalescing granulomas (b) were observed in some instances. Scale bar=150 μm. D. A microsporidian cyst (a) and encysted helminth parasite (b) within the anterior kidney (c) of a Site M3 bass. Scale bar=150 μm. H&E stain.

tumors were noted during gross examinations. Masses in the ovaries of two relatively old carp (ages 43 years and 56 years) from Site A1 (Fig. 6 A and B) were both diagnosed as leiomyosarcoma (Fig. 6 C and D). A lip papilloma was identified on a Site M3 bass, and a lipoma within the spleen was found in a Site P3 bass (Fig. 7 A). A Seritoli cell tumor was also found in the gonad of a 7 year old male Site S1 carp (Fig. 7 B–D).

3.3.2. *Organosomatic indices*

The HSI and SSI computed from body and organ weights were used as general indicators of overall fish health. Individual HSI values were similar in female (0.37–1.58%) and male (0.38–1.65%) bass. Few HSI values >1.0% were calculated for individual bass, but relatively low HSI values (≤0.5%) were common in bass from all sites. Mean HSI scores did not differ significantly among basins in female or male bass but were generally considered to be low (Table 6).

SSI values were greater for carp than for bass but did not vary significantly among basins for either species (Table 6). Individual SSI values were similar in female (<0.01–0.69%) and male (0.03–0.36%) bass. Individual SSI values were >0.30% in female bass from the MRB, SRB, and PRB, but histologically these tissues appeared normal. Conversely, the high SSI value (0.36%) in a Site P3 male bass was due to a large lipoma in the spleen. Individual SSI values were similar in female (0.13–3.78%) and male (0.11–1.84%) carp. Relatively high SSI values (>1.0%) calculated for carp from Sites M1, M4, and P3 may have been associated with severe parasitic infestations.

3.3.3. *Macrophage aggregates*

Macrophage aggregates parameters (MA-#, MA-A, and MA-%) were generally greater in bass than carp but only differed among basins in female fish (Table 7). Individual MA-# values were similar in female (0.6–11.2 MA/mm^2) and male (0.6–12.9 MA/mm^2) bass, and MA density (≥8 MA/mm^2) was generally greater in older bass (>5 y). MA size (MA-A) was also similar in individual female (811–15128 μm^2) and male (700–10078 μm^2) bass, and MA-% ranged from 0.05 to 11.07% in female bass and 0.04 to 9.15% in male bass. MA-A values >10,000 μm^2 in individual bass from Sites M2, M3, M4, and A1 and MA-% values >6% in bass from Sites M1, M2, M3, M4, A1, and S2 were considered anomalous. Mean MA-A and MA-% values among sites were generally greatest in female bass from Sites M4 and S2 (Fig. 8). MRB and ARB bass had greater numbers of MAs than SRB and PRB bass, but these differences among basins were not significant (Table 7). In addition, MRB bass had larger MAs that occupied more splenic tissue than SRB and PRB bass, but these differences were only significant in females (Table 7).

Mean MA parameters in carp were similar to those in bass. Individual MA-# values were 0.0–11.8 MA/mm^2 in female carp

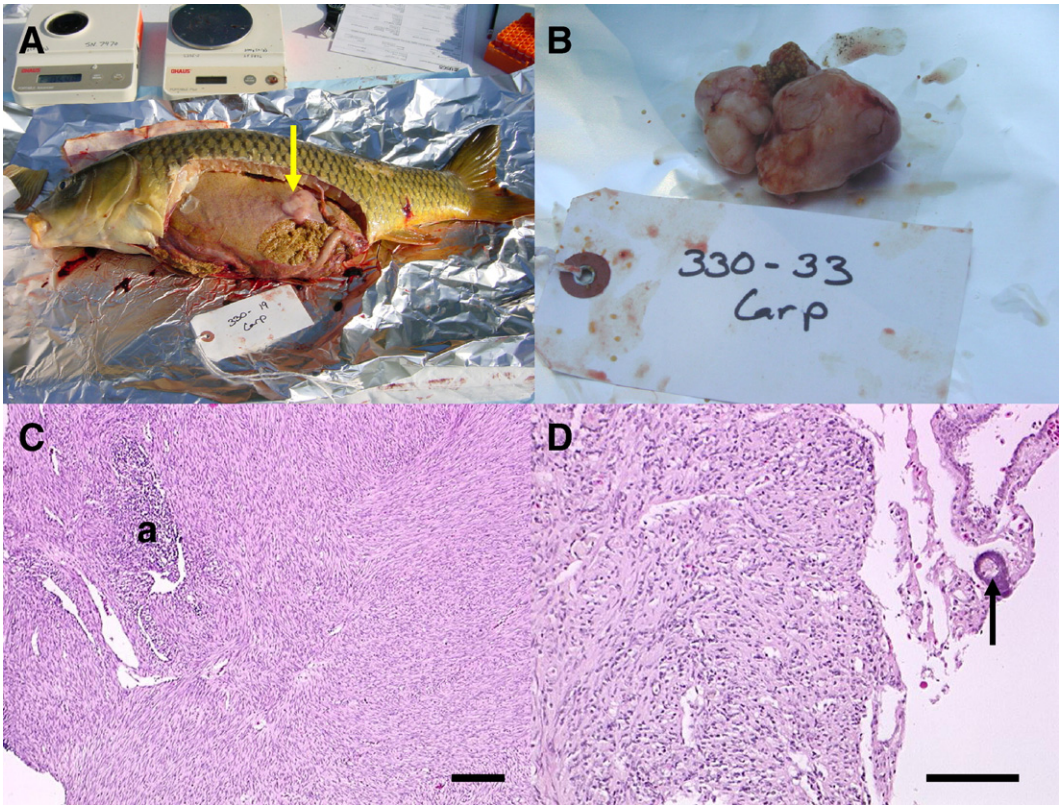

Fig. 6 – A. Raised, nodular mass, identified as a leiomyosarcoma, in the ovary of a common carp from Site A1. B. A second leiomyosarcoma, illustrating the circumscribed, nodular appearance of the mass, removed from the ovary of another female common carp from Site A1. C. Histologically, the tumor was composed of elongate smooth muscle cells in interlacing cords. Occasionally, areas of inflammation (a) were noted. Scale bar=150 μm. D. Higher magnification of the leiomyosarcoma with invasion into ovarian follicles. Immature oocytes (arrow) are present. Scale bar=100 μm. H&E stain.

