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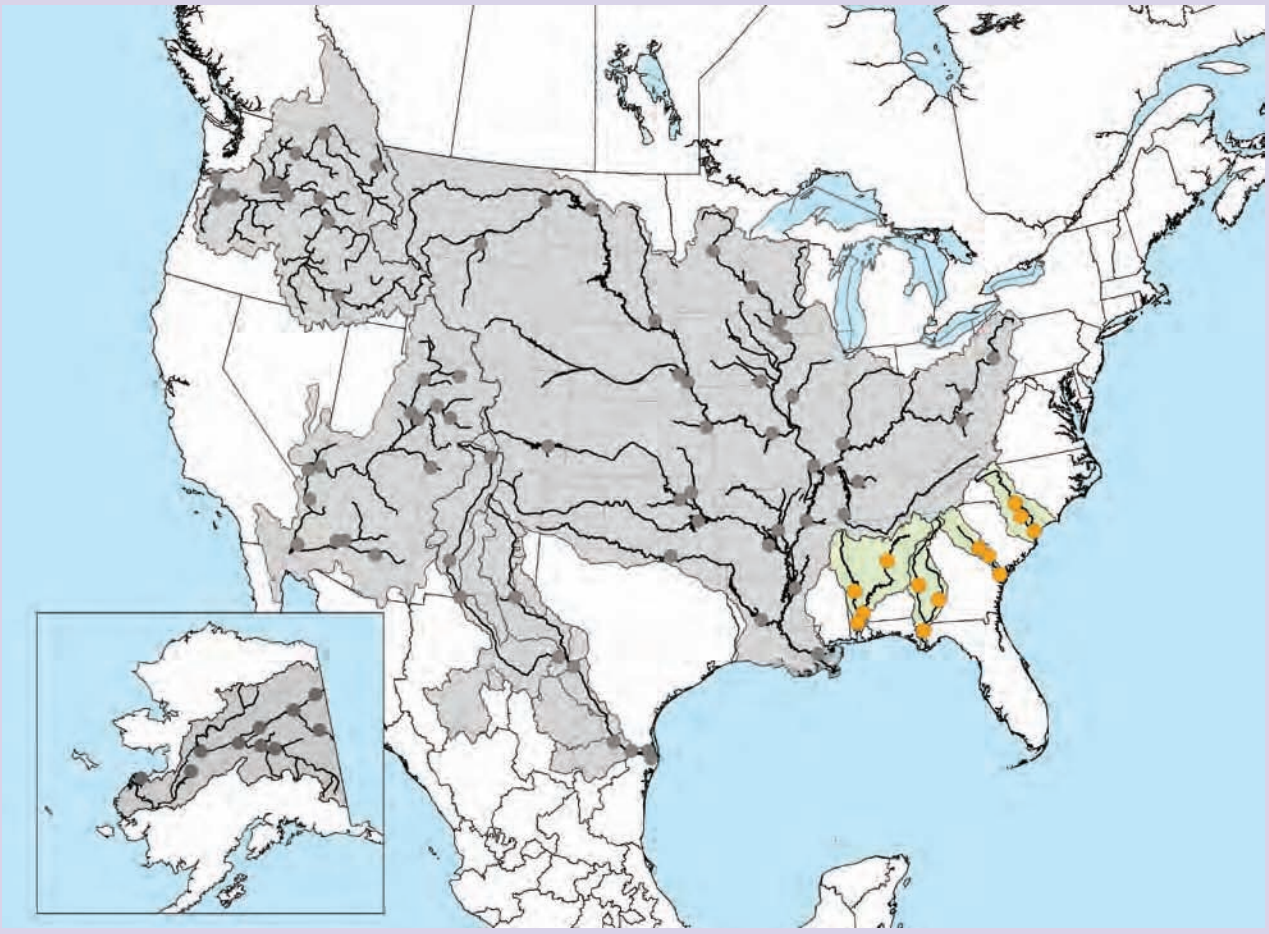
Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants, Health Indicators, and Reproductive Biomarkers in Fish from the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins



Scientific Investigations Report 2007–5176

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Cover. The U.S. map shows the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins (green) and stations sampled during this study (orange). Shown in gray are the major river basins and stations in the United States sampled during Biomonitoring of Environmental Status and Trends (BEST) investigations.

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Preface

This study was conducted as part of the U.S. Geological Survey (USGS) Biomonitoring of Environmental Status and Trends (BEST) Program's Large River Monitoring Network (LRMN). BEST evolved from previous Federal monitoring programs including the National Pesticide Monitoring Program (NPMP) of the 1960s, renamed the National Contaminant Biomonitoring Program (NCBP) in the early 1970s, which also screened for elemental contaminants. The U.S. Fish and Wildlife Service (USFWS) participated in the NPMP and maintained the NCBP by monitoring concentrations of persistent contaminants in freshwater fish and avian wildlife through 1986 (Schmitt and others, 1999). The BEST program was initiated in the 1990s to build on information produced by these earlier programs and to provide more biologically relevant information regarding potential contaminant effects on lands and species under USFWS management. The program was transferred to the National Biological Survey in 1993 and ultimately to USGS in 1996. The LRMN has principal emphasis to identify, monitor, and assess the effects of chemical contaminants on the fish health in the nation's large rivers. This study was one in a series of BEST-LRMN Program monitoring investigations. Previous studies include the Mississippi River Basin in 1995, the Columbia River and Rio Grande Basins in 1997, the Yukon River Basin in 2002, and the Colorado River Basin in 2003.

Acknowledgments

Staff from the U.S. Fish and Wildlife Service (USFWS) office in Daphne, Alabama (P. Tuttle, S. Floyd, A. Ford), D. Wilkins from the South Carolina Aquarium, and L. Dorsey from the North Carolina Wildlife Resources Commission: Division of Inland Fisheries participated in fish collections. J. Isley and T. Grabowski of the USGS-South Carolina Fish and Wildlife Research Unit at Clemson University, Clemson, South Carolina also collected and aged the fish. D. Cartwright (Leetown Science Center; LSC), A. Donahue (Columbia Environmental Research Center; CERC), A. Griffin (LSC), A. Fazio (Florida Integrated Science Center; FISC), J. Jeran (LSC), R. Lipkin (CERC), J. Scarborough (FISC), and T. Smith (FISC) participated in field dissections. Chemical analyses were conducted by CERC Environmental Chemistry laboratories (B. Brumbaugh, K. Feltz, J. Meadows, M. Tanner, G. Tegerdine, and M. Walther). Laboratory analyses for biomarkers were performed by D. Nicks (CERC), D. Bowling (LSC), and K. Kroll (University of Florida, Gainesville, Florida). D. Bowling (LSC) and K. Spring (LSC) assisted in the histology slide preparation, and B. Martin (LSC) assisted with the macrophage aggregate evaluation. R. Lipkin and K. Chojnacki (CERC) prepared the maps. W. Bryant (USGS), S. Finger (CERC), A. Ford (USFWS), and P. Tuttle (USFWS) reviewed the report and provided additional information.

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Conversion Factors

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square millimeter (mm^2)	0.00155	square inch (in^2)
square meter (m^2)	10.76	square foot (ft^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	35.31	cubic foot (ft^3)
cubic kilometer (km^3)	0.2399	cubic mile (mi^3)
	Flow rate	
cubic meter per second (m^3/s)	35.31	cubic foot per second (ft^3/s)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
	Concentration	
microgram per gram (μg/g)	=	part per million (ppm; 10^6)
nanogram per gram (ng/g))	=	part per billion (ppm; 10^9)
picogram per gram (pg/g)	=	part per trillion (ppm; 10^{12})
milligram per millimeter (mg/mL)	=	part per thousand (ppm; 10^3)
microgram per liter (μg/L)	=	part per billion (ppm; 10^9)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants, Health Indicators, and Reproductive Biomarkers in Fish from the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins

By Jo Ellen Hinck[1], Vicki S. Blazer[2], Nancy D. Denslow[3], Kathy R. Echols[1], Robert W. Gale[1], Tom W. May[1], Rachael Claunch[1], Carla Wieser[4], Patrick J. Anderson[5], James J. Coyle[5], Timothy S. Gross[4], and Donald E. Tillitt[1]

Abstract

Largemouth bass (*Micropterus salmoides*) and common carp (*Cyprinus carpio*) were collected from 13 sites in 4 river basins in the southeastern United States to document spatial trends in accumulative 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. Fish were field-examined for external and internal anomalies, selected organs were weighed to compute somatic indices, and tissue and fluid samples were preserved for fish health and reproductive biomarker analyses. Mercury concentrations in bass samples from all sites exceeded toxicity thresholds for mammals [>0.1 micrograms per gram wet weight (µg/g ww)], fish (>0.2 µg/g ww), and birds (>0.3 µg/g ww) and were greatest (>0.5 µg/g ww) in samples from the Alabama River at Eureka Landing, Alabama; the Mobile River at Bucks, Alabama; the Apalachicola River at Blountstown, Florida; the Savannah River at Sylvania, Georgia; and the Pee Dee River at Bucksport, South Carolina. Selenium concentrations were relatively high (>0.75 µg/g ww) in fish from the Tombigbee River at Lavaca, Alabama; the Mobile River at Bucks; and the Chattahoochee River at Omaha, Georgia compared to those from other sites. Concentrations of 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (*p,p*'-DDE) were high in fish from the Chattahoochee River at Omaha and the Mobile River near Bucks, which was near a 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (DDT) formulating facility that historically discharged into the lower Mobile River. Toxaphene concentrations in fish from the Flint River near Albany, Georgia (60-100 nanograms per gram (ng/g) ww) may pose a risk to fish. Concentrations of other formerly used (total chlordanes, dieldrin, endrin, aldrin, mirex, and hexachlorobenzene) and currently used (pentachlorobenzene, pentachloroanisole, dacthal, endosulfan, γ-HCH, and methoxychlor) organochlorine residues generally were low or did not exceed toxicity thresholds. Total polychlorinated biphenyls concentrations in samples from the Coosa River at Childersburg, Alabama; the Apalachicola River at Omaha; the Apalachicola River at Blountstown; and the Pee Dee River at Bucksport were >480 ng/g ww and may be a risk to piscivorous wildlife. Dioxin-like activity as measured by TCDD-EQ was greatest [>10 picograms per gram (pg/g)] in male fish from the Coosa River at Childersburg and the Mobile River at Bucks. Hepatic ethoxyresorufin *O*-deethylase activity generally was greatest in carp from the Mobile River Basin [means >10 picomols per minute per milligram of protein (pmol/min/mg)] and in bass from the Tombigbee River at Lavaca and Pee Dee River at Pee Dee, South Carolina (means >65 pmol/min/mg). Altered biomarkers were noted in fish from all basins. The field necropsy and histopathological examination determined that fish from the Mobile River Basin generally were in poorer health than those from the other basins. In bass, health assessment index scores were correlated with mercury and *p,p*'-DDE concentrations. High health assessment index scores in Mobile River Basin fish were

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widespread and caused primarily by parasitic infestations, which were most severe in fish from the Tombigbee River at Lavaca and the Alabama River at Eureka Landing. Tumors were present in few fish (n = 5; 0.01%). Ovarian tumors of the same origin (smooth muscle) were present in two older carp from the Chattahoochee River near Omaha, Georgia and may be contaminant related. Reproductive biomarkers including gonadosomatic index, vitellogenin concentrations, and steroid hormone concentrations were anomalous in fish from various sites but were not consistently related to any particular chemical contaminant. Intersex gonads were identified in 47 male bass (42%) representing 12 sites and may indicate exposure to endocrine disrupting compounds. The incidence of intersex male bass was greatest in the Pee Dee River Basin and least severe in the Mobile River Basin. Male bass and carp with low concentrations of vitellogenin were common in all basins. Comparatively high vitellogenin concentrations [>0.35 milligram per milliliter (mg/mL)] in male fish from the Coosa River at Childersburg, the Savannah River at Sylvania, and the Pee Dee River at Rockingham and Bucksport indicate exposure to estrogenic or anti-androgenic chemicals.

Introduction

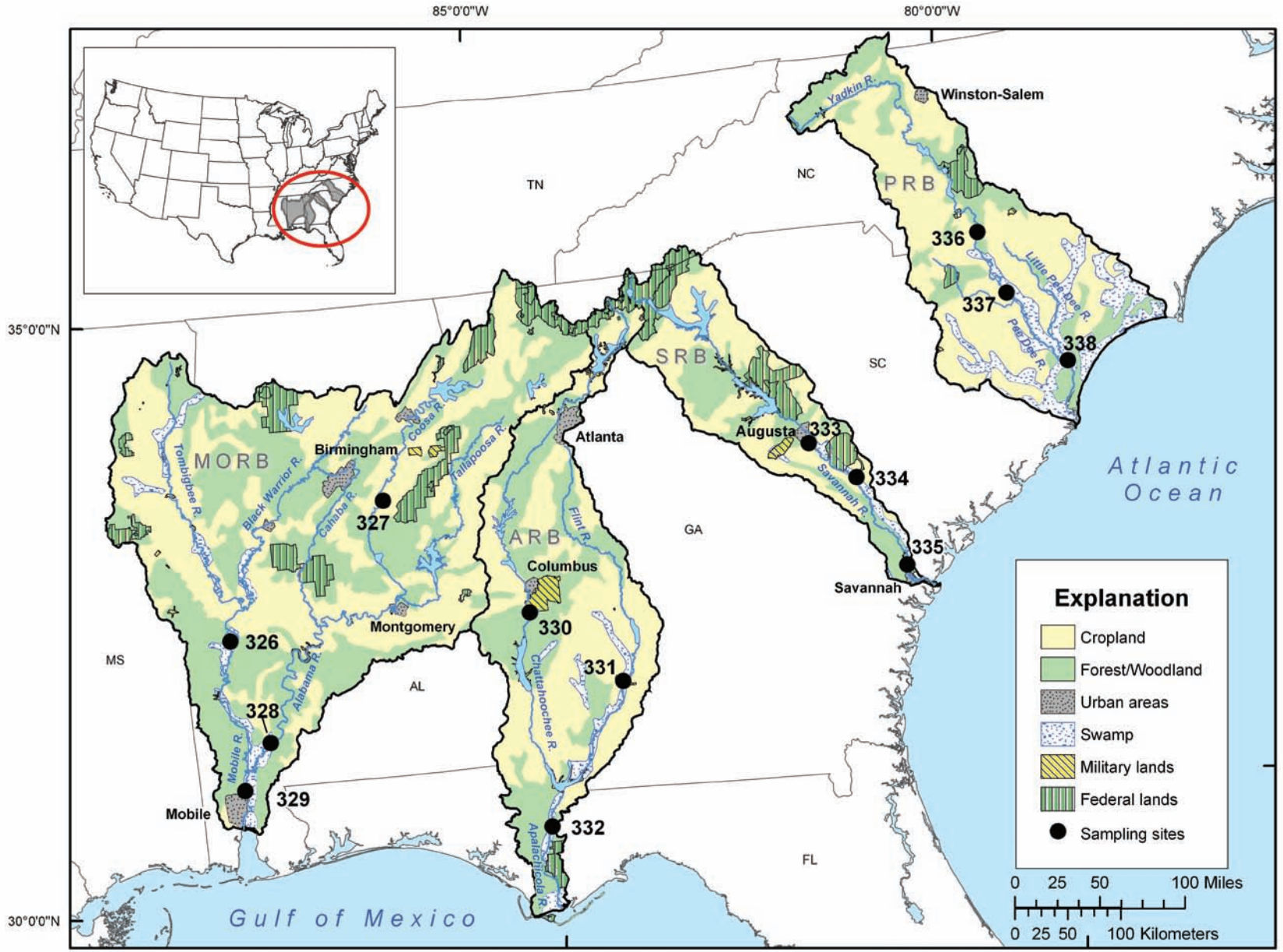
The rich soils, abundant forests, and warm climate of the southeastern United States have resulted in agriculture, forestry, mining, and manufacturing being important economic drivers that are dependent on local water sources. These industries have been associated with declines in water quality in the Mobile River Basin (MORB), Apalachicola-Chattahoochee-Flint River Basin (ARB), Savannah River Basin (SRB), and Pee Dee River Basin (PDRB). 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 previously have been associated with declines in water and habitat quality in one or more of these basins. As a result, many MORB, ARB, SRB, and PDRB waters have been listed as impaired. Elevated concentrations of pesticides, polychlorinated biphenyls (PCBs), and mercury (Hg) have been reported in water, sediment, and biota in these basins (Adair and others, 2003; Atkins and others, 2004; Gilliom and others, 2006; Johnson and others, 2002; U.S. Environmental Protection Agency (USEPA), 1992; 1995; U.S. Fish and Wildlife Service (USFWS), 1989a; 1989b; 1996), and fish consumption advisories for Hg and PCBs have been issued for large rivers and reservoirs in the MORB, ARB, SRB, and PDRB to protect human health. Previous contaminant studies in these basins focused on measuring chemical contaminant concentrations in biota, but few investigations have assessed the health of aquatic biota in these basins.

The Biomonitoring of Environmental Status and Trends (BEST) Program's Large River Monitoring Network (LRMN) studied the MORB, ARB, SRB, and PDRB in the fall of 2004. The LRMN is unique among national monitoring programs because of its emphasis on characterizing the effects of environmental contaminants on the health of fish and their supporting habitat. The LRMN accomplishes this task by measuring chemical concentrations and by evaluating the physiological, morphological, and histopathological responses of contaminant exposure by the organism. The primary objective of this study was to document the occurrence and distribution of contaminants, health indicators, and reproductive biomarkers in fish from the MORB, ARB, SRB, and PDRB and to evaluate the potential risk from these contaminants to other biota. Secondary objectives were to compare biomonitoring results from these river basins to other major rivers systems in the United States, to determine benchmarks for quantification and interpretation of biomarker results, and provide information on potential sources of chemical contaminants that exceed protective thresholds for fish and wildlife. These latter objectives were achieved by building on the results of similar LRMN investigations in the Mississippi River Basin (MRB) in 1995 (Schmitt, 2002), Rio Grande Basin (RGB) in 1997 (Schmitt and others, 2004; 2005), Columbia River Basin (CRB) in 1997 (Hinck and others, 2004a; 2006a), Yukon River Basin (YRB) in 2002 (Hinck and others, 2004b; 2006c) and Colorado River Basin (CDRB) in 2004 (Hinck and others, 2006b; 2007). An interactive national database at: *www.cerc.usgs.gov/data/best/search/index.htm* includes data from this study. Results from this study, together with those from similar investigations conducted in other river basins, will help resource managers and scientists assess contaminant impacts on fish and wildlife and human consumers of those fish. These data also will help resource managers and scientists to identify areas that warrant further investigation of contaminant threats.

Southeast River Basins Overview

Hydrology and Environmental Setting

The MORB is the sixth largest river basin in the United States in terms of drainage area [115,500 square kilometers (km^2)] and the fourth largest in terms of stream flow [2,250 cubic meters per second (m^3/s)] (Atkins, 1998; Hackney and others, 1992). The MORB is comprised of two large rivers, the Tombigbee and the Alabama, which meet to form the Mobile River in southwestern Alabama. The Mobile River eventually flows into the Gulf of Mexico (fig. 1). The Alabama River is 507 kilometers (km) long and drains 59,052 km^2. Large tributaries of the Alabama River include the Coosa (26,317 km^2 drainage area; 460 km long), Tallapoosa (12,108 km^2 drainage area; 431 km long), and Cahaba Rivers (4,727 km^2 drainage area; 307 km long; Atkins, 1998). The Tombigbee River, which is 644 km long and drains 52,318 km^2, is connected to Tennessee River via the Tennessee-Tombigbee Waterway and acts as a trade route between the Midwest and the Gulf Coast. The Tennessee-Tombigbee Waterway has been used as an



Base from U.S. Geological Survey National Atlas, 1:2,000,000
North America Albers Equal Area Conic projection

Figure 1. Land cover in the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee River (PRB) Basins. Major rivers and tributaries, federal lands (U.S. Fish and Wildlife Service, National Park Service, and U.S. Forest Service), military lands, and sites sampled in 2004 also are shown. See table 5 for station descriptions.

alternative shipping route to the Gulf Coast when the Mississippi River was closed to barge traffic during low flows. The Black Warrior River (16,255 km^2 drainage area) is the largest tributary of the Tombigbee River. Flow in the Alabama and Tombigbee Rivers is regulated by upstream reservoirs, navigational locks and dams, and hydroelectric power plants (Atkins, 1998; Hackney and others, 1992).

The ARB encompasses 52,836 km^2 in Georgia, Alabama, and Florida (U.S. Geological Survey (USGS), 2004). The Chattahoochee and Flint Rivers combine to form the Apalachicola River in Florida panhandle (fig. 1). The Chattahoochee River begins at the southern edge of the Blue Ridge Mountains, is 692 km long, and drains 22,714 km^2 (Leitman and others, 1991). Flow along most of the Chattahoochee River is controlled by hydroelectric dams (USGS, 2004). The Flint River begins south of Atlanta, is 563 km long, and drains 21,911 km^2 (Utley and Hemperley, 1975). The Apalachicola River is 171 km long, flows from the Jim Woodruff Lock and Dam to the Gulf of Mexico, and drains 6,734 km^2 (USGS, 2004; Livingston, 1992). Physical alterations including dredging, diking, and channelization have altered aquatic habitat in the Apalachicola River (Hackney and others, 1992). Mean annual flow is 555 m^3/s in the Apalachicola River, 234 m^3/s in the Chattahoochee River, and 114 m^3/s in the Flint River (USGS, 2004).

The SRB drains 27,400 km^2 in South Carolina, North Carolina, and Georgia (Hackney and others, 1992). The Savannah River is the boundary between South Carolina and Georgia, starting at the Hartwell Reservoir with the confluence of the Seneca and Tugaloo Rivers (University of Georgia, 2005). The Savannah River flows southeast for 476 km to Savannah, Georgia, where it drains into the Atlantic Ocean and has a mean annual flow of 302 m^3/s (Hackney and others, 1992).

The PDRB drains 27,553 km^2 in North Carolina and South Carolina and includes the Yadkin River, which becomes the Pee Dee River once it joins with the Uwharrie River in east central North Carolina (South Carolina Department of Health and Environmental Control (SCDHEC), 2003; Yadkin/Pee Dee River Basin Association, 2003). The Pee Dee River flows 700 km before it drains into Winyah Bay, South Carolina and has a mean annual flow of 243 m^3/s (South Carolina Department of Natural Resources, 2005).

The climate in the MORB, ARB, SRB, and PDRB is warm and humid and ranges from temperate in higher elevations to subtropical near the coast (Georgia Department of Natural Resources (GDNR), 1997a; 1997b; 2001; Hackney and others, 1992; Johnson and others, 2002; USGS, 2004). Warm, humid air masses from the Gulf of Mexico produce precipitation in the summer months, whereas arctic fronts from the central United States produce precipitation during the mild winter months. Most precipitation occurs as rainfall with even distributions throughout the year. In higher elevations, summers are milder and winters are colder (GDNR, 2001). Tropical depressions, storms, and hurricanes can move inland from the Gulf of Mexico in late summer or early fall and cause flooding. The Atlantic hurricane season was more active in 2004 than in previous years with 15 tropical storms and 9 hurricanes (National Oceanic and Atmospheric Administration (NOAA), 2004). Four hurricanes (Charley, Frances, Ivan, and Jeanne) made landfall in the southeastern United States in August–September 2004 causing more than $50 billion in damages and extensive flooding.

The MORB, ARB, SRB, and PDRB are part of three ecoregion provinces, Southeastern Mixed Forest, Outer Coastal Plain Mixed Forest, and the Central Appalachian Broadleaf Forest (Bailey, 1995). Most of each river basin lies within the Southeastern Mixed Forest province. This region consists of clay soils with flood plain soils being the best for agriculture. The Outer Coastal Plain Mixed Forest province includes the southern tip of the MORB, roughly one third of the ARB, and one half of each SRB and PDRB. Land in this area slopes toward the ocean, creating slow streams and many marshes, swamps, and lakes. Soils in the region consist of clay, gravel, and sand and normally are damp, acidic, and low in nutrients. The Central Appalachian Broadleaf Forest province includes the northern parts of all four basins. High elevations with mountainous topography and nutrient-poor clay soils characterize this ecoregion province.

Urban Areas and Economy

The human population of the southern United States was one of the fastest growing from 1990 to 2000 and also had the highest poverty rate in the United States (Bishaw and Iceland, 2003; Perry and Mackun, 2001). State populations increased > (greater than) 10 percent (%) in Mississippi, Alabama, and South Carolina and >20% Georgia, Florida, and North Carolina from 1990 to 2000 (Perry and Mackun, 2001). Georgia is one of the most populous states with the 10th largest population in the United States (Perry and Mackun, 2001; Georgia Department of Community Affairs (GDCA), 2006), but few towns and cities located in the MORB, ARB, SRB, and PDRB have populations >500,000. Birmingham, Montgomery, and Mobile, Alabama are the largest cities in the MORB with metropolitan populations of 921,106, 333,055, and 540,258, respectively (Alabama Development Office (ADO), 2006). Atlanta, Georgia, the largest metropolitan area in the ARB (4,610,000), also is one of the largest urban areas in the United States. Columbus, Georgia is the only other city in the ARB with a population >150,000. Augusta (195,182) and Savannah (131,510), Georgia are the largest cities in the SRB, and Winston-Salem, North Carolina (185,776) is the largest city located in the PDRB. Mobile and Savannah also are important ports that aid in the transportation of goods in and out of the southeastern United States.

The economies in the MORB, ARB, SRB, and PDRB are driven by manufacturing and agriculture. The manufacturing industry includes paper products, chemical products, textile goods, machinery, and food products and clothing (ADO, 2006; GDCA, 2006). Poultry, cattle, swine, cotton, soybeans, peanuts, tobacco, and nurseries and sod products drive the agricultural industry. Forestry also is important to the

economy of the ARB and SRB (GDNR, 1997a; 1997b; 2001), and mining occurs in the MORB (ADO, 2006). The forest industry, which includes the manufacture of paper, lumber, furniture, and a variety of other wood products, was $19.5 billion and employed >175,000 people in Georgia in 1997 (GDNR, 1997a; 1997b; 2001). Wholesale and retail trade and the tourism industry also are important to the economies of the southeastern United States.

Landownership and Land Use

The diverse fauna in rivers and streams of the southeastern United States have declined because of dam construction, channel modifications, water quality declines, and introduction of nonindigenous species (Lydeard and Mayden, 1995). Threatened and endangered aquatic species (TES) are present throughout the MORB, ARB, SRB, and PDRB (appendix 1), and National Wildlife Refuges (NWRs) have been designated in several areas (table 1; fig. 1). These areas provide important habitat for endemic aquatic species, nesting and brood habitat for duck and alligator populations, and wintering areas for migratory birds and waterfowl. Federal recreational resources have been designated in the MORB and PDRB. A 99 km segment of the Sipsy Fork of the Black Warrior River and a 130 km segment of the Lumber River, a tributary of the Little Pee Dee River, have been classified as wild and scenic rivers by the U.S. Forest Service (USFS) and National Park Service (NPS, 2006).

Table 1. Sampling locations located within approximately 75 kilometers of a National Wildlife Refuge.

[Station numbers are in parentheses]

Station	National Wildlife Refuge
Lavaca, AL (326)	Choctaw
Childersburg, AL (327)	Mountain Longleaf
Omaha, GA (330)	Eufaula
Port Wentworth, GA (335)	Savannah
Pee Dee, SC (337)	Carolina Sandhills
Bucksport, SC (338)	Waccamaw

Land use in the MORB is dominated by forests (69%) and agriculture (18%) including livestock, aquaculture, row crops, and pastureland; 3% of the MORB are urban areas (Johnson and others, 2002; Zappia, 2002; fig. 1). Most agriculture is concentrated in the Black Prairie Belt of the Coastal Plain Physiographic Province, and primary row crops include corn, soybeans, cotton, wheat, and sorghum (Johnson and others, 2002). Other major crops include peanuts, fruit, vegetables, and greenhouse seedlings. Farmers also raise several varieties of pine for harvesting lumber, turpentine, tar, and rosin. Poultry operations are concentrated in the northern parts of the MORB, and swine production is largely limited to the MORB. Most of the cattle are raised for beef on pastureland, with few large cattle feedlots operating in the MORB (Johnson and others, 2002).

Land uses in the ARB primarily are agriculture and forestry-related (fig. 1). Land use in the Chattahoochee River Basin is 68% forest, 19% agriculture, 9% urban, and 4% other, which includes wetlands (GDNR, 1997a). In the Flint River Basin, land use includes forest (48%), agriculture (42%), wetlands (5%), urban areas (3%), and other (2%; GDNR, 1997b). The upper ARB has more urban land use and includes the Atlanta metropolitan area, whereas the lower ARB has more agriculture. Forest acreage has decreased since the 1980s as a result of rapid urban growth and clearing for agricultural uses, although total farmland also has decreased during the same time (GDNR, 1997a; 1997b). Agriculture in the basin includes poultry, cattle (beef and dairy), crop, orchard, and vegetable production. Row crops such as peanuts, corn, soybeans, and cotton are harvested in the lower ARB, whereas pastureland and concentrated animal feeding operations (CAFOs) are present in the upper ARB. Poultry and livestock production is intensive with 203,000,000 broiler chickens, 364,000 head of cattle, and 176,000 head of swine. Orchard crop production also has increased since the late 1980s and includes pecans and peaches (GDNR, 1997a; 1997b), and approximately 24% of the Flint River Basin (5,260 km^2) is devoted to the production of crops, orchards, forages, nursery, and turf (GDNR, 1997b). Forestry in the basin provides turpentine, rosin, lumber, and pulpwood.

Land use in the SRB includes forests (57%), agriculture (9%), wetlands (9%), and urban areas (2%; GDNR, 2001; fig. 1). Agriculture in the SRB includes animal operations in the northern SRB and commodity production in the southern SRB. Total farmland has declined steadily in the SRB since the early 1980s. Most agricultural land use (75%) in the SRB is pastureland; the remaining 25% is cotton, peanuts, tobacco, wheat, sorghum, soybean, and millet. Livestock and poultry production is intensive in the SRB, especially in Franklin, Hart, Madison, and Oglethorpe Counties, with 202,000 head of cattle, 83,000 head of swine, and 265,000,000 broilers and layers (GDNR, 2001).

Land uses in the PDRB primarily are agriculture and forestry-related (fig. 1). Land use is >50% forest, 30% agriculture, 13% developed in the North Carolina part of the PDRB (North Carolina Division of Environment and Natural Resources (NCDENR), 2003). Land use includes forest (36%), agriculture (23%), wetlands (17%), and urban (2%) in South Carolina (SCDHEC, 2003). Agriculture includes cultivated crops (row, hay, orchard, nursery, and small grain crops), uncultivated crops (summer fallow and aquaculture in crop rotation), pastureland, and poultry, swine, and cattle operations. Cultivated cropland and forested land have decreased significantly in the PDRB, whereas notable increases in uncultivated crops, pastureland, and urban development have occurred during the past two decades (NCDENR, 2003). The forestry industry in the PDRB produces softwood trees for lumber, wood pulp, and paper; hardwood trees are used in the furniture industry. Livestock and poultry production

is intensive in North Carolina part of the PDRB with 41,000 head of dairy cattle, 157,000 head of swine, and 75,000,000 head of poultry; poultry and swine production have increased by 13% and 47%, respectively, from 1994 to 1998 (NCDENR, 2003). In South Carolina, improper land application of manure from CAFOs was a potential source of fecal coliform in some watersheds of the PDRB (SCDHEC, 2005c).

Dams

The creation of dams and reservoirs has altered the hydrology of the MORB, ARB, SRB, and PDRB. Dams, which are used for hydroelectric power, water supply, flood control, water storage, recreation, and navigation, have negative impacts such as blocked or delayed fish migration, increased fish mortality because of turbines or spillways, habitat alteration, and water quality changes (Larinier, 2001).

Dams in the MORB are used for navigation, flood control, hydroelectric power, and water supplies (fig. 2; U.S. Department of Interior (USDOI), 2002). Navigation dams include Stennis, Demopolis, and Coffeeville on the Tombigbee River and the Bankhead, Holt, and Oliver on the Black Warrior River. The locks and dams on the Tombigbee River create a part of the 377 km Tennessee-Tombigbee Waterway, which acts as a trade route between the Midwest and the Gulf Coast. The Bankhead and Holt Dams on the Black Warrior River have been equipped with turbines to generate hydroelectric power. Hydroelectric power dams also are located on Coosa (seven dams) and Tallapoosa Rivers (four dams). The R.F. Henry, Millers Ferry, and Claiborne Dams are navigation dams that impound 375 km (74%) of the mainstem Alabama River.

Dams in the ARB are used for hydroelectric power, navigation, flood control, and recreation (fig. 2). Hydroelectric power dams include Morgan Falls, Goat Rock, Oliver, Bartlett's Ferry, and Langdale on the Chattahoochee River and Muckafoonee Creek, Flint River, and Crisp County on the Flint River (USDOI, 2002). Buford Dam forms Lake Lanier and is the primary source of water to Atlanta. Navigation dams include Andrews and George Dams on the Chattahoochee River and the Jim Woodruff Dam on the Apalachicola River. The Apalachicola River flows unimpeded to the Gulf of Mexico after the Jim Woodruff Dam (USGS, 2004).

Dams in the SRB are used for hydroelectric power, navigation, and flood control (fig. 2). Large hydroelectric dams on the Savannah River mainstem include Hartwell, Richard B. Russell, and J. Strom Thurmond Dams near Augusta, Georgia; others also are located in the upper SRB on the Tallulah and Keowee Rivers (USDOI, 2002). The New Savannah Bluff Lock and Dam is located near Augusta, Georgia.

Dams in the PDRB are used for hydroelectric power, water supplies, and flood control (fig. 2). Hydroelectric power dams include Lake Robinson on Black Creek, Blewett Falls and Tillery on the Pee Dee River, and Yadkin Falls and Tuckertown on the Yadkin River (USDOI, 2002). Dams used for water supplies are present on many tributaries of the Pee Dee River, and a series of six reservoirs, known as the Yadkin chain lakes near Salisbury, North Carolina, impound a 80-kilometer section of the Yadkin River (NCDENR, 2001; North Carolina Wildlife Resources Commission, 2005).

Water Quality Impairments and Fish Consumption Advisories

States are required to list a water body as impaired if water quality standards for designated beneficial uses of that water are not attained pursuant to Section 303(d) of the Clean Water Act. Many water bodies in the MORB, ARB, SRB, and PDRB are listed as impaired because of low oxygen concentrations, metals, pathogens, bacteria, toxic chemicals, siltation, and acidity. The review of 303(d) listed waters for this report focused primarily on those associated with rivers and reservoirs in the MORB, ARB, SRB, and PDRB.

Impairment of water bodies has been attributed to agricultural, municipal, and industrial pollutant sources in the Tombigbee, Black Warrior, Cahaba, Coosa, Tallapoosa, Alabama, and Mobile Rivers of the MORB (Alabama Department of Environmental Management (ADEM), 2003; Mississippi Department of Environmental Quality (MDEQ), 2003). Multiple small creeks that flow into the Tombigbee River were considered impaired because of fecal coliform, excess nutrients, low dissolved oxygen, siltation, and pesticides. The Tombigbee River is listed as impaired near McIntosh because of Hg in sediments and in Pickens County because of iron (Fe) from an abandoned surface mine (ADEM, 2003). Impairments in the Black Warrior River, a tributary of the Tombigbee River, include pathogens from urban runoff and pasture grazing; excess nutrients and siltation from agriculture; industrial and municipal runoff; and altered habitat and metals (for example, aluminum, iron, arsenic (As)) from abandoned surface mines (ADEM, 2003). Common impairments in the Cahaba, Coosa, and Tallapoosa Rivers included excess nutrients from municipal and industrial sources, surface mining, and agriculture, siltation, pathogens from urban runoff and pasture grazing, and organics (for example, PCBs) from urban runoff, industrial and municipal sources (ADEM, 2003). Impairments in the Alabama River include pesticides from an unknown source; low dissolved oxygen; and excess nutrients from dam construction and flow modifications (ADEM, 2003). In the lower Mobile River, water bodies are impaired from fecal coliform, low dissolved oxygen, and excess nutrients from urban runoff and municipal sources, but multiple reaches have been designated as impaired because of Hg from unknown sources (ADEM, 2003).

Water quality impairments have been identified in the Chattahoochee, Flint, and Apalachicola Rivers of the ARB. Most impairments were for fecal coliform and poor health of fish populations, but potential sources or causes of these impairments were not defined (Florida Department of Environmental Protection (FDEP), 2004; GDNR, 2002; USEPA, 2006). Many small tributaries of these larger rivers also were

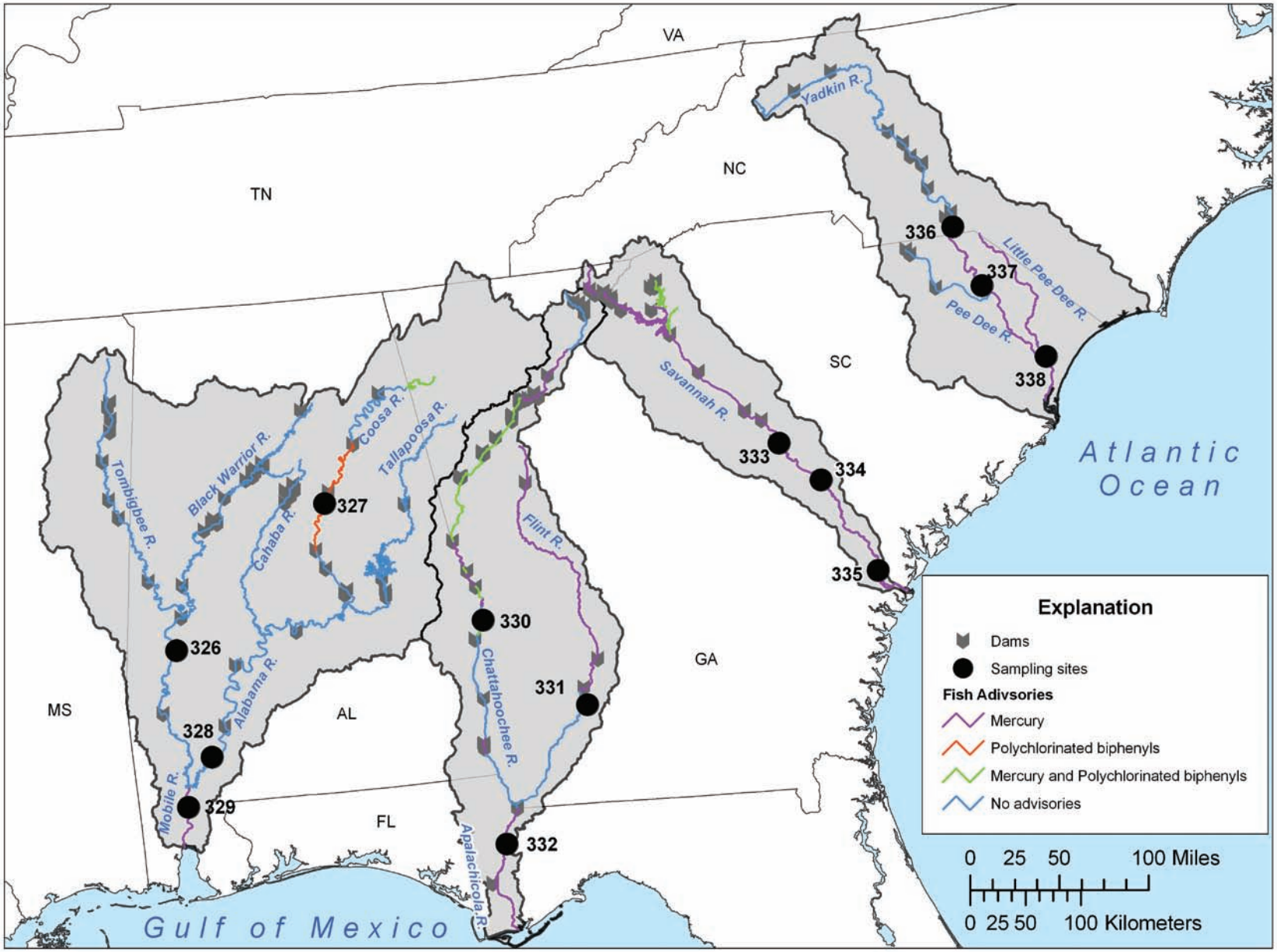


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Figure 2. Dams and fish consumption advisories to protect human health in the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins. Sites sampled in 2004 also are shown. See table 5 for station descriptions.

listed as impaired because of organic enrichment or low dissolved oxygen (GDNR, 2002; USEPA, 2006). The Chattahoochee River was impaired for PCBs from Morgan Falls Dam to Franklin, Georgia, and Oliver Dam to Upatoi Creek in Muscogee County and for Hg from Soquee River to Lake Lanier, Georgia (USEPA, 2006). Some tributaries of the Flint River also were listed as impaired by copper (Cu) and zinc (Zn) from urban runoff (GDNR, 2002; USEPA, 2006). The Apalachicola River had impairments for fecal coliform, low dissolved oxygen, and Hg (FDEP, 2004). Helms and others (2005) concluded that fish health decreased as watershed urbanization increased in the Chattahoochee River.

Most SRB waters listed as impaired were from fecal coliform, organic enrichment, low dissolved oxygen, and poor health of fish populations (USEPA, 2006). Sources include urban runoff and municipal sources. The mainstem of the Savannah River was impaired for Hg from J. Strom Thurmond Lake to downstream from Highway 17, and impairments for Cu also were listed for some tributaries (USEPA, 2006). The source of Hg in this area is thought to be atmospheric deposition (GDNR, 2001). Lake Hartwell and the nearby Seneca River in the upper SRB were impaired for PCBs from a capacitor manufacturer (Brenner and others, 2004).

Most impairments in the PDRB were for fecal coliform, low dissolved oxygen, organic enrichment, metals, and siltation (NCDENR, 2003; SCDHEC, 2002; USEPA, 2006). Potential sources of these impairments include urban runoff, agriculture, municipal sources, and nonpoint source pollution (NCDENR, 2003). Impairments for Cu and Zn were listed for small tributaries of the Pee Dee River, but the Little Pee Dee River from the North Carolina/South Carolina state line to it's confluence with the Pee Dee River, the Lumber River, and the Lynches River were listed as impaired for Hg (SCDHEC, 2002; USEPA, 2006). Atmospheric deposition has been given as a potential Hg source in this area.

Fish consumption advisories to protect human health provide a useful indicator of water quality. Consumption advisories because of Hg and PCBs have been issued for several large rivers and reservoirs in the MOBR, ARB, SRB, and PDRB. Potential sources of Hg in the MORB, ARB, and SRB include chemical manufacturing plants and coal-fired power plants (ADPH, 2005; GDNR, 2005; SCDHEC, 2005b). Sources of PCBs in the Coosa River of the MORB, the Chattahoochee River of the ARB, and Lake Hartwell and its connected waters in the SRB include chemical manufacturing, production of electrical capacitors and transformers, for pressure treating lumber, and for paper manufacturing (ADPH, 2005; GDNR, 2005; SCDHEC, 2005b).

Fish consumption advisories were limited to the Coosa and lower Mobile Rivers in the MORB (fig. 2; ADPH, 2005; GDNR, 2005). The upper Coosa River in Georgia has consumption advisories for PCBs in largemouth bass (*Micropterus salmoides*), striped bass (*Morone saxatilis)*, blue catfish (*I. furcatus*), smallmouth buffalo (*Ictiobus bubalus*), and channel catfish (*I. punctatus*) and Hg and PCBs in spotted bass (*M. punctatus*; GDNR, 2005). The Coosa River between Neely Henry Dam and Lay Dam has consumption advisories for catfish (*Ictalurus* spp.), striped bass, crappie (*Pomoxis* spp.), and spotted bass because of PCBs (ADPH, 2005). Consumption advisories for Hg in largemouth bass have been issued for several water bodies in the lower MORB (ADPH, 2005).

Many fish consumption advisories have been issued for the Apalachicola, Flint, and Chattahoochee Rivers (fig. 2; FDH, 2005; GDNR, 2005). A Hg advisory for the Apalachicola River affects flathead catfish (*Pylodictis olivaris*), largemouth bass, bluegill sunfish (*Lepomis macrochirus*), bowfin (*Amia calva*), and gar (*Lepisosteus* spp.; FDH, 2005). The Flint River has consumption advisories for Hg in largemouth bass and shoal bass (*M. cataractae*) from its headwaters south of Atlanta to Albany, Georgia (GDNR, 2005). Largemouth bass and channel catfish in Lake Blackshear and largemouth bass in Lake Chehaw also were included in these Hg advisories. The Chattahoochee River has multiple Hg advisories for various species including largemouth bass, spotted bass, striped bass, channel catfish, and jumprock sucker (*Moxostoma* sp.) from the Buford Dam to near Columbus, Georgia. Mainstem reservoirs of the Chattahoochee River including Lake Lanier, Lake Harding, Goat Rock Lake, Lake Oliver, and Lake Andrew have Hg consumption advisories for many species. PCB advisories have been issued in the Chattahoochee River from Morgan Falls Dam to West Point Lake and Oliver Dam to Upatoi Creek near Columbus, Georgia, for various species including common carp (*Cyprinus carpio*), bluegill, striped bass, and bullhead catfish and in mainstem reservoirs (for example, Lake Harding, West Point Lake, Goat Rock Lake, and Lake Oliver) for numerous species (GDNR, 2005).

The SRB has fish consumption advisories for Hg and PCBs (fig. 2). Most of the Savannah River has a Hg advisory issued from Lake Ruban and Lake Hartwell in the upper SRB to the mouth of the river for multiple species including largemouth bass, spotted sucker, white catfish (*Ameiurus catus*), and bowfin (GDNR, 2005; SCDHEC, 2005b). Consumption advisories for PCBs in bass and channel catfish have been issued for Lake Hartwell in upper SRB (GDNR, 2005; SCDHEC, 2005b). The 12 Mile Creek and Seneca River Arm of Lake Hartwell have PCB advisories for all fish (SCDHEC, 2005b).

Fish consumption advisories for Hg have been issued in the PDRB (fig. 2). The Pee Dee River from North Carolina and South Carolina state line to its mouth has consumption advisories issued for Hg in largemouth bass and bowfin (SCDHEC, 2005b). North Carolina has not issued any Hg advisories but suggests not consuming largemouth bass, chain pickerel (*Esox niger*), and bowfin (North Carolina Department of Health and Human Services, 2005). Mercury advisories also have been issued for chain pickerel, bowfin, flathead catfish, and largemouth bass in the Little Pee Dee River and Lynches River (SCDHEC, 2005b).

Primary Sources of Contaminants to the MORB, ARB, SRB, and PDRB

The rich soils, abundant forests, and warm climate of the southeastern United States have resulted in agriculture, forestry, mining, and manufacturing being important economic factors in the MORB, ARB, SRB, and PDRB. Low dissolved oxygen, pathogens, habitat alteration, sediment, turbidity, siltation, and toxic chemicals are some of the factors linked to these industries that have contributed to declines in water and habitat quality (ADEM, 2003; Western Center for Environmental Information, 2005; NCDENR, 2003). The lower MORB has one of the largest concentrations of primary industrial manufacturers ($n > 15$) along the Gulf of Mexico, four of which have been designated as Superfund sites by the USEPA, that have released organochlorine chemicals into the basin (USFWS, 1996). Mine drainage has been monitored in the MORB, and chemicals used in the forest industry to manufacture paper, lumber, and furniture have been released into the MORB, ARB, and PDRB (GDNR, 1997a; 1997b; 2001). Pesticides used in agricultural and heavily-populated residential areas of MORB, ARB, SRB, and PDRB enter water systems as runoff. The livestock and poultry production is intensive in the ARB, SRB, and PDRB with multiple CAFOs that produce large amounts of animal waste and byproducts that can enter nearby streams and rivers (Burkholder and others, 2007). All of these industries are major sources of contaminants to the MORB, ARB, SRB, and PDRB.

Agriculture

Alabama, Georgia, South Carolina, and North Carolina are among the top U.S. producers of peanuts, pecans, peaches, vegetables, melons, and tobacco (U.S. Department of Agriculture (USDA), 2006). Various herbicides, insecticides, and fungicides are applied in agricultural areas within the MORB, ARB, SRB, and PDRB (appendix 2) and have affected water quality in some areas (Atkins and others, 2004). Many pesticides are used on cotton in the lower ARB, SRB, and PDRB, soybeans and peanuts in the lower SRB and PDRB, corn in the lower SRB, tobacco in the lower PDRB (USGS, 2003). Pesticides including atrazine, simazine, metolachlor, tebuthiuron, fluometuron, nonflurazon, and 2,4-D have been detected in streams and ground water in the MORB (Frick and others, 1998; Gilliom and others, 2006; Hoos and others, 2002; McPherson and others, 2003), and certain organochlorine pesticides (for example, dieldrin, *cis*-chlordane, *p,p'*-DDE, *p,p'*-DDT, *cis*-nonachlor, heptachlor epoxide) frequently were detected and reflected land use (Zappia, 2002). Various pesticides including bentazon, paraquat, 2,4-DB, methanearsonate, alachlor, and pendimethalin commonly are applied in agricultural areas of the ARB from March to October, which overlaps the spawning time for many fish species. In addition, herbicides (imazapyr, dicamba, 2,4-D, 2,4-DP, glyphosate, sulfometuron, hexazinone, triclopyr, picloram, atrazine, and sethoxydim) and insecticides (for example, dimethoate, malathion, acephate, carbaryl, lindane, and chlorpyrifos) have been used extensively in silvicultural areas of the ARB (Stell and others, 1995).

Alabama, Georgia, South Carolina, and North Carolina are among the top U.S. poultry producers (USDA, 2006), and poultry, swine, and cattle production is intensive in the northern MORB, ARB, SRB, and PDRB with many CAFOs in these areas. Animal wastes from production facilities also can affect water quality in the basins by increasing nutrient loads (that is, nitrogen and phosphorus; Frick and others, 1998). Other pollutants including antibiotics, pathogens, pesticides, veterinary pharmaceuticals, natural and synthetic hormones, and heavy metals (Cu and Zn) also have been associated with manure-related discharges at CAFOs (Burkholder and others, 2007), and feedlot effluent has been shown to have adverse effects on fish (for example, Orlando and others, 2004) and mammals (for example, Gray and others, 2006).

Mining and Extractive Industries

Nonfuel mineral production is a large contributor to the economies of Alabama ($805 million), Georgia ($1,770 million), South Carolina ($507 million), and North Carolina ($758 million; Kramer and others, 2006). Most mining activities in the ARB, SRB, and PDRB are limited to construction minerals including clay, shale, sand, gravel, stone, cement, and dimension stones (USDOI, 2002). Other mining activities include bentonite, sulfur, silicon, and strontium plants in the MORB, kaolin extractions near Georgetown (ARB) and Augusta (SRB), Georgia, and lime facilities near Birmingham, Alabama (MORB; USDOI, 2002). Two other facilities near Birmingham produce iron oxide pigments, which are used in paints and coatings for architectural and industrial applications (USDOI, 2002). Coal mining is the predominant natural resource extracted from the MORB, and Alabama ranks 15th in coal production in the United States (Johnson and others, 2002). Four coal fields in Alabama (Plateau, Warrior, Cahaba, and Coosa) are part of the Great Appalachian coal basin which yield high-volatile bituminous coal (Johnson and others, 2002). The coal industry is estimated to contribute $1.97 billion to the Alabama economy (Alabama Coal Association, 2006). Most of the coal in Alabama (72%; 16 million tons) is mined underground, and total reserves were estimated to be 2.8 billion tons (U.S. Department of Energy (USDOE), 2004). Four of the top coal consumers (Georgia-Pacific Corporation, International Paper Company, Lafarge North America, Drummond Company Incorporated) have plants in Alabama (USDOE, 2004). Many abandoned strip mines are located in the upper Black Warrior and Coosa River Basins and are potential sources of acid mine drainage and sediment (Johnson and others, 2002).

Industrial and Municipal Sources

Federal law requires that permits be issued for companies to discharge wastewater into rivers. Industries that manufac-

ture, process, or use toxic chemicals are required to annually report releases of these chemicals. Chemical contaminants from chemical plants, pulp and paper mills, textile mills, electrical power plants, and wastewater treatment plants have been released into the MORB, ARB, SRB, and PDRB and have affected water quality (fig. 3). Most permitted point sources in the four basins were municipal wastewater treatment plants (USEPA, 2005a), but most non-permitted releases were from industrial sources including chemical and textile manufacturing facilities and pulp and paper mills (USEPA, 2005b). Releases described below are from 2003 and limited to water.

Chemical manufacturing plants with large releases of multiple chemicals are located in the MORB, ARB, SRB, and PDRB (fig. 3). Large quantities [>100 kilograms (kg)] of chemicals including nitrates, ammonia, glycol ethers, tert-butyl alcohol, diethanolamine, ethylene glycol, ethylbenzene, fomesafen, methanol, naphthalene, and acifluorfen sodium salt were released from chemical plants at Hamilton and Columbus, Mississippi, in the Tombigbee River and at McIntosh, Bucks, and Axis, Alabama, in the Lower Mobile River Basin (USEPA, 2005b). In addition, a chemical plant at Axis, Alabama, is the largest surface water discharger of the fungicide bis(tributyltin) oxide. Large quantities of creosote (54,431 kg) and polycyclic aromatic hydrocarbons (PAHs; 5,443 kg) were reported as single releases to surface waters of the Tombigbee River from the chemical plant at Columbus, Mississippi. Smaller quantities (10–100 kg) of aniline, methyl acrylate, triethylamine, molinate, acetonitrile, molybdenum trioxide, thiourea, atrazine, methanol, toluene, acrylamide, methyl isobutyl ketone, chlorine, chromium (Cr), and xylene were released from plants at McIntosh or Bucks. In the ARB, large quantities (>1,000 kg) of ammonia, ethylene glycol, triethylamine, methanol, and n,n-dimethylformamide were released from a chemical plant at Albany, Georgia. Smaller quantities (<150 kg) of sec-butyl alcohol, ammonia, n-butyl alcohol, and formaldehyde were released into a tributary of the Coosa River from a chemical plant at Cedartown, Georgia. Cyclohexane (18 kg), benzo-(*g,h,i*)-perylene (186 kg), polycyclic aromatic compounds (277 kg), Zn (338 kg), and ammonia (3,044 kg), and nitrates (1,415,010 kg) were released into the Savannah River from a chemical plant in Augusta, Georgia. Large quantities of 1,4-dioxane (3,779 kg), acetaldehyde (872 kg), methanol (151 kg), and ethylene glycol (151 kg) were released into the Pee Dee River at Florence, South Carolina (USEPA, 2005b).

The pulp and paper industry is an important economic factor in the southeastern United States. Pulp and paper mills in the MORB, ARB, SRB, and PDRB have released large quantities of chemicals (fig. 3; USEPA, 2005b). Chemicals that commonly were released from SRB pulp and paper mills in large quantities (>500 kg) included ammonia, nitrates, manganese, barium, Zn, methanol, formic acid, formaldehyde, and acetaldehyde; chemicals released in smaller quantities (<500 kg) included vanadium, lead (Pb), catechol, polycyclic aromatic compounds, methyl ethyl ketone, and xylene (USEPA, 2005b). In the MORB, pulp and paper mills are located at Columbus, Mississippi, and Childersburg, Demopolis, Jackson, Pennington, Perdue Hill, Pine Hill, Prattville, and Selma, Alabama. In the ARB, pulp and paper mills release chemicals into the Chattahoochee River at Cedar Springs, Georgia, and the Flint River at Oglethorpe, Georgia. Pulp and paper mills are located at Augusta, Port Wentworth, Rincon, and Savannah, Georgia, in the SRB and at Bennettsville and Florence, South Carolina, in the PDRB.

Coal-fired power plants have released chemicals including Hg into waters of the southeastern United States. In the MORB, coal-fired power plants are located at Bucks, Forkland, Gadsden, Leroy, Parrish, Quinton, and Wilsonville, Alabama, and Rome, Georgia (fig. 3). Large quantities (>300 kg) of Cu, Zn, barium, As, vanadium, and selenium (Se) were released from these power plants in 2003, and smaller quantities (<100 kg) of manganese, nickel (Ni), cobalt, Cr, Pb, and thallium also were reported (USEPA, 2005b). Coal-fired power plants in the ARB are located at Albany, Catersville, Newman, and Smyrna, Georgia (fig. 3). Large releases of chemicals (>400 kg) including Cu, Ni, manganese, Zn, vanadium, As, and barium only were reported from the plants at Catersville, Newnan, and Smyrna, Georgia. In the SRB, large chemical releases (>100 kg) from coal-fired power plants located at Beech Island, South Carolina, and Port Wentworth and Rincon, Georgia, were not reported in 2003, but 87 kg of Hg were released from the power plant at Beech Island. Coal-fired power plants in the PDRB at Hartsville and Conway, South Carolina, reported few chemical releases in 2003 (USEPA, 2005b).

Other facilities reporting fewer chemical releases in the southeastern United States included textile manufacturers, wood processing plants, military facilities, metal foundries, food manufacturers, and gasoline distributors. Chemicals associated with the textile industry were released into waters of the SRB and PDRB. Decabromodiphenyl oxide (663 kg) and antimony (205 kg) were released into a tributary of the Savannah River at Abbeville, South Carolina (USEPA, 2005b). A textile plant at Society Hill, South Carolina, released nitrates (99,790 kg), naphthalene (4,990 kg), ammonia (3,084 kg), 1,2,4-trimethylbenzene (435 kg), and glycol ethers (50 kg) into the Pee Dee River. Decabromodiphenyl oxide, a polybrominated diphenyl ether (PBDE) flame retardant, (380 kg) was released into the Yadkin River in the upper Pee Dee River Basin from a textile plant in Elkin, North Carolina (USEPA, 2005b).

Dioxins, which are toxic to aquatic organisms, also were released in 2003 (fig. 3). In the MORB, dioxins were released into Alabama waters at Bay Minette [>500 grams (g)], Brierfield (75 g), Huxford (>500 g), and Northport (41 g), and smaller quantities (≤1 g) were released at Demopolis, Jackson, Mobile, Pennington, Pine Hill, and Selma, Alabama, and Columbus, Mississippi (USEPA, 2005b). Dioxin releases were <3 g in the ARB at East Point and Oglethorpe, Georgia; the SRB at Augusta, Port Wentworth, and Savannah, Georgia; and the PDRB at Florence, South Carolina (USEPA, 2005b).

The Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) develops requirements for

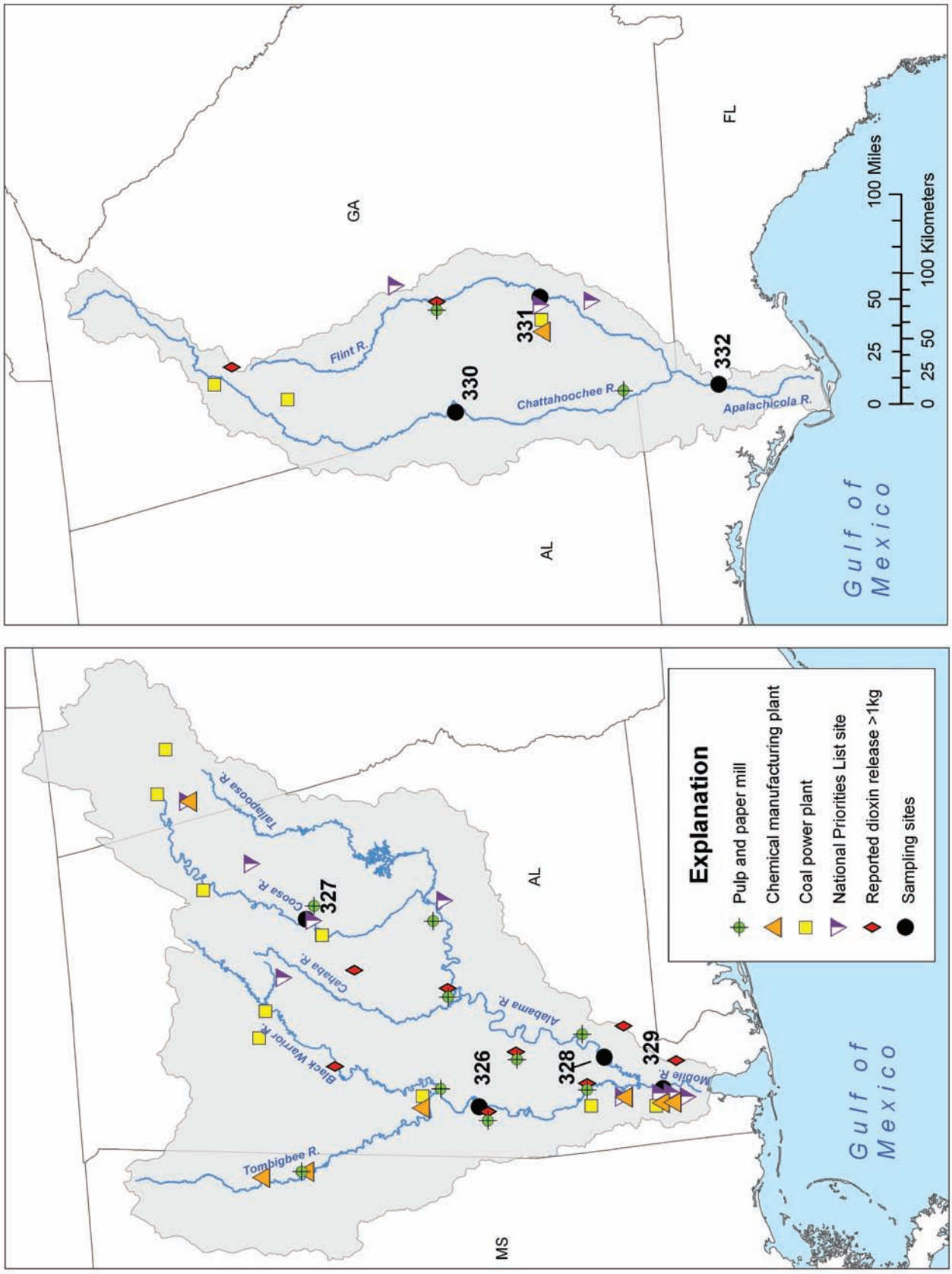
closed or abandoned hazardous waste sites and provides funds to clean up sites where potentially responsible parties are either not viable, unavailable, or refuse to participate. National Priorities List (NPL) sites are designated under CERCLA and identify media including soil, sediment, surface water, ground water, and sludge that is contaminated by chemicals (USEPA, 2005c). Ten sites in the MORB are on the NPL (fig. 3). These sites include chemical manufacturers at Axis, Bucks, McIntosh, and Montgomery, Alabama; military facilities at Anniston and Childersburg, Alabama; a lead smelter and battery recycling facility at Leeds, Alabama; a trucking terminal at Saraland, Alabama; and a lead smelter and an oil pitch at Cedartown, Georgia (USEPA, 2005c). An NPL-caliber site, a site that has not been designated as an NPL site but cleanup is underway, also has been identified at a historical PCB manufacturing site at Anniston. The ARB has five listed NPL sites (all in Georgia) including military facilities at Albany, pesticide manufacturers at Albany and Fort Valley, a tire manufacturer at Albany, and a wood preserving facility at Camilla. The SRB has two listed NPL sites, all of which are in Georgia, including a USDOE nuclear facility (Savannah River Site) at Aiken and a capacitor manufacturer at Pickens. The PDRB has three listed NPL sites (fig. 3). These sites include a wood treating facility in Florence, South Carolina, a chemical manufacturer for textiles in Salisbury, North Carolina, and an oil recycling and antifreeze manufacturer in Cordova, North Carolina (USEPA, 2005c).