and 0.6–16.5 MA/mm^2 in male carp. Relatively high MA-# values (>10 MA/mm^2) were measured in carp from Sites M2, M4, A1, and S1. MA size (MA-A) was greater in individual female (0–36299 μm^2) than in male (741–14317 μm^2) carp, and MA-% ranged from 0.00 to 11.15% in female carp and 0.04 to 11.02% in male carp. MA-A values >10,000 μm^2 in individual carp from Sites M2, M4, A1, P1, and P2 were considered anomalous. Mean MA-#, MA-A, and MA-% values among sites were generally greatest in carp from Sites M2, S1, and S2 (Fig. 8). The site mean for MA-A and MA-% in female bass from Site P1 was the greatest, but sample size (n=1) was small. Overall, MRB and ARB carp had larger and more numerous MAs that occupied larger proportions of splenic tissue than SRB and PRB (males only) carp; however, few of these differences among basins were statistically significant (Table 7).

3.4. *Reproductive biomarkers*

3.4.1. *Gonadal histopathology*

Gonadal stages 0 (10%), 1 (85%), and 2 (5%) were present in female bass (n=125). Several female bass from Sites M3 (n=3) and M4 (n=6) were stage-0 (immature) and ranged in age from two to six years old. Gonadal stages 0 (3%), 1 (20.5%), 2 (20.5%), and 3 (56%) were present in female carp (n=103). Gonadal stage was most variable (stages 0, 1, 2, and 3 present) in female carp from Sites M1 and M3. Female carp were generally more advanced in the ARB and PRB (most stage 3) than those from the MRB and SRB (many stage 1 and 2). Oocyte atresia was typically <10% in bass and <20% in carp. Oocyte atresia was relatively high (>15%) in individual bass from Sites A1, A2, A3, P1, and P2 but generally low in MRB bass. Oocyte atresia was >20% in individual carp from Sites M1, M2, A2, A3, S3, and P2. A sporozoan parasite within oocytes of female carp from Sites M1 and P2 caused inflammation and degeneration.

Gonadal stages 0 (2%), 1 (88%), and 2 (10%) were present in male bass (n=112). Stage-0 males were from Sites M3 and M4, where stage-0 female bass were also identified. Gonadal stages 0 (1%), 1 (2%), 2 (59%), and 3 (30%) were present in male carp (n=106). Stage-0 and-1 male carp were from Sites A3 and S1. Intersex gonads were identified in 47 male bass (42%) representing all sites except M4 (Fig. 9). A high proportion (≥50%) of male bass from Sites A3, S2, S3, P1, P2, and P3 were intersex. Whole gonads, rather than the three to five representative pieces of gonadal tissue typically collected, were collected and examined in PRB bass. Therefore, the greater occurrence of intersex in male PRB bass may be the result of examining a greater proportion of the gonadal tissue during the histological analysis. Most intersex bass were observed having few oocytes in testicular tissue, but moderate numbers of oocytes were identified in male bass gonads from Sites P2 and P3. The intersex condition was not found in male carp.