Extant Sources of Information on Contaminants in the Southeast River Basins

Water quality in the MORB, ARB, SRB, and PDRB has been studied because of concerns for threatened and endangered species, chemical discharges from various industrial manufacturers and power plants, and urban and agricultural runoff. Multiple Federal agencies including the USEPA, USFWS, and USGS conducted studies in the region to determine the extent and effects of contamination on water quality and on the species inhabiting the MORB, ARB, SRB, and PDRB. Federal monitoring programs within the USFWS and USGS include the National Contaminant Biomonitoring Program (NCBP) and the National Water Quality Assessment (NAWQA) Program. Most of these studies were limited to comparing chemical contaminant concentrations in fish tissues to literature-based toxicity thresholds. Few studies have been conducted exclusively to examine fish health in the MORB, ARB, SRB, or PDRB.

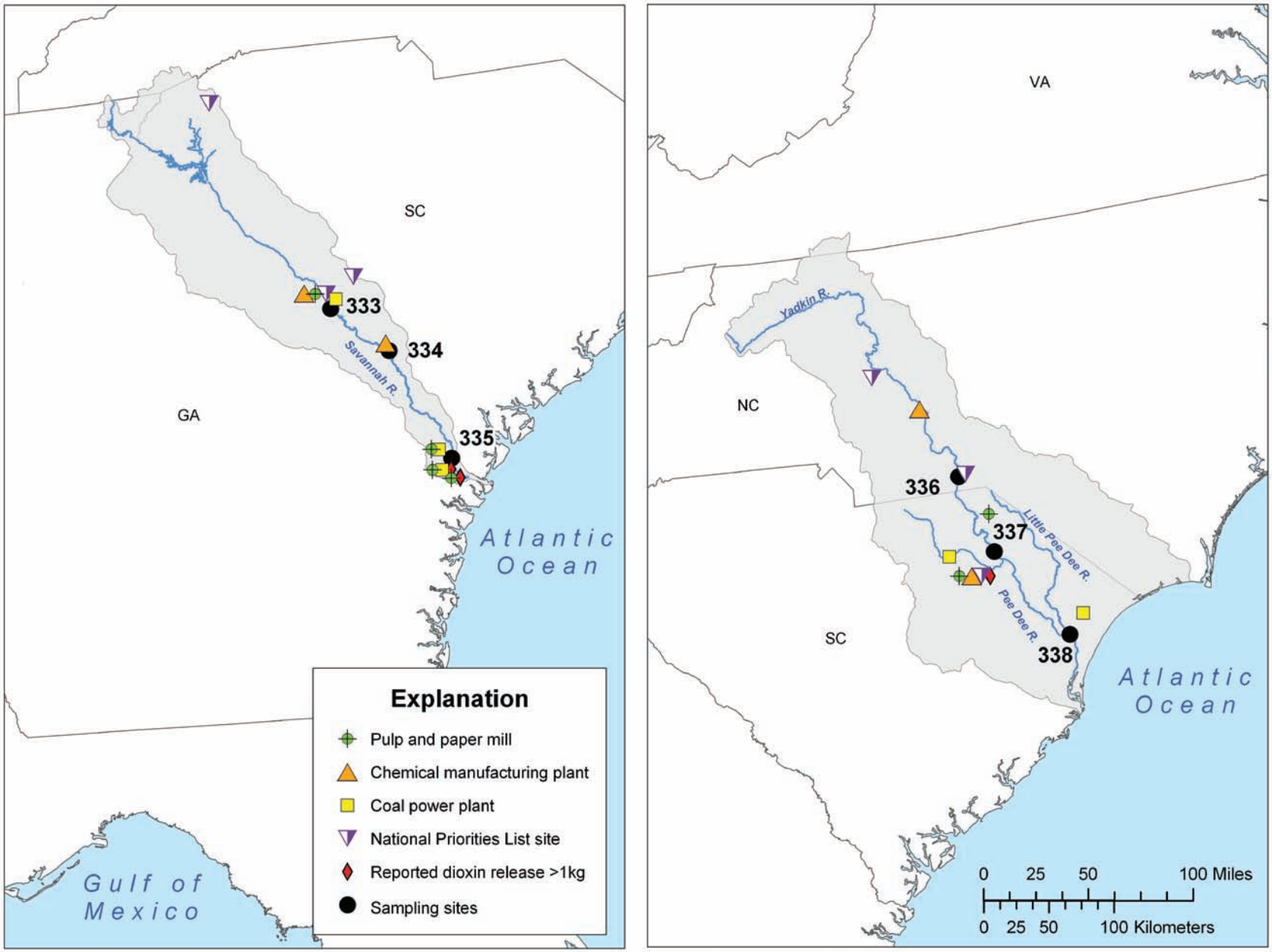
The main objective of the NCBP was to document temporal and spatial trends of organochlorine and inorganic concentrations in fish throughout the United States (Schmitt and others, 1999). The program reported concentrations of many persistent contaminants such as organochlorine pesticides, PCBs, and Hg were decreasing in whole-body fish samples from 107 locations across the United States by the mid-1980s. Historical NCBP concentration data from 1970 to 1986 are available for fish from locations in the MORB (Tombigbee River at McIntosh, Alabama; Alabama River at Chysler, Alabama), ARB (Apalachicola River at J. Woodruff Dam, Florida), SRB (Savannah River at Savannah, Georgia), and PDRB (Pee Dee River at Johnsonville, South Carolina; Schmitt and others, 1999). Most organochlorine residues and inorganic contaminant concentrations in fish have decreased since the 1970s, but chemical manufacturers and pulp and paper mills have contributed to high organochlorine chemicals in fish from some basins. In 1986, mean concentrations of total 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene [DDT; >0.5 microgram per gram (µg/g)], pentachloroanisole (PCA; 0.01 µg/g), and hexachlorobenzene (HCB; 0.24 µg/g) in fish from the Tombigbee River near McIntosh, Alabama, were among the highest measured in the United States. Also in the MORB, the second highest mean PCA concentration in the United States (>0.03 µg/g) was in fish from the Alabama River at Chysler, Alabama. Trace concentrations of mirex in fish from the Savannah River at Savannah, Georgia (0.01 µg/g), could be from insecticide applications to control invasive red fire ants (*Solenopsis wagneri*; Schmitt and others, 1999).

The NAWQA Program of the USGS examines water quality in river basins and ground water systems in the United States and has study units in the MORB and ARB. The NAWQA program studied water quality in the Mobile River Basin from 1998 to 2001 and reported elevated concentrations of nutrients and pesticides and environmental stress of biological communities (Atkins and others, 2004; Zappia, 2002). Atkins and others (2004) concluded that the degraded water quality conditions in the basin were the result of increased urban development and population density and runoff from urban and agricultural areas. Animal wastes and commercial fertilizers were the primary sources of nitrogen and phosphorus, and insecticide and herbicide applications on farmland and residential areas have resulted in widespread occurrence of multiple pesticides ($n = 69$) in streams. Herbicides including atrazine, simazine, metolachlor, tebuthiuron, and 2,4-D accounted for most of the pesticide detections in surface water, but pesticide concentrations generally were low [<0.05 microgram per liter (µg/L)] and varied seasonally (McPherson and others, 2003). Dieldrin (<0.01–0.72 µg/g), *cis*-chlordane (<0.01–0.07 µg/g), *p,p'*-DDE (<0.0–0.55 µg/g), *p,p'*-DDT (<0.01–0.39 µg/g), *cis*-nonachlor (<0.01–0.03 µg/g), heptachlor epoxide (<0.01–0.90 µg/g), and PCBs (0.05–0.90 µg/g) were the most frequently detected organochlorine contaminants in fish from the MORB, and detection frequency reflected land use (Zappia, 2002). Concentrations of *cis*-chlordane, *p,p'*-DDT, and PCBs exceeded protective guidelines for wildlife in some whole-fish tissue samples (Atkins and others, 2004; Zappia, 2002). Arsenic [34 µg/g dry weight (dw)], Cr (>120 µg/g dw), Pb (156 µg/g dw), Ni (49 µg/g dw), and Zn (514 µg/g dw) concentrations in streambed sediment exceeded toxicity guidelines for aquatic life (Zappia, 2002). Lead and Zn were associated with the steel industry in the Birmingham, Alabama, area. Chloroform, tetrachloroethylene, trichloroethylene, *cis*-1,2-dichloroethene, methyl *tert*-butyl ether, benzene,



Base from U.S. Geological Survey National Atlas, 1:2,000,000
North America Albers Equal Area Conic projection

Figure 3. Potential chemical contaminant sources in the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins from permitted and non-permitted release data (USEPA, 2005a; 2005b; 2005c). See table 5 for station descriptions.



Base from U.S. Geological Survey National Atlas, 1:2,000,000
North America Albers Equal Area Conic projection

Figure 3. Potential chemical contaminant sources in the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins from permitted and non-permitted release data (USEPA, 2005a; 2005b; 2005c). See table 5 for station descriptions.—Continued

and toluene were the most frequently detected volatile organic compounds in urban streams of the MORB.

The NAWQA Program studied the water quality of the ARB from 1992 to 1995 and reported that much of the organochlorine and elemental contaminant load in ARB waters was from storm water runoff from impervious surfaces in urban areas and local and regional industrial emissions (Frick and others, 1998). Pesticide distribution in sediments and aquatic biota was related to land use patterns in the ARB (Stell and others, 1995). Frick and others (1998) reported that pesticides (for example, atrazine, simazine, metolachlor, fluometuron, norflurazon) frequently were detected in urban streams, and some pesticide concentrations exceeded protective guidelines for aquatic life. Storm runoff is the primary pathway for pesticides in urban and agricultural areas to enter stream and rivers of the ARB. Relatively large amounts of pesticides (>15 pound per acre; 1,680 kilogram per square kilometer) were applied to peanut and cotton crops in the ARB, and approximately 3.2 million pounds (1.45 million kg) of pesticides were applied annually to other minor crops such as pecans, peaches, melons, and cucumbers (Frick and others, 1998). Hoos and others (2002) reported that <5% of the estimated mass of pesticides applied annually to agricultural areas in the Flint River Basin was transported to the Flint River, but the preemergent herbicides including atrazine, metolachlor, fluometuron, and nonflurazon had the greatest probability of being transported to water after application. Nutrient concentrations were high in waters draining urban and poultry land use areas but were generally lower in waters draining forests and croplands. Bed sediment concentrations of Hg, cadmium (Cd), Pb, and Zn in urban waters increased as the percentage of industrial land and transportation corridors increased (Frick and others, 1998), although concentrations of elemental contaminants in sediments have declined since the 1970s presumably as a result of emission regulations. Frick and others (1998) did not measure chemical contaminant concentrations in biota but did assess biological indicators of water quality. Fish populations were healthiest in watersheds draining forests and poorest in watersheds draining poultry and urban areas; macroinvertebrate communities in urban areas were severely degraded as a result of high stormflows, poor habitat, and the presence of insecticides (Frick and others, 1998).

From 1999 to 2002, the USGS measured concentrations of organic wastewater contaminants (OWCs), including fire retardants, animal steroids, disinfectants, high-use domestic pesticides, PAHs, solvents, and antioxidants, in water samples from treated effluent sites (for example, water pollution control plants and poultry processing plants), tributary streams, wastewater treatment plants, and mainstem river sites in the upper Chattahoochee River Basin (Frick and Zaugg, 2003). The greatest number of OWCs were detected in treated effluent samples, whereas samples from wastewater treatment plants had the fewest OWC detections. Although few OWCs have human or ecological health criteria, 12 samples exceeded aquatic life criteria for the insecticides diazinon (0.56 µg/L), carbaryl (0.4 µg/L), and chloropyrifos (0.1 µg/L), which are compounds with known or suspected potential to disrupt endocrine function (Frick and Zaugg, 2003).

The USEPA has conducted several national contaminant studies in freshwater fish. As a follow-up to the National Dioxin Study, the USEPA conducted a one-time screening study, the National Study of Chemical Residues in Fish (NSCRF; previously referred to as the National Bioaccumulation Study) to determine the occurrence and prevalence of selected bioaccumulative chemical contaminants (for example, dioxins, furans, *p,p'*-DDE, chlordanes, dieldrin, mirex, PCBs, and Hg) in freshwater fish from riverine systems. Contaminant tissue concentrations also were correlated with known chemical point sources (USEPA, 1992). Point sources included pulp and paper mills, wood preserving facilities, refineries, and wastewater treatment plants. Fish species including carp, spotted sucker, and largemouth bass were collected from multiple NSCRF sampling sites located within the MORB, ARB, SRB, and PDRB. The NSCRF concluded that the primary chemicals of concern based on presence were *p,p'*-DDE, PCBs, and Hg. Concentrations of PCBs and dieldrin were elevated at multiple sites, including the Coosa River at the Alabama/Georgia state line and the Chattahoochee River at Austell, Georgia, and posed the greatest risk to humans (USEPA, 1992).

In 1998, the USEPA initiated a national contaminant survey to estimate the distribution of selected bioaccumulative chemicals in freshwater fish from lakes and reservoirs (USEPA, 2004a; 2005d). Chemical contaminants in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB were included in this study. Concentrations of aldrin (<0.004 µg/g), α-hexachlorocyclohexane (HCH; <0.01 µg/g), γ-HCH (<0.002 µg/g), endosulfan I (<0.004 µg/g), endosulfan II (<0.04 µg/g), endosulfan sulfate (<0.01 µg/g), endrin (<0.01 µg/g), hexachlorobenzene (<0.333 µg/g), pentachlorobenzene (<0.666 µg/g), toxaphene (<0.1 µg/g), and As (<0.1 µg/g) were less than the detection limits in all samples from the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Concentrations of other organochlorine contaminants including *p,p'*-DDT (<0.002–0.005 µg/g), *cis*-chlordane (<0.004–0.02 µg/g), *trans*-chlordane (<0.004–0.006 µg/g), *cis*-nonachlor (<0.004–0.022 µg/g), *trans*-nonachlor (<0.004–0.017 µg/g), heptachlor (<0.002–0.010 µg/g), heptachlor epoxide (≤0.002 µg/g), oxychlordane (<0.004–0.007 µg/g), β-HCH (<0.004–0.005 µg/g), δ-HCH (<0.004–0.008 µg/g), dieldrin (<0.001–0.007 µg/g), methoxychlor (<0.02–0.11 µg/g), mirex (<0.004–0.005 µg/g), pentachloroanisole (<0.004–0.094 µg/g), and 2,3,7,8-TCDD (<0.1–0.3 picograms per gram; pg/g) were detected in few samples or generally were low. Concentrations of *p,p'*-DDD were low in piscivorous species (<0.002–0.005 µg/g) but were greater in benthivorous species in the MORB (<0.002–0.012 µg/g), ARB (<0.002–0.010 µg/g), and SRB (<0.002–0.018 µg/g). Concentrations of *p,p'*-DDE were <0.002–0.287 µg/g in benthivorous whole-body samples and <0.002–0.036 µg/g in piscivorous fillet samples; concentrations generally were greatest in samples from SRB reservoirs. Total PCB concentrations were 0.007–0.435 µg/g in benthivorous whole-body

samples and <0.001–0.033 µg/g in piscivorous fillet samples, and concentrations in samples from MORB reservoirs generally were the greatest. Relatively high Hg concentrations were measured in benthivorous whole-body samples (0.017–0.356 µg/g) and piscivorous fillet samples (0.087–0.822 µg/g) from all three basins.

The USFWS conducted contaminant studies in the Lower MORB. In 1989, two USFWS studies were initiated to determine if activities at chemical manufacturing facilities in McIntosh, Alabama, affected water quality and biota in the Tombigbee River (USFWS, 1989a; 1989b). The Olin Chlor Alkali Products McIntosh Plant, which produced chlorine, caustic soda, sodium hypochlorite, dilute sulfuric acid, hydrogen, and salt, discharged Hg as a waste product into unlined containment ponds where Hg concentrations in sludge were 111–498 µg/g (USFWS, 1989a). Sediments and fish (largemouth bass and channel catfish) were collected to determine if Hg present in river sediments near the Olin facility outfall was bioavailable to endemic river biota. The USFWS study concluded that Hg concentrations were greatest near the Olin facility outfall (0.95 µg/g in bass and 0.15 µg/g in channel catfish) compared to other sampling sites (0.20–0.26 µg/g in bass and 0.04–0.10 µg/g in channel catfish) and that Hg concentrations in largemouth bass could be harmful to piscivorous wildlife and humans (USFWS, 1989a). Directly upstream from the Olin facility is the Ciba-Geigy facility (currently known as Ciba Specialty Chemical), which produced specialty chemicals such as industrial organic chemicals, pesticides, agricultural chemicals, and synthetic resins. The facility opened in 1952, and DDT was manufactured until 1963, contaminating ground water and surrounding surface environment. Facility wastes were disposed into on-site landfills and an open burning area; DDT and its metabolites were detected in soils and sediment near the burning area (USEPA, 2004b). The USFWS study concluded that total DDT concentrations in bass were greatest near the Ciba-Geigy facility outfall (8.9 µg/g) compared to other sampling sites (0.95–4.44 µg/g), and total DDT concentrations in largemouth bass could be harmful to piscivorous wildlife and humans (USFWS, 1989b). The NCBP conducted a follow-up study at McIntosh, Alabama, in 1990 to further assess the area for Hg and DDT contamination (USFWS, 1992). The NCBP study reported that DDT concentrations were high in largemouth bass (0.34–34.52 µg/g) and exceeded protective criteria for wildlife and humans. Mercury concentrations in largemouth bass from the Olin outfall were lower (0.07–0.50 µg/g) than those from the 1989 USFWS study (0.95 µg/g), but continued to exceed concentrations in fish from reference areas.

The USEPA conducted a study of the Mobile River to assess contamination entering the Mobile-Tombigbee River system from four NPL sites including the McIntosh Plant of the Olin Corporation (McIntosh, Alabama), the McIntosh Plant of the Ciba-Geigy Corporation (McIntosh, Alabama), the LeMoyne Plant of the Stauffer Chemical Site (Axis, Alabama), and the Cold Creek Plant of the Stauffer Chemical Site (Bucks, Alabama) in 1993 and 1994 (USEPA, 1995). The study reported that Hg and *p,p'*-DDT and its metabolites were the primary chemicals of concern in sediment, water, and fish tissue, and total DDT concentrations in largemouth bass and channel catfish fillets exceeded the state criteria for human consumption. Mean whole-body concentrations of total DDT and Hg were 0.08–0.49 µg/g and 0.10–0.75 µg/g, respectively, in bass and 0.04–0.33 µg/g and 0.04–0.13 µg/g, respectively, in channel catfish. A fish health assessment indicated that fish were in good condition with the exception of encysted parasitic worms in spleen, liver, and kidney tissues. The USEPA concluded that the sites and nearby wetlands were potential contaminant sources to the Mobile-Tombigbee River system and recommended that monitoring be continued until Hg and *p,p'*-DDT (and its metabolites) concentrations decrease to acceptable levels (USEPA, 1995).

The USFWS also studied the uptake of contaminants, particularly Hg and DDT, in largemouth bass and sediment from the lower Tombigbee and Mobile Rivers (USFWS, 1996). Sampling locations were located upstream and downstream from the Boise-Cascade pulp and paper mill at Jackson, Alabama, the Olin and Ciba-Giegy floodplain at McIntosh, Alabama, and Stauffer Chemical Cold Creek Swamp site at Axis, Alabama. Mean Hg concentrations in largemouth bass were 0.18–0.48 µg/g with the highest concentrations (>0.44 µg/g) in fish upstream from the Boise-Cascade pulp and paper mill and downstream from Stauffer's Cold Creek Swamp. Mean total DDT concentrations in largemouth bass were 0.18–21.16 µg/g with the highest concentrations in fish downstream from the Olin and Ciba-Geigy flood plain. The USFWS recommended that monitoring of sediment and biota should continue at these locations and that biomarker studies of aquatic species were warranted to assess the risk of these bioaccumulative contaminants to higher trophic levels. In 1995 and 1996, Adair and others (2003) measured Hg concentrations in kidney tissue of prothonotary warblers (*Protonotaria citrea*) nesting sites near the Olin and Ciba-Giegy facilities at McIntosh, Alabama, and concluded that Hg concentrations in adult and nestling warblers posed minimal risk to their reproduction and survival.

Fish tissue monitoring also has been conducted by the Alabama Department of Environmental Management (ADEM, 1996). Mercury, PCBs, DDT, chlordane, and dioxin were named the primary contaminants of concern in regards to fish consumption advisories. Concentrations of contaminants did not exceed Federal Drug Administration (FDA) action levels in fish tissues from the Alabama, Apalachicola, Cahaba, Chattahoochee, Choctawhatchee, and Escatawpa Rivers. A consumption advisory was issued in the Coosa River from the Logan Martin Dam to Lay Dam because of PCB contamination. The ADEM concluded that three reservoirs (Lay Lake, Lake Jordan, and Lake Mitchell) in the Coosa River were the primary areas of PCB contamination from historical manufacturing at facilities in Anniston, Alabama. High Hg concentrations in largemouth bass were of concern in the Mobile River, and a consumption advisory was issued for the Cold Creek Swamp, which received Hg from the Stauffer Chemical facil-

ity. The ADEM also assessed fish condition as part of their monitoring program and noted that >91% of channel catfish, largemouth bass, and spotted bass had no external lesions. External lesions that were identified primarily were sores on the fins or body surface that probably were caused by bacterial infections; no sampling location was noted for having large numbers of fish with external anomalies (ADEM, 1996).

Materials and Methods

Monitoring Methods Overview

A suite of chemical and biological methods including reproductive biomarkers, measures of cytochrome P450 enzyme induction, fish health assessments, tissue histopathology, and chemical analyses of fish carcasses was used to characterize the exposure of fish to contaminants and the effects of exposure (table 2). Concurrent determination of tissue residue concentrations along with the suite of fish health, immune system responses, and reproductive assessments, supports the interpretation of relations between exposure and biological responses.

Multiple organochlorine chemical residues and elemental contaminants were measured in the whole-body fish composite samples (table 3). These analytes were selected to provide the maximum amount of information on accumulative contaminants of interest at minimal cost and to maintain continuity with the historical NCBP database. Instrumental analyses of specific planar halogenated hydrocarbons (PHHs), such as polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners, were excluded because of their high cost of analysis. Instead, extracts of the composite samples were screened with the H4IIE rat hepatoma cell bioassay (Tillitt and others, 1991; Whyte and others, 2004), which responds to planar PHHs (table 4). Ethoxyresorufin *O*-deethylase (EROD) activity, which indicates recent exposure to exogenous aryl hydrocarbon receptor (AhR) ligands including planar halogenated hydrocarbons (PHHs) and PAHs, were measured in the livers of individual fish (Kennedy and Jones, 1994; Pohl and Fouts, 1980; Whyte and others, 2000). Together these assays and analyses allowed for the estimation of the relative concentrations of potential biological effects of PHHs and PAHs without the expense of instrumental analyses for these compounds (table 4).

Measurements of fish health, immune system responses, and reproductive status were included in the suite of indicators to examine potential impacts from non-accumulative contaminants and contaminant mixtures (table 2). Fish health indicators included gross observations for abnormalities, condition and organosomatic indices, and histopathological examination (Goodbred and others, 1997; Hinton, 1993; Hinton and others, 1992). Gross observations and determination of indices based on relative fish and organ sizes such as condition factor (CF), hepatosomatic index (HSI), and splenosomatic index (SSI) are relatively simple and indicative of cumulative, organism-level changes (Grady and others, 1992). However, these health indicators are non-specific in terms of causal mechanisms yet may reflect early, subtle alterations and foreshadow subsequent effects at the individual- or population-level.

The SSI is an indicator of overall organism health, as well as a measure of immune system stress. Other immune system indicators included the measurement of macrophage aggregates (MA) in preserved spleen tissue samples (table 2). Macrophage aggregates, also known as melanomacrophage centers, are discrete aggregations of pigment-bearing macrophages present in spleen, kidney, and sometimes liver of advanced teleosts (Agius, 1980). Pigmented cell accumulations also can occur in the gonad and other tissues. These specialized cells are thought to be responsible for centralizing foreign material and debris for destruction, detoxification or reuse, storing waste products, contributing to the immune response, and storing/recycling Fe (Ellis and others, 1976; Ferguson, 1976). Macrophage aggregate measurements have responded to contaminant exposure in field and laboratory studies, although they may be affected by a variety of factors (reviews by Wolke, 1992 and Blazer and others, 1997).

Measures of reproductive condition included plasma vitellogenin (vtg) and sex steroid hormone concentrations, gonadosomatic index (GSI), and gonadal histopathology (table 2). Contaminants, particularly estrogen mimics, have been shown to impact reproduction in laboratory and field studies, although the reproductive condition in fish can be affected by many factors including gender, age, reproductive stage, season, and water temperature (Allen and others, 1999; Gimeno and others, 1998). Estrogen mimics are capable of stimulating the production of vtg, a precursor of yolk protein, in the livers of oviparous vertebrates, and several endocrine disrupting compounds have been shown to induce abnormal vitellogenesis (Servos, 1999; Tyler and others, 1998). Vitellogenin production normally is associated with female fish, but vtg can be produced in males if exposed to estrogen or an estrogen-like chemicals. The detection of concentrations typical of early- to mid-vitellogenic females in male fish has been associated with exposure to exogenous estrogens (Bowman and others, 2002; Denslow and others, 1999; Folmar and others, 1996; 2000; 2001), but low vtg concentrations in males may be normal for certain species (Gross and others, 2002). Vitellogenin was measured in male and female fish. The GSI and gonadal histopathology [reproductive stage, presence of atretic oocytes, and intersex condition (presence of female reproductive tissue in males or *vice-versa*)] also were assessed as measures of reproductive health and status. The GSI relates the proportional size of the gonad to the body size and may reflect changes resulting from a variety of physiological factors such as reproductive stage and environmental factors, including exposure to contaminants. Elevated occurrence of atretic (unfertilized, reabsorbed, or both) eggs has been noted in fish exposed to contaminants (Cross and Hose, 1988; Johnson and others, 1988), although other factors also may be involved. Feminiza-

Table 2. Methods used to characterize fish exposure to contaminants.

Method	Description	Tissue(s) examined	Sensitivity	Primary reference(s)
Histopathology	Microscopic examination for the presence of lesions; can provide early indication of chemical exposure	Liver, gill, gonads, spleen, and kidney	Overall organism health and contaminants	Hinton and others (1992); Hinton (1993); Goodbred and others (1997)
Ethoxyresorufin *O*-deethylase (EROD) activity	Enzyme induction by planar hydrocarbons	Liver	PCBs; chlorinated dioxins and furans; PAHs	Pohl and Fouts (1980); Kennedy and Jones (1994); Whyte and others (2000)
Macrophage aggregate analysis	Macrophages are important in the immune system, serving as a first line of defense for the organism and as an antigen processing cell	Spleen	Multiple contaminants including PAHs and metals	Blazer and others (1994; 1997)
H4IIE bioassay	A screening tool to determine the presence of certain classes of planar halogenated compounds	Whole fish (composite samples)	PCBs; chlorinated dioxins and furans	Tillitt and others (1991); Whyte and others (2004)
Vitellogenin	A precursor of egg yolk, normally synthesized in the liver of female fish	Blood plasma	Endocrine-modulating substances	Denslow and others (1999)
Steroid hormones (17β-estradiol and 11-ketotestosterone)	Determine reproductive health and status	Blood plasma	Endocrine-modulating substances	Guillette and others (1994); Goodbred and others (1997)
Chemical analyses	Organochlorine chemical residues and elemental contaminants	Whole fish (composite samples)	Specific analytes	Schmitt and others (1999)
Somatic indices	The relative mass of some organs is often indicative of chemical exposure	Gonads, spleen, liver	Overall organism health	Grady and others (1992)
Necropsy-based fish health assessment	Visual assessment of external/internal anomalies (for example, lesions, parasites, tumors), which may indicate contaminant-related stress	All	Overall organism health	Goede (1988, 1996); Adams and others (1993)

Table 3. Organochlorine chemical residues and elemental contaminants measured in whole-body fish composite samples

Contaminant class and analyte	Chemical name(s) or atomic symbol	Principal uses and sources to aquatic ecosystems
Organochlorine chemicals		
p,p'-DDE	2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene	DDT-metabolite
p,p'-DDD (TDE)	2,2-bis (*p*-chlorophenyl)-1,1-dichloroethane	Insecticide; DDT-metabolite
p,p'-DDT	2,2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane	Insecticide
o,p'-DDE	2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1-dichloroethylene	*o,p'*-DDT metabolite
o,p'-DDD (TDE)	2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1-dichloroethane	*o,p'*-DDT metabolite
o,p'-DDT	2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane	*p,p'*-DDT impurity
Total polychlorinated biphenyls (PCBs)	Mixture containing as many as 209 mono- through nona-chloro-substituted biphenyl congeners.	Dielectric, hydraulic, and transformer fluids; lubricants; extenders; de-dusting agents; carbonless copy paper
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene	Insecticide
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,8,8a-hexahydro-1,4-endnnno-exo-5,8-dimethanonaphthalene	Insecticide; aldrin metabolite
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahdyro-1,4-endo-endo-5,8-dimethanonaphthalene	Insecticide; isodrin metabolite
Heptachlor epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene	Heptachlor metabolite; technical chlordane constituent/ metabolite
cis-Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene (1-α,2-α,3a-α,4-β,7-β,7a-α)	Insecticide; technical chlordane constituent
trans-Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a- hexahydro-4,7-methano-1H-indene(1-α,2-β,3a-α,4-β, 7-β,7a-α)	Technical chlordane constituent
cis-Nonachlor	1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a- hexahydro-4,7-methano-1H-indene (1-α,2-α, 3-α, 3a-α, 4-β, 7-β, 7a-α)	Technical chlordane constituent
trans-Nonachlor	1,2,3,4,5,6,7,8,8-nonachloro-2,3,3a,4,7,7a- hexahydro-4,7-methano-1H-indene(1-α,2-β,3-α, 3a-α,4-β,7-β,7a-α)	Technical chlordane constituent
Oxychlordane (octachlor epoxide)	2,3,4,5,6,6a,7,7-octachloro-1a,1b,5,5a,6, 6a-hexahydro-2,5-methano-2H-indeno(1,2-b)oxirene (1a-α,1b-β,2-α,5-α,5a-β,6-β,6a-α)	cis-Chlordane metabolite
Heptachlor	1H-1,4,5,6,7,8-8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	Insecticide
Methoxychlor	1,1'-(2,2,2-trichloroethylidene)-bis[4-methoxybenzene]	Insecticide
Toxaphene	Chlorinated camphene mixture averaging 62% chlorine by weight	Insecticide; herbicide
α-Hexachlorocyclohexane (HCH)	1,2,3,4,5,6-hexachlorocyclohexane	Constituent of insecticide mixture containing various HCH isomers; also known as α-benzene hexachloride (BHC)
β-HCH	1,2,3,4,5,6-hexachlorocyclohexane	Technical HCH (BHC) constituent
δ-HCH	1,2,3,4,5,6-hexachlorocyclohexane	Technical HCH (BHC) constituent

Table 3. Organochlorine chemical residues and elemental contaminants measured in whole-body fish composite samples.—Continued

Contaminant class and analyte	Chemical name(s) or atomic symbol	Principal uses and sources to aquatic ecosystems
γ-HCH (Lindane)	1,2,3,4,5,6-hexachlorocyclohexane	Insecticide; technical HCH (BHC) constituent
Hexachlorobenzene (HCB)	Perchlorobenzene	Fungicide; industrial intermediate
Pentachlorobenzene	Chlorinated benzene	Fungicide; fire retardant
Pentachloroanisole	Chlorinated benzene	Metabolite of pentachlorophenol
Endosulfan I (α-Endosulfan)	6,9-methano-2,4,3-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a	Pesticide
Endosulfan II (β-Endosulfan)	6,7,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodiozathiepin-3-oxide, (3α, 5aα, 6β, 9β, 9aα)	Pesticide
Endosulfan sulfate	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano,2,4,3-benodiatiepin 3,3-dioxide	Endosulfan byproduct
Dacthal	dimethyl-2,3,5,6-tetrachlorobenzene-1,4-dicarboxylic acid	Herbicide; may contain dioxin (2,3,7,8-TCDD) and HCB as impurities
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachloro- octahydro-1,3,4-metheno-1H-cyclobuta(cd)pentalene	Insecticide; fire retardant
Elemental contaminants		
Arsenic	As	Industrial sources; herbicides; defoliants
Cadmium	Cd	Mining, smelting and other industrial sources; urban runoff; sewage discharges
Chromium	Cr	Mining, tanning, and other refractory and chemical industrial sources
Copper	Cu	Mining, smelting and other industrial sources
Lead	Pb	Mining, smelting and other industrial sources; urban runoff; atmospheric pollution; fishing sinkers; lead shot
Mercury	Hg	Herbicides; fungicides; pulp, paper, and textile effluents; open-cycle chloralkali cells; landfills; mining; atmospheric pollution
Nickel	Ni	Mining, smelting, and other industrial sources
Selenium	Se	Coal-fired powerplants; irrigation return flows
Zinc	Zn	Mining, smelting and other industrial sources; urban runoff

Table 4. Monitoring and assessment strategy for polycyclic aromatic and planar halogenated hydrocarbons.

[PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran; PAH, polycyclic aromatic hydrocarbon; GC-ECD, gas chromatography with electron-capture detection; + responds; – does not respond; EROD, 7-ethoxyresorufin *O*-deethylase; *AhR-active isomers and congeners only]

Endpoint (tissue)	Contaminants		
	PCBs	PCDDs & PCDFs	PAHs[a]
GC-ECD (carcass)	+	-	-
EROD activity (liver)	*	*	*
H4IIE bioassay[b] (carcass)	*	*	-

[a] And other planar organic compounds.

[b] H4IIE bioassay was performed after reactive cleanup to remove aryl hydrocarbon receptor-active PAHs.

tion of male fish (that is, intersex condition) has been reported in laboratory and field studies of contaminants (Allen and others, 1999; Gimeno and others, 1997; 1998; Jobling and others, 1998) and in previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). Reproductive biomarkers can vary with temperature, photoperiod, and annual reproductive cycle. To minimize the effect of these factors on the reproductive biomarkers, all fish were collected post-spawn.

Sampling and Field Procedures

Largemouth bass (henceforth bass; $n = 237$) and common carp (henceforth carp; $n = 209$) were collected at 13 sites from 4 river basins in the southeastern United States by electrofishing (table 5). Four sites were located in the MORB and three each were located in the ARB, SRB, and PDRB. Sites were chosen to represent a range of contaminant sources (for example, industrial, agricultural, and urban areas). Fish were collected post-spawn between October and December 2004 (table 5). These species were targeted because of their widespread distribution and the abundant contaminant, health indicator, and reproductive biomarker data available. Several sucker species (*Moxostoma* sp. and *Minytrema* sp.) also were collected from Station 334 but were not included in this report. Adult fish of similar size were targeted at each site to reduce variation because of age; however, the age of fish varied. Fish were held in aerated live-wells until processed (usually less than 3 hours). All collection, handling, and euthanasia procedures followed animal care and use guidelines (American Fisheries Society and others, 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 examined for abnormalities. Pieces of liver were collected and immediately flash-frozen in liquid nitrogen 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). Upon completion of the internal examination and dissection, otoliths (asterisci in carp, sagitta in bass) and scales were collected for age determination (Berg and Grimaldi, 1967; Casselman, 1990; Cowan and others, 1995). Remaining tissues (those not frozen or fixed) were replaced into the body cavity; the entire fish was wrapped in aluminum foil and frozen for analysis of organochlorine chemical and elemental contaminants and 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 liquid nitrogen 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.

Laboratory Analysis

Fish samples were shipped frozen on dry ice to the USGS Columbia Environmental Research Center (CERC) and stored frozen (-20 °C) until analyzed. CERC performed analyses of composite fish samples for organic and elemental contaminants and completed quality assurance (QA) and quality control (QC) procedures described in Hinck and others (2006b, 2006c). Fish were homogenized and lyophilized by Laboratory and Environmental Testing, Inc. (Columbia, Missouri) according to protocols provided by CERC. Cryogenically frozen liver samples for EROD analysis also were shipped on dry ice to CERC for analysis. Cryogenically frozen plasma samples were similarly shipped to the Center for Environmental and Human Toxicology of the University of Florida for vtg analysis and the Florida-Caribbean Science Center of the USGS for sex steroid hormone analysis. Preserved tissue samples were shipped to the National Fish Health Laboratory of the USGS Leetown Science Center for histopathological analysis. Information on these latter procedures are given by Blazer and others (2002) and McDonald and others (2002). Age determination was conducted by the USGS South Carolina Cooperative Fish and Wildlife Research Unit (Clemson, South Carolina). Transverse section of otoliths were processed for age determination following modified procedures from Cowan and others (1995)

Table 5. Sampling location, collection date, and number of fish collected in 2004.

[Stations are grouped by basin and ordered upstream to downstream]

Basin and river	Station number	Nearest city	Collection dates	Latitude, Longitude	Number of fish collected	Bass		Carp	
						Male	Female	Male	Female
Mobile River Basin									
Tombigbee	326	Lavaca, AL	10/12–10/13	32°15'53.60"N, 88°00'44.21"W	38	8	11	9	10
Coosa	327	Childersburg, AL	10/14–10/15	33°19'57.76"N, 86°21'55.87"W	39	10	10	11	8
Alabama	328	Eureka Landing, AL	10/6–10/7	31°23'14.06"N, 87°42'42.19"W	40	10	10	10	10
Mobile	329	Bucks, AL	10/8–10/9	31°03'15.85"N, 87°59'48.07"W	40	8	12	11	9
Apalachicola-Chattahoochee-Flint River Basin									
Chattahoochee	330	Omaha, GA	10/25–10/26	32°13'19.80"N, 84°55'35.10"W	37	10	10	7	10
Flint	331	Albany, GA	10/27–10/28	31°34'34.86"N, 84°08'49.80"W	36	10	10	7	9
Apalachicola	332	Blountstown, FL	11/2	30°25'58.20"N, 85°01'17.10"W	40	10	10	10	10
Savannah River Basin									
Savannah	333	Augusta, GA	11/30–12/1	33°22'00.18"N, 81°56'46.44"W	30	7	3	10	10
Savannah	334	Sylvania, GA	12/2–12/3	33°01'16.86"N, 81°31'04.50"W	25	4	5	9	7
Savannah	335	Port Wentworth, GA	12/6–12/7	32°13'26.34"N, 81°08'47.04"W	40	10	10	10	10
Pee Dee River Basin									
Pee Dee	336	Rockingham, NC	11/4–11/5	34°53'22.14"N, 79°51'24.89"W	17	3	13	0	1
Pee Dee	337	Pee Dee, SC	11/6–11/7	34°21'23.22"N, 79°41'35.19"W	39	11	11	10	7
Pee Dee	338	Bucksport, SC	11/8–11/9	33°42'18.09"N, 79°11'24.00"W	25	11	10	2	2

and Casselman (1987, 1990). Annual growth increments (annuli) were designated at the distal edge of an opaque zone imbedded between two translucent zones. As collection period varied and samples were examined in the blind, ages were reported as the number of increments regardless of time of year. Scales were processed for age determination when otoliths were unavailable by estimating age (years) from the number of completed annuli (Berg and Grimaldi, 1967; Hesthagen, 1985).

Composite Sample Preparation

Individual fish were partly thawed, cut into pieces, and ground to a fine texture. Fifteen percent of the total body weight was subsampled (18–1,186 g) to maintain the proportional size representation of each fish in a composite sample. The ground subsamples were grouped by site, species, and gender and re-ground to create a homogenous composite sample. The composite sample was then subsampled (~200 g) and re-frozen (-20 °C). All equipment was disassembled and chemically cleaned between composite samples to prevent cross contamination. Subsamples were used for analysis of moisture content and elemental contaminants (~100 g), lipid content and organochlorine chemical residues (~10 g), and the H4IIE bioassay (~10 g; Hinck and others, 2006c). 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 Station 336.

Elemental Contaminants and Moisture Content

Subsamples for elemental analyses (~100 g) were freeze-dried, and percent moisture was determined as weight lost during lyophilization. One part of the dried material was digested in nitric acid and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) for the determination of Cd, Cr, Cu, Fe, Pb, Ni, and Zn. A homogenized aliquot of each dried sample (~0.25 g) was heated with 6 mL of nitric acid in a sealed low-pressure Teflon® vessel in a microwave oven. The cooled digestate liquid was transferred to a 125 mL polyethylene bottle and ultrapure water (> 10 megOhm/cm) was added, yielding a final mass of 101.5 g (100 mL). The final acid matrix was 6% HNO_3. All samples were diluted 10 times by a CETAC ASD-500 auto-diluter as part of the analytical sequence. Internal standards were germanium (50 nanogram per gram; ng/g), rhodium (10 ng/g), and thorium (10 ng/g). The external standard consisted

of a NIST traceable reference solution to which five elements (praseodymium, terbium, thulium, tantalum, and gold) were added for improved calibration in the rare earth region of the mass spectral range. A second part (~0.5 g) was dry-ashed [magnesium nitrate-nitric acid-hydrochloric acid (HCl)] and analyzed by hydride generation atomic absorption spectroscopy for As and Se. The dry ashing procedure consisted of three steps: 1) boiling with nitric acid for solubilization and partial oxidation; 2) 500 °C ashing with magnesium nitrate to complete the oxidation and decompose remaining organic matter; and 3) heating with HCl to dissolve the ash and reduce Se^{+6} to the Se^{+4} oxidation state required for hydride generation. Digestates were diluted following the HCl reduction to ~100 mL with de-ionized water that yielded a final acid matrix of 10% HCl. The digestates were mixed with HCl carrier solution and reduced by sodium tetrahydridoborate, which was stabilized with sodium hydroxide. The resulting volatile hydrogen selenide or arsenide was transferred with argon carrier gas into a heated quartz cell mounted on an atomic absorption spectrophotometer for decomposition and measurement. A third part was analyzed directly for total Hg using thermal combustion, amalgamation, and atomic absorption spectroscopy.

Quality assurance (QA) measures for elemental determinations included the analysis of reagent blanks, replicate samples, certified reference materials, and fortified samples. Nominal limits-of-detection (LODs) were 0.03 µg/g dw for As, 0.05 µg/g dw for Hg, 0.06 µg/g dw for Se, 0.04 µg/g dw for Cd, Cr, and Pb, and 0.4 µg/g dw for Cu, Ni, and Zn (appendix 3). Elemental concentrations (including LODs) were converted from dw to wet-weight (ww) for statistical analysis and reporting using the moisture content of each sample, which ranged from 67 to 76%.

Organochlorine Contaminants and Lipid Content

One subsample (~10 g) of each composite was solvent-extracted and analyzed gravimetrically for lipid content (range: 2–11%) and by high-resolution capillary gas chromatography with electron capture detection (GC-ECD). The analytical procedure began with blending anhydrous sodium sulfate with the composite subsample. Targeted chemicals were then extracted from the dried sample with dichloromethane. The extract was quantitatively split into portions for H4IIE bioassay (80%), OCP/PCB/toxaphene analyses (8%), percent-lipid determination (2%), and archive (10%). The analytical portion was spiked with the following chemical standards to track method recoveries: PCB 029 (2,4,6-trichlorobiphenyl) for early-eluting PCBs (Cl_1 - Cl_3); PCB 155 (2,2',4,4',6,6'-hexachlorobiphenyl) for mid-range eluting congeners (Cl_4 - Cl_6), PCB 204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl) for later-eluting PCBs (Cl_7 - Cl_{10}), and d_8-DDD for pesticides. Quality assurance measures for the organochlorine analyses included the analysis of triplicate and fortified samples, use of internal standards to monitor recoveries of each sample, and the confirmation of residue identities by dual-column gas chromatography-electron capture detection (appendix 4).

The analytical part of the extract was purified by removing interfering co-extracted lipids and biogenic materials before the gas chromatographic quantification. Most interferences were removed by low-pressure size-exclusion chromatography (LPSEC). High performance SEC (HPSEC) was used to remove residual interferences. A two-layered octadecyl silica/activated silica gel column was used to separate the organochlorine pesticide residues from the PCBs before gas chromatography (GC) analysis.

Dual column GC-ECD allowed for confirmation of 29 organochlorine pesticide residues and total PCBs (appendix 4). Total PCBs were calculated and reported as the sum of 112 congeners. Total toxaphene concentrations were determined by quantifying 20 component peaks in a technical toxaphene standard. Nominal LODs were ≤2.4 ng/g for individual compounds, 61 ng/g for total PCBs, and 10 ng/g for toxaphene (appendix 4).

H4IIE Rat Hepatoma Cell Bioassay

Subsamples for H4IIE analysis were kept frozen at CERC until the initiation of sample processing. Samples were thawed, homogenized, and extracted from a column with methylene chloride. Percent lipid was determined on 1% portion of the extract. The remainder was concentrated and cleaned up by two-stage column chromatography. Extracts were evaporated, re-dissolved with isooctane and ampulated. Matrix QC of prepared samples (blanks and spikes) included ground tissues from laboratory-raised bluegill and samples of a CERC standard positive control tissue (carp from Saginaw Bay, Michigan).

The H4IIE rat hepatoma cells were seeded at 23,000 cells/well in 300 µL of D-MEM culture media (Tillitt and others, 1991) and allowed to proliferate for 24 hours. The cells were then dosed with sample extracts or standards in isooctane and incubated for 72 hours to allow for maximal EROD induction. A standardized TCDD solution was used to generate an analytical dose-response curve. A total of six dose-response curves were analyzed on each assay date. A linear regression was performed on each sample well to obtain the slope and estimate the rate of the reaction (picomols resorufin formed per minute; pmol/min). The amount of protein in each well was determined by the fluorescamine assay (Lorenzen and Kennedy, 1993). The reaction rate observed in each well was normalized according to the measured protein content, generating a value of specific activity (pmols resorufin formed per minute per mg of protein; pmol/min/mg). The reaction rate in each well was then divided by the measured dose given to each well (gram equivalents/mg) to result in specific activity per min per gram equivalent (g.eq.) dosed. Reported results are the average of four replicate concentrate doses. The mean EROD reaction rate (pmol/min/g.eq.) was divided by the average initial slope obtained for the TCDD standard curves, resulting in a measure of an equivalent dose of TCDD (TCDD-EQ; pg/g) for each sample. Assay LODs ranged from 0.3 to 1.2 pg/g, and the limits of quantification (LOQs) ranged from 1.0 to 2.5

pg/g. Dioxin-like activity was <LOQ in 39 of 50 samples; a TCDD-EQ could not be calculated for one composite sample (male carp from Station 337).

Hepatic EROD Activity

Cryogenically frozen liver samples were stored at -80 °C by CERC until the preparation of microsomal fractions, which were used the same day they were prepared. The kinetic microsomal assays were conducted in 96-well microtiter plates (Whyte and others, 2000). Triplicate determinations of EROD activity were performed on 10 μL portions of each microsomal preparation, and mean EROD activity was reported. Protein content was determined using the fluorescamine protein assay (Lorenzen and Kennedy, 1993) in the same 96-well microtiter plate as the EROD analyses. A positive control material, liver microsomes of male Sprague Dawley rats injected with 500 mg/kg of Aroclor 1254, also was analyzed. A linear regression was performed on the data from each well to determine an EROD rate (pmol/min) along with its associated estimate of variance. Protein content was used to normalize EROD activity (pmol/min/mg) in each well. The LOD was calculated by adding the average basal EROD rate to three times the standard deviation of that rate, and the LOQ was calculated by adding the average basal EROD rate to ten times the standard deviation of that rate. LODs ranged from 0.2 to 1.9 pmol/min/mg, and the LOQs ranged from 0.6 to 1.9 pmol/min/mg. Hepatic EROD activity was <LOQ in 66 of 445 samples.

Fish Health Indicators

General Histopathological Analyses

Tissues preserved in 10% NBF (liver, kidney, spleen, gill, gonad, and grossly visible lesions) were shipped to the USGS Leetown Science Center and prepared for routine histopathological analysis (Blazer and others, 2002). Tissue sections (5 to 6-μm, on glass slides) were stained with hematoxylin and eosin (H & E) for light microscopic examination.

Quantitative Organism-Level Indicators

Gross pathologies were selected for consistency with other monitoring programs that have used this type of assessment (Fournie and others, 1996). Gross abnormalities included grossly visible disorders of the eye (exophthalmia, hemorrhage, opacity, emboli, missing), opercles (shortening, deformities, parasites), and body surface (ulcers, parasites, discolored areas or raised growths). In addition, disorders of the fins and skeleton were included. Numerical values were assigned to internal and external observations of lesions recorded in the field, and a necropsy-based fish health assessment index (HAI) score was calculated for each fish by summing these values for all organs (Blazer and others, 2002). The HAI score can range from 0 to 220.

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) X 100; splenosomatic index (SSI) = spleen weight/(total body weight – gonad weight) X 100; gonadosomatic index (GSI) = gonad weight/total body weight X 100. The weight of the gonads was subtracted from the body weight to minimize the effect of the reproductive cycle on the HSI and SSI.

Macrophage Aggregates

Macrophage aggregates were quantified using computer-based image analysis. Macrophage aggregates in splenic tissues were stained with the Perl's Prussian Blue method (Luna, 1992), which enhances visualization of the pigments in the MAs. The total viewed area was 2 mm^2 per spleen section. MA measurements included the number of aggregates in a mm^2 of tissue (MA-#) and the area of each aggregate (MA-A). The percentage of tissue occupied by MA (MA-%) was computed from these measurements (Blazer and others, 2002).

Reproductive Biomarkers

Gonadal Histopathology

Pieces of gonadal tissue were preserved in 10% NBF. Transverse sections were processed for routine light microscopy (embedded in paraffin, sectioned at 5 to 6 μm, and stained with H & E). Female gonadal tissue was staged using developmental stages (designated 0–5) to classify each section (Blazer, 2002; McDonald and others, 2002; Nagahama, 1983; Rodriguez and others, 1995; Treasurer and Holliday, 1981). Fish ovaries typically contain oocytes in several developmental stages and were classified according to the maturity of the predominant stage of oogenesis in each tissue sample. Ovaries containing only undeveloped, previtellogenic oocytes were assigned stage 0 (immature). Samples containing only previtellogenic chromatin nucleoli and perinuclear oocytes, identified by cytoplasm that stained basophilic with H & E, were assigned stage 1 (previtellogenic). Ovaries containing previtellogenic oocytes as in stage 1 plus some cortical alveoli oocytes were assigned stage 2 (early vitellogenic). Those containing larger oocytes in which the cortical alveoli were pushed to the periphery of the cell, yolk globules filled the center, and the chorion of the developing oocytes were thicker than in earlier stages were assigned stage 3 (mid-vitellogenic). Ovaries containing oocytes with fused yolk globules were assigned stage 4 (late vitellogenic). Ovaries containing post-ovulatory follicles, which can be observed for some time after ovulation, were assigned stage 5 (spent). After the ovarian tissues were staged

they were examined further by light microscopy for atresia and other abnormalities. One hundred oocytes in each fish sample were counted when possible. Those showing morphological evidence of resorption or necrosis were quantified, and the percent of atretic oocytes were calculated.

Analogous to the procedure used to stage ovaries, male gonadal tissue was classified into developmental stages (0–4) according to the maturity of the predominant stage of spermatogenesis of each tissue sample (Blazer, 2002; Nagahama, 1983). Immature, undeveloped, or regressed testes containing only spermatogonia were assigned stage 0 (immature); those containing primarily spermatocytes and spermatids were assigned stage 1 (early spermatogenic). Testes containing approximately equal proportions of spermatocytes, spermatids, and spermatozoa were assigned stage 2 (mid-spermatogenic), and testes containing primarily mature spermatozoa were assigned stage 3 (late spermatogenic). Post-spawning or spent testes were assigned stage 4. Testicular tissue also was examined microscopically for any abnormalities such as intersex. Male fish were classified as intersex when individual or small foci of undeveloped oocytes were observed within testicular tissue (that is, when an ovotestis condition was detected).

Vitellogenin

Concentrations of plasma vtg were determined by direct Enzyme-Link Immunosorbent Assay (ELISA) using the monoclonal antibodies (mAb) 3G2 for bass and 2D4 for carp. The plasma samples were diluted 1:200 (1:100 for carp), 1:10,000, 1:100,000 and 1:1,000,000 with the wash buffer (10 millimolar (mM) phosphate, 150 mM sodium chloride, 0.02% azide; PBSZ) to which 10 Kallikrein inhibitor units (KIU)/mL Aprotinin, pH 7.6 (AP) had been added. Species-specific vtg standards (0, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 microgram per milliliter; µg/mL) containing 1:200, 1:10,000, 1:100,000, and 1:1,000,000 male plasma (in PBSZ-AP) were added to account for matrix effect (Denslow and others, 1999). Samples and standards were loaded onto a 96-well ELISA plate in triplicate and stored overnight at 4 ºC in a humidified container. The following day the plates were washed four times with PBSZ and then blocked with 1% bovine serum albumin in 10 mM tris, 150 mM NaCl, 0.05% tween, 0.02% azide, 10 KIU/mL Aprotinin, pH 7.6 (1% BSA/TBSTZ-AP) for 2 hours at room temperature. The plates were rewashed with PBSZ (4 times) and the monoclonal antibody was loaded into wells on each plate. The lowest dilution (1:200) was probed with 1–3 µg/mL of the mAb (depending on species) and dilutions of 1:10k and higher with 0.1 µg/mL. After the addition of the mAb, the plates were stored at overnight 4 ºC in the humidified container. The following day the plates were washed and the biotinylated secondary antibody (goat anti mouse immunoglobulin G-biotin) was added to each well at 1:1000 dilution in 1% BSA/TBSTZ-AP and incubated at room temperature for 2 hours. The plates were washed, and strepavidin-alkaline phosphatase was added at 1:1,000 dilution in 1% BSA/TBSTZ-AP and incubated for 2 hours at room temperature. After a final wash of the plates, the color was developed by adding 1 mg/mL p-nitro-phenyl phosphate in carbonate buffer (0.03M carbonate, 2 mM magnesium chloride, pH 9.6) and measuring the color using an ELISA plate reader (SpectraMax Plus384, Applied Biosystems) at 405 nanometer (nm). Concentrations of the unknowns were determined from the standard curves.

All assays were performed in triplicate and reported as the mean of the three measurements. The coefficient of variation was <10% for all samples analyzed. Inter and intra-assay variability was routinely measured by analyzing controls on several plates and different runs and was determined to be <10% and <5%, respectively. Vitellogenin concentrations were not measured from 12 fish (1 carp and 11 bass) because of the poor quality of the field sample. Vitellogenin concentrations were <LOD in 28 bass (0.001 mg/mL) and 115 carp (0.0005 mg/mL).

Sex Steroid Hormones

Concentrations of 17β-estradiol (E2) and 11-ketotestosterone (KT) in plasma samples collected from bass and carp were measured by radioimmunoassay (RIA). For analysis, samples were thawed and split. Duplicate plasma samples (50 µL) were extracted twice by adding 4 mL of ethyl ether, vortexing for 1 minute, freezing the aqueous layer in a methanol-dry ice bath, and decanting the ether layer containing the lipophilic sex steroids. Standard curves were prepared in phosphate buffered saline with gelatin and azide (PBSGA) buffer using variable amounts of unlabeled E2 or KT (1, 5, 10, 25, 50, 100, 250, 500 and 1,000 pg) and a constant concentration of radiolabeled hormone. Cross-reactivities of the E2 and KT antiserums with other steroids were low. Reactions were comprised of plasma extract (50 µL), radiolabeled sex steroid hormone (100 µL), and corresponding sex steroid hormone-specific antibody (100 µL) in PBSGA buffer (250 µL). The reaction solutions were allowed to equilibrate overnight, during which time the unlabeled hormone from the extract and a constant concentration of the corresponding radiolabeled sex steroid hormone competed for the same antibody binding sites. Following incubation, non-antibody bound radiolabeled hormone was removed by adding 250 µL of charcoal dextran and centrifuging at 3,000 times g for 10 minutes. Supernatant aliquots (0.4 mL) containing bound radiolabeled hormone were removed and placed in a vial with 4 mL of scintillation fluid. Radioactivity was measured using scintillation spectrophotometry. Sex steroid concentrations in plasma extracts were determined using a four-parameter logistics regression analysis of standard curves, which was then used to calculate concentrations for plasma extracts.

Pooled samples in triplicate were assayed serially in 10, 20, 30, 40, and 50 µL volumes (final volume of 50 µL with charcoal-stripped plasma). The resulting inhibition curves were parallel to the respective standard curve, with the tests for homogeneity of regression indicating that the curves did not differ. Further characterization of the assays involved mea-

surement of known amounts (1, 2, 5, 10, 25, 50, 100, 250 and 500 pg) of E2 or KT in 50 µL charcoal-stripped plasma. Inter- and intra-assay coefficients of variation were 9.7% and 11.4%, respectively, for E2 and 10.4% and 8.3%, respectively, for KT. Concentrations of E2 and KT were not obtained from one carp and 19 bass because the plasma sample had coagulated and analysis could not be performed. Sex steroid hormones in all other samples were >LOD (12.7 picogram per milliliter (pg/mL) for E2; 15.7 pg/mL for KT). 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.

The ratio of E2 to KT (E/KT) is an additional variable used to analyze sex steroid hormones (Folmar and others, 1996; Goodbred and others, 1997; Hileman, 1994). Typically, E/KT ratio is >1.0 in female fish and <1.0 in male fish, but exact ranges of normality and seasonal fluctuations in this variable have not been established.

Data Set Composition and Statistical Analyses

A total of 51 composite samples (26 bass and 25 carp) from 13 sites were analyzed for chemical contaminants in fish tissue. All results for whole-body composite samples were converted to, analyzed statistically as, and reported as ww concentrations. A value of one-half the LOD was substituted for censored values (that is, values <LOD) in the computation of un-weighted geometric station means and for statistical analyses and censored values in all graphs (Schmitt and others, 1999). Spatial differences in concentrations of As, Cu, Hg, Pb, Se, Zn, *p,p'*-DDE, chlordanes, dieldrin, mirex, toxaphene, PCA, and total PCBs were tested with analysis-of-variance (ANOVA) using Fisher's unrestricted least significant difference (LSD; Saville, 1990). Log-transformed concentrations of these contaminants were analyzed with a series of *t*-tests using a pooled error mean-square (MS_e) representing differences between samples of the same species. A conservative α-level of 0.01 was used in these comparisons to protect against experiment-wise error. Because concentrations of Hg in predatory fish increase with size, age, or both (Wiener and others, 2002), log-transformed length-adjusted (HgL) and weight-adjusted (HgW) concentrations also were tested using this procedure. Following the method of Brumbaugh and others (2001), the adjusted Hg values were computed by dividing the measured concentration in each composite sample by the mean length (m) and weight (kg) of the individual fish comprised by the sample.

Biomarker results were analyzed using ANOVA to test for differences among sites and to examine variation because of gender, age, and reproductive stage. Fish for which otoliths or scales were unreadable for age determination (2 bass and 33 carp) were excluded from all analyses that included age as a factor. Hepatic EROD activity, MA-A, MA-%, and vtg concentrations were $\log_{10}$-transformed to approximate normality and homogeneity-of-variance. A value of one-half the LOQ or LOD was substituted for censored EROD activities and vtg concentrations, respectively, in all statistical analyses. Males and females were analyzed and presented separately if ANOVA indicated that gender was a significant factor for a biomarker.

Relations between and among groups of biomarker variables combined with contaminant concentrations were examined through the use of Spearman rank correlation coefficients. Mean biomarker data for the fish in each composite sample were computed to compare contaminant concentrations in whole-body composite samples with mean biomarker data from individual samples. Males and females were analyzed separately if ANOVA indicated that gender was a significant factor. All computations and statistical analyses were performed with Version 9.1 of the Statistical Analysis System (SAS Institute, Cary, North Carolina). Histological descriptions of tissues were qualitative and not included in the statistical analyses. Toxicity thresholds and contaminant concentration data from other studies were converted to ww concentrations, assuming 75% moisture, if the original study documented concentrations in dw and percent moisture was not reported.

Results and Discussion

Accumulative Contaminants, H4IIE Bioassay, and Hepatic EROD Activity

Elemental Contaminants

Arsenic

Concentrations of As were >LOD (0.013-0.014 µg/g) in 48 samples (94%) representing all sites (table 6). The greatest concentrations were measured in bass from Stations 327 (0.22 µg/g) and 335 (0.24–0.28 µg/g), and As concentrations were lowest (<0.10 µg/g) in fish from Stations 328, 333, 334, 337, and 338 (fig. 4). Arsenic concentrations differed significantly among sites in bass and carp (table 7). Arsenic concentrations were significantly greater in bass from Station 335 than bass from most other sites, and As concentrations were significantly greater in carp from Stations 329, 332, and 336 than carp from Station 333 (table 7).

Concentrations of As in 2004 samples (<0.013–0.28 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical As concentrations in bass near Station 335 increased from <0.05 µg/g in the early 1970s to generally >0.20 µg/g

Table 6. Percentage of samples and stations that exceeded the limit of detection concentration for elemental contaminants in composite samples of whole fish.

[LOD, limit of detection; µg/g, micrograms per gram; F, female; M, male; NA, not applicable]

Analyte	Samples (% of 51)	Stations (% of 13)	LOD Range (µg/g)	Maximum concentration			
				µg/g	Station location and number	Gender	Species
Arsenic	94	100	0.013-0.014	0.28	Port Wentworth, GA (335)	F	Bass
Cadmium	37	85	0.010-0.013	0.19	Albany, GA (331)	M	Carp
Chromium	100	100	NA	2.35	Blountstown, FL (332)	F	Bass
Copper	100	100	NA	2.09	Bucksport, SC (338)	M	Carp
Lead	82	100	0.010-0.011	0.58	Omaha, GA (330)	M	Carp
Mercury	100	100	NA	0.78	Eureka Landing, AL (328); Bucksport, SC (338)	M	Bass
Nickel	96	100	0.10-0.11	1.64	Pee Dee, SC (337)	F	Bass
Selenium	100	100	NA	1.29	Bucks, AL (329)	M	Carp
Zinc	100	100	NA	98.4	Albany, GA (331)	F	Carp

from 1978 to 1986 (Schmitt and others, 1999), which was consistent with the relatively high As concentrations (0.24–0.28 µg/g) measured in bass from Station 335 in 2004. Arsenic concentrations were lower in 2004 than in historical NCBP samples of bass, carp, or both from Stations 328 (<0.05–0.26 µg/g), 332 (<0.05–0.26 µg/g), and 338 (<0.05–0.47 µg/g). Arsenic concentrations in fish collected by the NCBP from Station 329 (<0.05–0.75 µg/g) were similar (Schmitt and others, 1999). Arsenic was not detected (<0.1 µg/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d), and As concentrations in channel catfish (<0.07–<0.2 µg/g) and bass (<0.13–0.16 µg/g) from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996) were similar to those measured in 2004.

Concentrations of As in bass and carp were measured in previous LRMN studies. In bass, As concentrations were 0.03–0.12 µg/g in the CDRB (Hinck and others, 2006b), 0.22–0.53 µg/g in the CRB (Hinck and others, 2006a), 0.10–0.57 µg/g in the MRB (Schmitt and others, 2002), and 0.04–0.25 µg/g in the RGB (Schmitt and others, 2005). Concentrations in carp were 0.02–0.19 µg/g (Hinck and others, 2006b), 0.24–0.56 µg/g in the CRB (Hinck and others, 2006a), 0.12–0.32 µg/g in the MRB (Schmitt and others, 2002), and 0.05–0.55 µg/g in the RGB (Schmitt and others, 2005). Overall, As concentrations in fish from the MORB (0.03–0.22 µg/g), ARB (0.04–0.17 µg/g), SRB (<0.013–0.28 µg/g), and PDRB (<0.013–0.18 µg/g) were less than those from the CRB, MRB, RGB, and YRB and similar to those from the CDRB (table 8).

Arsenic concentrations in MORB, ARB, SRB, and PDRB fish (<0.013–0.28 µg/g) were not considered hazardous to fish or piscivorous wildlife (USEPA, 1984). A review by Jarvinen and Ankley (1999) included several laboratory studies in which As effects were evaluated relative to whole-body concentrations. Concentrations of 8.1–13.5 µg/g were associated with loss of equilibrium and 5.4 µg/g caused increased mortality in rainbow trout (*Oncorhynchus mykiss*) fingerlings (McGreachy and Dixon, 1990; 1992). Adult bluegill experienced reduced survival and growth at 11.6 µg/g (Gilderhus, 1966). Concentrations of As in all 2004 samples were below these thresholds and were not expected to adversely affect fish or wildlife in the MORB, ARB, SRB, and PDRB.

Selenium

Selenium was detected in all samples; the greatest concentration (1.29 µg/g) was measured in male carp from Station 329 (table 6). Concentrations generally were greater in carp (0.43–1.29 µg/g) than in bass (0.28–0.52 µg/g), and relatively high Se concentrations (>0.75 µg/g) were measured in carp from Stations 326, 329, and 330 (fig. 4). Selenium concentrations differed among sites in bass and carp (table 7). Selenium concentrations were lowest in bass from the PDRB, and concentrations in carp from Station 329 in the lower MORB were significantly greater than those from most other sites (table 7).

Selenium concentrations in 2004 samples (<0.013–0.28 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Selenium concentrations were greater in 2004 samples than in historical NCBP samples of bass, carp, or both from Stations 328 (0.10–0.65 µg/g), 332 (0.12–0.56 µg/g), and 335 (<0.05–0.73 µg/g), and NCBP concentrations in fish from Station 338

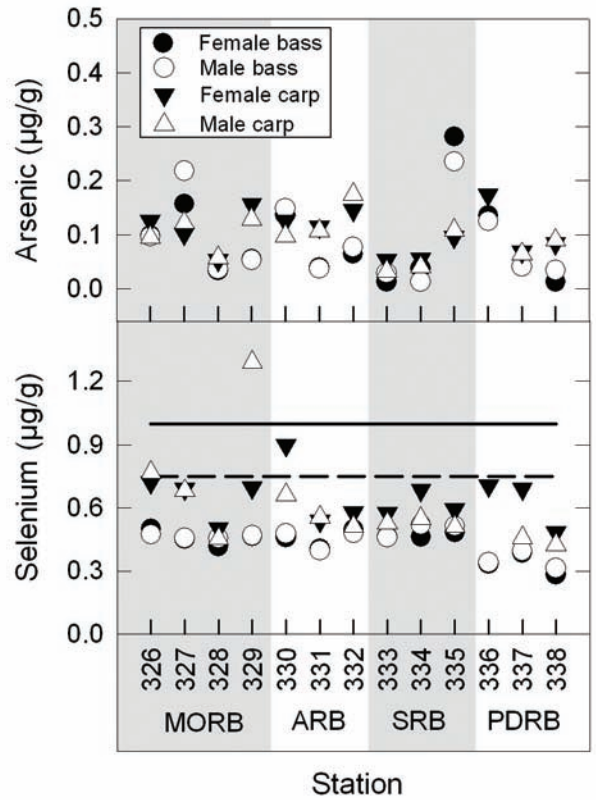


Figure 4. Concentrations (micrograms per gram (µg/g) wet weight) of arsenic and selenium by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Reference lines on the selenium graph include protective thresholds for piscivorous wildlife (0.75 µg/g) and larval fish (1.0 µg/g; Lemly, 1996; 2002). Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

(0.20–0.55 µg/g) were similar (Schmitt and others, 1999). Selenium concentrations were high in some channel catfish (<0.30–1.1 µg/g) and bass (<0.30–0.63 µg/g) samples from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996), which was consistent with the relatively high concentrations measured in 2004 samples from Station 329.

Selenium concentrations in bass and carp were measured in previous LRMN studies. Concentrations in bass were 0.52–2.72 µg/g in the CDRB (Hinck and others, 2006b), <0.02–0.81 µg/g in the CRB (Hinck and others, 2006a), 0.21–4.46 µg/g in the MRB (Schmitt and others, 2002), and 0.47–1.26 µg/g in the RGB (Schmitt and others, 2005). Concentrations in carp were 0.72–2.95 µg/g in the CDRB (Hinck and others, 2006b), 0.32–1.1 µg/g in the CRB (Hinck and others, 2006a), <0.1–4.66 µg/g in the MRB (Schmitt and others, 2002), and 0.23–1.54 µg/g in the RGB (Schmitt and others, 2005). Selenium concentrations in fish from the MORB (0.42–1.29 µg/g), ARB (0.40–0.90 µg/g), SRB (0.46–0.69 µg/g), and PDRB (0.28–0.71 µg/g) were similar to those from the CRB, MRB, and YRB and less than those from the CDRB and RGB (table 8).

Several Se studies were included in a review by Jarvinen and Ankley (1999) on the effects of inorganic chemicals to aquatic organisms. Various studies from this review reported that whole-body concentrations of Se between 8 and 16 µg/g dw (2–4 µg/g ww assuming 75% moisture) have caused reproductive failure in fathead minnows (*Pimephales promelas*; Schultz and Hermanutz, 1990) and bluegill (Coyle and others, 1993; Gillespie and Baumann, 1986; Hermanutz and others, 1992). Vidal and others (2005) reported reduced growth in larval rainbow trout at whole-body Se concentrations of 1.2 µg/g. In addition, concentrations of Se present in the egg stage or at hatch can affect larval survival (Coyle and others, 1993; Hamilton and others 2005b; 2005c), which emphasizes that multiple life stages need to be examined to correctly assess toxicity and tissue concentration relations (Jarvinen and Ankley, 1999). Selenium accumulation varies among tissues in fish. High Se concentrations have been measured in spleen, liver, kidney, and gonads relative to other tissues (Hamilton and others, 2005a). Selenium contamination also has been associated with histopathological effects in the gill, liver, kidney, and ovary of freshwater fish (Sorenson, 1988; Sorenson and others, 1984). Whole-body concentrations of Se should not exceed 4 µg/g dw (1.0 µg/g ww assuming 75% moisture) to avoid toxicity to the fish and 3 µg/g dw (0.75 µg/g ww assuming 75% moisture) to avoid toxicity to piscivorous wildlife (Hamilton, 2004; Lemly, 1996). Carp samples from Stations 326, 329, and 330 exceeded one or both of these thresholds (fig. 4); therefore, Se represents a risk to aquatic and piscivorous wildlife at these MORB and ARB sites.

Mercury

Mercury was detected in all samples, and the maximum concentration (0.78 µg/g) was measured in male bass from Stations 328 and 338 (table 6). Concentrations were >0.6 µg/g in samples from Stations 328, 329, 332, 334, and 338 and were greater in bass (0.22–0.78 µg/g) than in carp (0.05–0.31 µg/g; fig. 5). Mercury concentrations in predatory fish increase with size (that is, heavier and longer fish have greater concentrations; Brumbaugh and others, 2001); therefore, Hg concentrations of Hg adjusted for weight and length were examined. Relating concentrations of Hg in composite samples to individual length and weight measurements is difficult, although overall trends or patterns can be identified. The length- or weight-adjusted Hg concentrations (HgL and HgW, respectively) also were greatest in bass. The greatest HgL concentrations (>2.0 µg/g/m) were measured in female bass from Stations 328, 334, and 338 and in male bass from Station 338 (fig. 5). The mean length of fish in these samples were 300–392 mm, which was within the range of mean lengths in the other samples (254–457 mm) and indicates that not all differences in the Hg concentrations were related to length of

Table 7. Spatial trends of elemental contaminants in fish.

[Mean wet weight concentrations (± standard errors) are presented. Values within each group of species-station means followed by the same letter were not significantly different ($P > 0.01$ Fisher's unrestricted least significant difference). Also shown are analysis of variance F-values, degrees-of-freedom, and significance levels (*, $P \leq 0.01$). $n = 2$ composite samples for all species-station group except for carp from Station 336 where $n = 1$. Stations are listed upstream to downstream within a basin. µg/g, micrograms per gram; µg/g/m, micrograms per gram per meter; µg/g/kg, micrograms per gram per kilogram]

Species, station location, and station number	Elemental contaminant concentration																	
	Arsenic (µg/g)		Copper (µg/g)		Mercury (µg/g)		Length-adjusted mercury (µg/g/m)		Weight-adjusted mercury (µg/g/kg)		Lead (µg/g)		Selenium (µg/g)		Zinc (µg/g)	
Bass																
Lavaca, AL (326)	0.10 ± 0.00	bcde	0.26 ± 0.00	a	0.48 ± 0.04	bcde	1.28 ± 0.05	cd	0.69 ± 0.05	bc	0.03 ± 0.02	a	0.49 ± 0.01	c	11.7 ± 1.3	a
Childersburg, AL (327)	0.19 ± 0.03	de	0.27 ± 0.01	a	0.32 ± 0.07	abc	0.76 ± 0.12	ab	0.28 ± 0.01	a	0.03 ± 0.03	a	0.46 ± 0.00	bc	12.0 ± 0.8	a
Eureka Landing, AL (328)	0.04 ± 0.00	abc	0.28 ± 0.00	a	0.68 ± 0.10	de	1.71 ± 0.28	de	0.80 ± 0.18	c	0.03 ± 0.02	a	0.44 ± 0.02	bc	11.3 ± 0.0	a
Bucks, AL (329)	0.05 ± 0.00	bcd	0.27 ± 0.00	a	0.64 ± 0.05	de	1.54 ± 0.08	cde	0.70 ± 0.03	bc	0.01 ± 0.00	a	0.47 ± 0.00	bc	12.0 ± 1.5	a
Omaha, GA (330)	0.14 ± 0.01	de	0.28 ± 0.01	a	0.29 ± 0.05	ab	0.75 ± 0.11	ab	0.36 ± 0.01	ab	0.04 ± 0.02	a	0.47 ± 0.01	bc	11.1 ± 0.4	a
Albany, GA (331)	0.04 ± 0.00	abc	0.44 ± 0.14	abc	0.23 ± 0.02	a	0.65 ± 0.01	ab	0.36 ± 0.07	ab	0.01 ± 0.01	a	0.40 ± 0.00	abc	11.8 ± 0.2	a
Blountstown, FL (332)	0.07 ± 0.01	cd	0.29 ± 0.00	a	0.58 ± 0.07	cde	1.54 ± 0.06	cde	0.78 ± 0.19	c	0.03 ± 0.00	a	0.49 ± 0.01	c	11.6 ± 0.1	a
Augusta, GA (333)	0.02 ± 0.01	a	0.26 ± 0.01	a	0.37 ± 0.05	abcd	1.29 ± 0.14	cd	1.26 ± 0.02	cd	0.02 ± 0.00	a	0.46 ± 0.00	bc	13.4 ± 2.5	a
Sylvania, GA (334)	0.03 ± 0.02	a	0.41 ± 0.14	a	0.65 ± 0.02	de	1.95 ± 0.27	de	1.24 ± 0.57	cd	0.01 ± 0.00	a	0.49 ± 0.03	c	10.9 ± 2.8	a
Port Wentworth, GA (335)	0.26 ± 0.02	e	0.80 ± 0.00	bc	0.35 ± 0.11	abc	1.03 ± 0.28	bc	0.65 ± 0.09	bc	0.01 ± 0.00	a	0.50 ± 0.01	c	10.7 ± 0.0	a
Rockingham, NC (336)	0.13 ± 0.01	de	0.89 ± 0.30	c	0.23 ± 0.01	a	0.57 ± 0.02	a	0.27 ± 0.06	a	0.01 ± 0.00	a	0.34 ± 0.00	ab	11.9 ± 0.1	a
Pee Dee, SC (337)	0.04 ± 0.00	abc	0.42 ± 0.13	b	0.42 ± 0.06	abcde	1.60 ± 0.14	cde	1.64 ± 0.10	d	0.07 ± 0.01	a	0.39 ± 0.00	abc	12.7 ± 1.0	a
Bucksport, SC (338)	0.02 ± 0.01	a	0.43 ± 0.15	a	0.72 ± 0.07	e	2.36 ± 0.24	e	1.77 ± 0.18	d	0.02 ± 0.00	a	0.30 ± 0.02	a	12.6 ± 1.0	a
Carp																
Lavaca, AL (326)	0.11 ± 0.02	ab	0.93 ± 0.14	a	0.09 ± 0.01	abc	0.19 ± 0.01	abc	0.07 ± 0.00	cde	0.03 ± 0.00	ab	0.75 ± 0.02	cd	53.1 ± 0.03	ab
Childersburg, AL (327)	0.11 ± 0.01	ab	1.31 ± 0.11	a	0.15 ± 0.02	cde	0.23 ± 0.04	bcd	0.05 ± 0.01	abc	0.14 ± 0.05	bc	0.69 ± 0.00	cd	58.2 ± 1.6	ab
Eureka Landing, AL (328)	0.06 ± 0.00	ab	0.75 ± 0.01	a	0.05 ± 0.00	a	0.13 ± 0.01	a	0.05 ± 0.00	bc	0.01 ± 0.00	a	0.48 ± 0.02	ab	50.1 ± 0.4	a
Bucks, AL (329)	0.14 ± 0.01	b	0.84 ± 0.01	a	0.10 ± 0.01	bcd	0.20 ± 0.02	abc	0.06 ± 0.01	bcd	0.04 ± 0.02	ab	0.99 ± 0.30	d	55.7 ± 1.0	ab
Omaha, GA (330)	0.11 ± 0.01	ab	0.88 ± 0.01	a	0.16 ± 0.01	de	0.28 ± 0.05	cde	0.06 ± 0.02	bc	0.44 ± 0.14	c	0.78 ± 0.12	cd	73.6 ± 15.8	ab
Albany, GA (331)	0.11 ± 0.00	ab	1.12 ± 0.14	a	0.14 ± 0.01	cde	0.21 ± 0.01	abcd	0.03 ± 0.00	ab	0.06 ± 000	abc	0.55 ± 0.01	abc	80.7 ± 17.7	b
Blountstown, FL (332)	0.16 ± 0.01	b	0.87 ± 0.02	a	0.07 ± 0.00	ab	0.14 ± 0.01	ab	0.04 ± 0.00	abc	0.03 ± 0.00	ab	0.55 ± 0.03	abc	58.2 ± 1.0	ab
Augusta, GA (333)	0.04 ± 0.01	a	1.07 ± 0.06	a	0.13 ± 0.00	cde	0.29 ± 0.01	cde	0.09 ± 0.01	cde	0.04 ± 0.01	ab	0.55 ± 0.03	abc	53.4 ± 3.1	a
Sylvania, GA (334)	0.05 ± 0.01	ab	1.11 ± 0.04	a	0.16 ± 0.03	cde	0.36 ± 0.06	de	0.11 ± 0.02	de	0.04 ± 0.02	ab	0.62 ± 0.07	abc	55.3 ± 2.2	ab
Port Wentworth, GA (335)	0.10 ± 0.01	ab	1.06 ± 0.00	a	0.17 ± 0.01	de	0.39 ± 0.01	e	0.13 ± 0.01	e	0.06 ± 0.05	ab	0.55 ± 0.04	abc	53.2 ± 0.2	ab
Rockingham, NC (336)	0.18	b	0.82	a	0.11	abcde	0.13	a	0.02	a	0.14	bc	0.71	bcd	54.6	ab
Pee Dee, SC (337)	0.07 ± 0.00	ab	1.18 ± 0.06	a	0.17 ± 0.04	de	0.32 ± 0.02	cde	0.07 ± 0.02	cde	0.08 ± 0.05	abc	0.58 ± 0.12	abc	59.0 ± 3.1	ab
Bucksport, SC (338)	0.09 ± 0.00	ab	1.53 ± 0.56	a	0.23 ± 0.08	e	0.35 ± 0.10	de	0.08 ± 0.01	cde	0.09 ± 0.03	bc	0.46 ± 0.03	a	62.2 ± 2.4	ab
$F_{25,25}$	7.14*		11.55*		22.40*		50.07*		52.07*		2.90*		7.78*		65.30*	

Table 8. Mean concentrations of select elemental contaminants in piscivorous and benthivorous fish from Biomonitoring of Environmental Status and Trend (BEST) Program studies.

[Unweighted geometric means (all wet weight) are presented. Studies include the Mobile River Basin, Apalachicola-Chattahoochee-Flint River Basin, Savannah River Basin, Pee Dee River Basin, Colorado River Basin (Hinck and others, 2006b), Columbia River Basin (Hinck and others, 2004a), Mississippi River Basin (Schmitt, 2002), Rio Grande Basin (Schmitt and others, 2004), and Yukon River Basin (Hinck and others, 2004b). Censored values were replaced by one-half the limit of detection for the computations of basin means. µg/g, micrograms per gram; Pisc, Piscivrous; Ben, Benthivorous; Min, minimum; Max; maximum; <, less than]

Basin	Arsenic (µg/g)		Cadmium (µg/g)		Chromium (µg/g)		Copper (µg/g)		Mercury (µg/g)		Lead (µg/g)		Selenium (µg/g)		Zinc (µg/g)	
	Pisc[a]	Ben[b]	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben
Southeastern U.S. basins sampled (2004)																
Mobile																
Mean	0.08	0.10	0.01	0.01	0.53	0.47	0.27	0.93	0.51	0.09	0.01	0.03	0.46	0.69	11.7	54.2
Min	0.03	0.05	<0.01	<0.01	0.26	0.17	0.26	0.75	0.26	0.05	<0.01	<0.01	0.42	0.45	10.5	49.8
Max	0.22	0.16	0.01	0.03	0.84	1.01	0.28	1.42	0.78	0.17	0.06	0.20	0.50	1.29	13.4	59.8
Apalachicola-Chattahoochee-Flint																
Mean	0.07	0.12	0.01	0.06	0.64	0.47	0.32	0.95	0.34	0.12	0.02	0.09	0.45	0.61	11.5	69.1
Min	0.04	0.10	<0.01	<0.01	0.29	0.29	0.27	0.86	0.22	0.07	<0.01	0.02	0.40	0.51	10.8	57.2
Max	0.15	0.17	0.01	0.19	2.35	1.19	0.58	1.26	0.65	0.18	0.05	0.58	0.50	0.90	12.0	98.4
Savannah																
Mean	0.04	0.06	0.01	0.02	0.53	0.28	0.43	1.08	0.43	0.15	0.01	0.03	0.48	0.57	11.4	53.9
Min	<0.01	0.03	<0.01	<0.01	0.27	0.23	0.25	1.01	0.23	0.13	<0.01	<0.01	0.46	0.51	8.1	50.3
Max	0.28	0.11	0.01	0.03	1.32	0.54	0.80	1.15	0.67	0.19	0.02	0.11	0.52	0.69	15.8	57.5
Pee Dee																
Mean	0.04	0.10	0.01	0.03	0.58	0.19	0.51	1.11	0.41	0.16	0.02	0.09	0.34	0.56	12.3	58.4
Min	<0.01	0.06	<0.01	<0.01	0.30	0.06	0.27	0.82	0.23	0.11	<0.01	0.03	0.28	0.43	11.5	54.6
Max	0.14	0.18	0.01	0.12	0.82	0.60	1.19	2.09	0.78	0.31	0.08	0.14	0.40	0.71	13.7	64.6
Other basins sampled (1995–2003)																
Colorado (2003)																
Mean	0.05	0.08	0.02	0.05	0.36	0.41	0.56	1.00	0.11	0.06	0.16	0.14	1.11	1.75	18.1	67.1
Min	0.01	0.02	<0.02	<0.03	<0.24	<0.34	0.34	0.71	0.04	0.01	<0.24	<0.25	0.51	0.72	12.8	13.1
Max	0.12	0.19	0.04	0.24	2.38	0.73	2.80	1.39	0.37	0.25	0.33	0.35	2.72	2.95	36.1	99.6
Columbia (1997)																
Mean	0.16	0.17	0.03	0.09	0.80	1.69	0.70	1.42	0.19	0.11	0.06	0.12	0.36	0.34	16.3	43.0
Min	<0.22	<0.21	<0.04	<0.04	0.30	0.50	0.34	0.81	<0.05	<0.05	<0.09	<0.09	<0.22	<0.22	11.6	17.7
Max	0.53	0.56	0.14	0.51	3.70	11.2	2.32	3.92	0.61	0.22	0.29	9.29	0.84	1.10	22.9	105.6
Mississippi (1995)																
Mean	0.16	0.11	0.02	0.07	0.51	0.91	0.55	1.09	0.17	0.09	0.02	0.09	0.49	0.62	19.7	70.1
Min	<0.11	<0.12	<0.02	<0.03	<0.16	0.25	0.35	0.47	0.05	<0.04	<0.01	<0.01	0.20	<0.12	13.7	16.5
Max	0.57	0.33	0.22	0.51	2.29	7.48	3.84	2.68	0.45	0.34	0.49	0.69	4.46	4.66	41.6	150.0
Rio Grande (1997)																
Mean	0.12	0.16	0.01	0.03	4.23	6.31	0.57	1.03	0.10	0.10	0.07	0.10	0.54	0.63	18.3	54.2
Max	0.44	0.55	0.04	0.12	70.2	71.8	1.01	1.80	0.46	0.20	0.83	0.43	1.87	1.73	55.9	83.6
Min	<0.05	<0.10	<0.02	<0.02	0.28	0.38	0.29	0.56	<0.02	0.03	<0.03	<0.07	0.17	0.23	11.1	16.6
Yukon (2002)																
Mean	0.12	0.07	<0.01	0.07	0.30	0.52	0.53	0.99	0.27	0.13	<0.27	<0.27	0.46	0.55	39.6	19.3
Min	0.04	0.03	<0.01	0.04	<0.22	0.26	0.41	0.63	0.11	0.08	<0.27	<0.27	0.23	0.45	10.5	15.6
Max	1.95	0.17	<0.01	0.12	1.26	1.64	0.94	1.49	0.65	0.19	<0.27	<0.27	0.85	0.62	56.4	30.8

[a]Piscivorous fish samples sizes for all elemental contaminants were $n = 4$ for the Mobile, $n = 3$ for the Apalachicola-Chattahoochee-Flint, $n = 3$ for the Savannah, $n = 3$ for the Pee Dee, $n = 12$ for the Colorado, $n = 14$ for the Columbia, $n = 34$ for the Mississippi, $n = 9$ for the Rio Grande, and $n = 10$ for the Yukon.

[b]Benthivorous fish samples sizes for all elemental contaminants were $n = 4$ for the Mobile, $n = 3$ for the Apalachicola-Chattahoochee-Flint, $n = 3$ for the Savannah, $n = 3$ for the Pee Dee, $n = 14$ for the Colorado, $n = 16$ for the Columbia, $n = 46$ for the Mississippi, $n = 10$ for the Rio Grande, and $n = 5$ for the Yukon.

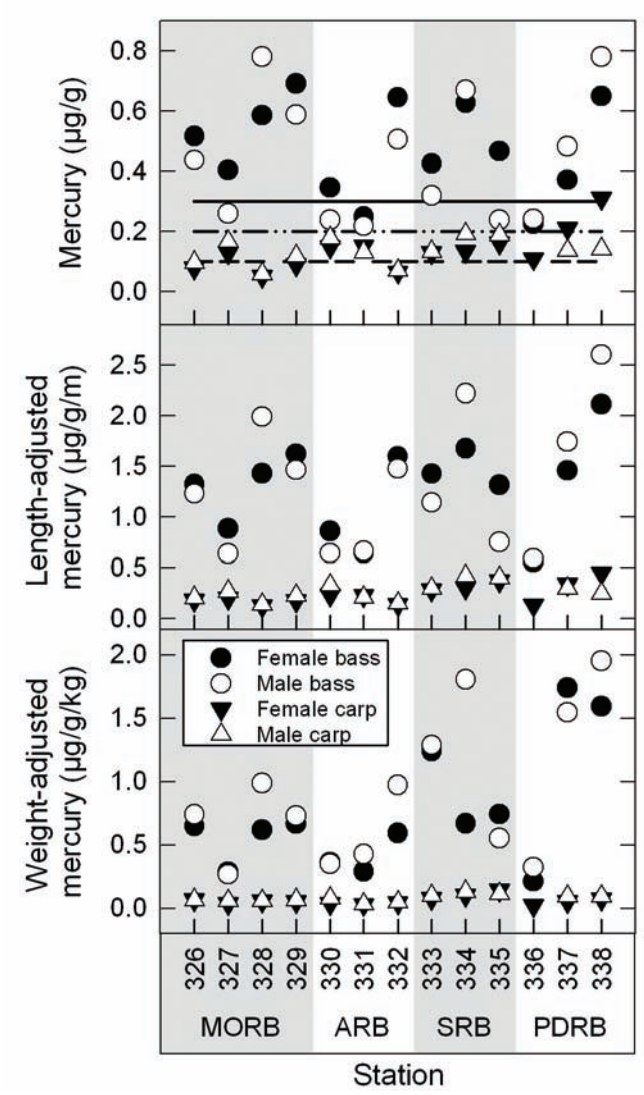


Figure 5. Concentrations (microgram per gram (µg/g) wet weight) of mercury by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Length-adjusted and weight-adjusted mercury concentrations are also presented. Reference lines on mercury graph include protective thresholds for piscivorous mammals (0.1 µg/g; Yeardley and others, 1998), juvenile and adult fish (0.2 µg/g; Beckvar and others, 2005), and piscivorous birds (0.3 µg/g; Barr, 1986). Stations are ordered from upstream to downstream and are grouped by basin. See text for computations and table 5 for station descriptions.

the fish. The greatest HgW concentrations (>1.5 µg/g/kg) were measured in bass from Stations 334, 337, and 338 (fig. 5). The mean weight of these samples (213–408 g) was in the lower range for all bass samples (213–1,405 g). Concentrations of HgW generally were greater in samples with lower mean weights, which indicated that some differences in the Hg concentrations were related to the weight of the fish. The current study design (that is, measuring concentrations in composite samples) cannot definitively account for the contribution of size to concentrations of Hg.

Concentrations of Hg in bass and carp differed significantly among sites (table 7). Concentrations of unadjusted Hg were significantly greater in bass from Station 338 than those from Stations 327, 330, 331, 333, 335, and 336 (table 7). In carp, unadjusted Hg concentrations were significantly greater in fish from Station 338 than those from Stations 326, 328, 329, and 332 (table 7). Spatial differences in HgL and HgW concentrations were similar to unadjusted Hg concentrations for bass and carp (table 7). Relative differences among concentrations remained after adjusting for fish size, which indicates that spatial differences were not entirely artifacts of fish size.

Mercury concentrations in 2004 samples (0.05–0.78 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical Hg concentrations from NCBP sites near Stations 328 (0.02–0.60 µg/g), 332 (<0.01–0.23 µg/g), 335 (<0.01–1.8 µg/g), and 338 (<0.01–1.03 µg/g) were lower in bass but similar in carp compared to concentrations in 2004 samples (Schmitt and others, 1999). Whole-body Hg concentrations in benthivorous fish were 0.19 µg/g near Station 329, 0.07 µg/g near Stations 330 and 331, 0.08–0.21 µg/g near Station 333, and 0.18 µg/g near Station 335 (USEPA, 1992). Mean Hg concentrations in bass (0.10–0.75 µg/g) and channel catfish (0.04–0.13 µg/g) samples from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996) were similar to those measured in 2004 samples.

Mercury concentrations in bass and carp were measured in previous LRMN studies. Concentrations in bass were 0.11–0.87 µg/g in the CDRB (Hinck and others, 2006b), 0.06–0.31 µg/g in the CRB (Hinck and others, 2006a), 0.05–0.45 µg/g in the MRB (Schmitt and others, 2002), and 0.07–0.45 µg/g in the RGB (Schmitt and others, 2005). In carp, concentrations were 0.03–0.54 µg/g in the CDRB (Hinck and others, 2006b), <0.05–0.20 µg/g in the CRB (Hinck and others, 2006a), 0.04–0.34 µg/g in the MRB (Schmitt and others, 2002), and 0.03–0.20 µg/g in the RGB (Schmitt and others, 2005). Overall, Hg concentrations in fish from the MORB (0.05–0.78 µg/g), ARB (0.07–0.65 µg/g), SRB (0.13–0.67 µg/g), and PDRB (0.11–0.78 µg/g) were greater than those from the CDRB, CRB, MRB, RGB, and YRB (table 8).

Fish populations are at greatest risk from Hg during embryonic and larval stages partially because of maternal transfer (Wiener and Spry, 1996). Behavioral effects in laboratory studies have been documented in fish containing whole-body concentrations of 0.7–5.4 µg/g (Kania and O'Hara, 1974; Wiener and Spry, 1996). Permanent impairment of grayling (*Thymallus thymallus*) fry feeding efficiency and competitive ability occurred at Hg concentrations of 0.27 µg/g (Fjeld and others, 1998). Dietary Hg exposure increased mortality (0.20–0.47 µg/g) and altered sex ratios (0.44–1.1 µg/g) of adult mummichog (*Fundulus heteroclitus*) and reduced fer-

tilization success (0.01–0.63 µg/g) of eggs (Matta and others, 2001). Jarvinen and Ankley (1999) reviewed various laboratory studies evaluating the effects of Hg on reproduction in freshwater fish. Included were studies that determined reduced reproduction at whole-body concentrations of 4.47 µg/g in fathead minnows (Snarski and Olson, 1982) and 9.4 µg/g in second-generation brook trout (*Salvelinus fontinalis*; McKim and others, 1976). In fathead minnows, dietary Hg concentrations of 0.87 µg/g dw (0.22 µg/g ww assuming 75% moisture) increased whole-body concentrations more than 10-fold, suppressed hormone levels, and inhibited gonadal development in females (Drevnick and Sandheinrich, 2003). Suppressed 17β-estradiol concentrations and smaller ovary size were related to increased ovarian follicular apoptosis in fathead minnows fed dietary Hg concentrations of 0.87–3.93 µg/g dw (0.22–0.98 µg/g ww assuming 75% moisture; Drevnick and others, 2006), and reproductive behavior in fathead minnows was altered at whole-body Hg concentrations of 0.71–4.2 µg/g dw (0.17–1.05 µg/g ww assuming 75% moisture; Sandheinrich and Miller, 2005). Whole-body concentrations associated with behavioral and reproductive effects were approximately 5 µg/g for brook trout and 10 µg/g for rainbow trout (Wiener and Spry, 1996; Wiener and others, 2002). However, caution should be used with these thresholds because many factors can contribute uncertainty to these critical tissue concentration estimates (Wiener and others, 2002). Using various systematic approaches to derive protective tissue residue-effect concentrations, Beckvar and others (2005) recommended that total Hg whole-body concentrations should not exceed 0.2 µg/g to protect juvenile and adult fish. Mercury concentrations in all bass samples and female carp samples from Stations 337 and 338 in the PDRB exceeded this protective guideline (fig. 5).

Dietary concentrations of Hg in wildlife as low as 0.3 µg/g have been associated with reproductive impairment in common loons (*Gavia immer*; Barr, 1986), and reproduction in mallards (*Anas platyrhynchos*) was affected at concentrations as low as 0.1 µg/g (Heinz, 1979). Dietary concentrations of 0.25–1.0 µg/g also may be toxic to piscivorous mammals (Wolfe and others, 1998). Neurotoxicity and mortality occurred in adult minks (*Mustela vison*) after chronic exposure to dietary Hg concentrations >1 µg/g (Dansereau and others, 1999; Wobeser and others, 1976; Wren and others, 1987). Consequently, guidelines for the protection of piscivorous wildlife range from 0.5 to 1.0 µg/g (Eisler, 1987; Thompson, 1996), and values as low as 0.1 µg/g for mammals and 0.02 µg/g for birds have been derived from water quality criteria and bioaccumulation factors (Yeardley and others, 1998). Selenium affords a degree of protection against Hg toxicity in wildlife by demethylation to inorganic Hg when Se and Hg are in molar ratio of 1:1 (Dietz and others, 1990; Heinz and Hoffman, 1998; Scheuhammer and others, 1998; Wiener and others, 2002). However, studies have shown Se-enhanced Hg embryo toxicity in birds (Heinz and Hoffman, 1998). Thus, although the significant amounts of Se may protect adult birds from the toxic effects of Hg, reproductive effects may be exacerbated. Mercury concentrations in bass samples from all sites except Stations 331 and 336 exceeded 0.3 µg/g, and concentrations in at least one sample from all sites exceeded 0.1 µg/g (fig. 5). Therefore, wildlife may be at risk from exposure to Hg in the MORB, ARB, SRB, and PDRB.

Lead

Concentrations of Pb were >LOD (0.010–0.011 µg/g) in 42 samples (82%) representing all sites (table 6). Concentrations were ≥0.20 µg/g in carp samples from Stations 327 and 330, and the maximum concentration (0.58 µg/g) was measured in male carp from Station 330 (fig. 6; table 6). Lead concentrations differed significantly among sites in carp but not in bass (table 7). Concentrations were significantly greater in carp from Station 330 than those from all other sites (table 7).

Lead concentrations in 2004 samples (<0.01–0.58 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical Pb concentrations in bass and carp from NCBP sites near Stations 328 (<0.10–0.42 µg/g), 332 (<0.10–0.51 µg/g), 335

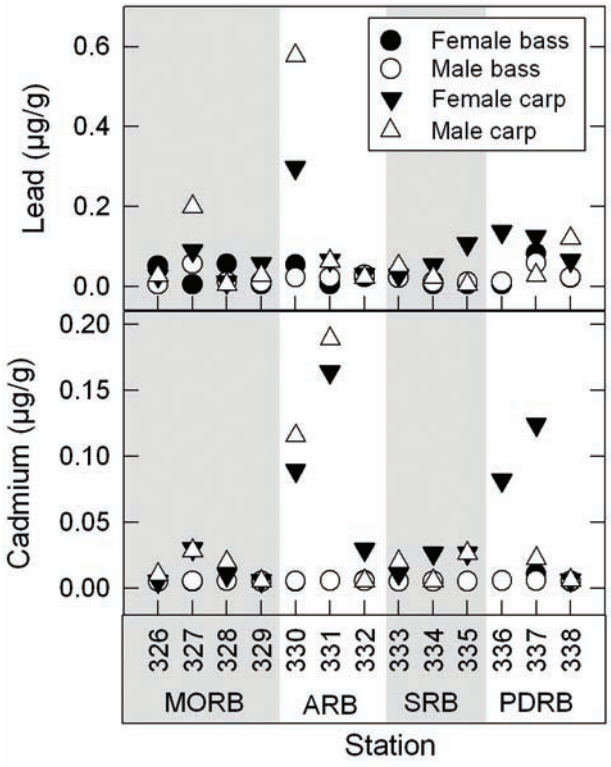


Figure 6. Concentrations (micrograms per gram (µg/g) wet weight) of lead and cadmium by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

(<0.10–0.21 µg/g), and 338 (<0.1–1.2 µg/g) were similar to concentrations in 2004 samples (Schmitt and others, 1999). Lead was detected in few whole-body channel catfish samples (n = 2) from the lower MORB near Stations 328 and 329, where mean concentrations were <0.05–0.80 µg/g (USEPA, 1995).

In previous LRMN studies, Pb concentrations in bass were <0.01–0.49 µg/g in the MRB (Schmitt and others, 2002), <0.1–0.83 µg/g in the RGB (Schmitt and others, 2005), and ≤LOD (0.1–0.3 µg/g) in the CDRB and CRB (Hinck and others, 2006a; 2006b). Lead concentrations in carp were <LOD (0.3 µg/g) in the CDRB (Hinck and others, 2006b), <0.1–0.34 µg/g in the CRB (Hinck and others, 2006a), <0.01–0.69 µg/g in the MRB (Schmitt and others, 2002), and <0.1–0.43 µg/g in the RGB (Schmitt and others, 2005). Lead concentrations in fish from the MORB (<0.01–0.20 µg/g), ARB (<0.01–0.58 µg/g), SRB (<0.01–0.11 µg/g), and PDRB (<0.01–0.14 µg/g) generally were less than those from the CDRB, CRB, MRB, and RGB (table 8).

Whole-body Pb concentrations of 0.4 µg/g reduced hatchability and 4.0–8.8 µg/g reduced growth in third generation brook trout at various life stages (Holcombe and others, 1976). Only one sample, male carp from Station 330, exceeded 0.4 µg/g. The risk of Pb to fish and wildlife from the MORB, ARB, SRB, and PDRB was expected to be minimal.

Cadmium

Concentrations of Cd were >LOD (0.010–0.013 µg/g) in 19 samples (37%) from 11 sites (table 6). Concentrations were >0.08 µg/g in carp samples from Stations 330, 331, 336, and 337, and the maximum concentration (0.19 µg/g) was measured in a male carp sample from Station 331 (fig. 6; table 6). Cadmium concentrations generally were greater in carp than in bass, which was consistent with results from previous LRMN studies (Hinck and others, 2006a; 2006b; Schmitt and others, 2002; 2005). Spatial differences in Cd concentrations were not determined because of the high number of samples that were <LOD.

Cadmium concentrations in 2004 samples (<0.01–0.19 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical NCBP concentrations also were low in whole-body fish near Stations 328 (<0.05–0.16 µg/g), 332 (<0.05 µg/g), 335 (<0.05–0.50 µg/g), and 338 (<0.05–0.55 µg/g) from 1970 to 1986 (Schmitt and others, 1999). Other studies reporting Cd concentrations in fish from the MORB, ARB, SRB, and PDRB were not found. In previous LRMN studies, Cd concentrations in bass were <0.02–0.22 µg/g in the MRB (Schmitt and others, 2002) and <LOD (0.02 µg/g) in the CDRB, CRB, and RGB (Hinck and others, 2006a; 2006b; Schmitt and others, 2005). Cadmium concentrations in carp were <0.02–0.24 µg/g in the CDRB (Hinck and others, 2006b), <0.04–0.51 µg/g in the CRB (Hinck and others, 2006a), <0.02–0.51 µg/g in the MRB (Schmitt and others, 2002), and <0.02–0.12 µg/g in the RGB (Schmitt and others, 2005). Cadmium concentrations in fish from the MORB (<0.01–0.03 µg/g), ARB (<0.01–0.19 µg/g), SRB (<0.01–0.03 µg/g), and PDRB (<0.01–0.12 µg/g) generally were less than those from the CDRB, CRB, MRB, RGB, and YRB (table 8).

Birds and mammals are comparatively resistant to Cd. Dietary toxicity thresholds were >100 µg/g in the studies reviewed by Eisler (1985). Eisler (1985) suggested that a Cd concentration of 2 µg/g in fish is evidence of contamination, that 5 µg/g is potentially life-threatening to the fish, and that 13–15 µg/g is a threat to higher trophic levels. A review by Jarvinen and Ankley (1999) included several laboratory studies in which whole-body Cd concentrations in juvenile and adult freshwater fish of 0.25–15.6 µg/g resulted in reduced survival, growth, or both, and concentrations of 2–8 µg/g caused decreased spawning and embryo production. Decreased growth and survival in juvenile bull trout (*S. confluentus*) were reported at whole-body Cd concentrations of 0.91 µg/g dw (0.23 µg/g ww assuming 75% moisture; Hansen and others, 2002). All Cd concentrations in fish from the MORB, ARB, SRB, and PDRB were below these benchmarks. Fish and wildlife from the MORB, ARB, SRB, and PDRB were not at risk from Cd using these toxicity criteria.

Zinc

Zinc was detected in all samples, and the maximum concentrations (>89 µg/g) were in female carp from Stations 330 and 331 (fig. 7; table 6). Concentrations were ≥50 µg/g in carp and <16 µg/g in bass (fig. 7). Carp partition Zn in their digestive tissue (Sun and Jeng, 1998), and previous studies have reported higher Zn concentrations in carp than other species (Hinck and others, 2006a; 2006b; Schmitt and others, 1999, 2002a, 2005). Concentrations of Zn differed significantly among sites in carp but not in bass (table 7). Concentrations were significantly greater in carp from Station 331 than those from Stations 328 and 333 (table 7).

Zinc concentrations in 2004 samples (8.1–98.4 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical NCBP concentrations of Zn in whole-body fish near Stations 328 (10.3–32.7 µg/g), 332 (10.7–21.1 µg/g), 335 (9.5–54.9 µg/g), and 338 (12.5–21.2 µg/g) from 1970 to 1986 were similar to those measured in 2004 samples (Schmitt and others, 1999). Mean Zn concentrations in bass (12–25 µg/g) and channel catfish (18–39 µg/g) samples from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996) were similar to those measured in 2004 bass samples from Stations 328 and 329.

Zinc concentrations in bass and carp were measured in previous LRMN studies. Concentrations in bass were 13–19 µg/g in the CDRB (Hinck and others, 2006b), 12–19 µg/g in the CRB (Hinck and others, 2006a), 14–37 µg/g in the MRB (Schmitt and others, 2002), and 11–20 µg/g in the RGB (Schmitt and others, 2005). In carp, concentrations were 52–100 µg/g in the CDRB (Hinck and others, 2006b), 54–106 µg/g in the CRB (Hinck and others, 2006a), 17–150 µg/g in

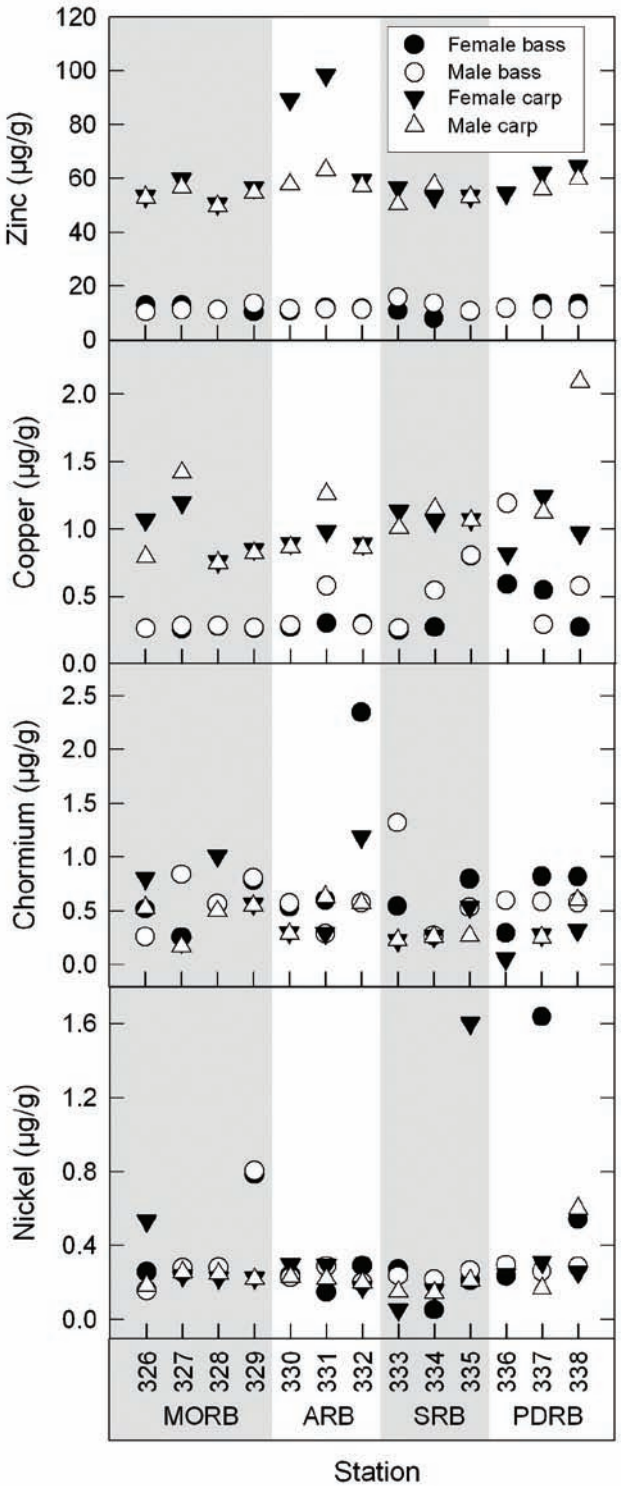


Figure 7. Concentrations (micrograms per gram (µg/g) wet weight) of zinc, copper, chromium, and nickel by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

the MRB (Schmitt and others, 2002), and 17–84 µg/g in the RGB (Schmitt and others, 2005). Zinc concentrations in fish from the MORB (11–60 µg/g), ARB (11–98 µg/g), SRB (8–58 µg/g), and PDRB (12–65 µg/g) were similar to those from the CDRB, CRB, MRB, and RGB (table 8).

Zinc is highly regulated in fish, and few studies have measured Zn concentrations in whole-body fish (Jarvinen and Ankley, 1999). Zinc concentrations of 40–64 µg/g affected the growth and survival of American flagfish (*Jordanella floridae*), a cyprinid, exposed over a life-cycle (larvae-to-adult; Spehar, 1976). Because Zn is an essential nutrient, it is unlikely to be potentially harmful to fish or piscivorous wildlife (Eisler, 1993). Fish and wildlife from the MORB, ARB, SRB, and PDRB were not expected to be at risk from Zn.

Copper

Copper was detected in all samples, and the maximum concentration (2.09 µg/g) was measured in male carp from Station 338 (table 6). All other concentrations were <1.42 µg/g. Copper concentrations generally were greater in carp (>0.85 µg/g) than in bass (most <0.60 µg/g; fig. 7). Concentrations of Cu differed among sites in bass but not in carp (table 7). Copper concentrations were significantly greater in bass from Stations 335, 336, and 337 than those from most other sites (table 7).

Copper concentrations in 2004 samples (0.25–2.09 µg/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical NCBP concentrations of Cu in whole-body fish near Stations 328 (0.25–3.65 µg/g), 332 (0.25–1.04 µg/g), 335 (0.32–2.25 µg/g), and 338 (0.26–2.26 µg/g) from 1970 to 1986 were slightly greater than those measured in 2004 samples (Schmitt and others, 1999). Mean Cu concentrations were <0.3–3.5 µg/g in bass and <LOD (≤0.6 µg/g) in channel catfish samples from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996).

Copper concentrations in bass and carp were measured in previous LRMN studies. Concentrations in bass were 0.34–1.16 µg/g in the CDRB (Hinck and others, 2006b), 0.34–1.01 µg/g in the CRB (Hinck and others, 2004a), 0.35–0.72 µg/g in the MRB (Schmitt and others, 2002), and 0.36–0.95 µg/g in the RGB (Schmitt and others, 2004). In carp, concentrations were 0.71–1.39 µg/g in the CDRB (Hinck and others, 2006b), 0.86–3.92 µg/g in the CRB (Hinck and others, 2004a), 0.47–2.68 µg/g in the MRB (Schmitt and others, 2002), and 0.56–1.80 µg/g in the RGB (Schmitt and others, 2004). Copper concentrations in fish from the MORB (0.26–1.42 µg/g), ARB (0.27–1.26 µg/g), SRB (0.25–1.15 µg/g), and PDRB (0.27–2.09 µg/g) were similar to CDRB, CRB, MRB, RGB, and YRB concentrations in benthivorous species but less than those in piscivorous species (table 8).

The ecological relevance of Cu in MORB, ARB, SRB, and PDRB fish was unknown, and tissue-based criteria for Cu were not available for the protection of avian and mammalian wildlife (Eisler, 1997). Cyprinids appear to be less sensitive to Cu toxic-

ity than salmonids, although elevated concentrations can cause more severe gill damage and epithelial swelling in carp (De Boeck and others, 2004). Chronic Cu exposure has been associated with physiological effects, including changes in oxygen consumption, ionic regulation, and cell types as well as endocrine disrupting effects such as adrenergic response and cortisol release (Handy, 2003). Copper accumulates in the kidney during chronic exposure, and fish exposed to dietary Cu may also have increased MA activity in the kidney (Handy, 2003).

Chromium

Chromium was detected in all samples, and the greatest concentrations (>1.0 µg/g) were measured in female carp from Stations 328 and 332, female bass from Station 332, and male bass from Station 333 (fig. 7; table 6). The maximum concentration (2.35 µg/g) was measured in female bass from Station 332 (table 6). Concentrations of Cr did not differ significantly among sites ($F_{26, 26} = 1.47$, $P > 0.05$).

Chromium concentrations in 2004 samples (0.06–2.35 µg/g) were compared to whole-body fish concentrations from other studies within the MORB, ARB, SRB, and PDRB and previous LRMN investigations. Chromium was not detected (<0.5 µg/g) in channel catfish samples from the lower MORB near Stations 328 and 329 (USEPA, 1995). Concentrations in bass were 0.31–2.38 µg/g in the CDRB (Hinck and others, 2006b), 0.30–3.70 µg/g in the CRB (Hinck and others, 2004a), 0.23–2.13 µg/g in the MRB (Schmitt and others, 2002), and 0.71–70.1 µg/g in the RGB (Schmitt and others, 2004). In carp, concentrations were <0.15–0.73 µg/g in the CDRB (Hinck and others, 2006b), 0.76–3.96 µg/g in the CRB (Hinck and others, 2004a), 0.25–7.48 µg/g in the MRB (Schmitt and others, 2002), and 0.38–71.8 µg/g in the RGB (Schmitt and others, 2004). Chromium concentrations in fish from the MORB (0.17–1.01 µg/g), ARB (0.29–2.35 µg/g), SRB (0.23–1.32 µg/g), and PDRB (0.06–0.82 µg/g) were similar to those from the CDRB, MRB, and YRB but less than concentrations in fish from the CRB and RGB (table 8).

Toxicity thresholds have not been established for Cr. Eisler (1986) suggested that Cr concentrations >4.0 µg/g dw (1.0 µg/g ww assuming 75% moisture) in the tissues and organs of fish and wildlife indicate environmental contamination, but the significance of such a value is unclear. Studies linking whole-body Cr concentrations to survival or growth effects in freshwater fishes were not found (Jarvinen and Ankley, 1999).

Nickel

Concentrations of Ni were >LOD (0.11 µg/g) in 49 of 51 samples (96%) from all sites (table 6). Concentrations >0.50 µg/g were measured in samples from Stations 326, 329, 335, 337, and 338, and the maximum concentration (1.64 µg/g) was measured in female bass from Station 337 (fig. 7; table 6). Nickel concentrations did not differ significantly among sites ($F_{26, 26} = 1.15$, $P > 0.05$).

Nickel concentrations in 2004 samples (<0.10–1.64 µg/g) were compared to whole-body fish concentrations from other studies within the MORB, ARB, SRB, and PDRB and previous LRMN investigations. Mean Ni concentrations were <0.02–35 µg/g in bass and <0.03–32 µg/g in channel catfish samples from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996). Many Ni concentrations were <LOD in previous LRMN studies. Concentrations in bass were <0.15–1.53 µg/g in the CDRB (Hinck and others, 2006b), <0.22–0.31 µg/g in the CRB (Hinck and others, 2004a), <0.20–2.48 µg/g in the MRB (Schmitt and others, 2002), and 0.23–3.29 µg/g in the RGB (Schmitt and others, 2004). In carp, concentrations were <0.17–1.72 µg/g in the CDRB (Hinck and others, 2006b), <0.25–0.75 µg/g in the CRB (Hinck and others, 2004a), <0.26–5.59 µg/g in the MRB (Schmitt and others, 2002), and 0.18–4.21 µg/g in the RGB (Schmitt and others, 2004).

Studies are lacking for linkages of whole-body concentrations to effects for Ni (Jarvinen and Ankley, 1999), and tissue-based criteria for the protection of fishes and piscivorous wildlife were not available for Ni. Concentration data for additional elements analyzed as a part of this study are available at <*http://www.cerc.usgs.gov/data/search.htm*>.

Organochlorine Contaminants

DDT and Primary Metabolites

The United States banned the use of DDT in 1972, although concentrations of this persistent organochlorine residue and its metabolites remain present in the environment from historical use as an insecticide on cotton, fruits, and vegetables, for mosquito control, near former sites of production and formulation, and as a consequence of atmospheric transport (Fernadez and Grimalt, 2003; Schmitt and others, 2002; Stell and others, 1995). The parent compound, *p,p'*-DDT, exceeded the LOD (>0.47 ng/g) in 37 of 51 whole-body composite samples (73%) from 10 sites (table 9), but all concentrations of *p,p'*-DDT were <12 ng/g. The greatest *p,p'*-DDT concentrations (>5 ng/g) were measured in bass from Stations 330 and 331 (fig. 8). The primary metabolite of *p,p'*-DDT, *p,p'*-DDE, was detected in all samples, and the maximum concentration (310 ng/g) was measured in male carp from Station 330 (table 9; fig. 9). Other samples with relatively high *p,p'*-DDE concentrations (>100 ng/g) included female and male bass from Station 330 (fig. 9). Concentrations of *p,p'*-DDE differed significantly among sites in bass and carp, with significantly greater concentrations in fish from Station 330 than those from other sites (table 10). Total DDT (*p,p'*- homologs) were greatest in fish from Station 330, although relatively high concentrations also were measured in samples from Stations 327, 329, and 336 (fig. 8). Concentrations of *p,p'*-DDD from *p,p'*-DDT breakdown and use as an insecticide were detected in all samples, and the greatest concentrations (12–19 ng/g) were measured in fish from Stations 330 and 331 (table 9).

Table 9. Percentage of samples and stations with concentrations exceeding the limit of detection for organochlorine chemical residues in composite samples of whole fish.

[ng/g, nanograms per gram; F, female; M, male; NA, not applicable]

Analyte(s)	Samples (% of 51)	Stations (% of 13)	Maximum concentration			
			ng/g	Station location and number	Gender	Species
p,p'-DDT	73	77	12	Omaha, GA (330)	F	Bass
p,p'-DDD	100	100	19	Omaha, GA (330)	M	Carp
p,p'-DDE	100	100	310	Omaha, GA (330)	M	Carp
Total *p,p'*-homologs[a]	NA	NA	330	Omaha, GA (330)	M	Carp
o,p'-DDT	24	38	1.7	Sylvania, GA (334)	F	Carp
o,p'-DDD	96	100	3.1	Bucks, AL (329)	M	Carp
o,p'-DDE	57	77	14	Bucks, AL (329)	M	Carp
Aldrin	16	23	0.20	Rockingham, NC (336)	M	Bass
Dieldrin	98	100	19	Albany, GA (331)	F	Bass
Endrin	14	46	1.7	Bucks, AL (329)	M	Carp
cis-Chlordane	82	85	22	Omaha, GA (330)	M	Carp
trans-Chlordane	100	100	11	Omaha, GA (330)	M	Carp
cis-Nonachlor	100	100	17	Omaha, GA (330)	F	Bass
trans-Nonachlor	100	100	23	Omaha, GA (330)	M, F	Bass
Oxychlordane	90	100	6.0	Albany, GA (331)	F	Bass
Heptachlor epoxide	94	100	5.0	Omaha, GA (330)	M	Carp
Heptachlor	0	0	NA	NA	NA	NA
Total chlordane-related residues[b]	NA	NA	75	Omaha, GA (330)	M	Carp
Toxaphene	84	100	100	Albany, GA (331)	F	Carp
Mirex	98	100	22	Omaha, GA (330)	M	Carp
Hexachlorobenzene	100	100	13	Childersburg, AL (327)	F	Carp
Pentachlorobenzene	35	54	1.2	Augusta, GA (333)	F	Carp
Pentachloroanisole	90	100	4.0	Rockingham, NC (336)	F	Carp
α- hexachlorocyclohexane	12	23	0.29	Blountstown, FL (332)	M	Bass
β- hexachlorocyclohexane	12	23	0.54	Blountstown, FL (332)	F	Carp
γ- hexachlorocyclohexane (Lindane)	8	15	0.41	Childersburg, AL (327)	M	Carp
δ- hexachlorocyclohexane	0	0	NA	NA	NA	NA
Total hexachlorocyclohexane (HCH)[c]	NA	NA	0.87	Blountstown, FL (332)	F	Carp
Dacthal	31	46	2.8	Albany, GA (331)	F	Carp
Endosulfan I	0	0	NA	NA	NA	NA
Endosulfan II	33	46	0.63	Sylvania, GA (334)	M	Carp
Endosulfan sulfate	55	85	1.2	Bucksport, SC (338)	F	Carp
Methoxychlor	0	0	NA	NA	NA	NA
Total PCBs	100	100	2700	Childersburg, AL (327)	M	Bass
TCDD-EQ	22	46	33.6	Childersburg, AL (327)	M	Bass

[a]Sum of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE with censored values represented as one-half the limit of detection.

[b]Sum of *cis*- and *trans*-chlordanes and nonachlors; oxychlordane; heptachlor; and heptachlor epoxide with censored values represented as one-half the limit of detection.

[c]Sum of α-, β-, γ-, and δ-hexachlorocyclohexane with censored values represented as one-half the limit of detection.

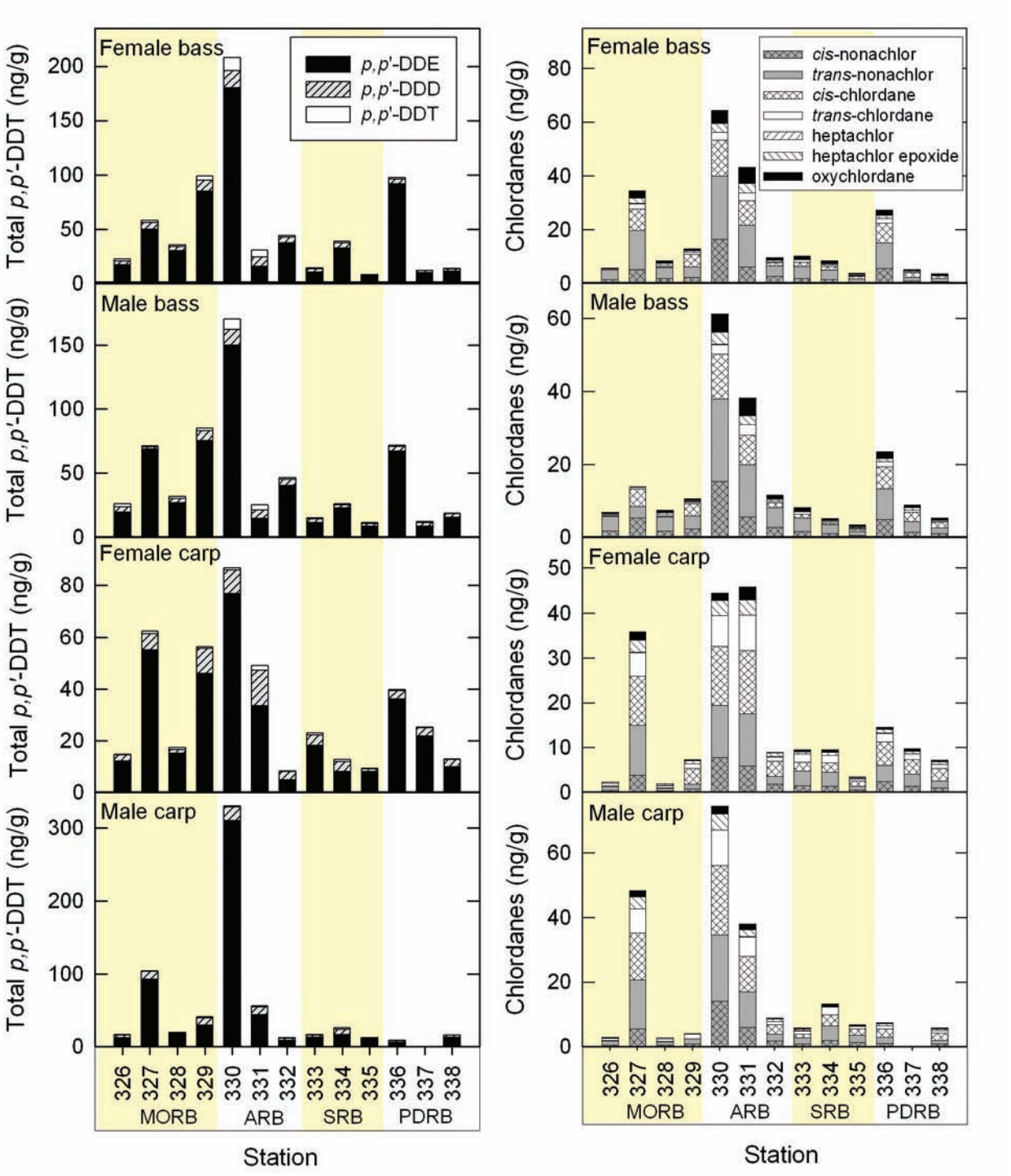


Figure 8. Unweighted geometric mean concentrations (nanograms per gram (ng/g) wet weight) of total *p,p'*-DDT (*p,p'*-DDT, DDE, and DDD) and chlordane-related compounds (*cis*- and *trans*-chlordanes and nonachlors, heptachlor, heptachlor epoxide, and oxychlordane) by station in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Censored values are represented by one-half the limit of detection in the computation of means and totals but are not shown in the figure. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

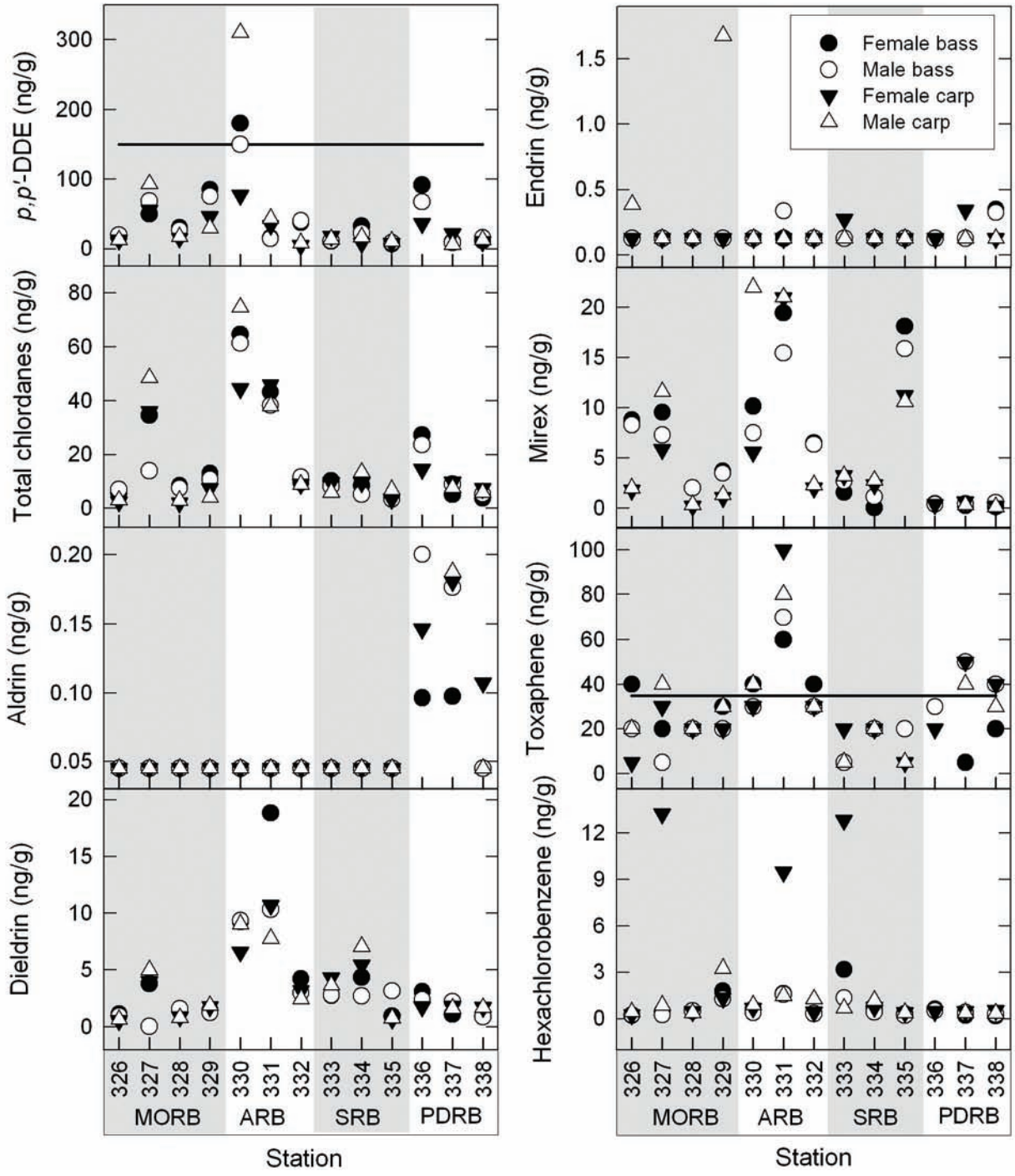


Figure 9. Concentrations (nanograms per gram (ng/g) wet weight) of formerly used organochlorine pesticides and their metabolites including *p,p'*-DDE, total chlordanes, aldrin, dieldrin, endrin, mirex, toxaphene, and hexachlorobenzene by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Total chlordanes are the sum of *cis*- and *trans*-chlordanes and nonachlors, heptachlor, heptachlor epoxide, and oxychlordane. Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. Literature-based toxicity thresholds were plotted if concentrations in one or more samples exceeded the threshold; toxicity threshold were not available for all organochlorine contaminants. The reference line on the *p,p'*-DDE graph represents the toxicity threshold for sensitive avian wildlife (150 ng/g; Anderson and others, 1975). The reference line on the toxaphene graph represents the lowest threshold where reproductive effects have been documented in fish (35 ng/g; Mayer and others, 1975). See table 5 for station descriptions.