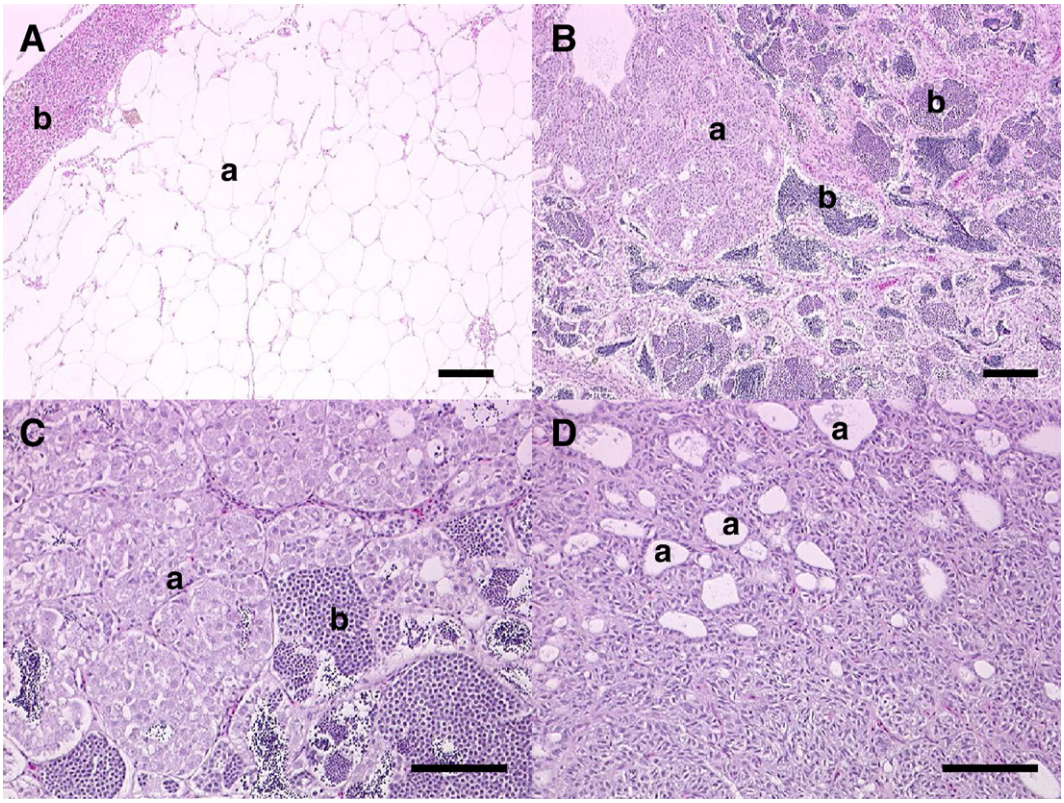

Fig. 7 – A. A section of a large lipoma (a) within the spleen (b) of a Site P3 bass. Scale bar = 150 μm. B–D. Sertoli cell tumor from a male common carp from Site S1. B. Foci of neoplastic cells (a) within normal testicular tissue (b). Scale bar = 150 μm. C. Higher magnification of the neoplastic cells (a), which were fairly uniform, large cells, adjacent to normal spermatocytes (b). Scale bar = 100 μm. D. The neoplastic cells formed tubule-like structures (a) in some areas. Scale bar = 100 μm. H&E stain.

3.4.2. *Gonadosomatic index (GSI)*

GSI values differed significantly among basins for male bass but not for female bass or carp (Table 8). Individual GSI values were 0.31–1.84% in female bass and 0.04–1.59% in male bass, and site means were greatest in female bass from Sites S3 and P1 (Fig. 10). Relatively high GSI values were calculated for stage-1 and -2

Table 7 – Mean (±standard error) macrophage aggregate (MA) density (MA-#), MA area (MA-A), and percent tissue occupied by MA (MA-%)[a] in bass and carp from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Species, basin	Female				Male			
	n	MA-# (MA/mm^2)	MA-A (μm^2)	MA-% (%)	*n*	MA-# (MA/mm^2)	MA-A (μm^2)	MA-% (%)
Bass								
MRB	43	4.6±0.7 a	4859±318 b	2.41±0.38 c	35	5.3±0.7 a	5012±448 a	2.97±0.54 a
ARB	30	4.6±0.1 a	3833±1024 ab	1.81±0.44 bc	30	5.2±0.6 a	3086±514 a	1.74±0.42 a
SRB	18	4.2±0.3 a	2598±280 a [b]	1.24±0.28 ab [b]	21	2.8±1.8 a	3045±785 a	1.11±1.08 a
PRB	33	2.7±0.6 a	2844±386 a	0.86±0.29 a	25	2.7±0.4 a	2429±459 a	0.81±0.28 a
ANOVA		$F_{3,9}$=3.78	$F_{3,9}$=5.62*	$F_{3,9}$=7.43*		$F_{3,9}$=1.80	$F_{3,9}$=3.14	$F_{3,9}$=2.30
Carp								
MRB	36	1.8±0.5 a	5189±1867 b	1.18±0.71 a	41	3.2±1.0 a	4283±1156 a	1.75±0.90 a
ARB	27	3.4±1.4 a	4572±1182 b	2.12±1.23 a	24	3.9±1.7 a	4987±1462 a	2.67±1.33 a
SRB	26	1.7±0.5 a	2276±438 a	0.60±0.36 a	29	1.8±0.3 a	2857±308 a	0.57±0.15 a
PRB	10	2.0±0.7 a [b]	5320±6503 ab	1.88±1.65 a	12	0.9±0.1 a	3288±647 a [b]	0.31±0.10 a [b]
ANOVA		$F_{3,9}$=2.86	$F_{3,9}$=5.10*	$F_{3,9}$=2.00		$F_{3,8}$=1.37	$F_{3,8}$=0.88	$F_{3,8}$=1.16

[a] Also shown are results of a nested analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P < 0.05$). Data were log-transformed for ANOVA.