Table 10. Spatial trends of organochlorine residues in fish.

[Mean wet weight concentrations (± standard errors) are presented. Values within each group of species-station means followed by the same letter were not significantly different ($P > 0.01$, Fisher's unrestricted least significance difference). Also shown are analysis of variance F-values, degrees-of-freedom, and significance levels (*, $P \leq 0.01$). $n = 2$ composite samples for all species-station group except for carp from Station 336 where $n = 1$. Stations are listed upstream to downstream within a basin. Chlordanes, sum of *cis*- and *trans*-chlordanes and nonachlors, heptachlor, heptachlor epoxide, and oxychlordane; PCA, pentachloroanisole; PCBs, sum of all congeners; ng/g, nanograms per gram]

Species, station location, and station number	*p,p'*-DDE (ng/g)		Chlordanes (ng/g)		Dieldrin (ng/g)		Mirex (ng/g)		Toxaphene (ng/g)		PCA (ng/g)		PCBs (ng/g)	
Bass														
Lavaca, AL (326)	18.5 ± 1.5	abcd	6.4 ± 0.7	ab	1.03 ± 0.08	ab	8.55 ± 0.25	cd	30 ± 10	bc	0.22 ± 0.01	bcd	185 ± 25	ab
Childersburg, AL (327)	59.5 ± 9.5	efg	24.2 ± 10.3	d	1.92 ± 1.88	a	8.40 ± 1.10	cd	13 ± 8	ab	0.72 ± 0.29	f	2100 ± 600	d
Eureka Landing, AL (328)	28.5 ± 1.5	cde	8.0 ± 0.5	bc	1.55 ± 0.05	abc	2.00 ± 0.00	bc	20 ± 0	abc	0.31 ± 0.01	de	230 ± 0	ab
Bucks, AL (329)	80.5 ± 4.5	gh	11.8 ± 1.1	cd	1.35 ± 0.15	ab	3.60 ± 0.10	cd	25 ± 5	bc	0.27 ± 0.01	cde	215 ± 35	ab
Omaha, GA (330)	165.0 ± 15.0	h	63.0 ± 1.7	e	9.30 ± 0.10	cd	8.75 ± 1.25	cd	35 ± 5	bc	0.46 ± 0.12	ef	965 ± 25	cd
Albany, GA (331)	15.5 ± 0.5	abcd	40.7 ± 2.5	de	14.50 ± 4.50	d	17.00 ± 2.00	d	65 ± 5	c	0.21 ± 0.01	bcd	270 ± 30	abc
Blountstown, FL (332)	38.5 ± 1.5	defg	10.6 ± 1.0	c	3.60 ± 0.60	bcd	6.40 ± 0.10	cd	35 ± 5	bc	0.15 ± 0.02	b	110 ± 0	ab
Augusta, GA (333)	11.0 ± 0.0	ab	9.3 ± 1.1	bc	2.80 ± 0.00	bcd	2.15 ± 0.55	bc	5 ± 0	a	0.05 ± 0.00	ab	195 ± 35	ab
Sylvania, GA (334)	27.5 ± 4.5	bcde	6.9 ± 1.7	abc	3.55 ± 0.85	bcd	0.63 ± 0.58	a	20 ± 0	abc	0.08 ± 0.03	ab	111 ± 20	ab
Port Wentworth, GA (335)	7.8 ± 0.9	a	3.7 ± 0.2	a	2.07 ± 1.13	abc	17.00 ± 1.00	d	20 ± 0	abc	0.05 ± 0.00	a	69 ± 5	a
Rockingham, NC (336)	79.5 ± 12.5	fgh	25.4 ± 1.8	d	2.75 ± 2.75	bcd	0.40 ± 0.02	a	30 ± 0	bc	0.49 ± 0.08	ef	385 ± 5	bc
Pee Dee, SC (337)	9.4 ± 0.7	a	7.0 ± 1.9	abc	1.65 ± 0.55	abc	0.36 ± 0.10	a	28 ± 23	abc	0.18 ± 0.02	bcd	155 ± 35	ab
Bucksport, SC (338)	14.0 ± 2.0	abc	4.5 ± 0.8	ab	0.89 ± 0.02	ab	0.36 ± 0.20	a	30 ± 10	bc	0.16 ± 0.00	bc	392 ± 319	ab
Carp														
Lavaca, AL (326)	12.5 ± 0.5	ab	2.7 ± 0.3	ab	0.60 ± 0.05	a	1.90 ± 0.10	bcde	13 ± 8	ab	1.19 ± 0.31	ab	68 ± 2	a
Childersburg, AL (327)	74.0 ± 19.0	de	42.2 ± 6.3	f	4.50 ± 0.50	bc	8.90 ± 3.10	efg	35 ± 5	bcd	3.00 ± 0.40	c	1575 ± 625	e
Eureka Landing, AL (328)	16.0 ± 1.0	abc	2.5 ± 0.5	a	0.82 ± 0.04	ab	0.27 ± 0.08	ab	20 ± 0	abc	1.00 ± 0.10	ab	96 ± 1	abc
Bucks, AL (329)	38.0 ± 8.0	cd	5.7 ± 1.6	bcd	1.85 ± 0.05	abc	1.20 ± 0.10	bcd	25 ± 5	bcd	2.75 ± 0.05	c	185 ± 35	abcd
Omaha, GA (330)	193.5 ± 116.5	e	59.5 ± 15.1	f	7.80 ± 1.20	c	13.80 ± 8.20	fg	35 ± 5	bcd	2.65 ± 0.05	c	850 ± 450	de
Albany, GA (331)	39.0 ± 5.0	cd	41.9 ± 3.9	f	9.40 ± 1.60	c	21.00 ± 0.00	g	90 ± 10	d	1.65 ± 0.15	bc	335 ± 85	cd
Blountstown, FL (332)	6.6 ± 1.9	a	9.0 ± 0.0	cde	2.80 ± 0.40	abc	2.15 ± 0.15	cde	30 ± 0	bcd	0.77 ± 0.09	a	415 ± 285	bcd
Augusta, GA (333)	15.5 ± 2.5	abc	7.7 ± 1.8	cde	4.00 ± 0.30	abc	3.20 ± 0.00	def	13 ± 8	ab	0.73 ± 0.04	a	124 ± 26	abc
Sylvania, GA (334)	12.1 ± 4.0	ab	11.4 ± 1.8	de	6.25 ± 0.85	c	2.55 ± 0.25	def	20 ± 0	abc	0.96 ± 0.14	ab	116 ± 34	abc
Port Wentworth, GA (335)	9.6 ± 1.5	a	5.1 ± 1.7	abc	0.70 ± 0.04	ab	11.00 ± 0.00	fg	5 ± 0	a	0.47 ± 0.01	a	77 ± 8	ab
Rockingham, NC (336)	36.0	bcd	14.5	e	1.70	abc	0.37	abc	20	abcd	4.00	c	310	abcde
Pee Dee, SC (337)	14.1 ± 8.0	ab	8.6 ± 1.1	cde	1.70 ± 0.10	abc	0.45 ± 0.15	ab	45 ± 5	cd	2.60 ± 0.20	c	215 ± 35	abcd
Bucksport, SC (338)	11.5 ± 1.6	ab	6.5 ± 0.7	cde	1.70 ± 0.00	abc	0.16 ± 0.03	a	35 ± 5	bcd	3.35 ± 0.25	c	120 ± 0	abc
$F_{25,25}$	14.91*		23.74*		3.44*		14.81*		3.54*		66.58*		6.40*	

Concentrations of *p,p'*-DDE in 2004 samples (4.7–310 ng/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. *p,p'*-DDT and its metabolites have been previously identified as chemicals of concern in sediment, water, and fish tissues in the lower MORB as a result of chemical manufacturing facilities in the basin (USFWS, 1996). Historical *p,p'*-DDE concentrations from NCBP sites near Stations 328 (40–1,700 ng/g), 332 (20–2,620 ng/g), 335 (10–1,200 ng/g), and 338 (10–480 ng/g) were greater in bass and carp compared to concentrations in 2004 samples (Schmitt and others, 1999). Concentrations of *p,p'*-DDE were much lower in carp from Stations 329 and 330 than those measured previously near these sites (409–590 ng/g; USEPA, 1992). Concentrations of *p,p'*-DDE were low in benthivorous whole-body (<2–17.7 ng/g) and piscivorous fillet (<2–5.1 ng/g) samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d) but were relatively high in channel catfish (6–1,100 ng/g) and bass (4–4,130 ng/g) from the lower MORB near Stations 328 and 329 (USEPA, 1995; USFWS, 1996). Overall, concentrations of DDT and its metabolites were lower in fish from Stations 328, 329, and 330 in our study compared to those in previous studies.

Concentrations of *p,p'*-DDE in bass and carp were measured in previous LRMN studies. Concentrations in bass were 1–2,700 ng/g in the CDRB (Hinck and others, 2006b), <10–1,200 ng/g in the CRB (Hinck and others, 2006a), <10–530 ng/g in the MRB (Schmitt and others, 2002), and <10–400 ng/g in the RGB (Schmitt and others, 2005). In carp, concentrations were 7–1,700 ng/g in the CDRB (Hinck and others, 2006b), <10–1,100 ng/g in the CRB (Hinck and others, 2006a), <10–8,300 ng/g in the MRB (Schmitt and others, 2002), and <10–670 ng/g in the RGB (Schmitt and others, 2005). Overall, *p,p'*-DDE concentrations in fish from the MORB (12–93 ng/g), ARB (5–310 ng/g), SRB (7–32 ng/g), and PDRB (6–92 ng/g) were greater than those from the YRB and less than those from the CDRB, CRB, MRB, and RGB (table 11).

Concentrations of total DDT in fish >150 ng/g are potentially harmful to the brown pelican (*Pelicanus occidentalis*), a sensitive avian species (Anderson and others, 1975). Protective wildlife criteria as low as 200 ng/g have been suggested by Newell and others (1987). Total DDT concentrations of 1,000–3,000 ng/g are potentially hazardous to most piscivorous birds (Blus, 1996), and whole-body concentrations as low as 500 ng/g have been associated with toxic effects to fish (Jarvinen and Ankley, 1999). Whole-body total DDT concentrations <5,000 ng/g have reduced survival of fry or fingerlings in freshwater fish species (Burdick and others, 1964; Cuerrier and others, 1967; Hopkins and others, 1969; Johnson and Pecor, 1969; Macek, 1968). Beckvar and others (2005) recommended that whole-body total DDT concentrations should not exceed 600 ng/g to protect juvenile and adult fish and 700 ng/g to protect early life stage fish (egg, embryo, and fry). Whole-body *p,p'*-DDE concentrations of 1,003 ng/g altered sex steroid hormone (E2 and KT) concentrations in blood plasma and gene expression involved in the endocrine pathway in adult largemouth bass (Garcia-Reyero and others, 2006). Concentrations of *p,p'*-DDE in male carp and female and male bass from Station 330 exceeded thresholds to protect sensitive avian species (150 ng/g; fig. 9); therefore, total DDT and *p,p'*-DDE concentrations pose a risk to wildlife and this site on the Chattahoochee River.

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 and others, 1999; 2002; Hinck and others, 2006b). Concentrations of *o,p'*-DDE (<0.81–14 ng/g), *o,p'*-DDD (<0.10–3.1 ng/g), and *o,p'*-DDT (<0.10–1.7 ng/g) generally were low in all samples but were greatest in samples from Station 329 (table 9). Concentrations of *o,p'*-homologs generally were not detected or low (≤10 ng/g) in bass or carp from the RGB and CRB (Hinck and others, 2006a; Schmitt and others, 2005), but *o,p'*-DDE (<0.1–17 ng/g), *o,p'*-DDD (<1–17 ng/g), and *o,p'*-DDT (<1–110 ng/g) concentrations in CDRB fish were greater than those measured in MORB, ARB, SRB, and PDRB samples (Hinck and others, 2006b; 2007). The *o,p*'-homologs historically were considered relatively benign, but studies have determined that these compounds are estrogenic (Ackerman and others, 2002; Donohoe and Curtis, 1996; Guillette and others, 1996; Metcalfe and others, 2000; Papoulias and others, 2003; Toppari and others, 1996). Dietary exposure to estrogenic chemicals including *o,p*'-DDT and *o,p*'-DDE produced hepatotoxicity in rainbow trout, potentially causing decreased HSI values, plasma vtg concentrations, and lipid levels (Donohoe and Curtis, 1996). Papoulias and others (2003) reported that low concentrations of *o,p*'-DDE (<0.5 ng/egg) may interfere with the binding of natural ligands to steroid binding receptors and proteins resulting in endocrine-disrupting effects such as decreased GSI values. Conversely, Ungerer and Thomas (1996) determined that increases in dietary *o,p*'-DDT concentrations (0.01–0.05 ng/ 100 g fish/day) were associated with increased GSI values in female Atlantic croaker (*Micropogonias undulatus*) and concluded that *o,p*'-DDT binds to different lipoproteins in the plasma and compartmentalizes in triglyceride-rich oil globules within the oocyte. However, Metcalfe and others (2000) suggested that continuous exposure to estrogenic compounds such as *o,p*'-DDT must begin *in ovo* and continue throughout early development to affect reproductive endpoints in fish. The total risk to fish and wildlife represented by concentrations of *o,p'*-DDT and its homologs was unknown.

Chlordane and Heptachlor

Chlordane is a mixture of cyclopentadiene-derived compounds that was widely used as a soil insecticide until 1988. Concentrations of these compounds typically have been greatest in fish from corn-growing regions, urban areas in the "termite belt" and the southeastern United States, and near production and formulation facilities (Schmitt and others, 1999; Schmitt, 2002). Seven chlordane-related constituents were measured: *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor, and

Table 11. Mean concentrations of select organochlorine residues in piscivorous and benthivorous fish from Biomonitoring of Environmental Status and Trends (BEST) Program studies.

[Unweighted geometric means (all wet weight) are presented. Studies include the Mobile River Basin, Apalachicola-Chattahoochee-Flint River Basin, Savannah River Basin, Pee Dee River Basin, Colorado River Basin (Hinck and others, 2006b), Columbia River Basin (Hinck and others, 2004a), Mississippi River Basin (Schmitt, 2002), Rio Grande Basin (Schmitt and others, 2004), and Yukon River Basin (Hinck and others, 2004b). Censored values were replaced by one-half the limit of detection for the computations of basin means. DDT is the sum of *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT. Chlordane is the sum of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor epoxide, and oxychlordane. Polychlorinated biphenyl (PCB) is the sum of all congeners. ng/g, nanograms per gram; pg/g, picograms per gram; Pisc, Piscivrous; Ben, Benthivorous; Min, minimum; Max, maximum; <, less than]

Basin	*p,p'*-DDE (ng/g)		DDT (ng/g)		Chlordane (ng/g)		Toxaphene (ng/g)		PCB (ng/g)		TCDD-EQ (pg/g)	
	Pisc[a]	Ben[b]	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben	Pisc	Ben
Southeastern U.S. basins sampled (2004)												
Mobile												
Mean	40	27	47	33	11	6	19	20	366	203	0.88	2.16
Min	17	12	22	15	6	2	<10	<10	160	66	<0.30	<0.30
Max	85	93	99	104	35	48	40	40	2700	2200	33.6	14.8
Apalachicola-Chattahoochee-Flint												
Mean	46	33	62	45	30	28	43	45	306	413	0.59	<0.30
Min	15	5	25	8	10	9	30	30	110	130	<0.50	<0.30
Max	180	310	208	330	65	75	70	100	990	1300	4.6	<0.50
Savannah												
Mean	13	12	17	16	6	7	13	10	113	101	<0.50	<0.50
Min	7	8	8	9	3	3	<10	<10	64	69	<0.50	<0.50
Max	32	18	26	39	10	13	20	20	230	150	<0.70	<0.70
Pee Dee												
Mean	22	14	25	18	9	9	24	34	237	182	0.68	<0.70
Min	9	6	12	9	6	4	<10	20	73	120	<0.70	<0.70
Max	92	36	97	40	27	15	50	50	710	310	13.7	<0.70
Other basins sampled (1995–2003)												
Colorado (2003)												
Mean	40	49	47	60	9	12	20	19	42	66	0.15	0.13
Min	1	4	3	8	2	3	<24	<24	<48	<48	<0.01	<0.01
Max	2700	1700	1743	2768	121	121	870	740	2100	1600	6.0	6.0
Columbia (1997)												
Mean	144	167	185	220	33	34	<30	16	76	83	2.4	1.97
Min	<10	<10	15	15	30	30	<30	<30	<30	<30	0.0	0.0
Max	1200	1100	1390	1305	72	125	<30	50	1300	750	8.0	43.0
Mississippi (1995)												
Mean	37	78	62	120	42	54	27	41	142	132	3.0	3.4
Min	<10	<10	15	15	30	30	<50	<50	<50	<50	<0.21	<0.40
Max	530	8300	840	1111	363	527	740	8300	2000	3300	62.0	68.0
Rio Grande (1997)												
Mean	75	100	103	121	41	33	46	33	<50	<50	1.7	1.2
Min	<10	12	15	22	30	30	<50	<50	<50	<50	<0.40	<0.35
Max	1600	670	1686	700	211	51	2400	670	<50	<50	6.0	3.0
Yukon (2002)												
Mean	1	1	3	3	2	3	10	8	27	37	<1.7	<1.7
Min	<1	<1	1	1	<1	<1	<12	<12	<10	24	<1.7	<1.7
Max	4	5	14	13	7	5	34	19	63	87	<1.7	<1.7

[a]Piscivorous fish samples sizes for all organochlorine chemical contaminants were $n = 4$ for the Mobile, $n = 3$ for the Apalachicola-Chattahoochee-Flint, $n = 3$ for the Savannah, $n = 3$ for the Pee Dee, $n = 12$ for the Colorado, $n = 14$ for the Columbia, $n = 34$ for the Mississippi, $n = 9$ for the Rio Grande, and $n = 10$ for the Yukon.

[b]Benthivorous fish samples sizes for all organochlorine chemical contaminants were $n = 4$ for the Mobile, $n = 3$ for the Apalachicola-Chattahoochee-Flint, $n = 3$ for the Savannah, $n = 3$ for the Pee Dee, $n = 14$ for the Colorado, $n = 16$ for the Columbia, $n = 46$ for the Mississippi, $n = 10$ for the Rio Grande, and $n = 5$ for the Yukon.

heptachlor epoxide (table 9). Oxychlordane is a metabolite of *cis*-chlordane. Heptachlor epoxide, a metabolite of heptachlor, is a minor constituent of chlordane and also was used historically as an insecticide; environmental concentrations result from both sources. Heptachlor was not detected (<0.1 ng/g) in any sample, and concentrations of oxychlordane and heptachlor epoxide were low (≤6 ng/g; table 9). Concentrations of c*is*-chlordane were >LOD in 42 of 51 (82%) samples from 11 sites (table 9), and concentrations were >10 ng/g in fish from Stations 327, 330, and 331 (fig. 8). *Trans*-chlordane was detected in all samples (0.22–11 ng/g), but concentrations were >10 ng/g in male carp from Station 330 only (table 9). *Cis*-nonachlor also was detected in all samples (0.33–17 ng/g), and the greatest concentrations were measured in fish from Stations 330 (7.8–17 ng/g) and 331 (5.6–6.2 ng/g; fig. 8). *Trans*-nonachlor concentrations were 0.57–23 ng/g, and concentrations were >10 ng/g in fish samples from Stations 327, 330, and 331 (fig. 8). Concentrations of total chlordanes (sum of seven compounds) ranged from 2 to 75 ng/g, and concentrations were greatest in fish from Stations 327, 330, and 331 (figs. 8 & 9). Total chlordane concentrations were significantly greater in samples from Stations 327, 330, and 331 than those from most other sites (table 10). *Trans*-nonachlor, *trans*-chlordane, and *cis*-chlordane were the primary constituents of total chlordanes at these sites (fig. 8). Relatively high total chlordane concentrations also were measured in bass from Station 336 (24–27 ng/g; fig. 8).

Chlordane constituents in 2004 samples were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Historical NCBP concentrations of *cis*-chlordane (10–70 ng/g), *trans*-chlordane (≤10 ng/g), *cis*-nonachlor (10–60 ng/g), *trans*-nonachlor (<10–70 ng/g), oxychlordane (≤10 ng/g), heptachlor (<10 ng/g), and heptachlor epoxide (≤10 ng/g) were low in bass near Stations 328, 329, 332, 335, and 338 from 1970 to 1986 (Schmitt and others, 1999) and were similar to concentrations measured at these sites in 2004. In a USEPA study, concentrations of *cis*-chlordane (8–42 ng/g), *trans*-chlordane (5–40 ng/g), and *trans*-nonachlor (9–53 ng/g) were greatest in benthivorous fish in close proximity to pulp and paper mills near Stations 327, 330, and 333 (USEPA, 1992). Concentrations of *cis*-chlordane (<4–9.1 ng/g), *trans*-chlordane (<2–6 ng/g), *cis*-nonachlor (<4–22 ng/g), *trans*-nonachlor (<4–17 ng/g), oxychlordane (<4–7 ng/g), heptachlor (<2–11 ng/g), and heptachlor epoxide (<2 ng/g) were low in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Concentrations of *trans*-chlordane (<20–40 ng/g), *cis*-nonachlor (<20–90 ng/g), and heptachlor epoxide (<20–320 ng/g) in bass from the lower MORB were greater than those measured in bass from Station 329 in this study (USFWS, 1996).

Total chlordane concentrations were measured in previous LRMN studies. Concentrations in bass were 1–33 ng/g in the CDRB (Hinck and others, 2006b), <30–72 ng/g in the CRB (Hinck and others, 2006a), <30–246 ng/g in the MRB (Schmitt and others, 2002), and <30–59 ng/g in the RGB (Schmitt and others, 2005). In carp, concentrations were 3–121 ng/g in the CDRB (Hinck and others, 2006b), <30–125 ng/g in the CRB (Hinck and others, 2006a), <30–527 ng/g in the MRB (Schmitt and others, 2002), and <30–51 ng/g in the RGB (Schmitt and others, 2005). Total chlordane concentrations in fish from the MORB (2–48 ng/g), ARB (9–75 ng/g), SRB (3–13 ng/g), and PDRB (4–27 ng/g) were generally greater than or equal to those from the CDRB and YRB and less than those from the CRB, MRB, and RGB (table 11). However, greater mean concentrations in samples from CRB, MRB, and RGB were partially the result of greater LODs for chlordane constituents in the CRB, MRB, and RGB studies (5 ng/g) compared to the other LRMN studies (0.1–0.3 ng/g). Total chlordane concentrations in fish from the MORB, ARB, SRB, and PDRB did not exceed 300 ng/g, concentrations that may pose a threat to predatory fish and fish-eating birds (Eisler, 1990). Fish and wildlife were not at risk from chlordane in the MORB, ARB, SRB, and PDRB using these criteria.

Aldrin and Dieldrin

Most environmental dieldrin is present because of the breakdown of aldrin, which has not been used in the United States since 1974. Aldrin was detected (10–20 ng/g) in eight of 51 (16%) samples from Stations 336, 337, and 338 in the PDRB (table 9; fig. 9). Concentrations of dieldrin were detected in 50 of 51 (98%) samples representing all sites and were ≥9 ng/g in carp and bass from Stations 330 and 331 (table 9; fig. 9). Dieldrin concentrations differed significantly among sites in bass and carp (table 10). Concentrations were significantly greater in bass from Station 331 than bass from most other sites, and dieldrin concentrations were significantly greater in carp from Stations 330, 331, and 334 than carp from Stations 326, 328, and 335 (table 10).

Dieldrin concentrations were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Dieldrin concentrations in 2004 samples were less than historical NCBP concentrations near Stations 328 (<5–40 ng/g), 332 (<5–1,590 ng/g), 335 (<5–550 ng/g), and 338 (<5–290 ng/g) from 1970 to 1986 (Schmitt and others, 1999). Whole-body dieldrin concentrations in benthivorous fish were <3 ng/g near Stations 329 and 330, 15 ng/g near Station 331, 21 ng/g near Station 333, and 4 ng/g near Station 335 in a national USEPA study (USEPA, 1992) and were similar to concentrations in 2004 samples from these sites. Dieldrin concentrations also were low in benthivorous whole-body (<1–7 ng/g) and piscivorous fillet (<1 ng/g) samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Previous LRMN studies from the CDRB (Hinck and others, 2006b), CRB (Hinck and others, 2006a), MRB (Schmitt and others, 2002), and RGB (Schmitt and others, 2005) reported low dieldrin concentrations in most bass (<10–80 ng/g) and carp (<10–250 ng/g).

Toxicity studies reporting whole-body dieldrin concentrations in fish were limited. Whole-body dieldrin concen-

trations of 360–2,130 ng/g in juvenile rainbow trout did not affect survival or growth, but concentrations of 5,650 ng/g reduced survival (Shubat and Curtis, 1986; Macek and others, 1970). Whole-body dieldrin concentrations of 204 ng/g decreased sex steroid hormone (E2 and KT) concentrations in blood plasma and altered gene expression involved in the endocrine pathway in adult largemouth bass (Garcia-Reyero and others, 2006). Dieldrin concentrations in fish from the MORB (<0.1–5 ng/g), ARB (2–19 ng/g), SRB (0.7–7 ng/g), and PDRB (0.9–3 ng/g) were less than these thresholds and were unlikely to represent a significant threat to either fish or piscivorous wildlife (Jarvinen and Ankley, 1999; Peakall, 1996).

Endrin

Endrin, one of the most acutely toxic organochlorine insecticides to fish (Johnson and Finley, 1980), was used on comparatively few crops historically. Endrin was detected in 7 of 51 (14%) samples (table 9), and concentrations were >0.5 ng/g in male carp from Station 329 only (fig. 9). Endrin concentrations in 2004 samples were less than historical NCBP concentrations near Stations 328 (<5–160 ng/g), 332 (<5–10 ng/g), 335 (<5–10 ng/g), and 338 (<5–10 ng/g) from 1970 to 1986 (Schmitt and others, 1999). Endrin concentrations were <LOD or low (<10 ng/g) in bass and carp from previous LRMN investigations (Hinck and others, 2004a; 2006b; Schmitt and others, 2002; 2004). Endrin concentrations were <LOD (10 ng/g) in fish from other studies within the MORB, ARB, SRB, and PDRB (USEPA, 1992; 2004a; 2005d). Toxicity studies documenting whole-body concentrations of endrin in fish were not found. The total risk to fish and wildlife from exposure to endrin was unknown, but endrin concentrations in fish from the MORB, ARB, SRB, and PDRB were considered low (≤1.7 ng/g).

Mirex

Mirex was used as an insecticide to combat red imported fire ants (*Solenopsis wagneri*) in the southern United States. Elsewhere, mirex also was used as a flame retardant and as a polymerizing agent (Kaiser, 1987). Mirex was detected in 50 of 51 (98%) samples (table 9), and concentrations were >10 ng/g in samples from Stations 327, 330, 331, and 335 (fig. 9). The greatest concentrations (>20 ng/g) were measured in male carp from Station 330 and female and male carp from Station 331 (fig. 9). Mirex concentrations differed significantly among sites in bass and carp (table 10). Concentrations were significantly greater in bass from Stations 331 and 335 than bass from Stations 328, 333, 334 336, 337, and 338, and mirex concentrations were significantly greater in carp from Stations 330, 331, and 335 than carp from most other sites (table 10).

Mirex concentrations were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB. Mirex concentrations in historical NCBP concentrations were <10 ng/g near Stations 328 and 338, <5–40 ng/g near Station 332, and 10–120 ng/g near Station 335 from1970 to 1986 (Schmitt and others, 1999). Whole-body mirex concentrations in benthivorous fish were 6 ng/g near Station 327, 5 ng/g near Station 329, 10 ng/g near Station 330, 8 ng/g near Station 331, <2.5 ng/g near Station 333, and 73 ng/g near Station 335 in a national USEPA study (USEPA, 1992), and concentrations also were low in benthivorous whole-body (<4–5 ng/g) and piscivorous fillet (<4 ng/g) samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Previous LRMN studies also reported low concentrations of mirex (most <10 ng/g; Hinck and others, 2004a; 2006b; 2006c; Schmitt and others, 2002; 2004). Toxicity studies reporting whole-body concentrations of mirex in fish were not found. The risk of mirex to fish and wildlife is unknown, but mirex concentrations were relatively high (>15 ng/g) in fish from Stations 330 and 331 in the ARB and Station 335 in the PDRB.

Toxaphene

Toxaphene was the most heavily used insecticide in the United States following the ban on DDT (Schmitt and Winger, 1980). Use of toxaphene in the United States peaked in the late 1970s, and the pesticide was subsequently banned. Although toxaphene was used mostly on cotton, this pesticide has been transported atmospherically to remote locations, and residues have been detected in fish from the Arctic and the Great Lakes (Muir and others, 1999; Schmitt and others, 1999). Toxaphene was detected in 43 of 51 (84%) samples representing all sites (table 9), and concentrations were ≥40 ng/g in samples from Stations 326, 327, 330, 331, 332, 337, and 338 (fig. 9). Toxaphene concentrations differed among sites in bass and carp and were significantly greater in bass and carp from Station 331 than fish from most other sites (table 10).

Toxaphene was used historically in cotton growing regions of the MORB, ARB, SRB, and PDRB. Toxaphene concentrations 2004 samples (<10–100 ng/g) were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB and previous LRMN studies. Toxaphene concentrations in 2004 samples were less than historical NCBP concentrations near Stations 328 (<5–9,300 ng/g), 332 (<5–3,500 ng/g), 335 (<5–1,000 ng/g), and 338 (<5–3,300 ng/g) from 1970 to 1986 (Schmitt and others, 1999). Toxaphene was not detected (<100 ng/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Concentrations in bass were <24–870 ng/g in the CDRB (Hinck and others, 2006b), <30 ng/g in the CRB (Hinck and others, 2006a), <50–740 ng/g in the MRB (Schmitt and others, 2002), and <50–110 ng/g in the RGB (Schmitt and others, 2005). In carp, concentrations were <24–740 ng/g in the CDRB (Hinck and others, 2006b), <30 ng/g in the CRB (Hinck and others, 2006a), <50–8,300 ng/g in the MRB (Schmitt and others, 2002), and <50–670 ng/g in the RGB (Schmitt and others, 2005). Overall, toxaphene concentrations in fish from the MORB (<10–40 ng/g), ARB (30–100 ng/g), SRB (<10–20

ng/g), and PDRB (<10–50 ng/g) were similar to those from the CDRB, CRB, MRB, RGB, and YRB (table 11).

Acute and chronic effects of toxaphene on freshwater fish have been reported at whole-body concentrations ≥400 ng/g (Eisler and Jacknow, 1985; Jarvinen and Ankley, 1999). Adult brook trout containing whole-body concentrations of 400 ng/g produced eggs with reduced viability, and lake trout (*S. namaycush*) and white sucker (*Catostomus commersoni*) containing toxaphene concentrations of 35–203 ng/g also produced eggs with reduced viability (Mayer and others, 1975). Survival and growth of several freshwater fish species at various life stages were reduced at concentrations >900 ng/g (Mayer and others, 1975; 1978). Toxaphene concentrations in fish from the MORB, ARB, SRB, and PDRB did not exceed 100 ng/g, but concentrations in fish from Station 331 (60–100 ng/g) in the ARB may affect fish reproduction.

Hexachlorobenzene (HCB)

HCB was produced for use as a fungicide and was a by-product of the production of other chlorinated hydrocarbons. This compound is less toxic to fish than many other persistent organochlorines but contains toxic impurities including polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (Schmitt and others, 1999; Villanueva and others, 1974). HCB was detected in all samples (table 9), and concentrations ranged from 0.2 ng/g to 13 ng/g (fig. 9). Concentrations >9 ng/g were measured in female carp from Stations 327, 331, and 333 (fig. 9). HCB concentrations in 2004 samples generally were less than historical NCBP concentrations near Stations 328 (<10–50 ng/g), 332 (<5–60 ng/g), 335 (<10–30 ng/g), and 338 (<10–20 ng/g) from 1970 to 1986 (Schmitt and others, 1999), and HCB was not detected (<333 ng/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). HCB concentrations generally were ≤10 ng/g in bass and carp from previous LRMN investigations (Hinck and others, 2006a; 2006b; Schmitt and others, 2002; 2004). HCB concentrations in whole fish ranged from <LOD (0.01–100 ng/g) to 27,000 ng/g in United States monitoring studies included in a review by Nowell and others (1999). Protective toxicity thresholds for HCB in fish are not available. Concentrations as low as 330 ng/g in whole fish have been suggested to protect piscivorous wildlife (Newell and others, 1987). HCB concentrations in fish from the MORB, ARB, SRB, and PDRB were well below this benchmark.

Pentachlorobenzene

Pentachlorobenzene is used as a precursor in the synthesis of the fungicide pentachloronitrobenzene and as a fire retardant. Pentachlorobenzene can enter aquatic systems through industrial discharge and as a degradation product of other organochlorine compounds such as HCB (Barber and others, 1997). Pentachlorobenzene was detected in 18 of 51 (35%) samples from seven sites (table 9). The greatest concentrations (>0.4 ng/g) were measured in samples from Stations 329, 330, 331, 332, and 333 (fig. 10).

Other studies reporting pentachlorobenzene concentrations in fish from the MORB, ARB, SRB, and PDRB were limited. Although LODs were higher, pentachlorobenzene was not detected (<666 ng/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Pentachlorobenzene concentrations were <1 ng/g in previous LRMN studies (Hinck and others, 2006b, 2006c), which was consistent with the findings in this study. Few studies have examined toxicological effects of pentachlorobenzene exposure in fish and piscivorous wildlife. Histological lesions in the kidney, liver, and thyroid have been associated with pentachlorobenzene exposure in mice and rats (McDonald, 1991). Overall, the risk of pentachlorobenzene to fish and wildlife in the MORB, ARB, ARB, and PDRB was unknown.

Pentachloroanisole (PCA)

PCA, a metabolite of the wood preservative pentachlorophenol (PCP), is toxic and potentially carcinogenic (National Research Council of Canada, 1982; Schmitt and others, 1999). Early formulations of PCP contained chlorinated dioxins and other toxic impurities (Schmitt and others, 1999). PCA was detected in 46 of 51 samples (90%; table 9), and concentrations were greater in carp (0.5–4.0 ng/g) than bass (<0.1–1.0 ng/g; fig. 10). Concentrations were greatest (>2 ng/g) in carp from Stations 327, 329, 330, 336, 336, and 337 (fig. 10). PCA concentrations differed significantly among sites in bass and carp (table 10). Concentrations were significantly greater in bass from Station 327 than bass from most other sites, and PCA concentrations were significantly greater in carp from Stations 327, 329, 330, 336, 337, and 338 than carp from most other sites (table 10).

Other studies measuring PCA in fish from the MORB, ARB, SRB, and PDRB were limited. In 1986, PCA concentrations in historical NCBP samples were ≤10 ng/g near Stations 332, 335, and 338 but were 30 ng/g in samples near Station 328 (Schmitt and others, 1999). PCA concentrations were <LOD (4 ng/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB with the exception of several catfish samples from the MORB where PCA concentrations were 4.2–9.4 ng/g (USEPA, 2004a; 2005d). PCA concentrations in previous LRMN studies were <0.1–21 ng/g in the CDRB (Hinck and others, 2006b) and <0.2–2 ng/g in the YRB (Hinck and others, 2006c). The risk of PCA to fish and wildlife in the MORB, ARB, SRB, and PDRB was unknown because studies examining toxicological effects of PCA exposure in fish and piscivorous wildlife were not available.

Hexachlorocyclohexane (HCH)

Four HCH isomers (α-, β-, γ-, δ- HCH) were measured in the composite samples. Although a mixture of isomers

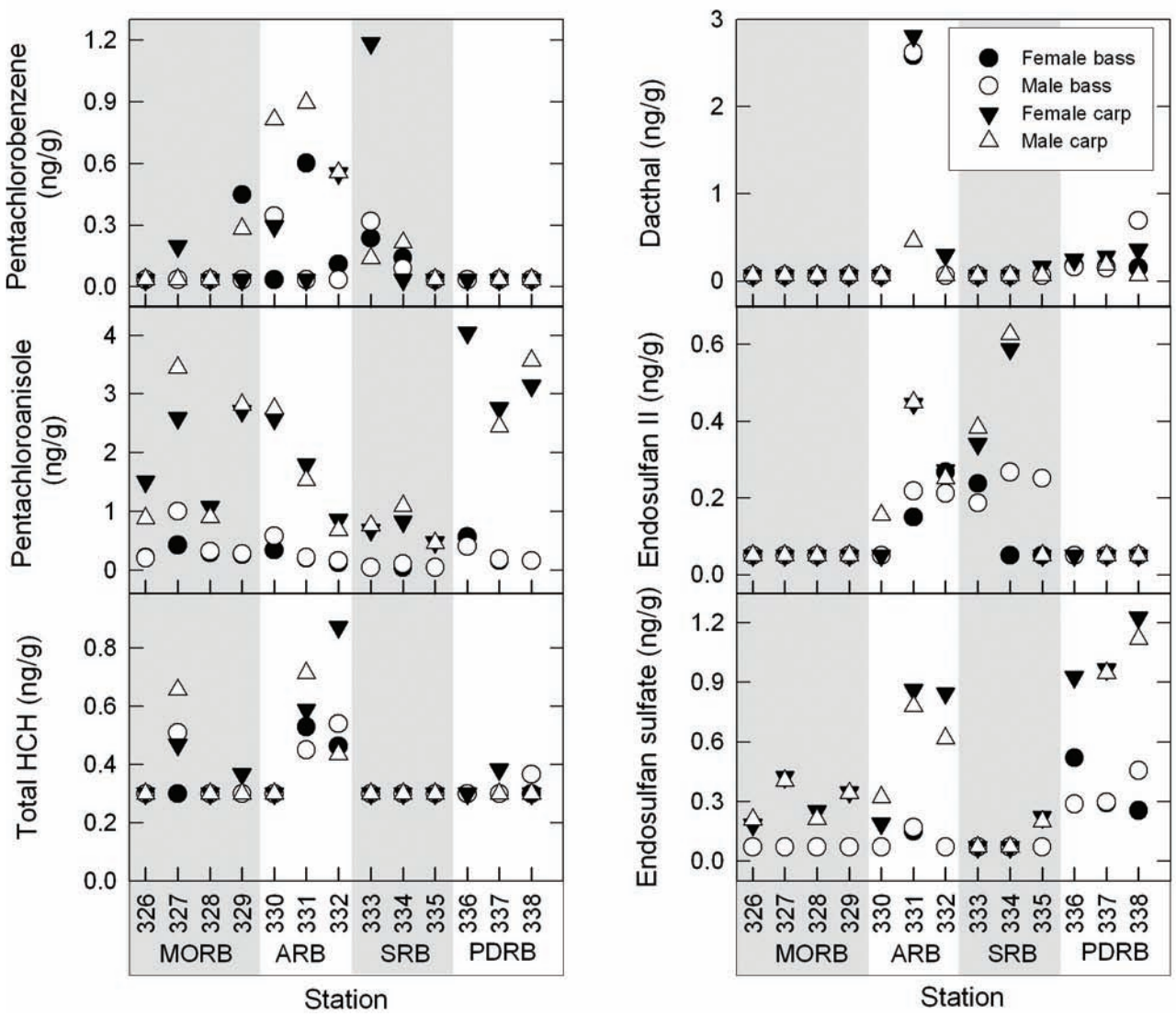


Figure 10. Concentrations (nanograms per gram (ng/g) wet weight) of currently used organochlorine residues and their metabolites including pentachlorobenzene, pentachloroanisole, total hexachlorocyclohexane (sum of α-, β-, γ-, δ-HCH), dacthal, endosulfan II, and endosulfan sulfate by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

was historically used on cotton and other crops in the United States, only γ-HCH (lindane) is still used in North America for some agricultural and domestic applications. Lindane is used in the MORB, ARB, SRB, and PDRB (USGS, 2003), and is applied heavily on pecan trees in the ARB and SRB (appendix 2; USGS, 2003). HCH isomers, which are relatively short-lived, were detected in few samples (table 9). Total HCH (sum of α-, β-, γ-, δ- HCH) were greatest (>0.4 ng/g) in samples from Stations 327, 331, and 332 (fig. 10).

HCH isomer concentrations were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB and previous monitoring studies. Historical NCBP concentrations of γ-HCH generally were ≤10 in fish near Stations 328, 329, 332, 335, and 338, and α-HCH concentrations in 2004 samples were less than historical NCBP concentrations near Stations 328 (<5–120 ng/g), 329 (<5–280 ng/g), 332 (<5–160 ng/g), 335 (<5–30 ng/g), and 338 (<5–100 ng/g) from 1970 to 1986 (Schmitt and others, 1999). Concentrations of α-HCH (<2.5–10.6 ng/g) and γ-HCH (<2.5–4.0 ng/g) also were low in benthivorous whole-body and piscivorous fillet samples near Stations 329, 330, 331, 333, and 335 in a national USEPA study (USEPA, 1992). Concentrations of α-HCH (<10 ng/g), β-HCH (<4–4.9 ng/g), γ-HCH (<2 ng/g), and δ-HCH (<4–7.6 ng/g) also were low in benthivorous whole-body and piscivorous fillet samples from reservoirs in the

MORB, ARB, and SRB (USEPA, 2004a; 2005d). Concentrations of α-, β-, γ-, and δ- HCH were ≤10 ng/g in most samples from previous LRMN studies (Hinck and others, 2006a, 2006b, 2006c; Schmitt and others, 2002, 2005), and γ-HCH concentrations were ≤120 ng/g in whole fish from monitoring studies across the United States (Nowell and others, 1999).

Few studies have examined the toxicological effects of HCH exposure in fish and piscivorous wildlife. Histopathological alterations in the gill, liver, and kidney of freshwater fish have been associated with γ-HCH contamination (Ortiz and others, 2003), and γ-HCH concentrations <100 ng/g in whole fish have been suggested to protect piscivorous wildlife (Newell and others, 1987). γ-HCH concentrations in fish from the MORB (<0.10–0.41 ng/g), ARB (<0.10 ng/g), SRB (<0.10 ng/g), and PDRB (<0.10–0.12 ng/g) were well below this benchmark. Fish and wildlife were not at risk from γ-HCH in the MORB, ARB, SRB, and PDRB based on these thresholds.

Dacthal

Dacthal remains registered as a broad-spectrum herbicide for use on ornamental plants, turf, and vegetable and field crops. The technical product of the dacthal can contain 2,3,7,8-TCDD and HCB as impurities (Cox, 1991; Schmitt and others, 1999). Dacthal use in the MORB, ARB, SRB, and PDRB has been limited (USGS, 2003). Dacthal was detected in 16 of 51 samples (31%; table 9), and concentrations were greatest (>2.5 ng/g) in fish from Station 331 (fig. 10).

Other studies reporting dacthal concentrations in whole-body fish samples from the MORB, ARB, SRB, and PDRB were limited. Historical NCBP concentrations of dacthal in whole-body fish were <5–60 ng/g near Station 332 and <10 ng/g near Stations 328, 335, and 338 (Schmitt and others, 1999). Dacthal concentrations in previous LRMN studies were <0.5–9.3 ng/g in the CDRB (Hinck and others, 2006b) and <0.3–2.8 ng/g in the YRB (Hinck and others, 2006c). Toxicity studies reporting effects of dacthal exposure in fish were not found; therefore, the total risk to fish and wildlife represented from dacthal was unknown.

Endosulfan

Endosulfan is a broad spectrum insecticide used on a wide variety of vegetables, fruits, cereal grains, cotton, and ornamental plants. Endosulfan is used in the MORB, ARB, SRB, and PDRB and is applied heavily on crops including cotton, fruits, and tobacco in the ARB, SRB, and PDRB (appendix 2; USGS, 2003). Technical-grade endosulfan contains two pure isomers, endosulfan I and II. Endosulfan sulfate is a reaction product of technical endosulfan and can be detected in organisms as a result of oxidation of endosulfan I and II (Agency for Toxic Substances and Disease Registry (ATSDR), 2000). Endosulfan I was not detected in any sample (table 9). Endosulfan II was detected in 17 of 51 samples (33%; table 9), and concentrations were >0.2 ng/g in samples from Stations 331, 332, 333, 334, and 335 (fig. 10). The greatest endosulfan II concentrations (0.59–0.63 ng/g) were measured in carp from Station 334 (fig. 10). Endosulfan sulfate was detected in 28 of 51 (55%) samples, and the greatest concentrations (>0.6 ng/g) were in carp from Stations 331, 332, 336, 337, and 338 (fig. 10).

Endosulfan concentrations were compared to whole-body fish concentrations in other studies within the MORB, ARB, SRB, and PDRB and previous monitoring studies. Endosulfan 1 (<4 ng/g), endosulfan II (<40 ng/g), and endosulfan sulfate (<10 ng/g) were not detected in benthivorous whole-body or piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). In previous LRMN studies, endosulfan I, endosulfan II, and endosulfan sulfate concentrations were low in the CDRB (<79 ng/g; Hinck and others, 2006b) and YRB (<2 ng/g Hinck and others, 2006c). Endosulfan concentrations were ≤170 ng/g in whole fish from monitoring studies across the United States (Nowell and others, 1999).

Toxicity studies reporting whole-body endosulfan concentrations in fish were limited, and the range of concentrations where effects were reported in other vertebrates differs by orders of magnitude. Amphibians, fish, birds, and mammals treated with endosulfan exhibited developmental and reproductive effects typically associated with endocrine disrupting chemicals (Dutta and others, 2006; USEPA, 2002). Dutta and others (2006) reported that significant damage to testicular tissue in adult bluegill after exposure to endosulfan may have deleterious effects on spermatogenesis and male fertility. Endosulfan exposure impaired genital tract development in birds (30,000–120,000 ng/g) and reduced hormone levels and sperm production in mammals (15,000–75,000 ng/g; USEPA, 2002). Effects in fish were observed at much lower concentrations, and growth and survival were the most sensitive endpoints (USEPA, 2002). Reproductive effects including decreased GSI, reduced oocyte size, and increased oocyte atresia were documented in tilapia (*Sarotherodon mossambicus*) after exposure to endosulfan (1 ng/g; Shukla and Pandey, 1986). Endosulfan may pose a risk to PDRB fish from Station 336 where the endosulfan sulfate concentrations in carp (1.1–1.2 ng/g) exceeded this threshold.

Methoxychlor

Methoxychlor, a derivative of DDT, is an insecticide used on field crops, vegetables, fruits, stored grain, livestock, and domestic pets (ATSDR, 2002). Methoxychlor can bioaccumulate in fish, insects, and mammals, although it is not as persistent as DDT. Historical pesticide application records indicate that methoxychlor was not heavily used in the MORB, ARB, SRB, or PDRB (USGS, 2003), and methoxychlor was not detected in any sample (table 9).

Total PCBs, H4IIE-Derived Dioxin Equivalents, and Ethoxyresorufin *O*-Deethylase (EROD) Activity

Total PCBs

PCBs, mixtures of 209 chlorinated compounds, were used as coolants and lubricants in electrical capacitors and transformers, for pressure treating lumber, and for paper manufacturing until the United States ban in 1977. Total PCBs concentrations were detected in all composite samples at concentrations ranging from 64 ng/g to 2,700 ng/g (table 9). Concentrations were >500 ng/g in samples from Stations 327, 330, 332, and 338 (fig. 11). Total PCB concentrations differed significantly among sites in bass and carp (table 10). Concentrations were significantly greater in bass and carp from Station 327 than those from most other sites (table 10).

Total PCB concentrations were compared to whole-body fish concentrations from other studies within the MORB, ARB, SRB, and PDRB. Historical NCBP concentrations (as Aroclor mixtures 1248, 1254, 1260) near Stations 328 (<10–8,700 ng/g), 332 (<10–2,500 ng/g), 335 (<10–3,200 ng/g), and 338 (<10–3,700 ng/g) were greater than concentrations in 2004 samples from these sites (Schmitt and others, 1999). Total PCB concentrations were 7.4–435 ng/g in benthivorous whole-body and 0.4–59 ng/g in piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Total PCB concentrations in bass near Stations 328 and 329 (60–350 ng/g; USFWS, 1996) were similar to those measured in 2004 bass samples from these sites.

Total PCB concentrations were measured in previous LRMN studies. Total PCB concentrations in bass were <48–180 ng/g in the CDRB (Hinck and others, 2006b), <30–640 ng/g in the CRB (Hinck and others, 2006a), 50–2,000 ng/g in the MRB (Schmitt and others, 2002), and <50 ng/g in the RGB (Schmitt and others, 2005). In carp, total PCB concentrations were <48–1,600 ng/g in the CDRB (Hinck and others, 2006b), <30–450 ng/g in the CRB (Hinck and others, 2006a), <50–3,300 ng/g in the MRB (Schmitt and others, 2002), and <50 ng/g in the RGB (Schmitt and others, 2005). Total PCB concentrations in fish from the MORB (66–2,700 ng/g), ARB (110–1,300 ng/g), SRB (64–230 ng/g), and PDRB (73–710 ng/g) were greater than those from the CDRB, CRB, RGB, and YRB but similar to those from the MRB (table 11).

Total PCB concentrations were compared to toxicity thresholds available in the scientific literature. Total PCB concentrations from all sites except Station 335 exceeded the New York State Department of Environmental Conservation (NYSDEC) wildlife guideline for fish (110 ng/g; Newell and others, 1987). The toxicity of individual PCB congeners ranges over several orders of magnitude (Ahlborg and others, 1994; van den Berg and others, 1998) and varies with the endpoint being considered (Hansen, 1998). Total PCB concentrations of 16,000–41,000 ng/g did not affect growth rates of young-of-year largemouth bass in a field study (Reiser and others, 2004), and Aroclor 1254 concentrations of 5,000 ng/g decreased fry survival in laboratory studies (Hansen and others, 1973; Schimmel and others, 1974). Whole-body PCB concentrations >100,000 ng/g can affect survival and reproduction in female fish, and concentrations >50,000 ng/g can reduce growth and survival in offspring (Niimi, 1996). However, these effects may occur at lower concentrations in more sensitive fish species (Niimi, 1996). PCB concentrations in MORB, ARB, SRB, and PDRB did not exceed these thresholds. Inferior reproductive performance and offspring

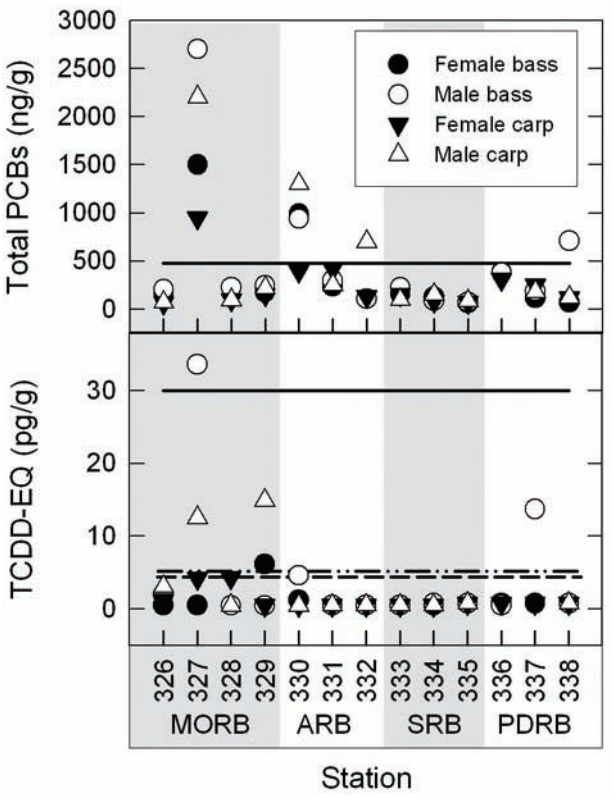


Figure 11. Concentrations of total PCB (nanograms per gram (ng/g) wet weight) and H4IIE bioassay-derived TCDD-EQ (picograms per gram (pg/g) wet weight) by station, species, and gender in whole-body fish composite samples from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. The reference line on the PCB graph represents the protective threshold for mink (480 ng/g; Hornshaw and others, 1983). Reference lines on the TCDD-EQ graph include dietary thresholds for mammals (4.4 pg/g; Heaton and others, 1995; Tillitt and others, 1996), birds (5 pg/g; Nosek and others, 1992), and fish (30 pg/g; Walker and others, 1996; Whyte and others, 2004). See table 5 for station descriptions.

survival were reported in mink fed fish or fish products with PCB concentrations of 480 ng/g (Hornshaw and others, 1983). Total PCB concentrations in samples from Station 327 in the MORB, Stations 330 and 332 in the ARB, and Station 338 in the PDRB were >480 ng/g and may be a risk to piscivorous wildlife.

H4IIE Bioassay

The H4IIE bioassay was performed on composite samples extracts that received additional cleanup to remove AhR-active PAHs. TCDD-EQs were detected in 11 of 50 samples (22%) from 6 sites (fig. 11); a TCDD-EQ could not be calculated for male carp from Station 337. Concentrations of TCDD-EQs ranged from 1.2 pg/g to 33.6 pg/g and were >6 pg/g in male bass from Stations 327 and 337, female bass from Station 329, and male carp from Stations 327 and 329 (fig. 11). Spatial differences were not statistically tested because of the low detection frequency of TCDD-EQ concentrations in the composite samples.

Previous studies have measured TCDD-EQ or dioxin concentrations in fish from the MORB, ARB, SRB, and previous LRMN investigations. Dioxin was named a primary contaminant of concern in Alabama after monitoring efforts documented relatively high dioxin concentrations in fish tissue (ADEM, 1996). Concentrations of 2,3,7,8-TCDD were 24–30 pg/g in whole-body carp samples near pulp and paper mills using chlorine, which were located in the vicinity of Stations 327 (Coosa River) and 328 (Alabama River; USEPA, 1992). Lower 2,3,7,8-TCDD concentrations (<2 pg/g) were measured in whole-body carp samples from other locations near Stations 330 and 331 (USEPA, 1992). Concentrations of 2,3,7,8-TCDD were low (≤0.1–0.3 pg/g) in benthivorous whole-body and piscivorous fillet samples from reservoirs in the MORB, ARB, and SRB (USEPA, 2004a; 2005d). Mean TCDD-EQ concentrations in fish from the MORB, ARB, SRB, and PDRB generally were less than those from the CRB, MRB, and RGB (table 10). Similar to TCDD-EQ concentrations in fish from the ARB, SRB, and PDRB, dioxin-like activity in fish from the CDRB and YRB were low (table 10).

TCDD-EQ concentrations were compared to toxicity thresholds available in the scientific literature. Concentrations of TCDD-EQ in male bass from Stations 327, 330, and 337, female bass from Station 329, and male carp from Stations 327 and 329 exceeded the dietary toxicity threshold for TCDD in mammals (4.4 pg/g; Heaton and others, 1995; Tillitt and others, 1996) and avian wildlife (5 pg/g; Nosek and others, 1992). A toxicity threshold of 30 pg/g has been suggested to protect fish (Walker and others, 1996; Whyte and others, 2004), which was exceeded by the male bass sample from Station 327. Risk from dioxin-like compounds was limited primarily to fish from the MORB, where previous studies have identified dioxins as a contaminant of concern; relatively high dioxin-like activity also was measured in male bass from Station 337 in the PDRB.

Ethoxyresorufin *O*-Deethylase (EROD) Activity

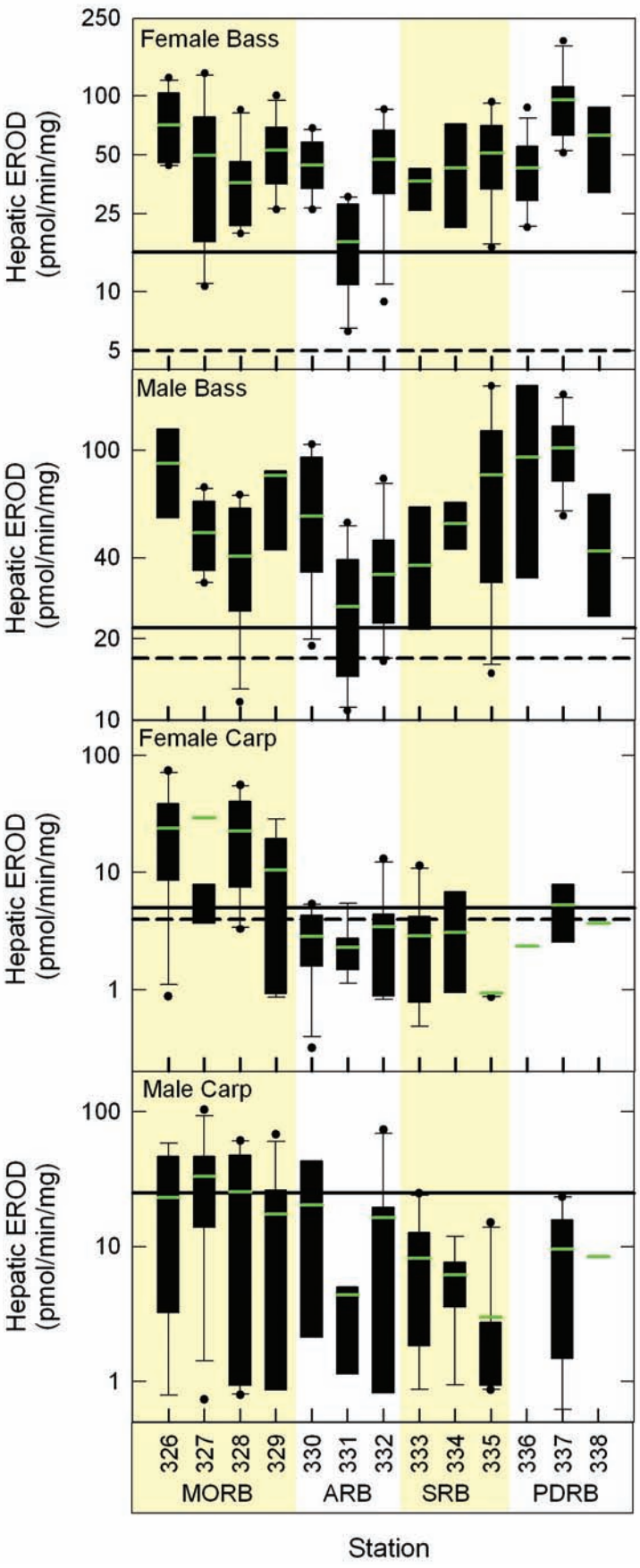
Many factors including species, gender, and gonadal stage affect hepatic EROD activity (Whyte and others, 2000). An ANOVA model that included station (location), gender, gonadal stage, and their interactions was significant in bass ($F_{42, 193} = 3.80$, $P < 0.01$) and carp ($F_{39, 169} = 3.58$, $P < 0.01$; appendix 5). Genders were analyzed and reported separately to maintain comparability with data from previous LRMN studies and the scientific literature.