[b] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

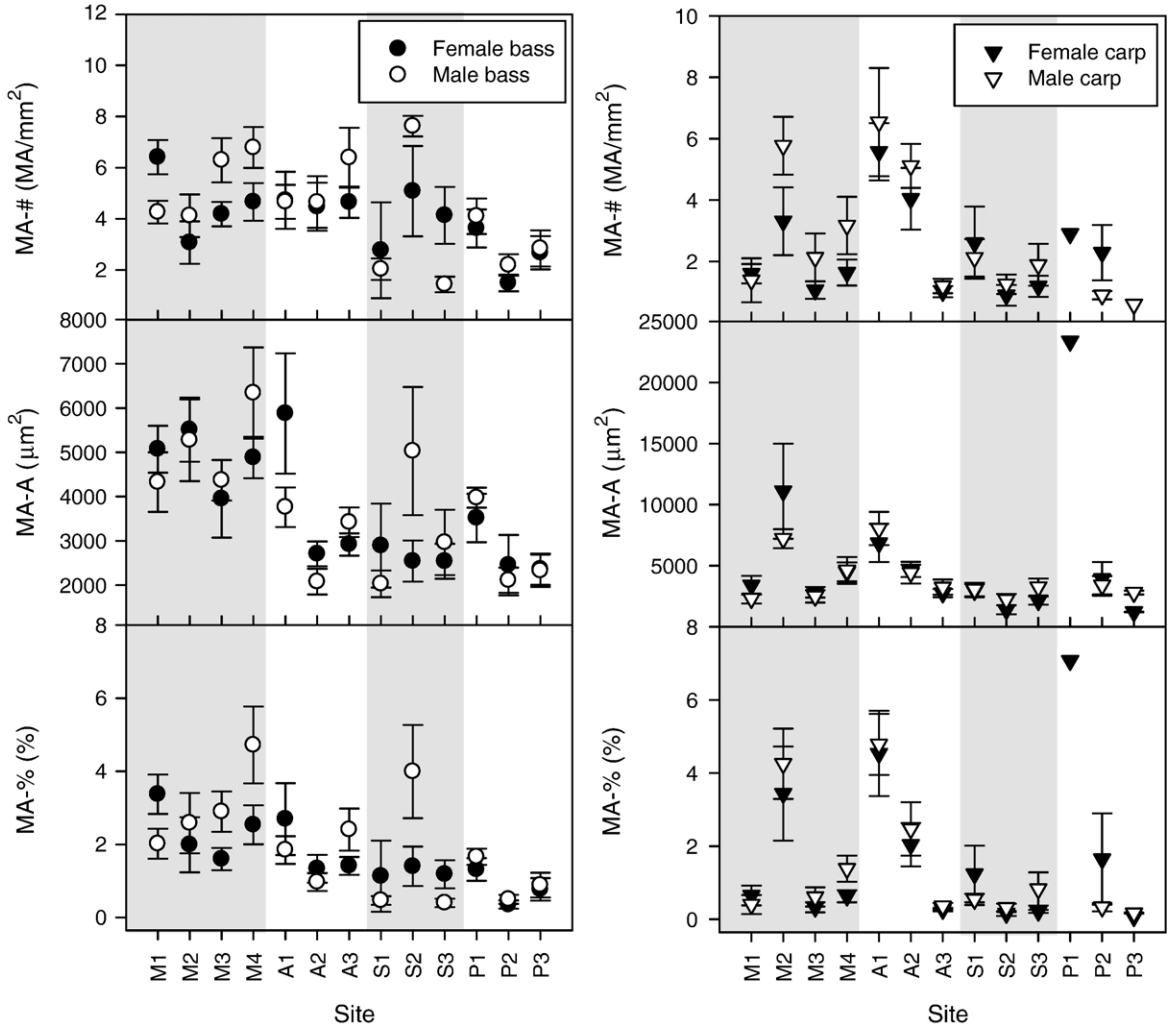

Fig. 8 – Mean (±standard error) splenic macrophage aggregate parameters in bass and carp. Parameters include macrophage density (MA-#), macrophage aggregate area (MA-A), and percent splenic tissue occupied by macrophage aggregates (MA-%). (Note: different scales are used for bass and carp plots).

female bass (>1.2%) from Sites S3 and P1 and stage-1 male bass (>0.6%) from Sites M4 and P3. Overall, mean GSI values among basins were greatest in female SRB and PRB bass (Table 8).

Individual GSI values were 0.44–23.52% in female carp and 0.07–27.40% in male carp. GSI values were greatest (site mean >12%) in female carp from Sites M2, A1, A2, P2, and P3 that were primarily stage 2 and 3 (Fig. 10). High GSI values (>11%) were calculated for individual male carp from Sites M2, A2, and P3. Mean GSI values were greater in female and male ARB and PRB carp than MRB and SRB carp, but basin differences were not significant (Table 8).

3.4.3. *Vitellogenin (vtg)*

Vtg concentrations did not differ significantly among basins for either female or male bass (Table 8); vtg concentrations differed among sites within each basin (Fig. 10). Vtg concentrations were >LOD (0.001 mg/mL) in 70 of 120 female bass (58%) and 44 of 105 male bass (42%). Concentrations ranged from 0.002 to 5.80 mg/mL in female bass and 0.001 to 2.84 mg/mL in male bass. Vtg concentrations were detected in male bass from all sites except A1. Concentrations were >1.0 mg/mL in individual females from Sites S3 and P1, where GSI values were also relatively high. Vtg concentrations were ≥0.01 mg/mL in few (16%) male bass. Relatively high vtg concentrations (0.35–2.5 mg/mL) measured

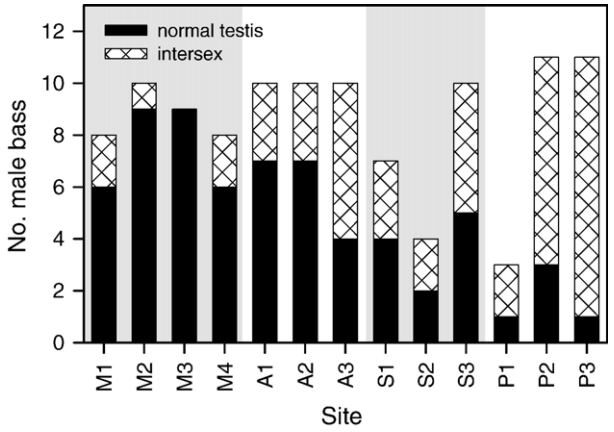

Fig. 9 – Intersex occurrence in male bass. The intersex condition was not found in male carp.

Table 8 – Mean (±standard error) gonadosomatic index (GSI), vitellogenin (Vtg), 17β-estradiol (E2), and 11-ketotestosterone (KT)[a] in bass and carp from the Mobile River Basin (MRB), Apalachicola–Chattahoochee–Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PRB)

Species, basin	Female					Male				
	n	GSI (%)	Vtg (mg/mL)	E2 (pg/mL)	KT (pg/mL)	*n*	GSI (%)	Vtg (mg/mL)	E2 (pg/mL)	KT (pg/mL)
Bass										
MRB	43 [b]	0.64±0.04 a	0.05±0.04 a	561±31 a	308±34 a	36 [c]	0.19±0.04 a	0.013±0.011 a	369±82 a	620±90 a
ARB	30 [d]	0.65±0.07 a	0.17±0.11 a	546±48 a	190±22 a	30 [e]	0.19±0.01 ab	0.012±0.005 a	259±11 a	748±9 a
SRB	18 [f]	1.01±0.29 a	0.52±0.25 a	621±64 a	336±51 a	21 [g]	0.23±0.03 bc	0.030±0.033 a	405±54 a	765±65 a
PRB	34 [h]	0.81±0.18 a	0.86±0.72 a	633±193 a	352±49 a	25 [i]	0.26±0.02 c	0.148±0.097 a	260±37 a	708±116 a
ANOVA		$F_{3,9}$=0.22	$F_{3,9}$=0.94	$F_{3,9}$=0.11	$F_{3,9}$=3.24		$F_{3,9}$=6.60*	$F_{3,9}$=2.02	$F_{3,9}$=0.79	$F_{3,9}$=0.85
Carp										
MRB	37 [j]	7.8±1.9 a	2.17±0.43 a	877±145 a	331±93 a	41	6.8±1.1 a	0.026±0.021 a	338±73 a	1186±145 a
ARB	29 [k]	11.9±2.8 a	2.38±0.36 a	920±116 a	357±66 a	24	8.0±1.6 a	0.005±0.001 a	519±123 a	1382±162 a
SRB	27	7.0±0.7 a	1.93±0.26 a	1001±220 a	401±71 a	29	5.8±0.6 a	0.021±0.11 a	413±71 a	1045±114 a
PRB	10	12.9±2.7 a	2.75±0.45 a [l]	911±149 a [l]	409±58 a [l]	12	7.2±7.3 a	0.005±0.015 a	189±29 a [l]	694±80 a [l]
ANOVA		$F_{3,9}$=1.01	$F_{3,9}$=1.01	$F_{3,9}$=0.14	$F_{3,9}$=0.37		$F_{3,8}$=0.51	$F_{3,8}$=1.21	$F_{3,8}$=0.91	$F_{3,8}$=2.10

[a] Also shown are results of a nested analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among basins (*$P \leq 0.05$). Within each species-basin group, means followed by the same letter are not significantly different ($P<0.05$). Data were log-transformed for ANOVA.
[b] $n=42$ for vtg, $n=41$ for E2 and KT.
[c] $n=33$ for vtg and KT, $n=32$ for E2.
[d] $n=29$ for vtg.
[e] $n=29$ for vtg, $n=27$ for E2, $n=28$ for KT.
[f] $n=16$ for vtg.
[g] $n=19$ for vtg, $n=20$ for E2 and KT.
[h] $n=33$ for vtg, $n=29$ for E2 and KT.
[i] $n=21$ for E2 and KT.
[j] $n=36$ for vtg, E2, and KT.
[k] $n=28$ for GSI.
[l] The overall standard error was negative (based on the variance component); therefore, an approximation of the standard error was computed as the square root of the total variance divided by the total sample size (*n*).

in male bass ($n=5$) from Sites M2, S2, P1, and P2 may indicate an estrogenic response in these fish. Three of the five males with high vtg concentrations were intersex; however, the intersex condition was also found in males with vtg concentrations <LOD. Mean vtg concentrations were greatest in female and male PRB bass (Table 8). The mean vtg concentration in male PRB bass was within the concentration range of early vitellogenic females and was considered anomalous.

Vtg concentrations did not differ significantly among basins for either female or male carp (Table 8). Vtg concentrations were >LOD (0.0005 mg/mL) in all female carp and 84 of 106 male carp (79%); concentrations ranged from 0.003 to 6.14 mg/mL in female carp and 0.002 to 0.90 mg/mL in male carp. Concentrations were >4.0 mg/mL in individual females from Sites M2, M3, A2, A3, and P2. Vtg concentrations were ≥0.01 mg/mL in 22% of male carp, and means among sites were greatest at Sites M2 and S2 (Fig. 10). Relatively high vtg concentrations (0.04–0.90 mg/mL) measured in males ($n=8$) from Sites M2, S2, and S3, and mean concentrations for these basins were high (>0.02 mg/mL; Table 8).

3.4.4. *Steroid hormones (17β-estradiol and 11-ketotestosterone)*

Steroid hormone concentrations did not differ significantly among basins for either female or male bass (Table 8). Individual E2 concentrations were 156–1236 pg/mL in female bass and 101–902 pg/mL in male bass. Mean E2 concentrations among sites were greatest in female bass from S2 and P1 and male bass from Site M1 (Fig. 10). E2 concentrations were relatively low (<300 pg/mL) in several female bass from Sites P2 and P3 and high (>600 pg/mL) in male bass from Sites M1, M4, and S1. Individual KT concentrations were 87–883 pg/mL in female bass and 168–1307 pg/mL in male bass. KT concentrations were low in female bass (<300 pg/mL) from Sites M2, A1, A2, and A3 and male bass (67–521 pg/mL) from Site M3 compared to all other sites (Fig. 10). E2 and KT concentrations were not anomalous in intersex bass. KT concentrations were generally lower in ARB female bass compared to those from other basins (Table 8).

Steroid hormone concentrations did not differ significantly among basins for either female or male carp, but concentrations differed among sites within each basin (Table 8; Fig. 10). Individual E2 concentrations were 209–1890 pg/mL in female carp and 44–991 pg/mL in male carp. Individual KT concentrations were 48–965 pg/mL in female carp and 143–2508 pg/mL in male carp. Both E2 and KT concentrations were lower in female carp from Sites M1, M2, A1, A2, S3, and P3 than those from other sites (Fig. 10). Mean E2 concentrations among sites were greatest in male carp from Sites A1, A2, S2, and S3 (Fig. 10). Mean E2 and KT concentrations were generally lower in PRB male carp compared to those from other basins (Table 8).