EROD in Bass

Hepatic EROD activity in most female bass (79%) was 20–90 pmol/min/mg, and mean EROD activity ranged from 15.7 pmol/min/mg at Station 331 to 89.2 pmol/min/mg at Station 337 (fig. 12; table 12). Hepatic EROD activity was lower in female bass from Station 331 (6.3–30.5 pmol/min/mg) than those from other sites, and relatively high EROD activities (51.2–191 pmol/min/mg) were measured in bass from Station 337 (fig. 12). Hepatic EROD activity was 20–90 pmol/min/mg in most male bass (70%), and mean EROD activity ranged from 23.0 pmol/min/mg at Station 331 to 97.9 pmol/min/mg at Station 337 (fig. 12; table 12). Hepatic EROD activity was greater in male bass from Stations 326, 336, and 337 than those from other sites with multiple male bass having EROD activities >75 pmol/min/mg, and relatively low EROD activity (<15 pmol/min/mg) was measured in males from Station 331 (fig. 12).

Hepatic EROD activity in bass was reported by previous LRMN investigations, but EROD data specific to the MORB, ARB, SRB, and PDRB were not found. Mean EROD activities in female bass from the MORB (32.3–66.2 pmol/min/mg), ARB (15.7–42.4 pmol/min/mg), SRB (35.8–45.6 pmol/min/mg), and PDRB (39.5–89.2 pmol/min/mg) were greater than those from the CDRB (2.5–13.2 pmol/min/mg; Hinck and others, 2006b), CRB (5.2–40.4 pmol/min/mg; Hinck and others, 2006a), and MRB (most <30 pmol/min/mg; Schmitt, 2002) and similar to those from the RGB (21.3–108 pmol/min/mg; Schmitt and others, 2005). In male bass, mean EROD activities from the MORB (36.0–83.6 pmol/min/mg), ARB (23.0–49.9 pmol/min/mg), SRB (31.4–62.9 pmol/min/mg), and PDRB (37.8–97.9 pmol/min/mg) generally were greater than those from the CDRB (6.7–61.2 pmol/min/mg; Hinck and others, 2006b), CRB (6.9–68.3 pmol/min/mg; Hinck and others, 2006a), and MRB (most <30 pmol/min/mg; Schmitt and others, 2002) and similar to those from the RGB (17.0–75.9 pmol/min/mg; Schmitt and others, 2005).

Previous studies have reported basal EROD activity in bass. Adams and others (1994) determined basal EROD activity to be 0–5 pmol/min/mg in female bass and 0–17 pmol/min/mg in male bass. Basal EROD activity was 0–16 pmol/min/mg and 0–22 pmol/min/mg in female and male bass, respectively, in the MRB (Schmitt and others, 2002). Hepatic EROD activity in bass from all sites exceeded the basal activities reported in previous studies. Overall, mean hepatic EROD



activity was generally greatest in bass from Stations 326 and 337. PCB and TCDD-EQ concentrations were low in bass from Station 326, which indicates induced hepatic EROD activity may have been caused by another AhR agonist (for example, PAH). TCDD-EQ concentrations in male bass from Station 337 were high (13.7 pg/g) and correlated with EROD activity, but TCDD-EQ concentrations in female bass from Station 337 were <LOD and not correlated with EROD activity (table 13). Elevated EROD activity in male bass from Station 337 may have been from dioxin-like compounds. Hepatic EROD activity was negatively correlated with dieldrin and HCB in female bass (table 13). Bass with the greatest PCB concentrations (Station 327) did not have relatively high EROD activity. Female bass from Station 327 had among the lowest EROD activities.

EROD in Carp

Hepatic EROD activity generally was lower in carp than in bass. Hepatic EROD activity in most female carp (84%) was <13.5 pmol/min/mg, and mean EROD activity ranged from 0.9 pmol/min/mg at Station 335 to 15.4 pmol/min/mg at Station 328 (fig. 12; table 12). Hepatic EROD activity was greater in female carp from Stations 326, 327, 328, and 329 in the MORB than those from other sites, and relatively low EROD activity (0.9 pmol/min/mg) was measured in females from Station 335 (fig. 12). Hepatic EROD activity was <24.8 pmol/min/mg in most male carp (80%), and mean EROD activity ranged from 1.8 pmol/min/mg at Station 335 to 19.8 pmol/min/mg at Station 327 (fig. 12; table 12). As in female carp, EROD activity was greatest (≥3.7 pmol/min/mg) in male carp from Stations 326, 327, 328, and 329 in the MORB, and EROD activity was lowest (≤1.4 pmol/min/mg) in male carp from Stations 331 and 335 (fig. 12).

Hepatic EROD activity in carp was reported by previous LRMN investigations, but EROD data specific to the MORB, ARB, SRB, and PDRB were not available. Mean

Figure 12. Hepatic 7-ethoxyresorufin *O*-deethlyase (EROD) activity (picomols resorufin formed per minute per milligram of protein; pmol/min/mg) by station in female and male bass and carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Censored values are plotted as one-half the limit of quantification. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions. Reference lines indicate basal or background EROD activity for bass (Adams and others, 1994; Schmitt and others, 2002) and carp (Schlenk and others, 1996; Schmitt and others, 2002) from previous studies.

Table 12. Mean hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity (pmol/mg/min protein) in fish.

[Censored values were represented by one-half the limit of quantification in the computation of geometric means. Stations are ordered upstream to downstream within a basin. *n*, sample size; Mean, geometric mean; --, not applicable]

Species, station location, and station number	Female			Male		
	n	Mean	Range	*n*	Mean	Range
Bass						
Lavaca, AL (326)	11	66.2	44.1–123.5	8	83.6	46.8–140.3
Childersburg, AL (327)	10	37.2	10.7–131.2	10	47.4	32.2–72.9
Eureka Landing, AL (328)	10	32.3	19.8–84.9	10	36.0	11.7–68.4
Bucks, AL (329)	12	49.0	26.3–100.9	8	61.5	29.5–277.4
Omaha, GA (330)	10	42.4	26.3–68.4	10	49.9	18.8–105.4
Albany, GA (331)	10	15.7	6.3–30.5	10	23.0	10.9–53.9
Blountstown, FL (332)	10	40.9	8.9–85.5	10	31.1	16.5–78.5
Augusta, GA (333)	3	35.8	26.0–42.5	7	31.4	9.6–64.3
Sylvania, GA (334)	5	36.3	20.4–77.7	4	52.6	41.2–65.8
Port Wentworth, GA (335)	10	45.6	16.8–93.4	10	62.9	14.9–173.4
Rockingham, NC (336)	13	39.5	21.3–87.4	3	75.9	33.6–174.1
Pee Dee, SC (337)	11	89.2	51.2–191.3	11	97.9	57.0–160.8
Bucksport, SC (338)	10	45.6	19.3–110.1	10	37.8	19.2–69.8
Carp						
Lavaca, AL (326)	10	13.9	0.9–74.4	9	10.9	0.8–58.7
Childersburg, AL (327)	8	6.2	0.9–203.2	11	19.8	0.7–103.6
Eureka Landing, AL (328)	10	15.4	3.3–56.2	10	8.7	0.8–60.9
Bucks, AL (329)	9	4.9	0.9–28.8	11	6.9	0.9–68.0
Omaha, GA (330)	10	2.3	0.3–5.4	7	10.7	0.8–43.7
Albany, GA (331)	9	2.0	1.1–5.5	7	2.4	0.5–17.4
Blountstown, FL (332)	10	2.3	0.8–13.1	10	7.0	0.8–73.7
Augusta, GA (333)	10	1.6	0.5–11.4	10	4.9	0.9–24.8
Sylvania, GA (334)	7	1.9	0.5–8.2	9	5.0	0.9–11.9
Port Wentworth, GA (335)	10	0.9	0.9–0.9	10	1.8	0.9–15.1
Rockingham, NC (336)	1	2.4	--	0	--	--
Pee Dee, SC (337)	7	3.3	0.5–17.1	10	5.4	0.6–23.4
Bucksport, SC (338)	2	3.4	2.2–5.2	2	3.6	0.8–16.0

EROD activities in female carp from the MORB (3.7–15.4 pmol/min/mg), ARB (1.3–2.3 pmol/min/mg), SRB (0.4–1.7 pmol/min/mg), and PDRB (2.4–3.3 pmol/min/mg) were similar to those from the CDRB (0.7–9.05 pmol/min/mg; Hinck and others, 2006b), CRB (0.3–10.3 pmol/min/mg; Hinck and others, 2006a), MRB (most <5 pmol/min/mg; Schmitt, 2002) and RGB (0.3–16.8 pmol/min/mg; Schmitt and others, 2005). In male carp, mean EROD activities from the MORB (5.6–19.8 pmol/min/mg), ARB (1.3–10.7 pmol/min/mg), SRB (1.4–4.9 pmol/min/mg), and PDRB (1.7–4.9 pmol/min/mg) were similar to those from the CDRB (1.2–10.7 pmol/min/mg; Hinck and others, 2006b), CRB (0.9–10.6 pmol/min/mg; Hinck and others, 2006a), MRB (most <10 pmol/min/mg; Schmitt, 2002) and RGB (0.3–32.6 pmol/min/mg; Schmitt and others, 2005).

Previous studies have reported basal EROD activity in carp. Schlenk and others (1996) determined basal EROD activity in female carp from uncontaminated sites to be 0–5 pmol/min/mg, which was similar to basal activity (0–4 pmol/min/mg) reported for carp by Schmitt and others (2002). Hepatic EROD activities were >5 pmol/min/mg in individual female carp from all sites except Stations 335 and 336, and mean EROD activity was >5 pmol/min/mg at Stations 326, 327, and 328 in the MORB (table 12). In male carp, individual EROD activities exceeded basal levels (25 pmol/min/mg; Schlenk and others, 1996; Schmitt and others, 2002) from all sites except Stations 331, 334, 335, and 338, but mean EROD activity did not exceed 25 pmol/min/mg at any site (table 12). In laboratory studies, EROD activities were 7.1–25 pmol/min/mg in reference juvenile carp (Kosmala and others, 1998; Taysse and others, 1998; Marionnet and others, 1997; 1998) and 2.7–41.9 pmol/min/mg in reference adult carp (Deér and others, 1996; Solé and others, 2000).

Carp from Stations 326, 327, 328, and 329 in the MORB were likely exposed to AhR agonists in the environment as multiple fish from these sites exceeded the reference values available for carp. Relatively high PCB and TCDD-EQ concentrations, which are known AhR agonists, were measured in carp from Station 327 but not in carp from Stations 326 and 328, and TCDD-EQ concentrations were elevated in male carp from Station 329. Hepatic EROD activity was not correlated with any contaminant including PCBs and TCDD-EQ in male

Table 13. Spearman rank correlation coefficients for the relation between biological endpoints and contaminant concentrations in female and male bass.

[Female (n = 13; n = 12 for SSI) and male (n = 13) bass were analyzed separately after analysis of variance indicated that gender was a significant factor. Chlordane is the sum of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, heptachlor epoxide, and oxychlordane. Polychlorinated biphenyl (PCB) is the sum of all congeners. Asterisks denote significant correlations ($P < 0.05$). HCB, hexachlorobenzene; PCA, pentachloroanisole; SSI, splenosomatic index; Vtg, vitellogenin; E2, 17β-estradiol; KT, 11-ketotestosterone; E/KT, 17β-estradiol and 11-ketotestosterone ratio; GSI, gonadosomatic index; NA, not applicable]

Contaminant	Biological endpoint									
	EROD	Length	Weight	SSI	Vtg	E2	KT	E/KT	GSI	Atresia
Female										
Dieldrin	–0.67*	0.32	0.52	–0.10	0.22	0.29	–0.69*	0.79*	–0.05	0.45
HCB	–0.70*	0.25	0.27	–0.20	–0.03	0.54	–0.13	0.69*	–0.27	0.26
PCA	–0.03	0.74*	0.65*	–0.55	0.05	0.21	–0.29	0.35	0.15	–0.11
Chlordane	–0.53	0.53	0.63*	–0.37	–0.16	0.31	–0.59*	0.81*	–0.08	0.31
o,p'-DDD	–0.53	0.38	0.48	–0.20	–0.05	0.34	–0.49	0.70*	–0.44	0.22
p,p'-DDE	–0.19	0.79*	0.87*	–0.52	0.06	0.47	–0.54	0.74*	–0.16	0.03
p,p'-DDD	–0.38	0.65*	0.70*	–0.37	0.06	0.24	–0.76*	0.76*	–0.20	0.02
p,p'-DDT	–0.30	0.64*	0.55*	–0.43	–0.15	0.12	–0.63*	0.52	–0.14	–0.12
Mirex	–0.13	0.31	0.24	–0.47	0.13	–0.13	–0.41	0.19	0.27	0.12
PCB	–0.42	0.65*	0.61*	–0.37	0.04	0.37	–0.39	0.60*	0.06	0.03
Toxaphene	–0.16	0.44	0.47	–0.55	0.16	–0.05	–0.68*	0.47	0.13	0.26
TCDD-EQ	0.37	0.29	0.22	–0.28	0.19	–0.25	–0.46	0.26	0.40	–0.29
Arsenic	0.29	0.41	0.49	–0.64*	0.43	0.13	–0.27	0.31	0.49	–0.04
Cadmium	–0.17	–0.02	0.02	–0.02	0.02	–0.21	0.12	–0.10	0.11	0.33
Chromium	0.33	–0.38	–0.41	0.18	–0.19	–0.63*	0.19	–0.53	–0.29	0.10
Copper	–0.01	–0.20	–0.09	–0.31	0.55	–0.07	0.06	–0.01	0.53	0.45
Mercury	0.30	0.08	–0.04	0.19	–0.44	–0.14	0.10	–0.36	–0.48	–0.48
Nickel	0.55	–0.03	–0.20	0.23	–0.65*	–0.51	0.40	–0.62*	–0.62*	–0.43
Lead	0.11	–0.24	–0.33	0.32	–0.56*	–0.24	0.30	–0.46	–0.35	0.02
Selenium	0.14	–0.13	0.09	–0.15	–0.33	0.10	0.06	–0.01	–0.20	–0.25
Zinc	0.42	–0.19	–0.24	0.37	–0.02	–0.69*	0.05	0.54	0.24	–0.07
Male										
Dieldrin	–0.26	–0.24	–0.29	–0.45	0.07	–0.21	0.49	–0.54	0.12	NA
HCB	–0.29	0.04	–0.01	–0.01	0.12	–0.24	0.52	–0.44	0.25	NA
PCA	0.14	0.81*	0.84*	0.04	–0.05	–0.28	–0.08	–0.25	–0.09	NA
Chlordane	–0.15	0.49	0.50	–0.14	0.13	–0.32	0.40	–0.61*	0.09	NA
o,p'-DDD	–0.28	–0.08	–0.14	–0.20	0.10	–0.13	0.65*	–0.44	0.29	NA
p,p'-DDE	0.00	0.80*	0.78*	–0.07	–0.22	–0.16	0.17	–0.30	–0.07	NA
p,p'-DDD	–0.08	0.31	0.31	–0.46	–0.33	–0.11	0.48	–0.39	0.11	NA
p,p'-DDT	–0.15	0.57*	0.54	–0.39	–0.26	–0.04	0.18	–0.26	–0.26	NA
Mirex	–0.23	0.11	0.09	–0.68*	–0.34	0.55*	–0.01	0.35	–0.25	NA
PCB	–0.13	0.48	0.52	0.10	–0.23	–0.05	0.17	–0.18	0.15	NA
Toxaphene	0.02	–0.24	–0.15	0.24	0.08	–0.45	–0.13	–0.53	0.41	NA
TCDD-EQ	0.56*	0.01	0.06	0.00	0.30	–0.01	–0.27	–0.03	–0.13	NA
Arsenic	0.43	0.54	0.58*	–0.48	–0.02	0.16	0.05	0.12	0.08	NA
Cadmium	–0.21	0.21	0.30	0.38	0.13	–0.72*	–0.17	–0.59*	–0.05	NA
Chromium	0.00	0.13	0.14	0.02	0.12	–0.02	0.32	–0.03	0.33	NA
Copper	0.09	–0.12	–0.08	0.27	0.37	–0.42	0.19	–0.46	0.52	NA
Mercury	–0.09	–0.16	–0.14	0.48	–0.21	–0.15	–0.41	0.19	–0.17	NA
Nickel	0.00	0.36	0.04	0.09	0.00	–0.08	0.26	–0.02	0.63*	NA
Lead	–0.22	–0.20	–0.14	0.31	0.29	–0.37	–0.06	–0.41	–0.06	NA
Selenium	–0.02	–0.03	–0.12	–0.45	–0.14	0.32	0.01	0.14	–0.31	NA
Zinc	–0.09	–0.29	–0.34	0.26	0.32	–0.16	0.53	–0.38	0.44	NA

carp (table 14). Hepatic EROD activity was positively correlated with TCDD-EQ concentrations in female carp (table 14). The relevance of this correlation was unclear because TCDD-EQ concentrations were >LOD in only two female carp samples (from Stations 327 and 328) and were below toxicity thresholds. Relatively high EROD activity may have been from PCBs, dioxin-like compounds, or both in carp from Stations 327 and 329 and other AhR agonists in carp from Stations 326 and 328.

Accumulative Contaminants, H4IIE Bioassay, and Hepatic EROD Activity: Summary

Concentrations of most organochlorine residues and elemental contaminants measured in MORB, ARB, SRB, and PDRB fish did not exceed toxicity thresholds; however, Se, Hg, *p,p*'-DDE, toxaphene, endosulfan sulfate, PCBs, and TCDD-EQ concentrations may pose a risk to fish, wildlife, or both from one or more sites. Selenium concentrations in carp samples from Stations 326, 329, and 330 exceeded protective criteria (>0.75 µg/g) for piscivorous wildlife (Lemly, 1996; 2002). Potential sources of Se in the MORB and ARB include coal and petroleum combustion (Sorenson, 1991). Mercury concentrations were >0.3 µg/g in all bass samples except those from Stations 331 and 336 and may represent a threat to piscivorous birds and mammals (Barr, 1986; Yeardley and others, 1998). Total Hg whole-body concentrations >0.2 µg/g may pose a risk to juvenile and adult fish (Beckvar and others, 2005). Mercury has been identified as a contaminant of concern in previous studies within the region. Multiple rivers and reservoirs in the MORB, ARB, SRB, and PDRB have fish consumption advisories for Hg in various fish species. Probable sources of Hg in these areas include releases from chemical manufacturing plants, coal-fired power plants, and atmospheric deposition (ADPH, 2005; GDNR, 2005; SCDHEC, 2005a; 2005b; USEPA, 2001). Elevated Hg concentrations in bass from the MORB previously were associated with shallow water depths, wetland surface area, low aqueous potassium concentrations, and high chlorophyll a concentrations (Warner and others, 2005). Paller and others (2004) also named wetlands as likely Hg sources in the SRB and documented that Hg concentrations in bass and sunfish (*Lepomis* spp.) increased significantly in fish sampled progressively lower in the SRB. Risk from Hg to non-aquatic wildlife also has been documented in the MORB. Mercury released into the Tombigbee River at McIntosh, Alabama (upstream from Station 329), from chlor-alkali and DDT facilities may cause reproductive effects, organ toxicity, and mortality in warblers inhabiting contaminated sites (Adair and others, 2003). Mercury concentrations that could cause adverse effects were reported in river otters (*Lutra canadensis*) from the lower coastal plain and Piedmont of Georgia (Halbrook and others, 1994), and Hg concentrations high enough to affect reproduction, growth, and behavior in mink along the coastal plain in Georgia, South Carolina, and North Carolina have been suggested as a contributing factor of mink population declines in the region (Osowski and others, 1995).

Organochlorine pesticides were used historically in agricultural areas of the MORB, ARB, SRB, and PDRB. Other organochlorine residues and metabolites, like PCA, were used in the lumber industry. DDT and toxaphene were used in cotton growing regions of the basins, but concentrations of these insecticides only exceeded toxicity thresholds in fish samples from the ARB. Total DDT (primarily from *p,p*'-DDE) concentrations >150 ng/g in male and female bass and male carp from Station 330 may represent a risk to wildlife (Anderson and others, 1975; Newell and others, 1987) but did not exceed toxicity thresholds for fish (Beckvar and others, 2005; Garcia-Reyero and others, 2006; Jarvinen and Ankley, 1999). Relatively high *p,p*'-DDE concentrations previously were measured in carp from this region of the Chattahoochee River (USEPA, 1992). Toxaphene concentrations were greater in composite samples from Station 331 (60–100 ng/g) than those from all other sites (<60 ng/g) and exceeded a toxicity threshold to protect fish (Mayer and others, 1975). Toxaphene concentrations in Station 331 samples were less than most effects criteria for freshwater fish, however (Eisler and Jacknow, 1985; Jarvinen and Ankley, 1999). Total chlordanes, dieldrin, mirex, pentachlorobenzene, total HCH, and dacthal also were relatively high in fish samples from the ARB but were less than effects thresholds. Endosulfan concentrations were greatest in carp from the ARB, SRB, and PDRB where it has been used as a broad spectrum insecticide on fruits, cotton, and tobacco. Endosulfan may pose a risk to fish from Station 336 where the endosulfan sulfate concentrations in carp (1.1–1.2 ng/g) exceeded toxicity thresholds for reproduction (Shukla and Pandey, 1986). Toxicity thresholds for most currently used organochlorine residues and their metabolites including pentachlorobenzene, PCA, total HCH, dacthal, and endosulfan were not available, although concentrations of these contaminants were low in fish from previous MORB, ARB, SRB, and PDRB studies (USEPA, 2004a; 2005d). Nevertheless, many of these currently used organochlorine chemical residues and their metabolites should continue to be monitored because they can cause reproductive and developmental effects in fish and wildlife (McDonald, 1991; Ortiz and others, 2003; Shukla and Pandey, 1986; USEPA, 2002; Versonnen and others, 2004).

PCBs were manufactured and used in electrical capacitors and transformers, for pressure treating lumber, and for paper manufacturing, which were the likely sources of PCBs in the Coosa River of the MORB, the Chattahoochee River of the ARB, and Lake Hartwell and its connected waters in the SRB. As a result, many waters of the MORB, ARB, and SRB have fish consumption advisories for PCBs in multiple fish species. Previous studies have documented elevated PCB concentrations in water and biota from the MORB, ARB, SRB, and PDRB (Atkins and others, 2004; Schmitt and others, 1999; USEPA, 2004a; 2005d; USFWS, 1996; Zappia, 2002). PCB concentrations were high in fish from some of these same rivers. PCB concentrations in fish from Stations 327 (950–2,700 ng/g), 330 (940–1,300 ng/g), 332 (700 ng/g), and 338 (710

Table 14. Spearman rank correlation coefficients for the relation between biological endpoints and contaminant concentrations in female and male carp.

[Female (n = 13) and male (n = 12; n = 11 for TCDD-EQ) carp were analyzed separately after analysis of variance indicated that gender was a significant factor. Chlordane is the sum of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, heptachlor epoxide, and oxychlordane. Polychlorinated biphenyl (PCB) is the sum of all congeners. Asterisks denote significant correlations (P < 0.05). HCB, hexachlorobenzene; PCA, pentachloroanisole; Vtg, vitellogenin; E2, 17β-estradiol; KT, 11-ketotestosterone; E/KT, 17β-estradiol and 11-ketotestosterone ratio; GSI, gonadosomatic index; NA, not applicable]

Contaminant	Biological endpoint								
	EROD	Length	Weight	Vtg	E2	KT	E/KT	GSI	Atresia
Female									
Dieldrin	–0.29	0.36	0.41	0.36	0.34	0.01	0.47	0.57*	0.07
HCB	–0.02	0.37	0.32	0.37	0.35	0.03	0.24	0.57*	–0.20
PCA	0.25	0.79*	0.78*	0.61*	–0.13	–0.01	–0.35	0.58*	0.33
Chlordane	–0.32	0.69*	0.73*	0.71*	0.14	–0.11	0.33	0.67*	0.42
o,p'-DDD	–0.18	0.35	0.35	0.29	0.34	0.10	0.34	0.47	–0.11
p,p'-DDE	0.13	0.52	0.53	0.44	–0.13	–0.24	0.11	0.47	0.32
p,p'-DDD	–0.12	0.52	0.55	0.48	0.23	–0.08	0.44	0.62*	0.21
p,p'-DDT	–0.05	–0.01	–0.01	0.02	0.24	–0.01	0.38	0.21	–0.19
Mirex	–0.43	–0.04	–0.02	0.18	–0.23	–0.51	0.67*	0.18	0.09
PCB	–0.08	0.74*	0.75*	0.66*	0.09	–0.10	0.24	0.66*	0.48
Toxaphene	–0.01	0.66*	0.69*	0.58*	0.00	–0.08	0.09	0.78*	0.30
TCDD-EQ	0.70	–0.22	–0.33	–0.03	–0.24	–0.09	–0.16	–0.30	–0.07
Arsenic	–0.07	0.45	0.46	0.28	–0.19	–0.14	0.28	0.10	0.51
Cadmium	–0.45	0.47	0.56*	0.56*	0.11	0.02	0.19	0.40	0.68*
Chromium	0.26	–0.53	–0.50	–0.63*	–0.31	–0.08	0.12	–0.45	–0.07
Copper	0.04	–0.07	–0.10	0.22	–0.25	–0.54	0.18	0.20	–0.09
Mercury	–0.43	0.38	0.42	0.47	–0.30	–0.32	–0.34	0.64*	–0.01
Nickel	–0.10	0.09	0.14	0.19	–0.85*	–0.65*	–0.12	0.09	0.30
Lead	–0.40	0.63*	0.66*	0.66*	–0.26	–0.22	–0.16	0.54	0.53
Selenium	0.15	0.10	0.14	0.13	–0.23	–0.30	0.22	–0.05	0.29
Zinc	–0.15	0.74*	0.77*	0.55*	–0.30	–0.41	0.18	0.84*	0.32
Male									
Dieldrin	–0.09	0.34	0.36	0.34	0.53	0.64*	0.03	0.45	NA
HCB	–0.02	0.28	0.43	–0.06	0.52	0.40	0.04	–0.05	NA
PCA	0.27	0.59*	0.40	0.22	–0.07	0.05	–0.05	0.69*	NA
Chlordane	–0.14	0.43	0.51	0.34	0.63*	0.43	0.36	0.43	NA
o,p'-DDD	0.08	0.41	0.41	0.33	0.47	0.49	0.00	0.57	NA
p,p'-DDE	0.37	0.49	0.54	0.31	0.46	0.77*	0.15	0.54	NA
p,p'-DDD	0.26	0.61*	0.66*	0.27	0.68*	0.58*	0.23	0.62*	NA
p,p'-DDT	0.11	0.19	0.28	0.07	0.59*	0.67*	0.26	0.34	NA
Mirex	–0.10	0.36	0.57	0.38	0.79*	0.61*	0.51	0.27	NA
PCB	0.23	0.50	0.52	0.06	0.29	0.45	–0.10	0.45	NA
Toxaphene	0.18	0.67*	0.62*	–0.19	0.22	0.26	0.08	0.57	NA
TCDD-EQ	0.08	0.41	0.38	0.29	–0.12	–0.65*	0.22	0.38	NA
Arsenic	0.15	0.58*	0.69*	–0.13	0.27	0.04	0.13	0.36	NA
Cadmium	–0.02	0.34	0.47	0.05	0.38	0.40	0.47	0.14	NA
Chromium	–0.20	0.38	0.25	–0.35	0.10	0.01	–0.07	0.38	NA
Copper	–0.47	0.50	0.31	0.59*	0.14	–0.17	0.24	0.62*	NA
Mercury	–0.43	0.20	0.22	0.69*	0.43	–0.08	0.58*	0.38	NA
Nickel	0.34	0.59*	0.45	0.10	–0.10	0.29	–0.08	0.53	NA
Lead	0.17	0.67*	0.54	0.36	0.24	0.17	0.14	0.65*	NA
Selenium	0.31	0.42	0.61*	0.21	0.61*	0.26	0.43	0.34	NA
Zinc	–0.36	0.66*	0.52	0.25	0.49	0.17	0.26	0.79*	NA

ng/g) may represent a risk to piscivorous wildlife (Hornshaw and others, 1983). PCB concentrations did not exceed toxicity thresholds in fish from the SRB, which was consistent with a previous study by Winger and others (1990). However, liver PCB concentrations in mink from the coastal plain of Georgia, South Carolina, and North Carolina were high enough to cause reproductive dysfunction (Osowski and others, 1995).

Dioxin releases have been reported in waters of the MORB, ARB, SRB, and PDRB (USEPA, 2005b). Many dioxin releases in these basins were associated with pulp and paper mills (USEPA, 1992). In this study, the risk from dioxin-like compounds was greatest to fish from the MORB, where previous studies have identified dioxin as a contaminant of concern (ADEM, 1996; USEPA, 1992). Dioxin-like activity in male bass from Stations 327, 330, and 337, female bass from Station 329, and male carp from Stations 327 and 329 exceeded the dietary toxicity threshold for TCDD in mammals (4.4 pg/g; Heaton and others, 1995; Tillitt and others, 1996) and avian wildlife (5 pg/g; Nosek and others, 1992). The TCDD-EQ concentration in male bass from Station 327 also exceeded the toxicity threshold suggested to protect fish (30 pg/g; Walker and others, 1996; Whyte and others, 2004). Dioxin-like activity in fish from Station 327 may be due to PCBs, which also were elevated at this site.

Hepatic EROD activity indicates recent exposure to exogenous AhR ligands, including some PCBs, dioxins, and PAHs. Hepatic EROD activity in bass from all sites exceeded the basal activities reported in previous studies (Adams and others, 1994; Schmitt and others, 2002), and mean hepatic EROD activity generally was greatest in bass from Stations 326 and 337. Because PCB and TCDD-EQ concentrations were not elevated, the relatively high EROD activity in bass from Station 326 was likely the result of induction by an AhR agonist other than dioxins and PCBs. However, the elevated EROD activity in male bass from Station 337 may have been from dioxin-like compounds as TCDD-EQ concentrations were relatively high in this sample. Individual carp from all sites except Station 335 exceeded the basal activities reported in previous studies (Schlenk and others, 1996; Schmitt and others, 2002), and mean hepatic EROD activity generally was greatest in carp from MORB sites. The relatively high PCB and TCDD-EQ concentrations in carp from Stations 327 and 329 may have contributed to the elevated hepatic EROD activity in these fish, but other AhR agonists may have caused the high EROD activity in carp from Stations 326 and 328 because neither PCBs or TCDD-EQ were considered elevated in these fish.

Health Indicators

Organism-Level Indicators

Length, Weight, and Age

Total length, weight, and age differed in bass. Total length in bass differed among sites ($F_{12, 211} = 14.24$, $P < 0.05$) and between genders ($F_{1, 211} = 14.02$, $P < 0.05$), and weight also differed among sites ($F_{12, 211} = 7.42$, $P < 0.05$) and between genders ($F_{1, 211} = 15.00$, $P < 0.05$). Age in bass differed among sites ($F_{12, 209} = 11.67$, $P < 0.05$) but not between genders ($F_{1, 209} = 2.56$, $P > 0.05$). Bass from the MORB and ARB generally were larger and older than those from the SRB and PDRB (table 15). The largest female and male bass (mean length and weight) were from Stations 327, 329, and 336, and the smallest bass were from Stations 333 and 337 (table 15). Bass from the MORB and ARB (most >2 y) generally were older than those from the SRB and PDRB (most <2 y), and the oldest bass were from Stations 327, 328, and 329 (table 15). The mean lengths, weights, and ages of MORB, ARB, SRB, and PDRB bass were similar to those from previous LRMN studies (Hinck and others, 2004a; 2006b; Schmitt, 2002; Schmitt and others, 2004).

In carp, total length, weight, and age differed. Total length differed among sites ($F_{12, 184} = 21.31$, $P < 0.05$) and between genders ($F_{1, 184} = 7.36$, $P < 0.05$), and weight also differed among sites ($F_{12, 184} = 17.47$, $P < 0.05$) and between genders ($F_{1, 184} = 19.44$, $P < 0.05$). Age in carp differed among sites ($F_{12, 151} = 33.90$, $P < 0.05$) but not between genders ($F_{1, 151} = 0.47$, $P > 0.05$). Carp generally were smallest (mean length and weight) from Stations 326 and 328 (table 16), and carp from Stations 327, 330, and 331 were older than those from other sites. Otoliths have been used to age carp in other studies, but the use of otoliths to estimate age in carp has not been validated for fish >14 years old (Brown and others, 2004; Vilizzi and Walker, 1999). Otoliths (asterisci) were used to estimate age in carp in from a previous LRMN investigation in the CDRB (Hinck and others, 2006b; 2007). Hinck and others (2006b; 2007) suggested that the age of some CDRB carp may have been overestimated if otolith rings represented changes in water temperature, periods of rapid growth, or lunar cycles rather than annual growth and that more information was needed to determine if otoliths were the appropriate structures to estimate age in carp. Nevertheless, the same methods to estimate age from otoliths were used for this study (fig. 13). Overall, the relatively old fish from Stations 327, 330, and 331 also were among the largest (longest) carp collected. Most carp otoliths were composed of aragonite, but vaterite, the crystallized form of calcium carbonate, was present in some carp otoliths. The cause of vaterite formation in many fish species was unknown but may be related to stress and increased metabolic activity (Sweeting and others, 2004).

Length, weight, and age were significantly correlated with contaminant concentrations, primarily organochlorine contaminants, in bass and carp. Length and weight were positively correlated with PCA, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, and PCB concentrations in female bass and PCA and *p,p'*-DDE concentrations in male bass (table 13). Other significant correlations included weight and chlordane in female bass, length and *p,p'*-DDT in male bass, and weight and As in male bass (table 13). Age was significantly correlated with PCA, chlordane, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, PCB, and Cu concentrations in bass (table 17). In carp, length and weight

Table 15. Mean length, weight, and age of bass.

[Stations are listed upstream to downstream within a basin. mm, millimeter; g, gram; *n*, sample size; Mean, arithmetic mean; SE, standard error]

Gender, station location, and station name	*n*	Length (mm)		Weight (g)		Age (years)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
All bass	237[a]	362 ± 5	215–551	724 ± 33	12–2880	3.3 ± 0.1	1–12
Female bass							
All stations	125[b]	380 ± 8	215–551	856 ± 54	120–2880	3.6 ± 0.2	1–12
Lavaca, AL (326)	11	391 ± 21	323–514	798 ± 160	385–1875	4.5 ± 0.3	3–7
Childersburg, AL (327)	10[c]	457 ± 16	375–550	1405 ± 143	760–2345	5.2 ± 0.9	3–12
Eureka Landing, AL (328)	10	410 ± 18	300–478	948 ± 120	300–1500	4.6 ± 0.3	3–6
Bucks, AL (329)	12	426 ± 12	370–509	1041 ± 118	650–2080	4.6 ± 0.4	3–8
Omaha, GA (330)	10	401 ± 28	318–544	949 ± 213	415–2105	4.2 ± 0.7	2–7
Albany, GA (331)	10	388 ± 19	281–478	864 ± 129	290–1575	2.1 ± 0.5	1–6
Blountstown, FL (332)	10	404 ± 29	291–549	1090 ± 266	280–2880	3.4 ± 0.4	2–7
Augusta, GA (333)	3	298 ± 29	261–356	343 ± 116	190–570	2.3 ± 0.7	2–3
Sylvania, GA (334)	5	374 ± 54	275–551	937 ± 486	260–2805	4.2 ± 0.5	2–11
Port Wentworth, GA (335)	10	355 ± 15	271–442	630 ± 98	240–1380	2.0 ± 0.6	1–3
Rockingham, NC (336)	13	405 ± 26	280–525	1137 ± 202	315–2270	3.6 ±0.3	2–6
Pee Dee, SC (337)	11	254 ± 9	215–300	213 ± 27	120–390	2.0 ± 0.1	1–3
Bucksport, SC (338)	10	307 ± 14	260–390	408 ± 69	225–820	1.9 ± 0.2	1–3
Male bass							
All stations	112[d]	343 ± 6	217–485	576 ± 31	120–1715	3.1 ± 0.2	1–12
Lavaca, AL (326)	8	356 ± 18	305–455	591 ± 115	305–1310	4.1 ± 0.4	3–5
Childersburg, AL (327)	10	407 ± 13	350–483	974 ± 110	615–1715	4.0 ± 0.4	3–7
Eureka Landing, AL (328)	10	392 ± 18	330–485	790 ± 141	385–1575	6.2 ± 1.4	2–12
Bucks, AL (329)	8	401 ± 11	346–426	807 ± 62	555–1065	5.4 ± 0.8	3–10
Omaha, GA (330)	10	373 ± 12	297–416	681 ± 62	325–955	3.2 ± 0.2	2–4
Albany, GA (331)	10	329 ± 9	291–369	512 ± 62	285–820	2.5 ± 0.3	2–4
Blountstown, FL (332)	10	342 ± 13	300–409	521 ± 79	235–995	2.6 ± 0.3	1–4
Augusta, GA (333)	7	279 ± 9	250–314	248 ± 27	175–360	2.0 ± 0.2	1–3
Sylvania, GA (334)	4	302 ± 18	276–355	371 ± 63	270–555	2.0 ± 0.0	2
Port Wentworth, GA (335)	10	317 ± 7	282–359	432 ± 28	295–625	1.3 ± 0.2	1–3
Rockingham, NC (336)	3	408 ± 20	371–440	1083 ± 163	370–1370	4.0 ± 0.6	3–5
Pee Dee, SC (337)	11	277 ± 15	222–404	312 ± 78	120–1035	1.6 ± 0.2	1–3
Bucksport, SC (338)	11	300 ± 17	217–399	400 ± 74	140–865	2.1 ± 0.1	2–3

[a] $n = 235$ for age.

[b] $n = 124$ for age.

[c] $n = 9$ for age.

[d] $n = 111$ for age.

were positively correlated with PCA, chlordane, PCB, toxaphene, Pb, and Zn concentrations in females and *p,p'*-DDD, toxaphene, and As concentrations in males (table 14). Other significant correlations included length and PCA, Ni, Pb, and Zn in male carp, weight and Cd in female carp, and weight and Se in male carp (table 14). Age was correlated with dieldrin, chlordane, *p,p'*-DDE, mirex, PCB, toxaphene, Cd, Cr, Pb, and Zn concentrations in carp (table 17).

Health Assessment Index and Histopathological Evaluation

The health assessment index (HAI) is a systematic method to identify external and internal lesions on individual fish during field necropsy. Lesions were categorized by location including occurrence on the body surface, eyes, opercles, gills, fins, liver, spleen, and kidney on fish from the MORB,

Table 16. Mean length, weight, and age of carp.

[Stations are listed upstream to downstream within a basin. mm, millimeter; g, gram; *n*, sample size; Mean, arithmetic mean; SE, standard error; --, not applicable]

Gender, station location, and station number	*n*	Length (mm)		Weight (g)		Age (years)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
All carp	209[a]	514 ± 8	334–823	2186 ± 113	470–7905	16.4 ± 1.2	3–62
Female carp							
All stations	103[b]	527 ± 12	334–823	2488 ± 193	470–7905	16.5 ± 1.7	3–62
Lavaca, AL (326)	10[c]	436 ± 19	364–587	1160 ± 204	645–2900	6.1 ± 0.7	4–11
Childersburg, AL (327)	8	656 ± 24	576–767	3814 ± 520	1240–6045	27.5 ± 6.1	3–50
Eureka Landing, AL (328)	10[c]	415 ± 17	334–520	948 ± 122	470–1810	7.8 ± 0.8	4–11
Bucks, AL (329)	9[d]	509 ± 20	471–667	1836 ± 298	1215–4170	6.1 ± 0.9	4–12
Omaha, GA (330)	10[c]	637 ± 22	545–778	4222 ± 520	2155–7905	46.2 ± 5.2	7–58
Albany, GA (331)	9[d]	676 ± 25	566–762	5335 ± 533	3135–7515	25.3 ± 3.6	7–37
Blountstown, FL (332)	10[d]	483 ± 9	448–533	1682 ± 79	1345–2135	10.9 ± 2.1	5–19
Augusta, GA (333)	10	466 ± 30	394–651	1669 ± 450	865–4805	12.8 ± 3.9	6–37
Sylvania, GA (334)	7[e]	446 ± 18	396–515	1343 ± 182	920–2150	7.4 ± 2.2	4–16
Port Wentworth, GA (335)	10[c]	433 ± 8	383–473	1145 ± 70	790–1555	7.8 ± 0.4	7–11
Rockingham, NC (336)	1	823	--	5835	--	62.0	--
Pee Dee, SC (337)	7[f]	617 ± 55	446–797	3991 ± 933	1240–6965	15.5 ± 4.8	5–34
Bucksport, SC (338)	2[g]	696 ± 114	582–810	4870 ± 2960	1910–7830	16.0	--
Male carp							
All stations	106[h]	502 ± 9	350–759	1893 ± 113	580–7545	16.3 ± 1.7	3–62
Lavaca, AL (326)	9	479 ± 28	362–671	1543 ± 351	580–4200	5.9 ± 0.5	4–8
Childersburg, AL (327)	11[d]	620 ± 13	537–717	2979 ± 214	2055–4490	42.1 ± 2.2	28–50
Eureka Landing, AL (328)	10	427 ± 21	372–577	1060 ± 182	650–2375	12.4 ± 3.7	4–44
Bucks, AL (329)	11	523 ± 18	427–608	1857 ± 172	915–2715	6.5 ± 0.7	3–12
Omaha, GA (330)	7[f]	542 ± 13	495–592	2306 ± 196	1775–3025	50.8 ± 6.0	22–62
Albany, GA (331)	7[i]	631 ± 25	561–759	4225 ± 632	2950–7545	24.0 ± 5.7	7–31
Blountstown, FL (332)	10[d]	470 ± 17	411–569	1530 ± 171	915–2425	17.5 ± 1.7	7–23
Augusta, GA (333)	10[j]	454 ± 23	391–638	1389 ± 226	910–3320	10.6 ± 2.8	6–27
Sylvania, GA (334)	9[j]	456 ± 24	420–643	1472 ± 291	1080–3795	6.1 ± 0.5	4–8
Port Wentworth, GA (335)	10[j]	470 ± 36	386–703	1682 ± 452	770–4875	10.7 ± 2.5	6–25
Rockingham, NC (336)	0	--	--	--	--	--	--
Pee Dee, SC (337)	10[f]	462 ± 23	350–620	1492 ± 211	600–3015	8.7 ± 1.7	5–13
Bucksport, SC (338)	2	579 ± 32	547–610	1533 ± 713	820–2245	6.5 ± 0.5	6–7

[a] $n = 76$ for age.

[b] $n = 91$ for age.

[c] $n = 9$ for age.

[d] $n = 8$ for age.

[e] $n = 5$ for age.

[f] $n = 6$ for age.

[g] $n = 1$ for age.

[h] $n = 85$ for age.

[i] $n = 4$ for age.

[j] $n = 7$ for age.

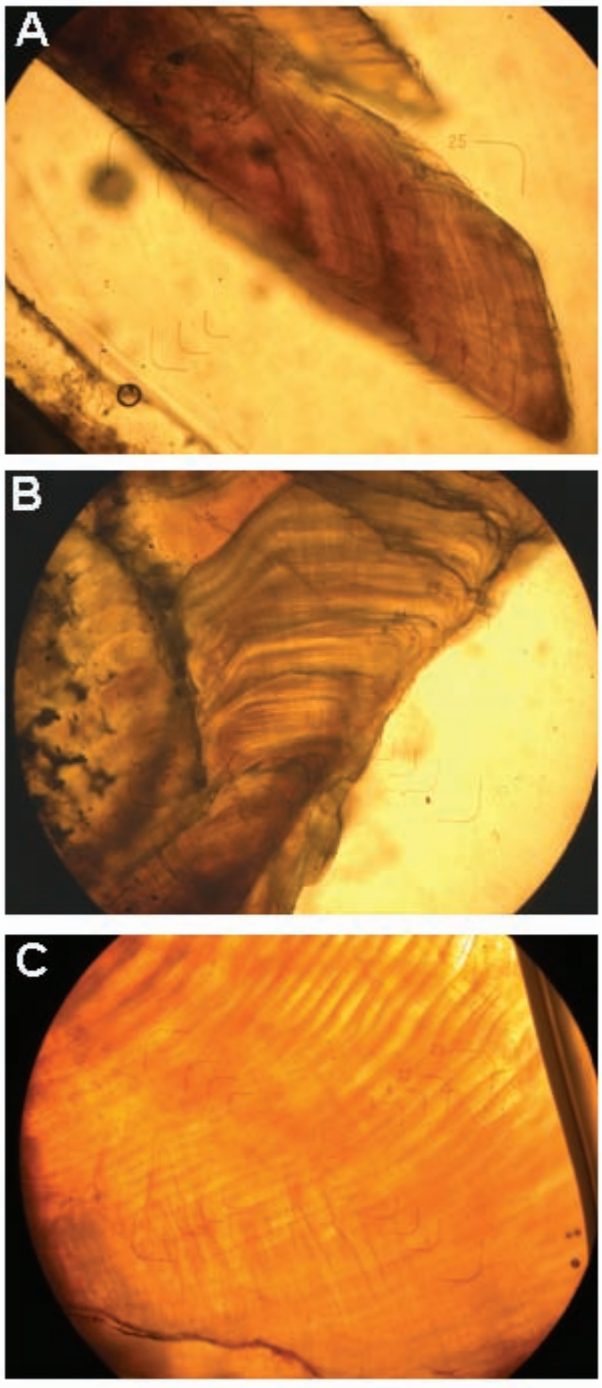


Figure 13. Select microscopic images of carp otolith cross-sections. *A*, Otolith section of nine year old carp from the Coosa River near Childersburg, Alabama (Station 327). *B*, Otolith section of 35 year old carp from the Coosa River near Childersburg, Alabama (Station 327). *C*, Otolith section of 50 year old carp from the Pee Dee River near Pee Dee, South Carolina (Station 337).

ARB, SRB, and PDRB. HAI scores differed between genders in bass ($F_{1, 194} = 4.64$, $P < 0.05$) but not carp ($F_{1, 169} = 1.02$, $P < 0.05$). The affect of gender on HAI is discussed in the following paragraphs. Generally, fish with high HAI scores were considered to be in poorer health than those with low HAI scores.

HAI scores in bass differed among sites. Mean HAI scores ranged from 29 at Station 333 to 114 at Station 329 and were greatest (>88) in fish from the MORB (table 18). Most (82%) HAI scores in individual bass were 0-100. Liver discoloration and granular liver, kidney, and spleen were the main contributors of elevated HAI scores in male and female bass from Stations 326, 327, 328, and 329 (fig. 14). Microscopically, liver discoloration was because of 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 tissues that were scored as granular or nodular at Stations 326, 327, 328, and 329, and severe infestations were present in some fish (fig. 15A). In addition, cysts containing myxozoan parasites occasionally were present within the kidney (fig. 15B). Small, focal granulomas also were present in liver, kidney, and spleen tissues but were more extensive in bass from Station 326. In one bass from Station 326, these granulomas were large and replaced much of the normal tissue (fig. 15C). Cysts of a microsporidia were observed in kidney tissue of fish from Stations 326 and 328 and were most common and severe at Station 328 (fig. 15D). HAI scores generally were lower in male bass than in female bass at most sites, although lesion type was similar in both genders (fig. 14). Frayed gills and parasites were the most common gill lesions, and most of these were observed on bass from the MORB and PDRB (table 18). Gill parasites included large metacercarial stages of digenetic trematodes, monogenetic trematodes, and myxozoan cysts (fig. 16). Frayed fins and parasites also were common fin lesions, although black spots were identified on fins of fish from Stations 326, 337, and 338 (table 18). Histological examination determined that the black spots were due to an accumulation of melanin around the metacercariae of digenetic trematodes. Opaque eyes were observed on bass only from the MORB (Stations 326, 327, 328, and 329), and exopthalmic (swollen, protruding) eyes were noted in three bass from Station 336; no samples were collected for histological examination. Fat-covered spleens were observed in multiple bass from Stations 328, 329, 331, 336, and 338, and enlarged spleens were noted in bass from Stations 334, 335, and 338. Swollen and granular kidneys were noted in several bass from Stations 330, 331, 332, 334, and 335. Microscopically, these kidney sections had large numbers of helminth and/or Myxozoan parasites present. HAI scores in bass were significantly correlated with *p,p'*-DDE and Hg concentrations (table 17).

HAI scores in carp were less than those in bass. Mean HAI scores ranged from 0 at Station 330 to 53 at Station 326 and generally were greatest in the MORB (table 18). Most

Table 17. Spearman rank correlation coefficients for the relation between biological endpoints and contaminant concentrations in bass and carp.

[Genders were combined for statistical analysis after analysis of variance indicated that gender was not a significant factor. Chlordane is the sum of *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, heptachlor epoxide, and oxychlordane. Polychlorinated biphenyl (PCB) is the sum of all congeners. Asterisks denote significant correlations ($P < 0.05$). Bass; $n = 26$. Carp; $n = 25$. HCB, hexachlorobenzene; PCA, pentachloroanisole; CF, condition factor; HSI, hepatosomatic index; SSI, splenosomatic index; MA-#, macrophage aggregate density; MA-A, macrophage aggregate size; MA-%, percent tissue occupied by macrophage aggregates; HAI, health assessment index; NA, not applicable]

Contaminant	Biological endpoint							
	CF	Age	HSI	SSI	MA-#	MA-A	MA-%	HAI
Bass								
Dieldrin	0.16	0.03	–0.45*	NA	0.18	–0.07	–0.02	–0.30
HCB	–0.12	0.34	–0.28	NA	0.24	0.03	0.20	0.03
PCA	0.28	0.63*	0.17	NA	0.23	0.61*	0.48*	0.27
Chlordane	0.25	0.43*	–0.25	NA	0.27	0.30	0.36	–0.05
o,p'-DDD	–0.13	0.22	–0.37	NA	0.46*	0.06	0.32	0.02
p,p'-DDE	0.18	0.70*	–0.04	NA	0.54*	0.70*	0.70*	0.44*
p,p'-DDD	0.03	0.58*	–0.38	NA	0.54*	0.34	0.43*	0.12
p,p'-DDT	0.02	0.68*	–0.43*	NA	0.61*	0.50*	0.57*	0.26
Mirex	0.32	0.10	–0.11	NA	0.19	0.24	0.20	–0.07
PCB	0.22	0.48*	0.14	NA	0.03	0.34	0.28	0.17
Toxaphene	0.38	0.04	–0.19	NA	0.29	–0.09	0.00	–0.38
TCDD-EQ	0.21	–0.07	0.16	NA	–0.15	0.23	0.05	–0.16
Arsenic	0.39	0.17	0.21	NA	0.00	0.40*	0.17	0.03
Cadmium	0.14	–0.07	–0.04	NA	–0.13	–0.27	–0.18	–0.09
Chromium	–0.03	–0.25	0.16	NA	–0.25	–0.26	–0.17	–0.29
Copper	0.49*	–0.49*	0.29	NA	–0.20	–0.38	–0.36	–0.38
Mercury	–0.28	0.21	0.12	NA	0.35	0.11	0.30	0.46*
Nickel	0.09	0.07	0.27	NA	–0.22	0.02	–0.09	–0.04
Lead	–0.10	–0.21	–0.11	NA	–0.13	–0.14	–0.20	–0.37
Selenium	–0.13	0.05	–0.25	NA	0.49*	0.36	0.40	0.19
Zinc	0.04	–0.21	0.19	NA	–0.11	–0.11	–0.20	–0.20
Carp								
Dieldrin	0.56*	0.50*	NA	–0.20	0.51*	0.34	0.37	–0.39
HCB	0.33	0.34	NA	–0.31	0.51*	0.38	0.39	–0.35
PCA	–0.47*	0.28	NA	0.52*	0.26	0.52*	0.37	0.16
Chlordane	0.37	0.73*	NA	–0.06	0.60*	0.55*	0.58*	–0.37
o,p'-DDD	0.35	0.17	NA	–0.08	0.47*	0.36	0.34	–0.27
p,p'-DDE	–0.09	0.50*	NA	0.05	0.86*	0.76*	0.83*	0.03
p,p'-DDD	0.33	0.36	NA	0.03	0.67*	0.54*	0.57*	–0.21
p,p'-DDT	0.35	0.10	NA	–0.09	0.51*	0.18	0.34	0.12
Mirex	0.57*	0.41*	NA	–0.30	0.63*	0.33	0.48*	–0.31
PCB	0.12	0.75*	NA	0.00	0.64*	0.75*	0.66	–0.30
Toxaphene	0.11	0.53*	NA	0.30	0.29	0.36	0.29	–0.13
TCDD-EQ	–0.62*	–0.35	NA	0.07	–0.12	0.15	–0.02	0.29
Arsenic	0..03	0.27	NA	0.01	0.31	0.56*	0.43*	–0.36
Cadmium	0.36	0.71*	NA	–0.10	0.54*	0.42*	0.54*	–0.24
Chromium	0.10	–0.41*	NA	–0.01	–0.36	–0.30	–0.38	–0.01
Copper	0.01	0.07	NA	0.21	0.03	0.03	0.00	0.13
Mercury	0.01	0.24	NA	0.15	0.11	–0.01	0.05	–0.35
Nickel	–0.37	0.23	NA	0.22	0.15	0.23	0.20	–0.17
Lead	–0.06	0.57*	NA	0.13	0.40*	0.48*	0.43*	–0.31
Selenium	–0.04	–0.02	NA	0.13	0.46*	0.46*	0.50*	–0.01
Zinc	0.22	0.48*	NA	0.10	0.23	0.30	0.23	–0.52

Table 18. Health assessment index and lesion location in bass and carp.

[Stations are listed upstream to downstream within a basin. HAI, health assessment index; *n*, sample size; Mean, arithmetic mean; SE, standard error]

Species, station location, and station number	*n*	HAI		Location (Number of fish with lesion)							
		Mean	SE	Body	Eyes	Opercles	Gills	Fins	Liver	Spleen	Kidney
Bass											
Lavaca, AL (326)	19	110	7	4	2	0	6	11	18	17	19
Childersburg, AL (327)	20	88	9	4	3	1	4	7	17	12	16
Eureka Landing, AL (328)	20	110	7	3	5	1	12	8	20	12	18
Bucks, AL (329)	20	114	6	6	2	0	11	15	20	13	18
Omaha, GA (330)	20	41	5	0	0	0	0	0	20	4	3
Albany, GA (331)	20	40	7	5	0	0	0	4	14	2	3
Blountstown, FL (332)	20	49	6	2	0	0	4	1	18	0	8
Augusta, GA (333)	10	29	5	1	0	0	0	2	6	1	1
Sylvania, GA (334)	9	64	11	2	0	0	1	1	8	2	6
Port Wentworth, GA (335)	20	42	7	12	0	0	1	9	3	3	6
Rockingham, NC (336)	16	63	9	1	3	3	8	4	13	4	2
Pee Dee, SC (337)	22	61	8	0	0	0	9	11	16	5	11
Bucksport, SC (338)	21	44	5	2	0	0	12	9	10	3	1
Carp											
Lavaca, AL (326)	19	53	7	5	0	0	4	15	8	7	3
Childersburg, AL (327)	19	42	10	3	3	0	6	8	9	1	2
Eureka Landing, AL (328)	19[a]	43	7	4	0	0	2	11	7	4	9
Bucks, AL (329)	20	33	5	0	1	0	2	12	9	4	2
Omaha, GA (330)	17	0	0	0	0	0	0	0	0	0	0
Albany, GA (331)	16	10	4	2	0	0	0	4	0	2	0
Blountstown, FL (332)	20	6	2	1	0	0	0	2	2	0	0
Augusta, GA (333)	20	13	4	2	0	0	0	5	5	0	0
Sylvania, GA (334)	16	20	7	3	0	0	0	5	4	0	2
Port Wentworth, GA (335)	20	8	3	2	0	0	0	1	3	0	0
Rockingham, NC (336)	1	10	0	0	0	0	0	1	0	0	0
Pee Dee, SC (337)	17	51	4	3	1	0	14	14	5	0	1
Bucksport, SC (338)	4	13	9	0	0	0	1	2	0	0	0

[a]HAI scores were not available for all individual fish.

(84%) HAI scores in individual carp were 0–40. Frayed fins, gill abnormalities, and liver discoloration were the main contributors of elevated HAI scores in male and female carp from Stations 326, 327, 328, 329, and 337 (fig. 14). HAI scores were similar in male and female carp, and the types of lesion found in carp were similar in both genders (fig. 14). Fin erosion was noted in carp from Stations 326, 329, and 332, and multiple carp from Stations 327 and 337 had deformed or notched fins. Frayed gills were observed on carp from Stations 326, 327, 337, and 338. Microscopically, the gill lesions of carp included abnormal cartilage (fig. 17A), proliferation of lamellar epithelial cells, leading to fusion of lamellae, and congestion or telangiectasis. Multiple carp from Station 337 also had discoloration (white areas) on gill tissue. These areas were cysts of a myxozoan parasite (fig. 17B). Opaque eyes were observed on three carp from Station 327, and body surface lesions were noted on carp from most sites (table 18). Other observations noted in carp from Station 327 were fluid-filled abdominal cavities, hard gonads, and thick otoliths; one male carp from Station 327 also had an opening between its pelvic fins that was connected to the intestine in addition to an anal pore. Microscopically, the "hard" gonads contained areas of calcified follicles or fibrosis. Enlarged spleens and granular spleen and kidney tissue also were noted in carp from Stations 326, 327, 328, and 329 (table 18). Microscopically, these organs contained small granulomas whose etiology was congestion, fibrosis, or unidentified. HAI scores in carp were not significantly correlated with any contaminant concentration (table 17).

Most of the fin or body surface lesions that were fixed for histology were areas of thickened epidermis or dermal inflammation, which often included congestion or hemorrhage. Many of these lesions appeared to be consistent with secondary bacterial infections; however, some contained trematode metacercariae. Ovaries from two carp at Station 337 contained degenerating eggs that were infected with a microsporidian

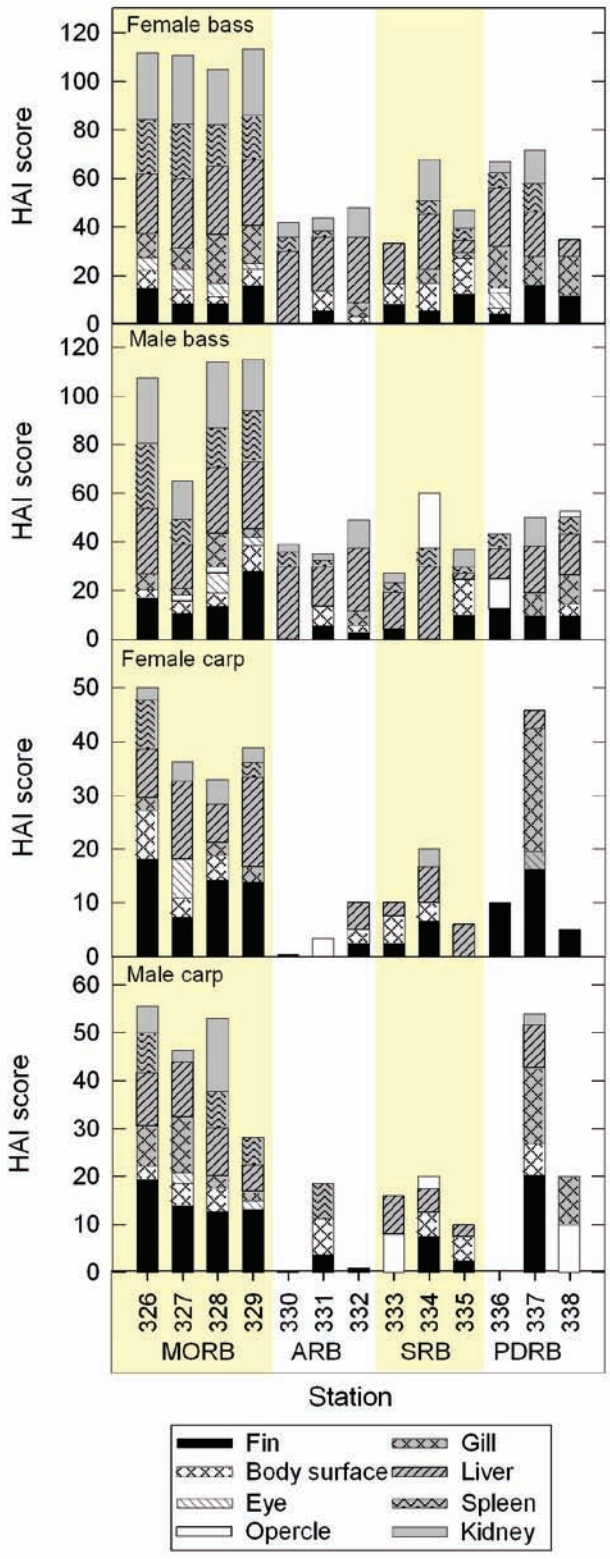


Figure 14. Mean health assessment index (HAI) scores by lesion location in bass and carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. No male carp were collected from Station 336. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

parasite. Several tumors were noted during gross examinations. Masses in the ovaries of two relatively old carp (43 and 56 years) from Station 330 (fig. 18A & B) were diagnosed as leiomyosarcoma (fig. 18C & D). A lip papilloma was identified on a bass from Station 328, and a lipoma within the spleen was present in a bass from Station 338 (fig. 19A). A Seritoli cell tumor was present in the gonad of a 7 year old male carp from Station 333 (fig. 19B–D).

HAI scores in bass and carp were reported by previous LRMN investigations. HAI scores in bass from previous LRMN investigations generally were lower than those in the MORB (88–114) but similar to those in the ARB (40–49), SRB (29–64), and PDRB (44–63; Blazer and others, 2002; Hinck and others, 2006a; Schmitt and others, 2005). HAI scores in carp from previous LRMN investigations generally were similar to those in the MORB (33–53) but greater than those in the ARB (0–10), SRB (8–20), and PDRB (10–51; Blazer and others, 2002; Hinck and others, 2006b; Schmitt and others, 2005). The USEPA reported HAI scores in largemouth bass (means 42–85) and channel catfish (27–43) in the lower MORB near Stations 328 and 329 and concluded that fish were in relatively good condition (USEPA, 1995). External lesions were observed rarely in this study, but internal abnormalities including encysted parasites in the liver, kidney, and spleen and liver discoloration were common in bass (USEPA, 1995), which was consistent with our findings in fish from Stations 328 and 329.

Condition Factor and Organosomatic Indices

These indices were calculated from body and organ weights in individual fish and considered general indicators of the overall fish health. Alterations of these indices may be indicative of effects resulting from exposure to contaminants, but these indicators also can vary among species, gender, and gonadal stage. ANOVA models containing the factors station, gender, and gonadal stage were tested to determine if these factors affected condition factor, HSI, and SSI in bass and carp (appendix 5).