4. Discussion

4.1. *Exposure indicators*

Methylmercury, which is the most toxic form of Hg, represents >90% of the total Hg that occurs in fish (Wiener et al., 2002).

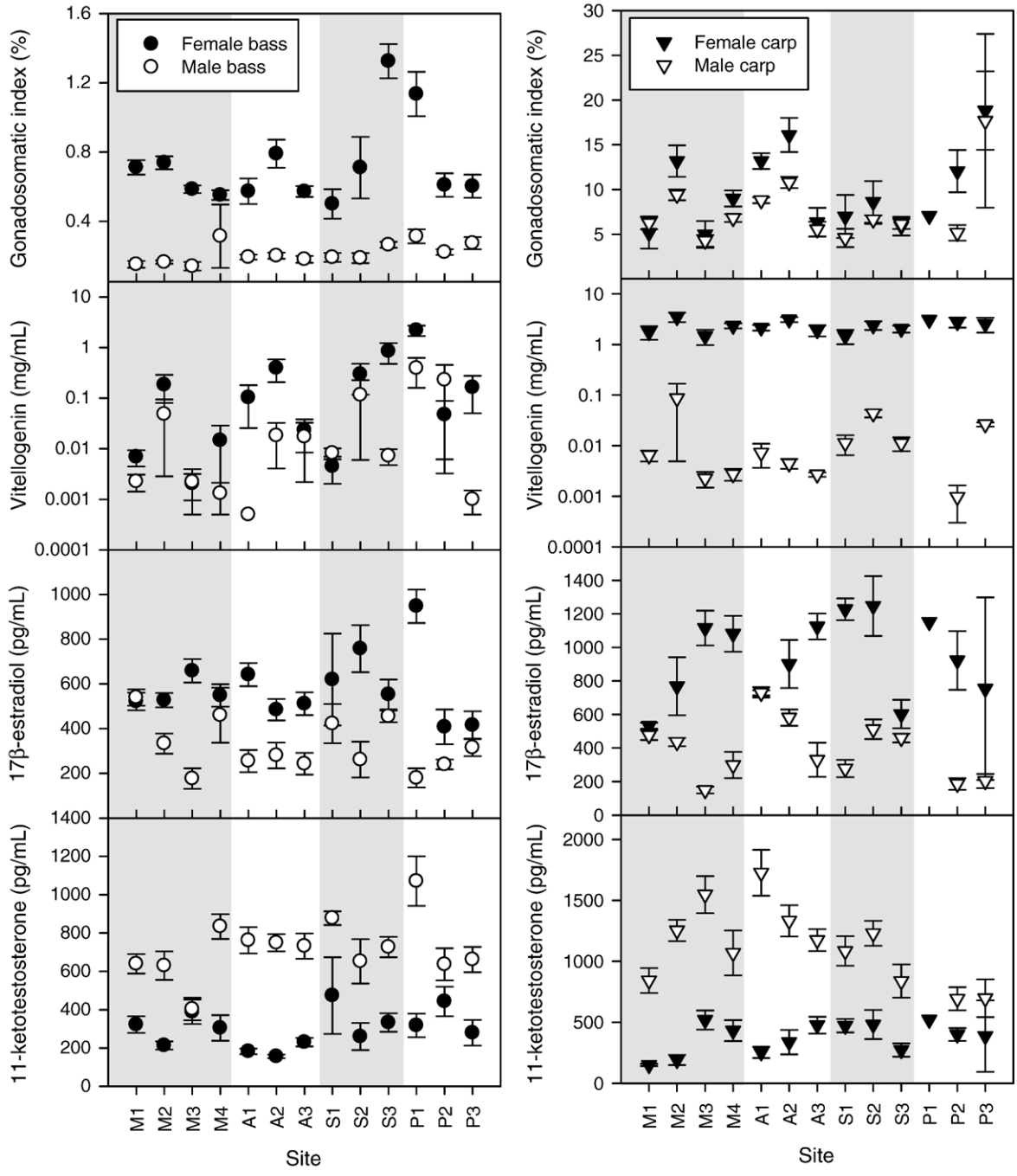

Fig. 10 – Mean (±standard error) reproductive biomarkers including gonadosomatic index, vitellogenin, 17β-estradiol, and 11-ketotestosterone in bass and carp. (Note: different scales are used for bass and carp plots, and censored vtg concentrations are plotted as 50% of LOD).

Total Hg concentrations in fish from our study were greater than historical concentrations measured in bass and carp from National Contaminant Biomonitoring Program (NCBP) sites in the region (Schmitt et al., 1999) and previous LRMN investigations (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a), and Hg concentrations in bass from all basins continue to represent a threat to fish and piscivorous wildlife (Barr, 1986; Yeardley et al., 1998; Beckvar et al., 2005). Mercury was previously identified as a contaminant of concern to aquatic species in the region, and multiple rivers and reservoirs in the MRB, ARB, SRB, and PRB have fish consumption advisories for Hg in numerous fish species. Probable sources of Hg in these areas include releases from chemical manufacturing and coal-fired power plants, atmospheric deposition, and wetlands (USEPA, 2001; Paller et al., 2004; Warner et al., 2005). Mercury has also been suspected to cause reproductive

effects, organ toxicity, and mortality in avian and mammalian wildlife in these basins (Halbrook et al., 1994; Osowski et al., 1995; Adair et al., 2003).

Diet is the primary route of Se exposure and toxicity in aquatic vertebrates (Lemly, 2002; Hamilton, 2004), and toxicity thresholds for Se tissue concentrations are relatively low due to the high bioaccumulation of organic forms of selenium in tissue which can lead to reproductive effects. However, Se concentrations exceeded protective criteria (>0.75 μg/g) for piscivorous wildlife in few samples from our study (Lemly, 1996, 2002). Potential sources of Se in the MRB and ARB, where concentrations in carp exceed 0.75 μg/g, include coal and petroleum combustion (Sorenson, 1991). Selenium concentrations in fish from our study were similar to historical concentrations measured in bass and carp from NCBP sites in the region (Schmitt et al., 1999) but less than those reported in waters with known selenium contamination (Schmitt et al., 2005; Hinck et al., 2007a).