Condition Factor and Organosomatic Indices in Bass

Condition factor values in bass did not differ among sites ($F_{9, 194} = 1.34$, $P > 0.05$) or between genders ($F_{1, 194} = 1.28$, $P > 0.05$). The study-wide mean CF value in bass was 1.30, and station means ranged from 1.14 at Station 333 to 1.48 at Station 336 (table 19). Relatively high CF values (>1.4) were calculated for most fish from Stations 327 and 336, and lower CF values (1.0–2.0) were calculated for fish from Station 333 (fig. 20). CF values in bass were significantly correlated with only one contaminant, Cu (table 17). Mean CF values in bass from the MORB (1.19–1.41), ARB (1.28–1.37), SRB (1.14–1.33), and PDRB (1.24–1.48) were similar to those reported in bass from the CDRB (1.0–1.7; Hinck and others, 2006b), CRB (1.3–2.1; Hinck and others, 2006a), MRB (0.8–2.4; Blazer and others, 2002), and RGB (1.4–1.8; Schmitt and others, 2005)

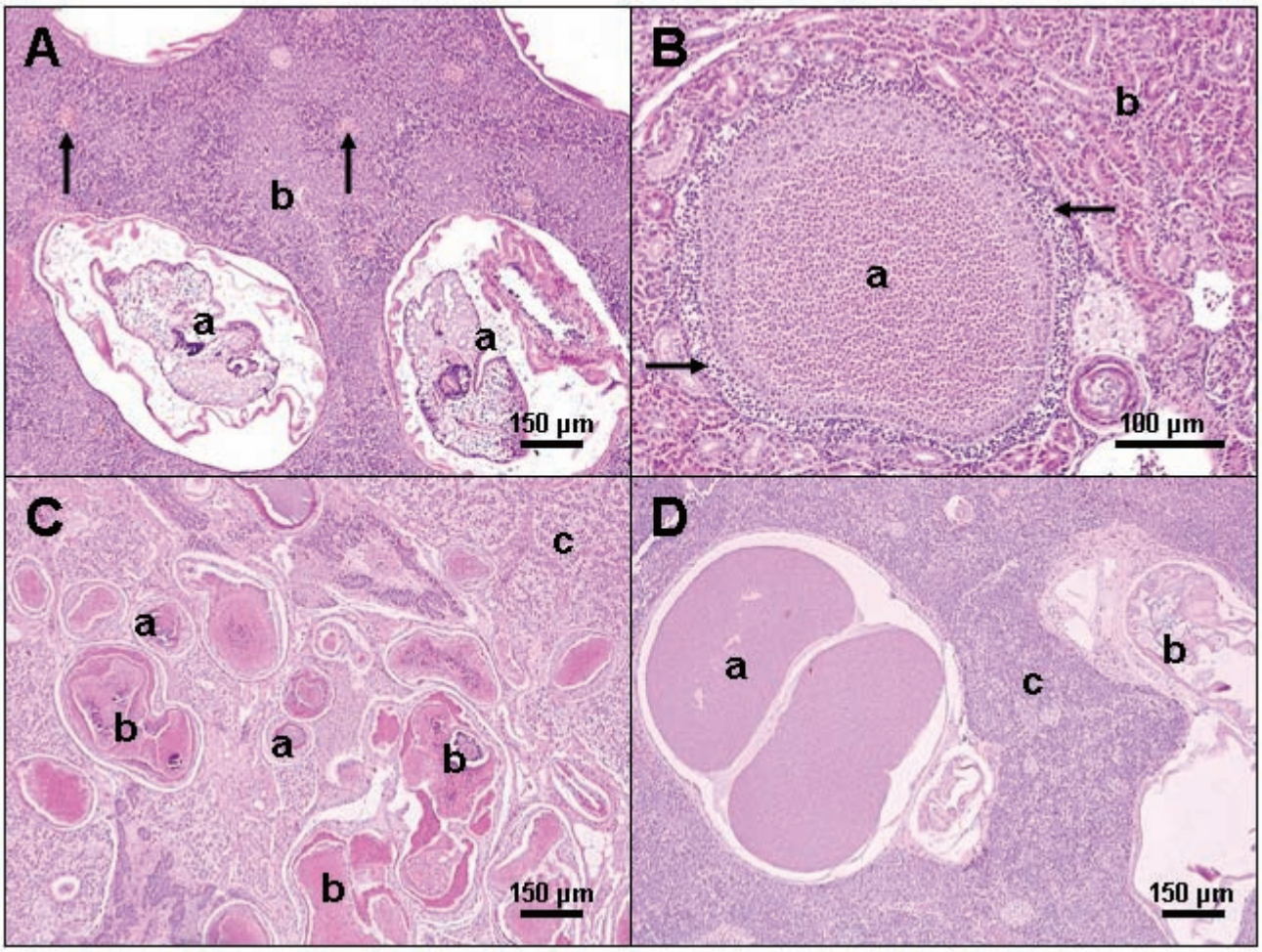


Figure 15. *A*, Helminth parasites (a) encysted in the anterior kidney (b) of a largemouth bass. Arrows illustrate macrophage aggregates. *B*, A cyst (a) containing spores of a myxozoan parasite within the posterior kidney (b) of a largemouth bass. The encysted parasites are surrounded by a cuff of inflammatory cells (arrows). *C*, Granulomas (a and b), a chronic inflammatory reaction, replacing much of the normal kidney (c) in a largemouth bass from the Tombigbee River near Lavaca, Alabama (Station 326). Small, focal granulomas (a) were most common; however, larger, coalescing granulomas (b) were observed in some instances. *D*, A microsporidian cyst (a) and encysted helminth parasite (b) within the anterior kidney (c) of a largemouth bass from the Alabama River near Eureka Landing, Alabama (Station 328). Hematoxylin and eosin stain.

and were within the range considered to be normal (1.0–2.0) for healthy largemouth and smallmouth bass (Blazer and others, 2002; Carlander, 1977; Hinck and others, 2006a; Schmitt and others, 2005; Sepúlveda and others, 2001).

HSI values in bass differed among sites ($F_{12, 211} = 4.02$, $P < 0.01$) but not between genders ($F_{1, 211} = 0.76$, $P > 0.05$). The study-wide mean HSI value in bass was 0.67%, and station means ranged from 0.56% at Station 332 to 0.82% at Station 338 (table 20). HSI values >1.0% were calculated for fish from Stations 327, 329, 331, 335, 337, and 338, but relatively low HSI values (≤0.5%) were calculated for fish from all sites (fig. 20). Liver tissue from most sites was described as discolored and granular because of a variety of parasites. HSI values were negatively correlated with dieldrin and *p,p'*-DDT concentrations in bass (table 17). Mean HSI values generally were less than those reported in bass from the CDRB (0.6–1.7%; Hinck and others, 2006b), CRB (0.9–2.3%; Hinck and others, 2006a), MRB (0.6–2.0%; Blazer and others, 2002), and RGB (0.7–1.0%; Schmitt and others, 2005). HSI values in bass from the MORB, ARB, SRB, and PDRB also were below the range considered normal (1–3%) for healthy fish (Gingerich, 1982) although HSI values <1.0% have been reported previously in bass (Blazer and others, 2002; Schmitt and others, 2005).

SSI values in bass differed among sites ($F_{12, 211} = 1.91$, $P < 0.05$) and between genders ($F_{1, 211} = 8.33$, $P < 0.05$); therefore, males and females were analyzed separately. The study-wide mean SSI value in female bass was 0.12%, and station means ranged from 0.08% at Station 335 to 0.30% at Station 333 (table 21). Individual SSI values were >0.30% in female bass from Stations 326, 333, 337, and 338 (fig. 20), and histologically these tissues appeared normal. Relatively low SSI values (≤0.03%) were calculated in females from Stations 327, 330, 332, and 335 ($n = 5$; fig. 20). SSI values generally were lower in male bass than in female bass. The study-wide mean

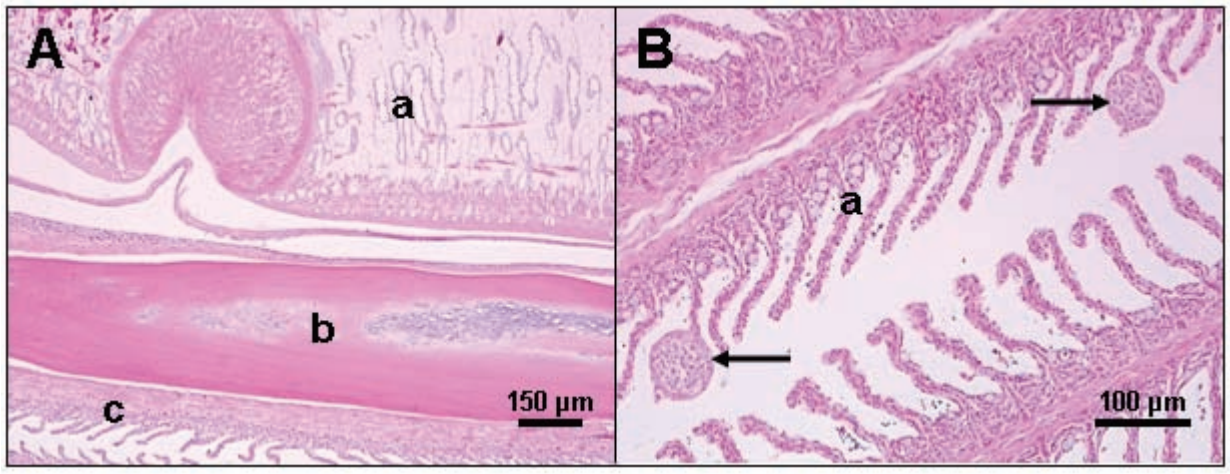


Figure 16. *A*, A large metacercaria or grub (a) attached to the gill (b) of a largemouth bass. The normal lamellar structure (c) is destroyed in the vicinity of the parasite. *B*, Encysted myxozoan parasites (arrows) within the gill lamellae (a) of a largemouth bass. Hematoxylin and eosin stain.

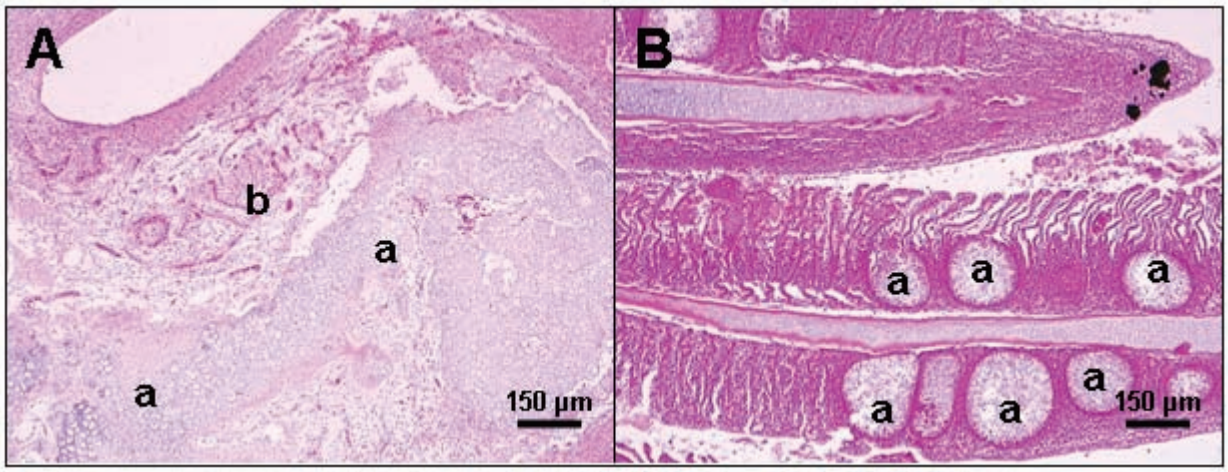


Figure 17. *A*, Abnormal proliferation of cartilage (a) destroying the normal structure of the respiratory surface (b) in the gill of a common carp. *B*, Cysts of a myxozoan parasite (a) within the gill tissue of a common carp. Hematoxylin and eosin stain.

SSI value in male bass was 0.09%, and station means ranged from 0.05% at Station 335 to 0.13% at Station 334 (table 21). The high SSI value (>0.30%) in a male bass from Station 338 was because of a large lipoma found in the spleen. SSI values generally were lower in male bass from Station 335 than those from other sites (fig. 20); histologically these spleens were normal and the lower SSI may have been because of lower parasite loads. SSI values were negatively correlated with As in female bass and mirex in male bass (table 13). Mean SSI values in bass from the MORB (0.08–0.14%), ARB (0.08–0.11%), SRB (0.05–0.30%), and PDRB (0.10–0.15%) were similar to those reported in previous LRMN investigations from the CDRB (0.03–0.16%; Hinck and others, 2006b), CRB (0.11–0.25%; Hinck and others, 2006a), MRB (0.09–0.24%; Blazer and others, 2002), and RGB (0.07–0.22%; Schmitt and others, 2005).

Condition Factor and Organosomatic Indices in Carp

Condition factor values differed among sites ($F_{9, 169}$ = 3.38, $P < 0.01$) but not between genders ($F_{1, 169}$ = 0.61, $P > 0.05$) in carp. The study-wide mean CF value was 1.39, and station means ranged from 0.92 at Station 338 to 1.66 at Station 331 (table 19). Relatively high CF values (>1.6) were calculated for carp from Stations 328, 330, 331, 332, and 333, and low CF values (<0.7) characterized carp from Stations 327 and 338 (fig. 21). CF values were significantly correlated with dieldrin, PCA, mirex, and TCDD-EQ concentrations in carp (table 17). Mean CF values in carp from the MORB (1.26–1.29), ARB (1.46–1.66), SRB (1.40–1.46), and PDRB (0.92–1.46) were similar to those reported in carp from the CDRB (1.0–1.9; Hinck and others, 2006b), CRB (1.2–1.9;

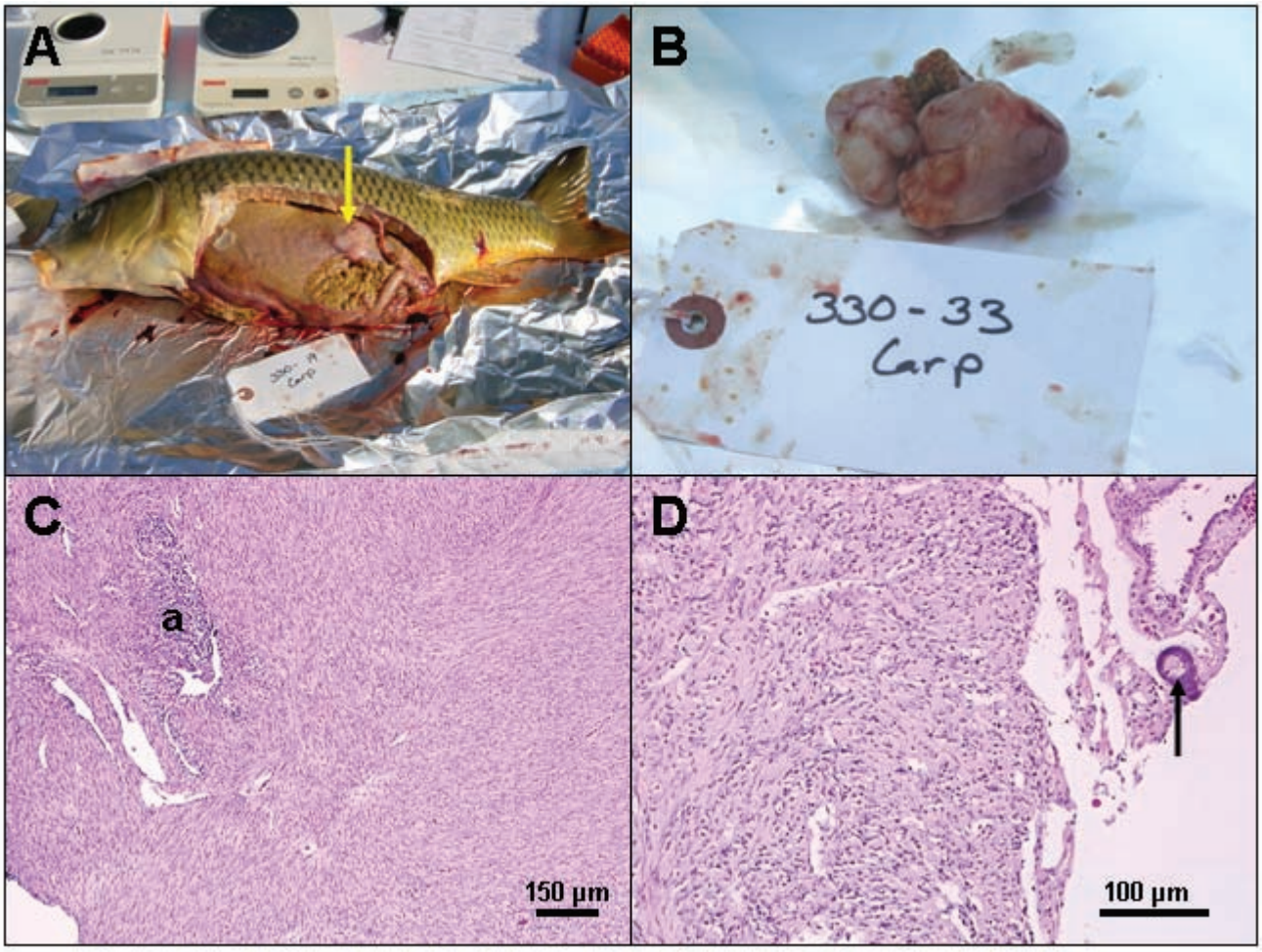


Figure 18. *A*, Raised, nodular mass, identified as a leiomyosarcoma, in the ovary of a common carp from the Chattahoochee River near Omaha, Georgia (Station 330). *B*, A second leiomyosarcoma, illustrating the circumscribed, nodular appearance of the mass, removed from the ovary of another female common carp from Station 330. *C*, Histologically, the tumor was composed of elongate smooth muscle cells in interlacing cords. Occasionally, areas of inflammation (a) were noted. *D*, Higher magnification of the leiomyosarcoma with invasion into ovarian follicles. Immature oocytes (arrow) are present. Hematoxylin and eosin stain.

Hinck and others, 2006a), MRB (1.1–1.5; Blazer and others, 2002), and RGB (1.2–1.5; Schmitt and others, 2005) and a national survey (1.2- >2.0; Carlander, 1969).

Relative spleen sizes in carp did not differ among sites ($F_{12, 183} = 1.40$, $P > 0.05$) or between genders ($F_{1, 183} = 2.63$, $P > 0.05$). The study-wide mean SSI value in carp was 0.31%, and station means ranged from 0.23% at Station 332 to 0.56% at Station 338 (table 21). Relatively high SSI values (>1.0%) were calculated for carp from Stations 326, 329, and 338 (fig. 21) and may be associated with severe parasitic infestations. SSI values <0.15% were reported in fish from Stations 328 ($n = 3$) and 331 ($n = 1$) but were microscopically normal. SSI values were positively correlated with PCA concentrations in carp (table 17). Mean SSI values in carp from the MORB (0.26–0.53%), ARB (0.23–0.31%), SRB (0.24–0.30%), and PDRB (0.33–0.56%) generally were greater than those reported in previous LRMN investigations from the CDRB (0.15–0.46%; Hinck and others, 2006b), MRB (0.04–0.87%; Blazer and others, 2002), RGB (0.10–0.40%; Schmitt and others, 2005), CRB (0.15–0.44%; Hinck and others, 2006a). Relative spleen sizes >1.0% in carp from Stations 326 and 338 were considered abnormal and may indicate physiologic stress in these individuals.

Macrophage Aggregates

Macrophage aggregates contain endogenous and exogenous waste products and are active in the immune response to these materials. Three MA parameters, density or number of aggregates per mm^2 (MA-#), mean size of aggregates in μm^2 (MA-A), and percent of tissue occupied by macrophage aggregates (MA-%) were analyzed for bass and carp. Female and male fish were analyzed together in bass and carp because MA parameters did not differ between genders (appendix 5). Age was a significant factor in some species and is discussed where appropriate.

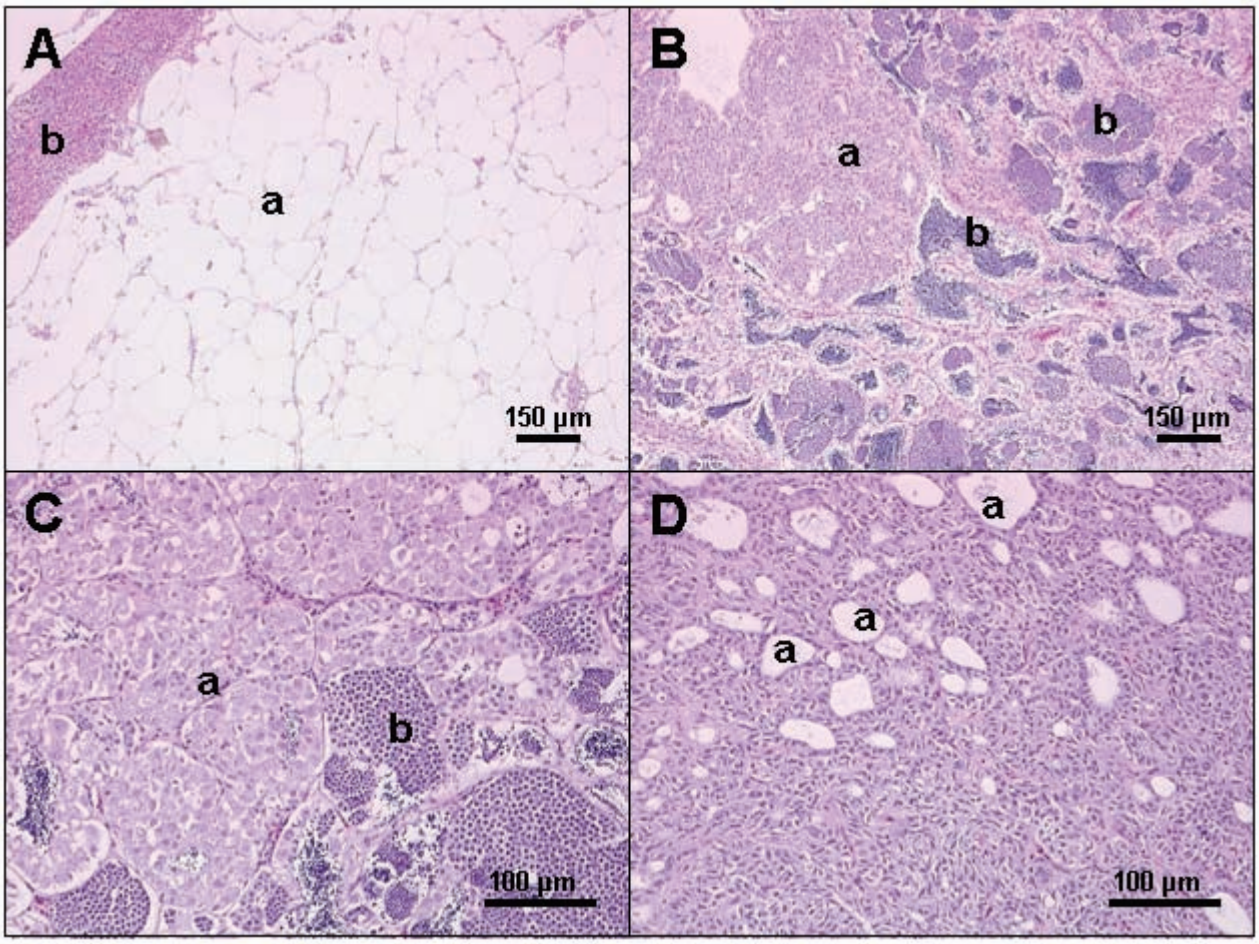


Figure 19. *A*, A section of a large lipoma (a) within the spleen (b) of a largemouth bass from the Pee Dee River near Bucksport, South Carolina (Station 338). *B–D*, Sertoli cell tumor from a male common carp from the Savannah River near Augusta, Georgia (Station 333). *B*, Foci of neoplastic cells (a) within normal testicular tissue (b). *C*, Higher magnification of the neoplastic cells (a), which were fairly uniform, large cells, adjacent to normal spermatocytes (b). *D*, The neoplastic cells formed tubule-like structures (a) in some areas. Hematoxylin and eosin stain.

MA Measurements in Bass

The number of MAs in bass differed significantly among sites ($F_{12, 182} = 2.25$, $P < 0.05$) and age ($F_{1, 182} = 18.29$, $P < 0.05$). Station means ranged from 1.9 MA/mm^2 at Station 337 to 6.2 MA/mm^2 at Station 334 (table 22). Mean MA-# values generally were lowest in bass from the PDRB (fig. 22). MA density (≥8 MA/mm^2) generally was greater in older bass. MA-# values were significantly correlated with *o,p'*-DDD, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, and Se concentrations in bass (table 17). Mean MA-# values in bass from the MORB (3.6–5.5 MA/mm^2), ARB (4.6–5.5 MA/mm^2), SRB (2.3–6.2 MA/mm^2), and PDRB (1.9–3.7 MA/mm^2) were similar to those reported in previous LRMN investigations from the CDRB (0.9–5.5 MA/mm^2; Hinck and others, 2006b), MRB (2.2–11.2 MA/mm^2; Blazer and others, 2002), RGB (4–8 MA/mm^2; Schmitt and others, 2005), and CRB (4.1–9.5 MA/mm^2; Hinck and others, 2006a).

The size of MAs in bass differed significantly by age ($F_{1, 182} = 5.05$, $P < 0.05$) but not among sites ($F_{12, 182} = 1.26$, $P > 0.05$). Mean MA-A generally were largest in bass from the MORB (4,158–5,420 µm^2), where bass were older (table 22). MA-A values >10,000 µm^2 were measured in bass from Stations 327, 328, and 329 in the MORB and Station 330 in the ARB (fig. 22) and were among the largest MAs measured in bass from any LRMN study (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). MA-A values were significantly correlated with PCA, *p,p'*-DDE, *p,p'*-DDT, and As concentrations in bass (table 17). Mean MA-A values in bass from the MORB (4,158–5,420 µm^2), ARB (2,392–4,819 µm^2), SRB (2,287–3,647 µm^2), and PDRB (2,274–3,603 µm^2) were similar to those reported in previous LRMN investigations from the CDRB (612–9,389 µm^2; Hinck and others, 2006b), MRB (1,049–4,440 µm^2; Blazer and others, 2002), RGB (3,000–5,000 µm^2; Schmitt and others, 2005), and CRB (2,118–5,095 µm^2; Hinck and others, 2006a).

Table 19. Mean condition factor in bass and carp.

[Genders were combined for statistical analysis after analysis of variance indicated that gender was not a significant factor. Stations are ordered upstream to downstream within a basin. *n*, sample size; Mean, arithmetic mean; SE, standard error; --, not applicable]

Species, station location, and station number	Condition factor (CF)		
	n	Mean ± SE	Range
Bass			
All stations	237	1.30 ± 0.01	0.5–1.9
Lavaca, AL (326)	19	1.19 ± 0.03	1.0–1.4
Childersburg, AL (327)	20	1.41 ± 0.03	1.2–1.7
Eureka Landing, AL (328)	20	1.25 ± 0.03	1.0–1.5
Bucks, AL (329)	20	1.27 ± 0.03	1.0–1.6
Omaha, GA (330)	20	1.28 ± 0.02	1.0–1.4
Albany, GA (331)	20	1.37 ± 0.04	1.1–1.9
Blountstown, FL (332)	20	1.31 ± 0.05	0.5–1.7
Augusta, GA (333)	10	1.14 ± 0.02	1.0–1.3
Sylvania, GA (334)	9	1.30 ± 0.06	1.1–1.7
Port Wentworth, GA (335)	20	1.33 ± 0.02	1.1–1.6
Rockingham, NC (336)	16	1.48 ± 0.04	1.2–1.8
Pee Dee, SC (337)	22	1.24 ± 0.03	1.0–1.6
Bucksport, SC (338)	21	1.32 ± 0.02	1.2–1.5
Carp			
All stations	209	1.39 ± 0.01	0.4–1.9
Lavaca, AL (326)	19	1.29 ± 0.02	1.1–1.5
Childersburg, AL (327)	19	1.26 ± 0.04	0.7–1.6
Eureka Landing, AL (328)	20	1.27 ± 0.03	1.1–1.7
Bucks, AL (329)	20	1.29 ± 0.02	1.1–1.5
Omaha, GA (330)	17	1.50 ± 0.03	1.3–1.7
Albany, GA (331)	16	1.66 ± 0.03	1.4–1.9
Blountstown, FL (332)	20	1.46 ± 0.03	1.2–1.7
Augusta, GA (333)	20	1.41 ± 0.03	1.2–1.7
Sylvania, GA (334)	16	1.46 ± 0.01	1.4–1.6
Port Wentworth, GA (335)	20	1.40 ± 0.01	1.2–1.5
Rockingham, NC (336)	1	1.05	--
Pee Dee, SC (337)	17	1.46 ± 0.02	1.3–1.6
Bucksport, SC (338)	4	0.92 ± 0.25	0.4–1.5

The percent of tissue occupied by MAs in bass differ among sites ($F_{12, 182} = 2.55$, $P < 0.05$) and age ($F_{1, 182} = 20.23$, $P < 0.05$). Mean MA-% values were lower in bass from the PDRB (0.4–1.4%) than the MORB (2.3–3.3%; table 22). Individual MA-% values were >6% in bass from Stations 326, 327, 328, and 329 in the MORB, Station 330 in the ARB, and Station 334 in the SRB (fig. 22) and were among the greatest measured in bass from any LRMN study (Blazer and others,

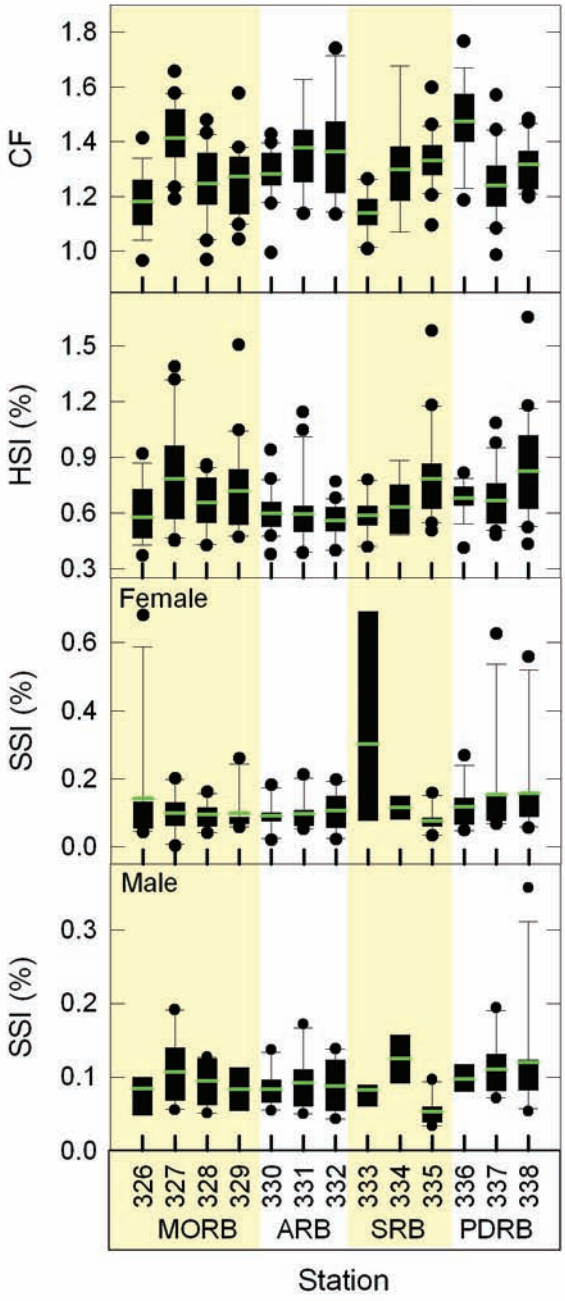


Figure 20. Fish health indicators by station in female and male bass from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include condition factor (CF), hepatosomatic index (HSI; percent), and splenosomatic index (SSI; percent). Females and males were plotted separately when analysis-of-variance modeling indicated that gender was a significant factor. Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

Table 20. Mean hepatosomatic index in bass.

[Genders were combined for statistical analysis after analysis of variance indicated that gender was not a significant factor. Stations are ordered upstream to downstream. HSI, hepatosomatic index; *n*, sample size; Mean, arithmetic mean; SE, standard error]

Station location and station number	HSI (%)		
	n	Mean ± SE	Range
All stations	237	0.67 ± 0.01	0.37–1.65
Lavaca, AL (326)	19	0.58 ± 0.04	0.37–0.92
Childersburg, AL (327)	20	0.78 ± 0.06	0.45–1.39
Eureka Landing, AL (328)	20	0.65 ± 0.03	0.42–0.86
Bucks, AL (329)	20	0.72 ± 0.06	0.47–1.50
Omaha, GA (330)	20	0.60 ± 0.03	0.38–0.94
Albany, GA (331)	20	0.59 ± 0.04	0.38–1.14
Blountstown, FL (332)	20	0.56 ± 0.02	0.40–0.77
Augusta, GA (333)	10	0.59 ± 0.03	0.42–0.78
Sylvania, GA (334)	9	0.63 ± 0.05	0.48–0.88
Port Wentworth, GA (335)	20	0.78 ± 0.06	0.50–1.58
Rockingham, NC (336)	16	0.68 ± 0.02	0.41–0.81
Pee Dee, SC (337)	22	0.66 ± 0.03	0.48–1.08
Bucksport, SC (338)	21	0.82 ± 0.06	0.43–1.65

2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). MA-% values generally increased as fish age increased and were significantly correlated with PCA, *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT concentrations in bass (table 17). Mean MA-% values in bass from the MORB (2.3–3.3%), ARB (1.2–2.3%), SRB (0.7–2.6%) and PDRB (0.4–1.4%) were similar to those reported in previous LRMN investigations from the CDRB (0.1–3.5%; Hinck and others, 2006b), MRB (0.3–3.8%; Blazer and others, 2002), RGB (2–3%; Schmitt and others, 2005), and CRB (1.1–4.0%; Hinck and others, 2006a).

Overall, MA parameters were greatest in bass from the MORB (Stations 326, 327, 328, and 329). However, the number and size of MAs in bass were related to the age of the fish with older bass having more numerous and larger MAs. All MA parameters were significantly correlated with *p,p'*-DDE and *p,p'*-DDT concentrations in bass.

MA Measurements in Carp

The number of MAs in carp did not differ significantly among sites ($F_{11, 124} = 1.33$, $P > 0.05$) or age ($F_{1, 124} = 0.03$, $P > 0.05$). However, station means ranged from 0.6 MA/mm^2 at Station 338 to 6.0 MA/mm^2 at Station 330 (table 22). Differences in mean MA-# values among sites were likely not significant because of the variation in samples size at each site (n = 1–20). Mean MA-# values were greatest in carp from Stations 327, 330, and 331, and relatively high MA-# values (>10 MA/mm^2) were measured in individual fish from Stations 327, 329, 330, and 333 (fig. 23). MA-# values were significantly correlated with Cd, Pb, Se, and most organochlorine concentrations in carp (table 17). Mean MA-# values in carp from the MORB (1.5–4.7 MA/mm^2), ARB (1.1–6.0 MA/mm^2), SRB (1.1–2.4 MA/mm^2), and PDRB (0.6–2.9 MA/mm^2) were similar to those reported in previous LRMN investigations from the CDRB (4.1–10.9 MA/mm^2; Hinck and others, 2006b), MRB (5.1–18.3 MA/mm^2; Blazer and others, 2002), RGB (1–16 MA/mm^2; Schmitt and others, 2005), and CRB (3.0–10.2 MA/mm^2; Hinck and others, 2006a).

The size of MAs in carp did not differ significantly among sites ($F_{11, 124} = 0.61$, $P > 0.05$) or age ($F_{1, 124} = 0.07$, $P > 0.05$), but mean MA-A values were greater in carp from Stations 327 and 330 (7,411–8,846 μm^2) than those from other sites (1,886–4,564 μm^2; table 22). The MA-A value in one carp from Station 336 was very high (23,366 μm^2); this carp had a few very large MAs. MA-A values >10,000 μm^2 were measured in carp from Stations 327, 329, 330, 336, and 337 (fig. 23). The MA-A value from Station 327 (36,300 μm^2) was the largest measured in a carp from any LRMN study (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). MA-A values were significantly correlated with PCA, total chlordanes, *p,p'*-DDE, *p,p'*-DDD, total PCBs, As, Cd, Pb, and Se concentrations in carp (table 17). With the exception of Stations 327, 330, and 336, mean MA-A values in carp from the MORB (2,649–4,564 μm^2), ARB (2,994–4,506 μm^2), SRB (1,886–2,999 μm^2), and PDRB (2,002–3,605 μm^2) were similar to those reported in previous LRMN investigations from the CDRB (2,349–11,463 μm^2; Hinck and others, 2006b), MRB (1,670–4,684 μm^2; Blazer and others, 2002), RGB (1,500–8,000 μm^2; Schmitt and others, 2005), and CRB (2,690–5,850 μm^2; Hinck and others, 2006a).

The percent of tissue occupied by MAs in carp did not differ significantly among sites ($F_{11, 124} = 1.56$, $P > 0.05$) or age ($F_{1, 124} = 0.03$, $P > 0.05$). Mean MA-% values were <1.0% at all sites except Stations 327, 329, 330, 331, and 336 (table 22). MA-% values >11.2% in carp from Station 327 were among the greatest measured in a carp from any LRMN study (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005; fig. 23). MA-% values were significantly correlated with total chlordanes, *p,p'*-DDE, *p,p'*-DDD, mirex, As, Cd, Pb, and Se concentrations in carp (table 17). Mean MA-% values in carp from the MORB (0.5–3.9%), ARB (0.3–4.7%), SRB (0.3–0.9%) and PDRB (0.1–7.1%) were similar to those reported in previous LRMN investigations from the CDRB (0.1–6.6%; Hinck and others, 2006b), MRB (1.2–6.4%; Blazer and others, 2002), RGB (1–13%; Schmitt and others, 2005), and CRB (0.9–4.7%; Hinck and others, 2006a).

MA parameters were relatively high in a few carp from Station 327 in the MORB, Stations 330 and 331 in the ARB, and Station 337 (n = 1) in the PDRB. All MA parameters were significantly correlated with total chlordanes, *p,p'*-DDE, *p,p'*-DDD, Cd, Pb, and Se concentrations in carp.

Health Indicators: Summary

The quantitative fish health indicators used in this study have been widely used and discussed in the literature and were

Table 21. Mean splenosomatic index in bass and carp.

[Male and female fish were analyzed separately if analysis of variance indicated that gender was a significant factor. Stations are ordered upstream to downstream within a basin. SSI, splenosomatic index; *n*, sample size; Mean, arithmetic mean; SE, standard error; --, not applicable]

Species, station location, and station number	SSI (%)		
	n	Mean ± SE	Range
Female Bass			
All stations	125	0.12 ± 0.01	0.00–0.69
Lavaca, AL (326)	11	0.14 ± 0.06	0.04–0.68
Childersburg, AL (327)	10	0.10 ± 0.02	0.00–0.20
Eureka Landing, AL (328)	10	0.10 ± 0.01	0.04–0.16
Bucks, AL (329)	12	0.10 ± 0.02	0.06–0.26
Omaha, GA (330)	10	0.09 ± 0.01	0.02–0.18
Albany, GA (331)	10	0.10 ± 0.01	0.05–0.21
Blountstown, FL (332)	10	0.11 ± 0.02	0.02–0.20
Augusta, GA (333)	3	0.30 ± 0.20	0.08–0.69
Sylvania, GA (334)	5	0.12 ± 0.02	0.08–0.18
Port Wentworth, GA (335)	10	0.08 ± 0.01	0.03–0.16
Rockingham, NC (336)	13	0.12 ± 0.02	0.05–0.27
Pee Dee, SC (337)	11	0.15 ± 0.05	0.07–0.63
Bucksport, SC (338)	10	0.15 ± 0.05	0.06–0.56
Male Bass			
All stations	112	0.09 ± 0.01	0.03–0.36
Lavaca, AL (326)	8	0.08 ± 0.02	0.04–0.18
Childersburg, AL (327)	10	0.11 ± 0.02	0.06–0.19
Eureka Landing, AL (328)	10	0.09 ± 0.01	0.05–0.13
Bucks, AL (329)	8	0.08 ± 0.02	0.04–0.17
Omaha, GA (330)	10	0.08 ± 0.01	0.05–0.14
Albany, GA (331)	10	0.09 ± 0.01	0.05–0.17
Blountstown, FL (332)	10	0.09 ± 0.01	0.04–0.14
Augusta, GA (333)	7	0.08 ± 0.01	0.04–0.15
Sylvania, GA (334)	4	0.13 ± 0.02	0.09–0.16
Port Wentworth, GA (335)	10	0.05 ± 0.01	0.03–0.10
Rockingham, NC (336)	3	0.10 ± 0.01	0.08–0.12
Pee Dee, SC (337)	11	0.11 ± 0.01	0.07–0.19
Bucksport, SC (338)	11	0.12 ± 0.03	0.05–0.36
Carp			
All stations	208	0.31 ± 0.02	0.11–3.78
Lavaca, AL (326)	19	0.53 ± 0.20	0.15–3.78
Childersburg, AL (327)	19	0.30 ± 0.02	0.16–0.47
Eureka Landing, AL (328)	20	0.26 ± 0.04	0.11–0.80
Bucks, AL (329)	20	0.30 ± 0.05	0.17–1.25
Omaha, GA (330)	17	0.31 ± 0.03	0.18–0.62
Albany, GA (331)	16	0.29 ± 0.03	0.14–0.53
Blountstown, FL (332)	19	0.23 ± 0.01	0.15–0.32
Augusta, GA (333)	20	0.24 ± 0.01	0.15–0.35
Sylvania, GA (334)	16	0.30 ± 0.02	0.17–0.51
Port Wentworth, GA (335)	20	0.25 ± 0.01	0.15–0.33
Rockingham, NC (336)	1	0.33	--
Pee Dee, SC (337)	17	0.37 ± 0.04	0.19–0.83
Bucksport, SC (338)	4	0.56 ± 0.22	0.25–1.21

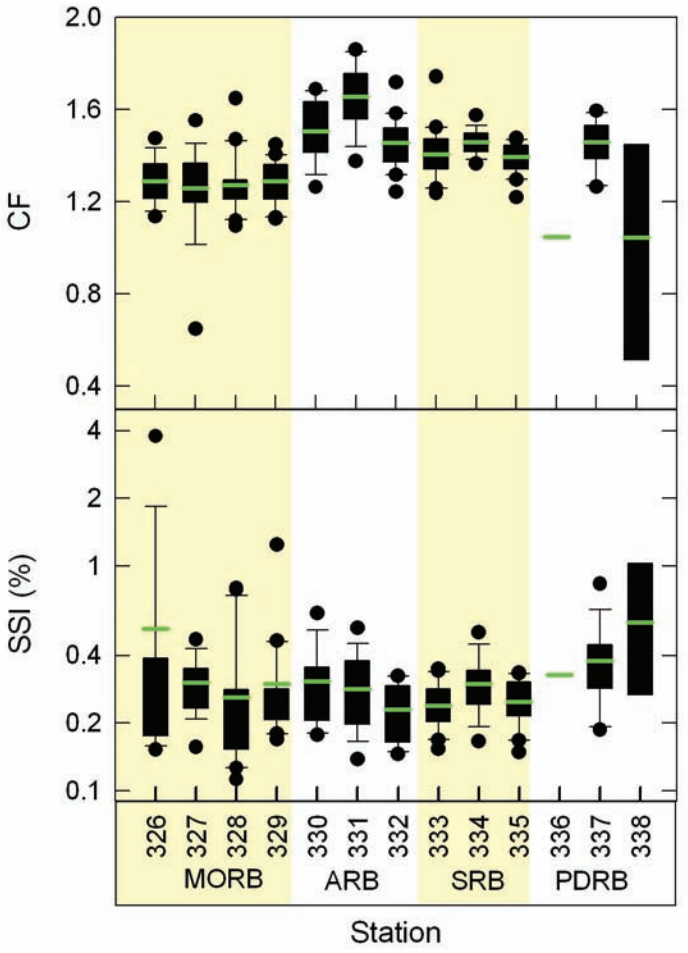


Figure 21. Fish health indicators by station in carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include condition factor (CF) and splenosomatic index (SSI; percent). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

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 MORB, ARB, SRB, and PDRB were in poorer health than others.

The HAI scores in bass from the MORB were greater than those observed in bass from other LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005) and would be considered unhealthy or contaminated by comparable criteria from other studies (Adams and others, 1993; Coughlan and others, 1996). Relatively high mean HAI scores were observed in bass (88–114) and carp (33–53) from all MORB sites (Stations 326, 327, 328, and 329) and carp from Station 337, which indicates greater numbers of external and internal lesions were observed on these fish. Liver discoloration and granular liver, kidney, and spleen were the main contributors of elevated HAI scores in bass, and frayed fins, gill abnormalities, and liver discoloration were associated

Table 22. Mean macrophage aggregate parameters in bass and carp.

[Genders were combined for statistical analysis after analysis of variance indicated that gender was not a significant factor. Stations are ordered upstream to downstream within a basin. MA-#, macrophage aggregate density; MA-A, MA area; MA-%, percent splenic tissue occupied by MA; *n*, sample size; Mean, arithmetic mean; SE, standard error; --, not applicable]

Species, station location, and station number	*n*	MA-# (MA/mm²)		MA-A (μm²)		MA-% (%)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Bass							
All stations	235	4.1 ± 0.2	0.6–12.9	3648 ± 145	700–15128	1.7 ± 0.1	0.04–11.07
Lavaca, AL (326)	19	5.5 ± 0.5	2.4–10.6	4757 ± 415	1648–8701	2.8 ± 0.4	0.40–7.37
Childersburg, AL (327)	20	3.6 ± 0.6	0.6–9.4	5391 ± 573	2459–10011	2.3 ± 0.6	0.16–8.82
Eureka Landing, AL (328)	20	5.2 ± 0.5	1.2–10.6	4158 ± 484	2243–11476	2.3 ± 0.3	0.53–6.03
Bucks, AL (329)	19	5.4 ± 0.6	1.2–8.8	5420 ± 493	1998–10079	3.3 ± 0.6	0.43–9.15
Omaha, GA (330)	20	4.7 ± 0.6	0.6–10.6	4819 ± 737	2100–15128	2.3 ± 0.5	0.14–11.07
Albany, GA (331)	20	4.6 ± 0.7	1.2–11.2	2392 ± 210	879–4376	1.2 ± 0.2	0.19–3.98
Blountstown, FL (332)	20	5.5 ± 0.7	1.2–12.9	3169 ± 212	1764–4810	1.9 ± 0.3	0.31–5.82
Augusta, GA (333)	10	2.3 ± 0.6	0.6–6.5	2287 ± 345	715–4618	0.7 ± 0.3	0.04–3.07
Sylvania, GA (334)	9	6.2 ± 1.1	1.2–11.2	3647 ± 775	1477–9130	2.6 ± 0.8	0.18–7.74
Port Wentworth, GA (335)	20	2.8 ± 0.6	0.6–10.0	2751 ± 410	1290–9234	0.8 ± 0.2	0.12–3.84
Rockingham, NC (336)	16	3.7 ± 0.6	0.6–8.2	3603 ± 443	1234–9087	1.4 ± 0.3	0.07–3.38
Pee Dee, SC (337)	21	1.9 ± 0.3	0.6–4.7	2274 ± 349	700–7235	0.4 ± 0.1	0.04–1.37
Bucksport, SC (338)	21	2.7 ± 0.5	0.6–7.6	2341 ± 248	905–4710	0.8 ± 0.2	0.08–3.28
Carp							
All stations	205	2.5 ± 0.2	0.0–16.5	4099 ± 271	0–36300	1.4 ± 0.2	0.00–11.15
Lavaca, AL (326)	19	1.5 ± 0.4	0.6–7.1	2875 ± 456	864–9913	0.5 ± 0.2	0.05–3.00
Childersburg, AL (327)	19	4.7 ± 0.8	0.6–10.6	8846 ± 1696	1446–36300	3.9 ± 0.8	0.09–11.15
Eureka Landing, AL (328)	20	1.6 ± 0.4	0.6–8.8	2649 ± 324	1389–6781	0.5 ± 0.2	0.09–2.57
Bucks, AL (329)	19	2.5 ± 0.6	0.6–10.6	4564 ± 701	1022–14318	1.1 ± 0.2	0.08–3.97
Omaha, GA (330)	15	6.0 ± 0.9	0.6–16.5	7411 ± 1024	1597–13523	4.7 ± 0.7	0.10–8.63
Albany, GA (331)	16	4.5 ± 0.6	0.6–8.2	4506 ± 466	2306–9331	2.2 ± 0.5	0.14–6.21
Blountstown, FL (332)	20	1.1 ± 0.2	0.6–2.4	2994 ± 349	972–6762	0.3 ± 0.1	0.10–0.85
Augusta, GA (333)	20	2.4 ± 0.7	0.0–11.8	2999 ± 374	0–7605	0.9 ± 0.4	0.00–7.95
Sylvania, GA (334)	15	1.1 ± 0.2	0.0–3.5	1886 ± 325	0–5490	0.3 ± 0.1	0.00–1.00
Port Wentworth, GA (335)	20	1.5 ± 0.4	0.6–7.6	2687 ± 397	642–7874	0.5 ± 0.2	0.04–4.79
Rockingham, NC (336)	1	2.9	--	23366	--	7.1	--
Pee Dee, SC (337)	17	1.5 ± 0.4	0.6–7.6	3605 ± 696	615–11583	0.9 ± 0.5	0.04–9.11
Bucksport, SC (338)	4	0.6 ± 0.0	0.6	2002 ± 463	1198–2957	0.1 ± 0.0	0.07–0.18

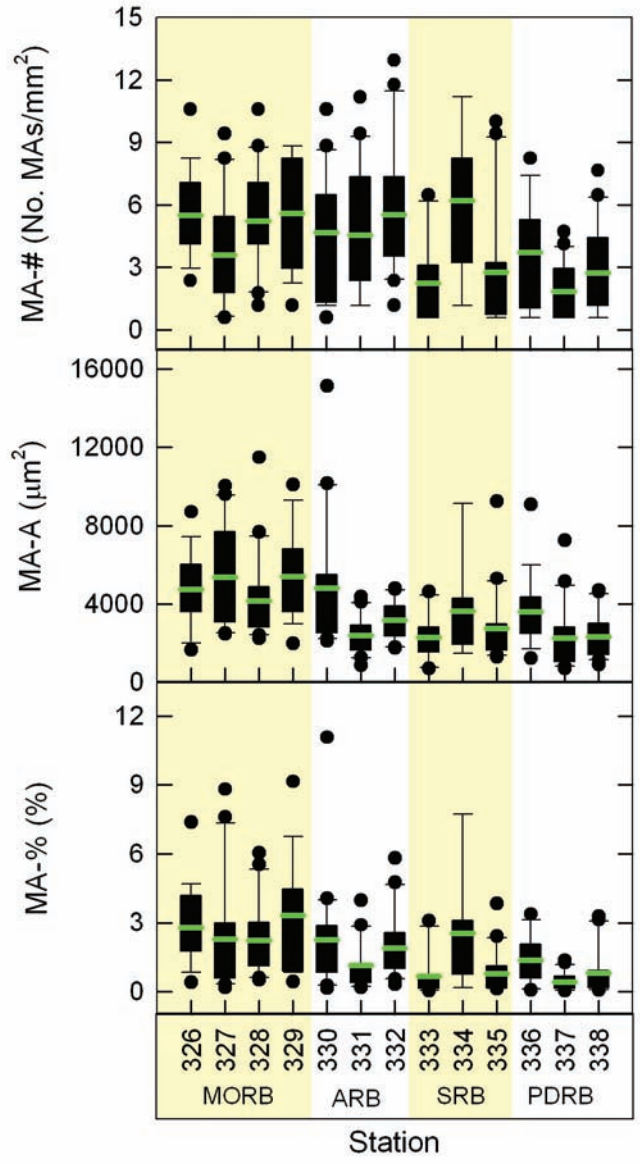


Figure 22. Splenic macrophage aggregate parameters by station in bass from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Parameters include macrophage aggregate density (MA-#; number of macrophage aggregrates per square millimeter), macrophage aggregate area (MA-A; square micometer), and percent of splenic tissue occupied by macrophage aggregates (MA-%). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

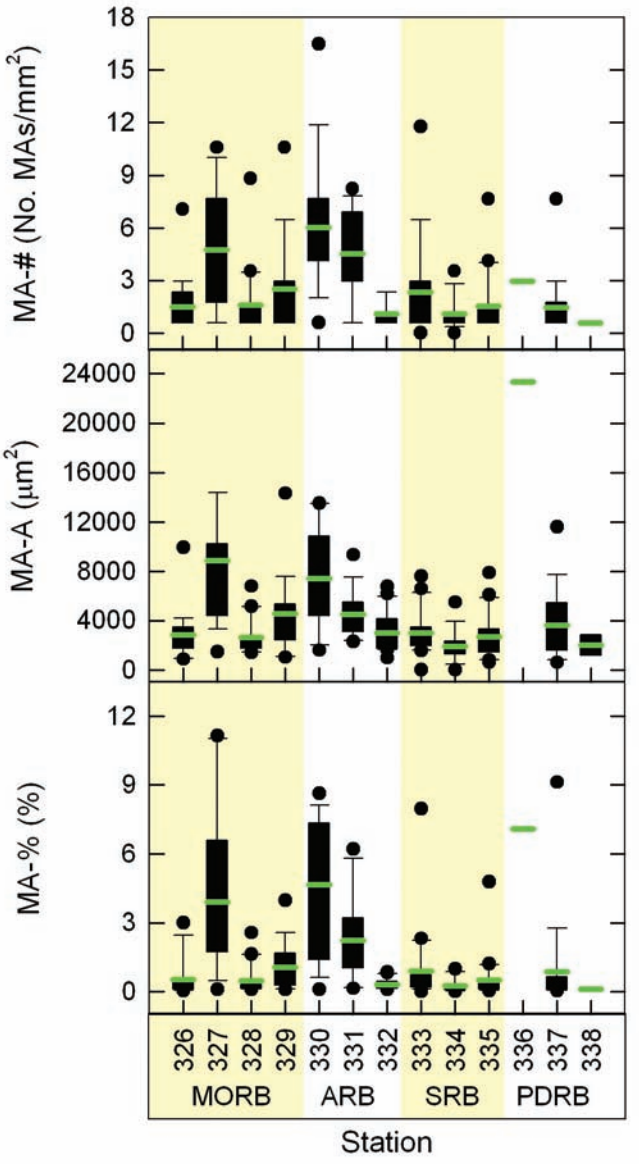


Figure 23. Splenic macrophage aggregate parameters by station in carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Parameters include macrophage aggregate density (MA-#; number of macrophage aggregrates per square millimeter), macrophage aggregate area (MA-A; square micometer), and percent of splenic tissue occupied by macrophage aggregates (MA-%). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

with elevated HAI scores in carp. Most abnormal tissues were associated with a variety of parasites. A USEPA study (1995) also reported parasitic infestations in spleen, liver, and kidney tissues in fish from the lower MORB. Tumors were present in a total of five fish (0.01%) representing Stations 328, 330, 333, and 338. Ovarian tumors of the same origin (smooth muscle) were present in two older carp from Station 330. Gonadal tumors of encapsulated teratogenic masses have been previously documented in carp and goldfish hybrids from the Great Lakes area (Harshbarger and Clark, 1990).

Few CF values were anomalous in MORB, ARB, SRB, and PDRB 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; Blazer and others, 2002), and similar to those reported in previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a; 2006b, Schmitt and others, 2005).

Mean HSI values in bass were 0.56–0.82% and were less than HSI values normally reported in most fish species (1–2%; Gingerich, 1982). Liver weights were abnormally low in bass from all sites except Stations 327, 329, 335, and 338 as reflected by mean HSI values <0.70%. HSI values in bass from the MORB, ARB, SRB, and PDRB also were less than HSI values reported in bass from previous LRMN investigations (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). The HSI can vary with season (Beamish and others, 1996; Delahunty and de Vlaming, 1980), temperature (Fine and others, 1996), nutrition (Daniels and Robinson, 1986; Foster and others, 1993), gender, and reproductive status (Fabacher and Baumann, 1985; Förlin and Haux, 1990; Grady and others, 1992), but decreased liver size also has been reported in various fish species after exposure to contaminants including metals and bleached kraft mill effluent (Adams and others, 1992a; Larsson and others, 1984; McMaster and others, 1991). Significant negative correlations were present between HSI and pesticide concentrations (dieldrin and *p,p'*-DDT) in bass from this study, but the cause of the low HSI values in bass from the MORB, ARB, SRB, and PDRB was unknown.

SSI values were anomalous in few fish from the MORB, ARB, SRB, and PDRB. Relatively low SSI values were observed in bass from Stations 327, 330, 332, and 335 (≤0.03%) and carp from Stations 328 and 331 (<0.15%). Reduced spleen size in fish has been associated with exposure to organic contaminants including PCBs and PAHs and bleach kraft mill effluent (Kiceniuk and Khan, 1987; Payne and others, 1978; Pulsford and others, 1995). PCB and PAH concentrations (as measured by hepatic EROD activity) were elevated in fish at Stations 327 and 330. High SSI values calculated for individual bass (>0.3%) and carp (>1.0%) from Stations 326 and 338 were considered abnormal and may indicate poorer health in these individual fish. An increase in relative spleen size is considered indicative of disease or immune problems (Goede and Barton, 1990) and rarely has been documented with contaminant exposure (Adams and others, 1992b). Parasitic infestations in the spleen were considered severe in fish from Station 326. The SSI values in bass were similar to those reported in previous LRMN studies, but SSI values in carp generally were greater than those from other LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005).

MA parameters were anomalous in some fish from the MORB, ARB, and PDRB. MAs were more numerous and larger in bass from Stations 326, 327, 328, and 329 and in carp from Stations 327, 330, 331, and 336 compared to those from other sites. MA parameters in individual bass and carp from Station 327 were among the greatest measured in any LRMN study and were considered to be abnormally high (Blazer and others, 2002; Hinck and others, 2006a; 2006b; Schmitt and others, 2005). Age affected MA parameters in bass with older fish having larger and more numerous MAs, but age did not affect MA parameters in carp. Increases in MA parameters have been associated with contaminants in laboratory and field studies (Blazer and others, 1994; 1997; Wolke, 1992), but can vary with fish size, nutritional status (Wolke and others, 1985), and age (Brown and George, 1985; Blazer and others, 1987; Couillard and Hodson, 1996). MA parameters were significantly correlated with *p,p'*-DDE and *p,p'*-DDT concentrations in bass and total chlordanes, *p,p'*-DDE, *p,p'*-DDD, Cd, Pb, and Se concentrations in carp.

Reproductive Biomarkers

Reproductive biomarkers including gonadosomatic index (GSI), gonadal histopathology (for example, oocyte atresia and intersex condition), vitellogenin concentrations, and steroid hormone concentration were examined. These endpoints provide information on reproductive health of the fish and were quantifiable measures of biochemical, physiological, and histological changes that occur throughout the reproductive cycle. To minimize the effects of temperature, photoperiod, and maturational stage, all fish were collected post-spawn within 9 weeks (October to early December). Reproductive biomarkers also can be affected by gender, age, and contaminants (Bromage and others, 1982; Chang and Chen, 1990; Denslow and others, 1999; Down and others, 1990; Goodbred and others, 1997; So and others, 1989). Reproductive biomarkers were tested using ANOVA models that contained the factors station (location), gender, gonadal stage, age, and their interactions (appendix 5). Although gender was not a significant factor in all ANOVA models, females and males were analyzed separately because of the known gender influence on the reproductive biomarkers measured in this study. In this section, gonadal stage distribution, gonadal histopathology, GSI, vitellogenin concentrations, oocyte atresia (females only), and sex steroid hormone concentrations are described for each species and gender.

Reproductive Biomarkers in Bass

Gonadal stage in bass was similar among sites. Most female bass were stage 1 (85%), and the remaining fish were

stages 0 (10%) and 2 (5%; fig. 24). Stage-0 fish were present at Stations 326, 328, 329, 330, 333, and 337, and stage-2 fish were from Stations 330, 331, 335, and 336 (fig. 24). Multiple females from Stations 328 (n = 3) and 329 (n = 6) were stage-0 (immature) and ranged from 2 to 6 years old. Most male bass were stage 1 (88%), and the remaining fish were stages 0 (2%) and 2 (10%; fig. 24). Stage-0 males were from Stations 328 and 329, where stage-0 female bass also were identified. Stage-2 male bass were from Stations 326, 327, 328, 330, 331, 335, and 336 (fig. 24).

Intersex gonads were identified in 47 male bass (42%) representing all sites except Station 328 (fig. 25). A relatively large proportion (≥50%) of male bass from Stations 332, 334, 335, 336, 337, and 338 were intersex. The occurrence of intersex was greatest in the PDRB and least severe in the MORB. Whole gonads from PDRB bass were collected during field necropsy to determine if the oocytes were distributed throughout the gonad or located in one area (for example, posterior end of the lobe). Therefore, the greater occurrence of intersex in male bass from the PDRB 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 Stations 337 and 338.

GSI values in bass differed between genders ($F_{1, 194}$ = 10.11, $P < 0.01$) but not among sites ($F_{9, 194}$ = 0.81, $P > 0.05$). The study-wide mean GSI value in female bass was 0.7%, and station means ranged from 0.5% at Station 333 to 1.3% at Station 335 (table 23). GSI values >1.2% were calculated for multiple female bass from Stations 335 and 336, and GSI values were <0.4% in female bass from Stations 329, 330, 337, and 338 (fig. 26; table 23). Mean GSI values in female bass from the MORB (0.6–0.7%), ARB (0.6–0.8%), SRB (0.5–1.3%), and PDRB (0.6–1.1%) were similar to those reported in post-spawn female bass from the CDRB (0.3–3.0%; Hinck and others, 2006b), CRB (0.5–2.9%; Hinck and others, 2006a), MRB (<2%; McDonald and others, 2002), and RGB (0.6–0.9%; Schmitt and others, 2005).

GSI values were <0.3% in most male bass, and the study-wide mean GSI value was 0.2% (table 23). Station means ranged from 0.1% to 0.3% (table 23), and GSI values were relatively high (0.6–1.6%) in stage-1 male bass from Stations 329 and 338 (table 23; fig. 27). These GSI values were similar to those documented in post-spawn male bass from the CDRB (0.1–0.5%; Hinck and others, 2006b), CRB (0.2–0.9%; Hinck and others, 2006a), MRB (<0.7%; McDonald and others, 2002), and RGB (0.2–0.4%; Schmitt and others, 2005). Few correlations between GSI values and contaminant concentrations were significant in bass; GSI was correlated with Ni concentrations in both female and male bass (table 13).