Organochlorine pesticides were used historically in agricultural areas of the MRB, ARB, SRB, and PRB, and other organochlorine residues and metabolites, like PCA, were used in the lumber industry. Toxaphene and DDT were applied to cotton fields within the basins, and we found that the greatest concentrations of these insecticides were in ARB fish. Toxaphene concentrations in Site A1 fish exceeded lower toxicity thresholds to protect fish but were less than most effects criteria for freshwater fish (Mayer et al., 1975; Eisler and Jacknow, 1985); therefore, the risk of toxaphene to fish in this region is expected to be minimal. Although *p,p'*-DDE concentrations in fish have declined in the region (USEPA, 1992; Schmitt et al., 1999), concentrations in Site A1 fish remain a risk to avian wildlife (Anderson et al., 1975). In addition, concentrations of *p,p'*-DDE in fish from our study were less than mean concentrations in bass and carp from other large U.S. river basins (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a). Technical DDT contains *o,p'*-DDT as an impurity (up to approximately 15%), and residues of this compound and its metabolites also remain widespread (Schmitt et al., 1999). Concentrations of *o,p'*-DDE in Site M4 fish were unusually high and were potentially from a former DDT formulating facility located upstream. The *o,p'*-homologs were historically considered benign in aquatic systems, but studies have found that these compounds are estrogenic in fish (Donohoe and Curtis, 1996; Metcalfe et al., 2000; Papoulias et al., 2003). The total risk to fish and wildlife represented by concentrations of *o,p'*-DDT and its homologs is unknown. Other organochlorine residue concentrations including total chlordanes, dieldrin, and mirex were also relatively high in fish from the ARB but were less than effects thresholds or toxicity thresholds were not available.

PCBs were manufactured and used in electrical capacitors and transformers, for pressure treating lumber, and paper manufacturing, which were the likely sources of PCBs near Sites M2 and A1. Elevated PCB concentrations have been documented in water and biota from the MRB, ARB, SRB, and PRB (Atkins et al., 2004; Schmitt et al., 1999; USFWS, 1996; Zappia, 2002) and identified as a potential cause of reproductive dysfunction in mink from the coastal plain of Georgia, South Carolina, and North Carolina (Osowski et al., 1995). PCB concentrations in fish from Sites M2, A1, A2, and P3 exceeded a concentration known to cause inferior reproductive performance and offspring survival in piscivorous wildlife (Table 5; Hornshaw et al., 1983), but all concentrations were less than those associated with reproductive and developmental effects in fish (Monosson, 2000). Total PCB concentrations in fish from our study were generally greater than concentrations measured in bass and carp from previous LRMN investigations (Schmitt et al., 2005; Hinck et al., 2006a, 2007a).

Many dioxin releases in the region have been associated with pulp and paper mills (USEPA, 1992). In our study, the risk from dioxin-like compounds was greatest to MRB fish where previous studies have identified dioxin as a contaminant of concern (USEPA, 1992). The TCDD-EQs in fish from Sites M2, M4, A1, and P2 exceeded toxicity thresholds for mammalian and avian wildlife (4.4–5 pg/g; Nosek et al., 1992; Tillitt et al., 1996), and the TCDD-EQ concentrations in Site M2 male bass also exceeded the toxicity threshold to protect fish (30 pg/g; Walker et al., 1996; Whyte et al., 2004). Dioxin-like activity in Site M2 fish may be due to PCBs, which were also elevated at this site.

Hepatic EROD activity indicates recent exposure to exogenous aryl hydrocarbon receptor (AhR) ligands including some PCBs, dioxins, and polycyclic aromatic hydrocarbons (PAHs). Compared to other studies, mean EROD activity was greater than basal levels for bass from all basins (0–22 pmol/min/mg; Adams et al., 1994; Schmitt, 2002) and for female and male MRB carp (0–5 pmol/min/mg in females and <25 pmol/min/mg in males; Schlenk et al., 1996; Schmitt, 2002). Overall, mean hepatic EROD activity was generally highest in MRB and PRB fish. High EROD activity was likely the result of induction by dioxin-like compounds in Site M2 male bass, as indicated by the TCDD-EQ concentrations in these fish, and an AhR agonist other than dioxins and PCBs in Site M1 bass. Exposure to PCBs, dioxin-like compounds, or both may have resulted in the elevated EROD activity in Sites M2 and M4 carp, and other AhR agonists may have caused the high EROD activity in Sites M1 and M3 carp.

4.2. *Health indicators*

The quantitative fish health indicators used in this study have been widely used and discussed in the literature and were selected to reflect overall organismal health of the fish and their populations. Evaluation of these endpoints indicated that bass and carp in some regions of the MRB, ARB, SRB, and PRB were in poorer health than others.

The HAI scores in MRB bass were greater than those observed in bass from other LRMN studies (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a,b, 2007a) and would be considered unhealthy or contaminated by comparable criteria from other studies (Adams et al., 1993; Coughlan et al., 1996). Internal organs of MRB bass had severe parasitic infestations, which had been previously documented in the MRB (USEPA, 1995). Few incidences of confirmed tumors or other grossly visible indications of exposure to toxic chemicals were found in fish. Tumors were found in a total of five fish (0.01%) representing Sites M3, A1, S1, and P3. Ovarian tumors of the same origin (smooth muscle) were found in two Site A1 carp. Gonadal tumors of encapsulated teratogenic masses were previously documented in carp and goldfish hybrids from the Great Lakes area but their cause was unknown (Harshbarger and Clark, 1990).