Vitellogenin concentrations in bass did not differ among sites ($F_{8, 184}$ = 1.14, $P > 0.05$) or between genders ($F_{1, 184}$ = 0.08, $P > 0.05$); however, female and male fish were analyzed separately to maintain consistency with the other reproductive biomarkers measured in this study. Gender differences were likely not statistically significant as the result of the analyti-

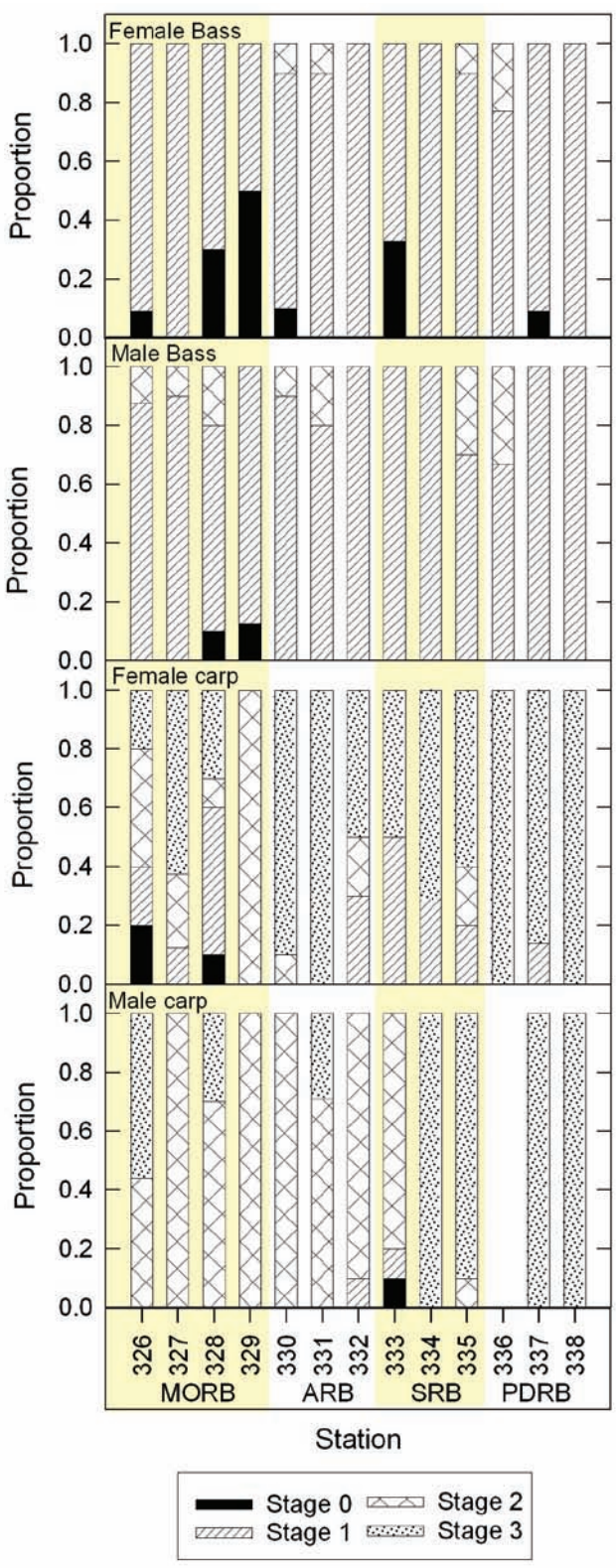


Figure 24. Gonadal stage proportions by station in female and male bass and carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions and collection dates.

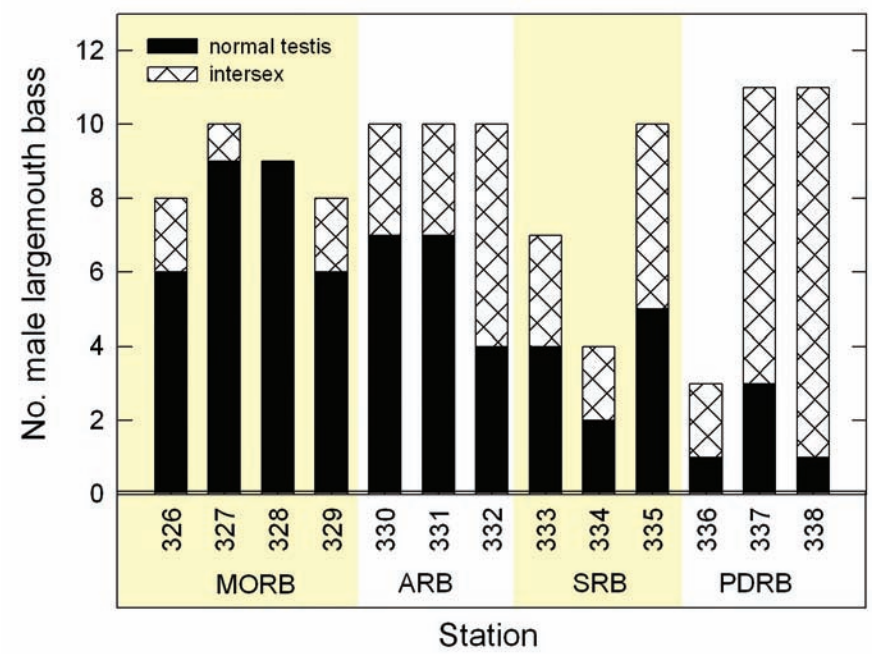


Figure 25. Intersex occurrence in male bass from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. The intersex condition was not found in male carp. See table 5 for station descriptions.

cal model, which computes the *F*-statistic after accounting for all other factors (station, stage) in the model (appendix 5). When stage was removed from the model, vtg concentrations differed between genders ($F_{1, 200} = 20.04$, $P < 0.05$). The study-wide mean vtg concentration was 0.36 mg/mL in female bass, and station means ranged from 0.002 mg/mL at Station 328 to 2.2 mg/mL at Station 336 (table 23). Concentrations were >1.0 mg/mL in multiple females from Stations 335 and 336, where GSI values were also relatively high (fig. 26). Mean vtg concentrations in post-spawn female bass from previous LRMN investigations were 0.01–8.4 mg/mL in the CDRB (Hinck and others, 2006b), 0.06–14.3 mg/mL in the CRB (Hinck and others, 2006a), ≤3.7 mg/mL in the MRB (McDonald and others, 2002), and 0.01–3.1 mg/mL in the RGB (Schmitt and others, 2005). Vitellogenin concentrations in female bass were not correlated with any organochlorine contaminant but were negatively correlated with Ni and Pb (table 13).

Vitellogenin concentrations were detected (>0.001 mg/mL) in 41% of male bass from all sites except Station 330 (fig. 27). Station means ranged from <0.001 mg/mL to 0.39 mg/mL, and vtg concentrations were ≥0.01 mg/mL in few (16%) males (table 23). Relatively high vtg concentrations (0.35–2.5 mg/mL) measured in males ($n = 5$) from Stations 327, 334, 336, and 337 may indicate an estrogenic response in these fish (table 23). Three of the five males with high vtg concentrations were intersex; however, the intersex condition was also present in males with vtg concentrations <LOD. Vitellogenin concentrations were reported in post-spawn male bass from previous LRMN investigations including the CDRB (<0.001–0.08 mg/mL; Hinck and others, 2006b), CRB (<0.002–0.67 mg/mL; Hinck and others, 2006a), MRB (<0.001–2.8 mg/mL; McDonald and others, 2002), and RGB (<0.002–3.2 mg/mL; Schmitt and others, 2005). Although vtg concentrations in most male bass remain low (<0.1 mg/mL), the number of male bass with detectable concentrations has increased in later LRMN investigations. Vitellogenin concentrations in male bass were not significantly correlated with any organochlorine or elemental contaminant (table 13).

An ANOVA model for oocyte atresia in female bass containing the factors station, gonadal stage, and their interactions was significant ($F_{21,124} = 2.82$, $P < 0.05$; appendix 5). The study-wide mean percent oocyte atresia was 5.6%, and station means ranged from 0.3% at Station 329 to 13.8% at Station 336 (fig. 26). Percent atresia was >15% in bass from Stations 330, 331, 332, 336, and 337 and generally lowest in female bass from the MORB (fig. 26). Female bass from Station 336 with high percent atresia (20–25%) also had relatively high vtg concentrations (1.5–5.8 mg/mL) and GSI values (0.5–1.8%). Mean percent atresia in female bass from the MORB (0.3–4.1%), ARB (5.3–10.9%), SRB (4.2–6.3%), and PDRB (3.5–13.8%) generally was similar to atresia reported in bass from the CDRB (0–5%; Hinck and others, 2006b), CRB (1–12%; Hinck and others, 2006a), MRB (0–6%; McDonald and others, 2002), and RGB (0–30%; Schmitt and others, 2005). Percent oocyte atresia in female bass was not significantly correlated with any organochlorine or elemental contaminant (table 13).

Concentrations of E2 in bass differed between genders ($F_{1, 176} = 9.43$, $P < 0.01$) but not among sites ($F_{8, 176} = 1.68$, $P > 0.05$) or gonadal stage ($F_{1, 176} = 0.26$, $P > 0.05$). KT concentrations differed among sites ($F_{8, 178} = 2.28$, $P < 0.05$) and between genders ($F_{1, 178} = 28.36$, $P < 0.01$) but not gonadal stage ($F_{1, 178} = 3.71$, $P > 0.05$). The study-wide mean E2 concentration in female bass was 584 pg/mL, and station means ranged from 408 pg/mL at Station 337 to 947 pg/mL at Station 336 (table 24). Concentrations were relatively low (<300 pg/mL) in multiple females from Stations 337 and 338 (fig. 26). The study-wide mean KT concentration was 293 pg/mL in female bass, and station means ranged from 156 pg/mL at

Table 23. Mean gonadosomatic index and vitellogenin concentrations in bass and carp.

[Censored values for vtg were represented by one-half the limit of detection in the computation of means. Stations are ordered upstream to downstream within a basin. GSI, gonadosomatic index; Vtg, vitellogenin. *n*, sample size; Mean, arithmetic mean; SE, standard error; mg/mL, milligrams per milliliter; #, number of males with vitellogenin concentrations >0.01 mg/mL; <, less than; --, not applicable]

Species, station location, and station number	Female – GSI (%)			Female – Vtg (mg/mL)		Male – GSI (%)			Male – Vtg (mg/mL)		
	n	Mean ± SE	Range	Mean ± SE	Range	*n*	Mean ± SE	Range	Mean ± SE	Range	#
Bass											
All stations	125[a]	0.7 ± 0.0	0.3–1.8	0.364 ± 0.083	<0.001–5.802	112[e]	0.2 ± 0.0	0.04–1.59	0.048 ± 0.025	<0.001–2.481	17
Lavaca, AL (326)	11	0.7 ± 0.0	0.5–1.0	0.007 ± 0.002	<0.001–0.026	8	0.2 ± 0.0	0.09–0.26	0.002 ± 0.001	<0.001–0.006	0
Childersburg, AL (327)	10	0.7 ± 0.0	0.5–0.9	0.184 ± 0.104	<0.001–0.963	10[c]	0.2 ± 0.0	0.10–0.22	0.048 ± 0.046	<0.001–0.367	2
Eureka Landing, AL (328)	10[b]	0.6 ± 0.0	0.5–0.7	0.002 ± 0.001	<0.001–0.010	10[b]	0.1 ± 0.0	0.04–0.32	0.002 ± 0.002	<0.001–0.016	1
Bucks, AL (329)	12	0.6 ± 0.0	0.4–0.7	0.015 ±0.014	<0.001–0.170	8	0.3 ± 0.2	0.07–1.59	0.001 ± 0.001	<0.001–0.007	0
Omaha, GA (330)	10	0.6 ± 0.1	0.3–1.1	0.103 ± 0.077	<0.001–0.766	10	0.2 ± 0.0	0.12–0.28	0.001 ± 0.000	<0.001	0
Albany, GA (331)	10[b]	0.8 ± 0.1	0.5–1.4	0.394 ± 0.187	<0.001–1.740	10[b]	0.2 ± 0.0	0.11–0.28	0.018 ± 0.014	<0.001–0.129	2
Blountstown, FL (332)	10	0.6 ± 0.0	0.4–0.8	0.023 ± 0.015	<0.001–0.155	10	0.2 ± 0.0	0.08–0.27	0.017 ± 0.015	<0.001–0.153	1
Augusta, GA (333)	3	0.5 ± 0.1	0.4–0.7	0.005 ± 0.002	<0.001–0.009	7[f]	0.2 ± 0.0	0.11–0.28	0.008 ± 0.002	<0.002–0.016	3
Sylvania, GA (334)	5	0.7 ± 0.2	0.4–1.4	0.297 ± 0.180	0.002–0.905	4	0.2 ± 0.0	0.15–0.27	0.116 ± 0.110	0.005–0.446	1
Port Wentworth, GA (335)	10[c]	1.3 ± 0.1	0.7–1.7	0.848 ± 0.373	0.004–3.156	10[b]	0.3 ± 0.0	0.17–0.37	0.007 ± 0.002	<0.001–0.025	2
Rockingham, NC (336)	13[d]	1.1 ± 0.1	0.5–1.8	2.191 ±0.514	0.018–5.802	3	0.3 ± 0.0	0.24–0.38	0.392 ± 0.233	0.010–0.814	3
Pee Dee, SC (337)	11	0.6 ± 0.1	0.3–1.0	0.047 ± 0.041	<0.001–0.455	11	0.2 ± 0.0	0.16–0.30	0.229 ± 0.225	<0.001–2.481	2
Bucksport, SC (338)	10	0.6 ± 0.1	0.3–1.0	0.163 ± 0.113	<0.001–1.048	11	0.3 ± 0.0	0.14–0.57	0.001 ± 0.001	<0.001–0.006	0
Carp											
All stations	103[g]	9.2 ± 0.6	0.4–23.5	2.22 ± 0.14	0.003–6.14	106	6.8 ± 0.3	0.1–27.4	0.017 ± 0.009	<0.0005–0.904	22
Lavaca, AL (326)	10	5.2 ± 1.8	0.5–18.6	1.77 ± 0.52	0.007–3.94	9	6.3 ± 0.7	1.6–8.4	0.006 ± 0.002	0.002–0.017	1
Childersburg, AL (327)	8[h]	13.2 ± 1.8	3.9–18.3	3.52 ± 0.75	0.031–6.14	11	9.4 ± 0.6	7.6–13.6	0.087 ± 0.082	<0.0005–0.904	1
Eureka Landing, AL (328)	10	5.0 ± 1.5	0.4–12.1	1.47 ± 0.50	0.003–4.36	10	4.3 ± 0.6	0.9–7.1	0.002 ± 0.001	<0.0005–0.007	0
Bucks, AL (329)	9	9.0 ± 0.9	5.5–12.4	2.35 ± 0.27	1.018–3.34	11	6.8 ± 0.5	3.7–8.5	0.003 ± 0.001	<0.0005–0.006	0
Omaha, GA (330)	10	13.2 ± 0.9	8.7–17.3	2.16 ± 0.28	1.066–3.27	7	8.8 ± 0.3	7.8–10.0	0.007 ± 0.004	0.003–0.029	1
Albany, GA (331)	9	16.1 ± 1.9	6.4–23.5	3.13 ± 0.36	1.429–4.58	7	10.8 ± 0.6	8.4–12.9	0.004 ± 0.001	<0.0005–0.008	0
Blountstown, FL (332)	10[i]	6.3 ± 1.6	1.2–12.2	1.93 ± 0.48	0.024–5.05	10	5.6 ± 0.8	0.7–9.4	0.003 ± 0.000	0.002–0.004	0
Augusta, GA (333)	10	7.0 ± 2.4	0.7–21.2	1.48 ± 0.47	0.004–3.55	10	4.6 ± 1.0	0.3–10.2	0.011 ± 0.005	0.002–0.049	3
Sylvania, GA (334)	7	8.6 ± 2.3	1.6–17.1	2.40 ± 0.47	0.032–3.66	9	6.6 ± 0.5	5.4–9.9	0.044 ± 0.007	0.016–0.085	9
Port Wentworth, GA (335)	10	5.9 ± 1.0	1.7–11.1	2.04 ± 0.33	0.038–3.54	10	6.2 ± 0.6	2.5–8.6	0.011 ± 0.003	<0.0005–0.031	5
Rockingham, NC (336)	1	7.1	--	3.07	--	0	--	--	--	--	
Pee Dee, SC (337)	7	12.1 ± 2.4	0.8–19.4	2.77 ± 0.62	0.696–5.18	10	5.2 ± 0.9	0.1–8.6	0.001 ± 0.001	<0.0005–0.007	0
Bucksport, SC (338)	2	18.8 ± 4.4	14.4–23.2	2.54 ± 0.83	1.713–3.37	2	17.7 ± 9.7	8.0–27.4	0.027 ± 0.003	0.024–0.029	2

[a] $n = 120$ for vtg.

[b] $n = 9$ for vtg.

[c] $n = 8$ for vtg.

[d] $n = 12$ for vtg.

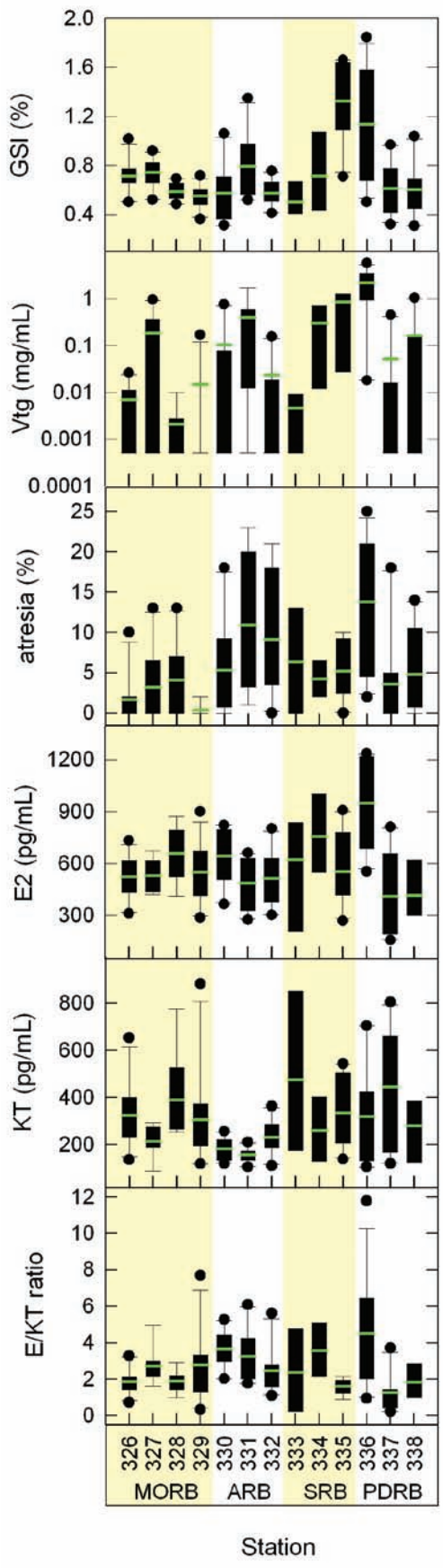
[e] $n = 106$ for vtg.

[f] $n = 6$ for vtg.

[g] $n = 102$ for vtg.

[h] $n = 7$ for vtg.

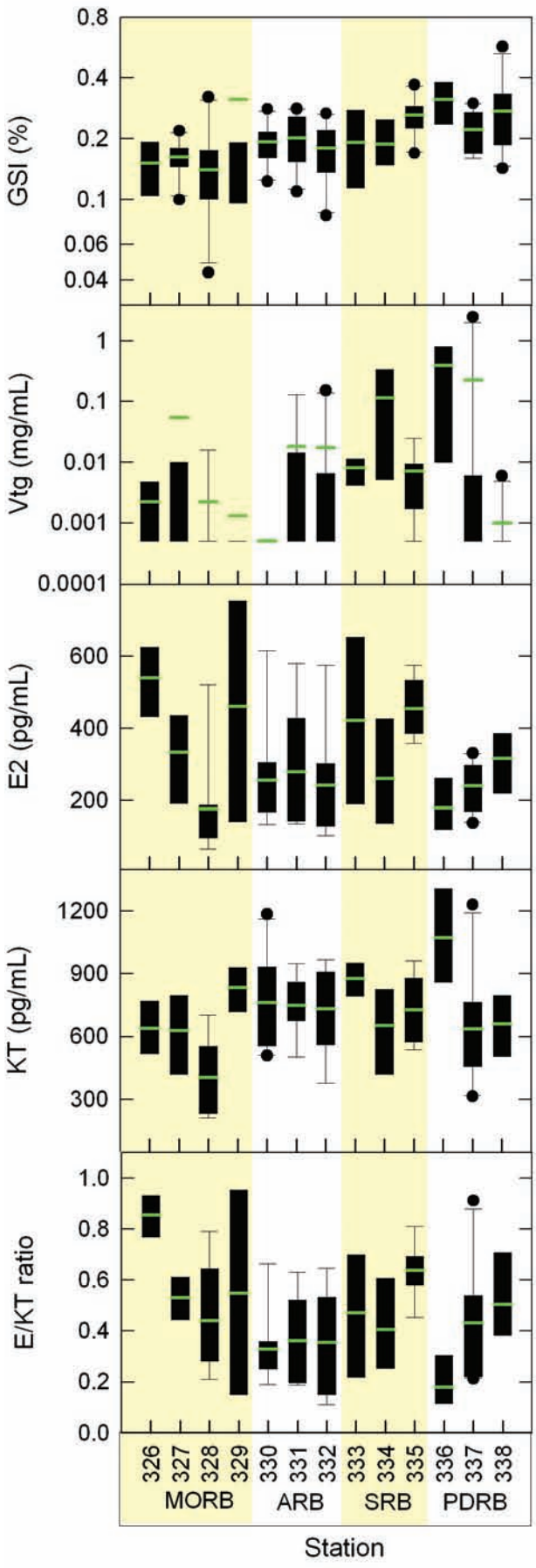
[i] $n = 9$ for GSI.



Station 331 to 474 pg/mL at Station 333 (table 24). KT concentrations in female bass were relatively low (<300 pg/mL) at Stations 327, 330, 331, and 332 (fig. 26). Mean concentrations of E2 and KT were similar to those reported in post-spawn female bass from previous LRMN studies in the CDRB (Hinck and others, 2006b) and MRB (McDonald and others, 2002). Concentrations of E2 generally were greater than KT concentrations in female bass as reflected by E/KT ratios >1.0 (fig. 26; table 24). Ratios <1.0 were reported in females from Stations 326, 329, 333, 335, 336, 337, and 338 (fig. 26). Ratios <0.5 in female bass from Stations 329 (n = 1), 333 (n = 1), and 337 (n = 3) were because of low E2 concentrations (156–314 pg/mL) and high KT concentrations (488–883 pg/mL). Low KT concentrations (104–187 pg/mL) resulted in ratios >6 in female bass from Stations 329, 331, 334, and 336. Several correlations between steroid hormone and contaminant concentrations were significant in female bass (table 13). E2 concentrations were negatively correlated with Cr and Zn, and KT concentrations were negatively correlated with several organochlorine contaminants including dieldrin, chlordane, *p,p'*-DDD, *p,p'*-DDT, and toxaphene (table 13). E/KT ratios were significantly correlated with dieldrin, HCB, chlordane, *o,p'*-DDD, *p,p'*-DDE, *p,p'*-DDD, PCB, and Ni concentrations in female bass (table 13).

The study-wide mean E2 concentration in male bass was 324 pg/mL, and station means ranged from 177 pg/mL at Station 328 to 539 pg/mL at Station 326 (table 24). Concentrations were relatively high in male bass from Stations 326, 329, 333, and 335 (fig. 27). The study-wide mean KT concentration was 702 pg/mL, and station means ranged from 404 pg/mL at Station 328 to 1,071 pg/mL at Station 336 (table 24). E2 and KT concentrations were relatively low in male bass from Station 328 compared to other sites (fig. 27); other reproductive biomarkers were not anomalous in males from this site. Most E2 and KT concentrations were similar to those reported in post-spawn male bass from previous LRMN studies in the CDRB (Hinck and others, 2006b) and MRB (McDonald and others, 2002). Concentrations of E2 generally were lower than KT concentrations in male bass as reflected by E/KT ratios <1.0 (fig. 27; table 24). Ratios >1.0 were rare (n = 2) and occurred only in males from Stations 326 and 329 (fig. 27). Unlike female bass, few correlations between steroid hormone

Figure 26. Reproductive health indicators by station in female bass from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include gonadosomatic index (GSI; percent), vitellogenin (vtg; milligrams per milliliter), oocyte atresia (percent), 17β-estradiol (E2; picograms per milliliter), 11-ketotestosterone (KT; picograms per milliliter), and the ratio of 17β-estradiol and 11-ketotestosterone (E/KT). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.



and contaminant concentrations were significant in male bass (table 13). E2 concentrations were correlated with mirex and Cd, and KT concentrations were positively correlated with *o,p'*-DDD (table 13). E/KT ratios were negatively correlated with chlordane and Cd concentrations in male bass (table 13).

Reproductive Biomarkers in Carp

Gonadal stage in female carp differed among sites. Female carp were stage 0 (3%), 1 (20%), 2 (20%), and 3 (56%; fig. 24). Gonadal stage was most variable in female carp from Stations 326 and 328 (fig. 24). Generally, female carp were more advanced in the ARB and PDRB than those from the MORB and SRB (fig. 24). Most male carp were stage 2 (59%) and 3 (30%), and the remaining males were stage 0 (1%) and 1 (2%; fig. 24). Stage-0 and -1 male carp were from Stations 332 and 333. Intersex gonads were not identified in any carp.

GSI values in carp differed between genders ($F_{1, 168}$ = 27.25, $P < 0.05$) but not among sites ($F_{9, 168}$ = 1.21, $P > 0.05$). The study-wide mean GSI value in female carp was 9.2%, and station means ranged from 5.0% at Station 328 to 18.8% at Station 338 (n = 2; table 23). GSI values were greater in female carp from Stations 327, 330, 331, 337, and 338 compared to those from other sites (fig. 28); these fish primarily were stage-2 or -3. Female carp with low GSI values (<1%) from Stations 326, 328, 333, and 337 were stage-0 and -1 (fig. 28). Mean GSI values in female carp from the MORB (5.0–13.2%), ARB (6.3–16.1%), SRB (5.9–8.6%), and PDRB (7.1–18.8%) were similar to those reported in post-spawn female carp from the CDRB (5.0–17.6%; Hinck and others, 2006b), CRB (0.6–20.5%; Hinck and others, 2006a), MRB (1–18%; McDonald and others, 2002), and RGB (5–20%; Schmitt and others, 2005).

GSI values were <10% in most male carp, and the study-wide mean GSI value was 6.8% (table 23). Station means ranged from 4.3% at Station 328 to 17.7% at Station 338 (n = 2; table 23). GSI values in male carp were relatively high (>11%) at Stations 327, 331, and 338 and low (<1%) at Stations 328, 332, 333, and 337 (fig. 29). These GSI values generally were greater than those documented in post-spawn male

Figure 27. Reproductive health indicators by station in male bass from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include gonadosomatic index (GSI; percent), vitellogenin (vtg; milligrams per milliliter), 17β-estradiol (E2; picograms per milliliter), 11-ketotestosterone (KT; picograms per milliliter), and the ratio of 17β-estradiol and 11-ketotestosterone (E/KT). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

Table 24. Mean sex steroid hormone concentrations in bass and carp.

[Stations are ordered upstream to downstream within a basin. E2, 17β-estradiol; KT, 11-ketotestosterone; E/KT, the ratio of 17β-estradiol to 11-ketotestosterone; *n*, sample size; Mean, arithmetic mean; SE, standard error; pg/mL, picograms per milliliter; --, not applicable]

Species, station location, and station number	*n*	E2 (pg/mL)		KT (pg/mL)		E/KT	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Female Bass							
All stations	118	584 ± 21	156–1236	293 ± 17	87–883	2.6 ± 0.1	0.3–11.8
Lavaca, AL (326)	11	522 ± 39	310–732	322 ± 44	135–655	1.8 ± 0.2	0.7–3.3
Childersburg, AL (327)	9	527 ± 32	421–676	213 ± 21	87–292	2.7 ± 0.3	1.6–5.0
Eureka Landing, AL (328)	9	658 ± 52	412–871	389 ± 64	252–778	1.9 ± 0.2	1.0–2.9
Bucks, AL (329)	12	549 ± 50	287–900	305 ± 67	117–883	2.8 ± 0.6	0.3–7.7
Omaha, GA (330)	10	641 ± 52	365–821	182 ± 15	119–256	3.7 ± 0.3	2.0–5.3
Albany, GA (331)	10	485 ± 48	275–660	156 ± 9	104–210	3.3 ± 0.5	1.8–6.1
Blountstown, FL (332)	10	511 ± 51	301–801	230 ± 22	109–364	2.4 ± 0.4	1.1–5.6
Augusta, GA (333)	3	619 ± 205	209–838	474 ± 200	175–854	2.4 ± 1.3	0.2–4.8
Sylvania, GA (334)	5	757 ± 106	512–1072	260 ± 70	105–508	3.6 ± 0.8	1.8–6.6
Port Wentworth, GA (335)	10	553 ± 67	268–908	333 ± 48	137–544	1.9 ± 0.3	0.9–4.6
Rockingham, NC (336)	12	947 ± 75	553–1236	318 ± 61	105–706	4.5 ± 0.9	0.9–11.8
Pee Dee, SC (337)	10	408 ± 78	156–810	443 ± 77	118–806	1.2 ± 0.3	0.2–3.7
Bucksport, SC (338)	7	415 ± 62	243–662	279 ± 67	105–617	1.8 ± 0.4	0.9–3.4
Male Bass							
All stations	102[a]	324 ± 18	67–902	702 ± 22	210–1307	0.5 ± 0.0	0.1–1.2
Lavaca, AL (326)	8	539 ± 36	412–669	639 ± 51	448–872	0.9 ± 0.0	0.7–1.1
Childersburg, AL (327)	8	333 ± 45	133–459	630 ± 74	326–891	0.5 ± 0.1	0.3–0.9
Eureka Landing, AL (328)	9	177 ± 45	67–521	404 ± 59	210–703	0.4 ± 0.1	0.2–0.8
Bucks, AL (329)	8[b]	460 ± 123	88–902	834 ± 64	609–1201	0.5 ± 0.2	0.1–1.2
Omaha, GA (330)	10[c]	255 ± 49	134–616	762 ± 69	508–1186	0.3 ± 0.0	0.2–0.7
Albany, GA (331)	9	280 ± 58	137–580	749 ± 45	502–950	0.4 ± 0.1	0.2–0.6
Blountstown, FL (332)	9	243 ± 49	104–576	732 ± 66	376–969	0.4 ± 0.1	0.1–0.7
Augusta, GA (333)	7	422 ± 88	158–669	878 ± 35	723–969	0.5 ± 0.1	0.2–0.8
Sylvania, GA (334)	4	261 ± 80	118–488	653 ± 116	321–863	0.4 ± 0.1	0.2–0.7
Port Wentworth, GA (335)	9	455 ± 26	358–576	727 ± 54	537–962	0.6 ± 0.0	0.5–0.8
Rockingham, NC (336)	3	180 ± 42	121–262	1071 ± 129	861–1307	0.2 ± 0.1	0.1–0.3
Pee Dee, SC (337)	10	240 ± 22	139–331	636 ± 84	314–1230	0.4 ± 0.1	0.2–0.9
Bucksport, SC (338)	8	316 ± 39	141–486	662 ± 66	402–966	0.5 ± 0.1	0.2–0.8
Female Carp							
All stations	102	925 ± 39	209–1890	365 ± 23	48–965	3.3 ± 0.3	0.9–24.4
Lavaca, AL (326)	10	533 ± 18	424–641	151 ± 9	115–212	3.7 ± 0.3	2.4–5.3
Childersburg, AL (327)	7	768 ± 173	419–1748	192 ± 42	82–356	4.9 ± 1.1	1.5–10.9
Eureka Landing, AL (328)	10	1115 ± 103	329–1657	519 ± 78	211–965	2.6 ± 0.5	1.3–5.4
Bucks, AL (329)	9	1081 ± 107	679–1667	432 ± 85	150–858	3.1 ± 0.5	1.4–5.6
Omaha, GA (330)	10	732 ± 31	598–936	254 ± 46	143–569	3.5 ± 0.4	1.5–5.7
Albany, GA (331)	9	901 ± 143	413–1563	338 ± 100	88–898	3.9 ± 0.7	1.3–6.6
Blountstown, FL (332)	10	1125 ± 77	644–1462	478 ± 69	48–828	4.4 ± 2.2	1.4–24.4
Augusta, GA (333)	10	1227 ± 65	822–1639	473 ± 56	215–734	3.0 ± 0.5	1.5–5.9
Sylvania, GA (334)	7	1247 ± 179	484–1890	482 ± 119	145–965	3.1 ± 0.5	1.6–5.3
Port Wentworth, GA (335)	10	602 ± 85	370–1232	272 ± 54	98–605	2.7 ± 0.4	1.5–5.3
Rockingham, NC (336)	1	1152	--	521	--	2.2	--
Pee Dee, SC (337)	7	922 ± 175	412–1407	400 ± 52	161–575	2.4 ± 0.3	0.9–3.5
Bucksport, SC (338)	2	754 ± 545	209–1298	388 ± 294	94–681	2.1 ± 0.2	1.9–2.2

Table 24. Mean sex steroid hormone concentrations in bass and carp.—Continued

[Stations are ordered upstream to downstream within a basin. E2, 17β-estradiol; KT, 11-ketotestosterone; E/KT, the ratio of 17β-estradiol to 11-ketotestosterone; *n*, sample size; Mean, arithmetic mean; SE, standard error; pg/mL, picograms per milliliter; --, not applicable]

Species, station location, and station number	*n*	E2 (pg/mL)		KT (pg/mL)		E/KT	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Male Carp							
All stations	106	383 ± 22	44–991	1136 ± 46	143–2508	0.4 ± 0.0	0.1–3.1
Lavaca, AL (326)	9	476 ± 29	362–594	843 ± 102	459–1421	0.6 ± 0.1	0.3–0.9
Childersburg, AL (327)	11	435 ± 25	321–582	1254 ± 87	718–1814	0.4 ± 0.0	0.3–0.6
Eureka Landing, AL (328)	10	149 ± 20	44–241	1548 ± 151	795–2160	0.1 ± 0.0	0.1–0.2
Bucks, AL (329)	11	298 ± 78	66–842	1070 ± 184	419–2162	0.3 ± 0.1	0.1–0.8
Omaha, GA (330)	7	728 ± 16	679–809	1727 ± 188	1021–2508	0.5 ± 0.1	0.3–0.8
Albany, GA (331)	7	582 ± 49	413–760	1333 ± 129	823–1900	0.5 ± 0.0	0.3–0.6
Blountstown, FL (332)	10	330 ± 102	101–991	1175 ± 90	799–1826	0.3 ± 0.1	0.1–0.6
Augusta, GA (333)	10	278 ± 52	121–708	1085 ± 121	520–1593	0.3 ± 0.0	0.1–0.5
Sylvania, GA (334)	9	512 ± 58	149–709	1231 ± 102	661–1761	0.4 ± 0.0	0.2–0.6
Port Wentworth, GA (335)	10	460 ± 26	340–614	838 ± 136	143–1659	0.8 ± 0.3	0.4–3.1
Rockingham, NC (336)	0	--	--	--	--	--	--
Pee Dee, SC (337)	10	186 ± 35	52–358	693 ± 95	245–1207	0.3 ± 0.1	0.1–0.7
Bucksport, SC (338)	2	203 ± 42	161–245	698 ± 155	543–852	0.3 ± 0.0	0.3

[a] $n = 100$ for E2 and E/KT.

[b] $n = 7$ for E2 and E/KT.

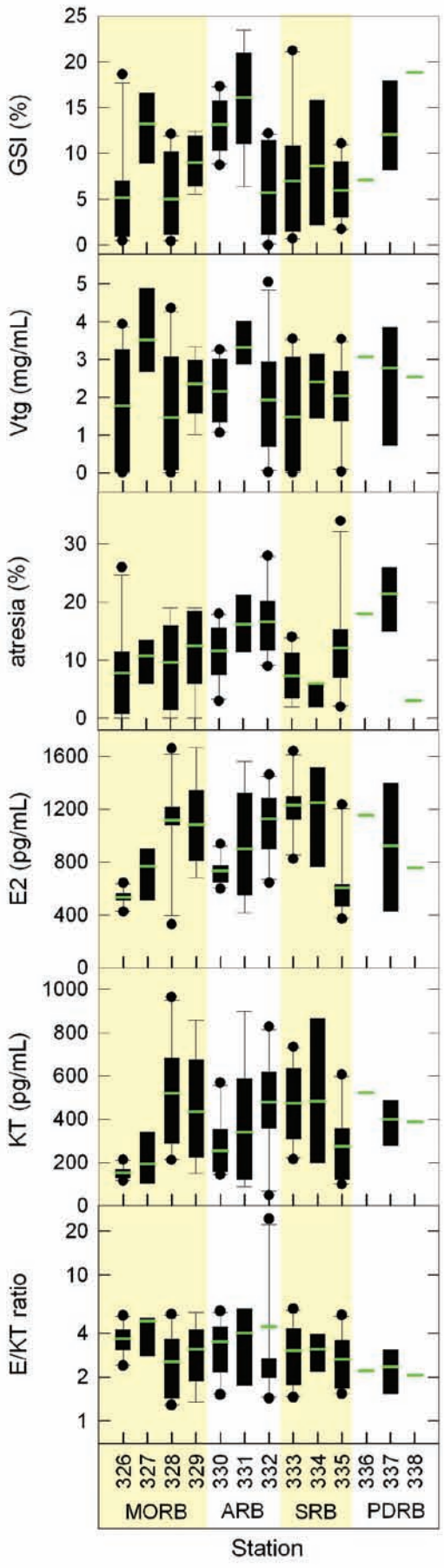
[c] $n = 9$ for E2 and E/KT.

carp from the CDRB (0.9–9.6%; Hinck and others, 2006b), CRB (<0.1–11.0%; Hinck and others, 2006a), MRB (2–12%; McDonald and others, 2002), and RGB (1–13%; Schmitt and others, 2005). Multiple correlations between GSI values and contaminant concentrations were significant in carp (table 14).

Vitellogenin concentrations in carp differed between genders ($F_{1, 168} = 13.39$, $P < 0.01$) but not among sites ($F_{9, 168} = 1.64$, $P > 0.05$). The study-wide mean vtg concentration was 2.2 mg/mL in female carp, and station means ranged from 1.5 mg/mL at Stations 328 and 333 to 3.5 mg/mL at Station 327 (table 23). Concentrations were >4.0 mg/mL in multiple females from Stations 327, 328, 331, 332, and 337, and vtg concentrations were <0.01 mg/mL in stage-0 ($n = 3$) and -1 ($n = 1$) females from Stations 326, 328, and 333 (table 23; fig. 28). Mean vtg concentrations in post-spawn female carp from previous LRMN investigations were 0.6–6.0 mg/mL in the CDRB (Hinck and others, 2006b), <0.005–7.4 mg/mL in the CRB (Hinck and others, 2006a), ≤2.9 mg/mL in the MRB (McDonald and others, 2002), and <0.005–3.7 mg/mL in the RGB (Schmitt and others, 2005). Vitellogenin concentrations were significantly correlated with PCA, chlordane, PCB, toxaphene, Cd, Cr, Pb, and Zn concentrations in female carp (table 14).

Vitellogenin concentrations were detected (>0.0005 mg/mL) in 79% of male carp representing all sites (fig. 29). The study-wide mean vtg concentration was 0.017 mg/mL in male carp (table 23). Station means ranged from 0.002 mg/mL at Station 328 to 0.087 mg/mL at Station 327, and vtg concentrations were ≥0.01 mg/mL in 22% of male carp (table 23). Relatively high vtg concentrations (0.04–0.90 mg/mL) measured in males ($n = 8$) from Stations 327, 333, and 334 may indicate that these fish were exposed to endocrine modulating chemicals. High vtg concentrations also were measured in male bass from Stations 327 and 334. Vitellogenin concentrations were reported in post-spawn male carp from previous LRMN investigations including the CDRB (<0.0005–0.06 mg/mL; Hinck and others, 2006b), CRB (<0.005–0.04 mg/mL; Hinck and others, 2006a), MRB (<0.001–2.7 mg/mL; McDonald and others, 2002), and RGB (<0.002–1.6 mg/mL; Schmitt and others, 2005). Concentrations >0.1 mg/mL were rare in bass (6%) and carp (<1%) from the MORB, ARB, SRB, and MORB. Although vtg concentrations in most male carp remain low (<0.1 mg/mL), the number of male carp with detectable vtg concentrations has increased in later LRMN investigations. Vitellogenin concentrations were significantly correlated with two elemental contaminants, Cu and Hg, in male carp (table 14).

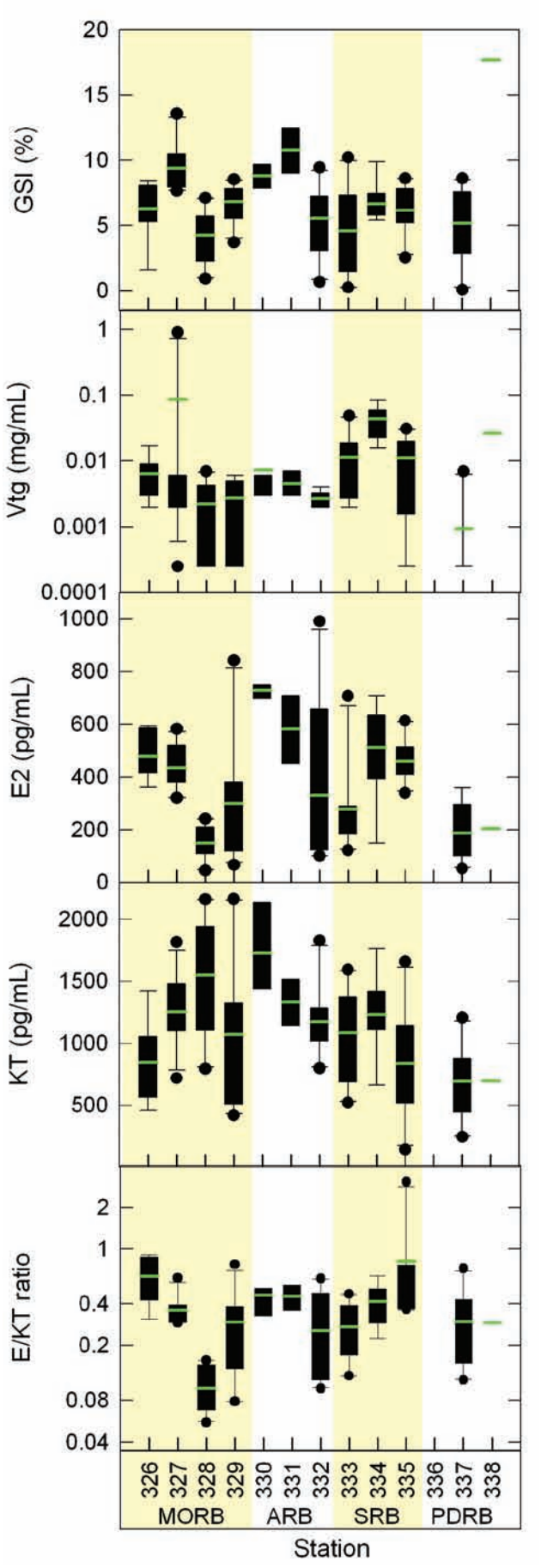
Percent oocyte atresia was greater in carp than in bass. Oocyte atresia in female carp differed among sites ($F_{1, 81} = 2.57$, $P < 0.01$) but not gonadal stage ($F_{1, 81} = 2.45$, $P > 0.05$). The study-wide mean percent oocyte atresia was 11.8%, and station means ranged from 3.0% at Station 338 to 21.4% at Station 337 (fig. 28). Percent atresia was >20% in individual carp from Stations 326, 327, 331, 332, 335, and 337 but low in female carp from Stations 334, 335, and 338 (fig. 28). A sporozoan parasite within oocytes caused inflammation and



degeneration in fish from Stations 326 and 337 (fig. 30). Mean percent atresia in female carp from the MORB (7.8–12.4%), ARB (11.6–16.8%), SRB (6.0–12.1%), and PDRB (3.0–21.4%) was similar to atresia reported in carp from the CDRB (7–18%; Hinck and others, 2006b), CRB (0–18%; Hinck and others, 2006a), MRB (0–25%; McDonald and others, 2002), and RGB (1–13%; Schmitt and others, 2005). Percent oocyte atresia was significantly correlated with only one contaminant, Cd (table 14).

Concentrations of E2 differed between genders ($F_{1, 168}$ = 6.27, $P < 0.05$) but not among sites ($F_{9, 168}$ = 0.52, $P > 0.05$), and KT concentrations also differed between genders ($F_{1, 168}$ = 14.89, $P < 0.01$) but not among sites ($F_{9, 168}$ = 1.84, $P > 0.05$). E2 and KT concentrations were lower in female carp from Stations 326, 327, 330, 331, and 335 than those from other sites (table 24; fig. 28). The study-wide mean E2 concentration for females was 925 pg/mL, and station means ranged from 533 pg/mL at Station 326 to 1,247 pg/mL at Station 334 (table 24). Mean E2 concentrations were >1,200 pg/mL in females from Stations 333 and 334 but were relatively low (533–602 pg/mL) in fish from Stations 326 and 335 (fig. 28). These differences did not reflect gonadal stage differences among female carp. The study-wide mean KT concentrations in female carp was 365 pg/mL, and station means ranged from 151 pg/mL at Station 326 to 519 pg/mL at Station 328 (table 24). The KT concentration in the female carp from Station 336 (n = 1) was 521 pg/mL. Concentrations were low (<100 pg/mL) in individual fish from Stations 327, 331, 332, 335, and 338 (fig. 28). Mean E2 and KT concentrations were similar to those reported in post-spawn female carp in the CDRB (Hinck and others, 2006b) but slightly lower than those in the MRB (McDonald and others, 2002). Concentrations of E2 were greater than KT concentrations in female carp as reflected by E/KT ratios >1.0 (fig. 28). The only E/KT ratio <1.0 was in a female from Station 337 (fig. 28). High E/KT ratios (10.9–24.4) in females from Stations 327 and 332 were the result of low KT concentrations (48–82) in these fish. Few correlations between steroid hormone and contaminant concentrations were significant in female carp (table 14). E2 and KT concentrations were negatively correlated with Ni, and E/KT ratios were significantly correlated with mirex.

Figure 28. Reproductive health indicators by station in female carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include gonadosomatic index (GSI; percent), vitellogenin (vtg; milligrams per milliliter), oocyte atresia (percent), 17β-estradiol (E2; picograms per milliliter), 11-ketotestosterone (KT; picograms per milliliter), and the ratio of 17β-estradiol and 11-ketotestosterone (E/KT). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Censored values are plotted as one half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.



The study-wide mean E2 concentration in male carp was 383 pg/mL, and station means ranged from 149 pg/mL at Station 328 to 728 pg/mL at Station 330 (table 24). Concentrations of E2 were high (>600 pg/mL) in males from Stations 330, 331, 332, 333, 334, and 335 and generally low (<100 pg/mL) in males from Stations 328, 329, and 337 (fig. 29). Mean E2 concentrations in male carp were similar to those from the MRB (203–1,209 pg/mL; McDonald and others, 2002) and greater than those from the CDRB (29–313 pg/mL; Hinck and others, 2006b). The study-wide mean KT concentration was 1,136, and station means ranged from 693 pg/mL at Station 337 to 1,727 pg/mL at Station 330 (table 24). Concentrations generally were greater in males from Stations 328, 329, and 330, and lower concentrations were measured in male carp from Stations 326, 329, 335, 337, and 338 (fig. 29). Mean KT concentrations in male carp were similar to those from the MRB (215–3,663 pg/mL; McDonald and others, 2002) and greater than those from the CDRB (121–1,141 pg/mL; Hinck and others, 2006b). Mean KT concentrations also were similar to those in non-mated male carp reported by Barry and others (1990). Concentrations of KT were greater than E2 concentrations in male carp as reflected by E/KT ratios <1.0 (fig. 29). One male carp from Station 335 had a ratio >1.0 as a result of a low KT concentration (143 pg/mL; table 24). Several correlations between steroid hormone and contaminant concentrations were significant in male carp (table 14). E2 and KT concentrations were positively correlated with multiple organochlorine contaminants and Se (E2 only), and E/KT ratios were significantly correlated with Hg (table 14).

Reproductive Biomarkers: Summary

The reproductive biomarkers used in this study are key measures of reproductive function and are used routinely to help evaluate contaminant effects or simply assess general reproductive health in fish. Age, species, water temperature, photoperiod, and other biotic and abiotic factors can affect these biomarkers during the course of their reproductive cycle. Therefore, care must be taken when interpreting these biomarkers, and these ancillary factors should be considered when possible. Our evaluations consider the age, species, and gonadal stage directly, and photoperiod and temperature

Figure 29. Reproductive health indicators by station in male carp from the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins in 2004. Indicators include gonadosomatic index (GSI; percent), vitellogenin (vtg; milligrams per milliliter), oocyte atresia (percent), 17β-estradiol (E2; picograms per milliliter), 11-ketotestosterone (KT; picograms per milliliter), and the ratio of 17β-estradiol and 11-ketotestosterone (E/KT). Shown for each group are the mean (green horizontal line), interquartile range (box), the 10th and 90th percentiles (whiskers), and outliers that are outside of the 10th and 90th percentiles (black circles). Censored values are plotted as one-half the limit of detection. Stations are ordered from upstream to downstream and are grouped by basin. See table 5 for station descriptions.

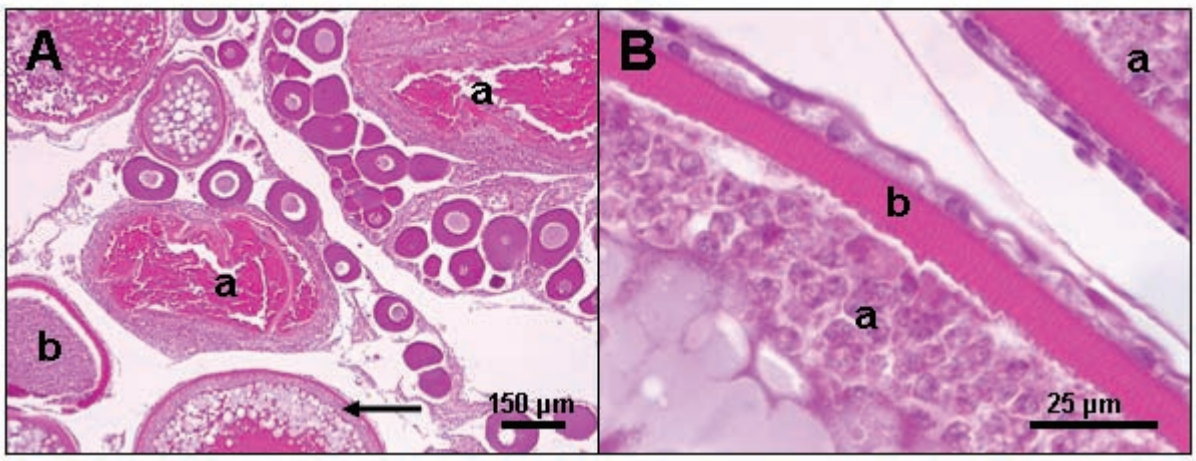


Figure 30. *A*, Atretic eggs (a) associated with a sporozoan (b) within the oocytes of female carp. The sporozoans (arrow) could be observed on the periphery of intact eggs. As the infection progresses, eggs undergo degeneration and are surrounded by inflammation (a). *B*, Higher magnification illustrating the sporozoans (a) within the intact eggs (b). Hematoxylin and eosin stain.

may be considered through indirect information about each station (collection date and available gaging station information, respectively). All fish samples were collected post-spawn within 9 weeks (October to December) to minimize the variation of reproductive biomarkers from temperature, photoperiod, and maturational stage. However, natural changes or fluctuations in reproductive biomarkers may have occurred during this collection period.

Gonadal histopathology was used to confirm gender, assign reproductive stage, and detect anatomical abnormalities such as the presence of intersex and excessive oocyte atresia. Collection date did not appear to affect gonadal stage. Gonadal stage in bass generally was consistent among sites, with most bass identified as stage 1 (previtellogenic), although multiple female and male bass from Stations 328 and 329 were stage-0 (immature). In carp, females were more advanced in the ARB and PDRB (primarily stage-3) than those from the MORB and SRB (stage-1, -2, -and -3). Most male carp were stage 2 and 3 (early to mid-vitellogenic).

Intersex gonads were identified in 42% of the male bass collected representing all sites except Station 328. The occurrence of intersex was greatest in the PDRB and least severe in the MORB. Greater proportions of gonadal tissue from PDRB bass were examined during the histological analysis, which may account for the greater occurrence of intersex fish from these sites. Nevertheless, evidence of intersex was observed in ≥50% of male bass from Stations 332, 334, 335, 336, 337, and 338. Other reproductive biomarkers in the intersex bass were not anomalous. 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 proportion of intersex bass at one site generally was lower (<40%; Hinck and others, 2006a, 2006b; McDonald and others, 2002; Schmitt and others, 2005). However, large proportions of intersex bass have been documented in the Yampa River (Hinck and others, 2006b) and the Mississippi River (McDonald and others, 2002). The background occurrence of intersex fish has not been established for bass, but the high incidence of intersex bass in the ARB, SRB, and PDRB is cause for concern. These results indicate that the intersex male bass had estrogenic responses although the cause is unknown. Such widespread occurrence of intersex has not been previously documented by the BEST Program, but these findings warrant further investigation to determine what is causing this condition in fish from the MORB, ARB, SRB, and PDRB.

Oocyte atresia is defined as the involution or resorption of oocytes by the ovaries and has been validated as a histopathological biomarker. Oocyte atresia is a normal physiological event in all fish but can become a pathological condition following exposure to certain environmental contaminants including Hg (Cross and Hose, 1988; 1989; Johnson and others, 1988; Kirubagaran and Joy, 1988). Other factors, such as water temperature also may affect, pathological oocyte atresia (June, 1970; 1977). Mean percent oocyte atresia was relatively high in bass from Stations 331 and 336 (>10%) and carp from Station 337 (>20%), but atresia in most fish generally were similar to those previously reported in bass and carp (Hinck and others, 2006a, 2006b; McDonald and others, 2002; Schmitt and others, 2005). Percent atresia in female bass from Station 336 (20–25%) was high, and vtg concentrations (1.5–5.8 mg/mL) and GSI values (0.5–1.8%) also were relatively high in these fish.

The GSI often is used to evaluate reproductive status and health, although interpretations of GSI values rely on understanding natural variations among fish of similar age, gender, and species. Environmental influences and behavioral patterns also may confound the interpretation of the data. Considerable variations in gonad size have been reported throughout

the reproductive cycle of most species of fish (de Vlaming and others, 1981). The gonads can constitute substantially differing proportions of the total body weight of a fish. For example, in this study, gonads made up a greater proportion of the total body mass in carp than in bass. Proportionately larger gonads present in female bass from Stations 335 and 336, female carp from Stations 327, 330, 331, 337, and 338, and male carp from Stations 327, 330, 331, and 338 were not related to reproductive stage. GSI values in female carp were positively correlated to many organochlorine residues including dieldrin, HCB, PCA, total chlordanes, PCBs, and toxaphene and may have been associated with maternal transfer of contaminants to the eggs. These correlations were not significant in female bass, however. Mean GSI values in female carp from Station 326, female and male carp from Station 328, and female bass and male carp from Station 333 were relatively low but were not considered abnormal. Decreases in GSI values have been associated with exposure to wastewater effluent (Diniz and others, 2005; Hinck and others, 2006b; Lavado and others, 2004), E2 (Mills and others, 2001; Zaroogian and others, 2001), and *o,p'*-DDT (Mills and others, 2001; Zaroogian and others, 2001) in fish. Patiño and others (2003) reported that environmental contaminants such as PCBs, dioxins, furans, and PBDEs may affect the reproductive development in male carp. Gonadal abnormalities including calcified follicles and fibrosis were present in carp from Station 327, where PCB concentrations were high. Endocrine disrupting chemicals were suspected to be associated with gonadal abnormalities including irregular ovarian plasma membranes, intrusion of muscle into the ovary, and MAs in ovarian and testicular tissue in white sturgeon (*Acipenser transmontanus*; Feist and others, 2005).

Vitellogenin is an important yolk precursor protein for developing embryos that is synthesized by the liver and delivered to the ovaries via the bloodstream during oocyte development. Vitellogenin is normally not produced in male fish because of the presumed absence of circulating estrogens (Harries and others, 1997). However, male fish can produce vtg in quantities near those of mature females if exposed to an exogenous estrogen source (Denslow and others, 1999). Relatively low plasma vtg concentrations in female bass were likely due to the immature gonadal stage (most stage 1) of bass from the MORB, ARB, SRB, and PDRB. Vitellogenin concentrations were greater (>1.0 mg/mL) in most female bass from Stations 335 and 336 than those from other sites but were not considered abnormal. Plasma vtg concentrations in female carp were not abnormally low at any station but were significantly correlated with PCA, chlordane, PCB, and toxaphene concentrations. Vitellogenin was detected in male bass and carp from all sites, but concentrations were generally low. Vitellogenin concentrations >0.01 mg/mL in male bass from Stations 327, 334, 336, and 337 and male carp from Stations 327, 333, and 334 indicate that these fish may have been exposed to estrogen mimics and were considered abnormal. Induction of vtg in male fish has been associated with sewage effluent (Folmar and others, 1996) and pulp and paper mill effluent (Mellanen and others, 1999; Soimasou and others, 1998; van den Heuvel and Ellis, 2002). Vitellogenin concentrations in fish collected near pulp and paper mills in the MORB, ARB, SRB, and PDRB were not uniformly elevated, which may be because of fish migrating throughout the year and not being constantly exposed to effluent at these sites. However, the elevated vtg concentrations in bass and carp from Stations 327 and 334 indicate further studies investigating endocrine modulating chemicals are warranted.

Sex steroids are a class of hormones derived from cholesterol and synthesized by the gonads in response to circulating levels of gonadotropin hormones that are important for gametogenesis, ovulation, and spermiation in mature fish (Redding and Patiño, 1993). Like many other reproductive biomarkers, concentrations of sex steroid hormones can vary by gender, age, geographical locations, species, and season (Barry and others, 1990; Bromage and others, 1982; Chang and Chen, 1990; Denslow and others, 1999; Down and others, 1990; Goodbred and others, 1997; So and others, 1989). Variation in sex steroid hormones concentrations within each species is expected (McDonald and others, 2002), but E/KT ratios >1.0 in females and <1.0 in males are considered normal (Folmar and others, 1996; Hileman, 1994). Mean E/KT ratios were normal for bass and carp at all MORB, ARB, SRB, and PDRB sites. However, E2 and KT concentrations were low in male bass from Station 329, female carp from Station 326, and male carp from Stations 337 and 338 and did not appear to be related to reproductive stage.

Geographic Summaries of Contaminant Concentrations, Health Indicators, and Reproductive Biomarkers

Geographic station summaries were made to emphasize relatively high contaminant concentrations, consistent biomarker responses, or both (table 25). The highlighted findings indicate contaminant concentrations or EROD activities that exceeded known thresholds or were outside expected ranges relative to other sites. The colors for the fish health indicators and reproductive biomarkers are relative and indicate the number, magnitude, or both of the anomalies at a station. The summaries are intended to draw attention to particular sites discussed in the text, possibly for further investigation. Increased frequencies of external lesions or elevated HAI scores, which represent the cumulative total number of grossly visible internal and external lesions, do not necessarily indicate direct contaminant effects. Many factors other than contaminants can indirectly affect fish health indicators and reproductive biomarkers, including nutrients, organic matter, and water temperature. Considerably more is known about risk to fish and piscivorous wildlife associated with bioaccumulative contaminants and EROD activities than about long- and short-term risks represented by the other biomarkers. Therefore, greater relative risk has been associated with elevated contaminant concentrations and EROD activi-

ties than with anomalous fish health indicators or reproductive biomarkers (table 25).

Mobile River Basin (MORB)

The MORB sites are located near potential sources of chemical contaminants including mine drainage, agricultural runoff, poultry and livestock production, chemical manufacturing plants, pulp and paper mills, and coal-fired power plants. Station 326 was located downstream from pulp and paper mills on the Tombigbee River, and Station 327 was located on the Coosa River where PCB contamination has occurred. Station 328, located near the terminus of the Alabama River, integrates drainage from the eastern part of Mobile River Basin. Station 329, located on the Mobile River upstream from Mobile Bay, integrates drainage from the entire Mobile River Basin. Several chemical manufacturing and other industrial facilities in the vicinity of Station 329 continue to discharge a variety of conventional and toxic pollutants (USEPA, 2005b), and five facilities in the vicinity have been added to the National Priorities List under the CERCLA. Previous studies have concluded that chemical contaminants from urban and agricultural run-off and releases from industrial sources have degraded water quality in the MORB (Gilliom and others, 2006; McPherson and others, 2003; USFWS, 1989a; 1989b; 1996; Zappia, 2002) and pose a risk to aquatic and terrestrial organisms including migratory birds that breed in the region (Adair and others, 2003). In addition, fish consumption advisories to protect human health have been issued for Hg near Station 329 and PCBs near Station 327.

Tombigbee River, Lavaca, Alabama (Station 326)

Bass and carp were collected in mid-October 2004 from Station 326, which was located on the Tombigbee River near Coffeeville Lake, Alabama. Several contaminants, health indicators, and reproductive biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). The Se concentration in male carp (0.77 μg/g) exceeded and the concentration in female carp (0.69 μg/g) approached the protective threshold for fish and piscivorous wildlife (Lemly, 1996). Mercury concentrations in bass (0.44–0.52 μg/g) may be harmful to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Relatively high EROD activity in bass and carp was likely the result of induction by an AhR agonist other than dioxins and PCBs as indicated by the relatively low PCB and TCDD-EQ concentrations in these fish. HAI scores were high in bass and carp. Liver discoloration in bass was associated with differential storage of lipid/glycogen within hepatocytes and large MAs. Granular or nodular livers, and granular kidney and spleens were because of a variety of parasites. Infestations of helminth parasites were apparent in all the tissues and severe in some fish. Small, focal granulomas and microsporidian cysts present in liver, kidney, and spleen tissues were more extensive in bass from Station 326 than those from most other sites. In carp, enlarged spleens and granular spleen and kidney tissue contained small granulomas whose etiology was congestion, fibrosis, or unidentified. The mean HSI value in bass (0.58%) was less than HSI values normally present in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). High SSI values calculated for individual bass (>0.3%) and carp (>1.0%) were considered abnormal and may indicate poorer health of these individual fish. Several reproductive biomarkers were anomalous in fish from this site. Two of eight male bass were intersex, which indicates that these fish may have been exposed to endocrine modulating chemicals. Mean GSI and sex steroid concentrations were low in female carp compared to those from other sites.

Coosa River, Childersburg, Alabama (Station 327)

Station 327 was located along the Coosa River at Childersburg, an area with known PCB contamination. Bass and carp were collected in mid-October 2004. Several contaminants and biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). Mercury concentrations in bass (0.26–0.40 μg/g) from this site may be harmful to fish, piscivorous wildlife, or both (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Total chlordanes, HCB, PCA, and total HCH concentrations were relatively high in fish but were either less than toxicity thresholds or no toxicity thresholds were available. PCB concentrations (950–2,700 ng/g) were the greatest measured in this study and may represent a risk to piscivorous wildlife (Hornshaw and others, 1983). TCDD-EQ concentrations in male bass (33.6 pg/g) and carp (12.5 pg/g) exceeded dietary toxicity threshold for TCDD in mammals (Heaton and others, 1995; Tillitt and others, 1996) and avian wildlife (Nosek and others, 1992), and TCDD-EQ concentrations in male bass exceeded protective thresholds for fish (Walker and others, 1996; Whyte and others, 2004). Exposure to PCBs, dioxin-like compounds, or both may have resulted in the elevated hepatic EROD activity in carp at this site. HAI scores were high in bass and carp. Liver discoloration in bass was because of differential storage of lipid/glycogen within hepatocytes and large MAs. Granular or nodular livers, and granular kidney and spleens were associated with a variety of parasites. Helminth parasites were apparent in all the tissues. In carp, enlarged spleens and granular spleen and kidney tissue contained small granulomas whose etiology was congestion, fibrosis, or unidentified. Other observations noted in carp from Station 327 were fluid-filled abdominal cavities, calcified or fibrosis gonads, and thick otoliths. Relatively high MA-# (>8 MA/mm^2), MA-A (>10,000 μm^2), and MA-% (>8.0%) values in multiple bass and carp were considered anomalous compared to fish from most other sites. Several reproductive biomarkers were anomalous in fish from this site. One of ten male bass was intersex. Vitellogenin concentrations were >0.01 mg/mL in one male bass and carp; these concentrations indicate that these fish may have been exposed to estrogen mimics and were considered abnormal.

Table 25. Summary of chemical and biological indicator results by station.

[Within each column, colors indicate the severity, prevalence, or both of the indicated condition or conditions at each station (green<yellow<red). These designations are relative; see text for explanations. Female (f) and male (m) bass (b) and carp (c) were collected from all sites unless otherwise noted. See table 5 and figures 1 and 2 for station and basin locations. Hg, mercury; Se, selenium; DDE, *p,p'*-DDE; Tox, toxaphene; Endo, endosulfan sulfate; PCB, total polychlorinated biphenyls; TCDD-EQ, dioxin-like activity as determined by H4IIE bioassay; EROD, ethoxyresorufin *O*-deethylase; HSI, hepatosomatic index; T, tumor; SSI, splenosomatic index; HAI, health assessment index; MA, macrophage aggregates (one or more parameters); GSI, gonadosomatic index; ovt, ovotestis; vtg, vitellogenin; E2, 17β-estradiol; KT, 11-ketotestosterone. – indicates smaller; all others larger]

Basin, station location, and station number	Chemical contaminants and EROD	Health indicators	Reproductive biomarkers
Mobile River Basin			
Tombigbee R. at Lavaca, AL (326)	Se (mc); Hg (fb, mb); EROD (fb, mb, fc, mc)	HAI (b, c); HSI (-b); SSI (b, c)	ovt (mb); GSI (-fc); E2 (-fc); KT (-fc)
Coosa R. at Childersburg, AL (327)	Hg (fb, mb); PCB (fb, mb, fc, mc); TCDD-EQ (mb, mc); EROD (fc, mc)	HAI (b, c); MA (b, c)	ovt (mb); vtg (mb, mc)
Alabama R. at Eureka Landing, AL (328)	Hg (fb, mb); EROD (fc, mc)	HAI (b, c); HSI (-b); MA (b); T (b)	GSI (-fc, -mc); E2 (-mb); KT (-mb)
Mobile R. at Bucks, AL (329)	Se (mc); Hg (fb, mb); TCDD-EQ (fb, mc); EROD (fc, mc)	HAI (b, c); SSI (c); MA (b)	ovt (mb)
Apalachicola-Chattahoochee-Flint River Basin			
Chattahoochee R. at Omaha, GA (330)	Se (fc); Hg (fb, mb); DDE (fb, mb, mc); PCB (fb, mb, mc); TCDD-EQ (mb)	HSI (-b); MA (c); T (c)	ovt (mb)
Flint R. at Albany, GA (331)	Hg (fb, mb); Tox (fb, mb, fc, mc)	HSI (-b); MA (c)	ovt (mb); atresia (fb)
Apalachicola R. at Blountstown, FL (332)	Hg (fb, mb); PCB (mc)	HSI (-b)	ovt (mb)
Savannah River Basin			
Savannah R. at Augusta, GA (333)	Hg (fb, mb)	HSI (-b); SSI (b); T (c)	ovt (mb); GSI (-fb, -mc); vtg (mc)
Savannah R. at Sylvania, GA (334)	Hg (fb, mb)	HSI (-b)	ovt (mb); vtg (mb, mc)
Savannah R. at Port Wentworth, GA (335)	Hg (fb, mb)	None	ovt (mb)
Pee Dee River Basin			
Pee Dee R. at Rockingham, NC (336)[a]	Hg (fb, mb)	HSI (-b)	ovt (mb), atresia (fb); vtg (mb)
Pee Dee R. at Pee Dee, SC (337)	Hg (fb, mb, fc); TCDD-EQ (mb); EROD (fb, mb)	HAI (c); HSI (-b); SSI (b); MA (c)	ovt (mb); atresia (fc); vtg (mb); E2 (-mc); KT (-mc)
Pee Dee R. at Bucksport, SC (338)	Hg (fb, mb, fc); Endo (fc, mc); PCB (mb)	SSI (b, c); T (b)	ovt (mb); E2 (-mc); KT (-mc)

[a]No male carp collected.

Alabama River, Eureka Landing, Alabama (Station 328)

Bass and carp were collected in early October 2004 from Station 328, which was located on the Alabama River near Eureka Landing, Alabama. Several contaminants and biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). Mercury concentrations in bass (0.59–0.78 µg/g) were among the greatest measured in this study and may be harmful to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Because PCB and TCDD-EQ concentrations were considered to be low, AhR agonists other than PCBs and dioxin-like compounds may have caused the high EROD activity in carp. Elevated HAI scores were noted for bass and carp. Liver discoloration in bass was because of differential storage of lipid/glycogen within hepatocytes and large MAs, and granular liver, kidney, and spleens were associated with a variety of parasites. Microsporidian cysts present in liver, kidney, and spleen tissues were more common and severe in bass from Station 328 than those from other sites. One tumor, a lip papilloma, was present on a bass. In carp, enlarged spleens and granular spleen and kidney tissue contained small granulomas whose etiology was congestion, fibrosis, or unidentified. The mean HSI value in bass (0.65%) was less than HSI values nor-

mally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). Relatively high MA-# (>8 MA/mm^2) or MA-A (>10,000 μm^2) values in multiple bass were considered anomalous. Mean GSI values in female (5.0%) and male (4.3%) carp were relatively low but were not considered abnormal. This was the only site where intersex bass were not found.

Mobile River, Bucks, Alabama (Station 329)

Station 329 was located along the Mobile River at Bucks, near several chemical manufacturing plants and a coal-fired power plant. Bass and carp were collected in early October 2004. Several contaminants and biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). The Se concentration in male carp (1.29 μg/g) was the greatest measured in this study and was considered potentially hazardous to fish and piscivorous wildlife (Lemly, 1996). Mercury concentrations in bass (0.59–0.69 μg/g) were among the greatest measured in this study and may pose a risk to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). PCA concentrations were relatively high in carp samples, but toxicity thresholds were not available for this contaminant. TCDD-EQ concentrations in female bass (6.1 pg/g) and male carp (14.8 ng/g) may pose a risk to mammals (Heaton and others, 1995; Tillitt and others, 1996) and avian wildlife (Nosek and others, 1992). Exposure to PCBs, dioxin-like compounds, or both may have caused high EROD activity in male and female carp at this site. HAI scores were high for bass and carp. Liver discoloration in bass was due to differential storage of lipid/glycogen within hepatocytes and large MAs. Granular or nodular livers, and granular kidney and spleens were associated with a variety of parasites. Helminth parasites were apparent in all the tissues. In carp, enlarged spleens and granular spleen and kidney tissue contained small granulomas whose etiology was congestion, fibrosis, or unidentified. An SSI value of 1.25% in a carp was considered abnormal. Relatively high MA-# (>8 MA/mm^2), MA-A (>9,000 μm^2), and MA-% (>6.0%) values in multiple bass were considered anomalous. Several reproductive biomarkers were anomalous in fish from this site. Two of eight male bass were intersex, which indicates that these fish may have been exposed to endocrine modulating chemicals. Both E2 and KT concentrations were low in male bass compared to those from other sites.

Apalachicola-Chattahoochee-Flint River Basin (ARB)

Potential chemical contaminant sources in the ARB include agricultural and urban runoff, poultry and livestock production, pulp and paper mills, coal-fired power plants, and military facilities (Frick and others, 1998). CAFO effluent, which may contain antibiotics, pathogens, pesticides, hormones, and trace elements, can adversely affect fish (Orlando and others, 2004) and mammals (Gray and others, 2006). Declines in fish health and aquatic invertebrate communities have been reported in urban areas and near poultry farms in the ARB (Frick and others, 1998). Mercury and PCBs from industrial sources have contaminated ARB waters, and fish consumption advisories to protect human health have been issued for Hg near Stations 330, 331, and 332 and PCBs in the upper Chattahoochee River.

Chattahoochee River, Omaha, Georgia (Station 330)

Bass and carp were collected in late October 2004 from Station 330, which was located on the Chattahoochee River upstream of Omaha, Georgia. Multiple contaminants and biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). The Se concentration in female carp (0.90 μg/g) may be harmful to fish and piscivorous wildlife (Lemly, 1996). Mercury concentrations in bass (0.24–0.35 μg/g) may be harmful to fish, piscivorous wildlife, or both (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Concentrations of *p,p'*-DDE in female bass (180 ng/g), male bass (150 ng/g), and male carp (310 ng/g) may represent a risk to wildlife (Anderson and others, 1975; Newell and others, 1987) but did not exceed toxicity thresholds for fish (Beckvar and others, 2005; Blus, 1996; Garcia-Reyero and others, 2006; Jarvinen and Ankley, 1999). Total chlordanes, dieldrin, mirex, pentachlorobenzene, and PCA concentrations were relatively high in one or more composite samples but were either less than toxicity thresholds or no toxicity thresholds were available (table 9). PCB concentrations in female bass (990 ng/g), male bass (940 ng/g), and male carp (1,300 ng/g) may represent a risk to piscivorous wildlife (Hornshaw and others, 1983), and TCDD-EQ concentrations in male bass (4.6 pg/g) pose a threat to mammals (Heaton and others, 1995; Tillitt and others, 1996). HAI scores were low for bass and carp from this site, but swollen kidneys, possibly from large numbers of helminth and/or myxozoan parasites, were observed in several bass. Leiomyosarcoma, tumors of smooth muscle origin, were present in the ovaries of two female carp. The mean HSI value in bass (0.60%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). Relatively high MA-# (>8 MA/mm^2), MA-A (>10,000 μm^2), and MA-% (>7.0%) values in multiple carp were greater than those in fish from most other sites. Three of 10 male bass were intersex, which indicates potential exposure to endocrine modulating chemicals.

Flint River, Albany, Georgia (Station 331)

Bass and carp were collected in late October 2004 from Station 331, located on the Flint River at Radium Springs, Georgia. Several chemical contaminants and biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). Mercury concentrations in bass (0.22–0.25 μg/g) exceeded criteria to protect juvenile and

adult fish (Beckvar and others, 2005). The greatest toxaphene concentrations (60–100 ng/g) were measured in fish from this site; toxaphene concentrations exceeded some toxicity thresholds to protect freshwater fish (Mayer and others, 1975) but were less than most effects criteria for freshwater fish (Eisler and Jacknow, 1985; Jarvinen and Ankley, 1999). Total chlordanes, dieldrin, mirex, HCB, pentachlorobenzene, total HCH, dacthal, and endosulfan concentrations were relatively high in fish but were either less than toxicity thresholds or no toxicity thresholds were available. HAI scores were low for bass and carp from this site, but swollen kidneys, possibly from large numbers of helminth and/or myxozoan parasites, were observed in several bass. The mean HSI value in bass (0.59%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). Relatively high MA-# (>7 MA/mm^2), MA-A (>6,000 μm^2), and MA-% (>5.0%) values in multiple carp were greater than those in fish from most other sites. Several reproductive biomarkers were anomalous in fish from this site. Three of 10 male bass were intersex, which indicates that these fish may have been exposed to endocrine modulating chemicals. Oocyte atresia was relatively high in female bass from this site.

Apalachicola River, Blountstown, Georgia (Station 332)

Bass and carp were collected in early November 2004 from Station 332, which was located on the Apalachicola River at Blountstown, Georgia. Several contaminants exceeded threshold criteria, but few biomarkers appeared anomalous in fish from this site (table 25). Mercury concentrations in bass (0.51–0.65 μg/g) may represent a threat to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Pentachlorobenzene, total HCH, and endosulfan were relatively high in one or more samples, but toxicity thresholds were not available for these organochlorine contaminants. PCB concentrations in male carp (700 ng/g) may pose a threat to piscivorous wildlife (Hornshaw and others, 1983). HAI scores were low for bass and carp from this site, but swollen kidneys, possibly a result of helminth and/or myxozoan parasite infestations, were noted in several bass. Histopathological examination determined that kidney tissue in these bass had large numbers of parasites present. The mean HSI value in bass (0.56%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). A large proportion of male bass were intersex (60%), which indicates that these fish may have been exposed to endocrine modulating chemicals.

Savannah River Basin (SRB)

Bass and carp were collected on the Savannah River near Augusta (Station 333), Sylvania (Station 334), and Port Wentworth (Station 335), Georgia. Potential sources of chemical contaminants near these sites include urban and agricultural runoff, CAFOs, chemical manufacturing, pulp and paper mills, and coal-fired power plants. The manure-related discharges from livestock and poultry production can include other pollutants (antibiotics, pathogens, pesticides, hormones, and trace elements) that can affect aquatic and terrestrial wildlife (Burkholder and others, 2007; Gray and others, 2006; Orlando and others, 2004). Mercury and PCBs from industrial sources have contaminated ARB waters, and fish consumption advisories have been issued for Hg and PCBs in the SRB.

Savannah River, Augusta, Georgia (Station 333)

Bass and carp were collected in early December 2004 from Station 333, which was located on the Savannah River at Phinizy Swamp upstream of Augusta, Georgia. Several chemical contaminants or biomarkers appeared anomalous or exceeded threshold criteria in fish from this site (table 25). Mercury concentrations in bass (0.32–0.43 μg/g) may represent a threat to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). HCB, pentachlorobenzene, and endosulfan concentrations were relatively high in one or more samples but were either less than toxicity thresholds or no toxicity thresholds were available. The mean HSI value in bass (0.59%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). An SSI value of 0.69% in a female bass was considered abnormal. Several reproductive biomarkers were anomalous in fish. Three of seven male bass were intersex, which indicates that these fish may have been exposed to endocrine modulating chemicals. Mean GSI values in female bass (0.5%) and male carp (4.6%) were relatively low and vtg concentrations were >0.01 mg/mL in multiple male carp. A Seritoli cell tumor also was present in the gonad of a male carp.

Savannah River, Sylvania, Georgia (Station 334)

Bass and carp were collected in early December 2004 from Station 334, which was located on the Savannah River at Burton's Ferry east of Sylvania, Georgia. Few chemical contaminants or biomarkers appeared anomalous or exceeded threshold criteria in fish from this site (table 25). Mercury concentrations in bass (0.63–0.67 μg/g) were among the greatest measured in this study and may represent a threat to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Endosulfan concentrations were relatively high but below available toxicity thresholds in carp samples. HAI scores were low for bass and carp, but swollen kidneys observed in several bass may have been from large numbers of helminth and/or myxozoan parasites. The mean HSI value in bass (0.63%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). Several reproductive biomarkers were anomalous in fish from this

site. Two of four male bass were intersex, and vtg concentrations >0.01 mg/mL in male bass (n = 1) and carp (n = 9) were considered abnormal. The reproductive biomarker responses indicate that these fish may have been exposed to endocrine modulating chemicals.

Savannah River, Port Wentworth, Georgia (Station 335)

Bass and carp were collected in early December 2004 from Station 335, which was located on the Savannah River at Port Wentworth, Georgia. Few chemical contaminants or biomarkers appeared anomalous or exceeded threshold criteria in fish from this site (table 25). Mercury concentrations in bass (0.24–0.47 µg/g) may be harmful to fish, piscivorous wildlife, or both (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Mirex concentrations in carp and bass samples were relatively high but below toxicity thresholds. HAI scores were low for bass and carp from this site, but swollen kidneys observed in several bass may be from large numbers of helminth and/or myxozoan parasites present. Endocrine modulating chemicals may be present as indicated by the large proportion of intersex bass (50%) at this site.

Pee Dee River Basin (PDRB)

Bass and carp were collected on the Pee Dee River near Rockingham, North Carolina (Station 336) and Pee Dee (Station 337) and Bucksport (Station 338), South Carolina. Potential sources of chemical contaminants near these sites include agricultural runoff, chemical manufacturing, pulp and paper mills, and coal-fired power plants. Degraded water quality has been documented at NPL sites near Stations 336 and 337. Mercury from industrial sources have contaminated PDRB waters, and fish consumption advisories have been issued for Hg in South Carolina near Stations 336, 337, and 338.

Pee Dee River, Rockingham, North Carolina (Station 336)

Bass and one carp were collected in early November 2004 from Station 336, which was located on the Pee Dee River near Hwy 74 west of Rockingham, North Carolina. Few chemical contaminants or biomarkers appeared anomalous or exceeded threshold criteria in fish from this site (table 25). Mercury concentrations in bass (0.23–0.24 µg/g) exceeded criteria to protect juvenile and adult fish (Beckvar and others, 2005). Aldrin, PCA, and endosulfan concentrations were relatively high in one or more samples but were either less than toxicity thresholds or no toxicity thresholds were available. HAI scores were low for bass and carp. The mean HSI value in bass (0.68%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). The MA-A (23,366 μm^2) and MA-% (7.1%) values in the only carp collected from this sites were among the greatest measured in any LRMN investigation. Several reproductive biomarkers were anomalous in fish from this site. Vitellogenin concentrations >0.01 mg/mL in all male bass (n = 3) and occurrence of intersex in two male bass indicated potential exposure to endocrine modulating chemicals. Oocyte atresia was relatively high in female bass from this site.

Pee Dee River, Pee Dee, South Carolina (Station 337)

Bass and carp were collected in early November 2004 from Station 337, which was located on the Pee Dee River near Pee Dee, South Carolina. Several contaminants, health indicators, and reproductive biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). Mercury concentrations in bass (0.37–0.48 µg/g) may represent a threat to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Aldrin, PCA, and endosulfan concentrations were relatively high in one or more samples but were either less than toxicity thresholds or no toxicity thresholds were available. TCDD-EQ concentrations in male bass (13.7 pg/g) may pose a risk to mammals (Heaton and others, 1995; Tillitt and others, 1996) and avian wildlife (Nosek and others, 1992). Elevated EROD activity in male bass may have resulted from exposure to dioxin-like compounds as indicated by the relatively high TCDD-EQ concentration in the composite sample, but high EROD activity in female bass were likely from another AhR agonist because both PCBs and TCDD-EQ concentrations were low in the female composite sample. Elevated HAI scores in bass and carp were related to heavy parasite loads. The mean HSI value in bass (0.66%) was less than HSI values normally reported in fish (1–2%; Gingerich, 1982) and bass from previous LRMN studies (Blazer and others, 2002; Hinck and others, 2006a, 2006b; Schmitt and others, 2005). An SSI value of 0.63% in a male bass was considered abnormal. Several reproductive biomarkers were anomalous in fish from this site and indicated that fish were exposed to endocrine modulating compounds. A large proportion of male bass (73%) were intersex, and one male bass had a vtg concentration (2.48 mg/mL) approaching concentrations measured in pre-vitellogenic females, which were considered abnormal. Oocyte atresia was relatively high in female carp from this site. Ovaries from two carp at Station 337 contained degenerating eggs that were infected with a microsporidian parasite. Both E2 and KT concentrations were low in male carp compared to those from other sites.

Pee Dee River, Bucksport, South Carolina (Station 338)

Bass and a limited number of carp were collected in early November 2004 from Station 338, which was located on the Pee Dee River near Bucksport, South Carolina. Several contaminants, health indicators, and reproductive biomarkers exceeded threshold criteria or appeared anomalous in fish from this site (table 25). Mercury concentrations in

bass (0.65–0.78 µg/g) were among the greatest measured in this study and may represent a threat to fish and piscivorous wildlife (Barr, 1986; Beckvar and others, 2005; Yeardley and others, 1998). Endosulfan sulfate concentrations in carp (1.1–1.2 ng/g) exceeded toxicity thresholds for reproduction (Shukla and Pandey, 1986) and may pose a risk to fish at this site. Aldrin, PCA, and endosulfan concentrations were relatively high in one or more samples but were either less than toxicity thresholds or no toxicity thresholds were available. PCB concentrations in male bass (710 ng/g) may pose a threat to piscivorous wildlife (Hornshaw and others, 1983). High SSI values calculated for individual bass (>0.3%) and carp (>1.0%) were considered abnormal and may indicate poorer health in these individual fish. A large lipoma within the spleen of this bass caused the high SSI value. Several reproductive biomarkers were anomalous in fish from this site. The greatest proportion of intersex male bass (91%) was present at this site, which indicates that these fish may have been exposed to endocrine modulating chemicals. Vitellogenin concentrations were low (<0.006 mg/mL) in all intersex male bass, however. Both E2 and KT concentrations were low in male carp compared to carp from other sites.

Conclusions

Overall, chemical contaminant concentrations and biological responses were most severe or prevalent in fish from the MORB. Selenium, Hg, PCBs, and TCDD-EQ were the main chemical contaminants of concern in the MORB, and hepatic EROD activity also was relatively high in fish from all MORB sites, which indicates that AhR agonists such as PAHs may be affecting fish. Mercury concentrations in MORB fish were among the greatest measured in any BEST-LRMN study and were possibly the result of releases from chemical manufacturing plants, coal-fired power plants, and atmospheric deposition. Chlor-alkaline facilities directly discharged Hg into the lower MORB until the 1980s and contaminated surrounding wetlands and biota inhabiting the area (USFWS, 1996). Although DDT concentrations did not exceed toxicity thresholds for fish or wildlife, concentrations were relatively high in fish from Station 329 (Bucks, Alabama). The lower MORB has a history of DDT contamination in water and biota associated with a DDT formulating facility upstream from Station 329 on the Tombigbee River (Atkins and others, 2004; USEPA, 1995; USFWS, 1996; Zappia, 2002), and biomarker studies of aquatic species previously were recommended for this area to assess the risk of DDT exposure to higher trophic levels (USFWS, 1996). A PCB manufacturing facility at Anniston, Alabama, and an electrical capacitor and transformer facility have contaminated the Coosa River with PCBs, which has led to fish consumption advisories for much of the river. Results indicate that PCB concentrations continue to threaten aquatic biota near Station 327 (Childersburg, Alabama). The high dioxin-like activity, as measured by TCDD-EQ, in fish from Station 327 was likely associated with high PCB concentrations, but the dioxin-like activity in fish from Stations 329 were likely from other sources such as paper manufacturing or wastewater treatment plants.

Fish health indicators and reproductive biomarkers were anomalous in MORB fish and may indicate responses to chemical contaminant exposure. The field necropsy and histopathological examination determined that fish from the MORB generally were in poorer health than those from the other basins. In bass, HAI scores were correlated with Hg and *p,p*'-DDE concentrations. High HAI scores in MORB fish were widespread and caused primarily by parasitic infestations, which were most severe in fish from Stations 326 and 328; liver weights in bass from these two sites also were abnormally low. The immune system of MORB fish responded to chemical or environmental stressors as indicated by the large and numerous MAs present in bass and carp. Fish from Station 327 (Childersburg, Alabama) appeared to have had the most severe responses. Splenic MAs in bass and carp and vtg concentrations in male bass and carp were abnormally high at Station 327. Carp gonads from this site also were abnormal, containing calcified follicles and exhibiting fibrosis. These biological responses may be the result of PCB exposure, but the relatively old age of these fish is also an important factor to consider. Wastewater treatment plant effluent or runoff from poultry and livestock production facilities are potential sources of endocrine disrupting compounds that may be associated with the occurrence of intersex male bass at most MORB sites.

Organochlorine pesticides were historically used in agricultural and urban areas of the ARB, and Hg and PCBs have contaminated much of the Chattahoochee and Flint Rivers. Station 330 on the Chattahoochee River near Omaha, Georgia was the most contaminated site in the ARB with Se, Hg, *p,p*'-DDE, PCBs, and TCDD-EQ concentrations exceeding protective criteria for fish, wildlife, or both. Possible contaminant sources near Station 330 include agricultural and urban runoff, military facilities, and paper manufacturing facilities. Total chlordanes, dieldrin, mirex, pentachlorobenzene, and PCA concentrations were relatively high in fish from Station 330 but were less than toxicity thresholds or no toxicity thresholds were available. Concentrations of formerly used pesticides (toxaphene, dieldrin, mirex, and total chlordanes) and currently used organochlorine residues and their metabolites (pentachlorobenzene, total HCH, dacthal, and endosulfans) were high in fish from Station 331 (Albany, Georgia), which is located in the rich agricultural area of Georgia.

Although organochlorine residue concentrations were relatively high in ARB fish, few health indicators or reproductive biomarkers were anomalous in fish. The ovarian tumors (leiomyosarcomas) present in female carp from Station 330 were of particular interest. These tumors are not common in carp and were not observed in carp from other sites in this study or in previous BEST-LRMN studies. However, gonadal tumors of encapsulated teratogenic masses have been widely documented in carp (Harshbarger and Clark, 1990). The cause

of the leiomyosarcomas was unknown but may be related to chemical contaminant exposure or age. The high incidence of intersex male bass at all ARB sites indicated that endocrine disrupting compounds may be affecting bass in the basin. Potential sources of these compounds include runoff from CAFOs in the ARB or wastewater treatment plant effluent from metropolitan areas such as Atlanta.

In the SRB, fish generally were healthier than those from the MORB, ARB, and PDRB. Mercury was the only chemical contaminant to exceed protective thresholds for fish and wildlife, and concentrations were among the greatest measured in any BEST-LRMN study. Atmospheric deposition is suspected to be the primary source of Hg in the SRB. Mirex, HCB, pentachlorobenzene, and endosulfan concentrations were high in one or more SRB samples but did not exceed toxicity thresholds for fish or wildlife. Fish health indicators were normal in most SRB fish. Anomalous reproductive biomarker responses, including high incidence of intersex male bass, elevated vtg concentrations in male fish, proportionally small gonad size, and a Seritoli cell tumor in a male carp gonad, indicated that SRB fish were exposed to endocrine modulating compounds.

Similar to the other basins studied, Hg was the primary contaminant of concern in fish from the PDRB. Mercury concentrations in bass and female carp exceeded toxicity thresholds for fish and wildlife, and atmospheric deposition is potentially the primary Hg source in the PDRB. Endosulfans, PCBs, and dioxin-like compounds may pose a risk to fish at a limited number of sites within the PDRB. The field necropsy and histopathological examination determined that the poor health of some PDRB fish was associated with parasitic infestations, although the infestations were less severe than in MORB fish. Reproductive biomarkers including high vtg concentrations in male fish, low steroid hormone concentrations, high atresia rates, and high incidence of intersex male bass were considered anomalous in PDRB fish but were not definitively associated with contaminant exposure.

The agricultural and manufacturing industries that support the economy of the southeastern United States historically have had deleterious effects on water and aquatic habitat quality through the production and release of organochlorine compounds and deposition of Hg. In addition, the intense poultry and livestock production facilities 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). Continued degradation of aquatic resources is possible as these industries increase the magnitude and type of chemical contaminants, including endocrine disrupting compounds that are released into aquatic ecosystems. Biological responses would be expected to increase in magnitude as chemical concentrations increase, which ultimately may place fish populations at risk. Results from this study and other investigations indicate that continued monitoring of chemical contaminant tissue concentrations and biomarkers is needed to follow conditions at degraded sites and identify those with emerging problems, especially with issues relating to CAFOs. Focused investigations also are needed in the southeastern United States to document chemical sources, interactions with other factors, and cause-effect relations.

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Appendices 1–5

Appendix 1. Selected aquatic species within the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins identified as having special status by the U.S. Fish and Wildlife Service.

[Data from the Threatened and Endangered Species System (TESS) webpage (*https://ecos/fws/gov/ecos/sec/species.do*) accessed 6/09/05]

Common name	Scientific name	State listed
Acornshell, southern	*Epioblasma othcaloogensis*	Alabama, Georgia
Bankclimber, purple	*Elliptoideus sloatianus*	Georgia
Blossom, turgid (pearlymussel)	*Epioblasma turgidula*	Alabama
Blossom, yellow (pearylmussel)	*Epioblasma florentina florentina*	Alabama
Catspaw (purple cat's paw pearlymussel)	*Epioblasma obliquata obliquata*	Alabama
Cavefish, Alabama	*Speoplatyrhinus poulsoni*	Alabama
Chub, spotfin	*Cyprinella monacha*	Alabama, North Carolina
Clubshell, black	*Pleurobema curtum*	Mississippi
Clubshell, ovate	*Pleurobema perovatum*	Alabama, Mississippi
Clubshell, southern	*Pleurobema decisum*	Alabama, Georgia, Mississippi
Combshell, Cumberlandian	*Epioblasma brevidens*	Alabama
Combshell, southern	*Epioblasma penita*	Alabama, Mississippi
Combshell, upland	*Epioblasma metastriata*	Alabama, Georgia
Darter, amber	*Percina antesella*	Georgia
Darter, bayou	*Etheostoma rubrum*	Mississippi
Darter, boulder	*Etheostoma wapiti*	Alabama
Darter, Cherokee	*Etheostoma scotti*	Georgia
Darter, Etowah	*Etheostoma etowahae*	Georgia
Darter, goldline	*Percina aurolineata*	Alabama, Georgia
Darter, Okaloosa	*Etheostoma okaloosae*	Florida
Darter, slackwater	*Etheostoma boschungi*	Alabama
Darter, snail	*Percina tanasi*	Alabama, Georgia
Darter, vermilion	*Etheostoma chermocki*	Alabama
Darter, watercress	*Etheostoma nuchale*	Alabama
Elktoe, Appalachian	*Alasmidonta raveneliana*	North Carolina
Fanshell	*Cyprogenia stegaria*	Alabama
Heelsplitter, Alabama	*Potamilus inflatus*	Alabama
Heelsplitter, Carolina	*Lasmigona decorata*	North Carolina, South Carolina
Kidneyshell, triangular	*Ptychobranchus greeni*	Alabama, Georgia
Lampmussel, Alabama	*Lampsilis virescens*	Alabama
Lilliput, pale (perylmussel)	*Toxolasma cylindrellus*	Alabama
Logperch, Conasauga	*Percina jenkinsi*	Georgia
Moccasinshell, Alabama	*Medionidus acutissimus*	Alabama, Georgia, Mississippi
Moccasinshell, Coosa	*Medionidus parvulus*	Georgia
Moccasinshell, Gulf	*Medionidus penicillatus*	Florida, Georgia
Moccasinshell, Ochlockonee	*Medionidus simpsonianus*	Florida, Georgia
Monkeyface, Cumberland (pearylmussel)	*Quadrula intermedia*	Alabama
Mucket, orangeacre	*Lampsilis perovalis*	Alabama, Mississippi
Mucket, pink (pearylmussel	*Lampsilis abrupta*	Alabama
Mussel, oyster	*Epioblasma capsaeformis*	Alabama

Appendix 1. Selected aquatic species within the Mobile, Apalachicola-Chattahoochee-Flint, Savannah, and Pee Dee River Basins identified as having special status by the U.S. Fish and Wildlife Service.—Continued

[Data from the Threatened and Endangered Species System (TESS) webpage (*https://ecos/fws/gov/ecos/sec/species.do*) accessed 6/09/05]

Common name	Scientific name	State listed
Pearlymussel	*Hemistena lata*	Alabama
Pearlymussel, littlewing	*Pegias fabula*	North Carolina
Pigtoe, dark	*Pleurobema furvum*	Alabama
Pigtoe, finerayed	*Fusconaia cuneolus*	Alabama
Pigtoe, flat	*Pleurobema marshalli*	Alabama, Mississippi
Pigtoe, heavy	*Pleurobema taitianum*	Alabama
Pigtoe, oval	*Pleurobema pyriforme*	Florida, Georgia
Pigtoe, rough	*Pleurobema plenum*	Alabama
Pigtoe, shiny	*Fusconaia cor*	Alabama
Pigtoe, southern	*Pleurobema georgianum*	Alabama, Georgia
Pimpleback, orangefoot (pearlymussel)	*Plethobasus cooperianus*	Alabama
Pocketbook, fat	*Potamilus capax*	Mississippi
Pocketbook, finelined	*Lampsilis altilis*	Alabama, Georgia
Pocketbook, shinyrayed	*Lampsilis subangulata*	Alabama, Florida, Georgia
Ring pink	*Obovaria retusa*	Alabama
Sculpin, pygmy	*Cottus paulus*	Alabama
Shiner, blue	*Cyprinella caerulea*	Alabama, Georgia
Shiner, Cahaba	*Notropis cahabae*	Alabama
Shiner, Cape Fear	*Notropis mekistocholas*	North Carolina
Shiner, palezone	*Notropis albizonatus*	Alabama
Shrimp, Alabama cave	*Palaemonias alabamae*	Alabama
Silverside, Waccamaw	*Menidia extensa*	North Carolina
Slabshell, Chipola	*Elliptio chipolaensis*	Alabama, Florida
Spinymussel, James	*Pleurobema collina*	North Carolina
Spinymussel, Tar River	*Elliptio steinstansana*	North Carolina
Stirrupshell	*Quadrula stapes*	Alabama
Sturgeon, Alabama	*Scaphirhynchus suttkusi*	Alabama, Mississippi
Sturgeon, gulf	*Acipenser oxyrinchus desotoi*	Alabama, Florida, Mississippi
Sturgeon, pallid	*Scaphirhynchus albus*	Mississippi
Sturgeon, shortnose	*Acipenser brevirostrum*	Florida, Georgia, North Carolina, South Carolina
Wedgemussel, dwarf	*Alasmidonta heterodon*	North Carolina

Appendix 2. Estimated pesticide use in agricultural areas of the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins.

[Data from the Pesticide National Synthesis Project (USGS, 2003). Crop to which the pesticide most commonly is applied also is listed. The average annual pesticide use is expressed as average weight (in pounds) of a pesticide applies to each square mile of agricultural land in a county based on state-level estimates of pesticide use rates for individual crops that were compiled by the National Center for Food and Agricultural Policy during 1995–1998 and on 1997 Census of Agriculture county crop acreage (Thelin and Gianessi, 2000). >, greater than; Y, yes; N, no]

Pesticide	Crop most commonly applied	Average annual use of active ingredient (lbs/mi²/yr)	Heavily used in agricultural areas? (Y/N)			
			MORB	ARB	SRB	PDRB
Herbicides						
2,4-DB	alfalfa hay, peanuts	> 0.177	N	Y	N	N
Acifluorfen	soybeans, peanuts	> 1.039	N	Y	N	Y
Ametryn	corn, sugarcane	> 1.577	N	N	Y	Y
Bentazon	soybeans, corn, peanuts	> 4.52	N	Y	N	N
Clomazone	soybeans, cotton, tobacco	> 1.571	N	N	N	Y
Cyanazine	corn, cotton	> 8.879	N	Y	Y	Y
Diclofop	wheat, barley	> 1.01	N	N	N	Y
Diuron	cotton, citrus, seed crops	> 1.264	N	Y	Y	N
Ethalfluralin	sunflower, dry beans, soybeans, peanuts	> 0.984	N	Y	Y	N
Fluazifop	soybeans, cotton	> 0.328	Y	N	N	Y
Fluometuron	cotton	> 10.305	N	Y	Y	Y
Fomesafen	soybeans	> 0.966	N	N	N	Y
Glyphosate	soybeans, corn	> 12.281	Y	N	N	Y
Imazapic	peanuts	> 0.077	N	Y	N	N
Lactofen	soybeans, cotton	> 0.333	N	Y	Y	N
MSMA	cotton	> 7.096	N	Y	Y	Y
Napropamide	tomatoes, tobacco	> 0.253	N	N	N	Y
Naptalam	watermelons, cucumbers	> 0.138	N	Y	N	N
Norflurazon	cotton, citrus	> 1.639	N	Y	Y	Y
Oryzalin	fruit, nuts	> 0.084	N	Y	Y	N
Paraquat	corn, soybeans, cotton	> 2.843	N	Y	N	Y
Pebulate	tomatoes, tobacco	> 0.36	N	N	N	Y
Pendimethalin	soybeans, cotton	> 12.456	N	Y	Y	Y
Prometryn	cotton	> 2.857	N	Y	N	N
Pyridate	corn, peanuts	> 0.389	N	Y	N	N
Pyrithiobac	cotton	> 0.427	N	Y	Y	Y
Sethoxydim	soybeans	> 0.801	N	Y	Y	Y
Sulfentrazone	tobacco	> 0.444	N	N	N	Y
Thifensulfuron	wheat, soybeans	> 0.055	N	N	N	Y
Tribenuron	wheat, barley	> 0.042	N	N	N	Y
Trifluralin	soybeans, cotton	> 11.11	N	Y	Y	Y
Vernolate	peanuts, soybeans	> 0.827	N	N	Y	Y
Insecticides						
Acephate	cotton, tobacco, vegetables	> 1.696	N	Y	N	Y
Aldicarb	cotton, peanuts	> 4.363	N	Y	Y	Y
Amitraz	cotton, pears	> 0.249	N	Y	Y	N
Chlorpyrifos	corn, cotton, fuits, nuts	> 4.516	N	Y	Y	Y
Cyfluthrin	cotton, citrus	> 0.062	N	Y	Y	Y
Cypermethrin	cotton, vegetables, pecans	> 0.222	N	Y	Y	N
Dicofol	cotton, dry beans, nuts, fruits	> 0.209	N	Y	Y	N
Diflubenzuron	cotton, citrus, soybeans	> 0.177	N	Y	Y	N
Dimethoate	wheat, alfalfa hay, cotton	> 0.556	N	Y	N	N
Disulfoton	cotton, potatoes, wheat	> 0.679	N	Y	N	Y
Endosulfan	cotton, fruits, tobacco	> 0.481	N	Y	Y	Y
Esfenvalerate	cotton, sunflower, soybeans	> 0.1	N	Y	Y	N
Ethoprop	vegetables, tobacco, peanuts	> 1.26	N	Y	N	Y
Fenamiphos	tobacco, fruits, peanuts	> 3.31	N	Y	N	Y
Fenpropathrin	cotton, peanuts	> 0.206	N	Y	N	N

Appendix 2. Estimated pesticide use in agricultural areas of the Mobile (MORB), Apalachicola-Chattahoochee-Flint (ARB), Savannah (SRB), and Pee Dee (PDRB) River Basins.—Continued

[Data from the Pesticide National Synthesis Project (USGS, 2003). Crop to which the pesticide most commonly is applied also is listed. The average annual pesticide use is expressed as average weight (in pounds) of a pesticide applies to each square mile of agricultural land in a county based on state-level estimates of pesticide use rates for individual crops that were compiled by the National Center for Food and Agricultural Policy during 1995–1998 and on 1997 Census of Agriculture county crop acreage (Thelin and Gianessi, 2000). >, greater than; Y, yes; N, no]

Pesticide	Crop most commonly applied	Average annual use of active ingredient (lbs/mi²/yr)	Heavily used in agricultural areas? (Y/N)			
			MORB	ARB	SRB	PDRB
Lamdacyhalothrin	cotton, corn	> 0.104	Y	Y	Y	Y
Lindane	pecans	> 0.052	N	Y	Y	N
Methomyl	vegetables, cotton	> 0.395	N	Y	Y	Y
Methyl parathion	cotton, corn	> 0.931	Y	Y	Y	Y
Oxamyl	cotton	> 0.423	N	Y	N	N
Phorate	corn, potatoes, cotton	> 1.188	N	Y	N	Y
Phosmet	fruit, nuts	> 0.26	N	Y	Y	N
Profenofos	cotton	> 1.791	N	Y	N	N
Propargite	corn, almonds, grapes	> 1.647	N	Y	N	N
Spinosad	cotton, vegetables	> 0.228	N	Y	N	N
Terbufos	cotton	> 3.239	N	N	N	Y
Thiodicarb	cotton, soybeans	> 0.6	N	Y	Y	Y
Tralomethrin	cotton, soybeans	> 0.043	N	Y	Y	Y
Fungicides						
Azoxystrobin	rice, peanuts	> 0.272	N	Y	N	N
Chlorothalonil	potatoes, peanuts, vegetables	> 2.415	N	Y	Y	N
Copper	citrus, rice, almonds, fruits	> 0.776	N	Y	Y	N
Dimethomorph	tobacco, potatoes	> 0.156	N	N	N	Y
Etridiazole	cotton	> 0.285	N	Y	N	N
Flutolanil	peanuts	> 0.573	N	Y	N	N
Mancozeb	vegetables, fruits	> 2.005	N	N	Y	Y
Metalaxyl	tobacco, citrus	> 0.354	N	N	N	Y
PCNB	cotton	> 1.041	N	Y	Y	N
Propiconazole	wheat, seed crops	> 0.17	N	Y	N	Y
Sulfur	fruit	> 3.059	N	Y	Y	N
Tebuconazole	peanuts, wheat	> 0.75	N	Y	N	N
Triadimefon	wheat, fruit	> 0.036	N	N	N	Y
Triphenyltin Hyd	sugarbeets, pecans	> 0.23	N	Y	Y	N
Ziram	fruit, nuts	> 0.416	N	Y	N	N
Other						
1,3-D	tobacco, potatoes, cotton	> 41.194	N	Y	Y	Y
Chloropicrin	tobacco, vegetables, fruits	> 2.586	N	Y	N	Y
Cyclanilide	cotton	> 0.46	N	Y	N	Y
Dimethipin	cotton	> 0.607	N	Y	Y	Y
Ethephon	cotton	> 3.868	N	Y	Y	Y
Flumetralin	tobacco	> 1.624	N	N	N	Y
Maleic hydrazide	tobacco	> 2.748	N	N	N	Y
Mepiquat chloride	cotton	> 0.394	N	Y	N	N
Methyl bromide	fruits, vegetables	> 5.626	N	N	Y	Y
Sodium chlorate	cotton	> 6.312	N	Y	N	N
Thidiazuron	cotton	> 0.583	N	Y	N	N
Tribufos	cotton	> 9.586	N	Y	Y	Y

Appendix 3. Quality assurance and limit of detection for elemental contaminants in whole-body fish composite samples.

[Data from quantitative analysis for arsenic, selenium, and mercury and an inductively coupled plasma mass spectrometry semi-quantitative scan of other elements. All concentrations are dry weight. QA, quality assurance; µg/g, micrograms per gram; <, less than; IAEA 407, International Atomic Energy Agency Reference Material 407: Trace Elements and Methymercury in Fish Tissue; IRMM 422, Institute for Reference Materials and Measurements CRM 422: Trace Elements in Cod Muscle; NRCC DORM-2, National Research Council Canada DORM-2 CRM: Dogfish Muscle; CCR, certified concentration range; NRCC DOLT-2, National Research Council Canada DOLT-2 CRM: Dogfish Liver; NIST RM50, National Institute of Standards and Technology Research Material 50: Albacore Tuna; NIST 2976, National Institute of Standards and Technology SRM 2976: Mussel Tissue; LOD, limit of detection; --, not applicable]

QA component	Element								
	Arsenic (µg/g)	Cadmium (µg/g)	Copper (µg/g)	Chromium (µg/g)	Mercury (µg/g)	Nickel (µg/g)	Lead (µg/g)	Selenium (µg/g)	Zinc (µg/g)
Blank equivalent concentrations[a]									
Minimum	<0.014	<0.04	<0.4	<0.04	0.003	<0.4	<0.04	<0.016	<0.4
Maximum	<0.03	<0.04	<0.4	0.2	0.007	<0.4	<0.04	<0.018	<0.4
Triplicate sample analyses (% relative standard deviation)[b]									
Minimum	3.7	0.0	0.0	13.0	0.7	0.0	0.0	1.4	0.0
Maximum	16.0	35.0	16.0	50.0	4.5	39.0	43.0	2.7	12.0
Reference materials (% recovery)									
IAEA 407; $n = 2$									
Minimum	100	86	100	100	98	165	100	100	98
Maximum	100	87	101	101	100	185	100	100	103
CCR	12.6 ± 0.3	0.189 ± 0.004	3.28 ± 0.8	0.73 ± 0.6	0.222 ± 0.006	0.60 ± 0.05	0.12 ± 0.02	2.83 ± 0.13	67.1 ± 0.8
IRMM 422; $n = 2$									
Minimum	99	--	113	--	--	--	57	108	64
Maximum	99	--	121	--	--	--	100	109	70
CCR	21.1 ± 0.5	0.017 ± 0.002	1.05 ± 0.07	--	0.559 ± 0.016	--	0.085 ± 0.015	1.63 ± 0.07	19.6 ± 0.5
NRCC DORM-2; $n = 8$									
Minimum	--	--	--	--	100	--	--	103	--
Maximum	--	--	--	--	100	--	--	108	--
CCR	--	--	--	--	4.64 ± 0.26	--	--	1.40 ± 0.09	--
NRCC DOLT-2 $n = 6$									
Minimum	--	--	--	--	100	--	--	--	--
Maximum	--	--	--	--	100	--	--	--	--
CCR	--	--	--	--	2.14 ± 0.28	--	--	--	--
NIST RM50; $n = 6$									
Minimum	--	--	--	--	100	--	--	--	--
Maximum	--	--	--	--	100	--	--	--	--
CCR	--	--	--	--	0.95 ± 0.1	--	--	--	--
NIST 2976; $n = 6$									
Minimum	--	--	--	--	100	--	--	--	--
Maximum	--	--	--	--	100	--	--	--	--
CCR	--	--	--	--	0.061 ± 0.004	--	--	--	--
Pre-digestion spiked samples (% recovery)[c]									
Minimum	99	94	103	96	108	110	105	103	97
Maximum	120	110	112	105	116	113	114	111	116
Nominal LOD	0.03	--	--	--	0.05	--	--	0.06	--
Reporting Limit[d]	--	0.04	0.4	0.04	--	0.4	0.04	--	0.4

[a] $n = 2$ for arsenic and selenium, $n = 6$ for all other elements.

[b] $n = 6$ for mercury, $n = 4$ for all other elements.

[c] $n = 6$ for mercury and selenium; $n = 8$ for all other elements.

[d] semi-quantitative scan.

Appendix 4. Quality assurance, methods detection limit, and methods quantitative limit for organochlorine residues in whole-body fish composite samples.

[All concentrations are nanograms per gram wet weight; Sample sizes were $n = 5$ for all compounds. RSD, relative standard deviation; PCB, polychlorinated biphenyl; QCB, pentachlorobenzene; HCB, hexachlorobenzene; PCA, pentachloroanisole; HCH, hexachlorocyclohexane; NA, not applicable; MDL, method detection limit; MQL, method quantitative limit]

QA component	Compound											
	Total PCB	Total toxaphene	QCB	HCB	PCA	α-HCH	β-HCH	σ-HCH	γ-HCH	Heptachlor	Heptachlor epoxide	Aldrin
Procedure blanks												
Minimum	18	3	0.01	0.08	0.05	0.00	0.04	0.00	0.00	0.00	0.00	0.02
Maximum	42	8	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	29	6	0.04	0.10	0.07	0.00	0.17	0.00	0.00	0.00	0.00	0.05
Matrix blanks												
Minimum	98	10	0.16	0.35	0.12	0.27	0.00	0.00	0.00	0.00	0.38	0.00
Maximum	98	10	0.16	0.35	0.12	0.27	0.00	0.00	0.00	0.00	0.38	0.00
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Triplicate sample analyses (% RSD)[a]												
Minimum	16	NA	NA	9	8	NA	NA	NA	NA	NA	13	NA
Maximum	16	NA	NA	9	8	NA	NA	NA	NA	NA	13	NA
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Reference material[b]												
Minimum	6700	180	6.8	15	0.91	1.8	< 0.30	1.5	< 0.10	< 0.10	2.0	< 0.09
Maximum	6700	180	6.8	15	0.91	1.8	< 0.30	1.5	< 0.10	< 0.10	2.0	< 0.09
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Long term conc.	6700	---[c]	5.4	12	3.0	6.1	1.6	2.5	0.4	0.1	3.9	<MDL
Fortified spiked sample (% recovery)												
Minimum	98	92	107	108	94	66	86	74	79	98	102	104
Maximum	98	92	107	108	94	66	86	74	79	98	102	104
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nominal MDL	61	10	0.07	0.14	0.10	0.10	0.30	0.10	0.10	0.10	0.10	0.09
Nominal MQL	140	30	0.21	0.28	0.20	0.30	0.89	0.30	0.30	0.30	0.30	0.25

Appendix 4. Quality assurance, methods detection limit, and methods quantitative limit for organochlorine residues in whole-body fish composite samples.—Continued

[All concentrations are nanograms per gram wet weight; Sample sizes were $n = 5$ for all compounds. RSD, relative standard deviation; PCB, polychlorinated biphenyl; QCB, pentachlorobenzene; HCB, hexachlorobenzene; PCA, pentachloroanisole; HCH, hexachlorocyclohexane; NA, not applicable; MDL, method detection limit; MQL, method quantitative limit]

QA component	Compound										
	Dacthal	Dieldrin	Endrin	Oxychlordane	*cis*-Chlordane	*trans*-Chlordane	*cis*-Nonachlor	*trans*-Nonachlor	*o,p'*-DDE	*o,p'*-DDD	*o,p'*-DDT
Procedure blanks											
Minimum	0.03	0.02	0.00	0.00	0.05	0.06	0.00	0.03	0.47	0.00	0.00
Maximum	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.33	0.00	0.00
Mean	0.08	0.04	0.00	0.00	0.16	0.10	0.00	0.05	0.60	0.00	0.00
Matrix blanks											
Minimum	0.43	1.9	0.00	1.2	0.00	0.04	1.1	4.4	0.62	0.01	0.00
Maximum	0.43	1.9	0.00	1.2	0.00	0.04	1.1	4.4	0.62	0.01	0.00
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Triplicate sample analyses (% RSD)[a]											
Minimum	NA	25	NA	18	5	7	11	7	NA	5	NA
Maximum	NA	25	NA	18	5	7	11	7	NA	5	NA
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Reference material[b]											
Minimum	1.1	8.1	< 0.25	1.4	7.6	4.4	4.9	9.8	3.0	23	< 0.10
Maximum	1.1	8.1	< 0.25	1.4	7.6	4.4	4.9	9.8	3.0	23	< 0.10
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Long term conc.	5.0	19	4.4	4.0	23	11	12	25	6.1	52	2.0
Fortified spiked sample (% recovery)											
Minimum	93	98	37	93	97	89	105	88	106	91	148
Maximum	93	98	37	93	97	89	105	88	106	91	148
Mean	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nominal MDL	0.08	0.25	0.10	0.28	0.15	0.10	0.09	0.81	0.10	0.10	0.13
Nominal MQL	0.22	0.70	0.30	0.82	0.35	0.30	0.23	1.6	0.30	0.30	0.38

Appendix 4. Quality assurance, methods detection limit, and methods quantitative limit for organochlorine residues in whole-body fish composite samples.—Continued

[All concentrations are nanograms per gram wet weight; Sample sizes were $n = 5$ for all compounds. RSD, relative standard deviation; PCB, polychlorinated biphenyl; QCB, pentachlorobenzene; HCB, hexachlorobenzene; PCA, pentachloroanisole; HCH, hexachlorocyclohexane; NA, not applicable; MDL, method detection limit; MQL, method quantitative limit]

QA component	Compound							
	p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	Endosulfan I	Endosulfan II	Endosulfan sulfate	Methoxychlor	Mirex
Procedure blanks								
Minimum	1.4	0.04	0.14	0.00	0.00	0.02	0.00	0.00
Maximum	1.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	1.8	0.10	0.27	0.00	0.00	0.08	0.00	0.00
Matrix blanks								
Minimum	1.7	0.22	0.22	0.00	0.00	0.09	0.00	0.04
Maximum	1.7	0.22	0.22	0.00	0.00	0.09	0.00	0.04
Mean	NA	NA	NA	NA	NA	NA	NA	NA
Triplicate sample analyses (% RSD)[a]								
Minimum	22	7	NA	NA	NA	6.3	NA	NA
Maximum	22	7	NA	NA	NA	6.3	NA	NA
Mean	NA	NA	NA	NA	NA	NA	NA	NA
Reference material[b]								
Minimum	190	99	0.55	< 0.10	0.58	< 0.14	< 0.10	2.0
Maximum	190	99	0.55	< 0.10	0.58	< 0.14	< 0.10	2.0
Mean	NA	NA	NA	NA	NA	NA	NA	NA
Long term conc.	440	260	3.3	5.8	3.1	3.2	9.7	3.7
Fortified spiked sample (% recovery)								
Minimum	92	94	96	103	97	113	161	114
Maximum	92	94	96	103	97	113	161	114
Mean	NA	NA	NA	NA	NA	NA	NA	NA
Nominal MDL	2.4	0.18	0.47	0.10	0.10	0.14	0.10	0.10
Nominal MQL	4.6	0.52	1.2	0.30	0.30	0.42	0.30	0.30

[a]Percent relative standard deviation.

[b]Reference material was a Columbia Environmental Research Center positive control fish tissue, carp from Saginaw Bay ($n = 16$).

[c]Toxaphene has not been evaluated historically in this fish material.

Appendix 5. Analysis-of-variance results investigating the effects of various factors on biomarker responses in bass and carp

[Degrees-of-freedom (df), *F*-values with levels of significance (*, $0.01 < P \leq 0.05$; **, $P \leq 0.01$), and coefficients of determination (R^2) are presented. NA, not applicable; ND, not determined]

Variable, source, and (transformation)	Bass			Carp		
	df	*F*	R^2	df	*F*	R^2
Ethoxyresorufin *O*-deethylase (log)						
Model	42	3.80**	0.45	39	3.58**	0.45
Station	9	1.94*	NA	9	3.51**	NA
Gender	1	0.51	NA	1	1.86	NA
Station*Gender	6	0.91	NA	4	2.44*	NA
Reproductive stage	1	0.85	NA	1	8.43**	NA
Reproductive stage*Station	9	0.82	NA	9	2.21**	NA
Reproductive stage*Gender	1	0.02	NA	1	0.79	NA
Reproductive stage*Station*Gender	6	1.41	NA	4	3.33*	NA
Error	193	NA	NA	169	NA	NA
Condition Factor						
Model	42	2.47**	0.35	39	6.57**	0.60
Station	9	1.34	NA	9	3.38**	NA
Gender	1	1.28	NA	1	0.61	NA
Station*Gender	6	1.38	NA	4	0.07	NA
Reproductive stage	1	1.23	NA	1	0.06	NA
Reproductive stage*Station	9	0.34	NA	9	2.55**	NA
Reproductive stage*Gender	1	2.03	NA	1	1.26	NA
Reproductive stage*Station*Gender	6	1.23	NA	4	0.17	NA
Error	194	NA	NA	169	NA	NA
Splenosomatic Index						
Model	25	1.50	0.15	24	0.93	0.11
Station	12	1.91*	NA	12	1.40	NA
Gender	1	8.33*	NA	1	2.63	NA
Station*Gender	12	1.29	NA	11	0.33	NA
Error	211	NA	NA	183	NA	NA
Hepatosomatic Index						
Model	25	2.64**	0.24	ND	ND	ND
Station	12	4.02**	NA	ND	NA	NA
Gender	1	0.76	NA	ND	NA	NA
Station*Gender	12	1.49	NA	ND	NA	NA
Error	211	NA	NA	ND	NA	NA
HAI (rank)						
Model	42	4.98**	0.52	39	6.06**	0.58
Station	9	4.11**	NA	9	2.96**	NA
Gender	1	4.64*	NA	1	1.02	NA
Station*Gender	6	0.86	NA	4	0.49	NA
Reproductive stage	1	0.00	NA	1	0.91	NA
Reproductive stage*Station	9	2.02*	NA	9	2.09*	NA
Reproductive stage*Gender	1	2.51	NA	1	1.80	NA
Reproductive stage*Station*Gender	6	0.55	NA	4	0.56	NA
Error	194	NA	NA	169	NA	NA

Appendix 5. Analysis-of-variance results investigating the effects of various factors on biomarker responses in bass and carp.—Continued

[Degrees-of-freedom (df), *F*-values with levels of significance (*, $0.01 < P \leq 0.05$; **, $P \leq 0.01$), and coefficients of determination (R^2) are presented. NA, not applicable; ND, not determined]

Variable, source, and (transformation)	Bass			Carp		
	df	*F*	R^2	df	*F*	R^2
MA-% (log)						
Model	50	5.44**	0.60	47	4.91**	0.65
Station	12	2.55**	NA	11	1.56	NA
Gender	1	2.09	NA	1	0.88	NA
Station*Gender	11	2.14*	NA	10	1.04	NA
Age	1	20.23**	NA	1	0.03	NA
Age*Station	12	2.09*	NA	11	0.99	NA
Age*Gender	1	1.24	NA	1	5.49*	NA
Age*Station*Gender	11	2.36**	NA	10	1.24	NA
Error	182	NA	NA	124	NA	NA
MA-A (log)						
Model	50	3.44**	0.49	47	3.22**	0.55
Station	12	1.26	NA	11	0.61	NA
Gender	1	0.05	NA	1	0.62	NA
Station*Gender	11	0.91	NA	10	1.16	NA
Age	1	5.05*	NA	1	0.07	NA
Age*Station	12	0.85	NA	11	0.72	NA
Age*Gender	1	0.42	NA	1	5.02*	NA
Age*Station*Gender	11	1.00	NA	10	1.42	NA
Error	182	NA	NA	124	NA	NA
MA-#						
Model	50	4.02**	0.52	47	3.53**	0.57
Station	12	2.25*	NA	11	1.33	NA
Gender	1	2.11	NA	1	0.21	NA
Station*Gender	11	2.03*	NA	10	0.43	NA
Age	1	18.29**	NA	1	0.03	NA
Age*Station	12	2.56**	NA	11	1.56	NA
Age*Gender	1	0.06	NA	1	1.14	NA
Age*Station*Gender	11	2.74**	NA	10	0.38	NA
Error	182	NA	NA	124	NA	NA
Gonadosomatic Index						
Model	42	17.19**	0.79	39	9.95**	0.70
Station	9	0.81	NA	9	1.21	NA
Gender	1	10.11**	NA	1	0.08	NA
Station*Gender	6	1.16	NA	4	0.21	NA
Reproductive stage	1	10.63**	NA	1	27.25**	NA
Reproductive stage*Station	9	1.01	NA	9	1.07	NA
Reproductive stage*Gender	1	5.16*	NA	1	0.77	NA
Reproductive stage*Station*Gender	6	2.38*	NA	4	0.38	NA
Error	194	NA	NA	168	NA	NA

Appendix 5. Analysis-of-variance results investigating the effects of various factors on biomarker responses in bass and carp.—Continued

[Degrees-of-freedom (df), *F*-values with levels of significance (*, $0.01 < P \leq 0.05$; **, $P \leq 0.01$), and coefficients of determination (R^2) are presented. NA, not applicable; ND, not determined]

Variable, source, and (transformation)	Bass			Carp		
	df	*F*	R^2	df	*F*	R^2
Vitellogenin (log)						
Model	41	5.67**	0.56	39	40.60**	0.90
Station	8	1.14	NA	9	1.64	NA
Gender	1	0.08	NA	1	13.39**	NA
Station*Gender	6	0.48	NA	4	1.69	NA
Reproductive stage	1	4.34*	NA	1	10.26**	NA
Reproductive stage*Station	8	0.41	NA	9	1.89	NA
Reproductive stage*Gender	1	1.86	NA	1	4.14*	NA
Reproductive stage*Station*Gender	6	1.21	NA	4	1.78	NA
Error	184	NA	NA	168	NA	NA
17β-estradiol						
Model	41	6.76**	0.61	39	8.97**	0.68
Station	8	1.68	NA	9	0.52	NA
Gender	1	9.43**	NA	1	6.27*	NA
Station*Gender	6	1.29	NA	4	1.59	NA
Reproductive stage	1	0.26	NA	1	0.40	NA
Reproductive stage*Station	8	2.25*	NA	9	0.18	NA
Reproductive stage*Gender	1	0.01	NA	1	0.18	NA
Reproductive stage*Station*Gender	6	1.03	NA	4	0.29	NA
Error	176	NA	NA	168	NA	NA
11-ketotestosterone						
Model	41	9.87**	0.69	39	11.73**	0.73
Station	8	2.28*	NA	9	1.84	NA
Gender	1	28.36**	NA	1	14.89**	NA
Station*Gender	6	3.45**	NA	4	4.12**	NA
Reproductive stage	1	3.71	NA	1	0.10	NA
Reproductive stage*Station	8	1.27	NA	9	2.31*	NA
Reproductive stage*Gender	1	0.74	NA	1	0.48	NA
Reproductive stage*Station*Gender	6	1.74	NA	4	3.94	NA
Error	178	NA	NA	168	NA	NA
17β-estradiol/11-ketotestosterone						
Model	41	6.58**	0.61	39	4.39**	0.50
Station	8	1.47	NA	9	0.53	NA
Gender	1	17.76**	NA	1	0.86	NA
Station*Gender	6	1.11	NA	4	0.25	NA
Reproductive stage	1	0.01	NA	1	0.10	NA
Reproductive stage*Station	8	0.95	NA	9	0.77	NA
Reproductive stage*Gender	1	0.69	NA	1	0.77	NA
Reproductive stage*Station*Gender	6	0.25	NA	4	0.35	NA
Error	176	NA	NA	168	NA	NA
Atresia (females only)						
Model	21	2.82**	0.36	21	2.52**	0.40
Station	8	1.19	NA	8	2.57*	NA
Reproductive stage	1	0.72	NA	1	2.45	NA
Reproductive stage*Station	8	0.54	NA	8	1.37	NA
Error	124	NA	NA	81	NA	NA

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