CF and SSI values were generally normal in MRB, ARB, SRB, and PRB fish. Most CF values in bass and carp were 1.0–2.0, which were considered normal for healthy fish of these species (Carlander, 1969, 1977; Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a,b, 2007a). HSI values in bass from all basins were low compared to those normally found in most fish (1–2%; Gingerich, 1982; Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a,b, 2007a). Decreased liver size has been reported in various fish species after exposure to contaminants including metals and bleached kraft mill effluent (McMaster et al., 1991; Adams et al., 1992).

Increases in MA parameters in fish from specific contaminated sites relative to reference sites have been documented in both laboratory and field studies (Wolke, 1992; Blazer et al., 1997). MAs were more numerous and larger in MRB bass. MA parameters in individual bass and carp from Site M2 were among the greatest measured in any LRMN study and were considered to be abnormally high (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a,b, 2007a). Increases in MA parameters have been associated with contaminants in laboratory and field studies (see reviews by Wolke, 1992; Blazer et al., 1997), but can vary with fish size, age, and nutritional status (Couillard and Hodson, 1996; Blazer et al., 1997).

4.3. *Reproductive biomarkers*

The reproductive biomarkers measured in this study are the key measures of reproductive function and are routinely used to help evaluate contaminant effects or simply assess general reproductive health in fish. However, factors other than exposure to chemical contaminants including disease, age, season, and geographic location can affect reproductive biomarker responses. All fish samples were collected post-spawn and within a nine week period (October to December) to minimize the variation of reproductive biomarkers from temperature, photoperiod, and annual reproductive cycle. However, natural changes or fluctuations in reproductive biomarkers may have occurred during this collection period. Nevertheless, gonadal stages in bass and carp were generally consistent among basins, and mean GSI values were generally greater in fish that were in more advanced gonadal stages. Gonadal abnormalities including calcified follicles and fibrosis were present in Site M2 carp and may be associated with PCB exposure or age of the fish.

Intersex gonads were identified in 42% of the male bass collected representing all basins. The occurrence of intersex was greatest in the PRB and least severe in the MRB; greater proportions of gonadal tissue from PRB bass were examined during the histological analysis which may account for the greater occurrence of intersex fish from these sites. Gonadal tissue of all intersex bass was primarily testicular tissue with mild to moderate numbers of immature oocytes. Previous LRMN studies have observed intersex in largemouth and smallmouth bass, but the occurrence of intersex bass was generally limited to a few individual fish at any one site (<40%; Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a). Conversely, the majority (>50%) of male bass from multiple sites (A3, S2, S3, P1, P2, and P3) were intersex in our study. The background occurrence of intersex in bass is unknown, but the high incidence of intersex bass in the ARB, SRB, and PRB is cause for concern and should be further studied.

Plasma vtg concentrations in female fish were considered normal. Vitellogenin was detected in male bass and carp from all basins but concentrations were generally low. Vitellogenin concentrations >0.01 mg/mL in individual male fish from Sites M2, S2, P1, and P2 were considered abnormal and indicate that these fish may have been exposed to estrogen mimics. Mean vtg concentrations in PRB bass were within the range of early vitellogenic females considered to be high in previous studies (Schmitt, 2002; Schmitt et al., 2005; Hinck et al., 2006a, 2007a). Induction of vtg in male fish has been associated with sewage effluent (Folmar et al., 1996) and pulp and paper mill effluent (Soimasou et al., 1998; Mellanen et al., 1999; van den Heuvel and Ellis, 2002). Vitellogenin concentrations in fish collected near pulp and paper mills (Sites M1, M2, S1, and S2) were not uniformly elevated, but exposure of fish to effluents from these sites is likely not constant due to migration. Concentrations of E2 and KT were generally considered normal in bass and carp. Relatively low E2 and KT concentrations were measured in Site M4 male bass, Site M1 female carp, and Sites P2 and P3 male carp.

5. Conclusions

Overall, Hg and PCBs were the primary contaminants of concern in our study. Elevated Hg concentrations were found in fish from all basins and were among the greatest measured in any BEST-LRMN study. Possible Hg sources include releases from chemical manufacturing plants, coal-fired power plants, atmospheric deposition, and wetlands. Total PCBs remain a concern in the MRB where a manufacturing facility and an electrical capacitor and transformer facility have contaminated the Coosa River, and fish consumption advisories remain for much of the river. It is unknown whether the cause of ovarian tumors in female carp and widespread intersex in male bass are related to chemical contaminant exposure, but our results indicate that studies designed to address these abnormal reproductive developments in fish are warranted within the region.

Historically, the agricultural and manufacturing industries that drive the economy of the southeastern United States have had deleterious effects on water and aquatic habitat quality through the production and release of organochlorine compounds and deposition of Hg. In addition, poultry and livestock production facilities in the region pose a risk to aquatic habitats through the release of nutrients (nitrogen and phosphorus), pathogens, heavy metals (Cu and Zn), veterinary pharmaceuticals, and natural and synthetic hormones (including estrogens). Further degradation of aquatic resources associated with these industries is possible if chemical contaminants, including endocrine disrupting compounds, continue to be released into aquatic ecosystems. However, conservation programs may be able to ameliorate some of the risk associated with these compounds by improving application and disposal technology and management practices of these industries. Biological responses may increase in magnitude as chemical concentrations increase, which may affect the quality of our aquatic ecosystems. Results from this study and other investigations indicate that site specific studies, continued monitoring, and technological advancements in the agricultural and manufacturing industries are needed to

better understand and minimize the impacts of chemical contaminants on our aquatic resources.

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